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FOOD ING

1st INTERNATIONAL CONVENTION
FOOD INGREDIENTS: NEW TECHNOLOGIES

Fruits & Vegetables

15/16/17 SEPTEMBER 1997 - CUNEO - ITALY

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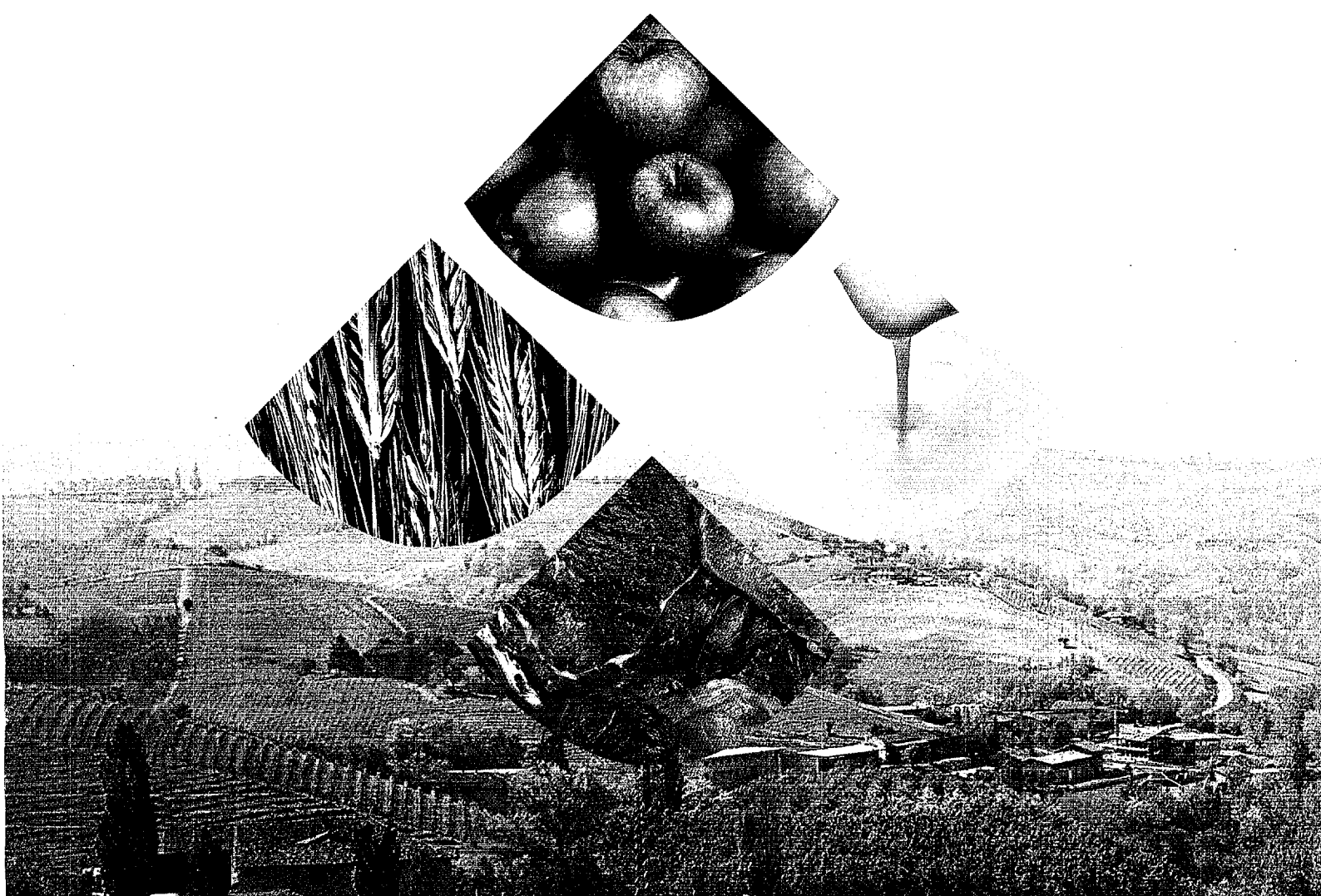

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I am glad to welcome and make my kindest wishes for an interesting and useful work to all the partecipants to "FOOD ING, 1st International Convention on Food Ingredients: New Technologies". In order to make the job easier and with the hope to be helpful, Allione Industria Alimentare S.p.A. is forwarding herewith a copy of the paperworks to be presented at the meeting.
I wish you all once more, best wishes for a nice job.

President FOOD ING
Adriana Allione

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Allione Ricerca Agroalimentare S.p.A. wishes to thank all the managers and technicians of the Allione Group for their involvement and kind help in the construction and realisation of the Research Center.

ENGLISH

FOOD ING

1st INTERNATIONAL CONVENTION
FOOD INGREDIENTS: NEW TECHNOLOGIES
Fruits & Vegetables

15/16/17 SEPTEMBER 1997 - CUNEO - PIEMONTE - ITALY

UNIDO
UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANISATION

programme at a glance

monday 15th of september 1997

09.00 OPENING CEREMONY AND OPENING SESSION

11.00 Effect of Cultural Practice on Quality of Harvested Fruit, Vegetables and Derivatives; Quality Control. Part 1

12.30 LUNCH

14.00 Effect of Cultural Practice on Quality of Harvested Fruit, Vegetables and Derivatives; Quality Control. Part 2

15.30 New Technologies for Packaging, Transport of Raw and Distribution of Fruit, Vegetables and Derivatives

17.45 CLOSE

20.00 GALA DINNER AND SHOW

tuesday 16th of september 1997

08.45 New Technologies for Processing and Preservation. Part 1: operations

10.30 New Technologies for Processing and Preservation. Part 2: operations

12.45 LUNCH

14.00 Development of New Processes and Products. Part 1: technologies

16.00 Development of New Processes and Products. Part 2: products

17.45 CLOSE

20.00 GALA DINNER AND SHOW

wednesday 17th of september 1997

08.45 Genetic Engineering: Advances, Needs of Food Industries & Social Concerns

11.45 New Technologies for Analysis/Description; Quality Control. Part 1

12.30 LUNCH

14.00 New Technologies for Analysis/Description; Quality Control. Part 2

16.15 CLOSE

16.30 TRANSFER MEETING CENTER "FONDAZIONE FERRERO ALBA"

17.30 Identifying the Important Economic Concerns of the Food Processing Industries World-wide as they prepare for the 21st Century

18.00 Examining World-wide Trends in Consumer Food Preferences and Needs in the 21st Century

18.30 Closing Session and Farewell

20.00 FAREWELL DINNER

programme

monday 15th of september 1997

09.00 - 11.00 OPENING CEREMONY: CENTRO INCONTRI, PROVINCIA DI CUNEO, CORSO DANTE N. 41, CUNEO (TEL. 0171/445460)

Welcome to Food Ing 97

Opening Ceremony Coordinator	*Giuseppe Grosso*
President of the Cuneo Province	*Giovanni Quaglia*
Mayor of Cuneo	*Elio Rostagno*
President of the Industrial Union of Cuneo	*Ottaviano Anselmino*
President of the Piedmont Region	*Enzo Ghigo*
UNIDO Director Agro-Industrial Branch	*A. R. Ben Brahim*
Agricultural, Forest and Food Resources Minister	*Michele Pinto*

11.00 - 12.30 EFFECT OF CULTURAL PRACTICE ON QUALITY OF HARVESTED FRUIT, VEGETABLES AND DERIVATIVES; QUALITY CONTROL. Part 1

Session Chairman: *W. Garrone (Dir. Assicurazione Qualità, Ferrero Italia S.p.A., Alba, ITALIA)*
Co-Chairman: *A.Mika (Res. Inst. of Pomology and Floriculture, Skierniewice, POLAND)*

11.00 - 11.30 **Plenary Lecture**
Fruit Quality and Orchard Management in the Integrated Fruit Production Pg. 1
P. Cravedi, Ist. di Entomologia, Univ. Cattolica di Piacenza, ITALIA
B. Marangoni, Dip. di Colture Arboree, Univ. di Bologna, ITALIA
G.Tonini, CRIOF, Univ. di Bologna, ITALIA

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Characterization of four Spanish Red Raspberry Cultivars for Industrial End-Use: Physico-Chemical and Chemical Aspects Pg. 9
M. P. Cano, B. De Ancos, E.M. Gonzales, Dept Plant Foods Science and Technology, Inst. del Frio, Ciudad Universitaria, Madrid, ESPAÑA
A. I. Olives, M. M. Camara, M. C. Matallana, Dept Food Science and Nutrition, Facultad de Farmacia, Ciudad Universitaria, Madrid, ESPAÑA
G. Reglero, Dept Caracterización, Inst. Fermentaciones Industriales, Madrid, ESPAÑA

tuesday 16th of september 1997

10.30 - 12.30 **NEW TECHNOLOGIES FOR PROCESSING AND PRESERVATION. Part 2: operations**

Session Chairman: *P. Cagnasso (Dir. Ricerca e Sviluppo Parmalat, Collecchio, ITALIA)*
Co-Chairman: *E. Drioli (Ist. di Ricerca su Membrane e Modellistica di Reattori Chimici, CNR, Univ. della Calabria, ITALIA)*

12.45 **LUNCH**

14.00 - 15.45 **DEVELOPMENT OF NEW PROCESSES AND PRODUCTS. Part 1: technologies**

Session Chairman: *C. Lerici (Dip. di Scienze Alimentari, Univ. di Udine, ITALIA)*
Co-Chairman: *G. Dall'Aglio (Stazione Sperimentale per l'Industria delle Conserve Alimentari, Parma, ITALIA)*

16.00 - 17.30 **DEVELOPMENT OF NEW PROCESSES AND PRODUCTS. Part 2: products**

Session Chairman: *E. Lavazza (Cav. del Lavoro, Presidente della Luigi Lavazza S.p.A., Torino e Presidente della AIIPA, Milano, ITALIA)*
Co-Chairman: *A. Amati (Ist. Ind. Agrarie, Univ. di Bologna, ITALIA)*

20.00 **GALA DINNER AND SHOW**

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Session Chairman: *W. J. Scholle (Managing Director of Scholle Corporation, Irvine, CA, USA)*
Co-Chairman: *J. Labavitch (Dept. of Pomology, Univ. of California, Davis, CA, USA)*

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S. Harlander, Green Giant Research and Development and Agricultural Research the Pillsbury Company, Minneapolis, USA

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A. Bennett, Vegetable Crops Dept., College of Agriculture and Enviromental Science, Univ. of California, Davis, CA, USA

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N.W. Kerby[1], R.J. McNicol[2], H.V. Davies[2], J.R. Hillman[1,2],
[1]Mylnefield Research Services Ltd, Dundee, SCOTLAND
[2]The Scottish Crop Research Institute, Dundee, SCOTLAND

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A. Pagella, Plasmon Dietetici Alimentari, Milano, ITALIA

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J. N. Labavitch, Pomology Dept., Univ. of California, Davis, CA, USA
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Co-Chairman: *G. Nano (Univ. di Torino, ITALIA)*

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Co-Chairman: *P. Sandra (Univ. of Gent, BELGIUM)*

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B) Inst. Fur Physikalische Und Theoretische Chemie, Tech. Univ. Munchen, GERMANY; C) Lehrstuhl Fur Okologische Chemie Und Umweltanalytik, Tech. Univ. Munchen, GERMANY; D) Nestlé Research Center, Losanna, SWITZERLAND

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S. Petersburg, RUSSIA

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B. W. Berman, Senior Agribusiness Specialist, Natural Resources Management Division, Country Dept. IV, Europe and Central Asia Region, World Bank, Washington, USA

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C. Bruhn (Consumer Food Marketing Specialist - Director of the Center for Consumer Research Univ. of California, Davis, CA, USA)

18.30 **Closing Session and Farewell**
A. Allione (Presidente di Food Ing - Membro del Consiglio di Amministrazione dell'Allione Industria Alimentare S.p.A. e Allione Ricerca Agroalimentare S.p.A., Tarantasca, ITALIA - Presidente del Consiglio di Amministrazione dell'Allione Central Europe Sp z o. o., POLAND)

20.00 **FAREWELL DINNER**

FRUIT QUALITY AND ORCHARD MANAGEMENT IN THE INTEGRATED FRUIT PRODUCTION

Cravedi Piero- Istituto di Entomologia -Università Cattolica di Piacenza, (Italy)
Marangoni Bruno- Dipartimento di Colture Arboree - Università di Bologna, (Italy)
Tonini Giuseppe- CRIOF- Università di Bologna, (Italy)

Summary

Fruit growers aimed for decades to achieve maximum yield pushing the use of chemicals to protect plants, to increase soil fertility and frequently disregarding the environment conservation and to assure healthy quality fruits. In recent years the concept of Sustainable Agricultural was adopted also in fruit crops and Integrated Fruit Production (IFP) guidelines have been suggested by National and International Scientific and Grower Associations. Orchard management is addressed to maintain technologies for storage and processing. The joint effort among Extension services, public research Institutions, Growers and Industry might be necessary to archieve a new way of sustainable fruit crops linked to the of fruit processing industry.

1- Introduction

The development of intensive fruticulture in the Western Countries has been oriented, till some decades ago, to maximize yield while not taking into account fruit quality and environmental aspects. High orchard productivity was pursued by increasing tree density, pushing chemical applications to improve soil fertility and the control of plant diseases. Also the market demand played an important role since the consumers preferred attractive fruits without considering their nutritional value, taste and healthiness. Marketing, skill farmers and fruit industry influenced the intensive frutculture area and fruits crops grown in specialized orchards were reduced, i.e. peach in Emilia-Romagna; apple in Trentino-Alto Adige, genotipic variability, germplasm and biodiversity. The prolonged presence of the same species and few varieties in the same environment led to the spread of diseases and enhanced the interaction among tree pathogens and pests.

The basic principles underpinning the interrelations between fruticulture and the environment can essentially be caracterized by the maintenance of own market orientation without depleting natural resources for future generations and following the new principle of Integrated Fruit Production (IFP).

The introduction of intensive fruit growing, exspecially in devoloping Countries, must be modelled on the specific conditions (e.g. socio-economic, soil climate, structural and political) in which orchard management is carried out in full recognition of local tradition and history.

Recently the integration of the fruit crops among the North and the South emisphere and the expanding world market system caused over production in Western Countries affecting also the availability of products for the fruit industry. This new world scenery showed take into account the mutual benefit between Farmers and Fruit Industry with the introduction of IFP and the important role of technical assistance to drive all the working phase in orchard management.

2- ORCHARD MANAGEMENT

The new perspective of the fruit crops induces a great evolution of tree and soil management in relation to the reduction of the prime cost, to improve the fruit quality and healthy and to preserve the natural environmental resources.

Tree training - Training systems and planting density focused on the concept of small tree size to permit the managing of plants from the ground. Small well-shaped trees allow a regular distribution of fruits inside the canopy, light and air circulation, as well as the chemical sprays should reach easily leaves and fruit surfaces. .A correct leaf density and training system is crucial to achieve the high photosynthetic efficiency, to improve yield quality and the resistance to plant diseases. Leaf density can be optimized by removing in summer vigorous unproductive suckers, by choosing suitable rootstock/variety combination and by exploiting natural the research such as temperature, solar radiation and rainfall (Deckers, 1992). Tree shape and pruning should be adapted to meet the demand of growers and industry to reach easily management and profitable results. However pruning, plant density and orchard modeling are tools to acquire a good quality of the fruit to make use for processing or fresh market and in relation to the consumer requests.

Plant nutrition and irrigation - Dressing schedules for fruit trees have formerly been based primarily on restitution of the individual minerals removed or, better, on estimations of crop uptakes over the growing season. The initial and residual supply of nutrients was not taken into account in this procedure. With developments of soil analysis data now able the amounts of available nutrients to show available nutrient supplies in the soil and the up take demands of the trees themselves, dressing strategies have subsequently focused on a nutritional budget distinguishing between supply and tree demand during the phenological stages. Orchard crops fertilization must take in to account that fruit trees stays on the same soil for a decades and that the permanent organs (e.g. roots, trunk, branches) can store reserves of mineral and organic nutrients in relation to their use under given enviromnental and plant growth conditions. The nutritional budget of fruit trees must also take into account nutrient uptake due to the annual yield as well as soil inputs from leaves, pruning wood fruit drop and, wherever practiced, grass mulching. The input for orchard crops, even those at high density, can be considerably reduced as the root system occupies a notable volume of soil and the plant itself can access the reserves stored in its woody organs. Effort to assess the nutritional status of orchards for timely and

directly targeted inputs (even micro) are increasingly focused on the analysis of tissues (leaves, petioles, plant parts) sampled at given dates and matched with corresponding soil data (Mengel and Kirby, 1987). The techniques just designed wich are capable to reduce nutrient inputs and enhancement of product quality, storage life and processing compatibility. There has also been a notable reduction of nitrogen's impact on the enviromnent (fig. 1) as to pollution of water tables in particular and of water resources in general.

Nitrogen and water supplies play an important role on vegetative growth and a correct fruit orchard management has to use them in correctly way. Excessive nitrogen fertilization in late spring are effective in promoting vegetative growth and shoots become dominant sinks in the competition with fruits. High rates of shoot growth increase the leaf density in canopy and decrease the fruit dry matter contents with negative effects on the quality, storage life and processing suitability. Also, the availability of new-growth watery tissues (leaves and shoots) becomes a target for insects and pathogens (Rease and Staiff, 1989). The lower flesh dry matter content and the high level of total nitrogen make fruits more susceptible to rot and storage diseases. At the same time excess of nitrogen reduce the thickness of fruit skin, that becomes a weaker barrier against pathogens and insects (Daane *et al.,* 1995). To avoid these problems, nitrogen fertilization should be supplied before and after the time of highest vegetative growth rate , and doses should never exceed tree requirements. Nitrates in soil solution contribute significantly to plant necessity and an accurate evaluation of N-NO3 availability could give helpful information about the time and the appropiate amount of fertilizer to be supplied (Tagliavini *et al.,* 1996). In apple and pear fruits high N/CA and K/CA ratio could promote physiological disorder such as *bitter pit* or rot fungus; calcium spray and a balanced soil application of nitrogen and potassium could be helpful to get a better quality and to reduce physiological fruit disorders.

A rational use of water may be an important tool in orchard management that contributes directly to the vegetative cicle of the tree and quality caracteristics of the products. The volume of water applied should be evaluated very carefully considering the tree performances, soil types, root density and climatic condititions expecially rainfall and depth of soil water table. In fruit trees an high supply of water, when the shoot growth rate is high as well, produces the same effect as previously described for nitrogen. Therefore, expecially in stone fruit species, it is suggested to impose a mild water stress during the fastest vegetative growth with the target to limitate the shoots development and reducing their competition toward fruit enlargement and buds differentiation.

3- DISEASES AND PESTS CONTROL

The production of high quality fruit involve a special attention to chemical residuals, heavy metal content, nitrate exceed and microbial toxins. Then it is necessary to reduce chemical residual and some natural substances also dangerous for health as well.

Biological productions are obtained without parasiticide but they do not assure the absence of dangerous natural substances. For this reason many food industry prefer integrated production with an Integrated Pest Management instead of biological ones.

This choice is coherent with the proposal of two of the greatest international scientific associations, the "International Organisation for Biological and Integrated Control of Noxious Animals and Plants" and the "International Society for Horticultural Science". Usually food industries fixt their parameters below the law provisions.

The use of monitoring method of the infestation and of parasiticide with a specific field-application need a cultural adaptation and a technical assistance that can not be find easily. The difficulty for food industries to find good products for processing should be overcome when the different European legislation on parasiticide will be regulated by the Directive 91/414/CEE and by the CE revision about the parasiticide actually sold. The major differences are in regard to the Extracomunitary Countries that must assure the food industries demands about the fruit quality for an healthy production.

4- POST HARVEST AND STORAGE

Post-harvest fruit quality is the primary objective for operators the fresh market (current or future, national, continental or extra-continental) and of the processing industry.

On the fresh market the task "more quality = more change to enlarge the market" is already achieved but it is not the same for the product processed and as a matter of fact, some of the fresh product rejected by consumers are used for processing. In the last decade the fruit and vegetable processing industry has developed the preparation of high quality product, like baby foods, reaching superior healthiness then the fresh ones. Therefore it is necessary to ensure fruit quality in the period between harvest and processing and, on the other side, to warrant a low level of pesticide and heavy metals in the fruits.

It is important to stress that all the orchard management has to be organised in order to obtain the highest fruit quality including harvest and transport of the product to the processing industry.

The final fruit quality depends on the level of ripeness when the fruits are picked. For this reason it is important to choose the right harvest-time. In fact if the pickers are unable to distinguish when the fruit are ready and/or make abrasion and wound on fruit (Tab. 1) the final product will

be of poor quality. The fruits have to be picked with the quality shown in Tab. 2 trying also to avoid wounds and scars especially if the product will not be processed.
If the harvested fruits are immediately marketed it's not necessary a treatment against desease and physiological desorder because the period between the harvest and the marketing is very short. If for some reasons (problem in the food industry) this period is prolonged further, it became very important to avoid the development of diseases, during the storage and the pre-transformation rest, like *Penicillium* spp., *Botrytis cinerea*, surface scald in pear and apple and rotting in peach, apricot, ect. Usually the fruit have to be sprayed in the field far from harvest. The pests must have a low residual referring to the processed product.
The fresh products for food industry can be immediately transformed or stored for long period. Good storage conditions like pre-cooling, controled atmosphere are very important for the final product quality. The technology used to preserve the fruit depends on the species and on the cultivar but also the storage has to be limited in length because fresh products are "alive" and can be stored in good condition according to the characteristic of the cultivar and with adequated parameters (Tab. 3).

5- CONCLUSIONS

The socio-economic changes of the last 20 years have significantly transformed the objectives and strategies of fruit production and had an enormous impact on the environment and its natural resources. There has been a gradual shift of emphasis from high yield to the quality of products and the effects of orchard management practices on the entire ecosystem. A decrease of pesticide sprays in fruit orchards could be more easily pursued if orchardist would understand the importance of "the biological equilibrium" concept, working to obtain and preserve it.
The development of integrated fruit production, including post-harvest and storage, has proved itself capable of maintaining yield, enhancing fruit quality and healthness and reducing cost outlays.
The agreement between Fruit Industry and Growers, with the support of Extension Service and Public Research Institutions, might be useful to achieve the goal of high quality level of processed fruit and enviromental preservation.

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Tab. 1 - Peaches and nectarines: harvest injury (%) (*)

Peaches				Nectarines			
Cultivar	Destalked (%)	Harvest injury (%)	Total injury (%)	Cultivar	Destalked (%)	Harvest injury (%)	Total injury (%)
Springcrest	11.8	2.9	14.7	Maygrand	2.5	1.1	3.6
Redhaven	2.1	2.8	4.9	Weinberger	1.0	7.0	8.0
Roza	1.0	2.0	3.0	Claudia	7.0	7.0	14.0
July Lady	28.0	5.0	33.0	Maria Laura	3.5	11.5	15.0
Glohaven	17.5	11.0	28.5	Indipendence	3.0	1.0	4.0
Maria Marta	2.5	6.5	9.0	Early Sungrand	1.0	5.5	6.5
Elegant Lady	27.0	3.0	30.0	Spring Red	2.0	2.7	4.7
K2	41.0	2.0	43.0	Super Star	13.0	0.0	13.0
Fayette	33.6	3.5	37.1	Stark Red Gold	7.4	5.7	13.1
Michelini	29.0	5.0	34.0	Caldesi 2010	27.0	3.0	30.0
Duchessa d'Este	36.0	4.0	40.0	Venus	5.5	6.5	12.0

(*) Multiyears average values from Romagna warehouses. (Cravedi *et al.*, 1995, modified)

Tab. 2 - Fruit for processing: harvest index advised for storage

Species and cvv	Firmness (kg)	Species and cvv	Firmness (kg)
<u>Apples</u>		<u>Pears</u>	
Golden Delicious (group)	6.5 ± 0.5 (1)	Conference	5.5 ± 0.5 (2)
Granny Smith	7.5 ± 0.5 (1)	Guyot	6.5 ± 0.5 (2)
Red Delicious (group)	6.5 ± 0.5 (1)	Passa Crassana	6.3 ± 0.5 (2)
Rome Beauty (group)	7 ± 0.5 (1)	William	6.5 ± 0.5 (2)
<u>Peaches and nectarines</u>	5 ± 0.5 (2)		

(1) Tip 11mm; (2) Tip 8mm.

Tab. 3 - Fruit for processing: storage requirements

Species and cvv	Temp (°C)	O_2 (%)	CO_2 (%)	Storage life	Species and cvv	Temp (°C)	O_2 (%)	CO_2 (%)	Storage life
Apples					Pears				
Golden D. (group)	1	-	-	5-7 m	Conference	-0.5	-	-	6-7 m
"	1	1	2	8-9 m	"	-0.5	2	0.8	7-8 m
"	1	2-3	2	7-8 m	"	-0.5	5	2	7-8 m
Granny Smith	0	-	-	5-6 m	Guyot	-0.5	-	-	15-20 d
"	0	1	1	8-9 m	Passa Cr.	-0.5	-	-	4-5 m
"	0	2-3	2	7-8 m	"	-0.5	2-3	5	4-5 m
Red D. (group)	0	-	-	6-7 m	William's	-0.5	-	-	2-3 m
"	0	1	1	8-9 m	"	-0.5	2-3	5	3 m
"	0	2-3	3	7-8 m	Apricot	0	-	-	10-15 d
Rome B. (group)	1	-	-	6-7 m	Nectarine	0	-	-	15-20 d
"	1	2-3	2-3	7-8 m	"	0	1.5-2	8	20-30 d
Peach (*)	0	-	-	10-15 d	Plum	0	-	-	15-30 d
"	0	1.5-2	5	15-30 d	" europ.	0	1.5-2	8	30-60 d

R.H. - For all the cvv >90%. m=months; d=days (*) freestone and clingstone

Fig 1 Soil trend of nitrate on peach orchards managed with traditional and integrated sistem

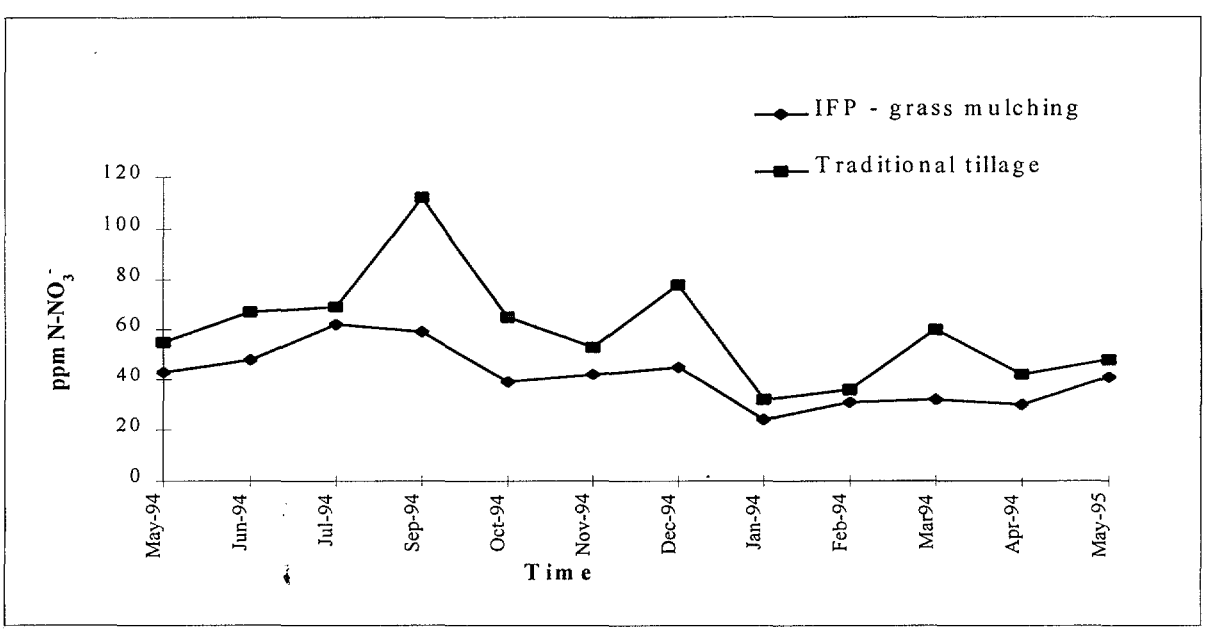


Fig 2 Total application of pesticides (Kg/ha) on peach orchards - cv Nectaross

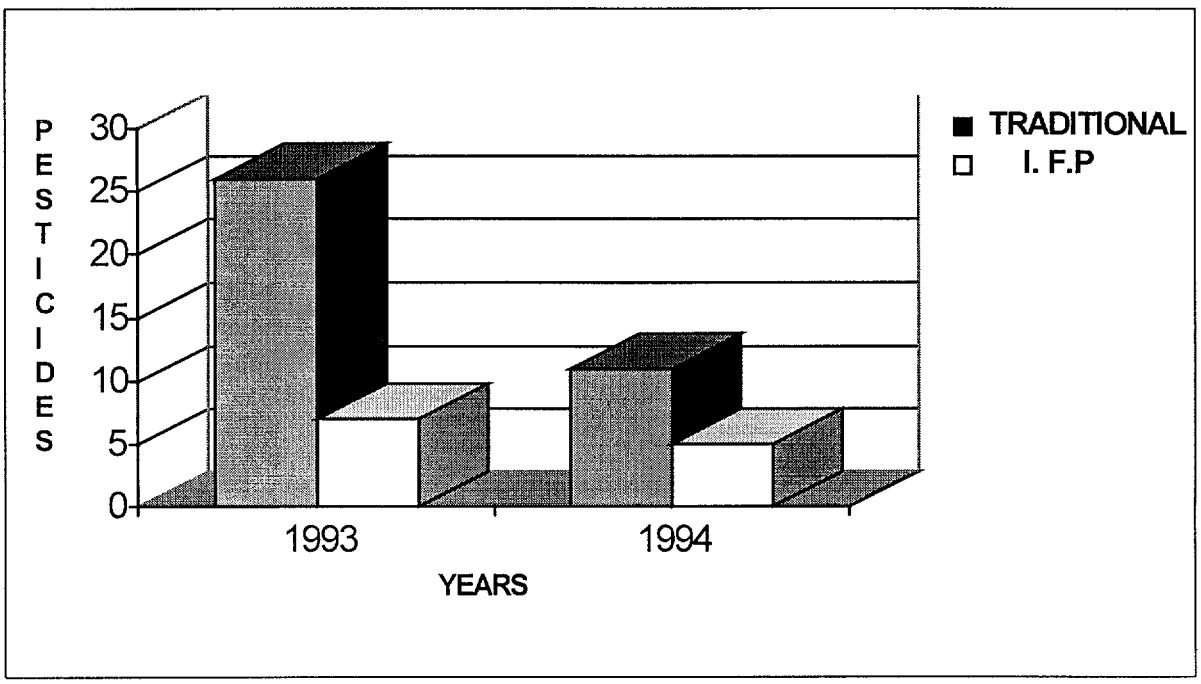
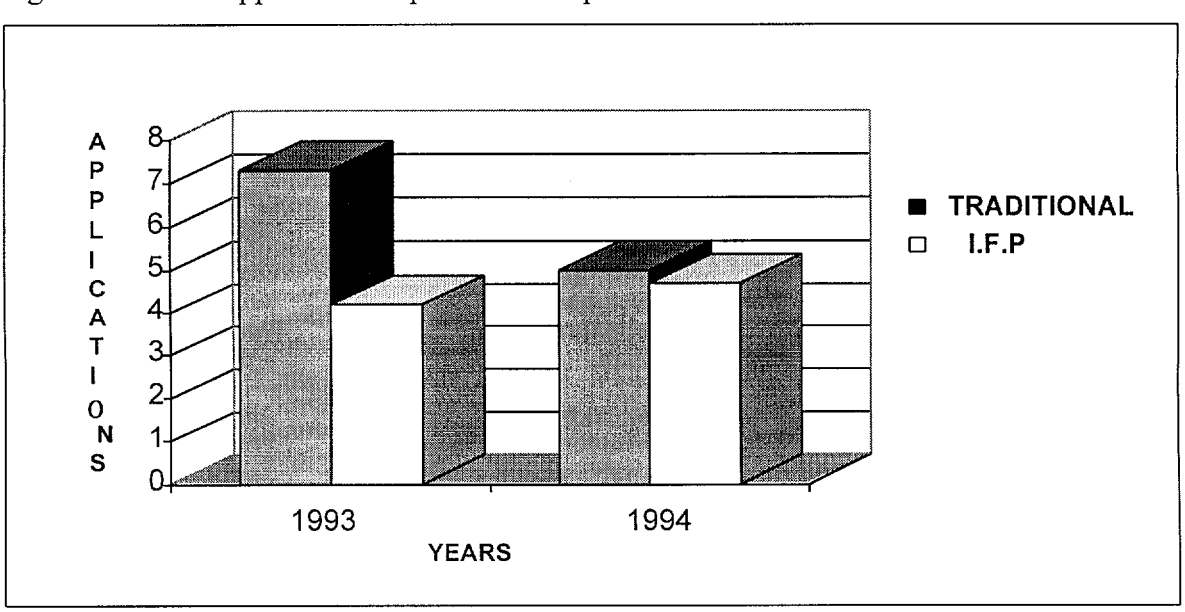


Fig 3 Number of application of pesticides on peach orchards - cv Nectaross



CHARACTERIZATION OF FOUR SPANISH RED RASPBERRY CULTIVARS FOR INDUSTRIAL END-USE: PHYSICO-CHEMICAL AND CHEMICAL ASPECTS

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Four spanish raspberry (*Rubus idaeus*, L.) cultivars: Autum Bliss, Heritage, Rubi and Ceva, were studied in order to determine the factors that could severely afect their suitability to freezing process or to their industrial end-use quality. Physico-chemical parameters (moisture content, soluble solids, acidity, pH) together with other physical ones (color, weight, texture) were studied. The main quality aspect to the industrial raspberry suitability were the differences found between cultivars in terms of chemical and biochemical parameters, which could be related to sensorial appreciation of fruits. Differences in terms of anthocyanins content (HPLC), aroma compounds (GC-ES), peroxidase and poliphenol oxidase (enzymes related to colour modifications), and micronutrients (total minerals, vitamins) were evaluated in order to select the most suitable cultivars to processing.

INTRODUCTION

Raspberries (*Rubus idaeus*) is the common European raspberry fruit, but it has largely been replaced in America by R. *strigosus*. In addition, the black raspberry R. *occidentalis* and to a lesser extent the yellow raspberry developed from the American red raspberry are also cultivated. Leinbach et al.[1] found 68-108 seeds per berry in red raspberries, indicating the number of drupelets on a plug. In contrast to the blackberry, the berries of raspberry can be readly separated from the receptacle when ripe. Raspberries are a very perisable commodity, partly due to high respiration and transpiration rates, a morphology that predisposes them to crushing, and suceptibility to gray mold fruit rot. Raspberries are infected with all fungal diseases affecting other berries and small fruits caousing serious postharvest losses..

Frozen fruits constitute a large and important food group in modern society. Fruit may be more extensively used if available during the off-season. In addition, frozen fruit can be transported to remote markets that could not be accessed with fresh fruit. Freezing also makes year-round futher processing of fruit products, such as jams, juice, and syrups from frozen whole fruit, slices, or pulps possible[2]. Factors such as variety, maturity, growing area, and seasonal variations influence frozen processing perfomance of fruits to an extend that may override the positive effect of a high freezing rate. Bushway et al.[3] evaluated five raspberry cultivars harvested at the red-ripe stage at harvest and during frozen storage at -20°C. In this study a significant decrease in shear values (firmness) for all raspberry varieties during 9-months storage period, corresponding to less firm fruits was observed.

However, there was not literature about the characterization of raspberry cultivars for processing employing the novel availabe analysis techniques (HPLC, GC-EM) for the evaluation of the most important raspberry constituents related to sensorial appreciation of the fruits. In the present work together with these parameters, the activities of the enzymes related to colour modification (polyphenol oxidase and peroxidase) were also characterized in order to obtain a most complete picture of raspberry tissue composition and suitability to processing.

MATERIALS AND METHODS

RAW MATERIAL

Raspberry fruits (*Rubus* idaeus, L.) of different cultivars (cvs. Autum Bliss, Heritage, Rubi and Ceva), were obtained from commercial orchards in the region of Valle del Jerte, (Cáceres, Spain), and they were brought to Instituto del Frío within 12 h after harvest. On arrival, undamaged fruits were selected, and 1 Kg of each cultivar

was homogeinized using a blender jar (Osterizer) and the pulps obtained were used inmediatelly for physical and physicochemical determinations. Another 1Kg of each cultivar was inmediatlly frozen in liquid nitrogen and stored at -70°C until chemical and biochemical analysis were carried out.

PHYSICAL PARAMETERS

Colour

Colour of fruit pulp was measured with a HunterLab D25 A-9 Tristimulus Colorimeter. A Standard white plate (No. C2-19913), having reflectance values of X=82.51, Y=84.46 y Z=101.23, was used as reference. Raspberry puree was placed on the light port using a 5 cm diamenter plastic dish with cover. Each value represents a mean of triplicare determinations of three different samples. Colour was measurement using the CIE-L*a*b *colour values, as L* (lightness), a* (+a is red, -a is green) and b* (+b is yellow , -b is blue) .Hue angle was calculted fron h=arctan b*/a* and Chroma (C) from $[(a^*)^2 + (b^*)^2]^{0.5}$

Weight

Fruit weight were carefully determinated using a sample size of 30 fruits of each cultivar.

PHYSICO-CHEMICAL PARAMETERS

pH and titratable acidity

Ten grams of raspberry pulp were minced and blended with 40 mL of deionized water in a Sorvall Onmimixer. The pH was measured at this temperature with a Crison pH-meter. After determination of the pH, the solution was titrated with 0.1 N NaOH up to pH 8.1. The results were expressed as percentage citric acid (g citric acid per 100 g FW)[4].

Soluble Solids

Soluble solids were measured with an Atago digital refractometer dbx-30 at 20°C. Results were reported as degrees Brix.

BIOCHEMICAL PARAMETERS

Enzyme extraction

The enzyme extracts for determination of peroxidase (POD) and polyphenol oxidase (PPO) were made by homogenization of 10g of each sample with 40 mL of 0.05M sodium phosphate buffer (pH 7.0) [containing 4% (w/v) insoluble polyvinylpolypirrolidone (PVPP) and 1% (v/v) Triton X-100) in an ultrahomogenizer (Omnimixer, mod. ES-207, Omni International, Inc, Gainsville, VA, USA) with external cooling, for 2 min. The homogenate was allowed at 25 °C during 1 hour with continously shaking and then was centrifugated at 8.000 g at 4°C for 15 min. The supenatant was filtered throught 6 nylon cloths and the obtained volume was carefully measured.

POD activity determination

Peroxidase activity was assayed spectrophotometrically using aliquots (0.300 mL) of extract and a reaction mixture composed of 2.4 mL 0.2M sodium phosphate buffer (pH 5,5) with 0.2 mL 1% (w/v) o-dianisidine as H-donor and 0.1 mL 1,5% (w/v) hydrogen peroxide as oxidant. The oxidation of o-dianisidine was measured using a double beam spectrophotometer (Perkin Elmer, mod.Lambda 15, Bodenseewerk, FRG) at 485 nm at 25°C.

PPO activity determination

Polyphenol oxidase activity was assayed using aliquots (0.3 mL) of extract and 2.7 mL of a solution 0.1M catechol in 0.2M sodium phosphate buffer (pH 5.5). The reaction was measured with the spectrophotometer at 420 nm at 25 °C. All enzyme activities were determinate by measuring the slope of reaction. The enzyme activity unit was defined as the change in absorbance/min/g fresh wt of sample.

Protein content

Soluble protein concentraton in all extracts was determined employing a Bio-Rad kit for the Bradford reaction[5] , with bovine serum albumin as standard.

CHEMICAL PARAMETERS

Vitamin C

Vitamin C was analysed by two methods (HPLC analysis with fluorimetric detection or fluorimetric method).Sample preparation was almost the same for the two methods. HPLC method (fluorimetric detection) was described by Speek[10], The emission at 425 nm with excitation at 355 nm were employed to HPLC-fluorimetric detection (HPLC apparatus described in anthocyanin analysis section). The fluorimetric method was described by Brubacher[11] and fluorophor had activation max at 350 nm and fluorescence max at 430 nm (Perkin-Elmer fluorimeter mod. LS3). Fluorescence intensity was proportional to concentration.

Total minerals

For total mineral analysis, a portion of fresh sample was freezedried and homogeneized. Aliquots were analysed for mineral content by dry ash technique. Samples were incinerated at temperature lower than 450°C until white ashes[6].

HPLC Anthocyanin Analysis

The sample preparation and HPLC separation and identification were carried out according to the procedure described by Hong and Wrolstad[7] with minor modifications.

Extraction of anthocyanins.

Raspberry sample (10 g) was hogeinized with 100 mL o 1% HCl in methanol. The slurry was filtered, and the solids were washed with an additional 100 mL of 1% HCl in methanol. The methanol extracts were combined and concentrated to ca. 5 mL in a rotary evaporator (30°C). The aqueous extract was placed in a volumetric flash and up to 50 mL with 0.01% HCl solution. The aqueous solution of anthocyanins (5 mL) was adsorbed onto an activated C-18 Sep-pack cartridge (Water Associates, Milford, MA) , activated with 3 mL of methanol and 3 mL of 0.01% HCl. The pigments adsorbed onto the cartridge were eluted withe 0,01% HCl in HPLC grade methanol. The solution was evaporated to dryness in a rotary evaporator and the extract were dissolved in 4% phosphoric acid and filtered through a 0.45 mm filter and infected 20 µl in HPLC system. All the analysis were performed in duplicate. The separation of anthocyanins was carried out by HPLC and the identification was performed by the analysis of spectra properties by an on-line photodiode array detector. Total pigment concentration was made using cyanidin-3-glucoside, previously separated by TLC, as an external standard. A standard curve was prepared by plotting different concentrations of cyanidin-3-glucoside versus area measurements in HPLC.

Apparatus

A Hewlett-Packard 1050 quatenary solvent delivery equipped with a Hewlett-Packard 1040A rapid scanning UV-visible photodiode array detector was employed. The data were stored and processed by means of a Hewlett-Packard Model 9000/300 computing system and Colour Pro-plotter. The absortion spectra of the pigments was recorded between 300 and 600 nm at the rate of 12 spectra/min. The HP-9000 computer with a built-in integration program was used to evaluate the peak area and peak height. Column separation was performed on a stainless stees (250 X 4 mm) ODS-Hypersil (5µm spherical particles) (Hewlett-Packard).

Chromatographic Procedures

Solvent A was 4% phosphoric acid and solvent B was 100% acetonitrile, and the program began with isocratic elution with 6% B from 0 to 10 min, and then a liear gradient to 20% of B from 10 to 55 min, and finally an isocratic elution at 20% of B from 55 to 60 min. Flow rate was 1.0 mL/min and the runs are monitored at 520 nm.

Total anthocyanin content

Total content of anthocyanins was evaluated by addition of the individual anthocyanin concentrations using cyanidin-3-glucoside as standard, or by spectrophometric measurement of the total anthocyanin extracts at 535 nm, using also cyanidin-3-glucoside ($\varepsilon = 29{,}000$).

Aroma analysis

Fresh raspberry samples of different cultivars were used to perform the study. 3 g of fruit puree were placed in a

20 mL vial and extracted by suing the SPME device during 30 minutes at 30°C. The SPME holder (Supelco, Bellefonte, PA, USA) was used to perform the experiments. A fused silica fiber coated with a 100μm layer of dimethylpolysiloxane was chosen to extract the volatile components of the raspberries.

Instrumental analysis of volatile compounds

A Perkin elmer Model 8500 gas chromatograph equipped with a PTV injector and FID detector was used to perform the analysis. The system was coupled to a Model 2600 chromatography software system (Perkin Elmer Nelson Analysitical). A 50m x 0.25 mm i.d. fused-silica capillary column (Chrompack, Middelburg, the Netherlands) coated with a 0.25μm layer of CP-Sil-5 CB was used. Helium was the carrier gas. Thermal desorption of the compounds in the fiber took place in the GC injector at 200°C for 15 min in splitless mode for 5 min. The detector operated at 250°C. The oven temperature was programmed from 50°C (3 min constant temperature) to 250°C at 5°C/min. The final temperature was maintained for 17 min.

GC/MS analysis was carried out by coupling the gas chromatograh described above to a Perkin Elmer ITD-50 ion trap detector (EI 70 eV). Capillary column and chromatographic program used as mentioned previously. Compounds were identified by comparison of the spectra with those in a general purpose library. Moreover, the identity of the components was confirmed by matching their mass spectrometric data with those obtained from the same equipment and corresponding to authentic reference compounds.

DATA ANALYSIS

Data were statistically analysed by an analysis of variance (ANOVA) and mean separation was by Duncan's multiple range test at $p \leq 0.05$, using the INSTAT program. Significant differences were indicated by different letters in the same row.

RESULTS AND DISCUSSION

Spanish raspberries, cvs. Autumm Bliss, Heritage, Rubi and Ceva, showed the physical and physicochemical characteristics showed in Table 1. There was not significant differences in raspberry fruit weigth, being 2.95g the media between cultivars. Fruit colour determined by objective CIELAB parameters, showed that Autumm Bliss and Heritage raspberries are the most luminous (higher L values). The darker ones are Ceva and Rubi cultivars. The L* value was reported that correlated well with the senssory depth of colour assessment. Differences in C reflect a bias towards the dominant colour component (a* or b*). In the present study , only Ceva raspberry cultivar exhibited a significant lower Chroma parameter (37.51), which indicated that these fruits were less reddish.

Table 1. Physical and physicochemical characteristics of spanish raspberry fruit cultivars

Characteristics	Raspberry cultivars			
	Autumm Bliss	**Heritage**	**Ceva**	**Rubi**
Fruit weight (g)	3.08±0.59a	2.08±0.29b	3.02±1.14a	2.53±0.65a
Colour				
L	25.89±2.04a	25.80±1.52a	18.29±0.42b	21.26±0.58ab
a*	35.03±0.70a	34.98±0.58b	33.03±0.50c	35.10±0.40d
b*	19.05±1.87a	18.34±2.42b	17.78±1.40c	18.63±2.73d
Angle Hue (h)	31.77±1.03a	30.74±1.8b	31.43±1.00a	31.06±1.56a
Chroma (C)	39.87±1.5a	39.50±1.8a	37.51±0.77b	39.74±1.23a
pH	3.65±0.1a	3.87±0.02b	2.88±0.02c	2.65±0.01d
Titratable acidity (g citric acid/100 g f.w.)	1.67±0.01a	1.76±0.01a	1.75±0.05a	2.32±0-12b
Soluble solids (Brix degrees at 20°C)	9.26±0.14a	9.80±0.06b	10.54±0.05c	10.00±0.09b
Moisture Content (g/100 g f.w.)	84.77±0.11a	85.31±0.63a	83.67±1.53b	82.02±3.01c
Total solids (g / 100 g f.w.)	15.23±0.02a	14.69±0.11a	16.33±0.30b	17.98±0.66c

Different letter in the same row indicate significat differences ($P \leq 0.05$); f.w. fresh weight; Values are the mean (±SD) of three determinations.

Titratable acidity and pH of values raspberry cultivars (Table 1) indicate no signifiacnt differences between cultivars, only Rubi fruits exhibited a higher acidity. However, these results were not reflect in their pH's values; being the pH of Heritage cultivar the highest one. Related to soluble solids, the Ceva and Rubi cultivars seemed to be the most sweet raspberry fruits attending to this parameter; meanwhile Autumm Bliss and Heritage showed the lowest soluble solids values. Again, the two raspberry early cultivars, Autumm Bliss and Heritage content the greater moisture content, while Ceva and Rubi fruits showed the lower and significative different values between them.

Chemical and biochemical characteristics of raspberries are showed in Table 2. Total anthocyanins were expressed as mg of cyanidin-3-glucoside/ 100 g fresh weight. cyanidin-3-sophoroside is the most abundant anthocyanin compound in raspberry fruits. In this work, the total anthocyanin content was determined by two methods: by addition of individual content of each anthocyanin compound analysed by HPLC, or by spectrophotometric measurement of the extract using the ε=29,000 of cyanidin-3-glucoside. Similar results were obtained using the two mencioned methods. Autumm Bliss raspberry showed the lower anthocyanin content (31.13 mg cy-3-glu/100g f.w.) followed by Heritage, Rubi and Ceva. The two raspberry cultivars, Ceva and Rubi, which were harvested in Autumm (november) showed the greater amounts of anthocyanins.

Vitamin C is other important parameter of raspberry quality, not only by nutritional point of view, also by the its antioxidant fuction which protect the anthocyanin degradation. In this sense, Rubi was the raspberry cultivar which showed the higher vitamin C content (31.14 mg ascorbic acid/100 g fresh weigth). Autumm Bliss and Ceva cultivars were not differ signifiicnaly attending to this parameter; and Heritage fruits content the lower amount of vitamin C. Fluorimetric determination of vitamin C rended lower values for all samples.

Total mineral content also is a very important nutritional parameter. In raspberry fruits Autumm Bliss cultivar was the richest one in terms of minerals, meanwhile the other three raspberry cultivars showed values of 0.43-0.47 g/100 g fresh weight.

Biochemical parameters as polyphenol oxidase (PPO) and peroxidase (POD) activities are very important regarding to colour stability of these fruits submitted to freezing preservation. Peroxidase (POD) was not present in raspberry tissues in quantity to represent a processing problem for frozen storage stability. However, polyphenol oxidase (PPO) represented the most important oxydoreductase in raspberry tissues. Autumm bliss and Ceva fruits showed the higher PPO activity, followed by Heritage and Rubi raspberries. This parameter together with the presence of ascorbic acid and the total content of anthocyanins could be an important factor to expect the severity of colour modification of frozen fruits during storage. Ceva raspberries showed the greater amount of total anthocyanins and also a high content of vitamin C. For this reason, in spite of the high PPO activity of Ceva tissue, the darkening due to anthocyanin degradation will be very low. In a similar way, Autumm Bliss cultivar which showed the lower anthocyanin content together with the same value of vitamin C that Ceva fruits, could suffer a severe darkening due to its high PPO enzimatic activity (Table 2). Rubi cultivar showed the grater protein content, while its PPO activity was the lower one.

Table 2. Chemical and biochemical characteristics of raspberry fruit cultivars

Characteristics	Raspberry cultivars			
	Autumm bliss	Heritage	Rubi	Ceva
Total anthocyanins* (mg cy-3-glu/100g f.w.)	31.13±098a	35.91±1.51b	96.08±2.02c	122.88±1.46d
Total anthocyanins** (mg cy-3-glu/100g f.w.)	32.59±0.08a	45.24±0.25a	98.72±4.30b	85.48±3.26a
Vitamin C* (mg ascorbic acid/100g f.w.)	29.36±1.11a	21.26±2.06b	31.14±1.92c	29.28±0.30a
Vitamin C** (mg ascorbic acid/100g f.w.)	19.29±0.01a	14.69±1.51b	15.11±0.72b	14.71±0.71b
Total minerals (g/100g f.w.)	0.54±0.02a	0.46±0.02bc	0.43±0.02b	0.47±0.01c
Proteins (μg/g f.w.)	82.24±10.46a	97.38±3.58ab	111.76 ±9.08b	97.60±0.18ab
PPO activity (O.D/min/g f.w.)	1.19±0.006a	0.83±0.006b	0.64±0.01b	1.21±0.05a
POD activity	ND	ND	ND	ND

Different letters in the same row indicate significant differences ($p \leq 0.05$);f.w., fresh weight; Values are the mean (±SD) of three determinations;* HPLC determination;** Total spectrophotometric or fluorimetric determination

Table 3 shows the anthocyanin patterns of the extracts obtained from the four studied cultivars. Also, Figure 1 represents a view of the four HPLC chromatograms, one for each cultivar. In all samples, cyanidin-3-sophoroside was the main anthocyanin compound identified in raspberry extracts. Other cyanidin-derivative, the cyanidin-3-glucoside was the second largest compound. Cyanidin-3-glucoside together with, cyanidin-3-sophoroside were most than 60% of the total anthocyanin content for all raspberry cultivars. Rubi and Ceva raspberries showed the greater amounts of cyanidin-3-glucoside (23.67 and 25.12 mg cy-3-glu/100g f.w., respectiviely); while Heritage (14.00 mg cy-3-glu/100g f.w.) and Autumm Bliss (10.53 mg cy-3-glu/100 g f.w.) showed the lower values of this compound. Cyanidin-3-sophoroside was the main anthocyanin in Ceva cultivar (63.86 mg cy-3-glu/100g f.w.), followed by Rubi raspberries with 55.77 mg cy-3-glu/100g f.w. the cultivar with low amount of cyanidin-3-sophoroside was the Autumm Bliss. These facts were related to the total anthocyanin content of raspberry cultivars, due to the main contribution of these two compounds.

Other minor anthocyanin components could be observed in the HPLC chromatograms, Figure 1. Cyanidin-3-derivatives (two), cyanidin-3-glucorutinoside, cyanidin-3-rutinoside, and pelargonidin-3-sophoroside, pelargonidin-3-glucorutinoside, pelargonidin-3-glucoside, together with low amounts of malvidin-3-glucoside and delphinidin-3-glucoside made up the anthocyanin pattern of raspberry fruits. Heritage cultivar showed the most simple anthocyanin pattern; and Ceva raspberries the most complicated one due to the presence of the more than seven minor compounds. In conclusion, the anthocyanin pattern of raspberries is composed by two main cyanidin componunds (cy-3-sophoroside, and cy-3-glucoside) and other minor compounds where the glicoside forms are glucoside, rutinoside, glucorutinoside and sophoroside.

Aroma of fresh raspberry are composed by a complex mixture of volatile compounds as can be observed in Figure 2. Table 4, shows the percent of the most abundant volatile compounds which represent the raspberry aroma. Linalol and terpinolene are the most important aroma compounds in all four cultivars. Heritage and Rubi showed a 5.75 % and 5.95% of linalol, followed by Ceva (3.4%) and Autumm Bliss (2.08%). Terpinolene is the main volatile compound in Heritage raspberries; meanwhile this compound only represents a 1.5% in Ceva cultivar. Aldehydes, as citral, also contributes to the aroma of raspberry, being most abundant in Heritage and Rubi cultivars. Cariophyllene and β-ionone represent other two important aroma compounds, being β-ionone the compound which characterize better the raspberry aroma.

CONCLUSION

Heritage and Autumm Bliss seemed to be the most resistant and suitable to freezing preservation, after the freezing studies carried out with these four raspberry cultivars. These conclusion was made taking into consideration fruit firmness, sugar/acidity ratio, colour maintenance and fresh fruit aroma.

Table 3. Anthocyanin pattern of Spanish raspberry cultivars

Anthocyanin (mg cy-3-glu/100g f.w.)	**Raspberry cultivars**			
	Autumm bliss	**Heritage**	**Rubi**	**Ceva**
cyanidin-3-derivative	5.28	ND	ND	ND
cyanidin-3-derivative	6.27	ND	ND	ND
cyanidin-3-sophoroside*	9.05	21.91	55.77	63.86
cyanidin-3-glucorutinoside	ND	ND	ND	11.58
pelargonidin-3-sophoroside	ND	ND	8.77	5.21
cyanidin-3-glucoside*	10.53	14.00	23.67	25.12
pelargonidin-3-glucorutinoside	ND	ND	ND	2.49
cyanidin-3-rutinoside	ND	ND	ND	6.22
pelargonidin-3-glucoside	ND	ND	4.23	2.67
malvidin-3-glucoside	ND	ND	3.64	2.85
delphinidin-3-glucoside	ND	ND	ND	2.88
TOTAL	31.13	35.91	96.08	122.88

* Main anthocyanin compounds

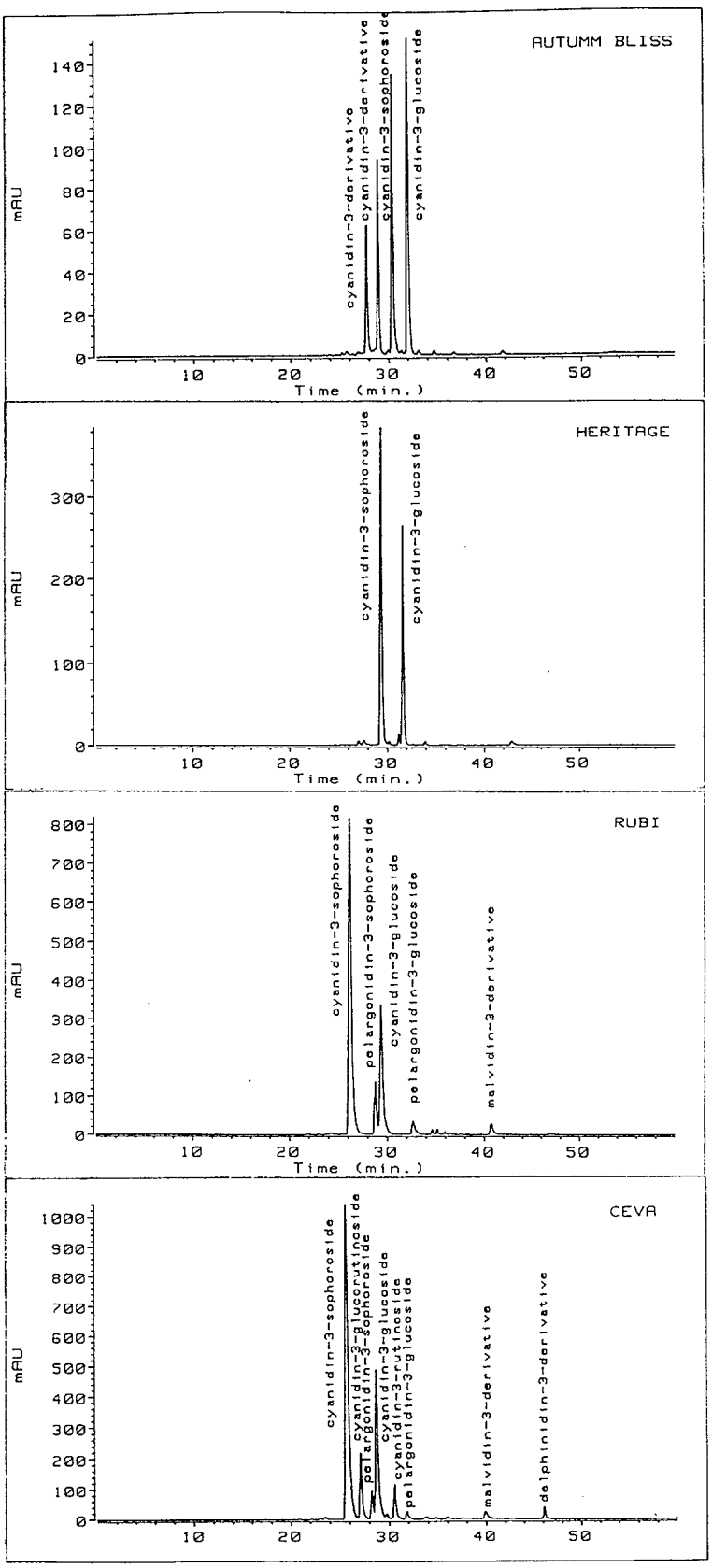

Figure 1. HPLC chromatograms of anthocyanin extracts from raspberry fruits

Table 4. Aroma compounds of spanish raspberry cultivars

Compound (%)	Raspberry cultivars			
	Autumm Bliss	**Heritage**	**Rubi**	**Ceva**
Linalol	2.08	5.75	5.95	3.40
Citral	0.23	0.69	0.70	0.30
Linalyl acetate	0.08	0.37	0.33	0.15
Terpinolene	3.36	5.86	2.70	1.50
Cariophyllene	0.41	0.31	0.74	0.30
β-inonone	0.24	1.05	2.03	1.18

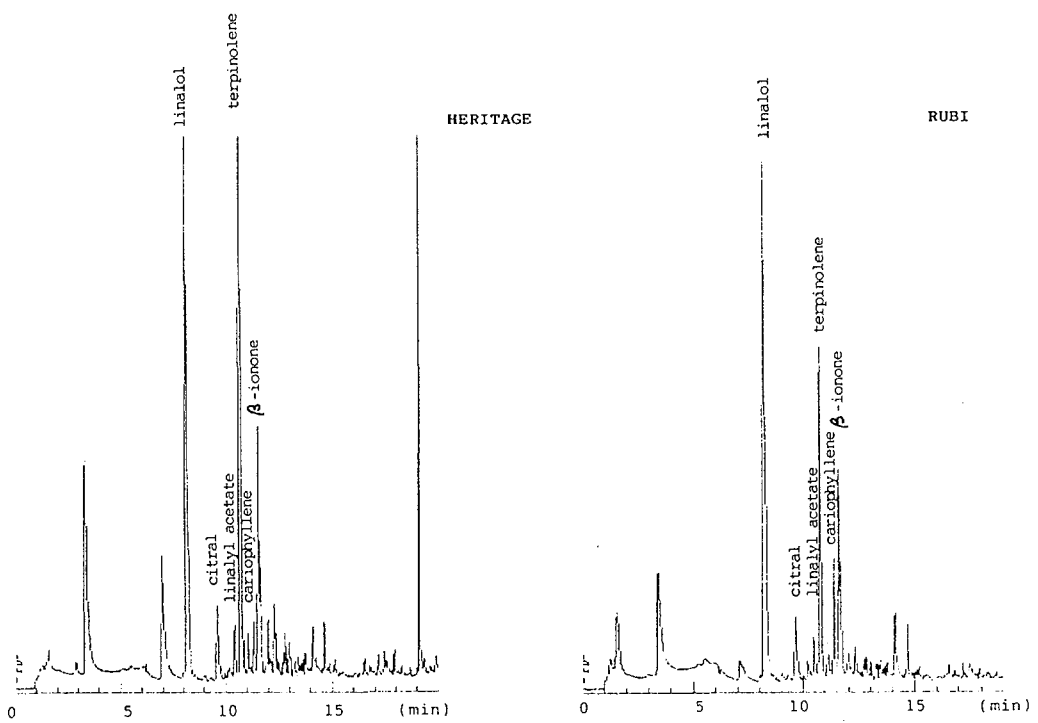

Figure 2. GC chromatogram of aroma compounds from raspberry fruits (cvs. Heritage and Rubi)

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CHERRY FRUIT PRODUCTION FOR FOOD INDUSTRY

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Synopsis
After a brief overview on the world cherry production, the principal types of cherry processing and some parameters to evaluate the fruit quality for industry are listed.
In order to improve the yielding and the fruit quality and to minimize the costs the more important agronomic traits of the cultivation of cherry which fruit destination is the processing are also presented.
In particular, starting from the preliminary kinds of decisions before plantation, such as the choice of varieties and their pollinizers and rootstocks, suggestions are given on agronomic practices among which lastly is the mechanical harvesting and its related techniques.

GENERAL ASPECTS

It is estimated that the global production of cherries, supplied by more than 55 Countries, varies from year to year between 2 and 3 million tons, more than 60% of which are represented by sour cherries. The fruit processing concerns on average 40% of the sour cherries and 15% of the sweet ones.

Among the most important world producers of cherries (in thousands of tons) are Germany (280), the U.S. (170), Turkey (177), Italy (140) and the former Soviet Union (130).

The products' destination to the processing industry in the individual Countries may reach 50% (U.S.A.), for the sweet varieties and up to 100% for the sour varieties (Belgium, Canada, Holland and U.S.A.).

Like other horticultural products, cherries going for the processing use must meet special requirements which are often quite different from those demanded for fresh consumption cherries. Even though they are not always essential and exclusive, among these we notice immediately the price, which as seen in Tab.1, is always the decidedly lower than that of fresh market cherries, at the same period of harvesting. In the 1970's this ratio was 1:3 at Avellino and 1:7 at Bari. It can therefore be maintained that at the present day the grower of cherries for industry is, at least in Italy, more disadvantaged than he was in the past. In particular years and markets, this price difference can turn out to be greatly amplified.

The grower often complains about the low price, but the fruit processing industry always looks for raw materials at low cost, so as to avoid, on account of "added value", a final product arriving at the price the consumer finds unattractive.

There are many possibilities for the processing of cherries, as may be seen in Tab.2. For each of these, particular physical, chemical and organoleptic quality characteristics are required of the fruits. Speaking generally, one may said that the qualitative "limits" for cherries, destined for the industrial processing, should concern internal and external parameters of the fruit such as those listed in Tab.3.

In the past, the grower sent to the processing industry the left-over (unsold) cherries from the fresh fruit market or sent them in directly when they were over-abundant. In this way for the industry processed fruits from varieties with a dual ability having characteristics that were not always optimal its purposes. Still today, even for the cherries directed solely to the industry, there are merchandised and offered rather heterogeneous assortments, in terms of variety, of ripening and hence with regard to size and other fruit characteristics.

At present, whenever possible, it is preferred to use varieties whose principal and often only destination is the food industry. The use of such specific varieties, obviously, allows for a greater standardization of the raw material.

While the industry's demand is fairly fixed and linked to any stocks from the previous year, the cherry grower's offer is rather variable, chiefly on account of climatic events. Among these we think of winter and spring frosts capable of destroying buds and flowers. All too often hailstones may destroy the crop. Thunderstorms, even though they may not deliver hail, cause the fruit cracking (5) before just the harvest. The course of the previous summer's weather may, on the other hand, be responsible for the presence of twin fruits (double cherries). This serious defect in the Bing and the Royal Ann varieties may affect more than 30% of the crop (8) with an appreciable drop in the commercial value of the entire yield. Unfortunately, climatic trends are outside the grower's control.

Let us now see, instead, the variables that the grower can control, within limits, and so control and make changes to ensure high quantitative and qualitative levels for the fruits which are suitable for the industry.

CHOICHE OF VARIETY

The suitability of different varieties of sweet and sour cherries for industrial processing has been the subject of numerous Italian and foreign research studies that, for the sake of brevity, are not reported here.

Each Country can avail itself of a more or less rich assortment of varieties often only local, suitable for one or more processing uses. Apart from this, the grower may have at his disposition particularly suitable varieties widespread and known all over the world, such as Bing, Rainer, Royal Ann (= B. Napoleon), Stark Glorious Gold among the sweet kind and Fanal, Montmorency, Pandy, Schattenmorelle and Stevensbear among the sour ones.

Of course, in choosing the variety for new orchards, he should look towards the most final processing destination for his future production. In this respect the cherry grower could protect himself if not with true and proper contracts, at lest with mutual unwritten agreements. In recent years, furthermore, it has happened that some food industries have asked the grower to establish new plantations with particular varieties whose yields they will purchase. In few cases, lastly, have the cherry orchards been managed directly and for their own purposes by these industries.

POLLINIZERS

In order to obtain a better productivity it will be necessary for the adopted varieties to have good pollinizers nearby. If in the cherry orchard several varieties are grown it is essential to check (13) their compatibility carefully both in genetic and phenological terms if, instead, the plantation of a single varieties there will be employed 2-3 pollinizers whose number account of the 8-15 % of the total trees. For example, one could use Stella variety or other universal pollen donors, all the better if they have the characteristic of the fruit suitable for the processing industry.

ROOTSTOCKS

Another variable that the grower can easily control, prior to establishing new plantations, is the kind of rootstock which - *coeteris paribus* - can, on average, be responsible for variations in yielding of more than 30%, as shown in Tab.4.

Apart from productivity, the rootstock also conditions the rapid overcome of the unproductive phase and the longevity of the orchard,just as is observed in Southern Italy for the *Magaleppo* compared to the *Franco* (seedling).

TREE SPACING

The criteria governing the choice for the investment per unit and for the arrangement of the trees must take into account the variety, the rootstock and their interaction, as well as the soil and climatic environment and the cultural practices (e.g. pruning, irrigation, etc.).

Lastly, whether or not it is necessary to go in for mechanical harvesting conditions the tree spacing. With regard to this last point it is to be pointed that it has been found useful, in practice, to make use of spacing a little wider than the minima suggested by manufactures of harvesting machines.

PRUNING AND TRAINING SYSTEM

In order to obtain the desired tree training system it is necessary to carry out pruning operations that can concern young trees from the nursery onwards, to avoid having subsequently to make "heavy" cuttings.

A training system that is very suitable is the vase with 3 - 4 branches, with or without the central leader. Most particularly in the sour cherry, with plants set in a rectangle, as time passed the plantation takes on the aspect of a true and proper hedge or productive wall.

This will be emphasized also by the fact that after having obtained the desired training system, the pruning can be a mechanized operating (hedging and topping).

No matter what training system may be adopted, pruning should be aimed, with a view to mechanical harvesting, at obtaining a straight trunk without scars from heavy cuttings. The branches on it must have a correct insertion and inclination. To allow the shaker clamps to grip the trunk well and not to interfere with positioning of the fruit collectors, the first branch must be found at not less than 70 - 90 cms. from the ground. Experimental researches for the sour cherry (6) and findings over the field on the sweet cherries (Roversi, unpublished data) have shown that the upright habits makes mechanical harvesting easier. Hence the training system should try, as far as the genotype allows, to stimulate a rising development of the scaffold.

SOIL MANAGMENT

It is generally considered that the root system of the cherry tree is shallow and "delicate". A practical consequence of this is that many cherry growers till the soil only in the first years of the plantation and/or only along the rows. All the same, in certain situation in some Countries, the tillage of the soil is customary and involves all the surface to make up for drought. In other, instead, the tillage is limited to digging furrows for irrigation. Controlled weeding with or without periodic cuttings is an alternative to till the soil. The frequency of this is linked to the locally available water supply (rain and/or irrigation). In either case, and especially with spontaneous floor vegetation, the grass covering will be cut early to avoid its blooming having the effect of distracting the pollinators from pollinate the cherry flowers. Usually a surface tillage is resorted to, along the row plant, using entering cutter bars or by using herbicides.

IRRIGATION AND FERTILIZATION

The cherry tree's specific needs for water, in particular those for the varieties suitable for processing, are not well known. However, the choice of the irrigation system depends rather on the soil and climate conditions, on the availability of water and other agronomic factors, as well as economic considerations. Hence in the various cherry growing Countries irrigation can turn out to be totally absent, be carried out in a traditional way by surface (furrows or border) mainly in old plantations, or may arrive at sophisticated thecnological levels with a completely and programmed drip irrigation. Drip irrigation costs between 1.500 and 3.000 U.S. $ per hectare, but permits notable saving to be made in water and eases the matching of the water supply to the need of the trees and to the different soil and climatic conditions. For all these reasons and for the certain productive increase it allows, this irrigation system must always be taken into consideration for new plantation.

With regard to fertilization it is to be observed that it depends on the soil fertility and on the possibility of checking its efficiency with a leaf diagnostic method (Tab.5). The standard (critical) nutrient levels available at present, unfortunately, almost always refer to variety for fresh consumption. Lastly it should be remembered that floor vegetation can give rise to competition for water and mineral elements with the tree culture.

INCREASING FRUIT SETTING

It is well known that a good level of fruit setting ensures a high yield per hectare. In some varieties and in certain years, in addition, it also governs the percentage of oversized fruits, not always desired.

To have a high level of fruit setting, apart from suitable pollinizers that furnish compatible pollen, there must be the pollinators to transport it. Here an important, perhaps indispensable role is filled by the bees (11). As for other species with abundant blossoms and small fruits, the cherry particularly requires a large numbers of bee hives by hectare. The use of hormonal and hormonal-like treatments to increase fruit setting, while having given good results (3 and 10), is still in experimental stage.

DISEASE AND PEST CONTROL

There are some biotic adversities (disease and pest) which affect the tree and its fruits and can reduce the yielding or lower the quality so much that the entire crop of cherries is unacceptable for the processing industry. Among these are to be numbered the notorious cherry fruit fly (Ragolethis cerasi) and, less commonly, the Balaninus cerasorum. In the case of cherries intended for luxury uses, such as cherries in alcohol, because of the high value of the conserving vehicle (up to 10 times the price of the fruits), it must be ensured absolutely that there are not larvae of this parasites present in order not to incur any risks. The techniques for prevention, monitoring (12) and or chemical control against the cherry's biotic adversities are well known to cherry growers and so are not discussed here. It is common knowledge that this techniques are to be applied with full respect for the regulation in force which, in protecting the consumer, rightly set out in a restrictive manner the spraying residues. It is satisfying to emphasize, lastly, the growing interest of the food industries towards crops obtained with the so called integrated pest management (7).

MECHANICAL HARVESTING

With the present world prices for cherries for processing (from 0.5 to 1.5 U.S. $), it would be folly to make new plantations without the opportunity of the integrally mechanical harvesting. Exception must be made, it is not yet clear for how long, for some Countries with special social, economic and political conditions that still today favour manual harvesting by reason of the low labor cost. For mechanical harvesting many different types of harvester are now available, to meet widely differing needs. These mechanical means differ mainly in the type of shaker, for the fruit collector, dimensions and maneuverability and the number of persons they require, Their cost varies between 50.000 and 200.000 U.S.$ and their harvesting capacities between 1 and 2 hectares a day. At the moment only some harvester are used successfully in Italy, while abroad their use is much more widespread and common, being linked to specialization and to the great size of cherry orchard. Often to improve the efficiency of the mechanical harvesting, in addition to the cultivation of varieties (2 and 9) with a low Fruit Retention Force (FRF) the spraying of ethylene releasers, such CEPA, is commonly used. However, its use has negative secondary effects such as defoliation and gummosis. Careful strategies in its use allow for a reduction in the negative effects without reducing the efficacy of the fruit removal stimulus. Indeed, the combined use of CEPA + Na-bicarbonate + glycerin has permitted (Roversi, unpublished data) the halving the dosage with the same results.

A useful, and sometimes indispensable auxiliary aid to mechanized harvesting is cooling the fruits, which reduces enzymatic activity and improves the cherries quality for processing. This concerns hydrocooling techniques. One of these, not however widespread consists (1) in sprinkling the fruits -still on the trees- 15 minutes before harvesting. After harvesting, instead, hydrocooling is on a large scale, either with blocks of ice in the bins, as can be seen effected in the south of France, or with true and proper cooling stations as usually made (4) in Michigan.

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Tab.1 Price rates between cherries for fresh consumption and processing recorded in Apulia during the period 1995-97 (Roversi, unpublished data).

Cultivar	**Price ratio**
B. Moreau / Forlì	2.2 - 2.8
Ferrovia / Montagnola	1.3 - 1.9

Tab.2 Some different types of cherry processing

Brining
Canning (water, syrup)
In alcohol
Drying
Freezing
Osmodehydration
Pulp (brined, canned, frozen)
Jams & jellies
Juices & nectares
Fermentation (wines)
Distillation (liquors)
Candied, glacè & *mostarda*
Graffioni sotto spirito
Yogurt and hamburger enriching

Tab. 3 Some parameters to evaluate the quality of cherries for processing

Stalk removal
Fruit size
Fruit shape
Color
External integrity
Pulp firmness
Internal oxidative browning
Stone size
Stone centernig
Stone removal
Ripeness degree
Soluble solids
pH
Titratable acidity
Anthocyanins
Flavour
Standing pesticide residues

Tab. 4 Average yield (kg/tree) by rootstock and variety, at the 8th year from plantation (Roversi, unpublished data)

Rootstocks	Varieties				Averages
	Cherie	*Colafemmina*	*Donissens*	*Montagnola*	
Cerasus	3.5	----	9.5	----	6.5
Colt	----	3.8	3.2	4.0	3.7
Franco	0.6	6.6	----	6.6	4.6
Mazzard	----	13.0	7.2	----	10.1
Mahaleb	----	---	----	11.0	11.0
Averages	2.05	7.8	6.6	7.2	-

Tab. 5 Mineral leaf composition of 4 sweet cherry varieties for processing recorded for orchards in good vegetative conditions and good yielding (Roversi, unpublished data).

Locations		Acquaviva delle Fonti (Bari)		Curicò (Chile)	
Elements		*Donissens*	*Stark G. Gold*	*D'Annonay*	*Black Tartarian*
N	%	2.0-2.2	2.3-2.5	2.1-2.3	1.9-2.3
P	"	0.22-0.28	0.21-0.22	0.13-0.15	0.14-0.28
K	"	1.9-2.4	1.8-2.1	1.6-1.7	1.6-2.0
Ca	"	3.1-4.9	3.5-4.2	2.3-2.8	2.8-2.9
Mg	"	0.59-0.66	0.57-0.64	0.36-0.38	0.38-0.47
Fe	*ppm*	263-335	242-341	----	----
Mn	"	75-120	67-88	33-40	33-35
Zn	"	47-77	33-39	9-12	10-13
B	"	38-41	44-47	62-71	66-78

THE PRESENT SITUATION AND FUTURE DEVELOPMENT OF ROSELLE FROM *HIBISCUS SABDARIFFA*

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Summary

The *Hibiscus Sabdariffa* is commonly called *Bissap* in West Africa and *Carcadé* in East Africa. It is a tropical annual plant whose different parts have a number of traditional uses: food, production of energy, production of ropes etc. The leaves and the calices of the flowers are used in several different forms to feed the population in the production areas. In Senegal there are two spreading varieties that distinguish each other by the colour of their calices (green or red). As a result of growing commercial interest at the national and international level, several new varieties have recently been introduced and production contracts between farmers and business men or industrialists are currently being drawn up.

The red calices, which are rich in anthocyanin and of growing interests to industry, are the object of an export production directed to European markets (particularly France and Germany). Their use in the production of beverages has achieved a remarkable increase during recent years. Their use in herbal teas and other warm or cold drinks or as food colouring, often offer new possibilities that contribute to an increased market interest and encourage production and research and development.

This document introduces the *Hibiscus Sabdariffa* and describes the different varieties and their main characteristics. It focusses on those varieties whose red calices are used in the food industry in the production of beverages, herbal teas (health tea), jams and jelly, concentrates, food colouring and fruit sauces. The pharmaco-dynamic qualities that are attributed to this plant are also discussed.

Basic data on the production of fruit juice will serve as a basis in order to identify the niche that the *Bissap* could take in this sector on the world market. The agricultural parameters, post-harvest techniques and formulation of industrial products are also taken into consideration. Techniques of transformation and flow-sheets are given for the main *Bissap* -based products of large consumption.

PALMITO, ITS PRESENT SITUATION AND FUTURE

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Summary

Palmito or heart of palm is a relatively unknown commodity in the international food market. For a long time it has been processed as an acidified preserve, being Brazil the major producer and consumer, and France the largest importer. Research has been conducted towards the development of new alternative products using pejibaye heart of palm and by-products. This includes marinated preserves, products cooked under vacuum, dehydrated palmito (pieces, powder and flakes), and fresh palmito with a longer shelf life. Palmito has a very promising future and it is up to the exporting countries to further promote it through consistent quality and adequate marketing strategies.

The tender apical part of a palm stem, including embrionary leaves, is known as palmito or heart of palm. Many different palm species have been used for palmito extraction since pre-Columbian times, and they are still used for this purpose in the American tropics. Palmito is commercially extracted today mainly from wild palms of the genus *Euterpe,* and from the pejibaye palm *(Bactris gasipaes Kunth)*. Euterpe palms are used in a large scale by Brazil and other South American countries, both for local consumption an for the international market. The use of wild palms has always been questioned because of its environmental effects. The pejibaye palm has a broad natural distribution ranging approximately from Honduras to Bolivia. Commercial plantations were first established in Costa Rica around 1975, and other countries like Ecuador and Bolivia have established commercial plantations in recent years.

Palmito is a relatively new product in the international market and the volume traded is yet small. There are about 60 importing countries in the world, most of them taking sporadically small amounts. Nevertheless the product has a high potential that has encouraged ever enlarging plantations in many Latin American countries.
Palmito is considered a gourmet product in the international market, it is litle known and it is expensive if compared to other canned vegetables.

Europe is the main exporter market, specially France. Other European countries can be considered as alternative and occasional markets. Brazil is the major producer and also the largest consumer. Most of its production is used to cover the domestic demand. Fluctuations in Brazil's local consumption influence the availability of the product for the export offer, affecting the international prices and the chances of other countries to increase their exports. North American and Asian countries seems to be promising markets, although consumption habits are not yet established. The United States have shown a growing demand in recent years [5]. A greater effort from the exporter countries will be required to reach the consumer and make him familiar with the product [4]

Heart of palm has been processed in acidified brine for many years, and this is the most known presentation in the international market. This traditional preserve has sometimes been criticised in export markets because of quality problems related basically to variations in acidity and to the presence of inedible or fibrous parts. Acidity variations are critical since they pose a health risk to the consumer (pH must be 4.5 or lower to prevent anaerobic pathogens from growing). These problems are less frequent when material from organised, technically [1] managed plantations is used and processing takes place in adequate facilities with the necessary quality assurance procedures in place.

Product characteristics vary with the acidifying agent (mostly citric plus ascorbic acid), the salt content, processing conditions and specially the raw material. Palmito from Euterpe palms is white, straight and has a neutral flavour, while palmito from pejibaye palms is yellowish, slightly conical, has a sweeter taste and a characteristic aroma.

Research has been conducted towards the development of new alternative products using pejibaye heart of palm and its by-products (loose leaflets and tender internodes).Palmito by-products have a higher yield than the heart and also a higher crude fibre and sugar content [2]. A variety of marinated preserves are already in the international market, together with the traditional acidified brine preserve. Marinated palmito, with a more crunchy texture is well accepted in the United States, while European market prefers a softer texture and plain acid taste.

Cook under vacuum technology was applied to develop a product closer to the natural palmito. Acceptance trials conducted in France indicated that the consumer was used only to the acidified preserve and preferred a more acid product. Thus a 'sous vide' product including lemon juice and salt was developed, showing acceptable sensory characteristics [6]. Palmito sous vide is a low acidity product requiring careful and accurate temperature management (under 3°C) being this the limiting factor for its distribution.

Dehydration studies using hot air have yielded a product (approximately 10% moisture content, stable at ambient temperature) that can be used in the preparation of hot dishes and salad. Powdered palmito and flakes have been obtained to formulate creams, sauces and soups. All these products have very good sensory characteristics.

Fresh heart of palm enables the preparation of a wider variety of dishes than the acidified preserve, since the natural taste is unchanged. A significant demand for fresh palmito exists in the United States market that can be covered from Latin American countries. Considering that this is a very perishable commodity, studies were conducted to increase its storage life under refrigeration. The use of rapid immersions in preserving solutions prior to packaging showed as a good alternative applicable at a relatively low technological level. Different substances alone and mixed were used. Levels were defined so that the concentration in the final products would be under acceptable limits when applicable. Product samples were evaluated for colour, appearance, microbiological parameters and sensory characteristics. Substances like sodium benzoate plus salt, lemon juice plus salt, kilol and some others, yielded much better products than the control after at least 15 days of storage under refrigeration [3]. This storage life was considered enough for export and distribution to specific market niches in the United States.

As mentioned above, the demand for heart of palm in the international market is increasing, and would be expected to grow at higher rate since exporting countries are making stronger efforts to

promote the product in traditional and new markets. The introduction of new products may help differentiate from the plain acidified preserve market moving to more specialised niches. Also the production of organic palmito is a good option since the pejibaye is a very resistant crop. Palmito has a very promising future and it is up the exporting countries to further promote it through consistent quality and adequate marketing strategies.

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FRUIT PRODUCTION IN POLAND PRESENT SITUATION AND THE FUTURE

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*Fruit Production *Fruit Juice Industry * Development Perspectives

Poland with the aeria of 360 000 km^2 and population of nearly 38 millions is an important producer of fruit. In last years we produced about 1600 thousand tons of apples, nearly 140 thousands tons of morello cherry, around 200 thousands tons of strawberries, 160 thousands tons of black currents and over 300 thousands tons of other fruit (tab.1). Total production in 1996 reached 2700 thousands tons. The production of apples and morello cherry increases rapidly. In 1997 we expect to harvest around 2 millions tons of apples. The production of strawberries vary from year to year and the production of other fruit shows a tendency to increase except of pears.

We have mainly young trees in our orchards. During very hard winter 1986/87, when temperature dropped below -35°C, we lost nearly 40% of fruit trees and 30% was badly damaged. Since that time each year has been planted 6,5-8,5 millions apple trees, 2-3,5 millions of sour cherry trees, nearly 1 millions of plum trees, over 0,5 millions pears and others. It is estimated that in aple orchards there is around 70% of young trees (age 1 to 8 years) and 30% of old trees. As the result of orchard renovation new cultivars and new rootstocks have been planted since 1998. In old orchards we grew mainly American apple cultivars: McIntosh, Spartan, Lobo, Bancroft, Cortland, Idared, Jonathan, Vista Bella, Jerseymac, Close, Red Delicious, and Russian: Antonovka, Yellow Transparent grafted on seedlings and planted at the density 300-400 trees/ha. In new apple orchards mainly semidwarf trees are planted grafted on M 26 (also on M 7, MM 106, P 2, P 16) at the density 1000 trees/ha, and also dwarf trees grafted on M 9, P 22 and planted at the density 2000-3000/ha. In new orchards we still have a large quantity of 3 old American cultivars: Idared, Lobo and Cortland. Among new cultivars the most popular are: Jonagold and Jonagold red mutations, Šampion from Bohemia, Gloster, Gala, Elstar, Boskoop, and Polish: Alwa, Ligol. In majority of pear, cherry and morello cherry orchards the same cultivars are planted as 10 years ago. Conference, Beure de Lucas and Klapp's Favourite are the leading pear cultivars. Shatten Morelle is almost only one cultivar in morello cherry orchards,

plums from _acak (Yougoslavia) are becoming very popular. On strawberry plantations one finds almost solely one cultivar, good for processiong - Senga Sengana.

Over 90% of fruit farms are small having 5-10 ha of land. The largest are having up to 200 ha of fruit plantation. We estimate that there are about 10.000 farms growing solely fruits. Bush fruit plantations are more dispersed among agriculture farms. Soft fruit are produced on small plantation, of 0,1 to 2 ha.Villages are overpopulated (nearly 30% of total population works in agriculture). Family farms grow fruit to employ spare hands and to obtain extra income.

Fruit production in Poland is heavily concentrated in some regions. Almost half of production comes from Radom region that is about 40 kilometers south to Warsaw (fig.1). This region supplies around 700 thousands tons of apples yearly. The other important fruit regions are around Warsaw, east of Poland, and Karpation region of south of Poland. Very little fruit is grown in western and northern part of the country due to lack of tradition or more severe climatic conditions.

Poland is a great exporter of processed fruits whereas export of fresh fruit is not high (tab.2). Above 60% of all fruit produced in Poland undergoes processiing, and after processing there are exported. In case of apples the figures since 1993 to 1996 are from 64 to 73%. One of the reason is a large number of old trees of old cultivars grown not only in cultivated orchard but also at agricultural farms and not sprayed (tab.3). There is also overproduction of McIntosh and Idared. In such case apples of poorer quality are processed. Almost all processed apples in Poland are turned into concentrated juice and exported.

The fruit juice processing industry is the most modern of all food industries in Poland. Most of processing plants with Bucher-Guyer presses belongs to Hortex S.p.A. The largest pressing capacity has processing plant in Tarczyn which can process 1200 t of apples daily, that is around 100 000 t per year. Apple juice is concentrated to 68-70° Brix, cooled to 0°C and stored in stainless steel tanks. Most of concentration juice is exported mainly to Germany and Austria. Soft fruits (morello cherries, currants, strawberries) are exported either as deep frozen or cooled fruit pulp.

Lately the country has been opened for foreign investments and especially joint ventures are promoted with Polish companies. Several fruit processing factories entered into busines for example Gerber - an American company and Allione Central Europe Sp z o.o., a newly established factory in Radom fruit region.

The future

The leading specialists in fruit processing industry W.P³ocharski and E.Rembowski from Res. Inst. of Pomology and Floriculture, Skierniewice see the following future of Polish fruit processing industry:

1. Fruit production in Poland shows high elasticity, typical for free market economy.
2. The links between fruit growers, advisory services and processing plants guarantee to some extent further development of fruit production and processing industry.
3. The future of the industry depends however on further development of international and home markets.
4. The partnership links between Western and Polish companies are the right basis for future cooperation.
5. Fruit production and fruit processing in Poland are modernized taking the example of Western Europe.
6. We need the knowledge of marketing and any help in this respect give by EEC countries, USA and Canada is greatly appreciated.

Literature

P³ocharski W., E. Rembowski (1991):Fruit and processing potential for fruit juice production in Poland. Publ. Res. Inst. of Pomol.and Floriculture, Skierniewice 1-13.

Table 1. Fruit production in Poland 1991-95 and 1996

Fruit species	Tree number (mln) or area in thousha	Production in thousands tons		% 1996 (1991-95=100)
		1991-95	1996	
Apples	85	1457	1900	130
Pears	8	67	50	74
Plums and prunes	15	86	109	127
Morello cherries	18	122	139	114
Sweet cherries	4	28	35	128
Others from trees	3	10	15	144
	Area (x 1000 ha)			
Strawberries	60	204	179	88
Raspberries	11	33	35	107
Currants	70	180	184	102
Gooseberries	13	44	41	94
Others from bushes	4	8	14	188
Total	-	2239	2701	-

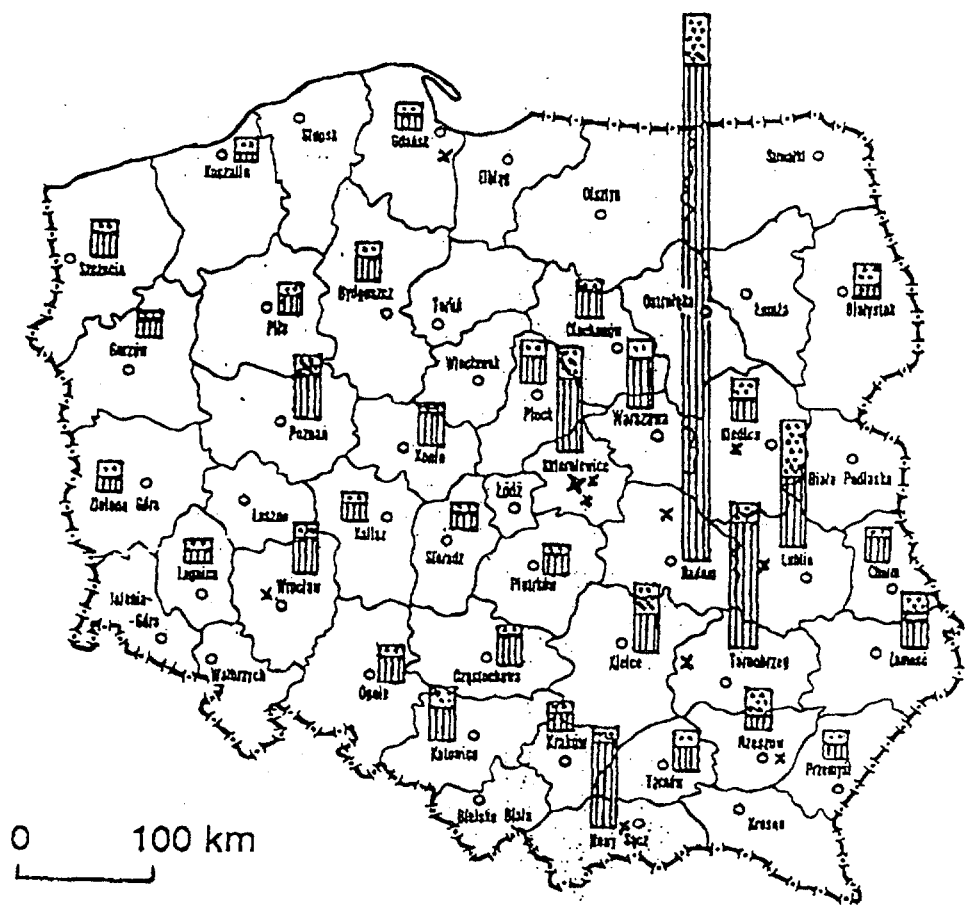
Table 2. Quality characteristics of industrial apple in Poland

Variety	Brix (°Bx)	Total solids (%)	Total acidity after harvest (%)	Firmness after harvest (kg)	Mean fruit diameter (cm)
Antonowka	11.0	12.43	1.16	-	7-8
Boiken	11.2	-	-	10.98	6,5-7
Cortland	12.9	14.10	0.63	7.20	7,5-8
Idared	12.7	14.80	0.77	8.13	7-8
James Grieve	12.9	15.82	0.81	-	7-8
Jonathan	12.2	13.40	0.81	7.21	6,5-7
Lobo	13.3	14.45	0.50	-	7,5-8
McIntosh	13.0	14.18	0.76	7.70	6,5-7
Spartan	10.8	-	0.44	7.66	6,5-7

Table 3. Export of apples from Poland (in thousands tons)

Kind of apples	1993	1994	1995	1996
dessert	176	115	245	96
concentrated apple juice	110	105	82	102
dried apple	6	12	12	12
Total export calculated as fresh apples	1.180	1.020	950	1.350
% of total production	64	71	73	71

FRUIT PRODUCTION REGIONS IN POLAND (1991)



100,000 tons

tree fruit

berry fruit

× Institute of Pomology

× and its research stations

Qbo: Quality by Objectives

dr. Walter Garrone

A company can only enjoy market success by Quality. This objective can be achieved with a process standardisation with clearly defined standards and rules. Agricultural raw materials are the basic product of the food industry and we must go right back to the source to guarantee a high quality product which is absolutely safe for the consumer

Much has been written and said recently on the subject of quality; we might even say that the consumer is disoriented by the daily abuse made of this term and, as often happens in the case of words that are continuously repeated, its real meaning is confused.

A company can only enjoy market success by seducing the customer and this can only be achieved with Quality. Before continuing with this analysis, it is essential that these terms are clearly defined, in an unequivocable and precise manner.

The customer is the final consumer, he is the person who expresses judgement on every purchase made, and he is also the strictest judge who does not justify any failing on our behalf.

Quality means satisfying the final consumer and "producing Quality" represents the series of operations that are necessary to supply the customer with the product he desires.

In operative terms, quality can be achieved by fulfilling a number of compulsory objectives:

- being familiar with the consumer's requirements;
- doing things well, designing carefully right from the outset;
- controlling the company processes that have a bearing on quality (purchases, production, distribution, sales) through compliance with clearly defined standards and rules.

The iteration of these three points represents the basis for assuring that a product will have constant characteristics guaranteed over time, a synonym for the real achievement of process standardisation at top quality levels.

Turning our attention to the topics of this conference, I would now like to focus on the important aspect of raw materials.

In a production cycle, any errors that are made on the lines may be corrected or avoided, but "raw material errors", on the contrary, are no longer correctable and will jeopardise the end result.

We must therefore think of raw materials as the outcome of a production line upstream of the factory, often also in remote areas. At Ferrero we are convinced that our objective must be to plan Quality starting from "the crops in the fields" and to follow it through every production sector, without resorting to compromises and overcoming any logistic problems.

Agricultural raw materials are the basic product of the food industry: Ferrero use some highly distinctive raw materials for its products, like hazelnuts, milk, coffee, cherries, etc.

In the case of milk, for example, limiting the guarantee for the consumer to a chemical and microbiological control on the incoming material would result in a negligible benefit. Instead, we feel that we must go right back to the source, selecting herds kept in healthy areas far from pollution. Only in this way can we guarantee a high quality product which is absolutely safe for the consumer.

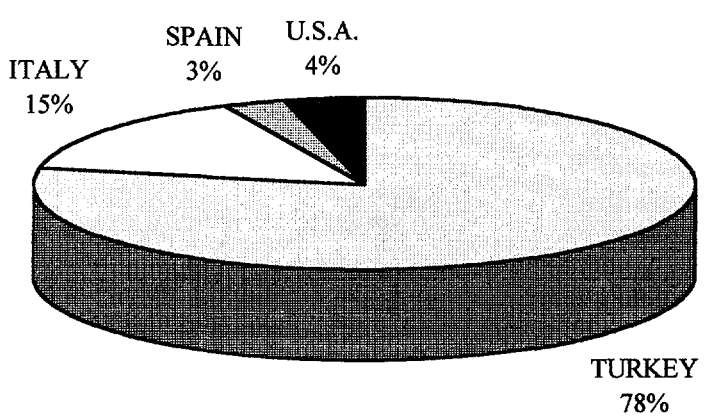
Let us now turn our attention to the raw material that is most typical of Ferrero products and gives them their distinctive character: hazelnuts. These nuts are particularly important to our company because since the 1950s we have believed in their special aroma and we have sold highly successful products, starting with "Pasta Gianduja" and moving on to Nutella and Rocher.

Before examining some aspects of this raw material in detail, it might be useful to give a brief description of its characteristics as a commodity.

The hazelnut is the fruit of the *Corylus avellana* tree and, as can be seen from chart no. 1, production is almost exclusively located in countries in the Mediterranean belt, in particular Turkey which alone accounts for 78% of the entire world production, whereas Italy and Spain represent 15% and 3 % respectively. A small quantity is produced in the United States, or more precisely in Oregon.

At an Italian level (chart no.2), Campania and Lazio are the regions which produce the largest crops, but it is important not to overlook the importance of production here in the province of Cuneo which, although not particularly large, is of fine quality.

CHART no.1
UNSHELLED HAZELNUTS - WORLD PRODUCTION (average 1992-1996)

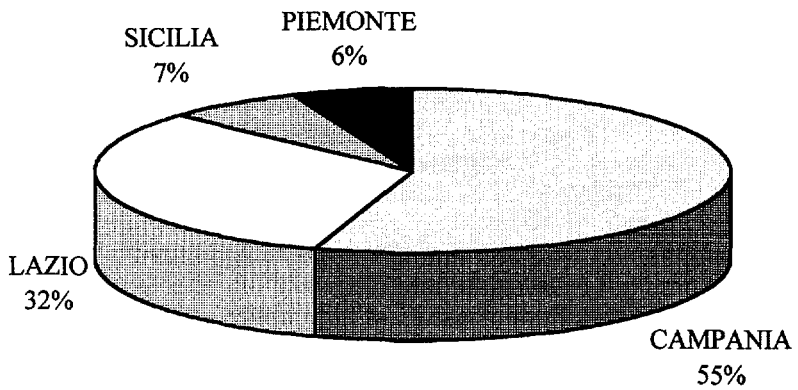


The fact that in Piedmont this crop has not reached significant production levels for the market, in spite of the particularly suitable environment, may be attributed to a wide variety of factors that lie outside the topic of today's meeting, but we will leave the subject open to political representatives and local experts.

Probably Ferrero is the biggest world consumer of hazelnuts. This fact, which is already striking from a quantitative point of view, is even more remarkable when its real qualitative value is considered given that Ferrero only uses certain types of hazelnuts that are suited to the characteristics of its products.

When a hazelnut is roasted, it will only express its utmost goodness provided that precise qualitative requisites are met: in particular, in order to ensure the optimal development of aroma, it is essential that the nut contains a clearly defined blend of molecules which act as precursors.

CHART no. 2
UNSHELLED HAZELNUTS - ITALIAN PRODUCTION (average 1992-1996)



The quality of hazelnuts starts with the selection of the most suitable crops and consists in the identification of those varieties that present the shape, structure, flavour and capacity to preserve organoleptic characteristics over time.

Having selected the optimal varieties, it is then necessary to choose the geographical zones in which the best quality crops are grown, taking into account the growing environment and farming (e.g.: soil preparation, fertilisation, pruning, phytosanitary protection, harvesting, etc.) and post-farming procedures (e.g. drying, preservation, husking).

In terms of the qualitative results, the harvesting, drying and preservation of hazelnuts are essential operations.

Nuts must be harvested when they are fully ripe and fall to the ground.

Unfortunately, hazelnuts that are left on the ground for long periods are subject to the negative effects caused by damp or rainy weather, normally frequent at harvesting time, causing the formation of mould or fermentation with severe repercussions on quality.

Harvesting is immediately followed by drying which must take place in the sun in order to complete the ripening process naturally.

During rainy weather and at nightime, the hazelnuts must be suitably covered using sheets or stored in dry, ventilated rooms. In the event of particularly difficult climatic conditions, it is possible to resort to artificial drying.

After drying, using either natural or artificial methods, the moisture content of the nuts must be reduced to less than 6%, an essential condition for their correct preservation.

Dried hazelnuts must be stored until shelling in dry, well ventilated and suitably hygienic warehouses.

In spite of its external appearance, the hazelnut is a delicate fruit; hazelnut oil is rich in nutritional properties and must be protected during storage.

All the workers responsible for handling nuts are fully informed and made aware of these aspects and they work carefully and delicately to avoid any severe damage, such as the loss of oil caused by bumping or crushing.

If the precautions briefly described above are not put into practice, the nuts will inevitably present defects that will jeopardise the quality of the finished products.

What has been said on the subject of hazelnuts is borne out in our everyday work. Only by using raw materials offering a high qualitative guarantee, can we succeed in obtaining high quality products throughout the production.

It should not be forgotten that hazelnuts are only harvested once a year, whereas Ferrero products are sold on all continents all year round.

Customers come to us expecting a product that always has the same level of quality and only by constantly checking raw materials at every single processing stage can we succeed in fulfilling this objective.

Every year our Quality department faces an exciting challenge: to provide the consumer with quality products whose hazelnut aroma is as fresh as if it had just been picked.

In Ferrero these principles are fully implemented not only in the raw materials sector, but in all corporate processes which have a bearing on final product quality: this has proved to be a winning philosophy and consumers have reconfirmed their trust in our products.

In the future, our job will be to maintain this level of commitment.

Thank you for your attention.

ITALIAN MINISTRY OF AGRICULTURE RESEARCH GROUP: RESIDUES OF PESTICIDES, ACTIVITY AND RESULTS

Giancarlo Imbroglini
Istituto Sperimentale per la Patologia Vegetale – Rome

The Ministry of Agricultural Policy (MiPA) has since 1992 promoted and financed actions aimed at reducing the use of plant protection chemicals, with the intention of improving the quality of Italian farm produce in terms of health aspects. For the purpose it created a network to monitor the use of plant protection chemicals and perform technical and scientific analyses to measure their residues in farm produce. In five years of activity over 52,000 samples of the principal crops have been analysed, and it is intended to continue for a further five years with a greater involvement of Regional governments in planning the crops and environments to monitor and especially in discussion and assessment of the results. The data so far collected on residues demonstrate the good quality of Italian produce, with only 3.6% of samples being over legal limits.

INTRODUCTION

The national plan for integrated plant disease control, prepared by the Ministry of Agriculture and Forests (today the Ministry of Agricultural Policy), was conceived as a planning instrument in the context of Law 752/86, from which it drew its origin.

The chief aims of the plan were to reduce the use of pesticides in agriculture and re-examine the question of the use of chemicals in agriculture from a strictly technical and scientific point of view in order to prevent repercussions that could be damaging to agriculture itself.

The plan identified and defined specific objectives for investigation and activities.

1) Monitoring and rationalisation of the use of pesticides both in the field and in store
2) Development and adoption of alternative means of protection
3) Improving communication between the world of agriculture and the chemical industry in order to promote research and manufacture of plant protection chemicals compatible with the environment and human health.
4) Promotion of the quality image of Italian food and agricultural products.

To achieve these objectives, the plan provided for "actions" which, although conducted at different levels, would be inter-related and complementary. The "actions" lie within the fields of competence of governmental bodies, involved in various ways, and are largely directed to strengthening technical assistance, extension and information services; reorganisation of local plant health services and institutions, and meticulous monitoring of the correct use of plant protection chemicals. The actions also aimed at launching a targeted project on biological and integrated disease control and establishing a national network for monitoring agrochemical residues on fruit and vegetables.

The action that provided for creation of a national network for monitoring agrochemical residues has become the basic research tool for study of questions associated with modalities of use, monitoring of the particular chemical and assessment of the impact on the environment, through a series of systematic tests and analyses.

THE NATIONAL MONITORING NETWORK ON PESTICIDE RESIDUE

The "National Monitoring Network on Pesticide Residue" set up under the Decree of 30.12.1991 on behalf of the Ministry of Agriculture and Forests (today the Ministry of Agricultural Policy "MiPA"), by the Plant Pathology Research Institute (ISPaVe) of Rome and by the Centro Operativo Ortofrutticolo (COO) of Ferrara, came into

operation from July 1992. Its activities have made it possible to quantify the residues phenomenon on some products.

Analysing samples with a known history (problems combated, principal chemicals used, doses, period of treatment, interval between the last treatment and taking of the sample) it is possible to get back to the causes of any problems and supply decision support information to agricultural technicians and the competent authorities.

The samples are taken in the period between harvesting and sale by technicians specially appointed or made available by producers associations. The samples come from farms that adopt different plant health protection measures (integrated, guided or traditional) and from storage organisations that adopt post-harvest protection systems.

The Ministry of Health remains responsible for checking compliance with maximum limits for residues in products offered for sale and the same Ministry imposes any necessary penalties.

Figure 1 – Structure of the Monitoring Network.

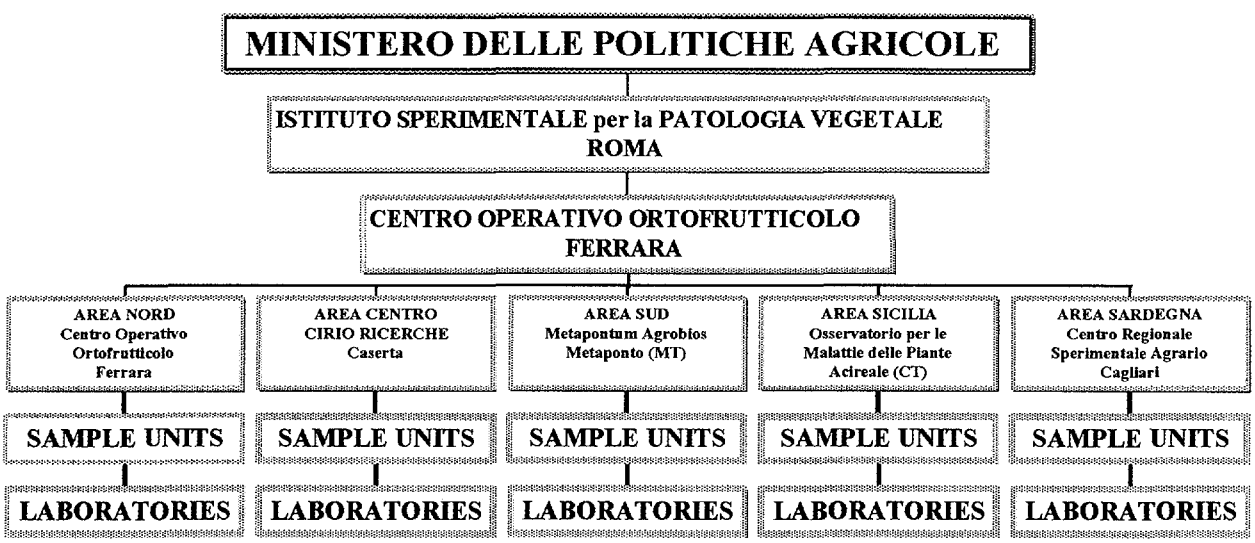


Table 1 – Monitoring Centres and Areas and Regions covered.

MONITORING CENTRES	AREA	REGIONS COVERED
Centro Operativo Ortofrutticolo Ferrara	NORD	Piemonte - Lombardia- Valle d'Aosta - Liguria - Trentino Alto Adige - Emilia Romagna - Friuli Venezia Giulia - Marche
CIRIO Ricerche Caserta	CENTRO	Toscana - Lazio - Umbria - Abruzzo - Campania
Metapontum Agrobios Metaponto (MT)	SUD	Molise - Basilicata - Calabria - Puglia
Osservatorio per le malattie delle piante Acireale	SICILIA	Sicilia
Centro Regionale Agrario Sperimentale Cagliari	SARDEGNA	Sardegna

Within the area it covers, each Centre organises sampling and analysis services in proportion to the residue monitoring programme. It identifies the best place to take samples (farms, stores, silos etc.) and arranges transport of the samples from the Sampling Unit to the Analysis unit, as well as for the transmission to the COO at Ferrara of the results of the analyses for assembly and initial processing. The tasks of the Monitoring Centre also include

providing for the training of sample-takers and participation in the organisation of the cross-check analyses required to monitor the laboratories included in the analysis service.

The Monitoring Network thus bases its activities on uniform methodologies standardised in respect of:

1) Training of sample takers
2) Taking of samples
3) Chemical analyses
4) Processing and evaluation of data

thus making it possible to obtain results than can be compared, even though they come from different areas.

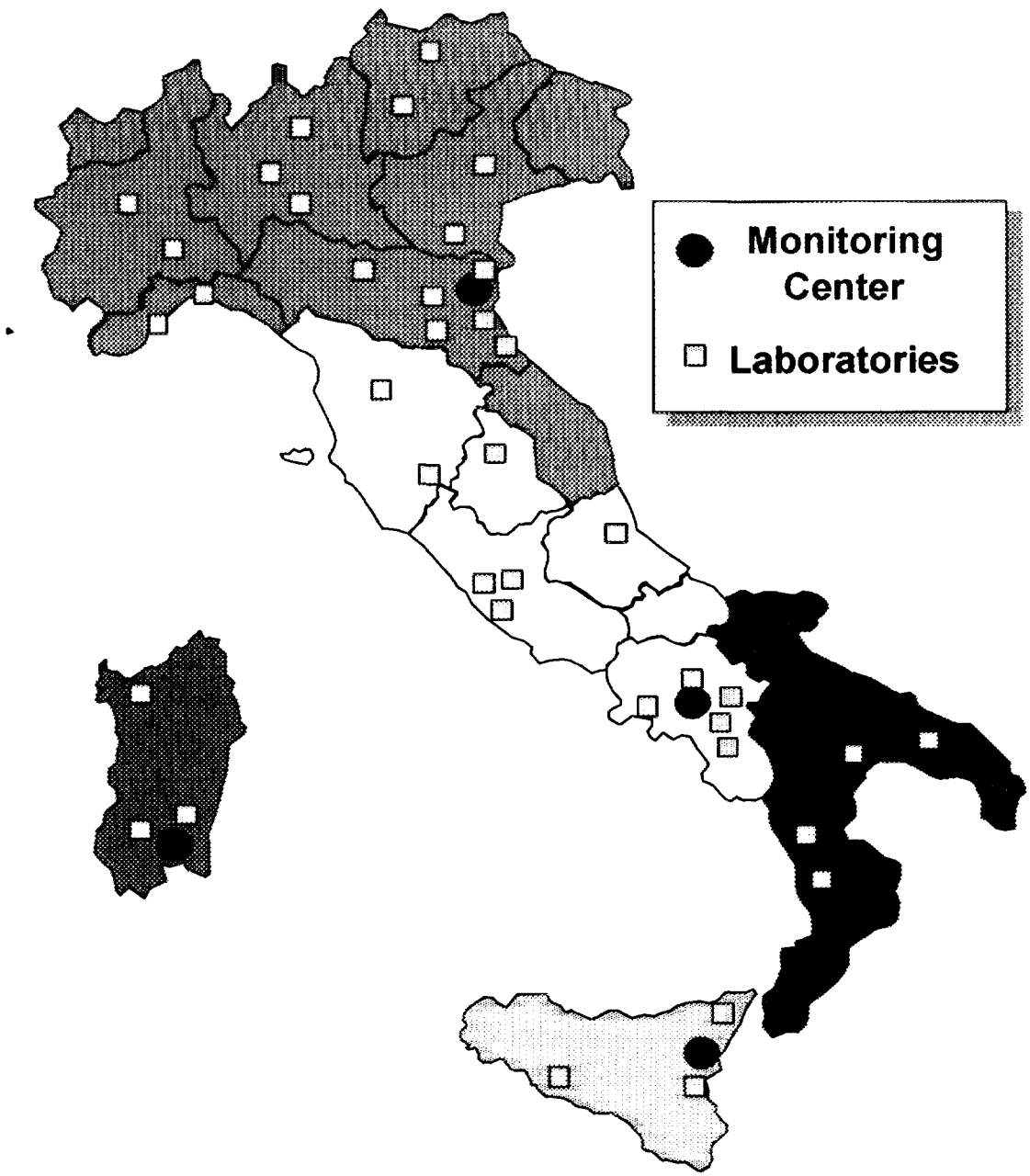
The laboratories are assessed by ISPaVe on the basis of specific organisational capability (instruments and personnel) and of experience over the years. Acceptance of a laboratory is also subject to its passing specific acceptance tests. During execution of the project ISPaVe arranged for cross-check analysis in order to assess the operational performance of the laboratories chosen.

Table 2 – Crops monitored in the five years of activity.

YEAR 1	YEAR 3	YEAR 4	YEAR 5
WHEAT (ph)	KIWI FRUIT	APRICOTS	KIWI FRUIT
APPLES	ORANGES	ORANGES	APRICOTS
APPLES (ph)	ARTICHOKES	ASPARAGUS	ORANGES
NECTARINES	CAULIFLOWERS	ARTICHOKES	ARTICHOKES
CAMMING PEACHES	BROCCOLI	CARROTS	CARROTS
PEARS	CHICORY	CHERRIES	CABBAGES
PEARS (pr)	CLEMENTINES	CLEMENTINES	CUCUMBERS
PEACHES	FENNEL	FENNEL	CHERRIES
	STRAWBERRIES	STRAWBERRIES	CLEMENTINES
8,440 samples	ENDIVE	LETTUCE	GREEN BEANS
	PERSIMMONS	LEMONS	FENNEL
	LETTUCE	AUBERGINES	STRAWBERRIES
	LEMONS	NECTARINES	SALAD
	MANDARINS	PEPPERS	PERSIMMONS
YEAR 2	APPLES	CAMMING PEACHES	LETTUCE
KIWI FRUITS	NECTARINES	PEACHES	LEMONS
KIWI FRUITS (ph)	POTATOES	TOMATOES	MANDARINS
APRICOTS	POTATOES (ph)	CELERY	AUBERGINES
ORANGES	CAMMING PEACHES	PLUMS	APPLES
ARTICHOKES	PEARS	ZUCCHINI	MELONS
CARROTS	PEACHES		NECTARINES
CHERRIES	TOMATOES	**3,958 samples**	POTATOES
STRAWBERRIES	RADICCHI		PEPPERS
APPLES	ESCAROLE		CAMMING PEACHES
APPLES (ph)	CELERY		PEARS
NECTARINES	SPINACH		PEACHES
POTATOES	GRAPES (table)		SMALL FRUITS
POTATOES (ph)			TOMATOES
CAMMING PEACHES	**12,494 samples**		LEEKS
PEARS			RADICCHI
PEARS (ph)			CELERY
PEACHES			PLUMS
TOMATOES			GRAPES (table)
PLUMS			ZUCCHINI
GRAPES (table)			WINE
15,909 samples	ph = post harvest		**11,200 samples**

One of the positive aspects of the programme is that it has resulted in laboratories able to perform pesticide analysis being set up in areas where there were few none before, such as the South and the Islands (Figure 2).

Figure 2 – Geographical distribution of the Monitoring Centres and laboratories included in the Network.



This makes it possible for the individual grower to use the laboratories when he wants to check-up on his own operations and the compliance of his produce with the regulations on pesticide residues.

The overall results of the first four years of residue monitoring are presented in Table 3.

Table 3 - Results of the first 4 years of activity (11,200 samples are to be taken in the current 5th year).

Samples collected	40,800
Chemical analyses performed	708,712
Information available in the data bases (crop, problem, type of defence, principal active ingredients, interval between application and harvest, results of analyses, samples over legal limits encountered)	23,751,000
Number of samples over limits	1,478 (3.6%)

When the tests were repeated two consecutive years on the same colture, the percentages of samples taken from crops treated with not admitted agrochemicals decrease, wich demonstrates the value of the discussions with technicians on the results obtained year by year (Table 4).

Table 4 – Percentage of samples of crops monitored both the 3rd and the 4th year, treated with not admitted agrochemicals.

	YEAR 3	YEAR 4
FENNEL	75.4	51.1
STRAWBERRIES	44.9	8.6
LETTUCE	24.2	9.8
LEMONS	6.3	0.0
PEACHES	0.2	0.0
TOMATOES	6.9	3.8
CELERY	39.3	39.3

The sensitivity of the farmers to the problem of pesticide residues increased and in consequence also their attention to the choice of active principles, correct dosage, compliance with use-sale intervals and checking of application machinery, which leads one to hope for still more reassuring results in future.

Both governmental and private laboratories have acted as Analysis Units, according to the needs in the area and the crops monitored.

The grid of chemical principles monitored included those most widely used in crop protection in the various areas covered (Table 5).

Table 5 – List of active principles tested for grouped by chemical category.

ACARICIDES group A	Procymidone	Forate
Bromopropylate	Vinclozolin	Formotion
Dicofol	Captan	Fosalone
Endosulfan	Chlorothalonil	Fosphamidone
Tetradifon	Folpet	Malathion
	Dichlofluanid	Methamidophos
ACARICIDES group B		Methidathion
Amitraz	**HERBICIDES group A**	Omethoate
	(solo su colture orticole)	Parathion ethyl
ACARICIDES group C	Metribuzin	Parathion methyl
Clofentezine	Propyzamide	Pyridafenthion
Exythiazox	Trifluralin	Quinalphos
		Trichlorfon
ACARICIDES group D	**HERBICIDES group B**	
Propargite	(solo su colture orticole)	**PYRETHROIDES**
	Diuron	Alphamethrine
ACILALANINES	Linuron	Cyflutrin
Benalaxyl	Metobromuron	Cypermethrin
Methalaxyl	Metoxuron	Deltamethrin
Oxadixyl	Monolinuron	Fenpropathrin
		Fenvalerate
BENZIMIDAZOLES	**DITHIOCARBAMATES**	Flucytrinate
Benomyl	Mancozeb	
Carbendazim	Maneb	**GROWTH REGULATORS**
Thiophanate methyl	Metiram	Diflubenzuron
	Propineb	Teflubenzuron
CARBAMATES group A	Thiram (TMTD)	Triflumuron
Carbaryl	Zineb	
Ethiofencarb	Ziram	**TRIAZOLES**
Pirimicarb		**PYRIMIDINES**
Propoxur	**ORGANOPHOSPHOROUS**	Bitertanole
	Acephate	Cyproconazole
CARBAMATES group B	Azynphos ethyl	Dichlobutrazole
Methomyl	Azynphos methyl	Exaconazole
	Chlorpyrifos ethyl	Myclobutanil
CYMOXANIL	Chlorpyrifos methyl	Penconazole
	Diazinon	Propiconazole
DICHLOROANILINES	Dimetoate	Triadimefon
THIOPHTALIMIDES	Heptenophos	Triadimenol
Chlozolinate	Fenitrotion	Fenarimol
Iprodione	Fenthion	Nuarimol

Law 578 of 5 November 1996 recognised the value of what had been done and extended the life of the National Monitoring Network on Pesticide Residue, though making some adjustments. In addition to continuing monitoring of fruit and vegetables and agricultural produce, it provides for monitoring of processed agricultural products (fruit and vegetables, milk and cheese, wine) as well as of soil and surface water in relation to weed control. The intention is to give greater attention to environmental protection in addition to maximising the value of Italian produce. The whole could contribute towards proper implementation of EEC Regulation 2078 and adoption of collective quality trade marks.

Residue monitoring activity may also provide technical support for testing in application of the various pesticides, permitting redirection of national crop protection programmes. The information and analysis data collected will serve as a support for drafting and revising production regulations aimed at marketing of products furnished with a certificate guaranteeing compliance with quality parameters. This envisages greater involvement by the Regions, which will be able to avail themselves of support from farmers' organisations.

The items to be sampled will be agreed at the inter-regional level, taking account of specific situations. The selection of the farms to be sampled will be done at the Regional level, selecting them from among those that practise monitored integrated crop protection and compile the "Register of treatments performed and stocks of plant protection products". Other farms will be sampled for comparison.

The active principles selected will essentially be those included in the same Register, but others may also be included if they are known to be used in the area concerned for protection of the crop being monitored.

In future activities the crop will again be monitored on harvesting, following the seasons: early, spring, summer, autumn, open field and protected. Crops stored for long periods and therefore treated post-harvest will be monitored at the time they leave the store.

The results obtained will be assessed and discussed, as in the past, by a Technical and Scientific Committee. In future this will be composed of 7 experts nominated by the Ministry and the Regions. The Committee will have the task of formulating recommendations on modifications to plant health protection strategies and to regulations in the light of what has been discovered.

EXPERIENCES IN THE CERTIFICATION OF PRODUCTION AND TRANSFORMATION OF FRUIT PRODUCTS

by Dr. Franco Taccani,
Plasmon Dietetici Alimentari S.p.A.

SYNOPSIS
This article describes how to design, manage and control a whole food production system in order to obtain infant foods that guarantee special safety and qualitative standards. The article also explains how to achieve third party certification of these products and the benefits which ensue.

In order to manufacture food products, such as homogenized fruit and fruit juices for first infancy, aimed at consumers with special needs and having to deliver special safety guarantees and quality standards, we thought essential to adopt innovative and modern (at least at the time) quality assurance systems.

One of our main objectives was the elimination of any risk linked to the presence of residues from phytochemical treatments conducted on fields or from post-harvest treatments applied to the fruit.

Our objective was attained in two parallel ways.

The first was the elimination of residues from those compounds which, as set out in internationally accredited guidelines (IARC, CCTN, EEC etc.) have, or are suspected to have, harmful (carcinogenic, mutagenic, teratogenic) effects on the human organism. Following a precise procedure, elaborated on the basis of the above guidelines, the compounds reported to be harmful, though still permitted by law, were identified and banned from our crops.

The second method consisted of a restriction of the treatments to a limited number of safe products, adopting strict limitations and "integrated pest management" techniques which, if used with care, leave virtually no residues, or residues approaching the analytical zero.

On the other hand crops normally found on the marketplace may still be largely contaminated by pesticides, as shown in table 1, although generally complying with the limits set by law.

The table sets out the data collected by the Italian Ministry of Agriculture and by the Italian Ministry of Health on pesticide residues in apples and pears productions versus data on the same type of fruit from crop productions on which a limited number of phytochemicals had been used.

Although the data from the two Ministries are not homogeneous, in both cases 2% of the samples were found to contain residues exceeding the limits set by the law. In one case 8% of the samples exceeds 50% of the law limits, in the other 69 % exceeds 20 ppb. As regards the controlled phytochemical crops, the little residues found are always below 20 ppb.

Through this careful balance of operations two fundamental results are obtained: the total absence of pesticide residues on one part, and the preservation of the most needed treatments on the other. This has permitted to achieve an optimum qualitative standard, with excellent results also in relation to possible natural contaminations from mycotoxins and infestations in general.

Such innovative and far-reaching systems saw the involvement of various company functions in the attainment of common objectives. Thanks to a precise company policy a strategy was defined, maximum safey levels were set and the necessary resources were identified.

After policies and strategies were finalised by the Management, priority was given to the management of the processes in the definition of the ways and methods to be adopted in order to provide the desired assurances. The verification of the system efficacy and validity was left to the analyses and tests to be performed on the final product. Such principles had been formulated for a while but they were seldom applied. In practice we aimed at the identification of the critical risk points of all the production processes and at the definition of the appropriate preventive measures.

Only recently has the application of these principles been made compulsory by law and directives, though only in relation to hygienic guarantees in food manufacturing; under the widespread denomination of HACCP they represent a very useful tool to achieve and ensure special qualitative standards.

To achieve the desired results we had to monitor the various processes carried out by different functions. We realized that simple analytical controls, as far-reaching as they can be, can neither provide the reliability necessary to guarantee a safe use to consumers, nor the prevention sufficient to ensure manufacturing continuity.

All the processes had to be monitored, from cultivation, gathering and preservation to preliminary transformation down to final production and distribution. Jointly with our suppliers, we developed a set of Quality Systems aimed at reaching a common objective.

In practice we had to establish relationships of close cooperation with our suppliers, defining the objectives and the ways to attain them. We agreed a precise course of action, selected the appropriate farms and farmers, verifying their willingness and ability, bound them through ad-hoc agreements and checked on field that contracts were respected. We mapped out the land areas and conducted an accurate education and information program to increase the growers' awareness. Production, fertilization and treatment techniques had to be agreed directly with the growers.

To realize the method of crop-growing which would be later called "Plasmon Environmental Oasis" specific procedures had to be worked out, assigning responsibilities and defining the operational methods to achieve the integration of the different quality systems.

The sequence of this procedure is briefly illustated in table 2.

This work-plan also requires the adoption of special cultivation agreements and compliance with various operational standards: crop-growing, collection , transportation, storage and transformation.

The monitoring and testing of the System's performance were carried out through numerous analytical controls both on samples taken on field and on the final product, but, primarily, through inspections of the overall System, performed by qualified Agronomists in cooperation with the suppliers.

Certification by an Indipendent Body was a qualifying action of the overall System, which also contributed to its further improvement.

Certification by the C.S.Q.A. (Certificazione Qualità Agroalimentare) of Thiene involved farms, warehouses, first transformation plants and, of course, the Plasmon production factory, its headquarters and factory labs and the relating offices.

An appropriate set of operational standards had to be defined to start off the certification process: they are called "**Technical Operational Standards for the Certification of Homogenized Fruits and Fruit Juices from Plasmon Environmental Oasis**".

The operational standard on which the certification is based identify the objectives, describe the techniques adopted for their achievement, the means and resources used, the controls provided as well as the system's features.

They also comprise: rationale, product characteristics, charts illustrating production, transformation and distribution processes, methods of identifying and tracing the product throughout its cycle, control plans, management of non-conformities, corrective actions, auditing and documentation of the critical points.

In Italy this is the first case of product certification covering the whole manufacturing process, from field to distribution.

Due to its rigour and competence not only has the Certification body testified to the actual effectiveness of the System, but it has also detected possibilities of improving it, increasing its efficiency and offering the parties involved an opportunity of growth.

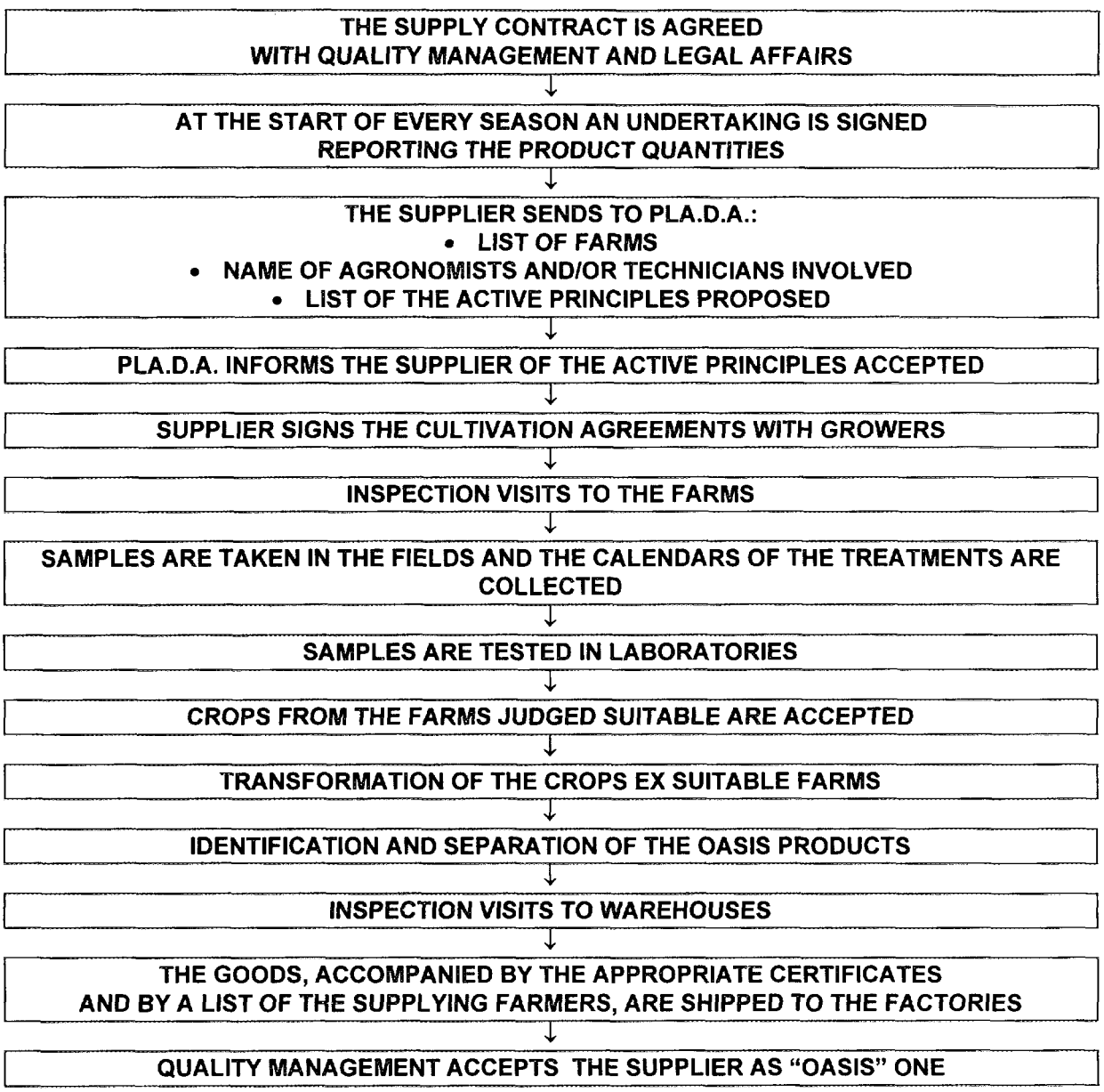
TABLE 1

	Crops of Plasmon Environmental Oasis	**NORMAL CROPS SOLD ON THE MARKET**	
		Italian Ministry of Agriculture	Health Ministry
Samples tested	66	1920	1421
Pesticides monitored	65	87	243
Total tests	2729	44019	66482
Tests per sample	41	23	47
PERCENTAGES ON 100 SAMPLES			
• > the limit set by the law	0	2	2
• > 50% of the limit set by the law	0	8	-
• > 20 ppb	0	-	69
• Residues from compounds banned from Plasmon crops	0	33	36

TABLE 2

FLOW DIAGRAM

SEQUENCE FOR THE IMPLEMENTATION OF PLASMON ENVIRONMENTAL OASES FOR RAW MATERIALS OF VEGETABLE ORIGIN



CONTROLLED ATMOSPHERES DURING MARINE SHIPMENT OF FRESH FRUITS AND VEGETABLES

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Summary
Shipment of perishable fruits and vegetables under controlled atmospheres in refrigerated marine containers is increasing in popularity. The potential benefits of controlled atmospheres include maintenance of produce quality, reduction in the rate of ripening, and a non-chemical means of decay and insect control. The number of companies providing controlled atmosphere services is increasing each year which should reduce costs making shipment under controlled atmospheres economically beneficial for a wider range of commodities.

OVERVIEW OF CONTROLLED ATMOSPHERES DURING MARINE TRANSPORT

Perishable produce has been moved by ship for many years, but it is only recently that controlled atmospheres (CA) as a supplement to refrigeration has become quite common. Years ago, most perishable cargo was generally transported in bulk or, more recently, on pallets within the hull of the ship. The concept of containerization began in the 1950's with the movement of refrigerated transportation containers (often called reefers) by ship. The movement of reefer containers was in part prompted by intermodalism, the movement of containers by water, rail and over the road. The benefits of intermodal transportation are that it provides the shipper with door-to door service with nearly uninterrupted temperature and atmosphere control, and a considerable reduction in handling for their perishable commodities.

Today, virtually any commodity may be transported globally in refrigerated ocean containers via combinations of water and land transit. Microprocessor-based controllers provide precise temperature control, the ability to continuously monitor temperatures remotely, and detailed electronic trip records. Refrigeration systems have high heat removal capacities and provide bottom airflow technology. CA systems can control oxygen, carbon dioxide, relative humidity and ethylene levels. A typical container vessel can carry 2,000 forty-foot containers at one time, limited by the number of electrical receptacles available.

Even with this technology available, the amount of produce shipped via CA container as compared with non-CA container is negligible. For example, of the 164,000 FEUs (forty foot equivalent units) of reefer imports and exports shipped to and from the US, approximately 4,000 FEUs of CA cargo was transported in 1996, just under 2.5% of the total volume. These 4,000 loads also represent more than 75% of the entire containerized CA volume worldwide.

From an economic standpoint, CA transport remains a niche market with relatively low volumes and only a few select products for which it is economically beneficial. To date, most CA loads have been charged a flat fee of $1,500 US in addition to the regular shipping charges. Low value commodities may increase in price by as much as 10% when shipped under CA while a higher value commodity increases in price by only 3% making the use of CA for the higher valued commodity more economical. Relatively high-valued commodities such as avocado, stone fruit, pears, mangoes, asparagus and tangerines made up over 70% of the volume shipped in CA containers in recent years. As additional information is developed regarding the optimal atmospheres to maintain postharvest quality for transportation of various commodities, and atmospheres to control postharvest decays and insect pests, shipment of a greater variety of commodities under CA may become economically beneficial. In

some cases, shipment under CA may open new markets by maintaining quality for the critical length of time needed to reach a distant market, by allowing for less expensive marine shipment in place of air shipment, or by allowing shipment to markets previously closed due to insect quarantine issues.

BENEFITS OF CONTROLLED ATMOSPHERES FOR PERISHABLE COMMODITIES

Maintaining the quality of perishable commodities (fruits and vegetables) requires that attention be paid to many factors. These include, harvesting at the proper maturity, careful handling to avoid damage, rapid cooling to the proper storage temperature for the specific commodity, storage at the proper temperature and humidity, and marketing or utilization prior to the end of the postharvest life. CA should be viewed as a supplement to the factors described previously which can greatly extend the storage life for some commodities.

CA generally contain reduced oxygen or enriched carbon dioxide atmospheres or both. Control of ethylene gas can also be a component of the CA system. The exact concentrations of oxygen and carbon dioxide which provide the optimal maintenance of the commodity varies by commodity, but are usually in the range of 2 to 3% oxygen (air has 21% oxygen) and 3 to 15% carbon dioxide (air has 0.03% carbon dioxide). It is very possible to provide an inappropriate atmosphere for the commodity resulting in product injury and a reduction rather than an extension in postharvest life.

In terms of the direct effect on the commodity, reduced oxygen and elevated carbon dioxide atmospheres that do not cause physiological stress to the product reduce respiration and ethylene production rates and retard compositional changes associated with color, firmness, flavor and nutritional quality. Fruits and vegetables are living plant parts in which metabolic processes continue after harvest. Stored food reserves are consumed during respiration to provide the necessary energy for survival. By reducing the respiration rate, CA (together with storing at the lowest safe temperature) results in slower utilization of the stored energy reserves and allows for a longer postharvest life. In addition, storage under CA reduces the rate of ripening, thereby maintaining the product in a physiological state which is more resistant to physical damage and attack by decay-causing organisms. CA also reduces the product's production of and response to ethylene gas which promotes both ripening and senescence (deterioration).

CONTROLLED ATMOSPHERES FOR DECAY CONTROL

CA with carbon dioxide concentration between 10 and 20% and/or oxygen concentration below 1% have been demonstrated to have fungistatic effects, i.e., they prevent (or greatly slow) the growth of the fungus while the atmosphere is present, but do not kill the fungus. This technique, utilizing high carbon dioxide atmospheres, has been used successfully for the shipment of strawberries, raspberries, and sweet cherries. Each of these three commodities has been demonstrated to tolerate exposure to 10 to 20% carbon dioxide. It has been thought that many other commodities could not tolerate such high concentrations of carbon dioxide. This mind set is based, for the most part, on research to develop CA for long-term storage of commodities (>1 month). Recent research in our laboratory has shown that peaches and nectarines may tolerate as much as three weeks of exposure to 15% carbon dioxide. While 15% carbon dioxide does not completely prevent the growth of *Monilinia fructicola*, the causal agent of brown rot on stone fruits, transportation under 15% carbon dioxide can significantly reduce the growth rate of this pathogen. There are numerous commodities for which this type of research is needed to determine the short-term tolerance to 10 to 20% carbon dioxide and/or 1% oxygen and the benefits in terms of decay control and product quality.

CA FOR INSECT CONTROL

The presence of live insects in loads of perishable products is often a concern for international as well as interstate shipments. Detection of insect pests upon arrival may result in fumigation of the load, resulting in extra costs and potential damage to the product. In some cases, the load is dumped or destroyed. For some shipments, product is required to undergo a specific treatment prior to entry to control insect pests. In other cases where a treatment has not been developed, the produce is prohibited from entry because of insect quarantine concerns.

Many types of treatments have been developed to control insect pests. These include heat treatment, cold treatment, irradiation, and fumigation. With the impending loss of methyl bromide, the most commonly used fumigant, alternative treatments are being sought. CA effects on insect pests have been studied for many years, particularly for control of stored product pests in grains. In recent years, research on the control of insect pests in fresh commodities has shown considerable promise for some commodities and pests. The effectiveness of CA for

insect control is dependent on temperature and exposure time and usually involves atmospheres containing 20 to 95% carbon dioxide and/or < 0.5% oxygen. The atmospheres are extreme and determination of product tolerance as well as insect control is essential. The short-term tolerance of many commodities to such extreme atmospheres is surprisingly good. Examples of successful treatments include a 5 day trial shipment of asparagus from New Zealand to Japan in 60% carbon dioxide at 0°C. The treatment provided complete control of New Zealand flower thrips and green peach aphids without damage to the asparagus. A 12 day treatment with 45% carbon dioxide at 2°C has been developed in my laboratory at UCDavis, for control of Pacific spider mite (*Tetranychus pacificus*), western flower thrips (*Frankliniella occidentalis*) and omnivorous leafroller (*Platynota stultana*), pests of California table grapes. This treatment, if approved, would allow shipment of table grapes to Australia from California for the first time. Pacific spider mite and western flower thrips are pests of many perishable commodities. This treatment may also be useful for shipment of other commodities for which these pests are of concern; however, product tolerance testing is needed. At UCDavis, we continue to develop alternative CA treatments which have application for economically important pests and the commodities on which they are of concern.

CONTROLLED ATMOSPHERE SYSTEMS FOR MARINE CONTAINERS

There are basically two types of CA systems available to ocean carriers, active and semi-active. Active systems give complete atmosphere control through their ability to purge with nitrogen obtained from a hollow fiber membrane or pressure swing absorption (PSA) system. In the hollow fiber membrane system, a membrane which has different permeability for oxygen and nitrogen is used to separate oxygen and carbon dioxide from the nitrogen in air. The resulting high purity nitrogen is introduced into the container to create a positive pressure within the reefer flushing out unwanted oxygen and carbon dioxide. In the PSA system, an activated carbon molecular sieve is used to adsorb oxygen at high pressure and is reactivated at low pressure. Two adsorbers are combined with one adsorbing and the other reactivating at any given time. One drawback to these systems is that they require a carbon dioxide source if the product needs high concentrations of carbon dioxide, but does not generate much carbon dioxide metabolically. For reducing carbon dioxide levels, two principles are used, flushing with nitrogen and carbon dioxide scrubbers. Flushing is generally used when a nitrogen generator is present. However, the concentration of oxygen present in the nitrogen produced by these generators is 2 to 3%, therefore the oxygen concentration cannot be reduced significantly below the 2 to 3% level when carbon dioxide is being flushed. Carbon dioxide scrubbers include the PSA system with charcoal, and lime scrubbers. When carbon dioxide scrubbers are used and less nitrogen flush is used, care must be taken to avoid a build-up of ethylene. Many systems offer special ethylene scrubbers to eliminate this concern.

The most prevalent CA system used today is the semi-active TransFRESH Tectrol^{TM} CA system. The container is injected with an initial gas mixture at origin and the system contains a controller which senses oxygen and carbon dioxide concentrations and can raise the oxygen by adding fresh air into the container and lower the carbon dioxide by activating lime scrubbers. This system cannot reduce oxygen or raise carbon dioxide once the initial atmosphere has been injected. Successful operation requires a well-sealed container. To achieve significant carbon dioxide concentrations, the product must produce significant levels of carbon dioxide metabolically. In 1996, about 25,000 containers were equipped with this system and around 5,000 FEUs had been shipped under CA since the service's inception in 1990. The system is attractive because of the low initial hardware costs and the service provided by TransFRESH.

MANUFACTURERS AND PROVIDERS OF CA CONTAINERS

TransFRESH

See above, semi-active system.

SABROE/FRESHTAINER

The Austrian firm Freshtainer offers the most sophisticated CA equipment available today, the INTAC IV. The system features a PSA nitrogen separator, an optional separate carbon dioxide scrubber and an ethylene scrubber. Humidification and dehumidification are available. Additional carbon dioxide can be added from cylinders. This system can supply down to 1% oxygen and between 0 and 80% carbon dioxide at a humidity of 60 to 98%. The unit is installed behind the refrigeration unit and 295 mm of storage room inside the container are lost. There are 450 containers built to date for this system.

CARRIER TRANSICOLD
Since 1994, this company has been offering its own CA system under the name of EVERFRESH. System uses a membrane-type nitrogen separator. Solenoid valves located behind the membrane make it possible to operate the system with three different purity ratios of oxygen to nitrogen. Control of carbon dioxide is achieved by flushing with nitrogen. Carbon dioxide can also be added from gas cylinders. A cylinder of reference gas is carried along for automatic calibration of the sensors en route. To date, 150 containers have been built.

BIMITSUSHI
New system on the market for summer of 1997. PSA nitrogen generator, nitrogen produced is collected in a container for use while PSA is regenerating. A separate carbon dioxide and ethylene scrubber is included, but it uses the same compressors as the nitrogen generator so that either CO2 and ethylene can be scrubbed or nitrogen can be produced. The unit is installed behind the refrigeration equipment and 295 mm of storage room are lost. A small supply of carbon dioxide in cylinders is available to raise carbon dioxide levels.

ISOLCELL
This Italian firm has been also offering a CA container since 1992. Isolcell is well known in the field of land-based CA storage facilities. Their container system is comprised of a nitrogen generator which operates with a Permea membrane. The system includes a carbon dioxide scrubber and a catalytically operated ethylene converter.

CONAIR-PLUS
G+H Montage is offering a CA system which was developed in cooperation with Isolcell. The system has a nitrogen separator which generates 30 m^3 nitrogen per hour with a residual oxygen content of 3%. The system includes a carbon dioxide scrubber utilizing activated carbon and a catalytic ethylene converter (scrubber). The system is designed to be used with porthole stations. To date, three CONAIR plus systems with a total of 25 porthole stations have been constructed, on the CAP Finistere of the Hamburg-Sud and the two Alianca vessels, the ALIANCA BRASIL and ALIANCA EUROPA. The main problem with the system is tightness of the containers. In contrast to integral-type containers, the containers are not permanently linked with a CA system and the containers are not particularly well sealed resulting in considerable leakage of air. In practice, it has been found that the system can achieve low oxygen contents but cannot increase the carbon dioxide to an appreciable degree.

NITEC
This CA system utilizes an empty container equipped with the controller, the nitrogen generator and a cylinder of carbon dioxide to provide CA control for up to 8 (potentially more in the future) fully loaded containers. The master container is then connected to each of the 8 containers on the vessel, providing the atmosphere modifications for each of the containers. Each container can be set to a separate atmosphere. This system is relatively new to the market and only a few units are presently available.

The list of equipment manufacturers investing in CA continues to increase yearly. The competition developed between these groups and the investments in this technology should lead to lower equipment costs which in turn will help bring down the cost of supplying CA service to produce shippers, therefore attracting additional volumes. With continued regulatory and consumer pressure on chemicals used postharvest, CA will become an important tool for decay and insect control for a variety of products during transport.

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FREEZE CONCENTRATION AND ITS APPLICATIONS IN THE FRUIT JUICE INDUSTRY

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The pursuit of quality in the liquid food industry has been the incentive for development of the freeze concentration technology. Freeze concentration can give an optimal solution in the demand for naturalness at acceptable cost and is therefore an alternative for single strength juices. Top quality juice can be presented to the market at a competitive price/quality ratio.

Due to an increasingly quality consciousness, quality improvement is a continuous concern of food processors everywhere, whether in the domestic or in the world export market. Pursuit of quality is becoming a life style and various trend can be observed.
Among these trends are:

- the increase in demand for high quality products brought by the rise in living standards;
- the increase in acceptance of chilled juices by consumer and distributor;
- the trend towards healthier drinks resulting from better consumer education;
- the rising cost consciousness in increasingly competitive markets;
- the continuing search for new products.

In the fruit juice sector, top quality has much to do with the "naturalness" of a product. In order to meet this demand, juice processors are trying to reduce quality loss by reducing conventional unit operations and are shipping fruit juices in single strength form. This gives relatively high packing- and transportation costs which result in high retail prices and, consequently, limits the market opportunities.
The development of the freeze concentration technology creates an alternative to bring top quality juices on the market at a competitive price/quality ratio.
Freeze concentration operates at freezing point temperature of the product due to which thermal damage of the product is avoided. The efficient separation of water guarantees total flavour and aroma retention while also product losses are eliminated.

Why concentrate?
The food liquid processor is facing the fact that most of the juices contain much water. Water which for most purposes prevents a long shelf life or gives much cost on storage, packaging and transportation. Various concentration technologies can cope with these facts, however, most techniques have the serious drawback of affecting the original quality.

The development of freeze concentration technology and the commercial implementation of this unique system offers the possibility to avoid degradation during concentrating food liquids. Reconstituted concentrate has proven to be indistinguishable from original liquid even over a long period of time.

Concentration

The aim of concentration is to add value to the product. The gross added value is the difference in market value between the concentrated and non-concentrated product. The net added value is the gross added value mines additional processing costs.
However, not only the obvious cost such as utilities, capital and packaging associated with concentration should be examined, but also the change in product quality due to the concentration process and the consumer's perception of the reconstituted product should be considered.

Consequences of concentration processes that negatively affect the added value are:

- selective physical losses such as losses of volatiles.
- physical changes
- chemical and biochemical reactions, such as enzymatic and non enzymatic reactions and destruction of vitamins and nutrients.
- chemical reactions producing precursors which are responsible for an increase in the rate of quality loss during storage.

Factors that positively affect the gross added value are:

- the concentration factor
- the shelf life stability
- the possibility for bulk handling at low temperature.

For concentration of liquid foods three different processes are commercially available with or without combination with other technologies like e.g. aroma recovery and/or distillation. These technologies can be specified as evaporation, reverse osmosis and freeze concentration.

Selective physical losses

Volatile compounds which would otherwise almost be lost in evaporative concentration or partly in membrane processes are at least partly recovered by either separating them from the feed prior to concentration. The volatile compounds can be concentrated by e.g. distillation.
However, for many products the recovery of aromas is not satisfactory (1). In this respect the retention in reverse osmosis is better (2), (3), (4).
Freeze concentration is a highly selective crystallization and separation process that produces pure ice crystals and gives complete separation of water.
In the rating of the three concentration processes the negative gross added value factor of selective losses is:

- no selective losses in freeze concentration
- low to moderate losses in membrane processes
- relatively high selective losses in evaporation.

Physical changes

Physical changes are affected by chemical and biochemical reactions. Since these reactions are strongly influenced by temperature it is obvious that due to its low processing temperature freeze concentration slows down or even prevents such changes.

Concentration factor

The maximum concentration that is achievable is determined by the product viscosity which increases with concentration and with temperature decrease.
For a depectinized and cleared fruit juice the product concentration which technically and economically can be obtained is as follows: evaporation 60 - 75 wt%, freeze concentration 40 - 55 wt% and reverse osmosis 15 - 25 wt%.

Costs of concentration

The investment in membrane processes is lower than that for evaporation with aroma recovery.
The investment for freeze concentration is 2 to 4 times higher than for evaporation.
The operating costs are the lowest for reverse osmosis while for evaporation and freeze concentration the differences are small. The cost of electricity versus steam has a great effect.

Freeze concentration

The freeze concentration process consists of a crystallization and a separation section.
In the crystallization section part of the water present in the product is converted into spherical and pure ice crystals.
In a scraped surface heat exchanger small ice crystals are formed instantly. These very small ice crystals then are pumped into a recrystallizer vessel where the crystals will recrystallize into large and spherical crystals. During this recrystallization process no inclusion of components into the ice occurs thus giving pure ice crystals.
The large pure spherical ice crystals are separated from the liquid in a wash column. The wash column squeezes the juice by mechanical filtration after which the ice is being washed to avoid any kind of losses. The ice is being removed mechanically, is melted and leaves the system as pure water. The losses of soluble solids in the removed water are in the ppm range.
The advantages of the freeze concentration process can be summarized as:

- due to the freezing point temperature during concentration all chemical, biochemical and microbiological reactions have stopped or have a low activity.
- due to the efficient water separation no losses occur.
- due to the closed system contact with oxygen and consequently, oxidation is prevented.
- it is a continuous process which requires no intermediate cleaning.

Consequently, the concentrated product after being reconstituted has the same quality as the original product.
The freeze concentration system is an unique process with maximum aroma retention.
Reconstituted freeze concentrated orange juice has proven by taste panels to be indistinguishable from the original liquid (9). After several months of frozen storage freeze concentrated juice shows even preference over not concentrated orange juice due to the better stability.

Pre-treatment requirement

The merit from freeze concentration is that there is no change in quality during concentration. Freeze concentration, therefore, has the best merits when the feed material is of the best possible quality and that this feed material has been given the optimal treatment.
There are some critical factors to be observed. Each product might need it specific pre-treatment. Improper handling of the product will have a negative influence on its quality. The typical pre-treatment of orange juice when freeze concentrating is extraction with controlled peel oil, finishing, pulp reduction to 4-6 %, de-aeration, short time/high temperature stabilization and fast cooling to +4°C.

There has been questions about the quality effect of heat stabilization on fruit juices.
Heat stabilization is a pre-treatment step that is required for single strength juices as well as freeze concentrated juices. Practice has learned that besides for micro biological reasons stabilization is also needed for inactivation of enzymes.
Experience has learned that the quality of e.g. pasteurized orange juice can be very preserved provided the pasteurization is done mildly e.g. for orange juice 5 seconds, 85°C and under well controlled conditions.

Post treatment requirement

At +4°C freeze concentrated products will have a shelf life of several weeks. For long time storage concentrates are frozen and stored at -18°C. Freeze concentration has the advantage that production is made at freezing point of the product which is generally in the range of -5 to -12°C. This saves energy and time for freezing.

Depending on its further use to have full effect of freeze concentration further heat treatment of the product should be avoided. If reconstitution is done under the proper hygienic conditions the product should be presented to the consumer in the cold chain at +4°C. In this way the shelf life will be limited to several weeks, however, maximum quality retention is obtained.

Economical evaluation

Mainly due to the higher capital cost reconstituted freeze concentrated products will be higher priced than reconstituted products made out of thermal concentrated products. In comparing the two products one should not only compare the concentrate production cost but also compare the retail prices. Evaluations (8) in the U.S.A. show that a 42°Brix blend containing 70% freeze concentrated 42°Brix orange juice is only 4.5% higher in retail price.

The major application for freeze concentrated products is in the segment of high quality juices like the not-concentrated juices. Not-concentrated fruit juices have succeeded to gain market share because it offers the consumer original quality characteristics. Due to its high water content the cost and therefore the market price is very high.

Freeze concentrated products offer quality characteristics like not-concentrated juice, however, thanks to the volume reduction major cost savings are achieved. It is due to the quality preservation and the cost savings that juice from freeze concentrated products are very competitive in the high quality segment of the juice market.

For the purpose of evaluating the economics of freeze concentration the return of investment (R.O.I.) has been calculated for four- and three fold concentration.

Four units have been taken operating during 3,600 hours per year.

The other assumptions are: electricity cost USD 0.06 per KWh, operator cost USD 30,000.00 per year, maintenance 1% of investment, drumming/handling/transportation USD 425 per Mton.

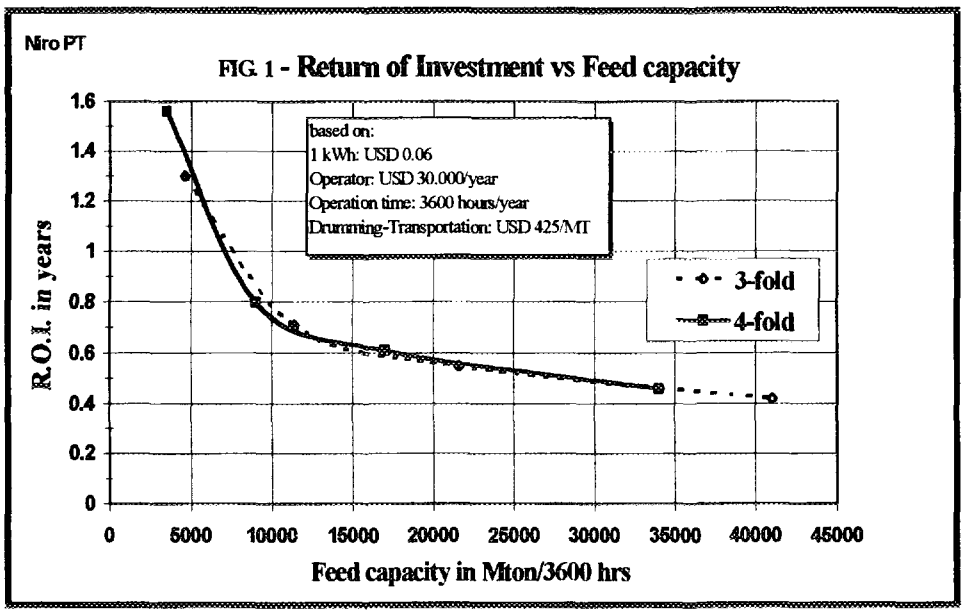
The R.O.I. is calculated from Total Investment/Net Savings.

The Net Savings are taken from the Feed Volume x USD 425/Mton - Operating Costs.

The results have been depicted in Graph 1.

The conclusions from this graph are:

- the difference between four- and three fold concentration is small which can be explained from the higher throughput at three fold concentration and its lower operating costs without additional investment.
- below 10,000 Mton feed capacity there is a strong influence of the capacity to the R.O.I.
- at capacities over 10,000 Mton the R.O.I. ranges from 9.5 month down to 5 months.
- actual drumming/handling/transportation costs have a big influence in the R.O.I.
- even at lower capacities the R.O.I. is relatively low and could make the process interesting for special high quality products.



Summary
Due its low processing temperature and effective water separation freeze concentration guarantees maximum quality retention. In combination with its cost savings for drumming, handling and transportation freeze concentrated products are an attractive and economical alternative for single strength juices.

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INFLUENCE OF THE MECHANICAL IMPACTS ON APPLE: PHYSICAL AND CHEMICAL ASPECTS

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During harvesting, handling and transport, apples are subjected to mechanical shocks. The effects of these stresses depend on physical parameter and on vegetal tissue characteristics. Impacts of intensity comparable to those occurred during harvesting and handling were reproduced using a drop test device with aluminium plate. To describe physical parameters which define impacts, an accelerometer fixed on the apple and a load cell mounted under the plate were used. Samples of two apple varieties were dropped by different heights. After 72 h storage, at ~20° C, the damaged tissues were evaluated, both as dimensions and as aspect. Moreover, samples of sound and damaged tissue were analysed to define some compositional modifications (carbohydrates, organic acids, polysaccharides and phenolic compounds) linked to mechanical damage. Considering that the new food technologies could preserve the fruit characteristics, we can suppose that also limited physical-chemical modifications in apple tissue could be detrimental for the quality of processed products.

INTRODUCTION

Several studies were carried out to correlate mechanical stresses with the damages of the fruits. Different equipments and methodologies to submit the vegetables to dynamical forces and to measure the main mechanical characteristics of the impacts were used in laboratory [8,9,10,11,13,14,19]. Moreover, impacts were measured, by "Instrumented sphere", in real conditions of fruit handling [3,4,5,20]. Many experiences have considered the influence of different parameters of fruits: flesh firmness, depending of maturity, kind and duration of storage, variety of fruit [12,14,16]. Peak of acceleration, velocity change, adsorbed energy were the most used parameters to characterize the impacts [6,15,16]. On the contrary, no exhaustive data were published about chemical modifications in damaged tissues of apples.

To evaluate the susceptibility of apples produced in Italy related to mechanical stresses and to obtain damages apple tissues for the chemical analysis discussed in the present paper, two varieties of apples (*Golden* and *Stark delicious*) were submitted to a series of drop tests and to subsequent analyses of carbohydrates, organic acids, polysaccharides and phenolic compounds using the following described equipment and methodologies.

MATERIALS AND METHOD

DROP TESTS

A very simple device (fig. 1) was used to produce impacts on the fruits by free fall onto a flat plate made of aluminium, from 20, 40 and 60 mm, or onto an other apple learned on the same plate, from an height of 60 mm ("apple vs. apple" test).

Homogeneous samples of 50 fruits - stored at controlled atmosphere (2° C) for 60 days - were used in the drop test from the three heights onto the plate and onto the other fruit (table 1).

The heights chosen for the drop test are considered representative of some hard handlings which the fruits are submitted during harvesting (impact onto the bottom of a bin, for example) and post-harvesting handling (drop onto a conveyor-belt). Particularly, the impacts generated can produce mechanical alterations on apples similar to the

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AMATI A., Professor; CAPRARA C., CASTELLARI M., RAGNI L., Researchers.
Prof. AMATI A. has coordinated the work; Dr. CAPRARA C. and Dr. RAGNI L. have carried out the physical-mechanical analysis, contributing in equal measure to this research; Dr. CASTELLARI M. has carried out the chemical analysis.

ones we can detect on the commercialized fruits by the market or at the reception of the canning industry. The apples submitted to test was suspended at the wished height by a very light sling that permits the mounting, on the apple surface - by modellable wax -, of a microaccelerometer, in the diametrical opposite point of the impact area.

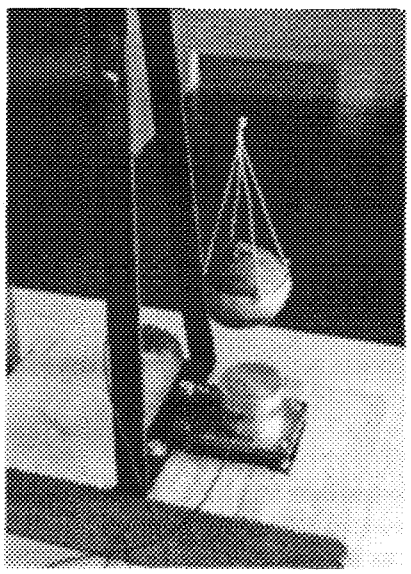

Fig. 1 - Equipment used in the drop test to measure the acceleration of the apple and the force transmitted to the aluminium plate.

Apple variety	Mass		Equatorial diameter[1] [mm]				Longitudinal diameter[2]		Pre-impact circum-ference radius[3]		M-T flesh firmness[4]		D.R.R.[5]	
	[g]	σ [±g]	min	σ [±mm]	max	σ [±mm]	[mm]	σ [±mm]	[mm]	σ [±mm]	[N]	σ [±N]	[° Brix]	σ [±° Brix]
Golden	216	9	77	2	80	2	74	3	36	9	37	5	13.7	1.6
Stark delicious	218	8	75	2	80	2	72	3	35	10	62	8	13.8	1.0

Table 1 - Main characteristics of the apples used in the drop test.

[1] On a horizontal plane; [2] on a vertical plane; [3] average of two orthogonal measures (according to a horizontal and a vertical plane); [4] Magness-Taylor flesh firmness, measured by a 11 mm diameter point; [5] dry refractometric residuum.

Different impact point were chosen on the apple surface: approximately 50 % on the crown, near the peduncle, 50 % on the maximum equatorial diameter of the fruit (in the "apple vs. apple" test, the impact point is always on this last position). Impact area was described as impact radius (average of two orthogonal measures - horizontal and vertical - carried out by a suitable callipers having 25 mm chord). Under the plate, a load cell was mounted to measure the impact force to compare and to control the data collected by the microaccelerometer.

To collect and analyse data the following acquisition chain was used:
- a piezoelectric microaccelerometer (mass of 0,65 g) (B & K, 4375), with lower frequency of 1 Hz;
- charge preamplifier for the microaccelerometer (B & K, 2635);
- piezoelectric load cell with a range of -100 daN + 500 daN (B & K, 8200);
- charge preamplifier for the load cell (Kistler, 5007);
- ADC for IBM PC, with software for Microsoft's Windows;
- a self made C++ programme to analyse files to determine peaks of acceleration and force, velocity change, impulses of force.

Impact characteristics were described as maximum acceleration and velocity change[1] (the last one is linked to the adsorbed energy), considering the first impact and the two following impacts due to the rebounds.

[1] Velocity change $= \int_{ti}^{tf} a \cdot dt$ [m / s],

where:
ti = time at the start of the impact [s]; tf = time at the end of the impact [s]; a = istantaneous acceleration [m/s^2].

After 72 h from the impact, the damages of the apples - stored at 18 ÷ 22 °C - were evaluated cutting the tissues of the impact area and measuring the diameter (maximum and orthogonal to the maximum one) and the maximum depth of the damage. Moreover, the degree of the alterations was checked and described on the basis of a severity numerical scale from 1 to 3 (flesh fracture, diffusion of the dark)(fig. 2).

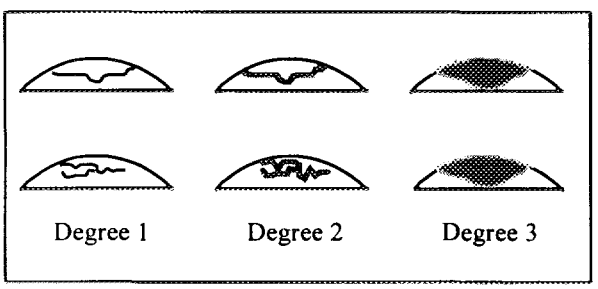

Fig. 2 - Degree of the damage of the flesh tissue according to a numerical scale.

CHEMICAL ANALYSIS

After the evaluation of the damage, the tissues were immediately placed in 100 mL of a $K_2S_2O_5$ solution (600 mg/L - 0 °C) and, then, homogenized using a Silverson laboratory homogenizer. Portions of sound tissue were taken from the same apples in the same way, treated as the damaged tissues and considered as control. Polysaccharides content was determined boiling 15 g of homogenized tissues in 60 mL of distilled water for 1 h. Samples were then made up to 100 mL with distilled water and filtered. Total polysaccharides (TPS) and acid polysaccharides (APS) were determined as proposed by Segarra et al. [17]. Phenolic composition and browning susceptibility were evaluated as proposed by Amiot et al.[1]. Total phenolic compounds were determined on methanolic extracts using the colorimetric procedure of Singleton and Rossi [18]. HPLC analysis of the methanolic extracts were also made using a Jasco 880 PU and a 870 UV-VIS detector equipped with an Inertsil (GL-Science) ODS2 RP column (250 x 4.0 mm). Elution was carried out with a binary mixture of A (water at pH 3.0 with ortophosphoric acid) and B (eluent A:MeOH - 50:50) at 0.7 mL/min, with detector setted at 280 nm. Reflectance measurements (L*, a*, b*, C*) were determined on the pellets with a Minolta Chroma Meter CR-300. Homogenized tissues (20 g) aliquots were centrifuged, twice resuspended with 20 mL of water and recentrifuged. Supernatants were mixed, made up to 100 mL and analysed. Sucrose, fructose, glucose and sorbitol were determined using a Jasco PU-980, equipped with an Alltech 700-CH column at 90 °C and with a 830-IR detector. Isocratic elution (0.70 mL/min.) was carried out with double distilled water. L-lactic acid and malic acid were quantified with enzymatic method [2].

RESULTS

Fig. 3 shows the average values (and the relative st. dev.) of the peak acceleration and velocity change of the first impact and the following two impacts due to the rebounds on the aluminium plate, for the three drop heights, and for the "apple vs. apple" test.

The trends of the different impacts underline that the most characteristic impact - related to the drop height - is only the first one. In fact, falling from a greater height, the apple reaches a greater rebound height, after the first impact, but its flesh tissues are less elastic, because more serious ruptures happened (fig. 4), so, a velocity change reduction of the second impact is observed. Moreover, a very high standard deviation affects the measures, although this is mainly due to the different impact area on the apple surface.

Table 2 shows the influence of the drop height on the average dimensions and the quality of the dàmages. From 40 and 60 mm, all the apples show flesh damages. The dependence of the average values of damage dimensions on the drop height is quasi-linear, the dependence of the damage degree on the drop height is quasi-linear for the *Golden* apple too, while it is exponential for the *Stark* variety.

Altogether, *Stark* variety appears more delicate than *Golden* variety, above all concerning the damage degree (it also increases when the circumference radius of the impact area decreases). Yet, about this last aspect, it is necessary to consider the differences between the peak acceleration values for the two varieties (higher values for the *Stark* apple), due, above all, to the different flesh firmness of the fruits.

The comparison between the values of the impulse (calculated by the force values measured by the load cell) and the variation of the motion quantities (calculated by the accelerometer) shows 2 ÷ 3 % average differences, that

confirm the correct mounting of the accelerometer and of the calibration of the chain.

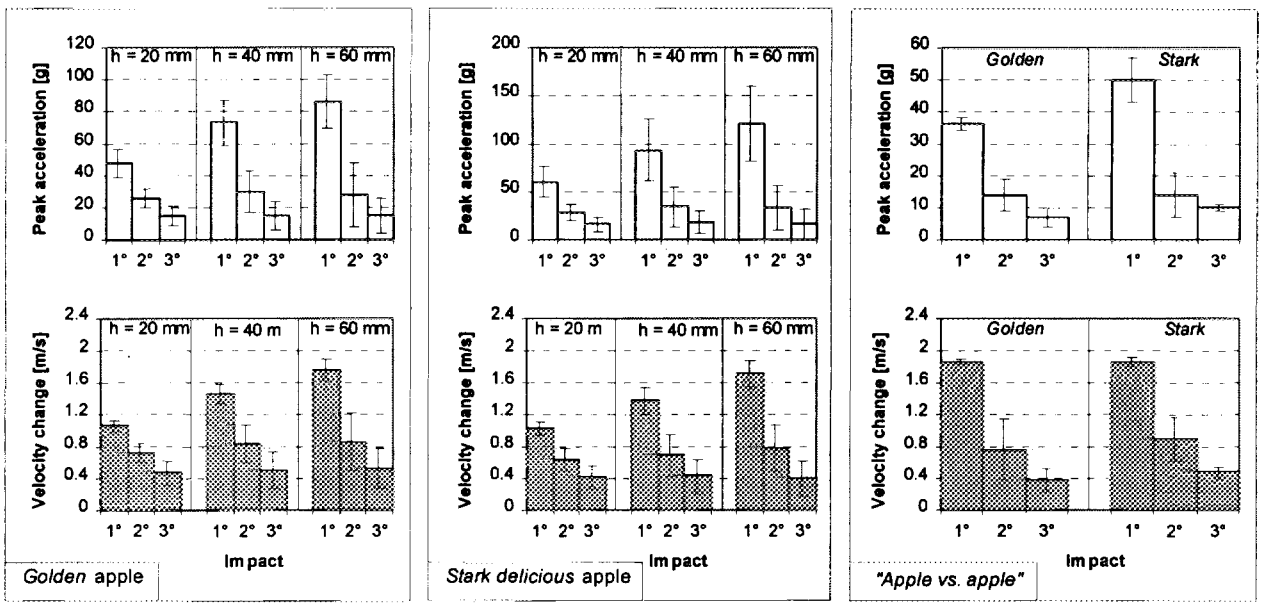

Fig. 3 - Peak acceleration and velocity change of the first impact (1°) and of the following two impacts (2°, 3°) due to the rebounds, for the three drop heights and for the "apple vs. apple" drop test (from an height of 60 mm).

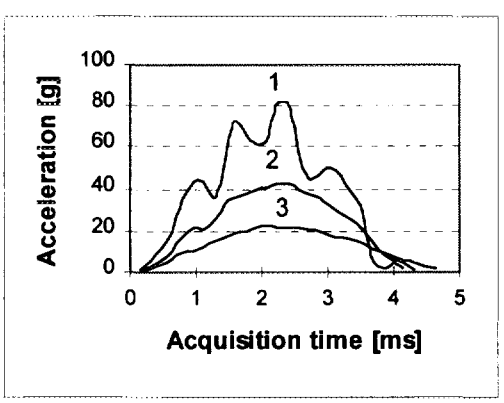

Fig. 4 - Typical trend of the instantaneous acceleration measured on an apple (*Golden* variety) during the drop test (height of 40 mm). During the first impact, we can observe the evident rupture points of the flesh tissues.

1) First impact; 2) impact after the first rebound; 3) impact after the second rebound.

Apple variety	Drop height	Damage diameter(1)		Max damage depth		Damage degree(2)		Undamaged apples
	[mm]	[mm]	σ [±mm	[mm]	σ [±mm]		σ	[%]
Golden	**20**	11	2	3.1	0.7	1.2	0.4	22
	40	14	3	3.9	0.8	1.3	0.5	-
	60	16	3	4.7	0.9	1.8	0.6	-
Stark delicious	**20**	12	2	3.0	0.7	1.5	0.6	7
	40	15	2	4.5	0.8	1.9	0.6	-
	60	17	3	5.7	1.2	2.2	0.7	-

Table 2 - Characteristics of the flesh damages vs. drop height, for the two apple varieties((1) Average of two hortogonal measures; (2) see fig. 2).

The results of the "apple vs. apple" test, from 60 mm of height (Table 3), shows a low percentage of damaged apples, for the *Golden* variety, while this percentage grows to 40 % for the *Stark* apple. In any case, the damage are represented by a light fracture of the tissue.

Apple variety	Damage diameter[(1)]		Max damage depth		Damage degree[(2)]		Undamaged apples
	[mm]	σ [±mm]	[mm]	σ [±mm]		σ	[%]
Golden	15	3	3.8	0.3	1.0	0.3	87
Stark delicious	14	3	3.3	0.6	1.1	0.5	60

Table 3 - Characteristics of the flesh damages in the "apple vs. apple" test, for the two apple varieties (drop height of 60 mm)([(1)] Average of two hortogonal measures; [(2)] see fig. 2).

HPLC analyses of apples homogeneized seem to indicate a higher content of sucrose and a lower concentration of fructose, glucose and malic acid in damaged tissues (table 4) than in the sound ones. Observing the absence of ethanol, the low level of l-lactic acid and the peel integrity, which suggest no microbiological contamination, it's possible that this results are due to different cellular activities between sound and damaged tissues after the drop test.

Also polysaccharides contents are influenced by the impacts (fig. 5). APS do not significantly differ between control and damaged tissues, while TPS increase in damaged tissues.

Apple variety	Sample	Sucrose [g/kg]	Fructose [g/kg]	Glucose [g/kg]	Sorbitol [g/kg]	Malic acid [g/kg]	L-lactic acid [g/kg]
Golden	Control	19.5	59.6	23.2	2.2	2.65	0.03
	Damaged	22.0	57.8	20.2	2.4	2.25	0.01
Stark delicious	Control	20.1	66.2	20.2	2.5	3.38	0.03
	Damaged	23.4	63.8	18.0	2.6	3.03	0.02

Table 4 - Principal fixed components of the apple tissues.

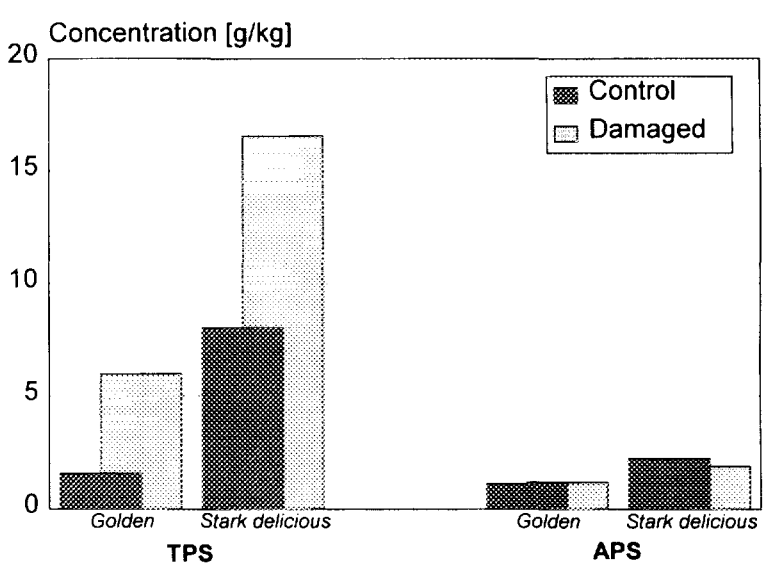

Fig. 5 - Polysaccharides contents in the apple tissues.

In table 5 are reported the results of reflectance measurements on the pellets after methanolic extraction. Its clear that in damaged tissues there is a decrease of lightness (L*) and an increase of the red-yellow components of colour (a*, b*) and chroma (C*) if compared to control tissues, linked to browning phenomena occurred in damaged tissues after the impacts.

Apple variety	Sample	L*	a*	b*	C*
Golden	Control	64.92	-5.47	18.21	19.01
	Damaged	58.08	-2.17	30.52	30.60
Stark delicious	Control	63.41	-5.91	17.84	18.79
	Damaged	62.51	-4.44	27.67	28.02

Table 5 - Reflectance measurements on the pellets after methanolic extraction (CIE, 1976)

Table 6 shows that in methanolic extracts of apple tissues the total phenolic compounds contents result lower in damaged samples; moreover the HPLC chromatograms of these extracts underline a different peaks distribution in HPLC separation (λ = 280 nm) between samples obtained from sound and damaged tissues.

Apple variety	Sample	Total phenolics [mg/kg]
Golden	Control	587
	Damaged	472
Stark delicious	Control	652
	Damaged	595

Table 6 - Total phenolic content in homogenized tissues (methanolic extract).

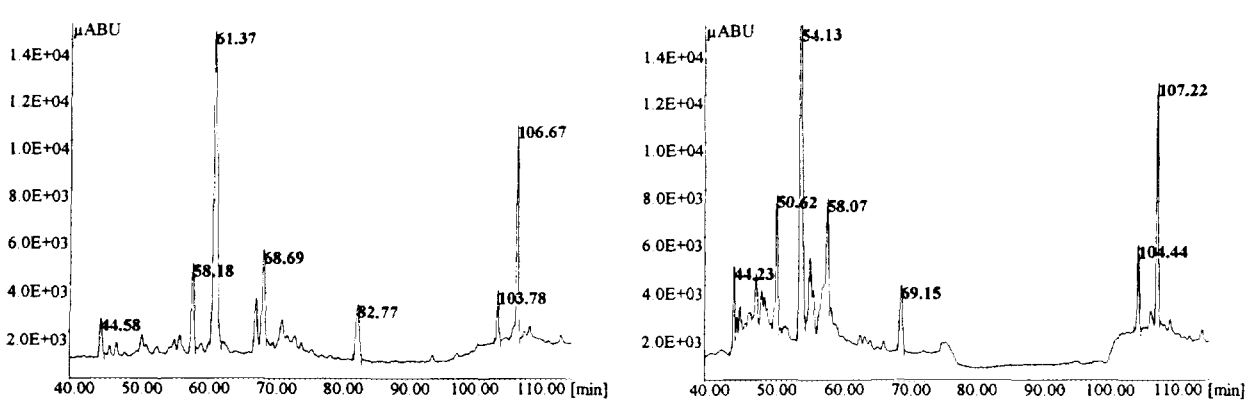

Fig. 7 - HPLC chromatograms of methanolic extracts of sound tissues (left) and damaged tissues (right).

CONCLUSIONS

During harvesting and handling, apples can be exposed to mechanical damage risk caused by impacts, because also a low drop height frequently causes fractures and blackening of the tissues. In the case of hard impact (onto metal, wood, plastic), over 20 mm drop height, damages of significant dimensions (> 10 mm) are detectable on a great percentage of fruits, with blackening diffusion increasing when the drop height increases. In the case of impact onto an other fruit (for the same drop height), a significant reduction of the acceleration peak (~ 60 %) was observed and only a percentage of fruit < 40 % shows light alteration. Chemical analyses underline that in damaged tissues, if compared to sound tissues, some modifications occur concerning sucrose, fructose, glucose and malic acid content, color parameter L*, a*, b*, C*, neutral polysaccharides and extractable phenolics.

If we consider that apple damages, due to compression force, also frequently occur during transports [13,14,17], it is clear that this kind of physical-chemical modifications can represent a significant problem for products obtained by innovative process, as deep-freeze, mild technology, high pressure, which preserve the quality of fresh fruits.

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MATERIAL SELECTION FOR THE RETAIL PACKAGING OF FRESH FRUIT AND VEGETABLES

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Vegetable parchment is a very safe and well known material, which has been used in contact with foods for more than a century. In this work the diffusional properties of parchment sheets were characterised in the perspective of using this material as permeability and selectivity modulator in a flexible package for minimally processed vegetables. A suitable packaging should be able to provide a combination of both high permeability and selectivity (ratio of CO_2 to O_2 permeability) according to the expected gas composition at steady state. The results obtained highlighted interesting features of the material, which has a permeance of both O_2 and CO_2 around 4-6000 $cm^3m^{-2}24h^{-1}$ $100kPa^{-1}$ and a selectivity of 0.8. Moreover, the porosity, and the consequent gas permeance, seem to be controllable during manufacturing by varying the surface pH, which can affect the tightness of the cellulosic network of the material.

INTRODUCTION

Plastic packaging in produce distribution has been used in the past mainly to facilitate product handling and provide brand identification but, recently, a greater emphasis on freshness and product quality has resulted in a revaluation of plastic films for fresh and minimally processed products [1-4]. Since gases permeate polymers through a well known phenomenon of activated diffusion [5], plastics films may be considered ideal materials for modified atmosphere packages [3,6-7].

Actually, modified atmosphere (MA) is naturally created in a package, as a direct result of counterbalancing of the vegetable aerobic respiration (O2 uptake and CO2 production) with the diffusion of gases across hte package walls. Today, the importance of low O2 and moderately high CO2 concentrations in the surrounding atmosphere in maintaining the quality of fruit and vegetables is well documented [8-11] and it is also known that a key parameter for a MA package is its selectivity, which refers to the ratio of CO2 to O2 permeability of the plastic film [3,12]. A suitable packaging material for a given produce should be able to provide a combination of both permeability and selectivity, according to the type of vegetable, its mass, temperature, package size and expected gas composition at steady state. The suitability of plastic films in maintaining modified atmospheres for fresh fruit and vegetables is however a hard task to achieve; testing a number of plastic films for various products [13], led to the conclusion that there could be a risk of low O2 permeability of many plastic films and that most of them provide only a narrow range of gas selectivity higher than the optimum values, which are in the range 1.5-4 [14].

The ultimate solution to these problems of selective gas transmission, could be the development of new polymers with the different diffusional properties required. Developing new polymers, however, is time consuming, it could be environmentally problematic and the potential results are uncertain, so the choice goes to combined systems. By using two or more devices having different selectivities, a broad range of effective selectivity and total permeability can be generated [12]. The development of these new forms of packaging systems for fruit and vegetables led to the idea of films characterised by microporosity and microperforations; in particular, a silicon membrane coupled with a porous membrane seems to be a good way of increasing the permeability in a controlled manner [3,12,15].

In our work we wanted to characterise the diffusional properties of vegetable parchment in the perspective of using this safe and traditional material as permeability and selectivity modulator in a flexible package for minimally processed salad. Parchment, commonly called vegetable parchment, has been manufactured since 1850 by chemically treating paper. By soaking absorbent paper in concentrated sulphuric acid, the cellulosic fibres are significantly swollen and partially dissolved [16]. In this state the plasticized fibres close their pores, they fill in voids in the fibre network and thus produce intimate contact for extensive hydrogen bonding; rinsing with water causes reprecipitation and network consolidation. As a result of this process, vegetable parchment has several distinctive properties: it is both odourless and tasteless, it is partially or completely grease resistant and, depending on its

weight, it allows the passage of air or is nonporous; thus it seems possible to design a modulable porous sheet to combine with permeable plastic film.

EXPERIMENTAL

VEGETABLE PARCHMENT

The sheets of parchment used for the experimental measurements of gas permeability, were supplied by a specialised paper-mill (CIMA spa, San Giovanni Bianco-Italy). The general characteristics of the parchment sheets, provided by the manufacturer are reported in Table 1.

Whenever it was possible, the measurements were performed on the same sample or samples obtained from the same sheet. The following procedure was used to modify the pH of the parchment sheet,: square samples of vegetable parchment (15 x 10 cm) were soaked at room temperature (24 $\pm$ 2°C) in appropriate solutions of diluted H_2SO_4 (for pH = 1.89), Citric acid (for pH = 2.31) or Citrate/Citric acid buffer (for pH = 3.31); after the wet samples were dripped carefully and allowed to dry in a vented oven at 40°C, stretched among four grips to avoid folds and maintain the specimens level. Some samples were also neutralised after the acid treatment by using NaOH 12N and washing the samples with plain water until a pH of 7 was reached. The pH measurements were always performed on the surface of the sheet with a slightly moist, combined electrode (Ingold, METTLER-TOLEDO, Switzerland).

Table 1 - Technical data of the vegetable parchment sheets used.

Property	Test Method	Value
Grammage (g m^{-2})	ISO 536	64 $\pm$ 4%
Thickness (μm)	ISO 534	70 $\pm$ 8%
Bursting strength (kPa)	ISO 2758	220 (min. 176)
Breaking load in M.D.(kN m^{-1})	ISO 1924	5.8 (min. 4.64)
Breaking load in C.D.(kN m^{-1})	ISO 1924	2.7 (min. 2.16)
Brightness (%)	ISO 2470	85 (min. 83)
Opacity (%)	ISO 2471	70 (min. 67)

PERMEABILITY MEASUREMENTS

The gas permeability was measured using the quasi-isostatic instrumental method (Gas Permeability Tester GPM 200, LYSSY, Zollikon-Switzerland) at various temperatures and partial pressures but always at 0% of relative humidity.

The instrument temperature was controlled by means of an external temperature controlled water bath (Haake F3, ENCO, Spinea-Italy), with an accuracy of $\pm$ 0.5 °C. The different gas mixtures were combined from pure gases (SIAD, Monza-Italy) mixed by means of a proportional gas blender (tri-gas blender mod. 013F, PBI DANSENSOR, Ringsted-Danemark) and checked by GC analysis. The terms *gas transmission rate* and *permeance* are used according to ASTM standard[17]. Whenever possible, the *selectivity* of gas permeability has been calculated as the ratio between carbon dioxide and oxygen permeance. The different conditions used for the permeability measurements are summarised in Table 2.

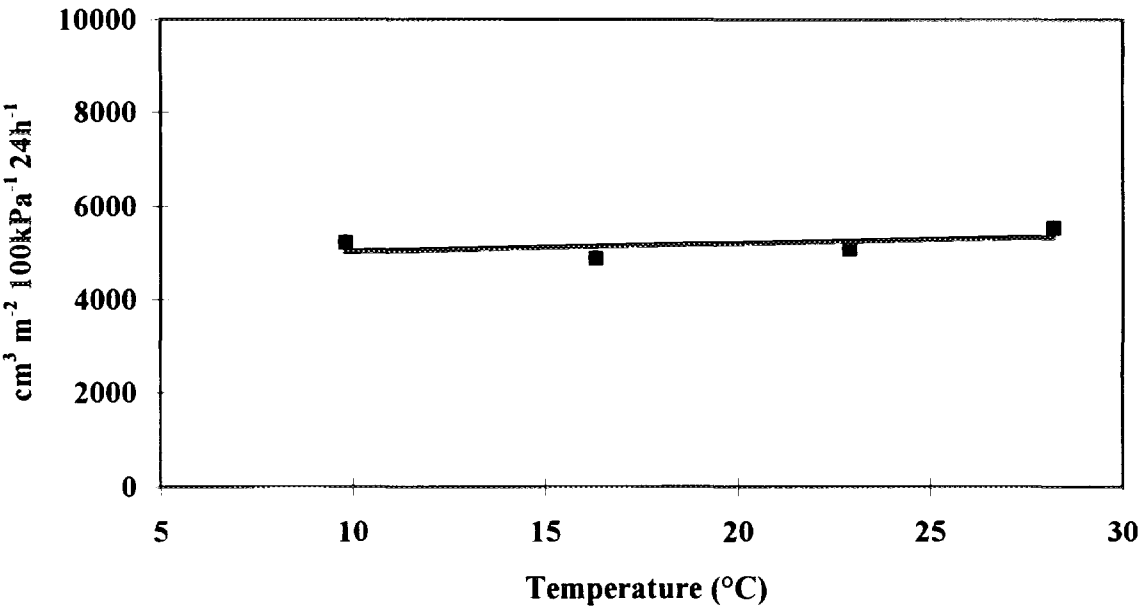
Table 2 - Conditions applied in measuring the O_2 and CO_2 permeability of the vegetable parchment sheets

Variable	Oxygen permeability	Carbon dioxide permeability
Temperature (°C)	9.8 - 16.3 - 22.9 - 28.2	-
Partial pressure (kPa)	100 - 49.9 - 17.4 - 7.5	100 - 48.8 - 21.9 - 7.93
Surface area (cm^2)	9 - 12 - 16 - 20 - 25	-
pH	7.00 - 3.31 - 2.31 - 1.89	7.00 - 3.31 - 2.31 - 1.89

RESULTS AND DISCUSSION

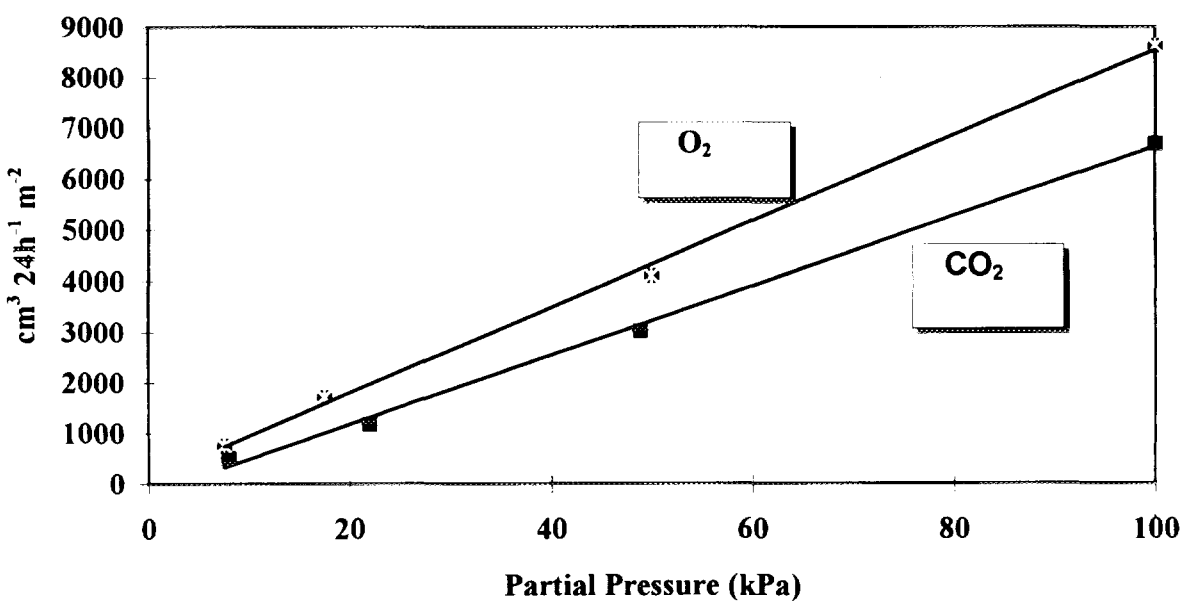
In order to characterise the diffusional properties of the vegetable parchment sheets, we started considering the temperature dependence of the oxygen transmission. These data are presented in Figure 1 as Oxygen permeance versus Temperature, in a range compatible with the shelf-life of a packed vegetable product (10-30°C). Two main comments should be made regarding these results. Firstly, it must be noted that the oxygen permeance of the sheets tested (about 5000 cm^3 m^{-2} 24 h^{-1} bar^{-1}), is quite close to that of many plastic films of suitable thickness (for instance a 30 μm thick polyolephyn film can have similar figures) and quite far from those of other common papers like glassine, kraft and greaseproof paper which can have a 10 or 100 times greater oxygen permeance.

Figure 1 - Oxygen Permeance versus Temperature



The second comment concerns the considerable independence of the gas transmission from the temperature, within the range considered. This clearly indicates that the transmission is not an activated diffusion phenomenon and demonstrates the porous nature of the material. The independence of gas transmission from possible temperature changes is not desirable for the packaging of fresh produce, since temperature deeply affects the respiration rate (the Q_{10} values are generally 2 to 3), much more than the gas transmission rate, whose Q_{10} values are normally 1 to 2. What should be expected is a packaging material able to modify its diffusional properties according to the changes of O_2 consumption rates due to temperature fluctuations; but this seems to be a quite difficult task and also a questionable need, considering the transient and rare nature of temperature changes during storage or transportation[3].

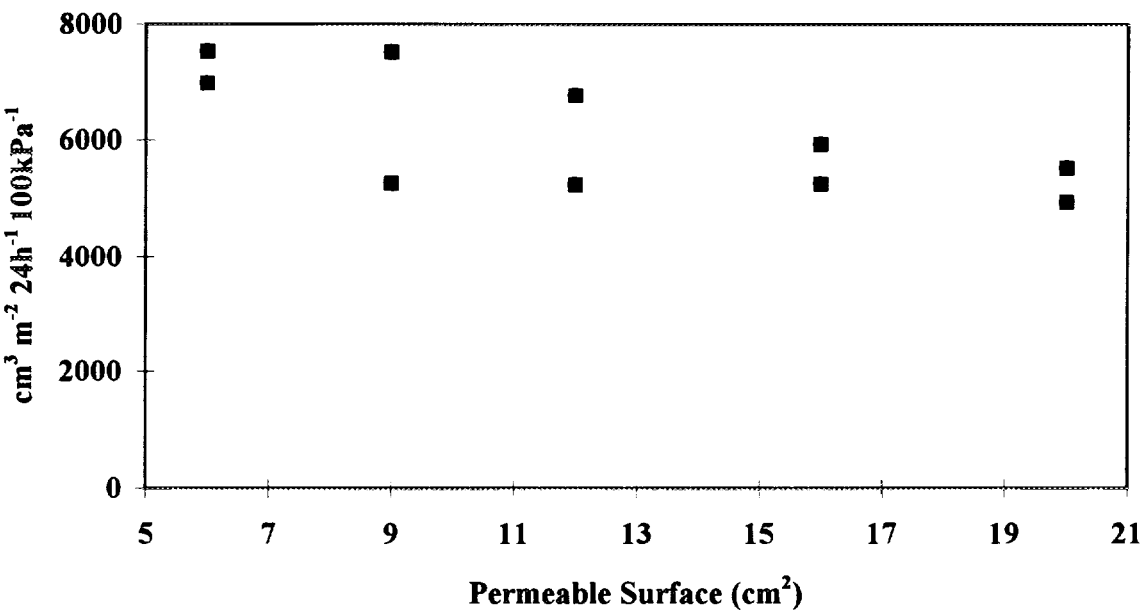
Figure 2 - Gas Transmission Rate versus Partial Pressure



Besides the oxygen permeability, for a fresh vegetable application is obviuosly very important to know the carbon dioxide permeability of the packaging material. Therefore, at a constant temperature of 10 °C, the permeability measurements were performed for both O_2 and CO_2, with the partial pressures ranging from 7 to 100 kPa.

These results are in Figure 2 as Gas Transmission Rate versus Partial Pressure. The straight lines which interpolate the experimental results, show the good correlation between the transmission rate and the driving force, a relationship which allows an accurate estimate of the gases exchange across the parchment sheet and consequently the correct forecast of passive atmosphere modification. Moreover the results highlight the faster transmission of oxygen in comparison with carbon dioxide. This phenomenon is in accordance with the values of diffusivity of CO_2 and O_2 in air without convective flows[15], which lead to a selectivity for a porous material of about 0.82. Averaging all the values of permeability collected in this work on parchment sheets (almost 40 measurements) we had a selectivity of 0.804 with a standard deviation of 0.123. At this point it is evident that combining a relatively small surface of parchment with a plastic package might represent a good method to obtain the desired high rate of gas exchange with the right selectivity of oxygen and carbon dioxide transmission also for vegetables such as minimally processed salads, with high respiration rates.

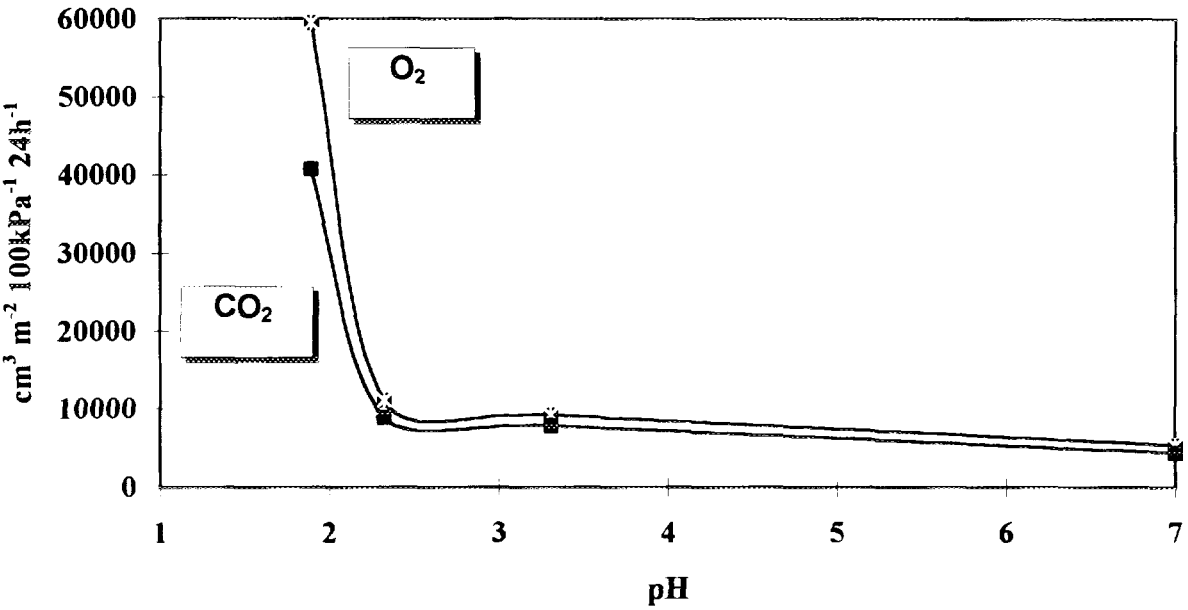
Figure 3 - Permeance versus Surface Area



The rather high variability noted in selectivity calculation is likely connected to the unpreventable heterogeneity that to some extent always affects cellulosic materials. During the parchmentizing process the cellulosic fibres become gelatinous and partially fuse together but, even if the fibrous structure is lost, the material cannnot be regarded as a continuous matter. As a consequence of this lack of homogeneity we observed a shift in oxygen permeance according to the surface area that was submitted to the test. Figure 3 shows these results: the progressive reduction of permeable area on the same sample of parchment sheet, led to a continuous increase in oxygen permeance which in two different samples exceeded 25%. Even if this evidence is in contrast with the need of an accurate estimate of gases' exchange, it is possible to suppose an improvement in parchment manufacturing to reduce the material heterogeneity.

As previously mentioned, the transformation of paper in parchment is a pH dependent process: the action of concentrate sulphuric acid establishes the extent and intensity of parchmentizing through the gelatinisation of cellulosic fibres; however this process must be controlled and stopped by means of washing and neutralisation of the acidified material, to avoid a complete dissolution of the cellulose and to stabilise the structural modification. Starting from this assumption, it was decided to investigate the effect of parchment pH modification on oxygen and carbon dioxide permeance and, in compliance with the procedure described in the experimental section, the pH of some samples of parchment sheets were brought from the natural value of neutrality to 3.3, 2.3 and 1.9 pH respectively, before performing the gas permeation measurements. The results obtained were plotted in Figure 4 and show a strong effect of the lowest pH value on the gas transmission, increased by ten times in comparison with the permeance of the unmodified material, without any change in selectivity.

Figure 4 - Permeance versus pH



This increase in gas permeance, however, is almost completely reversible; in fact washing and neutralising the previously acidified samples restored the original values of permeability (data not shown). The dramatic effect of pH modification on permeability may be explained with the dissolution of hydrogen bonds and consequent relaxing of the parchment structure and, above all, it could indicate the true possibility of modulating the gas transmission rates of parchment trough a variable that can be easily controlled during the manufacturing process. This opportunity possibly allows the easier and more flexibele design of a porous part of a package for respiring produce, increasing the possibility of realising an intelligent pacakging.

CONCLUSION

Vegetable parchment is a very safe and well known material. Free of additives and contaminants, parchment has been used in contact with foods for more than a century. It is a porous material but its porosity is tight, leading to very interesting values of gas transmission, similar to polyolephyn plastics but with a selectivity less than 1. Above all, the porosity and the consequent gas permeance seem to be easily controllable during manufacturing through process variables like temperature, time and, particularly, final pH of surface, which all can affect the tightness of cellulosic network and the diffusional properties of the material. It is likely, and at the moment under investigation in our laboratory, that by combining a surface of parchment with plastic films it could be possible to find an effective, environmental friendly end convenient way to obtain the optimum gas exchange conditions for many vegetable products.

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INFLUENCE OF STORAGE TEMPERATURE ON THE MICRONUTRIENT CONTENT OF GREEN BEAN (*Phaseolus vulgaris* L.)

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Water-soluble vitamins and mineral elements evolution of two varieties (flat pod and round pod) of green bean (*Phaseolus vulgaris* L.), under different storage temperatures: 4, 8, 12 and 20 °C have been studied. Water-soluble vitamins C, B_1 and B_2 were analysed by fluorometric method; and vitamin B_6, by spectrophotometry. Mineral elements (Na, K, Ca, Mg, Cu, Fe, Mn, Zn) were determined using flame atomic absorption spectrophotometry (AAS). Because of fungus growth on samples stored at 8, 12 and 20 °C, and chilling injury symptoms on those stored at 4 °C, the final sampling dates were: 22 days for flat pod green bean and 15 days for round one. To evaluate the storage temperature influence on the micronutrients content of this vegetable, the one-way statistical analysis of variance ANOVA has been used.
From the results, the micronutrients most affected by the storage are vitamins C and B_6, as well as the mineral elements Ca and K. To keep this micronutrients in the optimal levels, a maximum storage of eight days, under 8 °C is recommended.

INTRODUCTION

Concentrations of vitamins in fruits and vegetables can vary considerably depending on several factors such as cultivar, maturity harvest, post-harvest handling methods and storage conditions. The mainproblem for vitamins analysis is their low stability related to physico-chemical factors such as pH, temperature or light. The presence of different vitamers with different vitamin activity and behaviour respect to the analytical signals should be also considered (4).

The total amount of vitamins in a food sample may not correspond of that avaible to a human, as food processing can cause a loss of vitamin activity through the formation of reaction products that are unavailable to the organism.

Vegetables are the most significant sources of dietary vitamin C (19). Ascorbic acid is very susceptible to oxidation, and as oxidation is most dependent of pH; most studies report greatest stability of vitamin C at low pH and the presence of sugars (2). Variability in vitamin C content of vegetable products is influenced by processing factors, packaging and storage conditions.

Thiamin and riboflavin are water-soluble vitamins whose low intake can produce deficiency diseases. Although thiamin deficiency, beriberi, occurs infrequently, subclinical deficiencies are more common, characterized by loss of appetite and weight. Vitamin B_6 has an important physiological function, taking part on the methabolism of proteins in the human body (13).

The mineral fraction of fruits and vegetables has a great importance for the organic funtions where they take part. They are specially rich in potassium, and some microelements, depending on the kind of vegetable (3).

Watada *et al.*(1987) (18) studied the variations in some vitamins during the storage of some vegetables, finding that vitamin C decrease considerably during storage of green bean, thiamine tends to increase and riboflavin does not suffer important variations.

This work is aimed to evaluate the evolution of water-soluble vitamins and mineral elements during storage of green bean samples, in order to select the best storage temperature to keep the optimal content of this micronutrients. This study is part of a more complete work about the storage influence on the chemical composition of green bean.

MATERIALS & METHODS

SAMPLING

Two varieties of green bean (*Phaseolus vulgaris* L.), flat pod and round pod, proceeding from Almería (Spain) have been analysed. The following temperatures were selected for green bean storage during one month: 4, 8 and 12, and a control temperature of 20 °C.

Flat pods green bean began deteriorating after fifteen days of storage at 8, 12, 20 °C, because of the fungus growth; although samples stored under 4 °C did not seem to be contaminated by fungus, they showed chilling injury symptoms. For all these reasons the samples were discarded (considered as comercially useless) after twenty-two days of storage.

Round pods green bean stored at 20 °C showed high fungus presence after five days of storage, so they were discarded after this moment. For samples stored at 4, 8 and 12 °C, fifteen days was the maximun time of storage considered, because of the fungus growth and the appearance of chilling injury on samples stored at 4 °C.

Samples were analysed inmediately after receipt and on different days during the storage period. Each sampling date, 500 g of sample were taken for analysis. The ends of the pods, as well as the contaminated areas were discarded (analysing only the edible part). One part of the sample was freezdryed, to determine its mineral content, and the other one was homogenized as fresh sample for vitamins analysis. All the analysis were carried out on triplicate.

ANALYTICAL PROCEDURE

From the different methods for determining water soluble vitamins we have chosen the fluorometric method described by Brubacher (1985) (4) and recommended for COST 91, for the determination of vitamin C in our samples; fluorimetric one of the Association of Vitamin Assay (1985) for vitamin B_1 and B_2 and spectrophotometryc method for vitamin B_6 (13). The instrumental equipment used were: a Perkin-Elmer LS-3 Fluorescence Spectrofotometer and a Pharmacia LKB-Ultrospect Plus Spectrophotometer.

For mineral elements, the analysis have been carried out by atomic absorption spectrophotometry, wich has showed very good accuracy, precission and specificity (16, 10), using a Perkin - Elmer Spectrophotometer mod.2280 provided with air - acetylene flame.

Vitamin C

10 g of homogeneizated green bean were diluted with 40 mL of metaphosphoric acid solution (80 g metaphosphoric acid and 30 mL of glacial acetic acid, made up to 1L with water) to extract the ascorbic acid of samples, standing for 15 min. 20 mL of filtered homegeneizate were oxidazed to dehydroascorbic acid with 2 g of Norit ; the mixture were allowed to stand for 30 min and then filtered again.

The dehydroascorbic acid was transformed to a fluorescent quinoxaline compound by a subsequent reaction with 1,2 phenylenediamine (sample solution). Blocking of this reaction by boric acid a stable boric acid-dehydroascorbic acid complex is formed, and provides the relevant blank value (sample solution blank). For this reason, 5 mL of filtrate were diluted with 5 mL of sodium acetate solution (500g sodium acetate trihydrate dissolved in water to 1L) and allowed to stand for 15 min. Another aliquote of 5 mL of filtrate was diluted with 5 mL of boric acid-sodium acetate solution (3g boric acid dissolved in 100 mL of sodium acetate solution) and allowed to stand for 15 min. 2 mL of each of the samples solutions were diluted with 5 mL of 1,2 phenylenediamine solution (40 mg 1,2 phenylenediamine dissolved in water to 100 mL) and allowed to stand in the dark for 35 min. The emission at 430 nm with a 350 nm excitation was then measured of each sample solution and set to zero with its corresponding sample solution blank. A standard calibration curve of ascorbic acid, between 1 and 20 μg/mL, was used for the calculations: $y = 1.184\ x + 0.1368$ ($r^2 = 99.88$ %), were y = fluorescence signal and x = μg/mL of ascorbic acid.

Vitamins B

Accurately weight 20 g of samples were place in a 100 mL volumetric flask and added 60 mL of acetic acid/sodium acetate buffer pH 4.0-4.5. For the conversion of bound vitamins to their free form, 0.2 g of diastase and 0,2 g of papain (proteolytic enzyme) were also added. the mixture was kept in incubation at 37°C overnight, and after, cooled to room temperature, made up to 100 mL with water and vaccum filtered.

For thiamine (vitamin B_1) determination, 5 mL of filtrate were transfered to centrifuge tubes, and the following reagents were added: 4 mL of 25 % KCl, 3 mL of alkaline ferricyanide (30 mg potassium ferricyanide dissolved in 100 mL of 15 % NaOH) solution and 10 mL of isobutyl alcohol, and shaked vigorously. Thiamin

was oxidized by potassium ferricyanide in the presence of strong alkali, yielding thiochrome wich is soluble in isobutyl alcohol. When irradiated with ultraviolet light, thiochrome fluorescens emitting a blue light.

For each sample, a blank was prepared, placing a second aliquot of the extract into a centrifuge tube and treat identically except that 15 %NaOH is added rather than alkaline ferricyanide. Both, blanks and samples were centrifuged at 1500 rpm, for 5 min.

The clear colorless isobutyl alcohol solutions were decanted into matched cuvetes and read the fluorescence at 365 nm and 435nm, setting to zero with the corresponding blank of each sample. To make the calculations, a calibration curve of a standard solution of thiamine between concentrations of 0.025 and 0.080 μg/mL was used. The ecuation of the standard curve is as follows: $y = 1105.350\,x - 3.916$ ($r^2 = 99.08$), where y = fluorescence signal and x = μg/mL of thiamine.

For riboflavin (vitamin B_2) analysis, 10 mL of filtrate were placed in a amber glass bottle and added 1 mL of glacial acetic acid, mixing well. Riboflavin was oxidized with 0.5 mL of 3% $KMnO_4$, mixing and allowing to stand for 2 min. After that, 0.2 mL of 3% H_2O_2 was added and mixed thorouhgly. The red colour should disappear within 10 sec (sufficient H_2O_2 should be addded to just decolorize the liquid). A blank assay was carried out with 10 mL destiled water instead of the vitamin extract.

The fluorescence of the extract was measured at 440 nm (excitation) and 565 nm (emission), before and after adding 20 mg of $Na_2S_2O_4$ (to reduce the oxidazed vitamin, and detect the quantity of the posible interfering substances). The value of the fluorescence after the addition of $Na_2S_2O_4$, was substracted from the initial fluorescence of the sample, and the calculations were made comparing with a standard curve of riboflavin, between 0.012 and 0.400 μg/mL. The ecuation of the standard curve used was: $y = 8.139\,x + 0.022$ ($r^2 = 99.94$ %), where y = fluorescence signal and x = μg/mL of riboflavin.

For vitamin B_6 analysis, first the filtrate should be adjusted to pH 7, and added 4 mL of 25 % sodium wolframate 25 % and 1.5 mL of concentrate sulfuric acid. After 5 min, samples were centrifugated for 10 min at 5000 rpm, the supernatant was collected and the residue was washed with 5 mL of water adding it to the previous supernatants. Liquids with vitamin B_6 were adjusted to pH 3 with NaOH and added 0,5 of Filtrol grade 13. After 30 min at room temperature, shaking occasionally to resuspend the absorbent, the tubes were centrifuged at 6000 rpm for 10 min, discarding the supernatants, and the residue was washed twice with 15 mL of citrate buffer pH 3. The residues (Filtrol grade 13 + vitamin) were mixed with 20 mL of alcaline alcohol and heated at 65°C with magnetic stirring. After centrifugation at 6000 rpm, for 10 min, the supernatans were collected and the residues washed with 5 mL of alcaline alcohol (1 g NaOH dissolved in 96° ethanol), adjusting the total liquid to pH 7.3 with acetic acid, and transferring to a volumetric flask to make up to 25 mL with 96° ethanol. Liquids were filtered through Whatman n° 40. 10 mL of filtrated were added 4 mL of 50 % sodium acetate, 1 mL of diazoic reagent (0.4 mL $NaNO_2$ + 2.5 mL sulfanilic acid and made up to 10 mL) and 2 mL of 5,5 % solution sodium carbonate.

For blank preparation instead of filtrate, 10 mL of ethanol was added Absorbance was measured after 5 min, at 424 nm, and compared with a standard curve of piridoxal, between 0.5 and 3 μg/mL. The ecuation of this standard curve was: $y = 0.084\,x - 0.0127$ ($r^2 = 99.92$), where y = absorbance and x = μg/mL of piridoxal.

Minerals analysis

A portion of the fresh sample was freezdryed and homogenized; aliquot of it (0.5 g) were analysed for mineral content by dry ash technique. The samples were incinerated at temperature lower than 450 °C, aproximately for 10 hours, until white ashes. The ashes were reached with acids: 2 mL HCl 50% and 2 mL HNO_3 50% (v/v) and made up to 50 ml with water. For calcium and magnesium analysis 10 mL of Cl_3La_2 solution (5 % of lanthanunm) was added to 5 mL aliquot of samples and standards and complet with water to final volume 50 mL (10% dilution) in order to avoid interferences.

STATISTICAL ANALYSIS

The one - way analysis of variance applied (ANOVA) analizes the effect of the storage temperature on the green bean micronutrient composition. All the analytical results (triplicates of each sample) were used to conduct this analysis. The Fisher statistic (F value) obtained from our data and compared with a critical value (Fc at 95 % confidence level) shows the significance of the storage temperature influence on water-soluble vitamins and mineral composition changes, testing the hypotheses of no variation (12, 14).

RESULTS & DISCUSSION

As it was mentioned previously, the main problem of the storage of this vegetable is the fungus contamination.

Table 1

Water-soluble vitamins content of green beans (mg/100 g on wet basis)

T (°C)	Storage days	FLAT POD GREEN BEAN				ROUND POD GREEN BEAN			
		Vitamin C X ± SD	Vitamin B_1 X ± SD	Vitamin B_2 X ± SD	Vitamin B_6 X ± SD	Vitamin C X ± SD	Vitamin B_1 X ± SD	Vitamin B_2 X ± SD	Vitamin B_6 X ± SD
	0	16.817 ± 1.599	0.023 ± 0.002	0.079 ± 0.001	1.381 ± 0.001	5.146 ± 0.418	0.019 ± 0.000	0.088 ± 0.004	1.147 ± 0.135
	8	8.958 ± 0.562	0.011 ± 0.002	0.065 ± 0.006	0.729 ± 0.187	5.911 ± 0.019	0.056 ± 0.003	0.057 ± 0.000	0.687 ± 0.075
4	15	9.660 ± 0.291	0.012 ± 0.001	0.107 ± 0.007	0.355 ± 0.150	3.669 ± 0.299	0.052 ± 0.000	0.156 ± 0.011	0.946 ± 0.035
	22	5.305 ± 0.129	0.022 ± 0.003	0.081 ± 0.026	0.560 ± 0.127	-----	-----	-----	-----
	8	13.023 ± 0.080	0.011 ± 0.000	0.077 ± 0.012	0.652 ± 0.037	4.041 ± 0.104	0.047 ± 0.001	0.061 ± 0.011	0.687 ± 0.063
8	15	10.853 ± 0.287	0.022 ± 0.003	0.097 ± 0.003	0.522 ± 0.124	5.433 ± 0.331	0.046 ± 0.000	0.133 ± 0.028	0.785 ± 0.139
	22	5.148 ± 0.590	0.017 ± 0.000	0.097 ± 0.027	0.740 ± 0.038	-----	-----	-----	-----
	8	11.478 ± 0.315	0.015 ± 0.004	0.094 ± 0.000	0.476 ± 0.074	4.697 ± 0.860	0.052 ± 0.011	0.082 ± 0.025	0.644 ± 0.077
12	15	10.227 ± 0.927	0.014 ± 0.003	0.087 ± 0.003	0.410 ± 0.119	6.824 ± 0.150	0.047 ± 0.001	0.121 ± 0.009	0.528 ± 0.057
	22	2.988 ± 0.268	0.017 ± 0.002	0.089 ± 0.007	0.528 ± 0.106	-----	-----	-----	-----
	5	9.883 ± 1.003	0.023 ± 0.002	0.104 ± 0.003	0.822 ± 0.138	3.104 ± 0.046	0.024 ± 0.001	0.094 ± 0.010	0.956 ± 0.018
20	12	6.957 ± 0.070	0.017 ± 0.002	0.073 ± 0.005	0.505 ± 0.070	-----	-----	-----	-----
	19	6.090 ± 0.346	0.024 ± 0.001	0.057 ± 0.03	1.185 ± 0.259	-----	-----	-----	-----

X = mean of three determinations.
SD = standard desviation (n-1).

Table 2

Mineral content of flat pod green beans (mg/100 g on wet basis)

T (°C)	Storage days	Na X ± SD	K X ± SD	Ca X ± SD	Mg X ± SD	Cu X ± SD	Fe X ± SD	Mn X ± SD	Zn X ± SD
	0	2.493 ± 0.089	150.712 ± 12.599	49.079 ± 3.860	26.162 ± 0.524	0.235 ± 0.013	0.611 ± 0.037	0.333 ± 0.403	0.285 ± 0.034
	8	1.457 ± 0.376	156.603 ± 5.218	49.428 ± 0.090	27.308 ± 1.946	0.148 ± 0.012	0.505 ± 0.030	0.275 ± 0.006	0.258 ± 0.008
4	15	1.478 ± 0.199	114.178 ± 3.628	26.567 ± 0.259	16.647 ± 0.468	0.034 ± 0.004	0.370 ± 0.023	0.098 ± 0.003	0.129 ± 0.030
	22	1.697 ± 0.125	137.652 ± 13.616	43.956 ± 0.000	22.458 ± 1.494	0.059 ± 0.011	0.459 ± 0.025	0.149 ± 0.020	0.217 ± 0.044
	8	2.094 ± 0.119	150.260 ± 13.090	51.580 ± 0.001	26.147 ± 1.663	0.118 ± 0.015	0.501 ± 0.014	0.259 ± 0.013	0.269 ± 0.043
8	15	1.906 ± 3.945	114.968 ± 3.745	37.991 ± 3.618	19.730 ± 1.566	0.082 ± 0.001	0.374 ± 0.002	0.182 ± 0.009	0.218 ± 0.031
	22	1.507 ± 0.085	148.375 ± 3.805	38.815 ± 3.618	22.340 ± 1.300	0.182 ± 0.011	0.507 ± 0.026	0.193 ± 0.015	0.248 ± 0.031
	8	4.647 ± 0.134	122.719 ± 11.504	38.627 ± 2.637	23.385 ± 0.752	0.131 ± 0.008	0.520 ± 0.016	0.226 ± 0.016	0.281 ± 0.037
12	15	2.184 ± 0.067	157.305 ± 1.810	46.804 ± 2.159	26.671 ± 0.443	0.129 ± 0.012	0.486 ± 0.020	0.218 ± 0.012	0.220 ± 0.011
	22	0.834 ± 0.140	123.660 ± 4.415	34.052 ± 1.868	19.882 ± 0.102	0.072 ± 0.013	0.453 ± 0.007	0.124 ± 0.013	0.177 ± 0.024
	5	2.313 ± 0.200	121.599 ± 5.798	37.922 ± 2.357	22.653 ± 0.468	0.068 ± 0.012	0.534 ± 0.018	0.122 ± 0.005	0.194 ± 0.037
20	12	1.733 ± 0.114	155.320 ± 2.315	52.192 ± 0.312	27.095 ± 1.270	0.095 ± 0.007	0.675 ± 0.017	0.159 ± 0.009	0.174 ± 0.016
	19	1.829 ± 0.129	108.230 ± 6.886	57.249 ± 0.432	31.675 ± 1.975	0.089 ± 0.010	0.815 ± 0.037	0.196 ± 0.008	0.306 ± 0.039

X = mean of three determinations.
SD = standard desviation (n-1).

Table 3

Mineral content of round pod green beans (mg/100 g on wet basis)

T (°C)	Storage days	Na X ± SD	K X ± SD	Ca X ± SD	Mg X ± SD	Cu X ± SD	Fe X ± SD	Mn X ± SD	Zn X ± SD
	0	1.484 ± 0.681	139.338 ± 3.665	43.405 ± 5.318	21.094 ± 1.196	0.049 ± 0.006	0.700 ± 0.047	0.142 ± 0.003	0.230 ± 0.091
	8	3.772 ± 0.527	182.864 ± 8.712	48.317 ± 3.562	32.766 ± 1.312	0.084 ± 0.005	0.827 ± 0.072	0.144 ± 0.008	0.277 ± 0.012
4	15	3.029 ± 0.455	194.039 ± 2.496	50.505 ± 4.822	32.776 ± 1.345	0.073 ± 0.010	0.969 ± 0.035	0.154 ± 0.006	0.286 ± 0.017
	22	-----	-----	-----	-----	-----	-----	-----	-----
	8	3.326 ± 0.580	171.495 ± 8.966	39.496 ± 2.115	28.480 ± 0.491	0.064 ± 0.008	0.758 ± 0.024	0.147 ± 0.005	0.285 ± 0.031
8	15	3.216 ± 0.474	153.216 ± 16.553	41.177 ± 1.553	30.143 ± 1.889	0.057 ± 0.006	0.780 ± 0.031	0.143 ± 0.004	0.231 ± 0.020
	22	-----	-----	-----	-----	-----	-----	-----	-----
	8	2.469 ± 0.210	182.889 ± 6.357	48.994 ± 6.939	34.332 ± 1.098	0.075 ± 0.007	0.819 ± 0.036	0.182 ± 0.006	0.394 ± 0.040
12	15	2.243 ± 0.234	267.187 ± 5.322	42.856 ± 4.178	33.788 ± 3.889	0.076 ± 0.016	0.917 ± 0.103	0.167 ± 0.034	0.297 ± 0.016
	22	-----	-----	-----	-----	-----	-----	-----	-----
	5	1.448 ± 0.030	160.467 ± 0.390	44.938 ± 5.808	24.096 ± 0.433	0.047 ± 0.012	0.706 ± 0.124	0.144 ± 0.002	0.230 ± 0.000
20	12	-----	-----	-----	-----	-----	-----	-----	-----
	19	-----	-----	-----	-----	-----	-----	-----	-----

X = mean of three determinations.
SD = standard desviation (n-1).

Flat pod green bean stored under 8, 12, 20°C became deteriorated after fifteen days, while the sample stored under 4°C temperature still had a good aspect. Although chilling injury appeared in 4 °C samples, after twenty-two days of storage. Round pod green bean showed fungus contamintion after twelve storage days at 8 and 12 °C; and after five days at 20 °C. Samples stored under 4°C temperature did not present this contamination, but we could find symptoms of chilling injury on them, after fifteen days.

Tables 1 to 3 show the experimental results, mean value of three analysis for the vitamins and elements studied and its standard desviation (n-1).

WATER-SOLUBLE VITAMINS

The vitamin C content of our fresh flat green bean (16.817 mg/100 g) agree with Watada *et al.* (1987) (18), Souci *et al.* (1994) (15) and Katsaboxakis & Papanicolaou (1996) (11), who found 10-27 mg/100 g on wet basis, of vitamin C in green bean (Table 1). The round pod green bean analysed shows a smaller content (5.146 mg/100 g). This variation can be a consecuence of varietal differences, but also can be due to the different season in which the samples were harvested (February and June, for flat and round pods, respectively). Vázquez-Oderiz *et al.* (1994) (17) reported values of 4-7.4 mg/100 g for green bean harvested in summer time.

Thiamine content of our samples (0.019-0.023 mg/100 g) is smaller than those reported by Souci *et al.* (1994) (15), and for riboflavin (0.079-0.088 mg/100 g), our results agree with theese authors (Table 1).

Vitamin B_6 content of our fresh samples (1.147 - 1.381 mg/100 g) of green bean agree with Martínez Tamayo *et al.* (1964) (13) who found 1.350 mg/100 g, and it is higher than the content reported by Souci *et al.* , 1994 (15). (Table 1).

Storage temperature has a strong effect on the vitamin content of the analysed samples. We have found statisticaly significative variation ($p \leq 0,05$) due to storage temperature on vitamin C and thiamine content of all the samples. Vitamin C decrease progresively during storage, in a higher rate for the samples of flat pod green bean (with had a higher content), specially on samples stored under 20°C temperature. Watada *et al.* (1987) (18) also detected higher non linear losses of vitamin C in green bean after storage under 20 °C than on 10°C. When samples are more physically deteriorated, the decrease in vitamin C content is more abrupt (after fifteen days); untill this moment, samples stored under 8 and 12°C temperature keep higher levels of vitamin C than the other samples.

Thiamine shows an irregular behaviour, and its content on dry basis tend to increase in the samples, after the eighth day of storage. In round pod green bean, this behaviour is more homogeneous, increasing in all the samples untill eight days of storage and decreasing after this moment.

This fact accords with the observations of Watada *et al.* (1987) (18), who found increases in the vitamin B_1 content of green bean stored under 10°C and 20 °C temperature (and also in other vegetables like bell peppers and spinach). The increase of vitamin B_1 can be due to some residual sinthesis in the vegetable, and also because the microorganisms that grow in the pods during the last stage of the storage, could synthesize this vitamin. The statistical analysis aplied shows a significative influence ($p \leq 0.05$) of storage temperature on vitamin B_1 content variation.

Vitamin B_2 varies in a significative way with the storage temperature in flat pod samples. In the first stage of storage, we can find higher contents in samples kept under 12°C; and after fifteen days the content is higher in samples stored under 4°C temperature. However, in round pod samples the variation due to temperature is not statistically significative ($p \leq 0.05$). Watada *et al.* (1987) (18) found only little variations in vitamin B_2 content of green bean stored for eight days. In general, we have found higher vitamin B_2 content at fifteen days storage.

In flat pod samples, vitamin B_6 content decrease during the first eight to twelve days of storage, in all temperatures considered, until a loss of 35.87 - 69.68 % from its initial content. After this moment vitamin B_6 tend to keep this value, increasing only a little after a fifteen days storage, but we have not found a significative variation ($p \leq 0.05$) due to this factor. In round pod samples we have found statistically significative variation ($p \leq 0.05$), keeping higher contents in samples storage under 4 and 8°C, after fifteen days of storage.

MINERAL ELEMENTS

A previous study about the total mineral content of this samples, showed an increase during the storage (7). K and Ca are the most variable mineral elements in both varieties of green bean during the storage time ($p \leq 0.05$).

The storage temperature has a significative influence on Fe, Cu, Mn, Ca dry basis content of flat pod green bean, in every stages of this storage ($p \leq 0.05$). Zn seems to be the least affected by this factor, while K and Mg are affected by temperature in the last stage of storage. Na presents an irregular behaviour in this samples.

The mineral content of round pod green bean appears less affected by storage temperature. K and Ca are the only minerals that show statistically significative variations ($p \leq 0.05$) due to this factor during all the storage period. Cu, Na and Mg are the most stable with the storage temperature; and Mn, Zn and Fe behave in an

irregular way.

The variations detected in the mineral content of samples, can be due to a possible redistribution of this elements by the pod (the final and contaminated parts of it were discarded for the analysis).

In general, 8 and 12°C temperature seem to be those which keep a higher mineral content in green bean samples, better than 4 and 20 °C.

CONCLUSION

We can conclude that a maximum storage of fifteen days is appropiated for our green bean samples. After this moment, fungus growth makes commercially unacceptable this product. This can be avoided storing it under 4°C temperature, but the apparition of chilling injury makes its quality decrease. In this moment, vitamin C content are 50,18 - 89,82 % of the initial one and decrease abruptly after a fifteen days storage. Vitamin B_1 and B_2 show an irregular behaviour during storage, while B_6 reaches its lowest content after eight to twelve days stored (30,32-64,13 % of the initial one).

About the optimal temperature, water-soluble vitamins (and specially vitamin C) are the most affected micronutrients by this factor. We can consider 8°C temperature as the best one to get a higher retention for vitamin C and B_6, and also to keep the mineral content in the best levels in green bean, avoiding chilling injury of this vegetable.

For this reasons, the optimal moment for the consumption of this vegetable is before eight days of storage, under a temperature of 8 °C.

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EXPERIMENTAL MANUFACTURING OF FRESH-CUT FRUIT SALAD ACCORDING TO DIFFERENT PROCESSING TECHNIQUES

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SUMMARY
A fresh-cut fruit salad was prepared using apples, clingstone peaches and kiwifruits and was packaged according to different techniques: 1) normal atmosphere, 2) modified atmosphere, 3) normal atmosphere + ethylene absorber, 4) modified atmosphere + ethylene absorber.
The internal atmosphere of the packages as well as the colour and the firmness of the fruit pieces are reported.
The results obtained indicate that the shelf-life of the fresh-cut fruit salad should be 5-7 days if the temperature is kept continuously at 3°C±1°C, while the data reported don't allow to demonstrate that the modified atmosphere and the ethylene absorbers are particularly effective in maintaining the firmness and the colour of the fruit pieces.

INTRODUCTION

Fresh-cut fruit is a very innovative product which could play an important part in the increase of fresh horticultural food consumption.

Specialized magazines have already indicated that 14% of the fresh horticultural foods on the U.S.A. market is made up of fresh-cut fruits and vegetables (1); even if this might be an over-estimation, this figure is in line with the market survey published in 1995; it foresaw the spread of fresh-cut horticultural products from catering to home consumption (2). The same phenomenon is happening in Europe, even if the percentage of market share could be estimated at some points lower; in fact the supermarket chains provide more and more space for these products.
Various researches have been carried out on the behaviour and on the quality indexes of one species fresh-cut fruit but very little has been published on fresh-cut fruit salad. Usually one species fresh-cut horticultural foods are considered as convenience products owing to their many advantages: they satisfy the demand for nutritious, wholesome and tasty foods; they could be used in new ways such as in school canteens, old people's meals, snacks for long and medium distance travelling; raw material of small size or with slight external defects could be used for their manufacturing. But fresh-cut fruit salad adds another advantage: it could be thought of as a dessert, even considering seasonal fruits, making the fruit even more appreciated and remunerative.

If these products are to have a success they must be genuine and of high quality, with sensory characteristics as similar as possible to the fresh ones; moreover they should have a shelf-life that permits the transportation, the distribution and the sale.

This paper examines the problems connected to the most suitable technology to be adopted (modified atmosphere inside the package, utilization of ethylene absorbers) and defines some quality characteristics of both raw material and final products. For this purpose a fresh-cut fruit salad was made up of apples, kiwifruits and clingstone peaches without adding fruit juice or liquid filling.

MATERIAL AND METHODS

Apples (cv Golden Delicious), clingstone peaches (cv Carson) and kiwifruits (cv Hayward) were purchased at the Milan main distribution center and were processed immediately after their arrival at the laboratory. Manufacturing was performed in isolated rooms where the air was sanitized by UV lamps. Fruits of uniform size and external colour were selected, washed, peeled and sliced by hand; the apples and the cingstone peaches were longitudinally cut into 8 pieces, while the kiwifruits were sliced to 5 mm thickness. The apple and clingstone pieces were immersed in an Ascorbate 1% + Citrate 0,2% solution for 15 min as an antibrowning treatment while the kiwifruit slices were immersed in a $CaCl_2$ 80mM solution for 15 min (fruits/solution 1:3) as an antisoftening treatment. After draining and towel-drying, 60 grams of each species of fruit were placed in a polystirene tray (dimensions: 0,108m*0,193m*0,030m); then the trays (about 180 g of fruit) were wrapped with plastic film having barrier properties (OTR=4,0mL/m^2/24h; WVTR=15,5g/m^2/24h) and sealed thus forming a bag with a surface of 0,0682m^2.

The fresh-cut fruit salad samples were divided in four lots that were packaged according to different techniques: 1) normal atmosphere (NA), 2) modified atmosphere (MA), 3) normal atmosphere + ethylene absorber (NA+A), 4) modified atmosphere + ethylene absorber (MA+A).

Modified atmosphere was obtained by means of the gas flushing technique using food grade nitrogen until a 95% nitrogen and 5% oxygen internal atmosphere was reached. A vacuum sealing machinery was used (Vacuum Pump mod. CS27S) both for modified atmosphere packaging and for normal atmosphere packaging. The ethylene absorbers were paper mini-packets (dimension 55mm x 65mm x 2mm) containing 6 grams of granules of sepiolite clay (Hydrous magnesium silicate); they were placed inside the package and fixed with adhesive band so as they hanged over the fruit pieces. Immediately after packaging the samples were placed in a cold room at 3°C±1°C and stored for 7 days. At 0, 1, 4, 7 day the samples were submitted to the physical analyses of texture and colour as well as to the analysis of the atmosphere composition inside the package.

The internal atmosphere was determined by checking the concentrations of carbon dioxide (CO_2), oxygen (O_2) and ethylene (C_2H_4) by means, respectively, of Servomex IR Gas Analyser PA404, Servomex Oxygen Analyser 474 and Gas Chromatograph DANI 3400.

The colour measurement was performed with a reflectance colorimeter Minolta Chroma Meter CR 200, measuring the central part of both the sides of 20 pieces of fruit, randomly picked up from two bags. From the values of L*, a*, b* were calculated the hue (a*/b*), the saturation $(a^{*2} + b^{*2})^{1/2}$ and the colour difference (ΔE) with respect to time 0, according to the Hunter formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. The firmness of the slices of apple and of clingstone peach was determined with a dinamometer Instron mod. 4300 by measuring the force required to press a 8mm diameter probe into the pulp of 20 fruit pieces to 20 mm deepness with a speed of 0.2m/min. The firmness of kiwifruit slices was determined at the same way but with a 3mm thick blade.

The results of the analyses of colour and firmness were submitted to ANOVA and Tukey's test.

RESULTS AND DISCUSSION

INTERNAL ATMOSPHERE

In the packages without absorbers the ethylene reaches the peak on the 4th day with a value slightly higher in the NA samples; at the last checking time (7th day) the ethylene remains quite stable in both NA and MA packages (fig. 1), showing an evident slowing down of the respiration activity. On the contrary the samples packed with the absorbers have a constant almost negligible ethylene concentration.

The data obtained confirm the effectiveness of the ethylene absorbers in maintaining the ethylene concentration at a very low level, that was already referred to in a previous paper (3).

The oxygen concentration behaves differently according to the packaging techniques (fig. 2). In NA samples the oxygen reaches the minimum percentage on the 4th day and then shows a slight increase which means that the oxygen consumption is stable and confirms that the respiration activity has been slowed down. In MA samples, instead, the oxygen concentration rises continuously until it almost reaches the level found in the NA samples. The increase of the oxygen concentration in fresh-cut fruit salad packed in a low oxygen atmosphere is coherent with the figures observed in previous papers (3, 4) but it can be difficult to explain, as the packaging film has barrier properties. Two hypotheses could be put forward; the first regards the total surface of the package together with the film permeability, which could make the oxygen concentration increase; the second concerns the exit of the oxygen present inside the fruit tissues, both intra and extra the plant cells, owing to a gradient of the partial pressure of the oxygen.

The graphic of the carbon dioxide concentration (fig. 3) shows that in NA samples packed without absorbers the carbon dioxide accumulates until the 7th day of storage, while in the other samples the carbon dioxide concentration reaches the peak on the 4th day and then remains quite stable.

The data on the internal atmosphere composition indicate that fresh-cut fruit salad maintains a respiration activity strong enough at least until the 4th day of storage; probably this phenomenon is due to the physiological stress induced on the fruits by the unit operations such as peeling and cutting. Then the respiration activity slows down and the internal atmosphere is inclined to stabilize.

APPLE

The apple slices have a distinct tendency to softening that cannot be prevented by the modified atmosphere nor by the ethylene absorber (tab. 1). The firmness loss ranges from 54% to 63%; in the MA sample the maximum softening occurs on the 4th day, while in the other samples the texture reaches the minimum on the 7th day of storage.

The loss of firmness in the apple slices has already been studied and discussed in previous papers that, however, led to different conclusions. At first an evident softening was detected in MA samples where softening occurred on the 4th day (5), while a following trial did not reveal any difference between the types of packaging and between apples processed after various post harvest storage time (3). In this last experiment the most important fact was the effectiveness of the ethylene absorbers in maintaining stable the firmness of the apples processed after 4

months from the harvest. The results reported in the present paper agree with the experiments carried out at the IVTPA and elsewhere: in that the firmness loss is due above all to the action of the endogenous enzymes on the cell walls which cannot be inhibited by the antibrowning pretreatments nor by the packaging technique. The disagreements sometimes found over many trials carried out at IVTPA or elsewhere regarding the firmness behaviour could be ascribed to the raw material variability, as the fruits used did not come from the same lot.

The colour parameters of the apple slices show a good stability in every type of packaging (tab. 1). Sometimes some variations of the hue and of the saturation are noted and they could be ascribed to sliding of a* and b* values which however don't mean a browning but a shifting of the hue towards the green and a decrease of the yellow component. The appearance of green pigments cannot be attributed to a specific packaging technique, whereas it is probably related to the raw material ripeness grade.

On the whole the results of the physical analyses of the apple slices mixed in a fresh-cut fruit salad confirm that the Golden Delicious apples are well suited to this type of manufacturing and that there is no influence of the presence of other fruits.

CLINGSTONE PEACH

The physical analysis of texture shows a very good stability of the firmness: the same firmness was found at the end of storage as at the beginning (tab. 2).

The colour parameters denote few changes, even if significant differences are sometimes observed in few samples over the storage, above all regarding lightness and saturation (tab. 2). These changes are not large enough to signal a browning occurrence but they are linked to slight changes of the reflectance at the slice surface and to modest increases of the yellow and red components which are more evident in the area surrounding the stone.

There is no influence of the packaging techniques, even if, according to ΔE values, NA without absorber protects the colour more effectively in protecting the colour. Therefore the differences observed could be ascribed to the raw material variability.

These remarks confirm the findings already referred to in a previous paper (5) and demonstrate not only that clingstone peach (cv Carson) is ideally suited to this type of manufacturing but also that the behaviour is not affected by the presence of other fruit species.

KIWIFRUIT

The results of the firmness analyses carried out on the kiwifruit slices added to the fruit salad don't show any significant change of the samples differently packed over the storage time (tab. 3).

This finding disagrees with the results of a previous trial which underlined a significant loss of the kiwifruit slices firmness on the 4th day of storage (5). Moreover the figures reported in the present paper are 50% lower than those found at the previous time. Other Authors, too, reported a progressive softening which occurred, however, in different ways (7, 8).

This disagreement could be explained by the fact that the fruits used in this experiment had already reached their climateric peak and then the maximum ripeness stage. This hypothesis could be supported by considering that this experiment was carried out in summer and the kiwifruits undoubtely came from the southern hemisphere.

The colour parameters show a progressive decrease of the brightness and of the saturation which are quite evident after 7 days of storage, as can be seen from the value of the colour difference (ΔE); the hue shows a shift towards the green already on the 1st day of storage (tab. 3). This findings confirm what was referred to in a previous paper (5).

The modified atmosphere and the ethylene absorber don't have any significant influence on either the firmness or the colour parameters of the kiwifruit slices; however, as regards the colour, if only the colour difference values are considered (ΔE), the MA samples show the lowest figures compared to the other type of packaging.

CONCLUSIONS

The results confirm that the apples (cv Golden Delicious), the kiwis (cv Hayward) and the clingstone peaches (cv Carson) are suitable for manufacturing fresh-cut fruit as has already been reported. There are no negative aspects to mixing pieces of these types of fruits in order to obtain ready prepared fresh-cut fruit salad, since the behaviour of the fruits is similar to that of single fresh-cut fruit.

The shelf-life of fresh-cut fruit salad should be 5-7 days if the temperature is kept continuously at 3°C±1°C, which is the same as for the single fresh-cut fruit.

From the data obtained in this study the modified atmosphere and the ethylene absorber don't seem to be particularly effective in maintaining the stability of the firmness and of the colour.

However further considerations should be taken into account.

Many different species and varieties could be mixed but it would be necessary to study whether these different fruits are compatible with each other and whether they are suitable for this type of manufacturing. Above all it is important to determine the best grade of ripeness and chemical and physical parameters in order to obtain high level quality products.

Another point to be taken into consideration is the choice of fruits according to the season. In fact using early or late varieties and fruits coming from the southern hemisphere could signify a change in the quality parameters both for raw material and final product. This issue should be remembered when making up the mixture for the fresh-cut fruit salad.

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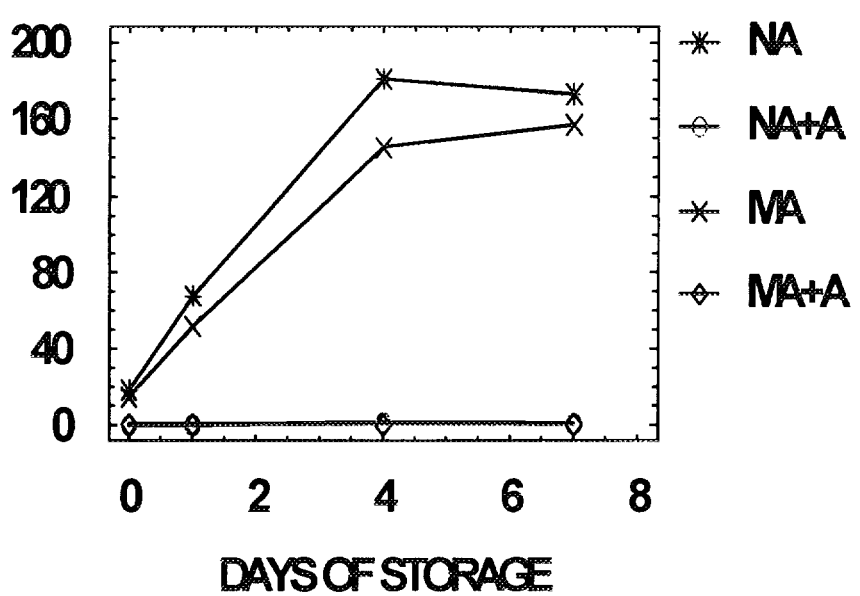
Tab. 1 - Physical parameters of apple slices in fresh-cut fruit salad packaged according to different techniques

	Firmness	L*	Hue	Saturation	ΔE
NA					
0 gg	5,88 a	81,38 a	-0,18 a	25,38 a	- -
1 "	6,12 a	80,75 a	-0,19 a	24,68 a	2,12
4 "	5,44 a	80,94 a	-0,19 a	23,22 a	2,85
7 "	2,91 b	79,54 a	-0,16 a	22,83 a	3,22
NA+A					
0 gg	5,96 a	82,46 a	-0,16 a	21,89 ab	- -
1 "	5,25 a	81,01 ab	-0,18 a	24,11 a	2,68
4 "	3,81 ab	78,08 b	-0,17 a	23,47 a	4,64
7 "	2,59 b	81,58 a	-0,17 a	19,05b	2,96
MA					
0 gg	5,61 a	82,18 a	-0,16 a	24,42 a	- -
1 "	4,73 a	80,70 a	-0,19 a	23,81 ab	1,54
4 "	2,99 b	81,12 a	-0,16 a	23,29 ab	1,65
7 "	2,78 b	81,44 a	-0,17 a	21,23 b	3,78
MA+A					
0 gg	5,53 a	81,70 a	-0,15 a	25,18 a	- -
1 "	4,79 ab	80,62 a	-0,18 a	22,44 b	2,98
4 "	3,11 ab	79,97 a	-0,19 a	21,30 a	4,30
7 "	2,01 b	81,13 a	-0,16 a	22,16 a	3,08

Different letters in the same column show statiscally significative differences ($p<0.01$)

Fig. 1 - Ethylene concentration (ppm) in fresh-cut fruit salad packaged according to different techniques



Tab. 2 - Physical parameters of clingstone peach slices in fresh-cut fruit salad packaged according to different techniques

	Firmness	L*	Hue	Saturation	ΔE
NA					
0 gg	2,60 a	64,80 a	0,06 a	47,07 ab	- -
1 "	2,48 a	65,30 a	0,07 a	45,12 b	1,51
4 "	1,97 a	63,80 a	0,10 a	47,20 ab	3,84
7 "	2,23 a	64,30 a	0,09 a	48,30 a	1,86
NA+A					
0 gg	2,21 a	63,23 b	0,08 ab	44,65 b	- -
1 "	2,58 a	62,83 b	0,14 a	49,50 a	4,41
4 "	2,35 a	63,99 ab	0,09 ab	44,94 b	2,77
7 "	2,52 a	65,46 a	0,06 b	48,13 a	4,46
MA					
0 gg	2,12 a	64,19 a	0,07 a	45,38 ab	- -
1 "	2,21 a	62,71 ab	0,09 a	48,15 a	3,08
4 "	1,91 a	60,35 ab	0,08 a	44,23 b	4,22
7 "	2,06 a	58,70 b	0,10 a	46,28 ab	6,15
MA+A					
0 gg	2,35 ab	62,37 a	0,06 a	47,41 ab	- -
1 "	2,73 a	56,93 b	0,08 a	46,07 ab	5,61
4 "	1,81 b	58,76 ab	0,09 a	45,11 b	3,71
7 "	2,53 ab	62,95 a	0,08 a	48,19 a	2,34

Different letters in the same column show statiscally significative differences ($p<0.01$)

Tab. 3 - Physical parameters of kiwifruits slices in fresh-cut fruit salad packaged according to different techniques

	Firmness	L*	Hue	Saturation	ΔE
NA					
0 gg	0,19 a	49,14 a	-0,47 a	32,39 a	- -
1 "	0,12 a	46,53 a	-0,55 ab	29,51 a	9,42
4 "	0,12 a	44,57 ab	-0,53 ab	23,74 b	12,18
7 "	0,12 a	37,87 b	-0,60 b	20,21 b	14,49
NA+A					
0 gg	0,16 a	55,26 a	-0,47 a	36,13 a	- -
1 "	0,12 a	44,87 bc	-0,57 b	25,45 bc	15,08
4 "	0,16 a	47,33 b	-0,56 b	27,24 b	12,09
7 "	0,13 a	41,38 c	-0,59 b	20,78 c	20,86
MA					
0 gg	0,14 a	45,97 a	-0,50 a	31,54 a	- -
1 "	0,13 a	43,72 a	-0,58 b	23,93 b	8,13
4 "	0,11 a	42,66 a	-0,55 ab	24,69 b	7,70
7 "	0,12 a	41,67 a	-0,59 b	21,55b	11,04
MA+A					
0 gg	0,13 a	49,56 a	-0,48 a	32,76 a	- -
1 "	0,12 a	44,33 b	-0,59 b	26,77 b	8,29
4 "	0,18 a	43,29 b	-0,59 b	24,04 bc	10,99
7 "	0,13 a	39,82 b	-0,59 b	20,30 c	15,93

Different letters in the same column show statiscally significative differences ($p<0.01$)

Fig. 2 - Oxygen percentage in fresh-cut fruit salad packaged according to different techniques

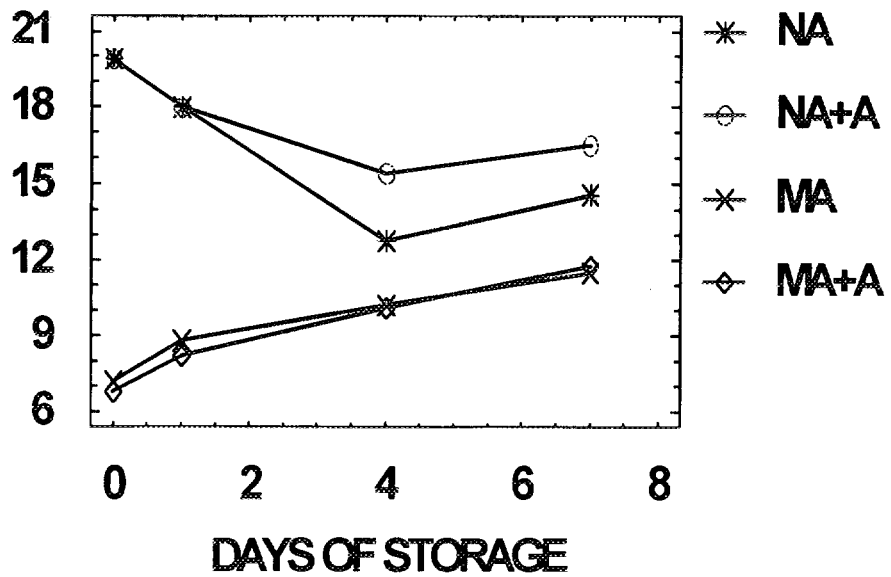
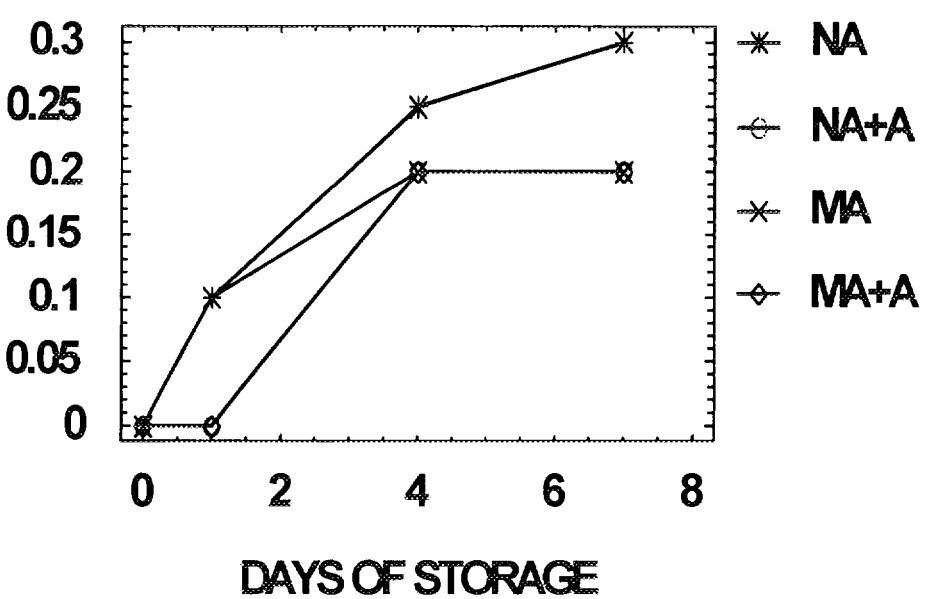


Fig. 3 - Carbon dioxide percentage in fresh-cut fruit salad packaged according to different techniques



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COCONUT PRODUCTS AND INGREDIENTS: THEIR PRESENT SITUATION AND FUTURE

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Summary

The coconut (Cocos nucifera L.) is often referred to as the Tree of Life with uses as food, drink, medicine, pharmaceutical and cosmetics, shelter, energy, clothing, detergent and handicraft, among others. The coconut fruit in particular, is used in many food preparation and manufactured products due to its unique range of oils, proteins, and carbohydrates including dietary fiber and sugars, minerals and vitamins.

The traditional commercial coconut food products include oil and desiccated coconut (DCN). The high degree of saturation and long stability of coconut oil makes it one of the most desirable natural oils for deep-frying of foods and as ingredients in confection, bakery goods, filled milk products, shortening compound, and margarine. DCN is produced in various forms that find application in baked products and confectionaries. DCN impart desirable texture and optimum coconut flavour to products and serves as an economical substitute for various kinds of nuts, as bulking agent, and as sanding material for cookies and confections.

Developments in coconut R & D for food application yielded a whole range of utilization of the coconut fruit (Kernel of meat and water). Products from the Kernel include dried granulated coconut, coconut chips / crisps, dehydrated buko kernel of 7-9 mo. Nut strips, buko in syrup, buko roll, instantized buko juice and buko in canned mixed fruit. Coconut milk/cream (prepared from macerated, comminuted and expelled from coconut endosperm where most fiber and residues excluded) products include, canned coconut milk, coconut milk powder, coconut jam/spread, coconut syrup, coconut tahu, coconut cheese, coconut yoghurt, coconut ice cream and coconut juice/shake/nectar.

Coconut skim milk products include instant coconut skim milk beverage, coconut protein isolate (CPI) simulated milk, CPI-based non-meat products (i. e. canned meatless spread, sausage, luncheon meat, dehydrated textured product), CPI-based coconut cereal weaning flakes, sweetened condensed coconut skim milk, coco honey and cultured coconut skim milk. On the other hand, the coconut residue is made into low fat flour, and serves as ingredient in bakery products, meat and fish analogue, noodles, snack foods, and soy sauce-type condiment. The products from coconut water (natural acqueous liquid endosperm of the coconut fruit or the liquid within the coconut kernel containing soluble proteins and sugars) include coconut vinegar, carbonated and non carbonated beverages, coconut wine, and coconut sparkling beverage. Coconut water alsoserves as substrate for the production of food yeasts, nata de coco, lactic acid, gluconic acid, dextran, and lysine, among others.

Many of these products have not been sufficiently commercially tested. Further in depth analyses of the products and process as to their economic viability and market acceptability is a challenge

to the coconut technologist and industrialist. Moreover, equipment development suited for a particular scale of production is equally important to assure products of high quality, uniformity, consistency, and reproduceability for them to be competitive in the global market. Exotic products from coconut like gourmet vinegar and natural flavoured beverages from coconut water and young coconut kernel will also find niche in the global market.

MANGO PRODUCTS AND MANGO BASED INGREDIENTS

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Summary

Mango (Mangifera Indica l:) is one of the most important tropical fruits valued for its succulence, exotic flavour and delicious taste. In addition, it is an excellent source of dietary fibre and carotenoids. The world production of mango during 1995 was 19 millions tonnes, India having a share of 53% producing about 10 millions tonnes. Most of the world production comes from developing countries. India dominates the world trade of processed mango products. The world trade of processed mango was about 60,000 MT valued at US $ 44,420,000 during 1993 of which India's share was 36,600 MT valued at US $ 27,100,100. The major export product is canned mango pulp, which has increased over the past decades by 3-5 times in value. The versatile properties of mango have found application for processing into several products both from ripe and unripe mangoes. The major products from ripe mangoes include: mango pulp (puree), concentrated, preserved slices, beverabes, jam, mango bar totee and mango powder. From raw mangoes products like pickles, chutneys, powder (amchur) beverages are also manifactured.

Several by-products are also produced from the waste of mango processing. The major wastes include: peel and pulper waste and seed (stone). From the peel waste pectin, carotenoids, fibre and juice are produced while the seed kernel is a good source of fat and kernel flour.

The food industry is veering away from artificial ingredients due to consumer interest in products that are as natural as possible. Many of the processed products from mango like pulp and concentrate find application as food ingredients in beverages, jams, infant food, dairy products, ice cream and yogurt. The by-products from mango processing waste such as kernel fat is an excellent substitute for cocoa butter. The kernel flour is used as ingredient in bakery products and pectin prepared from mango peel waste find application in the production of jams and jellies. Similarly carotenoids extracted from the peel waste can be used as a natural coloring material as well as vitamin supplement.

There are several technological options for the production of these products. The preservation techniques followed for the major products are also changing continuously. For example, mango pulp and concentrate were conventionally preserved by canning. The recent trends are aseptic bulk packaging and freezing. For the concentrate, aroma recovery has become an integral part of the process.

RECENT DEVELOPMENTS ON KIWIFRUIT DERIVATIVES

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SYNOPSIS

As it is well known, kiwifruit derivatives show very often unsatisfactory quality and this comes out from the peculiar sensitivity of kiwifruit to processing. The maintenance of the green colour has been for food scientists one of the most important goals in keeping the quality of the log-stable kiwifruit products.
Aim of this review paper is to present some of the research works on kiwifruit product development that in the past few years were carried out in Italy and in other European and non-European countries.

INTRODUCTION

Since the early 1970's, the possibility to process the fruit of *Actinidia chinensis* (or better, nowadays *Actinidia deliciosa*), commercially known as kiwifruit has represented a great interest by both fruit growers and food industries. Following the fast increasing of the world wide kiwifruit production in the 1980's, the kiwifruits became available 12 months of the year and the kiwifruit market collapsed[1]. Many researchers have put efforts to study the suitability of this fruit to give a commercial differentiation from the fresh market, following the falling of price paid to the growers.

Other than to try to solve the problem of the overproduction, the researches on the processing of kiwifruit must take into account also the proportion of fruit not suitable for the fresh market, that is generally around 20-30% of the total production, depending by the crop management and the climate. This is due to the particular biology of the kiwifruit plants, having male and female flowers on separate trees (dioecious trees) which give pollination problems and to the different varieties grown in same areas [2,3].

Nevertheless, the realisation of a good quality processed kiwifruit did not appeared to be easy, and the possibility to have an industrial valorisation of the fruit is yet away to be found.

The kiwifruit derivatives on the market are mostly semi-processed products, addressed to the food industry as ingredients or components for ice-creams, yoghurt, cakes and juice blending [4] . Furthermore, some products as well as syruped or dried kiwifruit slices are spread available only on the New Zealand market, and it is difficult to evaluate the consumer's acceptability.

However, the main goal of the kiwifruit processing is to obtain safe and stable products able to retain as more as possible the peculiarity of the fresh fruit, as well as green colour, aroma, nutritional value and structural characteristics.

The recent growing knowledge on the benefits due to the consumption of high vitamin fruits, could gives also to the processed kiwifruit an importance as functional food.

KIWIFRUIT PROCESSING: GENERAL ASPECTS

In order to study the qualitative changes of kiwifruit during processing, since the early 1980's some researches began in USA, New Zealand and in Italy, at first at the University of Bologna and then in Udine, in collaboration with the Experimental Station of Food Processing in Parma, and at IVTPA in Milan.

Since the beginning, some characters of the fresh fruit, as the green colour and the flavour were found to be difficult to preserve after processing [5]. Many authors used different approaches the suitability of kiwifruit to be processed using "conventional" techniques, performing the optimisation of the process parameters in the application of "traditional" processes or applying innovative technologies of stabilisation.

Some reviews on scientific and technical researches have been published [3,5,6,7,8].

Most evident phenomena occurring during kiwifruit process are due to the chlorophyll modification to pheophytin and to the decomposition of ascorbic acid. Studies carried out on the chlorophyll stability are reported by Robertson (1985) [9], demonstrating the thermal dependence of the ascorbic acid conversion. !00 per cent of the chlorophylls were degraded at 100°C for 5 min, while less than 30% are converted to pheopthytin at frozen state. A linear relationship was found between water activity and time necessary for the chlorophyll conversion; lower the Aw value, longer was the time for conversion [10]. The loss of the green colour can be slow down by using water removal processes at low temperature, reducing the water content and the Aw's value below 0.6. In alternative, the use of artificial colours is permitted by food laws but it leads to a negative impact to the consumers [3]. The browning of the kiwifruit concentrate (pulps and juices) was instead related to the decomposition of the ascorbic acid by Wong *et al.* (1992) [11].

Furthermore, the high acidity (1.5%) and the low pH (3.1-3.8) of the kiwifruit need to be by addition of sugars or edulcorants adjusted in the processed products (juices, canned, dried, etc.) in order to improve the final taste.

From the flavour characters, at the ripe stage the kiwifruit has a pleasant grassy-fruity aroma, of which the main components have been identified [12]; after thermal and mechanical treatments the aroma changes quickly owing to the activation of lipoxigenasic enzymes [13].

Finally, certain processed derivatives, but sometimes even the fresh fruits, are rejected by some consumers because an irritation phenomenon ("catch") due to the presence of oxalate crystals. Perera *et al.* (1990) [14] have evidenced how the oxalate crystals (80 μm, average length) are exposed with the disruption of the cell walls during a mechanical treatment or following a strong drying when the activity of the natural pectolytic enzymes is increased.

TRADITIONAL TECHNOLOGIES FOR KIWIFRUIT PROCESSING

As the first tentative of large-scale processing, in New Zealand, on 1981 were produced syruped pasteurised kiwifruit slices and frozen pulp [15].

Researches on the suitability of processing technologies for kiwifruit were carried out by several authors, since 1976, as extensively reported by Dalla Rosa e Bressa (1995) [5]:

- nectars and whole or sliced fruits appertized in syrup;
- frozen kiwifruit (whole or sliced);
- whole or dried kiwifruit;
- powder of dried purec;
- clarified juice, to be used in juice blending;
- jams;
- sugared and dried pulp (Kiwifruit *leather*);
- frozen pulp, juice and nectar;
- concentrated juices;
- fermented beverages and "wine".

Many of these products are industrially produced, even if in limited quantities, mostly in New Zealand. However, the presence on the market of the kiwifruit derivatives is quantitatively restricted; the absence of an efficient commercial promotion also for the fresh kiwifruit, especially in Italy, did not allow a progress of the kiwifruit derivatives consumption.

Peeling process appeared to be a critical step, and many authors studied the technical optimisation, in order to increase the yield and to reduce the presence of the brown peel residues in the peeled fruit. Also the fruit extraction, its refining and clarification were deeply studied [6,8], particularly in regard to chemico-physical stability of protein suspension during storage [3,16].

Different technologies have been proposed for kiwifruit peeling. Immersion in NaOH solution at boiling temperature was reported by Beutel *et al.* (1976) [17], whilst a superficial freezing-scalding sequence (*freeze-peeling*) or a process based on a quick steam pressurization-depressurization to break off peel from the pulp were studied by Dalla Rosa *et al.* (1980) [18].

In all cases, a problem is represented by the cutting of the fruit top; in some fruit this part is woody and a particular mechanical equipment have to be used to. Recently, on the market of the food plants have been proposed some machinery able to perform the mechanical peeling of kiwifruit, without any use of chemical or steam pressure, based on sharp cutting knives [19].

In any case, the peeling of kiwifruit is strongly influenced by the ripening stage of the fruits. In fact, the knowledge on kiwifruit ripening biochemistry shows that the increase of sugar concentration (indicated with the Brix degrees) during storage, after harvest, is influenced by ethylene and it is correlated to the flesh softening [20]. If, generally speaking, it would be preferable to process fully flavoured fruit, as well as the right stage of maturity, in the case of kiwifruit too soft flesh fruit shows a reduction in the peeling yield and difficulties in the subsequent cutting steps, when pieces or slices are required.

Then it is necessary to standardise as well as possible the ripening level of the fruit addressed to processing and however we need to be able to optimise to process parameter as a function of the difference of the fruit in term of firmness and Brix degrees.

PROPOSALS OF NON CONVENTIONAL TECHNOLOGIES FOR KIWIFRUIT PROCESSING

"Cold" stabilisation

Main objective of technological treatments is the maintenance of the product reducing the risks of microbial spoilage. Many authors have had as a principal target to obtain kiwifruit pulp without any thermal treatments.

The use of temperature below -10°C is a common storage for refined and concentrated pulp, even if chlorophyll discoloration and browning phenomena limit the shelf-life (12 months at -18°C). At such a low temperature, on the contrary, ascorbic acid content is not significantly changed [21,22,23].

Lodge *et al.* (1985) [24] studied the use of γ-rays irradiation of kiwifruit pulp to be stored at frozen state. This treatment, allowed only in some countries, showed to reduce significantly bacteria, moulds and yeast's population of frozen pulp, without evident colour changes.

In order to pursue the same goal, an intensive research activity is ongoing on the use of high hydrostatic pressure treatments (HPT) (over 500 MPa) to reach a certain microbial stability of kiwifruit derivatives avoiding thermal processing. [25,26,27]. HPT is one of the most interesting emergent technology; it appears to be one of the most attractive technology to obtain cold-pasteurised food products, and thus represents an interesting process to apply to kiwifruit. In fact, high-pressure processing of food materials can provide an effective non-thermal alternative to conventional thermal processing. After the first industrial application in Japan on '90's, HPT is widely studied in all country to obtain fruit products with the flavour and colour similar to the fresh.

High pressure effect on micro-organisms are known since the last century, but the application of this technique was limited because of the technology needed to produce economical high pressure equipment. After the evolution of the HPT in other industries (aircraft, metallurgy, ceramics, etc.) the technological transfer to the food industry has been possible. Hyperbaric treatments (200-900 MPa) showed to be able to food stabilisation and then to prolong the food shelf-life.

The HPT treatments resulted to be suitable for the microbiological stabilisation of kiwifruit products and using a consequent refrigerated distribution is possible to reduce the chemical and sensory modifications up to 30 days of shelf-life. On the other hand, HPT did not inhibit the enzymatic activity of kiwifruit and this results to be the main limiting factor of shelf-life [27].

The combination of the osmotic dehydration-impregnation process with the HPT, placing kiwifruit slices in different sugar solutions (30 and 60 °Brix) in closed containers and following different time-pressure combinations, up to 500 Mpa was reported by Bressa et al. (1996) [25] and Dalla Rosa et al. (1997) [26]. Aim of the research was to evaluate the mass transfer kinetics (weight reduction, water loss, solid gain) in comparison with the atmospheric pressure process, and to assess the effect of the combined process on chemical and microbiological stability of so treated kiwifruit slices during storage at ambient temperature. The microbial stability of HPTreated kiwifruit was found to be related to the pressure level, and a shelf-life more than 10 days was possible only for the samples obtained with 500 MPa HPT. Furthermore, the samples treated with the 30°Brix solution showed to have a higher microbial stability in comparison with the samples treated with the 60°Brix sugar solution.

Nevertheless, from the chemico-physical point of view, the colour of all the samples resulted to change during the storage at ambient temperature. The Hunter a* value increased according to the storage time, meaning a loss of greenness for all the samples both for pulp and core, even if the samples treated with 30°Brix solution maintain slightly more the original colour than the samples treated with the 60°Brix solution.

Beverages

Because of the difficulties in maintaining original colour and of the high acidity of the fruit juice, kiwifruit juices are mainly used as component of juice blends. In fact, frozen pulp and concentrated juices are used mixed with apple juice or other tropical juices (mango, papaya, passion fruit, etc.) for blended juice, nectar

and yoghurt preparation. The use of kiwifruit clarified juice blended with milk whey was studied by Sensidoni *et al.* (1995) [28], in order to obtain a "fortified" beverage to be used as mineral integrator. For use in dairy products, however, kiwifruit derivatives have to be thermally treated in order to inactivate the proteolityc enzymes [29].

The production and storage of a frozen kiwifruit pulp following a "cold preparation line" can allow to obtain a nectar - mixing the pulp with a isotonic sucrose solution - to be used as a ready chilled fruit beverage to be sold in the public exercise. The storage of this product in the "chilled dispenser" demonstrated to maintain for 3 days the original sensory characteristics; the limiting factor was found to be the lactic bacteria growth [30].

Other than the kiwifruit wine, proceed in USA and New Zealand and also proposed in Italy [31,32], could be interesting some researches on low-alcohol fermented juices obtained from non-conventional strain fermentation [33]. As well as the wine, this products from kiwifruit have lost the original flavour and colour, but acquire new pleasant fruity flavours.

Distillation under vacuum of kiwifruit pulp have been experimented by Sensidoni *et al.* (1997) [34].

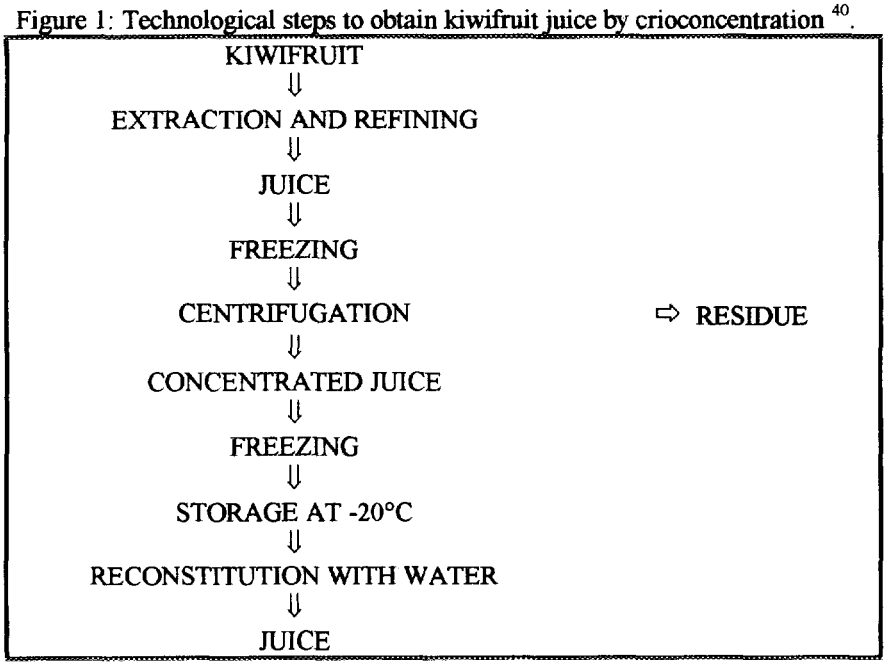
Among the non-alcoholic beverages, Crivelli *et al.* (1990) a coloured kiwifruit clarified juice with artificial colours permitted by EU legislation (E101 e E131) has been proposed in order to obtain a green juice to be mixed with apple and cherry juice [35].

Furthermore, the market of fruit syrups seems to be increasing in the European countries in the '90's, in particular for kiwifruit and melon [36]. The use of ultrafiltration technology or inverse osmosis to concentrate at low temperature the kiwifruit juice present some attractive potentials. Some authors have shown that membrane concentration allows to avoid some losses of flavour and ascorbic acid, but it is difficult to guarantee the maintenance of the original green colour [15,37]. Recently, membrane processing has been studied by Struebi (1995) [38] for kiwifruit juice clarification..

An ongoing research at Dipartimento di Scienze degli Alimenti of the University of Udine has been planned in order to use crioconcentration technology to concentrate kiwifruit juice to be stored at frozen state and to be reconstituted by water adding. (Figure 1).

All process goes at low temperature and at atmospheric pressure, without thermal damage and loss of volatiles, and the obtained juices presents excellent sensory characteristics [39]. From the technological point of view, the critical step is the ice-water separation [40].

Figure 1: Technological steps to obtain kiwifruit juice by crioconcentration [40].



Minimal processed or ready to eat fruit

Minimally processed fruit (MPF) are ready-to-eat packed foods showing several advantages in comparison with both fresh and long-term storage processed fruit:

a) high commodity content for both consumers and catering industry;

b) high adding value for the fresh fruit;

c) relatively low process cost;

d) *easy* substitution of whole fresh fruit in the consumer's habit.

On the other hand, MPF show some difficulties in maintaining the original quality of fresh peeled and cut fruit. Main problems can be listed as follow:

- superficial dehydration and shrinkage even if packed;
- activation of enzymatic reactions by processing (cutting, slicing, etc.) because enhanced enzyme-substrate contact;
- microbial contamination of the fruit surface;
- faster respiration processes than in whole fruit;
- optimum ripening stage for eating is often far from optimal technological maturity (for processing or manipulation).

In the case of kiwifruit this kind of product could actually have a great interest for food industry. In fact, the green round slices of kiwifruit are used for garnishing in many cakes and pastry preparation. On the contrary, owing to the acceleration of the enzymatic reaction due to the manipulation [41,42], the texture changes are very strong in sliced kiwifruit [43].

The shelf-life of sliced kiwifruit, pre-treated with dipping in calcium salt and citric acid, then packed in modified atmosphere, can reach 10 days, in comparison to the 3 days of the non-treated packed slices [44]

In order to extend the shelf-life and to improve the sensory quality of minimally processed kiwifruit slices "ready to eat", Dalla Rosa et al. (1992) [45] proposed an impregnation-direct osmosis step in the processing line to obtain minimally processed kiwifruit slices (MPKF). In Figure 2 the technological flow-chart and in Figure 2 the process layout are reported.

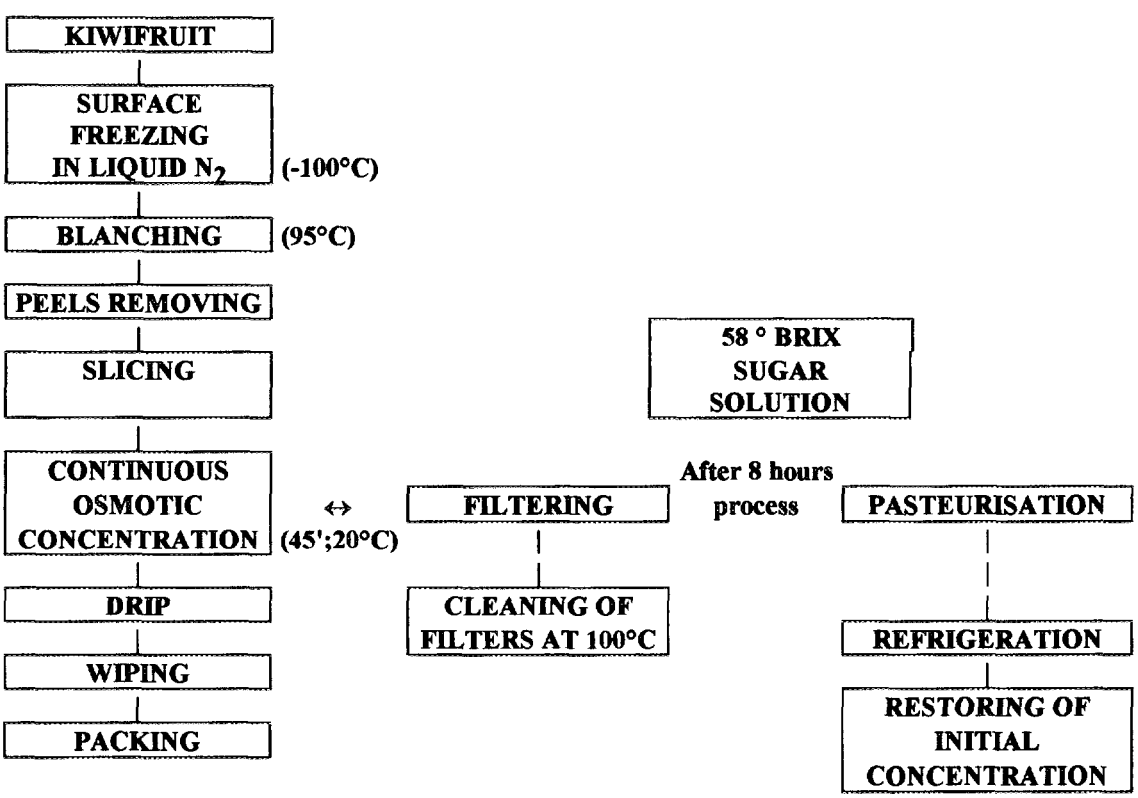


Figure 2 - Flow chart of direct osmosis process used to minimally prepared Kiwifruit slices [45].

This processing permitted to improve the sensory taste of refrigerated kiwifruit slices (stored between 0 and 4°C) in particular when the fruits were processed at an early ripening stage (less than 14 °Brix), maintaining the original colour and flavour and the microbial load at acceptable level up to 10-14 days of shelf-life. Full results of this research have been reported by Bressa et al. (1997) [46]. Other research groups are studying this technical opportunity to improve the quality of minimally processed fruit [47].

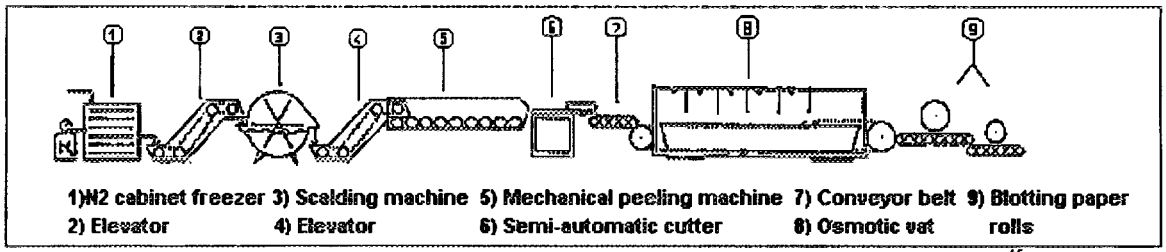


Figure 3 - Pilot plant layout set up to obtain minimally prepared Kiwifruit slices [45].

Osmotic treatments

Many research works have been shown how an immersion in sugar solution can improve the quality of kiwifruit slices. A slight inhibiting action in respect to the enzymatic activities has been demonstrated for sugar as glucose, fructose and sucrose. Increasing the viscosity of the liquid phase would act indirectly on enzyme-substrate system decreasing its mobility [48]. Furthermore, a significant protection would be done by the sugar deposit on fruit surface against oxygen action during immersion in sugar solutions.

These effect are exploited by osmotic dehydration treatments, where the water removal is performed by the chemical potential between fruit (diluted system) and a hypertonic solution. For kiwifruit this technology was proposed firstly by Dalla Rosa [49,50] and later other authors carried out researches on the process optimisation, studying the behaviour of main chemical and physical parameters [51-54]. The presence of the sugar solution in the processed kiwifruit, other than improve the sensory aspects, reduces the irritation phenomenon so called *catch*. Perera and Venning [55] patented a process based on this technology for kiwifruit slices. However, the dehydration level obtainable with the osmotic treatments not allows to reach the product stability so than other stabilisation treatments must be done to have a shelf-stable derivative (air-drying, freezing, canning, etc.). Vial *et al.* (1991) [56] have found how the treatments in Calcium solution modifies the cellular structure, linking the pectic components, reducing the amount of colour modification. The nature of the sugar used for the treatment did not seem to influence the colour and ascorbic acid modification, while Torreggiani *et al.* (1994) [57] demonstrated how the osmotic treatment of kiwifruit slices can modify the fruit composition and then can influence the colour change during frozen storage. The samples treated with maltose (higher molecular weight sugar→lower system mobility) showed less colour changes respect to the untreated fruits after storage at -10° C.

Separation of kiwifruit components

Other than for the production of derivatives addressed to the consumers or for the food industry, the kiwifruit can be exploited as a source of interesting components.

Some enzymatic fractions with industrial interest can be extracted from kiwifruit. In examples: use of kiwifruit extracts (rich in ascorbic acid) as antibrowning agents, actinidin proteinase with potential use as meat tenderising agents [58] or a glycoprotein with demonstrated inhibitory activity against pectin methylestherase [59] and β-galactosidase, β-amilase, polygalacturonase, lysozime, polyphenoloxydase [60].

Furthermore, Australian researchers, Steele and Johnson [61] proposed a non-conventional process for utilisation of kiwifruit, by means the separation of four fractions:

- clarified juice, rich in vitamin C and soluble substance;
- chlorophyll powder, storable at ambient temperature;
- dried seeds;
- concentrated flavours.

The method is based on the extraction of the chlorophyll from the peel, the remainder being processed to juice by conventional methods with the sacrifice of its chlorophyll content. Up to 70 % of the chlorophyll content of the fruit was recovered. An other method facilitated processing by washing screens used for separating fruit tissues from chloroplast-containing juice. It was shown that chloroplast extracts when stored in a domestic freezer at temp. of -15 to -10 degree C were stable for 1 year. Recombined frozen kiwifruit products such as sorbet and ice-cream were chlorophyll-colour stable for 7 and 14 months, respectively. It is concluded that these new processing techniques make feasible the creation of processed kiwifruit products which retain the visual appeal of fresh kiwifruit without using artificial colours. The limitation of this process is that recombined frozen or chilled products have relatively short shelf lives.

CONCLUSIONS

In conclusion, a large amount of derivatives could be produced to exploit the processing capability of kiwifruit. An adequate technological and marketing knowledge should permit a promotion of kiwifruit processed products in order to reach a significant number of consumers.

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INTEGRATED MEMBRANE OPERATION IN THE AGRO-FOOD PROCESSES

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Summary

Membrane operations are of wide potential use in the agro-food industry.They are very attractive for their simplicity and flexibility; they can operate molecular separations particularly compact and simple in their design and scale-up. A large number of membrane operations are today commercially available and new systems are under investigation or already tested at semi-industrial level. Pressure driven membrane processes aretraditional in the stabilization, clarification, fractionation and concentration of liquid foods and beverages competing with other technologies, such as centrifuges or multistage vacuum evaporators.

The introduction of these innovative techniques contributes to solve problem srelated to the loss of fresh juice flavors, color changes, cooked taste,minimizing moreover energy consumption. New molecular separation asnanofiltration, liquid supported membranes, pervaporation, membrane distillation, membrane contactors are today also available and of significant interest for possible applications in agro-food production cycles. Nanofiltration, for example, is offering the possibility to eliminate solvents and low molecular weight compounds as electrolytes from sugar solutions, aminoacids or in general intermediate molecular weight compounds (1000 - 1500 molecular weight).Osmotic distillation and membrane distillation, processes based on microporous hydrophobic polymeric systems do not present the traditional limitation of reverse osmosis in the concentration of the electrolyte solution. The possibility to concentrate musts or in general fruit juice at values approaching 70° Brix at temperature not higher than 25 - 30 degreesis becoming realistic.Pervaporation has been suggested for the removal or concentration of aroma compounds and alcohol solutions. Membrane contactors are becoming more and more important in various treatments such as the extraction of CO2 from liquid streams, the continuos extraction of biactive compounds from fermentation broths, etc.The importance of this compact group of innovative technologies is today recognized in chemical engineering processing and most of them are considered basic unit operations. The possibility to combine molecular separation with chemical conversion is one of the most attractive new potentiality for membrane engineers and different examples of catalytic membrane reactors are today under investigation. Various different configurations have been suggested based on the integration of traditional fermentors or chemical reactors with one of the available membrane operation or by the preparation of catalytic membrane systems acting themselves as reactors.Integrated membrane production can be realized today combining moremembrane operations in the same cycle with other traditional technologies. Interesting examples can be found in downstream processing of biotechnological compounds and in various agro-food productions, as, for example, the preparation of orange and apple juice, tomato concentration,etc. Membrane operation are also contributing to the development of a new innovative storage system of vegetables and fruits in controlled atmosphere. Polymeric membranes able to separate CO_2 or oxygen from nitrogen are available at cost competitive with other traditional techniques.

CHEMICAL-PHYSICAL CHARACTERISTICS OF OSMO-DEHYDROFROZEN SWEET CHERRY HALVES: INFLUENCE OF THE OSMODEHYDRATION METHODS AND SUGAR SYRUP COMPOSITION

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Osmodehydrofreezing is a combined process where freezing is preceded by partial dehydration through osmosis in concentrated sugar solution in order to prepare high and low moisture fruit ingredients. The purpose of this research is to study the influence of the syrup composition and of the pre-dehydration methods before air-drying on the chemical-physical parameters of two osmodehydrofrozen white pulp lines and one red pulp cv. of sweet cherry. The pectic composition was not modified by processing. Owing to the higher intake of sugars, osmo-air-dehydrated cherry showed lower consistency and higher color stability than the osmo-blanched ones at the same a_w. By incorporating sorbitol softer products with greater stability of color and anthocyanins were obtained, showing the osmotic syrup composition as a key point of the proposed process.

INTRODUCTION

Osmodehydrofreezing has been proposed by Torreggiani et al. (1) for the preparation of fruit ingredients with controlled water activity and functional properties suitable for specific food systems. The technique is a "combined" process in which a limited air-drying step is preceded by an osmotic treatment and followed by freezing of the product for long term preservation. Osmotic dehydration is the partial concentration of fruit by immersion in concentrated sugar solutions. One of its main benefits is that it simultaneously allows a rapid removal of water, without the disturbance of phase change experienced during air drying, and a soluble solids uptake. Some of the osmotic syrup may also not actively migrate into the cells, but may simply penetrate into the intercellular spaces. The technological aspects of osmotic dehydration and its wide prospects as a pre-step to further processing are summarized in a recent review by Torreggiani (2). Within osmodehydrofreezing the specific role of osmosis is mainly the enrichment of the soluble solids content rather than the removal of water. In this way a lowering of the water activity, which is dependent on soluble solids concentration, is obtained with only a limited decrease in the water content and thus a limited increase in consistency. Maltini et al. (3) found, for reduced moisture fruit, a correlation between the square of the percentage of insoluble solids and the consistency. Owing to the soluble solids intake during osmosis, the percentage of insoluble solids decreases leading to products that, at the same water activity as air-dehydrated fruit, are softer. These products are more pleasant to eat as a snack item or could be suitable for products such as pastry or ice cream, without a previous rehydration step. Besides, as reported by Giangiacomo et al. (4), a controlled rate of moisture uptake by the partially dehydrated fruit pieces in yogurts could avoid whey separation. If a concentrated fruit juice is used as osmotic solution (5), an even softer product is obtained because of the higher content of monosaccharides in the fruit juices compared to that contained in syrups from starch hydrolysis and because of the higher relative water content at a determined a_w. The product is totally of fruit origin and this feature may be relevant under merceological aspects. If sorbitol is added to a fructose syrup, softer frozen intermediate moisture apricot and clingstone peach cubes could be obtained, when compared with apricot and clingstone peach cubes osmodehydrated in fructose alone (6). The process has also been applied to vegetables. Red pepper cubes have been osmodehydrated in a new type of osmotic solution: concentrated hydrolyzed lactose syrup from cheese whey ultrafiltration permeate (7). The addition of sorbitol to the syrup leads to a lower consistency in frozen intermediate moisture red pepper cubes. Moreover, sorbitol showed a specific protective effect on the color of red pepper during the air drying step. The chemical changes most strongly related to the acceptability of cherry products are those associated with polyphenols (colour) and pectins (texture). Polesello and Bonzini (8) and Pizzocaro et al. (9) studied the anthocyanin composition of 13 cultivars of cherry grown in Italy, and confirmed that

Cy-3-rhamnoglucoside was the major anthocyanin present. Forni et al. (10) studied the changes in anthocyanins in cherries during osmodehydration, pasteurization and storage. Osmotic dehydration contributes slightly to the lightening of the red color of processed cherries, while the thermal process caused a loss of about 50% and a further fading of the color.

The aim of the present work is to study the influence of syrup composition and the pre-dehydration methods before air-drying on the chemical-physical characteristics of two osmodehydrofrozen white pulp lines and one red pulp cultivar of sweet cherries.

MATERIALS AND METHODS

RAW MATERIAL

Cherries of the cultivar Vittoria and of the lines i137 and i221, harvested at a commercial maturity stage, were pitted and halved. Each cultivar or line was divided into four lots and processed.

PRETREATMENTS

1) Osmoblanching (SC) in a 30% (wt/wt) fructose syrup for 2 min at 90°C; syrup/fruit ratio 3/1.
2) Vacuum osmodehydration (IV) in a 30% (wt/wt) fructose syrup containing 1% of ascorbic acid and 0.2% of citric acid as antioxidant agents (11), for 30 min at 25°C; syrup/fruit ratio 2/1.
3) Osmodehydration in a 65% (wt/wt) fructose syrup (OF) containing 1% of ascorbic acid and 0.2% of citric acid, for 45 min at 25°C; syrup/fruit ratio 3/1.
4) Osmodehydration in a 65% (wt/wt) fructose/sorbitol (1/1) syrup (OFS) containing 1% of ascorbic acid and 0.2% of citric acid, for 45 min at 25°C; syrup/fruit ratio 3/1.
5) Air-drying in an upward air-circulated drier (Simplicior mod. Babcock-BSH, Badhersfeld, D), at a dry-bulb temperature of 65°C and an air speed of 1.5 m/s. Two levels of solid content were obtained corresponding to 50 and 70% weight loss (D5, D7). Air-drying was applied only to Vittoria cherries after the other pretreatments as shown in Figure 1.

FREEZING

Pretreated cherry halves and pretreated-air-dried Vittoria cherry halves were frozen in an air-blast tunnel at -40°C and 4m/s air speed. The frozen products were packaged in sealed polyethylene plastic bags and stored at -20°C.

ANALYSIS

Dry matter (DM), pH and total titratable acidity (AC) were determined in accordance with AOAC Methods (12).

Refractometric index (°Bx) was measured with a Refractometer RFM (BS Instrument, UK).

Sugars were quantified by HPLC on a Polyspher CHCA (Merk) column (0.65x30 cm) at 90°C eluting with deionised water, with a Jasco 830 refractometric detector (13).

Alcohol insoluble solids (AIS) were determined according to Barbier and Thibault (14). The extraction of pectin fractions, soluble in water (W), soluble in oxalate (O) and insoluble (R, protopectin), from AIS was performed according to Forni et al. (15). Galacturonic acid content of pectin fractions was determined on enzymically depolymerized pectins (16) by HPLC on an ION 300 column (300x7.8mm) Altech at 66°C eluting with 0.0085 N H_2SO_4, with a Jasco 830 refractometric detector (17).

Anthocyanins were determined by spectrophotometric analysis according to Forni et al. (10).

Texture was measured using an Instron model 1140 Universal Testing Machine measuring the maximum force (kg) with a standard Shear Press Cell Model CS1, with a crosshead speed of 20 cm/min, on 30g of cherry halves.

Water activity (a_w) was measured using a Thermoconstanter Hygrometer (Novasina, Zurich, Switzerland).

Colour measurements were made by means of Minolta Chroma Meter CR 2000 (Minolta Camera Co. Ltd, Osaka, Japan). Reported data are the mean of 25 determinations. The readings were made on a double layer of cherry halves arranged on the transparent glass bottom of a black-walled cylinder of 10 cm diameter. From Hunter values of L*, a*, b* the color differences (Delta E) between the color of the fresh cherry and the products just after processing were calculated using the formula (18):

$$\text{Delta } E = (\text{Delta } L^{*2} + \text{Delta } a^{*2} + \text{Delta } b^{*2}).$$

The data reported are the average of four determinations. The analyses were conducted on fresh cherries, after pretreatments SC, IV, OF and OFS and freezing for i221 and i137 cherry halves, after pretreatments and after pretreatments+air drying and freezing for Vittoria cherry halves.

Following Giangiacomo et al. (19), solid gain (SG), water loss (WL) and material balance (Cp) were calculated after the different pretreatments.

RESULTS AND DISCUSSION

The composition of raw sweet cherries i221, i137 and Vittoria is reported in Figure 2. Cherry i221 shows the highest dry matter and total sugar content (TS). The alcohol insoluble solid substances (AIS) are the lowest in i221 and pectin composition, for this line, shows the highest protopectin content (R). No differences are noticed in the sugar distribution. The sweet cherry composition is in the range of the values previously observed by Giangiacomo et al. (19) and Torreggiani et al. (20,21).

Water loss (WL), solid gain (SG) and material balance, together with fruit composition after the different pretreatments are reported in Figure 3, 4 and 5 for i221, i137 and Vittoria , respectively.

Osmoblanching (SC) and vacuum osmodehydration (IV) cause a decrease of the soluble solids content in all the lines and cultivar considered. The decrease is higher in the SC pretreatment and the material balance indicates that it is mainly due to the flowing out of glucose into the blanching syrup. In the osmodehydration pretreatments (OF and OFS) the increase of dry matter is mainly due to water loss because of the low solid gain, as already observed by Forni et al. (10). This behaviour could be related to the small exchange surface and to the waxy peel of the sweet cherries. Except for osmoblanching, the cultivar Vittoria shows the highest water loss irrespectively from the different pretreatments.

Total titratable acidity slightly decreases while AIS content and pectin distribution among the different fractions do not significantly change during the different pretreatments. Total sugar intake is higher for line i221 in both the osmodehydration pretreatments. The use of sorbitol as an osmotic substance, together with fructose, does not influence the dehydration power of the syrup. Only in the cultivar Victoria the presence of sorbitol in the extracting syrup reduces the solids gain because of the lower intake of fructose not totally replaced by the sorbitol intake.

The anthocyanin content of fresh and processed Vittoria cherry halves are reported in Figure 6. The highest loss of anthocyanin pigments is observed after vacuum osmodehydration (-0.68 g/100g of initial fresh weight) and osmoblanching (-0.60), while the osmodehydration pretreatments are less damaging (-0.32 for OF and -0.33 for OFS). No differences were observed in the anthocyanin contents of the differently pretreated and air-dried cherry halves. The color data are reported in Figure 7. The hue values of both i221 and i137 are not modified by the different pretreatments, while the saturation values are decreased mainly by the osmoblanching and vacuum osmodehydration. Both hue and saturation values of the cultivar Vittoria are not modified by the different pretreatments and air-drying, confirming the high stability of the color attributes of this cultivar (10). The L* values of the fresh and pretreated cherries , and of the air-dried Vittoria cherries are reported in Figure 8. Both i221 and i137 lines show a decrease of the L* values, that is higher after osmoblanching and vacuum osmodehydration when compared with both osmodehydration treatments at atmospheric pressure. The L* values of the cultivar Vittoria are stable both after the different pretreatments and air-drying. Delta E values, shown in Figure 9, confirm that the highest color modifications are caused by vacuum osmodehydration (IV) and osmoblanching (SC). Even if the pigment loss is around 80%, the color of the dried cherries did not change probably because of the concentration effect of the air-drying step.

Owing to the intake of sorbitol, osmo-air-dehydrated (OFSD5, OFSD7) Vittoria cherry halves show a lower refractometric index (thus an higher relative water content) and a lower consistency than the other differently pretreated-air-dried cherry halves at the same water activity (Figure 10). The consistency is linearly correlated both with the isoluble matter, expressed as % AIS ($R^2 = 0.98$), and the insoluble protopectin fraction R ($R^2 = 0.92$), as already reported for osmodehydrated fruit by Maltini et al. (3). By using osmoblanching as a pretreatment (SCD5, SCD7) tougher dried products are obtained if compared to the other pretreatments.

CONCLUSION

The solid-liquid exchanges in cherry halves are quite low when compared to other fruit, showing the great importance of the tissue structure, as already observed by Forni et al.(10). The color of the two white pulp lines i221 and i137 is more sensitive to the different pretreatments than the red pulp cultivar Vittoria, osmoblanching (SC) and vacuum osmodehydration (IV) causing the highest fading of the red color. These two pretreatments cause also the highest loss of anthocyanin pigments in the Vittoria cherry halves. Due to the lack of solid gain and the high temperature of the treatment, osmoblanched-air-dried Vittoria cherry halves are tougher than the other differently pretreated cherries.As already observed for apricot and clingstone peaches (6) and red pepper cubes (7) the presence of sorbitol in the syrup leads to softer fruit when compared to fruit osmodehydrated in fructose alone. It must be further underlined that the key point of this proposed process is the sugar composition of the osmotic syrup. In fact, through the incorporation of a specific solute into the fruit by osmosis, it is possible, to a certain extent, to change its functional properties, achieving a specific formulation of the product without modifying its integrity.

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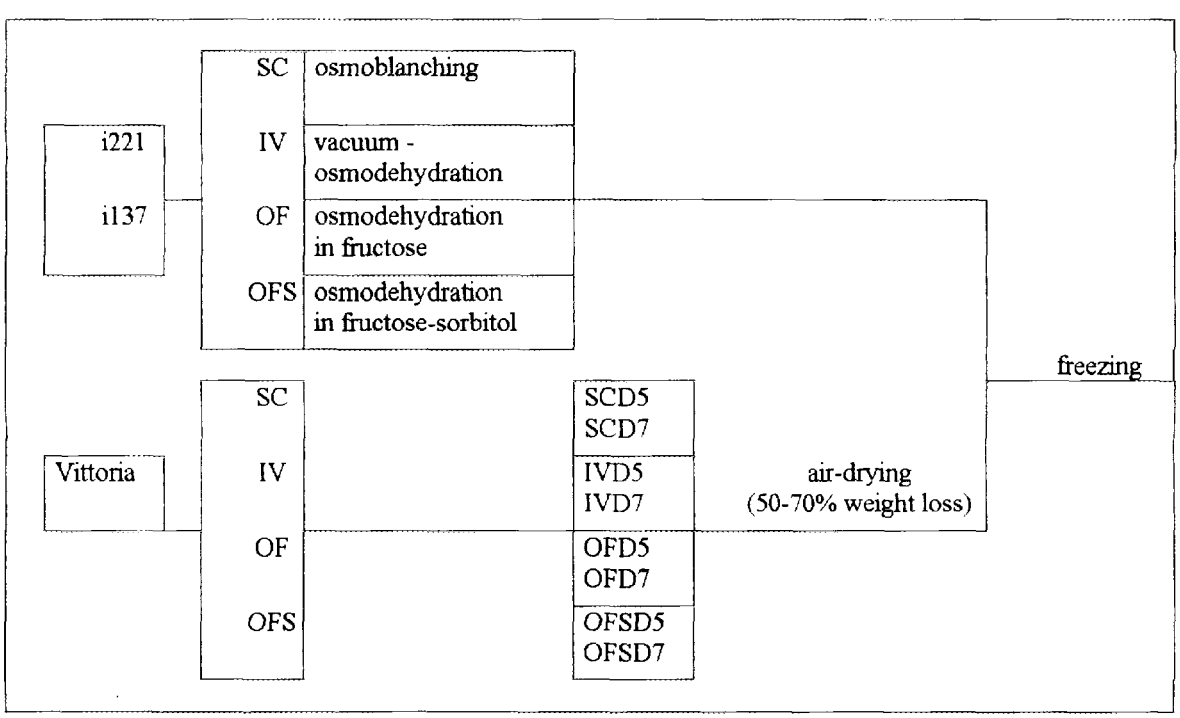

Figure 1: Flow sheet of the process.

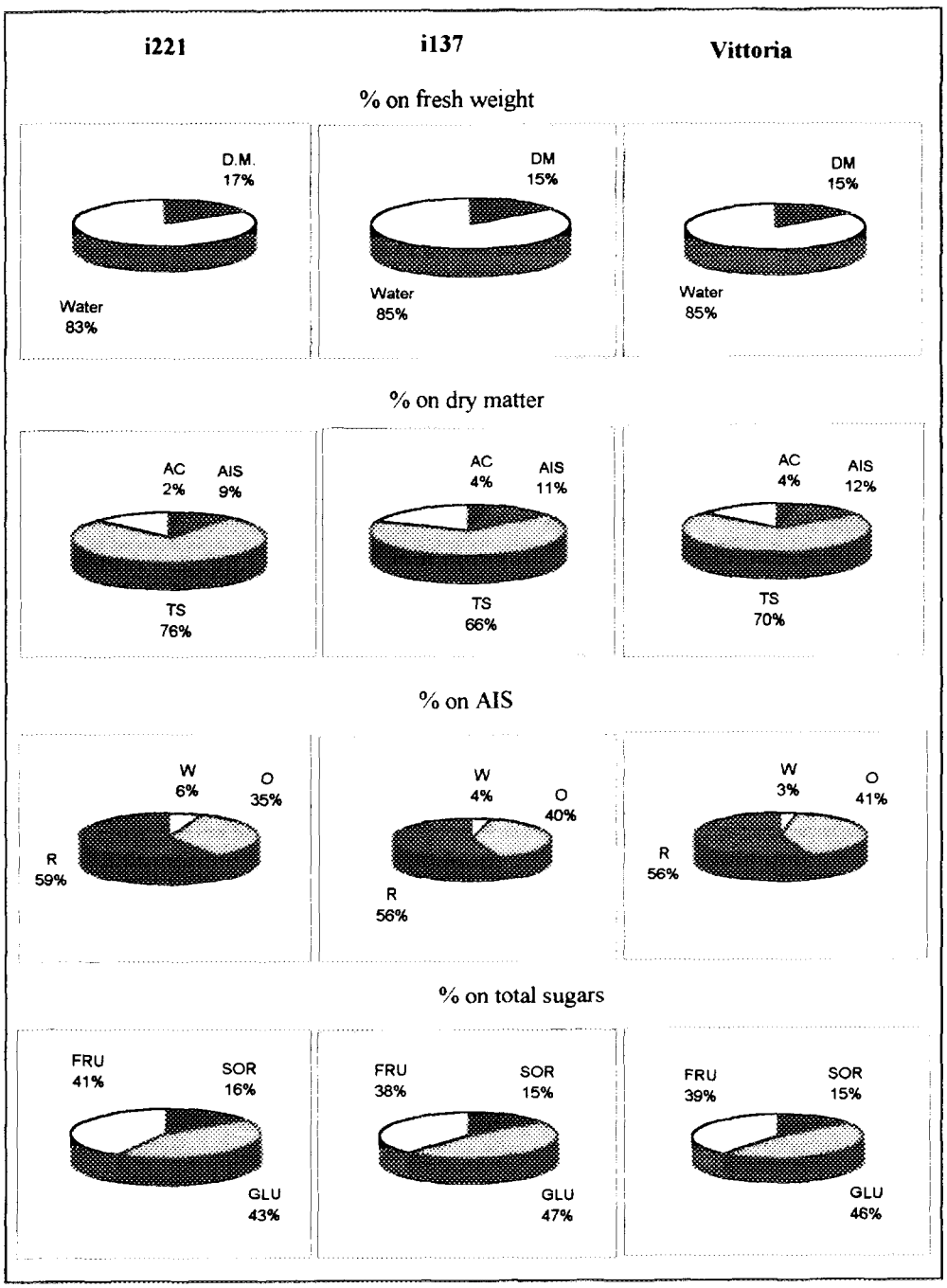

Figure 2: Chemical characterization of fresh cherry

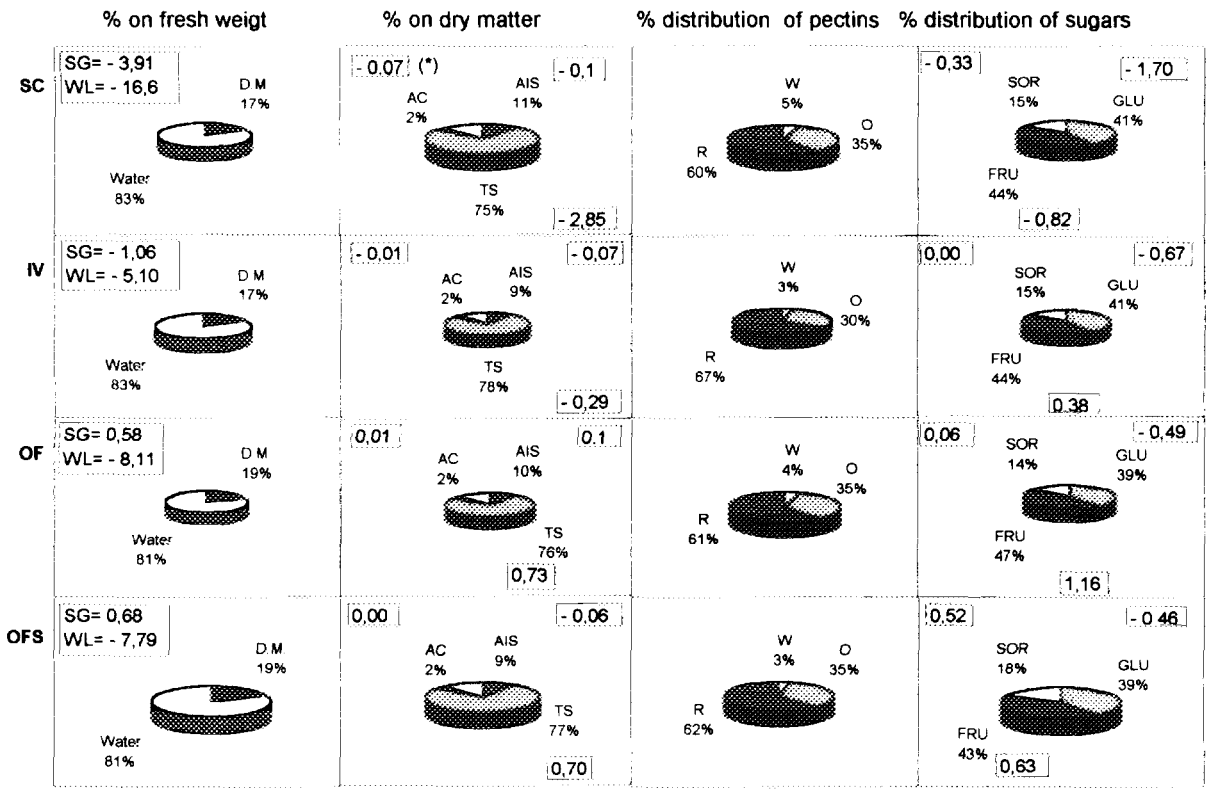

Figure 3: Water loss , solid gain (SG), material balance of the chemical components (*) and pectin and sugars percentage distributions of i221 cherry.

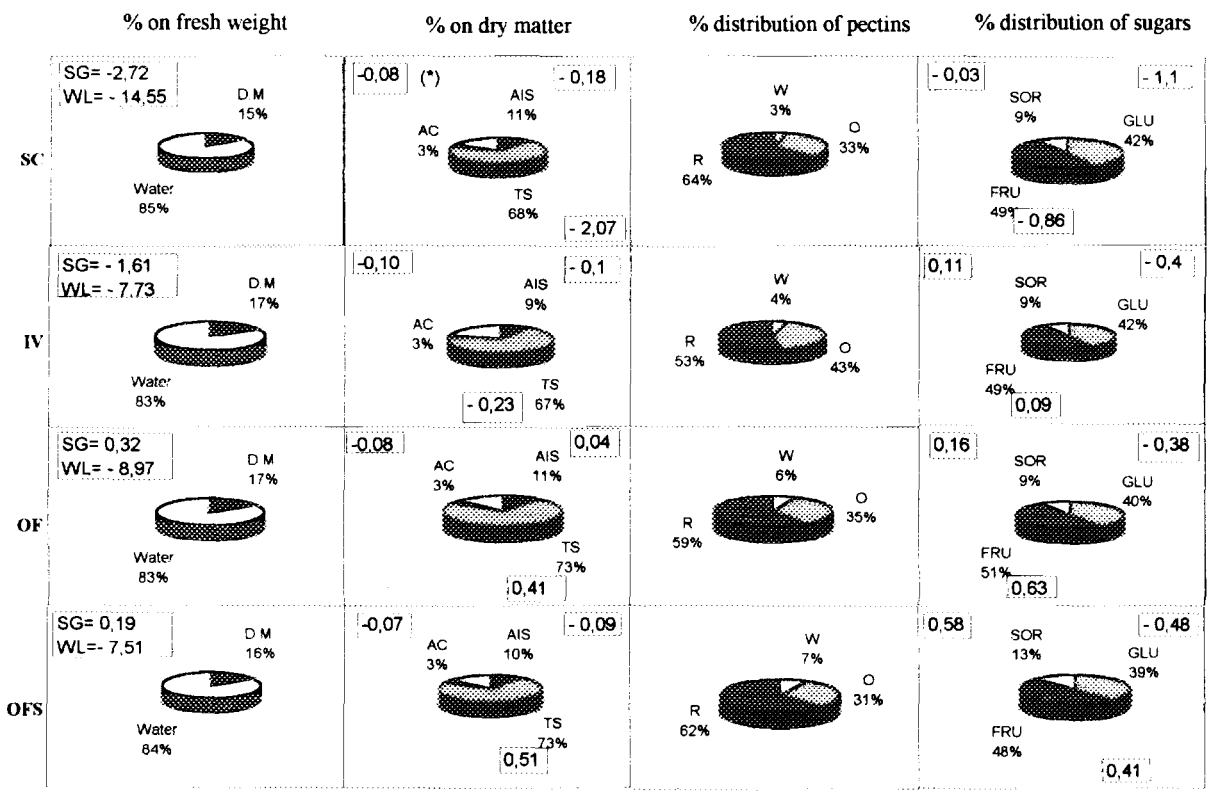

Figure 4: Water loss , solid gain (SG), material balance of the chemical components (*) and pectin and sugars percentage distributions of i137 cherry.

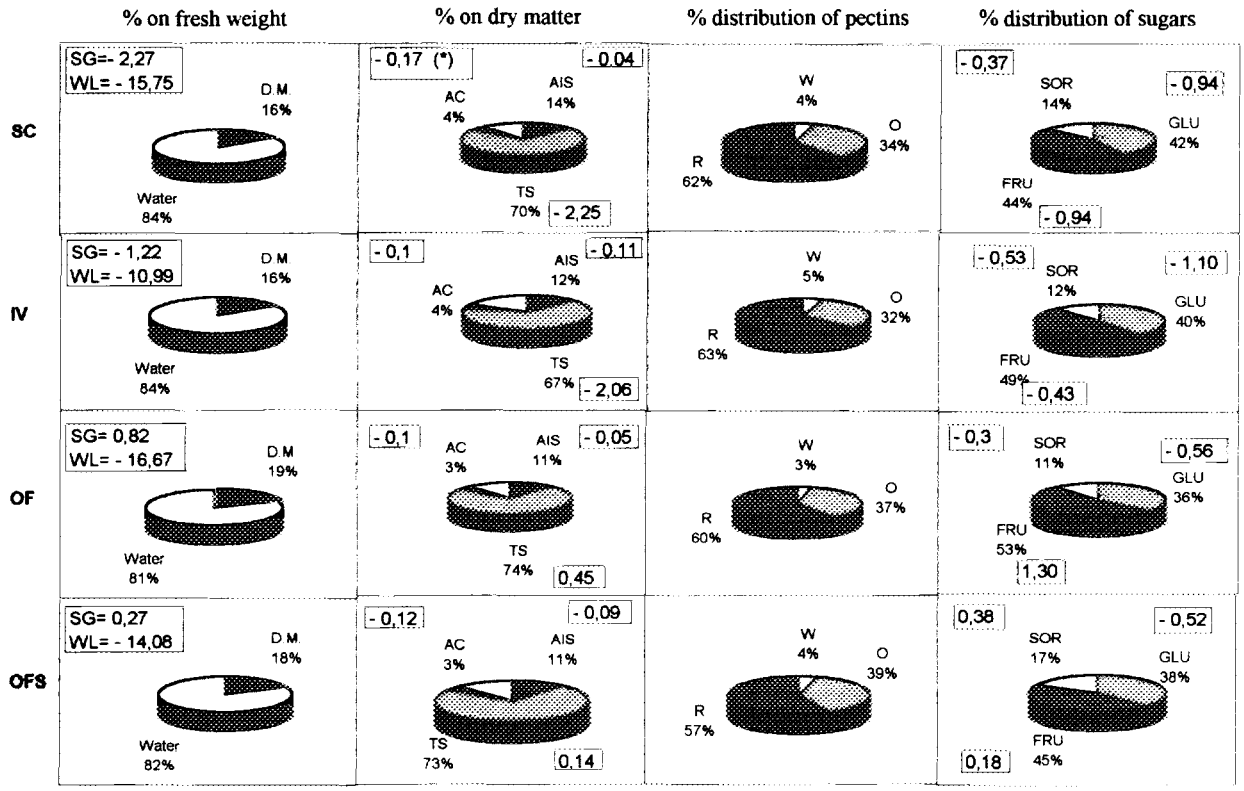

Figure 5: Water loss(WL), solid gain (SG), material balance (*) of the chemical components and pectin and sugars percentage distribution of Vittoria cherry.

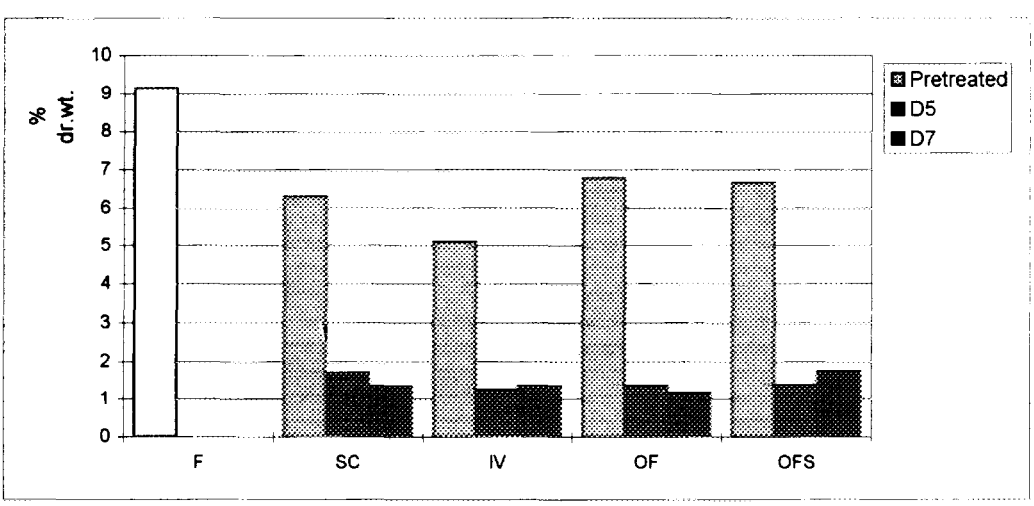

Figure 6: Anthocyanin content of fresh and processed Vittoria cherry

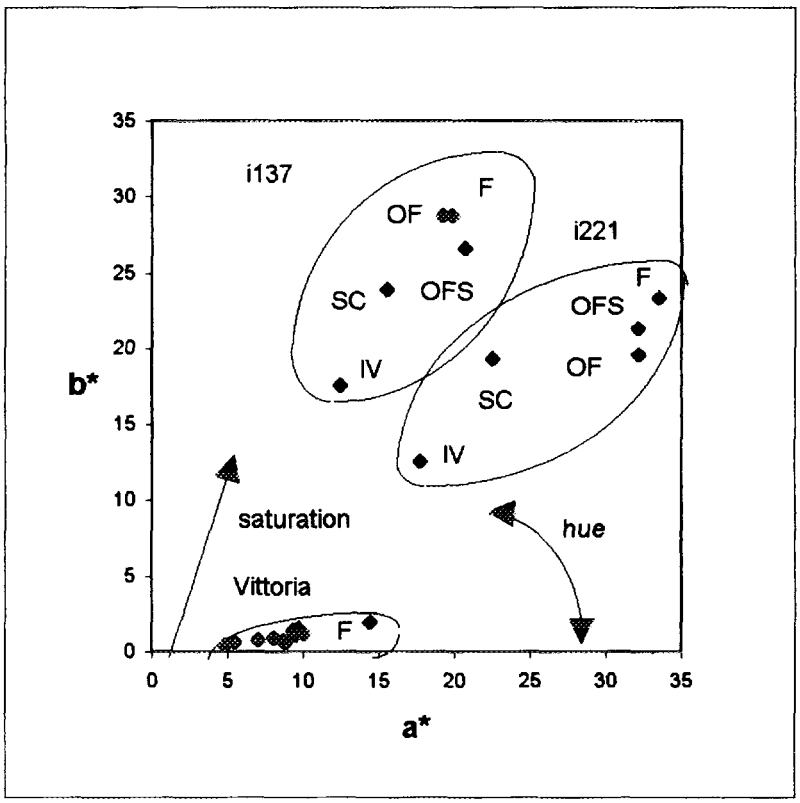

Figure 7: Color data of fresh and processed cherry.

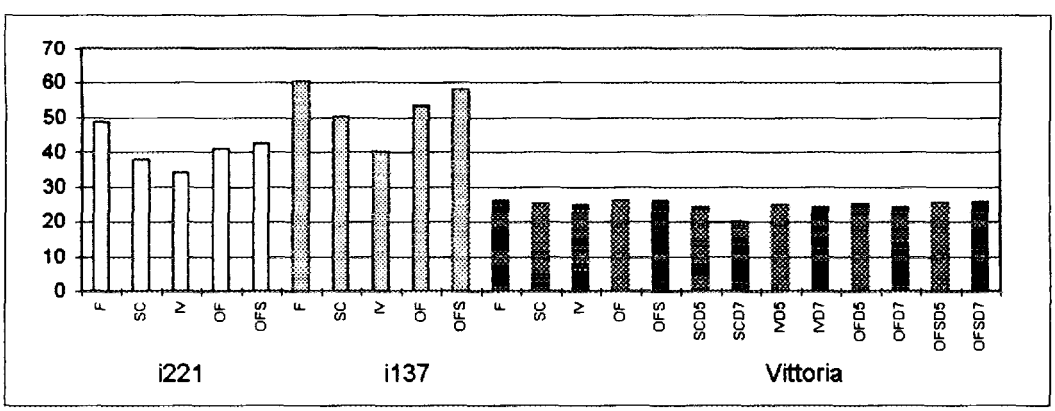

Figure 8: L* values of fresh and processed cherry.

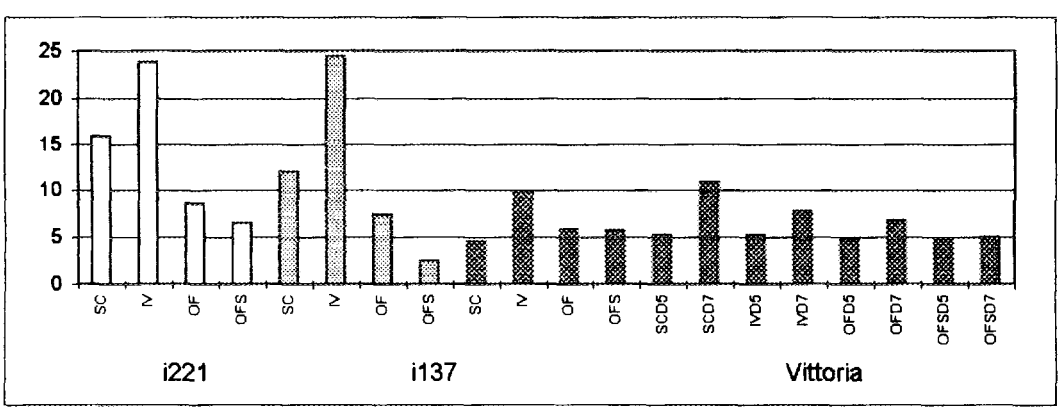

Figure 9: Delta E values of processed cherry.

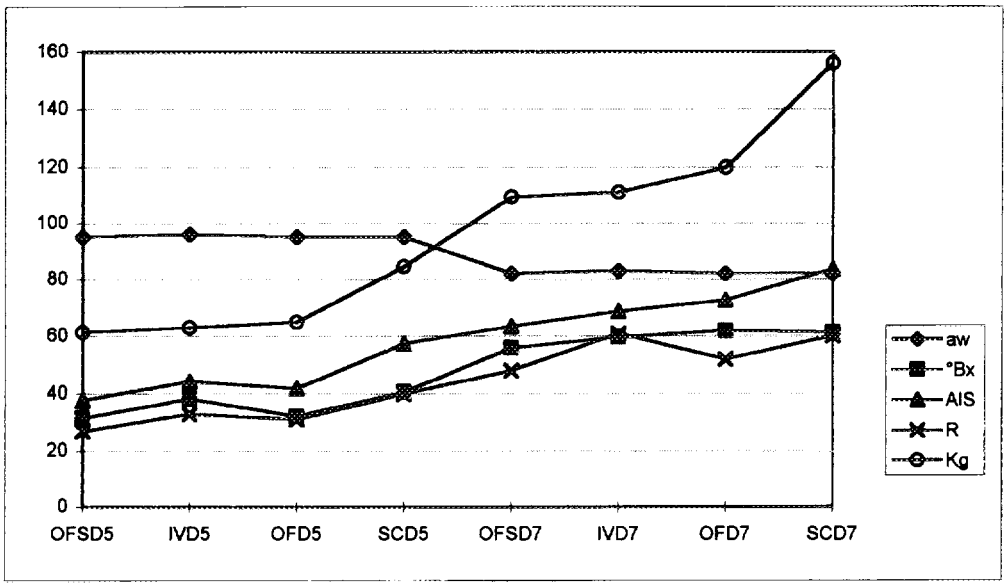

Figure 10: Water activity (awx100), refractometric index (°Bx), alcohol insoluble substances (AISx10), protopectin fraction (Rx100) and consistency (Kg) of Vittoria cherry after pretreatments and air-drying.

Analysis of the Effects of Ultrafiltration on the Quality of Freshly Squeezed Orange Juice

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ABSTRACT

Crossflow ultrafiltration by tubular PVDF membranes (MWCO=$1.8 \cdot 10^4$) was studied for clarification and debittering of orange juice. The effects of separation processes on the distribution of aroma compounds between permeate and fresh juice was investigated. In particular the limonin concentration in the permeate was reduced of about 65% with respect to the fresh juice. In addition, most of the more water soluble compounds passed through the membrane. Hydrocarbons and less polar aroma compounds, mainly remained in the retentate. A mathematical model of ultrafiltration system is also presented. It describes the accumulation of suspended particles on the membrane, accounting for back-diffusion transport mechanisms. The additional resistance of particles layer was evaluated by the Blake-Cozeny-equation. The theoretical predictions in terms of permeate flux as a function of time resulted in a good agreement with the experimental data.

INTRODUCTION

Pressure-driven membrane operations represent today an interesting answer for innovations in fruit juices processing. Juice clarification, stabilisation, depectinisations and concentration are typical steps where molecular separation processes as microfiltration, ultrafiltration, nanofiltration, reverse osmosis and membrane distillation have been successfully utilised[1]. An important limitation in the performance of such membrane processes is that the permeate flux is adversely effected by the transient build-of a layer of rejected species at the upstream interface of the membrane. The general effect of this phenomena, known as concentration polarisation phenomena, is a rapid permeate flux decay during early period of filtration, followed by a long and gradual flux decline towards a steady or quasi-steady-state limit value. However a more important aspect of concentration polarisation phenomena which has to be considered is related to the physicochemical interactions with the membrane of the accumulated material. In this case a fouling mechanism such as adsorption on the membrane pore walls and pore plugging by solute penetration occurs rather than the build-up of a particle cake layer at the interface.

Membrane fouling in cross-flow membrane separation processes is a key factor affecting economic and commercial viability of a membrane system which depends, essentially, on the obtained permeate fluxes and their stability. Fluxes, in fact, are a function of time, and depend strongly on the transmembrane pressure difference (TMP) which is the driving force for permeation; on the crossflow velocity, on the nature of the membrane and on the nature of the feed solution. Nevertheless the flux increases with pressure up to a limiting value of TMP which, for a given suspension, depends on cross-flow velocity. Some experimental evidences in microfiltration of orange juice revealed that any attempt to increase the permeate flux by simply increasing TMP, fails if it is not accompanied by an increase in crossflow velocity[2].

The advantages of membrane operation, however, are that the separation process is athermal and involves no phase change or chemical agents. These features are becoming very important factors in the production of new fruit juices with natural fresh tasting and additive-free. In this work the effects of ultrafiltration process on the quality of freshly squeezed orange juice was investigated.

In particular one of the objectives has been determine whether ultrafiltration offers any advantages in the reduction of bitterness of orange juice. Bitterness of citrus juice is due to the presence of limonoids. In particular the bitterness gradually develops after extraction and is referred as "delayed bitterness". The intact

fruit do not normally contain limonin but rather a nonbitter precursor limonoate A - ring lactone (LARL). This nonbitter precursor, originally present in the insoluble fruit sections, diffuses into the juice during fruit processing and is converted to limonin under acidic conditions. This conversion is also accelerated by action of limonin D - ring lactone hydrolase which has been shown to be present in citrus[3].

Many attempts have been made to solve the problem of limonin formation by exposure of the fruit to ethylene[4], enzymatic treatment of the juice[5], adsorption of the limonin already formed on cellulose acetate[6], use of enclosure complex forming agents with limonin like b-cyclodextrin[7].

Up to now, there are no entirely satisfactory method for preventing or removing limonin bitterness. The problem becomes more acute, as citrus production and processing increase, especially in Italy, were the orange-type fruits are characterised by high concentration of components responsible for the bitterness. In the present work the ultrafiltration (UF) was used to separate from juice the suspend pulp where the nonbitter precursor is located. In this way the contact time between pulp and serum was minimised thus allowing to control the conversion of nonbitter precursor in limonin. The nonbitter clarified juice was continuously collected as permeate stream. Moreover the effects of pulp removal on the flavour constituents of juice have been analysed by enriched head space analysis, exploiting the coupling of Purge and Trap (PT), gascromatography (GC) and mass spectrometry (MS) analytic techniques.

Finally a mathematical model to predict the effects of operating conditions on the permeate flux is presented. The model is based on a mass balance with respect to the suspended particle convected towards the membrane, accounting for their back transport modelled in terms of brownian motion, lateral migration and shear-induced diffusion mechanisms. The model estimates the thickness of particles layer accumulated on the membrane surface, thus allowing the evaluation of the additional transport resistance across the membrane by Blake-Cozeny-equation. The theoretical predictions of transient behaviour of permeate flux were compared with experimental results obtained at different values of transmembrane pressure and feed velocity. In the next sections the results of this analysis will be presented and discussed.

MATERIAL AND METHODS

The juice was obtained in laboratory by pressing of fresh Navel oranges. Samples of juice were collected and stored at -20°C to be analysed later. In each experimental run, about 10 l of juice were processed on a laboratory scale ultrafiltration . The membrane system consists of a tubular membrane module (Tri-Cor HF 18). A gear pump of 0.75 HP was used to recirculate the juice at different values of velocity. A pressure gauge and a tube and shell heat exchanger placed after the membrane module allowed to regulate the TMP at the desired value and kept the temperature at about 20 °C.

Two kind of experiments were performed. The first was devoted to the investigation of the effect of the operating conditions on the flux performance of the system. In this case the permeate was continuously recycled to the feed tank to ensure a steady or nearly-steady state in the volume and composition of the feed. In particular the experiments were carried out under turbulent conditions, characterised by Reynolds number of 7000, 10.000 and 15.000 and TMP in the range of 0.5-1 bar.

In the second one the UF system was operated to concentrate the juice up a factor of 50 %. In this experiments, the effect of the ultrafiltration process on the limonin content was analysed by comparison between the time evolution of limonin concentration in the permeate, the retentate and the untreated juice kept at ambient temperature. As far as the aroma compounds is concerned, the effect of ultrafiltration was carried out by comparison between the overall permeate collected and the untreated fresh juice.

Analysis of limonin was carried out by high performance liquid chromatography (HPLC) after extraction according to the method described in literature[8].

A Waters (Bedford, MA) 510 pumping system, a Waters 484 variable-wavelength detector set at 207 nm and a Waters 745 integrator were used. The limonin was quantified by peak area. Average values from the two injections were used.

The general distribution of aroma compounds in the fresh juice and the permeate were analysed by dynamic headspace Purge and Trap procedure with GC-MS identification (Fisons Instruments P&T 850, GC 8060, MS MD 800).

The Purge and Trap system was equipped with a glass ampulla in which a known aliquot of the juice sample was introduced. A stream of helium (30 ml/min, 7 psi) was used as carrier to transport the aroma compounds within a trap consisting of a packed Tenax column. Here the organic compounds were at first adsorbed at room temperature then thermally desorbed at 200 °C. The desorbed analytes were cryofocused at -90°C to focus directly on the head of a capillary SPB1 column (60 m x 0.32 mm id; 2.5 μm thickness film;

Supelchem) fitted in a 8060 gas chromatograph (Fisons Instruments) which was interfaced to a MD 800 ion trap mass spectrometer (Fisons Instruments).

Through the use of multiramp programming the analytes were resolved. An initial temperature of 50 °C was held for 5 min, then ramped at 10 °C/min to 250 °C, and held for 5 min. At the conclusion of the GC run, the eluants passed into the mass spectrometer. Mass spectra were obtained by electron ionization at 70 eV and an ion source temperature of 200 °C. Spectra were acquired for 30 min. The different components were identified by comparison of mass spectra with library spectra (NIST). Finally, through a judicious selection of mass spectrum relative to the most representative ions of molecules, a comparative quantitative analysis was possible. Quantitative data were derived from the trace obtained from the TIC monitor during GC-MS. All determinations were performed in duplicate.

MATHEMATICAL MODEL

Orange juice was considered like a biphasic system made up of a liquid phase, usually indicated as "serum", consisting essentially of water salts and sugars and a suspended solid phase consisting of pulp and a protein-pectin complex termed "cloud".

In the cross-flow ultrafiltration of juice, the serum and the suspended solids are all convected towards the membrane surface. Here, according to a sieving mechanism, the mechanical separation of suspended materials from the serum takes place. Thus the serum, which represents the carrier fluid, permeates freely across the membrane whereas the solids are retained on the membrane promoting a "cake" formation. As a consequence of their rejection the solid particles accumulates on the membrane thus providing a hydraulic resistance to serum flow in addition to that of the membrane. The permeate flux, in these conditions, typically decays sharply over the first 10 to 30 minutes.

However for cross-flow filtration, not all the particles deposited remain at the membrane surface. Depending on the cross-flow velocity and transmembrane pressure difference, the cake will grow until the rate of deposition is balanced by the rate of removal by effect of diffusion and crossflow velocity.

Taking into account the limiting effects on the permeation flux of cake formation, the following equation can be used to estimate the permeate flux:

$$J_p = \frac{TMP}{\mu(R_m + R_c)} \tag{1}$$

where Jp is the permeate flux in m/s, usually expressed in $L/m^2 \cdot h$, and μ the viscosity of serum. R_m is the intrinsic clean membrane hydraulic resistance and R_C an additional contribute to the transport resistance of permeate flux due to the accumulation of particles in a thin layer adjacent to the membrane surface.

In a recent work an analysis of fouling mechanisms involved in the orange juice microfiltration has been carried out[2]. The results pointed out that the fouling mechanism is strongly affected by fluid-dynamic operating conditions. In particular it was observed that, changing the fluid-dynamic regime in the interval of Reynolds number 5000-15000, the dominating fouling mechanism from "cake filtration" becomes "complete pore blocking" with a negligible flux decay.

In this paper, in order to describe the dynamic behaviour of permeate flux the particles transport was schematised as in Figure 1:

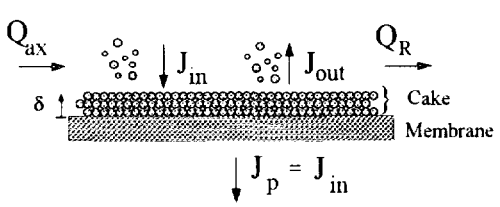

Figure 1. Schematic of the particles transport towards and from the membrane

According to the scheme of Figure 1, a lumped parameter mathematical has been formulated as follows:

$$(1-\varepsilon)\frac{d\delta}{dt} = \sum_i \left[J_p C_i - J_{out_i}(1-\varepsilon) \right] \qquad \text{I.C.} \quad t=0\ ,\ \delta=0 \tag{2}$$

Eq.2 takes into account the particles size distribution and their transport towards and from the membrane as due essentially to convective ($J_p C_i$) and diffusive (J_{out}) fluxes respectively. In particular, the former is generated by the transmembrane pressure difference applied across the membrane whereas the latter is due to the superimposition of brownian motion, lateral migration and shear-induced diffusion transport mechanism[9], as expressed by Eq 3:

$$J_{out_i} = \frac{kT}{3\pi\mu R_f D_{p_i}} + \frac{u^2 D_{p_i}^3}{32\nu R_f^2} + \frac{0.05 u D_{p_i}^2}{4R_f^2} \tag{3}$$

Eq.2 and Eq.3 allow to predict the time evolution of cake thickness, δ, under the hypothesis that all the particles whose representative diameter is larger than mean pore size of the membrane are completely rejected. Then the predicted thickness of the particles layer can be used to evaluate the additional transport resistance R_c, in Eq. 1, by Blake-Cozeny equation[10]:

$$R_c = \frac{150}{\overline{D}_p^2} \frac{(1-\varepsilon)^2}{\varepsilon^3} \delta(t) \tag{4}$$

where:

$$\overline{D}_p = \frac{1}{\sum_i \frac{\omega_i}{D_{p_i}}} \tag{5}$$

Combining Eq. 4 with Eq.1 we obtain:

$$J_p(t) = \frac{TMP}{\mu \left(\frac{150}{\overline{D}_p^2} \frac{(1-\varepsilon)^2}{\varepsilon^3} \delta(t) + R_m \right)} \tag{6}$$

Eq.6, through δ, establishes an implicit relationship between the permeate flux and the time, being the functionality of δ with time evaluable by Eq. 2.

In conclusion, Eqs. 2, 3 and 6 represent the equations model whose solution allows to calculate the permeate flux at any time as a function of the operating conditions, related to the axial feed velocity and applied transmembrane pressure. An interactive simulation program has been developed *ad hoc* by using the Interactive Simulation Language (ISIM, ChemEng, Simulation Sciences, Manchester).

In particular Eq. 2 has been solved numerically by using an adaptive-step-size Runge-Kutta algorithm of 5^{ht} order. The particles size distribution was subdived in five classes of particles whose mean pore size and the relative concentration has been measured experimentally. These informations were obtained by a cascade sequential filtration of a sample juice. With this sieving technique the mean diameter of each particles class, was estimated as the average between the largest filter diameter that retained suspended solids and the smallest filter diameter that did not. In Table 1 the size interval for each particle class and the relative concentration are summarised.

Table 1 Classification of particles in the juice

Class	Size interval (mm)	Phase	C_i (Vol. particles/Vol. serum) (mL/mL)
A	1.5 < Dp < 3	solid	0.095
B	0.3 < Dp < 1.5		0.069
C	0.125 < Dp < 0.3		0.047
D	0.002 < Dp < 0.125		0.105

E	$0.0003 < Dp < 0.002$	serum	0.728
Permeate	$0 < Dp < 0.0003$		0.272

Other data input for the program were: TMP, Q_{ax}, J_p (t=0), T, μ.

The only degree of freedom of equation system was the overall cake voidage, ε. which was used as adjustable parameter to obtained the best fit of experimental data.

RESULTS AND DISCUSSION

EFFECTS ON LIMONIN BITTERNESS.

In Figure 2 the time evolution of limonin concentration in the fresh juice, retentate and permeate is shown. The limonin concentration in the fresh juice increases during first hour, then attains a steady-state value. The overall increasing of concentration was estimated as 13%. In the retentate, the limonin concentration can be considered to remain approximately unchanged during all the experiment.

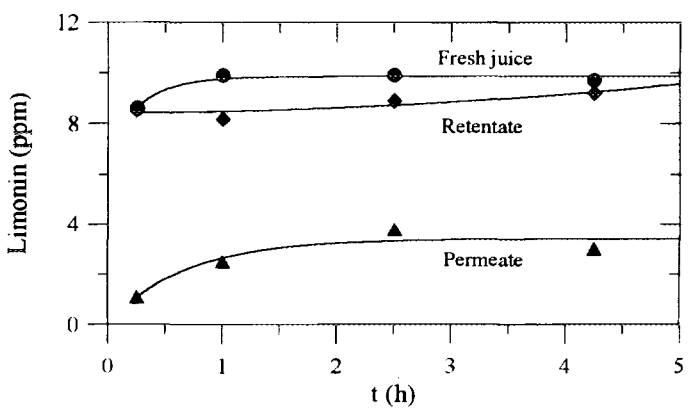

Figure 2. Time evolution of limonin concentration

The limonin concentration in the permeate increases during the time to achieve a level of 3.5 ppm. It is interesting to observe that the overall reduction of limonin concentration in the permeate, with respect to the untreated juice, is approximately 65%. This observation is apparently surprising because the molecular weight of limonin is 500 and it should flow freely through the membrane.

The continuous removal of serum from the pulp was faster then the diffusion of LARL and its conversion in bitter limonin. Accordingly, the higher level of limonin in the retentate can be accounted for the presence of pulp that, as known, mainly contains the limonin and its precursor.

It may be concluded from our results, that the procedure of ultrafiltration described above for debittering orange juice, although only studied at a laboratory scale, has the great advantage over the previous methods in that it does not require chemical modification of the juice. It is possible therefore to obtain by ultrafiltration a better quality product, from an organoleptic viewpoint, because the concentration of limonin in the permeate is lower than 5-6 ppm and so too small to cause bitterness[11].

EFFECTS ON AROMA COMPONENTS DISTRIBUTION.

According to the procedure developed in this study, it was found that most of the more water soluble compounds, aldehydes, esters, and alcohols, passed through the membrane and are associated with the serum; hydrocarbons and less polar aroma compounds like limonene, tended to remain in the pulp fraction during the ultrafiltration process.

In Table 2, the distribution data relating to some more important volatile flavours in fresh juice and permeate is given. This general distribution of aroma compounds was in agreement with the results for aroma distribution in pulp and serum reported by other authors[12].

The Table 2 shows that the ultrafiltered juice had the lowest level of total aldehydes thus reflecting the variable flavour quality in this product with respect to the fresh juice. Hexanal is not believed important to fresh

orange juice flavour, except for some possible contribution to a green flavour note. Octanal and decanal are generally considered important contributors to orange flavour and one of the standards of identity for orange peel oil[13]. Decanal was not detected in fresh juice nor in processed juice.

The esters identified in this study, methyl acetate, ethyl acetate, methyl butyrate, and ethyl butyrate, are known to contribute to the "top-note" of fruit flavours, including citrus[13].

Our data have suggested levels in ethyl acetate and ethyl butyrate of approximately 2-fold in the fresh juice in comparison with the permeate. These differences could be a major factor in the fruity top-notes present in fresh juice that are often missing in processed orange juice. Methyl acetate is present in the ultrafiltered juice at level within the range found in fresh juice.

Table 2 - Distribution ratio between ultrafiltered and fresh orange juice of the more important volatile flavour compounds and representative ions used for their calculations

Component	Ratio of peaks area (permeate / fresh juice)	representative ions (m/z)
Metanol	0.19	31 + 56
Methyl Acetate	0.88	43 + 74
Propan-1-ol	0.91	31
Ethyl Acetate	0.46	43
Butanol	0.31	31 + 56
Ethyl Butyrate	0.3	88 + 89
Hexanal	0.46	56
Hexenal	0.61	55 + 69
Eptanal	0.49	81 + 86
Octanal	0.27	44 + 57 + 84

From the GC profiles we found that the most abundant group of volatatile aroma was the monoterpene hydrocarbons. They are almost exclusively associated with the pulp. This finding is in agreement with previous studies[12] and are also supported by the results of similar analyses of the distribution of volatiles in ultrafiltered mango puree[14]. It may be concluded from the results reported here that even though the ultrafiltration process resulted in some volatile flavour losses, it can be used advantageously to obtain an orange juice of improved appearance without substantial impairment of flavour because the greatest contributors to orange flavour, such as alcohols, aldehydes and esters, pass through the membrane. In addition, the pulp, having a relatively small volume, can conveniently be processed separately.

MATHEMATICAL MODEL

In this study the tubular design of the membrane for the ultrafiltration of orange juice was chosen because of the presence of high level of pulp in the juice. Other configurations like spiral wound or hollow fibber, in spite the fact of having an higher surface/volume ratio are fouled very rapidly when concentrated suspension are treated.

Since ultrafiltration is a pressure-driven membrane separation processes, the pressure, applied to the solution or suspension, provides the driving force to induce the solvent to flow across the membrane.

For small pressure the solvent flux is proportional to the applied pressure. However, as the pressure is increased, flux shows a deviations from the linear flux-pressure behaviour and becomes independent of pressure. In these conditions depending on the crossflow velocity a limiting flux is reached and any further pressure increase no longer results in a permeate flux increasing.

The common explanation of the existence of a limiting flux in crossflow membrane processes is related to the concentration polarisation phenomenon. This phenomenon arises as the working solution is convected towards the membrane where the separation of suspended and soluble solids from bulk solution takes place. The rejected material accumulates on the membrane and generates a concentration profile from bulk solution to membrane surface.

If the solute concentration at the interface achieves the value of thermodynamic equilibrium a phase change (i.e., precipitation or gelation) occurs. In these conditions the formation of a viscous and gelatinous-type layer responsible for a further resistance to the flow of permeate in addition to that of the membrane takes

place. Once this gel layer is formed, even increasing the transmembrane pressure after a transient rise the permeate drops back to the previous level.

The most straightforward assumption to explain the limitation of the driving force is that the effect of the pressure increasing merely results in a more close-packed solute layer arrangement which exhibits an higher transport resistance able to drop the flux to the previous value. In this situation the flux can be enhanced by increasing the crossflow velocity in such a way to control the thickness of polarised layer by effect of induced shear back diffusion as already observed in the microfiltration of suspensions[15].

In this work in order to develop a mathematical description of the effects of crossflow velocity and transmembrane pressure on the permeate flux, a dynamic transport model was formulated and solved numerically using the cake voidage as adjustable parameter.

The predicted effects of the operating conditions on the morphology and composition of the cake, as due to the contribute of the different particles, are summarised in Table 3.

Table 3. Morphology and composition of the cake as a function of operating conditions.

Re	7000			10000			15000		
TMP (bar)	0.5	0.7	1.0	0.5	0.7	1.0	0.5	0.7	1.0
ε	0.42	0.37	0.31	0.52	0.41	0.34	0.62	0.56	0.455
δ_O (mm)	0	3.979	3.948	0	3.186	3.735	0	3.847	3.414
δ_{SS} (mm)	4.322	4.324	4.669	3.916	4.178	4.568	4.454	4.228	4.653
ω_A (%)	0.080	0.059	0.048	0.115	0.074	0.061	0.139	0.070	0.053
ω_B (%)	0.059	0.043	0.035	0.084	0.055	0.045	0.102	0.051	0.039
ω_C (%)	0.040	0.029	0.024	0.057	0.037	0.030	0.070	0.035	0.026
ω_D (%)	0.088	0.065	0.053	0.126	0.082	0.067	0.153	0.077	0.058
ω_E (%)	99.733	99.804	99.840	99.618	99.752	99.797	99.536	99.767	99.824

As it can be seen from the weight percentage of each class of particles in the cake, particles larger than 2 μm in diameter do not contribute significantly to the cake formation. This result is a consequence of the proportionality of lateral migration and shear-induced diffusion to the particle diameter cubed and squared, respectively. By effect of these mechanisms, the back diffusion rate of largest particles prevails with respect to their convective transport towards the membrane surface.

In Figure 3 the comparison between the model predictions in terms of permeate flux and experimental data is reported.

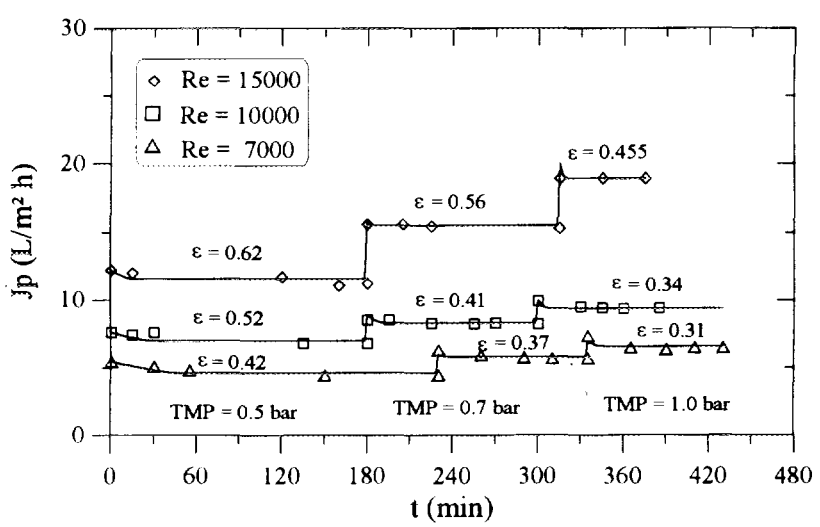

Figure 3 Comparison between theoretical predictions, from Eq. 6, and experimental results

As it can be seen the agreement between theoretical and experimental data is very satisfactory. It can be noticed, also, that the optimum value estimated for the cake voidage, increases as the Reynolds number is increased and decreases as the transmembrane pressure is increased. This observation appears to be consistent from a physical point of view. In fact, according to Eq. 3, the transfer rate of particles from the membrane towards the bulk solution increases as the crossflow velocity is increased leading, probably, to a less compact cake. On the contrary the effect o transmembrane pressure on the cake voidage can be explained if the hypothesis of compressible cake is considered. According to this hypothesis, a higher pressure value leads to a more compact cake which means lower cake voidage.

CONCLUSIONS

Crossflow ultrafiltration by tubular PVDF membranes was used with success in preventing the bittering of freshly squeezed orange juice. In particular the limonin concentration in the permeate was reduced of about 65% with respect to the fresh juice. Most of the more water soluble compounds passed through the membrane whereas hydrocarbons and less polar aroma compounds remained in the retentate. A mathematical model of the ultrafiltration system has been presented. The theoretical predictions in terms of permeate flux as a function of time resulted in a good agreement with the experimental data.

LIST OF SYMBOLS

$\overline{D}_p$	weighted mean diameter of particles in the cake (m)
C_i	volumetric fraction of a generic particles class in the juice (m^3/m^3)
Dp_i	diameter of a generic particles class (m)
i	generic class of particles (i = A, B, C, D, E; as defined in Table 1)
J_{in}	volumetric flux directed from the bulk solution towards the membrane ($m\ s^{-1}$)
J_{out}	velocity of particles back-diffusion directed from the membrane towards the bulk solution ($m\ s^{-1}$)
J_p	volumetric flux of permeate ($m\ s^{-1}$)
k	Boltzmann's constant ($kg\ m\ s^{-1}$
MWCO	molecular weight cut-off
o	refer to initial conditions
PVDF	poly-vinylidene-fluoride
Q_{ax}	axial feed flow rate ($m^3\ s^{-1}$)
Q_R	retentate flow rate ($m^3\ s^{-1}$)
R_c	cake resistance (m^{-1})
R_f	actual radius of tubular membrane [$R_f = R_{fo}-\delta(t)$] (m)
R_m	membrane resistance (m^{-1})
ss	refer to steady-state conditions
t	time (s)
T	absolute temperature (K)
TMP	transmembrane pressure (Pa)
u	centerline maximum velocity in the tubular membrane ($m\ s^{-1}$)

GREEK SYMBOLS

δ	cake thickness, (m)
ε	cake voidage
ν	kinematic viscosity of solution ($m^2\ s^{-1}$)
ω_i	mass fraction of a generic particles class in the cake

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RHEOLOGICAL PROPERTIES OF FRUIT PULPS IN ULTRAFILTRATION PROCESSES

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Rheological properties of fruit concentrates during UF processes are studied in a pilot plant that allows either UF operations or rheological determinations in a parallel loop. The paper demonstrates that the rise in apparent viscosity of concentrates can be correlated with the amount of removed permeate and therefore the time lenght of the operation can be known in advance. The major constituents, aside from not-soluble solids, distribute evenly between concentrate and permeate.

INTRODUCTION

Fruit pulps suffer a deterioration in storage that reduces their consistence (1,2). In order to get the original consistence they are dehydrated, but this way a loss of product must be borne and the increase in consistence takes place together with a corresponding increase in concentration of all the components. So, apart from consistence, the final product is rather different from the original one.

Ultrafiltration is often used for dehydration with very good results, but does not avoid the above -mentioned drawbacks. However a recent work (3) has shown that the use of UF membranes with high molecular weight cut-off (MWCO≥200,000) can produce concentrates with higher consistence, but with a content of the major constituents similar to the one of the original pulps and taste and colour practically unmodified; besides, the permeates too have a rather high content of the soluble constituents of the original pulp and, being sterile, their use in beverage and yoghurts industry may be considered.

In this note the findings of new experimental runs carried out in the laboratories of Allione Industria Alimentare SpA are reported and discussed with particular attention to the rheological behaviour of concentrates. In particular, as a first step a correlation between Bostwick degree and apparent viscosity of concentrates is suggested, then the increase of the apparent viscosity of concentrates during ultrafiltration is tentatively correlated with the amount of permeate removed and, at last, this amount is correlated with time. So, if a consignment must be done with a minimum Bostwick degree, the duration of the UF treatment should be predictable in advance. This is very useful in industrial practice. It should be possible to correlate directly the decrease of the Bostwick degree with the amount of permeate removed, but apparent viscosity is a more useful parameter for engineering calculations (for instance scale-up from pilot to industrial plant).

EXPERIMENTAL

The UF pilot plant used in the experimental work is described elsewhere (3). It was modified as shown in Fig. I in order to complete it with a loop for the study of the rheological behaviour of concentrates (2). In fact, operating the groups of valves V_1 and V_2, the flow was diverted from the UF circuit (black arrows) into the loop (white arrows) and the flow properties measured according to the method described later on.The most important dimensions of the plant are provided in Table I.

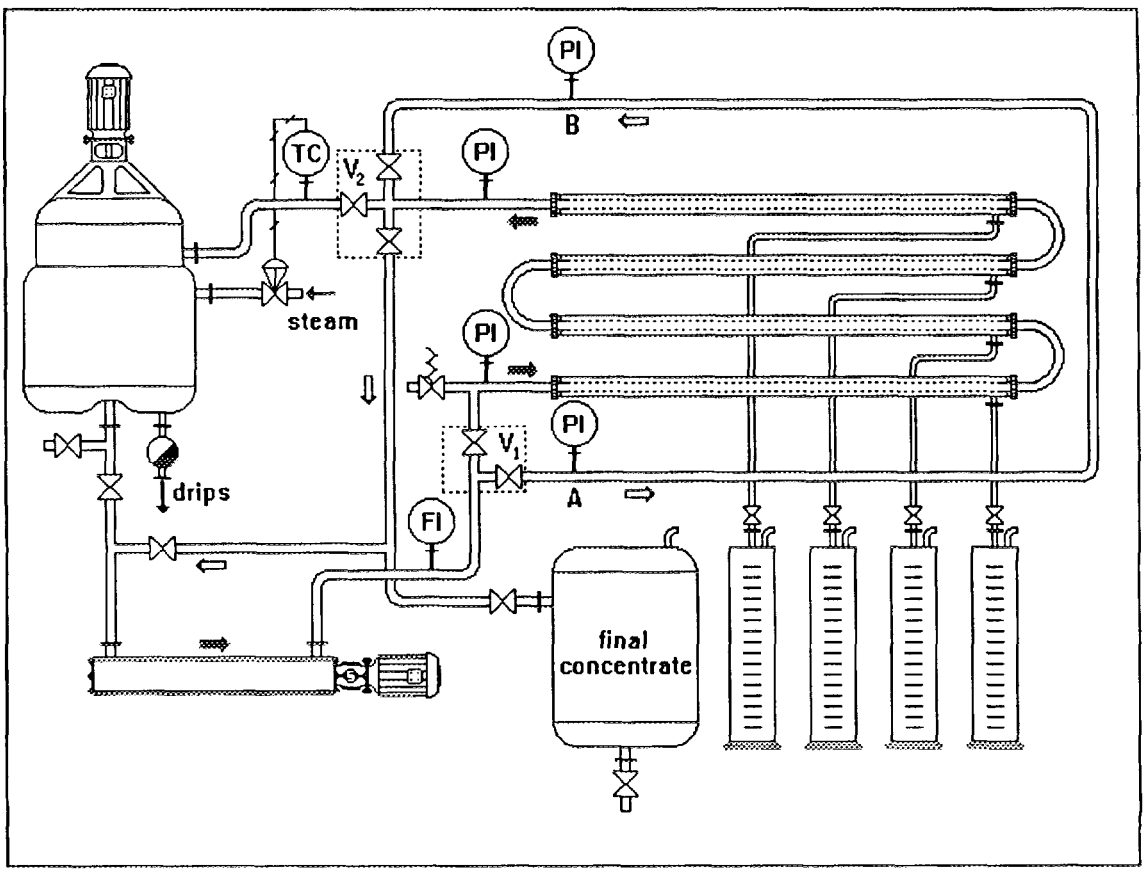

Fig. I. Schematic diagram of pilot plant.

In laminar flow of newtonian fluids in pipes the shear stress at the wall τ_w is:

$$\tau_W = \Delta P \frac{D}{4L} \qquad (1)$$

where D is the inside diameter of the pipe and ΔP the pressure drop in a pipe lenght L. The average velocity in the pipe v is:

$$v = \frac{\Delta P}{32\mu} \frac{D^2}{L} \qquad (2)$$

where μ is the viscosity of the fluid. Thus we have:

$$\mu = \frac{\pi D^4}{128L} \frac{\Delta P}{Q} \qquad (3)$$

and the measurements of the flow rate Q and of the pressure drop ΔP in a pipe lenght L make it possible the calculation of the viscosity of the fluid.

Fruit pulps have high consistence, so laminar flow is costumary (4), but exhibit non-newtonian behaviour (2); so Eq. 3, instead of the newtonian viscosity μ, allows the calculation of an apparent viscosity μ_a of the fluid which depends on the shear rate.

In this work apricot and pear pulps are considered; the first one is a Bingham plastic, while the second one exhibits a more complex rheological behaviour that is well described by the Herschel-Bulkley model. The following shear-velocity relationships have been suggested (2):

apricot pulp	at 18°C	$\tau = 5.1 + 0.06\,\gamma$	$R^2 = 0.995$
pear pulp	at 18°C	$\tau = 17.0 + 2.23\,\gamma^{0.44}$	$R^2 = 0.997$.

Both the apricot and pear pulps are practically time-independent (2) and for this reason they have been preferred to other fruit pulps extensively syudied in the past. The method adopted to study the variation of their consistence

during ultrafiltration is probably questionable since it is based on newtonian considerations while reliable shear-velocity relationships are available, but the use of a single parameter (the apparent viscosity) is more effective and easier to be used for industrial purposes.

Table I. Pilot plant details and working conditions.

vessel volume	0.5 m^3
recirculating pump	helicoidal screw type with maximum flow rate 5 m^3/h at a head of 6 bar
membranes	4 polyvinylidenfluoride modules with 0.025 m diameter and 3 m lenght
pipes diameter	0.025 m
A - B lenght	7.65 m
pressure transducers (loop)	2 WIKAtronic line, series 9021221, max. error 0.001 bar
working temperature	50°C
flow rate range	3.6 ÷ 4.8 m^3/h
velocity range	2.04 ÷ 2.72 m/s

As regards the laboratory examinations of concentrates and permeates, the set of determinations listed in Table II was selected for the first step of the research (3); afterwards consistence of concentrates and sugar content, pH and total acidity of concentrates and permeates were determined as routine analyses. As reported in Table II, the pulps and concentrates consistence was determined in laboratory by means of a Bostwick consistometer; this instrument is particularly suitable for fruit pulps, purees of vegetables and baby foods (5) and is widely used in industry. In this instrument the sample, at 20°C, is initially placed in a container; then the opening of a gate let it flow in a channel. The distance (cm) covered in 15 s represents the Bostwick degree. So lesser Bostwick values correspond to higher consistences.

Table II: Laboratory examinations.

Sugars content	Brix degree	Refractometric method. Since pulps contain more sugars than any other soluble constituent, the measurement is commonly considered a guide of sugars content and of soluble solids
pH		
Total acidity	as monohydrate citric acid	
Amino-acid content	Formol index	Sorensen method
Vitamin C	as ascorbic acid	iodine titration
Total solids		
Color		Munsell system and spectrometric method (adsorbance at 420 nm)
Mould content		Howard mould count
Consistence	Bostwick degree	

References: D. K. Tressler and M. A. Joslyn: The Chemistry and Technology of Fruit and Vegetable Juice Production, The Avi Publ. Co., New York, 1954; Fédération Internationale des Producteurs de Jus de Fruits, Paris, 1984; RSK-Values, The Complete Manual, VDF Association of the German Fruit Juice Industry, Verlag Flüssiges Obst GMBH, Bonn 1987; Metodi Ufficiali di Analisi delle Conserve Vegetali, Industria Conserve, No. 2, 3 and 4, 1974 and No. 1 and 2, 1975.

Since the pilot plant was equipped with a volumetric pump, during UF runs the flow rate Q of the concentrate was constant while, owing to the removal of permeate, the consistence increased and with it also the pressure drop. So every run was characterized by the value of Q.

In order to get reliable information, every run was repeated at least four times; owing to the variability of the starting product, a moderate scatter of data was observed.

RESULTS

Fig. II shows how apparent viscosity is related to Bostwick degree at pulp flow rates from 3.6 to 4.8 m^3/h. The following empirical correlation fits rather well experimental findings both for pear (left side of the graph) and for apricot (right side); nevertheless a stratification of experimental points in consequence of the variation of the pulp flow rate is evident; therefore the influence of this parameter should be considered when its value is changed:

$\mu_a = 1200\ B^{-0.86}$ $\quad R^2 = 0.949$ $\quad (\mu_a$ = apparent viscosity, cP; B = Bostwick degree, cm)

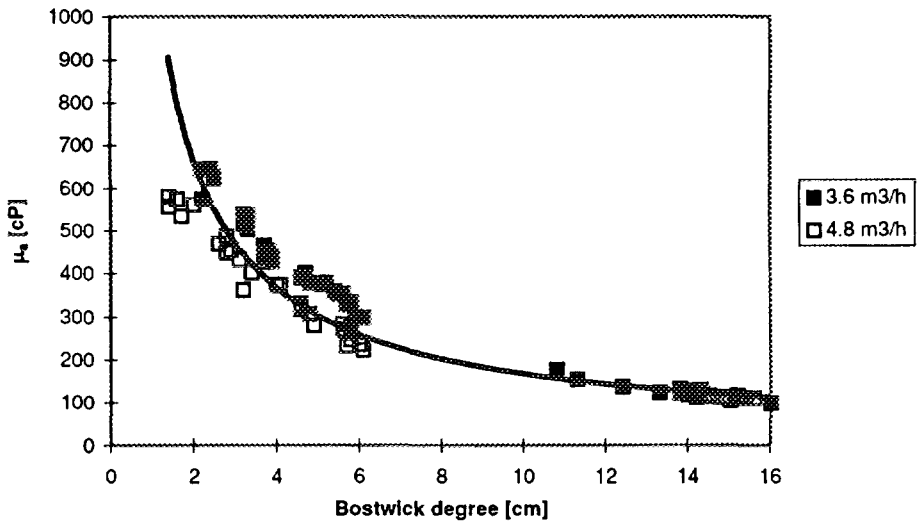

Fig.II. Apparent viscosity vs. Bostwick degree for apricot and pear pulps.

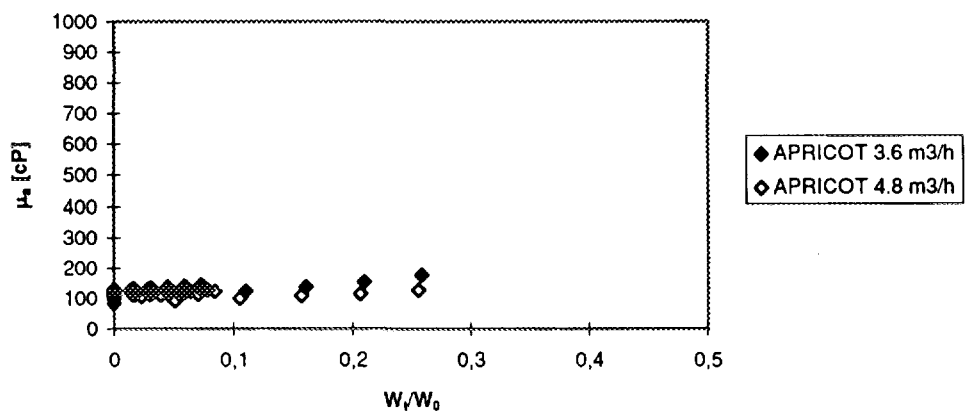

Fig. III. Apparent viscosity of apricot concentrates vs. the fraction of permeate removed.

In Figs. III and IV the apparent viscosities of the concentrates of apricot and pear are plotted versus the ratio W_f/W_0 between the amount of permeate removed at time t and the liquid content of the original pulp. The rise in apparent viscosity of the pear concentrates is more evident, but, if the ratio $\mu_a/\mu_{a,t=0}$ between the values of the apparent viscosity at time t and at t = 0 is plotted versus W_f/W_0 (Fig. V) for both pear and apricot concentrates and at the lowest and at the highest pulp flow rates tested, data points groups rather well. This means that a relatively strong correlation exists between the increase of apparent viscosity and the increase of the content of not-soluble solids in the concentrates. A tentative correlation can be suggested, i. e.:

$\mu_a/\mu_{a,t=0} = e^{2.65W_t/W0}$ $R^2 = 0.941$,

that corroborates the findings of other Authors about the effect of concentration of fruit pulps on apparent viscosity (4), but requires more experimental support.

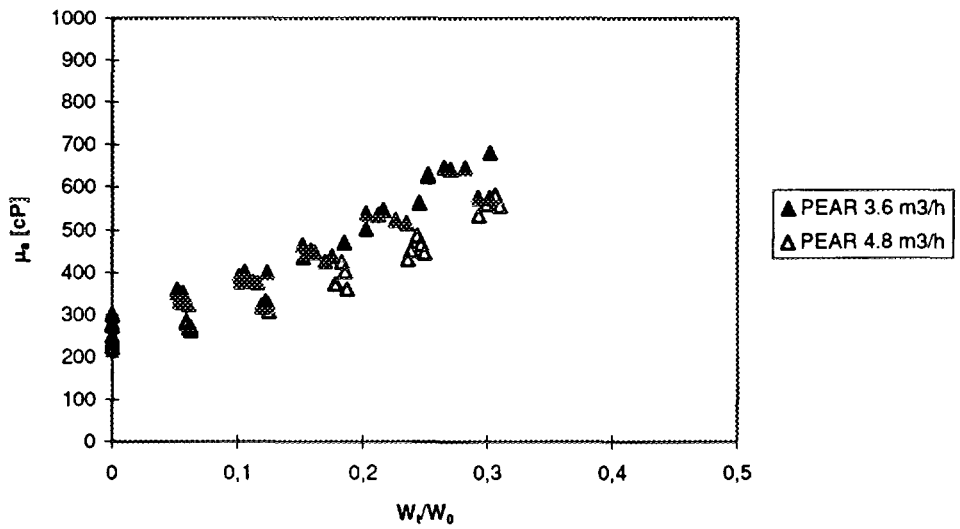

Fig. IV. Apparent viscosity of apricot concentrates vs. the fraction of permeate removed.

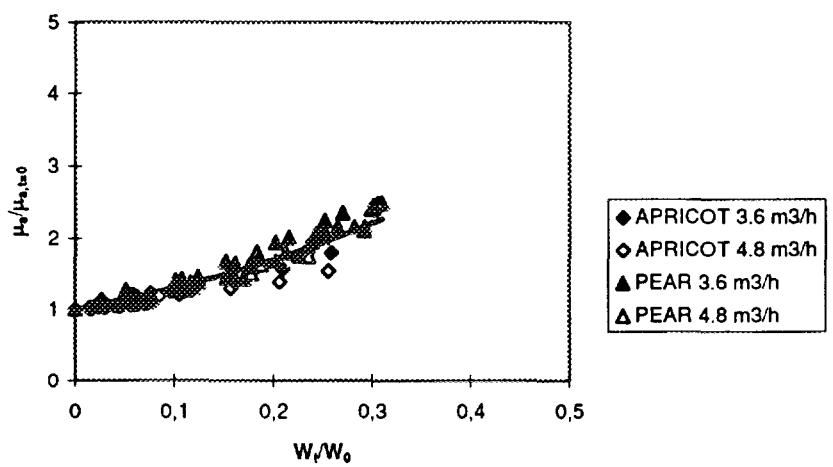

Fig. V. Effect of concentration on apparent viscosity.

In a previous work it was shown that at high flow rates Q, the permeate production was almost constant (3). It was also observed that, aside from pear pulp, permeate flux was influenced by Q and the anomaluos behaviour of pear was ascribed to the mechanical sweeping action of the clusters of cells of a woody nature present in the pulp. Fig. VI confirms the constancy of the permeate flux and also its absolute values that are higher for pear and lesser for apricot; however it shows that Q has a not negligible influence with pear pulp too. This notwithstanding this result might be influenced by the apparent viscosity of the original pear pulp used in the runs at 4.8 m^3/h, that was lower than the one of the other pear pulps, as it is evident in Fig. IV. Therefore further experimentation should be advisable.

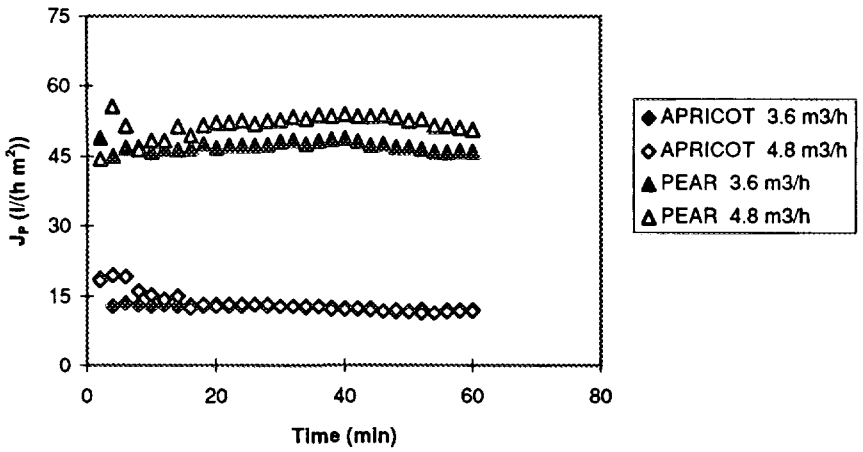

Fig. VI. Permeate flux vs. time.

The chemical analysis confirmed the findings of the previous work (3) about the distribution of major constituents in concentrates and permeates: some results are listed in Table III.

Table III. Distribution of major constituents in concentrates and permeates; data at time t = 0 min refer to th original pulp.

		Concentrates				Permeates		
	t (min)	0	20	40	60	20	40	60
Apricot	Brix degree	11.6	11.5	11.7	11.7	10.8	10.9	10.9
3.6 m^3/h	total acidity (%)	1.87	1.89	1.89	1.88	1.85	1.86	1.87
	pH	3.26	3.26	3.26	3.25	3.24	3.24	3.21
Apricot	Brix degree	11.6	11.7	11.8	11.9	11.2	11.1	11.2
4.8 m^3/h	total acidity (%)	1.89	1.90	1.92	1.89	1.84	1.84	1.87
	pH	3.24	3.24	3.24	3.28	3.28	3.28	3.28
Pear	Brix degree	13.7	13.5	14.0	14.3	12.1	11.9	11.8
3.6 m^3/h	total acidity (%)	0.33	0.33	0.32	0.34	0.30	0.30	0.30
	pH	4.01	4.01	4.01	4.01	3.88	3.88	3.88
Pear	Brix degree	13.7	14.0	14.3	14.5	12.4	12.3	12.4
4.8 m^3/h	total acidity (%)	0.32	0.32	0.33	0.33	0.28	0.28	0.28
	pH	3.87	3.87	3.87	3.85	3.87	3.87	3.88

CONCLUSION

This report shows that the planning of an UF operation in order to increase the consistence of a fruit pulp is possible. Making use of the diagrams or of the correlations, it is possible to find the values of apparent viscosity corresponding to the Bostwick degree of the original pulp and to the one that must be achieved at the end of the operation (Fig. II). From the corresponding value of the ratio $\mu_a/\mu_{a,t=0}$ it is possible to determine the amount of permeate that must be removed (Fig. V). Finally, knowing the permeate flux, it is possible to calculate the time lenght of the operation (Fig. VI). This should avoid the frequent determination of the Bostwick degree, that, owing to the lenght of the procedure (for instance cooling till 20°C), is difficult to carry out during the operation. Besides the quality of the products is very good.

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NOMENCLATURE

B = consistence of pulps and concentrates (Bostwick degree, cm)

D = pipe inside diameter (m)

J_P = flux of permeate (l/hm^2)

L = pipe lenght (m)

ΔP = pressure drop (N/m^2)

Q = flow rate of concentrates (m^3/h)

R^2 = correlation coefficient

v = velocity (m/s)

W_t = amount of permeate removed at time t (m^3)

W_0 = liquid content of the original pulp (m^3)

γ = shear rate (1/s)

μ = newtonian viscosity (Pa s)

μ_a = apparent viscosity (cP)

τ = shear stress (N/m^2)

τ_w = shear stress at the wall (N/m^2)

IMPORTANCE OF THE RHEOLOGICAL BEHAVIOUR ON THE TEMPERATURE DISTRIBUTION IN CONTINUOUS THERMAL TREATMENT

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The relationships between the rheological properties of fruit and vegetable juices and purees, the flow conditions and the temperature distribution in tubular continuous heat exchangers were investigated. Experiences were carried out in controlled conditions, on both lab model apparatus and pilot tubular plant. The results show that the distance from heating surface, the thermal gradient and the viscosity of the product affect the radial profiles of temperature. A model is proposed in order to describe the temperature variation in the time: $t = a + b \cdot \log(T_p - T) + c \cdot \log(k - k_p)$. This model takes into account the changes of the consistency index (k) with the temperature. Tests on the pilot plant showed thermal gradients in the radial section, with temperature decreasing towards the inner center of the tube. These gradients increased when higher viscous products were processed.

Production of fruit and vegetable juices, purees and concentrates needs always heat treatments to allow microbiological and enzymatic stabilization. The current tendency in the food industry is to apply continuous heat treatments and aseptic filling, especially for the preparation of semi-finished products, i.e. purees and concentrates. Treatment effectiveness depends on the optimization of heat process: low treatments may give microbiological hazards while strong treatments may damage product. It is very important to heat homogeneously all the product to avoid its overheating. For highly viscous products tubular heat exchangers are generally used; in these conditions laminar flow and conductive heat exchange are mainly observed.

Higher flow rate and greater distance from the exchange surface make that the product flowing in the inner center of the tube reaches the fixed temperature much more slowly than the one flowing in the peripheral layers; most of the processed product is consequently subjected to stronger heat exposure. All these phenomena are clearly connected to the rheological properties of the product and to the process conditions; it is well known that viscosity depends on the product temperature and, in the case of non-newtonian fluids, on flow rate and section. The increase of flow rate and the narrowing of flow section produce a decrease of apparent viscosity. At the same flow conditions the product viscosity changes continuously with temperature during heating and cooling steps. In a previous work it has been showed that these connections are very important to calculate correctly pressure losses in a continuous heat process including heating, holding and cooling steps.

Variations of rheological properties vs. temperature affect heat exchange process since heat diffusion rate inside the product depends on its apparent viscosity.

Temperature of a fluid flowing inside an heat exchanger will depend on several parameters such as: temperature and volume of heating fluid, inlet product temperature, distance from heating surface, retention time inside the exchanger (pipe length/flow rate), rheological properties of the product and their temperature variations.

The purpose of this work was to verify how to connect heat diffusion rate and the above mentioned parameters. Tests were carried out on both laboratory equipment and pilot plant. In laboratory a modified rotational viscosimeter with concentric cylinders was used to evaluate on different types of samples (juices, purees and tomato concentrates) dependence of heat diffusion rate on apparent viscosity of the product, at different distances from heating surface and in different conditions of thermal gradient between product and heating fluid. On semi-industrial scale samples of tomato juice and concentrate at different °Brix were heated in a tubular equipment, by monitoring product temperature at different distances from heating surface in a pre-established point of the exchanger. Trials at different flow rates were also carried out.

Materials and methods

Tests were carried out on samples with different rheological behaviour: tomato concentrates at different °Brix and fruit juices and purees at different pulp content.

Rheological measurements

Rheological characterization was carried out on each sample at 5 different temperatures between 10 and 75°C. Measurements were done by a coaxial cylinder viscosimeter Haake RV 12 equipped with a NESLAB RTE-8 circulating bath.

SVI (∅ 20,2 mm, h = 61,4 mm) rotor and MV (∅ 42 mm) stator were used for the determinations.

Rheological behaviour was described according to the power law $\tau = k \cdot \dot{\gamma}^n$ where n = 1 in the case of newtonian products.

The equation

$$\tau = k_0 \cdot k_T^{1000/T} \cdot \dot{\gamma}^{n_0 + n_T \cdot 1000/T}$$

was used to describe rheological parameters dependence on temperature.

Laboratory tests

A suitable system was developed to evaluate heat diffusion rate according to the rheological properties of the product and flow conditions.

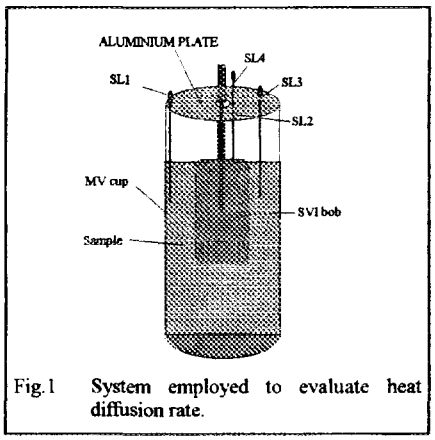

Fig.1 System employed to evaluate heat diffusion rate.

The viscosimeter was suitably modified by employing the same measurement equipment (MV stator and SVI rotor) closed in the upper part by an aluminium plate having a hole in the center to allow rotor movement. The plate presented 4 holes in order to insert probes for the measurement of the temperature at different distances from the heating surface: 0.13 cm (probe SL1), 0.43 cm (probe SL), 0.73 cm (probe SL3) and 1.00 cm (probe SL4). In the Fig.1 a layout of the system is illustrated. Four Cu-Constantan thermocouples (∅=1.4 mm., L=31 mm) were employed as probes,

connected to a CMC 821 UC Ellab data acquisition system.

Temperature of the viscosimeter jacket (T_b) was controlled by the connected circulating bath. Rotor-stator measurement system containing the sample was brought to the fixed initial temperature (T_i) by dipping in a thermostatic bath for 1 hour. Temperature was registered at 30 sec intervals after stator introduction containing the sample.

For each sample tests were carried out by planning the following rotation speeds: 10, 50, 150, 300 and 450 rpm.

The following models were applied to describe the time/temperature relationship

$$t = j \cdot \log(T_b - T_i) - Fh \cdot \log(T_b - T) \quad (4) \qquad \textbf{model 1}$$

$$t = A - B \cdot \log(T_b - T) + C \cdot \log(k - k_b) \qquad \textbf{model 2}$$

where k is consistency index of the product at temperature T and k_b is consistency index of the product at temperature T_b.

Since the first model describes asintote conditions and not the initial part of the curve, in this case the elaboration was carried out taking into consideration temperature values for times higher than 180 sec.

Pilot plant tests

Tests were carried out on a tubular heat exchanger consisting of four pipes (5 meter long, 75 mm internal diameter and 100 mm external diameter, including thermal jacket), joined each other by three 180° curves. Product was fed from a 400 *l* tank by a pump. 80°C countercurrent water was used as heating fluid.

Four Cu-Constantan thermocouples (1,4 mm ∅ and 73 mm length) were assembled on the curve coming out from the third pipe, having the tip towards the inner of the tube. Thermal probes were installed at four different distances from the heating surface (Fig.2), respectively 16 mm (SP1), 20 mm (SP2), 25 mm (SP3) e 35 mm (SP4) and connected to the acquisition data system CMC 821 UC through a stuffing box assembled on the curve.

Other two thermocouples were inserted in the center of pipes at the inlet and at the outlet of heating system.

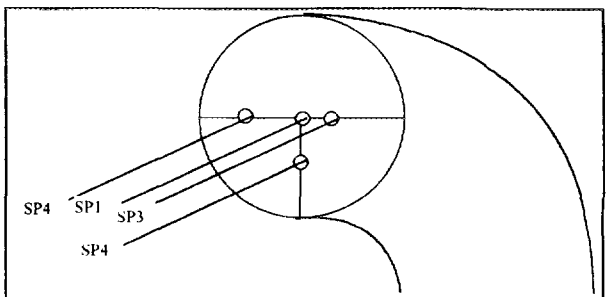

Fig 2 Arrangement of thermal probes in the pipeline for temperature monitoring at different distances from heating surface.

Product temperature was registered when thermal balance of the system was attained. The product coming out from the heating system was cooled by a continuous plant made up of 4 spiral tubes (2 m long and 5 mm internal diameter). Countercurrent well water was used as cooling fluid. The cooled product was recirculated into the feed tank.

The test was carried out on tomato paste samples after dilution at 13.0, 11.0, 8.0 and 4.5 °Brix and at three different flow rate of the product (between 0.015 e 0.07 m/s).

Results and Discussion

Laboratory tests

The rheological measurements showed that the samples presented laminar flow conditions in the whole

rotation speed range and at all the examined temperatures. Furthermore, by comparing measurements carried out with and without thermal probes it has been observed that their presence did not give any change to the flow conditions. Figures 3 and 4 show flow curves with and without thermal probes observed respectively for clear pear juice and apricot puree.

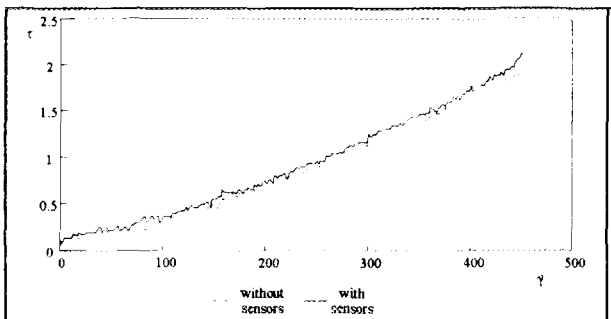

Fig.3 Flow curves of clarified pear juice

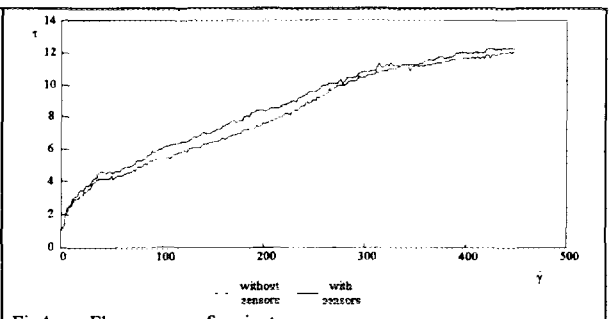

Fig4 Flow curves of apricot puree

For each sample, constants k_o, k_T, n_o and n_T were obtained from the rheological measurements at different temperatures.

Heat diffusion tests showed that in the process of high viscous products, i.e. apricot puree (Fig. 5-6), the temperature decreased with the distance from the heating surface at all the examined rotation speeds; this indicates a mainly conductive heat transmission mechanism. Thermal gradient flattened as rotation speed increased.

Low-viscous products, i.e. clear pear juice (Fig. 7-8), presented a mainly convective mechanism; after 2 minutes temperature profiles were flat both at high and low rotation speeds and after 5 minutes the sample reached a thermal balance conditions.

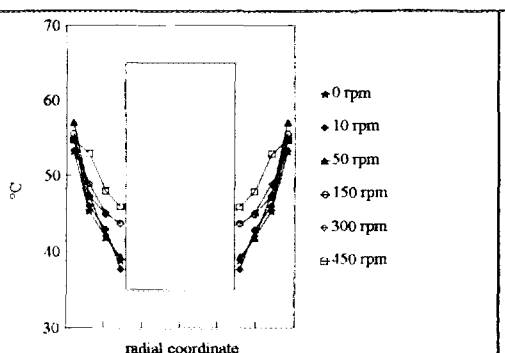

Fig.5 Temperature profiles after 3 min. Sample: apricot puree; T_b= 63.5°C; T_i=25°C.

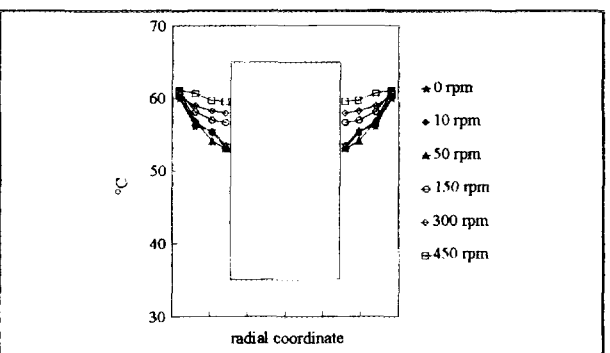

Fig.6 Temperature profiles after 10 min. Sample: apricot puree; T_b= 63.5°C; T_i=25°C.

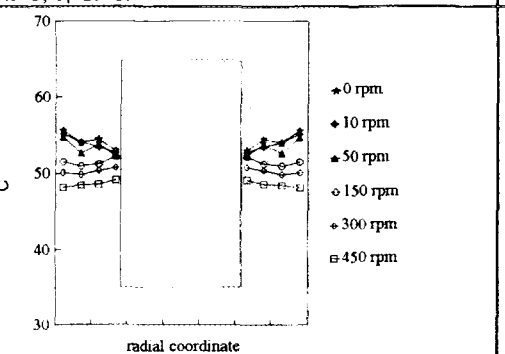

Fig.7 Temperature profiles after 2 min. Sample: clarified pear juice; T_b= 63.5°C; T_i=25°C

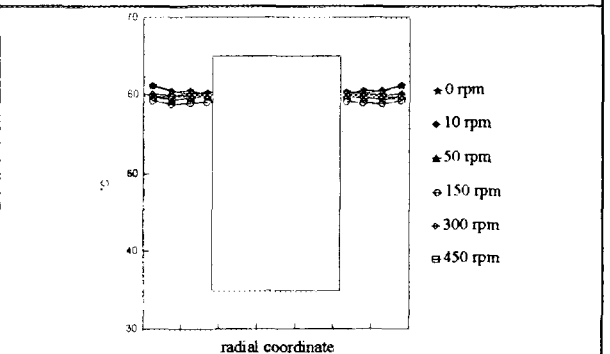

Fig.8 Temperature profiles after 5 min. Sample: clarified pear juice; T_b= 63.5°C; T_i=25°C

The results of time/temperature elaboration carried out by applying model 1 and model 2 shows that model 2 fits better to experimental values. As an example the graphics of the thermal probes SL1 and SL4 at 0 and 450 rpm for clear pear juice (Fig. 9 - 12) and for apricot puree (Fig. 13 - 16) are presented.

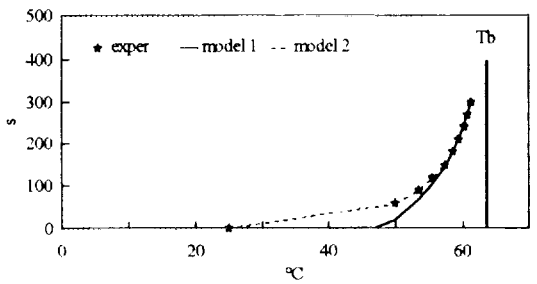

Fig.9 Temperature changes at 0 rpm recorded by sensor SL1. Sample: clarified pear juice.

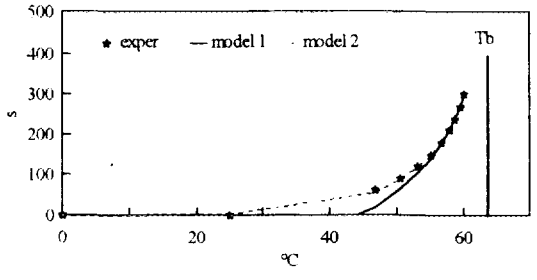

Fig.10 Temperature changes at 0 rpm recorded by sensor SL4. Sample: clarified pear juice.

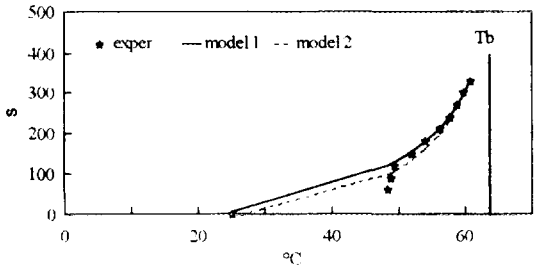

Fig.11 Temperature changes at 450 rpm recorded by sensor SL1. Sample: clarified pear juice.

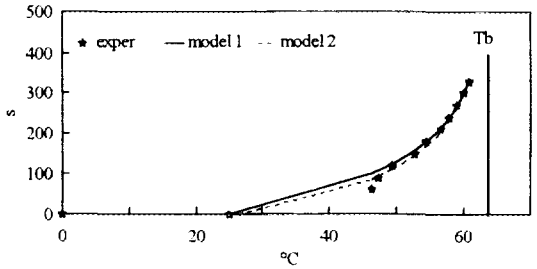

Fig.12 Temperature changes at 450 rpm recorded by sensor SL4. Sample: clarified pear juice.

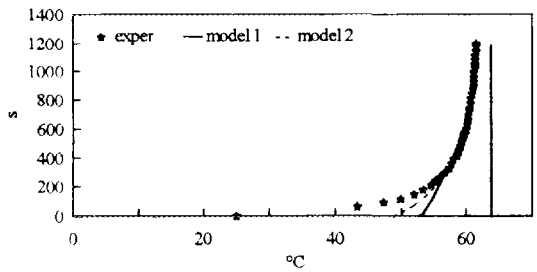

Fig.13 Temperature changes at 0 rpm recorded by sensor SL1. Sample: apricot puree.

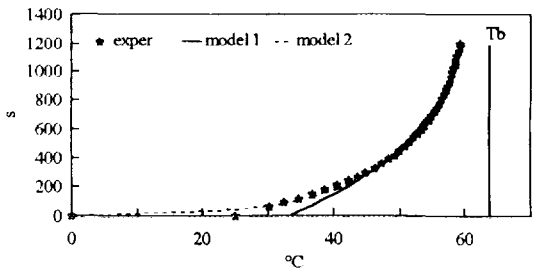

Fig.14 Temperature changes at 0 rpm recorded by sensor SL4. Sample: apricot puree.

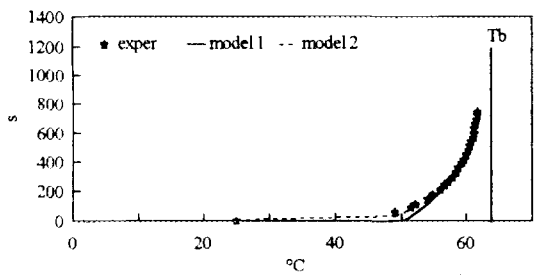

Fig.15 Temperature changes at 450 rpm recorded by sensor SL1. Sample: apricot puree.

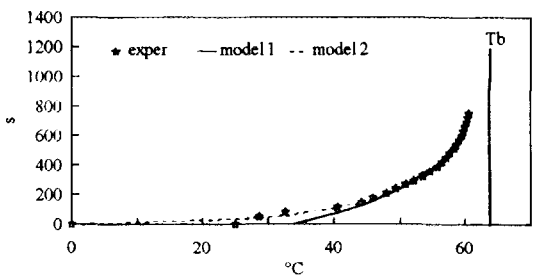

Fig.16 Temperature changes at 450 rpm recorded by sensor SL4. Sample: apricot puree.

Since the absolute values of B and C constants (model 2) are related by a linear dependence ($C=C_o+C_1 \cdot B$, $R=0.991$), A parameter can be calculated from the equation

$$A = B \cdot \log(T_b - T_i) - C_0 \cdot \log(k_i - k_b) - C_1 \cdot B \cdot \log(k_i - k_b).$$

All the model constants can be reduced to the B parameter, which depends on T_i, T_b, distance from the heating surface, rotation speed and k_o, k_T, n_o and n_T rheological constants.

Pilot plant tests

In the Fig. 17 velocity profile inside the pipe concerning the test carried out with 8 °Brix tomato juice at 0.042 m/s average speed is represented together with the positions of the four sensors. Significant temperature differences between the four thermal probes were appreciated.

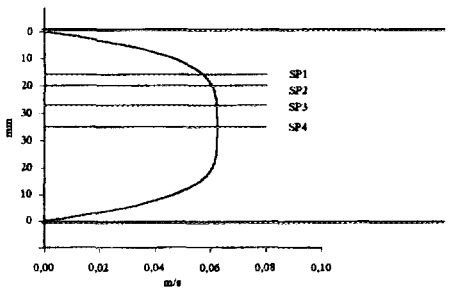

Fig.17 Position of sensors in the pipe and velocity profiles of tomato juice at 8 °Brix and 0.042 m/s volumetric flow rate.

The trials at different flow rate confirmed that the changes in product velocity influenced not only the residence time in the exchanger but also the viscosity of the non newtonian tomato concentrates, with opposite effects on the heating process. In fact, if diffusion heat velocity should be describable only as depending on wall temperature and residence time (model 1), variations of Log (T_b - T) vs time should have had a linear pattern; on the contrary from the graphic in Fig. 18 (temperatures recorded by SP4 sensor in the test on 13 °Brix tomato juice) it can be observed that thermal gradient is not linearly related to the product flow rate. By increasing the flow rate, the viscosity decreases and the heat exchange improves; this partially counterbalances lower residence time of the product in the exchanger.

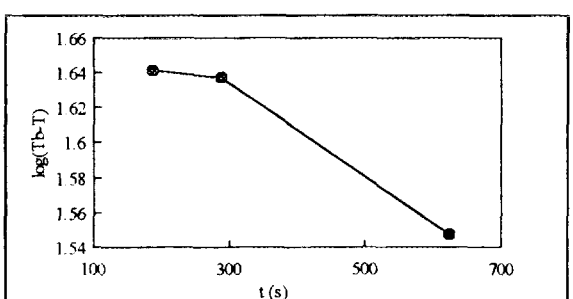

Fig.18 Thermal gradients recorded by SP4 sensor in tests on 13.0 °Brix tomato juice processed at different flow rates.

It has been observed that more viscous samples (11.0 and 13.0 °Brix) presented a mainly conductive exchange mechanism; temperatures recorded by sensors decreased by increasing flow rate and heating surface (Fig. 19).

Heat diffusion became mainly convective in the case of low viscous products (8.0 and especially 4.5 °Brix); the product reached on average higher temperatures and the lowest temperature values were recorded not in the center of the pipe but in the lower part. Moreover the temperature of the different sensors did not depend on the flow rate; probably the effects of viscosity changes on the heat diffusion counterbalanced completely the different residence time.

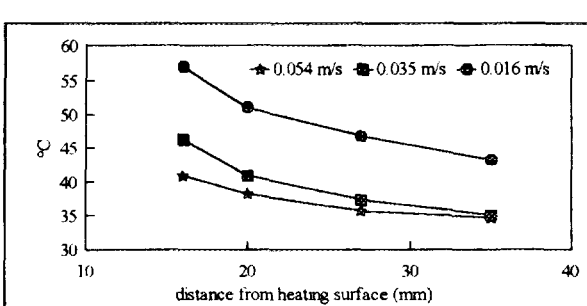

Fig.19 Thermal profiles obtained processing 13.0 °Brix tomato juice

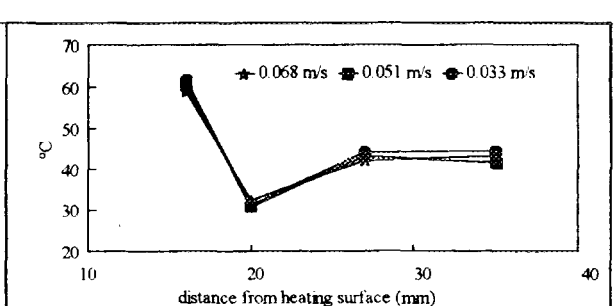

Fig.20 Thermal profiles obtained processing 4.5 °Brix tomato juice

B constant values of the model 2, illustrated by the equation

$$t = B \cdot \log(T_b - T_i) - C_0 \cdot \log(k_i - k_b) - C_1 \cdot B \cdot \log(k_i - k_b) - B \cdot \log(T_b - T) + C_0 \cdot \log(k - k_b) + C_1 \cdot B \cdot \log(k - k_b)$$

are related to product flow rate, distance from heating surface e k_0, k_T, n_o and n_T rheological constants.

Conclusion

The study allowed to verify that heat diffusion curves can be described by a model taking into consideration the rheological behaviour of the product and the effects due to the temperature variations.

In the pilot plant tests, temperature differences observed between the sensors show a noticeable non-homogeneous treatment with consequent great risks regarding either the product salubrity, if temperature in the slowest heating point is not reached, or heat overexposure of part of the product.

Finally it has been observed that, in the case of low viscous products, the lower temperatures are no more in the inner center of the tube and this involves modifications in the control of process temperature.

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HIGH PRESSURE STABILIZATION OF LEMON JUICE

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SYNOPSIS

A laboratory scale apparatus has been designed, set up and operated to investigate the effect of high hydrostatic pressure on the stabilization of foodstuffs. Experiments have been performed using lemon juice, whose stabilization process presents some peculiar difficulties. Preliminary results confirm that the technique is suitable for producing high quality lemon juice, with satisfactory shelf life, provided that a minimum pressure of 3.000 bar is applied.

INTRODUCTION

Stabilization of lemon juices is required to reduce microbial activity as well as the activity of pectinase enzyme, which is likely to cause gelification phenomena, degeneration of taste properties and reduction of the characteristic turbidity of the juice. However, stabilization is not a straightforward operation, due to the highly acid nature of the juice. When heating such product, the acid hydrolysis of pectins can take place. This in turn promotes gelification phenomena, and produces a significant change in juice transparency. As a consequence, while thermal processing is able to reduce the activity of pectinase enzymes, it is likely to produce directly phenomena not much different from those due to the presence of such enzyme[1,2]. This is the main reason why commercial lemon juices are currently stabilized through the addition of chemicals, such as sulphur dioxide and other sulphur compounds. In order to eliminate potentially dangerous chemicals in food processing, research efforts toward the set up of non-thermal, non-chemically aided stabilization processes of acid juices are produced.

EXPERIMENTAL

The experimental apparatus used, designed specifically for food processing, is sketched in Figure 1. It consists of an autoclave with a volume of 250 ml and can be operated up to pressures of 7.000 bar. Pressurization is obtained by the use of an intermediate fluid, which is in turn pressurized by two diaphragm pumps in series[3]. This is a modification of the conceptual design of commercial units, in which the fluid medium is direclty compressed in the autoclave or indirectly by a pressure intensifier. Both methods produce an intrinsically slow pressure rise, being set up for different needs. The use of pumps, while limits the maximum achievable static pressure, allows fast

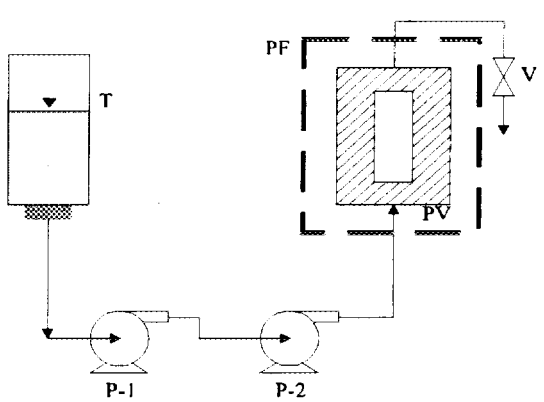

Fig. 1: Diagram of the experimental apparatus.

pressurization and can be the basis for the development of continuous apparatus.

Pressure can be released by an expansion valve. The valve can also be operated in order to produce pressure fluctuations superimposed to the static value during the process, provided that the pumps are continuously in operation. This feature is used to detect the effect of dynamic pressure components on the stabilization of food samples. Instantaneous pressure is measured by a strain gage and recorded continuously during each test.

The apparatus is controlled by a microprocessor, which allows to program specific pressure cycles. Preliminary tests, however, are carried out with simple pressurization cycles, the operative variables being the maximum pressure level and the process time.

Samples of lemon juice are obtained by plain squeezing of lemons of the same cultivar, supplied by the Istituto di Frutticultura in Caserta. After physical filtration for the elimination of coarse suspended solids, the juice is thermally sealed in 10 ml pouches made out by poliethylene-aluminium coupled material.

Pressure levels tested are: 3.000, 4.000. 4.500 bar, with a treatment time of 2, 5 and 10 minutes. Preliminary tests demonstrated that the effect of process time, in the range investigated, was not significant. Accordingly, most experiments were carried out with a process time of 5 minutes, and experimental data reported refer to such time.

Some samples of each batch of lemon juice are stored in cold conditions at 8° C without any treatment, for comparison purposes. They are referred to in the following as non pressurized juice (N.P.J.). The other are processed under the conditions stated above. Processed samples are stored at room temperature. Chemical and microbiological determinations are performed on processed and non processed juice just after the test and after 10 and 40 days, in order to follow the evolution of significant components.

The main analytical determinations are the following:

-pH, acidity, carbohydrates, according to the official Italian regulations [4].

-C vitamin and naringine [1]

-aromatic components by GM cromatography [5]

-microbiological determinations for yeasts and moulds [3]

RESULTS AND DISCUSSION

Results of microbiological analysis, limited to yeasts and moulds which are the only biological entities able to survive in very acid juices, are reported in Table 1 for samples processed at different pressure levels, in comparison with non processed samples, after a storage of 40 days. It appears that from this point of view, full stabilization is already obtained at a pressure level of 3.000 bar and maintained after 40 days. In spite of the highly acid ambient, non processed juice is heavily spoiled by yeasts and moulds after 10 days.

Results of chemical determinations are reported in Table 2. These these data demonstrate that the most significant components from the taste and nutritional point of view are stable after processing. In this case, the pressure level produces some effect, which can be detected by comparison of the compositon as a function of the preservation time. The reduction of the naringine level can be considered as a fair achievement, being this

	Yeasts c.f.u./g	Moulds c.f.u./g
N.P.J.	<10	<10
3000	-	-
4000	-	-
4500	-	-
after	**10**	**days**
N.P.J.	10^4	10^3
3000	-	-
4000	-	-
4500	-	-
after	**40**	**days**
N.P.J.	$>10^7$	$>10^7$
3000	-	-
4000	-	-
4500	-	-

Table 1: Result of microbiological analysis of lemon juice

component responsible for the bitter taste of lemon juice. As predicted, key nutritional components such as C vitamine are rapidly degenerating in non processed juice.

	Moisture Content %	pH	Acidity g Citr. Ac./(100 ml F.J.)	Carbohydrate %	Sucrose %	Citric Ac. g/100 g	Malic Ac. mg/100 g	Naringin ppm	Ascorbic Ac. mg/100 g
N.P.J.	90	2.8	7.50	3.75	1.90	6.60	270	280	69.10
3000	90	2.6	8.20	3.75	1.80	6.70	272	270	68.50
4000	90	2.6	8.20	3.70	1.90	6.60	270	250	68.60
4500	90	2.5	6.60	3.71	1.90	6.60	268	258	68.30
				after	**10**	**days**			
N.P.J.	90	2.8	6.76	3.70	1.80	6.20	260	300	60.10
3000	90	2.8	7.60	3.70	1.80	6.50	268	270	67.70
4000	90	2.9	7.43	3.70	1.90	6.50	268	260	67.00
4500	90	2.9	7.60	3.70	1.80	6.50	263	258	65.30
				after	**40**	**days**			
N.P.J.	90	2.8	6.76	3.70	1.80	6.20	260	300	60.10
3000	90	2.8	7.63	3.70	1.80	6.40	269	270	65.70
4000	90	2.6	7.40	3.70	1.90	6.30	260	251	61.90
4500	90	2.6	7.50	3.70	1.90	6.40	260	226	60.00

F.J.=Fresh Juice N.P.J.=Not-pressurized Juice

Table 2: Effects of pressure treatments on main lemon juice components.

Determination of aroma components by gas mass chromatography is reported in Table 3. It can be verified that most aroma components are slightly affected by pressurization and keep almost stable during storage.

	Mircene-β µl/l	d-limonene µl/l	linalool µl/l	terpineol-α µl/l	terpineol-4 µl/l
N.P.J.	1.6	27.3	3.2	0.7	1.1
3000	-	-	-	-	-
4000	-	-	-	-	-
4500	-	-	-	-	-
		after	**10**	**days**	
N.P.J.	1.5	22.6	2.7	0.7	1.1
3000	1.6	25.0	2.4	0.3	0.9
4000	1.5	24.9	2.0	0.4	1.0
4500	1.6	17.9	2.0	0.6	1.0
		after	**40**	**days**	
N.P.J.	0.9	8.7	1.1	0.9	0.8
3000	1.5	18.8	1.8	0.5	1.0
4000	1.5	13.3	1.6	0.8	1.0
4500	1.5	11.6	1.4	0.9	1.0

N.P.J.=Not-pressurized Juice

Table 3: Effects of pressure treatments on main aroma components of lemon juice.

In particular, d-limonene which is considered as the less stable aroma component, undergoes a slight reduction during pressurization and than its level keeps constant. The reduction seems to be larger at higher pressure levels. On the contrary, more drastic effects are observed on non processed juice samples for the longer preservation times.

CONCLUSIONS

Preliminary tests performed so far demonstrate that high pressure treatment is a valuable technique in the stabilization of lemon juice, preventing the development of moulds and yeasts and preserving almost at the initial value the level of key chemical components. It means that a valuable product can be obtained, with full aroma, taste and nutritional value. The high added value of such product, which has no current competitors, could make for the application on large scale of the high pressure stabilization technique.

While it can be stated that a pressure level of 3.000 bar is sufficient to ensure an effective stabilization, further work is necessary and is currently performed to determine the effect of pressure level above this value, which cannot be completely elucidated by present data. This work also aims at defining the optimal processing cycle and at determining the maximum shelf life of such product.

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NEW TRENDS IN HIGH PRESSURE EQUIPMENT

Mr Jean BIGNON - Process Engineer

Cold pasteurisation of fruit products is possible thanks to the high pressure technology. GEC ALSTHOM ACB proposes commercial industrial units (HYPERBAR cold pasteurizers) with capacities up to 3 000 l/h, with two technologies :

- in flexible container processing ;

- in bulk.

GEC ALSTHOM ACB has a first reference in freshly squeezed premium orange juice in France.

HYPERBAR : A TECHNOLOGY TRANSFER

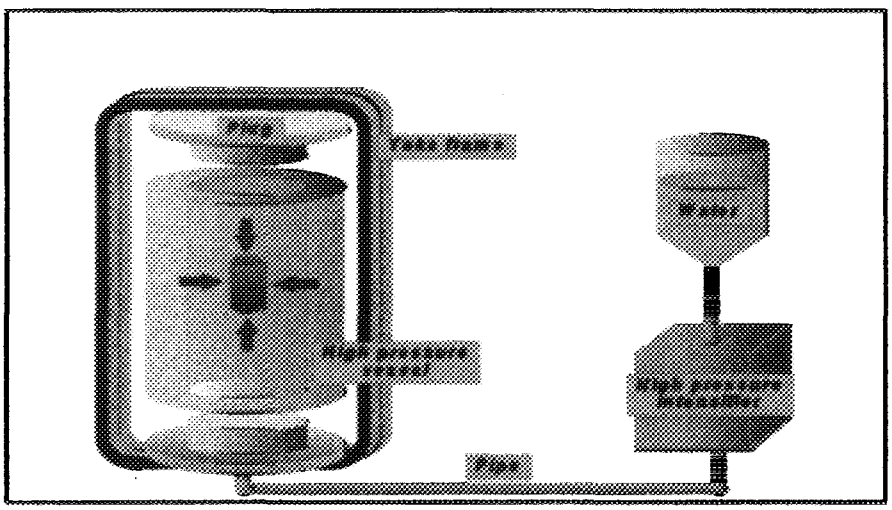
GEC ALSTHOM ACB is a world leader manufacturer of continuous sterilizers for the food industry (dairies, canneries) and the pharmaceutical industry, as well as a manufacturer of very high pressure machines for powder metallurgy, ceramics and quartz industry.

Considering the growing demand of the food processing industry for minimal processing, GEC ALSTHOM ACB has developed a new high pressure equipment specifically designed for food processors (the HYPERBAR cold pasteurizer) taking into account :

- High production rates for food products compared to other applications. This led GEC ALSTHOM ACB to design and patent a new feature for quick opening and closing of the treatment chamber ;
- Use of food-compatible materials when the product is directly in contact with the machine ;
- Bulk treatment for liquid foods with a cleaning ability. This led GEC ALSTHOM ACB to develop a new patented feature for direct compression of the liquid without package.

BASIC DESIGN OF A HYPERBAR COLD PASTEURIZER

See scheme 1



Scheme 1 : Main parts of a high pressure equipment

APPLICATION OF HIGH PRESSURE TECHNOLOGY TO COLD PASTEURIZATION OF FRUIT PRODUCTS

The main application of HYPERBAR processing on fruit products is the cold pasteurization, thus limiting sensorial damages such as colour changes, texture modification and flavour profiles changes compared to any heat pasteurization. Possible new products can also be made such as non cooked jams, by compression of fruits + pectines + sugar at ambient or low temperature, leading to pectin gelification with the taste and the colour of fresh fruits.

Cold pasteurization of fruit products can be achieved with pressures between 4 000 and 5 000 bars (60 to 75 000 p.s.i.) during 1 to 5 minutes. At such pressures, most vegetative micro-organisms are killed, with a reduction level between 10^4 and 10^7 CFU, and enzymes are slightly inactivated (Polyphenol oxydase, Pectin-Methyl Esterase). Complete enzyme inactivation can be achieved at higher pressure but investment cost for such machines is much higher, and this is economically of interest only when long shelf life is needed (> 1 month). An alternative for complete enzyme inactivation seems to be a combination of moderate heat treatment (50°C) with pressures of 4 000 to 5 000 bars. Gelification of pectin mixed with sugar and fruits requires lower pressures (2 000 bars).

Current industrial applications for fruits products are : Freshly squeezed orange juice, avocado puree, fruit preparation for dairy products, flash pasteurized clementine and grapefruit juice, non cooked jam (kiwi, strawberry, apples, ...).

MACHINES FOR IN CONTAINER PROCESSING OF FRUIT PRODUCTS

The high pressure cylindrical vessel is horizontal, thus allowing loading at one side and unloading at the opposite side (and facilitating maintenance).

The unique ACB QUICK LOCK SYSTEM : All our HYPERBAR pasteurizers are equipped with a unique feature for quick closing and opening of the vessel. This innovative system makes loading and unloading easy and fast, without any move of any heavy part of the machine, thus increasing productivity and cost savings.

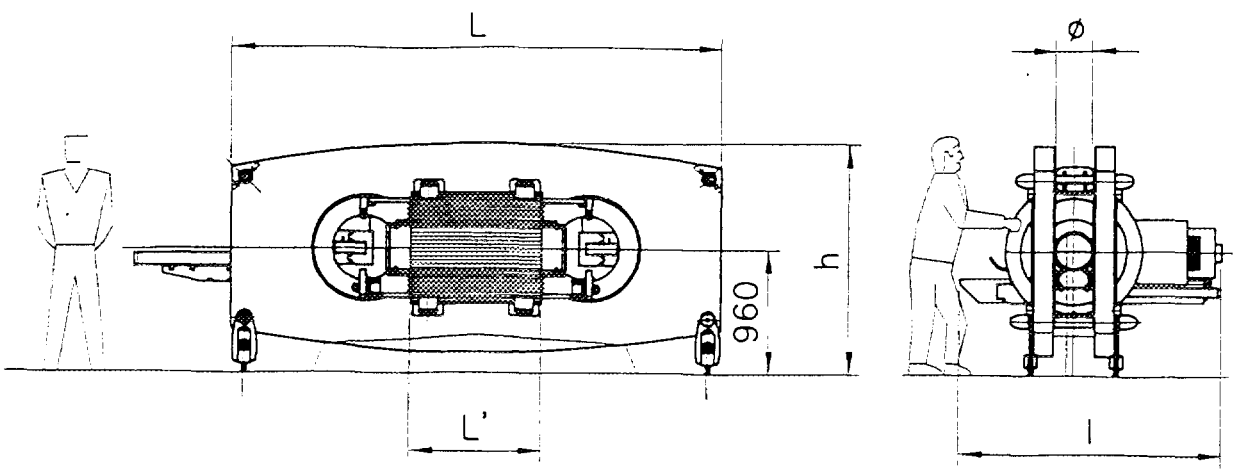
The high pressure compression system is fitted on a separated skid connected to the vessel with high pressure resistant pipes, and can be physically in a closed room, in order to avoid noise. The fluid used for compression is standard water, with possible temperature regulation and recycling.

An exemple of a cycle break down is as follow :

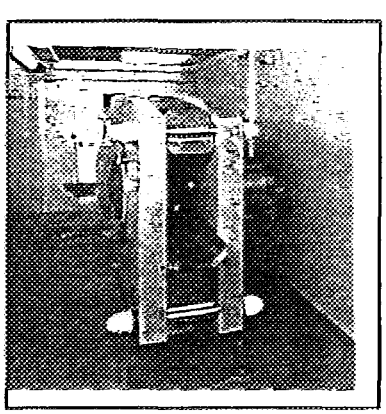
- loading of the baskets (filled with the products) into the chamber (direct introduction is also possible for bottled products) simultaneously to unloading of the processed products	0,5 min
- closing of the vessel	0,5 min
- water filling of the vessel	1 min
- pressure size	3,5 min
- holding time	Depend on the process
- pressure release	0,5 min
- opening	0,5 min

All these operations can be fully automated. Processing capacities of our HYPERBAR cold pasteurizers can go up to 3 000 l/h at 4 000 bars (see scheme 2 an 3).

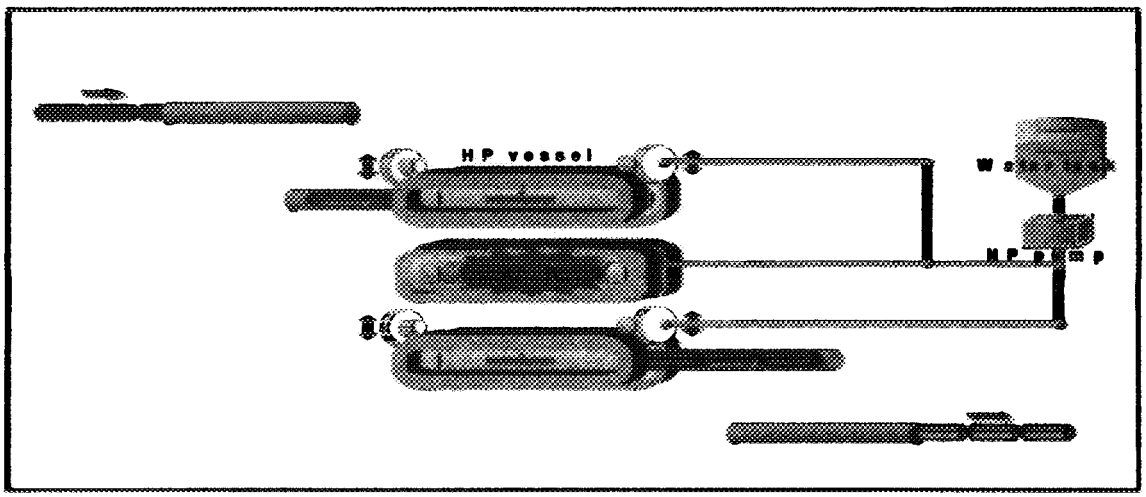
Scheme 2 : Industrial HYPERBAR cold pasteurizer from GEC ALSTHOM ACB



Ref.	L	l	h	Ø	L'	Weight	Power
HYPERBAR 2 000	10,2 m	2 m	1,8 m	280 mm	8 m	40 tons	260 kW
HYPERBAR 1 000	6,2 m	2 m	1,8 m	280 mm	4 m	25 tons	130 kW



Scheme 3



The packaging material needed to separate the product to treat from the compression fluid can be any flexible packaging type :

- Bottles or cups in PEHD, PVC, PP, PET, multilayers ... ;
- Bag in box ;
- Certain metal cans can also be used.

BULK HYPERBAR PROCESSING OF PUMPABLE PRODUCTS

Regarding high pressure processing of pumpable products, different factors can affect the cost and productivity of industrial equipments :

- Maximum pressure ;
- Holding time ;
- Filling ratio of the high pressure vessel (see graphic 1) ;
- Loading (filling) and unloading (emptying) into the high pressure vessel.

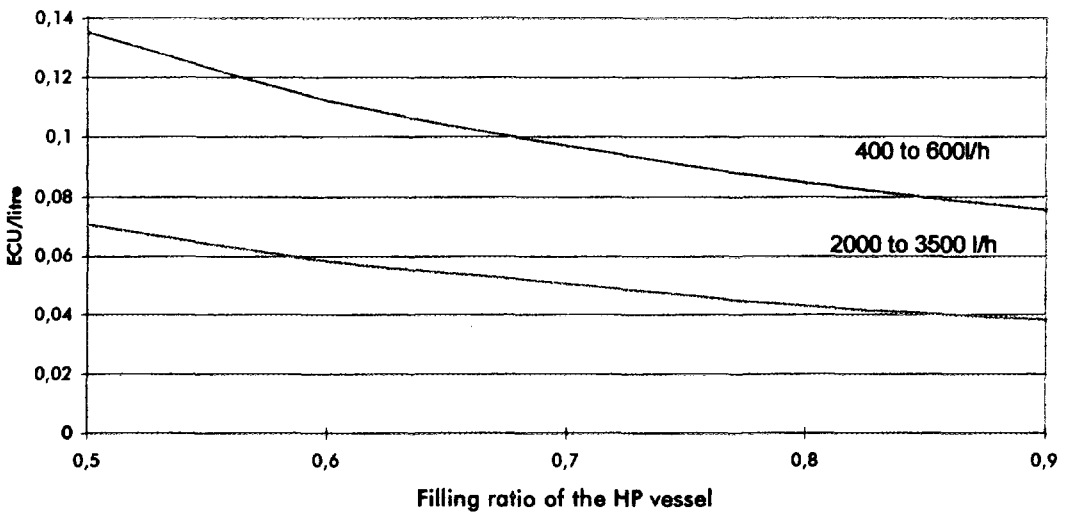
Factors n° 1 and 2 depend on the product only, whereas factors n° 3 and 4 mainly depend on the industrial design of the machine. As a consequence, bulk high pressure processing of liquids can lead to cost savings by increasing filling ratio and reducing filling/emptying duration compared to in container processing (see table 1).

Table 1 : Filling ratio of the high pressure vessel. Comparaison between in container and in bulk processing.

	In container	In bulk
Filling ratio	60 %	100 %

Graphic 1

Influence of filling ratio on the cost price per litre of HYPERBAR processing



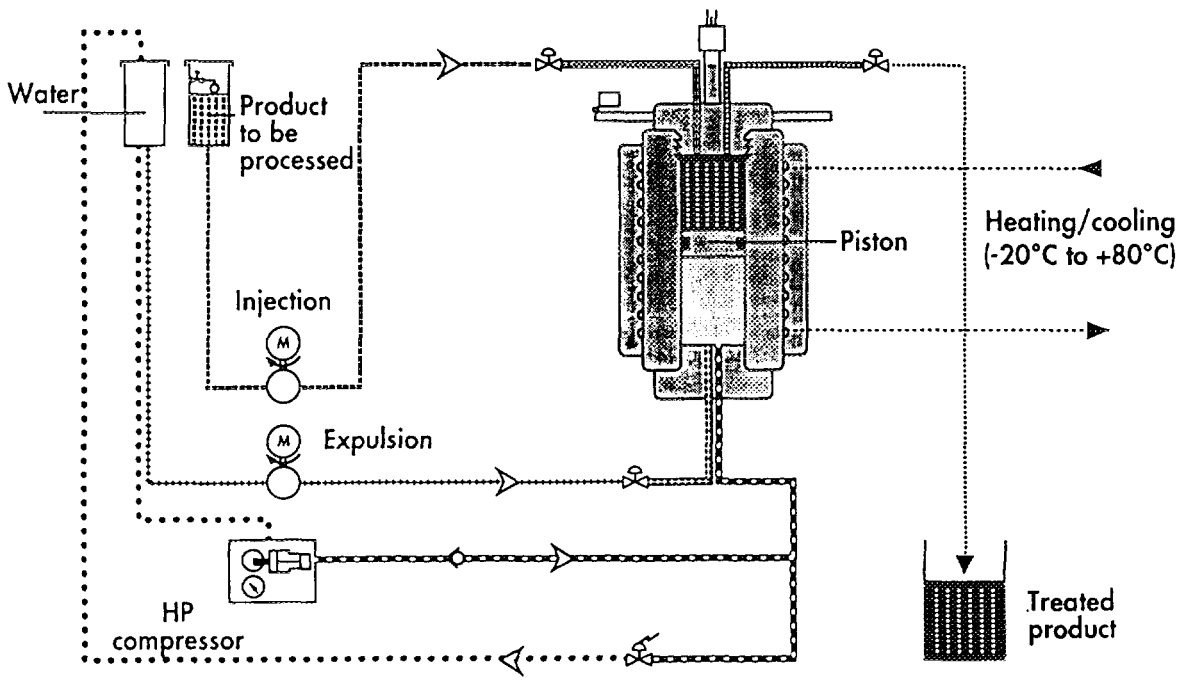
GEC ALSTHOM ACB has built a pilot HYPERBAR cold pasteurizer for liquids in bulk (capacity ≈ 100 l/h). Regarding industrial consideration, the chosen design is a high pressure vessel made of stainless steel including a floating piston, 3 high flow valves and 1 small valve. The floating piston separates the product side from the compression fluid (water), each being at the same pressure at any time (see scheme 4). Basically, it works like the cylinder of a car engine :

1. Filling into the high pressure chamber through a low pressure pump, inlet high pressure valve open and outlet high pressure valve closed.
2. Inlet valve closing and pressure rise due to water compression through the high pressure pump and the small valve.
3. Small valve closing. Holding time.
4. Pressure release thanks to the opening of the small valve on the water side.
5. Opening of the outlet high pressure valve, product side. Emptying of the chamber by low pressure compression through the low pressure pump and the high flow valve (water side).

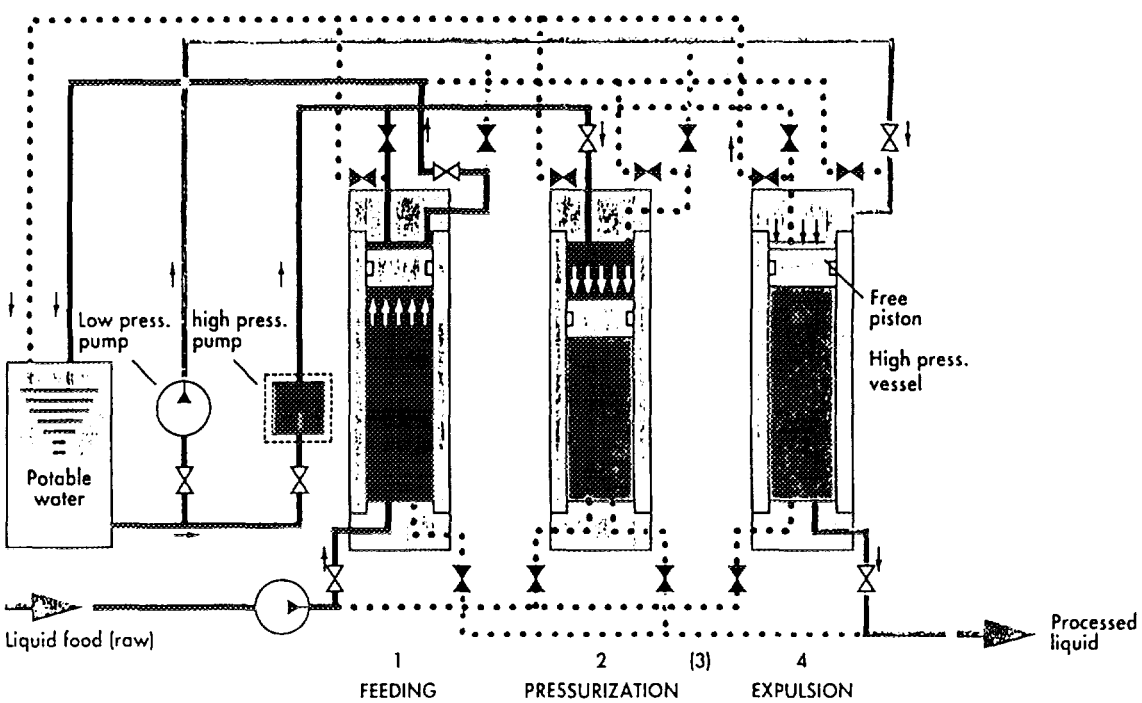
The specific high capacity high pressure valves have been developed and patented in order to decrease filling and emptying durations whereas the small valve is only for water compression and decompression which do not need a high flow. A first versus of this pilot HYPERBAR cold pasteurizer has been tested for cleanability, showing critical points to be improved (piston, circuity, feeding tank). A new versus have been rebuilt including a fully aseptic designed circuit and valves. Tests for cleanability have been performed with success as well as efficiency tests.

This design is perfectly upscalable to industrial units made of 3 distinct vessels with a commun high pressure compression pump for units of 3 000 l/h (see scheme 5).

Scheme 4 : Bulk high pressure processing of liquids. Bloc diagram



Scheme 5 : Industrial HYPERBAR 3 000 l/h



STERILIZATION PROCESS FOR CANNED TOMATOES

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Thermal treatment by sterilization is one of the most important steps in the processing of canned foods. A proper selection of the process parameters is important to achieve two conflicting objectives: maximum destruction of the microorganisms and minimum loss of organoleptic value of the product with a minimum consumption of energy in the process. In both cases it is fundamental the knowledge of the temperature history of the product. To this end, this work was addressed to develop a computationally efficient procedure for describing the heat transfer mechanism in canned peeled tomatoes as a function of the operating variables and for predicting thermal responses and lethality effect to be used for the better conduction of the sterilization process. An empirical correlation for the heat transfer coefficient that take into account the influence of the headspace in the container and the velocity of rotation of the cans, is presented. The equation was derived from experimental data on the sterilization effect taken from an industrial plant and obtained by recording the temperature values at the geometric centre of the food containers.

INTRODUCTION

Significance. Thermal treatments in the conservation of biological degradable substances like foods are intended to obtain a microbiologically safe final product by the inactivation of undesiderable contaminating microorganisms and with a minimum nutrient and quality factor degradation of the product. Thus, it is fundamental the knowledge of the temperature history of the material treated which must be measured at least at one point, usually chosen as the geometric centre for conductive-heating canned foods. In fact the common statistical control can highlight only the macroscopic situations of insufficient sterilization but it doesn't give complete assurance about the reduction of microorganisms to an acceptable degree of safety. On the other end also the sterilization programs based on empirical indicators (like "baremes") can cause insufficient or excessive processing times. It is better to make use of the concept of target sterilization value F_t, but to calculate on this value it is necessary to have experimental measurements concerning the heat transmission inside the can. Frequently, the lack of these informations and safety considerations lead to an overprocessing of the product with energy and quality waste.

Objective and methods. The experimental part of this work has been carried on in the production plant of CASAR S.R.L. (Serramanna-Cagliari) during the working period of the year 1995. The flowsheet of the canned peeled tomatoes section of the plant is shown in Figure 1.

The process starts from raw tomatoes and is part of the whole tomato's treatment. We focus our interest on the sterilization section where the canned tomatoes are stabilized by leaving the food containers in a hot environment for enough time to allow the inactivation of an amount of microorganisms sufficient to reach the commercial sterility.

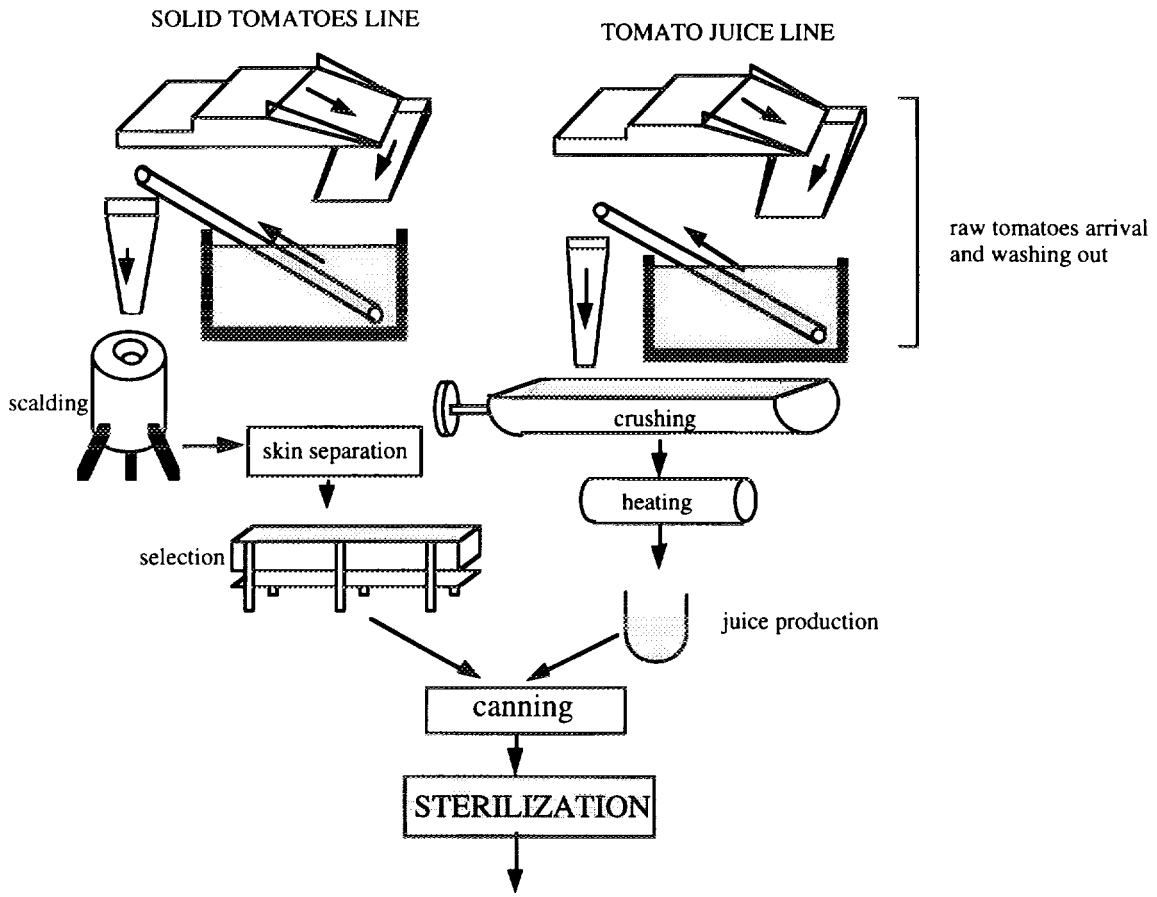

Figure 1. Flowsheet of the canned peeled tomatoes production.

The canned tomatoes are continuously fed to the sterilization equipment (a Dall'Argine & Ghiretti sterilizer) heated by steam at 100 °C. The cans enter the chamber at 40 °C on a conveyer belt and are subject to a periodical axial rotation to improve the heat penetration.

The destruction rate of microorganisms, at any temperature, is usually described, in food material, by a first-order reaction with the reaction rate constant related to the decimal reduction time, D:

$$-\frac{dC}{dt} = k_T \cdot C = \frac{2.303}{D} \cdot C \qquad (1)$$

The dependence of D from the temperature is frequently[1] written using the Arrhenius equation and since for most bacterial spores the temperature interval difference that causes a tenfold change, D, is small, the following simplified relationship is often used:

$$D = D_r \cdot 10^{(T_r - T)/Z} \qquad (2)$$

A complete sterilization can not be achieved because the dead of microorganisms follows an exponential law and it is common practice to consider a commercial sterility, that is to obtain a reduction in the concentration of the most important microorganism of 10^{10} - 10^{12}.

If n is the number of the requested decimal reduction in the concentration of microorganisms, the target sterilization effect, in terms of D, is given by:

$$F_t = n \cdot D \tag{3}$$

The sterilization effect of the thermal process for the whole range of temperature applied, may be indicated as an equivalent length of time, F_c, with respect to the reference temperature, T_r, that in this case has been considered equal to 100°C. The mathematical expression for F_c is[1]:

$$F_c = \int_0^t 10^{-(T_r - T(t))/Z} dt \tag{4}$$

Thus, the sterilization effect of the canned product can be calculated by solving this equation by means of experimental or theoretical time-space-temperature relationships in the can.

Scope. This work was performed to collect experimental data on both raw material characteristics and main process variables that could influence the heat penetration into the container and to evaluate theoretically the Temperature-space-time profiles by correlating the sterilization effect with the variation of these parameters. In this paper the temperature history only at the centre of the can was considered[2] and a set of raw material and process data were recorded in a number of sample cans. In particular for each sample can were measured: the net and dry weight of product, the headspace, the rotational velocity, the number of yellow and total crops, the optical absorption, the initial temperature of crops.

EXPERIMENTAL

The experimental work was carried out by utilizing, every ~5000 cans fed to the sterilization chamber, a sample container in which the choosen set of variables was recorded and a Datatrace system was applied for the measurement, processing and storage of the T-t data. The temperature was measured by means of a thermistor (NTC, d=4.5 mm) located at the geometrical centre of the container (Fig. 2).

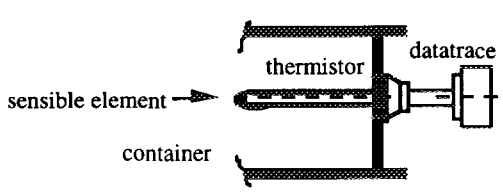

Figure 2. System for Temperature-time experimental data collection.

The data collected were transferred to a computer and the sterilization effect was calculated through the Patashnik's method[3]. The system for the collection of data has been proved to be one of the more suitable for a proper recording of heat penetration profiles in canned food products[4,5] because does not need cable connections and the limitation is the impossibility to have a real time measurement of the temperature behaviour.

A series of experimental runs have been performed utilizing three different sizes for the sample cans (weight of product 0.5, 1 and 3 kg)[6], but most of the data were collected with the size of 1kg and only these data, reported in Table 1, were utilized in the processing of the results. In Table 1 are also reported, for each run, the corresponding values of the sterilization effect F_e calculated from the T-t data taken in the centre of the container.

Run	wt_n	wt_{di}	H_s	v_p	n_c	wt_{df}	r_{oi}	r_{of}	T_{ic}	P	n_{yc}	F_e
1	806	592	7	14.5	12	489	7.5	5.7	47.1	5.0	3	18.5
2	809	596	7	14.5	10	502	7.5	5.7	43.4	5.0	2	14.5
3	810	598	7	14.5	11	509	7.2	5.6	47.2	7.8	0	8.84
4	781	610	10	14.5	11	523	7.8	6.4	45.1	7.2	3	13.3
5	795	590	8	14.5	10	469	7.8	6.1	46.2	7.2	2	30.4
6	811	700	7	14.5	15	629	8.6	5.6	43.6	7.0	2	20.5
7	781	590	12	14.5	10	524	8.6	6.0	42.1	7.0	1	28.3
8	804	645	8.5	14.5	11	562	8.2	5.8	40.0	7.0	2	15.7
9	796	690	10	10.3	13	549	8.0	5.3	44.2	7.6	1	16.1
10	787	600	11	10.3	10	524	8.0	5.3	47.2	7.6	0	15.9
11	786	600	9	10.3	11	535	7.6	6.2	47.6	7.8	2	12.7
12	800	690	8	10.3	15	630	7.6	6.2	47.6	7.8	2	12.7
13	806	600	6	8.7	11	527	7.6	5.6	37.5	7.5	0	7.58
14	803	700	6	8.7	13	572	7.6	5.4	38.8	7.5	0	17.9
15	782	600	11	12.5	11	531	8.0	5.6	45.7	7.8	1	22.3
16	799	680	8	12.5	12	595	8.0	5.5	39.3	7.8	1	20.8
17	787	610	10	12.5	12	551	8.0	5.5	40.0	7.5	0	18.0
18	799	690	8	12.5	12	565	8.0	5.5	38.1	7.5	0	10.5
19	790	600	9	12.5	11	528	8.1	6.0	39.6	7.2	1	24.7
20	787	700	11	12.5	12	625	8.1	5.1	39.8	7.2	2	14.2
21	797	700	9	12.5	10	609	8.2	5.5	42.7	7.5	1	28.7
22	805	700	6	12.5	11	604	8.2	5.5	41.4	7.5	0	20.4
23	788	600	10	10.5	10	514	8.3	5.6	42.9	7.5	0	21.3
24	802	700	8	10.5	13	591	8.3	5.6	36.3	7.5	0	18.3
25	785	600	9.5	10.5	11	516	8.4	5.6	36.5	7.5	1	17.2
26	800	700	8	10.5	14	576	8.4	5.5	40.3	7.5	1	11.4
27	779	600	11	14.5	12	515	8.0	5.6	39.2	7.5	0	21.2
28	799	700	10	14.5	14	606	8.0	5.6	39.6	7.5	0	18.1
29	787	590	10	14.5	10	502	8.0	6.0	39.1	7.5	0	23.3
30	811	700	7	14.5	13	574	8.0	6.0	39.8	7.5	0	16.9
31	792	600	9	8.7	11	526	8.0	5.5	36.8	7.5	0	16.3
32	794	700	9	8.7	14	590	8.0	5.5	37.0	7.5	0	18.1
33	796	700	8	8.7	12	609	8.0	5.6	33.2	7.2	1	10.0
34	792	600	9	8.7	12	523	8.0	5.6	36.1	7.2	0	11.2
35	794	600	9	14.5	13	534	8.0	5.6	41.1	7.1	0	24.5
36	800	700	8	14.5	12	568	8.0	5.6	37.9	7.1	0	17.9
37	787	600	11	8.7	12	524	7.5	5.6	38.2	8.5	0	22.9
38	791	700	9	8.7	13.5	544	7.5	5.6	38.5	8.5	1	19.4
39	789	600	10	14.5	12	509	7.3	5.6	40.6	7.8	0	24.3
40	788	700	9	14.5	15	576	7.3	5.6	39.5	7.8	0	28.3

Table 1.
Experimental data for 1 Kg sample cans.

HEAT TRANSFER MODEL AND RESULTS

The transient temperature profiles inside the food container can been obtained by considering the energy balance in cylindrical coordinates (in our case, can sizes: R = 5 cm, L = 11.8 cm):

$$\rho \cdot c_p \cdot \frac{\partial T}{\partial t} = k \cdot \left[\frac{\partial^2 T}{\partial z^2} + \frac{1}{r} \cdot \frac{\partial T}{\partial r} + \frac{\partial^2 T}{\partial r^2} \right] \tag{5}$$

subject to the following initial and boundary conditions:

$$T(r,z,0) = T_0 \; \forall \; r,z \tag{6}$$

$$\left[k \cdot \frac{\partial T}{\partial z} \right]_{z=L} = h \cdot (T_{ex} - T) \tag{7}$$

$$\left[k \cdot \frac{\partial T}{\partial r} \right]_{r=R} = h \cdot (T_{ex} - T) \tag{8}$$

$$\left[k \cdot \frac{\partial T}{\partial r} \right]_{z=0} = 0 \; \forall \; r,t \tag{9}$$

$$\left[k \cdot \frac{\partial T}{\partial z} \right]_{r=0} = 0 \; \forall \; z,t \tag{10}$$

Constant values obtained from literature data are utilized for h[7], c_p[8] and ρ[8] and the energy balance in eq. (5), with the conditions in eq. (6-10), was solved numerically: the geometrical part was transformed in an algebraic system using the method of finite differences; the differential part, due to its high stiffness, was integrated using the routine DIVPAG of the IMSL library.

The thermal conductivity k of the food was utilized as a fitting parameter correlated to the experimental values of H_S and v_p. The considerable importance on the sterilization effect of these two variables is highlighted from the experimental data reported in this work and in the literature[9]. A linear dependence of k from H_S and v_p was considered, according to the following equation:

$$k = a + b \cdot H_S + c \cdot v_p \tag{11}$$

The constants, a, b and c were evaluated by the error minimization between the value of the sterilization effect calculated from the theoretical T-t curves and that obtained in the experimental runs reported in Table 1.

Thus, the objective function utilized is:

$$\Sigma_i \left(F_e^i - F_c^i \right)^2 \tag{12}$$

and the solution achieved by means of the subroutine OPTNOV[10]. The following final values were obtained for the optimized constants (after about 800 iterations and with a variation less than 0.1% in two successive iterations): a= 7.84E-4 KJ/msK, b = 7.92E-2 KJ/m^{2}sK, c = 3.85E-5 KJ/msK(1/rpm). The T-t profiles calculated with these values for the constants have been compared with those measured with the Datatrace system. The results obtained have been sufficiently good, considering the limited number of experimental data and the number of variables that influence the heat penetration. A good agreement, as shown in the Figs. 3 and 4, was obtained especially in the runs with a proper value of the sterilization effect, F. More difference in the temperature profile was verified for those sample cans in which the sterilization effect is low, like, for instance, the run 12 reported in Fig.5, where the sterilization effect measured is 12.7 and the heat penetration calculated is higher than that experimentally verified.

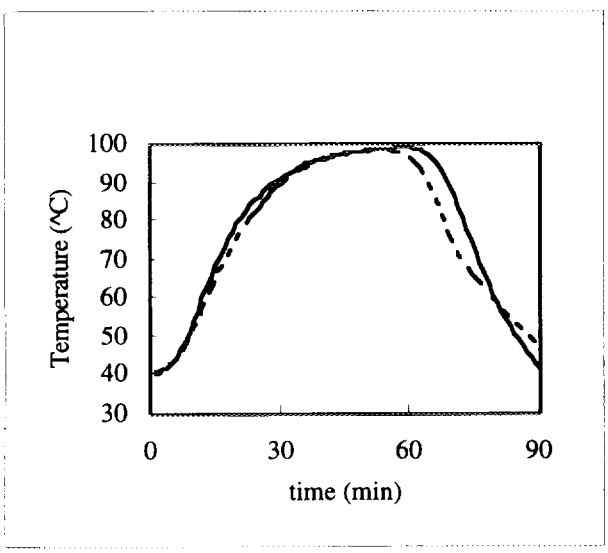

Figure 3. Experimental (- - -) and calculated (——) temperature profile for run No.8 (F_e=15.7).

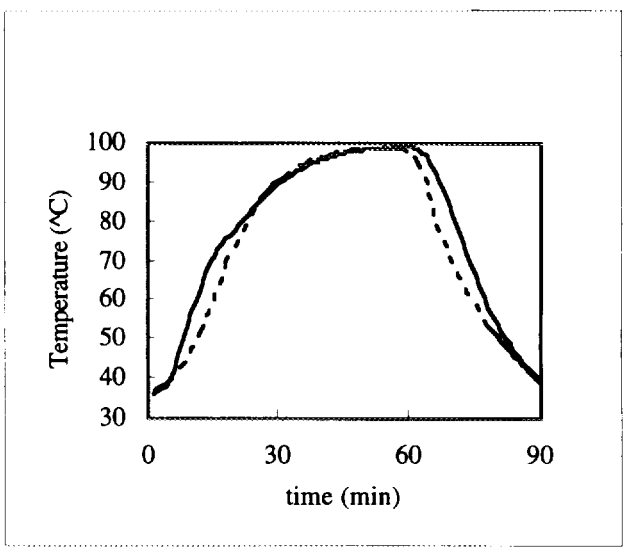

Figure 4. Experimental (- - -) and calculated (——) temperature profile for run No.24 (F_e=18.3).

Due to the uncertainty in the values of cp and ρ, the low influence on the results of the values utilized was verified up to changes of ±10%. The importance of the rotation speed applied to the containers was verified by comparing the T-t profile, obtained for the run 24 in Table 1, with that obtained for a sample can in the same conditions but sterilized in a static apparatus for the same time. As shown in Fig. 6 in the static apparatus the maximum temperature reached is lower and the sterilization effect is about 60% of that obtained in the dynamic apparatus. This result confirms the importance of the convective contribution to the heat penetration in the can and could be the cause of the poor agreement verified in the case of insufficient sterilization.

CONCLUSIONS

From the analysis reported in this work, it appears that despite the large number of simplifications made on account of all the variables that influence in the process, the metodology applied can give useful information on the

sterilization process of canned tomatoes. In this preliminary work only the influence of H_S and v_p on the heat penetration into the can was taken into account and further experimental data are necessary to better quantify this influence so as that of other parameters. In particular, it seems that also the amount of juice and the number of crops in the can should have some importance[9] since they affect the amount of heat transferred by convective mechanism.

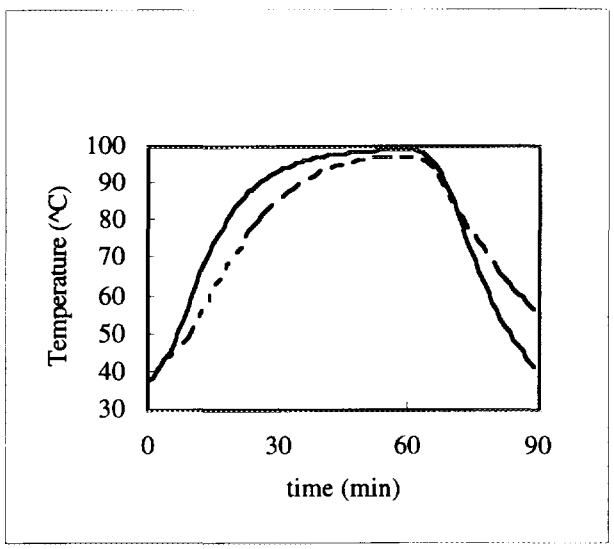

Figure 5. Experimental (- - -) and calculated (----) temperature profile for run No.12 (F_e=12.7).

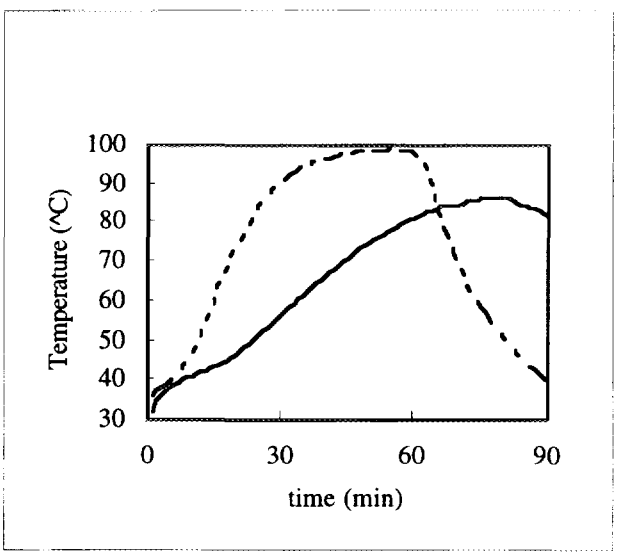

Figure 6. Experimental temperature profiles in the dynamic (- - -) and static (---) sterilizer for run No.24.

Also the concept of the less favourite point applied in this analysis needs to be examined more carefully because it is certainly true for conductive heat transfer, while in case of both conductive and convective mechanism this assumption should be better considered.

Acknowledgment: The technical support provided by CASAR S.R.L. is gratefully acknowledged.

NOMENCLATURE

- a, b, c constants in equation (11)
- c_p specific heat of food material, kJ/kgK
- D_r decimal reduction time (time required of concentration species to be reduced by a factor of ten at reference temperature), min
- F_c sterlization value with reference temperature of 100 C and Z=10, min
- F_e experimental sterlization value with reference temperature of 100 C and Z=10, min
- F_t target sterlization value, min
- h heat transfer coefficient, kW/m^2K

- H_S headspace in the can, mm
- k thermal conductivity of food, kW/mK
- n_C number of tomato crops in the container
- n_{YC} number of yellow tomato crops in the container
- P steam pressure at the sterilization apparatus inlet, atm
- r_{Of} optical absorption after the sterilization, Brix
- r_{Oi} optical absorption before the sterilization, Brix
- t time, min
- T_{ex} temperature of heating medium, ^C
- T_{ic} initial temperature of tomato crops, ^C
- T_r reference temperature, ^C
- v_p rotational speed of containers in the sterilization apparatus, revolutions per minute
- wt_{df} dry weight of product after the sterilization, g
- wt_{di} dry weight of product before the sterilization, g
- wt_n net weight of product, g
- Z temperature interval difference that causes a tenfold change in D, ^C
- ρ food density, kg/m^3

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REFINING OF LEMON ESSENTIAL OILS BY SUPERCRITICAL EXTRACTION WITH CARBON DIOXIDE

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This paper examines the possibility of obtaining deterpenated lemon oils by means of a supercritical extraction process using carbon dioxide as solvent. While this problem has already been tackled by other authors,their work focuses on experimental tests carried out on syntetic lemon essential oils. The experimental tests presented in this paper were instead carried out on a natural lemon oil from Sicily, which was placed in contact with supercritical carbon dioxide utilizing a semibatch apparatus.
The tests were carried out at different pressures and temperatures, the best result being an enriched oil with a concentration of oxygenated compounds ranging between 10% and 16%. The interest of these results lie in the fact that the initial concentration of the oil was about 6 %, that the temperatures used were in any case low and thermal degradation was therefore avoided, and that the yield was quite high in that the concentration of oxygenated compounds in the extract was very low.
The best results were obtained at temperature of 40°C and pressure of 8 MPa and at temperature of 43°C and pressure of 8.5 MPa.

INTRODUCTION

Large quantities of lemon oils are used as flavouring agents in the food and perfume industries. These products are characterized by a high number of compounds, which can be grouped from the chemical viewpoint into three main categories: terpenes, sesquiterpenes and oxygenated compounds. Small quantities of compounds with high molecular weight, such as waxes and coumarin, are also present [1,2].

The oxygenated compounds are the most important group as regards aroma, while the terpenes play a merely supporting role, despite the fact that they are the leading group in quantitative terms. Terpenes are even to be regarded as harmful in that they can, in the presence of light and air, give rise to phenomena of biodegradation [3,4] which impair the "bouquet" of the essential oil. The industrial solution to such problems is to produce deterpenated essential oils by means of different processes. Those most widely used are vacuum evaporation or distillation, or conventional extraction processes with a hydro-alcoholic solvent.

Both groups of processes present drawbacks which limit their use. The thermal processes must be carried out at the lowest possible temperatures so as to avoid degradation phenomena, which are in any case always present, while the conventional solvent-based extraction processes lead to the production of an enriched oil which always proves to be polluted, to a greater or lesser degree, by the solvent used in the operation.

The possibility has recently been examined of subjecting lemon essential oils to a process of extraction using a supercritical solvent (generally carbon dioxide)[5,6,7] to obtain a refined phase that is rich in oxygenated compounds and an extract in which the terpenes are concentrated. In such a process the operating temperature can be lower than that generally used for distillation processes, thus minimizing the problems of thermal decomposition. At the same time, it is also easier to separate the solvent from the refined phase than in conventional extraction processes. Such a process is made possible by the fact that the solubility of terpene compounds in carbon dioxide has proved the be greater than that of oxygenated compounds. When an essential oil is placed in contact with carbon dioxide, terpenes are therefore extracted preferentially while oxygenated compounds are concentrated in the refined phase.

The literature contains many articles on supercritical extraction applied to synthetic oils [5,8], but very few data on extraction as applied directly to natural essential oils [9,10].

A series of articles have recently been published on the adsorption of "synthetic" essential oils on a solid adsorbent matrix (generally silica gel)[11,12]. By causing carbon dioxide to flow through this bed it is possible to obtain the separation of terpenes from oxygenated compounds for the preferential desorption of terpene compounds followed by an analogous process also for oxygenated compounds.

This paper furnishes experimental data on a series of experimental runs carried out directly on an industrially

produced Italian essential oil. The oil was subjected to a direct process of extraction with supercritical carbon dioxide in a semibatch apparatus at pressures ranging from 7.5 to 8.5 MPa and temperatures ranging from 37°C to 43°C.

The results obtained are very promising and confirm the possibility of the process being used for industrial purposes.

EXPERIMENTAL SECTION

METHODS OF ANALYSIS

All the samples obtained during the experimental runs were subjected to gas chromatographic analysis in order to determine their composition. The instrument used was a Hewlett Packard HP GC 580 gas chromatograph equipped with a thermal conductivity detector. In accordance with the procedures indicated in the literature [13], separation of the compounds for analysis was effected by means of an HP 5 capillary column with copolymer filling: (5%)-diphenyl-(95%)-dimethylpolysiloxane (length = 30 m, internal diameter = 0.25 mm, thickness of film = 0.25 μm).

The analytic procedure adopted involved the following temperature program:

- initial temperature of column 75°C (maintained for 5 minutes)
- temperature increased by 4°C per minute up to a temperature of 200°C;
- temperature maintained at 250 °C for 20 minutes before returning to initial temperature (75°C).

The injection temperature was constant at 250°C.

The first phase involved analysing the essential oil to be used during the experimental runs. The chromatogram obtained is shown in figure 1 together with the assignation of the main peaks. Analysis of the figure shows that the main component is limonene (accounting for approximately 66%), followed by β pinene and by γ terpinene. As regards the oxygenated compounds, neral and geranial proved the most important in quantitative terms.

The instrument was then calibrated by injecting solutions of essential oil and ethanol at known concentrations and registering instrument response in terms of the total area of the peaks shown by the integrator. The samples at unknown levels of concentration were then injected into the GC and the concentration was worked out on the basis

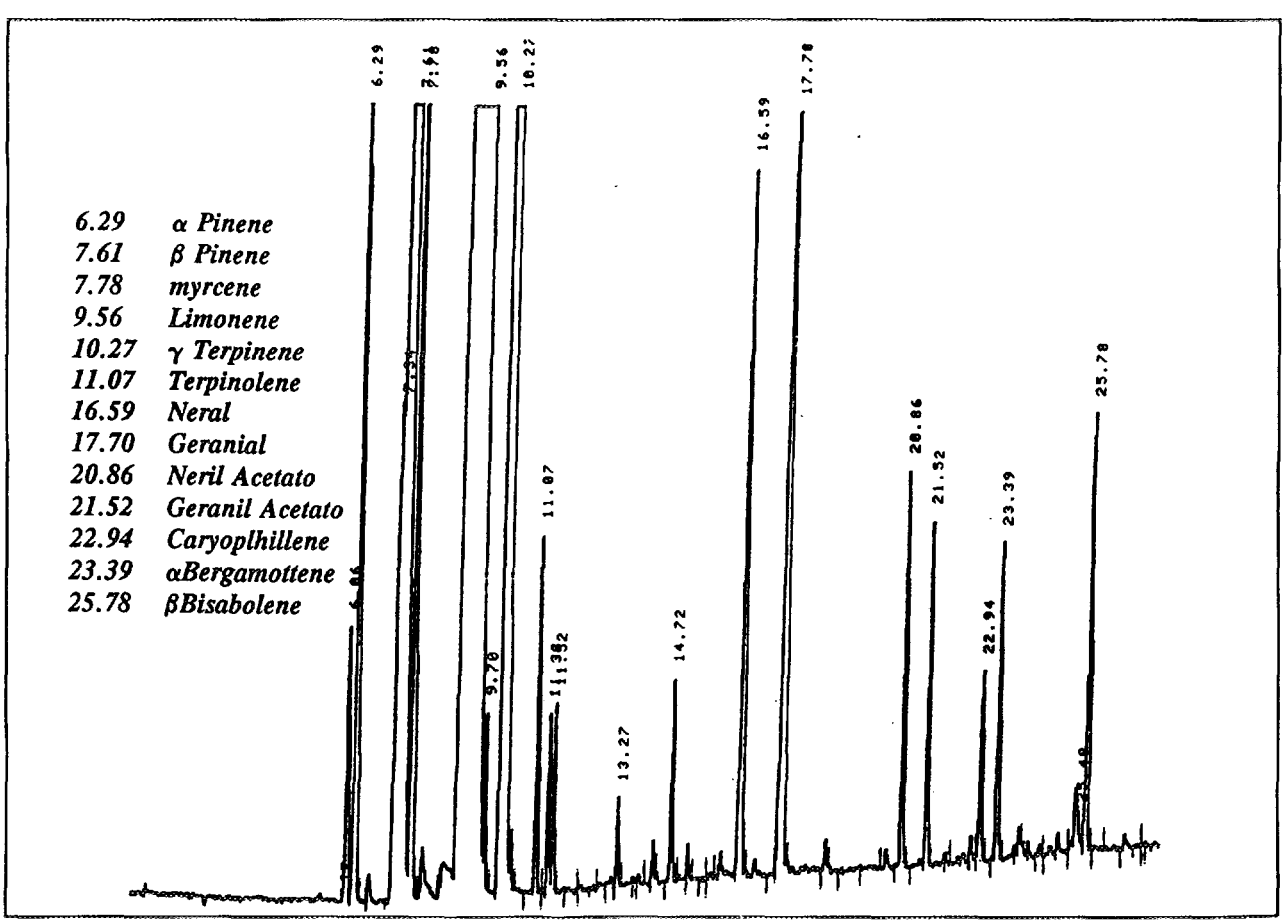

Figura 1 - Chromatogram of lemon essential oil utilized in this work

of total area through the calibration curve. The registered degree of error proved less than 5%.

Each analysis was repeated at least three times and the mean value of the total area was used to calculate the unknown concentration.

DESCRIPTION OF APPARATUS

The experimental apparatus used is produced by the Novi Swiss company and consists essentially of a lined vessel for contact between the gas phase and the liquid phase and a pumping system for the carbon dioxide. The detailed specifications of the apparatus, which is schematically illustrated in figure 2, are given elsewhere [14], while the present article furnishes only information on the experimental method adopted. Extraction runs were carried out with lemon oil at different pressures and temperatures in order to optimize the extracting power of the supercritical solvent with a view to establishing which operating conditions guaranteed the greatest selectivity. Each trial was carried out at constant values of temperature and pressure, equal respectively to 37°C, 40°C, 43°C and 7.5 MPa, 8.0 MPa, 8.5 MPa. Though primarily referring to experimental runs on synthetic essential oils, the experimental data given in the literature do in fact indicate the above values as those at which the extraction yield is maximized.

Each experimental run was carried out with a constant ratio between the quantity of solvent introduced and the quantity of oil initially present in the extraction vessel (about 32 g/g). For temperature and pressure values at

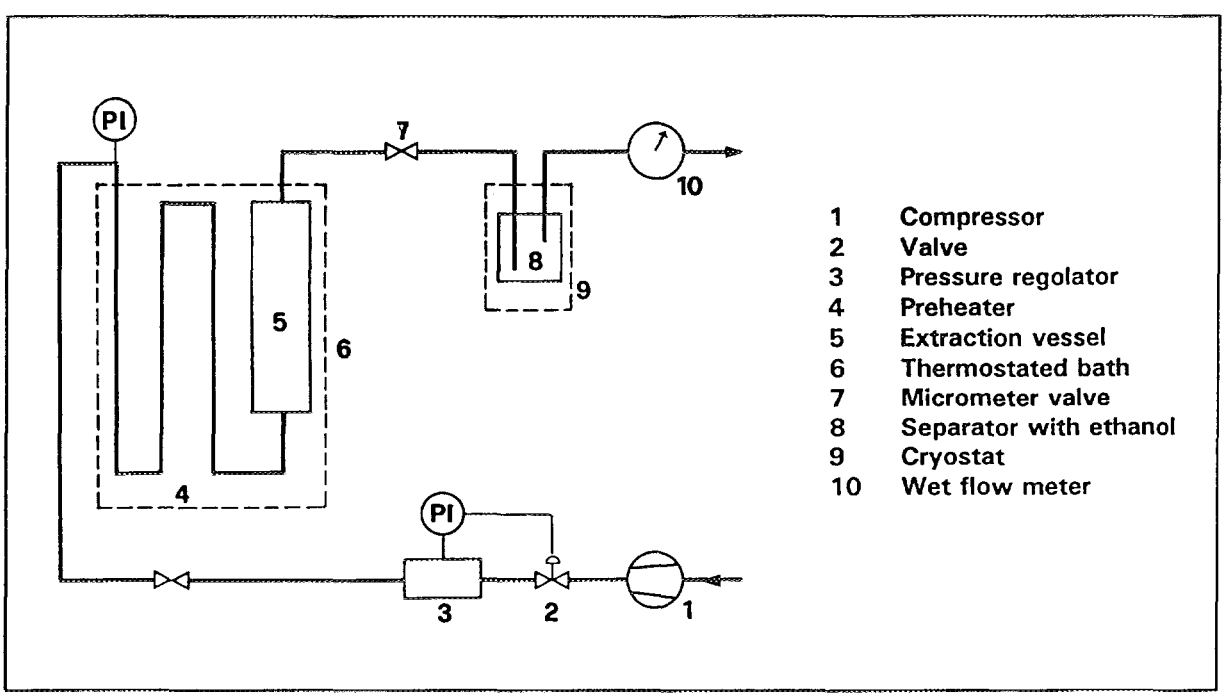

Figura 2 -Experimental Apparatus

which the quantity of solvent proved excessive (in that all the oil initially present in the reservoir was solubilized by the carbon dioxide), the run was repeated after halving the quantity of solvent introduced.

EXPERIMENTAL PROCEDURE

The experimental runs were carried out by following a standard procedure outlined below.

The contact vessel was first filled with glass beads in order to increase the liquid-gas interface. In the first phase, the vessel was loaded with a set quantity of oil, and carbon dioxide was then introduced until the required extraction pressure was obtained. Once the desired pressure had been obtained, the vessel was left in static conditions for about 12 hours.

In the second phase, the valve introducing the carbon dioxide was opened at the same time as the valve for extraction of the supercritical phase in such a way as to obtain a flow rate of carbon dioxide equal to approximately 200 ml/min. During this phase, the stream of gas leaving the extractor was bubbled through a cold trap pre-loaded with ethanol. In this way, the vapours of essential oil were brought to a liquid state through a process of condensation and absorption. The flow of carbon dioxide was halted when the meter placed after the trap indicated that a quantity equal to 100 N litres had gone through.

In the third phase, the extractor was degassed and the quantity of refined oil obtained was determined

gravimetrically. A sample of the residual oil was then taken to perform, after dilution with ethanol, gas chromatographic analysis so determining its composition. Some preliminary runs were performed to determine the margin of error involved in overlooking the quantity of oil sticking to the walls of the vessel and the filling. To this end, the reactor was washed with a weighed quantity of ethanol and the solution thus obtained was subjected to GC analysis. The previously determined calibration curve was used to calculate the quantity of oil adhering to the surface of the extractor and the filling. It was estimated between 0.5 and 0.8 g.

In the last phase, a sample was taken of the solution formed within the cold trap in order to determine both the composition and the quantity of the extract by means of gas chromatographic analysis.

RESULTS AND DISCUSSION

Table 1 and table 2 summarize the results obtained during the experiments discussed in this paper. Table 1 shows the quantities of extract and refined obtained whereas table 2 reports the overall composition of the above mentioned streams. In this paper the results will be discussed in terms of total concentration of single classes, despite the fact that an analysis of the problem in greater depth would entail taking into consideration the behaviour of each of the components involved. In this connection, figure 3 compares the composition of the extract and the refined phase in the run carried out at T=40°C and P=8.0 MPa. A considerable difference can be observed between the composition of the extract, which shows enrichment in all the terpene components, and the composition of the refined phase, where the oxygenated compounds are concentrated.

Analysis of the chromatograms also showed that only the compounds characterizing the original oil were present subsequent to the extraction process in both the extract and the refined phase, which thus makes it possible to rule out the presence of any undesired phenomena of degradation. Moreover, all the compounds belonging to the same class were observed to display more or less the same behaviour with respect to the solvent. The ratios between the concentrations of components belonging to the same class thus remained constant in both the extract and the refined phase.

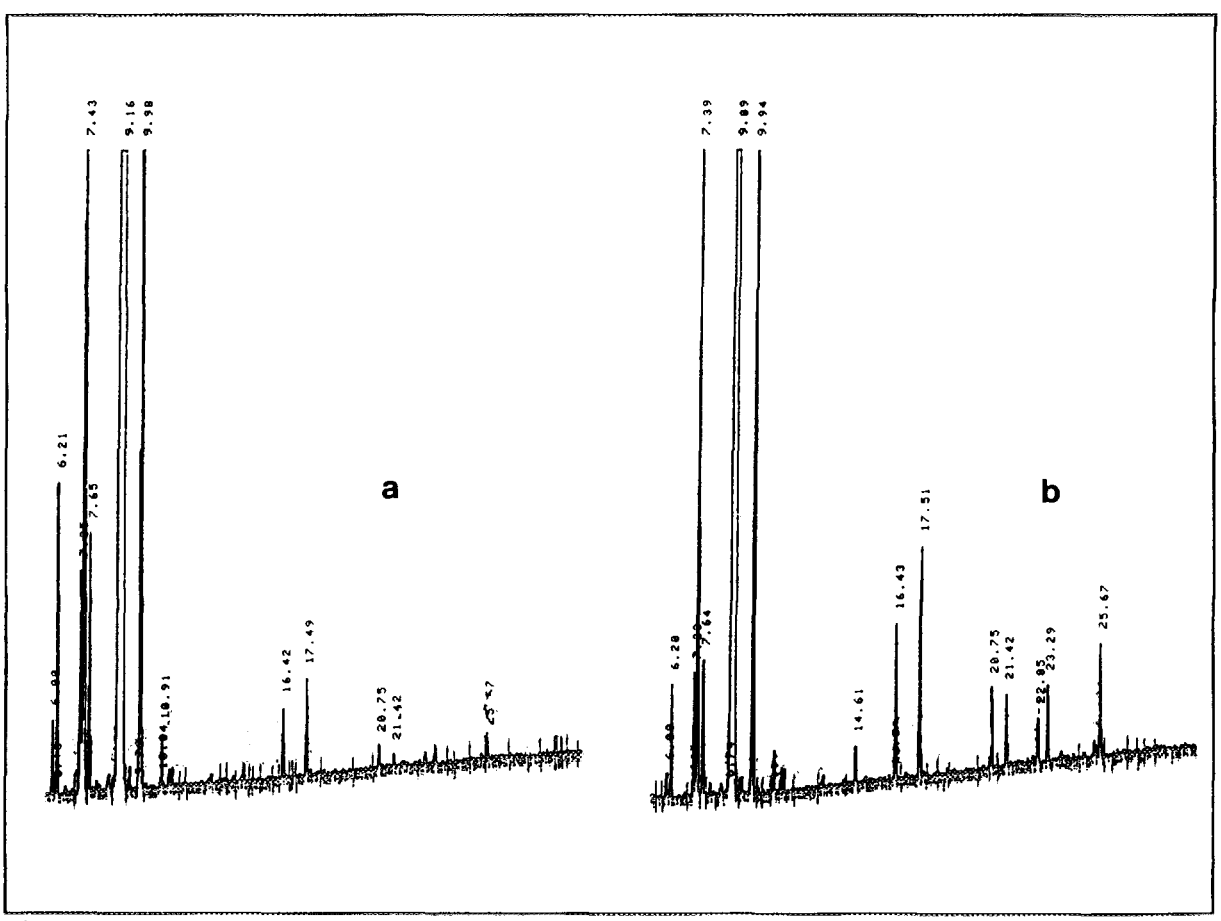

Figura 3 Chromatograms of extract (a) and refined (b)

Table 1 Extract and refined amounts

Temperature,°C	Pressure,MPa	Mass of extract,g	Mass of refined,g
37	7.5	1.30	4.3
	8.0	6.00	0.0
	8.5	6.00	0.0
40	7.5	1.25	4.60
	8.0	1.90	3.90
	8.5	5.80	0.13
		4.26*	1.47*
43	7.5	1.20	4.60
	8.0	1.70	4.1
	8.5	5.8	0.20
		1.73*	3.86*

* Data concerning 50 N liters of solvent

In general, the solubility of the components of the oil in supercritical carbon dioxide was observed to increase together with the rise in the pressure at which the operation was carried out. This is naturally due to the simultaneous increase in the solubility of all the compounds in the solvent when the pressure rises.

Analysis of the data shown in the tables makes it immediately possible to identify the values of pressure and temperature at which the system is in a non-equilibrium condition. At a temperature of 37°C and pressures of 8.0 and 8.5 MPa, the composition of the refined phase was in fact practically identical to that of the original oil, which obviously indicates the absence of any selective extraction of terpenes with respect to oxygenated compounds.

Extraction at 37°C furnishes satisfactory results only if the pressure is low. At 7.5 MPa, a terpene-rich extract is thus obtained together with a refined phase rich in oxygenated compounds. The yield of the process is also

Table 2 Extract and refined compositions

Temperature,°C	Pressure,MPa	Oxygenates, weight %	
		Extract	Refined
37	7.5	1.2%	8.5%
	8.0	6.5%	6.5%
	8.5	6.5%	6.5%
40	7.5	1.3%	8.2%
	8.0	1.2%	10.0%
	8.5	6.0%	14.0%
		5.5%*	9.2%*
43	7.5	1.5%	8.5%
	8.0	1.6%	9.4%
	8.5	5.5%	16.5%
		2.0%*	9.4%*

* Data concerning 50 N liters of solvent

interesting in that about 95% of the original oxygenated compounds are recovered in the refined phase. An increase in the quantity of solvent added would lead to greater enrichment of the refined phase, but obviously at the expense of the yield of the process. This result is very interesting in that for industrial application the temperature of the process must be kept as low as possible in order to avoid phenomena of thermal degradation which could impair the fragrance of the enriched oil. Extractions at 8.5 MPa were instead characterized by high solubility of the oil together with a small quantity of refined phase. In view of the fact that the latter in any case displayed substantial enrichment in oxygenated compounds (with a concentration of 14% at 40°C and 16.5% at 43°C), the tests were repeated with a smaller amount of solvent (50 N litres) to ascertain the influence of the solvent/feed ratio on yield. The use of a smaller amount of solvent was found to result in a lower concentration of oxygenated compounds in the refined phase, as is logical, but also an increase in the recovery of the same compounds. In particular, at 40°C the addition of 50 N litres of carbon dioxide gave 35% recovery of oxygenated compounds in the refined phase, whereas the addition of 100 N litres gave under 10% recovery. The situation shows marked improvement at 43°C, with nearly 90% recovery being achieved with the smaller quantity of solvent as against 10% with 100 Nlitres. The need is thus evident to optimize performance by determining which operating conditions (temperature, pressure, quantity of solvent added) furnish a high concentration of oxygenated compounds in the refined phase together with a yield of at least 70-80%.

The tests carried out at 8.0 MPa also proved satisfactory, being characterized by fairly high levels of extraction performance. At a temperature of 40°C, we thus obtained a 10% concentration of oxygenated compounds in the refined phase and 82% recovery. Similar results were obtained at 43°C, with a slightly lower concentration of oxygenated compounds in the refined phase but approximately the same rate of recovery.

Feasibility tests carried out on the process should take into account the fact that for industrial purposes the concentration of oxygenated compounds in deterpenated oils must be over 10% at the very least.

CONCLUSIONS

Supercritical fluid extraction of natural lemon oil was carried out in a semibatch apparatus into which the oil was introduced at the outset whereas the carbon dioxide flowed continuously.

The influence of temperature and pressure were examined during the tests, while a constant solvent/feed ratio was maintained throughout. The results obtained show clearly that this process can be used to obtain deterpenated essential oils with concentrations of oxygenated compounds in the refined phase ranging between 10% and 16%. In order to assess the feasibility of the process it would obviously be necessary to take into consideration also the yield to be obtained as determined by the ratio between the quantity of oxygenated compounds initially present in the essential oil and the quantity of the same recovered in the refined phase. Under the experimental conditions adopted, the yield ranged from a minimum of about 0.1 to a maximum of 0.95 (obtained together with a concentration of oxygenated compounds in the refined phase equal to about 10%). The problem of determining the operating conditions (temperature, pressure and solvent/feed ratio) needed to optimize performance will be tackled in a later phase of the study.

Acknowledgements

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HOT AIR DEHYDRATION IN PROCESSING BASIL

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Thermal drying of basil is studied in a pilot plant shelf dryer. The aim of the work is to suggest operative conditions that make it possible an enhancement of the quality of the dry product and to find correlations useful in scale-up. The findings of this study suggest that in order to get full flavour, an attractive green colour and a suitable particle size distribution after grinding, not too high air temperatures, 60 °C or so, should be used, preferably starting from blanched basil. In this condition a k_LA value equal to 25 kg dry air/hr may be suggested with an error within ±40% while UA may be calculated with the following correlation: $UA = 0.534V_G$ with an error depending on the reliability of the measurement of the temperature of the drying surface of the material.

INTRODUCTION

Thermal removal of moisture from food products is a very common practice; the main objective is the extension of storage life, but very often some other appreciable results are achieved, namely easier packaging, handling and transportation, improved milling and sometimes quality enhancement. Nevertheless some drawbacks come together, mainly changes in colour, flavour and sometimes in appearance. In order to enhance benefits and minimize disadvantages, preliminary experimentation in pilot plants is suitable to define the best working conditions and to determine the parameters for process calculations (1). Owing to the importance of flavour, colour and storage life, this practice is of particular consequence in processing of spices and herbs.

This paper reports experimental results of hot air dehydration of basil (*Ocimum basilicum*) operations carried out in a batch pilot plant in order to obtain products with long shelf-life period (residual moisture lower than 7 % and water activity lower than 0.6) and very similar in organoleptyc characteristics to the fresh product.

EXPERIMENTAL SET-UP

The experimental runs were carried out in a shelf batch pilot dryer (Fig. I). 1.3 kg of basil were put upon the drilled bottom of the shelf (0.5 x 0.8 m^2) and hot air was circulated through the material ("through drying"). Air was recycled to a great extent: recirculation ratios in the range 5÷15 were tested. Before entering the shelf, the air was heated by a battery of electric resistances: experimental runs with inlet air temperatures in the range 40÷75 °C were carried out.

The dryer was equipped with a centrifugal fan with 3000 m^3/hr (t = 20 °C) maximum flow rate. The runs were performed at costant head loss through the shelf (i.e. shelf bottom plus basil bed); therefore, owing to the variations in porosity of the basil bed, the air flow rate was consequently modified.

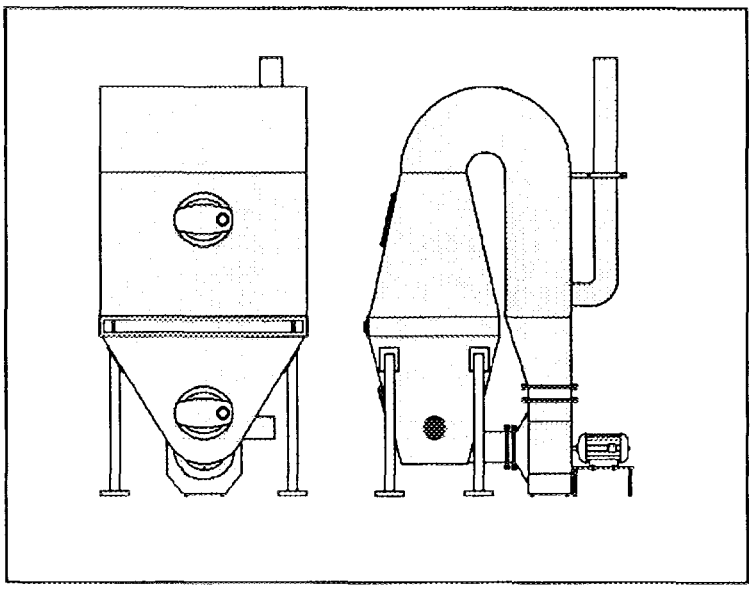

Fig. I. Pilot dryer.

According to the scheme in Fig. II, in addition to the measurement of the head loss through the shelf (ΔP), measurements were done of air flow rate (F), air humidity (H) and air temperature (T) at the inlet of the dryer and over the basil bed and of temperature at the outlet of the shelf. Moreover moisture content, water activity and temperature of basil were recorded. The drying process was considered complete when moisture content was lower than 7% and water activity was steadily about 0.1 (1). The final product was grinded and sieved.

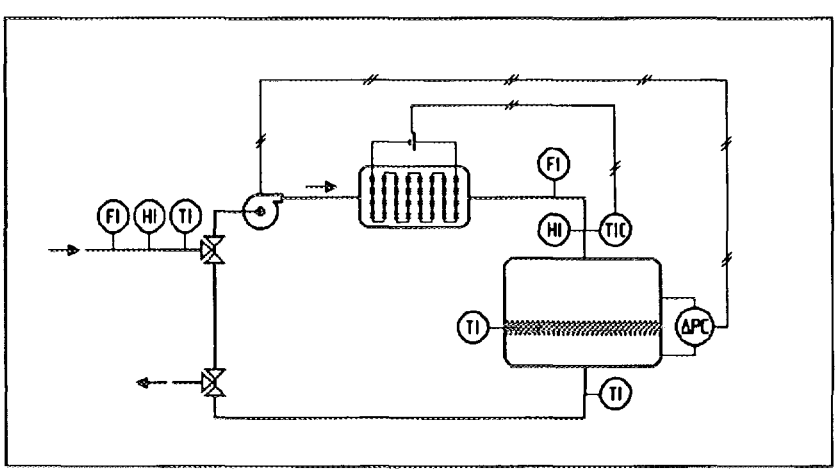

Fig. II. Flow sheet.

A comparison with commercial products was done about colour: the applied system was the tristimulus colorimetry.

DRYING RATE AND DIFFUSIVITY

The total amount of moisture removed in a time interval t_2 - t_1 is given by Eq. 1; therefore it can be calculated from the findings about air humidity and flow rate.

$$m = \int_{t_1}^{t_2} (y_{OUT} - y_{IN})\, m_G\, dt \qquad (1).$$

It can be experimentally determined too by weighing the basil in the shelf at the beginning and at the end of the interval t_2 - t_1. So also the residual moisture content M of the basil at the end of each time interval can be determined. The corresponding drying rate R_D can be calculated in the same way. Fig. III shows the rate-of-drying-versus-moisture-content plot at three air inlet temperatures (60, 70 and 75 °C). These drying curves are related to the mechanism by which drying occurs; in particular it can be observed that at the lowest temperature R_D is high and continuously decreasing while at the highest temperature R_D is lower and, after a constant-rate period, a very sharp decrease follows. This behaviour might be due to the resistance offered by the essential oils and vax compounds to the water transfer: at high temperature, these products form a film that is destroyed only after a long exposition to hot air.

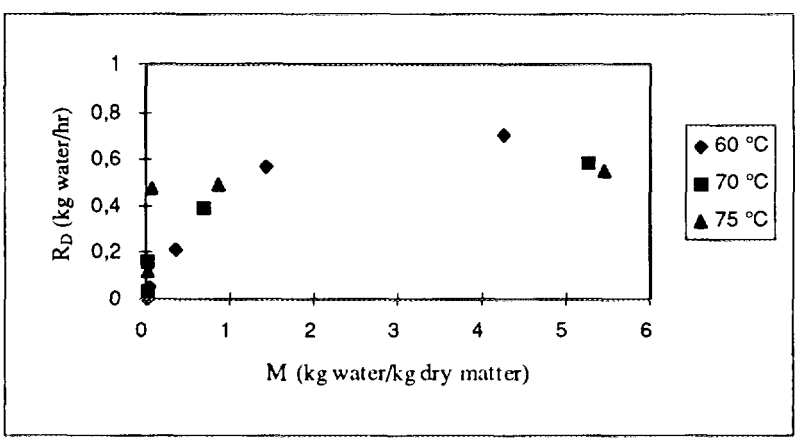

Figure III. Drying rate.

It is well known that in many cases the rate of moisture movement can be studied by means of a modified Fick's law (the leaf is assumed an infinite slab) (2):

$$\frac{dM}{dt} = D\frac{\delta^2 M}{\delta x^2} \qquad (2).$$

Diffusion coefficient *D* can be considered constant and the process mono-directional; so the integration of Eq. (2) gives (3):

$$\frac{M - M_e}{M_c - M_e} = \frac{8}{\pi^2}\left[e^{-kt} + \frac{1}{9}e^{-9kt} + \frac{1}{25}e^{-25kt} + \ldots\right] \qquad (3),$$

with $k = D(\pi/l)^2$ = dehydration coefficient and l = distance from face to center of the slab. Eq. 3 can be truncated after the first term:

$$\frac{M - M_e}{M_c - M_e} = \frac{8}{\pi^2}e^{-kt} \qquad (4)$$

obtaining a correlation that adequately fits the drying-rate curves. This way the value of k can be determined (4).

Unfortunately, owing to the difficulty in giving a value to the distance l, the value of the diffusion coefficient *D* cannot be estimated with sufficient accuracy. This problem is well known (5) and is particularly evident in the studied case since the modifications that occur in the structure of basil during the drying dramatically affect the process.

However the evaluation of the dehydration coefficient k provides useful information about the development of the process. Fig. IV shows the plot of the productivity of dry matter $R_I = kW_S$ (kg dry matter/hr) versus time at the three temperatures.

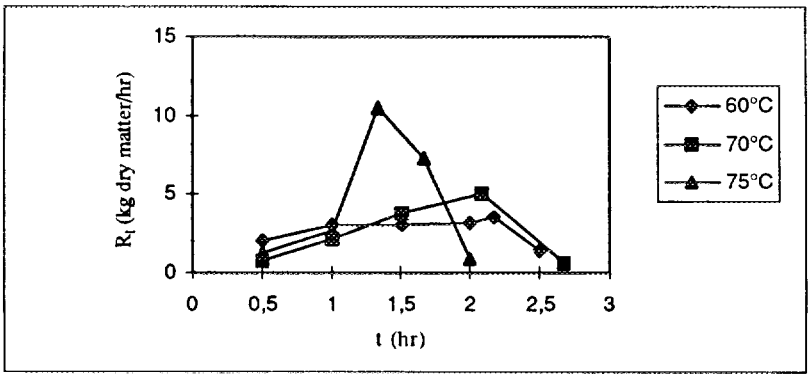

Figure IV. Diffusion versus time.

MASS AND HEAT TRANSFER COEFFICIENTS

In drying simultaneous mass transfer and heat transfer occur. During the constant rate drying period the drying rate R_D depends upon the mass transfer coefficient and the heat transfer coefficient from the solid surface to hot air according to the relation:

$$R_D = k_L A(y_P - y_A) = UA(T_A - T_P)/\lambda \qquad (5).$$

According to Fig. III, real constant rate drying periods are absent in basil processing; in spite of this, under an industrial point of view, a data processing making use of Eq. 5 provides useful information for scaling up and improvement of working conditions (6). In fact the value of y_P can be calculated from the experimental value of water activity of basil a_w, the value of T_A can be assumed equal to the arithmetical mean of those determined at the inlet and at the outlet of the shelf, y_A can be obtained from the humidity chart and the temperature of basil T_P is directly measured during the runs. Since some measurements are often not sufficiently accurate, considerations on sensitivity are added later on.

The effect of air flow rate on $k_L A$ for air inlet temperatures in the range 60°+75 °C is shown in Fig. V: in the ranges of air flow rate and air temperature tested $k_L A$ is almost constant and a tentative value of 25 kg dry air/hr may be suggested with an error within ±40%. The sensitivity analysis proves that this result can be used with good reliability in scale-up calculations: for instance, assuming that the measurements of air humidity and water activity are affected by an error equal to ± 10%, the $k_L A$ error ranges between -56%÷+84%.

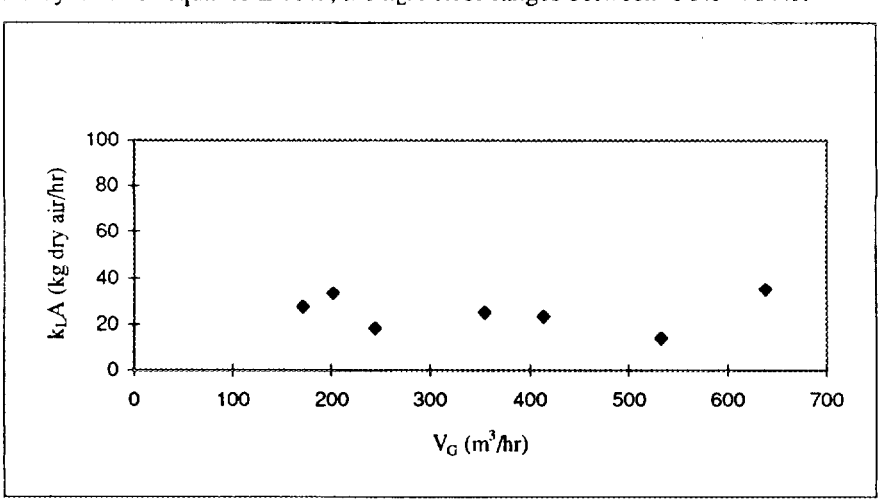

Figure V. Mass transfer coefficient versus air flow rate.

Fig. VI shows the plot of UA versus air flow rate at four air inlet temperatures. The plot suggests a weak influence of temperature probably linked to air viscosity variations and a linear dependence on air flow rate. This finding confirms that air flow is laminar. The correlation shown in Fig. VI is:

$$UA = a\, V_G \qquad (6)$$

with a = a(T) given in Table I.

air inlet temperature T (°C)	45	60	70	75
a (kcal/m^3 °C)	0.570	0.534	0.520	0.514

Table I. a = a(T).

The sensitivity analysis has been done for heat transfer coefficient with reference to possible errors in measurement of product temperature T_P. In fact this measurement is seldom fully reliable since the temperature of the drying surface should be determined without inconveniences from the inner material (colder) and from the air (warmer). This is particularly evident in basil drying since it consists of different materials (leaves and stems) and their size is rather small. So, even if checks can be done (wet bulb temperature, heat balance), there are reasons for being doubtful about the results. Moreover in the tested working conditions the system is very sensible: a 10% error in T_P may lead to a 100% error in UA. This suggests prudence in the use of UA data.

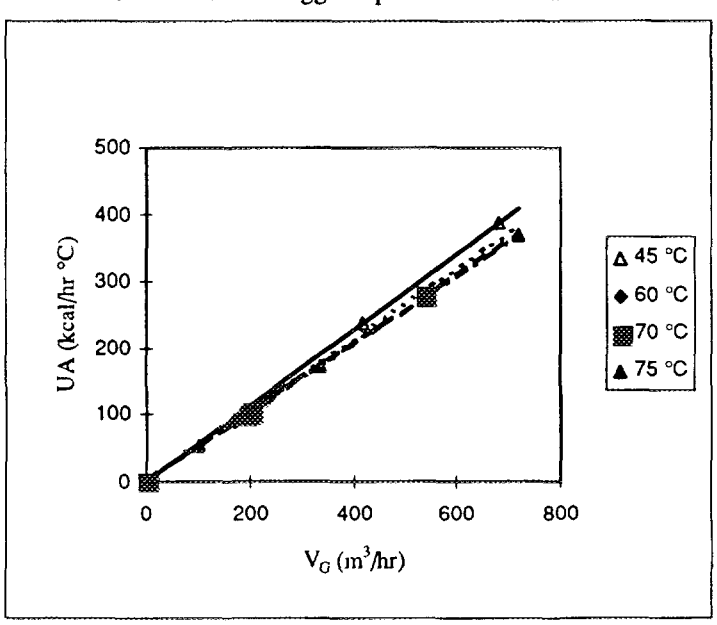

Figure VI. Heat transfer coefficient versus air flow rate.

CHARACTERIZATION OF DRY BASIL

From an industrial point of view, the physical characterization of dry basil must be done on the basis of the achievable particle size distribution and of the colour, relating the results to the operative conditions.

GRINDING AND SIEVING

Dry basil has been grinded in a test laboratory mill and then sieved through the following sieve series:

sieve number	1	2	3	4	5	6
sieve opening (mm)	5.00	3.35	2.50	1.40	1.00	0.50

obtaining 7 screenings. The results obtained with runs at different air inlet temperature are shown in Fig. VII; it shows that processing temperature has a considerable effect on achievable particle size distribution. The material that remains above the 5.00 mm sieve is considered refuse, so operations at high temperature should be preferred, but the higher efficiency is mainly due to stems that in high temperature operations become more brittle and therefore are size reduced under 5.00 mm. Since stems are the part of basil with lesser quality, their substantial presence in the final product has a negative effect. On account of this air inlet temperature may be regarded as a discriminating factor in order to attain high efficiency or high quality; in fact, in order to reconcile these results it would be necessary a preliminary grading of the basil which is generally an expensive operation (and, at any rate, reduces the global efficiency of the production).

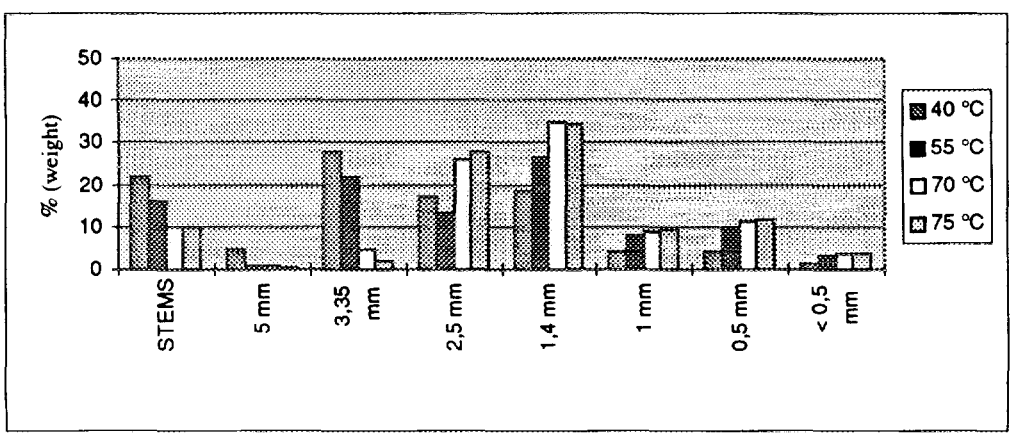

Figure VII. Granulometric distribution of basil dried at different air temperatures.

COLOUR

Processing temperature is also responsible for the final product colour. The differences are visible at a first sight, but these findings must be demonstrated scientifically. In this frame, the tristimulus colorimetry method has been proved and the results have proved it to be perfectly suitable. Each sample has been tested with a lighting source D65 (corresponding to daily light of central Europe), and the three coordinates of CIELAB system, a*, b* and L, have been measured. The achieved values are average ones, since milled and sieved basil fragments have different chromatic characteristics; at any rate each sample has been tested three times after remixing, and the results have shown a very good reproducibility.

Fig. VIII shows the results of CIELAB system (L = constant); it compares the dry basil produced in runs carried out at different air inlet temperatures with a commercial product and with a previously blanched basil (15 s with saturated steam at 130 °C).

The representative point of blanched basil is placed at the left of the diagram in the domain of green; going from left to right an always more evident red component is present which, in the range considered in the diagram, gives to the product a darker shade. The vertical coordinate (up = jellow, down = blue) is almost ineffective, therefore low temperatures are preferable to higher ones.

In order to analyse the effect of the duration of the process, the run at 70°C considered in Fig. VIII lasted 5 hours against the 3 hours of the run at 75°C; the findings show that the duration of the exposure to hot air is very important too and higher temperatures for shorter times may give a better product than the opposite conditions.

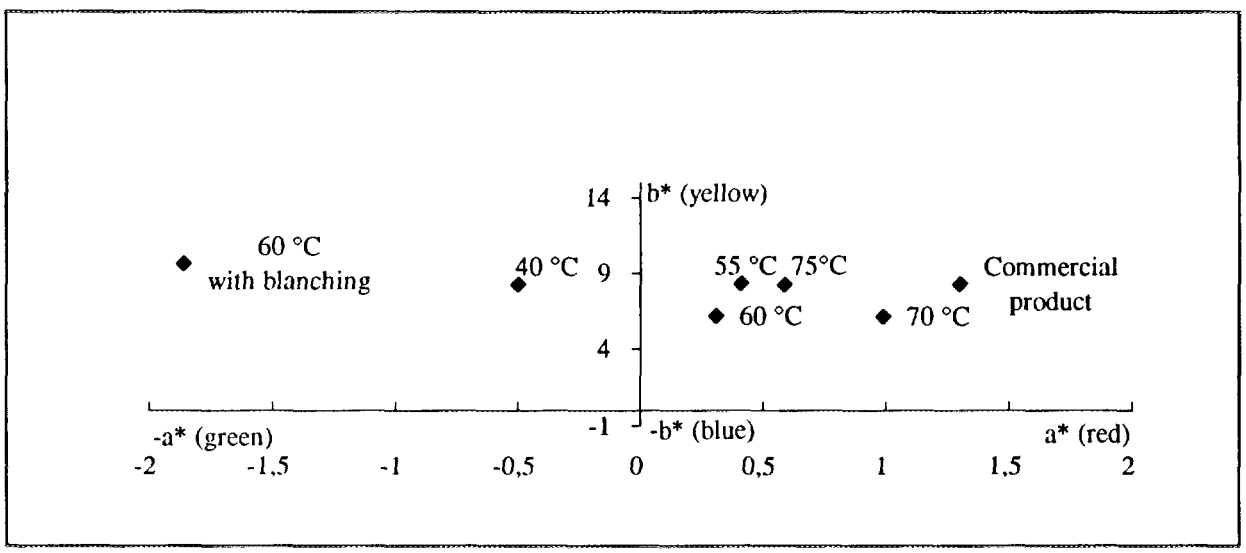

Figure VIII. Colour results.

CONCLUSIONS

Drying of basil presents the difficulties normally met with foodstuffs. In particular problems arise from being a mixture of materials with different characteristics: stems of small, but well defined, surface area and poor content of valuable products and leaves of large surface area, frequently overlapping and rich of aromatics. Besides the dry product should conserve full flavour and an attractive green colour and present a suitable particle size distribution after grinding. At last an enrichment in leaves ought to be possible, after grinding, only by sieving the material. The findings of this work suggest that the best way to approach these results all together is to work at not too high temperatures, 60°C or so, preferably starting from blanched basil. In this condition a k_LA value equal to 25 kg dry air/hr may be suggested with an error within ±40% while UA may be calculated with the following correlation: $UA = 0.534V_G$ with an error depending on the reliability of the measurement of the temperature of the drying surface of the material.

ACKNOWLEDGEMENTS

The authors would like to thank dr. Ernesto Allione for the financial support to the research and for the valuable contribution to the development of the work.

SIMBOLOGY

a = constant in Eq. (6) (kcal/m^3 °C)

a*, b*, L = CIELAB system coordinates (-)

a_w = water activity (-)

A = mass and heat transfer surface (m^2)

D = diffusion coefficient (m^2/s)

k = dehydration coefficient (s^{-1}) = $D(\pi/l)^2$

k_L = mass transfer coefficient (kg dry air/m^2 hr)

l = distance from face to centre of the leaf (m)

m = removed moisture (kg water)

$\dot{m}_G$ = mass air flow rate (kg dry air/hr)

M = water content on dry basis (kg water/kg dry matter)

M_c = critical water content on dry basis (kg water/kg dry matter)

M_e = equilibrium water content on dry basis (kg water/kg dry matter)

R_D = drying rate (kg water/hr)

R_I = dry matter productivity (kg dry matter/hr)

t = time (hr)

T = temperature (°C)

T_A = arithmetical mean between temperatures of air entering and leaving the shelf (°C)

T_P = basil temperature (°C)

U = heat transfer coefficient (kcal/m^2 hr °C)

V_G = air flow rate (m^3/hr)

W_S = dry matter (kg dry matter)

x = distance from the plane passing in the middle of the leaf (m)

y_A = moisture of air at T_A (kg water/kg dry air)

y_{IN} = moisture of process air entering the dryer (kg water/kg dry air)

y_{OUT} = moisture of process air leaving the dryer (kg water/kg dry air)

y_P = moisture of air in equilibrium with product (kg water/kg dry air)

λ = latent heat of vaporization of water (kcal/kg)

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DRYING OF PARTICULATE FOODSTUFFS IN A CONFINED FLUIDIZED BED

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SYNOPSIS

A novel drying apparatus, based on the confined fluidization technique, is presented. It consists of a fixed bed of coarse particles whose interparticle voids, filled with the fine powder to be dried, constitute the fluidization environment. This system, particularly suitable for heat-sensitive products, can be considered as an alternative to fluid bed driers with immersed heating surfaces. Drying experiments have been performed on two food powders, semolina and corn flour, and the effect of different variables on the drying kinetics has been investigated. Experimental results obtained allow to conclude that confined fluidized bed driers are very efficient units in which the drying time is reduced also with respect to conventional fluid bed driers. More work is needed to extend the application of this drying technique to food powders having high initial moisture content and small particle size.

INTRODUCTION

Many raw materials as well as intermediate and finite products of agriculture and food industry are produced in form of powders. An important role in their processing is played by drying, which allows to extend the foodstuff storage life, to enhance their quality and to improve packaging, handling and transportation stages.

Indeed, due to the drying process, the residual moisture content in foods and agricultural products is lowered and, consequently, water activity decreases.

It is well known that the rate of bacteria, yeasts and moulds growth, enzymic and non-enzymic reactions and lipid oxidation, which cause food spoilage, are inhibited or reduced at low water activity, i.e. at low moisture content. The shelf life of dried foods is thus assured, provided that materials impermeable to external atmosphere are used for packaging.

Drying also improves some characteristics of foods like palatability and digestibility and, as side effects, also the colour , the flavour and the aspect of foods are changed as a consequence of the process. Moreover, packaging, handling and transportation of dried products are easier and cheaper, due to the reduction of weight and volume of products with respect to wet materials, also because of the improvement in their flowability. Finally, further processing stages on dried products, like milling, take far less energy than that required to process wet products.

In the last decades, the utilization of fluidized beds has been recommended for drying of granular agricultural and food products, due to the excellent heat and mass transfer rates attainable, to the reliable thermal control of the drying process consequent to the rapid mixing of solids and to the low maintenance costs of the apparatus having no mechanical moving parts[1,2].

In fluid bed driers rapid heat and mass transfer between particles and gas allows to avoid overheating of products, so that volatile and heat sensitive components are retained in the products. That increases sensory and nutritional properties of dried foodstuffs. Moreover, fast solid particle mixing leads to nearly isothermal conditions thoughout the fluidized bed, thus avoiding uneven treatments or overprocessing of foods.

However, relatively few industrial scale drying units are presently in operation, due to the poor fluidization properties exhibited by foods and agricultural products, which are generally characterized by very fine or coarse sizes and irregular shape. Very fine solids are cohesive and tend, when fluidized, to undergo channelling and slugging if the gas velocity is kept low to prevent excessive elutriation of fines. On the other hand, coarse and odd-shaped particles require high gas velocities to be sustained and, when fluidized, give rise to disuniformities in gas permeation as well as in solid circulation which damp the aforementioned excellent transfer properties of fluidization.

Different kinds of fluid bed driers have been deviced: conventional plug-flow, vibrofluidized units, two-component fluid bed , fluid bed driers with immersed heating surfaces.

In plug flow fluid bed units the residence time distribution of particles is uniform, so that homogeneous processing of powders is achieved. Due to their flow characteristics, very low moisture contents can be obtained even though the thermal efficiency of the apparatus is not very high as most of water release takes place near the gas distributor. Furthermore, the gas flow rate flowing across the bed in form of bubbles bypasses the contact with the solids, lowering further the thermal exchange efficiency of the process.

In vibrofluidized beds particles are mantained in the fluidized state by the combined action of air flow and vibration. These units are used to dry cohesive and sticky powders, which tend to form agglomerates that would block driers employing unassisted fluidization. Powders with a wide particle size distribution and fragile or abrasive materials can be treated in such systems as well.

Treatment of coarse particles, as in the case of most agricultural products, can be conducted in two-component fluid bed driers[3]. Coarse particles undergoing drying are immersed in a fluidized bed of fine solids in which they can move freely. The difference of density between the granular products and the fluidized bed is such that they undergo no segregation effect. The heat and mass transfer coefficients between the dense phase of fines and the immersed particles are of the same order of magnitude of those measured between gas and particles in conventional fluidization[4].

Fluidized bed driers equipped with immersed heating surfaces are very suitable for drying heat-sensitive foodstuffs, when severe limitations in gas velocities and temperature levels in the bed have to be accepted to prevent thermal damages. In this case, if the heat needed to dry the products were supplied with the sole inlet air, an excessively large distributor would be required. To overcome this difficulty, part of the heat is transferred to the powder by means of external heating surfaces immersed in the solid bed.

In addition to the several fluidization modes used in powdered food drying operations mentioned above, this paper aims at presenting some preliminary experimental results obtained by a different technique, the confined fluidization.

THE CONFINED FLUIDIZATION TECHNIQUE

In the conventional fluidization technique, when the system is operated at gas velocities much higher than the minimum fluidization value, a large part of the gas flow rate finds its way through the bed in form of bubbles, which promote the mixing within the particulate mass; the bubble flow, anyway, constitutes a way through which much of the gas bypasses the fluidized bed, thus putting a limit to the heat and mass transfer efficiency of the operation.

To rise the effectiveness of the contact between gas and particles an alternative fluidization technique has been proposed, with the objective of suppressing or at least minimizing the presence of bubbles in the particulate system while however allowing a certain circulation of fine solids[5,6] ; it consists in fluidizing a bed of relatively fine particles within the voids of a fixed bed of a coarser spherical material, which acts as a confining environment. For that reason the technique is called "confined fluidization".

The basic principles of confined fluidization are illustrated in Fig.1.

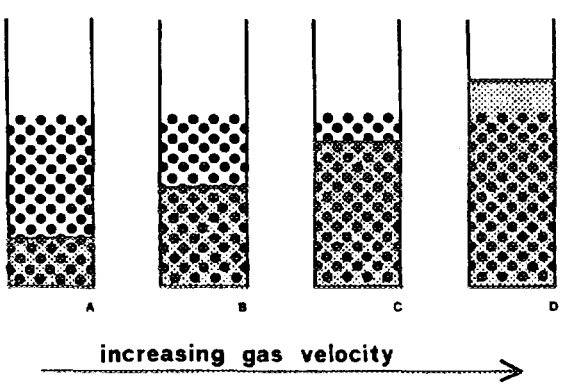

Fig.1. Behaviour of a fine particle bed of particles undergoing confined fluidization.

Provided that the size ratio between the two components of the system is high enough to allow free percolation, the fluidizable material forms a fixed layer at the bottom of the coarse packing as soon as it is poured into the column (A). By admitting and subsequently rising the gas flow rate through the bed, a superficial gas velocity $U_{mf,c}$ is reached at which the fine particles attain their incipiently fluidized state (B); this can be calculated as[5]

$$U_{mf,c} = k \frac{\rho_P g \varepsilon_c^2 d^2}{150 \mu_g} \frac{\varepsilon_f^3 (1 - \varepsilon_f)}{\left[\varepsilon_c (1 - \varepsilon_f) + (1 - \varepsilon_c) \frac{d}{D} \right]^2} \quad (1)$$

and for any higher velocity the confined fluidized bed undergoes a progressive expansion (C), described by a power law of the type[6]

$$U = \alpha \varepsilon^n \quad (2)$$

that can make its voidage assume values even higher than 0.9. Equation (2) holds also after the fluidized bed has exceeded the packing height forming a segregated bubbling layer on the top of it (D), but that is not a desirable mode of operation.

Altogether, the distinctive feature of a confined fluidized bed is that of reacting to any gas flow rate increase past minimum fluidization with its expansion, rather than with bubble formation. Compared with the conventional fluidization technique, where the interstitial velocity within the dense phase is practically unchanged by a velocity increase, the prevention of bubbling is accompanied by an unusual increase of the gas-particle slip velocity. This can easily grow, at constant total pressure drop, up to levels that can greatly accelerate any process whose kinetic is determined by the velocity of mass transfer phenomena occurring between particle surface and gas phase. This is the case of drying processes that operate on particulate solids for which the fluidization technique is however advisable, expecially when the drying mechanism is controlled by the superficial heat transfer and the rate of moisture removal is constant. In such a situation, a secondary benefit provided by the confining sphere packing is that of acting as a thermal flywheel for the fluidized particulate mass.

EXPERIMENTAL SET UP AND PROCEDURE

Experiments reported in this paper were performed in different types of driers: a natural convection oven, a forced convection oven with temperature and humidity control, a conventional fluidized bed and a confined fluidized bed. Food powders subjected to the drying treatment were semolina and corn flour, whose physical properties are reported in Table 1. The drying temperature was always chosen between 40 and 60°C to avoid starch gelatinization.

Table 1
Properties of food powders

Material	d_P [μm]	ρ_P [kg/m^3]	U_{mf} [cm/s]	X_0 [kg H_2O/kg s.s.]
Semolina	344	1450	5.50	0.103
Corn flour	393	1540	4.60	0.134

The fluidized bed drier consists of a perspex column 70 mm ID and 600 mm high. A porous plate at the base of the column, made of sintered brass, provides a uniform distribution of the gas flow throughout the bed cross section.

The fluidizing gas is air, preheated to the test temperature by an electrical heat exchanger, which in turn is regulated by a PID controller. The air flow rate is measured by a set of rotameters and the column is insulated with glass wool. The temperature of the inlet gas and of the bed is measured with thermocouples, one of which drives the temperature controller. A water manometer, fitted to a pressure tap at the base of the column just above the gas distributor, is used to measure the pressure drop across the bed.

The same apparatus is used in the confined fluidization drying experiments. The confining packing is made of stainless steel spheres 10 mm in diameter. An external rule aside the column allows reading the height of the confined bed of fines.

Fluidization experiments are preliminarly made, to determine the minimum fluidization velocities of the two powders in both the unconfined and the confined condition.

Subsequently, fluid bed drying experiments are carried out batchwise as follows: a predetermined weight of powder, of known initial moisture content, is introduced into the fluidized bed at ambient temperature. Every ten minutes, powder samples are taken from the fluid bed drier and introduced in an oven, operating at a temperature of 105 °C, to determine, by differential weighing, the residual moisture content. The same experimental procedure is used to determine the drying kinetics having place in the confined fluidized bed drier.

For the sake of comparison, drying experiments are also performed in a natural convection oven and in a temperature and humidity controlled oven, which simulate the drying conditions in conventional driers. The test temperature is set at the same values as in the fluid bed experiments and the relative humidity in the climatic chamber is always kept at 3%.

RESULTS AND DISCUSSION

The effect of some of the principal variables influencing the drying process has been investigated by carrying out a set of experiments on the two solids in the drying systems previously described.

Figure 2 shows typical drying curves for semolina at 45 °C in different apparatus. It can be observed that in the confined fluidized bed drier the drying kinetics in the first drying period, i. e. the constant rate period, are very fast with respect to that measured in the natural convection oven and in the fluid bed drier. In the second drying period, the falling rate period, drying rates become very slow and the process is controlled by the rate of diffusion of moisture inside the particles. That means that the reduction of the external resistance to heat and mass transfer, which is one of the important characteristics of the fluidization operation, does not play any role in this case. The drying kinetics measured in the confined fluid bed drier and in the fluid bed drier in the falling rate period are similar and still faster than that measured in the natural convection oven.

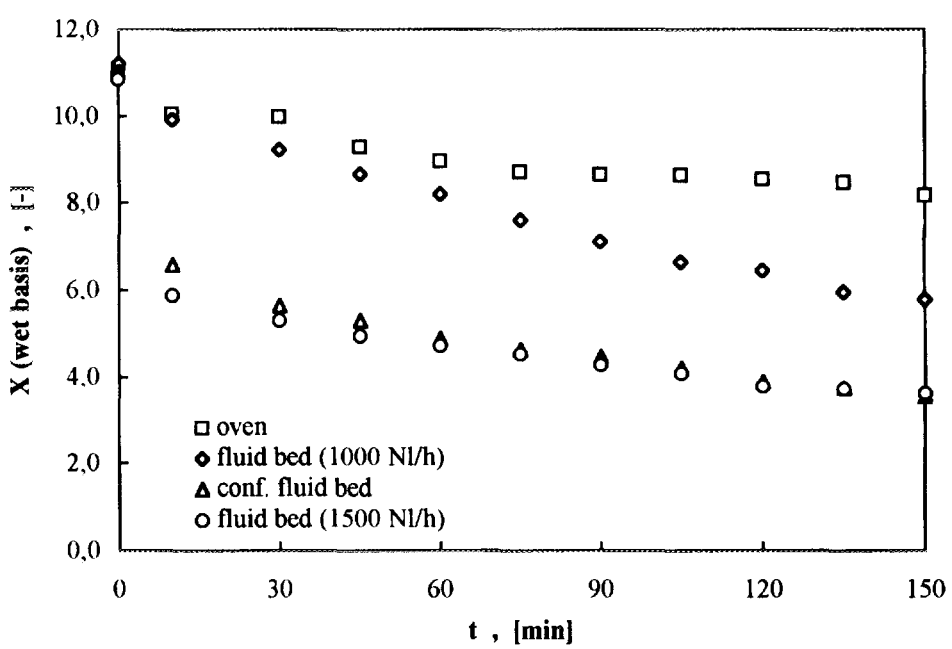

Fig.2. Curves of moisture content of semolina (wet basis) vs. time in different driers.

The performance comparison between conventional and confined fluidized bed driers is made both at equal superficial and interstitial gas velocity. In the first condition, i.e. at equal gas throughput and total energy expense, the confined system appears much more efficient, owing to the higher gas-particle slip velocity, which is expected to be the key variable when the external resistance is the one controlling the heat and water transfer rates. This

circumstance find its confirmation when the two systems are operated at the same interstitial gas velocity: in this case the drying kinetics in the two driers are nearly coincident.

When reported in terms of ratio of residual over initial free moisture content, as done in Fig.3 for semolina, the influence of process equipment and operating conditions on drying kinetics can more easily be evaluated.

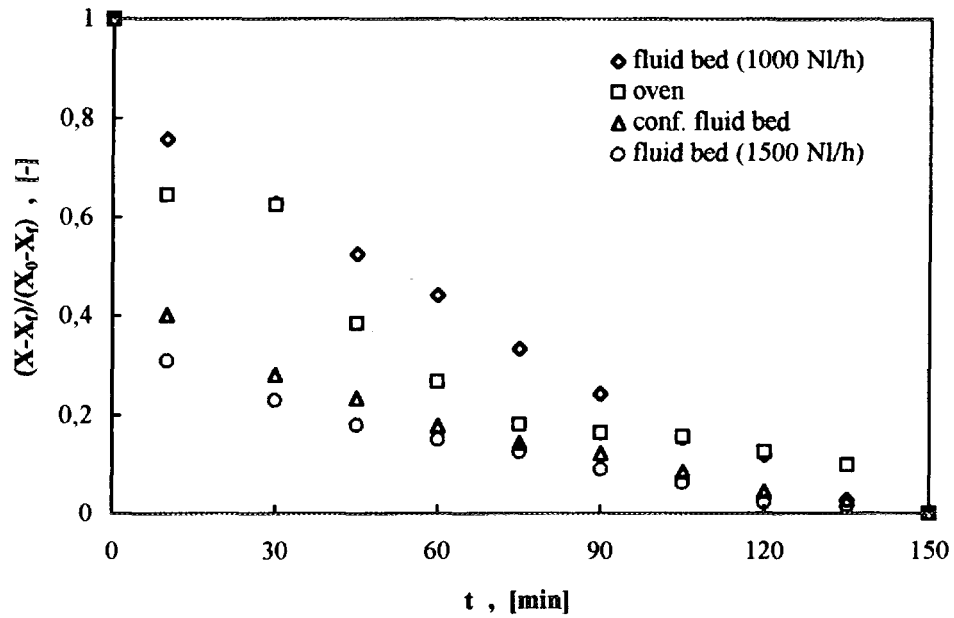

Fig.3. Dimensionless moisture reduction vs. time for semolina in different driers.

The advantage of fluidization and the role of operative velocity on drying kinetics in the two fluidized bed driers can again be observed, and a specific feature of the confined technique of fluidization, affecting the first minutes of the constant rate period of drying is highlighted: due to thermal flywheel action of the coarse packing constituting the confining environment, the fresh material entering the fluidized bed unit undergoes a heating action which is

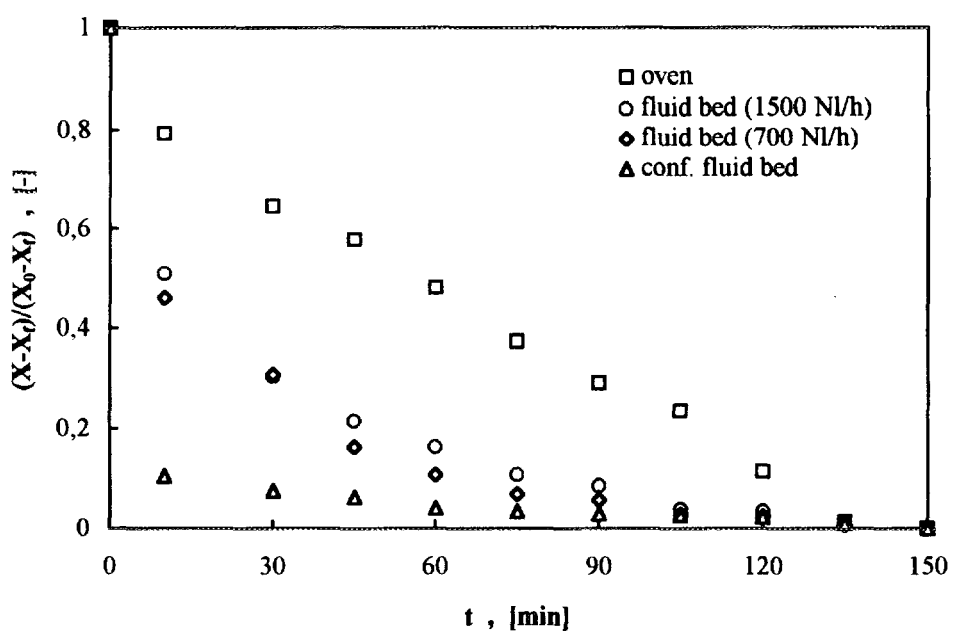

Fig.4. Dimensionless moisture reduction vs. time for corn flour in different driers.

much faster than in the conventional fluidization, where the fluidizing medium is the only heat source available for the solid.

Though not reported here, experiments confirmed the well known result that temperature affects the drying rate. As the temperature of the bed increases, the drying rate in the fluid bed drier increases. The same trend in also observed in the confined fluid bed drier as well as in the natural convection and in the forced convection oven.
Analogous results obtained with corn flour are illustrated in Fig.4. The higher efficiency of the confined fluidization technique is here more evident, even when the conventional fluid bed is operated at the same interstitial gas velocity. Drying is much faster in the first period, whereas the efficiency of the process decreases in the subsequent period of velocity fall.

CONCLUSIONS

Experimental results obtained thus far allow the following conclusions:

a) confined fluidization is a promising technique which can be used to dry particulate foodstuffs. In particular, it appears to provide a decrease in the drying time with respect to that measured in conventional fluidization, at least in the period of constant drying velocity;

b) the practical absence of bubbles, through which the excess gas flow rate tends to bypass the fluidized bed, seems to be the reason of its enhanced thermal efficiency, as the gas-particle contact is improved;

c) the presence of the confining spheres reduces the bed section available for the gas flow. Thus, at the same gas flow rate, a higher interstitial velocity is reached with respect to a conventional fluidized bed unit of the same cross section. A faster particle circulation and a better contact between gas and solid, which are promoted by the increase of gas velocity, determine a parallel increase of drying rates.

More work is anyway needed to clarify other fundamental aspects of dynamics and of heat and mass transfer in confined fluidized beds, together with the drying characteristics of various types of food and agricultural products, in order to predict drying kinetics of foodstuffs in these fluidized systems.

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SYMBOLS

d fine component diameter
D coarse component diameter
d_P mean particle diameter
g gravity acceleration
k parameter in Eq.(1)
n expansion index
t time
U superficial gas velocity
U_{mf} minimum fluidization velocity
$U_{mf,c}$ minimum fluidization velocity in the confined state
X moisture content of the solid
X_0 initial moisture content
X_f final moisture content

α parameter in Eq.(2)
ε fluidized bed voidage
ε_f, ε_c voidage of fine, coarse particles
ε_{mf} minimum fluidization voidage
μ_g gas viscosity
ρ_P particle density

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MODELING OF PRECONDITIONERS IN EXTRUSION COOKING: MEASUREMENT OF RESIDENCE TIME DISTRIBUTION

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Preconditioning has long been used in pasta and pet food manufacturing areas and has been introduced to breakfast cereal manufacture in the last decade as a means to increase twin screw extruder throughput.
The use of preconditioners is based on empirical data, with no basic understanding of related processing functions.
Our research program has the goal of developing a fundamental mechano-chemical model for the preconditioning and extrusion process. The first step in understanding the processes occurring in the preconditioner is the development of a model which describes the conditions experienced by the material as it is moved through the equipment.
The residence time distribution is useful for determining processing time characteristics, flow patterns as well as mixing efficiency in the preconditioner. Residence time distributions were measured using corn meal containing manganese dioxide as tracer. After introducing a well-defined plug of manganese dioxide into the preconditioner, samples of the output were collected, and analyzed for manganese content by Neutron Activation Analysis, using the University of Toronto's SLOWPOKE nuclear reactor facility.
The effect of several parameters such as feed rate, paddles configurations and shaft speed were investigated. The results will be compared with theoretical models derived for melt extrusion and other mixing unit operations.

INTRODUCTION

Preconditioners are used extensively to preheat and prehumidify biopolymer-based raw materials such as flours and grits, by mixing them with steam and water.

Benefits of preconditioning include increased product capacity by reducing the amount of mechanical energy required to cook the dough in the extruder, reduced extruder wear through reduced dough viscosity and improved product quality through more uniform cooking of dough. Larger particle size may be utilized with little change noted in finished product characteristics.

Preconditioners can have two distinct designs: single-paddled shafts and counter - rotating twin paddled shafts, inside an atmospheric chamber to which water and steam may be injected.

The flow of material in a paddle preconditioner is complicated, as these devices are conveying particulates solids. While a fluid is a continuum, particulates behave in a very different discontinuous fashion (Levine [1]). As a consequence to successfully and completely mix powders every section of the powder's bulk must come in contact or very nearly in contact with part of a moving paddle.

Although preconditioners are often used, they were developed and applied through empirical approaches, with no basic understanding of related processing functions (Bouvier [2]).

Thus this research proposes an engineering analysis of preconditioning and a description of the major processing functions carried out in these devices.

The residence time distribution (RTD) is a measure of the length of time process material spends in the preconditioner.To obtain experimental data on the residence time in the equipment an inert tracer is added to the feed material as an "instantaneous" plug

Mathematically, Danckwerts ([3]) has defined RTD function E(t) such that E(t)dt is the fraction of the mass at the exit which has spent a time between t and (t+dt) in the system.

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \cong \frac{C(t)}{\sum_0^\infty C(t)\Delta t} \qquad (1)$$

Where C(t) is the tracer concentration appearing at the outlet at time, t.

By integrating E(t), the cumulative residence time distribution function F(t) can readily be obtained.

$$F(t) = \int_0^t E(t)dt \cong \frac{\sum_0^t C(t)\Delta t}{\sum_0^\infty C(t)\Delta t} \qquad (2)$$

The two most important measures to characterize a distribution are the location, given by the mean residence time $\bar{t}$

$$\bar{t} = \int_0^\infty tE(t)dt \cong \frac{\sum_0^\infty tC(t)\Delta t}{\sum_0^\infty C(t)\Delta t} \qquad (3)$$

and the spread of the distribution, measured by the variance σ^2.

$$\sigma^2 = \int_0^\infty t^2 E(t)dt - \bar{t}^2 \cong \frac{\sum_0^\infty t^2 C(t)}{\sum_0^\infty C(t)} - \bar{t}^2 \qquad (4)$$

Although the RTD data is very useful in its own right, comparison of experimental data with theoretical models is a useful means of defining the behavior and performance characteristics of a preconditioner.
Two models suggested by Levenspiel ([4]) one being the tanks in series (5) model and the other being the dispersion model (6) were considered in the present study.

$$E(\vartheta) = \frac{N(N\vartheta)^{N-1}}{(N-1)!} \cdot e^{-N\vartheta} \qquad (5)$$

where N is the number of perfectly mixed tanks in series and ϑ is the reduced time

$$\vartheta = \frac{t}{\bar{t}}$$

$$E(\vartheta) = \frac{1}{2\sqrt{\pi\left(\frac{D_e}{uL}\right)}} \exp\left(-\frac{(1-\vartheta)^2}{4\left(\frac{D_e}{uL}\right)}\right) \qquad (6)$$

Where D_e is the axial dispersion coefficient, u is the average axial velocity and L is the barrel length.
$\frac{D_e}{uL}$ is termed the reactor dispersion number, varying from zero for plug flow to infinity for back mix flow.
Sometimes one-parameter models are unable to describe satisfactorily the flow systems then more complicated models must be considered. These usually consider the real system to consist of different regions (plug, dispersed plug, mixed, deadwater) interconnected in various ways (bypass, recycle or crossflow) (Levenspiel[4]).
For a combination of plug flow and perfect mixing a RTD model has been developed by Wolf and Resnick ([5]):

$$F(\vartheta) = 1 - \exp\left[-\left(\frac{1}{1-P}\right)(\vartheta - P)\right] \qquad \vartheta \geq P \qquad (7)$$

$$F(\vartheta) = 0 \qquad 0 < \vartheta < P$$

where P is the fraction of material in plug flow.

The experimental results were compared with all the theoretical RTD function described here in order to analyze and characterize the flow in the preconditioner. The experimental distribution function were calculated by using the data of output concentration and the equation [(1),(2)].

MATERIALS AND METHODS

Experiments were carried out in a counter-rotating twin shaft preconditioner (Extru-tech Inc.,Kansas,USA).The following operating conditions were varied : (a) feed rate (200 kg/h, 300 kg/h, 400 kg/h, 500 kg/h), (b) screw speed (20 Hz , 40 Hz, 60 Hz), (c) paddles configuration.

For all process conditions three replication were performed to evaluate the reproducibility of the residence time measurement technique.

The feed material was yellow corn meal, packed by King Milling of Canada (Chatham, Ontario, Canada). Manganese dioxide was used as a tracer for residence time distribution, as described by Wolf, D. & White,D.[6] The reasons for choosing this particular tracer is that it is inert in this system and it is readily detected by neutron activation analysis with little or no interference from food components and its relatively short half-life, which avoids longtime radiation contamination problems The half-life for the γ emitting Mn^{56} isotope is 2.576 hr.The Mn^{56} is obtained by radiation of the stable isotope Mn^{55} in a nuclear reactor[7]. The reaction is :

$$Mn^{55} \xrightarrow{(n,\gamma)} Mn^{56}$$

Sample of product (< 1 g) were placed in polyethylene vials and irradiated at the SLOWPOKE reactor at the University of Toronto according to the method of Davidson et al.[8]
An irradiation time of 180 sec was used at a neutron flux of $5x10^{11}$ neutrons cm^{-2} sec^{-1}. The amount of Mn^{56} isotope produced was determined by counting the γ ray emittance of each sample for 200 seconds with a Canberra 8180 Multichannel Analyzer equipped with a Ge detector after a delay time (time between end of irradiation and starting of counting) of 20 minutes. Emission energies at 847 and 1811 keV were measured.

The experimental procedure for the determination of the residence time distribution functions involves an impulse stimulus tracer in the feed stream. This is also know as a Dirac pulse or delta function in the input.
After the preconditioner was operated at steady state with regard to the flow rates, pressure and temperature , 1.2 g of manganese dioxide was added istantaneously into the feed stream and the time of injection was recorded. The output was collected every 25 seconds over a period of 15 minutes at 20 Hz, every 25 seconds for 10 minutes at 40 Hz, every 20 seconds for 5 minutes at 60 Hz.

RESULTS AND DISCUSSION

To evaluate the effects of several controlling variables on the RTD a series of experiments were carried out under selected operating conditions.

The operating conditions along with the mean time $\bar{t}$ and the variance σ are presented in table 1.
The mean residence time decreases with increasing shaft speed and feed rate. Shaft speed also affect the shape of the distribution as indicated by its variance while the effect of feed rate is negligible.
The results also indicate, as shown in the following figures, that a small amount of material is retained in the system for long time.

The effect of shaft speed

The RTD dependence on shaft speed is shown in figures 1 and 2. Two sets of data at different feed rates are reported to illustrate the effect of increasing the speed. It is easily seen that raising the shaft speed shifts the RTD to the left and shortens the mean residence time. These data also seem to indicate that the shaft speed changes the shape of the distribution as indicated by the variance and by its tendency to narrow the RTD.

The effect of feed rate

The throughput of the preconditioner can be varied over a wide range and is independent of paddle speed.The influence of feed rate on RTD is shown in figure 3 . The higher the rate, the more uniform the distribution. It seems that at the lower rates when the preconditioner is less filled, there is less tendency for the preconditioner to provide good mixing.

Table 1. Operating conditions and computed data for mean residence time and variance .

Experiment	Feed rate (kg/h)	shaft speed (Hz)	$\bar{t}$ (s)	σ^2 (s^2)
1	200	20	365	31765
2	200	40	228	19858
3	200	60	120	5470
4	300	20	298	36759
5	300	40	201	20632
6	300	60	109	5481
7	400	40	189	20556
8	400	60	100	5019
9	500	40	165	19610
10	500	60	94	4982

Figure 1.The effect of shaft speed on RTD at 200 kg/h

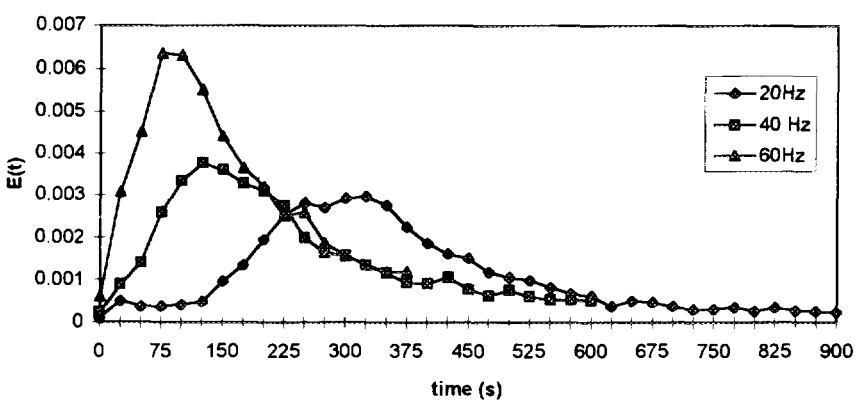


Figure 2. The effect of shaft speed on RTD at 300 kg/h

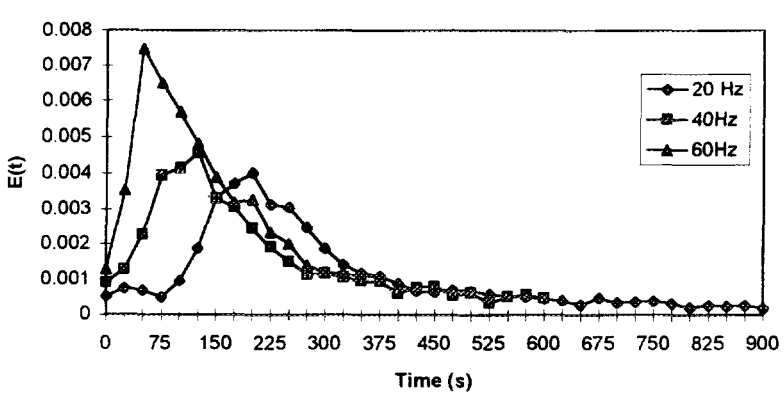
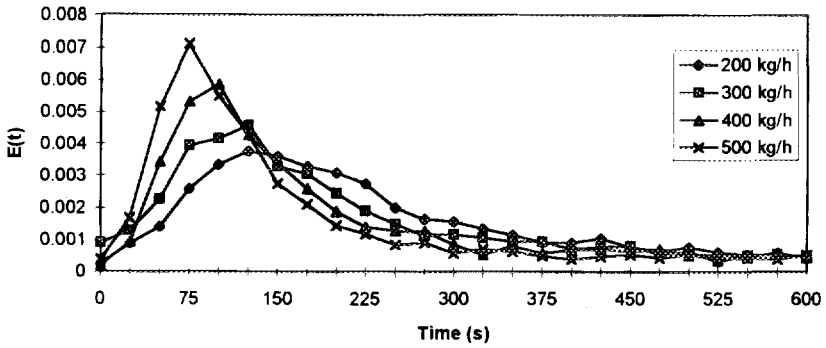


Figure 3.The effect of feed rate on RTD at 40 Hz



The effect of paddles configurations

In figures 4 and 5 the results for two different paddles configurations are reported. The second configuration is obtained adjusting the paddles in position 5 and 6 from neutral to reverse on all rows.

The data in table 2 and the distributions in figures 4-5 indicate that the flow and mixing patterns are similar for both configuration.

Additional paddles configurations need to be tested to determine whether the effect is coincidental or is a feature of preconditioner.

Table 2. Operating conditions and computed data for mean residence time and variance for changed configuration

Feed rate (kg/h)	shaft speed (Hz)	$\bar{t}$ (s)	σ (s^2)
500	60	96.73	4920
400	40	192	19393

Figure 4 The Effect of paddle configuration on RTD at 500 kg/h and 60 Hz

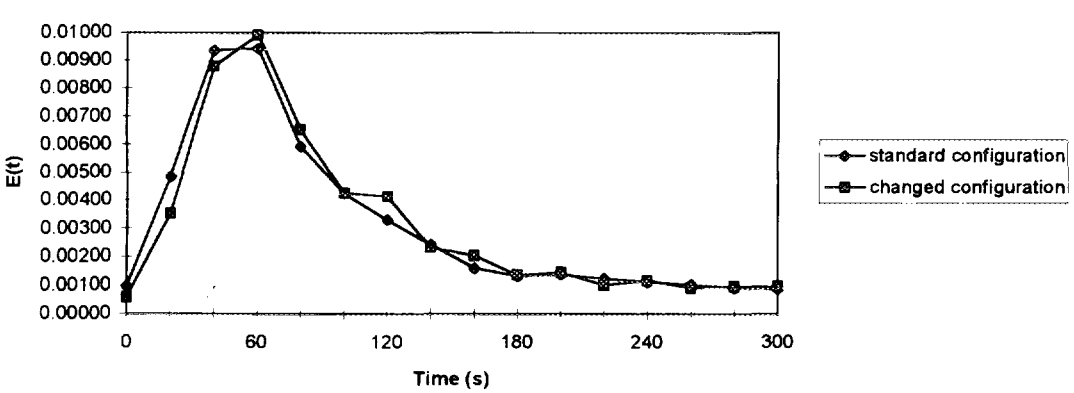
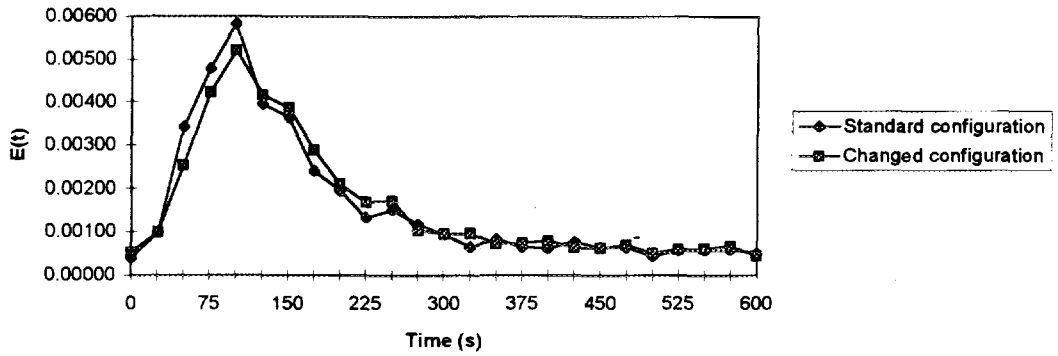


Figure 5. The effect of paddle configuration on RTD at 400 kg/h and 40 Hz



Comparison with theoretical models

The experimental RTD curves were initially compared for their fit to the dispersion and tanks in series model (equations 5 and 6).
No satisfactory fit was obtained as shown in figures 6 and 7, except for few conditions including 300 kg/h and 60 Hz where the tanks in series model with N=2 matches the experimental points quite well (figure 8).
The deviations observed in the data from the theoretical dispersion and tanks in series model suggested the use of a mixed model which divide the real system into different flow behavior sections(equation 7).
Figure 9 illustrates that a good fit is obtained at P value (fraction of plug flow) of 0.1.
The P value can be considered a measure of conveying performance, while (1-P) can be considered as a measure of the mixing performance.(Agur[9]).
These results indicate that ~ 10% of the mass moves by a plug flow while ~ 90% of the mass is perfectly mixed.

Figure 6. Comparison of experimental distribution E(θ) to tanks in series model

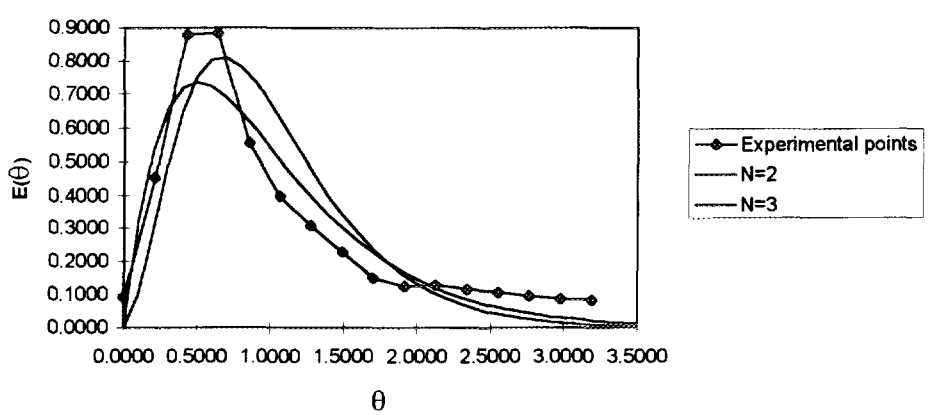


Figure 7. Comparison of experimental distribution E(θ) to dispersion model

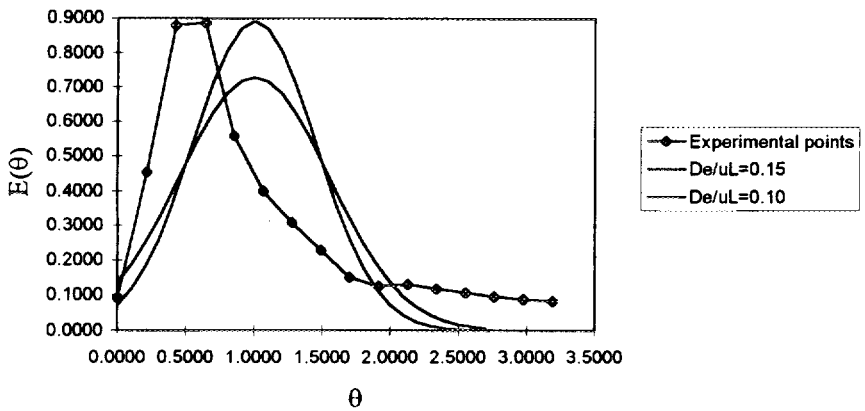


Figure 8.Comparison of experimental distribution E(θ) to tanks in series model

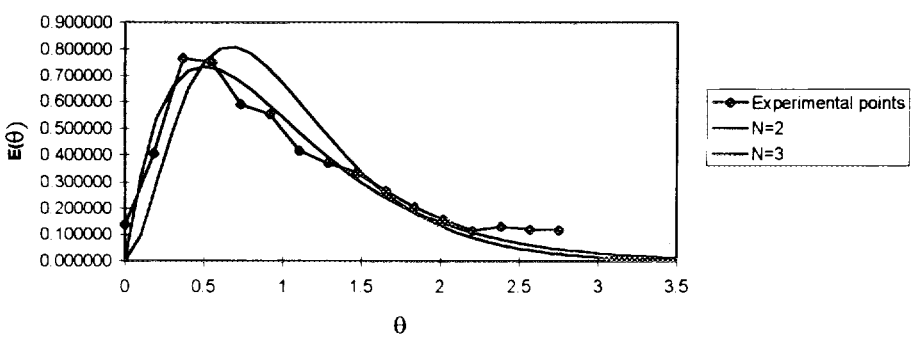
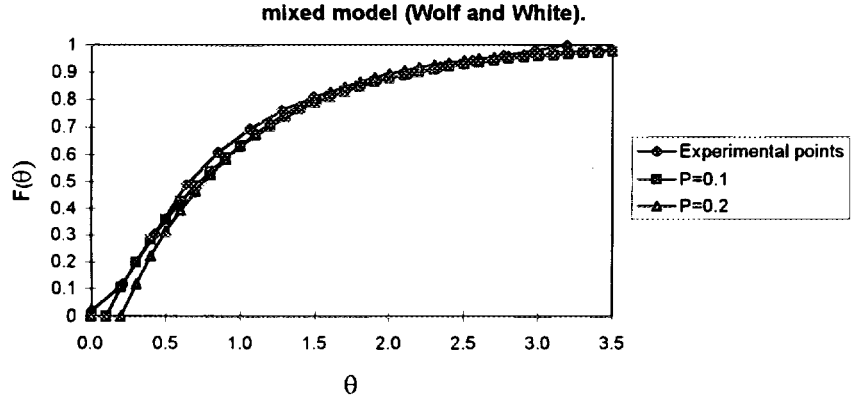


Figure 9. Comparison of experimental distribution F(θ) to mixed model (Wolf and White).



Conclusions

The residence time distributions were measured experimentally to determine the effect of operating conditions on the flow patterns in a preconditioner.The following results emerged:

- RTD depend on feed rates and paddle speed.

- The effect of changed configuration does not seem very evident.The one change in paddle configuration investigated had very little effect on RTD. Additional paddles configurations need to be tested.
- The model of Wolf and Resnick (equation 7) which assumes that the flow pattern is a combination of plug flow and perfect mixing was shown to adequately describe the flow behavior of the system.

The results clearly indicate that the premixer does not provide complete mixing and some of the material passes through the machine without mixing. A small amount of material is retained in the system for very long periods of time.This has some implications in controlling microbial contamination in these machines and this should be investigated further, to ensure that product safety is mantained under all actual operating conditions.

NOTATION

C(t)	Tacer concentration at time t (p.p.m.)
D_e	Axial dispersion coefficient (cm^2s^{-1})
E(t)	Residence time distribution function
E(θ)	Normalized residence time distribution function
F(t)	Cumulative residence time distribution function
F(θ)	Normalized cumulative residence time distribution function
L	Barrel length
N	Number of CSTRs in series
P	Fraction of plug flow
t	Time(s)
u	Axial velocity
$\bar{t}$	mean residence time (s^2)
θ	Normalized time
σ^2	variance of residence time distrubution (s^2)

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INCREASING OF ESSENTIAL OILS LOW YIELDS IN STEAM DISTILLATION METHOD BY INORGANIC SALTS ADDITIVES

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The possibilities of low yields essential oils increasing in "traditional" steam distillation method are discussed. One of the ways to solve this problem is to replace the water by saturated solutions of inorganic salts (preferably chlorides). The use of sodium chloride increase the yield of oils up to 1.2 - 4 times. Some variations of composition of essential oil caused by salts additives are illustrated on the example of *Mentha piperita* L.

INTRODUCTION

The steam distillation method of essential oils preparation is that being known for a long time and considered as any "standard" action. In the Former Soviet Union (FSU) it was named as Ginzberg method [1]. Its role in industry at present decreases due to usage of modern technologies, but it remains the importance in the laboratory practice, particularly for the preliminary investigation of essential oil's content in plant samples and their composition.

One of the most principal restriction of steam distillation is insufficient effectivity of this technique for the samples with low oil content (approx. less than 0.1 - 0.5 % by weight). The small quantities of oils may dissolve in the water after condensation that leads to the impossibility of their separate layers formation. The typical example of this situation is the attempt to quantify the eugenol in the underground parts of Geum Urbanum L. [2], when the content of this compound in the sample is only about 0.11 %. The comparison of this value with solubility of eugenol in water (approx. 0.8 g per l [3]) indicate that the formation of separate layer of oil in the contact with water is really impossible.

The similar problem exists with the principal component of rose essential oil, - 2-phenyl ethanol, that is important for the essential oil industry. It is interesting to consider some historical aspects of different attempts to increase the oil yield from rose petals in FSU.

There was numerous proposition to use solutions of sodium chloride instead of water for the conservation of raw material and following steam distillation (see, for instance, [4,5]). The recommended concentrations of NaCl in these solutions varied within the window 15 - 25 %. This modification of "traditional" method permit authors to increase the total yield of essential oil from 0.04 % three times more.

The usage of inorganic salts additives seems like general approach for modification of commonly used steam distillation procedure relative to following aspects:

i. To increase the bath temperature;

ii. To decrease the total time of distillation, and

iii. So far as the molar part of water in salt's solutions become less comparing with pure solvent, than it is possible to expect the relative enrichment of vapour phase by vapours of oil's components. It is the reason to decrease the distillation time (ii) and the relation of organic and water phases in the heterophaseous system after conpensation.

All these effects are displayed in most degree for the saturated solutions of inorganic salts. The guarantee of solution's saturation at the boiling point is the presence of small abundance of solid salt in the bath. So, the goals of present work is the examination of influence of inorganic salts additives on the essential oils yield and composition for different species, particularly with low oil content.

EXPERIMENTAL

The samples of essential oils have been prepared by standard procedure of steam distillation [1] from 30-100 g of dry plant material and 100-400 g of water or equivalent quantites of saturated salt's solutions (the excess of solid salt at the boiling point of solution was about 5 - 10 %). Gas chromatographic analysis of oil's samples have been carried out with Biochrom-1 gas chromatograph (Inst. of Org. Chem., Moscow) with flame ionization detector and WCOT quartz column (25 m, 0.2 mm i.d. with OV-101, "ChromTech", Moscow) in the temperature programming regime (initial temperature 50 ^{0}C, final temperature 220 ^{0}C, heating rate 3 deg per min). The parameters of GC signals have been measured with integrator TR 2213 (Takeda Riken, Japan). The identification of components have been based on their randomized GC retention indices on standard non-polar polydimethyl siloxanes [6,7] (the RI values are presented in the table 3, see below) and standard mass-spectra (LKB-2091 gas chromatograph - mass-spectrometer with the same column, ionization energy 70 eV).

DISCUSSION

The comparison of physico-chemical properties of saturated solutions of different inorganic salts according to listed above criteria includes the following parameters: boiling point of saturated solution, the limit of salt's solubility in the water at the boiling point, and estimation of relative price of these salts according to Fluka catalogue (see table 1). Most preferable compounds are only inorganic chlorides of metals of I or II groups of Periodic Table. Really, sodium chloride is most cheap reagent, that confirm the widespread application just this salt in practice. However, the boiling point of NaCl saturated solution is about 109 ^{0}C, that may be increased by usage of other chlorides ($MgCl_2$, $CaCl_2$ and, theoretically, LiCl).

Table 1. Some characteristics of water solutions of inorganic salts (chlorides)

Salt	Boiling point of saturated solution, ^{0}C	Solubility (g) per 100 g of water at boiling temperature	The price relative to NaCl (from Fluka catalogue)
NaCl	108.8	40.7	1.0
$MgCl_2$	130	62.9	2.4 - 3.3
$CaCl_2$	140	138	1.1 - 1.2
LiCl	168	151	4.8

The increase of boiling point of solution even in the case of NaCl makes it possible to decrease the total time of process approximately twice and increase the essential oil yield in 1.2 - 4.0 times. This effect is more important just when oil content in plant material is low (at least less than 1 %). When this parameter is about 3 % (for some species of Mentha piperita), the yield increasing become negligible. The comparison of results of steam distillation with water and NaCl solution is presented in the table 2.

The actual problem of standard steam distillation method modifications is possible changes of sample's composition. The evaluation of reliable conclusions needs to compare a large variety of samples being prepared under different conditions of distillation. It is possible to expect any variations within group of most high boiling compounds, because the boiling water is enough reactive system for numerous thermolabile compounds (for instance, sesquiterpenes and their derivatives). Extra variations of pH of solution by additives bases or acids may influence on the relative content of acid components

Table 2. The comparison of essential oil yields from some species produced by standard steam distillation and with saturated NaCl solution

Species	The yield in standard steam distillation, %	The yield with saturated NaCl solution, %
Asteraceae		
Achillea millefolium L.	0.3	0 4 - 0.5
Balsamita major Dest.	0.4 - 0.8	0.6 - 1.3
Tanacetum vulgare L. (leaves)	0.4 - 0.5	0.5 - 1.7
Tanacetum vulgare L. (flowers)	0.8	1.0
Ericaceae		
Ledum palustre L.	0.5 - 1.0	1.3 - 1.7
Lamiaceae		
Lavandula vera L.	0.4	0.7
Mentha piperita L.	0.7 - 3.2	2.8 - 3.2

Table 3 includes the results of identification and quantification of *Mentha piperita* L. essential oil components (specially selected kind of plant with high content of menthol). The samples under comparison have been prepared by simple steam distillation (I), with saturated NaCl solution (II) and with the same solution at pH $\approx$ 13 (extra additives of 1 % KOH). The differences between percentage of the majority of listed compounds lie within permissible random errors of samples preparation and GC analysis. Only few exceptions have been revealed for two oxygen derivatives of sesquiterpenes (distinguished by typing in the table 3). The content of α-caryophyllene alcohol increase from 0.3 % (regime I) up to 0.6 % (II) and 0.9 % (III). These differences are more than normal statistical deviations. Similarly, the content of unidentified sesquiterpene alcohol $C_{15}H_{24}O$ with RI = 1588 ± 2 reflect the same tendency. It may be explained by extra or principal formation of these compounds during heating of plant material in the contact with water solutions from any hydrocarbon precursors. As the precursors for α-caryophyllene alcohol two sesquiterpenes (α- and β-caryophyllenes) may be proposed, because the percentage of these compounds decrease three times from 3.6 + 0.6 = 4.2 % to 1.4 + 0.2 = 1.6 % (distinguished by typing in the table 3 also).

Table 3. The comparison of composition (%) of *Mentha piperita* L. essential oils produces by commonly used steam distillation (I), with saturated NaCl solution (II) and with this solution in the presence of 1 % KOH (III)

Compound	Retention indices (OV-101)		Percentage, %		
	experimental	from database	(I)	(II)	(III)
α-Pinene	927 ± 1	930 ± 5	tr	tr	tr
β-Pinene	968 ± 1	969 ± 4	tr	tr	tr
Myrcene	981 ± 1	983 ± 3	tr	tr	tr
Limonene	1018 ± 1	1021 ± 3	0.3	0.5	0.4
Menthone	1135 ± 2	1139 ± 8	2.5	2.3	2.8
Isomenthone	1142 ± 2	1151 ± 6	1.1	1.0	1.4
Menthol	1160 ± 10	1165 ± 7	83.2	83.2	81.5
Piperitone	1242 ± 2	1235 ± 10	0.8	0.7	0.8
Menthyl acetate	1283 ± 1	1280 ± 4	2.9	2.9	3.0
Σ sesquiterpenes			8.5	7.3	7.1
β-Elemene	1392 ± 1	1394 ± 12	0.3	0.2	0.2
β-Caryophyllene	1414 ± 1	1417 ± 6	**3.6**	**2.6**	**1.4**
α-Caryophyllene	1442 ± 1	1447 ± 6	**0.6**	**0.4**	**0.3**
Alloaromadendrene	1466 ± 1	1464 ± 10	0.2	0.3	0.2
mixture of $C_{15}H_{24}$ including D-Germacrene	1472 ± 1	1474 ± 5	0.8	0.5	0.6
α-Elemene	1485 ± 1	1486 ± 3	0.4	0.3	0.3
γ-Cadinene	1510 ± 1	1510 ± 13	0.2	0.2	0.2
α-Caryophyllene alcohol	1555 ± 2	1556 ± 8	**0.3**	**0.6**	**0.9**
$C_{15}H_{24}O$ (tent. α-bisabolole)	1579 ± 2	1580 ± 21	1.0	0.9	0.9
$C_{15}H_{24}O$ (tent. alcohol)	1588 ± 2	-	**0.3**	**0.5**	**0.6**

Notes: tr. - traces (0.01 - 0.05 %), tent. - tentatively identification.

On the basis of this feature it is possible to imagine the principally secondary origin of α-caryophyllene alcohol not only in the II and III regimes of steam distillation, but in regime I also (without salt additives). This compound may be formed by chemical addition of water to mentioned sesquiterpene hydrocarbons. However, there are only restricted practice at present to subdivide the components of essential oils on primary substances (which really present in the plant samples) and products of their secondary transformations. It is interesting to note, that steam distillation with alkaline NaCl solution don't influence on the menthyl acetate content, which is constant in all regimes I-III (2.9 ± 0.1 %), although this compound theoretically may be hydrolyzed with menthol formation by prolonged heating in water basic or acid solutions.

CONCLUSION

The preliminary experiments permit us to conclude that "traditional" steam distillation method may be modified successfully for the species with low contents of essential oils. This modification include the replacement of water by saturated inorganic salts solutions (preferably different chlorides). It leads to the increase of oil yield in 1.2 - 4 times and decrease the total time of process. Any variations in sample's composition comparing with standard method concern only content of separate components, which may be the secondary products being formed after hydrolysis of different precursors.

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STUDY OF ENZYME MEMBRANE REACTOR FOR APPLE JUICE CLARIFICATION

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INTRODUCTION

Membrane processes such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), have been used in the food industry for clarification, depectinisation and concentration of fruit juices[1]. Ih the present work the possibility to hydrolyze pectins present in refined apple juice and raw apple pulp has been investigated. Pectins are linear polymers which are responsible of turbidity and viscosity of fruit juices[2]. They are composed, essentially, of a 1,4-linked D-galacturonic acid units, characterised by a certain extent of methylation of their carboxylic group. The authors have studied pectins hydrolysis and their separation in 1) an enzyme reactor combined with an ultrafiltration membrane unit, wherein the enzyme was compatimentalized by the UF-membrane in the reaction mixture and 2) an enzyme reactor with an UF-membrane acting as catalytic and separation unit (the enzyme was gelified on the inner membrane surface). Experiments have been carried out using capillary, tubular and spiral-wound (s-w) ultrafiltration membranes. Rapidase enzyme has been used as biocatalyst. The effect of the enzyme concentration on the permeate flux and depectinization degree has been evaluated. It has been observed that permeate flux of the refined juice treated with free rapidase increases with s-w membranes with respect to the tubular configuration. Furthermore, the permeate flux improved with increasing the enzyme percentage from 0.05% to 1 % (v/v). Finally, it decreased operating with 2% of rapidase. The permeate flux behavior of refined juice treated with both, free and immobilized rapidase, was also studied and compared. It has been observed that the permeate flux of juice treated with immobilized enzyme increased with respect to the juice treated with free enzyme.
Hydrolysis of pectins in raw apple pulp showed a good clarification level and a lower membrane fouling.

Key words: Enzyme membrane reactor, hydrolysis of pectins, pulp clarification, permeate flux.

MATERIALS AND METHODS

Refined apple juice (liquified by partial treatment with enzyme and depulped) and raw apple pulp, provided by Valle Ballina y Fernandez, from Spain have been used.
The enzyme was commercially liquid solution of rapidase (Rapidase liquid plus from Gist Brocades). It was added directly to the substrate solution or immobilized on the inner membrane surface by cross-flow filtration. The reaction mixtures or the juice have been recirculated through the lumen side of UF membranes modules, in order to remove the hydrolysed pectins. Capillary, tubular and spiral-wound membranes have been used to clarify refined juice. Capillary membranes were made of polyamide (PA), with NMWCO of 10 kDA. The membrane module was 180 mm long, the inner membrane surface area was $34 \cdot 10^{-4}$ m^2. Tubular membranes were made in polyvinylidene fluoride (PVDF), with NMWCO of 18 kDA. The membrane module was 0.5 m long and the membrane surface area was $5 \cdot 10^{-2}$ m^2. Spiral-wound membranes were made of polysulphone (PS), with NMWCO of 10 kDA. The membrane module was 0.4 m long and membrane surface area was $25 \cdot 10^{-2}$ m^2. Tubular membranes made of PVDF, with NMWCO of 15 kDA have been used to clarify raw apple pulp. The membrane module was 0.5 m long and the membrane surface area was $23 \cdot 10^{-2}$ m^2.

RESULTS AND DISCUSSIONS

UF OF REFINED JUICE TREATED WITH FREE RAPIDASE

Experiments have been carried out treating refined juice with different percentage (v/v) of rapidase (0.05-0.5-1.0-2.0) in a batch reactor for 1 hour at 25 °C. The reaction mixtures were then unltrafiltered through s-w membrane module of PS 10 kDA, having a surface area of $25 \cdot 10^{-2}$ m^2 . The permeate flux and pectins concentration vs time have been evaluated as a function of enzyme percentage. During ultrafiltration axial flow rate was 800 L/h, TMP was 0.7 kPa and temperature was 25 °C. The Fig. 1, shows the

normalized permeate flux at the steady state for the different enzyme percentages: the flux increased by increasing the enzyme percentage from 0.05 and 1.0% and decreased with 2.0% of rapidase. Pectins concentrations (measured with the m-hydroxydiphenyl method)[3] vs ultrafiltration time, as a function of three different enzyme percentages are reported in Fig. 2.

Others experiments have been carried out using: tubular membrane of 18 kDA and capillary membrane of 10 kDA. Refined juice treated with rapidase was ultrafiltered through membrane module in the same conditions discussed above for s-w module. In Fig. 3, is reported the normalized permeate flux during ultrafiltration for different membrane configurations: it was high in the case of s-w membranes with respect to the tubular and capillary membranes

In order to evaluate long-term membrane stability, several UF experiments have been carried out using PS s-w membranes of 10 kDA and $25 \cdot 10^{-2}$ m^2. A volume of 15.6 liters of refined juice has been treated in a batch reactor with rapidase enzyme 1% (v/v) for 1 hour at 25 °C. After this time, the reaction mixture has been ultrafilterd through membrane system. The permeate has been recycled to the feed stream until t=350 minutes. Afterwards the permeate has been removed and the feed concentrated until t= 520 minutes. Operating conditions were: axial flow rate = 700 L/h; TMP = 1.2 kPa; T = 25 °C. Viscosity and density of reaction mixture during batch treatment, and of feed and permeate solutions during ultrafiltration, were also detected.

Permeate flux and viscosity of feed solution during UF are reported in Fig. 4. As it can be seen, the permeate flux decreased during the first 200 minutes and then was constant until the end. On the other hand, the feed viscosity was constant during the batch recycle step and increased significantly during concentration step.

UF OF REFINED JUICE TREATED WITH IMMOBILIZED RAPIDASE

In this contest, membrane system has been used as catalytic and separation unit. Experiments have been carried out using PS s-w membranes of 10 kDA. Membrane surface area was $25 \cdot 10^{-2}$ m^2.

Both, 0.5% and 1.0% of enzyme solutions (V=6L) were prepared in a distilled water and ultrafiltered at axial flow rate of 400 L/h and TMP of 0.25 kPa. The enzyme has been immobilized by cross-flow filtration at room temperature. The amount of immobilized rapidase has been determined by mass balance between the enzyme present in the feed solution at the beginning and the one present in the retentate and permeate at the end. The enzyme concentration has been measured by BCA assay protein test from Pierce.

About 0.5 g of enzyme has been gelified in the case of UF of solution with 0.5% of rapidase and about 0.84 g in the other case (the percentage of immobilized enzyme with respect to the initial mass was 25.26% and 26.22 respectively).

After immobilization procedure, the refined juice has been ultrafiltered through the membrane module for about 2 hours. Axial flow rate was 800 L/h; TMP was 0.7 kPa and temperature was 25 °C. The permeate flux vs time has been measured for each ultrafiltration experiment. As reported in Fig. 5, the normalized permeate flux observed during UF of refined juice treated with 0.5% of immobilized rapidase increased with respect to the flux observed both at 0.5 and 1.0 percent of free enzyme.

PECTINS HYDROLYSIS IN RAW APPLE PULP

The possibility to use apple pulp instead of refined juice in pectins hydrolysis has been investigated.

Experiments have been carried out in a semi-pilot scale. Tubular membranes made of PVDF with NMWCO of 15 kDA have been used. The membrane module was 0.5 m long and the membrane surface area was $23 \cdot 10^{-2}$ m^2.

Raw apple pulp (V=6 L) has been treated with 1% of rapidase for 1 hour in a batch reactor at 25 °C. After this time, the reaction mixture appeared very liquefied and it has been ultrafiltered at 1000 L/h and at 0,67 kPa of TMP, through membrane module for about 3.5 hours, until the permeate flux was constant (see Fig. 6). It has been observed that the membrane fouling was lower with respect to the experiments with the refined juice. After cleaning procedure it was possible restore the initial water permeate flux. Pectins concentration was similar to the concentration of samples obtained from refined juice clarification.

CONCLUSIONS

The study of the performance of an enzyme membrane reactor in terms of permeate flux and depectinizzation

degree, as a function of rapidase concentration has been carried out. EMRs have been realized by using PVDF tubular and PS s-w membranes in a semi-pilot scale. The increase of enzyme concentration from 0.05 to 1% improved the permeate flux through the s-w membranes.
During ultrafiltration of juice treated with immobilized rapidase it has been observed an increase of the permeate flux with respect to the ultrafiltration of juice treated with free enzyme. Pectins hydrolysis carried out in the ultrafiltration enzymatic reactor appears of interest for the level of clarification and also for the fouling control reached.

AKNOWLEDGMENTS

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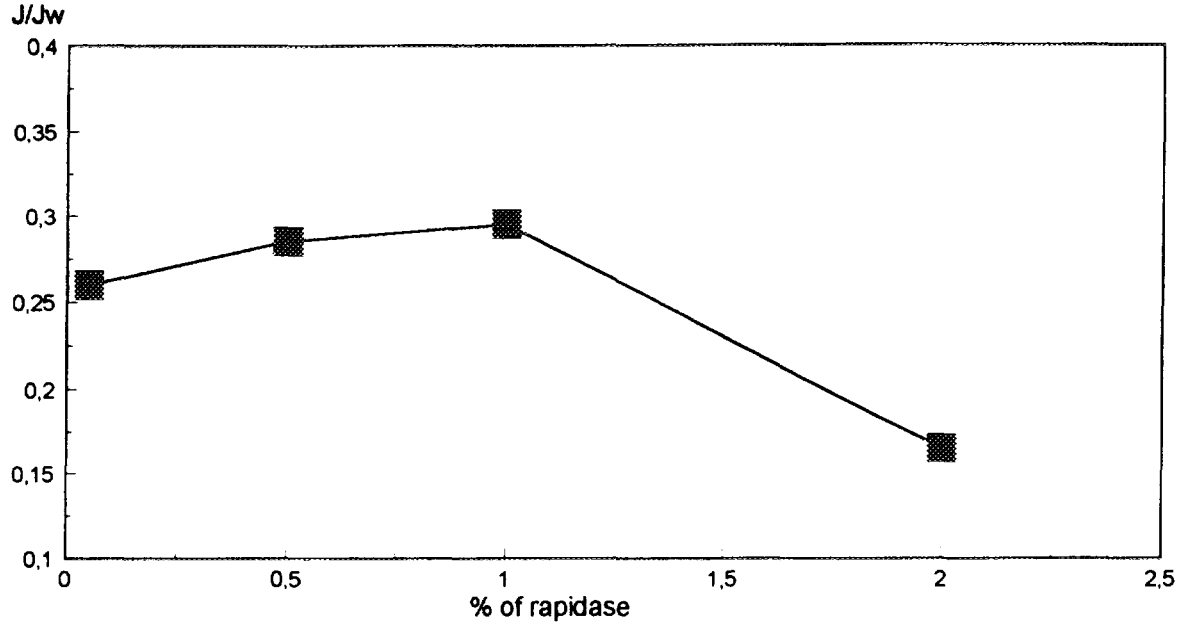

Fig. 1. Ultrafiltration of refined juice treated with different percentages of rapidase. Qax = 800 L/h; T = 25 °C; TMP = 0,7 kPa.

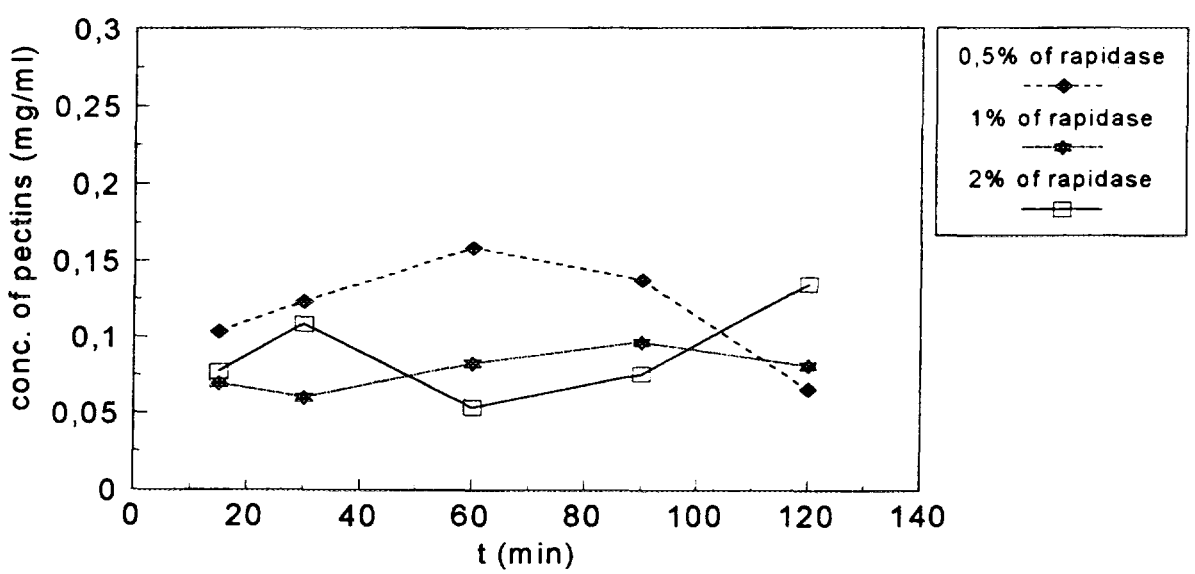

Fig. 2. Pectins concentration in the permeate during UF of juice treated with different percentages of rapidase. Qax = 800 L/h; T = 25 °C; TMP = 0,7 kPa.

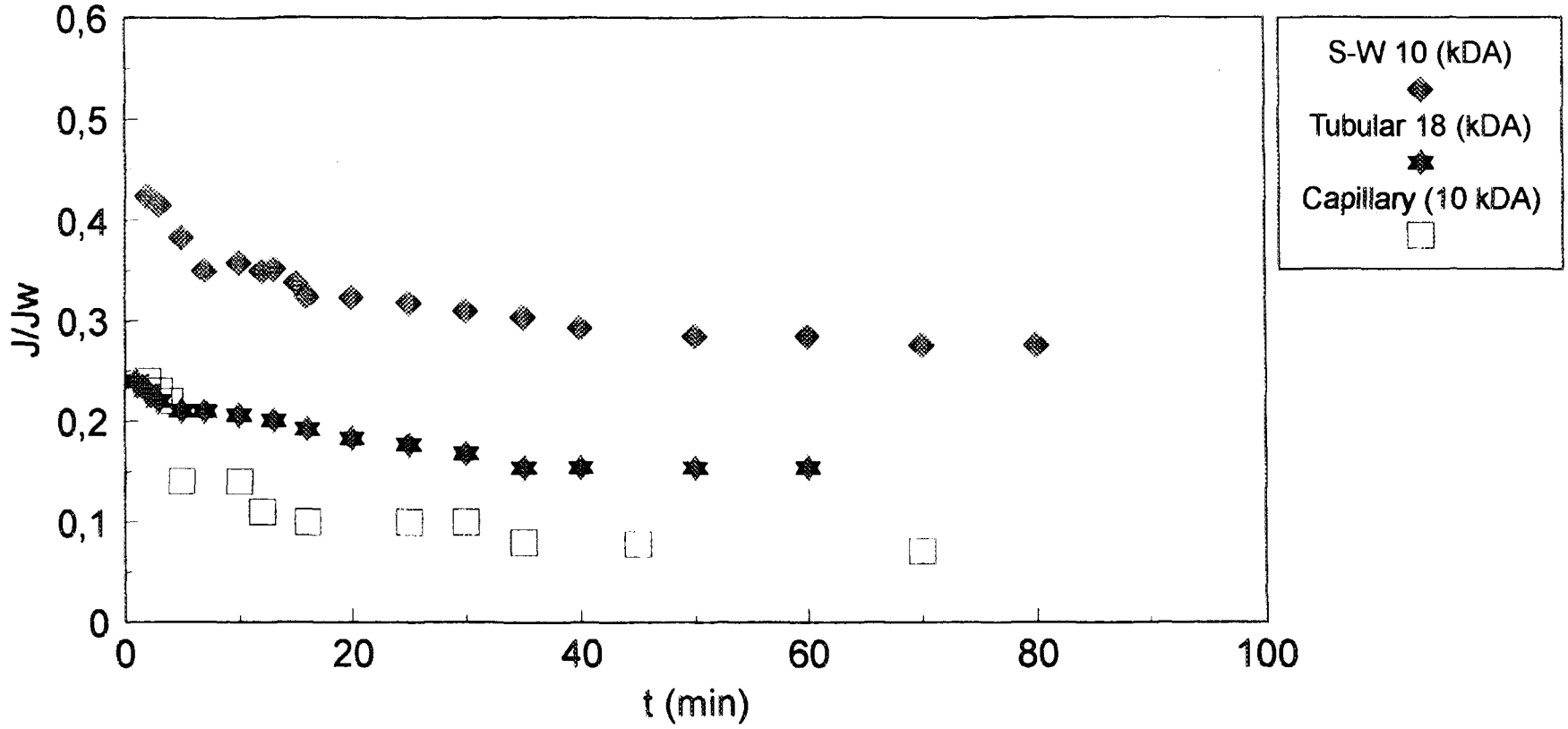

Fig. 3. Ultrafiltration of treated juice through different membrane configurations. Qax = 800 L/h; T = 25 °C; TMP = 0,7 kPa.

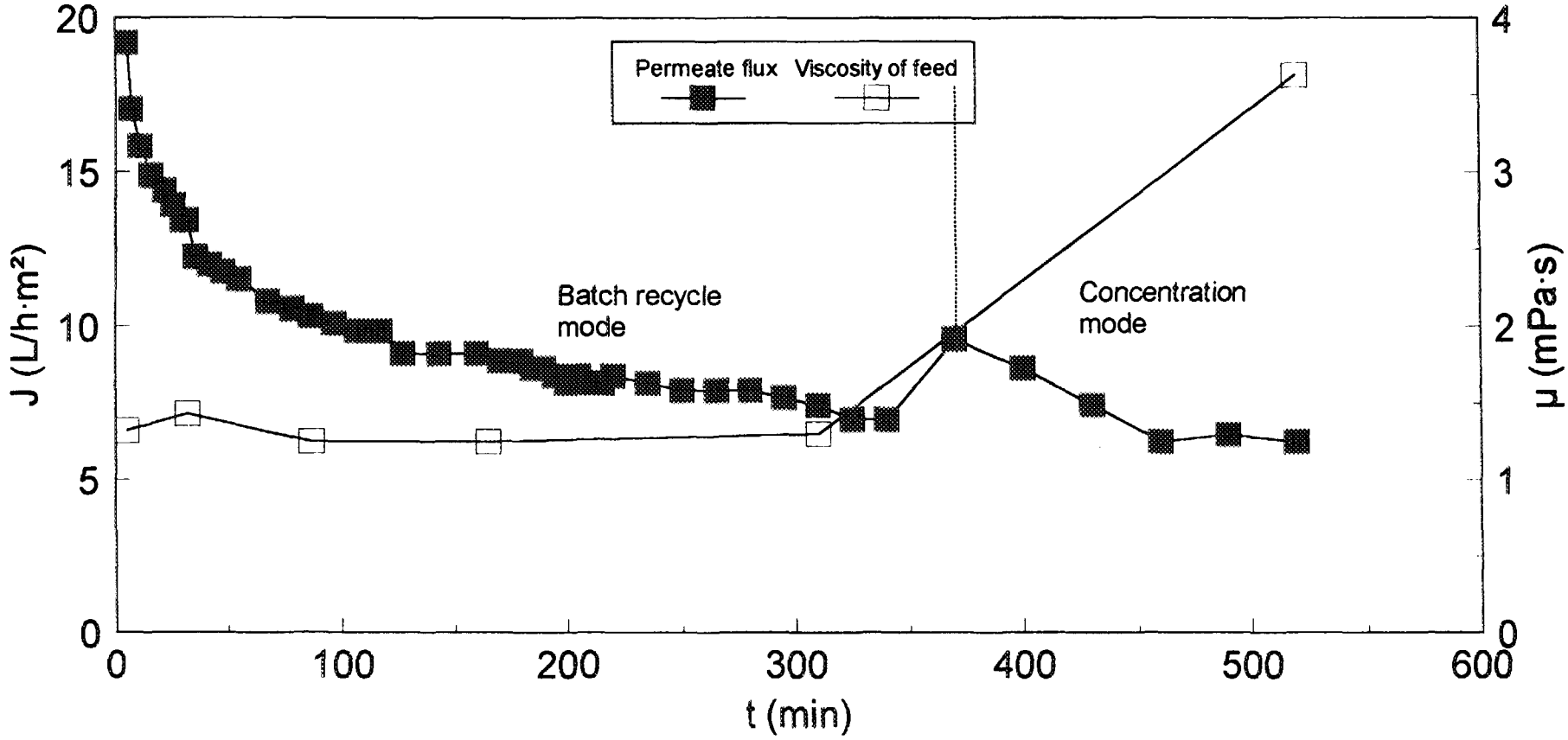

Fig. 4. Permeate flux and viscosity of refined raw juice in UF-EMR
Operating conditions: Vi = 15.6 L; 1% v/v rapidase; T = 25°C;
Qax = 700 L/; TMP = 1.2 kPa.

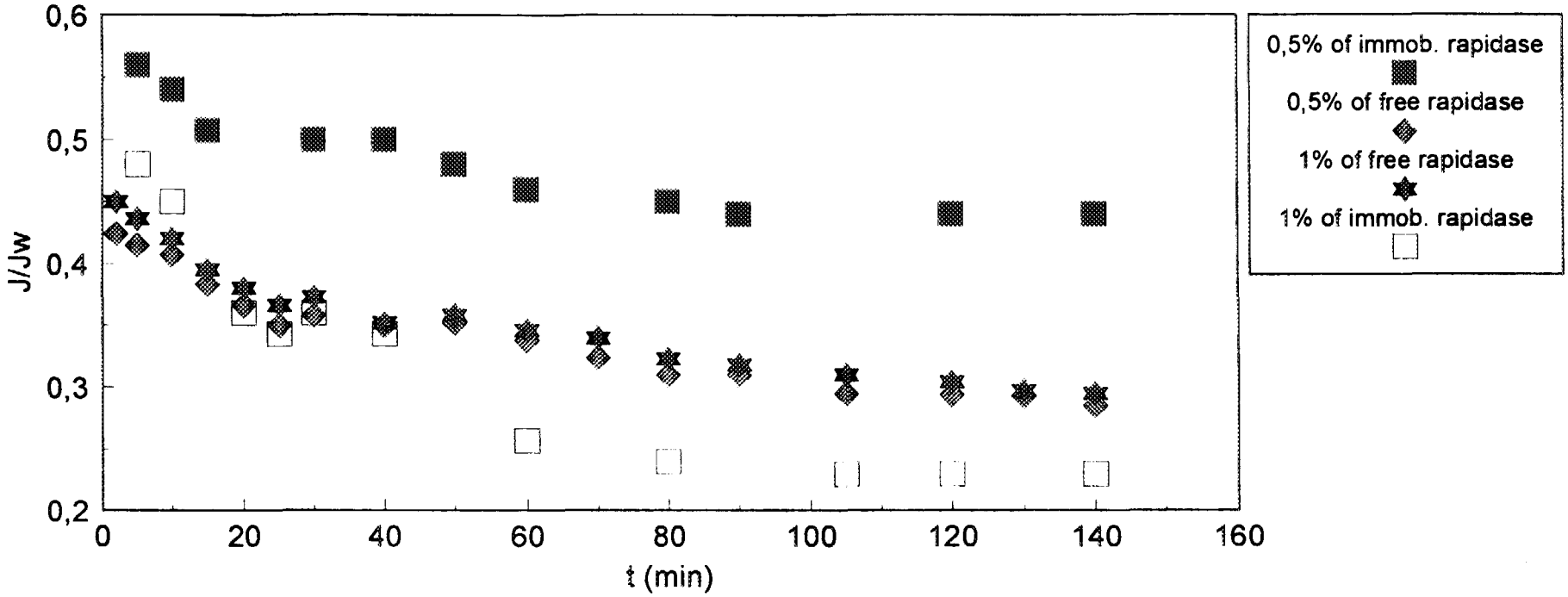

Fig. 5. Permeate flux through s-w module of juice treated with free and immobilized rapidase. Qax = 800 L/h; T = 25 °C; TMP = 0,7 kPa.

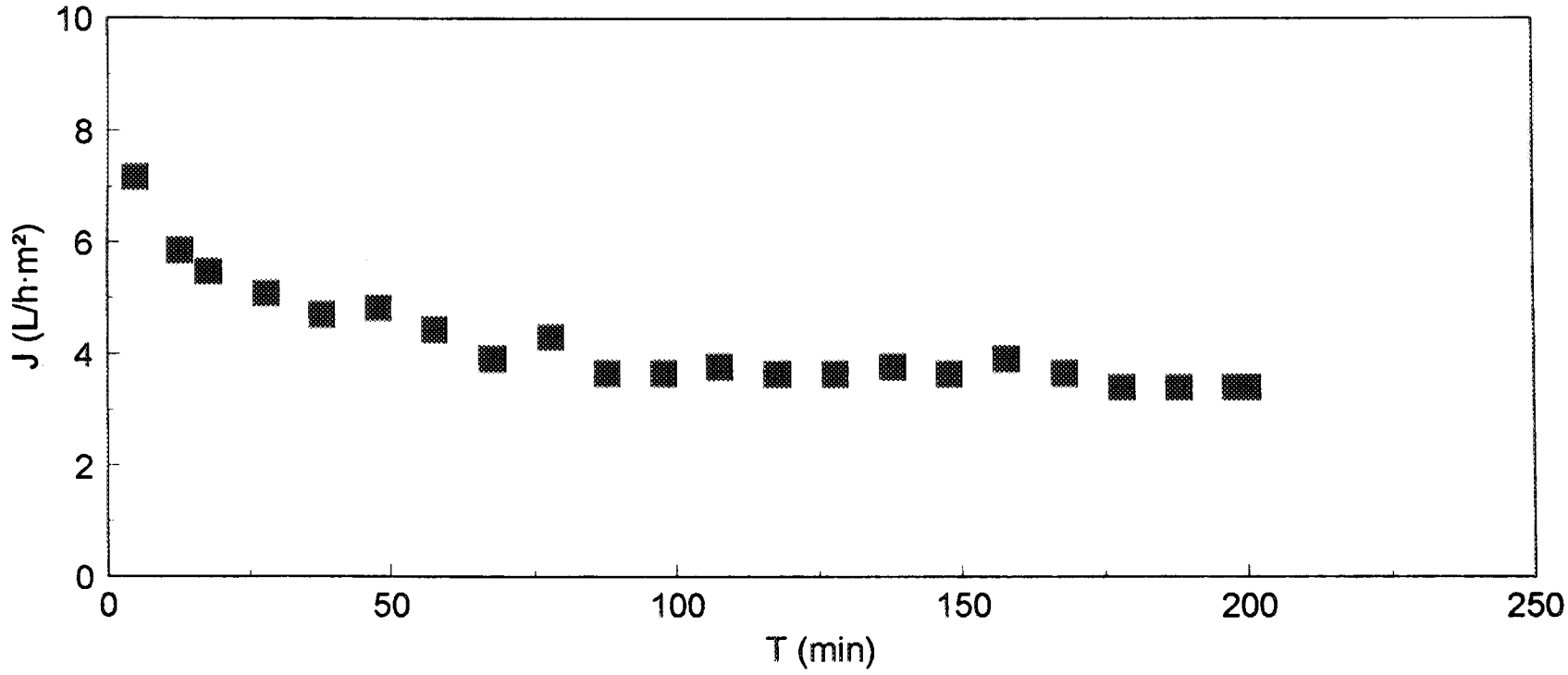

Fig. 6. Permeate flux of apple pulp in UF-EMR. Operating conditions: Vi = LI; 1% rapidase; Qax = 1000 L; T = 25°C; TMP = 0,67 kPa.

FOOD ENGINEERING ACHIEVEMENTS IN THE IMPROVEMENT OF PHYSICAL PROCESSES FOR FOOD PRESERVATION

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New trends in food engineering concerned with the physical processing of foods are presented, with reference to mild techniques whose industrial utilization can be envisaged in the next future. In particular, high pressure stabilization and combination technologies are considered and research developments needed for the scale up of laboratory or demonstration apparatus into equipments suitable for production are discussed.

INTRODUCTION

Food engineering can be considered as a relatively new applied science and research field, deriving from the extension to the analysis and development of food processes of the scientific background of process engineering, mainly developed with reference to the chemical industry in a wide acception. The use of an engineering methodology in food processing allows a more general approach to plant design, on the basis of the conceptual definition of unit operations as stages of a given production line. With reference to research problems, this approach promotes a deeper insight into transport phenomena which develop in specific apparatus, and to the possibility of optimising the design of single processors, even for well established unit operations such as the preservation techniques of food based on physical principles [1, 2].

Physical preservation techniques have been employed since the first stages of human civilization: dehydration through thermal effects by natural or artificial sources or through salt addition was the first method to ensure the extension from one season to the other of the life of vegetables as well as of animal foods and thus the survival itself of human beings. For a long time, no sensible innovation occurred in the basic design of apparatus concerned with these techniques, even if the understanding of some fundamental mechanisms of microbial spoilage brought in the last century to a scientific approach in the evaluation of the effectiveness of treatments. In the last years, the increasing interest of consumers toward minimum processed foods, which in turn rises the prediction of a rapid and substantial reduction of the use of chemicals in food processing, stimulated a diffused research effort towards the set up of low impact treatments, through the intensive utilization of the so called mild technologies [3, 4].

As a consequence of the reassessement of the role played by the optimal design of unit operations used in the physical treatment of foods, the role of food engineering is now fully recognized and research programs directed to the utilization of all potentialities of physical preservation techniques are developed extensively. In the following, a short illustration of some recent achievements in this field will be given.

First of all, a rough classification can be proposed in listing the main innovations suggested in last years :

- developments of conceptually new unit operations to perform in a more efficient way and/or under higher quality requirements a specific stage of a food production line
- set up of new processes, based on the integration in a different flow sheet of conventional unit operations and on the integration and optimisation of their performances

The first item refers to the use of physical principles not previously employed in food processing, such as hydrostatic pressure[1,2] pulsed electromagnetic fields [5] ,or to the extension to food industry of techniques already used in other fields, such as the interactions between supercritical solvents and specific components.

The second item mainly refers to the introduction of the concept of "combination technologies" in some productions [6] .

NEW UNIT OPERATIONS

Not all newly proposed unit operations for food processing are discussed in the following. Only those which, in the opinion of the Authors, will meet a significant interest in next years, are considered in some detail, with particular reference to the research needs stimulated in the field of food engineering.

Electroheating techniques

The so called electroheating techniques include: ohmic heating, microwave heating and the more recent radiofrequency heating. A latest development is the application of pulsed electric fields to the product, which is not necessarily conncted with a real heating.

Ohmic heating relies on the electrical resistance of the product to cause its temperature to rise when a suitable voltage is applied to it and consequently a sufficient current intensity passes across [7, 8] . This technique is well known, being based on basic principles, but its application to heating of foods is not yet widely accepted. Its main potential advantage is the ability of controlling accurately temperature rise reducing temperature gradients for almost any size of the product.

When applied to the sterilization of liquid foods containing particulates, the additional possibility of controlling residence times in flowing fluid systems, makes for minimising overprocessing of liquid in respect to particles, being the local development of heat related to electrical resistance instead of particle size.

The drawbacks of this technique are the following:
- the alreay mentioned limitation to liquid and semiliquid products or to suspensions, due to the required uniform contact with electrodes.
- the dependance of heat generation on the resistivity of the product to be processed. This limits application to materials with a narrow range of resistivities, due to economic and safety considerations when dealing with very high current intensities (required for heating low resistance products) or very high voltages (required for heating high resistance products).
- the strict dependence of temperature rise on product properties makes a given apparatus, with a certain geometry of electrodes and of the gap between them, able to process almost a single product, with a very limited range of composition fluctuations

Research developments needed are the set up of more flexible process cycle, able to work with a wider range of products. This can be performed by coupling static ohmic heating with pulse control of voltage. Other interesting developments concern the design of new electrodes consisting of a

patchwork of insulated sections, in order to allow under some conditions the processing of solids. Sections experiencing poor contact are automatically excluded, while only those operating in the proper range of current intensity are connected to the circuit [8].

Microwave heating is, at the moment, the more widely used electroheating technique in industrial applications. It consists in submitting the product to br heated to an electromagnetic field in the wave length of microwaves (frequencies of 1.000-3.000 MHz). When a material containing suitable molecules, like proteins or water, is exposed to an electromagnetic field, heat is generated by rapid reversal of polarization of individual molecules. Accordingly, heating occurs inside the product eliminating any problem of external heat transfer, and its amount depends on local dielectric properties of the product and on the power of the field. This technique, in respect to ohmic heating, does not require high voltages and is less dependent on the geometry of the apparatus, due to the easier propagation. waves in respect to electric current. This is the reason for many applications, particularly for solid or pastes. Industrial applications are reported for thawing of frozen foods, roasting of cocoa, sterilization of flour and other powders [9, 10].

The drawbacks of the technique, which damp further diffusion, are:
- the strict dependance on the local dielectric properties of the product, and in turn on the chemical composition with particular reference to the content of water in free or bonded form. This produces local overheating in foods with uneven composition, like most complex products.
- the sensitivity to the size of the products to be processed. This depends on the weakening of the electromagnetic field occurring as a consequence of absorption properties when passing across a body. As a consequence, the heating is not uniform in large bodies with high electromagnetic absorptance.

Research needs for wider applications are a deeper insight on wave propagation in non homogeneous media and the analysis of heating of flowing liquid foods. The use of microwave heaters for sterilization of liquids in continuous apparatus could be a new tool for solving some problems of temperature and residence time control in systems not suitable for ohmic heating in connection with their very low or very high resistivity.

Radiofrequency heating is the latest developed electroheating techniques, and is based on the same principles as microwave heating, provided that the frequency of the ectromagnetic field is kept at lower frequencies, usually in the range of 10-20 Mhz. The reason for this is the higher penetration depth of lower frequency waves, even if also lower efficiency in energy transfer is attained (not higher than 60% in experimental apparatus).

As for microwaves, preferential applications include the processing of solid and semisolid products, which are excluded from ohmic heating due to difficulty in creating a proper contact betwwen electrodes and product surface. On the contrary, the wave propagation mechanism does not require a direct contact between electrodes and product,and thus makes for easy application to solids. In the choice between microwaves and radiofrequency waves, the dependence of wave absorption properties of different products on wave frequency, must be accounted for. As a consequence of relevant dielectric properties, each product is heated more efficiently by a given wave frequency. However, due to Government regulations on the use of electromagnetic fields in connection with possible interferences with communications, limited frequency ranges are allocated for industrial use, and selection must be operated among them. Radiofrequency in its available range is well suited for meat products. Industrial applications in ham cooking and similar processes dealing with products in large pieces are reported [11, 12].

The drawbacks are almost the same as for microwaves, with the addition of a minor overall efficiency. This is partially compensated by a slightly minor sensitivity to composition uneveness.
Research developments needed are the fundamental analysis of wave propagation in non homogeneous media; this could help in designing multipurpose apparatus with more sensitive control devices and thus in the setting up of more flexible process cycles, able to work with a wider range of products. The coupling of static ohmic heating with the pulse control of voltage could be the tool for open to applications this reliable but neglected technique.

Pulsed electric fields (PEF) is a treatment technique which is somewhat in between electroheating and other non thermal techniques. It has been recently proposed as non thermal preservation technique for the production of minimally processed foods has been recently proposed. It consists in the application of very short bursts (of the order of microseconds) of high voltage to foods placed between two electrodes, in an arrangement closely resembling that for ohmic heatimg. The principle is as follows: the very short application time of electric voltage generates a correspondingly short pulse of current. This in turn generates a very limited local heating, but, due to biological phenomena connected with a transient high electric field already described in literature [13], a certain extent of inactivation of microrganisms occurs and pasteurization may be produced. Effective pasteurization for different liquid foods at bulk temeperatures as low as 50 C is reported [5].

The drawbacks of the technique are the following:

- application is limited to liquidlike products or suspensions, due to the requirement of uniform contact with electrodes.
- efficiency is rather poor at the present stage of development
- the extent of pasteurization must be carefully determined for each product through optimization of process conditions. Small composition variations, or degree of microbial spoilage, can produce poor pasteurization.

Research needs are rather wide, due to the scarce development of this process. They include, fundamental studies of the mechanism of interactions between pulsated electric fields and different microrganisms, as well as posssible interactions with enzimes and other complex components of food, and, from the technology side, the set up of effective control devices in order to ensure the proper duration and transient characteristics of current bursts to make sure that the desired effect is obtained. Also the scale up criteria of the process must be thoroughly investigated.

Continuous purification of liquid foods through supercritical solvents

Supercritical extraction is already considered an almost established operation in food industry. It is mainly applied for aroma recovery from vegetables or for the elimination of undesired components from solid foods, as in caffeine separation from coffee. All the reported applications are based on the same contact technique, which consists in the percolation of the supercritical fluid through the solid matrix. Due to the low concentration of valuable components to be extracted, the size of extractors is very large, being sized on the batch of the raw material. This makes the process very expensive, and limits its application to high added value products.

Very recently, an innovation has been proposed in the supercritical technology: the use of the supercritical solvent in the purification of a liquid mixture, coming from a conventional extraction stage. The advantage is that of utilizing the high solubility and selectivity of the supercritical fluid towards a concentrated product. This reduces of about two orders of magnitude the volumes of the apparatus and simplifies high added value operations, such as the purification of essential oils. The supercritical process is used only in the more difficult operation, as a stage of the overall process.

The reason why this technique has not yet been widely used, is that the contact must be now established between the supercritical solvent and a liquid, with some critical problems of fundamental fluid dynamics not yet fully solved.

The key for solving these problems is the use of a continuous device, closely resembling a countercurrent distillation tower with reflux. The first laboratory apparatus of this kind has been successfully tested and experiments on the fractionation of a citrus peel oil mixture demonstrated that the separation of terpenes can be performed by using supercritical carbon dioxide. By changing the reflux ratio, it was possible to change the composition of the oil in order to cut out different kind of terpenes [14].

Research needs are almost wide, as for a completely new operation. They include:

- the fluid dynamics of countercurrent flow of supercritical fluids and liquids
- the determination of transport properties in such systems
- the set up of design criteria of large scale apparatus

High pressure stabilization of foods

In its present application, this technique consists in the pressurization up to several thousands bars of the food product. This takes place through hydrostatic pressurization by means of a service fluid in a press, whose engineering derives from other field of application, such as metallurgy and also the first series of machines purely resembles the isostatic press. These machines are operated batchwise and characterized by a long pressure rise and release time, due to the use of the so called pressure intensifiers as pressure boosters.

On the basis of the available experimental results, pressurization cannot be considered as a pure sterilization or pasteurization process [3] . It induces complex modifications in the food product, including protein denaturation and major chemical degradation of complex molecules. This produces sensible variation in :chemical composition, nutrition properties, rheologic behaviour. This last feature can be important in the consumer acceptance of specific products.

Even if some products are available in commercial status, this operation is not yet considered for large application

Wide application can be envisaged in presence of a substantial cost reduction, i.e. under the following conditions:

- reduction of the pressure level, which produces a dramatic cost reduction
- continuous operation at least for flowable products, which highly increases the throughput of the apparatus per unit volume with the same effect on unit costs

The first aim can be reached by coupling to the pressure effect a second stabilization principle, such as moderate heating, electromagnetic waves (or u.v. irradiation has been recently proposed). The application of the second physical effect can be performed in the same apparatus, or in a separate stage.

Another possibility of reducing pressure level is that of producing fast pressure rise and release. Dynamic components of pressure are likely to enhance the microrganism mortality at a given maximum pressure level. This operation requires a different design of the apparatus, removing the pressure intensifier and producing pressure rise by direct pumping of the service fluid. This scheme has already been tested in laboratory scale, according to the sketch presented in Figure 1 [15]. A double stage diaphragm pump is used to boost the pressure up to 7.000 bars. The continuous pressure rise, not limited by the capacity of the pressure intensifier which is an intrinsically discontinuous device allows, in principle, to produce pulsations of wide intensity and frequency by operating discharge valves of suitable design. The problem has not been yet solved completely, but does not appear as impossible.

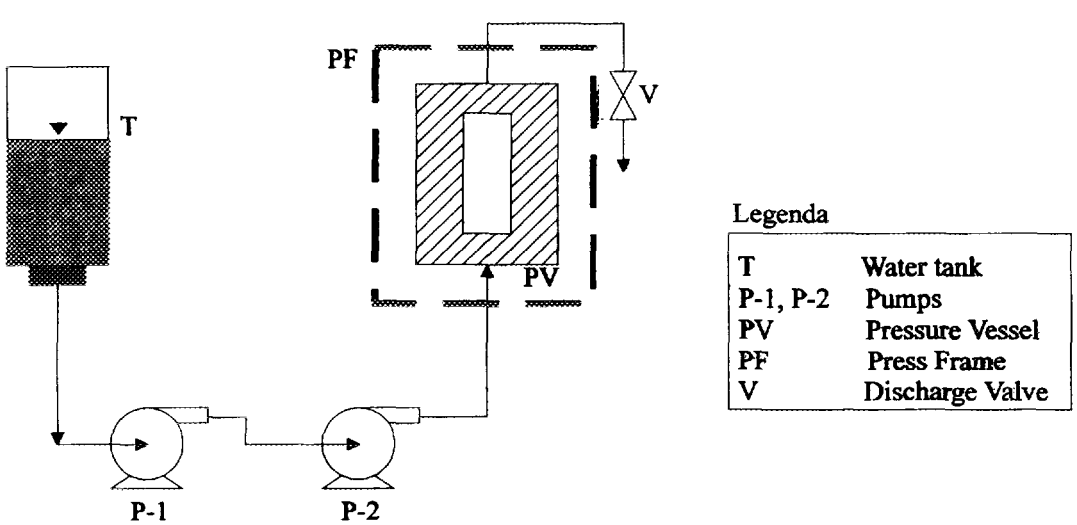

Fig. 1 - Sketch of the experimental apparatus for high pressure stabilization of foods

Continuous operation can be obtained in principle by eliminating the service fluid and feeding directly to the pumps the food to be processed, provided that its flow properties are as good as to be treated by a pump. Also in this case, the main problem is that of developing specific components (high pressure pumps and lamination valves) able to stand continuous operation without producing neither appreciable food degradation nor undesired thermal effects.

NEW PROCESSES

New processes can be almost infinite in number, deriving from the coupling of existing unit operations. In principle, a new process is designed whenever a new product is defined. In the following some attention is paid to a specific family of new processes which are designed acccording to principles not connected to the need for new products. This family of processes which are gaining importance in food processing can be identified as the so called combination technologies.

The principle is very simple: each process is usually performed in steps which are designed according to the availability of suitable unit operations able to accomplish the tratment in a minimum number of stages. If the point of view changes from the minimization of the number of stages to the optimization of the quality level of the product or to the minimization of the overall costs of the process, different conclusions about the design of the stages can be drawn, and in certain cases the splitting of a conventional stage in two or more operations may be considered, provided that this splitting can improve the quality level of the product or reduce the processing costs.

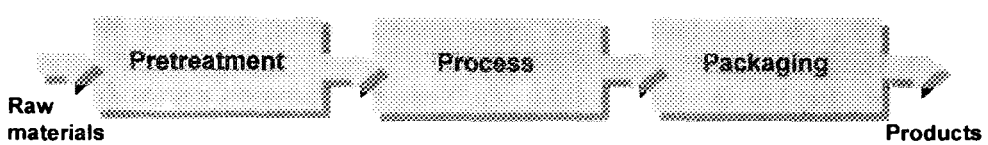

Fig. 2 - The principle of combination technologies

The analysis of the criteria for combining together unit operations in a more effective way is the basis of the combination technologies.

In spite of the simple principles involved, valuable results can be achieved, particularly in the field of high quality and high added value foods which are at the moment produced by the so called mild technologies. Mild technologies are usually expensive technologies, like freeze drying, high pressure stabilization or PEF pasteurization. In tis case, the basic consideration is that it is not always necessary that the whole process is carried out under mild conditions, provided that a the quality level is not dramatically affected if a conventional process, say thermal treatment, is applied for short time and under soft conditions. If a thermal stage, applied previously, performs part of the job due as dehydration or pasteurization, the duty of the mild technology stage is relieved and its cost reduced. Figure shows a typical scheme of a combination process.

By limiting consideration to freeze drying, it has been demonstrated that, with careful control, it is possible to perform dehydration up to a certain degree by using conventional thermal drying and apply freeze drying only to the tail of the process, when the sensitivity of the product to process conditions is at its maximum, without any major effect on the structure of the product [16].

The problem is to decide what is the moisture content level at which the first stage must stop. This is not easy, and requires a deep study on the properties of the product as a function of moisture content and on the type of process.

The interest on combination technologies has stimulated research works on the determination of the dependence of the evolution some structure properties of food materials on process conditions, according to the consideration that the quality premium of freeze dried products is bound to the preservation of the original structure properties of the food material. The analysis of pore structure, with reference to pore size and degree of interconnection of the pore network, can be useful to fix the optimal switching point from the conventional drying system to freeze drying [17]. This is the main research need raised by combination technologies, together with the extension of the concept to different processes. In particular, a similar approach is suggested to minimize the cost of other expensive technologies, such as high pressure stabilization or PEF. This could lead to change the ranking on economic basis of the latter unit operations and contribute to their wider diffusion.

CONCLUSIONS

The innovation of food industry is strictly connected with the achievements of food engineering. This science is growing towards two directions: the set up of new unit operations and apparatus, and the application of new principles in the design of the processes. In both directions, there are many results which deserve attention from the point of view of industrial applications.

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HIGH PRESSURE TECHNOLOGY: TOMATO PROCESSING

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ABSTRACT

The application of the High Pressure technique (HPP) coupled or not with mild heat treatments has already been the object of research in several experimental tests; some industrial plants are nowadays used for the processing of products such as fruit juices and nectars, fruit pieces, jams and sauces.

In the present work, the physico-chemical effects of the HPP treatment on a tomato product (in this case "passata di pomodoro") were evaluated.

In particular, the behaviour of tomato juice preheated at different temperatures and then subjected to different time-pressure combinations was investigated. The results obtained have evidenced that the HPP treatment does not influence the main chemical features of the product (the so called "quality markers") but has, however, a strong effect on its sensorY characteristics. In fact aroma, viscosity and consistency are deeply influenced by the different treatment combinations. On the basis of the data recordeed during the first part of this work different ways to process tomato juices were tested and the effects on the finished product were evaluated. A scheme for the processing line is suggested in order to obtain a new generation of tomato products. The conclusions drawn show that the HPP technique can be used as an effective technological instrument to prepare new products with improved quality.

INTRODUCTION

The application of High hydrostatic pressures on food products to improve their shelf-life has been considered since 1899. The difficulty in technical application of high levels of pressure (more than 200 MPa) with a high product and processing capacity and reliability was overcome only in the last few years. In the 1990's the first applications of this technology appeared on the Japanese market. In the following years a deep basic investigation (*1,2*) into

process effects and application was carried out mainly in Europe. By means of different research projects (one supported also by the European Community) many different companies have developed a wide knowledge on the application of HPP.

The last year the first non Japanese processing equipment started the massive production of a fresh vegetable spreading ("Guacamole") sold in the USA supermarkets. At the same time in Europe the first production of Orange Juice, by HPP, appeared on the market. The common characteristic of all those foodstuffs is the low impact of the technology on product sensory characteristics. In fact the high fresh-like sensation given by these processed foods, even if as stable as to be distributed through the conventional refrigerated chains, allows the processor to avoid flavouring or colouring agents. Moreover, the non-destructive mechanical action, in spite of the pressure levels reached during processing (500-700 Mpa), enables processing of whole berries or fruit pieces on smmoty brines that could not be conventionally carried out without any pre-icing.

The technology has shown a great effect on such macromolecules as starch, proteins and pectins. The water dispersions of these substances are can gel under pressure resulting in increased in viscosity and good water retention; the gel matrix obtained proved to be completely different from the corresponding thermal one. On this basis, the technology seems to be very promising not only as food pasteurising treatment but also as proved tool for food processing.

OBJECTIVE OF THE WORK

A preliminary series of tests (*3*) were carried out on tomato pulp focusing mainly on HPP effects on a pre-heated matrix. The different temperature of pre-processing, pressure and time of pressurising combinations performed helped to define the behaviour of the product during processing. On the basis of the data recorded during this first part of the investigation a possible lay out for a new processing line was defined and tried using a small scale processing test. In the present work we report a general summary of the data collected in the first part and the results regarding the comparison between a new (HPP) line and a traditional one.

MATERIAL AND METHODS

SAMPLE PREPARATION

Commercial tomatoes cv. Brigade Hy were purchased on the market. They were kept under cold storage conditions until processing. Ten kg of raw tomatoes were pitted and washed. The operations carried out during the sample preparation are represented in the following flow sheet (fig. 1).

HIGH PRESSURE BLANCHING (HPP) AND JUICE EXTRACTION

Half tomatoes were packaged in nylon/PE pouches and High Pressure Processed with a ABB (Vasteras-Sweden) QFP-6 press. The processing parameters were 600 Mpa at 25°C (press temperature) for 3 min. The processed material, because of the batch operations were kept refrigerated until juice extraction. The pouches were opened; the tomatoes were cut in cubes 10X10X10 mm using a Bertuzzi (Milan) mod California cutter and then extracted using a Rev-mec (Pram) cold pilot refiner (0.3 mm ∅ sieve). The collected juice was kept under reftrigerated conditions (0-3°C) until further processing.

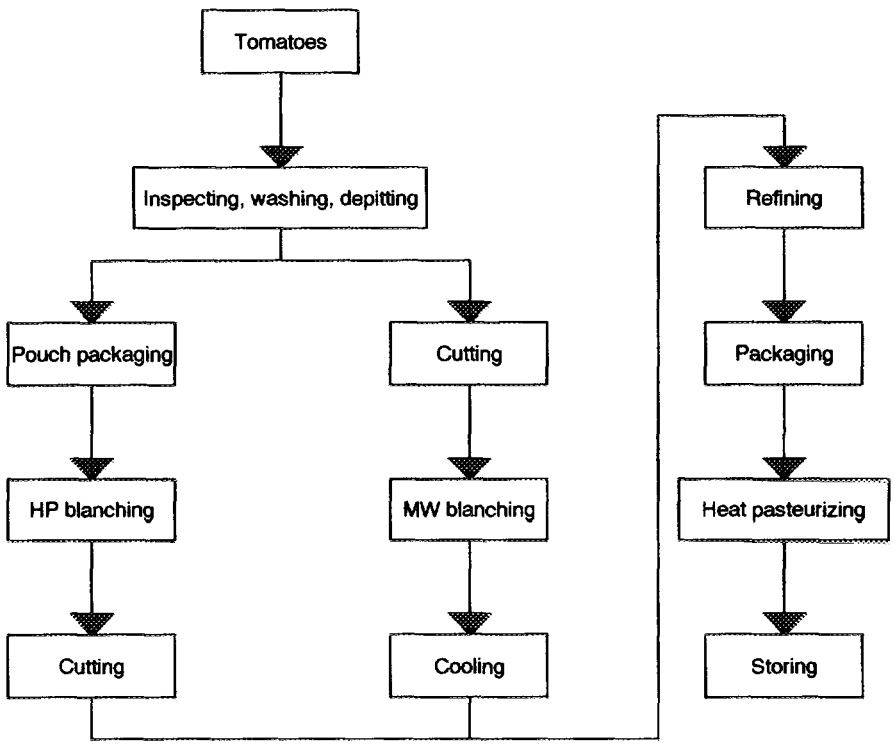

Fig. 1: Experimental flow-sheet

REFERENCE BLANCHING (MW) AND JUICE EXTRACTION

The reference samples were prepared using the remaining tomatoes. The fruits were divided into 0.5 kg batches. Each batch was cut into cubes 10X10X10 mm and quickly heated at 90°C using a pilot Omac MW 1540 microwave blancher (Reggio Emily) This temperature was reached by applying 2 kW in 3.5 min. The processed batches were quickly cooled at room temperature using an air cooler. The juice was extracted using a cold refiner (0.3 mm sieve). The collected juice was kept under refrigerated conditions (0-3°C) until further processing.

PACKAGING AND PASTEURISATION

The juices were packaged in plastic (PP/EVOH/PE) cups (100 ml) and vacuum sealed with a PE/Al/PE lid using a Mondini (Brescia) pilot sealer.

The cups were pasteurised using a Stork rotomat 900 (Neusmunster - Germany) 85°C at the slowest heating point F_{100}=5.

DETERMINATIONS

After 3 month's storage the following parameters were determined on the samples:

- Soluble solids(*4*)
- pH (*4*)
- acidity (*5*)
- Total sugars (*5*)

•Hydroxy-methyl-forfural content (*5*)
•Pectic acids (*6, 7*)
•Pectates (*8, 9*)
•Protopectins (*8*)
•Total pectins (*8*)
The following physical determinations were carried out:
•Serum colour (*5*)
•Gardner colour (*10*)
•Bostwick index (*11*)
•Blotter test (*10*)
A sensorial test was performed. A trained panel tested the juices one by one, without any direct comparison. The two samples were judged according to the following parameters:

- Sweetness
- Acidity
- Sapidity
- Colour
- Cooked taste
- Overall preference

PRELIMINARY TRIALS

In the previous set of test we investigated the effect of the High Pressure Processing on the tomato system. To understand the effect of processing on crushed tomatoes we introduced a new variable to modulate enzyme activity: blanching temperature (*12, 13, 14, 15*).

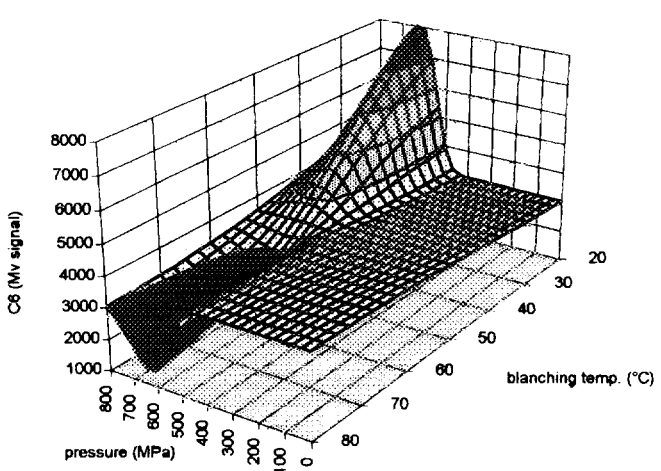

Fig. 2: C6 compounds variation in sample head-space (*16*) after heat blanching and HPP (5 min.)

In practice the tomatoes were crushed and quickly heated at 50 and 85°C then cooled (25°C) before HPP. In this way it was possible to affect at different levels the enzymes' systems responsible of structure and aroma changes. The HPP performed on such modified

matrix showed interesting effects. On the processed samples many different physico-chemical evaluations were carried out. From the statistical evaluation of the interactions between the results and processing variables, it was evidenced that only on structure and aroma were affected. At the same time no effect was evidenced on the main chemical characteristics of the processed matrix.

Table1 reports the statistical evaluation of the data collected.

	A	B	C	
	time	pressure	temperature	interaction
total sugars	ns	*	*	ns
% of total sugars on soluble ss	ns	ns	**	ns
Blotter	ns	**	**	BC**
Bostwick	*	**	**	BC**
a/b	ns	ns	**	ns
acidity	ns	*	**	BC*
hexanal	ns	ns	**	ns
hexenal	ns	ns	**	ns
C6 sum	ns	ns	ns	ns
total pectins	ns	ns	**	BC**
water soluble pectins	ns	ns	**	BC*
NaOH soluble pectins	ns	ns	*	BC**
hexametaphosphate soluble pectins	ns	ns	*	BC**

ns= not significant difference
* significant difference with $p<0.05$
** significant difference with $p<0.01$

Tab. 1: Statistical evaluation of the effects of the three variable tested and of their interaction on the analytical parameters considered

The main effect on the aroma fraction (Hexanal and C6 compounds) was given by blanching temperatures. In fact no effect came from High Pressure Processing (pressure level or time). Some of the results are reported in figure 2. This situation led us to assume that High Pressures are unable to inactivate the degradation of fatty acids to C6 compounds. The only way to avoid the detrimental effects (*17*) is to interviene in the early processing stages. In fact, other experiences (*18*) showed that the lipase enzyme group was sensitive to pressure inactivation. This suggested the possibility of operating only on whole fruit trying to avoid the reaction start induced by cutting or crushing.

At the same time it was evident how the HPP was effective on the physical characteristics of the tomatoes. In fact, Blotter and Bostwick values (fig.3)deeply changed especially if the product was not blanched before HPP. This change led us to investigate also into the pectic fractions. It was evident how the HPP affects the pectic fractions ratio resulting in the global effect responsible for physical parameter modification. The possible explanation for these

phenomena was that the HPP can not inactivate the enzymes responsible for pectin de-methylation (PME) while it can inactivate those responsible for pectin hydrolyse (glacturonases) (*19*). At the same time, HPP showed the ability to gel the low methylated pectins increasing their water retention.

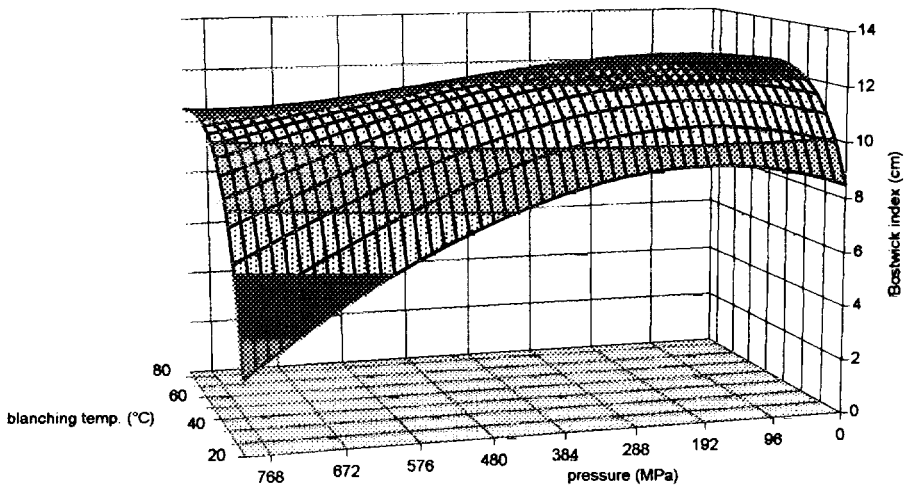

Fig. 3: Changes in Bostwick values variation after heat blanching and HPP (5 min.)

This behaviour will be of big technological interest if the supposed ability of HPP to inactivate the early steps of aroma degradation will be proved.

The practical follow-up of these observations and hypothesis was the use of the High Pressure Processing as whole tomato blanching step on a "conventional" processing line.

RESULTS AND DISCUSSION

The analytical results reported in table 2 show that there are no differences in the main chemical parameters taken into consideration. In practice SS. pH, acidity, sugars and colour parameters have the same values in both the HPP and MW samples.

At the same time, as was supposed, there is a big difference between the two samples when comparing the viscosity parameters. In fact, the lower Bostwick and Blotter values of the HPP sample indicate higher viscosity and higher water retention than in the RAW and the MW samples. Assuming that none of the samples was concentrated, because of the preparation steps adopted, the viscosity change, correlated with the pectic ratio change, can only be explained by the effect of the HPP.

Sensory evaluation result show that there is no difference in the two samples (MW and HPP). In fact, the score differences found are lower than the standard deviation (fig. 4). In any case the MW sample obtained a higher attribute score probably due to the comparison instinctively done by the panellists with the traditional product idea. In any case no off-flavours were founded in the samples.

sample	RAW	HPP	MW
Soluble Solids	5,03	4,56	5,11
pH	4,31	4,36	4,32
total acidity (%)	0,41	0,34	0,40
Bostwick index (cm)	ND	8,33	12,43
Blotter (mm)	ND	5,68	17,37
Serum color (a.u.)	0,25	0,24	0,23
Gardner color L	25,14	17,89	25,71
a	30,84	29,51	29,63
b	14,40	14,82	14,54
a/b	2,14	1,99	2,04
Sugars (%)	2,97	2,45	2,90
Hydroxy-methyl-furfural	6,77	6,36	6,75
Pectic acids (g/100g)	0,09	0,03	0,08
Pectates (g/100g)	0,09	0,13	0,14
Protopectins (g/100g)	0,02	0,04	0,05
Total pectins (g/100g)	0,20	0,21	0,27

Tab. 2: Physico Chemical-parameters of different tomato juices. The data reported are the mean of 3 different samples. (nd= not determinable)

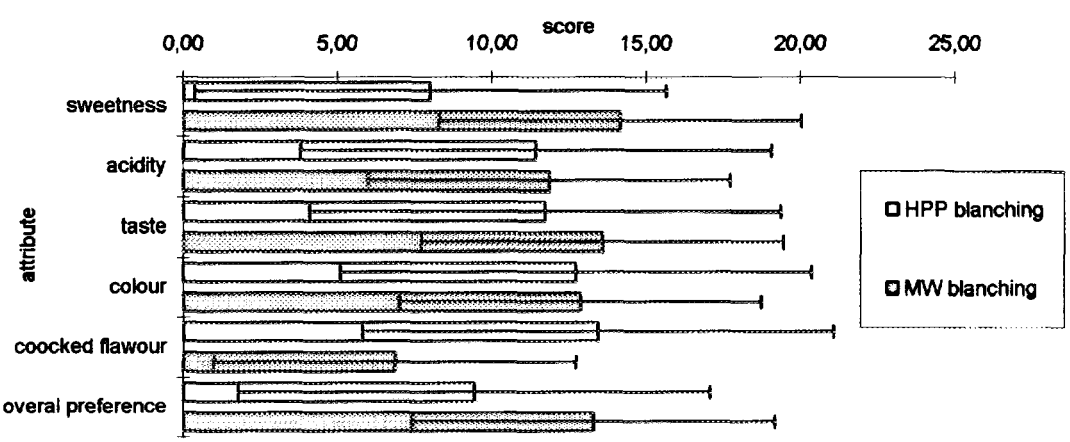

Fig. 3: Panel test evaluation of different samples. The score is the mean of 10 judges.

CONCLUSIONS

The present work has shows how much the HPP is an interesting tool in food processing. In fact, HPP blanching, with is ability to modify the enzyme pattern activity in tomatoes, could be an interesting operation. It has to be remembered that in the last years big interest aroused on the biotechnological changes of these parameters, opening up a lot of new questions.

The results show at the same time the ability of the process to avoid aroma degradation if performed at the right moment.

More work has to be done to optimise the processing flow but we believe we have demonstrated the potential of an interesting new processing idea: HPP blanching.

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THERMAL TREATMENT BY OHMIC HEATING OF HALF PROCESSED FRUIT IN PIECES

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The Ohmic heating process applies the effect of resistance heating within a continuous flow of an elettrically conducting food. The technique permits rapid and uniform heating of both the carrier fluid and particulate, avoiding the use of hot heating surfaces. In this paper a description is given of the Ohmic heater and the pilot plant for the production of aseptic particulate fruit products, developed by emmepiemme s.r.l.. Tests carried out on apple cubes in apple puree show that the pilot plant allows half-processed high-particulate fruit products to be obtained which are microbiologically and enzymatically stable and with minimal sensory and structural changes.

Thermal treatments for the stabilization of processed foods cause more or less marked modification of their organoleptic and nutritional characteristics; this is the reason why innovation in the field of sterilization is mainly aimed at reducing thermal damage while preserving the sterilizing effect.

With a temperature increase of 10°C, the kinetics of the thermal death of microrganisms increase 10 to 100 times, while the kinetics of thermal degradation are only doubled. For this reason, high temperature short timetreatments (HTST) cause less damage to qualitaty characteristics than processes where lower temperatures are applied for proportionally longer times. However in practice the benefit of HTST treatments can only be effective if the product is heated and cooled in a quick and uniform way.

In traditional technology, foods are sterilized after they are packaged so the container acts as a heat exchanger; in this respect however the containers normally used are not very efficient because of their low surface/volume ratio. This means that in order to achieve the desired sterilising effect throughout the product mass most of it will be oveheated and its degradation will be therefore greater than strictly necessary.

In aseptic packaging technology, the product undergoes a thermal treatment before being packed in a container; The heat treatment is therefore independent of thhe container used. This makes it possible to combine the most adequate thermal treatment conditions for the product with a wide choice of containers.

However, for food with a high content of particulates at, the use of conventional continuous heat exchangers may result in an overcooking of liquid phase to ensure that the centre of each particle reaches the prefixed sterilising effect and in a mechanical damage of the particulate.

These heat transfer and mechanical problems might be overcome using the Ohmic heating which operates by direct passage of alternating current through the continuous flow of an electrically conducting product.
Although the concept of Ohmic heating is not new, in the last decade some researchers renewed the interest in this technique (1, 2, 3), wich, when combined with an aseptic bag in box filling system, enables stable high-quality semi-finished products to be obtained.

Design of the aseptic Ohmic pilot plant

The pilot plant, developed by emmepiemme s.r.l , is represented in Fig.1. It consists of:

- *Feed tank-pump:* the product introduced in the feed tank is pumped into the pipes by a piston moved by a gear electric motor; a speed governor permits the flow rate to be regulate from 10 to 100 litres / hour.

-*Ohmic heater:* this is composed of two glass pipes and three annular electrodes. The common 50 Hz power supply is transformed into 25 kHz high voltage electric current and then connected to the central electrode; the lateral electrodes are connected to ground potential. A plug commutator permits the choice the most convenient voltage as a function of the characteristics of the products. The voltage ranges from 500 to 4000 Volts and the maximum deliverable power is 3 kW.

-*Holding tube*: being changeable in length, it permits a wide range of holding times in combination with the flow rate.

-*Recycle valve*: this common aseptic three way valve convoys a non sterile product into the recycling tank.

-*Cooler*: a tube in tube heat exchanger is used to cool the sterile product. The 40 mmm inner diameter permits the passage of products with particles up to 15 mm without mechanical damage.

-*Aseptic tank*: this pressurized vessel serves to store the cool sterile products before the filling operation; a counterpressure of filtered sterile nitrogen permits heat treatments up to 135 °C.

-*Aseptic filler*: the bag-in-box system is able to prepare sterile packaged samples ranging from 3 to 20 kg. Sterility of aseptic filling area is maintained by pressurized saturated steam.

Principle of Ohmic heating and determination of processing parameters

Ohmic heating occurs when electric alternating current passes through a moving column of electrically conducting product. The discriminating parameter in the applicability of Ohmic heating is product electrical conductivity. Most pumpable products containing water in excess of 30% and dissolved salts have been found to conduct well enough for Ohmic heating to be applied. Non ionised materials such as syrups, fats, oils and so on are not suitable.

From the electrical point of view, the Ohmic heater previously described, can be considered as two resistances in parallel; the total resistance R_t of the system is:

$$R_t = R_1 R_2 / R_1 + R_2$$

where: R_1= Electrical resistance (Ohm) of the product flowing into the first tube

R_2= electrical resistance of the product flowing into the second tube

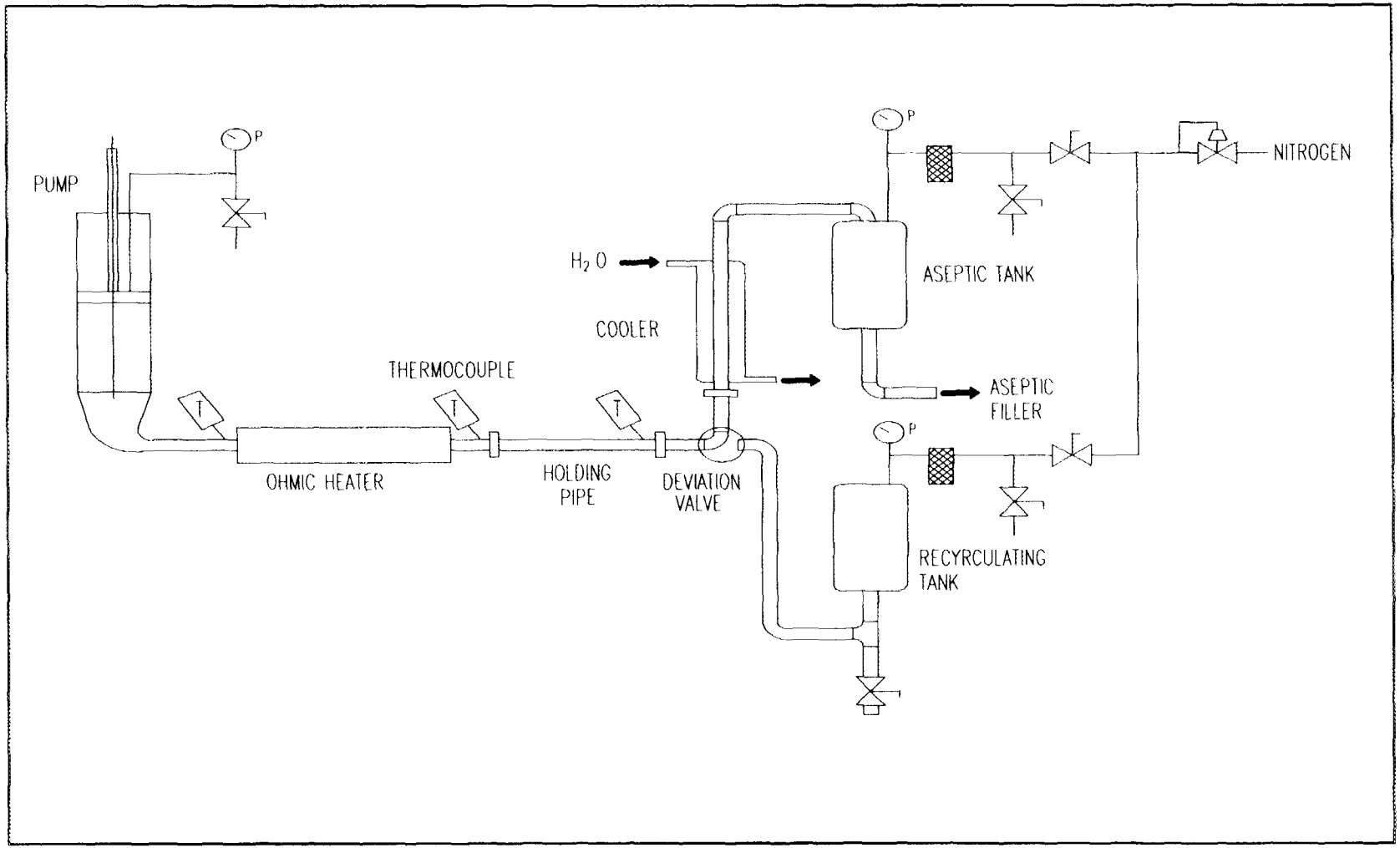

FIG 1: Ohmic heating pilot plant for production of aseptic half processed particulate fruit products

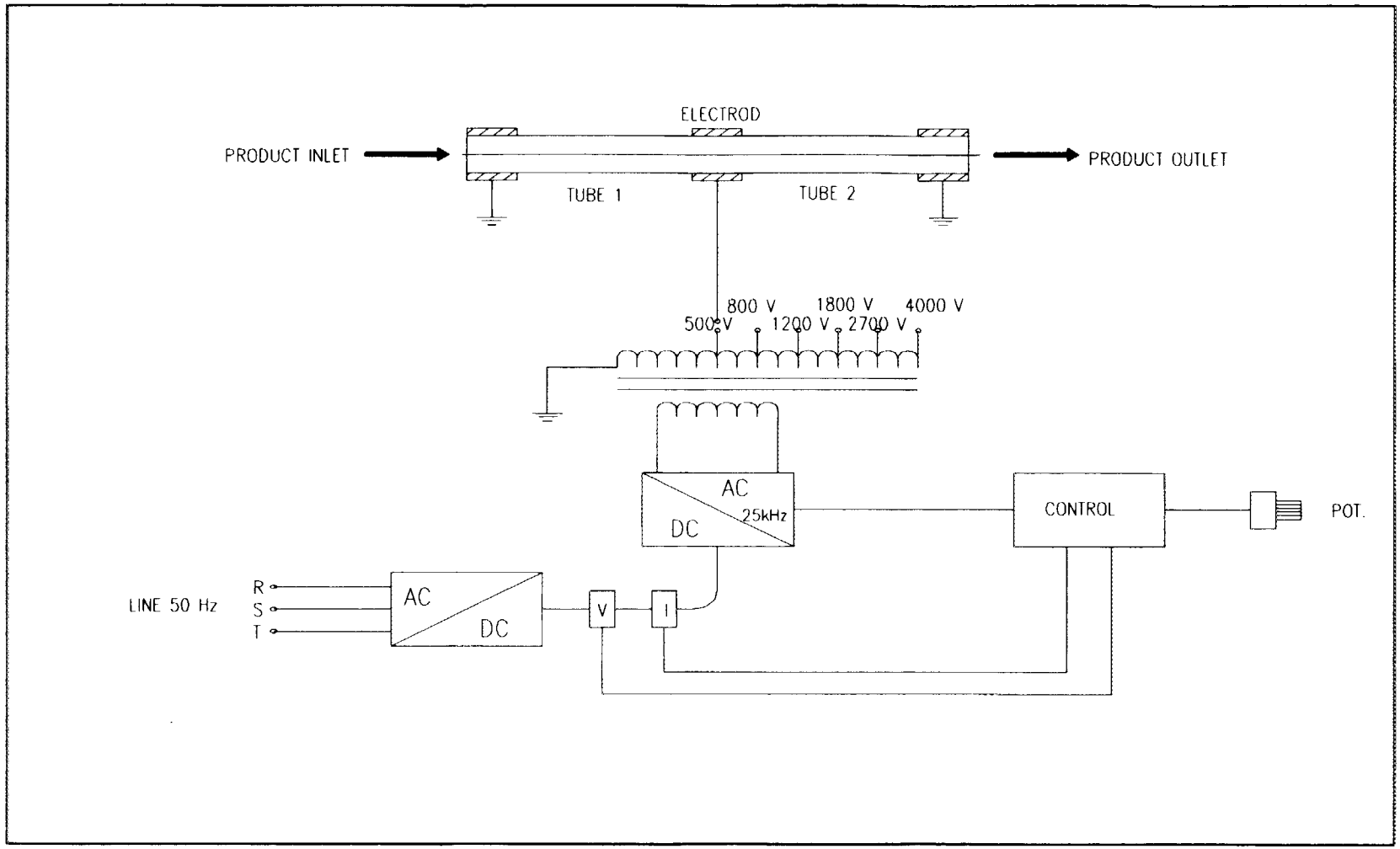

FIG 2: Electrical configuration and construction of the Ohmic heater

Since the electrical resistance decreases with increasing temperature, the specific resistivity at different temperature must be known.

The reiterated application of the following simple equations permits the process parameters to be determined:

$P = V^2 / Rt$

$T_2 - T_1 = 0.86\ P/Q$

$tr = vr / Q$

$ts = vs / Q$

where: P= Power (watts)

V= Nominal operating voltage (Volts)

R_t= Total resistance of the flowing product (Ohms)

T_2= Final temperature of the product (°C)

T_1= Initial temperature of the product (°C)

tr= Heating time (s)

ts= Holding time (s)

v_r= Volume of heating tubes

v_s= Volume of holding tubes

Q= Flow rate (kg/h)

The specific heat and density of the product are approximated to unit.

Test on apple cubes in apple puree

Experiments were conducted in the above mentioned pilot plant using apple cubes (1 cm size) in apple puree as carrier fluid. The specific resistivity of the product as a function of the temperature is shown in fig.3.

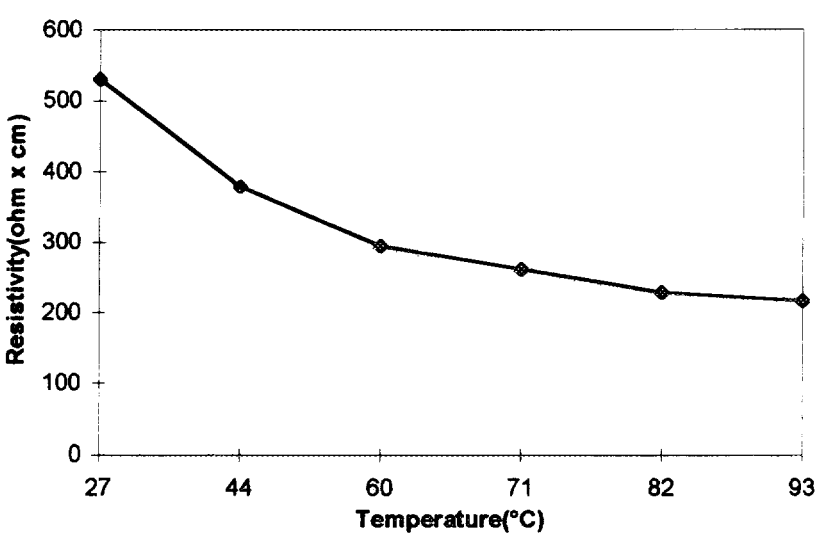

Fig.3: Specific resistance of apple puree at different temperatures

The product (70% particulates, 30% puree) was heated to 100 °C and mantained at this temperature for 45 s in order to achieve the microbiological and enzymatic stability by applying the following process parameters:

Q=30 kg/h; P=2.4 kw; V=1200 volts. After cooling the product was packaged into 2 kg sterile bags.

Ten bags were incubated at 30°C for 15 days and after this period all samples were found to be microbiologically stable.

Peroxidase (POD) activity of peroxidase was determined spectophotometrically by Putter's method (5) before and after heat treatment. The results obtained, reported in Fig.4, showed that the treated product had no residual POD activity.

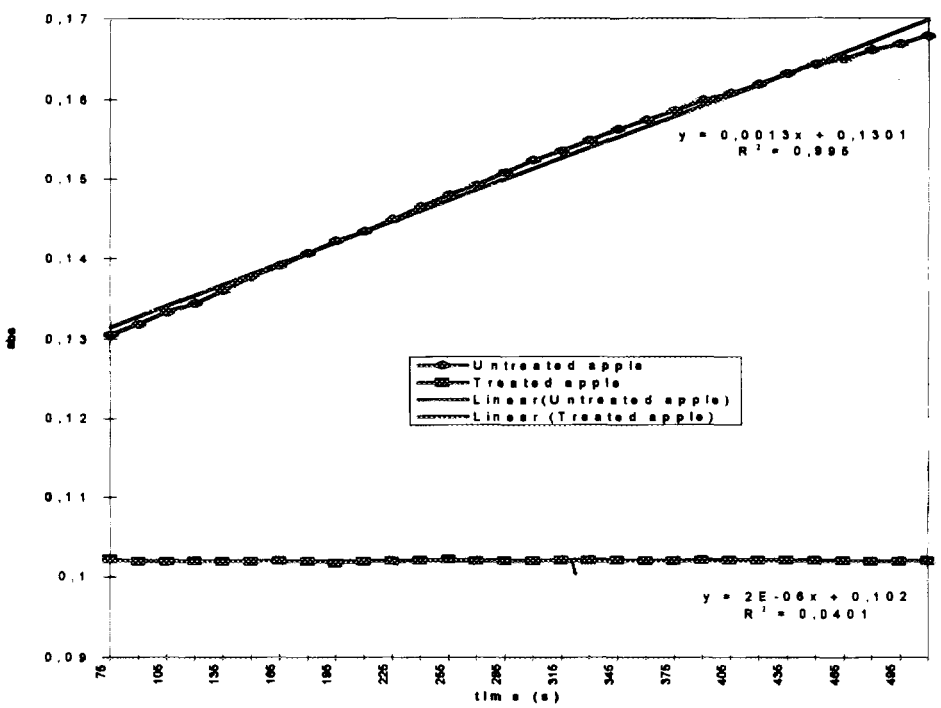

Fig.4: Determination of POD activity in apple cubes by Putter's spectrophotometric method

The product was also characterized in terms of colour and consistance using respectively a Gardner colorimeter and and FMC tenderometer. Table 1 shows that the color parameters of the treated product change slighty in comparison to untreated one; the final product retains about 50% of the initial consistance.

	Untreated apple	Treated apple
L	71,80	64,88
a	-9,31	-6,08
b	26,63	22,89
a/b	-0,35	-0,27

Tab.1: Color parameters before and after heat treatment

The coocking value (Co) of the Ohmic treatment calculated taking into account the heating, holding and cooling phases is 1.5 minutes. This value results 4-5 times less than the coocking value of the same products treated by traditional in can pasteurization.

Conclusions

The results of these first tests show that Homic heating may be a suitable methodo for heat treatment of fruit products containing large-sized particulates, since the products so treated are microbiologically and enzymatically stable and their sensory properties are almost unaffecteed.

Future experiments will be done on peaches, pears and tomato products to confirm the results obtained on apple cubes.

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QUALITY OF MINIMALLY PROCESSED FRUIT

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In the last decade great interest has been shown in the production of ready-to-use fresh fruits, mainly for their commodity and freshness attributes. In fact, the fruit products in question are presented for sale conveniently peeled, cored or sliced in prepacked packages. However, operation carried out to prepare the products (e.g. peeling, cutting, slicing, etc.) can accelerate microbial and enzymatic spoilage because of the physical rupture of plant cells. The extension of the shelf life of the minimally processed fruit can be achieved minimising the mechanical injuries as well as adopting, also in combination, various preservation technologies such as refrigeration, modified atmosphere packaging, high pressure treatments, addition of chemicals for the inibition of the enzymatic browning reactions, reduction of pH and water activity, etc.
In this paper the effects of different treatments on the shelf life of fresh fruit is discussed. In particular, the effects of low intensity heat treatments, high pressure treatments, modified atmosphere packaging, refrigeration and the low amounts of chemicals (ethanol or L-cysteine), in combination or not, were evaluated on the enzymatic and microbiological stability of the products.

TECHNICAL AND ECONOMICAL ASPECTES OF USING ELECTRON BEAM FOR DECONTAMINATION OF DRY FOOD AND FROZEN AROMATIC HERBS

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Abstract

Radiations are used for decontamination of foodstuffs. More and more industrialistics are interested in accelerators because they are electric machines.
This paper examines the effects of radiation, advantages of accelerator and costs.

I) INTRODUCTION

THOMSON-CSF Linac is a subsidiary of THOMSON-CSF, one of the largest electronics company in the world. THOMSON-CSF is well known in high technology and employ over 35 000 people throughout the world.

THOMSON-CSF Linac is in charge of manufacture, marketing, sales, installation and after-sales service of linear accelerators used in the field of radiography, scientific and industrial.

Industrial accelerators are used in different applications such as sterilization of medical supplies, polymerization of composite materials, decontamination of packaging and preservation of food stuffs.

II) GENERAL POINTS

Using radiation for preservation of food has started in the begining of the century. Indeed, in the 1960's and 1970's, many investigation have been carried out by scientists all around the world [1].

This investigation shows the following elements :

Conclusion of 30 years scientific experimentation

Conservation of food value ?		YES
«	vitamines ?	YES
«	taste ?	YES
«	flavour ?	YES
«	morphology ?	YES

INDUCED RADIOACTIVITY ?	NO
MUTAGENIC EFFECT ?	NO
TOXICAL EFFECT ?	NO

In 1980, a joint FAO / AEA / WHO Experts Commitee recommended the utilisation of radiation for it represents no danger.

III) IONISING RADIATION

Gamma rayse emitted by ^{60} Co; è beam produced by an accelerator up to 10 MeV and Xrays can be used for decontamination of agricultural produce.
Although these radiations are different from the origin, nature and dose rate, they produce the same effect on the product. These effects are limited to biochemical and biological and there is no secondary effects.

The product volumes which can be treated by a typical EB machine are shown in table 1.

TABLE 1.TREATEMENT OF FOOD PRODUCTS BY CIRCE 10 MeV 20 kW

PRODUCT	DOSE (kGy)	THROUGHPUT (tons / hour)
Onions, bulbs,garlic, ginger, potato	0.05-0.15	
Cereals, nuts, dried and fresh fruit, vegetables, flour	0.15-1	30
Fresh fruit, fresh vegetables, frozen goods	0.5-1	30
Seafood	3	14
Poultry	5	8
Meat	5	8
spices	10	4

One industrial example of food decontamination in France is radiation of mecanical separated poultry meat. Indeed, soft tissues i.e. meat and fat can be mechanically separated from bones and sustaining tissues by grinding then pressing through a sieve, poultry carcases and necks resulting from manual carving [2].

At present, radiation is used for different product in France. Some of the different kinds of foodstuffs pasteurized in Europe are shown in table 2 [3].

TABLE II. FOOD STUFFS AUTHORIZED FOR RADIATION TREATEMENT IN DIFFERENT COUNTRIES IN EUROPE

PRODUCTS	AUTHORIZED					
Vegetables					U	
Fruits					U	
Potatoes	B	F		I	U	
Strawberries	B	F				P
Mushrooms						P
Onions	B	F		I		P
Garlic	B	F		I		P
Shallots	B	F				
Condiments					U	
Black/White Pepper	B					
Paprika Powder	B					
Gum Arabic	B	F	N			P
Spices	B	F	N		U	P
Dried Vegetables	B	F	N			
Muesli-Like Cereal		F				
Mechanically Separated Poultry Meat		F				
Dried Fruits		F	N			
Frog legs		F	N			
Shellfish					U	
Schrimps	B	F				
Herbs	B	F	N			P
Poultry		F	N		U	
Fish					U	
Deep-Frozen meals for patients who need sterile diet			N		U	
Herbal Teas	B					
Edible Casein, Rennet Casein, Caseinates		F				
Raisins		F				
Dried Dates/Figs/Apricots		F				
Cereal Grains					U	
Cereal Flakes			N			
Cereal Germ		F				
Rice Flour		F				
Onion Powder		F				
Garlic Powder		F				
Animal blood, Plasma, Cruor		F				
Bovine Colostrum		F				
Egg White		F				
Bulbs, Roots, Tubers					U	

Notes : B (Belgium), F (France), N (The Netherlands), I (Italy), U (United Kingdom), P (Poland).

IV) TECHNICAL DESCRIPTION OF A STERILIZATION FACILITY 10 MeV 10 kW.

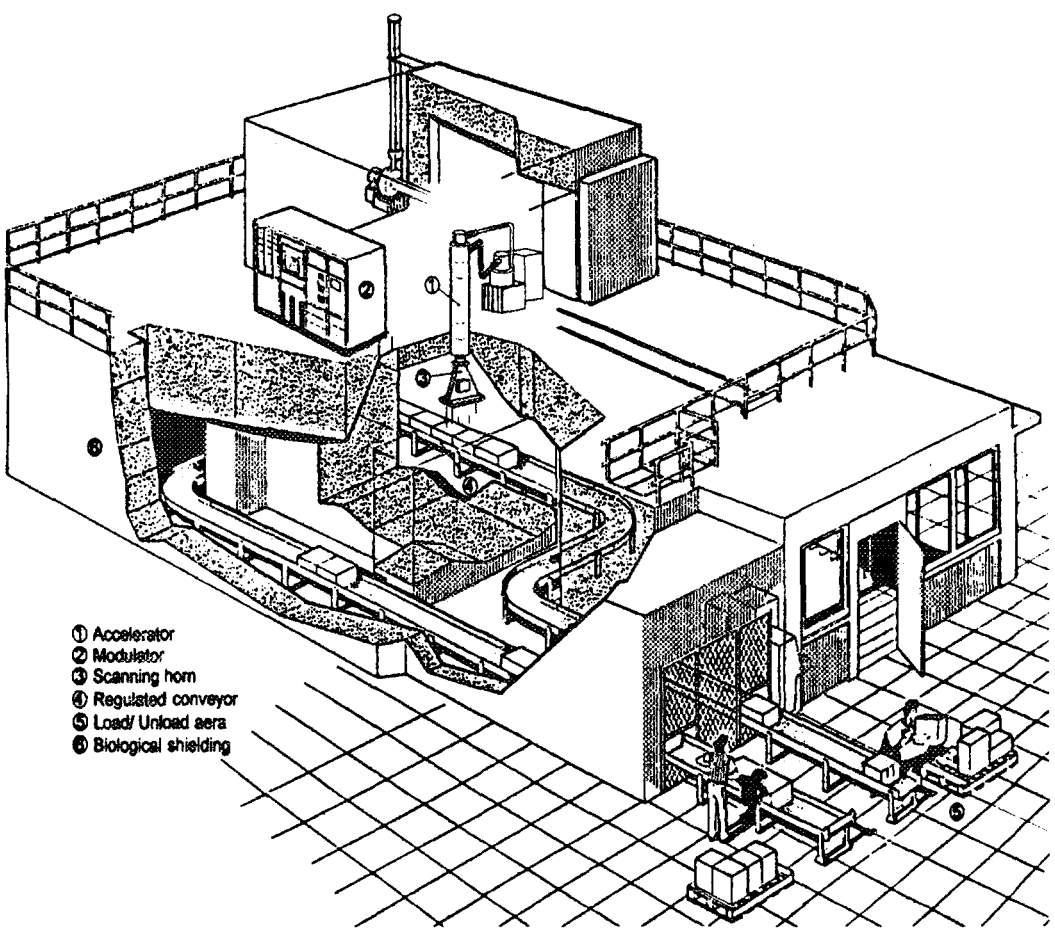
This technical description describes the elements that constitute a high energy sterilization facility built around an accelerator of type CIRCE II (10 MeV - 10 kW).

1. General description of a Sterilization Facility 10 MeV - 10 kW.

A typical sterilization facility is composed of the following elements :

- a blockhouse assuring the biological protection,
- an accelerator delivering an energy of 10 MeV and a power of 10 kW,
- a conveyor system enabling the products to be moved under the accelerator,
- the auxiliary circuits ensuring the functioning of the total assembly (cooling circuit, safety circuit, evacuation of ozone, interface circuit).

The following figure gives a view of the total sterilization facility as descriibed above.



2. Biological protection

Biological protection is ensured by a blockhouse built in ordinary concrete (density = 2.35), of which the waéll thickness and the geometry of the access points, have been calculated to guarantee a radiation dose rate of ≤ 0.1 mrem/h (≤ 1 µSv/h) at all accessible points when the facility is working.

3. Interface circuit

The interface circuit is assured principally by the supervisor system. The role of the supervisor system is to collect all the different signals coming from the different sub-assemblies (accelerator, cooling system, ozone extraction ...).

This network is connected to a supervisor from which the whole installation is piloted.

The accelerator and the conveyor are equipped with a programmable PLC. The other elements of the system (safety circuit, safety locks, ozone extraction, cooling system) are equipped with PLC block devices to which the signals coming from these elements are connected.

The supervisor is a computer of type PC Pentium equipped with a 15" monitor. A program has been developed to control an ionisation facility. All the operating instructions are managed from graphic representations on the screen accessible to the operator by using the keyboard and mouse.

An alarm manager aids the diagnostics. The treatement data is shown continually on the screen and all the data iq available on hard disk, saved in daily files for a period of at least one year. It is possible to transfer the daily files to another system.

4. Breakdown of investment details

The following table shows the details of the breakdown of investment for a sterilization facility as described above :

TABLE III. BREAKDOWN OF INVESTMENT FOR A STERILIZATION UNIT INSTALLED IN FRANCE AND EQUIPPED WITH A CIRCE II ACCELERATOR OF 10 MEV - 10 KW*

ITEMS	AMOUNT (kFF)
BUILDING	1100
Blockhouse	
EQUIPMENT FOR PROCESS	
Accelerator	8500
(with one year guarantee, technician training)	
Installation and tests	700
Interface	500
ANCILLARY EQUIPMENT	
Conveyor	1000
Cooling system, ozone exaust, ventilation	450
ENGINEERING	1100
TOTAL	13350

* Based on an installation in France. Amounts can vary depending on the country of installation.

V) PRESENTATION OF A COMPACT STERILIZATION SYSTEM : STERBOX

Many industrialists in the world have a production lower than throughput shown in table 1. That's why we have designed and manifactured a small accelerator which equip a black box named STERBOX.

STERBOX is a compact sterilization system using electrons to sterilize or to decontaminate goods.
The dimensions of STERBOX are : 4 m x 3 m x 2.8 m (13ft x 10 ft x 9 ft) including :

- the machine,
- the convoyor system
- the shielding.

STERBOX can be easily integrated in a production line to treat :

- medical disposable products in their individual packaging,
- cosmetic and pharmaceutical products,
- meals for hospital,
- food stuffs.

STERBOX is :

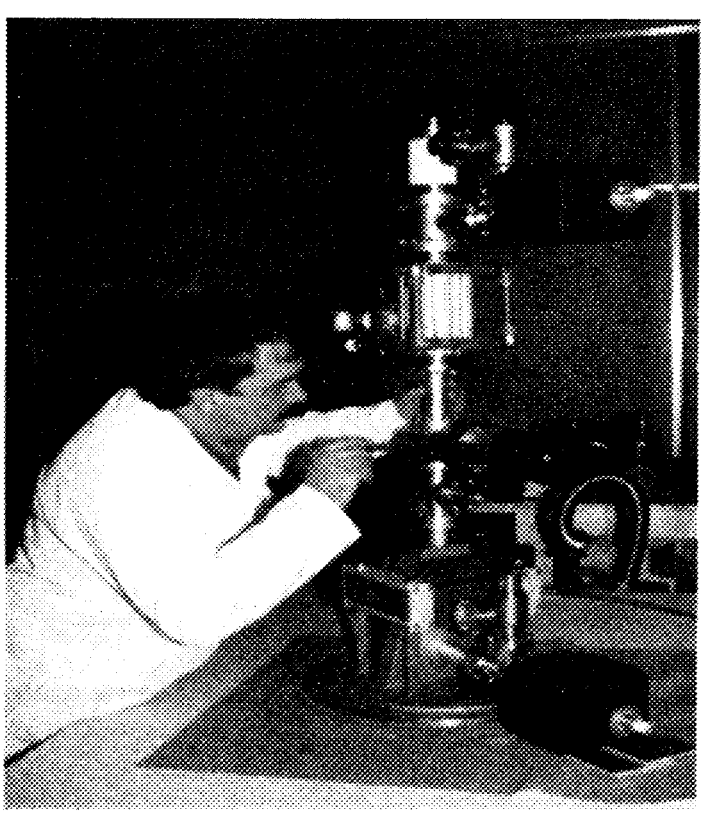
- Cost effective,
- Safe,
- Efficient,
- On-line,
- Rapid,
- No residues,
- Environmentally friendly,
- Simple to use.

STERBOX replaces :

- Contract Service Centers,
- In house EtO system,
- EtO equipment suppliers.

STERBOX advantages :

- Low capital investment,
- Full quality control,
- Improve your turn-round time,
- Products not delayed at a Service Center,
- No transport costs,
- No external suppliers.



Turn key project	1 M U$ (1997)	
Running costs including maintenance, dosimetry, parts, electricity and water consumption.		80 000 U$
Financial costs	amortization	100 000 U$
	interest	60 000 U$
	Total running cost / year	240 000 U$
Availability	(300 days/year x 24 hours/day) 95%	6640 hours/year
Cost / hour	$\frac{240000}{6640} =$	36 U$ / hour

Reduction of delay (Just In Time)
Reduction of stock level
Reduction of full control of the process

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A COMBINED TECHNOLOGY FOR THE PRODUCTION OF DRIED VEGETABLES: OSMOTIC DEHYDRATION/FREEZE DRYING

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SYNOPSIS

The use of a combination of osmotic dehydration and freeze-drying processes in the production of high quality dehydrated vegetables has been analysed. Drying kinetics of the osmodehydration process under different conditions have been determined as well as the effects of the pretreatment on the kinetics of the freeze-drying stage. Rehydration of osmo-freeze-dried samples has been evaluated and compared to that of the freeze-dried products. The experimental work has demonstrated that the combination process analysed is sound to obtain dehydrated vegetables at lower costs without severe deterioration of the nutritional and sensory characteristcs of products.

INTRODUCTION

Combined technologies have gained attention in recent years in food processing industry to achieve high quality products. Two or more unit operations are coupled and substitute the single stage process to obtain final products. Single stages operate in the near optimal conditions and to minimize biological, chemical and physical degradation of foods /1,2,3/.
An example is given by the combination of osmotic dehydration, which allows to obtain intermediate moisture foods, with a conventional drying process.
Interesting results have been achieved by coupling osmotic dehydration at low temperature /4,5,6/ with mild dehydration processes, such as freeze-drying. Nutrients, aroma components and sensory properties of products are retained while processing time and energy requirements are reduced in respect to the single stage freeze-drying process.
Osmotic dehydration is carried out by immersion of foods in a hypertonic sugar solution (10-60% in weight often in presence of salts). A considerable water loss and a simultaneous solid gain occurs to the product. The solid uptake from the solution, even though modifies chemical composition of foods, positively affects the quality of the final product /8/ by promoting:
- the inhibition of the enzymic and non-enzymic browning;
- the retention of volatile components during the following dehydration stages.

A further improvement of the quality of processed foods, i.e. texture, chemical and physical characteristics etc., is obtained by performing the osmotic dehydration stage at low temperature. Moreover, the rate of microbial growth in concentrated solution is reduced at low temperature and, thus, products contamination is avoided.
Intermediate moisture foods obtained by means of osmotic dehydration are usually not shelf stable products. A further dehydration stage (i.e. air drying, freeze-drying) is thus necessary to achieve the stability of the processed products /9,10/.
In this paper the combined osmotic dehydration/freeze-drying process of apple and potato is analysed. The aim of the experimental study is to investigate the effect of osmotic dehydration pretreatments at low temperature on drying kinetics of freeze-drying.

MATERIALS AND METHODS

Sample preparation

Stark delicious apple and bintjie potato are used in the experiments. After peeling , slabs of 1x1x2 cm are cut from the fruits.

Osmotic media

Glucose solution (50% in weight) and sucrose/sodium chloride solution (45/15 % in weight) are used as osmotic media of apple and potato respectively. A ratio 25:1 (in weight) of osmotic medium and samples is used during experiments to avoid significant dilution of the solution during dehydration.

The osmotic solution is gently agitated by means of a mechanical stirrer (100 r.p.m.) to increase the mass transfer rate and prevent the formation of a diluted film on the surface of the sample. This is particularly important at low temperature due to the increase of the viscosity of the solution /11/.

Osmotic dehydration

Osmotic dehydration of both products is carried out at three different temperature of the solution: 25 °C, 4 °C and -1 °C. The sample is completely immersed in the osmotic solution by means of a plastic mesh. A processing time of 72 h and 24 h for apple and potato respectively is necessary to reach the equilibrium conditions /12/, i.e. constant solid and liquid mass transfer rates.

To determine the kinetics of the process, samples are taken from the solution (every 30 min.), dried on blotted-paper to remove the excess solution from the surface and weighed. Samples are then dried under vacuum at 60 °C for 24 h to determine the total solid content.

The initial solid content of both products is also determined, according to standard methods.

Water loss WL, moisture content X_w and solid uptake SG, can be evaluated as follows:

$$SG = \frac{W_s - W_{s0}}{W_{s0} + W_{w0}} \qquad WL = \frac{W_{w0} - (W_t - W_s)}{W_{s0} + W_{w0}} \qquad X_w = \frac{W_t - W_s}{W_{w0}}$$

being W_t, W_w,W_s, total weight, water and solid weight in the sample (subscript 0 refers to fresh sample).

WL and SG are evaluated assuming that the leaching of soluble and unsoluble solids from the samples to the solution during osmotic dehydration is negligible /5, 7, 11/.

Triplicate dehydration runs are performed for each experimental conditions.

Freeze-drying

To evaluate the effect of the osmotic dehydration on freeze-drying kinetics of apple and potato, the pretreatment is stopped before the equilibrium conditions in the sample are reached, i.e. after 25 h and 3.5 h for apple and potato respectively.

Osmodehydrated samples are wrapped in a polyethylene film (to avoid freeze-dehydration) and frozen at -24 ° C for 24 h. Freeze-drying is performed in a Christ freeze-drier (model Alpha 1-4) at a residual pressure in the chamber of 0.2 mbar and a shelf temperature of 25 °C.

To determine the kinetics of the freeze-drying process, samples are taken from the freeze-drier, weighed and completely dried in a vacuum oven to estimate the solid uptake. Triplicate dehydration experiments are performed also in this case.

Rehydration kinetic

Rehydration of osmo-freeze-dried apple and potato is performed by immersion in water at 50 °C until a constant weight of the samples is reached. Relevant rehydration rates are compared with those of samples dehydrated utilizing the freeze-drying stage only.

RESULTS AND DISCUSSIONS

Osmotic dehydration

Figures 1 and 2 show the WL and SG curves of apple osmodehydrated at the three temperature of the solution tested. In the WL and SG curves, two different periods can be observed: the dynamic

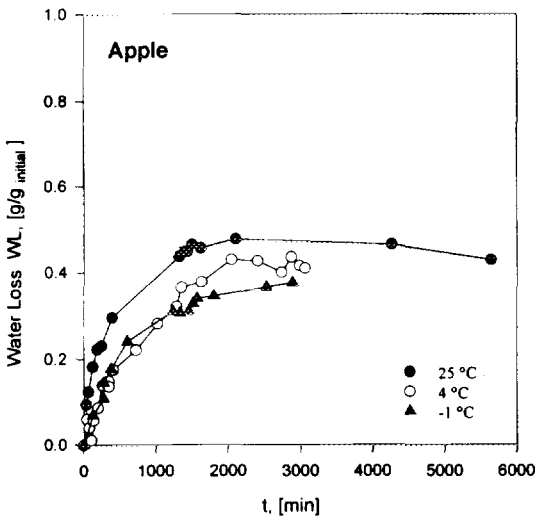

Fig. 1: Water loss of apple sample osmosed at 25, 4, -1 °C.

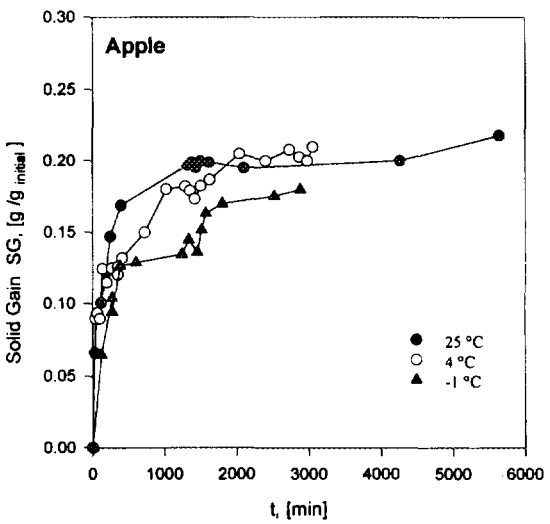

Fig. 2:Solid gain of apple sample osmosed at 25 , 4 and -1 °C.

period and the equilibrium period. In the first the mass transfer rates of water and solid increase rapidly until the equilibrium period is reached. In the equilibrium period the net mass transfer rate is zero. The dehydration kinetics are faster at higher temperature, as expected. The equilibrium period is reached, for all temperature levels, after a soaking time of 1500 min. The water loss at the beginning of the equilibrium period has almost the same value (0.4 g/g $_{initial}$) for all temperatures tested.

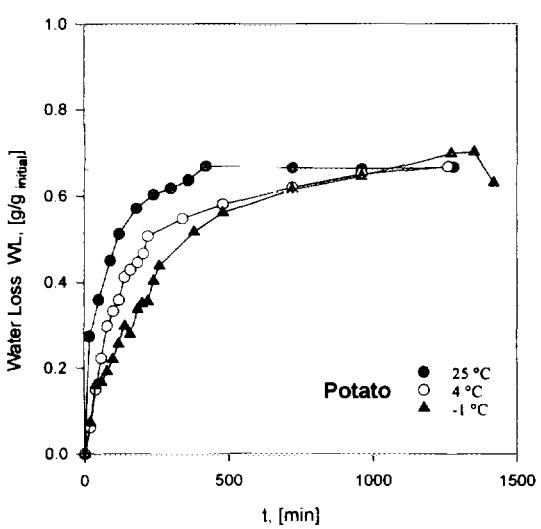

Fig. 3: Water loss of potato sample osmosed at 25 , 4 and -1 °C.

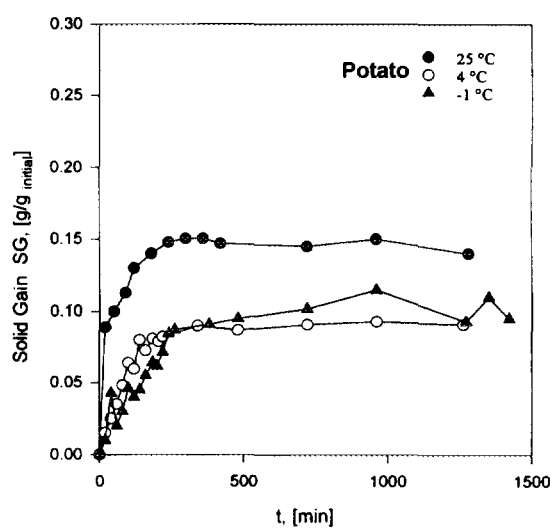

Fig. 4:Solid gain of potato sample osmosed at 25 , 4 and -1 °C.

Also the SG curves show the same trend. The equilibrium value of SG is about 0.20 g/g $_{initial}$ for all

temperature tested. In the dinamic period a lower solid uptake occurs, if the osmotic dehydration is performed at -1°C.
WL and SG curves of potato are shown in Figures 3 and 4. In the dynamic period a more marked effect of the temperature of the osmotic solution on WL can be detected. The equilibrium moisture content has the same value for all temperature tested. It can be noted in this case that a higher value of the solid gain is detected at higher temperature. The equilibrium solid gain is about 0.10 g/g $_{initial}$ at 4 °C and -1 °C and 0.15 g/g $_{initial}$ at 25 °C.

Freeze-drying
The effect of the osmotic dehydration pretreatment on the freeze-drying kinetics of apple is shown in Figure 5. Drying curves of fresh and pretreated apple slabs are reported for the three temperatures

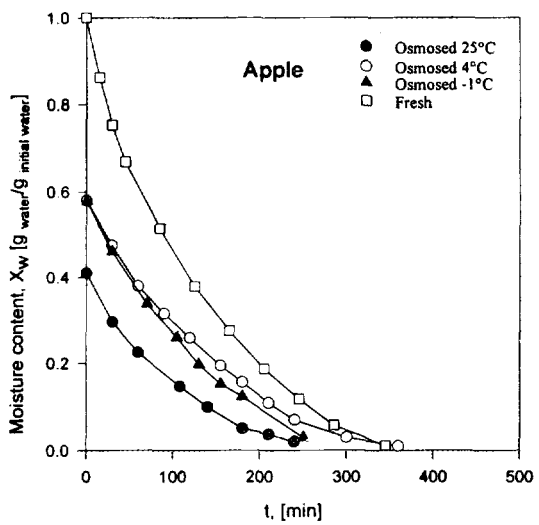

Fig. 5: Freeze-drying curves for osmosed apple compared to fresh freeze-dried samples

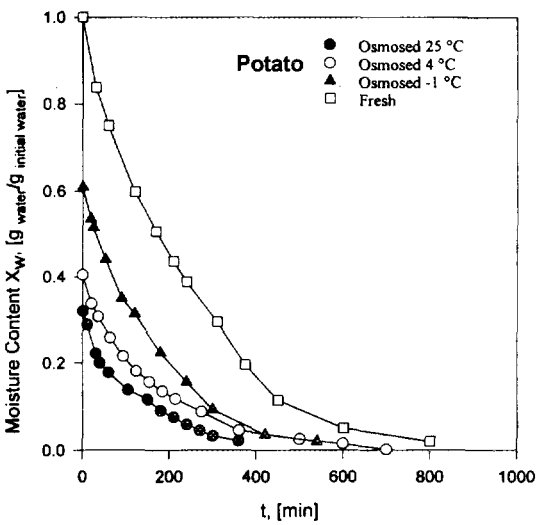

Fig. 6: Freeze-drying curves for osmosed potato compared to fresh freeze-dried samples

of the osmotic solution. The different initial moisture content of the samples is due to the different water loss occurring at the end of the osmotic dehydration process. This affects the processing time, the lower the initial moisture content, the shorter the freeze-drying time.
The same trend is also detected for potato samples, as shown in Figure 6. The effect of the pretreatment observed is due, also in this case, to the decrease of the initial moisture content of samples.

Rehydration
The comparison between the rehydration behaviour of sample dehydrated with the single freeze-drying stage and the combined process is shown in Figures 7 and 8 for apple and potato respectively. The water uptake (on total solid basis) is reported as a function of time.
Samples dehydrated with the combined process show a lower rehydratability than those dehydrated with the single stage process. This can be related to the solid gain occurring during the osmotic dehydration and to the shrinkage of the sample. The rehydration of apple, dehydrated with the single freeze-drying process, is sensibly higher than that of pretreated samples.

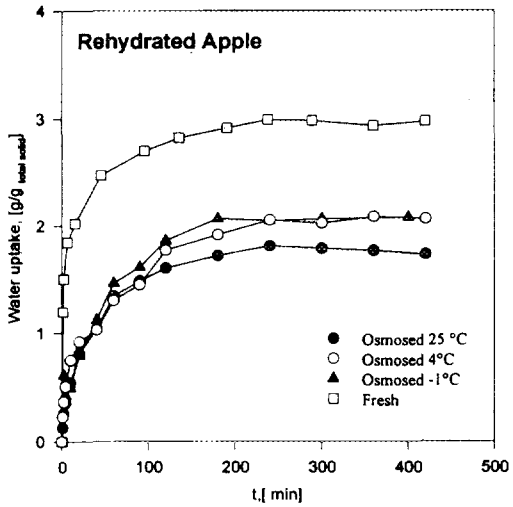

Fig. 7: Water uptake vs. time of freeze-dried pre-treated apple.

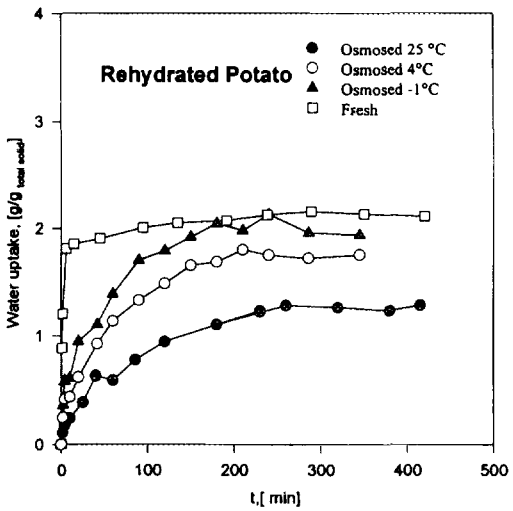

Fig. 8: Water uptake vs. time of freeze-dried pre-treated potato.

CONCLUSIONS

The work performed so far demonstrate that the combined process osmodehydration/freeze-drying is a very promising technology in the production of high quality dehydrated vegetables. Osmotic dehydration, performed at low temperatures, is an effective dehydration process determining high water loss and solid gain but moderate deterioration of chemical, physical and biological characteristics of products.

The effect of the osmotic dehydration pretreatment on freeze-drying kinetics is related to water removal. In fact, due to their lower initial water content, osmodried samples show a sensibly lower freeze-drying time.

The solid gain from the osmotic solution influences directely the rehydration kinetics of the two vegetables used in the experiments.

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PERSPECTIVES OF SUPERCRITICAL CO_2 USAGE IN THE FOOD INDUSTRY

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The use of a supercritical fluid as a solvent offers many interesting possibilities in food technology. Its solvent power is adjustable via mechanical compression and/or changing temperature; its diffusivity is considerably larger than liquid solvents; it enables fast extraction fluxes; there is the possibility to extract at low temperature; a non-toxic extraction media, such as carbon dioxide can be used; it is easy to recycle the extraction fluid and to separate extracted substances: by pressure reduction, change of temperature, external agent, or use of a solid or liquid phase trap; it can be used for both solid and liquid matrices, and for fractionation and/or purification of the extract by adjusting the solvent power during the extraction; slight addition of organic modifier can change solubility. The less-invasive, more benign conditions for extraction with supercritical carbon dioxide are suitable to food products compared to liquid-solid extractions.

1. WHAT IS A SUPERCRITICAL FLUID (SF).

A pure component is considered to be in a supercritical state if its temperature and pressure are higher than critical values, and the liquid and gaseous states are indistinguishable from one another (figure 1). The critical temperature is the highest temperature at which a gas can be converted to a liquid by an increase in pressure alone; while the critical pressure is the highest pressure at which a liquid can be converted to a gas by an increase in temperature alone.

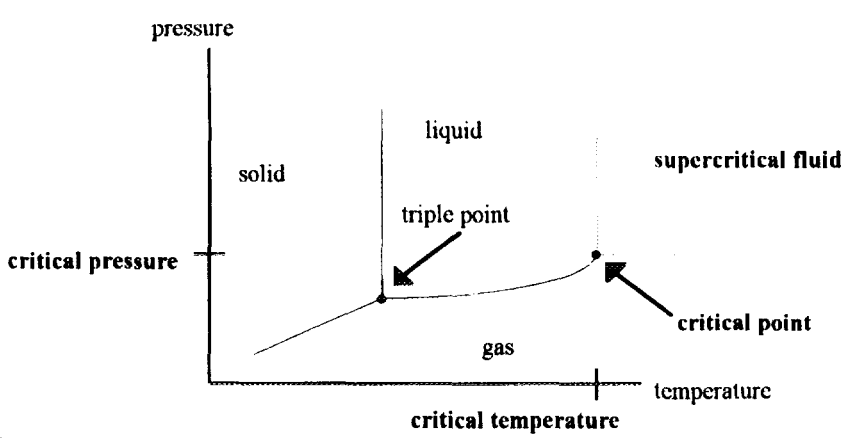

Figure 1. Pressure/Temperature diagram of state.

The interdependence of volume, temperature and pressure are of utmost importance for SF extraction, since properties of supercritical compounds change greatly with prevailing conditions, and these variations are the basis for many applications (1). The physical properties of a SF are intermediate between those of a typical gas or liquid (table 1).

Table 1. Characteristics of SFs.

	Density (g/ml)	Diffusivity (cm^2/sec)	Viscosity ($g*cm^{-1}*sec^{-1}$)
Liquid	0.7 - 1	10^{-6} - 10^{-5}	10^{-2}
Gas	10^{-3}	10^{-1}	10^{-4}
SF	0.2 - 1	10^{-3}	10^{-4} - 10^{-3}

For example, the density of a SF can be changed by varying the pressure applied to the fluid. Hence, a SF can have a density which ranges from those exhibited by gases to liquid-like values when the fluid is highly compressed. A SF maintained at a relatively high density is capable of dissolving a variety of materials, just as conventional liquids do. By changing pressure and temperature values of a SF it is possible to greatly modify its density, diffusivity and viscosity, thus greatly modifying its solvent power. Therefore using the same SF at different temperature and pressure conditions, it is possible to perform different kinds of extractions (2).

2. WHY TO USE A SUPERCRITICAL FLUID.

The useful features of SF are the following (2):
- solvent power adjustable via mechanical compression and/or changing temperature;
- diffusivity considerably larger than liquid solvents;
- fast extraction fluxes;
- possibility of extraction at low temperature;
- use of non-toxic extraction media, such as carbon dioxide;
- easy recycling of extraction fluid;
- easy separation of extracted substances: by pressure reduction, change of temperature, external agent, or use of a solid or liquid phase trap;
- can be used for both solid and liquid matrices;
- can be used for fractionation and/or purification of the extract by adjusting the solvent power during the extraction.
- slight addition of organic modifier can change solubility.

3. WHY TO USE SUPERCRITICAL CARBON DIOXIDE (SC-CO_2).

All substances can theoretically exist in the supercritical state, but not all have an easily reached critical point. Among them, CO_2 presents a series of advantageous characteristics (2):
- it is non-flammable, non toxic, and non explosive;
- no waste disposal is needed;
- it has a low critical temperature (table 2), allowing non destructive extraction of thermally labile compounds;
- it is commercially available and moderately priced at the required purity level;
- no negative impact either on the environment or the operator;
- a small quantity of modifier can be added to alter SF properties.

Table 2. CO_2 supercritical state.

	T_C (°C)	P_C (bar)	D_C (g/ml)
CO_2	31.01	72.9	0.472

For each application CO_2 has to be used at maximum solvent power towards the target compound (figure 2).

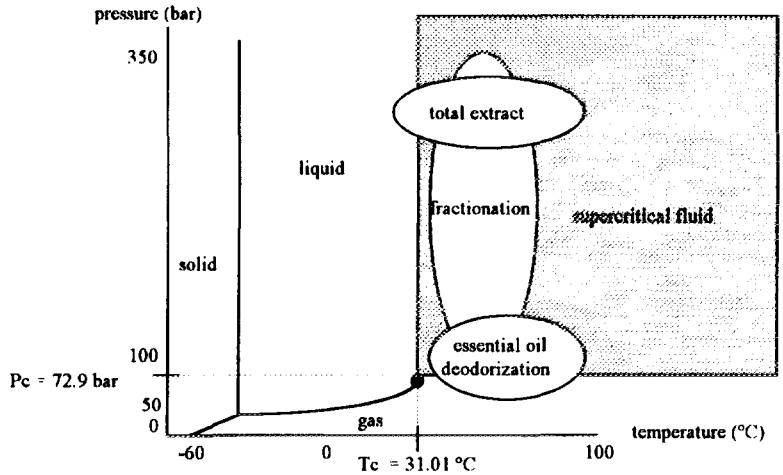

Figure 2. P/T CO_2 diagram of state, and solvent power areas (3).

The less-invasive, more benign conditions for extraction with supercritical CO_2 compared to liquid-solid extractions are suitable to food products (4).

4. PRESENT AND FUTURE APPLICATIONS OF SFs.

The first use of supercritical fluid technology was in 1936 (5) in the propane deasphalting process for refining lubricating oils (6). In 1955, Todd and Elgin (7) had highlighted the concept of Supercritical Fluid Extraction (SFE) for industrial scale operations; however, it was the work of Zosel (8) and co-workers who studied supercritical fluid extraction in many applications in the food, petroleum, and chemical sectors, that was responsible for creating wide spread interest in the technology. In their studies they identified that supercritical fluids, especially CO_2, exhibited many advantageous extraction and purification characteristics in the decaffeination of coffee beans. In fact, the first literature regarding food applications was produced early in the 70s (patent on decaffeination of green coffee), and the ex-FGR Gesundheits-ministerium prohibited the use of methylene chloride for decaffeinating coffee. Hag AG became the pioneer of the industrial food field (9). In 1976 Stahl and Shiltz (10) studied the extractability with supercritical carbon dioxide of numerous individual compounds from various classes (e.g. polyaromatics, phenols, aromatic carboxyl acids, anthraquinones, pyrones, hydrocarbons and other lipids; this furnished the first clear picture of the solubilities of natural substances in supercritical carbon dioxide. The extraction of fats and oils from a variety of matrices with supercritical fluids has been performed for some time. Supercritical fluids are of particular interest to the fats and oil analyst because of the extraordinarily high solubility exhibited by lipid material in SC-CO_2. For example, it is possible to obtain lipid solubilities ranging from a few percent to over 25 wt %, depending on the pressure and temperature conditions chosen. Other lipids, such as fatty acids, tocopherols, and sterols, exhibit similar trends in solubility. Unfortunately, the high overall solubility of many lipid compounds compromises the molecular specificity of SFE; however, the use of lower pressure and/or temperature permits supercritical fluids to be applied to many analytical applications. A pre extraction acid treatment of the sample was instituted to enhance the accessibility of lipids associated with carbohydrates in cheese. In some cases, ethanol was added to the pre weighed sample at 10-30% levels before extraction (4). For food samples that contain appreciable amounts of water (butter, hamburger, carrots, lettuce, etc.), hydromatrix has been shown to be an extraction enhancer. It absorbs at least twice its weight in water and effectively disperses the sample (11). A large body of experimental data has been accumulated on the solubility and extractability of natural products, such as steroids, alkaloids, anti cancer agents, oil from seeds, and caffeine from coffee beans, in various supercritical solvents such as CO_2, ethane, ethylene, and N_2O. Carbon dioxide is probably the most widely investigated supercritical fluid since its critical temperature makes it an ideal solvent for extracting materials that are thermally labile (6). This information, together with experience from more than two decades, has given rise to the rules of thumb listed below for extraction with SC-CO_2 (12):

- it extract easily (up to 300 bar) lipophilic organic compounds with a low molecular mass (up to 300-400), such as hydrocarbons, ethers , esters, lactones, ketones and similar compounds;
- the presence of polar functional groups (e.g., hydroxyl, carboxyl) lowers, and may even completely prevent, extraction;

- it doesn't extract polar substances, such as sugars, glycosides, amino acids, lecithins, etc., and polymers including proteins, cellulose, polyterpenes and plastics. Non-polar oligomers are only sparingly soluble;
- water shows increasing solubility in SC-CO_2 when temperature increase (ca. 0.3% by weight at 50°C);
- fractionation of liquids or solids is possible when the constituents of the mixture exhibit large differences in molecular mass, vapour pressure or polarity.

The different solubility powers of the various gases cannot be explained directly through physical properties, such as the gas density, critical point or dipole moment. Simple and generally valid rules about the solubilities of lipophilic natural substances can be no better established for the various supercritical gases than for the classical solvents. The field of application of SFs as solvents and extraction agents is extensive. Methods of substance separation have become highly important in all spheres and consequently this new separation procedure has led to diverse research activities. Most of the applications are still in the development stage and are being carried out only on the laboratory or pilot plant scale to explore procedural techniques. However, intensive efforts are being made in connection with industrial applications. This market interest is demonstrated by the considerable number of patent applications during the last years. In short, the wide range of SC-CO_2 applications (table 3), can be summarized as following: **food**; environment; medical; pharmaceutical; aerospace; mineral oil industry.

Table 3. Nowadays industrial application of SC-CO_2 technology (9,13).

Process	Raw material
caffeine removal	coffee, tea
denicotinization	tobacco
essential oil recovery	spices (e.g. hops, red pepper, vanillin), officinal plants, coniferous woods
recovering of fat/oil	oil seeds (e.g., soybean, peanuts, sunflower, rapeseed, corn germ, coconut, cocoa)
cholesterol removing	animal fats
fractionation	lemon essential oil
fractionation	cod liver oil, fatty acid esters
oil refining	recovering of free fatty acids, hydrogenation, separation
deodoration	vegetal oils, animal fats and oils, beer yeast
octacosanol extraction	sugar cane
aroma extraction	e.g. apples
remediation	waste waters
remediation	solid wastes
remediation	refinery wastes
pharmaceuticals recovery	botanicals or not
acetone residue extraction	antibiotics
cleaning, inspection, repair, recycle operations	missile and aircraft gyroscope components
removing oil traces	fibre optics rods
deasphalting	mineral oils
fuel production	residual oil
removing of lipids and protein (xenograft production)	bones

However, it is not uncommon to find interrelated applications among these and eventually other fields.

There are several ways of using SFE in the **food and beverage** area:

- extraction and separation of useful components from different materials. For example: extraction of lipids (fats/oils), extraction of essential oils (flavours/volatiles), etc.;
- removal of unwanted components (e.g., decaffeination of coffee beans, deterpening of citrus oils, etc.).
- sanitation of food (removing or killing of viruses, microorganisms, etc.);
- reactions (e.g. enzymes activity).

The extraction and refinement of edible oils is a predominant domain of application of supercritical technology.

Successes in the mid to late 80s and early 90s (e.g., coffee and tea decaffeination, hop extraction) coupled with the many papers and books being published on the subject of SFs, heightened the awareness of industry towards the potential of applying SF processing in many non-food operations. Some of these operations concerned many types of non-extractive processes. For example (9):

- Supercritical Fluid Nucleation (Rapid Expansion of Supercritical Solutions);
- Supercritical Fluid Infiltration (materials into micro porous solids);
- Supercritical Fluid Anti-solvent Recrystallization (14);
- Supercritical Fluid Infusion (into non porous polymers);
- Chemical Reactions in Supercritical Fluids;
- Unicarb® paint spraying and coating process.

The results obtained with supercritical fluids for these processes are not easily obtainable with liquid solvents, or by distillation (9). Still more recently, research is going on new fields such as:

- Supercritical Water Oxidation (fuel waste, environmental, effluents, waste water, sludge) (15);
- Enzymatic synthesis in Supercritical fluids (16);
- Supercritical paper deacidification (17);
- Supercritical fluids Particle Tailoring (18);
- Supercritical Carbon Dioxide Microcoating (19);
- Supercritical Carbon Dioxide Silica Aerogels Production (20);
- Supercritical Viral safety (21,22);
- Supercritical drying of porous silicon (23).

5. TECHNOLOGICAL FOOD APPLICATION OF SC-CO_2.

5.1. PRESENT INDUSTRIAL APPLICATIONS.

The process development work being carried out at the United States Department of Agriculture's (USDA) Northern Regional Research Center (Peoria, IL) on the extraction of vegetable **oils** with supercritical fluid solvents has been widely reported in the literature (24,25). Other research groups studied feasibility of lipid extraction from various matrices. Rossi et al. (26,27) studied cocoa butter extraction and characterization. Froning and co-workers (28) extracted cholesterol and other triglycerides from egg powder. King et al. (24) used SFE on dehydrated foods and meats to remove free lipid fractions and total fat. A similar study was conducted on cheese matrices by Lembke and Engelhardt (29). SC-CO_2 is being considered as a replacement for hexane in soybean oil extraction. Scrutiny by the FDA and awareness of the health and safety hazards associated with the use of hexane have prompted the examination of carbon dioxide as an extracting solvent (6). The use of SC-CO_2 offers here the chance of combining several complicated stages of classical chemical engineering, leading to an essentially simplified overall procedure. The Nutritional Labeling and Education Act in the United States now requires the total saturated and unsaturated fat packaged in foods to be listed on their labels. In addition, food manufacturers require consistent determination of fat for quality purposes (4).

The sphere of **flavouring materials** (aromas, spices and essential oils) is of practical interest in the world of nutrition (and also for the pharmaceutical domain and the perfume and cosmetic industries). Concentrates can be obtained under mild thermal conditions with a physiologically unobjectionable solvent, a procedure which is usually superior to classical extraction or steam distillation. Further, most of the substances concerned are highly soluble even at medium gas pressure of 100 bar. They are therefore attainable with a comparative minimum of effort and possess, in addition, a high market value.

The increasing demands of the food (and cosmetic industries) for **natural plant colouring materials** renders interesting the extraction of red and yellow carotenoids from plant material. Through their high extractive selectivity, SC-CO_2 can yield carotenoid fractions of great purity, largely free of undesirable material.

A number of publications, mainly in the patent literature, have been devoted to the **"detoxification"** of tea, coffee and tobacco; individual variations are distinguished through details in the technical procedure. Coffee decaffeination with carbon dioxide has been the object of a large amount of effort in research and development at the Max Planck Institute for Coal Research in Germany and at other academic and industrial laboratories in Europe and the United States. The selective extraction of the alkaloids can be favoured by a particular water-content of the plant material; examples are the extraction of caffeine from green coffee beans or tea leaves and of nicotine from tobacco leaves (12).

King and associates (30) have used SC-CO_2 extensively to prepare food samples for **organochlorine pesticide** analysis.

5.2. APPLICATIONS UNDER INVESTIGATION.

This section is dedicated to fields of the food area in which the studies are still in course, and whose industrial application has yet to be realized. Their industrial application wait to become a future purpose. Montanari et al.

(25) studied the selective extraction of phospholipids from soybean. Amongst lipids there exist some high value compounds, so Crudele et al. (31) conducted studies on extraction of gamma-linolenic acid from borage. Perretti et al. (32) extracted oil from millet to verify quality and technological perspectives of minor cereals. Montanari et al. (33) characterized vegetable butter extracted from an African tree, the shea (or karitè), which has an high unsaponifiable antioxidant fraction. Always more important is the field of industrial by-products for the recovery of high value compounds. Favati and others (34) studied the extraction of lycopene and β-carotene from by-products of the tomato industry. Further example are: selective sterol extraction (35); squalene extraction (36); tocopherol extraction (37); phenol extraction (38). There are many other applications, too numerous to mention.

6. ANALYTICAL FOOD APPLICATIONS OF THE SC-CO_2.

An interesting field of application of SFs is that of chemical-analytical. In recent years, in addition to the research on technology of industrial extraction, much research has been aimed at using SFC (Chromatography with Supercritical Fluids) and SFE for analytical ends. It happened, because the technological field of SF applications is very linked with the analytical one and *vice versa*. As health care becomes more important, the analytical field of SF is ever more influenced and enhanced by health regulations. Development and growth of the SFC have been preceded by a few very important developments in technology of separation. In recent decades, the chromatographic instruments field has been dominated by methods that use as the movable phase liquids (HPLC) or gas (GLC). One of the principal advantages of capillary SFC is that it can use numerous GLC and HPLC detectors. Another advantage of using this technique is the possibility of working with low oven temperature (generally not over 100°C) allowing therefore analysis of thermolabile compounds (pesticides, isocyanates, explosives, etc.), and separation of complex mixtures with medium-high molecular weight (tensioactives, polyglycoles, oligomers), where GLC techniques reach their limit in terms of molecular weight and HPLC has no sensitive and selective detectors. The above mentioned advantages, together with different covering polarity of capillary columns, and the possibility to modulate separation through all the parameters used in GLC and HPLC, mean that SFC techniques are highly promising for such investigation. Another field of analytical use of SC-CO_2 is the SFE. With respect to traditional solvents it is quicker, allows a quantitative reduction of organic solvents, and allows a more effective elimination of the extraction solvent. Everything reflects positively on costs and on preparation time of samples to be analysed afterwards. There are different application methodologies of SFE in analytical field. The simpler methodology of just extracting a single product is scarcely used because it is not always possible to obtain a higher extraction selectivity with a complete absence of other compounds in the extract. It is clear that by SFE it is possible to extract a pool of compounds, which then can be evaluated with appropriate analytical instruments inserted on-line to the extraction operation, or separately analysed in a succeeding time (off-line). In more complex methodologies, SFE technique is completed with adsorption. SFE technique offers the operator the possibility of eliminating the long and often troubling steps of preparing the sample, arriving at direct injection of the matrix in the SFC system, allowing elimination of substances without prior preparation before the analysis; besides, with this technique, the use of traditional organic solvents in preparative and/or analytical phase is avoided, with great advantage for operators and with a complete residuals absence and/or recycle (39). Analytical prescriptions and methods adopting SC-CO_2 at present time are (2):

- oil/protein seeds (soybean, peanuts, etc.)
- peanut butter
- milk powder
- corn flour
- hamburger
- cacao
- soy flour
- pork and bovine meat
- feed for domestic animals
- chips
- mayonnaise
- pre-cooked.

Furthermore, methods are in progress for (2):

- chicken
- pre-cooked
- pop-corn
- cereal kernels
- cheese
- fish
- foods for childhood
- olive
- cookies
- residuals of processing of fish and meat
- salami.

Future analytical applications will be disposable for tocopherols, carotenoids, phospholipids and other lipophilic substances in foods (2).

7. PRESENT MAIN RESEARCH AT THE ISTITUTO DI INDUSTRIE AGRARIE (AGRO-FOOD INDUSTRIES INSTITUTE), UNIVERSITY OF PERUGIA.

- Extraction, purification and fractionation of phospholipids from soybean.

- Extraction of borage oil and its enrichment in gamma-linolenic acid.
- Extraction, purification and fractionation of butter from karitè (*Butyrospermum parkii* or shea).
- Extraction, purification and fractionation of oils with high biological value from kernels of minor cereals.
- Quick determination of lipid content in product for childhood and Mediterranean food products (olives, salami, rough ham, mozzarella cheese, etc.)
- Extraction, purification and fractionation of antioxidants from organic complex matrices.
- Extraction and purification of sterols from organic complex matrices.
- Quick determination of nicotine in tobacco leaves and human skin.
- Extraction of high value pharmaceutical compounds from vegetal matrix (e.g., sesquiterpens).
- Up-grade of pilot plants to get SC-CO_2 extraction from solid and liquid matrices.

8. CONCLUSIONS.

SF technology is still too immature to expounds any hard and fast rules about its use and limits. In fact, the potential for this technology is bounded only by the imagination of the researcher who speculates on its potential application. But, as can be demonstrated throughout the wide literature and patent lists, imagination should not cloud sound engineering judgement. Certainly the literature should be used to see whether SF processing has either already been applied to the problem or whether the technology will *a priori* work for the application. Therefore, even though certain useful generalities has been developed, it is still important to evaluate each case separately. Respectable levels of detection and precision have been achieved using analytical SFE on a wide variety of sample types. Recorded precision levels vary over a large range which depends on the nature of the analyte, the type of matrix, and the concentration of the analyte. In many cases, the precision of SFE is as good as the measurement method used for quantification. SFE has not yet reached a stage in which the analyst can put just any sample in at one end and get results out the other, it is not a fully mature technology, but it will become so. A knowledge of the chemical properties of both the analyte and the matrix is important for SFE. In addition, one must ensure that the mechanics of SFE have been optimized (e.g., sufficient SF flow, minimal dead volume, efficient trapping, sufficient number of vessel volume sweeps, etc.). Bruce (40) indicated that SFE needs to be more fully developed in five areas to be successful in tomorrow's environmental laboratories: general rugged extraction methods; ease of use; automation; cost effectiveness; the ability to interface with existing laboratory instrumentation and computer systems. The developments in multisample capability, electronically controlled variable restriction, user-friendly vessels, higher operating pressures, tandem trapping, and in-line modifier addition have helped meet most of these needs (4).

But the question most frequently asked is: how much will it cost ? To indicate why there is no single answer, listed below are just a few of the parameters that influence the cost of a supercritical fluid process: pressure level; pressure reduction ratio; distribution coefficient; solubility level; amount of material to be extracted. All these items must be specified and they all affect capital to be invested and operating costs. Today food oils (olive, soybean, canola, safflower, etc.) extracted or refined with SC-CO_2 would be expensive relative to oils that are now produced commercially by pressure or first pressed and the pressed cake then extracted and refined with hexane. But also, today, hexane has (in the U.S.A.) been included in the Environmental Protection Agency Hazardous lists and thus in the future foods oils and other foods processed with hexane will have to be so labeled. Hexane extracted oil would probably look and taste the same as SC-CO_2 extracted oil, and even with no residual hexane in the oil, it is likely that a label proclaiming that hexane was used in the process, will influence the consumer. Consumer awareness, perception, and acceptance, all of which are market forces, may well drive SC-CO_2 extraction to be applied in food oil extraction and refining. Changes in government regulations, like the kind involving methylene chloride in Germany that motivated the development of SC-CO_2 extraction of coffee and like the kind concerning elimination of chlorofluorocarbons that motivated development of supercritical fluid cleaning of electronic components, will be another driving force.

This document attempts to give an overview of this, no longer unconventional, technology, which in certain areas has been successful, in others, not. There are SF processes that operate at the multi-million dollar per year level, while there are some processes that have not yet reached the industrial level.

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COMBINED TECHNIQUES TO PREPARE FRUIT INGREDIENTS: A SYNTHESIS OF PRINCIPLES AND METHODS

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Fruit pieces are widly used as basic material or as additional components in many food formulations; examples include, among others, pastry and cofectionery products, ice-cream, frozen desserts and sweets, fruit salads, cheese and yogurt (Table 1).
Proper application of combined factors technologies may give solution to most specific requirements. The basic techniques involved include: blanching - partial air dehydration - "osmotic dehydration" now defined as "dewatering-impregnation-soaking in concentrated solutions". Refrigeration and freezing are applied to reach stability.
Some technical and methodological criteria are presented here in order to obtain fruit ingredients suitable for specific applications. For fruit pieces with water activities > 0.85 dehydrofreezing is usually the most appropriate technology. This technique is a combination of partial air dehydration anf freezing.
The air dehydration step is usually carried out up to 50% weight loss, corresponding to 60% water reduction, a 10% relative increase of total solids on the fresh material, equal to a doubling of the dry matter. The dehydrofrozen product after storage and thawing does not show the quality changes, typical of frozen foods, such as texture degradation, structure collapse and juice dripping.
Dehydrofreezing may be still applied up to a weight loss of 75-80%, with solid contents ranging from 20 to 50%. Such products may be used for pastry products where wetting has to be avoided, and are suitable for yogurt preparations where they can adsorb moisture avoiding the separation of whey.
A further reduction of the a_w can be better obtained by combining osmotic dehydration with air dehydration. By dipping the fruit pieces in a concentrated syrup, to a limited dewatering effect is associated a substancial gain of soluble solids and a reduction of the a_w. Within this technique, the role of osmosis is the enrichment of the soluble solid content rather than the removal of water. Owing to the intake of low molecular weight saccharides, the overall effect of osmosis is a lowering of the a_w with only a limited increase of consistency, as consistency depends, primarely, on the insoluble matter and water content, rather than on the soluble solids and a_w. By appropriate combinations of osmotic treatments and air dehydration, a wide range of fruit ingredients with different a_w and defined functional properties could be obtained.
As methodological criteria, diagrams relating the functional properties of the processed fruit to its phase composition (insoluble solids, soluble solids and water) may be developed. As shown in the example reported in Figure 1 the diagrams give for each water activity:
(a) The range of phase compositions which give that water activity.
(b) The range of consistencies corresponding to that phase composition and water activity.
(c) The freezing temperature, as related to the water activity.
(d) The freezable fraction of water at any temperature or differential fraction of freezable water between two temperatures, which can be calculated with reference to the corrisponding phase composition and freezing points. Information on the equilibrium freezing properties are useful for the formulation of fruit components for use in ice-cream, sherbet and frozen desserts. Choices are possible among fruit ingredients which do not form ice at a determined temperature, i.e. with a_w lower or equal to the equilibrium a_w at that temperature, or fruit with water activities higher than the equilibrium one, in which a definite amount of ice will separate according to the freezing behaviour.
(e) The amount of water which can potentially be adsorbed by fruit pieces with water activities lower than that of the food system, assuming a determined rehydration ratio.
(f) The mass of processed fruits obtained from a unit mass of raw material.
Accordingly these guide lines could be followed:
(a) Choice of the a_w level of the fruit ingredient suitable for a given food formulation. Choice of piece form and size.
(b) Dehydrofreezing of the fruit pieces without osmosis, up to the desired a_w (dry bulb temperature not exceeding 65°C will give the best results). The product obtained will exhibit the maximal consistency at that a_w.

(c) Osmosis in a standard fructose syrup up to 2 hours, followed by air dehydration up to the desired a_w. The product will exhibit the minimal possible consistency for that a_w.
(d) Choice of the best consistency between the maximal and minimal values and definition of the corrisponding osmosis time (if required).
(e) Definition of the weight loss to be reached, after osmosis, by air dehydration.
(f) Re-adjustment of all the process parameters, with a possible choice of a different osmotic agent.

Table 1. Examples of foods where fruit pieces can be used as ingredients

Sample	aw
Fruit salad	0 98-1
Yogurt	0 97-0 99
Fresh cheese	0 97-0 99
Frozen foods (thawed)	0 90-1
Ice cream (melt)	0 89-0 92
Frozen foods, ice cream, frozen dessert (-18°C)	0 84
Pastry, pie filling, baked foods, etc.	0 75-0 85

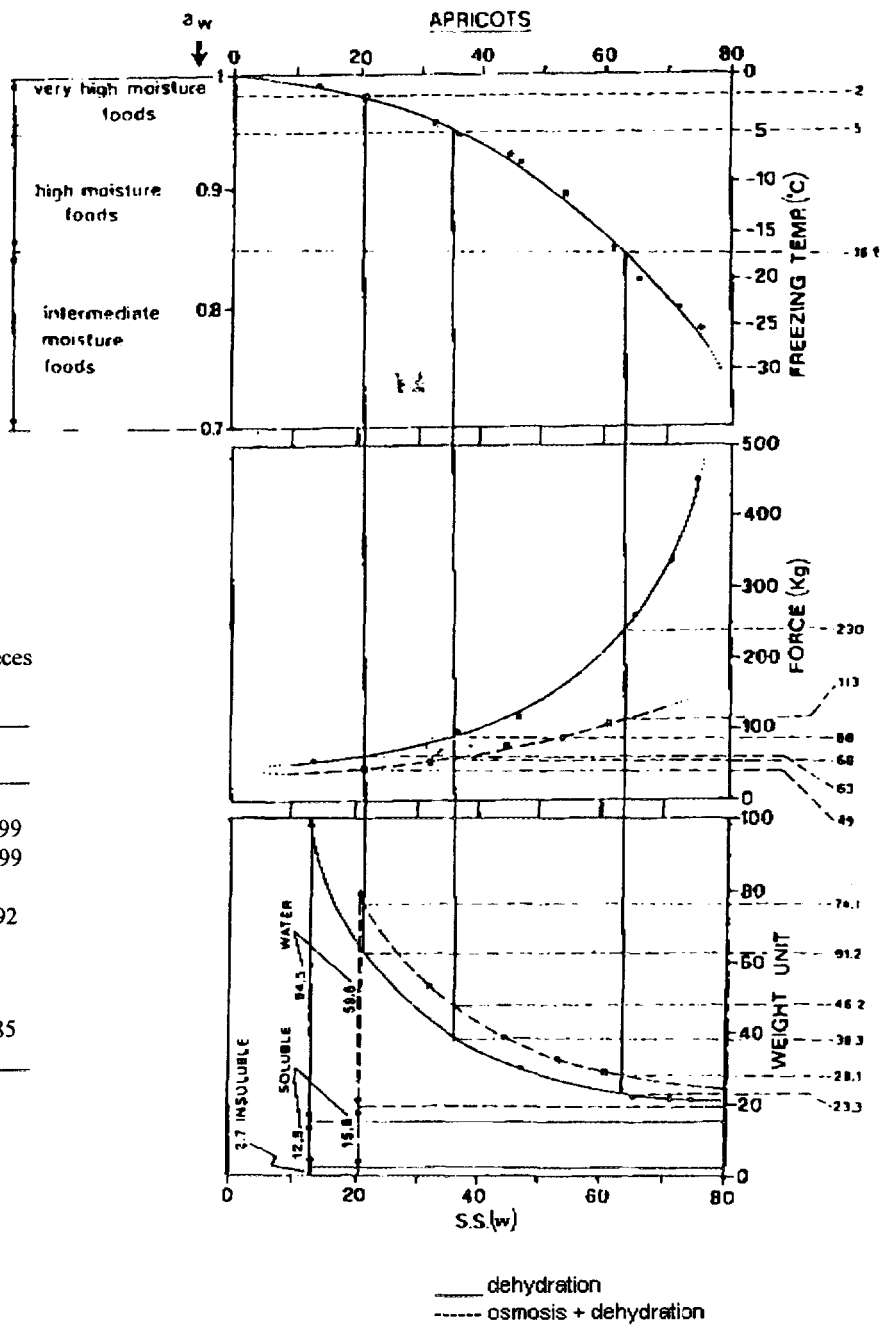

Fig. 1: Water activity (aw), freezing temperature, consistency (force) and phase composition (weight units) of 10 mm apricot cubes, as related to percentage soluble solids in the water phase (SS (w))

FUNCTIONAL PROPERTIES AND VERSATILITY OF DRIED FRUIT PIECES REHIDRATED IN SUGAR SOLUTIONS

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SYNOPSIS

Sugar solutions with different sugar composition and concentration have been used to reconstitute dried apple cubes. The mass transfer between fruit pieces and solution during the rehydration process and the physicochemical characteristics of the reconstituted products have been evaluated.
The results indicate that, varying the concentration of the sugar solution and reconstitution time, it is possible to obtain, from the same dried product, rehydrated apple pieces with different and peculiar characteristics such as water activity, freezing point, amount of freezable water and firmness. In addition, these characteristics were linearly correlated with soluble solids content in the rehydrated products. In this way the reconstituted dried apples are able to be used as ingredient in formulated foods at different level of water activity and also in frozen dessert or ice cream.

INTRODUCTION

Fruit ingredients, besides sensory characteristics related to the acceptability and to the role of "characterising components", should show well defined functional properties, in order to make them compatible with the food system without affecting shelf-life [1-3].

The "compatibility" of the fruit with other components of a formulated food depends basically on the equilibrium of the respective water activity (a_w) values. In fact, by adjusting a_w, it is possible to limit the mass transfer (water or solutes) among different food components [2, 4].

Fruit pieces with medium-low water activity values could be obtained by single or combined drying techniques [5-8]. Moreover, in order to enhance the soluble solids uptake and to reduce the a_w value, a direct osmosis treatment prior to air drying of fruit could be adopted [9-11].

Nevertheless, it has to be underlined that for formulated foods producers, it is not easy to find half-manufactured fruit pieces for specific uses. Hence in these situations could be profitable to have a standard product, such as dried or freeze dried fruit, which could be reconstituted under appropriate conditions in order to obtain specific a_w, and/or freezing point and/or firmness. The "modulation" or the "adjustment" of dried fruit pieces characteristics can be obtained by the appropriate choice of the reconstitution medium, such as water, water-sugar solutions or concentrated sugar solutions [3].

In literature, few works about the rehydration of fruit and vegetables can be found and many of these consider only water uptake kinetics and process variables [12-14].

The aim of this research work was to evaluate some physico-chemical characteristics of dehydrated apple pieces (a_w, freezing point, amount of freezable water and firmness) after their reconstitution in solutions at different sugar compositions and concentrations. Moreover, the mass transfer between fruit and solution in terms of water uptake, soluble solids gain or loss and amount of soluble solids in the water phase were determined.

MATERIALS AND METHODS

Materials and samples preparation
Trials were performed on apples (*Malus domestica*, cv. Golden delicious), all fruits were refrigerated at 2-3°C and before the trials equilibrated to room temperature (22-23°C) for 3 hours.

Apples, after hand-washing, peeling, coring and cutting to give uniform cubes of 1x1x1 cm, were air dried using a static suction-flow type equipment (Sandvik Process Systems, Milan, Italy), where the air flow runs downward perpendicular to the product. Circulating air speed was about 3 m/sec with at a constant temperature of 70°C [15].

Dehydrated product were packed in polyethylene laminate pouches and held at room temperature until reconstitution.

Reconstitution procedure

As reconstitution media were used :

W = distilled water;

Gluc15 = 15% (w/w) water solution of glucose;

Gluc30 = 30% (w/w) water solution of glucose;

Glic15 = 15% (w/w) water solution of corn syrup with high maltose content (Glicosa - Cerestar, Ferrara Italy) (Lerici et al., 1985);

Glic30 = 30% (w/w) water solution of corn syrup (Glicosa - Cerestar, Ferrara Italy).

Fixed amounts of dehydrated fruit pieces (30g) and reconstitution medium (600 ml) (weight ratio 1:20) were placed in 1000 ml glass beakers and the product was submerged by a plastic screen. All trials were performed at room temperature and solutions were submitted to a constant stirring (100 revolutions per minute). Fruit pieces were withdrawn from the solutions at 5, 10, 20, 40 and 60 minutes, drained for 5 min on a plastic screen and blotted.

Analytical methods

Dry matter (DM) determinations were carried out in vacuum oven for 12 hours [16].

Insoluble solids were determined in accordance with Barbier and Thibaults [17] and Maltini et al., [2].

Water activity was evaluated at 25°C by means of an electric hygrometer (Hygroskop DT Rotronic, Zurich, Switzerland) previously calibrated with Rotronic standard solutions at theoretical a_w values of 0.50, 0.65, 0.80 and 0.95 [18]. Prior to a_w determination, the samples were cut into small pieces and equilibrated in the hygrometer sample cell for 12 hours.

The firmness of fruit pieces after reconstitution was measured with an Instron Universal Testing Machine model 4301, (Instron International LTD, High Wycombe, UK), equipped with a temperature cabinet (model 3119-005), using a standard Kramer Shear press cell (model CS1) with a crosshead speed of 200 mm/min on 50 g of fruit. Extrusion force was taken as the maximum peak of recorded force on the charts expressed as Newtons (N) [2].

The amount of frozen water at -15°C was determined by thermal differential analysis, using a differential scanning calorimeter (DSC) Mettler TA 4000 (Mettler, Greifensee, Switzerland) with a DSC-30 measuring cell, supplied with a processor Mettler TC-11 TA. The equipment was calibrated for temperature and heat flow with indium, lead and zinc. For the calorimetric analysis, about 15 mg of sample were put in 40 µl aluminium DSC pans. An empty aluminium pan (equal to the one used for the sample) was used as reference. Heating rate was 5°C/min in all experiments. A nitrogen gas flow of 20-30 ml/min was used to avoid water condensation in the measuring cell.

Water uptake (WG), Solid loss (SL) or solid gain (SG) due to rehydration and amount of soluble solids in the water phase (SSw) was calculated in accordance to Mastrocola et al. [3].

Data analysis

The values of WG, SL or SG and SSw as a function of the reconstitution time and syrup concentration have been evaluated by means of least square fit of response surface (Response Surface Metodology, RSM) [19, 20]. The quadratic polynomial model used had the general formula:

$$Y = \beta_0 + \beta_1 t + \beta_2 C + \beta_{11} t^2 + \beta_{22} C^2 + \beta_{12} tC$$

were:

Y = values of WG, SL or SG and SSw;

t = reconstitution time (min);

C = syrup concentration (°Bx);

β_0 = intercept;

$\beta_1 t$ = linear effect of time;

$\beta_2 C$ = linear effect of syrup concentration;

$\beta_{11} t^2$ = quadratic effect of time;

$\beta_{22} C^2$ = quadratic effect of concentration;

$\beta_{12} tC$ = interaction effect of time and concentration.

In order to consider only the linear phase of the rehydration process the first value of each data set was taken after 5 min.

Linear regressions of the variations of a_w, frozen water at -15°C and firmness at -15°C of reconstituted samples as a function of soluble solids content have been carried out.

RESULTS AND DISCUSSION

In Figures 1 and 2 the amounts of water gained (WG) and solids lost (SL) or gained (SG) as function of reconstitution time and syrup concentration for air-dried apple cubes are reported.

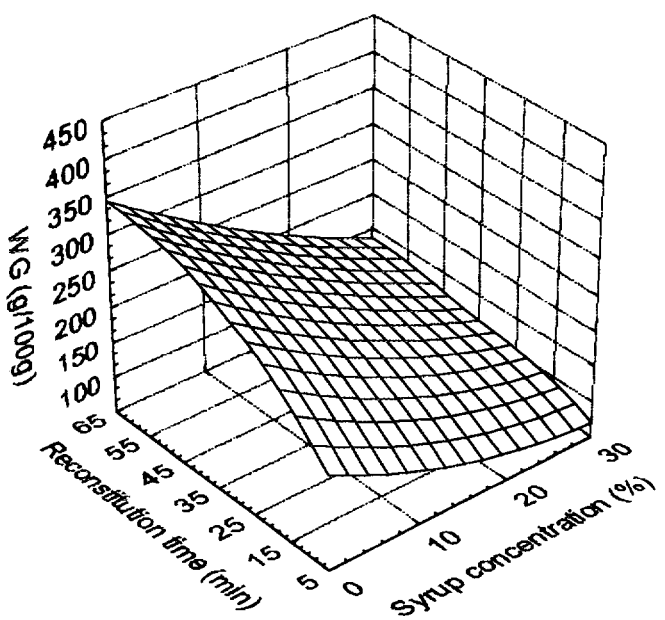

***Figure 1** : Response surface plot of water uptake (WG) as function of reconstitution time and glucose solution concentration for air-dried apple cubes.*

The shapes of response surfaces obtained by elaboration of experimental data, indicate that the mass transfer phenomena among dehydrated fruit pieces and sugar solutions take place mainly during the first period of the rehydration process (10-20 minutes). In fact, during the first phase of the reconstitution, diffusive phenomena predominate, because the product becomes impregnated with sugar solution and - when diluted solutions are used - lose very high quantities of soluble solids. When the vegetal tissues become partially rehydrated, the cell walls act like semipermeable membranes [7, 10], thus absorbing water and carrying out a selective action towards soluble solids, depending on their molecular weights.

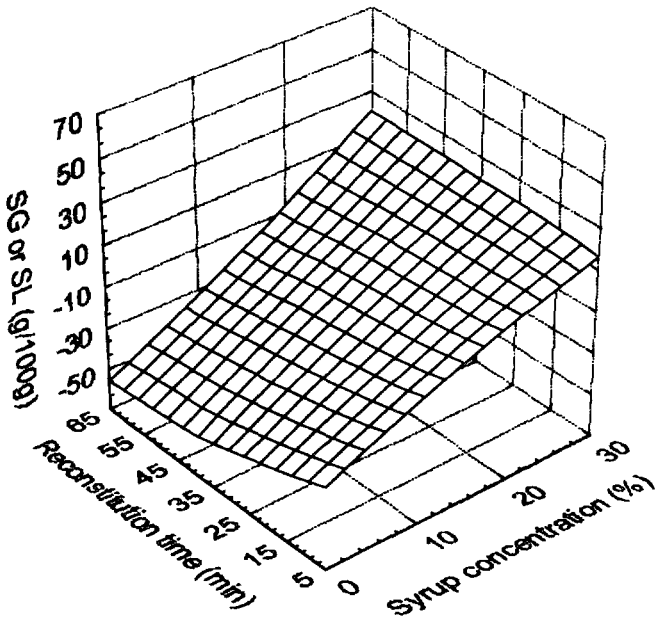

Figure 2 : Response surface plot of solid gain (SG) or solid loss (SL) as function of reconstitution time and glucose solution concentration for air-dried apple cubes.

In our trials, when the concentration of rehydrating solution increased, air-dried apple pieces showed a decrease in water uptake and soluble solids loss. On the contrary, by using solutions containing more than 20% of sugar, a solid uptake occurred, and the soluble solids gain was linearly correlated to the concentration of rehydrating medium (Figure 2). In general air-dried apple cubes showed a gradual mass transfer. This behaviour can depend on drying process. In fact, during air drying, soluble solids migrate to the surface of fruit pieces and lead to the formation of a barrier which is able to limit the water uptake and to determine an higher soluble solids loss during reconstitution.

In our trials, the a_w values of rehydrated products resulted well correlated to the soluble solids content, independently from time and reconstitution media (Figure 3).

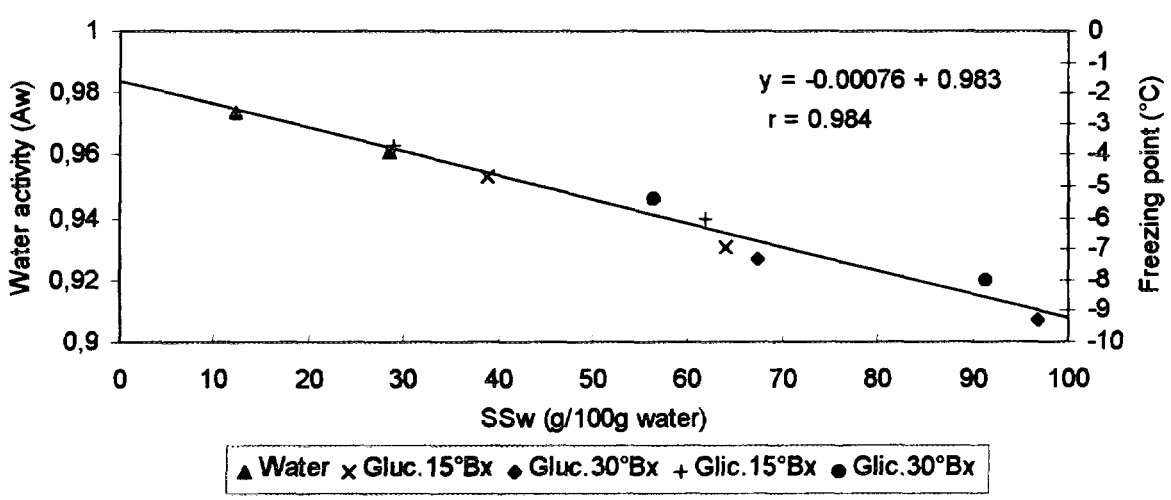

Figure 3 : Water activity (a_w) and freezing point as functions of soluble solids content in air-dried apple cubes reconstituted in water or different sugar solutions for 10 or 60 minutes.

This result is quite interesting in that, for a given a_w value necessary for the inclusion of fruit pieces in a formulated food, it directly allows to calculate the corresponding soluble solids content and to determine the concentration of reconstitution medium and the time necessary for reconstitution (Figure 4).

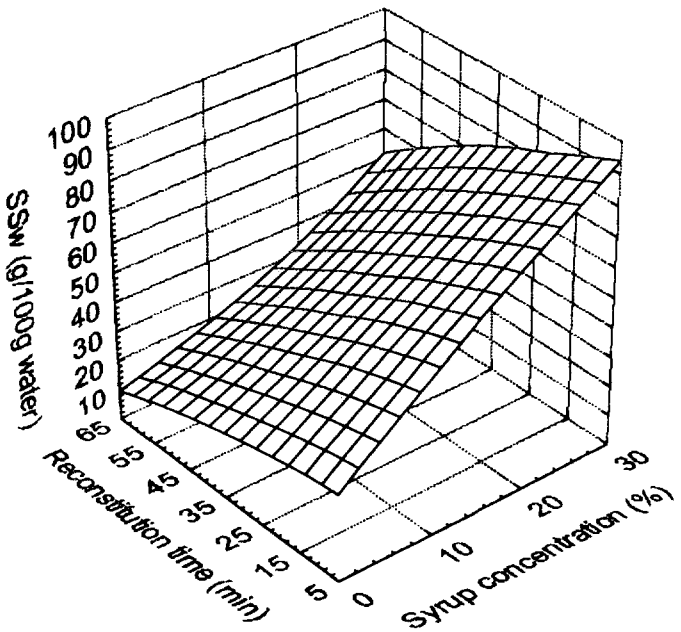

***Figure 4** : Response surface plot of soluble solids content (SSw) as function of reconstitution time and glucose solution concentration for air-dried apple cubes.*

Data on the freezing point, calculated from a_w values [21], could be useful when rehydrated fruit pieces are mixed with frozen products such as ice-creams, sherbets and frozen desserts. Moreover in the case of inclusion of this kind of products in ice-creams, generally consumed at an average temperature of -15°C, it is interesting to know their amount of frozen water at this temperature (Figure 5).

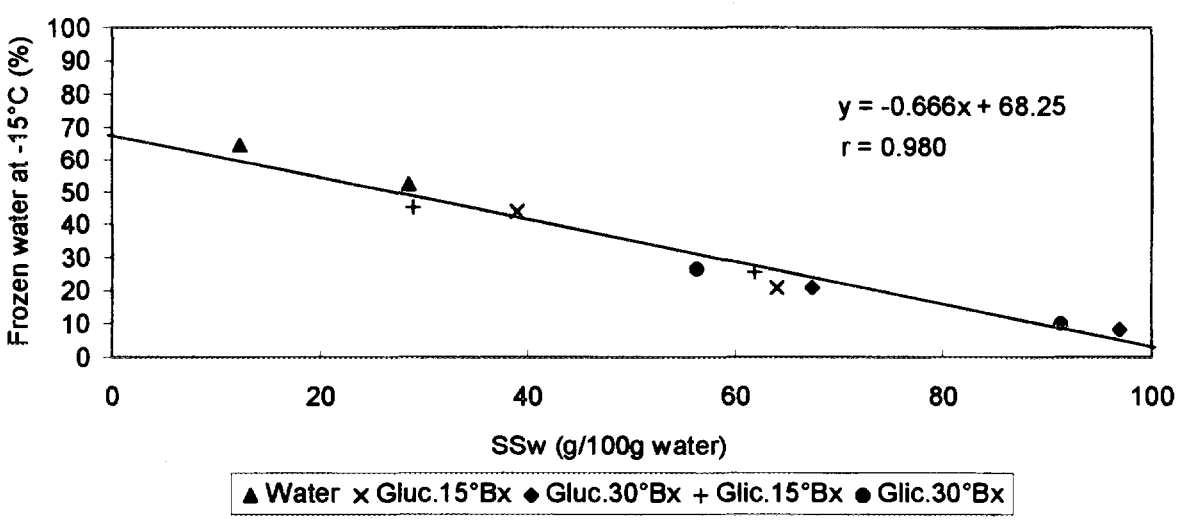

***Figure 5** : Percentage of frozen water at -15°C as functions of soluble solids content in air-dried apple cubes reconstituted in water or different sugar solutions for 10 or 60 minutes.*

In fact, even if the freezing temperature of reconstituted fruit is higher than that at which the ice-cream is consumed, for soluble solids contents higher than 50g/100g of water, these products show a very low quantity of freezable water (lower than 35%).

Another important functional properties of semi-processed fruit pieces is the texture. This characteristic basically depends on the swelling and plasticizing properties of pectin and cellulose when hydrated. For some authors the texture, at room temperature, directly depends on the total insoluble solids content of foods [2], or results well correlated to the total dry matter content [3]. When reconstituted fruits are kept at freezing temperatures, the texture is strongly influenced by frozen water and, only for low water contents, by the compositive characteristics of solids and by their hydration level. In the case of air dried apples, it is not possible to split the hardness effects due to the partially hydrated fruit tissues from those deriving from the presence of ice crystals (Figure 6).

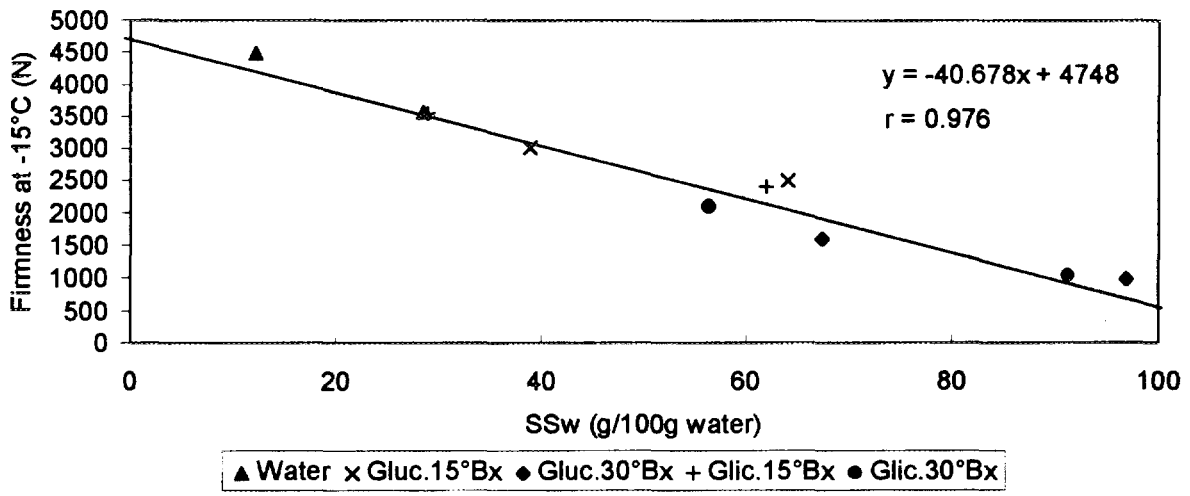

***Figure 6** : Firmness at -15°C as functions of soluble solids content in air-dried apple cubes reconstituted in water or different sugar solutions for 10 or 60 minutes.*

CONCLUSIONS

The results obtained in this study outlined that it is possible to modulate the water uptake and the soluble solids balance of a reconstituted fruit pieces by acting on the time, type and concentration of the reconstitution media. By starting from the same fruits, it is also possible to obtain products with different functional properties for specific uses in different food formulations. In this way dehydrated fruits can be considered as half-manufactured products which can be "modified" upon necessity directly by formulated food producers, only by an appropriate reconstitution procedure.

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FREE SUGARS AND SORBITOL CONTENT OF COMMERCIAL LIGHT AND DIETETIC JAMS (STRAWBERRY AND PLUM).

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This particular work aims to study the soluble sugars content of six different commercial Spanish brands of light and dietetic jams from strawberry and plum by using High Pressure Liquid Chromatography. Three different batches of each commercial brand were analyzed, all the analysis were carried out on triplicate. The results show great differences among the different brands considered: two of them have fructose as main sugar, glucose content varies from one brand to another and some of them have important quantities of sorbitol. These jams prepared with substitute sugars cuold be recommended for dietetic purposes.

This work is part of a wider research which includes the whole study of carbohydrate fraction of traditional, light and dietetic jams.

INTRODUCTION

Jam is a product often used in our daily diet. Historically, jams and jellies may have originated as an early effort to preserve fruit for consumption in the off-season (1, 2 , 3). There has been an enormous change in the human way of life recently. There is a growing concern over how healthy the food we eat really is. The importance of having a slim figure and the obsession for the bodycare has led to the development of low calory or light products, as many consumers have become aware of the possible risks to health from eating a diet high in calories (4). So, in order to satisfy peolple's inborn desire for sweet taste, but with less calories, there has been a reduction of the sugar content of industrially prepared foods. Traditional-made jams contain a great amount of calories due to a high percentage of sugars on its composition, therefore, in order to prepare light jams, there has been a replacement of sucrose by other sweeteners such as sorbitol or fructose (5, 6). The substitute sugar must have the same characteristics of sucrose: clean sugar taste, harmless,healthful, strong in sweetness potency, versatile in its applicability, and economically profitable (7). These light, diet or dietetic jams have some differences in the gelling process comparing with traditional-made jams which affect the final texture. Despite those differences, dietetic jams can be used in people with problems in the carbohydrates metabolism, such as diabetics, who should avoid glucose and sucrose on their diet (2, 8, 9).

High Performance Liquid Chromatography has become the method of choice for quantitative analysis of sugars in foods. Among all the quantitative chromatographic techniques for sugar analysis, HPLC is more attractive than GC since derivatives need not to be formed prior to analysis, and sample preparation time may therefore be reduced (10, 11, 12, 13).

SAMPLING

Light jams have been recently incorporated to the Spanish food market, therefore there have not been deep composition studies of them yet. For that reason this work aims to study total soluble sugars of commercial strawberry and plum light jams. Six different commercial brands of diet and light jams, made from strawberry and plum, obtained from Spanish local markets were selected (table 1). Brand A does not commercialize light plum jam.

Table 1.- Abreviations used for the samples analysed.

BRANDS	LIGHT			DIET OR DIETETIC		
	A	B	C	A	D	E
Strawberry	ASL	BSL	CSL	ASD	DSD	ESD
Plum	------	BPL	CPL	APD	DPD	EPD

METHOD

High Pressure Liquid Chromatography was chosen for the quantification of soluble sugars. For all the samples three batches (different lots) of each sample (commercial brand) were analysed on triplicate.

For HPLC determination of free sugars, the following method, previously reported by Mollá and col., 1994 (14), was carried out. First, 3g of jam were blended with 40 ml methanol in an Omni-mixer homogeneizer. The resulting slurry was heated in a magnetic mixer, for 45 min . Then the samples were centrifuged for 40 min at 6000 rpm (at ambient temperature); and the supernatants were cooled and brought to 50 ml with methanol. This extract was reduced in volume by using a rotary vacuum evaporator, to evaporate the methanol. The concentrate was made up to 25 ml with distilled water. Then the samples were filtered through a sep-pack C18 cartridge, 2 ml of filtrate was mixed with 8 ml acetonitrile. Before injection the samples were filtered through a 0.45 μm Millipore membrane. The injection volume was between 25 to 100 μl filtrate, depending on the final sugars concentration.

A Waters Associates liquid chromatograph equiped with a 6000A pump, a U6K injector and a differential refractometer R401, was used. The column used was a Waters μBondapak/carbohydrate analysis. The mobile phase was acetonitrile-water (80:20). Operating conditions were flowrate: 0.9 ml/min, temperature ambient. All chromatograms were recorder on a Waters Data Module 745 integrator.

Standard sugar solutions were prepared to contain 50-100 μg/ml, aliquots (25μl) of these solutions were injected into the chromatographic system and the resulting peak areas were plotted against concentration for the calibration curve.

VERIFICATION OF THE METHOD

Regarding the specificity and accurancy of the method, the work of Cámara and col., 1996, (15) was followed. Recovery assays, the standard addition method was used to calculate the recovery rates of the sugars, under the same experimental conditions, applying the method of known addition of individual sugars to a jam sample with a previously determined concentration. Results are shown on table 2, the recovery percentage ranged between 71.796% for saccharose and 100.708% for sorbitol.

Table 2.- Recovery assays results of sugars added to a light jam (mg of sugar).

FREE SUGAR	PARAMETERS			
	C initial X±SD	C added X±SD	C found X±SD	Percentage X±SD
Fructose	56.990±2.039	25	80.880±1.126	95.424±4.746
		50	102.211±1.899	90.442±3.799
Glucose	51.101±2.363	25	74.749±1.698	94.595±6.792
		50	99.522±2.614	96.841±5.227
Sorbitol	105.592±2.413	50	155.946±7.272	100.708±10.284
		150	227.189±0.020	81.065±0.013
Saccharose	184.867±2.746	50	220.765±2.085	71.796±4.170
		100	277.682±7.361	92.815±5.205
Maltose	2.705±0.018	10	12.774±0.614	95.686±4.340
		25	24.172±0.875	84.867±3.855
Maltothriose	17.882±0.375	10	26.613±1.174	87.312±8.302
		20	34.057±1.544	80.873±5.458

C, concentration; X, mean value of three analysis; SD, standard deviation (n-1).

RESULTS AND DISCUSSION

The obtained results are shown on table 3, for strawberry jams, and table 4, for the plum jams. According to them, samples ASD and APD have a very low sugar content, which affects the final external texture caused by a low fruit content. This can be confirmed by the high sorbitol content of these samples, which has been added as an artificial sweetener. Samples from brands D and E (both strawberry and plum) have fructose as main sugar, with a low glucose and saccharose content, DPD and EPD also present sorbitol in as much quantity as glucose.

Maltose has been detected as trace levels in brand A samples and quantified in B and C. The presence of maltose in these samples can be explained by the addition of glucose proceeding from starch hidrolysis. Maltothriose is only present

TABLE 3.- LIGHT, DIET OR DIETETIC STRAWBERRY JAMS

BRAND	BATCH	FRUCTOSE X±SD	GLUCOSE X±SD	SORBITOL X±SD	SACCHAROSE X±SD	MALTOSE X±SD	MALTOTHRIOSE X±SD
ASD	1	1.486±0.205	0.847±0.092	5.452±0.705	0.198±0.044	0.267±0.038	---------
	2	1.628±0.193	0.898±0.097	5.811±0.666	Traces	0.243±0.004	---------
	3	1.751±0.126	0.973±0.039	5.870±0.088	---------	0.237±0.040	---------
ASL	1	3.362±0.580	4.887±0.771	---------	11.607±0.191	1.226±0.210	---------
	2	7.653±0.659	9.592±0.715	---------	6.692±0.955	1.178±0.171	---------
	3	8.131±1.269	10.105±1.206	---------	5.624±0.748	1.453±0.151	---------
BSL	1	3.117±0.153	4.659±0.458	---------	7.790±0.018	2.358±0.883	Traces
	2	3.767±0.085	5.422±0.131	---------	8.695±0.413	2.979±0.522	---------
	3	3.680±0.073	5.296±0.156	---------	7.712±0.286	2.704±0.313	---------
CSL	1	5.806±0.609	8.348±0.688	---------	5.384±0.493	1.970±0.218	0.593±0.099
	2	5.505±0.423	7.828±0.589	---------	4.705±0.617	1.786±0.133	0.737±0.117
	3	5.347±0.514	7.875±0.723	---------	6.524±0.793	2.140±0.258	0.520±0.167
DSD	1	28.504±0.613	0.708±0.044	Traces	Traces	---------	---------
	2	29.514±1.544	0.846±0.051	Traces	Traces	---------	---------
	3	29.095±0.030	0.703±0.052	Traces	Traces	---------	---------
ESD	1	25.472±0.505	1.086±0.076	Traces	0.494±0.044	---------	---------
	2	24.955±0.241	0.817±0.047	Traces	0.219±0.009	---------	---------
	3	19.691±0.906	0.532±0.038	Traces	0.186±0.004	---------	---------

X, mean value of three analysis; SD, standard deviation (n-1).

TABLE 4.- LIGHT, DIET OR DIETETIC PLUM JAMS

BRAND	BATCH	FRUCTOSE X±SD	GLUCOSE X±SD	SORBITOL X±SD	SACCHAROSE X±SD	MALTOSE X±SD	MALTOTHRIOSE X±SD
APD	1	1.372±0.023	2.038±0.092	3.939±0.229	Traces	Traces	----------
	2	0.788±0.047	1.489±0.121	3.666±0.123	Traces	Traces	----------
	3	1.205±0.039	1.699±0.148	3.755±0.236	Traces	Traces	----------
BPL	1	4.159±0.242	9.281±0.398	----------	5.077±0.316	3.285±0.163	Traces
	2	3.971±0.264	9.210±0.392	----------	5.078±0.198	2.540±0.134	Traces
	3	4.444±0.228	8.864±0.734	----------	4.982±0.362	3.126±0.237	Traces
CPL	1	5.053±0.293	9.857±0.312	----------	6.209±0.245	1.831±0.136	Traces
	2	5.661±0.312	10.913±0.538	----------	4.894±0.401	2.416±0.042	Traces
	3	5.513±0.098	11.065±0.298	----------	7.303±0.059	2.416±0.094	Traces
DPD	1	24.934±1.541	1.638±0.216	1.059±0.132	Traces	----------	----------
	2	23.095±1.298	1.458±0.229	1.056±0.072	Traces	----------	----------
	3	22.900±1.985	2.067±0.115	0.576±0.106	0.286±0.032	----------	----------
EPD	1	19.979±0.175	1.673±0.193	1.014±0.153	0.204±0.004	----------	----------
	2	20.138±1.455	1.680±0.212	1.040±0.131	0.364±0.056	----------	----------
	3	22.026±0.486	1.455±0.116	1.130±0.048	0.447±0.024	----------	----------

X, mean value of three analysis; SD, standard deviation (n-1).

on brands B and C samples, but just in a very low quantity quite difficult to evaluate.

The different sugar composition of each sample considered is shown in figure 1, which includes the HPLC sugar profiles for strawberry and plum representing samples from brands A and B. Figure 2 includes HPLC profiles of strawberry jams from brands C, D and E. Finally figure 3 represents plum jams from brands C, D and E.

The one-way analysis of variance (ANOVA) was applied to evaluate the effect of the different fruit used for each brand. All the analytical results (triplicates of each sample) were used to conduct analysis of variance. The Fisher statistic (F value) obtained from our data and compared with a critical value (F_c at the 95% confidence level) shows the significance of the fruit used on the soluble sugars composition of the jams studied (16, 17). According to the results, glucose shows statistically significant differences in all tha brands studied. The same happens to sorbitol in the two kinds of fruit in brand A, saccharose in samples BSL/BPL and maltose in the relationship ASD/APD. There is no significant variation on fructose content, except between DSD/DPD. Significant variation of saccharose in brand B could be due to the action of invertase, which also could be the cause for the different values of fructose and glucose.

Table 5.- Analysis of variance. Influence of the kind of fruit on the soluble sugars content of jams.

		Fructose	Glucose	Sorbitol	Saccharose	Maltose
ASD/APD	$F_{1,4}$=	6.9407	25.9639	157.431	-------	738.1071
BSL/BPL	$F_{1,4}$=	7.4312	220.3100	-------	90.7182	1.1007
CSL/CPL	$F_{1,4}$=	0.3997	39.1685	-------	0.4660	1.3485
DSD/DPD	$F_{1,4}$=	57.5523	26.9442	-------	-------	-------
ESD/EPD	$F_{1,4}$=	1.8388	20.1579	-------	0.1023	-------

F_c1,4= 7.7086 (95% confidence level).

We can conclude that ASD and APD samples have very low fruit content, and in order to maintain sweet flavor, high amount of sorbitol has been added, but they have just a few calories, as the total soluble sugars content is around 6g/100g (APD) which means 24 Kcal= 100.32 KJ and 8g/100g for ASD (32 Kcal= 133.76 KJ). The other plum jams have from 20.799 g/100g of total soluble sugars in BPL (83.196 Kcal= 347.759 KJ) up to 27.637 g/100g in DPD (110.548 Kcal= 462.091 KJ). Strawberry jams vary from 17.924 g/100g total soluble sugars in BSL (71.696 Kcal= 299.689 KJ) up to 30.360 g/ 100g in DSD (121.440 Kcal= 507.619 KJ).

The jams with added glucose are the ones which show maltose on its composition (brands B and C). In jams with a low fruit content there is also low glucose content, therefore these jams have increased values of sorbitol (brands A, D and E) or fructose (brands D and E) which are used as substitute sugars. These samples from A, D and E brands (except ASL sample) from both strawberry and plum could be the ones most recommended for dietetic purposes.

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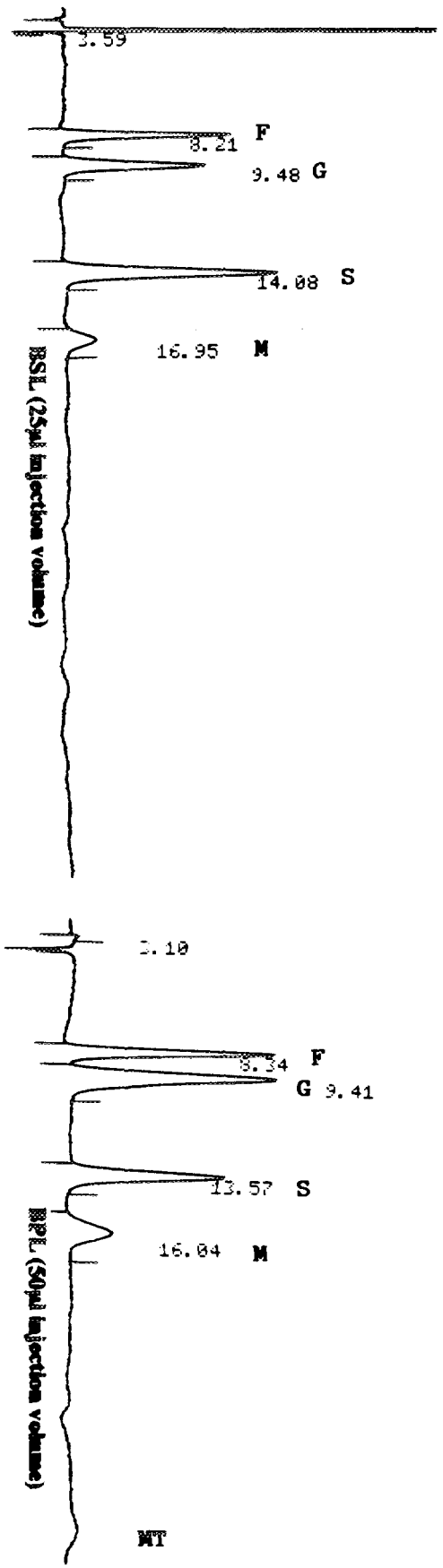
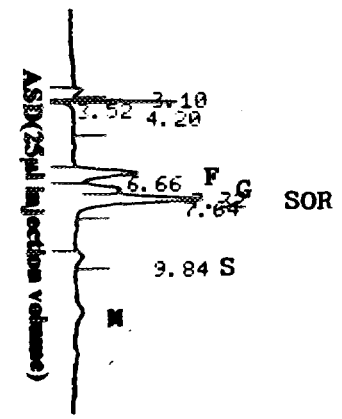
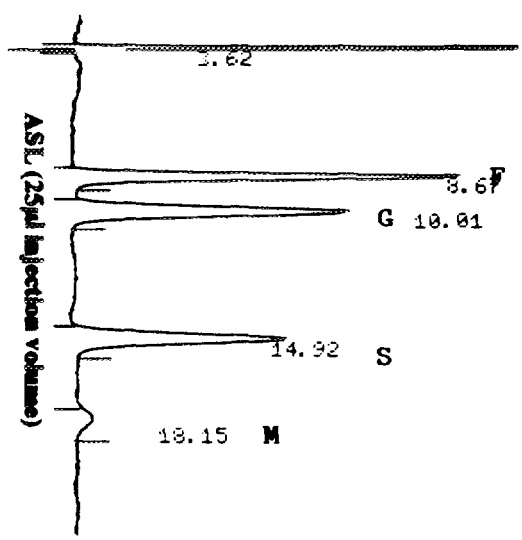






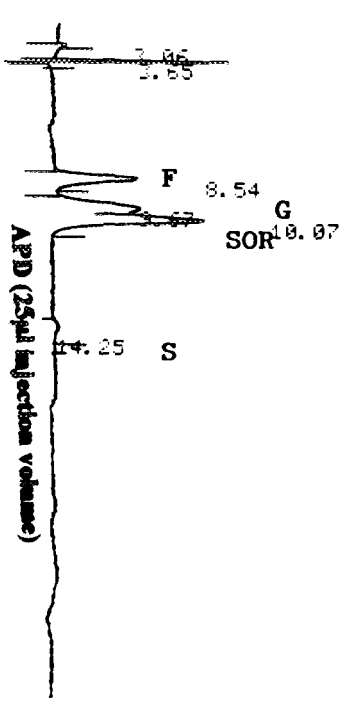


Figure 1.- HPLC profile of light, diet and dietetic strawberry and plum jams, brands A and B. *F: fructose; G: glucose; S: saccharose; M: maltose; MT: maltotriose.*

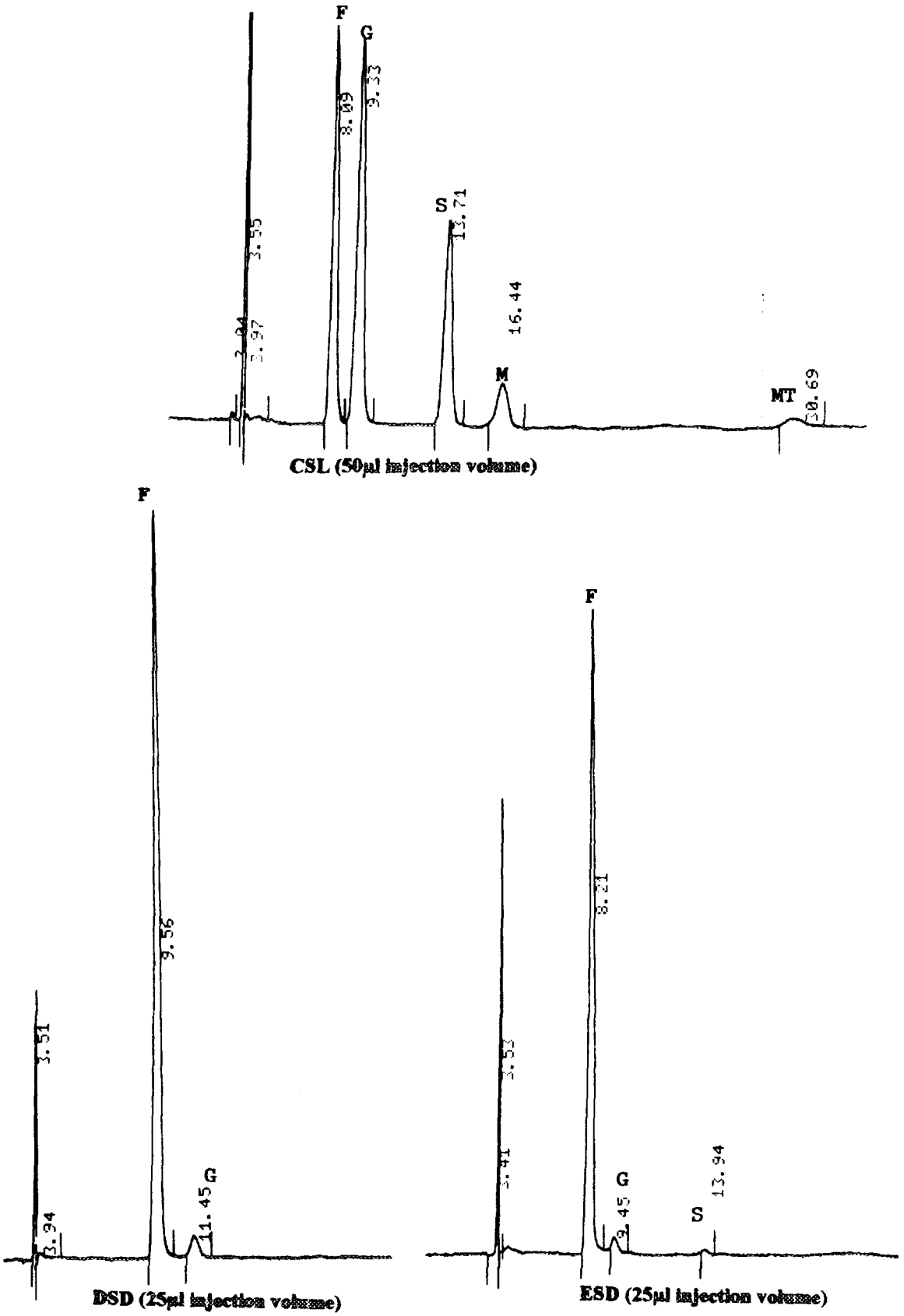


Figure 2.- HPLC profile of light, diet and dietetic strawberry jams, brands C, D and E. ***F: fructose, G: glucose; S: saccharose; M: maltose; MT: maltothriose.***

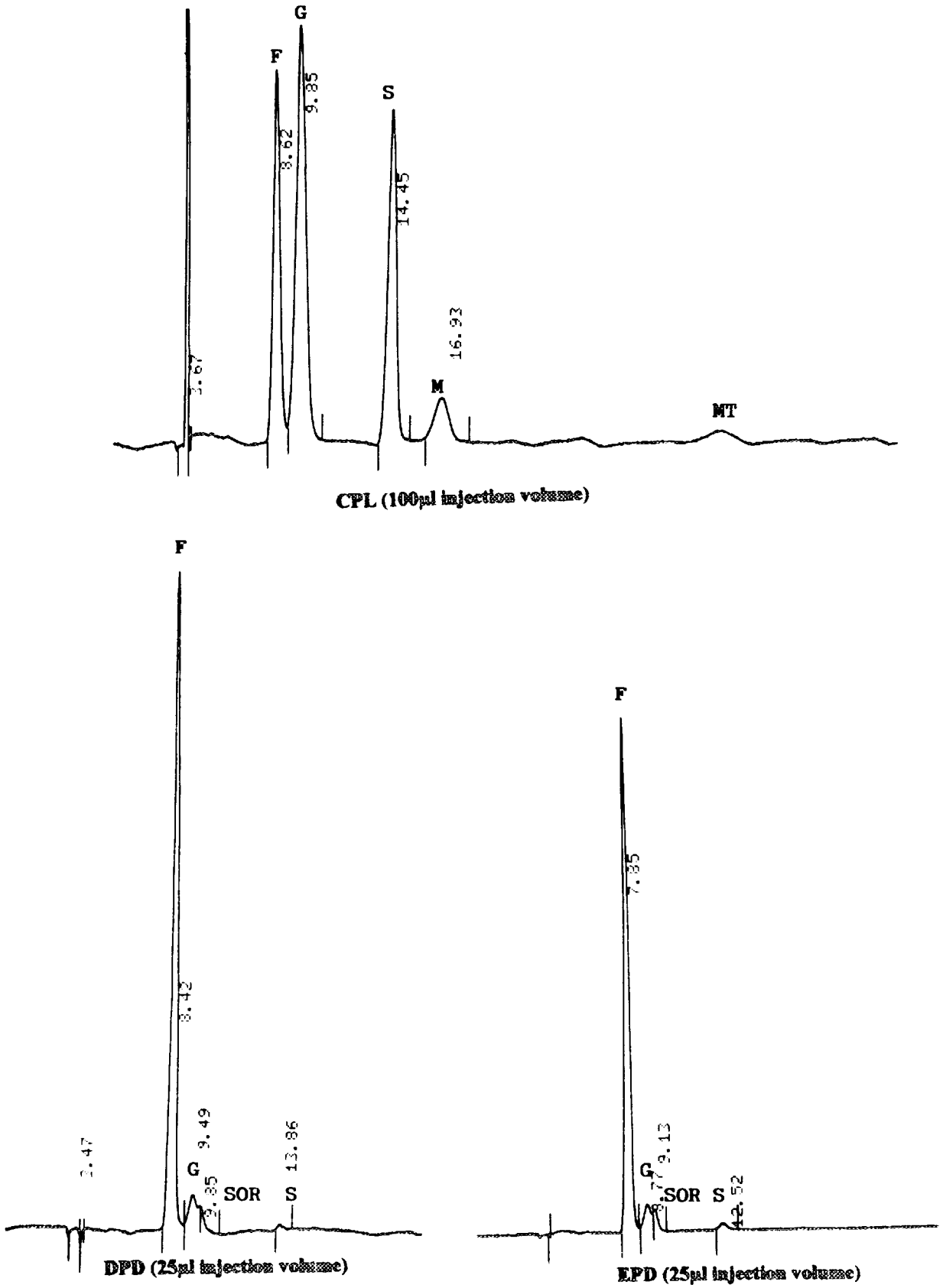


Figure 3.- HPLC profile of light, diet and dietetic plum jams, brands C, D and E. ***F: fructose, G: glucose; S: saccharose; M: maltose; MT: maltothriose.***

EFFECT OF RESIDUAL OXYGEN ON BEVERAGES PRESERVATION

L. Prost

Processes of liquids deoxygenation are commonly used by industrials to improve the quality and the preservation of beverages like wine, fruit juice and non carbonated soft drinks. Traditionnaly, a high purity Liquid Nitrogen source (99,99 %)is used for beverages deoxygenation ; the recent development of N_2 on site generators which deliver N_2 with a 92 to 99,5 % purity level has led to the study of low quantity residual oxygen on beverages preservation.
The study explains what is the performance of this new N_2 source on deoxygenation and what is the impact of the residual O_2 on liquids' quality parameters, such as vitamin C.

The oxygen dissolved into the liquids or present in the headspace of beverages is leading to several consequences :

- chemical/biochemical oxydation reactions :
  - . loss of vitamins (i.e. vitamin C)
  - . loss of aromas (i.e essential oils)
  - . color changes (i.e. enzymatic browing)
  - . development of off-flavors (i.e. lipid oxydation)

- metal corrosion
  - . metallic cans (packaging).
  - . pipes/tanks (processing plant)

- microbial growth : dissolved oxygen sustains the growth of strict and facultative aerobes.

These reactions give rise to a decrease of the shelf-life, the nutritional value and the organoleptic properties of the beverages.
Traditionnaly, cryogenic purity nitrogen is used to deoxigenate the liquids to avoid these degradations.

AIR LIQUIDE has developed N_2 on site generators that deliver N_2 with a 92 to 99,9 % purity level, at a lower cost than liquid nitrogen's.

The customer's concerns are :

- the impact of membrane N2 on his product
- the product tolerance threshold to O2

that is why AIR LIQUIDE has studied the effect of low quantity residual oxygen on non-carbonated soft drinks (fruit juices, tea/juice drinks) by

- computer modeling : theoretical determination of residual dissolved oxygen
- laboratory scale evaluation . tests on commercial products
  . test on model solutions
- industrial scale evaluation : field testing/technical data compiling.

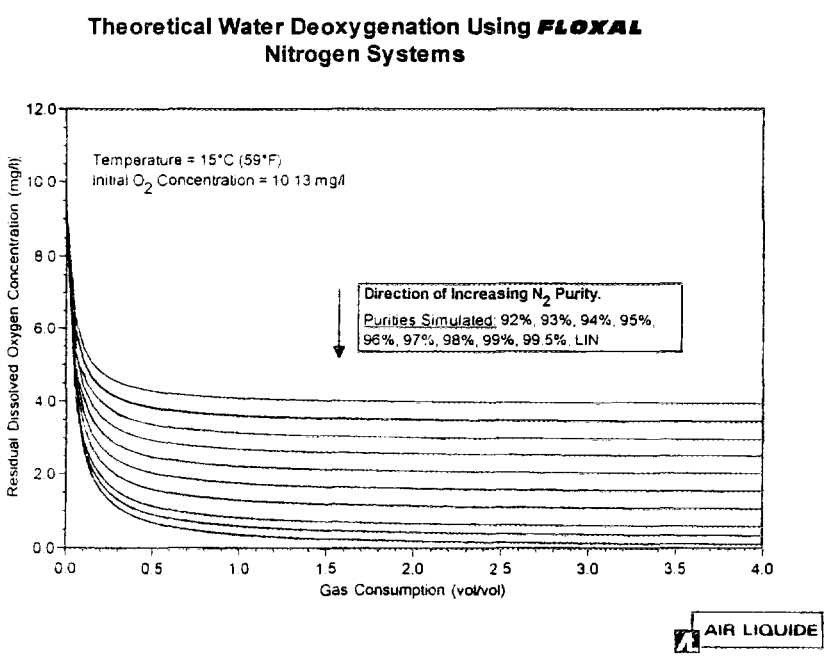
COMPUTER MODELING

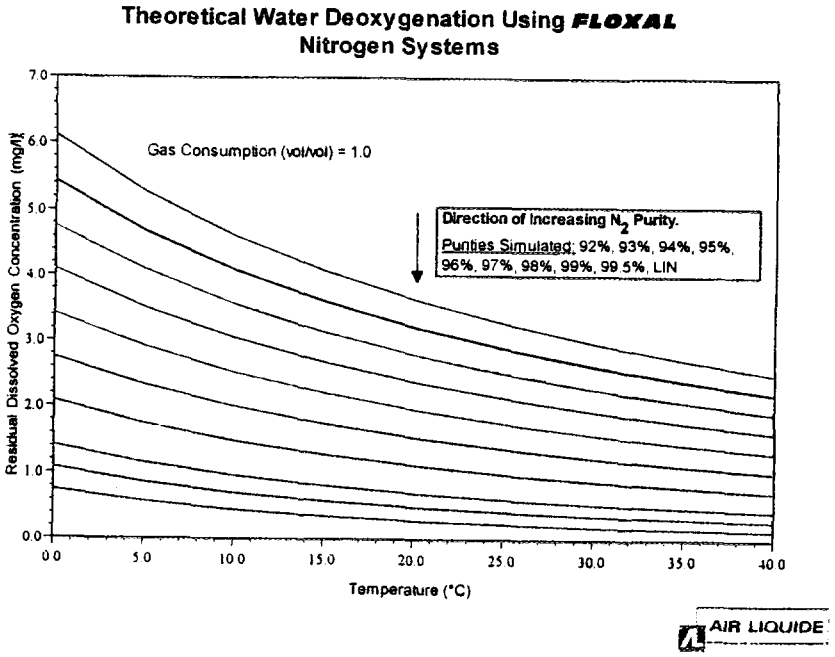
The purpose of the computer modeling was to predict the effect of gas flowrate and composition on equilibrium concentration of dissolved gases when being converted from the liquid nitrogen source to a membrane system for single-stage deoxygenation of pure water. The different variables were :

- nitrogen purity (92-99,5 %) vs liquid nitrogen source baseline (99,998 %)
- temperature (0-40° C)
- gas consumption (0-4 vol. gas/vol. liquid).

The process simulator generates and solves algebraic equations based on :

- mass balances
- energy balances
- thermodynamic equilibrium conditions.





LABORATORY SCALE EVALUATION

The product tested was a commercial fruit drink enriched in vitamin C (ascorbic acid). The purpose was to determinate the impact of residual O2 on the product's quality parameters over an 8 week storage period.

Storage conditions : - ambient temperature (22° C)
- amber glass vials (100 ml)

Several nitrogen purity levels were tested (99,98,96,94,92 %) vs. the air control.

Materials and methods

. dissolved oxygen concentration (Orion D.O. meter)

. Ascorbic acid concentration

- HPCL
- Column : micro Bondapak (Waters Millipors)
- Detector : photodiode array
- Wavelength : 254 nm
- Mobile phase : 0.5 M KH2PO4 pH 2.3
- Flow rate : 1 ml/min

. Yeast and mold counts :

- filtration (0.45 µm)/OGY agar (selective medium) and/or serial dilutions/3 M Y/M papers
- reading after 5 days incubation 25°C

. Absorbance at 495 nm (uv/vis spectrophotometer)

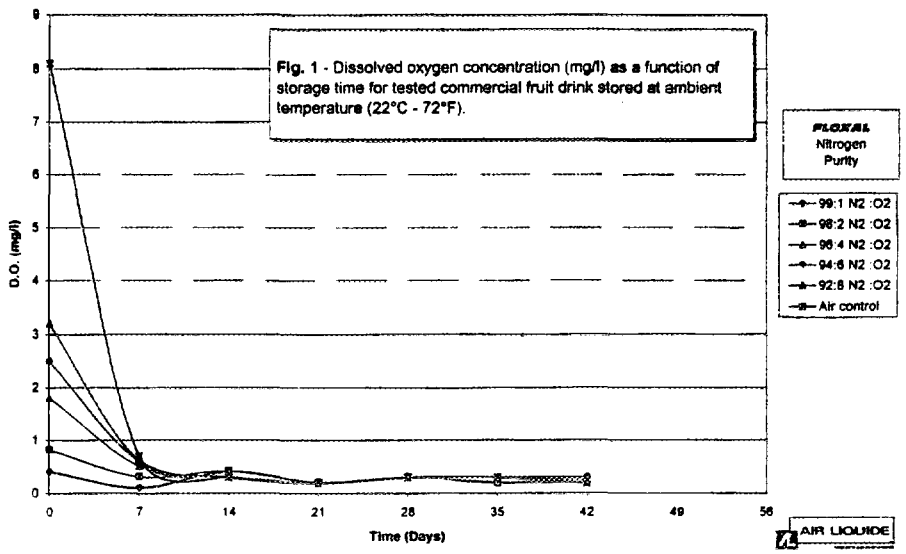


Fig. 1 - Dissolved oxygen concentration (mg/l) as a function of storage time for tested commercial fruit drink stored at ambient temperature (22°C - 72°F).

After 3 days, the residual O2 has been consummed by vitamin C, yeasts and molds for all nitrogen purity levels

After 35 days, 50 % of the ascorbic acid has been degrated when the drink is not deoxygenated, where as a deoxygenation with a 98 % nitrogen purity allows a 80 % retention of ascorbic acid.

The development of yeasts and moulds is delayed thanks to the high purity nitrogen deoxygenation.

The transition from an aerobic metabolism to an anaerobic metabolism can be observed between the 3rd and the 4th week.

This study has shown the performances of this new nitrogen source on beverages' deoxygenation, and the impact of the residual O_2 on vitamin C's degradation, yeasts and molds' development and the color of a commercial fruit juice. The compiling of the technical data as well as the on-site customer testing conducted by AIR LIQUIDE allow to choose an on-site generator solution with a determined nitrogen purity for beverages deoxygenation.

Other parameters should be taken into account for an industrial application to protect the result of this deoxygenation :

- to avoid air intake during the liquid's transfert and the storage,
- to purge the packaging with nitrogen before and after the filling.

The oxygen in the headspace of the packaging should be injurious to the product :

- to select a packaging with a high O2 impermeability

MODIFICATION OF ANISE (*Pimpinella anisum* L.) ESSENTIAL OIL COMPOSITION DURING STORAGE

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ABSTRACT

The importance of anise (*Pimpinella anisum* L.) grains storage conditions (time, temperature, packaging material) was investigated, focusing on the oil extraction yield and essential oil composition.

The average yield of steam-distilled oil was 1.8% and the main component was *trans*-anethole (up to 95%). *cis*-Anethole, pseudoisoeugenol-(2-methylbutyrate) and its epoxy derivative represented the remaining part.

During conservation, some oxidation reactions were observed.

Keywords: anise, storage, freezing, yield, composition.

INTRODUCTION

Anise (*Pimpinella anisum*) is an Oriental that is widely spread all over the world, and it is used for its aromatic properties. In Italy its cultivation is mainly concentrated in Middle-North regions (Emilia-Romagna and Marche)[1]. The essential oil (EO) is the main product of these cultivations, and it is employed in the food [2-3] and pharmaceutical industry [5].

The yield in EO from dried grains ranges around 1.9-3.1%[6]. Its composition is characterised by few anethole isomers as main constituents (up to 98%), *trans*- and *cis*-anethole, and estragole[7-8]. The quality of the oil is guaranteed by a high *trans*-anethole content[9], which vary within a relatively wide range (75-96%).

However, minor compounds contribute to sensory characteristics of anise EO. In fact, *cis*-anethole gives a woody-pungent note[10], whereas estragole has a fennel grass-like smell[11].

On the other hand, storage conditions (time, temperature and packaging materials) can also influence the sensory profile of this EO[12].

In this paper, the influence of some of these parameters on the EO yield and composition was studied.

MATERIALS AND METHODS

Storage conditions and sampling

A batch of anise grains from Ascoli Piceno district was divided into 4 parts and submitted to different storage conditions:

a 5 kg were stored in one a non-hermetic container;

b 5 kg were vacuum stored in 1 kg polyethylene airtight bags;

c 3 kg were freezed (-18 °C) in 1 kg polyethylene bags;

d 5 kg were immediately steam-distilled.

The first two lots (*a*, and *b*) were sampled after 30, 60, 105, 150, and 195 days of storage, while *c* was sampled after 105, 150, and 195 days of storage.

EO extraction

Oil extraction was carried out by steam distillation of 1 kg of grains using 2 L of water at an Alberici S.p.A. (Verona, Italy) pilot plant. The distillation time was standardised at 4 hours.

GC and GC-MS analyses

Samples (1 μL, 1000 ppm) were subjected to GC analysis in a Fisons gas chromatograph HRGC 5160 Mega Series equipped with a 25 m x 0.25 mm, 0.25 μm film thickness SE 52 (Mega S.r.L., Legnano, Italy), operated from 70 °C (10') at 5 °C/min to 150 °C (10') and then at 50 °C/min to 200 °C (10'), and finally to 350 °C at 20 °C/min. Splitter injector (split ratio 1:100) was held at 250 °C, while detector (FID) at 350 °C. Carrier gas (H_2) flowed at 3 mL/min.

GC-MS analysis were carried out at 70 eV electron impact mode, source temperature 180 °C. Chromatographic conditions were the same as those described above, except for the carrier gas (He) whose flow was set in such a way to have the same retention time as FID GC trace.

Reference compounds

Standard substances were supplied by different firms. Epoxianethole was prepared by making the anethole react with 3-chloroperbenzoic acid.

RESULTS AND DISCUSSION

Yield of extraction

The average extraction yield of the different tests was 1.8%, within a range of 1.4 to 2.1%. It was slightly lower than that reported in literature[6], with a few differences among the tests (fig. 1). Grains

stored at room temperature showed the highest variability during storage, as a response to relative humidity variations. However, at the end of the studied period, no significant differences were observed.

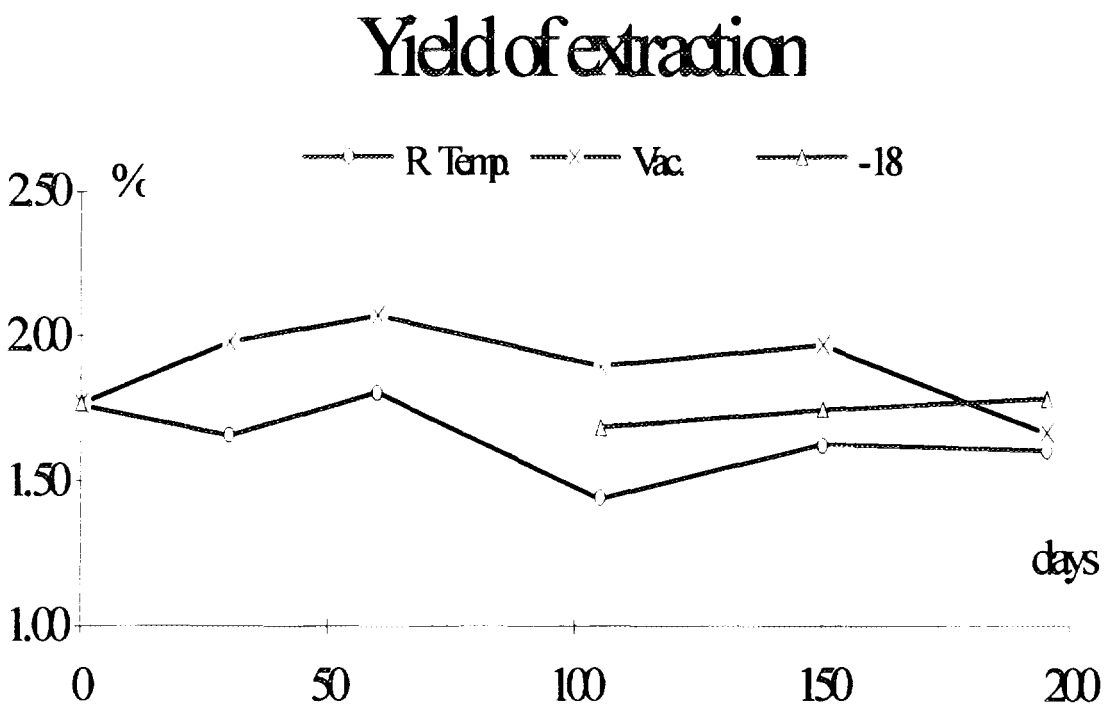

Figure 1. Yield of extraction of the different tests.

Oil composition

trans-Anethole was the major compound (up to 95%), while the *cis*- isomer and estragole were about 1.2% and 0.4% respectively. *p*-Methoxybenzaldehyde (MBA) was below 0.5%, but it showed a great variability. It is important to point out that pseudoisoeugenol-(2-methylbutyrate) (PiB) and its epoxy derivative (EPiB) were also present, which concentration level equal to 1% and 0.2% respectively (Table 1). Finally, trace amounts of monoterpenes (limonene, β-pinene, *p*-cimene, etc.), a few sesquiterpenes, and epoxyanethole were also detected.

Preservations tests

Table 1 reports the variation of the different compounds during storage. ***trans*-Anethole** showed the lowest variation, but it was the precursor of most of the other substances.

In spite of the stability of *trans*-anethole, some **oxidation** occurred. In fact, some degradation products were found. MBA was the major degradation product, which may have been generated via a hydroperoxyde intermediate. Another oxidation product was epoxyanethole, which was detected only as

trace. The aldehyde showed the highest increase during storage. On the other hand, anethole isomers were almost constant during the preservation time here tested.

Table 1. Percentual composition of anise grains stored in different conditions.

	Room temperature					
Time (days)	0	30	60	105	150	195
Estragole	0.35	0.38	0.36	0.41	0.39	0.42
cis-Anethole	1.19	1.19	1.17	1.24	1.23	1.23
p-Methoxybenzaldehyde	0.21	0.25	0.33	0.34	0.39	0.47
trans-Anethole	95.1	95.9	94.6	94.7	94.3	94.4
Pseudoisoeugenol-(2-methylbutyrate)	0.86	0.85	1.05	1.23	1.15	1.06
Epoxypseudoisoeugenol-(2-methylbutyrate)	0.14	0.09	0.19	0.25	0.24	0.19
	Vacuum sealed					
Time (days)	0	30	60	105	150	195
Estragole	0.35	0.36	0.38	0.40	0.29	0.33
cis-Anethole	1.19	1.18	1.22	1.23	1.24	1.20
p-Methoxybenzaldehyde	0.21	0.29	0.33	0.31	0.14	0.15
trans-Anethole	95.1	94.7	94.7	94.0	93.2	93.7
Pseudoisoeugenol-(2-methylbutyrate)	0.86	1.10	1.09	1.35	1.94	1.56
Epoxypseudoisoeugenol-(2-methylbutyrate)	0.14	0.21	0.20	0.27	0.42	0.34
	-18 °C					
Time (days)	0	30	60	105	150	195
Estragole	0.35	---	---	0.33	0.32	0.36
cis-Anethole	1.19	---	---	1.23	1.25	1.19
p-Methoxybenzaldehyde	0.21	---	---	0.16	0.35	0.16
trans-Anethole	95.1	---	---	95.3	95.5	94.6
Pseudoisoeugenol-(2-methylbutyrate)	0.86	---	---	0.64	1.15	1.11
Epoxypseudoisoeugenol-(2-methylbutyrate)	0.14	---	---	0.07	0.26	0.26

Storage conditions

directly affected the extent of these modifications. Storage at room temperature produced the highest degradations of the oil, which resulted in a high MBA content, while the other systems of preservation did not give rise to such modifications.

PiB increased during anise conservation, particularly under vacuum storage conditions. **EPiB** exhibited the same behaviour, having a greater variability due to its higher reactivity, though.

CONCLUSIONS

Oil yields were slight different, and probably they were also influenced by environmental condition, at least in grains stored at room temperature.

During the storage time here considered, small variation of *trans*-anethole were observed.

Until the first 195 days of storage, environmental conditions did not significantly influence the stability of the EO.

Longer preservation periods may have led to greater degradations in anise grains. These effects could be reduced by extracting the EO and storing it at less stressful temperature and light conditions, together with a possible addiction of antioxidants.

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THE STABILITY OF 'ACEROLA' JUICE BY COMPLEXATION WITH PROPOLIS FLAVONOIDS

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Synopsis
The colour, besides other parameters, influence the consumers in their evaluation of the quality of foods and juices. The brilliant red colour of 'acerola' juice is given by anthocyanins, present in a high content in the fruits. However, because the presence of ascorbic acid, the colour stability is affected. The complexation of anthocyanins with propolis flavonoids could enhance the juice colours imparting a marked stabilization.

INTRODUCTION

The fruit 'acerola' is a plant from Malpighiaceae family, having acquired a world importance after the discovery of its high content of ascorbic acid (ranging from 1028 to 4676 mg/100gr) (1). The red fruit is a unique source of vitamin C, which by its antioxidative capacity, has been consumed to remove free radicals and preventing cancer. Its juice has been suggested to fortify others fruits juices (2), (3).

However, the brilliant red colour of the fruit juice, given by anthocyanins, can be lost, possibly by the indirect interaction induced by the ascorbic acid (4), also resulting a decrease of the content of vitamin C. Besides the anthocyanins, the fruit pose carotenoids (fitofluene, α–, β-criptoxantin and β-carotene) (5) and flavonoids, this latest class consisting of flavonols, flavones, catochins and flavanones, which demonstrate a wide range of biochemical and pharmacological effects (6), (7). In our studies, we investigated the addition of propolis as a source of flavonoids (8), (9), (10), (11), to stabilish the copigmentation effects. The aime is to increase the colour stability of the pigments, decreasing the ascorbic acid oxidation effect which has a detrimental effect on the anthcyanins of the fruit juice (12).

MATERIALS AND METHODS

Pasteurized juice

The extracted pulp from acerola fruits was thermically treated to 90°C / 15 min and refrigerated at the same changer until a temperature of 25°C. The juice was aconditioned in cans.

Commercial juice

A commercial pasteurized juice was obtained at a local market.

Fruit extract

Fresh fruits of acerola were macerated overnight with 0,5% citric acid in water under N_2 at a temperature of 5°C. The extract was decanted, filtered and concentrated in a rotatory evaporator under reduced pressure at 30°C and partially purified by ionic exchange resin (Amberlite IR-50)

Flavonoids extract

Propolis (2 gr) was cut into small pieces and extacted with boiling ethanol (200 ml) for 2 hr. The extract was filtered warm, concentrated and diluited with 110 ml water and then extracted with ethyl acetate (3 x 50 ml). The obtained flavonoids were purified by HPLC as described in the literature (13).

Apparatus

The spectral data were obtained using a Beckman DU-70 Spectrophptometer.

The liquid chromatography was performed in a Shimadzu chromatograph, model LC-10 AD, equipped with a UV-Visible detector SPD-10 AV, using a chromatographic column prepacked with μ–βondapack/c_{18}

Experiments of stability

Both commercial and pasteurized juice were used after filtration, centrifugation and appropriated dilution. The corrected pH were obtained by adding HCl. The aqueous extract of fresh fruits was liofilized and redissolved in bufferred solutions citrate-phosphate at pH 2,0 and 3,0. The purified flavonoids were liofilized and redissolved in 10% ethanol.

For the experiments of stability, the buffered anthocyanins solutions (2,6 x 10^{-4} M) and both commercial and pasteurized juices were complexed with three different concentrations of the flavonoids extract (pigment/ copigment molar ratio of 1:1, 1:5 and 1:10). The solutions were distributed into screw-caps tubes of 10 ml capacity.Half of the tubes were stored at 220°C in the dark and half exposed to light. Control solutions were exposed at the conditions. Measurement of absorbance, at λ_{max}, wereobtained after intervals of 24 hs, during 336 hs of exposition.

RESSULTS AND DISCUSSION

Colour can give information on quality and condition, and its contribution to the appearance and attractiveness is unique because we expect food to have own characteristic appearance including colour and flavour (14). In this sense, to keep the colour is a challenge to the manufacturers

Anthocyanins degradation is observed at the same time of the decrease of content of ascorbic acid in stored juices. The exact mechanism is not clear but it has been found a linear relationship between the anthocyanins fading rate and ascorbic acid concentration (15), In other side, flavonoids could be used to complex anthocianins producing an increased at the absorbance, improving the stability and the extention of the shelf-life of fruit juice (16).

In the systems containing propolis flavonoids, ascorbic acid and anthcyanins in the both commercial and pasteurized juices and fresh fruit juice, the anthocyanins degradation was slowed down.Flavonoids added at the pigment/copigment molar ratio of 1:1, 1:5, and 1:10 exerted protective effects as shown in the table 1 and table 2, comparing the half-lives values ($t_{½}$) for the systems.

Table 1. Half-lives values (t ½) (hr) for the anthocyanins present in the commercial juice (a), pasteurized juice (b) and fruit extract , with and without (control) flavonoids extract, at the pigmeent/copigment molar ratio of 1:1, 1:5 and 1:10, at pH 2,0 and pH 3,0, under exposition to light.

				T1/2 (hr)				
Systems		**pH 2,0**			**pH 3,0**			
	Control	***1:1***	***1:5***	***1:10***	***Controll***	***1:1***	***1:5***	***1:10***
1	120,0	132,04	144,7	169,2	110,0	110,6	114,1	122,7
2	96,0	98,1	112,0	123,0	86,7	88,8	98,7	111,0
3	300,0	350,0	386,0	374,0	230,2	244,3	256,9	270,0

Table 2. Half-lives values (t ½) (hr) for the anthocyanins present in the commercial juice (a), pasteurized juice (b) and fruit extract , with and without (control) flavonoids extract, at the pigmeent/copigment molar ratio of 1:1, 1:5 and 1:10, at pH 2,0 and pH 3,0, in the dark..

				T1/2 (hr)				
Systems		**pH 2,0**			**pH 3,0**			
	Control	***1:1***	***1:5***	***1:10***	***Controll***	***1:1***	***1:5***	***1:10***
1	143,0	160,0	178,8	202,0	124,1	126,0	131,1	139,8
2	99,4	109,5	112,6	124,3	88,7	90,2	94,3	99,4
3	356,0	403,2	432,2	455,0	276,5	294,0	334,6	355,1

The pH 2,0 , protection against excessive light and the addition of flavonoids in a pigment/copigment molar ratio of 1:10 were the most protective conditions to all the systems. The Brazilian propolis can be considered as having a composition of flavonoids whic could colaborated in the maintenance of the colour juice.

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FUTURE FOOD: THE IMPACT OF BIOTECHNOLOGY

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This presentation will provide an overview of the state of plant biotechnology in the U.S., as well as a glimpse into future applications of food biotechnology. Potential benefits and challenges to production agriculture and the food processing industry as a result of the biotechnology revolution will be discussed. In addition, the most recent information on the perception and acceptance of genetically modified foods by U.S. consumers will be provided.

INTRODUCTION

After years of speculation and promise, biotechnology-derived foods are becoming a reality in the U.S. and throughout the world. Over 15,000 independent field tests have been conducted in at least 34 countries with 56 different crops[1]. The first genetically modified plants were produced in 1982, and since that time, the U.S. Department of Agriculture (USDA) has approved over 4000 permits for field testing of approximately 48 different crops in the U.S. The U.S. Food and Drug Administration (FDA) has reviewed twenty-eight products, with many more in various stages of the approval process. On a global basis, in 1996 alone, 7 products were approved in Japan, 5 in Canada, 4 in the EU, 3 in Mexico and 1 each in Argentina and Australia. Approximately 3-6 million acres of genetically modified crops were planted for commercial production in the U.S. in 1996 and it is anticipated that 20-30 million acres have been planted in 1997[2]. The first biotechnology-derived whole foods are making their way to supermarket shelves and for the first time consumers are able to see, touch, feel, taste and compare engineered tomatoes, potatoes, and squash. Biotechnology will have a profound impact on the food supply, and will also drive fundamental changes in the agricultural and processing industries responsible for producing and manufacturing the vast array of fresh and processed foods available to consumers.

WHY BIOTECHNOLOGY?

Traditional breeding methods have been successfully used for the improvement of food crops since the domestication of plants. By exploiting the genetic diversity of food crops throughout the world, yield, disease and pest resistance, nutrient content and overall quality of plant species has been dramatically improved using traditional methods. So, why do we need biotechnology? Traditional breeding methods involve random sorting and uncontrolled recombination of thousands of uncharacterized genes, while genetic engineering can be used to transfer or modify one or a few well-characterized genes. Traditional breeding requires extensive back crossing to eliminate undesirable traits that are closely linked to the trait of interest. Species barriers also limit conventional breeding – a valuable property in wheat cannot be readily transferred to corn due to natural breeding barriers that exist between unrelated plant species. Because the genetic information in all living organisms is structurally and functionally identical, genetic engineering can be used to transfer genes between unrelated species. Thus, genetic engineering is more predictable, precise, and controllable than conventional breeding, can be accomplished in a much shorter time frame, and allows for infinite expansion of the gene pool.

Demographics are also impacting the need for technological improvements in food production. The global population is expanding at a rate in excess of 90 million people per year. By the year 2040, world population is expected to double to approximately 10 billion people. Much of this growth is occurring in developing countries that are already challenged to produce sufficient food. Current technologies are reaching limits in improving crop yields and natural resources are limited. There is a critical need for better ways to maximize production efficiency in more sustainable ways with reduced environmental impact. Biotechnology provides an avenue for addressing these critical needs.

HOW WILL BIOTECHNOLOGY IMPROVE THE FOOD SUPPLY?

There are numerous examples of how biotechnology will impact the entire food chain from the seed (farmers) to the store (food processors) to the stomach (consumers). The first applications of biotechnology in the food supply will benefit production agriculture and the primary beneficiaries will be farmers. Crops will be substantially equivalent to traditional crops and the benefits of improved field performance, enhanced yield, and reduced need for pesticides/herbicides will be transparent to consumers. As the technology matures, biotechnology will have a greater impact on value-added agriculture where tangible benefits are readily recognizable by processors and consumers. A sampling of examples for each area are discussed below.

PRODUCTION AGRICULTURE

Insect resistance

Insect pests are responsible for significant crop yield losses. The annual cost of chemical control of insect pests has been estimated at $3-$5 billion worldwide; over $400 million is

spent each year in the U.S. for control of lepidopteran pests alone[3]. Therefore, the genetic engineering of insect resistance into crops has become an attractive target for agricultural biotechnology. The insect toxin gene from *Bacillus thuringiensis* has been successfully introduced into a number of plant species including tomato, cotton, potato, and corn. The commercialization of insect resistant crops will result in decreased use of chemical insecticides, reduced exposure of farmers to insecticides, and less chemical residues in soil and water.

Virus resistance

Reduced dependence on agricultural chemicals can also be achieved through the use of virus-resistant plant varieties. Viruses can reduce crop yields of yellow squash, for example, by 20-80% depending on geographic location and production season[3]. Virus-resistant varieties allow farmers to grow crops consistently, and without the use of chemical insecticides required to control virus spread by insect vectors. Virus resistance is being engineered into other important crops including tomato, potato, melon, rice, papaya, and sugar beet.

Fungal and disease resistance

Engineering fungal and bacterial disease resistance into food crops is a relatively new, but critically important area of focus. Recent efforts have focused on the expression in plants of various hydrolytic chitinases and glucanases that degrade fungal cell walls; however, in many cases these enzymes are not potent enough to confer complete resistance. Expression of plant defensins or other proteins induced upon fungal infection appear to be promising alternatives[4].

Herbicide tolerance

Herbicides are used to control weeds that compete with food crops for valuable nutrients. In addition to decreasing yield, weeds decrease harvest efficiency and seed quality, and can serve as a reservoir for crop pests. In industrialized countries, herbicides are used on essentially 100% of the acreage of major agronomic crops and account for 60 to 70% of pesticides sold for agricultural use, by volume[5]. Since herbicides also damage food crops, the most effective herbicides must be applied before emergence of food plants. Using genetic engineering, herbicide tolerant plants are being developed so farmers can spray selected herbicides with preferred environmental properties on an "as needed" basis during the growing season. There is a common misconception that the use of herbicide-tolerant plants would allow farmers to use larger amounts of herbicide; however, actual experience with the first commercialized Roundup (glyphosate) Ready soybean resulted in a 9 – 39% reduction in herbicide use. In addition, three out of four growers were able to control weeds effectively with a single application of Roundup, while achieving a 5% yield increase due to superior weed control. Examples of herbicide tolerant crops include soybeans, canola, cotton and corn tolerant to Roundup, glufosinate-tolerant canola, oilseed rape and corn tolerant to phosphinotricin, and cotton tolerant to sulfonylureas[2].

VALUE-ADDED AGRICULTURE

Applications of biotechnology that benefit production agriculture are often transparent to consumers and they may not feel immediate and personal benefits. The products are substantially equivalent in all respects to their traditional counterparts and the benefits may not be readily apparent. Value-added agriculture, on the other hand, provides personal and tangible benefits to food processors and consumers.

Tomato

The first genetically modified whole food to enter the U.S. market place was Calgene's FLAVR SAVR™ tomato, which was introduced in 1995 after a lengthy review process by FDA. FDA has reviewed four additional genetically modified tomatoes. The tomatoes have been genetically modified to alter the ripening and/or softening processes by either inhibiting breakdown of cell wall components or by directly altering the synthesis of a plant-derived compound, ethylene, that triggers fruit ripening. These tomatoes are particularly geared to the fresh market. Other applications of biotechnology are of interest to tomato processors. For example, it has been estimated that for every one- percent increase in tomato solids, the tomato processing industry would save $80 million per year from reduced raw product volumes, raw material transportation costs, and processing energy costs[6]. Future consumer-driven improvements include modifications in texture and flavor. In the future it may also be possible to increase the level of important phytochemicals, such as lycopene which have been implicated as protective against prostate cancer.

Potato

Potato is the #1 vegetable and fourth largest crop in the world, with an annual farmgate value of over $35 billion. In the U.S., potato generates $27 billion at retail with about 50% of this coming from French fries. The first transgenic potato was reported in 1986 and in 1995 the first transgenic potato, Monsanto's NewLeafTM potato was approved for sale in the U.S. This Russet Burbank potato, the dominant fresh market and processing variety in North America, contains a gene which confers complete control of the Colorado Potato beetle, the most devastating insect pest on potato in North America and much of the world. Monsanto is actively engaged in potato improvement and will introduce resistance to other viruses and bacterial and fungal pathogens in the near future[7].

Modifying the starch content and composition has been a major focus of value-added agriculture. The total amount of starch has been increased in potatoes by introducing a bacterial gene, which controls a key regulatory enzyme controlling starch biosynthesis in plants. The increased starch content is achieved at the expense of water, resulting in higher yields of processed potato products as well as reduced absorption of oil during frying. Modifications in the composition of starch have also been achieved. Amylose-free, amylopectin-enriched potatoes produce a starch, which is preferred for specific industrial applications. Redirecting the flow of carbon from starch biosynthesis can result in increased sugar content[4]. Improved storage stability may be achieved by preventing starch breakdown at low temperatures and engineering resistance to bruising into potatoes.

Oilseeds

A major focus for improvement of oilseed relates to altering the fatty acid composition via modulation of plant biosynthetic pathways. Insertion of genes involved in lipid biosynthesis result in altered chain length, degree of saturation or chemical modification of fatty acids in the storage lipids of seeds. Products derived from these oilseeds have improved stability and a more desirable nutritional profile. One of the first genetically improved oil to be tested, a high laurate canola oil serves as an alternative to tropical oils. Oil with increased levels of oleic acid derived from genetically modified canola has improved oxidative stability and requires less hydrogenation during oil processing. In addition, this new oil is free from *trans* fatty acids, which have been linked to cardiovascular disease[8].

Cereals and legumes

All cereals are deficient in specific amino acids; therefore, a focus of biotechnology has been improving the amino acid profile in rice, maize, triticale, wheat and barley. Amino acids can either be introduced in their free form or added as part of seed storage proteins that are present at relatively high levels in grain. It is particularly important to increase lysine content in cereals and methionine content in legumes. Improving the consistency of breadmaking quality of wheat flour is another target amenable to genetic engineering. The ability to improve dough-making quality of flour depends largely on the visco-elastic properties conferred to dough by the gluten proteins. Variation in the amount and composition of higher molecular weight gluten subunits is associated with elasticity and quality among various types of wheat[9].

Vegetables

One could imagine numerous applications of biotechnology for improvement of vegetables in general. Improved nutritional content (e.g., increased levels of fiber or vitamins, reduced fat, improved bioavailability); elimination of anti-nutrients (e.g., phytates in cereals, anti-trypsin factor in soy, allergens); extended shelf life; improved flavor, texture, color, and aroma; increased levels of phytochemicals (e.g., lycopene in tomatoes, beta-carotene in carrots, isoflavones in soy) implicated in disease prevention, are just a few examples of how vegetables could be improved.

ARE BIOTECHNOLOGY-DERIVED FOODS SAFE?

While consultation with FDA is not mandatory in the U.S., developers of genetically modified crops have routinely consulted with the agency prior to commercialization. Biotechnology-derived foods are evaluated extensively prior to commercialization. Data typically provided to U.S. regulatory agencies to support the food, feed and environmental safety of genetically modified plants and plant products include the following: For food/feed safety assessments, data provided include molecular characterization, protein safety assessment (including potential allergenicity), nutritional equivalence, and toxicological assessment. In terms of environmental safety assessment, data include the potential for and impact of outcrossing and gene flow, potential for weediness, competitiveness and survivability, morphological and phenotypic characteristics, insect and

disease susceptibility, impact on non-target organisms, agronomic performance and resistance management, where appropriate. Based on these data, FDA evaluates food safety issues and determines whether or not an engineered product is substantially equivalent to its traditional counterpart. This science-based consultation procedure protects both consumers and developers; therefore, there is a strong incentive to consult with FDA prior to marketing products. To date, no genetically modified crop has posed a new risk to food safety.

HOW WILL BIOTECHNOLOGY IMPACT U.S. FOOD PROCESSORS?

LABELING

FDA does not require special labeling of foods developed through genetic engineering or any other new method if the composition of the food is substantially equivalent to its conventional counterpart. If the new food contains a protein derived from a food that commonly causes allergic reaction and the developer cannot demonstrate that the protein is not an allergen, labeling would be required to alert sensitive consumers. Labeling would also be required if the nutritional composition of an engineered food were significantly different than its conventional counterpart. FDA does not require disclosure in labeling based solely on the basis of consumers' desire to know. This is clearly not the case in other countries, including the EU where the consumer's right to know is considered in labeling decisions, and mandatory labeling of all foods containing a genetically modified organism may be required. With so many foods traded internationally, there is a clear need to reach a harmonized approach to labeling of genetically modified foods or potential trade barriers will be created.

The labeling of genetically modified whole foods, where improved crops are segregated throughout the production and distribution systems would be relatively easy to accomplish. In fact, Calgene and Monsanto elected to voluntarily label the FLAVR SAVR™ tomato and NewLeaf™ potato, respectively, to inform consumers of the benefits of their products. Segregation becomes more challenging, if not impossible, with commodity crops and ingredients derived from these crops.

SEGREGATION AND IDENTITY PRESERVATION

Production agriculture-driven biotechnology applications (i.e., insect, disease and virus resistance, herbicide tolerance) will be broadly available to farmers and typically will involve plants grown on hundreds of thousands of acres. Many of the affected crops such as corn and soybean are handled as commodities in the U.S. Corn and soy are processed into a variety of common ingredients (e.g., starch, sweeteners, oil, flour) used extensively in processed food products. Many of these ingredients are also incorporated into ingredient blends and mixtures. A typical food processing company purchases thousands of ingredients from hundreds of suppliers. It has been estimated that soy-derived oil, flour, and/or protein are present in approximately 60% of all processed foods[10]. To label processed food products with regard to ingredients derived from genetically engineered crops would require segregation and identity preservation of commodity crops. Ensuring accurate segregation and labeling from seed production through intermediate and final

processing channels when there are no visible distinguishing characteristics between engineered and traditional crops would be logistically challenging and cost prohibitive on low margin commodity ingredients.

In order to reap the benefits of value-added agricultural applications, on the other hand, identity preservation and segregation would be desirable, if not mandatory. Co-mingling of a nutritionally improved oil containing a specific fatty acid profile with traditional oil from a non-modified plant would dilute the value and health benefit of the improved oil. Value-added applications of biotechnology are likely to be more specialized and proprietary in nature. For example, if a fast food company developed, either independently or through an alliance relationship, an improved potato for French fries, it would be to their advantage to use the variety exclusively and not make it readily available to competitors. This creates niche opportunities for farmers to grow smaller acreage specialty crops. It also creates challenges for farmers and processors who will need to segregate crops and their derivatives throughout the food chain.

STRUCTURAL CHANGES IN AGRIBUSINESS

Biotechnology is driving tremendous consolidation within the agricultural chemical and seed industries. Many of the large seed companies (Asgrow, Northrup King, Dekalb, and Ciba Seeds), and biotechnology boutiques (Calgene, Ecogen, Agracetus, Plant Genetics Systems, Mycogen, and DNA Plant Technology) have been purchased by agricultural chemical companies such as Monsanto, DowElanco, and DuPont. As a result, the technology is being consolidated within a limited number of very large companies who will control access to specific biotechnology applications. These large conglomerates are forming relationships with processors and marketers who will ultimately be responsible for delivering biotechnology-derived food products to the marketplace.

WILL CONSUMERS ACCEPT BIOTECHNOLOGY-DERIVED FOODS?

A national survey of 1,004 U.S. adults released in March 1997 found high awareness of food biotechnology, strong support for its benefits, and endorsement for the current FDA labeling requirements. Approximately 79% of respondents were aware of biotechnology and nearly half realized foods produced through biotechnology were already in supermarkets. Even when presented with the position of some critics of FDA's labeling policy, who believe all food biotechnology products should be labeled, nearly 6 in 10 still maintained their support of the current labeling policy[11]. Consumer acceptance of biotechnology-derived foods is a critical issue. In the U.S., consumers have been exposed to significant media coverage of food biotechnology and a limited number of products are available in the marketplace for consumers to taste and compare. In general, the U.S. public has confidence in federal regulatory agencies and trust they are safeguarding the food supply. There is also strong political support for science-based regulation of biotechnology. As more products are available to consumers that provide personal and tangible benefits such as decreased use of pesticides, improved nutritional quality, and extended shelf life, acceptance is likely to increase in the U.S. The same is not true in other countries, where consumers are less aware of biotechnology, where regulatory systems are

less well developed and agencies do not enjoy the same confidence level as in the U.S., and where politicians are more divided on how to manage biotechnology issues.

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METABOLIC ENGINEERING FOR IMPROVED FRUIT QUALITY

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Fruit quality is a complex trait that is defined by the flavor, texture, color, solids content and nutritional value of the fruit. Research strategies to improve fruit quality have used both classical and molecular genetic approaches to modify the general program of fruit development to allow the simultaneous enhancement of multiple quality parameters as well as on strategies to modify specific chemical constituents of fruit for specific applications. In one case, the modification of sucrose content of tomato was achieved by both classical and molecular genetic strategies. This provides an opportunity to compare and contrast how classical methods of plant breeding are related to molecular genetic approaches that are now available to engineer the composition and quality of food products.

FRUIT QUALITY TRAITS AND GENETIC MODIFICATION

Traits that define fruit quality are quite numerous and highly variable depending on the intended use of the raw product. Nevertheless, there are some quality traits such as flavor, nutritional content, postharvest shelf-life and resistance to pathogens that are of high value for essentially all intended food uses of fresh fruit. These traits have been the target of genetic modification for a very long time and continue to receive significant attention from plant breeders and, more recently, from plant genetic engineers. Plant breeding, in its simplest form, has been important for over 10,000 years in selecting improved plant material for human uses. The process of plant breeding has focused its efforts in recent years on new strategies that utilize molecular markers to improve its precision and speed. However, the process of plant breeding is ultimately limited by the variation in traits that can be introduced from closely related plant relatives. The application of recombinant DNA technologies provides the opportunity to greatly expand the genetic resources that can be brought to bear to address the improvement of a specific trait. In this paper I will describe some of the initial strategies that have been successful in enhancing fruit quality and focus on one example which allows a direct comparison between plant breeding and molecular genetic strategies for fruit quality improvement.

MODIFYING THE PROGRAM OF FRUIT DEVELOPMENT

A significant class of fruit complete their development and initiate their senescence with a pronounced activation of metabolism in which many fruit quality parameters are naturally enhanced. Thus, this period of ripening is critical to achieving maximal fruit quality but

because it also initiates the senescence phase of the fruit, it often dictates that the fruit be harvested before achieving full maturity. Several strategies have been developed to control the ripening process by regulating the genes that encode for ethylene biosynthetic enzymes or for receptors that perceive ethylene and transduce the ethylene signal to initiate ripening.

ACC synthase and ACC oxidase: The two terminal biochemical reactions in ethylene biosynthesis are the production of aminocyclopropane-1-carboxylic acid (ACC) and its oxidation to ethylene; reactrions which are catalyzed by ACC synthase and ACC oxidase, respectively. The genes encoding both of these enzymes have been isolated from a number of plant species (1). In order to control and retard fruit ripening senescence, each of these genes have been re-inserted back into plants in an antisense orientation which suppresses expression of the endogenous genes (2,3,4). In each case where this has been attempted, ethylene production is suppressed and the shelf-life of the fruit vastly extended. In the case of melon fruit in which ACC oxidase expression was suppressed, the ability of the fruit to remain longer on the vine resulted in increased sugar accumulation (3). However, because of the importance of ethylene in triggering biochemical processes that enhance fruit quality, it is not yet clear whether "ethylene-suppressed" fruit will achieve all of the quality parameters associated with non-engineered fruit.

Ethylene receptors: The endogenous plant responses to ethylene involve both endogenous ethylene biosynthesis as well as its perception by cellular receptors and transduction to activate cellular responses (5). The gene encoding the ethylene receptor was recently isolated from the model plant species, *Arabidopsis thaliana*, a specific mutation in this gene was shown to confer ethylene-insensitivity (5). This provided an alternative strategy to control ethylene responses by modifying the ability of the plant to perceive the presence of ethylene rather that altering its production. This strategy has the advantage that fruit ripening can not be accidentally activated by exogenous sources of ethylene but suffers for the relative difficulty in reversing its inhibitory effect and allowing the fruit to ripen and attain their full quality. Interestingly, because the ethylene receptor functions in vivo as a dimer (6), the expression of a single defective ethylene receptor gene is sufficient to "poison" the entire ethylene receptor complex. Thus, a single mutant ethylene receptor gene from *Arabidopsis* or tomato can be used in essentially all other plant species to confer ethylene insensitivity (7).

MODIFYING SPECIFIC FRUIT CONSITUENTS

Rather than modifying the entire program of fruit development, a number of research efforts have focused on the engineering of specific biochemical reactions and pathways to achieve a targeted change in some fruit constituent. In most cases, the biochemical changes have been highly predictable based on known biochemical pathways although the physiological consequences of those changes have not always met with expectations.

Carotenoids: Pigments are an important determinant of both the cosmetic and nutritional quality of fruit. In general, the major fruit pigments are either water-soluble anthocyanin derivatives or lipid-soluble carotenoids. While both of these pigments have potential health benefits, the carotenoid pigments are well-known antioxidants and certain carotenoids (ß-carotene) are readily converted to vitamin A following consumption. Thus, enhanced levels of either class of pigment is likely to increase overall fruit quality. In tomato, as well as many other fruit, carotenoid biosynthesis is ethylene-regulated and is activated as part of the ripening developmental program (8). It was demonstrated several years ago that a key enzyme in carotenoid biosynthesis, phytoene synthase, was induced at the onset of

ripening (9). Subsequent research identified the gene encoding phytoene synthase and re-inserted this gene into tomato in order to test the possibility that overexpression of the pytoene synthase gene would lead to an overall enhancement in carotenoid levels. The results conclusively demonstrated that overexpression of a single gene was sufficient to elevate total carotenoid levels, but the overall carotenoid composition was unpredictable. A number of additional genes encoding enzymes that regulate specific steps in the carotenoid biosynthetic pathway have now been isolated and much more detailed research is in progress to specifically modify the total level and composition of carotenoid pigments in plants (10, 11). The results of these experiments will also set the stage for the modification of related biosynthetic pathways, such as the pathway responsible for (-tocopheryl (vitamin E) production.

Cell wall polymers: The structure of cell wall polymers are major determinants of fruit softening as well as the texture of processed fruit products. In spite of this major contribution to fruit quality, the exceedingly complex structure of plant cell walls has complicated strategies to engineer specific changes to cell wall polymer structure and to predict the physiological outcome of such changes (see Labavitch, this volume). The best-known example of engineering cell wall polymer structure is the suppression of expression of the gene encoding polygalacturonase in tomato fruit, leading to the commercialization of the FLAVR SAVR^{TM} fresh tomato in North America and of processed tomato products in the U.K. (12, 13). Suppression of this single gene resulted in reduced pectin disassembly during fruit ripening and increased consistency of processed tomato products from these transgenic lines (14). Surprisingly, there was little effect on fruit softening, suggesting that disassembly of other cell wall polymers are likely to play a previously unrecognized role in the softening process. A great deal of research is currently testing the possibility that disassembly of other cell wall polymers, particularly hemicellulose, can be modified by similar single-gene suppression strategies (15).

Carbohydrate composition: Carbohydrate content of fruit is highly correlated with the total fruit solids, a key quality parameter of essentially all fruit. Starch is the major storage form of insoluble carbohydrate in plants and in some fruit starch serves as a transitory reservoir of carbohydrate which can be broken down to release soluble sugars (16). Starch biosynthesis and accumulation levels are regulated primarily by the activity of a single enzyme, ADP-glucose synthetase, which produces the immediate precursor to starch. Plants have been genetically engineered by the insertion of a single bacterial encoding a mutant ADP-glucose synthetase which is not feedback regulated (17). In potato, starch levels and total solids were increased dramatically by this strategy. The application of similar approaches to fruit have been tested but without definitive results. Alternative strategies to modify soluble sugar composition have been developed and one of these is discussed in detail below.

BREEDING OR GENETIC ENGINEERING - TWO MEANS TO THE SAME END

Although genetic engineering approaches are likely to be of increasingly importance to fruit quality enhancement, classical genetic strategies or plant breeding can sometimes achieve a similar result. It is interesting to note that there continues to be some public concern over the consumption of genetically engineered foods, yet there is no similar concern regarding human consumption of food modified by classical genetic approaches. There exists one example where a fruit quality trait was modified by both classical genetic and molecular genetic approaches and this example provides the basis to consider whether there exists a significant genetic basis for the difference in public perception of classical genetic and

molecular genetic approaches to the modification of foods. This example is summarized below.

Carbohydrate composition of tomato: Tomato fruit typically accumulate the hexose sugars, glucose and fructose throughout development. Sugars are the major component of fruit total soluble solids (TSS) and this parameter is the single most important contributor to the processing quality of tomatoes. Because processed tomato products are concentrated by evaporation to a fixed level of TSS, increases in sugar concentration in the raw fruit can lead to large increases in processed product yield and to reduced processing costs. Estimates indicate that increases in TSS of 0.1% have a value of approximately $7 M to the California tomato processing industry alone. In addition, sugar content is an important determinant of flavor and therefore contributes to the quality of fresh tomatoes as well.

A biochemical component that has been suggested to contribute to elevated sugar concentration in tomato fruit is the modification of sugar composition by increasing sucrose accumulation (18,19,20,21). Because tomato fruit normally accumulate hexose sugars and very low amounts of sucrose, this change alters the sucrose/hexose ratio in favor of sucrose. The basis for suggesting that sucrose accumulation would contribute to elevated total sugar levels is based on the decreased osmotic contribution of sucrose relative to hexose, which predicts that sucrose-accumulating fruit would accumulate less water during development and maintain a higher sugar concentration than the corresponding hexose-accumulating fruit (20,22). To test this proposal, tomatoes were genetically modified to accumulate sucrose by both classical genetic means and by genetic engineering.

Introgression of sucrose accumulation from a wild tomato species: Two close relatives of tomato, *L. chmielewskii* and *L. hirsutum*, were identified to accumulate sucrose, rather than hexose as in domesticated tomato, *L. esculentum* (18,22,23). In addition to accumulating sucrose, both of these wild relatives of tomato also accumulate very high levels of total sugar, consistent with the idea that this trait could contribute to elevated total sugar concentration. Previous research had also demonstrated the feasibility of using *L. chmielewskii* and *L. hirsutum* as parents in breeding programs with *L. esculentum* (24,25). Collectively, these observations indicated that there was a source of gene(s) that could be accessed by classical genetic strategies to confer sucrose-accumulation in tomato. The trait of sucrose accumulation in *L. chmielewskii* was transferred to tomato, *L. esculentum*, by crossing individuals of the two species (20). The first progeny of a cross between these two parents all accumulated hexose sugars, indicating that the trait of sucrose accumulation was recessive. Subsequent generations were obtained by backcrossing to a *L. esculentum* parent in order to progressively increase the relative contribution of *L. esculentum* to the genome. In the early generations, the segregation ratios indicated that the trait of sucrose accumulation was controlled by a single recessive gene which was named *sucr* (21). This conclusion meant that the trait could be transferred in a relatively straightforward manner into *L. esculentum*.

Introgression of traits by classical genetic means is characterized by the transfer of large blocks of DNA that may encompass entire chromosomes or large segments of chromosomes. Because the *L. chmielewskii* genome encodes many undesirable traits, our goal was to transfer the minimum segment of the *L. chmielewskii* genome to *L. esculentum* that conferred the sucrose accumulating trait. The use of molecular markers corresponding to the genome of the donor and recipient species provides the basis to achieve this goal, but requires that we first map the trait of sucrose accumulation to a specific chromosomal region. This was accomplished by screening a series of restriction fragment length polymorphism (RFLP)

markers in two populations segregating for the trait of sucrose accumulation. The RFLP analysis indicated that the trait resided on chromosome three and was tightly linked to the DNA marker, TG102, providing a means to indirectly select for the trait of sucrose accumulation by selection for individual plants carrying the *L. chmielewskii* allele of TG102. This indirect selection allowed screening of seedlings in both F_1 and F_2 populations for the fruit-specific trait and greatly accelerated the process of introgression.

In addition to accelerating the backcross process, the identification of molecular markers linked to the trait of sucrose accumulation also provided the basis to select plants which carried the minimum segment of the *L. chmielewskii* genome required to confer the trait of sucrose accumulation. In order to specifically introgress the *sucr* locus of *L. chmielewskii* into *L. esculentum*, sucrose-accumulating individuals of a third generation backcross family (BC_3) were identified and their detailed genotype of chromosome three determined by scoring for a number of RFLP markers along the chromosome. Approximately one third of chromosome 3 was comprised of *L. chmielewskii* DNA, and this region encompassed the TG102 locus which had been previously shown to be linked to *sucr*. This individual plant was backcrossed twice more to the recurrent *L. esculentum* parent and sucrose-accumulating progeny of the fifth generation backcross were scored for RFLP markers along chromosome three. Recombination events occurred at several positions which yielded individual progeny with reduced segments of the *L. chmielewskii* genome, that nevertheless encompassed the TG102 locus (26).

The use of molecular markers in the introgression of the *sucr* locus accelerated the rate of introgression by providing a basis for scoring individual plants in the seedling stage and for identifying heterozygotes in F_1 populations. In addition, the markers provided a basis to identify the chromosomal region carrying the *sucr* locus and to select plants carrying the minimum *L. chmielewskii* genomic segment necessary to confer sucrose accumulation. As indicated above, introgression of traits by classical genetic means is characterized by the transfer of large blocks of DNA. Even using molecular markers to minimize the size of the introgressed chromosome segment, we have determined that the introgressed *L. chmielewskii* chromosome segment carrying the *sucr* locus is between 0.5 and 7 centiMorgans. Although there is no strict relationship between genetic and physical distances in the tomato genome, we estimate that this chromosome segment could comprise up to five megabases of DNA and could potentially encode hundreds of genes other than the those corresponding to the *sucr* locus.

Tomato lines derived from marker-aided introgression of the *L. chmielewskii* TG102 locus accumulated sucrose to levels equivalent to the parent wild species but also exhibit negative horticultural traits, such as reduced fertility and scattered fruit set (27). The associated traits may be pleiotropic effects of the introgressed *sucr* gene or may result from the action of linked genes carried on the introgressed *L. chmielewskii* chromosome segment.

Genetic engineering of sucrose accumulation in tomato: Cloning of the gene responsible for sucrose accumulation in tomato was initiated by identifying the biochemical basis of the trait (20,22,28). Analysis of a number of enzymes in tomato fruit indicated that the level of a single enzyme, invertase, was greatly reduced in sucrose-accumulating *L. chmielewskii* and in sucrose-accumulating backcross derivatives of this wild species (20). Because invertase catalyzes the breakdown of sucrose to fructose and glucose, the finding of low invertase levels was consistent with its conferring the trait of sucrose-accumulation. Antibodies and cDNA probes that specifically react with invertase protein and mRNA were

used to determine that the reduced invertase enzyme activity resulted from the absence of the invertase protein and mRNA in sucrose-accumulating fruit (20,28). This result strongly implicated the gene encoding invertase as being responsible for the trait of sucrose accumulation, with the *L. chmielewskii* invertase gene differing from the *L. esculentum* invertase gene by having no expression in fruit.

The identification of the invertase gene as the genetic determinant of sucrose accumulation suggested an alternative strategy to engineer this trait in transgenic plants by down-regulating the endogenous *L. esculentum* invertase gene. The down-regulation of endogenous genes in plants has been accomplished by the expression of antisense transgenes (12,13) or by the phenomenon of co-suppression where expression of sense transgenes also resulted in suppression of endogenous gene expression (29). We constructed chimeric antisense invertase genes for expression in tomato in order to test whether direct suppression of endogenous invertase gene expression would be sufficient to confer the trait of sucrose accumulation. In these experiments, a antisense gene was constructed regulated by a constitutive promoter (35S) The chimeric gene is comprised of approximately 2.0 kb of the invertase coding sequence (cDNA) and was cloned into an *Agrobacterium*-based transformation vector, pBIN19 (30). This chimeric gene and the vector have been sequenced in their entirety (31).

Expression of the chimeric antisense invertase gene was effective in reducing expression of the endogenous invertase gene and had no deleterious effects on plant growth and development when plants were grown either in the greenhouse or field. When sugar composition of transgenic fruit expressing the antisense gene was analyzed, several transgenic lines accumulated sucrose to levels comparable to *L. chmielewskii* (32). This confirmed that low invertase gene expression was sufficient to confer the trait of sucrose accumulation. Further analysis of these transgenic lines indicates that they also accumulate a higher concentration of total sugars and, although they exhibit reduced fruit size, they do not suffer from negative horticultural traits such as the low fertility observed in lines derived from crosses with *L. chmielewskii*.

GENETIC "CERTAINTY" AND SAFETY OF MODIFIED FOODS

Here we have described a single genetically-determined biochemical modification that alters the ratio of soluble sugars in tomato fruit to favor sucrose accumulation. The unique feature of this trait with respect to the assessment of the safety of genetically modified foods is that the same trait has been conferred by both classical plant breeding as well as by genetic engineering. Because the same trait was conferred in both cases (e.g. the product of each change was substantially identical), it is possible to focus on the genetic changes associated with the process conferring the phenotypic change.

In considering safety aspects of genetic modifications there are chiefly two concerns:

1. That the added gene products are themselves safe and do not confer toxicity or allergenicity to the modified food product. To assess the safety of the modified food product in this context, it is important to have a high degree of certainty of the identity and sequence of the added gene.
2. That the genetic modification did not produce unexpected results either by pleiotropy, where a gene of known function indirectly effects other plant traits, or by insertional mutagenesis, where the added gene may integrate at the site of an endogenous functional gene and disrupt its activity. Safety assessment in this context suggests that it would be important to

have a high degree of certainty of the chromosomal location of the introduced gene. Collectively, the certainty of genetic changes that are introduced when plant traits are modified by either plant breeding or genetic engineering can be characterized by knowledge of the introduced gene identity (sequence) and the chromosomal location of its insertion.

Using the best techniques available, the trait of sucrose accumulation was conferred by introgression of a relatively large chromosomal segment from *L. chmielewskii* or by the direct transfer of a very small antisense gene. In the case of the antisense gene, its sequence and the sequence of all of the transferred genes was precisely known and so can be readily evaluated for potentially toxic or allergenic gene products. In the case of the introgressed *L. chmielewskii* chromosome segment, the transferred genetic material is sufficient to encode several hundred genes. Because the sequence of this chromosome segment is not precisely known, it can not be readily evaluated for the potential introduction of toxic or allergenic gene products.

Conversely, the chromosomal location of the introgressed *L. chmielewskii* chromosome segment is precisely known because this chromosome segment always integrates at its native site on chromosome 3. In contrast, the antisense gene inserts randomly into the recipient genome and random insertion of a transgene into an active gene could potentially interrupt a critical biochemical or regulatory pathway and lead to unanticipated changes in the plant phenotype. Thus, the certainty of the chromosomal location of the introgressed chromosome segment is high, and that of the antisense gene is low. In each method of genetic modification; plant breeding which is characterized by the transfer of large undefined genomic segments at a precise chromosomal location or genetic engineering which is characterized by the introduction of precisely defined genetic elements at random sites; a degree of uncertainty over the genetic makeup of the resulting food product is introduced.

CONCLUSIONS

Crop improvement by an iterative process of evaluation and selection is the foundation of agriculture. This practice has been greatly advanced by the application of genetic principles and most recently by the use of DNA-based molecular markers that increase both the precision and speed of introgression of traits from related species. This practice of plant breeding is generally regarded as safe, and indeed we have thousands of years of experience with relatively few introductions of unsafe food as a result of plant breeding. Nevertheless, such examples of the introduction of unsafe foods do exist, most likely due to the introduction of undesirable genes linked to the introgressed genomic segments. The example of sucrose-accumulation in tomato provides a unique example where a single trait has been conferred by both classical and molecular genetic approaches. From this comparison, it was possible to evaluate the precision of the genetic change in relation to the size and chromosomal location of the introduced DNA. Both approaches introduce a degree of uncertainty in the final genetic makeup. Classical genetic introgression introduces uncertainty in the identity of the introgressed genomic segment and genetic engineering introduces uncertainty in the chromosomal location of the introduced gene. However, because the majority of plant genomes are comprised of non-coding DNA, the likelihood of random inactivation of a functional gene by integration of a transgene is low, whereas the introgression of uncharacterized genes by classical genetic methods is virtually assured. Thus, at a minimum, there appears to be no inherently greater risk associated with molecular genetic improvement of crops when compared to genetic improvement using classical genetic approaches. It is appropriate then that the safety of genetically engineered food products be evaluated on the

same terms as food products modified by traditional, and widely accepted, plant breeding approaches that achieve similar ends.

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ENHANCING COMPETITIVENESS THROUGH PLANT BIOTECHNOLOGY

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Economic viability and success are highly dependent on enhancing competitiveness. Likewise, biotechnology aims to make plants more competitive against pests, diseases, weeds, and their environment. Successful innovation is essential for competitiveness. Industry, academia and Government need to work in partnership to provide wealth creation and enhance our quality of life. Technology-transfer companies stimulate the number of academic industry links resulting in the commercialisation of science as well as increasing access to private and public sector resources, money, research and development.

Genetically modified crops of corn, soya, potatoes and cotton exhibiting superior insect resistance, herbicide tolerance and altered ripening characteristics are now available. The willingness of consumers to buy the products of biotechnology will be highly dependent on clearly demonstrable benefits to the consumer. Such benefits include taste, price, nutrition, convenience and safety.

INTRODUCTION

Competitiveness is central to many Government policies and competitive products or services should either be sufficiently low in price or high in quality to be successful against commercial rivals. The 1994 UK White Paper "Competitiveness: Helping Business to Win" (Cm 2563) was the first comprehensive Government audit of the UK's competitiveness position and identified encouraging signs of growing competitiveness despite a lower Gross Domestic Product (GDP) *per capita* than other major industrialised countries. According to the World Economic Forum's Global Competitive Report 1997, the UK is currently in seventh place worldwide below Singapore, Hong Kong, USA, Canada, New Zealand and Switzerland. The global economy is becoming more competitive.

Innovation is essentially the successful exploitation of new ideas and is an engine of change that generates competitive advantage. Innovation is not solely about research but is a combination of skills which also include management, marketing and finance. Innovation depends on investment by the private sector and individuals. It results in products which should not only meet consumer needs but also are of a lower cost. Science, engineering and technology (SET) profoundly affect every aspect of our lives and are fundamental to future prosperity and quality of life. SET are a driving force of change and develop at an ever- increasing rate. The social and economic implications of this are the main reasons why Governments and companies invest in SET. However, the costs of research are considerable and outcomes by nature uncertain. Private-sector companies have to justify their investment in research through greater profits while consumers require greater diversity of quality products at competitive prices. If the private-sector is to invest in research and generate profit from research then an adequate framework for protecting intellectual property (IP) is essential. Equally, the need for protection and subsequent exploitation of IP in Government-funded research organisations is increasing and a clear route to exploitation is a pre-requisite for all grant applications.

FORESIGHT

The UK Government published its first White Paper on SET for 25 years which was aptly entitled "Realising our Potential. A Strategy for Science, Engineering and Technology" (Cm 2250, May 1993). It was the product of wide-ranging consultations between the scientific and business communities. Firstly, the White Paper sought to protect and enhance the science base and, secondly, to harness SET in wealth creation and improving the quality of life. The importance of partnership and greater dissemination of ideas between Government, academic institutions, industry and the financial sector was clearly recognised not only to stimulate mutual understanding but also to

improve competitiveness and quality of life. Technology Foresight, an initiative of the White Paper, focused on gaining early notice of emerging key technologies and forging new partnerships and networks. Government also uses the findings of Foresight in decision-making and setting the future direction of science programmes. Sixteen sector panels (Chemicals; Construction; Health and Life Sciences; Energy; Financial Services; Transport; Agriculture, Horticulture and Forestry; Defence and Aerospace; Manufacturing, Production and Business Processes; Marine; Food and Drink; Materials; Retail and Distribution; Natural Resources and Environment; IT, Electronics and Communications; Leisure and Learning) have been created and their initial findings published. To date over £350 m has been committed to initiatives reflecting Foresight priorities. However, the long-term benefits of Foresight have yet to be clearly demonstrated and may require attitudinal and cultural changes. Clearly the remits of certain panels overlap, for example in respect of food that of Agriculture, Horticulture and Forestry are contiguous with Health and Life Sciences and with Food and Drink and other panels. This specific example has been addressed by the establishment of the Food Chain Group. Good communication between sector panels will be essential for the full potential of Foresight to be realised. Common recommendations of these panels have included the following:- the application of biotechnology to underpin the development of new agricultural products and processes; applying molecular biology to investigate key metabolic pathways and for the biological manufacture of industrial products; improved technology transfer and uptake of new ideas, particularly to small and medium-sized enterprises (SMEs); promoting public understanding of science and particularly the balance between risks and benefits in adopting new technologies; a better coordination and management of research.

In order to be competitive, understand and fulfill the demands of industry, Government-funded research institutes need to establish technology-transfer organisations effectively to identify and commercialise innovative research. The Scottish Crop Research Institute (SCRI), a non-departmental public body, recognised this requirement well before the publication of the Science White Paper and in 1989 established Mylnefield Research Services (MRS) Ltd as its commercial arm. SCRI is a major international centre for research on agricultural, horticultural and industrial crops. A broad multidisciplinary approach to R&D is a special strength of the Institute and the range of skills available is unique within the UK research service. MRS Ltd and SCRI are aware of the importance of effectively managing and exploiting intellectual property. Profit-making activities by affiliated commercial departments or companies will permit a continued commitment of resources into R&D.

THE NEED FOR NEW TECHNOLOGY

In spite of unfulfilled promises and a slower-than-expected realisation of potential, biotechnology and particularly recombinant DNA technology has matured into an important discipline which will underpin much of our biological and medical advances in the new millennium. It is expected that the world population will have doubled by 2020 to around 8,000 m human inhabitants and we will have to utilise fully this technology if we are to meet the challenge of providing sufficient food without further damage to the environment. Cereal demand is expected to increase by 55% and meat demand by 75%. Most of these increases will have to come from yield increases since the total agricultural land area under food cultivation is likely to remain the same or slightly decrease due to demands for urban and industrial building land, the production of plant fibres and other plant-based products such as oils and even pharmaceuticals. Technical factors such as improved varieties, sound management of agrochemicals and fertilizers, good soil stewardship and up-to-date agronomic practices will be pivotal in achieving food production increases. Timescales are short and the most efficient plant improvement technology will have to be deployed. However, such technical advances alone will not achieve these goals without appropriate political and economic climates and consumer acceptance of products. R&D are the only route to decouple economic and population growth from environmental degradation.

Over the past 40 years conventional plant breeding has more than doubled the yields of crops. The creation of new and improved cultivars by conventional techniques requires careful long-term planning. The creation of tomorrow's new improved varieties requires the best available technologies and multidisciplinary breeding teams with complementary expertise in genetics, molecular biology, pest and pathogen biology, *in vitro* cultivation, glasshouse and field cultivation, best agronomic practices and latest agro-industry technologies. Well-maintained and characterised germplasm collections are essential for successful breeding and habitat-reconstruction programmes.

It is estimated that there are several hundred public and private organisations whose mission is plant improvement with an annual budget of approximately $1 bn. To remain competitive, these organisations require: (i) mechanisms to protect IP; (ii) efficient national regulatory regimes that safeguard the environment and consumer without stifling innovation or international competitiveness.

INTELLECTUAL PROPERTY IN AGRICULTURE

Both the improvement of plants by conventional plant breeding and the development of relevant biotechnological knowledge, skills and techniques are costly and require long-term investment. Government support over the past decade has been limited. The production of new and improved plant cultivars is seen as "near market" and therefore not eligible for UK Government support. The traditional short-term funding cycle (3-5 years) and even shorter review cycles are unsuitable for proving the new technologies in a meaningful way. In the past, plant and yield improvements were financially supported by Government to meet a societal need. The responsibility has transferred to private enterprise which is investing in the development of new cultivars and technologies, and will require a recovery of development costs and a return of profit to its investors. It is imperative that intellectual property can be effectively protected to capture value of innovative products and processes.

Patents provide an exclusive right and monopoly to inventors for a finite period of time (20-25 years). In return for such a monopoly the inventor makes a complete disclosure of the technical invention to the State. The Patent Co-operation Treaty has been adopted by a large number of countries (currently 49) and simplifies the protection of an invention in a number of countries. In Europe, the European Patent Office (EPO) exists in parallel with national Patent Offices. The EPO provides an optimal route by which a single Application results in Patents being granted in up to 20 European countries. Two major differences that exist between patent regulations in the US and Europe are: (i) in the US it is the first to invent rather than the first to file which establishes the priority date for claims, and (ii) in the US a period of grace for up to 12 months is permitted between a disclosure and subsequent filing. Under the European system any public disclosure destroys the novelty of an invention. In these two aspects, the US System is patently better than the European System.

A state of confusion currently exists in Europe with respect to the legal protection of biotechnological inventions. An earlier proposal (COM(95) 661) was rejected and delayed by the European Parliament. However, an amended version is currently being considered by the Council of Ministers prior to a second reading by the European Parliament. The current uncertainties over the legal protection of biotechnological inventions are hampering investment and a harmonised directive is urgently required. The European Directive recommends that: (i) plant and animal varieties and (ii) essentially biological procedures (excluding microbiological) for the breeding of plants shall not be patentable, unless the application (practicability) of the invention is not technically confined to a particular plant or animal variety.

Under US Utility Patent Law, which is distinct from the Plant Variety Protection Act (PVPA), protection has been applied to living organisms. US case law determined that corn varieties with enhanced amino acid profiles were patentable under US Utility Patent Law. It was deemed that a significant human intervention had been required to generate the product.

Current patent applications include DNA markers for "marker-assisted breeding"; methods of plant transformation, engineered plants and seeds, genes and their products, gene promoters etc. Several dominant generic patents have been issued while others are still pending and are subject to challenge. The breadth of some granted claims causes concern especially in the absence of reduction to practice.

The 1978 and 1991 International Convention for the Protection of New Varieties of Plants (UPOV) provides the appropriate framework for the protection of plant varieties. On the one hand, it safeguards the legitimate interests of plant breeders while, on the other, it permits free access to genetic variability which facilitates the continued development of new varieties (known as "breeders exemption"). Resulting new varieties (unless dependent under UPOV 1991), which result from crosses using material protected by Plant Variety Rights (PVR), may be freely sold regardless of Rights in the original varieties. Nonetheless, a plant patent gives much wider protection than PVR. A patent prevents unauthorised use of the invention and exploitation requires a licence. In Europe, experimental acts are exempt whereas experimental exemption is not recognised in the US. Thus, US plant breeders who patent parent lines or hybrids are entitled to prevent others from using the material in other breeding programmes. In order to encourage research, particularly in the academic sector and Government institutes, it is imperative that experimental exemption is recognised. Plant breeding programmes require "breeders exemption" otherwise they will become very narrow and genetic diversity will be lost at worst or not be fully exploited at best.

In the US, the Plant Patent Act of 1930 offered plant breeders protection of asexually propagated crops which was extended under the Plant Variety Protection Act of 1970 to cover sexually propagated crops. An exclusion was the "soup vegetables" (carrots, celery, cucumber, okra, peppers and tomatoes) for fear of proprietary

protection inhibiting research. Soup vegetables were subsequently protected in a 1980 amendment.

NATIONAL REGISTRATION AND REGULATION OF BIOTECHNOLOGY

Regulation serves to assure food product safety, efficacy and consistency as well as safeguarding the environment. A comparison of national regulations raises questions of international competitiveness particularly between the US and Europe (Kok *et al.*, 1996; Kraus,1996). Additionally, over-regulating the biotechnology industry stifles innovation. Kraus (1996) concluded that the US plant biotechnology industry has been positively affected by domestic regulations and is at a competitive regulatory advantage compared with its European counterparts. Even so, the US industry has been negatively affected by European regulations and is hesitant to export and invest in Europe because of biotech-regulatory uncertainty. The major differences between Europe and the US relate to the regulatory structure and requirements, stringency, review periods, costs, guidelines etc.

In Europe, a GMO product cannot be placed on the market without the agreement of (a majority) of other Members States. Companies that want to conduct field trials in several EU countries have to apply for authorisation from regulatory agencies in every individual country. In certain countries public hearings are required (*e.g.* Germany), in others advance warning in the press must be given (*e.g.* UK), while in others permission of Parliament must be sought (*e.g.* Denmark). The cost of field trial permits vary greatly between countries (up to 18,000 Ecus) while in the US there are no such costs. The US Animal and Plant Health Inspection Services (APHIS) has streamlined its Genetically Modified Organism (GMO) permit process for importation, interstate movements and field/testing of certain genetically engineered crop plants such as corn, soybeans, cotton, potatoes, tomatoes, and tobacco. Instead of applying for permits, applicants can just notify APHIS that movement or a field test is planned. This notification process now accounts for approx 90% of all field trials in the US. Furthermore, a petition process facilitates the deregulation of plant cultivars. As of August 1997, 23 transgenic plants are no longer regulated and are allowed on the open market providing that they also comply with Environmental Protection Agency (EPA) and for Food and Drug Administration regulations. The crops deregulated include: one papaya line with virus resistance; five tomato lines with delayed ripening, one with insect resistance and four with hebicide tolerance; two soybean lines with herbicide tolerance; one rapeseed line with increased laurate content; two squash lines with disease resistance; two potato lines with increased insect resistance; and six corn lines, three with herbicide tolerance and three with insect resistance.

A new EU Regulation establishing a pre-market approval system for novel foods and novel food ingredients, including genetically modified organisms, came into force on 15 May 1997. The new procedures are similar to those currently operating in the UK. A safety assessment is conducted by an independent body, the Advisory Committee on Novel Foods and Processes, with input from other committees such as the Food Advisory Committee. The EU Regulation applies to foods and food ingredients which are intended to be placed in the EU market but which have not previously been consumed in significant quantities by humans in the EU.

PLANT BIOTECHNOLOGY

Biotechnology allows the precise manipulation of metabolic processes to achieve novel outcomes. Due to the strong similarities in the character of DNA found in diverse living organisms, a common technology has developed that can map, sequence, track, and manipulate microorganisms, plants and animals. The enabling technology of delivering genes to all major plant species has now been developed and is being applied to make plants more competitive by: conferring pest and disease resistance; improving quality and nutritional value; increasing shelf-life; synthesising novel products; *etc.* The development of varieties more resistant to pests and diseases will enable plants to realise their yield potential and decrease agrochemical inputs. Stable new varieties must have to be adapted to different agro-climatic conditions and the products from genetically modified plants must meet consumer needs.

The potential "agbiotech" market is huge, Ernst and Young (San Francisco) estimate that the world agricultural and biotechnological market could reach $46 bn by the year 2000. According to Arthur Andersen (Binding and Fairpo, 1997), in 1996 in the UK there were 219 biotech companies (31 "agbio", 76 "biopharm", 50 diagnostic and 62 suppliers) employing more than 10,500 staff with a revenue of £702 m and an R&D spend of £190 m. By the end of 1998 it is predicted that there will be 265 companies (45 "agbio", 125 "biopharm", 45 diagnostic and 50 suppliers) employing more than 13,750 staff with forecast revenues of the existing companies in excess of £1.5 bn and an R&D spend of £319 m. Agbiotech shows the greatest percentage growth in this time.

Table 1 lists genetically modified crops that are currently being commercialised, together with the companies currently developing them.

Table1	Commercialisation of Genetically Engineered Crops	
Crop	Developer	Status
Tomatoes with delayed ripening and higher solids content	Calgene/Monsanto Zeneca DNA Plant Technology	Introduced US 1994 Introduced UK 1995, US 1996 Introduced US 1996
Virus resistant squash	Asgrow	Introduced US 1995
Bromoxynil-tolerant cotton	Calgene and Rhône-Poulenc	Introduced US 1995
Glufosinate-tolerant canola	AgrEvo	Introduced Canada 1995
High-laurate canola	Calgene	Introduced US 1995
Insect-resistant cotton	Monsanto/Delta & Pine Land	Introduced US and Australia 1996
Insect-resistant maize	Mycogen, Novartis, Monsanto, DEKALB, others	Introduced US 1996 Introduced US 1997
Insect-resistant potatoes	Monsanto	Introduced US 1996
Glyphosate-tolerant soybeans	Monsanto	Introduced US 1996
Glufosinate-tolerant maize	AgrEvo, DEKALB, others	Introduced US 1997
Glyphosate-tolerant canola	Monsanto/Pioneer	Introduced Canada 1997

Fruits and vegetables are important targets for genetic modification since they are biologically programmed to rot as part of their seed dispersal mechanism and ripe fruits and vegetables make excellent hosts for pathogens. Unfortunately, these natural processes are in conflict with modern society's requirement for a constant supply of fruit and vegetables which can survive complex distribution around the globe. To follow are details of the application of plant biotechnology to three important crop types.

TOMATO

The common cultivated tomato (*Lycopersicon esculentum*) provides a major focus for the improvement of crop quality through genetic engineering. The identification of ripening-related cDNAs was pivotal in the genetic manipulation of fruit ripening. Gene-silencing technology has been used to modify the expression of ripening genes. Antisense RNA inteferes with the accumulation of mRNA from the homologous gene target and the amount of the corresponding protein is reduced. Additional copies of a sense gene can also lead to gene silencing (co-suppression); however, the precise mechanisms remain unclear. Furthermore, antisense technology has been used to identify cDNA clones and subsequently modify genes.

The first biotechnology product based on gene silencing was the "Flavr Savr" tomato marketed in the US during 1994 by Calgene (US). The target was polygalacturonase (PG) which is involved in pectin degradation. This and other pectolytic enzymes (*e.g.* pectinase and pectin methylesterase) cause fruit to soften during ripening and levels of these pectolytic enzymes have been reduced in transgenic tomato plants (see Picton *et al.*, 1995; Thakur *et al.*, 1996). Low PG has also been achieved by sense gene suppression and is the basis for the genetically modified tomato puree, developed by Zeneca Plant Science (UK). This tomato puree was the first genetically modified plant product marketed by UK supermarkets (Safeway and Sainsbury's). Tomatoes with reduced levels of PG are more robust and can be left to ripen longer on the vine, developing superior flavour with reduced risk of spoilage. When low PG is incorporated into processing varieties the product can be processed more efficiently with less waste. Currently, the GMO product is cheaper and outsells the conventional product by a ratio of 5:4 (Sainsbury's, personal communication).

The plant hormone ethylene (ethene) in climateric fruits (*e.g.* bananas, mango, melon, papaya, tomato) increases sharply at the onset of ripening, and is thought to trigger the changes in colour, texture and flavour that make fruit acceptable for eating thereby facilitating seed dispersal. Two small multigene families encoding the

enzymes 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase control the biosynthesis of ethylene and can be effectively downregulated. Another strategy to reduce ethylene production is the overexpression of foreign genes for S-adenosyl methionine hydrolase or ACC deaminase which deplete cells of the required precursors. These strategies have been developed by Agritope (US) and Monsanto (US).

The development of GMO tomatoes recently suffered a highly visible setback when Calgene and DNAP Holding Corp. (US) removed their MacGegor and Endless Summer tomatoes, repectively, from the market in 1996 for further refinement. In the case of DNAP, patent issues were the major reason whereas Calgene's MacGregor brand needed refinement in the choice of germplasm and consistency of flavour (Dutton, 1997).

SOFT FRUIT

SCRI has the greatest concentration of expertise in the world of scientific research on soft fruit and probably the largest soft-fruit breeding programme. The Institute is leading the field in the development of soft fruit with improved pest and disease resistance. We have, using an *Agrobacterium*-mediated transformation protocol, inserted into strawberry the Cowpea Protease Trypsin Inhibitor gene (CpTi) (Graham *et al.*, 1995). Purified CpTi incorporated into artificial diets has been shown to be anti-metabolic against a wide range of insects including *Heliothis, Spodoptera, Diabrotica* and *Tribolium* (Hilder *et al.*, 1987). Preliminary glasshouse trials indicated that the presence of this gene in transgenic strawberries greatly reduced the ability of vine weevil (*Otiorhynchus sulcatus*) larvae to digest their food. In our 1993 bioassay, the best transgenic line had a 362% increase in root weight compared with the non-CpTi-containing transgenic control. In the following year, these preliminary results were confirmed with a 240% increase in root weight over the appropriate control. The first European field trial of a genetically modified strawberry was initiated in 1996, and preliminary data collected in 1997 show a strong correlation between the activity of trypsin inhibitor extracted from field-grown transgenic strawberry plants and root biomass. Useful field levels of vine weevil control appear to have been achieved.

SCRI has isolated two polygalacturonase inhibitor protein (PGIP) genes from raspberry. One gene has been inserted in strawberry and raspberry as a strategy to control one of the most important soft fruit spoilage organisms *(Botrytis cinerea)* without the use of fungicides. The activity of the PGIP in extracts of raspberry flowers and fruits shows the greatest activity in immature fruits and decreases during fruit ripening as susceptibility to grey mould increases. Genes encoding PGIPs have been isolated from bean, soybean, pear, tomato and kiwifruit. Both pear and tomato PGIP genes have been used in attempts to improve tomato disease resistance. Transformation of tomatoes with the pear PGIP gene, under the control of the 35S promoter, lead to an 8-fold decrease in susceptibility to infection by *B. cinerea* (Powell *et al.*, 1994).

POTATO

The potato, as the fourth most important world food crop, continues to stimulate research activity in commercial and academic sectors alike. Genetic engineering of potato offers significant potential for improved pest and disease resistance and quality traits (see Davies, 1996).

The genetic engineering of potato for virus resistance represents one of the major success stories in plant biotechnology. New sources of antiviral transgenes which give resistance continue to be discovered. One of the best documented approaches is coat protein (CP)-mediated resistance, now widely effective against PVX, PVY, PLRV and mop top, though not apparently, for TRV (Barker *et al.*, 1993; Kavanagh & Spillane, 1995). There have been many large-scale field trials of transgenic potato with CP-mediated resistance which confirm the durability of the trait in true-to-type lines. Other approaches involve modifying the expression of viral movement proteins, viral proteases involved in processing polyprotein gene products, and viral RNA-dependent polymerase or 'replicase' proteins (Kavanagh & Spillane, 1995 and references therein). As far as plant-derived genes are concerned Lodge *et al.* (1993) successfully engineered potato to express high levels of pokeweed antiviral protein (PAV) which conferred resistance to PVX and PVY when mechanically inoculated. PAV is an example of a gene encoding antiviral proteins (ribosome inactivating proteins (RIPs)), which can modify ribosomal RNA and interfere with translation. Natural host resistance genes are being sought and the potato *Rx* gene is being isolated using MAP-based cloning strategies. The *Rx* gene confers extreme resistance or immunity to PVX. Once induced by the virus, the mechanism can suppress replication of completely unrelated viruses (Kohm *et al.*, 1993).

Bacteria and fungal pathogens have been controlled by expressing lytic enzymes derived from bacteria or insects, thereby reducing the severity of pathogens such as *Erwinia caratovora* and *Pseudomonas solanacearum* (Watanabe *et al.*, 1993). Resistance to *Erwinia carotovora* and *Phytophthora infestans* is also improved by

expressing an *Aspergillus niger* glucose oxidase gene to stimulate hydrogen peroxide production within the plant.

The classical example of improved insect resistance in transgenic potato is the expression of the *Bt* (*Bacillus thuringensis*) toxin gene to provide a high degree of protection against tuber moth and Colorado beetle. Transgenic resistance to potato cyst nematodes is another important target with a range of approaches currently in place including the expression of lectin genes (Concanavalin A), cytotoxic genes and enzyme inhibitors. Results from glassshouse and field trials are expected within the next one to three years.

Nutritionally, potato tuber protein suffers from deficiencies in the sulphur amino acids methionine and cysteine. Preliminary SCRI data indicate that expression of the gene encoding the 2S (*BN2S*) protein from Brazil nut, which has a 19 % methionine and 18% cysteine content, in microtubers results in a 2-3-fold increase in the mole % contribution of methionine to the protein amino acid balance (Randhawa and Machray, personal communication).

Blackspot bruising occurs when physical damage to the tuber initiates enzymic browning, catalysed by polyphenol oxidase (PPO) and results in the production of black, brown and red pigments. PPO activity has been reduced by gene silencing in potato by Keygene and other groups in the USA and elsewhere; published data indicate a high degree of success in elimination of visible bruising (Bachem *et al.*, 1994; De Both *et al.*, 1996).

Starch is the primary storage compound in tubers, accounting for up to 70% of tuber dry matter. Starchy foods are the world's most abundant staples and the most important source of calories in the animal and human diet. High-amylopectin-starch potato transgenics have been produced by antisense-silencing of the granule-bound starch synthese (GBSS1) gene (Visser et al., 1991; Müller-Röber & Kossmann, 1994). The transgenics contained almost pure amylopectin which can be used directly in foods as thickening agents.

Stark *et al.* (1992) at Monsanto produced transgenic tubers of Russet Burbank with a starch content elevated by up to 60% (35% average) through over-expressing an *E. coli* glgC 16 gene encoding the bacterial ADPglucose pyrophosphorylase. Research at SCRI has shown that these tubers also store well as far as processing quality is concerned which is relevant to the French fry and crisping industries. The increase in tuber starch and dry matter content reduces oil uptake, minimising wastage.

The SCRI is also engaged in a major programme to deliver transgenic lines which will process well out of low-temperature storage. The current practice is to store tubers at *ca.* 10°C with a chemical sprout inhibitor to prevent starch breakdown and the development of a high reducing sugar content. High reducing sugar levels result in dark-coloured and unpalatable crisps and French fries. A major goal is to store tubers at temperatures around 3 to 5° C to eliminate the use of chemical sprout inhibitors. However, tubers still accumulate high reducing sugar levels at such temperatures (the low-temperature sweetening phenomenon). SCRI, in collaboration with the European Chip and Snack Association is currently testing the effectiveness of 13 genetic constructs to modify carbon metabolism and minimise low-temperature sweetening. Promising lines are already emerging.

CONSUMER ACCEPTANCE

Despite the early US influence on the development of biotechnological food products, the production, processing, and distribution of these products is an international business. Therefore, to be commercially successful the products of biotechnology must be acceptable internationally to consumers, industries and Governments. The consumer needs to be well informed and have a better appreciation of potential risks associated with genetic engineering. For instance, is it better to either continue using toxic and potentialy environmentally damaging agrochemicals to control pests and diseases or to use plants that have been genetically engineered for pest and disease resistance that require lower agrochemical inputs? We must provide scientific facts to the consumer and counter the high-profile activist groups that rely on fear, scare-mongering, unrealistic"what-if" scenarios and impossible demands to prove an absolute negative (i.e. nothing **ever** goes wrong). Historically, between two-thirds and three-quarters of US consumers support biotechnology and are willing to accept biotechnological food products. Outside the US, acceptance of biotechnology varies widely, with certain countries (Germany and Austria) clearly opposed to plant biotechnology (Hoban, 1997). Consumers' lack of acceptance of biotechnology may be most strongly correlated with the efforts of activist groups which are most vigorous in countries such as Germany and Austria. Interestingly, these countries also have the greatest awareness of biotechnology. In Europe, consumers perceive the following risks to be more serious than genetically engineering plants:- bacterial contamination, pesticide residues, antibiotic hormones, mould, product alteration, food irradiation and expired "sell-by date".

Consumers require both benefits and choice. Above all, the benefit has to be to the consumer rather than a farmer, perhaps in a distant country, that may more liberally apply a particular herbicide. Benefits could include taste, price, nutrition, convenience, safety and environmental cost of producing a product. With respect to soya, retailers may not be able to offer choice to consumers because at this juncture genetically modified soya has not been segregated from "normal" soya. If commodity crops could be harvested and stored separately there remain substantial difficulties in maintaining separation throughout primary and secondary processing, food manufacture and distribution. As from 31 July 1997, the new EU legislation made it compulsory for products containing genetically modified organisms to be labelled as such. Manufacturers who have already launched products in the market will be encouraged to follow this legislation voluntarily. The final consumer of the product must be informed of the composition, nutritional value or nutritional effects and intended use of the food which makes it different from an existing food or food ingredient. The characteristics or properties modified, including the method by which it was obtained, must also be shown in a non-pejorative way. Further measures are expected to be introduced as the revision of EU labelling legislation is completed.

CONCLUSIONS

Regardless of the actions of the various non-governmental organisations and individuals to impede biotechnology, the inperative to feed an extra 2 billion mouths before 2025 will create the demand for the benefits of plant biotechnology. Conventional breeding programmes now depend on biotechnology-derived crossing and selection systems. Biotechnology is the underpinning system to monitor and reconstruct biodiversity and habitats. The market-place will ensure that plant biotechnology enhances competitiveness in the western world. However, we have grave concerns about the exploitation of the technology in less developed countries.

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BIOTECHNOLOGIES: NEW OPPORTUNITIES FOR THE FOOD INDUSTRY

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WHAT IS FOOD BIOTECHNOLOGY?

Food biotechnology employs the tools of modern genetic in the age-old process of improving plants, animals and microorganisms for food production. Biotechnology is becoming an important part of the overall effort to produce an abundant supply of better tasting and more nutritious foods. For thousands of years, humans have been selecting, sowing, harvesting and breeding plants and seeds that produce food products that will sustain them. They also have been baking bread, brewing beer and making wine and cheese. Modern biotechnology allows food producers to do the same thing today, but with greater understanding and selectivity, because birds and bees have their limits!

Traditional methods to modify food crops have serious limitations. First, sexual crosses can only occur in the same or nearly related species. That greatly limits the genetic resources breeders can draw upon in enhancing desirable characteristics of plants. Second, when two plants are crossed, each having some 100,000 genes or so, all the genes from both plants get jumbled together. Because of this, traditional crop breeders must spend years "back-crossing" the jumbled up plant with the plant they started with, again and again, to slowly breed out the tens of thousands of unwanted genes. Traditional breeding takes a long time, typically 10 to 12 years.

The real solution is biotechnology. Instead of mixing hundreds of thousands of genes to improve a crop plant, modern crop breeders can select a specific trait and move the needed genetic information for it into the genetic code of another plant. This is the basis of modern biotechnology.

Simple plant breeding – Some examples:

Three notable achievements in plant breeding have been the development of low-erucic-acid rapeseed cultivars

(canola, Daun 1986); production of high-oleic-acid sunflower oil (Fick, 1983); and conversion of linseed oil from an industrial to an edible oil (Green, 1986). These modifications cannot be achieved by hydrogenation, which can only convert unsaturated to saturated. Another important advantage is that, unlike hydrogenation, breeding does not lead to formation of TRANS fatty acids, but in some crops there are limitations to the amount of modification that can be done using naturally occurring variation, or hybrid lines remain nonviable. In these cases, to induce changes in fatty acids, breeders have to consider using:

- **mutagenesis** (e.g. with mutagenous materials or with gamma rays and then analyzing a large number of offspring seeds from treated parents for fatty acid profile);
  or:
- **Biotechnological Approach.**

Biotechnology includes such techniques as recombinant DNA, gene transfer, tissue culture, and plant regeneration. Recombinant DNA technology is a process in which a piece of DNA is cleaved, altered, and inserted into a self-replicating genetic element. Gene transfer is a technique that transfers a piece of foreign DNA into a cell so that it become a permanent part of the genome. Tissue culture and plant regeneration involve in-vitro culturing of protoplasts isolated from plant tissues.

Biotechnology is Selective and precise. The same can be said for possible benefits in other areas of food production, such as brewing, baking and cheese making. Biotechnology may help improve products vital to food production, such as enzymes, proteins and vitamins.

For example, an enzyme used to make cheese, called rennet, is taken from the lining of calves' stomachs after slaughter. Biotechnology enabled researchers to copy the specific gene that produces rennet and reproduce it in bacteria. This allowed the production of rennet by bacteria, a process known as fermentation. Now, nearly 50 percent of rennet is produced by fermentation.

WHAT BIOTECHNOLOGY CAN DO

- ***Nutrition, Health and Food Safety***

A combination of biotechnology and rapid plant breeding techniques overcomes specific plant diseases that reduce crop production, and is now able to modify certain components (starch, proteins and oils) to provide nutritional values that are not naturally available.

Fruits can be developed with additional vitamin A and C, or more or less sucrose, or more or less solids.

Vegetables can contain higher levels of antioxidant vitamins or solids or specified proteins. So biotechnology is transforming plants into the pharmaceutical manufacturer of the future, as well as the producer of materials for medical and functional food.

Another example can be eliminating allergy-causing proteins from foods. Research to reduce the allergenic proteins in rice will help Asian nations, which have high rates of rice allergy.

What some of these advances really mean:

Fruits, potatoes and vegetables – The interest in producing vegetables and fruits that are easier to prepare and hold has increased. Seed companies have begun to produce vegetables with higher solids content, making them useful for minimally processed products. Some work has also already been done on potatoes, celery and carrots with higher solids levels.

Potatoes with higher solids levels for use in pre-cuts for French fries have been developed. A higher starch potato will mean fries and chips made from these potatoes will absorb less oil when fried.

Then, understanding of the structure of the gene that triggers production of sucrose synthase began to suggest to researchers that this enzyme might be useful in changing the plant structure. Sucrose synthase, in addition to increasing the amount of reducing sugar available from starch in plants, seeds, and tubers, has interesting effects on other structures. According to a recently published international patent application, researchers at Monsanto Co. found that potatoes with increased sucrose synthase had better cold tolerance and stored better without bruising.

Another example: Better-tasting tomatoes year round. Because the fruit softens more slowly, due to the lack of the pectinase enzyme, tomatoes bred through biotechnology can stay on vines longer before shipping to market, thereby gaining added flavor and color. This also will be possible with other fruits such as peaches, bananas and strawberries.

Dairy Products & Proteins – Genetic elements have been found that produce, directly in milk, those proteic components, such as human lactalbumin and lactoferrin, that make cow's milk more like human milk, improving immune system function. Work on transgenic mice, cows, and goats is beginning to look promising as a way around the extremely high price tag attached to conventional ways of obtaining these proteins from human milk.

Another possible way, on which some companies are working, is to insert the right gene into a microorganism, e.g. moulds or bacteria, to obtain from them the production of these human proteins. While infant formula are the obvious use of these proteins, products for the growing numbers of immune compromised persons are expected to become commercially important.

Fats and Oils – Biotechnology has been extremely active in developing plant, animal, and microbial sources of special fats. Not only are conventional oilseeds such as corn, soy, canola, and sunflower being rearranged to produce specific fatty acid ratios, but radically new approaches to fatty acid arrangements, that are not normally produced in plants but may be helpful in diets, may be provided.

Enhancing vegetable oil quality

Vegetable oils continue to be one of the world's most important plant commodities, with current annual production in the region of 65 million tons. Food technologists are searching new processing methods to enhance quality of

the existing fats and oils, by the methods of hydrogenation, interesterification and fractionation. Plant breeders are working hard to produce new vegetable oils with altered fatty acid composition: the tools used by them have been selections, crossing, and mutation.

Why this need for oil quality enhancement ? See the following three aspects:

- Evolution in Fat Nutrition. Example: A concern has been the stereo configuration of an individual fatty acid, such as trans fatty acids, which are formed mainly during hydrogenation of linoleic and linolenic acids.
- Functionality and Oxidative Stability. Functionality refers to such physical properties as melting points and behaviors of oils. It is determined mainly by the chain length of fatty acid components, and the degree of unsaturation, stereo configuration, and glyceride structure. Oxidative stability refers to resistance of an oil to oxidation during storage and processing.
- Industrial Demands for Unusual Fatty Acids. The majority of industrial uses require unconventional fatty acids, such as short- or medium- or very-long chain fatty acids, or those having double bonds at an unusual position. Oils with such fatty acids often sell at prices considerably higher than common oils. Therefore, introduction of unconventional fatty acids into common oilseed crops should reduce the cost of these materials.

General effort is being made to increase value of an oilseed crop by producing a specialty oil in which a particular fatty acid predominates or diminishes.

For example:

- a low-saturated oil is aimed at meeting consumers' dietary needs;
- a low-linolenic oil is aimed at increasing oil oxidative stability;
- a high-oleic (lowsaturate and low-linolenic) oil is aimed at improving both health image and oxidative stability;
- a high-stearate oil is aimed at replacing hydro-genated oils for production of margarine.

A second effort is being made to introduce some unconventional fatty acids into common oilseed crops to meet specific nonfood demands.

For example:

- an oil with high lauric acid content will meet demands of the cosmetic industry;
- an oil with a high erucic acid content serves as liquid wax demanded by the chemical industry;
- an oil with high petroselinic acid, isomer of oleic acid in which the double bond is inserted in 6 rather than the 9 position, can be converted by oxidative ozonolysis into lauric acid and adipic acid, both industrially valuable materials.

Gamma-linolenic acid (GLA), a precursor for arachidonic acid, is a precursor for prostaglandin production in humans. It is usually produced by metabolism of linoleic acid; reduced consumption of linoleic acid in low-fat or fat free foods reduces the production of GLA in humans.

There is now the possibility of producing GLA in oilseed plants that normally don't produce GLA, by inserting them of an isolated gene for delta-6-desaturase, which produces GLA in some others plants. These oilseed plants

will have the added advantage of chill resistance, conferred by the same gene, giving them better long-season tolerance. Chill resistance is thought to be a function of phase transitions of lipids in cell membranes, so increasing the degree of unsaturation of the lipids may induce or improve resistance to low growing temperatures.

- ***Environmental Protection***

Biotechnology, combined with appropriate plant breeding, further improves crop yields and extends growing areas by modifying plants to resist drought and pollution better.

Gene selection is also able to improve a plant's ability to thrive on lower-quality soils.

Some examples:

- Resistant crops to ward off destructive insects and diseases, reducing the need to use pesticides.
- Herbicide, insecticide and fungicide tolerant crops, that allows more selective application of agricultural chemicals.
- Reduced Fertilizer Use: modified plants to draw nitrogen from soil and air, reducing the need for fertilizer.
- Plants enhanced to withstand low-temperatures by modifying their production of linoleic acid.

NEW EXPECTED BENEFITS BY YEAR 2000

Biotechnology will positively influence world food production, by:

- Improved quality of seed grains
- Increased levels of proteins in forage crops
- Drought and flood tolerance
- Salt and metals tolerance
- Heat and cold tolerance

Along with additional food, this could provide greater productivity and self-reliance to economies of developing nations.

CONSUMER INFORMATION

Consumers are often interested in knowing more about where their food comes from and how it is produced. While the product label cannot provide all the answers, there should be are additional sources of information available: consumers should talk to grocery store managers and food companies. Other resources could include community health professionals, dietitians, public institutes and media.

CONCERNS

Genetic manipulation / genetic engineering / genetic modification methodologies have brought not only advances, but also increasing concerns about the safety of such an approach to the world's food problems.

Although the scientific community takes every possible factor into account when using genetic manipulation techniques for the creation of new "improved" crops and animals, it is inevitable that public concerns will receive high media attention.

Moral and ethical concerns focus on the transfer of "animal" DNA to "plants" or between animal species, and environmental contamination issues. Attitudes to genetic modification obviously vary and public concerns inevitably prevail in the developed rather than the developing world.

Example:

The most common genetically modified products used in the food industry is microbial chymosin, used as a rennet substitute and particularly useful for the manufacture of vegetarian cheeses. However, this recombinant enzyme is not completely accepted in Europe, and manufacturers have to be aware of the differing consumer attitudes. In particular, in Germany, consumer groups and environmentalists have campaigned strongly against the introduction of genetically modified foods and, at present, cheeses manufactured with microbial chymosin are not sold in Germany.

Consumer concerns will impact on the market for these foods, and the successful products will be ones which are produced by manufacturers who are sympathetic and sensitive to the feelings and beliefs of their customers.

LABELING

The need to provide information to consumers about these foods becomes an increasingly important issue with obvious concerns about the labeling of these products. But labeling foods as genetically modified does not necessarily mean that consumers will not buy these products. Surveys carried out prior to the introduction on UK market of a genetically modified tomato puree, showed that 90% of people questioned would not accept genetically modified foods; however, when the product was introduced, it took 60% of the tomato puree market and increased overall sales in this area.

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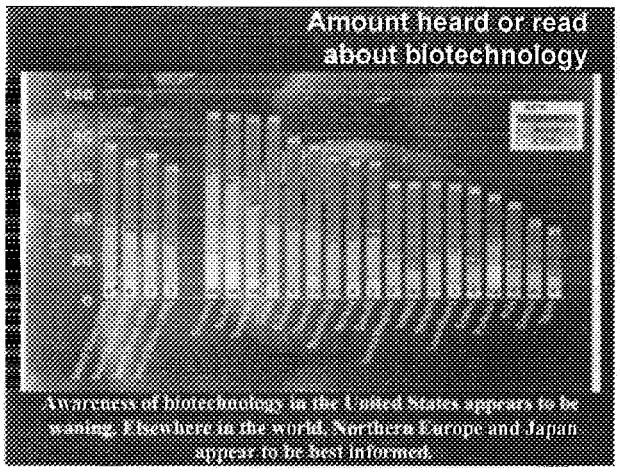

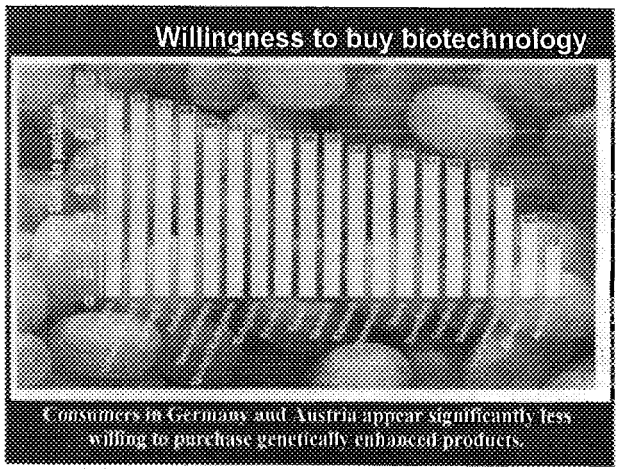

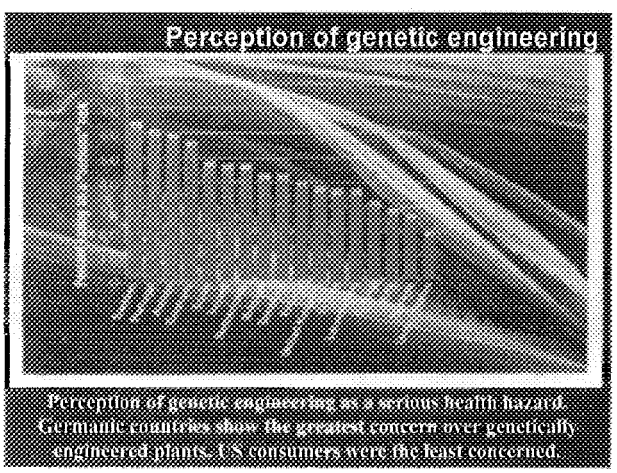

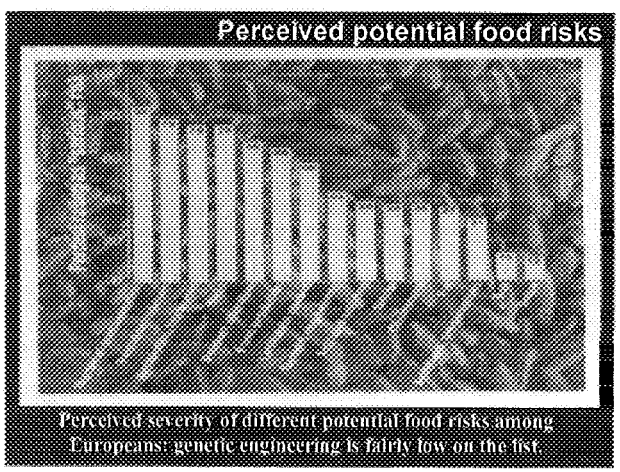

Table 1: Examples of genetically modified products which have (or have applied for) regulatory approval

Country	*Crop*	*Trait*	*Company*
USA	Tomato	Slow ripening (Flavr Savr™)	Calgene Inc
		Delayed ripening	Monsanto Inc.
		Delayed ripening	DNA Plant Technology Inc.
		Delayed ripening	Zeneca
	Maize	Glufosinate tolerant	AgroEvo
		Insect resistant	Ciba-Geigy corp.
		Insect resistant	Mycogen
		Insect resistant	Sandoz
	Cotton	Bromoxynil tolerant	Calgene Inc.
		Insect resistant	Monsanto Inc.
	Soybean	Glyphosate tolerant	Monsanto Inc.
	Squash	Virus resistant	Asgrow Seed Co.
	Oilseed rape	Modified oils	Calgene Inc.
		Glufosinate tolerant	AgrEvo
	Potato	Insect resistant	Monsanto Inc.
	Maize	Hybrid system/glufosinate tolerant	Plant Genetic Systems
		Insect Resistance	Monsanto Inc.
	Cotton	Glyphosate tolerant	Monsanto Inc.
JAPAN	Canola	Glufosinate tolerant	AgroEvo
		Glufosinate tolerant	Plant Genetics Systems
		Glyphosate tolerant	Monsanto
	Soybean	Glyphosate tolerant	Monsanto
	Potato	Insect resistance	Monsanto
	Maize	Insect resistance	Sandoz
		Insect resistance	Ciba Geigy
CANADA	Oilseed	Hybrid system/glufosinate tolerant	Plant Genetic Systems
		Glyphosate tolerant	Monsanto
		Glufosinate tolerant	AgroEvo
	Flax	Sulphonylurea tolerant	University Saskatchewan
	Tomato	Ripening delayed	Calgene

Adapted from the Bulletin of the Biotechnology Information Club, Issue number 4, Campden and Chorleywood Food Research Association, Chipping Campden, Gloucestershire, UK

Table 2: UK approvals of genetically manipulated food products at a glance

PRODUCT / FEATURE		
Bakers' yeast Saccharomyces cerevisiae faster liberation of Co2	*Gist Brocades*	*March 1990*
Bovine chymosin from genetically modified Kluyveromyces lactis (yeast)	*Gist Brocades*	*January 1991*
Bovine chymosin from genetically modified Aspergillus niger var. awamori (filamentous fungus).	*Genecor*	*May 1991*
Bovine chymosin from genetically modified Escherichia coli K12 (bacterium)	*Pfizer*	*March 1992*
Brewers' yeast Saccharomyces cerevisiae. Ability to break down dextrins/starch.	*BRF International*	*February 1994*
Tomato paste products from genetically modified tomato. Thicker tomato pastes.	*Zeneca*	*February 1995*
Processed material from genetically modified soybean. Herbicide (glyphosate tolerant)	*Monsanto*	*February 1995*
Oil from genetically modified oilseed rape. Male sterile/fertility restorer and herbicide (glufosinate) tolerant.	*Plant Genetics Systems*	*February 1995*
Oil from genetically modified oilseed rape. Herbicide (glufosinate) tolerant.	*AgrEvo*	*May 1995*
Oil from genetically modified oilseed rape. Male sterile/fertility restorer and herbicide (glufosinate) tolerant.	*Plant Genetics Systems*	*September 1995*
Oil from oilseed rape. Herbicide (glyphosate) tolerant.	*Monsanto*	*January 1996*
Tomato paste from genetically modified tomato. Thicker tomato pastes.	*Zeneca*	*February 1996*
Fresh Tomato (Flavr Savr) Improved flavour	*Calgene Inc.*	*February 1996*
Processed maize products from genetically modified maize. Insect resistant and glufosinate tolerant.	*Ciba-Geigy Ltd.*	*May 1996*
Hemicellulsase from genetically modified Aspergillus niger var. awamori (filamentous fungus).	*Ouest International*	*1996*
Hemicellulsase from genetically modified Bacillus subtilis (bacterium)	*Rohm GmbH*	*1996*
Riboflavin from genetically modified Bacillus subtilis using fermentation technology	*Hoffman La-Roche*	*January 1997*
Oil from genetically modified cottonseed. Herbicide (bromorynil) tolerant.	*Calgene Inc.*	*January 1997*
Processed maize products and unprocessed animal feed from genetically modified maize. Insect resistant.	*Pioneer Hi-Bred Int'L*	*February 1997*
Processed maize products and unprocessed animal feed from genetically modified maize. Insect resistant.	*Monsanto*	*February 1997*
Processed maize products and unprocessed animal feed from genetically modified maize. Herbicide (glufosinate ammonium) tolerant.	*AgrEvo*	*February 1997*
Processed maize products and unprocessed animal feed from genetically modified maize. Herbicide (glufosinate ammonium) tolerant and insect resistant.	*Northrup King Co.*	*February 1997*

From the Biotechnology Information Transfer Club, Campden & Chorleywood Food Research Association, Chipping Campden. Gloucestershire, UK

Table 3: Traits engineered into genetically modified plants (international data).

TRAIT	Potato	Oilseed rape	Sugarbeet	Corn	Tomatoes	Soybeans
Herbicide tolerance	O	O	O	O	O	O
Quality improvement	O	O		O	O	O
Virus resistance	O	O	O	O	O	
Insect resistance	O	O		O	O	
Marked genes	O	O		O	O	
Fungal resistance	O	O		O		O
Multiple traits	O	O				
Bacterial resistance	O	O				
Unspecified	O	O		O	O	

Adapted from Bio/Technology 13 (5) 454-458 (1995) (Table 3) and 11 (12) 1524-1524 (1993) (Table 4)

Table 4 : Willingness to purchase genetically engineered products. A survey of residents of New Jersey USA.

Commodity	Very	Somewhat	Not very	Not at all	Do not buy	Do not Know
Apples	16	38	14	29	-	3
Corn	17	39	11	31	-	2
Tomatoes	17	39	12	29	-	3
Milk	13	25	16	44	-	2
Beef	12	25	20	41	-	2
Baby Foods	9	20	13	51	5	3

Figures represent percentages.

Adapted from Fig 3 Bio/Technology 14 (1) 35 38 (1996)

FRUIT AND VEGETABLE FOODS OF THE FUTURE - IDENTIFYING THE MOST IMPORTANT TARGETS FOR GENETIC ENGINEERING

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Genetic engineering offers the possibility of modifying plants and the foods derived from them in exciting and useful new ways. This paper examines the possibility of altering non-food characteristics of plants in ways that will improve the utility of the foods derived from the modified plants for processors and the food quality that consumers desire. An important message is that research aimed at better understanding of plant metabolism and the ways that plants develop and respond to their environments is needed in order to identify reasonable targets for genetic engineering efforts.

Advances in our understanding of the ways in which genes direct the production of proteins and, hence, the characteristics of living cells have brought us to the threshold of an exciting and challenging era. "Genetic engineers" can alter the complement of genes in a variety of organisms and thereby cause significant changes. These changes will certainly have an impact on the fruit and vegetable foods we will eat in the 21st Century .

BEING A FOOD FOR HUMANS IS NOT WHAT THE PLANT HAS "IN MIND"

In other presentations in this Congress we have been told of a number of food improvements that have already been made by genetic engineers or are under investigation (see papers by Bennett, Harlander, Kerby and Pagella). It is important to recognize that the most important contributions to the food industry that geneticists involved in crop improvement might make may not be specifically connected to the organoleptic quality of a food or to its processing characteristics.

Furthermore, because biologists still have a great deal to learn about the overall integration of plant metabolism and plant development, it is important to be aware that a change which specifically improves an aspect of the food quality of a fruit or vegetable may be linked to unacceptable changes in other aspects of the plant's performance. For example, cassava is a staple food in the diets of many millions of people. Cassava cells contain hydrolytic enzymes and compounds, called cyanogenic glycosides, which, if mixed (for instance, when cells are broken during chewing), will produce toxic cyanide. Therefore the root is generally ground and fermented prior to being eaten so that the cyanide has time to form and be dissipated so that the cassava can be eaten safely. It is conceivable that either the glycosides or the hydrolytic enzymes could be altered by a

genetic engineering approach that would eliminate the formation of cyanide and improve the "food quality" of the root. While this would make cassava that is more readily eaten it would also eliminate an important aspect of the plant's biochemical defenses against insects. In an environment with a great deal of pressure from feeding insects, the "improved" cassava plants might not survive. We must be aware of the connections between a fruit or vegetable's food quality (which is based on our, non-plant perspective) and aspects of a plant's structure or chemistry which are needed for its survival. of This connection between cassava's food quality and its overall performance is not subtle. The plant is what it is because of eons of evolutionary pressures; it is a food only because of the more recent intervention of human beings.

In this short paper I would like to make a brief survey of some of the genetic engineering targets with which I am most familiar. My intent is to point out some of the characteristics of a plant's development and its responses to its environment that are strongly-associated with its value as a food. After all, the progress of a fruit or vegetable from the field to the dining table is long and involved. If processors are to be in a position to take advantage of "improved" foods (that is, make a profit from them) then they will depend on those who grow, harvest, and store these foods and ship them to the processing plant. These people will also be driven by a profit motive.

TAKING ADVANTAGE OF "NATURAL" PLANT DEFENSES AGAINST PESTS

Losses of crops to plant pests are a very important factor that reduces the harvestable amount and quality of a crop, from the time of seed germination, through the growth of the plant, its harvest and postharvest handling. This is true in agriculture worldwide. The losses are often much greater in developing countries because the widespread use of chemical pesticides limits pest damage in the more developed countries where chemical costs are an important part of the food cost. However, perhaps because we in the developed countries often do not have to worry about food shortages, we are increasingly concerned about the impacts agricultural chemicals have on our health and the environment. This has led to increasing government regulation of chemical use and quarantine requirements that limit shipping of many commodities across national borders (see the paper by Mitcham). These pressures have brought a substantial effort aimed at understanding the natural defenses of plants against pests so that they can be exploited using genetic engineering techniques. Successes in this area will affect several aspects of food production, from growers whose needs for chemicals will decrease to producers who can boast of chemical-free fruits and vegetables with "enhanced" natural protection.

A bacterial gene may be useful in protecting fruits and vegetables against insect damage The genes of the bacterium *Bacillus thuringiensis* (Bt) direct the production of a crystalline protein inclusion which is toxic to the larvae of certain Lepidopteran insects (moths and butterflies) which ingest it[1]. Tests of this Bt toxin have indicated that it is not harmful to humans and so it has been used as a natural insecticide. Genetic engineers have now isolated the Bt genes which code for the Bt toxin and used a number of biochemical "tricks" to get crop plants to produce this natural insecticide for themselves. Many crop plants (including tomato, tobacco and cotton) have been transformed in this way. This includes work at the University of California which has

introduced the toxin encoding genes into walnuts, apples and persimmons. Walnuts[2] and persimmons[3] which produce the Bt toxin are better able to resist feeding by larvae of the codling moth (*Cydia pomonella*) This work has important implications not only for the farmers who grow these crops but also for those who store, ship and market them. This sort of genetic engineering may reduce the use of potentially harmful chemicals on many of the plant foods we eat. The possibilities are very intriguing. *Would you eat an apple that expresses a bacterial gene which causes it to make a toxic substance?*

The mysteries of plant defenses against diseases Diseases caused by bacteria and fungi cause substantial damage to food crops and a great deal of work has been done to identify genes that allow plants to resist infection. Work of the last 10 years, much of it making use of molecular biology techniques, has identified an assortment of genes which may be responsible for natural resistance to pathogens. In some cases the protein products of these genes have been identified and in other cases they have not[4].

The engineering of improved natural defenses against disease organisms requires a great deal of research information. Polygalacturonase (PG) is an enzyme that hydrolyzes the pectin polysaccharides in plant cell walls. Many pathogenic fungi produce PG in order to help them break through the cell wall defense barrier and establish infection. Many plant tissues contain glycoproteins (PGIPs) which inhibit pathogen PG action. My colleagues and I have spent several years trying to determine if PGIPs play a role in protecting fruits against fungal diseases. We have cloned the gene that codes for PGIP in pear fruits and have introduced it into tomatoes so that they produce a great deal of the pear PGIP[5]. We then tested whether the transformed tomatoes were better able to resist infection by *Botrytis cinerea*, the fungus that causes grey mold on fruits. In some tests the pear PGIP appeared to contribute to an improved resistance; in other cases the transformed tomatoes fared no better than their non-transformed counterparts. We are currently trying to understand how the pear PGIP gene functions when it is expressed in tomatoes in an effort to determine what this protein's role in defense is. We are now working to modify strawberries so that they also express the pear PGIP at a high level. We will then determine the impact of this transformation on strawberry resistance to fungal disease. *Would you eat a tomato or strawberry that contained an inhibitory protein from pears?*

Our experience illustrates an important fact. We often do not know enough about the complex plant functions we wish to modify to make genetic engineering efficient. More research into developmental plant physiology and biochemistry is necessary if genetic engineering is to be used to address many of the plant food "problems" that need solutions. Resistance of plants to disease is often the result of the combined action of several individual defense factors (each controlled through the action of one or more genes). Under one set of disease conditions one of these defense factors (perhaps PGIP) may be of over-riding importance, but under another set of conditions that same factor may have little impact on resistance. Many other aspects of plant performance are just as complex. We must understand this complexity in order to make the best choices of targets for genetic engineering.

MANY IMPORTANT ASPECTS OF PLANT DEVELOPMENT ARE CONTROLLED BY A GAS

Plants control their life processes with an assortment of natural chemicals. Many of these are of such importance that they have been identified as hormones. Among the important plant hormones is the gaseous two-carbon, unsaturated hydrocarbon, ethylene. Ethylene is produced in plants via a cyclic biosynthetic pathway that has the net effect of converting two of the carbons of the sulfur-containing amino acid methionine into the volatile ethylene molecule. The conversion requires three enzyme-catalyzed steps and the identity of each of the enzymes is known[6]. The important elements of the ethylene biosynthetic pathway are illustrated below.

Methionine + ATP —> S-adenosyl methionine(SAM) —>
1-aminocyclopropane-1-carboxylic acid (ACC) —> Ethylene

Ethylene is very important in the control of many aspects of development in many fruit crops[7]. The ethylene that plants produce stimulates fruit abscission (i.e., the separation of the fruit from the parent plant [called abscission] - a developmental process that is crucial for proper harvesting). For many fruits (e.g., apple, tomato, pear *et al.*) ethylene also stimulates fruit ripening - the process that includes changes in many metabolic pathways that together convert fruits into the tasty and nutritious foods that we all enjoy.

Ethylene is also a controlling factor in some changes that detract from the quality of fruits and vegetables. These changes include the "shattering" of bunches of grapes and excessive ripening (accompanied by loss of texture and increased susceptibility to pathogens). Ethylene is also very important in the emerging "fresh cut" or "lightly processed" produce industry. Among the factors that limit the shelf life of fresh cut produce are the discoloration and deterioration that accompany the wounds inflicted on the living plant tissue during processing. We still do not fully understand the way that the occurrence of a wounding event is "signaled" throughout a damaged plant tissue, but an increase in tissue production of ethylene is often part of the signaling system[8].

It is clear, therefore, that improved control of plant ethylene production can have an important impact on fruit and vegetable food quality. Genetic engineers have taken advantage of the fact that the enzymes which catalyze the crucial steps of the ethylene biosynthetic pathway (depicted above) are known to develop several ways to slow down tissue production of this hormone[9]. Some of these efforts to slow ethylene production have utilized the concept of antisense mRNA. In this technique, a new gene with a nucleotide sequence that is the exact complement of the gene to be "negated" (i.e., whose protein product is to be reduced in amount) is introduced into the target plant. When the antisense gene is turned on it "neutralizes" the action of the complementary gene. Thus the protein that is normally encoded by that complementary gene is produced in abnormally low amount. Genetic engineers have used antisense mRNA to reduce the amount of the enzyme that converts SAM to ACC (ACC synthase)[10] and the enzyme that converts ACC to ethylene (ACC oxidase)[11] in tomatoes. These transgenic tomatoes produce much less ethylene than normal and the impact of this is striking. Since ethylene

promotes fruit abscission, the modified tomatoes remain on the vine longer and can accumulate a greater amount of the sugars that are produced during photosynthesis in the leaves. Since ethylene promotes tomato fruit ripening, the modified tomatoes ripen very slowly and thus remain stable longer. Because ethylene can be provided to tomato fields and harvested fruits from external sources ("ethylene-releasing" chemicals or compressed gas bottles), we can now make tomato abscission and ripening follow a time course that we have chosen. With the improved control of the ripening process we can expect that the postharvest quality of fruits and vegetables delivered to the market and the processor will be improved and that consumer satisfaction with both fresh and processed products will increase.

This work represents one of the clearest examples of the power of genetic engineering to improve the utility and ultimate quality of fruits. The success is due to the superb research in biochemistry and enzymology that led to the description of the ethylene biosynthetic pathway. It probably won't trouble too many consumers to eat a food that has been engineered by decreasing the normal expression of a gene, but another approach to engineering reduced ethylene production in tomatoes has been to introduce a bacterial gene that codes for an enzyme that reduces cellular ACC content (see the biosynthetic pathway, above) by converting it to another metabolic intermediate[12]. *Would you eat tomatoes that had been altered in this way?*

Because ethylene production by plants affects so many important processes, we can be confident that genetic engineers will use similar approaches in an attempt to control other aspects of food plant development, including the relatively rapid deterioration of some fresh cut products. Unfortunately, the ripening of many fruits (e.g., strawberry, cherry, citrus *et al.*) is controlled by factors that are not known and so manipulation of the ethylene biosynthetic pathway will not have universal significance *vis-a-vis* the control of ripening. A great deal of research is needed to understand the regulation of development in these fruits.

PLANT CELL WALL METABOLISM AND THE TEXTURE OF FRUITS AND VEGETABLES

Each plant cell is surrounded by a rigid envelope called the cell wall. The cell wall has a complex structure that includes several classes of polysaccharides (cellulose, pectins and hemicelluloses), structural and enzymically-active proteins, simple and polymerized phenolics and ions (the most important of which may be Ca^{2+} and BO_4^{3-})[13]. The cell wall serves as a defensive barrier, preventing the ingress of insects and pathogens, and gives the individual cells and tissues their rigidity. For a long time it has been thought that metabolic changes in cell walls were responsible for changes in fruit and vegetable texture. One can isolate from ripening fruits an assortment of enzymes which, *in vitro*, can digest fruit cell walls[14].

Postharvest physiologists and biochemists had accumulated a great deal of data which suggested that the PG produced by many (but not all) ripening fruits was responsible for the pectin solubilization that occurred during their ripening and, hence, ripening-associated fruit softening. To a degree, the softening of fruits makes them more palatable, but if softening occurs prematurely fruits are less able to tolerate storage and long distance travel. If softening goes too far, unsatisfactory textures result, particularly in

processed products. Therefore, genetic engineers have used antisense mRNA to substantially reduce the amount of PG produced by ripening tomatoes with the aim of creating a fruit which softened more slowly. In some cases the strategy worked[15] and in other cases it did not[16]. One of the most important results of this particular genetic engineering effort was the lesson that even when one focuses on a specific target that has been selected after substantial research, the target may not be appropriate. It is now understood that fruit softening does not depend solely on the action of PG. A great deal of research aimed at understanding the changes that lead to ripening-related fruit softening is now examining other potential cell wall polymer hydrolases that target non-pectin cell wall elements (see paper by Bennett). An unforeseen result of the work utilizing antisense mRNA to reduce tomato fruit PG production was that the PG-deficient fruit had improved resistance to some fungal pathogens[17]. This is a very important observation. Careful research combined with careful observation generally leads to valuable, and occasionally surprising, results.

THE PROMISE OF GENETIC ENGINEERING

Genetic engineering gives us the opportunity to modify many aspects of fruit and vegetable biochemistry and have important impacts on production and quality. The extent of our success will depend, in large measure, on the quality of the physiological and biochemical data that are needed for identification of which genes to modify in order to meet a particular goal.

This paper has not addressed one of the most important issues that relates to the marketplace success of engineered foods. The public must be convinced that the "new" foods are both improved and safe. Developers of these foods will have to be willing to take the time to explain what genetic engineering is in terms that consumers can understand and consumers will have to set their skepticism aside and be willing to listen. Those are big challenges in a society that has grown used to receiving information in emotion-tinged "sound bites".

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EVALUATION OF CHARACTER IMPACT FLAVOUR COMPOUNDS OF FOODS

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The volatile fraction of foods is very complex, consisting of hundreds of compounds. Two bioassays are used to screen the compounds contributing strongly to the aroma of foods. In aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry of headspace samples (GCOH) the potent odorants are localized in the capillary gas chromatograms. As AEDA reveals the medium and higher boiling odorants and GCOH the low boiling ones, both screening methods are complementary to each other. After identification and quantification of the odorants calculation of odour activity values (OAV, ratio of concentration to odour threshold) are the next steps in the analysis of food odours. Finally, sensory studies of model mixtures are performed to verify that the compounds with high OAV are actually the key aroma compounds of the food. To demonstrate these methods the analysis of the character impact flavour compounds of parsley and strawberry juice is reviewed.

INTRODUCTION

The volatile fraction of a food consists of hundreds of compounds, of which only a small number are of significance in determining the flavour. Therefore, a major task in flavour chemistry is to distinguish the compounds causing the flavour from those not involved.

The methods which are suitable to identify the character impact flavour compounds of foods are summarized in Table 1. Their application will be illustrated by discussing the results of studies on the character impact odorants of parsley leaves (*Petroselinum crispum*) and strawberry (*Fragaria vesca*) juice.

SCREENING FOR POTENT ODORANTS

Parsley leaves were homogenized in the presence of an aqueous solution of $CaCl_2$ which inhibits the enzymes[5,6]. The homogenate was extracted with methylene chloride and then the volatile fraction and the solvent were separated from the non-volatile material by distillation in vacuo. After concentration, the volatiles were separated by capillary-GC, and the effluent from the capillary was examined by sniffing. During this procedure odours were perceived at 20 positions of the gas chromatograms.

A single GC run is usually insufficient to indicate the potent odorants, because the result depends on such factors as quantity of the food sample, degree of dilution of the volatile fraction by the solvent and volume size of the sample which is injected on the GC capillary and then separated.

The result was improved by evaluation of a dilution series. The extract was diluted stepwise with the solvent in a volume ratio e.g. of 1:1 and after each dilution step gas chromatography-olfactometry (GCO) was repeated. This procedure, denoted AEDA (Table 1), was continued until no odorant was perceivable by GCO.

The compounds listed in Table 2 were identified in parsley as odorants showing high FD-factors. The highest dilution at which a substance is still smelled during GCO is defined as its flavour dilution (FD) factor[1,2]. The parsley-like smelling p-mentha-1,3,8-triene (**1**), already suggested by Garnero et al.[7] as character impact flavour compound of parsley, was among the potent odorants (Table 2).

Table 1
Strategy for the analysis of food aromas[1-3]

The character impact flavour compounds are evaluated by carrying out the following investigations:

- Localization of potent odorants in capillary gas chromatograms by
  aoma extract dilution analysis (AEDA) and/or aroma extract concentration analysis (AECA)[4]
  and
  gas chromatography-olfactometry of headspace samples (GCOH);
- Identification of potent odorants
- Quantification
- Calculation of odour activity values (OAV, ratio of concentration to odour threshold)
- Sensory study of mixtures containing the potent odorants in the concentrations found in the food samples

Table 2
Potent odorants of parsley - Results of AEDA[5,6]

Compound	Odour quality
p-Mentha-1,3,8-triene (**1**)	Parsley-like
Myrcene (**2**)	Metallic, herbaceous
2-Isopropyl-3-methoxypyrazine (**3**)	Musty
2-*sec*-Butyl-3-methoxypyrazine (**4**)	Musty
Myristicin (**5**)	Spicy, nutmeg-like
1-Octen-3-one (**6**)	Mushroom-like
(Z)-1,5-Octadien-3-one (**7**)	Geranium-like, metallic
Linalool (**8**)	Floral
(E,E)-2,4-Decadienal (**9**)	Fried fat
(Z)-6-Decenal (**10**)	Cucumber-like

As AEVA is limited to odorants boiling higher than the solvent used for the extraction and dilution steps, GCOH (Table 1) was performed to evaluate the potent highly volatile odorants of parsley[6]. According to Table 3 fifteen odorants, of which thirteen were identified, were perceived in a headspace volume of 5 mL. Methanethiol (**11**), (Z)-3-hexenal (**12**), an unknown compound, myrcene (**2**) and myristicin (**5**) were detectable in the smallest volume of 0.6 and 1.25 mL, respectively. Higher headspace volumes of 2.5 mL and 5 mL were necessary to detect the odorants **1**, **4**, 7, **13-17** by GCO.

QUANTITATIVE ANALYSIS AND CALCULATION OF OAV

AEDA and GCOH are only screening procedures, as the results are not corrected for the losses of the odorants during the isolation procedure and the separation by capillary gas chromatography. Furthermore, in AEDA and GCOH, the odorants are completely volatilized and then evaluated by sniffing, whereas the volatility of the odorants in foods depends on their solubility in the aqueous and/or oily phase as well as on their binding to non-volatile food constituents. To correct these errors quantification of the levels of potent odorants and calculation of their odour activity values (OAV) are the next steps in the analytical procedure (Table 1). The OAVs of the compounds are calculated on the basis of their odour thresholds in a medium predominating in the food of interest.

In aroma analysis we use an isotope dilution assay to quantify levels of labile odorants like p-mentha-1,3,8-triene (**1**), carbonyl compounds with a cis-double bond, e.g. (Z)-6-decenal (**10**), and polar substances, which are isolated in low yields by extraction from aqueous solution and/or are more or less degraded during capillary GC. In addition, when the concentration of the odorant is very low and, hence, several clean-up steps are required before the measurement can be performed, an isotope dilution assay is the most accurate method.

Table 3
GCOH of parsley leaves from the cultivar „Hamburger Schnitt"[a]

Odorant[b]	RI[c]	Volume (mL)[d]
Methanethiol (**11**)	<500	0.6
(Z)-3-Hexenal (**12**)	800	0.6
Unknown	1360	0.6
Myrcene (**2**)	962	1.25
Myristicin (**5**)	1533	1.25
p-Methylacetophenone (**13**)	1193	2.5
(Z)-3-Hexenyl acetate (**14**)	1005	2.5
Unknown	1167	5
2-*sec*-Butyl-3-methoxypyrazine (**4**)	1175	5
(Z)-3-Hexenol (**15**)	858	5
(Z)-1,5-Octadien-3-one (**7**)	984	5
ß-Phellandrene (**16**)	1035	5
1-Isopropenyl-4-methylbenzene (**17**)	1088	5
p-Mentha-1,3,8-triene (**1**)	1115	5

[a] Parsley leaves (0.5 g) were minced, and after 5 min the sample was placed into a vessel (250 mL) which was sealed with a septum and then held in a water-bath at 23°C. Headspace samples were analysed by GCO.

[b] Numbers **1**, **2**,**4**-**7** refer to Table 2.

[c] Retention index (RI) on capillary RTX-5.

[d] The lowest headspace volume which was required to perceive the odorant at the sniffing port of the gas chromatograph.

In such an assay the corresponding odorant labelled with a stable isotope (^2H, ^{13}C) is used as internal standard for the determination of the unlabelled analyte[1]. During the purification procedure the analyte and its isotopomer are equally enriched, as they agree in their chemical and physical properties apart from small isotope effects.

Of the compounds listed in Table 4 nos. **1**, **3**-**7**, **9**-**12**, **14** and **15** were determined using the corresponding isotopomers as internal standards. The remaining compounds were quantified by a conventional method.

According to the results summarized in Table 4, the parsley cultivar „Hamburger Schnitt" (HS) contained high levels of p-mentha-1,3,8-triene (**1**), ß-phellandrene (**16**), myristicin (**5**) and myrcene (**2**).

OAVs were calculated on the basis of the odour threshold values in water. The results in Table 4 indicate that the „terpeny, parsley-like" smelling p-mentha-1,3,8-triene (**1**), the „metallic, herbaceous" myrcene (**2**), the „musty, earthy" 2-*sec*-butyl-3-methoxypyrazine (**4**), „spicy" myristicin (**5**), the „fatty" (E,E)-2,4-decadienal (**9**), „green, cucumber-like" (Z)-6-decenal (**10**) and „terpeny" ß-phellandrene are important contributors to the aroma of parsley on the basis of higher OAVs.

SIMULATION OF PARSLEY FLAVOUR

To verify that the compounds showing higher OAV were the key odorants of parsley, a model mixture was formulated on the basis of the quantitative data obtained for the cultivar HS, merely methanethiol (**11**) and ß-phellandrene (**16**) were omitted despite their relatively high OAV (Table 4). The concentration of (Z)-3-hexenyl acetate (**14**) was reduced to that found immediately after chopping the parsley leaves (Table 5). In spite of these changes the overall odour of the model was described by the assessors as clearly parsley-like. This result revealed that the compounds in the mixture belonged to the key odorants of parsley. However, there were some differences between the odour profile of the model and that of HS (Table 6). The model was stronger in particular with regard to the intensity of the „spicy" odour note and weaker with regard to the „green, grassy" note.

Table 4.
Concentrations and odour activity values (OAV) of 17 odorants in the parsley cultivar "Hamburger Schnitt"[6]

Odorant	Concentration[a]	Threshold (µg/kg)[b]	OAV[c]
p-Mentha-1,3,8-triene (**1**)	393285	15	26219
Myrcene (**2**)	17980	14	1284
2-Isopropyl-3-methoxypyrazine (**3**)	1.4	0.004	350
2-*sec*-Butyl-3-methoxypyrazine (**4**)	7.7	0.003	2567
Myristicin (**5**)	57800	30	1927
1-Octen-3-one (**6**)	3.0	0.05	60
(Z)-1,5-Octadien-3-one (**7**)	1.0	0.0012	833
Linalool (**8**)	694	6	5290
(E,E)-2,4-Decadienal (**9**)	5898	0.2	17347
(Z)-6-Decenal (**10**)	5898	0.34	17347
Methanethiol (**11**)	260	0.2	1300
(Z)-3-Hexenal (**12**)	200	0.25	800
p-Methylacetophenone (**13**)	624	24	26
(Z)-3-Hexenyl acetate (**14**)	164	8	21
(Z)-3-Hexenol (**15**)	433	39	11
ß-Phellandrene (**16**)	204190	36	5672
1-Isopropenyl-4-methylbenzene (**17**)	15850	85	187

[a] Values in micrograms per kilogram (fresh weight). The data are means of at least two assays.

[b] Nasal odour threshold of the compound in water.

[c] OAV were obtained by dividing the concentration of the compound by the odour threshold.

Table 5.
Changes in the concentrations of odorants in the chopped parsley cultivar "Hamburger Schnitt"[6]

Odourant[a]	Concentration[b,c] after				
	0 min	1 min	5 min	15 min	30 min
p-Mentha-1,3,8-triene (**1**)	222	168	133	107	104
(E,E)-2,4-Decadienal (**9**)	0.74	0.34	0.26	0.17	0.12
(Z)-6-Decenal (**10**)	2.5	2.2	1.6	1.1	0.9
(Z)-3-Hexenal (**12**)	0.48	2.5	3.5	1.6	1.0
(Z)-3-Hexenyl acetate (**14**)	0.01	0.27	1.9	2.2	5.9
(Z)-3-Hexenol (**15**)	0.45	9.3	17.6	25.4	30.4
ß-Phellandrene (**16**)	148	110	91	78	76
1-Isopropenyl-4-methylbenzene (**17**)	6.9	7.0	6.9	7.2	7.2

[a] Numbering refers to Table 4.

[b] At the times given in the table enzymatic reactions were inhibited by the addition of an aqueous saturated solution of $CaCl_2$ to the chopped leaves.

[c] Values in milligrams per kilogram (fresh weight).

Table 6.
Flavour profiles of the freshly chopped parsley cultivars "Hamburger Schnitt" (HS) and the aroma model for parsley[6]

Attribute	Odour intensity[a]	
	HS	Model
Green, grassy	1.4 (2.5)[b]	0.8
Fruity	0.2 (1.5)[b]	0.5
Spicy	1.4	2.0
Green, cucumber-like	1.9	1.8
Earthy, musty	0.3	0.2
Terpeny, parsley-like	2.5 (1.4)[b]	2.6
Fatty	0.9	0.4

[a] The intensity of the attributes was scored on the scale: 0, absent to 3, strong.

[b] After storage of 5 min.s

The parsley-like character of the aroma model was completely lost when p-mentha-1,3,8-triene (**1**) and myrcene (**2**) were omitted. This confirmed the suggestion of Garnero et al.[7] that **1** is a character impact compound of the parsley flavour.

STABILITY OF THE PARSLEY FLAVOUR

The flavour profile of chopped parsley changed rapidly at room temperature. After only 5 min, the intensities of the „green, grassy" and the „fruity" notes had increased, while that of the „terpeny, parsley-like" note had decreased (Table 6). To describe this change more objectively, the concentrations of eight odorants were followed in the cultivar HS during a period of 30 min after chopping. The results in Table 5 indicate that p-mentha-1,3,8-triene (**1**) decreased rapidly; only 60 % was still present after 5 min. We suggest that this decrease contributes to the losses of the "terpeny, parsley-like" note in the flavour of HS (Table 6). Also (E,E)-2,4-decadienal (**9**) and (Z)-6-decenal (**10**) were sensitive to storage of chopped HS. After 30 min, the concentrations had fallen to 16 % (**9**) and 36 % (**10**), respectively (Table 5). At the same time (Z)-3-hexenal (**12**), the corresponding alcohol (**15**) and ester (**14**) increased drastically. Although after 5 min (Z)-3-hexenol (**15**) was 5-fold higher than the aldehyde (**12**), the latter might more strongly contribute to the increase in the "green, grassy" note of chopped and stored HS as its odour threshold was by a factor of 156 lower than that of **15** (Table 4). The stronger intensity of the "fruity" character (Table 6) might be due to the increase of (Z)-3-hexenyl acetate (**14**) which reached a concentration of 5.9 mg/kg after 30 min.

KEY ODORANTS OF STRAWBERRY JUICE

A study performed in our laboratory by Schieberle and Hofmann[8] on the character impact flavour compounds of strawberry juice is a further example showing a successful application of the methods listed in Table 1.

Screening experiments of which the results are summarized in Table 7 revealed 15 potent odorants in strawberry juice. The highest concentrations were found for acetic acid (**25**) followed by furanone (**18**) and methyl butanoate (**19**), while, e.g., ethyl 2- and 3-methylbutanoate (**21**, **22**) were present in comparatively lower amounts.

Calculation of OAV (Table 7) revealed six compounds as the most odour-active, namely furanone **18**, (Z)-3-hexenal (**12**), esters **19**, **20**, **30** and 2,3-butanedione (**26**).

The odour profile of the fresh juice was compared with that of a solution (pH 3.5) of the 12 odorants listed in Table 8. The result in Figure 1 indicated that the odour profile of the model was very close to that of the original.

To gain some insights into the relative importance of single odorants, 11 model mixtures were prepared, each lacking in only one odorant. Omission of furanone **18** (exp. I, Table 8) or (Z)-3-hexenal (exp. II), respectively, led to a strong change in the overall odour of the mixture, which was easily detectable by all six or five members of the

Table 7.
Concentrations, odour thresholds, and odour activity values (OAV) of potent odorants in fresh strawberry juice[8]

Compound[a]	Concentration (µg/kg)	Threshold (µg/kg, water)	OAV[b]
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (**18**)	16239	10	1624
(Z)-3-Hexenal (**12**)	333	0.25	1332
Methyl butanoate (**19**)	4957	5	991
Ethyl butanoate (**20**)	410	1	410
Ethyl 2-methylbutanoate (**21**)	} 7	0.15	} 42
Ethyl 3-methylbutanoate (**22**)		0.20	
Methyl 2-methylbutanoate (**23**)	} 48	0.25	} 150
Methyl 3-methylbutanoate (**24**)		0.40	
Acetic acid (**25**)	74513	60000	1
2,3-Butanedione (**26**)	1292	3	431
Butanoic acid (**27**)	1831	2730	<1
3-Methylbutanoic acid (**28**)	} 2237	540	} 3-4
2-Methylbutanoic acid (**29**)		740	
Ethyl 2-methylpropanoate (**30**)	43	0.1	430
4-Methoxy-2,5-dimethyl-3(2H)-furanone (**31**)	20	25	<1

[a] Number **12** refers to Table 4.

[b] Refer to footnote „c" in Table 4.

Table 8.
Odour of the model juice affected by the absence of one component[8]

Expt. no.	Odorant omitted in the model[a]	No. of 6 panelists detecting an odour difference
I	4-Hydroxy-2,5-dimethyl-3(2H)-furanone (**18**)	6
II	(Z)-3-Hexenal (**12**)	5
III	Methyl butanoate (**19**)	4
IV	Ethyl butanoate (**20**)	4
V	Ethyl 2-methylbutanoate (**21**)	4
VI	Methyl 2-methylbutanoate (**23**)	3
VII	Acetic acid (**25**)	3
VIII	2,3-Butanedione (**26**)	3
IX	Butanoic acid (**27**)	2
X	2-Methylbutanoic acid (**29**)	2
XI	Ethyl 2-methylpropanoate (**30**)	2
XII	4-Methoxy-2,5-dimethyl-3(2H)-furanone (**31**)	1

[a] Model juices lacking in one component (no. **12**, **18-21**, **23**, **25-27**, **29-31** in Table 7) were singly compared to the model juice containing the complete set of 12 odorants by using the triangle test. Numbering of the odorants refers to Table 7.

panel, respectively. To the contrary, a lack of the acids **27** and **29**, ester **30** and furanone **31** (expt. IX-XII) was only perceived by one or two panelists, respectively, indicating a much lower flavour impact of these compounds.

CONCLUSION

The results obtained in studies on parsley and strawberry juice clearly indicate that a systematic approach has been developed for the identification of the character impact flavour compounds of foods. These compounds are useful as indicators, e.g. to select varieties in plant breeding or to objectify flavour changes in products caused by raw materials, processing conditions, or storage.

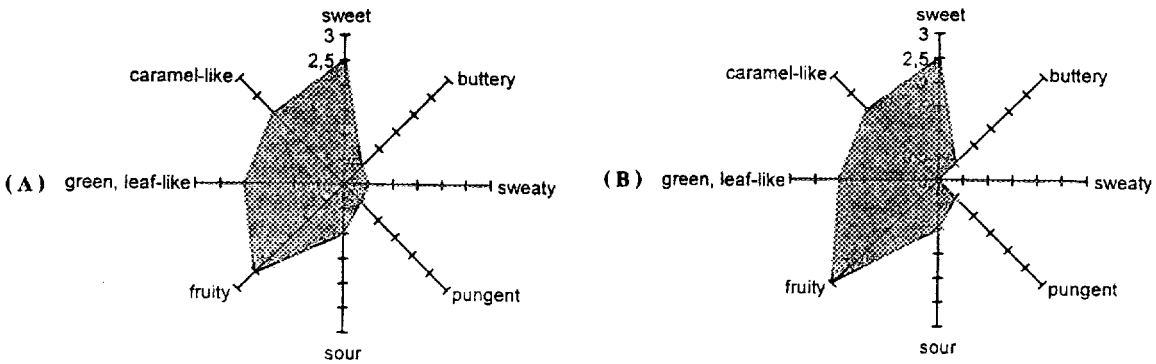

Figure 1
Flavour profiles of the strawberry model juice (A) and a freshly prepared strawberry juice (B)

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A NEW C_{30} HPLC METHOD FOR THE DETERMINATION OF *CIS-TRANS* ISOMERS OF CAROTENOIDS AND PROVITAMIN A NUTRIENTS IN FRESH AND PROCESSED FRUITS AND VEGETABLES.

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A reverse phase high performance liquid chromatography (HPLC) method used in this study was developed to optimize separations of carotenoids. The polymeric C_{30} stationary phase provides excellent resolution of all-trans carotenoids and possesses outstanding shape selectivity toward predominant geometrical isomers. Several geometrical isomers of carotenoids were found in extracts of various fresh and processed fruit and vegetables. Several cis isomers of lycopene were also resolved. Increases in the quantity of geometrical isomers were observed in relation to the severity of thermal processing. Nutritional and physiological implications for the presence of these phytochemicals in the food supply are considered.

Certain carotenoids are metabolic precursors of vitamin A and exhibit biological activity as singlet oxygen quenchers. Among dietary carotenoids, various geometric (*cis* and all-*trans*) isomers either occur naturally, or are formed during thermal processing of fruits and vegetables[1]. With provitamin A carotenoids, *cis* isomeric forms are less efficiently converted to vitamin A than their all-*trans* counterparts[2]. The measurement of geometric forms of provitamin A carotenoids is, therefore, dependent upon resolving and quantifying *cis*-carotenoids in foods. With respect to nutritional and other health-related aspects of carotenoid metabolism, the possibility that unique or altered physiological roles could be associated with *cis* versus all-*trans* carotenoids has not yet been rigorously addressed. Thus, the ability to accurately determine *cis-trans* carotenoids present in fresh and processed fruits and vegetables is a prerequisite to acquiring a better understanding of these phytonutrients in the diet. Recently a new polymeric C_{30} stationary phase was developed to optimize separations of carotenoids[3]. The new system has been shown to possess outstanding shape selectivity toward carotenoid geometric isomers.

The aim of this report is to describe a new C_{30} HPLC methodology for the analysis of *cis-trans* carotenoids present in fresh and processed fruits and vegetables. In addition, the developed methodology is applied to monitor the chemical changes which occur to carotenoids during thermal processing. This information is useful in assessing both nutritional and physiological implications of these phytochemicals in the diet.

MATERIAL AND METHODS

Standards of all-*trans* β-carotene, α-carotene and lycopene were purchased from Sigma Chemical Co. (St. Louis, MO) and all-*trans* β-cryptoxanthin was a gift from Hoffmann-LaRoche (Nutley, NJ). All extraction and HPLC solvents (Fisher Scientific Colk Fairlawn, NJ) were certified HPLC or ACS grade. The fruits and vegetables evaluated were purchased from local markets and processed according to standard time and temperature requirements[4].

EXTRACTION AND SAPONIFICATION: The following extraction procedure was carried out under subdued light to prevent isomerization and photodegradation. Fresh and processed carrot were diced and 10.0 g samples were homogenized in 50 mL methanol with 1.0 g $CaCO_3$ and 3.0 g Celite. Samples were successively extracted with mixture of 1:1 acetone/hexane and vacuum-filtered through Whatman paper No. 1 and 42. The filtrates were combined in a separatory funnel and water was added to induce phase separation. The hexane layer was removed and brought up to volume. Triplicate 5 mL samples were dried by flushing with nitrogen.

CHROMATOGRAPHY: The reverse-phase HPLC system used in this study consisted of a Waters (Milford, MA) 2690 separation module. Separations were achieved using analytical (250 x 4.6 mm I. D.) 3μm and 5μm polymeric C_{30} columns which were prepared at the National Institute of Standards and Technology (Gaithersburg, MD)[5]. Guard columns packed with either C_{18} or C_{30} stationary phase were used in-line for all separations. The mobile phase was 20% methyl-*t*-butyl ether (MTBE) in methanol, flowing isocratically at 1.0 mL per minute. Lycopene isomer separation was carried out at 1.0 mL per minute using a linear gradient of 40 to 50% MTBE in methanol for 35 min. Extracts were analyzed in duplicate.

RECOVERY DETERMINATIONS: Recovery experiments during extraction, saponification and chromatography procedures were measured. A known quantity of purified all-*trans* β-carotene standard was added to homogenized plant tissue. Extraction, saponification, and all chromatography procedures with and without added standard β-carotene were analyzed in duplicate. Results of recovery determinations were found to be greater than 95%. *Cis*-isomer content of samples with added standards remained the same as control extracts indicating that isomerzation did not occur during the handling and chromatographic procedures.

IODINE ISOMERIZATION: Lycopene standard was solubilized in 2 mL of hexane and treated with 100 μL of 1.0% iodine hexane solution. Following a brief exposure to direct sunlight, the mixture was dried, resuspended in mobile phase and injected.

PEAK IDENTIFICATION: Column effluent was monitored via a Waters 996 Photodiode Array Detector at 200-800 nm with a scanning rate of 1 scan per second and 1.2 nm spectral resolution. The detector was linked to a Digital (Maynard, MA) S133 Venturis computer with Waters Millennium 2010 chromatography software (LC Version 2.15.01). Quantification of geometric isomers was achieved using a standard curve of the corresponding all-*trans* isomers (Sigma, St. Louis, MO) and their molar absortivity coefficients[6]. This method approximates the *cis*-isomer content, as the molar extinction coefficient values for individual lycopene isomers are not known.

Chromatographic peak identification was based on comparison to previously reported separations on polymeric C_{30} columns and UV-visible absorption spectral libraries. Peaks which are identified as *cis*-lycopene isomers have been ascertained to not be oxygen-addition products of lycopene using electrospray mass spectroscopy[7]. The all-*trans* lycopene isomer peak was assigned based on retention time and co-chromatography of authentic standard.

RESULTS AND DISCUSSION:

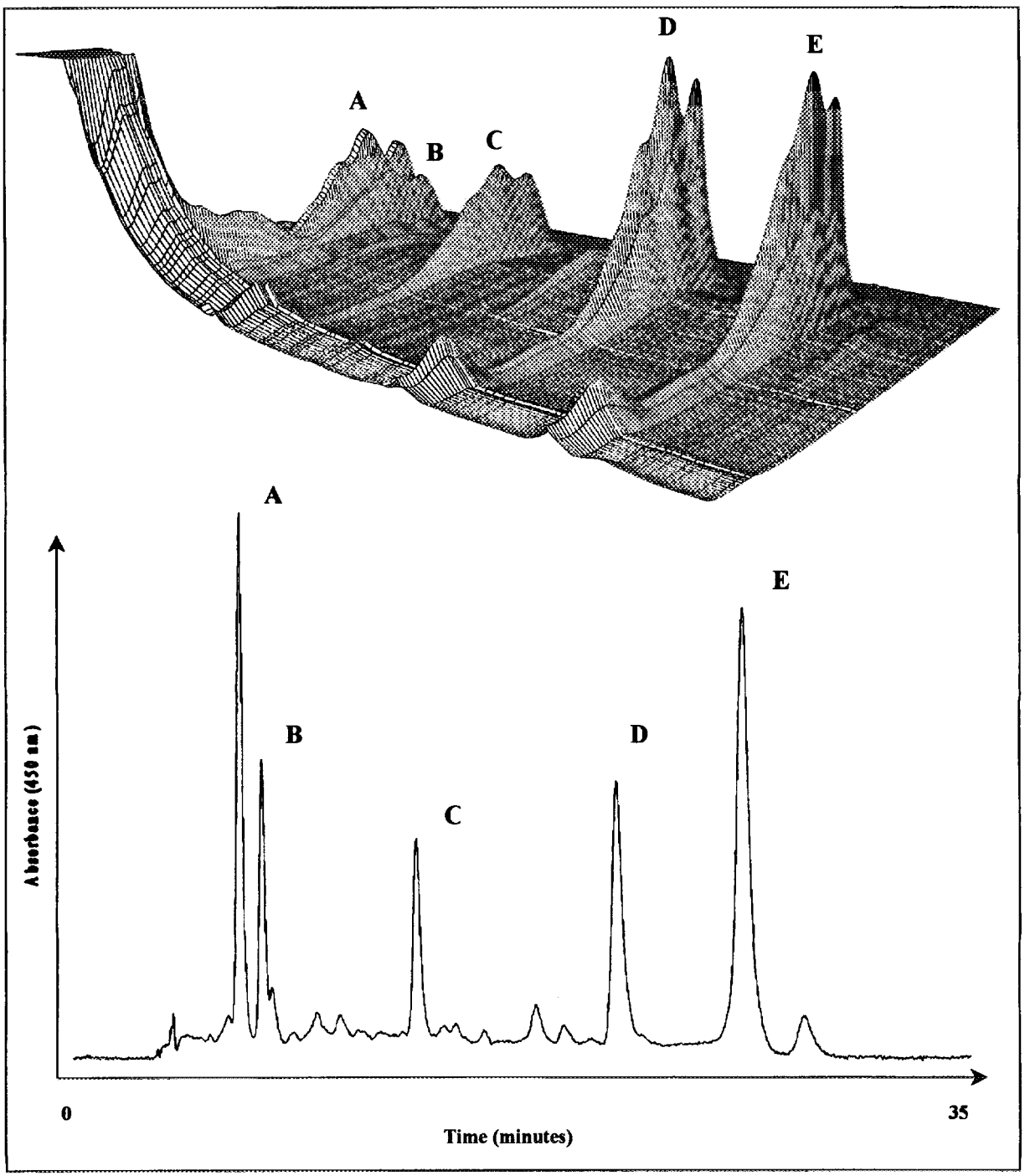

Figure 1. Separation of A. Lutein, B. Zeaxanthin, C. β-Cryptoxanthin, D. α-Carotene and E. β-Carotene Standards by Reverse-phase C_{30} HPLC with Photodiode Array Detection.

Figure 1 illustrates the chromatographic separation of common carotenoids found in fruits and vegetables. The mixture consists of all-*trans* standards of lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene. The peaks are identified using spectral data generated by the photo diode array (PDA) detector. The PDA capability allows for full ultraviolet and visible spectra to be obtained during analysis and is particularly useful to identify the pigments as well as their corresponding *cis* isomers. Baseline resolution of the carotenoids was achieved

within 35 minutes. In addition, excellent separation of lutein and zeaxanthin, two carotenoids which often co-elute during reversed-phase chromatography, is also demonstrated. Lycopene is more strongly retained on the C_{30} stationary phase and does not elute within a reasonable time using the mobile phase conditions of 20% MTBE in methanol. Analysis of lycopene requires greater concentrations of MTBE for elution or the use of gradient programming for rapid separation and analysis of these components.

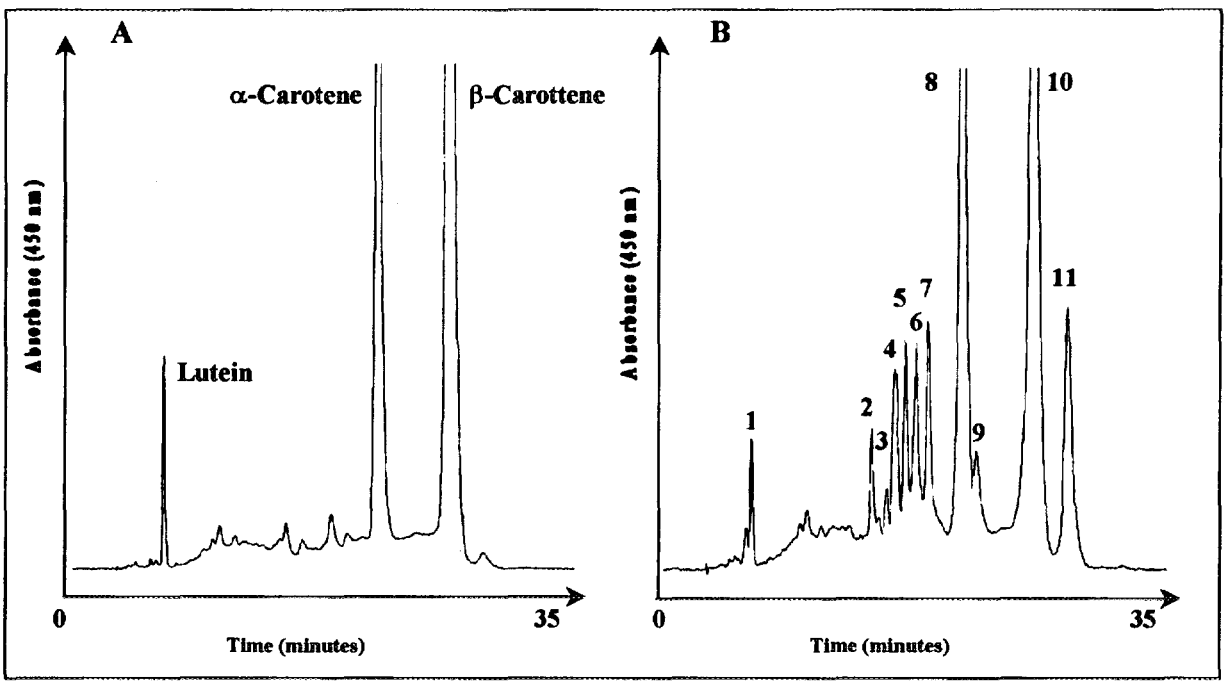

Figure 2. Carotenoid Pattern of Raw (A) and Processed (B) Carrot Extracts. 1. all-*trans* Lutein, 2. 13-*cis* α-carotene, 3. a *cis*-α carotene, 4. 13'-*cis*-α carotene, 5. 15-*cis* β-carotene, 6. a *cis* β-carotene, 7. 13-*cis* β-carotene, 8. all-*trans* α-carotene, 9. 9-*cis* α-carotene, 10. all-*trans* β-carotene, 11. 9-*cis* β-carotene.

Chromatography of extracts of fresh carrots resulted in the chromatogram depicted in Figure 2a. The carotenoids shown are well separated and are typical for the natural occurrence of lutein, α-carotene and β-carotene. These carotenoids are present in their all-*trans* configuration. Although several other components were also detected when monitoring at 450 nm and separated under the chromatographic conditions, these components do not predominate and were not identified. Typically, carrots contain other hydrocarbon carotenoids in addition to α and β carotene such as γ-carotene, ζ-carotene, neurosporene, phyofluene, and phytoene[8]. These carotenoid compounds all have similar structures as well as physical/chemical properties and thus would be expected to be present within these extracts.

Figure 2b represents a chromatogram typical of thermally processed canned carrots. The extracts contain substantial amounts of geometric isomers that were generated during the heat treatment used for canning. Such isomerization reactions induced by thermal treatments are well known and have been previously observed in processed foods[9]. The β-carotene isomers observed consisted of the 15-*cis*, 13-*cis*, all-*trans* and 9-*cis* components which were identified in the processed carrot extract. Many more isomers of α-carotene were detected because the structure of this carotenoid is asymmetrical in contrast to β-carotene which has a symmetrical structure. The 13-*cis*, 13'-*cis*, all-*trans* and 9-*cis* isomers of α-carotene are well resolved (Fig. 2b). The 9'-*cis* isomer of α-carotene is not shown because this compound coelutes with

all-*trans* β-carotene. Resolution of this geometric isomer from other α-carotene isomers can be achieved using the C_{30} system[10]; however, if all-*trans* β-carotene is present, detection of this compound is difficult. *Cis* forms of lutein were also noted to form during the thermal treatment. However, since the mobile phase used was optimized to separate the hydrocarbon carotenoids, elution of the xanthophylls occurred within the early eluting peaks which prevented tentative identification.

The chromatograms shown in Figure 2 are illustrative of several important considerations regarding the application of the C_{30} column to carotenoid separations. The chromatographic profile of carotenoids found in fresh carrots is quite simple relative to those found in processed samples. For applications where the carotenoid composition is complex and geometrical isomers are to be analyzed, the high degree of selectivity for these compounds on the C_{30} stationary phase may complicate identification due to coelution problems. Employing gradient elution schemes may provide a solution when quantitation of the geometric isomers in complex mixtures is necessary. Applications do exist where separations of the geometric forms are not warranted. However, for carotenoid separations where this information is needed, the C_{30} system exhibits excellent resolving capabilities for the geometric forms and better overall performance for this analysis relative to existing reverse phase columns.

Cis isomers of the provitamin A carotenoids (i.e. β-cryptoxanthin, α-carotene and β-carotene) have provitamin A activities which are approximately 50% or less than the corresponding all-*trans* carotenoids [2,11]. Therefore, in order to quantitate the provitamin A content of foods and the effects of processing on their nutritional value with respect to vitamin A, the various isomeric forms of these carotenoids present in both the fresh and processed state must be accurately determined. The chromatographic methodology described in this study will assist in providing more accurate information of vitamin A content for nutritional tables and databases. In addition, given the current interest of these antioxidants in health and disease, understanding the role and presence of carotenoid isomers in the diet may impact studies involving these compounds with specific physiological and biological activities [12, 13, 14].

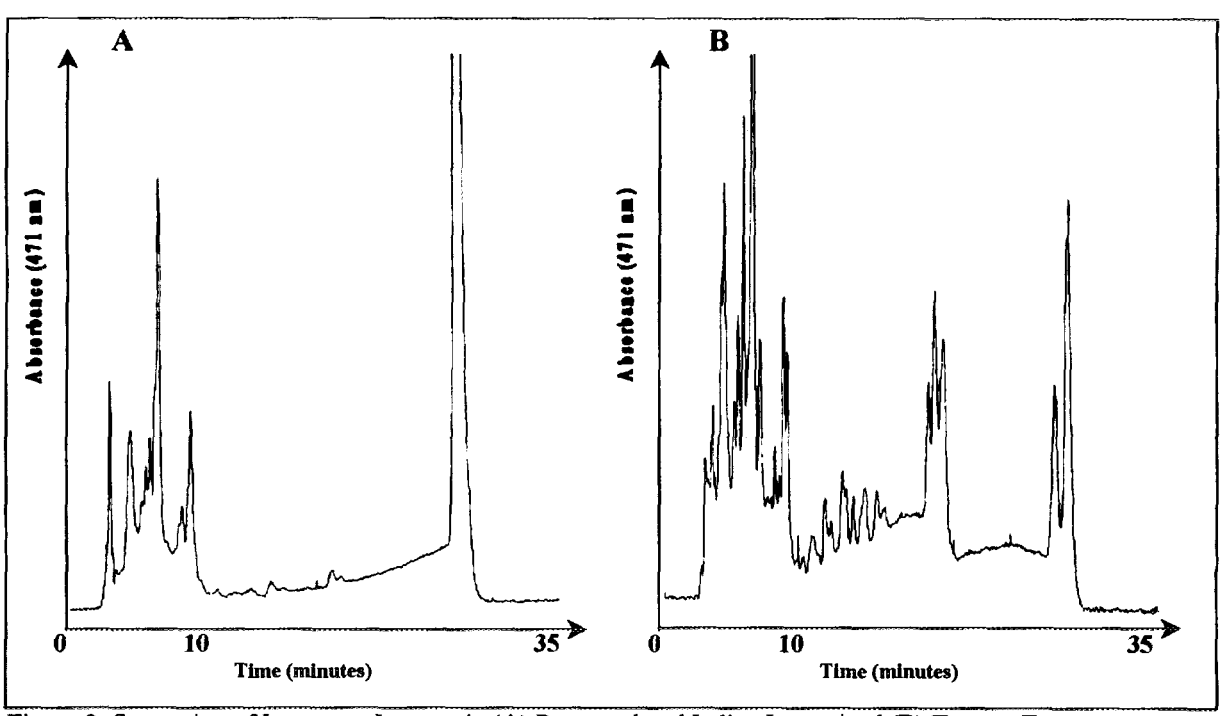

Figure 3. Separation of Lycopene Isomers in (A) Processed and Iodine Isomerized (B) Tomato Extracts.

Figure 3 illustrates the separation and distribution of lycopene isomers obtained from an isomerized mixture. The chromatogram depicts the resolving capabilities of the C30 stationary phase to separate *cis-trans* isomers of lycopene. Other reverse phase separations of lycopene will generally not resolve *cis* isomers and typical chromatograms will commonly show the *cis* forms eluting as a shoulder present with the all *trans* peak. The geometric configurations of the isomers shown on the C_{30} system have not yet been identified and will require further work to isolate the pure isomers and obtain nuclear magnetic resonance (NMR) spectra. However, all of the separated lycopene isomers shown in Figure 3 (retention time> 10 minutes) exhibited similar ultra-violet visible absorption spectral characteristics for lycopene. In addition, mass spectral determinations for the isolated lycopene fractions all confirm molecular weights of 536 amu providing additional evidence for their identity as lycopenes[15]. Although a large percentage of lycopene found in biological tissues such as in human sera exists in the *cis* configuration, it does not appear that the *cis* forms of lycopene are present in processed foods or processed tomato products (Figure 3). While other carotenoids (i.e. β-carotene) readily isomerize during the processing of tomato products, lycopene seems relativity stable to thermally induced isomerization reactions. Thus, the presence of the *cis* lycopenes measured in biological tissues cannot be attributed to the consumption of *cis* lycopenes from tomato products in the diet.

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SEARCH FOR RELATIONSHIPS BETWEEN CHEMICAL AND SENSORY MEASUREMENTS. THE EXAMPLE OF APRICOT PUREES.

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Apricot puree chemical and sensory data were analysed for relationships by multiple regression analysis in order to find predictive equations for sweet, acid, bitter taste and fragrant flavour. Results enabled selection of relevant chemical components for use with routine chemical control of incoming apricot material. The same chemicals were also used to anchor sensory scale of bitter taste intensity, resulting in improved consistency among panelists in successive assessment of the product.

Selected chemical components have long been used as quality markers for the control of raw materials and of end products. This is often the case in the quality control departments, where proximate analysis data, which can be easily achieved and interpreted by both the plant's technical and managing staff, are widely employed to grade products and intermediates, or classify products according to basic composition.
In recent years, efforts to describing of food properties in chemical and sensory terms have delivered comprehensive profiles for several fruits[1-3] and vegetables[4,5] and their derivatives.[6-8] However, the huge amount of information available from profiling studies can be rarely transferred to the manufacturing industry because of difficulties in on-line collection and interpretation of large data files, when fast and simple techniques would otherwise be required for decision making purposes.
Having as a main target the needs of a plant unit equipped with standard instrumental facilities and data analysis skills, we processed a wide apricot sensory and chemical record file in order to develop an affordable, easy-to-run procedure for use in the quality control of apricot as prime material.

MATERIALS AND METHODS

This work relates to previously collected data of apricot purees[9]. They were sampled, stored and analysed for proximate composition and for the additional chemical classes listed in table 1. Headspace volatiles were also determined by GC-MS.
A sensory evaluation was performed by two trained panels, working at different plant locations (control of processing and quality assurance, respectively). Both panels accomplished independent assessment of the same samples following recommended guidelines for quantitative attribute rating. Basic steps in sensory evaluation were as follows.

Sensory analysis

Samples (20) were assessed in five sessions of four samples, presented in random order and numbered with 3-digit codes. Each group of four was randomly assigned to the five testing sessions, held every three days, over two weeks. There were no replicates, nor were standard substances used to anchor the rating scales. Scores were expressed by numeric scales (0-9) with their extremes meaning no perception of the attribute (score 0) or maximum perceived intensity (score 9), respectively.
The panels were as follows: panel 1, made up of 6 subjects, selected among quality assurance department and panel 2, comprising 9 subjects, chosen from those in charge of the control of processing. They were all recruited, selected and trained following standard procedures for attribute rating. Both panels were aware of and familiar with the product under examination. They were asked to analyse given samples for these attributes: sweet taste, acid taste, bitter taste and fragrance, the latter being defined as the flavour that is specific to fresh apricot.

Data analysis

Average sensory scores from each panel were merged to the analytical data and independently used in regression analysis of sensory-to-chemical measurements. Comparison between panels and chemical data was eventually

performed by Generalised Procrustes Analysis of one data set comprising the joined sensory (two panels) and the chemical data.

RESULTS AND DISCUSSION

Ranges for chemical measurements were greater than for sensory scores, as shown by coefficients of variation (CV) reported in table 1 for the variables examined. As a trend, CVs for panel 1 were larger than for panel 2.

Table 1. Chemical and sensory measurements of apricot purees and CV values (Coefficients of Variation).

VARIABLE	CV	VARIABLE	CV
Chemical		Sensory (Panel 1)	
pH	6.2	sweet	11
dry matter	8.8	acid	18
ashes	3.7	bitter	19
formol value	23	fragrant	21
refractive index	5.0		
glucose	93	Sensory (Panel 2)	
fructose	71	sweet	19
citric acid	51	acid	23
malic acid	28	bitter	31
oxalic	59	fragrant	29
lactic	49		
Na^+	47		
K^+	22		
Mg^{2+}	12		
Ca^{2+}	23		
phosphate	24		

Regression analysis

Equations were obtained by multiple regression of attribute scores to the chemical measurements. Results, reported in table 2 as standardised coefficients of significant variables, exhibit high R values for sweet, acid and bitter taste and a lower coefficient for fragrant flavour. Equations for sweet and acid taste were consistent for both panels, indicating that perception and rating for these attributes relied on the same mechanism, as suggested by similar coefficients for the same chemical compounds, of which sugars, dry matter, organic acids and formol played a major role.

Table 2. Multiple regression of sensory attributes vs chemical measurements. Coefficients in standardised form.

Attribute	Panel 1	Panel 2	Attribute	Panel 1	Panel 2
1-SWEET TASTE			3-BITTER TASTE		
refractive index	0.32	0.29	phosphate	0.30	0.21
pH	0.30	0.33	magnesium	0.30	0.21
dry matter	0.23	0.11	pH	-0.20	-0.30
calcium	-0.19	-0.09			
			R	0.70	0.58
R	0.88	0.77			
2-ACID TASTE			4-FRAGRANCE		
pH	-0.39	-0.34	refractive index	0.27	0.03
phosphate	0.38	0.29	pH	0.18	0.19
calcium	0.26	0.31	sodium	0.11	0.20
R	0.73	0.65	R	0.33	0.29

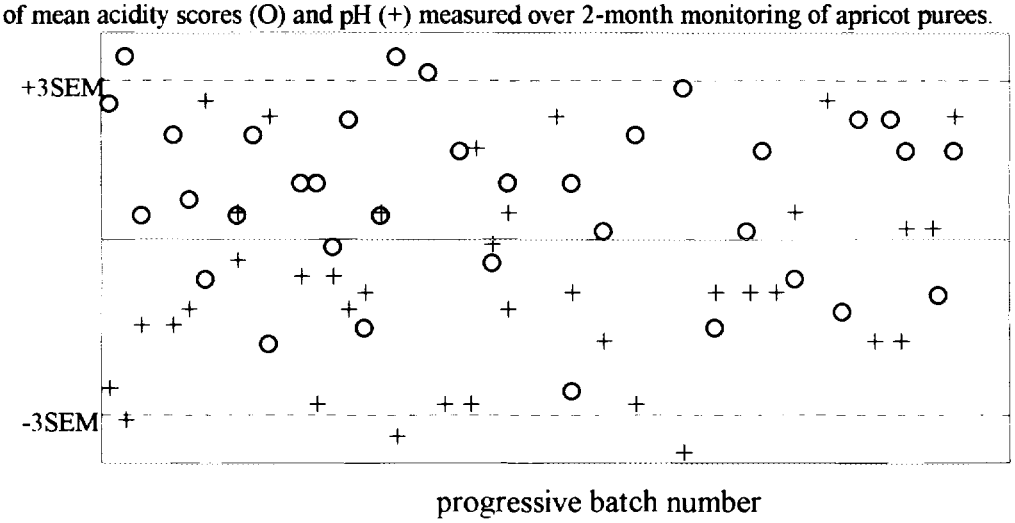
Table 3. Multiple regression of fragrant flavour vs chemical measurements, including headspace substances.

Attribute	Panel 1	Panel 2
BITTER TASTE		
phosphate	0.19	0.25
magnesium	0.38	0.27
2-Methylpropan-1-ol	-0.41	-0.38
β-Myrcene	0.23	0.21
R	0.71	0.72

In contrast, the panel behaviour toward bitter taste was less consistent, with panel 2 ratings yielding less significant regression coefficient. Finally, results were unsatisfactory with fragrant flavour scores, as shown by negligible quotes of variance for both panel data (R^2=0.11 and 0.08 for panel 1 and 2, respectively).
The fragrance regression models could be expectedly improved by including headspace analysis data into the equation. As result, R^2 was raised to more than 0.70 for both panels, with selected components having a major part (Table 3). In contrast, the taste equations did not benefit from the addition of volatile compounds, in accordance with the opinion, shared by most panelists, that apricot fragrance was mainly perceived at the olfactory level, while the three tastes were less affected by these nuances. Further comments on how results from this study might be used to better profile apricot derivatives are beyond the scopes of this paper.
What seems more relevant to the objectives of the quality control in the fruit industry is that the regression equations evidence those chemicals that really affect the sensory qualities and can be used for control purposes. The following two applications are straightforward.

1- Use of the relevant chemicals in routine control. Real case.
Taste regression data were employed to control incoming apricot material for excessive acidity. Based on panel 1 data, samples were monitored for sensory bitterness (equation and subsequent testing from panel 1) and for the first regressor (i.e. pH) over a 6-month span. Results, shown in fig. 1 as plot of the means, display the expected linkage between the two measurements. Typically, those samples exceeding the upper extreme or +3S.E.M. for acidity scores were also below -3S.E.M. for the chemical variable, enabling an easy selection of outlying units. On the long term (six months), a record of 37 correctly classified of 40 samples examined was obtained for excessive acidity prediction.
Figures were likewise fair with bitter taste when both chemical data (phosphate and magnesium) exceeded 2S.E.M., but the same correspondence failed to be obtained when chemicals were used one at a time, even at the 3S.E.M. level.
In conclusion, replacement of lengthy sensory assessment by selected chemical components is a cost-to-benefit matter relying substantially on how dependable the response should be in terms of processing requirements.

Fig. 1. Plot of mean acidity scores (O) and pH (+) measured over 2-month monitoring of apricot purees.

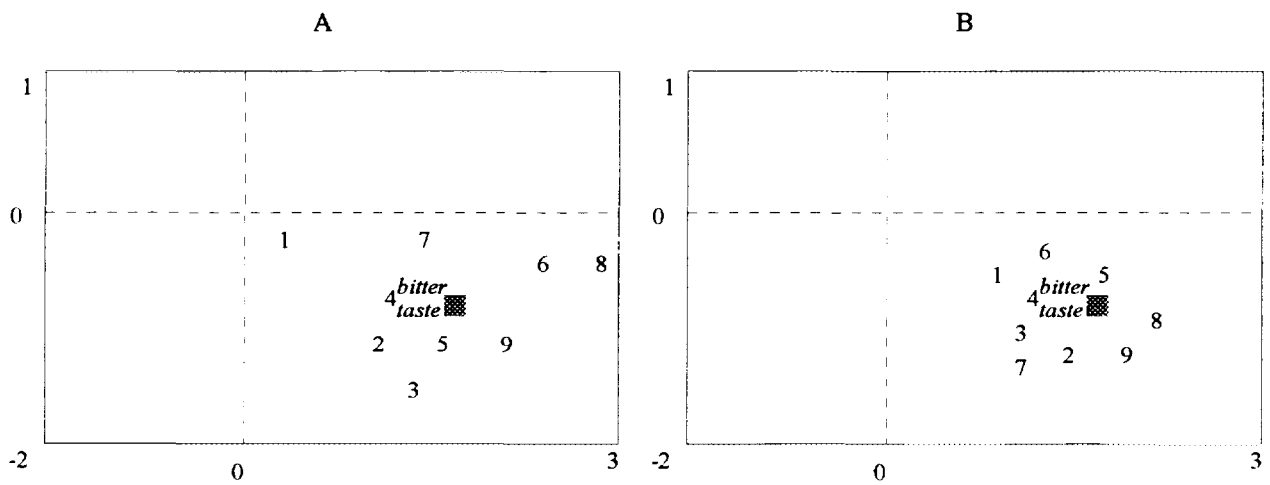


2- Use of the relevant chemicals to anchor panel's response.

Differences in regression equations for bitter taste and fragrant flavour witnessed possible differences in perception attitude of panels. This was likely to be due to absence of standard substances in the training and selection of assessors. It is not uncommon for the panel leader to lack suitable substances that can provide consistent guidance to recognition of descriptors and quantification of intensities.

In the case of bitter taste, we used apricot samples with both magnesium and phosphates exceeding 2S.E.M. to anchor bitter intensity scale to the upper extreme (score 8). When reaximined after training with the aid of the standard, the panels exhibited significant convergence, as clearly represented by individual correlations to bitter taste, obtained by Procrustes analysis of both chemical and sensory data (fig. 2).

Fig. 2. Generalised Procrustes Analysis of sensory + chemical data. Individual's correlations to bitter taste before (A) and after (B) training with the aid of a bitter puree standard. Data for panel 2 members. Numbers 1 to 9 indicate assessors.



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A METHOD USED TO SCREEN FOR ENDOCRINE DISRUPTERS AND MORE THAN 400 PESTICIDES

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A gas chromatographic (GC) method has been developed that can be used to screen for more than 400 pesticides and suspected endocrine disrupters. In principle, it can be used to screen for any GC-amenable pesticide, metabolite, or endocrine disrupter. The method relies on a new technique called retention time locking, a procedure that allows the chromatographer to reproduce analyte retention times independent of GC system, column length, or detector so long as columns with same stationary phase, nominal phase ratio and diameter are used. The chromatographer first locks the GC method so that all retention times match those listed in a 408-compound pesticide retention time database. Using GC/AED or other element-selective detectors, the analyst enters a peak's retention time and known elemental content (presence or absence of heteroatoms) into a dialog box. Software then searches the pesticide database for those compounds that elute at the correct retention time and have the right elemental content. Confirmation is usually performed by GC/MS.

INTRODUCTION

More than 700 pesticides are currently registered for use in the world (1) and many more continue to persist in the environment, even after being banned. For the protection of human health and the environment, acceptable limits in food and water have been set by governmental bureaus such as the United States Environmental Protection Agency (USEPA) and the Codex Alimentarius Commission (2). Numerous methods have been developed to screen for pesticide contamination in food (3-7) and the environment (8-10) to ensure that these standards are met.

Certain pesticides and other synthetic chemicals have been suspected of behaving as pseudo hormones, disrupting normal functions of the endocrine system in wildlife and humans. Maladies such as birth defects, behavioral changes, breast cancer, lowered sperm counts, and reduced intelligence have been blamed on exposure to endocrine disrupters (11). The 1996 publication of *Our Stolen Future*, a book by Colborn, Dumanoski, and Myers (11), brought these concerns to the attention of the public. Recently-passed legislation in the US calls for more testing of suspected endocrine disrupters and monitoring of them in food (12) and water (13) supplies. In order to facilitate more research into the endocrine disrupter issue, methods are needed to detect suspected compounds at trace levels.

There is still no universal method to analyze for all GC-amenable pesticides or suspected endocrine disrupters. While GC/MS methods are gaining in popularity, there are still some limitations. When GC/MS employs selected ion monitoring (SIM) or tandem mass spectrometry (MS/MS), method development is more tedious and any shift in GC retention times requires that individual analyte retention time windows be shifted accordingly. These methods are only capable of detecting compounds on the target list; there are still hundreds of pesticides, metabolites, and suspected endocrine disrupters that could be missed. On the other hand, methods based on scanning GC/MS alone require more sample cleanup to avoid interferences from coextracted indigenous compounds. Typically, these methods do not screen for many pesticide metabolites, endocrine disrupters, or other environmental contaminants. A method that could be used to screen for endocrine disrupters and almost all of the volatile pesticides and metabolites would offer a better means of monitoring the food supply and the environment.

This paper describes a universal method that, in principle, could be used to screen for any pesticide, metabolite, or endocrine disrupter that can be eluted from a gas chromatograph. As a first test of the concept, a method was developed to screen for 408 pesticides. The method is being expanded to include virtually all of the volatile pesticides, metabolites, and suspected endocrine disrupters. The screening procedure relies on a new gas

chromatographic technique called "retention time locking" (14) with database searching based on retention time and elemental content. This technique is used to narrow an analyte's identity to a few possibilities. Confirmation is performed by GC/MS or by calculation of a compound's elemental ratio using GC with atomic emission detection (GC/AED).

EXPERIMENTAL

SAMPLES. Fruit and vegetable extracts were obtained from the Florida Department of Agriculture and Consumer Services (Tallahassee) and from the Canadian Pest Management Regulatory Agency, Laboratory Services Subdivision (Ottawa). Samples from Florida were extracted using the Luke procedure (15-17) while those from Canada were prepared according to the method described by Fillion, et al. (5)

INSTRUMENTATION. Table 1 lists the instrumentation and chromatographic conditions used for GC/AED screening and GC/MS confirmation.

Table 1. Instrumentation and conditions of analysis.

GC/AED System	
Gas chromatograph	HP 6890
Automatic sampler	HP 6890 Series Automatic Sampler
Atomic Emission detector	HP G2350A Atomic Emission Detector
Computer for data acquisition & analysis	HP Vectra XM Series 4 5/150
Software	HP G2360AA GC/AED Software running on MS Windows 3.11
Column	30 m X 0.25 mm X 0.25 µm HP-5MS
GC Inlet	Split/splitless, 250°C or 280°C
Injection volumes	2 µL splitless or 5 µL pulsed splitless
Inlet pressure (splitless)[a]	27.6 psi, constant pressure for 2-µL injections
Inlet pressure program (pulsed splitless)[a]	60 psi (2.01 min), 10 psi/min to 27.9 psi (hold)
Oven temperature program	50°C (1.13 min), 30°C/min to 150°C (2 min), 3°C/min to 205°C (0 min), 10°C/min to 250°C (20 min)
AED transfer line temperature	260°C
AED cavity temperature	260°C
AED elements & wavelengths (nm)	Group 1: C 496, Cl 479, Br 478 Group 2: C 193, S 181, N 174 Group 3: P 178
GC/MS System	
Gas chromatograph	HP 6890
Automatic sampler	HP 6890 Series Automatic Sampler
Mass spectral detector	HP 5973 MSD
Computer for data acquisition & analysis	HP Vectra XU 6/200
Software	HP G1701AA Version A.03.00 running on MS Windows 95
Column	30 m X 0.25 mm X 0.25 µm HP-5MS
Inlet	Split/splitless, 250°C
Injection volume	2 µL
Inlet pressure[a]	14 psi (constant pressure)
Oven temperature program	Same as GC/AED
MSD Parameters	
Acquisition mode	Scan (35-550 amu)
Temperatures	Transfer line = 280°C, MS quad = 150°C, MS source = 230°C

a) The column head pressures shown are typical values. Exact values were determined as part of the retention time locking procedure.

SOFTWARE FOR METHOD TRANSLATION. Software for use in translating the GC method from one GC column to another column (same phase but different dimensions) was obtained from Hewlett-Packard Co. (Wilmington, DE); the software is available on the world wide web at the following address: http://www.dmo.hp.com/apg/servsup/usersoft/main.html

RESULTS AND DISCUSSION

RETENTION TIME LOCKING. Key to the development of this method is a new concept in gas chromatography called retention time locking. This is a procedure that allows the chromatographer to match analyte retention times from run to run, independent of the GC system, detector, or manufacturing variations in column dimensions; the only requirement is that the columns used have the same stationary phase and the same nominal diameter and phase ratio. For example, with retention time locking, it is possible to match analyte retention times on a GC/AED and a GC/MS even though the column outlet pressures are much different: 1.5 psi above ambient pressure for the AED and vacuum for the MSD. The procedure also compensates for differences in GC column length resulting from variations in manufacturing or from column cutting required during routine maintenance.

Retention time locking is accomplished by adjusting the GC column head pressure until a given analyte, such as an internal standard, has the required retention time. When this is done, all other analytes in the chromatogram will have the correct retention times as well. Software has been developed that can be used to determine the column head pressure that will correctly lock the retention times after a single "scouting" run.

With retention time locking, it is possible to measure pesticide retention times using a given GC method and then reproduce those retention times in subsequent runs on the same or different instruments. With this increased retention time precision and predictability, raw retention times become a far more useful indicator of analyte identity. With modern instrumentation and retention time locking, it seemed that raw retention times could be used for compound identification in much the same way that retention indices (18-19) have been used in the past, albeit with much less effort. The chromatographer could simply scan a table of pesticide retention times, eliminating all possibilities but those with close elution times under the same locked GC conditions.

Pesticides almost always contain heteroatoms and often have several in a single molecule; the most frequently encountered heteroatoms are O, P, S, N, Cl, Br, and F. GC/AED has been shown to be a useful tool for pesticide screening because it is selective for all of the elements found in these compounds (20-22). Thus, GC/AED screening provides valuable information about the elemental content of an unknown molecule. By including this elemental information along with the retention time, it should be possible to narrow pesticide "hits" to just a few possibilities.

PESTICIDE RETENTION TIME TABLE. To test this pesticide screening concept, a table of pesticide retention times and molecular formulas was required. Stan and Linkerhagner (7) recently published a list of 408 pesticides with their molecular formulas and their GC/AED retention times using a 25 m X 0.32 mm X 0.17 μm HP-5 column. While their retention time table and GC method could have been used as published, their column was not an ideal choice for GC/MS. Therefore, the GC method and all of the pesticide retention times were translated to a 30 m X 0.25 mm X 0.25 μm HP-5MS column which could be used for both GC/AED screening and GC/MS confirmation. These conversions were made using software for GC method translation developed by Blumberg (23-24). Further adjustments to the 408 retention times were made by curve fitting the actual retention times for 60 known compounds and applying the correction to the table.

PESTICIDE SCREENING METHOD. First, retention time locking was used to match GC/AED and GC/MS analyte retention times to those listed in the translated pesticide database. Prototype software for retention time locking was used to determine the column head pressure needed to produce a retention time of 25.216 min for p,p'-DDE. Using the GC/AED, element selective chromatograms were obtained for C, Cl, Br, N, S, P, and sometimes F and I. Prototype software was then used to search the database by retention time and elemental content.

Figure 1 is a screen capture from this software showing the dialog box used to input the search criteria. One must choose a search time window wide enough to be sure to include the correct analyte, but narrow enough to eliminate as many extraneous "hits" as possible. A value of 0.8 min was chosen because tests with several dozen compounds showed that, under locked conditions, pesticide retention times always fell within +/-0.3 min of the tabulated value. This time window would be smaller if one were to use a database generated on the same column under locked conditions. Of course, a narrower time window would generate fewer hits and a more accurate screening method.

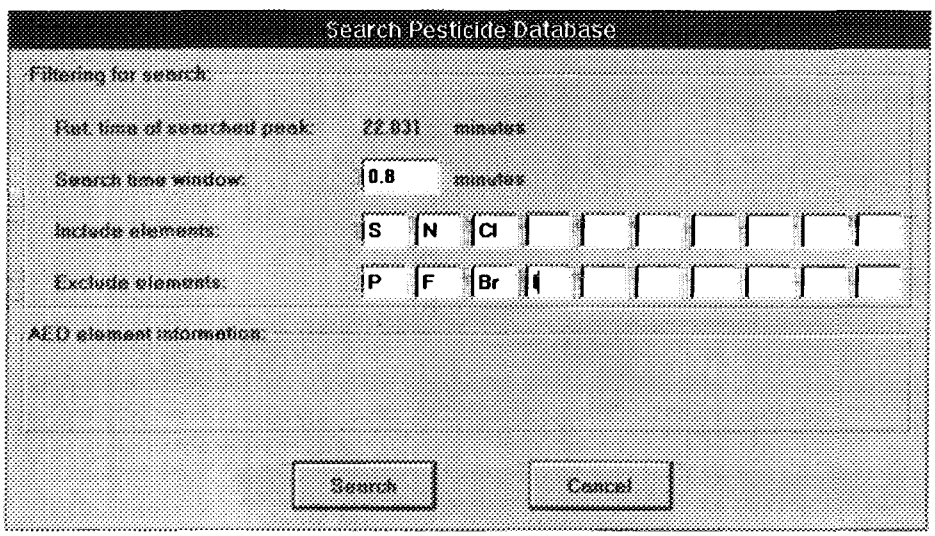

Figure 1. Dialog box used in the pesticide database searching software.

From the GC/AED chromatograms it is usually possible to determine which heteroatoms are present or absent in the suspected pesticide peak. All available information is added to the dialog box and this is used to focus the search on those pesticides that fall within the retention time window and have the specified elemental content. The search produces a list of pesticides that meet these criteria.

Confirmation is usually done by GC/MS under locked conditions so that all GC/MS retention times match the GC/AED values. Alternatively, when there is adequate signal to quantitate the analyte in multiple AED element-selective chromatograms, it is often possible to confirm a pesticide's identity simply by calculating its heteroatom ratio. GC/AED software for element ratioing facilitates this procedure. Although not yet tried, it should also be possible to use a second column with a different phase for confirmation. This would require developing a new retention time database on the confirmation column, but that is not yet available.

Figure 2 shows a set of GC/AED element-selective chromatograms obtained for a strawberry extract. Peaks in the S, N, P, and Cl chromatograms suggest the presence of several pesticides. The peak at 22.031 min contains S, N, and Cl but does not appear to have any P, F, Br, or I. When the database was searched only on the basis of the peak's retention time using a 0.8 min window, 20 possibilities were reported (Figure 3). However, when the elemental information was included in the search, only three of the 408 pesticides in the database met the criteria (Figure 4).

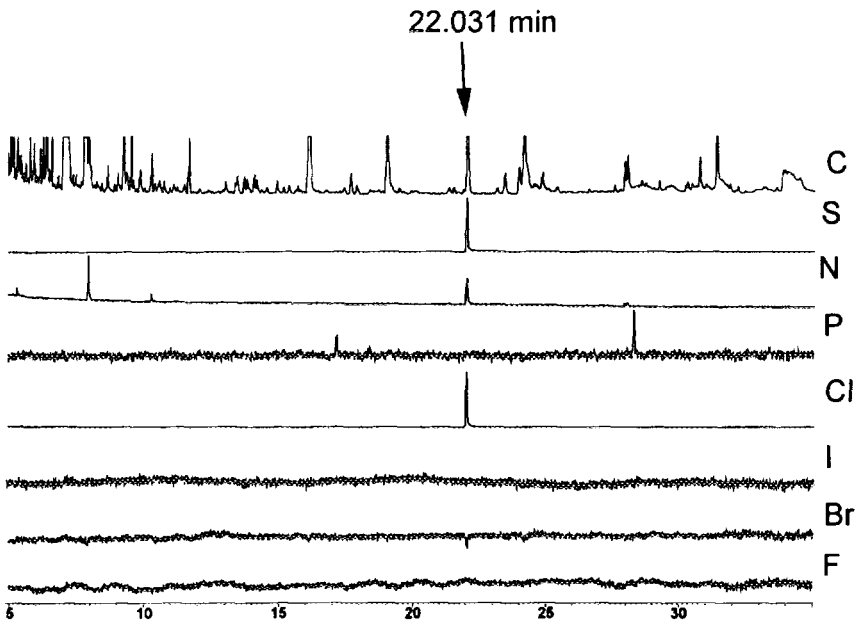

Figure 2. GC/AED element-selective chromatograms obtained for a strawberry extract. The GC/AED method was locked to the pesticide database.

Pesticide Search Results

#	Ret_Time	Mol_Formula	Compound_Name	Mol_Weight
1	21.76	C,15,H,23,N,3,O,4,	Isopropalin	309.37
2	21.79	C,14,H,16,Cl,1,N,5,O,5,S,1,	Triasulfuron	401.82
3	21.8	C,10,H,5,Cl,7,O,1,	Heptachlorepoxide-cis	389.32
4	21.81	C,14,H,16,Cl,1,N,3,O,1,	Metazachlor	277.75
5	21.84	C,19,H,26,O,3,	Allethrin	302.41
6	21.9	C,9,H,10,Br,1,Cl,1,N,2,O,2,	Chlorbromuron	293.55
7	21.9	C,10,H,5,Cl,7,O,1,	Heptachlorepoxide-trans	389.32
8	21.93	C,11,H,10,Cl,1,N,1,O,3,S,1,	Benazolin-ethyl	271.72
9	21.96	C,13,H,19,N,3,O,4,	Pendimethalin	281.31
10	21.99	C,12,H,14,N,4,O,4,S,2,	Thiophanate-methyl	342.39
11	22.06	C,13,H,15,Cl,2,N,3,	Penconazole	284.19
12	22.06	C,14,H,18,N,4,O,4,S,2,	Thiophanate-ethyl	370.44
13	22.08	C,11,H,21,N,5,S,1,	Dimethametryn	255.38
14	22.1	C,12,H,27,P,1,S,3,	Merphos I	298.5
15	22.11	C,9,H,5,Cl,3,N,4,	Anilazine	275.52
16	22.15	C,14,H,12,Cl,2,O,1,	Chlorfenethol	267.15
17	22.18	C,11,H,13,F,3,N,2,O,3,S,1,	Mefluidide	310.29
18	22.18	C,10,H,13,Cl,2,F,1,N,2,O,2,S,2,	Tolylfluanid	347.25
19	22.23	C,9,H,8,Cl,3,N,1,O,2,S,1,	Captan	300.59
20	22.36	C,13,H,11,Cl,2,N,1,O,5,	Chlozolinate	332.14

Figure 3. Database search results for the peak at 22.031 min (Figure 2). The database search used only retention time; no elemental information was entered into the search dialog box.

Pesticide Search Results

#	Ret_Time	Mol_Formula	Compound_Name	Mol_Weight
1	21.79	C,14,H,16,Cl,1,N,5,O,5,S,1,	Triasulfuron	401.82
2	21.93	C,11,H,10,Cl,1,N,1,O,3,S,1,	Benazolin-ethyl	271.72
3	22.23	C,9,H,8,Cl,3,N,1,O,2,S,1,	Captan	300.59

Figure 4. Database search results for the peak in the strawberry extract found at 22.031 min (Figure 2) when both retention time and elemental content were entered into the dialog box as shown in Figure 1.

The same sample was analyzed by GC/MS under locked conditions so that all suspect pesticides would have GC/MS retention times very close to their GC/AED values. Captan was found at 21.979 min, just 0.052 min away from its GC/AED retention time and close to the database value of 22.23 min (Figure 5). With retention time locking, it was possible to make the GC/AED, GC/MS, and database retention times all agree to within 0.25 min. This made it much easier to find the suspected pesticide in the total ion current chromatogram (TIC), because the compound's retention time was already known. Moreover, since the possibilities had been narrowed to just three compounds, their characteristic ions could be extracted to reduce the background contribution from coextracted indigenous materials.

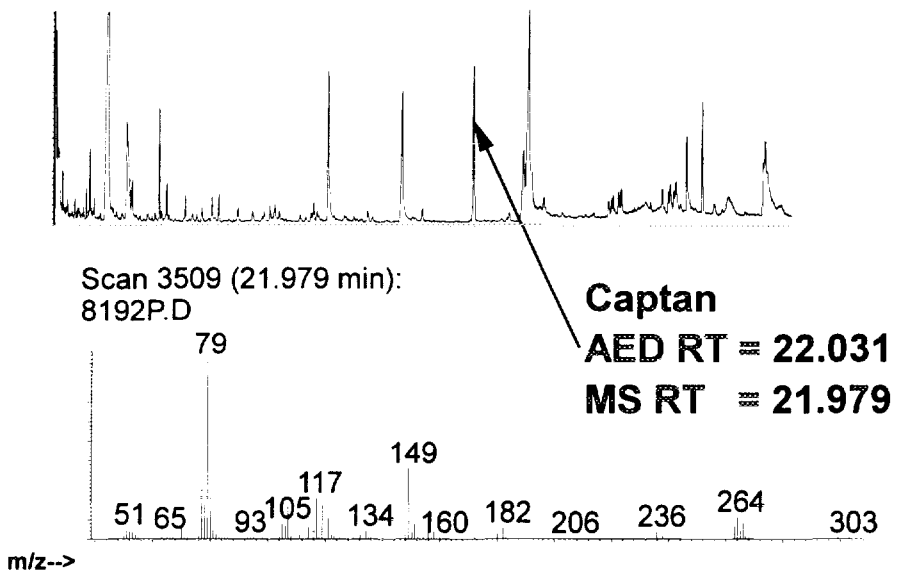

Figure 5. Scanning GC/MS analysis of the strawberry extract shown in Figure 2. The GC/MS method was locked so that retention times would match both the pesticide database and the GC/AED. Captan was identified at 21.979 min. Its retention times in the GC/AED and pesticide database were 22.031 min and 22.23 min, respectively.

Figure 6 shows S-, N-, P-, and Cl-selective chromatograms of an orange extract. When the database was searched for the chlorine-containing compound labeled 1, two possibilities were listed - aldrin and PCB-152 (Table 2). However, GC/MS could not confirm either of these possibilities nor were any other compounds suggested. Several explanations are possible for this discrepancy: a) the compound is not a pesticide or PCB, b) the compound is a pesticide or metabolite that does not appear in either the retention time or mass spectral databases, or c) one or both of the suggested compounds is not contained in the mass spectral libraries.

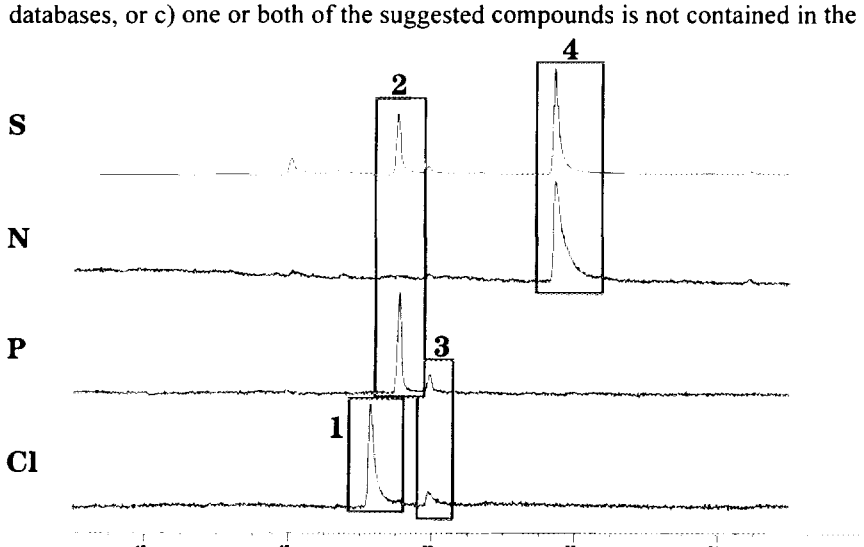

Figure 6. GC/AED element-selective chromatograms for an orange extract. The GC/AED method was locked to the pesticide database retention times. Peaks labeled 1-4 are identified in Table 2.

Table 2. Pesticides initially suggested by searching the pesticide database on the basis of retention times and elemental content. Identities as determined by GC/MS are shown along with pesticide concentrations determined using compound-independent calibration. Peak numbers refer to peaks labeled in Figure 6.

Peak Number	GC/AED Retention Time	Database Search Results Using AED Element Information	MS Confirmation	Concentration Using CIC (ppm)
1	19.152	Aldrin PCB-152	not found	0.6 (Cl only)
2	19.519	Malathion	Malathion	0.8
3	19.957	Chlorpyrifos Dicapthon	Chlorpyrifos	0.26
4	21.717	Thiophenate-methyl Thiophenate-ethyl Dimethametrynel	Thiabendazole (not in database)	2.28

Malathion (Table 2) was the only choice suggested for peak 2 (Figure 6) and this was easily confirmed by MS library searching. On the basis of retention time and elemental content, chlorpyrifos and dicapthon were suggested for peak 3. Chlorpyrifos was confirmed by GC/MS. Three possibilities were offered for peak 4 - thiophenate-methyl, thiophenate-ethyl, and dimethametrynel. However, at that retention time, GC/MS found thiabendazole. Thiabendazole was not in the original retention time database and this is why it was not given as a possibility. The database searching software allows one to add or edit entries, so thiabendazole was added to list. Even though thiabendazole was not in the original retention time database, it was still much easier to find and identify this compound in the TIC because retention time locking ensured that its GC/MS and GC/AED retention times were nearly the same.

Compound-independent calibration (CIC) is a GC/AED technique that allows one to use a single analyte as a calibration standard for all others that contain the same elements. Using chlorpyrifos as the element-selective calibration standard, the concentrations of malathion, chlorpyrifos, and thiabendazole in the orange extract were determined (Table 2). Even though peak 1 could not be identified, CIC could still be used to determine the concentration of Cl (0.6 ppm). For regulatory purposes this might be very useful. If the Cl level is found to be very low further investigation may not be required; however, if the Cl level is high, it may be necessary to work harder at compound identification.

SUMMARY

Most pesticide screening procedures are capable of finding only a fraction of the pesticides that are registered for use. This procedure has the capability of screening for virtually any volatile pesticide, metabolite, or endocrine disrupter. Although confirmation is usually required, GC/MS analysis is made much easier and more reliable because the pesticide's retention time is already known.

The pesticide database used to demonstrate the feasibility of this method was originally developed by Stan and Linkerhagner on a different gas chromatograph (HP 5890 Series II), using a different column, and without the benefit of retention time locking. Experience in this laboratory shows that retention time locking and the new generation of electronic pneumatic control should allow one to prepare a database with even more precise retention times. Moreover, retention time locking would allow chromatographers to match those retention times with far more accuracy than was routinely possible in the past. By creating a database from the beginning on the preferred GC column under locked conditions, it should be possible to narrow the required search window which would result in fewer hits and greater accuracy. A project is underway in this laboratory to create such a database on two different GC columns that will contain several hundred volatile pesticides, metabolites, and suspected endocrine disrupters.

ACKNOWLEDGMENTS

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AUTOMATIC NITROGEN / PROTEIN DETERMINATION IN FRUITS AND VEGETABLES USING THE DYNAMIC FLASH COMBUSTION METHOD AS AN ALTERNATIVE TO THE KJELDAHL METHOD.

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The determination of nitrogen and protein concentration in food products is becoming more and more important as it provides useful information for product characterization, nutritional studies, and product packaging labeling.
The traditional method for the nitrogen/protein determination is based on a classical wet chemistry procedure developed by Kjeldahl in 1883 (1). The flash combustion method proposed in this paper as the alternative to the Kjeldahl method (2, 3) offers simpler, faster and more reliable results without all the inherent problems of the classical method.

KEY WORDS: Nitrogen/protein determination, Combustion method, Fruits and vegetables, Automatic analyzer

The nutritional quality of fruits and vegetables plays an important dietary role for human beings. It depends on one of the most important nutrients: the protein content.
Determination of total nitrogen is the simplest way to establish the total protein content as the direct method for total protein determination is very difficult due to the complexity and variety of the protein molecules present in food.

The first step of the analytical process is the dynamic flash combustion, this is a reaction that quantitatively convert nitrogen bonded into elemental nitrogen. The sample is weighed in a tin container and loaded into the autosampler. It is then dropped into a quartz combustion reactor heated at 900 °C and filled with oxidation catalyst, the sample enters the reactor that is constantly flushed with helium carrier gas and its atmosphere is enriched by a fixed amount of oxygen just seconds before the sample drop.

As soon as the sample enters the oxygen-enriched reactor, an instantaneous highly exothermic oxidation reaction occurs: the heat generated by the tin oxidation increases the local temperature to a value of 1800 °C, that assures a complete quantitative combustion of any type of sample.

The helium stream sweeps the gases yielded by the combustion to a second reactor filled with pure copper, that ensures the reduction of the nitrogen oxides to molecular nitrogen and the removal of the excess of oxygen left by the combustion. A first soda lime filter and a second anhydrone trap adsorb carbon dioxide and water respectively.
The nitrogen is then carried to an activated charcoal GC column and to a thermal conductivity detector.

Figure 1 illustrates a schematic analytical diagram.

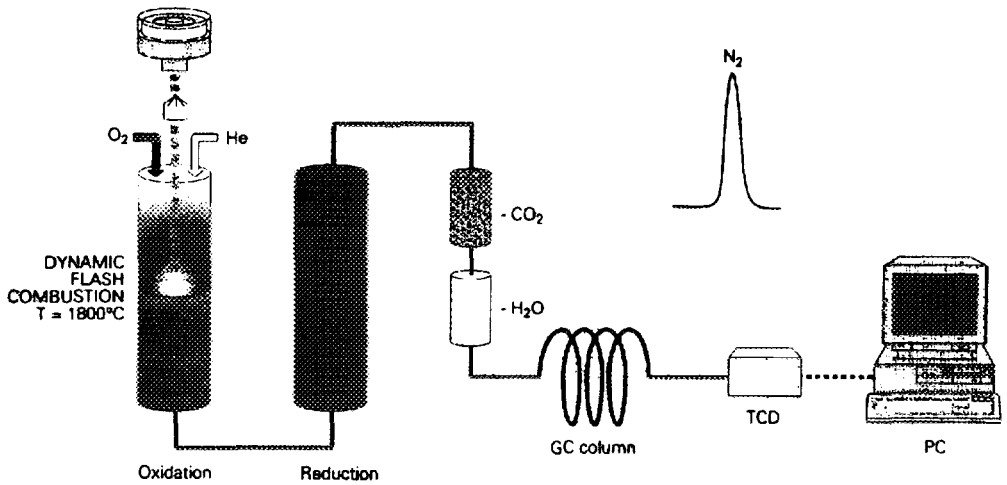

FIGURE 1. ANALYTICAL LAYOUT OF THE COMBUSTION ANALYZER NA 2100 PROTEIN

A series of analytical results for total nitrogen and protein determination in several different fruits and vegetables using the NA 2100 Protein Analyzer is shown in Table 1. To calculate the sample protein content from the nitrogen values, the corresponding conversion factor indicated by the United Nations Food and Agriculture Organization (FAO) (4) was used.
Table 2 demonstrates the reproducibility of ten consecutive analyses of a yoghurt sample with the statistic data.
Table 3 shows a comparison between the combustion and the classical Kjeldahl methods for nitrogen determination.

TABLE 1 - DETERMINATION OF NITROGEN / PROTEIN IN VARIOUS PRODUCTS

Sample	Nitrogen	Protein %	RSD%
Fruit Cake	1.430 %	8.937	0.980
Fruit Cake with nuts	1.030 %	6.437	1.840
Fruit Biscuits	1.254 %	7.837	0.513
Fruit biscuits with nuts	1.142 %	7.137	0.856
Yoghurt 1	0.719 %	4.497	0.706
Yoghurt 2	0.441 %	2.813	2.046
Baby Food Vegetable 1	0.156 %	0.975	0.370
Baby Food Vegetable 2	0.231 %	1.475	1.798
Chocolate with fruit	2.044 %	12.77	0.412
Apple Juice	109 ppm	0.068	1.554
Juice	150 ppm	0.094	2.137
Red Wine	274 ppm	0.171	1.125
White Wine	230 ppm	0.144	1.207
Tomato leaves	3.053 %	19.081	0.302
Potato leaves	0.309 %	1.931	0.692

TABLE 2 - NITROGEN/PROTEIN DETERMINATION REPRODUCIBILITY IN A FRUIT YOGHURT SAMPLE

	Nitrogen %	Protein %
	0.724	4.529
	0.728	4.549
	0.713	4.456
	0.719	4.494
	0.725	4.535
	0.712	4.453
	0.717	4.484
	0.718	4.487
	0.719	4.498
	0.718	4.492
Average %	0.719	4.497
SD	0.005	0.032
RSD %	0.706	0.706

TABLE 3 - NA 2100 COMBUSTION ANALYZER VS KJELDAHL METHOD

Sample	Kjeldahl N %	NA 2100 N %	RSD %
Fruit Cake	1.44	1.43	0.980
Fruit Cake with nuts	0.98	1.03	1.840
Yoghurt	0.46	0.44	2.047
Chocolate with fruit	1.15	1.14	0.870
Juice	0.010	0.011	2.058

CONCLUSIONS

Samples belonging to the main categories of fruits and vegetables were analyzed to evaluate the influence of various matrices. No memory effect was observed when type of sample was changed.
The results obtained with the automatic elemental analyzer are perfectly comparable with the classical Kjeldahl method and the reproducibility obtained was more than satisfactory taking into account the different levels of nitrogen present in the samples analyzed and the various matrices.
In conclusions, the combustion method can be successfully employed for all type of fruits and vegetables, therefore it has proven to be a viable and ideal alternative to the Kjeldahl method.
The AOAC (Association of Official Analytical Chemists) has approved the combustion method as the alternative to the traditional procedure for protein in animal feed (AOAC Method 990.03) and the analysis of crude protein in meat and meat products (AOAC Method 992.15) (5). Furthermore, the combustion method has been also approved and adopted by the American Association of Cereals Chemists (AACC) Method 46-30 for crude protein in cereal grains and cereal products; by the American Oil Chemists' Society (AOCS) Method Ba 4e-93 for crude protein in oil seeds; and by the American Society of Brewery Chemists (ASBC) for the analysis of beer and wort.

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Neural networks based analysis of NIR spectra: determination of SSC in kiwi fruit

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ABSTRACT

Few years ago there was a raising interest on new applications of NIR spectroscopy characterized by the use of more advanced statistical methods (e.g. the development, inside the NIR research community, of PLS regression).
This paper report on the most recent techniques based on the use of flexible non-linear regression tools such as *neural networks* and *Bayesian neural networks*.
Our case study is about the determination of SSC (Soluble Solids Content) in kiwi fruit. *Gaussian process regression*, equivalent in a particular limit to an infinite dimensional net but easier to understand in his behavior, is used to achieve an high accuracy, not easy to reach by more standard methods (R^2=0.98, *SEC*=0.40, *SEV*=0.47).

INTRODUCTION

NIR spectroscopy is an effective nondestructive optical method for determining the quality of fruits, vegetables, and cereals[35,24,20].

During the 80*s* a strong interest grew in the use of advanced statistical techniques in the calibration procedures: PLS (Partial Least Squares) regression, ideated by H. Wold[36] and largely unknown to statisticians, was specifically developed by chemometricians[13].

In the first part of this work we review the main developments in neural networks-based approaches to regression and classification emphasizing, in particular, the role of the emerging *Bayesian* framework. Recently, thanks to the work of D.J.C. MacKay, H.H. Thodberg, R.M. Neal and C.E. Rasmussen among others, the theory is advancing at a rapid pace and, at the same time, successful applications are being developed.

In an effort to make contact with real life problems, Gaussian process regression, demonstrably equivalent, inside the Bayesian framework, to three layered networks with an infinite number of hidden neurons, has been developed. It combines the flexibility of the usual techniques with a more intuitive parameterisation.

In the last part of the work we present an application of Gaussian process regression to NIR spectroscopy: a problem of calibration for SSC in kiwi fruit. Our small data set (a total of 86 samples) had proven quite difficult to deal with using "classic" linear calibration techniques.

NEURAL NETWORKS AND THEIR NIR APPLICATIONS

The real relationships between a spectrum and the corresponding chemical constituents are in general of a complex non-linear nature: this can be seen, typically, when the *range* of the latter is very large. Often the solution adopted then is to split the range into smaller regions over which linear approximations are adequate.

In fact many *non-linear regression* methods exists, quite popular in the modern chemometrics. For instance, *flexible* techniques such as MARS (Multivariate Adaptive Regression Splines) and "Projection pursuit" regression or, for regression and *classification* problems, CART (Classification and Regression Trees)[26]. Also, non linear variants of PLS have been proposed that use "regularizers" or "splines" in the regression but follows closely the original algorithm in the general structure[10,37].

Neural networks are a family of techniques, of loose biological inspiration (see e.g.,[6]), quite popular today[26,2].

A *feed-forward* net, the most common type, consists of a set of simple computation units, called *neurons*. Each of them computes a weighted sum of his inputs, transform it by a *sigmoidal* (non linear) function[a] and outputs the result. If M is the number of inputs, p_i a weight coefficient and *offset* a constant, the input-output function of a single neuron is, therefore, the following:

$$output = sigmoid(offset + \sum_{i=1}^{M} p_i input_i) .$$

Neurons are organized in *layers*: those of a layer have inputs connected only to the units of the preceding layer and feed only the units of the following layers, most of the times only the next one. In the example of Figure 1 we have five neurons in the input layer, three in the central one (a, so called, "hidden" layer, being not directly connected to the inputs and the output), and one in output.

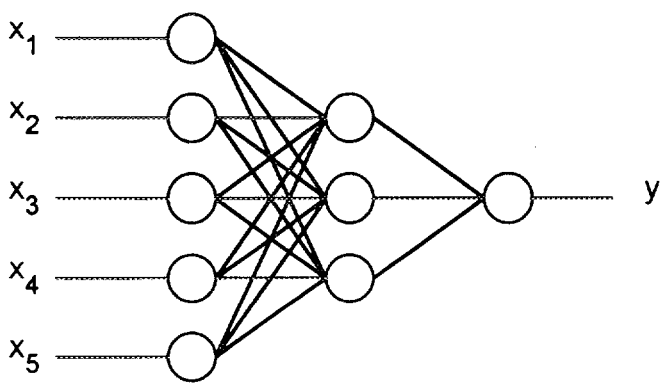

Figure 1 A three layers feed-forward net 5:3:1.

To adapt ("learning phase") a generic net to a particular task (of regression, classification or other) the weights and offset of each neuron are changed, in addition, of course, to select the topology of the net itself (number of layers and number of neurons in each layer).

The study of these non-linear systems knew a big explosion in the second half of the 80*s*, after the discover of an algorithm ("error back-propagation" procedure[27]) for the automatic determination of a good set of weights for a given task. The latter is indirectly specified using a set of input-output calibration pairs, in complete analogy with linear chemometrical techniques.

Thanks to the big number of parameters, these architectures[b] are very flexible but, on the other hand, are prone to "overfit" if the set of calibration samples is too small. Cross-validation techniques[31,9] can be effectively employed in order to determine the minimal complexity nets among those able to solve a given problem: this can be done controlling the number of layers (generally not bigger than four or five), the number of neurons, and the number of weights fixed to the null value[c]. Many alternative approaches have been devised inside this area of research: for up to date reviews see[26,2] and references therein.

As regards NIR spectroscopy applications, Wu and others recently presented a pair of detailed studies on the selection of input variables[38] and on the design of the training set[39].

In fact, back in 1992, Borgaard and Thodberg[4] already successfully employed standard cross-validation techniques in the *simplification* (i.e. pruning) of three layered nets. Direct connections between the input and the output (equivalent to standard multiple linear regression) were a priori allowed: the training could choose the best mix of linear and nonlinear behavior. Both a synthetic problem and a real-life one were studied, the determination of fat content in minced pork meat samples, and the results were very encouraging: errors reduced even by 50-75% with respect to more traditional approaches based on linear PCR (Principal Component Regression) and PLS.

[a] A typical sigmoidal function can be, for instance, the hyperbolic tangent.
[b] Quite often, in fact, the neural nets are *simulated* via software and not realized in hardware as massively parallel architectures.
[c] In this case the corresponding connection between the neurons is in fact *pruned*.

Bochereau et al.[3] predicted the soluble solids content in apples and studied the *direct* margin of improvement on PCR: the nets were trained by the residual error of calibration of the linear regression. In this particular application the improvement amounted to about 9%.

Delwiche et al.[7] and Song et al.[30], at the "Beltsville Agricultural Research Center", also used neural nets for the classification of varieties of wheat, in small quantities (80 grams) and in single kernels. In the former contribution, artificial neural networks performed better than PLS and various established chemometrical techniques: they were able to tell "hard red winter" from "hard red spring" with accuracies up to 95-98%. Moreover, the number of input nodes could be reduced from 223 to 111 with minor losses of accuracy provided the full wavelength range was preserved. In the latter, a neural net gave a fast solution, only 7 mins to calibrate and validate the model, and still good accuracies using the restricted wavelength range 899-1049 nm.

Anyway, the architectures employed had just two layers (functionally equivalent to classical linear techniques) and no advanced overfitting control techniques were used.

In the chemometrics field analytical methodologies have been recently suggested which try to integrate neural networks-based approaches with "traditional" PLS and PCR.

Frank[11] studied a structure that computes the factors by traditional linear techniques but uses flexible regressors or splines in the regression. The optimal number of regressors is automatically set by the procedure using cross-validation techniques. The author tried the algorithms on three chemical data sets and compared the fit and the prediction of the models with those of PCR and PLS. The first one, in particular, consisted of 60 NIR spectra of soybean samples to be regressed against the oil content measurements. The new methods outperformed the classical techniques in terms both of R^2 and cross validated R^2.

Another interesting study is that by Malthouse et al.[19]. PLS was extended (NonLinear Partial Least Squares) using three layered neural nets both in the determination of regression factors and in the regression itself. PLS, NLPLS, and "vanilla" neural nets were compared on a set of artificial examples and the respective strengths evidenced.

BAYESIAN STATISTICS AND BAYESIAN NEURAL NETWORKS

The Bayesian school of statistics differs from the "frequentist" one, today prevailing, because employs probabilities to quantify *degrees of belief* in values of parameters or in models[5,14].

The well known *Bayes formula*,

$$P(A|D) = \frac{P(D|A)P(A)}{P(D)},$$

acquire therefore a more profound meaning becoming a mean to evaluate the variation of degree of belief in an hypothesis A in the light of new experimental evidence D.

At a *first* level of *inference* we assume that a model, for example the i^{th} one, is true and we compute the new probabilities of the model parameters w in the light of the evidence D:

$$P(w|D,H_i) = \frac{P(D|w,H_i)P(w|H_i)}{P(D|H_i)}.$$

At a *second* level we want to determine what model is the more plausible in the light of the data. The *posterior* probability of the i^{th} model is given by:

$$P(H_i|D) \propto P(D|H_i)P(H_i).$$

The $P(D|H_i)$ term is called *evidence;* the term $P(H_i)$ is the probability subjectively attributed *a priori* to the i^{th} model before the new evidence D arrives.

If we assume to assign equal a priori probabilities to the various models, we have one of the basic tenets of the Bayesian school: *rank the models in accordance with the evidence assigned by the data.*

Empirically the evidence anticorrelates *well* with the generalization of the model but at present the relation between evidence and generalization (i.e. validation set error) cannot be proved in a closed mathematical settings.

Thodberg[32] argues that perhaps it is fundamentally impossible to prove this relationship, see[2] for an interesting discussion.

The evidence can be formally expressed as:

$$P(D|H_i) = \int P(D|w,H_i)P(w|H_i)dw .$$

This integral can be approximated in various ways, e.g. using stochastic "Monte Carlo" techniques, reviewed in[21], see also[18] for an introduction.

D.J.C. MacKay applied the preceding framework in the training of neural networks, in particular of the common feed-forward type (see e.g.[16,2] and references therein). In this case the various models are the possible topologies of the net (number of layers, number of neurons in each layer, pruned weights) and the parameters to be inferred are, of course, the weights of each neuron.

These are called, therefore, *Bayesian neural networks*, meaning that the choice of the net topology and the following training are managed as problems of inference accordingly to the Bayesian statistical paradigm. Also, the *use* of the networks should be Bayesian in spirit: the set of most probable a posteriori networks should be considered to perform the regression.

MacKay also developed some interesting techniques for the efficient implementation of Bayesian neural networks based on Gaussian approximations (see[32,33] for clear and detailed reviews). However different approaches are actually possible: as part of his PhD work Neal[22] implemented variants of "hybrid Monte Carlo" methods[d].

Thodberg[32,33] proposed the first application in the analysis of NIR spectra. The real-life problem studied is the same as in[4] and the approach is *fully* Bayesian: in the optimization of the topology, in the inference of parameters, and in the use of the net: in[32] a *committee* of 12 networks, favoured by the evidence and approximating the set of most probable a posteriori nets, is considered to perform the regression. PCR, "quadratic regression" and less advanced neural techniques were largely outperformed and error bars on the prediction could be accurately evaluated.

GAUSSIAN PROCESS REGRESSION

In the Bayesian treatment of neural networks, a question immediately arises as to how many hidden units are believed to be appropriate for a task. Neal[22] has argued compellingly that for real world problems, there is no reason to believe that neural networks models should be limited to nets containing a "small" number of hidden units. He has shown that it is sensible to consider a limit where the number of hidden units in a net tends to infinity, and that good predictions can be obtained from such models using the Bayesian machinery. He has also shown ([22], see also[34]) that many Bayesian regression models based on neural networks converge to Gaussian processes in the limit of an infinite network.

The empirical work of Rasmussen[25] has demonstrated that Gaussian process models have better predictive performance than several other flexible regression methods over a wide range of tasks[e].

The characteristics of a Gaussian process model can easily be controlled by writing the covariance function in terms of "hyperparameters". In a fully Bayesian approach, the hyperparameters are given prior distributions. Predictions are then made by averaging over the posterior distribution for the hyperparameters, which can be done using Monte Carlo methods[23].

The covariance function is usually specified as a sum of one or more terms of the following types:

1) A constant part, which is the same for any pair of cases, regardless of the inputs in those cases. This add a constant component to the regression function, with the prior for the value of this constant component having the variance given by the constant term in the covariance function.
2) A linear part, which for the covariance between cases i and j has the form:

$$\sum_u x_u^{(i)} x_u^{(j)} \sigma_u^2 .$$

This produces a linear function of the inputs x_u, or adds a linear component to the function, if there are other terms in the covariance as well.

[d] The software developed is publicly available via the following "URL": `http://www.cs.utoronto.ca/~radford/`.
[e] For a general introduction to Gaussian process models also see[23,15].

3) Any number of exponential parts, each of which, for the covariance between the cases i and j, has the form:

$$\eta^2 \prod_u \exp\left(-\left(\rho_u \left| x_u^{(i)} - x_u^{(j)} \right|^R \right) \right).$$

R must be in the range 0 to 2. The value $R = 2$ produces a function that is infinitely differentiable.

The parameters of these terms in the covariance function (such as the σ_u or the ρ_u) are usually treated as hyperparameters, with given prior distribution.

For problems with more than one input variable, the σ_u and ρ_u parameters control the degree to which each input is relevant to predicting the target. If ρ_u is close to zero, input u will have little effect on the degree of covariance between cases. Two cases could therefore have high covariance even if they have greatly different values for input u : i.e. input u is effectively ignored.

CASE STUDY: SSC IN KIWIFRUIT

We applied Gaussian process regression to a real life problem of NIR spectroscopy, adapting software developed by R. Neal and publicly available via his own "WWW" pages [f].

A total of 86 kiwi fruits were considered, randomly split in a 60 samples calibration set and a 26 samples validation set.

The NIR data were recorded by a rapid scanning (1.8 scans/sec) Vis-NIR (400-2500 nm) spectrophotometer *NIRSystems* 6500 using a fiber optic interactance probe. To collect the spectra each peeled fruit was hand placed against the probe at random locations on the equator for about 15 seconds, corresponding to 20 averaged scans. Two averaged lectures were performed on each fruit.

Following the optical measurement, the reference soluble solids content of tissue cut from the same location where the scan was conducted was determined using a temperature compensated *Officine Galileo* refractometer. The SSC range was 9.98-16.30 °Brix.

The NIR spectral data was lightly preprocessed selecting a spectral line every two acquired (resulting in data points 4 nm spaced) and removing the first 12 (i.e. 48 nm) and final 75 (i.e. 300 nm) data points, affected by some noise due to the long (2 m) fiber optic probe.

Preliminarily, we studied this data set using the *NIRS-2* Version 4.00 package by *Infrasoft International* and experimented with the various options available in the "spectra calibration" software module, e.g. regression type ("stepwise", "PLS", "Modified PLS"), pretreatments ("derivativizing", "SNV", "detrend", "MSC"), and so on. We were able to obtain R^2 as high as 0.95-0.97 on the calibration set but the regression always performed quite bad on the validation set, with SEV*s* never below the 0.8-0.9 °Brix level[g].

Therefore, as a first approach to Gaussian process calibration, we processed by principal component analysis our NIR data and we used the first 7 principal components as inputs to the Gaussian process regression software.

Various possibilities for the parameters were considered and eventually we set on the following choices for the model and the Monte Carlo sampling phase:

```
gp-spec $1 7 1 5 0.05:0.5 0.001 / 0.05:0.5 x0.05::1
model-spec $1 real 0.05:0.5::4
mc-spec $1 sample-variances heatbath hybrid 20 0.008
```

The covariance function comprised a constant part, a linear part and an exponential part. The σ_u in the linear part were all equal, the ρ_u in the exponential part a priori all different but equally distributed[h].

[f] `http://www.cs.utoronto.ca/~radford/`.

[g] The bad performances of the standard linear calibration techniques (most of the times very satisfying) on this data set was the original motivation for trying Gaussian process regression on NIR problems.

[h] See the documentation accompanying the software package for detailed explanations of the various options.

Each run consisted of a few hundred "leaps" and take an acceptable total time of under one hour on a *Pentium-133, Linux-2.0* system.

We obtained an R^2 larger than 0.98 on the samples selected for calibration, corresponding to a SEC (Standard Error of Calibration) of about 0.40 (see Figure 2), and a SEV (Standard Error of Validation) of 0.47 on the validation samples.

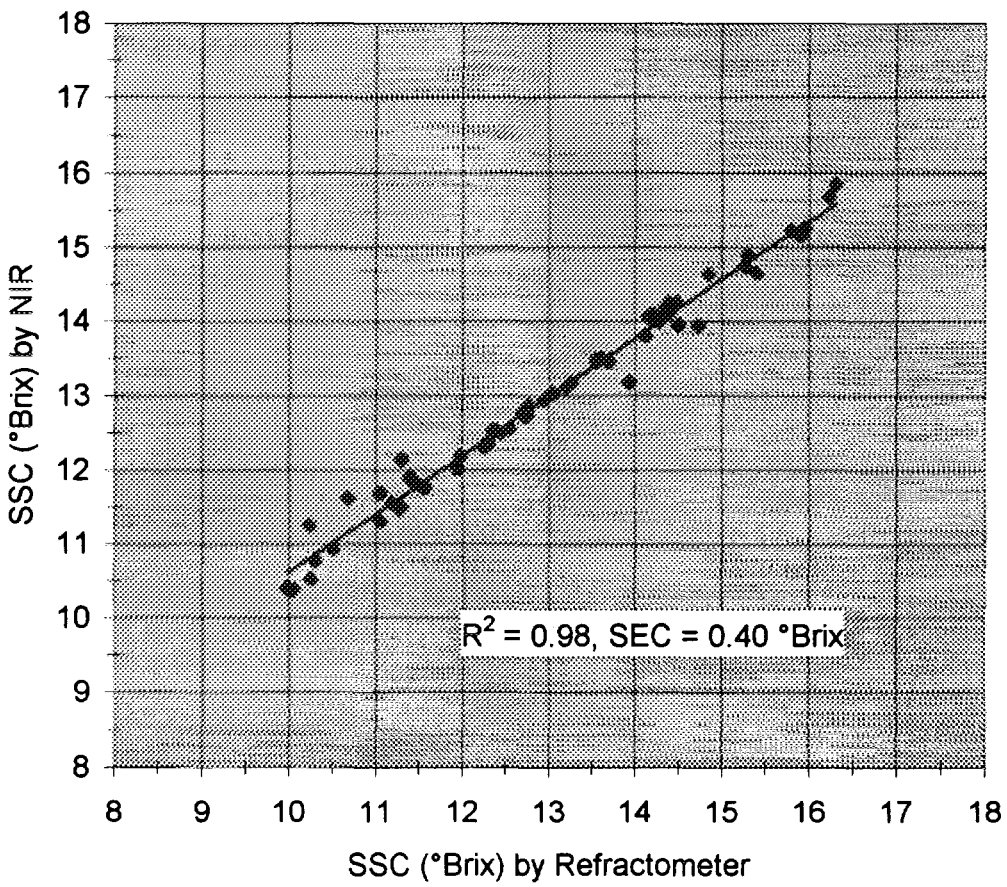

Figure 2.

CONCLUSIONS

On our small problem, Gaussian process regression performed appreciably better than available linear calibration techniques. However the full potential in NIR spectroscopy of Bayesian neural networks, and, in particular Gaussian process regression is still almost unexplored.

In the near future we would like to extend our experimentation to many more problems, in particular in the determination of constituents (e.g., total SSC, simple sugars, etc.) without damaging the fruit, a well known successful application of NIR spectroscopy in post-harvest quality evaluation.

Moreover, we intend to study in detail the effect on the calibration of the possible data pretreatments. A structure similar to that studied by Frank[11] but involving Gaussian process regression in the inner part could be tried out.

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On-Line Monitoring of Food Volatiles by Laser Ionization-Mass Spectrometry (REMPI-TOFMS): First Results for Coffee Roasting, Tea and Cocoa

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ABSTRACT

The combination of laser induced resonance-enhanced multiphoton ionization (REMPI) and time-of-flight mass spectrometry (TOFMS) represents a highly selective as well as sensitive analytical technique, well suited for species selective real-time, on-line monitoring of trace-gases. Here we present a newly designed, mobile REMPI-TOFMS instrument and discuss some applications on food products. This includes on-line analysis of off-gases during the roasting of coffee beans and headspace measurements from coffee, tea and cocoa.

INTRODUCTION

The typical flavour of coffee, cocoa or tea is not present in the fruits or leaves as they are harvested (i.e. in green coffee beans, cocoa beans or unfermented tea leaves). The multitude of the characteristic flavour compounds is formed during the technical processing of the raw material. For example, many flavour active compounds in coffee are formed by pyrolysis of precursor compounds or thermal induced chemical reactions in the coffee beans during the roasting process[1,2,3]. The formation of flavours during the coffee roasting process is highly dynamic. For on-line process analysis in this field it is important to record the concentrations of key flavour compounds or suitable indicator compounds in a fast and continuous manner. However, due to the high complexity of the process gas, an on-line registration of specific organic compounds in the ppbv concentration range is not feasible with conventional analytical techniques.
The laser induced resonance-enhanced multiphoton ionization (REMPI) time-of-flight mass spectrometry (TOFMS) technique is well suited for on-line analysis of complex gas mixtures and therefore preferably can be applied for process gas analysis[4,5,8]. The high overall selectivity is due to the combination of mass- and optical selectivity within the REMPI-TOFMS approach (i.e. REMPI-TOFMS represents a two dimensional analytical method). Achievable REMPI detection sensitivities are in the ppbv-range (in gas phase) or better for several compounds.
The results presented in this contribution emphasize an application of REMPI-TOFMS for continuos on-line monitoring of trace chemicals in process gases from industrial food processing plants. The final aim would be the realization of a feed back process steering that allows an optimization of quality, safety and economic efficiency of food production processes and products.

TECHNIQUE and EXPERIMENTAL SETUP

The resonance-enhanced multiphoton ionization technique (REMPI) introduces tunable substance selectivity into mass spectrometry. The principle of REMPI and its use as ion source for time-of-flight mass spectrometry (TOFMS)

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is described elsewhere[4,5,6]. Briefly, the optical selectivity of REMPI with pulsed lasers is given by the resonance condition for multiphoton absorption processes. The intensity of e.g. a two-photon absorption is enhanced by several orders of magnitude if the wavelength of the laser is in resonance with the excitation energy of an „UV-spectroscopic" transition of the target molecule. If the energy of the absorbed photons exceeds the molecular ionization potential, two-photon photo-ionization occur. By this resonance condition, laser UV-spectroscopy is directly involved in the ionization process. With REMPI often selective and soft (i.e. without fragmentation) ionization of target compounds even from complex substance mixtures is feasible. The formed ions are subsequently mass analyzed in a time-of-flight mass spectrometer. Figure 1 exhibits a schematic representation of the experimental setup as it was used for the on-line REMPI-TOFMS measurements presented within this article. The degree of optical selectivity depends on the sample inlet technique. Ultra high selectivity is achievable with the supersonic molecular beam (SMB) inlet technique. The SMB-inlet (or „Jet"-inlet) performs a sample cooling in the gas phase without condensation. As the UV-spectroscopic transitions of cold molecules exhibit a distinct vibronic fine structure, highly selective ionization is possible under SMB conditions, making even isomeric compounds distinguishable[6].

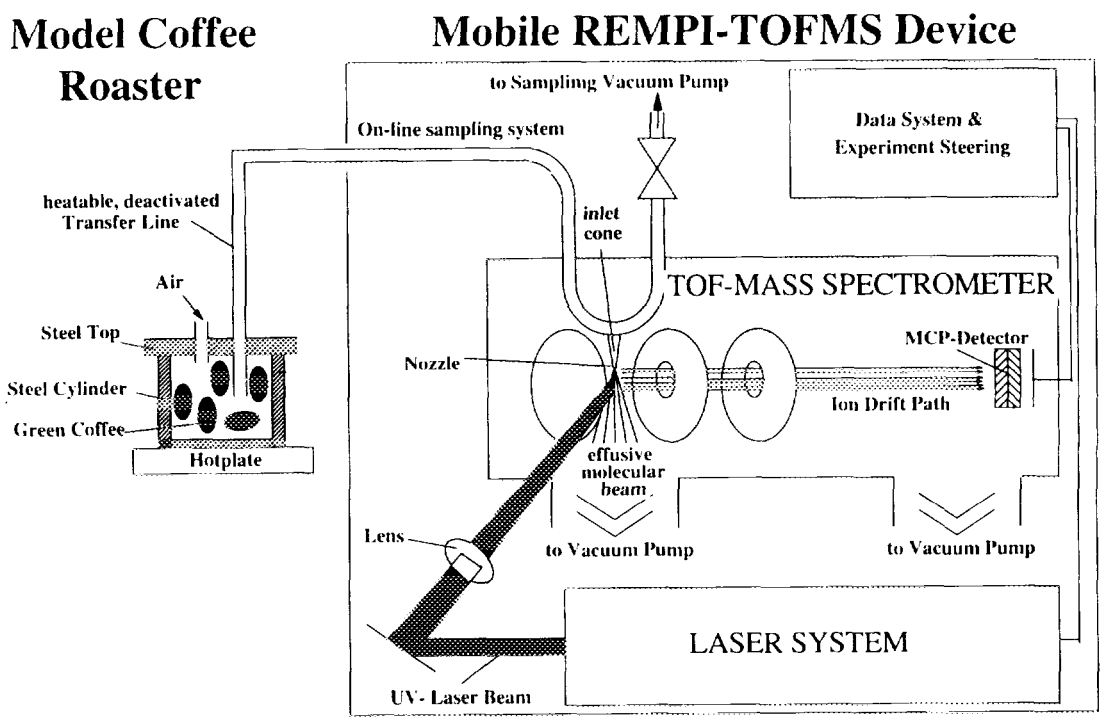

Figure 1
Schematic representation of the mobile REMPI-TOFMS device. The apparatus consists of a linear TOF-mass spectrometer, an ionization laser, data acquisition/analysis and steering, the vacuum system and, crucial for on-line applications e.g. of coffee-roasting off-gases, our newly developed on-line sampling/TOFMS inlet system. All components, expect of the data acquisition computer, are mounted in one cabinet (dimensions: 1.15 m x 0.85 m x 1.45 m, approximated whight: 250 kg

With an effusive molecular beam inlet (EMB) medium optical selectivity is obtained. The EMB inlet is easy manageable and therefore advantageous for practical REMPI-TOFMS applications under industrial conditions. Our mobile on-line REMPI-TOFMS monitoring device was designed for application at industrial settings (i.e. for process analysis). Therefore a compact and robust setup was realized. Nevertheless, the flexibility of the system still is high. The TOFMS can be converted between linear (highest sensitivity but restricted mass resolution) and reflectron (less sensitive but considerably enhanced mass resolution) mode, SMB- (free jet or skimmed jet[7], for highest optical selectivity) or EMB-inlet (less sophisticated but still medium optical selectivity) and can be used with an build-in fixed wavelength laser (*Lambda Physik* excimer laser MINEX™, 248 nm or *Continuum* Nd:YAG laser SURELIGHT™, 266 nm) or an external, tunable laser system (sophisticated, but allows highest selectivity in combination with SMB inlet). For the presented measurements a linear TOFMS set up was combined with an effusive sample inlet (EMB) and an attached fixed-wavelength laser. Although mass- and optical-selectivity are restricted in the linear TOFMS setup with EMB inlet, the overall selectivity still is sufficiently high for successful applications on volatiles from food products. Data acquisition and analysis is performed via a 500MHz transient recorder (*Signatec DA500A* on board in a personal computer). The acquisition rate of for entire mass spectra 16 kbyte is 10 Hz. Data

analysis (e.g. averaging for increase of the detection limits) can be performed on the preserved original data. However, for real-time display of the mass spectrometric information during the measurements a digital storage oscilloscope (*Le Croy 9361,* 300Mhz oscilloscope) is used. The dimension of the entire on-line REMPI-TOFMS device, including the laser, is 1.15m x 0.85m x 1.45 *m*. The apparatus is on castors and exhibits hooks for crane-lifting. The on-line REMPI-TOFMS device recently was tested under rough conditions during a measurement campaign at a technical waste incineration pilot plant, where traces of polycyclic aromatic hydrocarbons were measured in the flue gas by REMPI-TOFMS as a function of process parameters[5,4,8].

EXPERIMENTAL RESULTS

Recently we presented first results from REMPI-TOFMS investigations on coffee brew headspace and first time resolved measurements on coffee roasting process off-gas[9,4]. Further other food products as tea leaves, smoked ham and cocoa product have been investigated. In all cases characteristic volatiles/flavour compounds could be detected by on-line REMPI-TOFMS.

The headspace over roast and ground coffee or coffee brew can be analyzed by REMPI-TOFMS. Using 266 nm laser light for ionization (REMPI@266-TOFMS), several volatile phenolic compounds and nitrogen containing heterocyclic compounds are detectable in trace quantities (ppmv to ppbv range). The figure 2 exhibits an on-line registered REMPI@266-TOFMS mass spectrum of coffee brew headspace. A cup of pure arabica coffee was brewed by pouring 50 ml boiling water onto one slightly heaped spoon roast and ground coffee (coffee-grounds remained in the brew). The sampling tip of our on-line REMPI-TOFMS device was exposed to the headspace region. The measurement time for the on-line REMPI-TOFMS spectrum was one second.

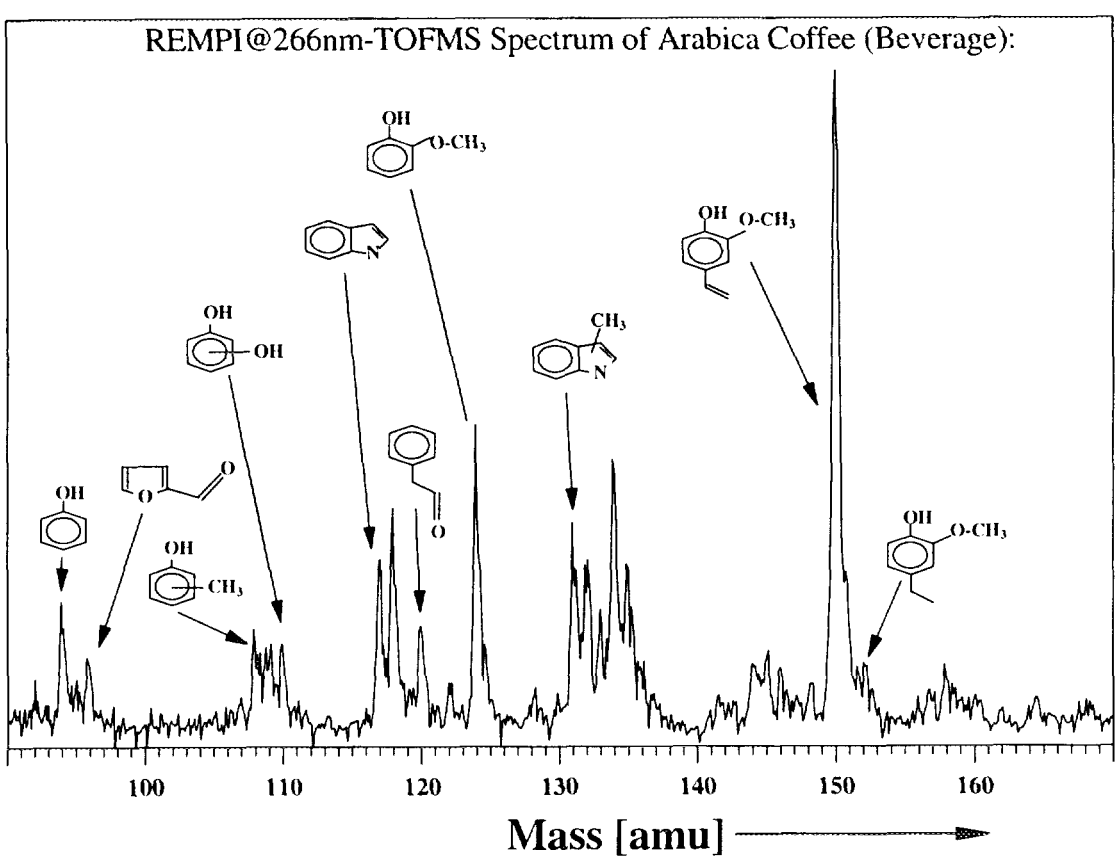

Figure 2
On-line registered REMPI@266-TOFMS mass spectrum of coffee brew headspace. A cop of pure arabica coffee was freshly brewed (grounds remained in brew) and the headspace region over the liquid surface was on-line sampled. Ten mass spectra were averaged (1 sec sampling time at a laser repetition rate of 10 Hz). With the chosen wavelength of 266 nm several volatile phenolic- and nitrogen containing heterocyclic-compounds are detectable.

Our investigations pointed out, that it is possible to discriminate different coffee species by their REMPI-TOFMS headspace spectrum. This can be done either from the headspace of roast and ground coffees at room temperature or from the coffee beverage headspace. The figure 3 shows REMPI@266-TOFMS headspace spectra obtained from two different coffee beverages. The upper mass spectrum is due to the headspace of coffee beverage prepared form pure arabica, whereas the lower, inverted spectrum corresponds to robusta coffee brew headspace. The spectra show, that the robusta coffee is characterized by an high content of phenolic compounds (in comparison to the arabica coffee). These phenolic compounds are responsible for an earthy, smoky flavour component. Especially 4-vinylguaiacol is registered at considerably increased levels in the case of the robusta coffee. Our investigations further indicate, that different coffee brands as well as coffee products (i.e. roast and ground- versus soluble-coffee) can be distinguished via their volatile pattern by REMPI-TOFMS. The on-line analysis of coffee product headspace by REMPI-TOFMS may be, in addition to sensory evaluations, an useful technique for quality control.

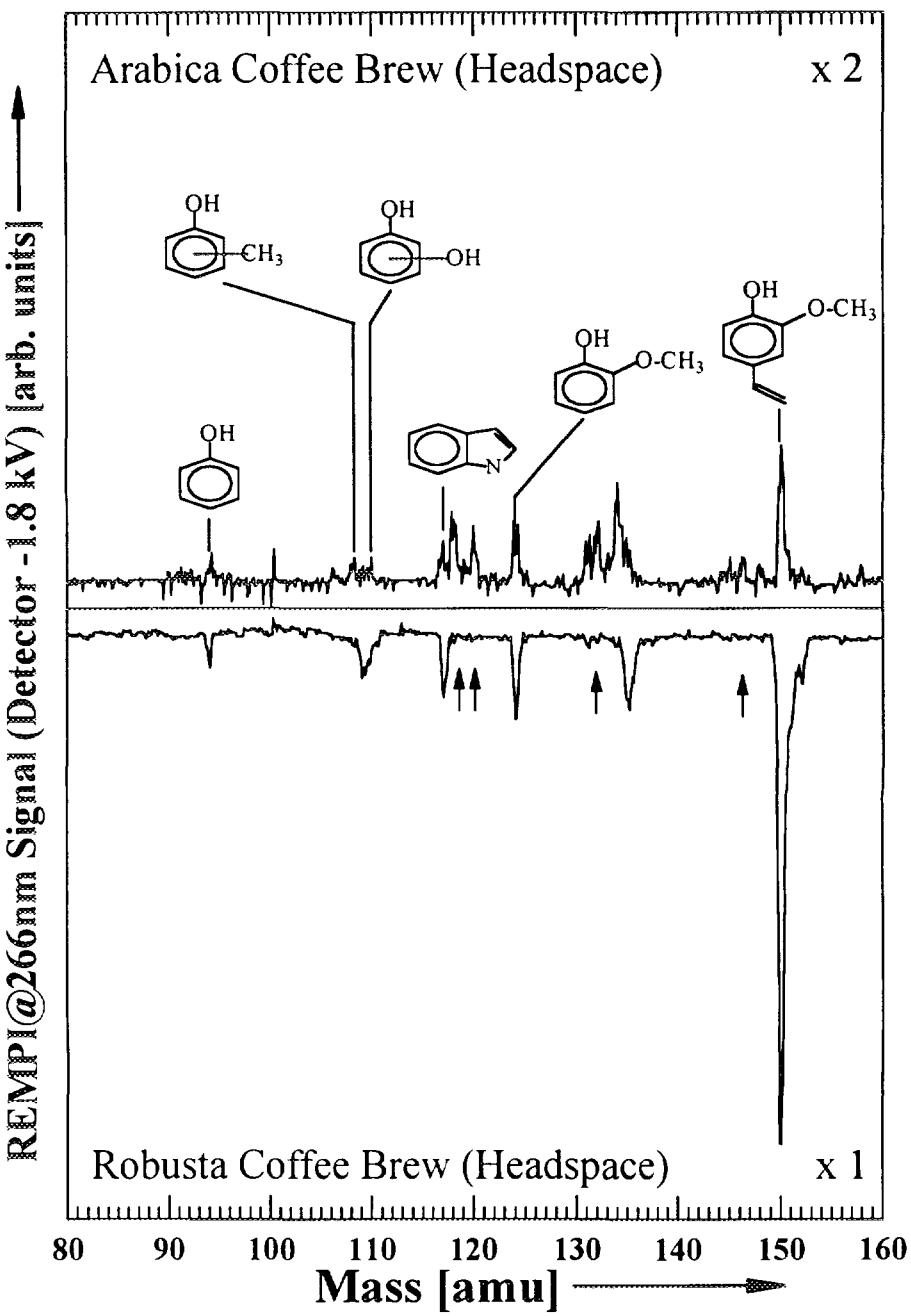

Figure 3
On-line registered REMPI@266-TOFMS mass spectra of arabica (upper trace) and robusta (lower trace, inverted) coffee brew headspace under comparable conditions. The robusta REMPI-TOFMS spectrum is characterized by an increased content of phenolic compounds These compounds are responsible for an earthy, smoky flavour note.

However, one of the most advantageous properties of the on-line REMPI-TOFMS approach is the high time resolution. We demonstrated previously, that highly dynamic concentration changes of trace key compounds in technical processes can be analyzed by REMPI-TOFMS with high time resolution[5,8]. The coffee- and cocoa roasting process is particular interesting in this context, as the flavour formation during the industrial roasting process is highly dynamic. Recently we presented the first, highly time-resolved on-line measurements of coffee-roasting off-gases using a REMPI-TOFMS device with a quadrupled Nd:YAG laser (266 nm, REMPI@266)[9] and an excimer laser (KrF: 248 nm, REMPI@248)[5] for ionization. More than 10 compounds were identified. Further on, the time-concentration profiles of these compounds were registered during the roasting progress. It is important to consider, that several hundred compounds are present in the coffee roasting off-gas within the ppb to ppm concentration range. Therefore any non selective ionization technique would generate congested mass spectra (due to multiple mass interferences), being of minor meaningfulness. Only the high REMPI selectivity enables a registration of isolated molecular species. In coffee roast gas phenol, furfural, methylphenols, dihydroxybenzenes, indole, phenylacetaldehyde, guaiacol, methylindols, vinylguiacol, ethylguiacol, dimethoxystyrene and caffeine were detected. The figure 4 shows a typical REMPI@266 mass spectrum of coffee roasting off gas. The inserts exhibit the time intensity profile of guaiacol and 4-vinylguaiacol during the coffee roasting process. It was interesting to recognize, that these two compounds exhibit significant time-intensity profile differences. After an initial latency period, caused by the warming up time of the beans under water vaporization, the concentrations of guaiacol and 4-vinylguaiacol start to increase.

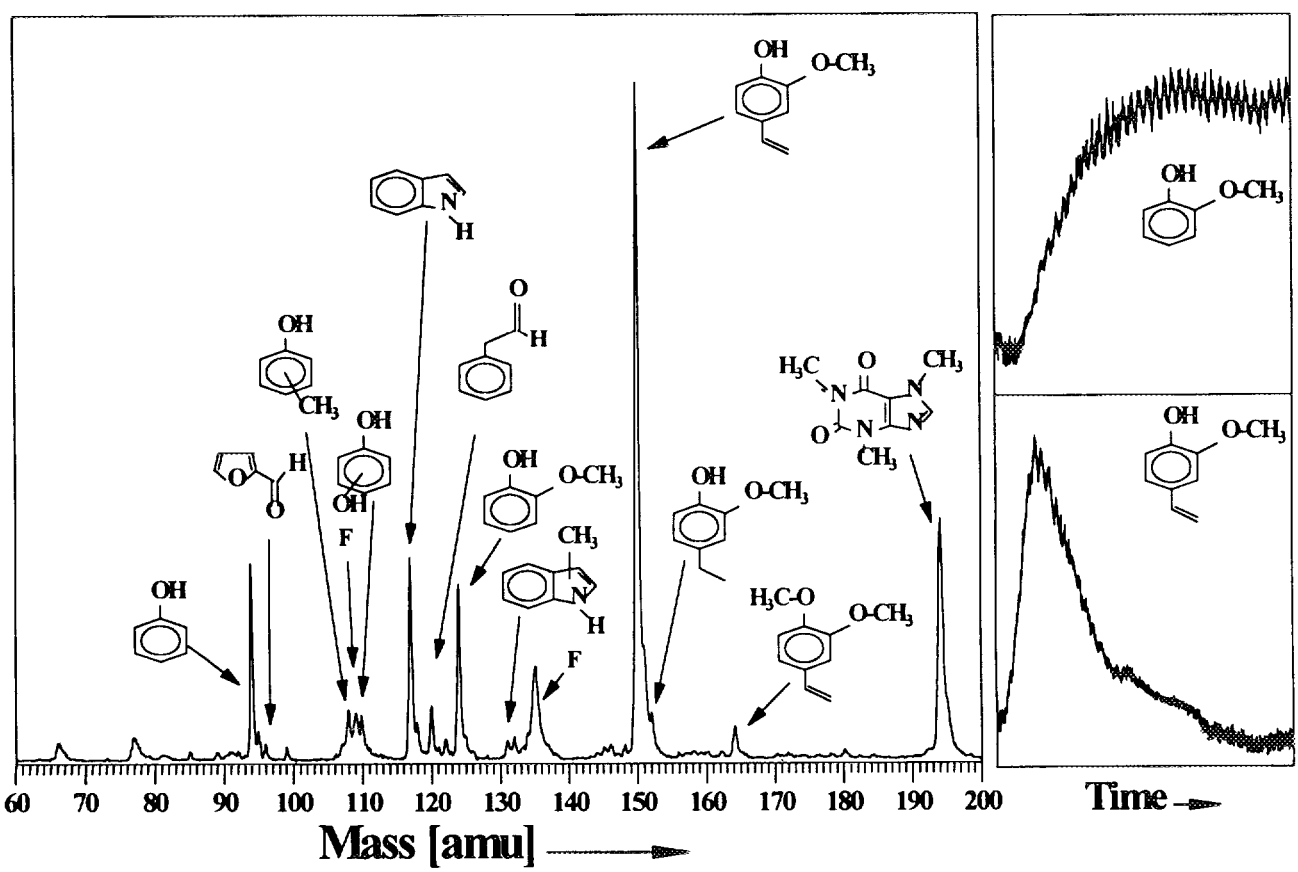

Figure 4
On-line registered REMPI@266-TOFMS mass spectrum of coffee roasting off-gas. The spectrum is averaged over 10 single laser shot mass spectra at a repetition rate of 10 Hz (i.e. one second measurement time). Several phenolic compounds have been identified (phenol, cresols, dihydroxyphenol, guaiacol, 4-vinylguaiacoland, dimethoxystyrene). Further on, the nitrogen containing heterocyclic compounds indole, methylindoles (e.g. skatole) and caffeine as well as the oxygen containing heterocyclic compound furfural are detected. The inserts show the REMPI@266-TOFMS time-concentration profiles of guaiacol and 4-vinylguaiacol, registered during the coffee roasting process. The different time intensity behavior of e.g. these two compounds suggest an application of the on-line REMPI-TOFMS technique for real-time control of technical coffee roasting processes as the relation between guaiacol and 4-vinylguaiacol is known as an indicator for the roast degree[1].

In the first phase the increase of the 4-vinylguaiacol signal was steeper than the increase of the guaiacol signal. Subsequently the 4-vinylguaiacol concentration drops again (probably caused by a decreasing formation rate and thermal induced decomposition), while the guaiacol concentration remains high. The relative concentrations of guaiacol and 4-vinylguaiacol in the off-gas of coffee roasters is already known as an indicator for the roast degree[1]. The on-line capability of REMPI-TOFMS implicates its application for on-line roast degree monitoring in industrial coffee roasting plants.

The high time resolution and sensitivity of the technique is demonstrated in figure 5. This figure shows a REMPI@248 time intensity profile of caffeine from a special roasting experiment. Only a few beans (3-4) where placed in the preheated steel cylinder. The temperature of the cylinder was chosen higher than usual (approximately 400°C). After a short latency period, the beans started to emit cracking sounds indicating „popping" effects. During these „popping" caffeine enriched carbon dioxide is ejected (i.e. caffeine does not pass well through the unruptured plant wall). The appropriate design of our inlet system allows the REMPI-TOFMS registration of even such short, spontaneous caffeine eruptions, without disturbance from memory effects. Figure 5 shows the REMPI@248-TOFMS time-concentration profiles of caffeine, registered during coffee roasting at high temperatures.

In addition to the REMPI-TOFMS application on coffee, also investigations on volatiles in the headspace of smoked ham, red wine, black tea and a cocoa product were performed[12]. Figure 6 shows typical REMPI-TOFMS results from headspace experiments on black tea and the cocoa product.

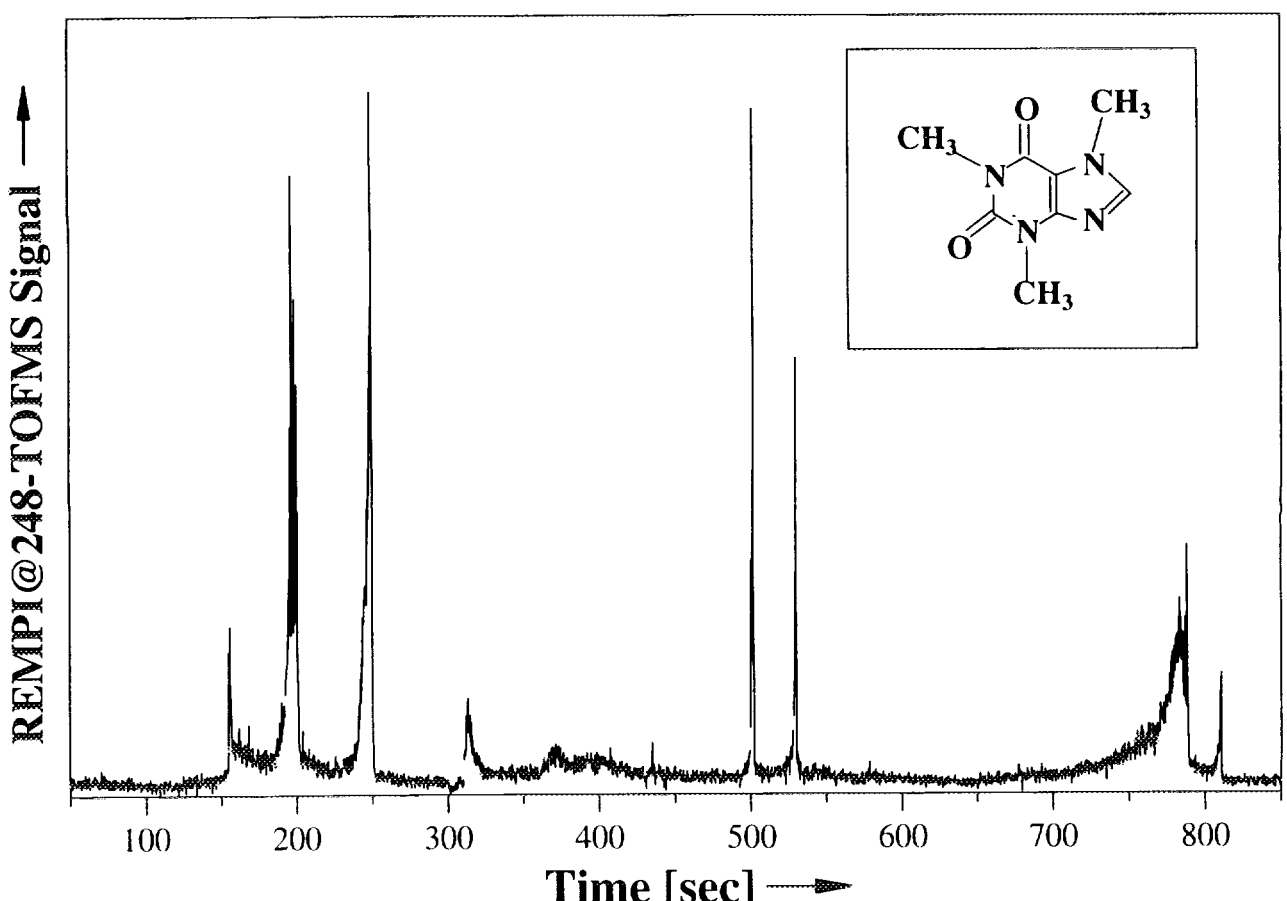

Figure 5

On-line registered REMPI@248-TOFMS time-concentration profiles of caffeine, registered during a coffee roasting process at very high temperatures. The coffee beans get cracks and „pop up" due to the carbon dioxide formed according to the pyrolysis of e.g. quinic acid. During each „popping", caffeine enriched carbon dioxide gas is emitted. These highly dynamic caffeine injections are clearly visible in the REMPI@248-TOFMS time-intensity profile.

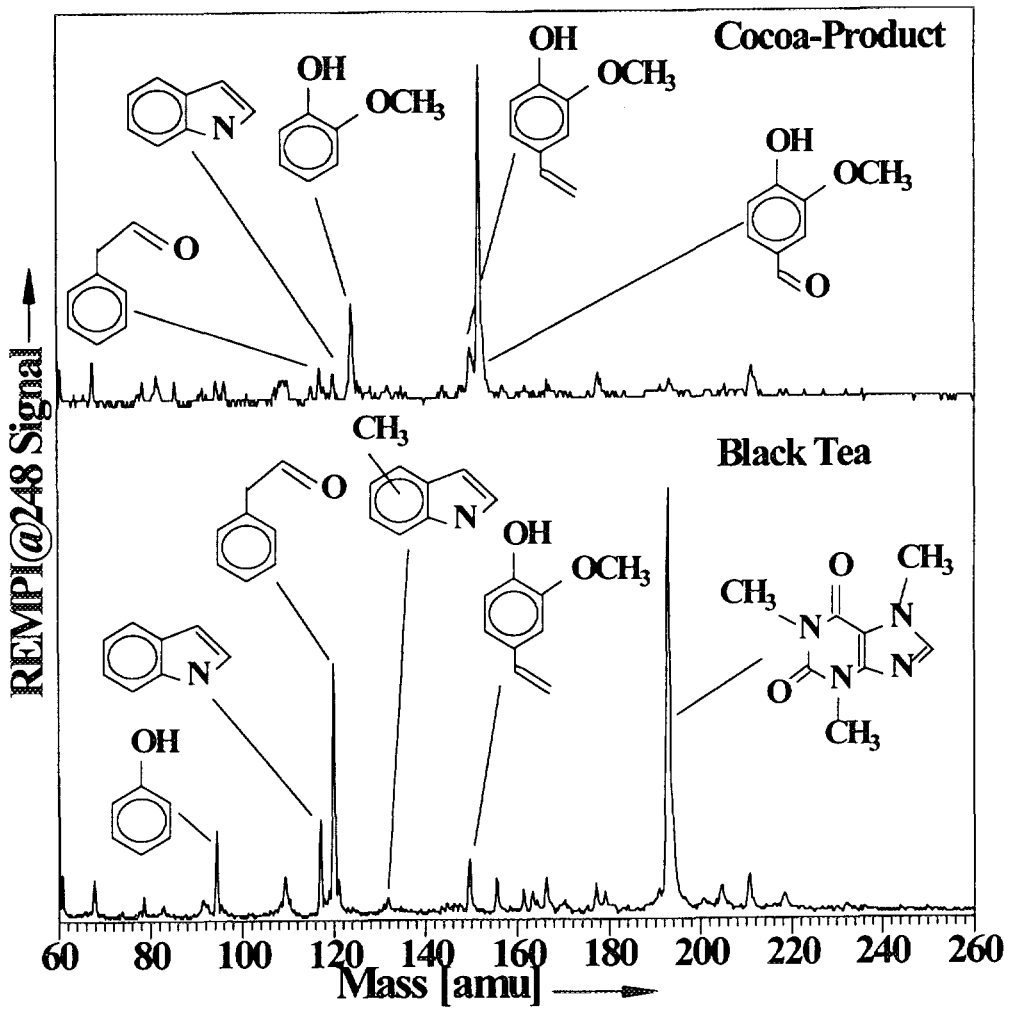

Figure 6
On-line registered REMPI@248-TOFMS mass spectra of the headspace from tea and a cocoa product (slightly heated).
top) *The REMPI@248-TOFMS spectrum of the cocoa product headspace (instant powder„Ovomaltine") exhibits small indol- (117 amu), vinylacetaldehyde- (120 amu) and vinylguaiacol-peaks (150 amu). The vinylguaiacol probably is originated from the malt content. The two more prominent peaks at 124 and 152 amu are due to guaiacol and vanillin. Vanillin is not present in cocoa, but is added as artificial flavouring.*
bottom) *In the headspace from black tea leaves we detected phenol (94 amu), indol (117 amu), vinylacetaldehyde (120 amu), methylindol (131 amu), vinylguaiacol (150 amu) and caffeine (194 amu) by REMPI@248.*

SUMMARY AND OUTLOOK

We demonstrated, that REMPI-TOFMS can be used for on-line analysis of the headspace of food products or food processing off-gases. The on-line capability of the REMPI-TOFMS technique, featured by its unique selectivity and sensitivity, allows the study of highly dynamic processes. Recent results demonstrate, that our REMPI-TOFMS device can be operated successfully even under rough industrial conditions (i.e. during a measurement campaign at a waste incineration pilot plant)[5,8]. One interesting future application would be the realization of an on-line control for the coffee roasting process, e.g. for the correct roasting-time determination in batch wise operated roasters. Numerous further applications, taking other food and non-food products and processes into account, are possible (e.g. for quality control). For example REMPI-TOFMS analysis of process gas from instant coffee manufacturing plants sampled at different sites may be helpful for improving the process design or process operation.
However, in addition to technical routine applications in the field of process analysis or quality control, more fundamental studies e.g. in the field of flavour research are feasible. For example, investigations of food aging effects, determinations of liquid - gas phase partition coefficients and studies on the formation kinetics of flavour compounds from respective precursors[1] cab be performed. Recently we reported a first, preliminary precursor experiment, concerning the formation of 4-vinylguaiacol from ferulic acid. The 4-vinylguaiacol molecule is one of the key flavour

compounds[1,2] in roast and ground coffee. During the roasting process 4-vinylguaiacol is formed by pyrolysis of ferulic acid, which is already present in green coffee. Under the elevated temperature, ferulic acid readily decarboxilates under formation of 4-vinylguaiacol[5]. In our first preliminary precursor experiment a sample of ferulic acid was placed in a steel cylinder, which subsequently was heated to a temperature of approximately 170-200 °C. The recorded REMPI-TOF mass spectrum corresponds exactly to the headspace spectrum of a pure 4-vinylguaiacol sample. No other pyrolysis products like phenol or guaiacol are detectable by REMPI-TOFMS (266 nm) at the moderate temperatures of the experiment.
The possibility of time resolved measurements of products originated from special precursors or precursors-mixtures under different physical and chemical conditions (e.g. temperature programs, different buffer gases, water- and oxygen content etc.) allows interesting studies on the flavour formation and release kinetics. Further, headspace or mouthspace investigations on e.g. the flavour release can be performed. If the REMPI-TOFMS device is combined with a fast headspace sampling unit for repetitive fast enrichment and release, the detection sensitivities even can be further improved (i.e. for measuring cycles from 20s to some minutes)[10]. Additional also a gas chromatograph can be coupled for special analytical challenges (GC-REMPI-TOFMS)[11]. The GC-REMPI-TOFMS represents a three dimensional analytical technique, performing ultra high selectivity and sensitivity. The high selectivity may be used for a reduction of the clean up effort in e.g. food product residual analysis.

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AUTHENTICITY EVALUATION OF SOME FRUIT JUICES BY MEANS OF HPLC-DAD DETERMINATION OF PHLORETIN GLUCOSIDES

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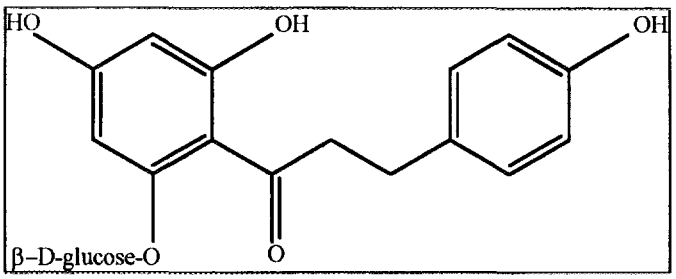
The adoption of an HPLC coupled with a diode array detector (DAD) allows to evaluate phloretin glucoside (phloridzin) and phloretin xyloglucoside. These compounds are dihydrochalcones and they are typical of apples and their derivatives (juices, jams, purees). Dihydrochalcones have not been detected in other fruits or juices (e.g. strawberry, pear, raspberry) and hence their presence, only in apple products, may help in the detection of apple juice addition to other products.
In this paper, it has been determined, by HPLC-DAD, the average content of phloretin glucoside and xyloglucoside in several commercial and experimental apple juices. From the results, it has been quantified a limit threshold of apple juice addition (5-7 %). Above these values, it is possible to evidentiate the authenticity of other fruit juices.

INTRODUCTION

Polyphenols are important constituents of fruits. They are involved in many aspects regarding fruits, for instance the taste, the colour, the antimicrobial or antioxidant activity and so on. In some cases, it has also been verified that polyphenols, as marker compounds, may be of interest in identification and characterization of fruits and their derivatives (nectars, purees, etc.,).
The phloretin-2'-β-glucoside (phloridzin) and the phloretin xyloglucoside are dihydrochalcones basicly occurring in apple skin and seeds (figure 1). They are flavonoids and their analytical determination can represent a parameter of authenticity for beverages quality evaluation [1-4]. The different dihydrochalcones concentration in apple fruits may depend both on the variety and on the ripening degree [5]. From a technological standpoint, the dihydrochalcones content in apple juices may increase up to 10 times if a hot diffusion process (50-70°C) is carried out, in comparison to the standard pressing-extraction process [6-7]. Moreover, the treatments of pasteurization, enzymatic clarification, filtration and concentration do not substantially modify the dihydrochalcones content in apple juices. In fact, also for apple jams, the phloretin content remains unchanged and its presence can be used as an authenticity marker for this class of products [8]. On the other hand, several Authors [5-7] verified a dihydrochalcones decrease varying form 40 to 50 %, during a 9 months storage at 25°C.
Taking into account that these compounds are characteristic of apples since they have not been detected in any other fruits, their analytical determination may result an useful tools for assesing food authenticity [9].
The HPLC method of analysis, coupled with a diode array detector (DAD), results effective for dihydrochalcones determination; in fact, phloretin derivatives show a characteristic UV-VIS adsorption spectrum [10-14].
The sugar molecule which esterifies the aglycone do not substantially modify the adsorption spectrum of the substance but it is responsible of polarity changes of the sugar-aglycone complex; as a consequence, the retention time gives an useful indication about the different sugars involved in dihydrochalcones [5].
The phloridzin detection limit in standard solutions is about 0.1 mg/L for an UV-VIS detector, while it is about 10 times lower (0.01 mg/L) if an electrochemical detector is used [13].

The aim of this work (i) to quantify the phenolic HPLC profile of apples, pears, strawberries and raspberries juices and (ii) to evaluate a threshold concentration of phloretin glucoside and xyloglucoside due to apple juice addition.

FIGURE 1 - The molecule of phloretin-2'-β-glucoside (phloridzin)



MATERIALS and METHODS

Apples, pears, strawberries and raspberries fruits (about 500 g each), coming from different countries and producers were processed for juice extraction [9]. All samples were centrifuged, diluted and filtered according to Versari et al., 1997.

The HPLC standard [(+) Catechin, chlorogenic acid, (-) epicatechin, quercetin, phloretin and phloridzin] were purchased from Sigma (Sigma Chemical Company, Dorset, U.K.). The HPLC equipment was a Dionex DX 500 (Dionex Corporation, CA, USA) equipped with a GP 40 LC pump, an AS 3500 autosampler and a diode array detector UVIS 206. The column (15 *x* 4,6 mm, 5μm), was an Hicarbosphere 3ODS (Hichrom Ltd, Berkshire, UK), thermostated at 30°C by means of a temperature control module (AS 3500). The phenolic compounds were separated by gradient elution as reported in table 1.

TABLE 1

Time	solvent A (%)	solvent B (%)
(min.)	KH_2PO_4 (0.08 mol/L)	H_2O/CH_3CN (30/70, v/v).
0	90	10
5	90	10
65	35	65
70	35	65
75	90	10
90	90	10

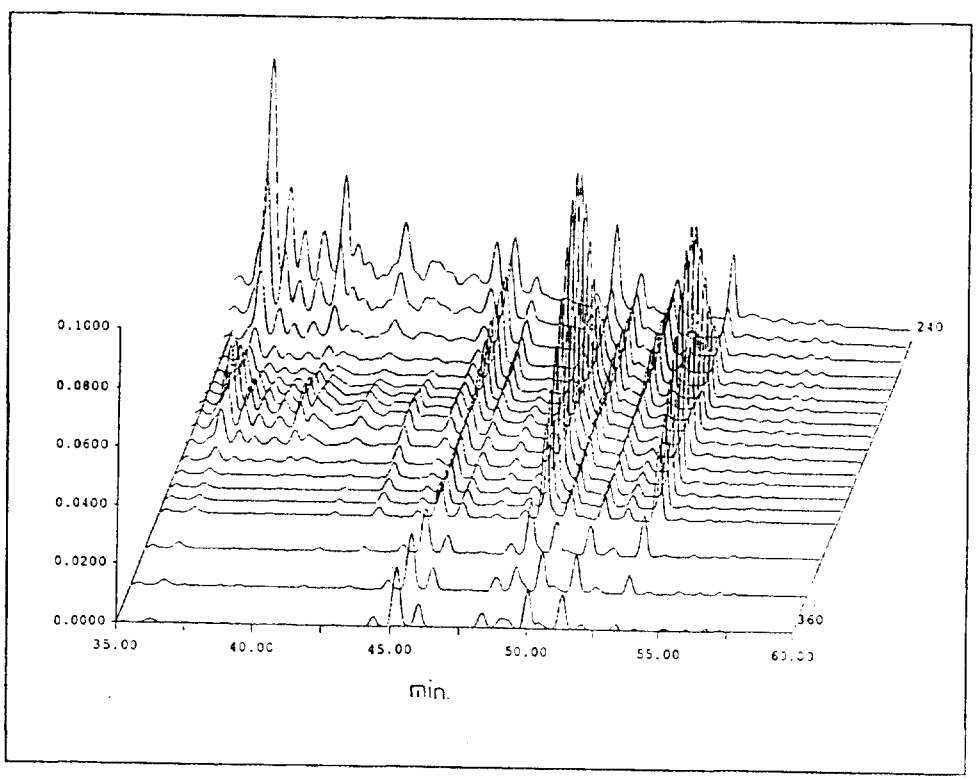
Phenolics identification in HPLC analysis was based on informations (retention times and UV spectra) available from the literature [1-4, 9, 15] and compared with those of our standards.

A single wavelength (λ_{max} = 285 nm) was reprocessed from the spectra for the quantification of identified phenolic compounds based on the external standard method using calibration curves; concentrations were expressed as mg/L of juice.

RESULTS and DISCUSSION

The HPLC analysis with a diode array detector revealed that only in apple juices, phloretin glucoside and phloretin xyloglucoside are always present. These two compounds, by reprocessing the spectrum data at λ = 285 nm, showed a retention time respectively at 52 and 56 minutes. In the case of strawberry, raspberry and pear juices, these peaks never appeared. As an example, in figure 2 is reported the HPLC spectrum of an apple juice.

FIGURE 2



In figures 3 and 4, the phloretin glucoside and the phloretin xyloglucoside contents (mg/L) for apple juices are respectively reported. A and B represent experimental juices, extracted in laboratory from apple fruits, respectively coming from the industry and from the market. C and D are apple juices, directly processed by some Italian and English factories.

The content of dihydrochalcones is low in the case of experimental juices (A and B samples) as well as their coefficient of variation (CV, error bars), both for the phloretin glucoside and for the xyloglucoside. For commerical juices (C and D samples), it has been determined an high dihydrochalcone content, with corresponding high CV values (about 12-15 %).

FIGURE 3

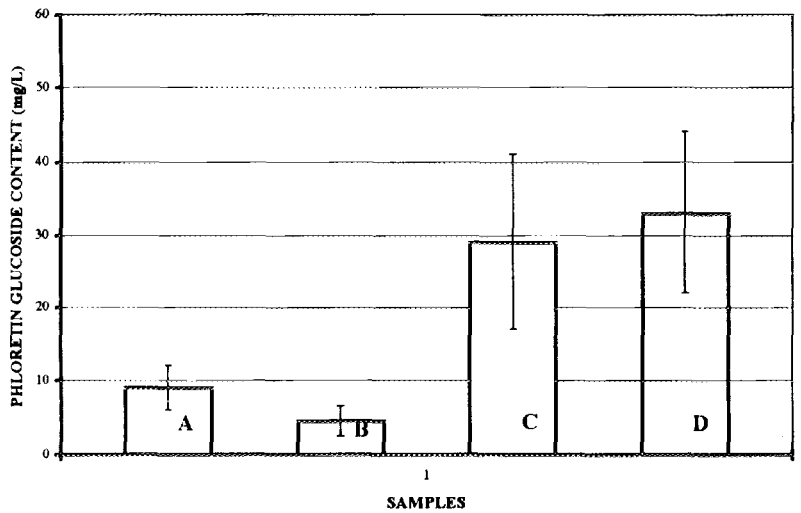
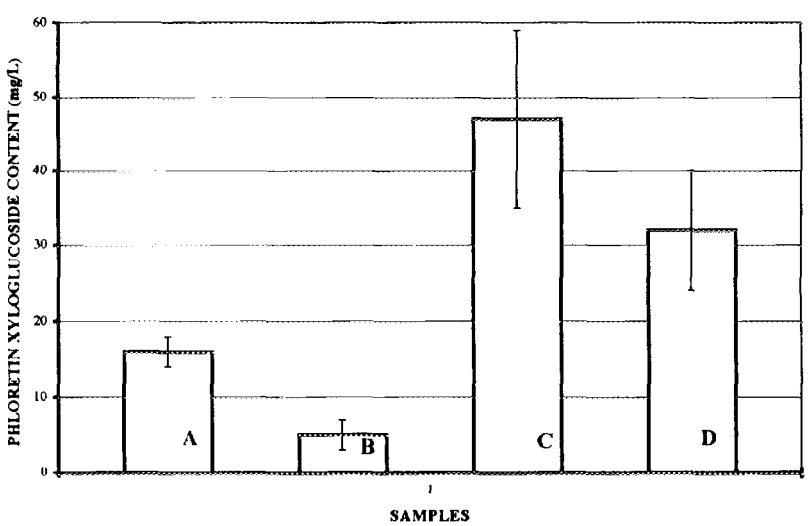


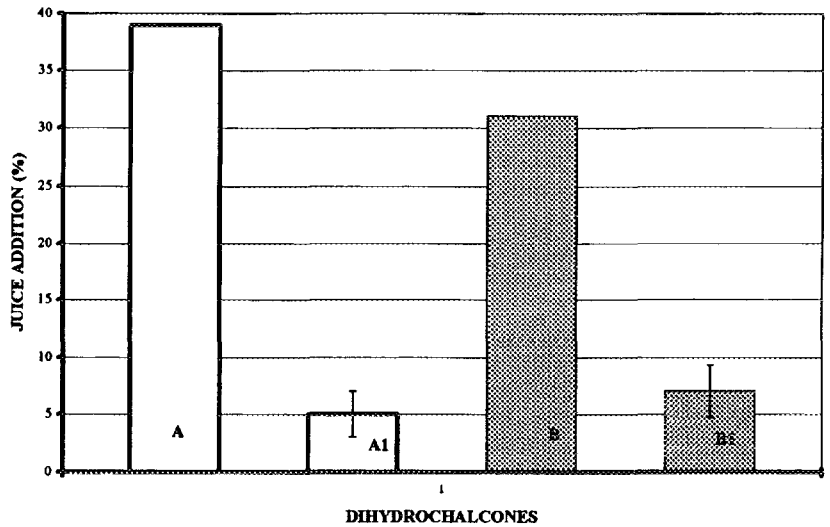
FIGURE 4



Taking into account (i) the average content of phloretin glucoside and xyloglucoside found in the commercial apple juices and (ii) the detection limit of 2 mg/L verified for phloretin glucoside in juices, it was possible to determine a "limit threshold" value. Above this value, it is possible to put in evidence the apple juice addition to another product, as shown in figure 5. A is the mean value of phloretin xyloglucoside in commercial apple juices and A1 is the minimum quantity of added juice which permits the phloretin xyloglucoside detection (over 2 mg/L). In the same way, B is the phloretin glucoside content of commerical apple juices and B1 the minimum detectable addition.

The highest quantity of phloretin xyloglucoside found in apple juices allows to reduce the limit threshold of added juice to 5 %. Moreover, the adoption of maceration or hot extraction of juices, increasing the extraction yields and the dihydrochalcone content, can lead to a further reduction of the limit threshold of detection. In addition, the use of an electrochemical detector, by reducing the instrumental limit of detection of about 10 times in respect to an UV-VIS one, may furtherly reduce this limit.

FIGURE 5



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Safety, Quality and Authenticity of Plant Foodstuffs. Role and Activities of the Joint Research Centre for the European Commission.

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The Food & Drug Analysis/Consumer Protection Unit of the Joint Research Centre for the European Commission provides technical and scientific support to regulatory actions of the Commission for the preparation and implementation of EU Directives on food, feeding stuff and cosmetics. The activities of the Unit are directed towards the areas of food safety (toxicants and contaminants either occurring naturally or resulting from technological treatment) and food quality (authenticity proof, detection of frauds, compliance with labeling). As a neutral and communitary laboratory, the Unit conducts research in support of food legislation for the harmonisation analytical methods for foods, feeding stuff and other consumer goods. An overview of selected project specifically focusing on plant foodstuffs is presented. Examples include quantification of toxicants, determination of botanical and geographical origins, and detection of frauds.

INTRODUCTION

Quality control of food is essential for both consumer protection and for the food industry. The consumer expects a wide range of competitively priced food products of consistently high quality. As agriculture and food technology have advanced and as populations have increased, the analytical problems concerning food have become very complex. Production processes are aiming to the preservation of nutritious foods for longer period of storage while preventing chemical and microbiological deterioration, insect infestation and bacterial contamination. Market competition led food producers to introduce increasingly innovative products, and modern food technology can lead to the production of mimic components in food which possess new significant biological effects and results in the formation of unknown chemical compounds. Today the major food control concerns all areas of the food industry and focuses on the safety of added chemicals and with chemical contamination from environmental pollution, natural contaminants and from the use of pesticides and veterinary drugs. Feeding stuff, being a prime route to the food chain, represents a potential source of residues and contaminants in food. Many countries have national food and feeding stuff policies, responding appropriately to their health situation and economy. Mostly, the regulations dealing with food and feeding stuff are very similar. The strategy of the European Union's Commission is to favour the adoption of harmonised rules at the Community level, applicable to all food, feeding stuff, cosmetics and other consumer goods marketed in the Community. The final scope in many cases would be the mutual recognition of national regulations and standards without the adoption of Community legislative measures. This presumes the harmonisation of analytical methods and requires a neutral and communitary laboratory in order to play an active role in handling possible technical disputes between Member States.
The Food & Drug Analysis/Consumer Protection Unit was established in the Environment Institute of the European Commission's Joint Research Centre (JRC) in 1989 in order to provide scientific and technical support or expertise to General Directorates such as DG III (Industry), DG VI (Agriculture), DG XXI (Customs Tariff) and DG XXIV (Consumer Protection). In 1993 the "European Office for wine, alcohols and spirit drinks" (BEVABS) has been institutionalised in order to detect adulteration of wine and other beverages. The Food and Drug Analysis / Consumer Protection Unit aims to resolve problems concerning safety, quality and origin of foods and consumer products. It also serves as a scientific and technical support to regulatory actions of the Commission for the preparation and implementation of EU Directives. The main activities are peri-normative research and support, dealing with food safety and food quality. A crucial

activity is thus method development, aimed at the harmonisation or evaluation of analytical methods for food safety, legislative purposes or resolution of technical disputes among Member States. Modern as well as alternative analytical techniques are also developed and compared for the investigation of food origin and quality, for example in the authenticity proof of foodstuffs and the detection of frauds and compliance with labelling. Descriptions of selected projects which are currently carried out on safety, quality and authenticity of food and feeding stuff of plant origin are given in this presentation. The activities are based either on "ad hoc tasks" (advisory work, short term technical and analytical support) or on programmed or strategic tasks (method development, collaborative trials).

ANALYTICAL MEANS OF THE FOOD AND DRUG ANALYSIS UNIT

Various chromatographic techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC) and thin layer chromatography (TLC) besides classical chemical methods are used to evaluate existing and to develop new analytical methods for the determination of additives, contaminants, ingredients and residues in food. In most cases of method development for control laboratories and industry quality control, the work is carried out using modern yet common means of analysis such as HPLC with either fluorescence, ultraviolet, refractive index, evaporative light scattering [1], electrochemical or mass selective detectors. GC is also widely used with varied detectors such as flame ionisation, nitrogen/phosphorous, electron capture or mass selective. Ion chromatography (IC) with conductivity or ultraviolet detectors are used in some cases. Nuclear magnetic resonance (NMR) and isotopic ratio mass spectrometer (IRMS) equipped for D, ^{13}C, ^{15}N, $^{18}0$, ^{34}S measurements are used in studies related to the adulteration of beverages and the authenticity of plants. Projects aimed at more sensitive determinations or at the development of screening procedures can employ more innovative techniques such as capillary electrophoresis with laser densitometer, ultraviolet or fluorescence detectors, pyrolysis mass spectrometry (PyMS), inductively coupled plasma spectrometry (ICP) or atomic absorption (AAS) spectrometry. Modern sample preparation methods such as supercritical fluid extraction [2,3] (SFE), solid phase extraction (SPE), high performance gel permeation, and microwave extraction are used and the extraction yields are compared to those obtained by classical solvent extraction methods.

ACTIVITIES IN FOOD SAFETY

The role of the Food & Drug Analysis / Consumer Protection Unit is to develop and harmonise analytical methods either chemical or microbiological, to evaluate existing analytical methods, and to evaluate chemical or toxicological data. Food and feed from plant origin can be contaminated by various types of compounds depending on the source of contamination. The sources of contamination can be broadly divided into the contaminants occurring inadvertently from the raw food sources, such as pesticides, mycotoxins, alkaloids, and the contaminations resulting from technological physical or chemical treatments which lead to chemical reactions of compounds within the food matrix.

Naturally occurring contaminants and toxicants in food and feeding stuff

Contamination of food and feed can occur during the growth of the original raw food material. For example, it can be the remaining presence of pesticides. Another case is the growth of toxic plants growing together with feeding stuff and leading to the presence of plant toxins such as alkaloids. Fungal species may use the feeding stuff as substrate, resulting in the contamination with mycotoxins. Suitable methods are in development for the analysis of various alkaloids and mycotoxins [4] (fumonisins, aflatoxins, zearalenone, ochratoxin A) in various types of feeding stuff.

• *Contaminations from mycotoxins in food and feeding stuff*

Mycotoxins are toxic substances produced by certain micro-organisms and can be present in food and feeding stuff. Mycotoxins can often give rise to chronic and irreversible forms of intoxications in humans and animals and, in some cases, these products may be carcinogenic. Some of the mycotoxins such as aflatoxins have a high cancerogenic potential to humans already in low concentrations. Although the occurrence of mycotoxins in human food presents a health risk to man there are no harmonised EU controls although most individual EU Member States do have regulations in place controlling maximum levels permitted. There is a well established methodology for most mycotoxins of concern in food, and yet these methods have not, in general, been either validated at the low levels required in some Member State regulations nor have been validated for the full range of foodstuffs covered by these regulations. The Unit is

involved in the determination of the microbiological contamination of food and feeding stuff as well in the chemical analysis of the corresponding mycotoxins. In particular, the Unit is developing and evaluating analytical chemical methods for the determination of several mycotoxins (fumonisins, aflatoxins, ochratoxin A, patulin) present in very low concentrations in various food matrices together with other laboratories from the Member States. The fumonisin contents have been monitored in various food products such as corn flour (polenta), snack products as well as feeding stuff [5] (Figure 1).

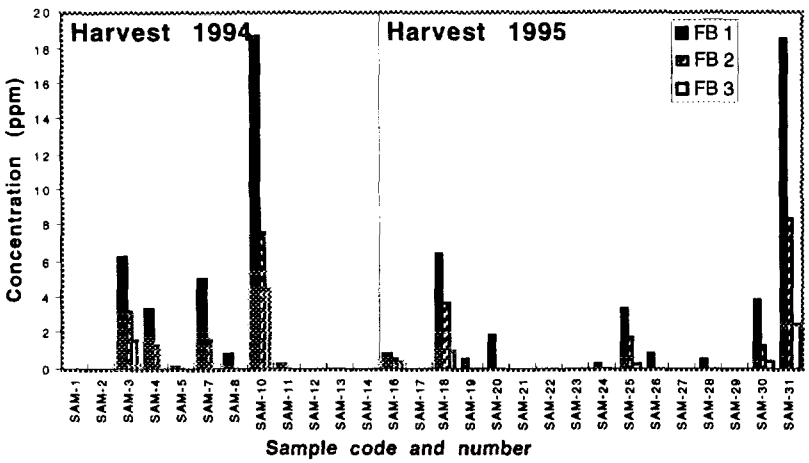

Figure 1: Corn and mixed feed samples harvested in 1994 and 1995 from the northern part of Italy (Province of Varese) have been analysed regarding the fumonisin B1, B2 and B3 content. Analysis was done by HPLC with fluorescence detection after derivatisation with o-phtaldialdehyde (OPA). All feed samples consisting of only maize were found to be positive for fumonisin contamination (FB1 0.4-19 ppm, FB2 0.6-8 ppm, FB3 0-5 ppm). FB3 was detected in samples containing high concentrations of FB1 and FB2. The fumonisin concentrations did not vary considerably between harvests.

In addition, suitable analytical methods for the analysis of mycotoxins are under development and evaluation. Ispra is responsible for the aflatoxins (B1, B2, G1, G2) determination in peanut paste, figs, paprika and pistachios (2 ppb) and infant formulae (0.1 ppb). The project has been started in 1996 and has a duration of three years. Analysis of ochratoxin in wine samples as well as other possible plant matrices such as coffee, barley, wheat is carried out in order to monitor potential contaminations, particularly in wines. From a microbiological point of view, it would be extremely important to know the environmental factors which could favour the growth of toxigenic strains of moulds, their diffusion and the synthesis of mycotoxins in food and foodstuffs. A study is under development to assess what categories of mycotoxins are synthesised by more than one mycetic species [6-8].

• *Contaminations from alkaloids present in Datura species in food and feeding stuff*

Feeding stuff may be contaminated by alkaloids from Datura species (ferrox, stramonium). Procedures for sample preparation and for the quantification of the alkaloids from Datura in feeding stuff are under development.

• *Contaminations from pesticides in food and feeding stuff, particularly fruits and vegetables*

Pesticides in food are monitored for reasons of food safety. Collaborative trials are co-ordinated as well as the on-going monitoring measurements will be evaluated. Methods, suitable for screening, have to be evaluated, especially with respect to new emerging pesticides. One example is the possible presence of ethyl carbamate, suspected to be carcinogenic which may naturally occur in wines depending on various factors. A survey the actual levels of ethyl carbamate in European wines can give a sound scientific basis prior to any draft of low tolerance limits in wines.

Detection of technological treatments

A typical example which can be given for plant foodstuffs is irradiation proof. There are already existing detection methods for irradiation of various food matrices. A study will be carried out in order to

review the methods published in the literature and to evaluate them (suitability). The aim is to identify possible gaps in this field. A method based on the determination of the thermoluminescence is suitable to detect γ–irradiation of food containing relatively high amounts of minerals. The Unit has participated in two collaborative trial studies and a meeting with the partners in order to evaluate the thermoluminescence method for detection of γ–irradiation of dried vegetables (carrots, asparagus, leak, onions) and fruits (apples). The work is in continuation. The thermoluminescence method is suitable to detect γ–irradiation of food containing relatively high amounts of minerals. During the γ–irradiation the minerals store the energy which can be released after isolation of the minerals material from the food matrix and subsequent heating in form of enhanced luminescence.

Additives and contaminants resulting from technological treatments in foods

• *3-Monochloropropandiol in seasonings*

Protein hydrolysates are widely used as seasonings and ingredients in processed savoury food products. They are commonly produced by hydrochloric acid hydrolysis of proteins deriving from plant materials (rape seed meals, maize gluten, soy bean etc.). During the production process several chloropropanols are formed of which one is 3-monochloropropandiol (3-MCDP), presenting a toxicity risk to humans. The JRC participated in a collaborative trial concerning the analysis of 3-MCDP in protein hydrolysates in order to evaluate the suitability of existing methods for determination of this compound in low concentrations. The method was based on GC with electron capture detection (ECD) or flame ionisation detection (FID) after derivatisation.

• *5-Hydroxymethylfurfural determination of contents in vinegar especially balsamic vinegar*

The compound HMF (5-hydroxymethylfurfural) is a Maillard reaction product which can be formed during heat treatment of food containing sugars especially in acid conditions or in the presence of amino acids. This compound is nearly "naturally" occurring. However, there are discussions about setting limits for certain food products. Vinegar is a fermentation product which can contain HMF formed during the production process from sugar and acetic acid. Vinegar samples, especially long processed ones, from various European Member States were investigated in view to their HMF content using reverse phase HPLC and diode array detection to identify the separated substances [9]. The preparation was limited to a filtration and dilution of the samples and the separation could be achieved within 10 min. The results for balsamic vinegars of increasing maturation is shown in Figure 2.

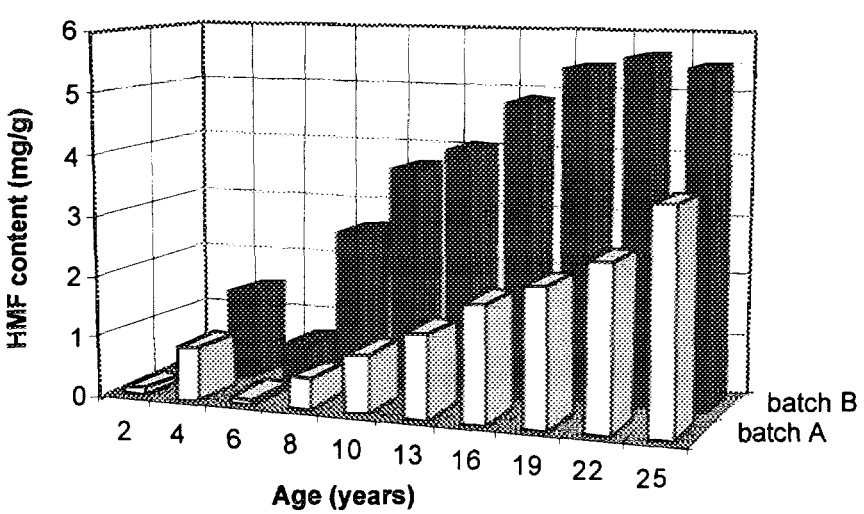

Figure 2: Variation of HMF concentration with aging. Commercial balsamic vinegars are stored for ripening to 7 years, whereas the traditional are aged up to 25 years. A nearly linear relation was observed between age and HMF content The 2 series A and B consist of samples taken at various time from 2 separate during aging.

ACTIVITIES ON FOOD ORIGIN & QUALITY

The Food & Drug Analysis / Consumer Protection is involved in various projects dealing with authenticity proof and detection of frauds and compliance with labelling. The proof of food authenticity is very important for consumer protection and to keep the European Union from economic losses. Producers of fraudulent food are developing more and more sophisticated methods. Therefore the task of food controllers becomes more difficult. There are two important lines to monitor frauds: 1) use of valid, new and sensitive methods for the accurate and rapid characterisation of frauds; 2) development of data banks collecting data on authenticity. Various multidisciplinary techniques are applied in order to detect adulteration of food and to proof authenticity. For example, attention is presently given to as vinegar, vanilla extracts, honey, cocoa butter etc., and to the adulteration of fruit juices by sugaring or blending.

Geographical origin and authenticity of plant foodstuffs

More and more frequently, innovative techniques such as pyrolysis MS are helping in the development of screening methods for the determination of origin and authencity of plant foodstuffs [10,11]. For example, this technique has been developed with success in cases involving vegetable fats [12], vinegars [13], and wines [14].

• *Origin of vinegar (including balsamic vinegar)*

A screening method based on pyrolysis MS with subsequent data evaluation by using neural nets is in development in order to classify vinegar from various sources (e.g. malt, wine, balsamic, apple). Pyrolysis MS applies thermal degradation (530°C) of the samples. The pyrolysate is subsequently analysed by a quadrupole mass spectrometer. The mass spectra are then evaluated by different types of chemometric analysis. A method was developed for the differentiation between vinegars [12] as depicted in Figure 3 and 4.

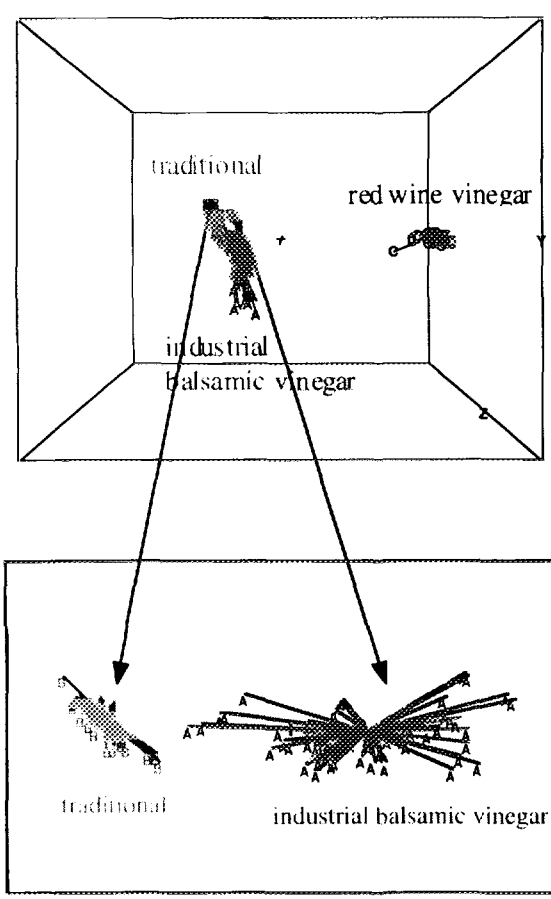

Figure 3: Differentiation of red wine vinegars and balsamic vinegars after pyrolysisMS of the samples. Chemometric data evaluation (canonical variance analysis followed by principal component analysis) was applied for the evaluation of the mass spectra. In the figure the loadings of the first three principal components are given. A clear discrimination of red wine vinegar from balsamic vinegar, either traditionally or industrially made could be achieved.

Figure 4: discrimination of traditionally vs. industrially made vinegars. Traditionally made balsamic vinegar is made by a lengthy and tedious fermentation process of up to 25 years, whereas industrially made balsamic vinegar is usually aged to 7 years. Py-MS discriminates distinctively the two groups.

• *Origin of spices*

A screening method based on pyrolysis MS with subsequent data evaluation by using neural nets is in development in order to classify spices from various sources.

• *Geographical and botanical origin of honey*

A project is co-ordinated in order to evaluate the methods for the determination of the geographical and botanical origin of honey. Methods under investigation consider several constituents of the honey, such as pollen analysis, sugars, carbohydrates, flavonoids and trace elements as well as physical parameters such as crystallisation and melting behaviour [15]. Suitable methods giving concurrent determinations on both the botanical as well as geographical origins of honey included the analysis of flavonoids by GC, HPLC or Micellar Elektrokinetic Capillary Chromatography (MECC), the analysis of aroma compounds by Simultaneous Distillation Extraction (SDE), GC-MS or Stable isotope Dilution Assay (SIDA), and possibly the analysis of amino acids or proteins. The combination of methods (multicomponent analysis) seems to be a promising approach for resolving the problem of authenticity proof for the origin of honeys, especially if combined with modern statistical evaluation techniques (e.g. principal component analysis (PCA), neural nets). The activities comprise the co-ordination of a project involving laboratories from several EU Member States.

Food quality and detection of frauds in plant foodstuffs

• *Addition of water or sugars in beverages, particularly fruit juices and wines*

Adulteration of wine by addition of sugars of different botanical origin to increase the alcoholic content, and watering of wine are major problems in the wine-producing countries. The BEVABS (Bureau Européen des Vins, Alcools et Boissons Spiritueuses - European Office for Wine, Alcohol and Spirit Drinks) acts as a laboratory devoted to the service of EU and Member States in the field of classical and advanced methods for wine, alcohol and spirit analysis. Its main task is the establishment of a databank for the isotopic content of the European wines in order to enable the detection of possible frauds [16,17] (addition of water, false geographical origins and excessive addition of sugar). This work is carried out in collaboration with specialised laboratories of the Member States. Nuclear magnetic resonance (NMR) and isotopic ratio mass spectroscopy (IRMS) allow to detect wine adulteration with a good degree of certainty when used with information from an appropriate data bank. The NMR method is based on the determination of the isotopic ratio deuterium/hydrogen (D/H) in the different sites of the ethanol molecule and allows the identification of the botanical origin of sugars present in the original must before fermentation. Additional information concerning sugaring and watering can be obtained using the IRMS method determining the $^{13}C/^{12}C$ ratio of ethanol and the $^{18}O/^{16}O$ ratio of wine water respectively. BEVABS (part of the Food & Drug Analysis/ Consumer Protection Unit) is maintaining a wine data bank containing the results of NMR analysis concerning the deuterium contents in specific sites of the ethanol molecule and all information concerning the location of the vineyard, the type and growth of grape of authentic European wines. This data bank will be enlarged by data on other natural isotopes [18,19] such as ^{13}C and ^{18}O. The NMR and IRMS techniques are also used for the analysis of liqueurs, spirits, fruit juices and sugars after fermentation and distillation. In addition to its role in the maintenance of the data bank, the BEVABS is responsible for the validation of measurements from the Member States and the isotopic determination for Member States which do not have the required facilities. The role of the BEVABS extends also to improvement of analytical methods [21,22], organisation of interlaboratory trials, and support to the Commission for any question regarding the analytical control of wine, alcohol and spirits. It also gives scientific advice in case of ambiguities between the Member States.

• *Addition of vegetable fats other than cocoa butter in chocolate*

The European Commission has recently drafted an amendment for its EEC Directive on chocolate in which the addition of 5 % of other vegetable fats than cocoa butter is permitted. However, there is up-to-now no suitable analytical method available. Due to the natural variations of cocoa butters as well as to the fact that the vegetable fats used in chocolate production are very similar in their physical and chemical composition to cocoa butter, this task is very difficult. Together with laboratories from Member States, JRC Ispra is improving existing methods and developing new methods for this purpose. The resulting suitable method (most probably multi-method) will be evaluated [23]. Methods under investigation concern the analysis of the triglyceride main constituents using techniques such as high temperature GC (or HRGC) and high pressure GC [24,25], HPLC with light scattering [26], pyrolysis MS [27]. The minor constituents and the physical behaviour of the samples are also analysed. Among the techniques that are being evaluated, a screening method by high resolution GC of triglyceride profiles has been tested on model systems made of cocoa butters and cocoa butter equivalents added to different levels from 0 to 20% on a cocoa butter (CB) basis, in

order to generate a blueprint of both natural variations (Figure 5) and variations induced by the introduction of other fats (Figure 6).

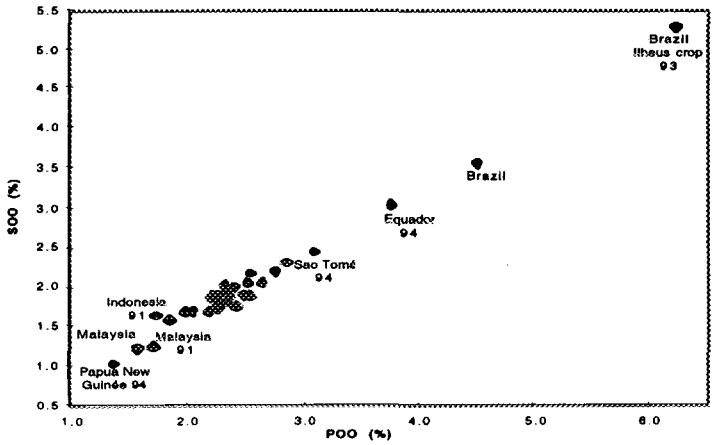

Figure 5: Determination the natural variations present in cocoa butters from different origins. POO: 1-palmitoyl-2,3-oleoylglycerol; SOO: 1-stearoyl-2,3-oleoylglycerol. It shows the differentiation between commercial cocoa butters blends and cocoa butters from specific crops or geographical origin. Pure CBs are distributed on the plot according to their geographical origin. In particular, Brazilian CBs, generally softer, exhibited a significantly higher percentages of lower melting SOO and POO. By the same token, south-eastern Asian CBs, harder, (such as Malaysian) were characterised by lower percentages of POO and SOO. Commercial CB mixes, both undeodorised or deodorised were clustered in a region without significant variations.

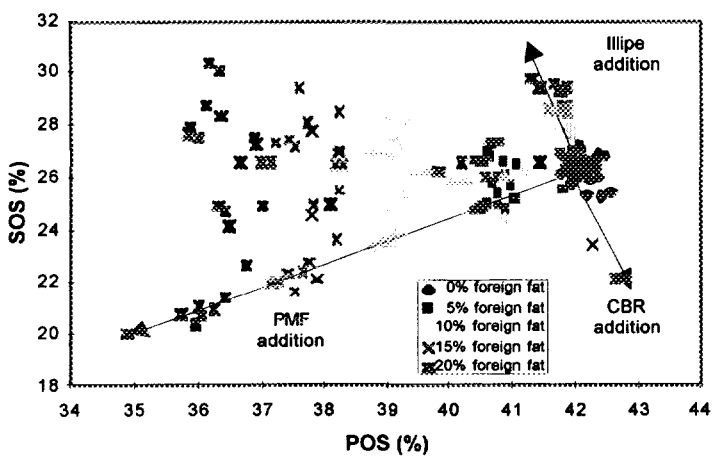

Figure 6: Quantification of the addition of vegetable fat other than cocoa butter on model systems of cocoa butters /vegetable fat mixtures. POS: 1-palmitoyl-2-oleoyl-3-stearoylglycerol; SOS: 1,3-stearoyl-2-oleoylglycerol; PMF: palm oil mid fraction; CBR: cocoa butter equivalent. Using different triglyceride indicators and statistical packages, additions of foreign fats could be detected and their level predicted down to 5% on a cocoa butter basis. The results suggest that in chocolate, which contains 20-25% cocoa butter, the detection reaches down to 1-2%, thus well below the proposed 5% regulatory level.

• *Detection of genetically modified organisms (GMO) in food towards labeling as "GMO free food"*

The evaluation of a screening method (direct method for detection) based on DNA analysis by means of polymerase chain reaction (PCR) is co-ordinated for the detection of GMO, for example in corn and soy beans. The principle of the method is the detection of certain promoter and terminator sequences being present in most GMO's (for 25 out of 28 approved GMO's). This method will be validated by laboratories of many Member States. Standard materials are in preparation in collaboration with the Institute for Reference Materials and Measurements of the European Commission and will be made available to the public after the evaluation. Indirect methods for detection of GMO in food are also in development for food products where DNA is no longer present (e.g. edible oils) or those which are highly purified (e.g. lecithin). Those are based on ingredient pattern analysis, as for example in soy and corn oils.

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The Volumetry of Viscous and Non-homogenous Fluids; Description of a new technique and its influence on our understanding of circulatory transport dynamics.

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Summary
A new instrumental technique that measures off 100 µl of a viscous, tenacious fluid or a volume of the particles (cells) in non-homogenous fluid such as blood is described. An exact volume is obtained, its specific density determined, and the volume used as reference point for further quantitative analysis in food technology, cosmetics industries, petroleum, oil soap and paint industries. Blood introduced into the instrument is fractionated into plasma and 100 µl of 98% pure red cells which remain under almost natural conditions. Transport processes between blood and tissues, e.g. absorption of nutrients into the circulation can mainly occur on red cells. Hence analysis of the cell fraction is indispensable, e.g. to estimate bioavailability.
Key words: Centrifugation, semiliquid, densitometry, volumetry, bioavailability.

Introduction

All quantitative analyses commence with a volumetric procedure, but the volumetry of tenacious or viscous fluid is difficult. The volumetry of semiliquids can be improved by a new technique, which relies on a flux of the semiliquid when subjected to a force of 10.000 g at room temperature.
Non-homogenous fluids can be problematical, as the isolation of their components during sampling can lead to inaccuracies. Sometimes it is more appropriate to analyse fluid constituents after their isolation, provided that this procedure does not affect the system, and consequently the results. Using the new technique, suspended particles which are denser than the suspension medium, have a diameter of less than 0.2 mm, and which possess suitable streaming properties, can be harvested in a fixed volume.
The technique has many applications, and was initially developed to measure off an exact volume of pure particles (cells) from blood, without affecting the existing partition of compounds between the particles and suspension medium; this is important, as nutrients, drugs and toxins, on entering the circulation, distribute throughout various blood components which possess very different uptake capacities. A partial analysis of blood can easily lead to a lack of clarity concerning the absorption and transport processes occurring between blood and tissues.

The volumetry of semiliquids

The measurement of liquid volume is a basic routine procedure performed in all laboratories. Pipetting techniques (1) depend on the flow of a liquid into a space of defined volume in response to the application of negative pressure. The volume so collected is then expelled by gravity (transfer pipettes) or by force (graduated pipettes and syringe pipettes).
Viscous fluids e.g. heavy oils, emulsions, butter, honey, syrup or molasses with a high resistance to flow, cannot enter a pipette or syringe easily. Attempted aspiration of these fluids usually generates multiple air bubbles within the pipette. Moreover the expulsion by gravity, and even by force, can leave a residuum in the pipette. Exertion of a significant force on the piston of a syringe may lead to seepage between the piston and the barrel with loss of material.
A relatively thick non-homogenous fluid such as blood presents even greater difficulties as a quasi homogenous state must be maintained during volume measurement. The distribution of particles (cells) in blood may not remain uniform during volumetry, a problem more likely to occur in patients with a high red blood cell sedimentation rate. The results obtained with blood are also affected by variations in the corpuscular elements. Normally the ratio of red to white cell is approximately 1000, but can be considerably different in some

pathological states. It is for such reasons that free-flowing plasma, not containing cells and transparent, is analysed in preference of blood.

Description and instrumentation of a new volumetric technique: See figures 1 and 2

Principle: flow resistant material is introduced into an instrument, and a chamber of calibrated volume filled using gravitational force. Surplus material is then forced out through an opening (2). The MESED (measuring sediment) tube (fig.1) consists of two parts, a reservoir A and a container B, which are initially fitted together with aperture '4' closed (fig. 1). After the introduction of a volume of @ 0.15 ml of semiliquid or 0.3 ml of blood, into the reservoir (A) followed by centrifugation, the lower part of the reservoir and a chamber of known volume (3) are totally filled. The two parts are then separated slightly (fig. 2), thereby opening '4', so that on further centrifugation the surplus material above '5' and in the outlet channel is vented, leaving a known residual volume of 100 µl ('3').

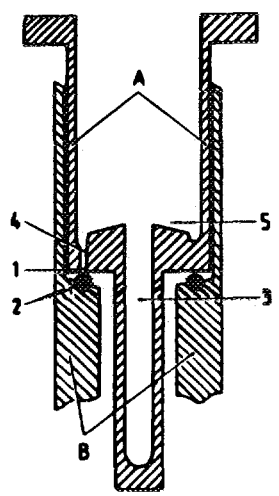

Fig. 1: The Mesed instrument: closed position

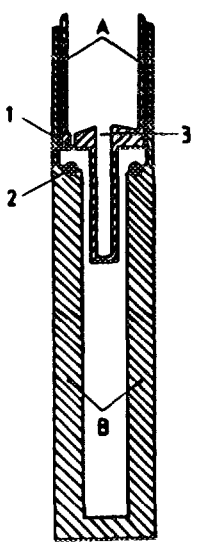

Fig. 2: The Mesed instrument: opened position

The weight of this accurate volume depends on the density of the substance, (e.g. approximately 140 mg of honey or 109 mg of red cells), and the volume provides the reference point for further quantitative analysis. In this way small volumes of semiliquids can be measured off reliably and their specific density determined, a valuable technique in monitoring the progress of a concentration process.

When a non-homogenous fluid such as blood is introduced into the instrument, separation by centrifugation produced a cell sediment and supernatant plasma which can be sampled separately. The centrifugal force concentrates a known volume of red cells in the calibrated chamber with a small constant percentage of plasma trapped between the cells. This percentage depends on the centrifugal force applied and has a minimum value of 2% v/v. The excess volume of red cells, together with all the white cells and platelets (the buffy coat), and surplus plasma, is vented in a second centrifugation. In this way a known volume of almost pure red cells is obtained, without disturbing the equilibrium between the cells and the surrounding plasma, a significant advantage over the more usual methods.

When using the instrument, certain facts need to be borne in mind. Particles can occasionally block opening '4' (fig. 1), so that the surplus material cannot be vented. Given a certain aperture, the likelihood of blockage depends on the diameter and streaming profile of the particle, e.g. plain peanut butter flows readily and is quantifiable, unlike peanut butter containing nut fragments. In the case of an emulsion, the centrifugal force must be reduced to prevent cracking. With tenacious material, care must be taken to avoid the trapping of air bubbles, which also will be harvested in the calibrated chamber. Remnants of viscous substances can sometimes be seen on the plastic surface of the calibrated chamber and reservoir after emptying. Faint red streaks indicating the lines of flow followed by red cells expelled during the processing of blood can comprise approximately 1% of the mass collected in the calibrated chamber. The extent of the loss depends upon the physical properties of the sample, including its viscosity, the smoothness of the reservoir wall, and the expelling centrifugal force.

Use of the instrument

1. Food technology and cosmetic industries.

Figure 3 shows the result of the measuring off process for butter, olive oil, egg yolk, mustard, marmite, ketchup, toothpaste and "beyond moisture creme". The surplus material in the container and the measured off volume in the reservoir are visible. The density of the mass measured off can be obtained by weighing the reservoir, whilst centrifuging the inverted reservoir quantitatively expels the mass, which than can be subjected to further analysis. As the volume of the semiliquid can be as low as 150 µl, the procedure is valuable in monitoring the progress of small scale processes.

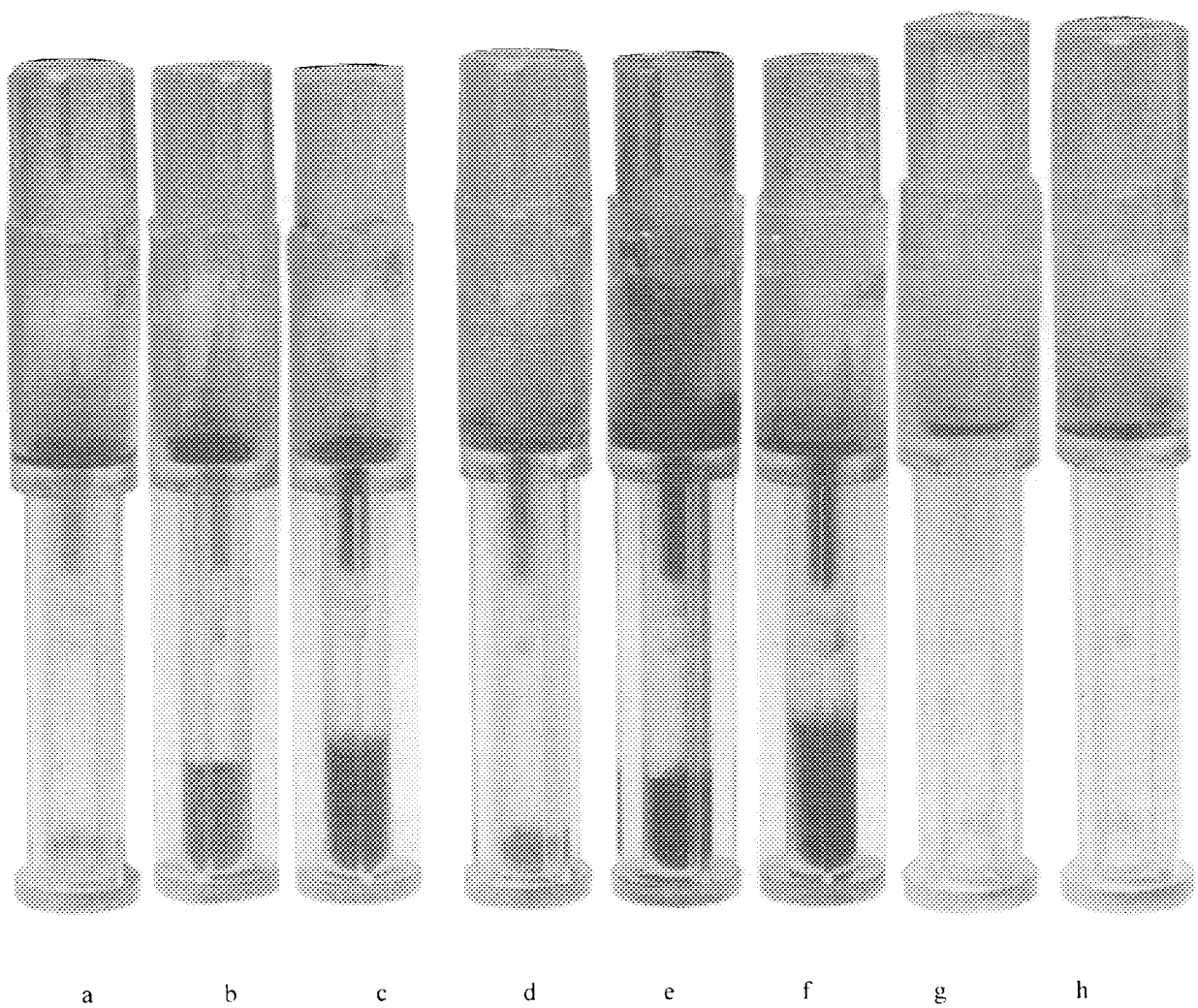

Fig. 3: Measuring of process for butter (a), olive oil (b), egg yolk (c), mustard (d), marmite (e), ketchup (f), toothpaste (g) and beyond moister creme (h).

2. Petroleum, oil, soap and paint industries

Figure 4 shows the results of the measuring off process for clean motor oil, motor oil drained from an engine after use, and green soap. Measuring off paint and tar is disappointing, as a significant quantity of material remains adherent to the wall of the reservoir and calibrated chamber.

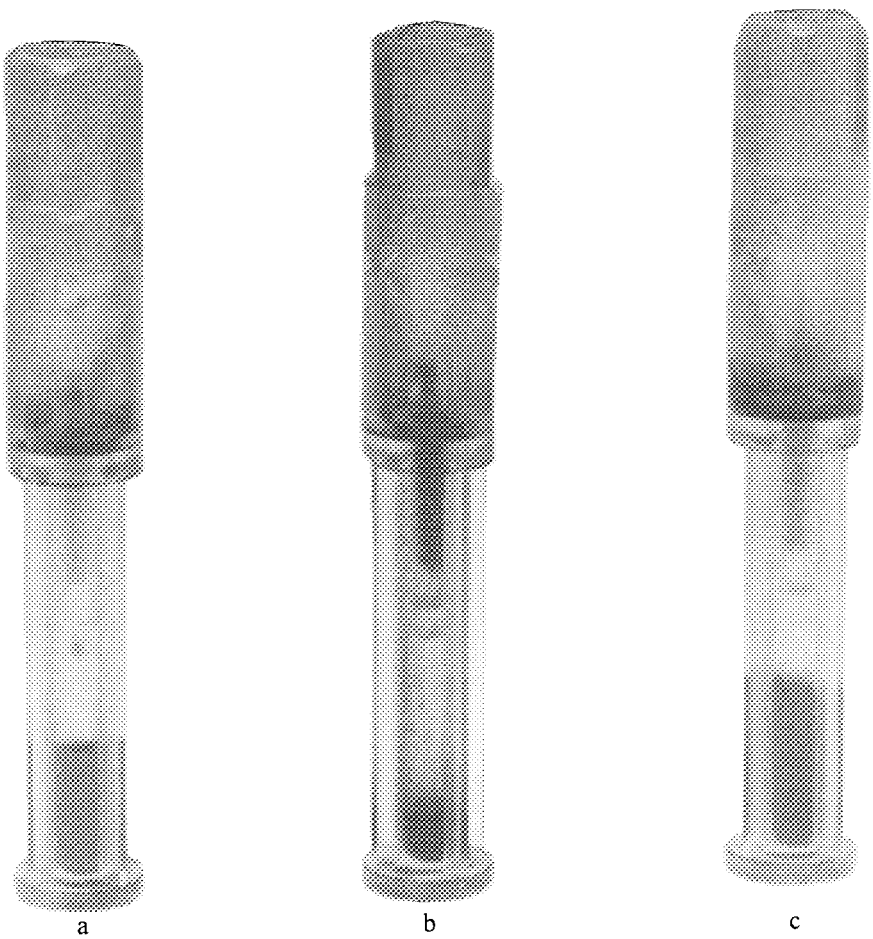

Fig. 4: Measuring of process for clean motor oil (a), motor oil drained from an engine (b) and green soap (c).

3. Environmental studies
As an aid to monitoring environmental pollution, attempts have been made to collect particles from harbour mud in the calibrated chamber. These were unsuccessful, however, owing to poor particle streaming characteristics and inadequate drainage of surplus wet sandy material.

4. Biochemistry, clinical and veterinary chemistry, physiology, pharmacology and toxicology
The impact of the new technique is perhaps greatest in the broad field of biology. To appreciate this, some comments regarding the routine handling of blood samples are useful.
Blood is collected from humans or animals to obtain information concerning biological processes, not only occurring within the circulation, but also within tissues with which blood is in equilibrium. Blood is a dynamic tissue containing many different particles (cells) suspended in a liquid matrix called plasma. Water forms the bulk of plasma, with proteins the second largest constituent, whereas red cells (erythrocytes) comprise the majority of the particles. As mentioned earlier, for practical reasons equilibria between blood and tissues are usually investigated only from the point of view of the plasma matrix and not the cells. However, with apologies to George Orwell, all blood components are equal but some are more equal than others in monitoring equilibria.
The analysis of substance associated with blood cells is usually undertaken after isolation and purification of the cells from the surrounding plasma by washing, a procedure that also removes compounds not tightly bound to

the cells and also disregards the fact that there are no washed cells in the circulation. An alternative method is to calculate the amount associated with red cells by determining the concentration in whole blood and substracting the amount present in the plasma fraction. However, as well as the previously mentioned difficulties in measuring the volume of whole blood, a serious disadvantage here is the use of two numbers with a high value (concentration in whole blood and that in plasma) to derive one with a value at times one or more orders of magnitude smaller. This indirect method leads to erroneous results and occasionally apparently negative concentrations are obtained on red cells (3). The technique described provides the opportunity to obtain plasma and pure red cells simultaneously from the same blood sample. As will be seen later, small concentrations on red cells are worth determining correctly.

The implications for the investigation of circulatory transport,
and the estimation of the concentration in tissues can be envisaged as follows:
By determining concentrations solely in plasma, only a limited understanding of the transport processes in blood and the equilibrium with tissues can be achieved. About 45% of blood volume is occupied by cells. In an adult, the total surface area of erythrocytes is about 3000 km^2, approximately one million times larger than that of the total capillary membrane through which tissues come in contact with blood. The inner surface of the capillary membrane (endothelium) is therefore in a dynamic equilibrium with the much larger red cell surface with which it comes in contact and with which it can fuse transiently during passage of red cells (4).
The association of endogenous compounds drugs or nutrients with red cells, is often weaker than their binding to plasma proteins, and therefore the residence time on these cells is shorter (3). The level of this more mobile red cell pool - rather than the plasma concentration - will be most representative of the equilibrium between blood and tissues, particularly in the case of compounds that pass readily through the capillary membrane.

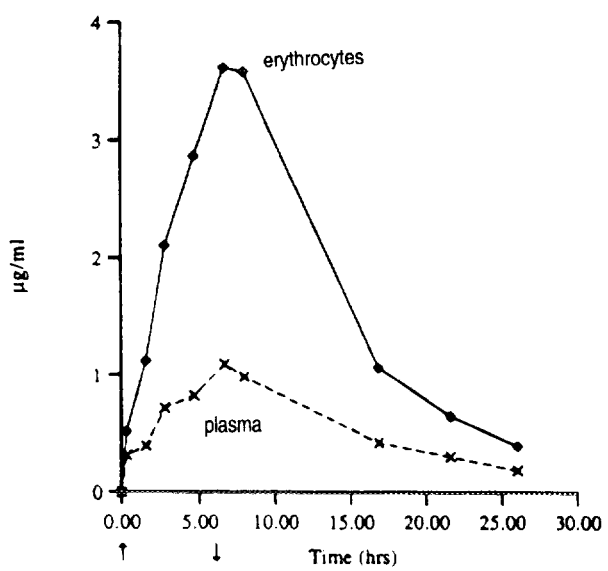

Fig. 5: Example of a compound located mainly in the red cell fraction, i.e. ifosfamide mustard (see ref. 6 for details) (↑, start of infusion; ↓, end of infusion).

The analysis of the red cell load therefore is of undisputed value in two situations. Firstly when the concentration on red cells is high, sometimes considerably greater than in plasma, and the residence time in the red cell fraction is shorter. If this site of cellular transport is ignored, a substantial percentage, if not all, of a circulating compound is overlooked (5, 6, 7). Figure 5 shows an example of such a situation. Secondly, when the red cell load is only small compared to that of plasma, and the compound is mainly carried bound to plasma proteins. One must appreciate that a circulating compound not fully soluble in plasma water, and with a plasma protein binding stronger than its association with red cells is the rule rather than the exception (3). However, the quantity transferred by red cells can still be important, provided that the turnover of the red cell fraction is significantly faster than that of the plasma protein fraction (8). It is in this scenario that the analysis of the erythrocyte compartment, is even more vital, as changes in the red cell pool, and therefore in the more dynamic equilibrium with tissues, is not evident in the plasma fraction (9, 10).

An assessment of the transport of ingested nutrients and drugs from the gastrointestinal tract into the blood, and the determination of the percentage reaching the body, the oral bioviability, is limited by the absence of the contribution of the blood cell fraction. A general method is to compare the plasma concentration-time curves obtained following oral and intravenous administration of a fixed quantify of a compound (e.g. drug, vitamin or nutrient). One situation exemplifying potential difficulties, which perhaps lead to erroneous results, can be outlined. If the compound is administered intravenously over a period of minutes, the plasma protein binding capacity will be exceeded, and there is a significant uptake by the cells, these will transport the excess which will be undetected. A slower absorption process from the gastrointestinal tract, lasting a number of hours, may not fully saturate the capacity of the plasma protein binding sites, and as a result less of the compound will enter the blood cell fraction after oral administration. Consequently, by comparing only the two plasma concentration data sets, there is a risk that the oral bioviability will be overestimated.

In summary, the facilitation of the volumetry of both viscous and non-homogenous fluids, provided by the technique described, will lead to greater insights into the equilibria of many substances between blood and tissues, identifying as yet unknown transport processes. An example is the study of the lipid equilibrium between erythrocyte membrane and the vascular endothelium, particularly of interest in the investigation of the origin and disappearance of fatty streaks in the intima of blood vessels (11). In monitoring the use of pesticides, those more loosely bound to red cells than to plasma proteins may be analysed more effectively in the blood cell fraction, which may be more sensitive than the plasma fraction to changes in the equilibrium between fat depots and blood.

Conclusion

As the need for viscous and non-homogenous fluid volumetry extends through most scientific disciplines, the instrument described (12) will find a place not only in biological research and service laboratories but also in institutions within other fields.

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ELECTROINJECTION ANALYSIS - THE NEW FIA VARIANT.

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Summary

The new combination of flow - injection analysis (FIA) and capillary zone electrophoresis (CZE) was proposed [1,2] and called electroinjection analysis (EIA). According to EIA sample and reagent are injected from the opposite ends of the capillary simultaneously electrokinetically, they meet in the capillary, go one through another and react. Product of reaction is detected photometrically. Due to the presence of electroosmotic flow it is possible to mix in this way not only oppositely charged sample and reagent but even the reactants with the same sign of the charge but different electrophoretic mobilities. Due to the absence of flow nonuniformity and the dispersion connected with sample reagent mixing, peak broadening in this new method, called EIA, is much smaller than in FIA and that is why sensitivity is higher.

FIA is widely used for food and beverage analysis and we hope that most of these reactions can be realized in EIA variant with higher sensitivity and with only one main limitation : either sample or reagent must be present in the charged form. EIA has much in common with EMMA [3] and it is shown [1] that these methods are mutually complementary. Two mathematical models for EIA and EMMA are developed. First one has the analytical solution and is for the case when the concentration of reagent is much higher than the concentration of the sample. The second one is for the arbitrary concentration ratio and is able to describe the case when the formed product is relatively unstable. The effect of kinematical focusing of product that occurs when the electrophoretic mobility of the product is close or equal to the electrophoretic mobility of the reagent is described by both models. This effect gives the possibility to further enhance the sensitivity of EIA determinations.

EIA determinations of Fe(III), Ti(IV), Cu, Zn, Co, Cr(VI) ions in water are realized. The 2 ppb level that is good enough for drinking water analysis is achieved. Another advantages of EIA and EMMA over FIA namely the intrinsic sample separation due to CZE and the possibility to preconcentrate the sample by electrostacking were also exploited.

The authors are interested in discussions on the possibilities of EIA applications for food and beverage analysis.

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Identifying the Important Economic Concerns of the Food Processing Industries World-Wide as They Prepare for the 21st Century

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This paper summarizes the food-relevant macro trends in demographics and socio-economic development expected towards 2020. It draws implications for the growth in world, and especially the developing world's, demand for food products. It suggests some challenges for the agribusiness community in meeting this rising food demand, providing the needed technologies, and responding to the concerns of food security and improved nutrition.

The Socio-economic Context

Last year's World Food Summit sponsored by FAO focused on the rapidly rising global demand for food, and the constraints on the global production systems, especially land and technologies, to meet this demand. The Summit highlighted the issues of new production technologies and the worsening plight of some of the developing countries.

Hunger is, and will remain, the primary challenge confronting developing countries.

> About 800 million people -- 20% of the developing world's population -- were food insecure in 1995: they lack economic and physical access to the food required to lead healthy and productive lives. *(IFPRI, 1995)*

The prospect of inadequate food supplies is real. This has implications for every nation's food security. Inadequate food supplies, and high food prices, have too often been the cause of civil unrest. Every nation is concerned that this basic need -- adequate access to food -- will not just be ensured but enhanced during the coming quarter century of economic development.

Many nations are now actively formulating and pursuing food and nutrition policies. The core of these policies is the state's responsibility to create an enabling environment for business and commerce, of which agriculture production and agribusiness are a part. A modern state is responsible for the creation of safety nets for its citizens who might otherwise not survive at an acceptable level under such an environment. It is also the responsibility for the modern state to be concerned with regulation of health standards for food, and with health policy, the impact nutrition on productivity and its delivery of health services.

These national policies will have implications for agribusiness active in that economy. It is recognized that these policies cannot be successfully pursued without a partnership with agribusiness. Each government will expect from agribusiness corporate actions that demonstrate understanding of, and directly contribute to furthering national food security.

Agribusiness can expect governments' support, providing appropriate public sector services, infrastructure, a "market-appropriate" regulatory framework, and polices that foster the development of the private sector.

Conditions for fast expanding food markets are now being created through the economic policies being actively pursued by the developing countries. A recent study[1] in the middle-income developing economies shows investments in agribusiness have growth rates in the order of 8% annually, far higher that growth rates of many other industries in these economies.

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* The views expressed in this paper are those of the author, and do not necessarily represent the views of the World Bank.

1 communicated (*John Mellor & Associates, Washington DC, 1997*)

Major Demographic Trends

There are four major trends that identify both the challenges and the opportunities for agribusiness to 2020:

- Population growth
- Expanding urban population
- Growth in household (or per capita) incomes
- Women's participation in the labor force

World population is projected to grow from 5.7 billion in 1995 to 7.9 billion in 2020, a 38% increase, or 2.2 billion new mouths to feed. The developing economies will account for 84% of this increase (*United Nations, 1995*). We can think of this as an additional 88 million people annually for the next quarter century, or just over the 1995 population of Germany.

Urban population growth projections provide the indicator for the growth and size of future market demand for food. By 2020 the global urban population will grow by 78%, and the developing world's urban population will grow by about 110%. For the developing countries this will be an urban food market-place of 3.6 billion consumers (*United Nations, 1995*).

Real incomes are growing. This is accompanied by a rise in the quantity and the diversity of food demanded.

Food demand will therefore grow at a rate faster than the population growth rate.

Compare developing countries' population growth at 1.3% annually with growth in demand for livestock products in the 2.8 to 3.5% range, and cereals in the 2% range (*Rosegrant et al, 1995*).

Projections are not available for the growth in demand for fruits, vegetables, beverages or agro-processed products. These products' growth rates usually exceed those of livestock products in upper-middle income countries, and lie between that of livestock and cereals in lower-middle income countries.

Urban food demand in 2020 in developing countries will grow to 240% of 1995 levels.

By 2020 food demand in developing countries is expected to have increased by about 97% over 1995 levels. Global food demand will rise by about 86%, when the developed world's lower growth in food demand is included.[2]

Food volumes to be delivered to developing countries' urban areas through formal marketing channels -- the business of agribusiness -- will have to increase annually at an average of about 3.6% to meet this demand.

Projected Income Annual Growth Rates (1995-2020)

Regions	*Annual Growth*
Asia*	4.0% to 7.0%
East Africa	4.5%
Central & West Africa	3.8%
Northern Africa	3.3%
Southern Africa	3.2%
Other Latin America**	3.5%
Developed Economies***	***2.2%***
Eastern Europe	1.6%
Former Soviet Union	1.6%

Source: IFPRI (communicated): underlying assumptions for *Rosegrant et al* projections

* excluding Laos, Cambodia and Pacific

** excluding Argentina, Mexico and Brazil at 2.8%, and Colombia at 3.7%

*** except Japan at 2.8%

[2] Endnote 1 shows assumed and derived annual growth rates in food demand.

This projection of the rate of growth in demand in *the market places* in developing countries is probably conservative because these projections do not capture two other important trends, *viz.*:

Food Demand 2020 as % 1995

	Rural	*Urban*	*Total*
Developed Countries Average	85%	133%	124%
Developing Countries Average	145%	242%	197%
Global Average	141%	218%	186%

- ***The increasing demand of rural populations for purchased foods.*** If poverty alleviation and rural development strategies are successful, there will be a decline in farming populations and an increase in rural population engaged in non-farm employment. This trend too will increase the *rural market-place demand* for purchased and processed foods; and

- ***Women's increasing participation in the labor force and increasing demand for processed foods.*** Changing consumer food demand will be strongly influenced by the increasing numbers of women participating in the labor force -- a 64% increase in developing countries by 2020, with an 115% increase in the upper middle income sub-group. This reduces women's time available for food preparation, and increases demand for processed foods.

Women's Participation in the Labor Force

(millions)	*1995*	*2020*	*Increase*		
			Number	*%*	*Annual Growth*
World	1,092	1,731	640	59%	1.9%
Developing Countries	910	1,494	583	64%	2.0%
of which:					
Upper Middle Income	62	133	71	115%	3.1%
Lower Middle Income	203	346	143	71%	2.2%

Source: 1995: World Development Indicators 1997 (*World Bank, 1997*);
2020 projection derived from historic growth trends 1980-1995

Price and International Trade Trends

Historically there is a continuous downward trend in food commodity prices. This is most obvious in the staple grains, livestock products and sugar. This same trend is evident in tropical fruits and vegetables, and their related processed products. As new bio-technologies are applied to the food production systems (*World Bank, 1990a*)[3] these downward price trends will continue. But the cost of raw materials is almost irrelevant in determining retail consumer food prices. There will be continuing competitive pressures to reduce distribution chain costs.

Projections show increases in absolute and relative volumes of primary commodities in international trade moving from the developed to the developing countries (*Rosegrant et al, 1995*). Liberalization will also increase production and trading specialization and thus increase the quantities of traded goods as comparative advantage emerges. However, I wish to suggest a variation on this scenario. If we take into account consumption of fruits, vegetables, beverages and processed foods, then that in relative terms over the next 25 years and, in spite of increasing flows of tropical products to the developed economies, food volumes in international trade will decline as a proportion of the total volume of marketed food domestically and internationally. Countries' food security policies will, irrespective of the arguments for comparative advantage, expect increases in local production and processing wherever possible and profitable.

The recent global reforms in international trade policy as a result of the Uruguay Round (*FAO, 1996*) and the World Trade Organization (WTO) are already increasing competitive pressures on all food producers and traders. This competitive

[3] see Endnote 2

pressure from quality product will translate into a demand for comparable quality of local products and cost effective advanced technologies.

The next twenty five years will see large increases in investment in agribusiness in the developing economies to serve local markets. This is a different investment strategy from the past, when many agribusiness investments were made by foreign investors primarily to serve export markets in the developed economies.

The Nutrition Factor

Malnutrition is a major concern in the developing countries. If food security and nutrition polices are not successfully implemented we can expect that world malnutrition will increase. The World Bank and other development agencies are supporting and actively promoting nutrition educational programs and the inclusion of a nutrition component in governments' development policy. Nutrition education campaigns are becoming more widespread in the developing countries as governments recognize the important economic contribution of good nutrition.

> Prospects for reducing malnutrition among the world's children are grim. About 18 million children under the age of 6 are seriously underweight for their age. *(IFPRI, 1995)*

There are direct links between good nutrition and worker productivity, the incidence reduction of certain diseases, and the mental development of children *(IFPRI, 1995)*. Yet with rapid growth in urbanization and personal incomes, obesity increases. Obesity, and other nutrition related diseases, are already significant concerns for health policy in Latin America *(World Bank, 1994a)* In China obesity is forecast to increase 15% by 2020 *(IFPRI, 1995)*. Recent research suggests that over-consumption of fast foods, both as a response to pressure to remain working longer hours, and also as a result of a lack of understanding of the nutritional implications, could be a factor *(Rozowski et al, 1997)*.[4]

In the developed economies improved consumer awareness of health and nutrition is resulting in an increasing demand for food of high nutritional value, e.g. "farm-fresh" product; reduced salt and fat content; moving away from over-processed foods, chemical additives and preservatives.

With the increasing international trade in foods, including processed products, many of these products -- including "health foods" and organic foods -- are now being marketed to the upper income strata of the developing world. Over time this awareness and demand will move into the other income strata. During the next quarter century demand for these products will increase in developing countries, at rates closely comparable to this market segment's growth rates in the developed economies.

I wish to suggest one additional factor that is presently not receiving adequate attention. The very significant increasing volumes of food that will physically need to be delivered to urban areas, raises the question: *"Is it efficient and affordable to continue to deliver required daily nutritional requirements through increasing quantities of traditional foods?"*

There are foods already known, some even pre-historic, that are more nutritious than current substitutes. Some are now reintroduced and marketed, but almost exclusively in upper income specialty markets. The Inca's staple, *Quinoa,* is a good example: so is *Amaranth*.[5] Such foods do not need significant investment in new technologies to bring them to mass markets. What is needed is a recognition that such products should be marketed and distributed to a broader socioeconomic spectrum, and that agribusiness accepts the challenge to actively promote and market such "enhanced-nutrition foods."

It will be good business, and will also be responsive to the nutritional aspects of national food and nutrition policies, if agribusiness were also to actively pursue the challenges of:

- nutrition priority in food production, complemented by pro-active nutrition content in product marketing and advertising;
- plant breeding for increased nutritional content in staple and perishable foods; and

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4 See Endnote 3.

5 see Endnote 4.

- quantity and nutritional value preservation during post-harvest, processing and distribution.

Responsive Agribusiness Strategy

The usual business strategy analysis of market niches opportunities suggests that priorities for agribusiness investment would be in those regions and countries where:

- income levels are relatively high, and the middle class is growing fast;
- there is a large food-purchasing population (i.e. primarily the urban populations); and
- there is a rapid increase in the level of women in the work force.

But this approach would not move us much away from already relatively well served markets in the upper-middle income developing countries -- the larger Latin American economies[6] and the so-called "Asian Tigers."[7] It would perhaps expand on the established agribusiness presence in China, India, and in the "transition economies" of Central Europe and the former Soviet Union. In 2020 this would account for an urban market of only about 600 million, or just 17% of the urban population in the developing world.

This "business as usual" approach would not be responsive to the social-economic concerns for national food security, either in smaller middle income developing countries, or in the expected large food markets of the lower income developing countries -- an expected urban market of about one billion by 2020.

Noteworthy are the appearance of Lagos(#3), Karachi (#7) and Dhaka (#9) among the top ten cities by 2015, and each with over 19 million inhabitants. Istanbul (#20), Lima (#26), Teheran (#27), and Kinshasa (#28) are among the top 30, all with over 9.5 million inhabitants each (*United Nations, 1995*).

What is perhaps even more noteworthy is the large increase in the number of "urban aggregations" of more than half-a-million each in developing countries -- 779 in 2020 by comparison with 478 in 1995, for a total urban population in these large centers of 1.65 billion. These urban aggregations offer new opportunities for economies of scale and efficiencies in food distribution.

The challenge to agribusiness is to adopt corporate development strategy to *profitably* serve these lower income markets, thus assuring consumers adequate supplies of affordable and nutritious food. Some have suggested this cannot be done; that serving lower income food markets will not be profitable; and therefore this should be the role of Governments. With current technology and marketing techniques they are partially correct, these markets are not profitable to commercial companies. But, it is unlikely that governments will be able to deliver food to these income strata at a lower cost than the private sector.

Current distribution systems are too costly to adequately service the lower income strata. Improvements in telecommunications, adopting "just-in-time" inventory management, introduction of improved logistics and quality management, and taking advantage of the scale economies of the large urban centers, will significantly reduce marketing chain costs. These systems improvement, when combined with more efficient processing technologies, will permit affordable food to be profitably delivered to lower income strata.

Partnerships between agribusiness and grassroots organizations can further reduce costs. These include farmers' associations, exporters'

Developing World 10 Largest Cities: 2015

	Millions	*Rank**
Bombay	27.4	2
Lagos	24.4	3
Shangahai	23.4	4
Jakarta	21.2	5
Sao Paulo	20.8	6
Karachi	20.6	7
Beijing	19.4	8
Dhaka	19.0	9
Mexico City	18.8	10
Calcutta	17.6	12

Source: *United Nations, 1995*
(*) Rank place includes developed country cities: Tokyo is #1 with 28.7 million, and New York #11 with 17.6 million.

6 Argentina, Brazil, Chile, Colombia and Mexico

7 Malaysia, Singapore, South Korea, Thailand

associations, consumer cooperatives, as well as the fast expanding non-Governmental Organizations and development foundations.[8]

Governments too will be partners, with the provision of improved telecommunications and transport infrastructure, improved legal systems, and other policies that reduce risk and barriers to business entry.

So I wish to suggest to you that serving the lower income markets is possible. It can, and must be done by the private sector. Let me quickly add that I am not suggesting that serving social welfare cases is a profitable business strategy. Instead, I urge agribusiness to develop business strategies to reach the lower, not lowest, income groups. I am sure that by 2010, for many developing countries this can be done by agribusiness *without subsidies*, if they now accept this as a priority and a challenge.

To meet real social concerns of food security and the stability of civil society, it is a social imperative that your industry now accepts this challenge. This is a very different challenge to agro-technology development, from that of providing increasingly sophisticated foods to the increasingly sophisticated upper income markets of the developed economies.

The Technology Challenge

Increasing concerned over the environment will reduce the levels of pesticide use. Consumer concerns over chemical residues in food will change the post-harvest chemical treatments that have been used to ensure longer shelf-life. There will be consumer resistance to hormonal treatments of food products. All these factors are a part of the context in which agribusiness will have to operate. Adherence to environmental, health and nutrition standards will be demanded in developing country markets, as it is today in the developed markets.

With the increasing competition from international trade, quality imported product will become more available in the developing countries. Therefore, to compete requires the latest and best technologies in the developing countries' post-harvest, distribution and processing chains. This requires transfers of advanced technologies to the developing world. This is a change. In the past, mostly second or third generation technologies were transferred to developing countries. Gone are the days when the relative isolation of the local markets from international competition would permit such a "low technology" strategy to be viable.

While joint venture approaches have predominated in the past as the vehicle for the transfer, protection, and management of advanced technologies, this too is changing. There is an increasing cadre of local business interests willing to buy technologies and not needing a strategic partner. We will see an increasing role for technology transfer agents -- primarily the equipment manufacturers and the turn-key contractors. So too will "design, build and operate" contracts play a larger role in the introduction of advanced technologies into the developing countries agro-industries.

Managing these new technologies will require a technically well trained labor force. This is an area for cooperation between governments and agribusiness. We can expect that the national food policies will require expanding the availability of technological and managerial skills. In the medium term, the local food industry will strive to decrease dependency on foreign technologies.

Given the dynamic market shift in demand to the developing countries, it is inconceivable that the current technology-rich and technology-poor split between the nations can continue. There are both social and economic imperatives to increasing the levels of technological skills in the developing countries, not just to receive new imported technologies, but as developers of new technology. We will see an increase in the number of governments adopting science and technology development policies and incentives, to redress the present imbalance and technological dependency on the developed countries.

A responsive strategy of agribusiness will be to:

- support the development of the higher education systems in developing countries for science, technology and business skills;

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[8] see Endnote 5.

- increase cooperative R&D with developing country institutions, and ultimately relocate some R&D efforts to the developing world; and
- in the short term pursue "new-technology" transfer strategies through a process that actively builds local skills and capabilities.

Of course this requires adequate protection of intellectual property rights. The recent activities of the UN and WTO in this area will promote and protect these rights. However, the most effective incentive for presently technology-poor countries to uphold these international conventions will be when their own scientific and high-tech communities have a stake in protecting their own inventions and patents. It is good business to help build and promote these local stakeholders.

Cooperation with the World Bank Group

The Bank Group has recently adopted a new policy of outreach and cooperation with the private sector. Public information sources are available through the World Wide Web,[9] and through the Business Partnerships Office.[10] We welcome direct contacts in Washington. We also have offices in London, Paris and Tokyo, and in most of the developing countries. Our local managers welcome discussions with potential business partners.

The **World Bank**, through its loans to governments, and its recent re-commitment to rural development, will be increasing its support for the public sector infrastructure for food supply. We support national agriculture research institutions, and the network of international research centers for agriculture under the umbrella of the CGIAR.[11]

We continue our lending for infrastructure in urban and rural areas -- in power, water, transport and telecommunications -- which in turn will support food market development.

Private sector investments are promoted through the Bank's provision of lending resources to the local financial institutions, and through its active involvement with governments to strengthen these financial institutions. The World Bank also engages in a pro-active dialogue with its member governments on the development of policies, and the management of the macro-economy, to encourage private investment, both local and foreign sourced.

The **International Finance Company** (IFC) is the World Bank Group's private sector investment organization. IFC is a partner in almost every developing country with local and foreign investors. IFC's portfolio in food and agribusiness is already at $1.3 billion, and it is growing at a rapid pace. IFC would be pleased to consider investment proposals in agribusiness.[12]

The **Multilateral Investment Guarantee Agency** (MIGA)[13] provides investment guarantees to the private sector against a range of political risk -- e.g. the risks of currency transfer restrictions, expropriation, war and civil disturbance.

It is my role in the Bank to be the focal point for agribusiness and marketing. This new office is developing a consultative and out-reach network with agribusiness world-wide. The objective of this network is to exchange information and views, and to permit us to discover what you are doing and thinking, and for you to know the same of us.[14] We hope through this dialogue to improve the products we deliver to our member governments, and to better serve the private sector interests in agribusiness.

I look forward to our continuing dialogue and thank you for this opportunity to share with you my personal vision of the opportunities and challenges for agribusiness for the next quarter century.

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9 *www.worldbank.org*

10 *www.worldbank.org\html\extdrv\business\bpcent.htm*

11 CGIAR -- Consultative Group on International Agricultural Research: *www.cgiar.org*

12 The IFC web page -- *www.ifc.org* -- contains information on emerging markets, and can be used to send investment proposals and inquires.

13 *www.miga.org*

14 electronic mail address: *bberman@worldbank.org*

Endnotes.

1. Assumed developing country food demand average growth at 2.5% annually, developed countries and rural areas in developing countries at 1% annually, the following annual growth rates evolve:

Food Demand Growth Rates 1995-2020

% annual average growth rate	*Rural*	*Urban*	*Total*
Developed Countries	-0.7%	1.2%	0.9%
Developing Countries	1.5%	3.6%	2.75%
Global Averages	1.4%	3.2%	2.5%

Sources: derived from *Rosegrant et al, 1995* and *United Nations, 1995.*

2. Market research predicts that US sales of bio-engineered products in agriculture and the environment will rise from US$475 million in 1995 to US$3 billion in 2000 and US$8.5 billion in 2005. Most of the product will be bio-engineered seed and crops, with enhanced pesticide-resistance, insect resistance. Other products will be bio-engineered pesticides and animal health products. It is interesting to note that surveys of the leading biotechnology researchers in agriculture, do not indicate any major trends in new product development with biotechnology applications for post-harvest applications, or for food nutrition improvement.

   With similar levels of research investments being made in western Europe and Japan on biotechnology, similar levels and growth of product sales in these markets can be expected.

3. Prevalence in Latin America appear to be on the increase and that levels can get as high as 50% in some countries like Uruguay (Body Mass Index>25 kg/m2). In Chile they have shown levels of 20% in men and 40% in women (sample from Santiago 1992, Body Mass Index>27.3 for women and 27.8 kg/ m2 for men) (*Rozowski et al, 1997*).

4. ***Chenopodium Quinoa*** (Linn.), cultivated in the Andean region since at least 3000 BC. It was displaced as a basic food with traditional grains since the Spanish conquest. It was displaced as a basic food with traditional grains since the Spanish conquest
   Genus ***Amaranthus***: forms with white seeds cultivated in pre-history for use as grain crop. It is a traditional food in India, Mexico, Peru and some other countries. The grain has an unusually nutritious balance of amino acids (*Seguar-Neito et al*, 1994).

5. **Fundacion Peru** is an example of a development foundation pursuing innovative approaches in agricultural product marketing in conjunction with achieving social and economic development objectives, and working in close collaboration with domestic and foreign private sector partners.

Demographic & Socio-economic Projections

Table 1: Population Projections

(billions)	*1995*	*2020*	*Increase*		
			Number	*%*	*Annual % Growth*
World	5.716	7.888	2.172	38%	1.3%
Developing Countries	4.550	6.656	2.106	46%	1.5%
of which:					
Middle Income	3.975	5.604	1.629	41%	1.4%
Lowest Income	0.575	1.052	0.477	83%	2.4%

Source: *World Urbanization Prospects: The 1994 Revision* (United Nations, New York, 1995)

Table 2: Urban Population Projections

(billions)	*1995*	*2020*	*Increase*		
			Number	*%*	*Annual % Growth*
World	2.584	4.599	2.015	78%	2.3%
Developing Countries	1.715	3.582	1.867	109%	3.0%
of which:					
Middle Income	1.586	3.165	1.579	100%	2.8%
Lowest Income	0.129	0.417	0.288	223%	4.8%
Urban as % Total					
World	45%	58%			
Developing Countries	38%	54%			
of which:					
Middle Income	40%	56%			
Lowest Income	22%	40%			

Source: *World Urbanization Prospects: The 1994 Revision* (United Nations, New York, 1995)

Table 3: Urban Population Projections by Developing Country Regions

(millions)	*1995*	*2020*	*Increase*		
			Amount	*%*	*Annual % Growth*
East Asia & Pacific	591.3	1,195.5	604.2	102%	4.8%
South Asia	332.8	789.4	456.7	137%	5.9%
Sun-Saharan Africa	176.6	529.2	352.6	200%	7.6%
Latin America and Caribbean	357.7	564.2	206.5	58%	3.1%
Middle East and North Africa	191.0	393.1	202.1	106%	4.9%
Central, Eastern Europe and Central Asia	61.1	110.7	49.5	81%	4.0%
Total Low and Middle Income Countries	***1,710.5***	***3,582.1***	***1,871.6***	***109%***	**5.1%**

Source: *World Urbanization Prospects: The 1994 Revision* (United Nations, New York, 1995)

Table 5: 30 Largest Cities: 2015

	1990		*2015*		*% Increase*
	(Millions)	*Rank*	*(Millions)*	*Rank*	
Tokyo	25.0	1	28.7	1	15%
Bombay	12.2	6	27.4	2	125%
Lagos	7.7	22	24.4	3	217%
Shangahai	13.5	5	23.4	4	73%
Jakarta	9.3	16	21.2	5	128%
Sao Paulo	14.8	4	20.8	6	41%
Karachi	8.0	21	20.6	7	158%
Beijing	10.9	8	19.4	8	78%
Dhaka	5.9	30	19	9	222%
Mexico City	15.4	3	18.8	10	22%
New York	16.1	2	17.6	11	9%
Calcutta	10.7	9	17.6	12	64%
New Delhi	8.2	19	17.6	13	115%
Tianjin	9.3	15	17	14	83%
Metro Manila	8.0	20	14.7	15	84%
Cairo	8.6	18	14.5	16	69%
Los Angeles	11.5	7	14.3	17	24%
Seoul	10.6	11	13.1	18	24%
Buenos Aires	10.6	10	12.4	19	17%
Istanbul	6.5	25	12.3	20	89%
Rio de Janeiro	9.5	13	11.6	21	22%
Lahor			10.8	22	
Hyderabad			10.7	23	
Osaka	10.5	12	10.6	24	1%
Bangkok	5.9	29	10.6	25	80%
Lima	6.5	26	10.5	26	62%
Teheran	6.4	28	10.2	27	59%
Kinshasa			9.9	28	
Paris	9.3	14	9.6	29	3%
Madras			9.5	30	
Moscow	9.3	17			
London	7.3	23			
Chicago	6.8	24			
Essen	6.4	27			

Source: *World Urbanization Prospects: The 1994 Revision* (United Nations, New York 1995)

Table 5: Number of Cities and Population, By City Size Class

	1995		*2015*	
	Agglomerations	***Pop. (Millions)***	***Agglomerations***	***Pop. (Millions)***
Cities of Populations of:				
10 Million or More				
World	15	207.5	27	449.6
Developed	4	66.2	4	71.2
Middle Income Developing	11	141.4	23	378.5
Lowest Income Developing	0	0.0	1	19.0
All Regions	***15***	***207.6***	***28***	***468.7***
5 million to 10 million				
World	23	167.4	44	281.7
Developed	6	44.5	8	56.2
Middle Income Developing	17	123.0	36	225.6
Lowest Income Developing	1	7.8	7	45.3
All Regions	***24***	***175.3***	***51***	***327.1***
1 million to 5 million				
World	287	552.4	472	941.0
Developed	102	204.2	120	239.7
Middle Income Developing	185	348.2	352	701.3
Lowest Income Developing	13	27.3	37	77.1
All Regions	***300***	***579.7***	***509***	***1,018.1***
500,000 to 1 million				
World	338	237.8	422	293.2
Developed	112	78.3	123	83.7
Middle Income Developing	226	159.6	299	209.5
Lowest Income Developing	25	18.8	24	16.8
All Regions	***363***	***256.7***	***446***	***310.0***
All Cities Over 500,000				
World	663	1165.1	965	1965.5
Developed	224	393.2	255	450.8
Middle Income Developing	439	772.2	710	1514.9
Lowest Income Developing	39	53.9	69	158.2
All Regions	***702***	***1,219.3***	***1,034***	***2,123.9***

Source: World Urbanization Prospects: The 1994 Revision (*United Nations, New York, 1995)*

Table 6: Women's Participation in the Labor Force

(millions)	*1995*	*2020*[a]	*Increase*		
			Number	*%*	*Annual % Growth*
World	1,092	1,731	640	59%	1.9%
Developing Countries	910	1,494	583	64%	2.0%
of which:					
Upper Middle Income	62	133	71	115%	3.1%
Lower Middle Income	203	346	143	71%	2.2%

Source: *World Development Indicators 1997* (World Bank, Washington DC, 1997)

a) Projection derived from historic growth trends 1980-1995

Table 7: Women's Participation in the Labor Force by Developing Country Regions

(millions)	*1995*	*2020*[a]	*Increase*		
			Number	*%*	*Annual % Growth*
Central, Eastern Europe & Central Asia	703	1,379	676	96%	2.7%
East Asia & Pacific	314	677	363	116%	3.1%
Sub-Saharan Africa	108	212	104	96%	2.7%
South Asia	176	271	96	55%	1.8%
Latin America & Caribbean	83	158	76	91%	2.6%
Middle East & North Africa	23	61	38	167%	4.0%
Total Low & Middle Income Countries	***1,406***	***2,758***	***1,352***	***96%***	***2.7%***

Source: *World Development Indicators 1997* (World Bank, Washington DC, 1997)

a) Projection derived from historic growth trends 1980-1995

Table 8: Projected average annual growth rates in total demand for major commodities, 1990-2020: Baseline Scenario

Commodity	Developed Countries	Developing Countries	Latin America	Sub-Saharan Africa	West Asia and North Africa	Asia
			(percent)			
Beef	0.37	2.81	1.86	3.29	2.83	4.11
Pigmeat	0.44	3.40	2.36	3.42	2.74	3.51
Sheepmeat	0.63	3.10	1.94	3.12	2.63	3.62
Poultry	0.93	3.28	2.42	3.41	3.03	3.90
Total Meat	0.55	3.20	2.12	3.29	2.85	3.65
Eggs	0.77	3.27	2.58	3.57	3.04	3.48
Wheat	0.58	2.19	1.73	3.07	2.30	2.16
Rice	0.51	1.67	1.97	3.22	2.31	1.60
Maize	0.80	2.18	1.79	3.02	1.86	2.23
Other coarse grains	0.87	2.03	1.24	3.08	1.98	1.58
Total Cereals	0.75	2.00	1.71	3.07	2.18	1.88
Roots and Tubers	0.64	1.77	1.52	2.96	1.91	1.02
Soybeans	1.10	2.76	2.28	3.19	2.65	3.32

Source: *Rosegrant et al*, 1995

Table 9: Income Growth Rate Projections (1995-2020)

Country/Region	*Annual Average Growth*	*Country/Region*	*Annual Average Growth*
Developed Economies		**Middle East and North Africa**	
USA	2.20%	Egypt	3.20%
EC12	2.20%	Turkey	4.60%
Japan	2.80%	*Northern Africa*	3.30%
Other Western Europe	2.25%		
Australia	2.20%	**South Asia**	
Other developed countries	2.20%	India	5.50%
		Pakistan	5.00%
Europe and Central Asia Transition Economies		Bangladesh	4.50%
Eastern Europe	1.60%	*Other South Asia*	5.00%
Former Soviet Union	1.60%		
		South East Asia	
Latin America and Caribbean		Indonesia	6.50%
Mexico	2.80%	Thailand	7.00%
Brazil	2.80%	Malaysia	6.50%
Argentina	2.80%	Philippines	5.00%
Colombia	3.70%	Vietnam	5.00%
Other Latin America	3.50%	Myanmar	4.00%
		Other SE Asia	4.00%
Sub-Saharan Africa			
Nigeria	3.20%	**East Asia**	
Madagascar	3.80%	China	6.00%
Central & West Africa	3.80%	South Korea	5.00%
Southern Africa	3.20%	*Other East Asia*	1.20%
East Africa	4.50%		
		Rest of the world	4.90%

Source: IFPRI (communicated): underlying assumptions for food demand projections in *Rosegrant et a, 1995*

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EXAMINING WORLD-WIDE TRENDS IN CONSUMER FOOD PREFERENCES AND NEEDS IN THE 21ST CENTURY

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Consumers world-wide want good tasting food which is healthful for their body and fits into their lifestyle. These requirements present challenges and opportunities for the food industry. Innovative processing of perishable food, including fresh cut produce, has been driven by the demand for convenience. Health conscious consumers will seek new, flavorful, convenient ways to include produce in the diet in response to increased understanding of the health promoting attributes of produce. Pesticide use, food safety and the environment will continue to be a concern with conflict between those seeking to use traditional approaches and those embracing biotechnology. Consumer and media attention to microbiological safety of produce will increase. The industry will strive to extend shelf life and maintain safety through traditional and newer methods, including the potential use of food irradiation. The need for consumer education in an increasingly urbanized world will grow, specially when consumers are faced with conflicting information on safety, environmental impact and effectiveness.

Consumers world-wide want good tasting food which is healthful for their body and fits into their lifestyle. These requirements present challenges and opportunities for the food industry.

Good flavor is paramount to food enjoyment. Each country retains its own cuisine, the result of history, geography, and culture. Although we differ in traditional flavor preferences, increased travel and exposure to different cuisines and the desire for new flavor combinations have opened opportunities for blending of cuisines. This means new opportunities for exotic fruits and vegetables with grains, nuts, and more.

In the last thirty years many places in the world has seen a transition from a consumption-oriented consumer to a demanding consumer.[31] The pattern of marketing what agriculture produces has shifted to providing what consumers demand. While there are differences in spending patterns among countries, the global trend highlights nutrition and health, moderation, and quality. The consumer has become multifarious, shopping at several store formats, buying both top of the line and lower quality, depending on the item and circumstances of use.

There are opportunities to improve fruit and vegetable quality. U.S. consumer name several products they would purchase more frequently if the quality was higher. The sales potential for a better tasting tomato produced through biotechnology, for example, has been estimated at $450 billion in the next few years and possibly $ 1 billion annually there after.[16]

CONVENIENCE

Increased opportunities and demands for time drive the need for convenience. Consumers want to enjoy favorite foods without placing excessive demand on the scarce resource of time. For example, the demand on personal time when United States women in the work force went from 30% in 1950 to 59% in 1994 led consumers to seek convenience in meal preparation.[6,14] Consumers are purchasing partially or fully prepared items from the supermarket and they are increasing the number of meals purchased from restaurants. In 1970 about 33% of the U.S. food dollar went for food away from home.[10] By 1995 that proportion increased to 47% . Lunch is eaten out most frequently, however the greatest dollar amount is spend on dinners.[3]

The entire food service industry has experienced growth, but the greatest growth is in the fast food segment. Many people don't want to sit in the restaurant and eat. Restaurants are now featuring meals ready-to-go to capture this increasing share of the food expenditure dollars. Restaurant takeout and delivery food sales increased to 10.2% of total restaurant sales, while takeout and delivery sales increased to 50.5% of total fast food sales.[3]

Similarly in Europe the percentage of consumers eating out increased from 80% in 1992 to 85% in 1995.[31] Frequency of eating out differs by country. As few as 67% of Spanish consumers and as many as 96% of Germany of consumers eat out monthly. With the exception of Luxembourg, the number of meals consumed outside the home in Europe has significantly increased, even doubling in certain countries.

Restaurant meals are an opportunity for the food industry both as direct sales and to introduce the consumer to new flavor combinations which are replicated at home.[28] Promotion to the food service industry will bring both direct returns from that industry and indirect benefits through increased consumer at-home use.

United States supermarkets help the consumer meet the demand for convenience. The deli department offers to put the meal together and it is ready-to-go when the customer finishes shopping. Deli sales in the United States are up 6.5 %, approaching the 20 billion mark [29] Markets also make shopping easier by displaying grocery items which constitute a meal together.

Food processors help the consumer manage time and meal preparation by featuring fresh-cut produce. Pre-cut produce sales in the U.S. are expected to reach $19 billion by the year 2000.[29] The U.S. industry has seen growth in prewashed greens for salads, peeled carrots, and other cut, washed, and packaged vegetables with or without sauce or accompaniments. Carrots consumption has responded significantly to added convenience of pre-peeled baby carrots available in small, medium or large packages and individual packets carrots with dip. Surveys in the U.S. indicate consumers perceive fresh-cut produce as a good value because it saves time and there is less waste.[25]

Marketers are combining produce with grains or meat products to create a complete meal. Examples include individual salads with cheese or shrimp with a fork, boned chicken meat the consumer cooks with pasta and a prepared sauce, and frozen pasta and vegetable combinations to which the consumer adds meat or fish. Some manufacturers are adding additional value by featuring award winning recipes by famous chefs. A produce company that only features produce is not capturing this market.

Consumers value perishable products with a long shelf life. About half, 53%, of shoppers who buy convenience items rate a products ability to be stored for a long time as the most desirable product attribute.[29] Requiring little or not preparation time is important to 43%. Almost three quarters, 73% of consumers, say they would pay more for healthier versions of food they always eat and 54% will accept higher prices for more convenient versions of traditional foods.

Expect a change in consumer cooking skills. Although some love food and enjoy its preparation, the availability of economical fully-prepared meals either through the restaurants or supermarkets gives others an opportunity to forgo cooking. In the U.S. basic food handling is no longer a mandatory school subject. The traditional person who prepares meals is working outside the home and has less time to cook or teach cooking to the children. United States consumers say they eat out primarily because they don't have time to cook, but 18% simply don't want to cook and 5% acknowledge they don't know how![12] This suggests that convenience foods must look easy and directions should be very basic.

DIET AND HEALTH

People want to live a long, active life. They look to food to help them achieve this goal. Food must appeal to the pallet and also promote health of the body. Knowledge of nutrients which promote health will increase and people will seek health promoting components, as long as the food taste goods.

A significant world wide trend in the nutritional arena is the focus on dietary fat. The Food Marketing Institute's annual survey indicates consumer volunteered nutritional concerns over time. In the United States, concern about dietary fat reached a peak in 1995 with 65% identifying this as a concern and decreased to 56% in 1997.[2] Concern about sodium content of food is less important, mentioned by 23% of the sample. Dietary cholesterol concern peaked in 1990 at 44% and is identified in 1997 by 20% of those sample. "As natural as possible" is volunteered by only 3% of the sample. These attitudes reflect media coverage of nutrition issues in the U.S.

In Europe, concern about the fat content of food is the highest volunteered concern, mentioned by 34% of respondents.[31] Freshness is volunteered by 29% of respondents, followed by nutrient value and naturalness, by 16% each. Cholesterol level and sodium contest are volunteered by significantly fewer people, at 7% and 4% respectively. Australians are also concerned about dietary fat.[21]

The greatest potential impact of increased health consciousness is on fruit and vegetable consumption. When US consumers are asked the changes they have made for a healthy diet, over seventy percent of the people surveyed say they are eating more fruits or vegetables [1,15] While actual consumption has increased gradually, it has not been at the rate predicted by consumer intentions.[23,24] Nutritional scientists continues to find positive relationships between produce consumption and health.[30] .The health benefits of consuming fruits and vegetables and the phytochemicals they contain will drive increased consumption.

FOOD SAFETY

Consumers expect food to be safe. People are generally confident that the food they purchase in the supermarket is safe, however there are areas where consumers have concerns.

PESTICIDE RESIDUES

In the U.S. concern about pesticide residue is the most frequently volunteered food safety concern associated with produce, followed by mishandling and cleanliness.[25] Concern about pesticide residue, highest in 1989 at the time of the controversy over use of growth regulator, Alar on apples. Since that time confidence in the safety of produce and belief in the health-enhancing value of produce has increased due to a concerted educational efforts by the produce industry and health professionals.[1] Some supermarkets advertise use of a certification system to verify that produce meets legal pesticide residue minimums or contains no residues detectable by test sensitivity. Many supermarkets also offer organic produce.

Similarly concern about pesticide residues is high in Europe, where pesticide residues are considered a serious hazard by 79% of consumers in 1995.[31] This area of concern is second only to bacterial contamination, classified as serious by 85% of consumers.

Interest in organic production has grown in recent years. Attitude studies in the U.S. indicate interest in organic production is highest among those with less formal education and lower income.[11,24] Organic produce has been perceived by consumers as a pesticide-free production method. The United States Department of Agriculture National Organic Standards Board clearly indicates that organic is a method of production, not a pesticide free claim.[13] The greatest appeal for organic products is the perception that it is environmental beneficial; the greatest detriment is limited availability, high cost, and consumer misconception that organic produce is grown without the use of pesticides.[25] Each country develops its own definition of organic. Care must be taken to accurately position the organic approach to agriculture to avoid the appearance of deception.

MICROBIOLOGICAL SAFETY

Increased media coverage of food borne illness throughout the world will be responsible for increased consumer concern about microbiological safety. As scientists are better able to trace the cause of food borne illness, consumers will become aware of the link between consuming certain foods and becoming ill. Concerns about pathogenic bacteria, protozoa, and viruses carried by produce will impact international trade. While some will prefer traditional approaches, the acceptance of newer technologies to reduce risk will be an issue.

Consumer concerns about microbiological safety exceed other food safety areas. This concern has been the most frequently volunteered in the U.S. for the last several years and reached 69% in 1997.[2] When specific food safety areas are identified, microbiological safety concern are described as a serious hazard by 82% of U.S. people surveyed. Pesticide residues are considered a serious hazard by 66% of those surveyed. Similarly in Europe bacteria contamination is classified as serious by 85% of the people surveyed.[31] Among Australian consumers, volunteered concerns that relate to microbiological threats, germs and un-hygienic handling by staff or shoppers, sum to 53%, up from 31% in 1992.[21] When specifically asked, bacterial contamination is classified as the greatest public health risk by 73% of Australians.[8]

These relative concerns reflect the media coverage given food borne illness outbreaks. In the past, most people associated food borne outbreaks with foods of animal origin. Today, however, *E. coli* 0157:H7, *Cyclospora*, and other pathogens have been associated with produce. *E. coli* O157:H7 apparently from radish sprouts was responsible for widespread illness in Japan. Alfalfa sprouts were implicated in an outbreak in Virginia. *Salmonella* has been associated with pre-cut tomatoes, cantaloupe, and watermelons. *Cyclospora* has been traced to raspberries from Guatemala, fresh basil in the U.S., and a contaminated water supply in Australia. Hepatitis A in frozen strawberries was the source of an outbreak in the US in 1997. Although outbreaks can originate from conditions in the host country, it is easy to blame an importer. The summer of 1997 the Washington Post carried an article, "Is imported produce safe to eat?"[5]

Outbreaks have ramifications for produce production in general as well as lightly processed products. When fresh produce was suspected as the source of bacteria responsible for food borne illness, consumers responded by avoiding the product. Care must be taken in production and processing to avoid or destroy potential pathogens. This may conflict with consumer preference for natural and minimally processed food. Newer method of controlling food borne pathogens and extending shelf life of lightly processed food are being explored. These include use of special bacteria cultures and irradiation either alone or in combination with other food treatments. How do consumers respond to the concept of spraying bacteria on fresh fruits and vegetables? Response could be positive or negative depending on consumer information. There is a base of knowledge on consumer response to irradiation.

THE PROMISE AND CHALLENGE OF NEW TECHNOLOGIES FOOD IRRADIATION

Food irradiation is an effective quarantine treatment for some insects, it prevents sprouting of tubers like potatoes and onions, it extends the shelf life of some produce, and at the appropriate treatment, it can serve as a pasteurization treatment against many food-borne pathogens.[9] The food appearance, flavor, and nutrient value is essentially comparable to fresh. This process is approved by the appropriate regulatory bodies in over 30 countries and is endorsed for safety by health authorities such as the World Health Organization and the American Medical Association.

Most U.S. consumers are unfamiliar with irradiation with only 5% in 1996 indicating they knew a lot about the technology with 17% indicating some knowledge.[1] The Food Marketing Institute annual survey indicates those in the United States expressing concern for this process have decreased from 42% in the 1980's to 33% in 1997.[2] Likelihood to buy varies by product attribute. The Food Marketing Institute found about 60% very or somewhat likely to buy strawberries irradiated to keep them fresh longer and 70% would buy poultry irradiated destroy bacteria.[1] Other surveys indicate on average 60% would purchase irradiated produce, with the percentage increasing to 80% or more after an educational programs and to 90% or more among after sampling irradiated food.[7]

Irradiated processing can expand the market for quality fruit from insect quarantine areas. In the 22 month period between April 1995 and June 1997, over one hundred thousand pounds of tropical fruit from Hawaii including papaya, atemoya, rambutan, lychee, starfruit, and Chinese taro sold in U.S. Midwest markets in collaboration with a study to determine quarantine treatment.[33] Fruit was well received by consumers, however one retailer withdrew due to threats from an activist organization. Irradiated poultry is also sold in some markets. Use of irradiation in the United States may increase when expected approval for red meats is issued by the Food and Drug Administration and U.S. Department of Agriculture.

The irradiation process is approved and labeled irradiated products are available in several countries. Those leading in the use of this technology include the Netherlands, South Africa, and France.[7,9] Although activists will continue to oppose this process, advantages in food safety are so great that use is likely to increase. Consumers will likely become more familiar with irradiation through its use as a replacement for fumigants in spices or a method to enhance safety in meat, fish, frog and poultry products. Irradiation advantages for fresh or processed produce include enhanced safety and extended shelf life.

BIOTECHNOLOGY

While nutritionists and doctors uncover the secrets of health and long life, plant scientists will find ways to deliver these promises in new plant varieties. Similarly as individual country and world populations increase, efforts to maintain land, water and air quality will increase. There will be a clash between those striving to preserve the

traditional approaches to health and farming and those seeking to use new tools of science and technology to realize improved environmental stewardship and healthier food products. The clash is taking place now with divergent views on labeling, efficacy, and appropriateness of products modified by biotechnology.

Biotechnology can be used to create new varieties that increase quality or reduce pesticide use. Nearly 8 out of 10 (79%) Americans were aware of biotechnology, with more than half (54%) saying biotechnology has already provided benefits to them and 3 out of 4 consumers (78%) predicting they will benefit from biotechnology in the next five years.[19] Nearly half of the respondents realized foods produced through biotechnology were already in supermarkets. Almost two-thirds, 62% indicated they are very or somewhat likely to buy a product modified to taste better or fresher with 17% of these very likely.[1] Additionally 74% were very or somewhat likely to buy a product modified to resist insect damage and require fewer pesticide applications.

Among Canadian consumers, about two-thirds expect society will receive benefits from biotechnology, and the same proportion believe that society will also be at increased risk.[32] Most Australians believe genetic engineering is a "good idea" with as many as 90% supporting medical and environmental applications and about two thirds supporting food and nutritional applications.[20] Almost all, 93%, Japanese consumers interviewed believed biotechnology will provide benefits to them or their family in the next five years.[17]

Concern about personal or environmental safety has led to opposition among some consumer and activist groups. Never-the-less, marketing experience in the United States with labeled tomatoes was positive, although the product was not widely available. Squash from plants modified to be virus resistant, potato naturally resistant to the potato beetle and oil crops resistant to herbicides are in U.S. markets. Potatoes specially marketed as bioengineered sold at a premium in the supermarket. Farmers' demand for seed have exceeded supply.

In other areas of the world, response is varied. China has embraced biotechnology for years. In July of 1997, the Egyptian Ministry of Health barred imports of genetically altered commodities.[4] When asked about the severity of potential food risks, 44% of Europeans considered Genetic Engineering a serious risk. [18] This is about in the middle of potential food risks, with bacterial contamination at the top with 85% of consumers and sugar at the bottom with 12%. With the exception of Austria and Germany, half or more European consumers indicate they will purchase a product modified by genetic engineering.

Labeling of biotechnology modified products in the European market is controversial at this time. In July 1997, the European Union Standard Committee on food accepted a proposal which calls for mandatory labeling on products which may come from genetically modified (GMO) sources.[22] The guidelines call for voluntary labeling for certified genetic modified organism-free foods so they can be labeled "contain no GMOs." Mandatory labeling is required of all products that are or contain GMOs whether or not they are intended for human consumption. The label statement "may contain GMOs" will be required when material of GMO origin can not be excluded but there is not evidence of its presence.

It is unclear if this labeling will have a negative or positive impact on consumers. If consumers perceive use of biotechnology as a safe technique which increases quality or improves the environment, identification on the label could be positive. If they see biotechnology as an unnatural innovation in which they are exposed to increased risks while others get benefits, labeled products will be avoided. Acceptance depends on providing information to the public and consumer philosophic orientation. Researchers have concluded that generally those concerned about other food safety areas, will be concerned about genetic engineering.[20]

SUMMARY

Consumers want good tasting, healthy, convenient, and inexpensive food. Concerns about food safety will remain high as the challenge of controlling food borne pathogens is great. Areas for growth include novel delicious produce, convenient quality fruit or vegetable products, and use of new technologies to meet demands for safety and quality.

Growers will develop plans to reduce opportunities for pathogen contamination. We can advance into the 21st Century in concert or conflict. Although people will always have an individual approach to style of life, if the industry wishes to have available the full breadth of technologies to address consumer demand, it is important to invest in consumer education. Information is needed on potential benefits and risks associated with growing techniques, processing methods, and consumption of consuming specific foods. Technological advances can help achieve increased trade and enhanced environmental and human health.

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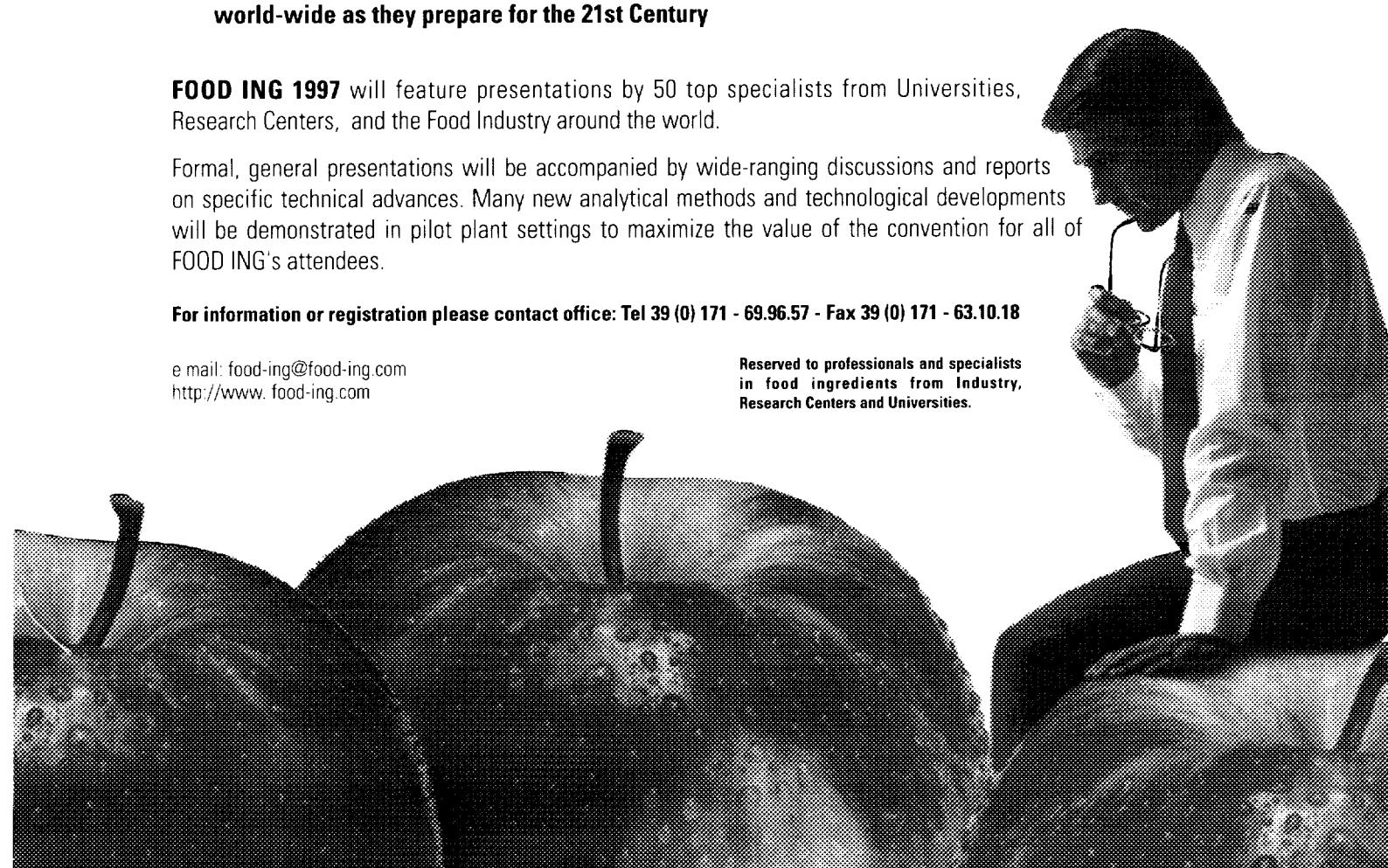

Peach orchard with view on Monviso mountain (Alps)