OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

This document has been produced without formal United Nations editing. The designations employed and the presentation of the material in this document do not imply the expression of any opinion whatsoever on the part of the Secretariat of the United Nations Industrial Development Organization (UNIDO) concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries, or its economic system or degree of development. Designations such as "developed", "industrialized" and "developing" are intended for statistical convenience and do not necessarily express a judgment about the stage reached by a particular country or area in the development process. Mention of firm names or commercial products does not constitute an endorsement by UNIDO.

FAIR USE POLICY

Any part of this publication may be quoted and referenced for educational and research purposes without additional permission from UNIDO. However, those who make use of quoting and referencing this publication are requested to follow the Fair Use Policy of giving due credit to UNIDO.

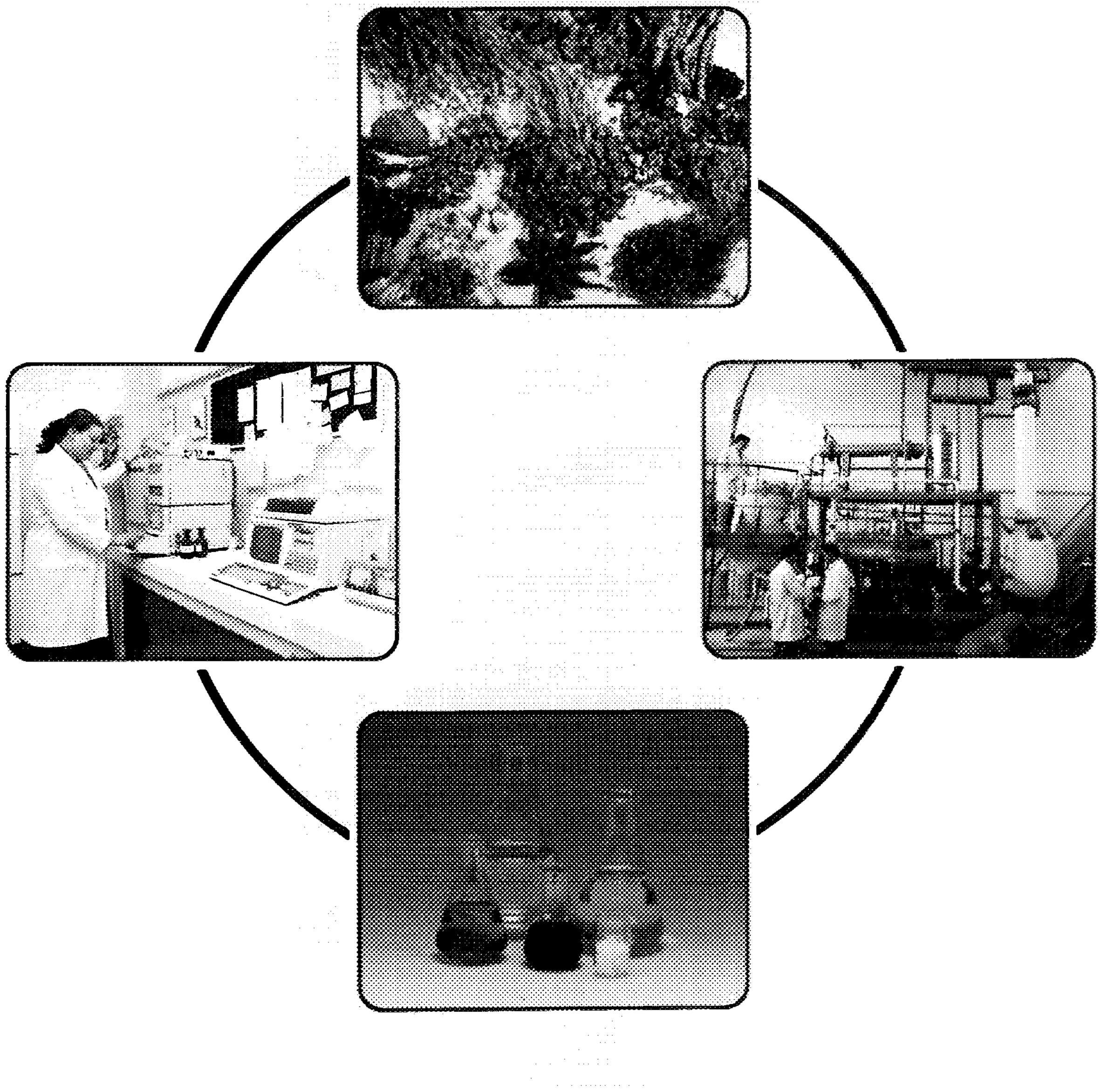
CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

A MANUAL ON
THE ESSENTIAL OIL INDUSTRY



Edited by K. Tuley De Silva

232 p
tables
graphs
diagrams
illus.

A MANUAL
ON
THE ESSENTIAL OIL INDUSTRY

EDITOR

K. TULEY DE SILVA

PRESENTATIONS MADE BY THE RESOURCE PERSONS AT THE THIRD UNIDO WORKSHOP ON ESSENTIAL OIL AND AROMA CHEMICAL INDUSTRIES HELD AT THE ANADOLU UNIVERSITY MEDICINAL AND AROMATIC PLANT AND DRUG RESEARCH CENTRE IN ESKIŞEHIR, TURKEY

NOVEMBER 1995

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

VIENNA, AUSTRIA

Cover design by Dr.Ergun Tunçkan

CONTENTS

FOREWORD

FOREWORD

Many developing countries are rich in aromatic plants and this renewable resource could be industrially utilized to produce essential oils for economic benefit.

UNIDO has been actively assisting developing countries to establish or upgrade essential oil industries. Many countries have increased their share of the export market in this commodity while diversifying the use of essential oils in local food and fragrance industries.

UNIDO organised workshops and seminars on the industrial utilization of medicinal and aromatic plants to update the know how of scientific and technical personnel of developing countries. In fact three workshops on the essential oil industry alone have been held during the past two decades. The first resulted in the publication of a Practical Manual on the Essential Oil Industry which proved to be very useful to scientists and technologists engaged in the production of essential oils. The third workshop which was held in Eskişehir in Turkey in collaboration with the Government of Turkey and the Anadolu University Medicinal and Aromatic Plant and Drug Research Centre, extended the coverage to aroma chemicals and marketing as well. It provided the opportunity for scientists from least developed countries to learn from the experiences of the resource persons and exchange information amongst them.

It is a fact that the developing countries face difficulties in gaining access to new developments in technologies and analytical techniques as a result of lack of funds to purchase recent books and journals. UNIDO has contributed in a small measure to disseminate some of the much needed technical information by holding workshops and by publishing manuals on specific subjects.

The presentations made by the resource persons at the third UNIDO workshop on essential oil and aroma chemical industries are published in this manual. It includes the latest developments in the essential oil industry. I hope that this manual will serve the scientists and technologists of developing countries to initiate programmes to develop essential oil industries in their countries. I thank the resource persons who readily agreed to share their knowledge and experiences by contributing to this manual.

The practical know how included in this manual will enable countries rich in aromatic plants to set up rural based small scale essential oil industries which would contribute to biodiversity conservation and sustainable development.

Dr. A. Tcheknavorian-Asenbauer
Managing Director
Industrial Sectors and Environment Division
United Nations Industrial Development Organization
Vienna International Centre
Austria

CHAPTER 1

DEVELOPMENT OF ESSENTIAL OIL INDUSTRIES IN DEVELOPING COUNTRIES

TULEY DE SILVA
Chemical Industries Branch
Industrial Sectors and Environment Division
United Nations Industrial Development Organization

1. INTRODUCTION

Developing countries are endowed with vast resources of aromatic plants which have been used by their people for centuries as food, health care products, flavours and fragrances. Apart from the traditional ways of using these plants, many are exported to industrialized countries as raw materials for drugs, fragrances and flavours. The value-added products are then imported back costing the countries several times more than the original revenue gained from exporting the raw materials. The actual value of these resources has not been retained in many countries for want of know how and trained manpower. Only a few developing countries are producing essential oils and even some of these countries are unable to produce good quality conforming to international specifications.

With a some exceptions, harvesting aromatic plants in developing countries for both local use and export has been from natural wild resources. As a result many plant species have been lost and some are in danger of extinction. It has also caused biodiversity conservation problems. It is therefore vital that systematic cultivation of these plants be introduced in order to conserve the biodiversity and protect endangered species.

Systematic cultivation of this type of plants could only be initiated if there is a continuous demand for the raw materials. Hence small scale processing units too have to be established in order the farmer is assured of a market for the raw material. Thus cultivation and processing should be started simultaneously in rural areas.

The lack of information on the social and economic benefits to be derived from the industrial utilization of aromatic plants has been a major factor impeding the development of the essential oils industry in the developing countries. Except for the use of these plants for domestic purposes, not much information was available on the market potential and trading possibilities. As a result, the real potential of these plants was not known to the governments or the entrepreneurs. Furthermore inventories on the types of useful plants and their abundance are not available for assessing the possibilities for their industrial utilization.

Today the promotion and development of plant based products have gained momentum due to certain ground realities:

- Green consumerism and the current resurgence on the use of "Naturals" in developed countries which has given a fresh impetus to the development of plant based products.

- Free market economy bringing in more openness and expanding markets and demand for new resources, materials and products.

- A growing acceptability of the social responsibility of minimizing socio-economic inequalities in favour of rural people resulting in opening up of additional job and income opportunities for the poor people.

- Poor economic conditions in developing countries restricting imports thereby placing increasing reliance on substitutes using indigenous plant resources.

- Increasing awareness regarding biodiversity conservation and therefore sustainable and protective use of plant resources.

The growing demand in industrialized countries for natural products in place of synthetic compounds has created a niche market for essential oils. Furthermore the requirements of essential oils for use in aromatherapy is increasing, creating a demand for organically produced exotic oils.

The development of the essential oils industry is therefore important to many developing countries which have rich resources of raw materials or the climatic conditions for the initiation of cropwise cultivation programmes. Though a lot of research has gone into synthetic substitutes for essential oils, the demand for natural oils has not declined. Hence the production of essential oils could still be a good source of foreign exchange revenue for developing countries.

Even those countries which are producing essential oils have many constraints to be competitive in the world market. Some of the problems associated with the essential oils industry in developing countries are:

- Poor propagation and agricultural practices
- Poor raw materials due to indiscriminate harvesting and poor post harvest treatment
- Lack of research on development of high yielding varieties, domestication etc.
- Inefficient processing techniques leading to low yields and poor quality products
- Lack of quality control of raw materials,finished products and cGMP
- High energy losses during processing
- Insufficient R & D on product and process development
- Difficulties in marketing (lack of access to market information and contacts)
- Lack of downstream processing facilities
- Lack of trained personnel, equipment and upto date technologies
- Non availability of locally fabricated equipment
- Lack of commitment and support from governments
- Lack of financial resources, loans, credit facilities

Synthetic substitutes have been produced from petrochemicals to compete with plant products such as resins, aroma chemicals, phytochemicals, rubber, leather etc. In the case of essential oils and flavours, real substitutes having all aroma characteristics of the natural products have not been fully accomplished. Nevertheless, the low cost and certain improvements on the natural, have given the synthetic chemicals a major share of the aroma chemicals market. Back to nature movement which has revived the interest on natural products will be beneficial for the developing countries which are endowed with vast resources of aromatic plants.

The entry into world markets depends not only on the demand but on the competitive price of production, quality and the ability to provide the quantities required by the purchaser. Of course the cost of production of the various products will vary according to the raw materials, facilities for processing and labour and energy costs. The bottleneck in many developing countries has been the lack of sufficient raw materials to supply the ordered quantities. The expansion of the resource base is therefore essential which may in certain instances mean large scale cultivations.

2. DEVELOPMENT ASSISTANCE PROGRAMMES OF UNIDO

Mindful of the vital role that UNIDO can play in strengthening the multi-disciplinary activities required and in market promotion of finished products to support the development of industrial production of essential oils, UNIDO launched an integrated programme two decades ago to assist developing countries to initiate development programmes for the industrial utilization of medicinal and aromatic plants. The main features of the programme were to provide specific inputs in terms of technology, training, equipment, consultant services, trade promotion and broad basing local use.

The main thrust of UNIDO's activities in this subsector has been the provision of technical assistance. The areas of technical assistance rendered cover a wide spectrum of activities, ranging from selection of plants, through cultivation, processing,quality assessment, formulation, packaging to marketing. The degree of assistance varies according to the needs and the state of development of this area of activity in a developing country. Though they have had the will, enthusiasm and interest to venture into this area of industrial processing, many developing countries have not had the financial resources nor the technological capabilities to initiate such activities. The timely intervention of UNIDO has contributed to initiating and catalysing activities in this area of agro-based industrial development.

Agrotechnology

Decisions on the scale of exploitation of aromatic plants from wild sources have to be based on accurate inventories about the kinds of plant resources, abundance and the feasibility of harvesting without damage to the ecosystem. In case potential candidates identified are not abundantly available, cultivation of them through agroforestry and community forestry programmes will have to be initiated as a means of conserving and ensuring continuous availability of the quantities required for processing. Taking into consideration the requirements of the plants selected, an agrotechnological package has to be developed to suit the infrastructural facilities available.

Organic farming is another practice that is gaining wide acceptance as the demand particularly in developed countries for organically grown crops is rapidly on the increase. Farmers have to be trained in all aspects of organic farming including obtaining certification from companies or associations that do the monitoring starting from cultivation to final harvesting. As chemicals cannot be used as fertilizers and pest control agents, the cultivations are labour intensive requiring labour for weeding and other farming activities. Hence developing countries which have cheap labour and unpolluted land can opt for organic cultivation. Organic manure has to be prepared which leads to environment friendly methods of organic waste disposal. Organic farming will thus reduce environment pollution, toxic effects due to use of pesticides and minerals, and contribute to biodiversity conservation.

Process Technology

Technologies and processes required for the industrial utilization of aromatic plants are described in detail in Chapter 2. The products that could be derived and the related processes are depicted in figure 1. Technical assistance has been provided to developing countries for the introduction and improvement of their technologies to develop essential oil industries. Their production on a large industrial scale requires significant variations in the technologies used. In case of rural small scale processing these technologies have to be adapted to suit the existing situation at field level.

Polyvalent pilot plant

There has been considerable interest and research activity in developing countries on potentially useful plants endemic to developing countries. Though a lot of time and money has been spent on strengthening institutions and training scientific staff, their work has not contributed much to industrial development. This can be mainly due to their inability to develop process parameters for large scale production and the inability to convince entrepreneurs or governments as to the feasibility and cost-effectiveness of production.

UNIDO has intervened in many countries by providing pilot plant processing facilities which require the services of many disciplines.The introduction of a polyvalent pilot-plant has been the most significant contribution of UNIDO to the development of the industrial utilization of medicinal and aromatic plants in developing countries. The gap that prevented the transfer of processes and products developed on a laboratory/bench-scale to industry was bridged by this exercise. The multidisciplinary nature of industrial activity was further demonstrated. Many research and development institutions in developing countries lacked the support of their engineering counterparts and most research therefore was confined to academic pursuits. The vital role played by chemical engineers in translating bench-

Processes Used and Products from Aromatic Plants

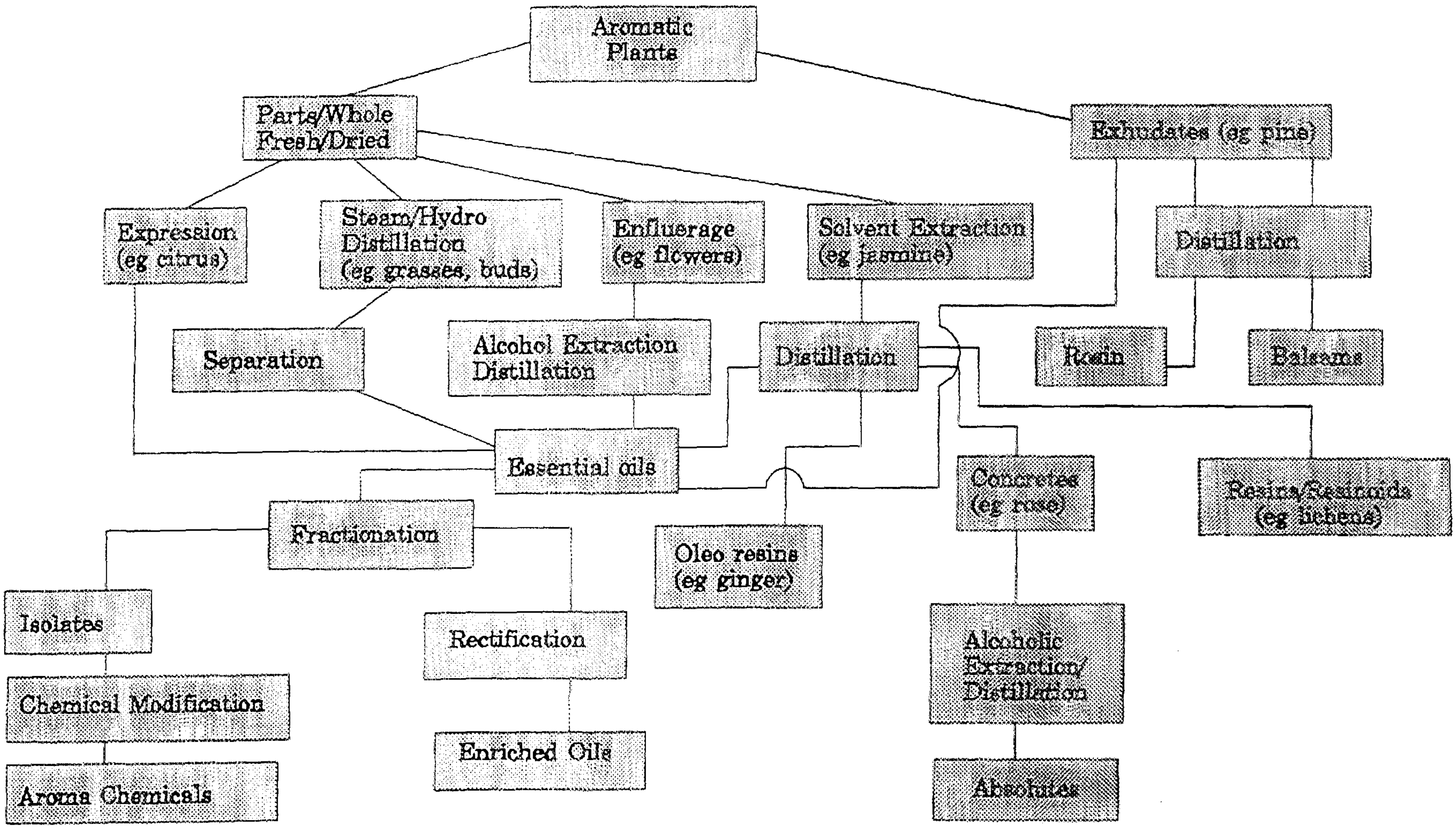


Figure 1

scale findings to industrial-scale outputs through pilot-scale process parameter development was a novel concept to most developing country research institutions.

The pilot plant has enabled the R & D work to be upscaled to produce sufficient quantities of finished products which could be market tested. University-industry links have thus been strengthened and the Universities have realized the hidden potential of industrialization of their R & D work.

Having recognised the competence and facilities available in some developing countries to fabricate equipment, UNIDO has now designed a polyvalent pilot plant (figure 2). The design includes all engineering drawings, together with specifications and bills of quantities, so that the pilot plant can be fabricated in countries where the facilities for stainless steel welding are available. UNIDO assists in procuring standard pumps, control units and any other material such as column-packing material. The design has just been published with the aim of enhancing self reliance and engineering capabilities in the local fabrication of equipment.

Processing of aromatic plants in rural areas

Up to now small scale processing of aromatic plants in rural areas whereby more economic benefits could be directed to the rural poor has been neglected for various reasons such as lack of financial resources,entrepreneurial capabilities and the low priority given by the governments.

As for the small scale producers in rural areas, integration with central downstream processing factories in the country would be essential to have a regular market for the primary products. These factories will produce requirements for other industries such as cosmetics, detergent, paint, paper and pharmaceuticals and should be encouraged. This link between the downstream processing companies and consumer industries too should be strengthened so as to have a complete chain of operations from the farmer to the consumer as a viable alternative to export.

Downstream processing

Essential oils could be further processed or rectified to add value. Rectification of essential oils is sometimes needed depending on their end uses. Rectification usually done by a process of fractionation may consist of one or more of the following:

- removal of moisture, colour and sediment
- removal of some compounds in order to improve the odour characteristics,and stability
- isolation of compounds that are highly valued
- enrichment of the oils by removing some unwanted fractions.

Additional training and equipment are required to carryout the rectification of oils Hence It would be better to carry out the fractionation of oils at a central facility which can afford to invest the funds and personnel required for this activity.

Another stage in the development of the industry is the initiation of downstream processing of the oils to produce isolates and chemically modified aroma chemicals. This is possible in countries producing large quantities of oils and where poor and cheap oils could be converted to other value added products. These products could also be used in the development of local perfumery industries. This would require the setting up of laboratories for compounding local perfumery formulations and testing them in various applications in cosmetics and toiletteries. The know how and the techniques for these activities have been transferred to local scientific personnel.

These isolates could further be processed using chemical methods to produce high value aroma chemicals which have an export market. These aroma chemicals can also be used in blending of perfumes and flavours for local industries.

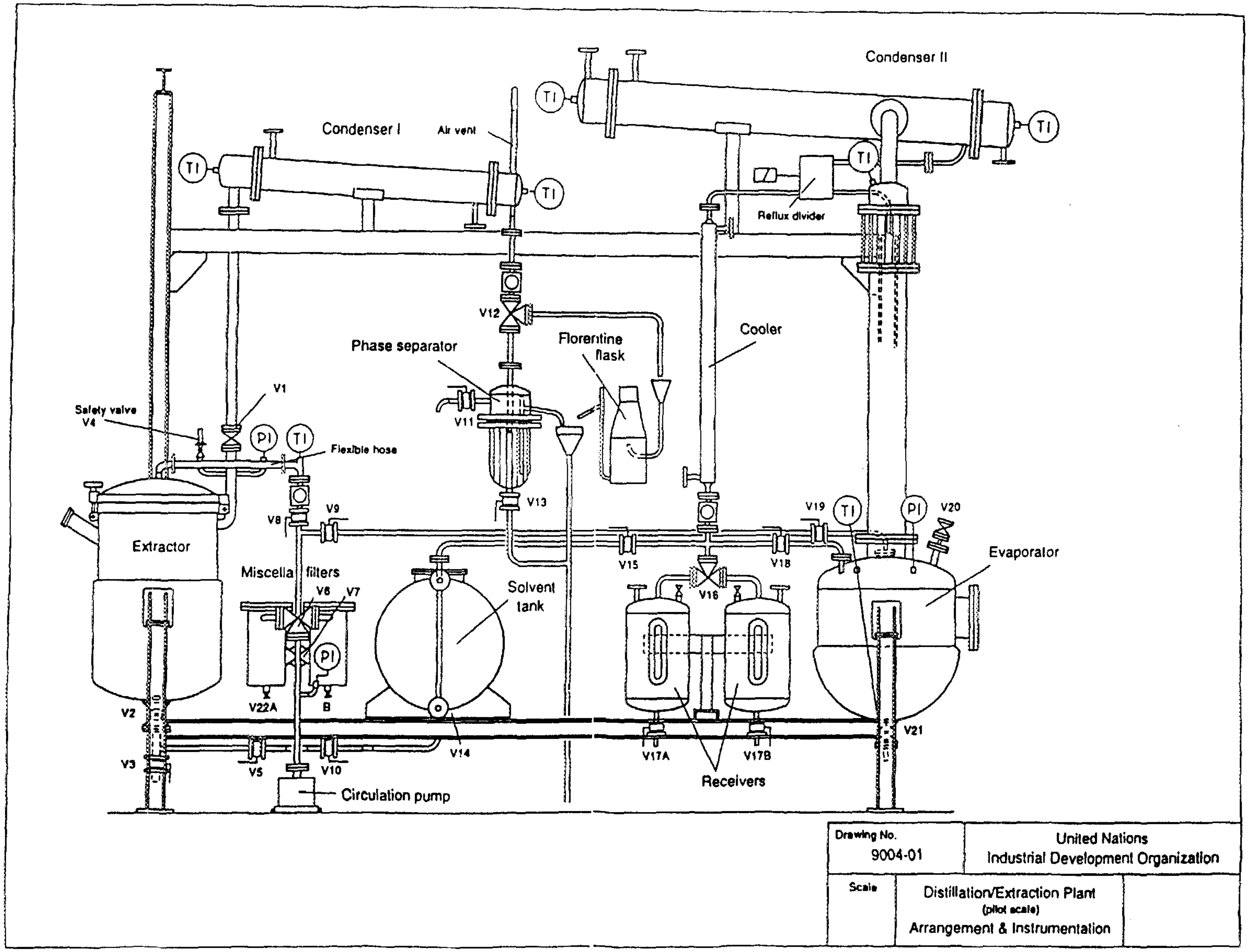

Condenser II
Condenser I
Air vent
TI
TI
TI
TI
TI
Reflux divider
Cooler
V12
Phase separator
Florentine flask
V1
Safety valve V4
PI
TI
Flexible hose
V11
V13
V8
V9
V15
V18
V19
TI
PI
V20
Extractor
Evaporator
Miscella filters
V6
V7
PI
Solvent tank
V16
V22A
B
V2
V3
V5
V10
V14
V17A
V17B
V21
Receivers
Circulation pump
Drawing No.
9004-01
United Nations
Industrial Development Organization
Scale
Distillation/Extraction Plant
(pilot scale)
Arrangement & Instrumentation

Figure 2

Quality Assurance

Quality has to be built into the whole process of production beginning from the selection of propagation material to the final product reaching the consumer. It is therefore a management system where all steps involved in the industrial utilization process have to be properly and strictly controlled to produce the desired quality products. ISO 9000 gives the requirements for the introduction of proper systems needed for certification.

The control of the quality of the raw materials, finished products and of processes is an absolute necessity, if one is to produce goods for world markets and human consumption. Monographs have to be prepared for each product to include all the specifications developed. Modern analytical techniques can be extensively used to develop identity, purity and quality parameters. The machinery and processes used in industries have to be validated to comply with International Standards.

It is imperative that the processed products comply with national and international specifications. There are International Standard Specifications (ISO)for most of the essential oils. In addition, countries and buyers can have their own requirements. The products could thus be tailor made to conform to the buyers requirements. Sometimes the requirements of the buyers are more stringent and specific, demanding the application of good manufacturing procedures.

Essential oils are evaluated not only by their chemical and physical characteristics that are measurable but more importantly by their sensory characteristics. Hence the control of the quality of essential oils through sensory evaluation is essential to developing countries so that they could obtain the correct market prices. Unido has successfully introduced sensory evaluation laboratories in several developing countries and trained their scientists in the techniques used.

Associated with quality management is the compliance with current good manufacturing practices. Without GMP products cannot be expected to be of required standards and quality. The concept of safety is almost non-existent in many developing countries. Safety requirements, with respect to buildings, machinery and staff have to be introduced. Safety manuals may have to be published in order to focus the attention of the management and staff to these issues.

Stringent requirements are being introduced presently to safeguard the environment, to reduce pollution caused by use of synthetic chemicals and reduce health risks due to side effects of using synthetic materials. The products have to conform to ISO specifications and other pharmacopoeial or buyer specifications. Furthermore eco-audit procedures will be required for safeguarding environmental damage. Organic production will reduce the risks of contamination of products and the environment with synthetic chemicals.In fact ISO 14000 requirements may have to be met in the future if the consumers insist on ecolabelling. This could mean that essential oils produced should have certificates ensuring that no ecological damage whatsoever has been caused during their production.

Human Resource Development

An integrated approach to the development of plant based products requires the involvement of multidisciplinary competence, extending from the farmer to the highly skilled professional scientist. The success of interaction and cooperation of these skills will determine the extent to which the industry could develop. A major constraint in developing this industry in is the non-availability of suitable personnel capable of undertaking training and the lack of tailor-made training schemes for specific activities. UNIDO has been successful in providing the required training to enable the development of essential oil industries in developing countries.

Many developing countries have a core of trained personnel in the fields of chemistry, biology, agriculture, pharmacology and pharmacy: they lack such resources in fields such as chemical engineering and technology. This can be considered as a major constraint on industrial development. Most of the agronomists are involved in the cultivation of cash crops and not of other useful plants.

UNIDO therefore had to develop the human resources required for this specific area of industrial development by conducting:

- Training abroad at appropriate institutions
- Training at site by international experts
- In service group training
- Tailor made group training courses on a interregional basis
- Workshops and seminars in specific areas
- Study tours and participation at international seminars/ congresses/ workshops and
- Provision of up-to-date know-how through books and journals

Training programmes have also to be conducted to enhance the knowledge of farmers to introduce proper agricultural practices including post harvest treatment. Harvesting methods, protective and sustainable in the case of forest plants have to be introduced during training programmes.

As mentioned earlier, one of the constraints on human resource development in the area of industrial utilization of aromatic plants has been the lack of tailor made training schemes suited to the needs of developing countries. The training obtained in the developed countries in the use of modern techniques and equipment can sometimes be too sophisticated and advanced to be adapted to the facilities available in some developing countries. Hence, in-plant group training programmes specifically designed to the needs of the developing countries have to be introduced.

Research and Development

Industrial development has to be accompanied if not preceded by research and development. Research and development is a high cost investment that most of the developing countries cannot afford. Nevertheless, research in the area of chemistry and bioactivity of indigenous flora of developing countries has been ongoing for quite sometime. These efforts have been funded through bilateral aid or non-governmental donor organisations. A systematic and a concerted approach to R & D has not been maintained for want of sophisticated equipment and high-cost chemicals. Moreover, most of the R & D work has been mainly academic pursuits. The concept of applied research to enable the industrial use of plants has not received much attention. In other words, the contribution of R & D to industrial development in the developing countries has been negligible.

UNIDO's approach to R & D has been to promote activities that could eventually support industrial development. Emphasis has been placed on multidisciplinary research incorporating engineering skills for process development. Research work to encompass all activities ranging from the development of superior propagation materials, agrotechnology, low cost, clean and efficient process technologies to improve the quality and the yield, new formulas, new products and marketing of finished products has been supported.

Advances in genetic engineering and transgenic manipulations have contributed to the development of new high yielding and disease resistant clones. These clones could easily be adapted to the climatic conditions of the developing countries to be used as propagation material leading to improved yields and low costs of production.

The downstream processing of essential oils require a considerable effort in R & D which has to be carried out by already established research institutes and Universities. Sometimes these institutions may have to be strengthened to undertake R & D work on essential oils. One of the constraints in this regard is the motivation of the scientific personnel who are bent on academic research and publications.

Increase of awareness of the usefulness of applied research to the country and some incentives for such work could help in getting the scientists involved in R & D work directed at value addition to raw materials.

In order to combat any threat from synthetic products intensive R & D work has to be conducted to make the products economically competitive. For essential oils this will mean research on breeding new and better oil yielding varieties, improvements on agro and process technologies, by-product utilization, development of value added products and, new formulations to improve their competitiveness vis a vis synthetic substitutes.

Marketing

Marketing is an unsurmountable problem besetting the development of the essential oils industry in developing countries. The prices are dictated by the buyers who control the market leading to unreliability and low prices. As a result the poor producers have suffered and even abandoned the production of essential oils in favour of other crops.

Marketability of the oils is a crucial factor in determining the failure or success of industries. Information on market prices and demand is difficult to get in view of the protective nature and limitations of the market. Furthermore the market is characterized by price fluctuations and high competition and substitute synthetic products. As such in the first instance, it may be useful to conduct market studies on local demand rather than the export potential. In developing countries, essential oils can be produced for import substitution resulting in saving the much limited foreign exchange. Some products could reach the consumer directly while others have to be either further processed or used as additives in other industries. Hence user industries have to be promoted so that the locally produced essential oils can be used as substitutes for the imported additives. Further processing to yield value added products will be limited by the local demand situation unless they could be produced at prices to be competitive in the world market. Even if the cost of production is low and quality of the products are good, aggressive market promotion has to be undertaken in order to penetrate the world market.

Market tie ups with progressive entrepreneurs from the developed world would be a convenient and a realistic option for securing markets for the finished products. Joint ventures or trade agreements could be initiated with companies that are expanding their production to meet the ever increasing demand for green products.

Having recognized the importance of encouraging the export of essential oils produced in the developing countries, assistance has been rendered by providing expert advice on the assessment of market data, including demand and supply situation, price trends, most feasible types and qualities of essential oils, that could be marketed, identifying different marketing arrangements and important trading houses, and recommending short-and long-term strategies for export promotion. In some instances, consultants together with senior counterpart staff have visited trading houses to establish contacts. Participation in international seminars focusing on marketing and trading aspects has also been sponsored. UNIDO workshops and seminars have always allocated programme time to the establishment of contacts and joint ventures.

Technical Information

Large volumes of literature are available on the chemistry and pharmacology of plants in various regions of the world. These focus mainly on research. There is very little authentic information on the extent of their traditional uses nor on world market trends. Agronomical data on industrially useful plants are rather scanty as compared to those on other agricultural cash crops. Many developing countries lack the resources to subscribe to research journals or acquire access to data bases. The few data bases which offer a free service are now finding it difficult to continue the free service. Moreover, the data required by the scientific personnel in developing countries with respect to technologies and methods used for processing and formulation of useful plants are not readily available in the literature nor in the data bases as some of these are patent protected.

Having recognized the lack of access to and non-availability of information on industrial use of medicinal and aromatic plants, UNIDO has played a small but a significant role in trying to fill this gap by publishing some documents so that the know how can be disseminated to a larger clientele, some as the proceedings of workshops or issue papers for consultations, others as specific outputs of technical assistance projects. In addition, one can have access to the large number of technical reports submitted by consultants who assisted in the development of activities in very specific areas of the plant based I industries.

In view of the greater demand for information on traditional uses and proper identification of plants used in developing countries and the tremendous volume of information presently being generated, a user friendly information storage and retrieval system will be of considerable importance.

As such a computer data base would be of invaluable assistance to R & D work on industrial utilization of local plant resources, UNIDO has published the guidelines for setting up a data base and developed the soft ware to assist countries to set up national data bases on useful plant resources. The data base once developed will be able to provide the following information:

- list of plants
- distribution and availability
- traditional uses
- information on Research conducted in the country
- bibliographies and research publications
- Research Institutes carrying out projects
- results of scientific researchers including scientific data.
- formulations in use
- users of plant preparations
- trade and marketing aspects

Investment Promotion

The Industrial Investment section of UNIDO is fully engaged in assisting the expansion of private and public sector industrial production in developing countries. This is achieved through various forms of joint industrial ventures between local sponsors and foreign partners.

One of the main programmes has been one of direct contacts with potential investors. Projects with investment potential in a country which need external assistance are identified and formulated into detailed project proposals. These are then promoted through the UNIDO network of Investment Promotion Offices established in industrialized countries. Direct contacts between the local project sponsors and the potential foreign partners are then arranged through investment project promotion forums or investors' forums held in the host country.

3. CONCLUSIONS

The development of any industrial processing activity has to be linked to the specific needs, socio-cultural background, resource potential and the technological capabilities of each country. Consequently, any integrated development approach adopted has to exploit the full potential of the renewable plant resources by providing a proper scientific, technological, economic and an industrial base.

Technical assistance has been provided to rectify one or many of the constraints for the development of the essential oil industry depending on the country situation and assistance requested.Furthermore, new crops have been introduced as required. As for technology development, UNIDO has secured improvements in distillation methods, including improved stills both for field and centralized processing and given assistance in the fabrication of equipment to suit specific requirements of the oils to be distilled. It has introduced quality assessment of the raw materials, processes and finished products. Fractionation of essential oils has also been introduced using the polyvalent processing plant. UNIDO has also supplied pilot-scale processing equipment, analytical equipment and some agronomical instruments, chemicals and books. Training has been provided to local staff in cultivation, post-harvest treatment, processing, analysis, packing and marketing, both locally and abroad. Reference standards, standard analytical profiles and journals have been provided. Where appropriate, technologies for the production of oleoresins, aroma chemicals and fragrance substances have been provided. Perfume blending has been introduced as a further stage of development.This has resulted in the establishment of local cosmetic industries.

Systematic crop-wise cultivation has led to environmental conservation the and protection of some endangered plant species. Para-processing activities have created employment opportunities for the rural poor, particularly women.

UNIDO has been able to initiate or catalyze the development of programmes on industrial utilization of aromatic plants resulting in the establishment of small and medium scale agrobased industries.

The main contribution of UNIDO has been to build up technological and scientific personnel capable of sustaining, improving and developing the industrial utilization of aromatic plants in the developing countries. Foci for activities in all areas ranging from cultivation to marketing have been created. Interaction and cooperation between research institutes and industry has been initiated, as a result of which research activity is expected to increase as their findings are translated into practical realities for the benefit of the society at large. Above all, research and development capabilities of the developing countries, including human resources, have been greatly enhanced so as to ensure the growth and sustenance of industries based on aromatic plants.

It is indeed imperative that the industry particularly in the developing countries should be planned, and the technology purpose oriented and designed to suit the requirements. In many respects countries such as India and China have led the way in re-establishing this industry in their countries and also competing with industrialized countries in the processing of downstream products and even formulated fragrances and flavours.

The sustainable development of industries based on plants requires multi-disciplinary activities and close collaboration between scientists, government officials, NGO's and international organizations. Such efforts have not received much attention resulting in the slow development of the economic uses of the renewable resources. Hence it is important that more action oriented plans are initiated to exploit the full potential of these resources, bearing in mind the conservation issues such that real rural household incomes and national economies could be augmented.

CHAPTER 2

AGROTECHNOLOGY OF AROMATIC PLANTS

K. K. S. BHAT
Department of Horticulture and Plantation Crops, CAB INTERNATIONAL,
Wallingford, Oxon OX10 8DE, United Kingdom

1. INTRODUCTION

In the course of evolution, many members of the plant kingdom have developed the ability to accumulate volatile odoriferous substances in various organs in order to attract beneficial insects and other animals which are essential for pollination and seed dispersal, to repel others which are harmful, and to protect themselves against pathogenic micro-organisms. These plants have been exploited by man for their culinary, perfumery, medicinal and other values since ancient times. With the spread of knowledge about these plants and the increasing demand for them in places far away from where they are collected, international trade in aromatic plants and products derived from them which have undergone various degrees of processing, extraction and alteration has grown over the centuries.

The widespread use of aromatic plant products by consumers around the world and increasing international trade have been accompanied by attempts to identify new species of potential interest, to bring them into cultivation from the wild, and to introduce them to distant locations away from their places of origin. Among the aromatic substances of natural origin which are currently used by the pharmaceutical, perfumery, cosmetic and food industries, some are derived from plants which have been cultivated for a long time whereas others are still obtained from species which grow abundantly in the wild.

Applications of aromatic plants and volatile oils extracted from them include: (i) direct culinary use: as fresh or dried herbs, spices and condiments; (ii) in perfumery, cosmetics, and household and personal hygiene products: either directly as major or minor ingredients or as raw materials for the extraction and/or synthesis of specific aroma chemicals; (iii) in food, drink and confectionery: as flavouring agents; (iv) in human and veterinary medicine: as components of pharmaceutical preparations; (v) in crop protection: as pesticides and insect repellents; and (vi) as antibacterial and antifungal agents in a range of situations.

Based on the commercial value of produce, it has been estimated that developing countries and Eastern Europe together account for approximately two-thirds of the total world production of volatile oils (Verlet, 1993). Among developing countries, the largest producers are China, India, Brazil, Morocco, Egypt, Indonesia and Turkey, these seven countries accounting for 85% of total production.

Whereas trade statistics indicate a strong growth in the overall demand for volatile oils during the 1980s, the figures for individual products have been variable. Potential threats to the market for aroma materials of natural origin include competition from (a) synthetic substitutes and (b) secondary metabolites produced *in vitro* using advanced techniques of tissue and cell culture.

2. CULTIVATED AROMATIC PLANTS: THEIR TAXONOMIC AND GEOGRAPHIC DISTRIBUTION

Depending on their end use, crops cultivated for the economic value of the flavour and fragrance substances they accumulate can be classified into the following categories although many of the species concerned will come under more than one category:

(1) those which yield plants or plant parts which are directly used for culinary purposes a herbs, spices or condiments, e.g. parsley, thyme, coriander, cumin, ginger, turmeric, etc.

(2) those which contain volatile oils of low boiling point which can be expelled by steam distillation, e.g. peppermint, lavender, lemongrass, vetiver, patchouli, etc.; and

(3) those from which the aromatic components are optionally or exclusively extracted using organic solvents. Solvent extraction using petroleum ether, hexane or toluene yields a *concrete* which can be further refined by extraction with ethyl alcohol to produce an *absolute*. These two types of extracts are together known as *oleoresins*. Oleoresins remain solid at room temperatures because they contain waxes and other non-volatiles which are also extracted by the solvents used. Ginger and *Capsicum* oleoresins are typical examples.

Other techniques used for the extraction of volatile components include absorbing into fixed oils and fats (*enfleurage*), fractional distillation, head space techniques, and extraction with liquid carbon dioxide.

The chemical compositions and other characteristics of volatile oils extracted from a large number of species, both cultivated and wild, have been published. The volume of phytochemical literature on aromatic plants has grown very rapidly in recent years. A list of cultivated plants from which aroma substances with industrial use have been extracted is given in Annex 1. This list is compiled from the world's largest bibliographic information database (CAB ABSTRACTS) on aromatic plants, and only contains species which have been reported as being cultivated or agronomically managed (e.g. forest trees) in some part of the world although some of them may grow in the wild elsewhere. In addition to herbs, spices and condiments, and other species grown exclusively as sources of essential oils, this list also contains (a) fruit (e.g. *Citrus*), vegetable (e.g. celery) and ornamental (e.g. rose, jasmine) species, (b) forest trees (e.g. *Cedrus, Eucalyptus, Pinus*) from which essential oils are commonly extracted and (c) incense plants (e.g. *Boswellia*) which yield essential-oil-containing natural oleoresins used in the flavour and fragrance industries. However, species for which there is no evidence of being under cultivation are excluded from this list.

It is clear from Annex 1 that essential oil bearing plants are not confined to a particular taxonomic group , but spread across a wide range of families of conifers as well as flowering plants. However, it is possible to identify a few families to which most of the commercially important aromatic plants belong. These are: Pinaceae (from which turpentine oil, the world's largest essential oil in terms of volume produced, is obtained) and Cupressaceae (cedarwood) among Gymnosperms; Gramineae (lemongrass, citronella, vetiver) and Zingiberaceae (ginger, turmeric, cardamom) among monocotyledons; and Compositae (chamomile, davana, tarragon), Geraniaceae (geranium), Labiatae (mint, lavender, thyme, basil), Lauraceae (bay, cinnamon, camphor, *Litsea*), Myristicaceae (nutmeg, mace), Myrtaceae (*Eucalyptus*, clove), Oleaceae (jasmine, lilac), Piperaceae (pepper), Rosaceae (rose), Rutaceae (*Citrus*), Santalaceae (sandalwood), and Umbelliferae (coriander, cumin, parsley) among dicotylendons.

Essential oil bearing plants can be found amongst the natural plant populations of nearly all vegetation-covered regions of the world. Among commercially important crops, many species of temperate and subtropical origin (mainly belonging to Labiatae, Umbelliferae and Compositae) can successfully be grown in the tropics whereas most crops of tropical origin cannot easily be grown in other geoclimatic regions.

Aromatic plants include herbs, shrubs and trees of all sizes, and may be annual, biennial or perennial. The oil-accumulating organ or tissue varies between species and no single part of the plant is excluded from the list of organs which can serve as oil stores.

Production statistics for volatile oils derived from over 80 species including the most important members of Labiatae, Umbelliferae and Compositae have been compiled by Lawrence (1993). Verlet (1993) has estimated that approximately two-thirds of the total quantity of volatile oils produced in the world are obtained from woody perennials (more than half of this volume being from *Citrus* spp.). The remaining third of the world's production comes from cultivated herbaceous plants. The contribution of wild plants to the total production is no more than 1-2%. In quantitative terms, the following 17 species account for approximately 90% of the total world production: orange, lemon, lime, *Mentha arvensis*, *M* x *piperita*, *M.* x *spicata*, cedar (*Juniperus virginiana* and *Chamaecyparis funebris*), citronella, *Eucalyptus globulus*, *Litsea cubeba*, clove, sassafras (*Ocotea pretiosa* and *Cinnnamomum micranthum*), *Lavandula intermedia*, *Osmanthus fragrans* and patchouli. In cash terms, in addition to most of the above crops, the following species which yield high-value products are also among the top 20 or so species which account for approximately three-quarters of the total value of produce: rose, jasmine, sandalwood, vetiver, bergamot, geranium and coriander.

3. PLANT IMPROVEMENT: BREEDING, SELECTION AND *IN VITRO* TECHNIQUES

Compared with arable crops, fruits, vegetables and ornamentals, plant improvement work in aromatic crops is still in its infancy. For many essential oil crops, including some of the important commodities traded on the world markets, named cultivars do not yet exist.

Breeding and selection

The primary objective of any breeding and selection programme is to deliver the highest marketable yield of the commodity concerned. In the case of most major agricultural and horticultural commodities, this generally means achieving the highest possible biomass production coupled with such traits as pest and disease resistance, drought and frost tolerance, and improving the aesthetic quality of the fresh produce. However, in the case of aromatic plants, the objectives vary depending on the intended end use of the commodity concerned. For commodities to be marketed as culinary herbs and spices, improvement in yield, disease resistance, aesthetic quality (e.g. visual appearance of decorative herbs) and immediate organoleptic properties (e.g. fibre content in ginger, pigment content in turmeric) are still regarded as important aims. However, in commodities destined for the essential oils and aroma chemical industries, biomass production is only of secondary relevance.

For crops in the latter category, the overwhelming consideration in breeding and selection is to achieve the desired content and composition of volatile oils in the harvested produce. From the perfumer's point of view, in order to reproduce on a continuing basis the odour qualities of the perfume he has created, it is vital that the raw materials he uses are of the same quality batch after batch. As far as the consistency of quality and composition of the essential oil is concerned, perfumery houses are therefore one of the most demanding of customers.

A typical essential oil consists of a small number of major chemical constituents which together make up the bulk of the oil, a larger number of minor constituents which are present in smaller but significant proportions, and a very large number of other molecules which are present only in trace amounts. However, regardless of their relative amounts, every chemical entity present contributes to the final odour qualities of the oil concerned.

In natural populations of many species of essential oil bearing plants, large differences commonly occur in the chemical composition of oil extracted from morphologically similar plants growing on different sites or even between individual plants growing on the same site. Depending on the major constituents of the oil, different *chemotypes* have been identified in the wild populations of many species. The composition of essential oil varies with the plant's genetic make up, stage of growth and environmental factors. Whereas the presence or absence of a particular chemical constituent at any stage of growth is determined exclusively by the genes, its concentration is governed by both genetic and environmental factors. For many crops, the optimum period for harvesting does not coincide with the maximum dry matter yield. The oil content in the aerial parts in most (but not all) species of Labiatae peaks at the flowering stage.

The extreme heterogeneity of the starting material available to the breeder, coupled with the elaborate instrumentation required for the chemical analyses of essential oils extracted from successive generations of plants make the breeder's task particularly long and tedious. Nevertheless, considerable progress has been made in recent years in our understanding of the basis of inheritance of genes controlling the biosynthetic pathways responsible for the accumulation of specific compounds in a limited number of species. Research work has been aimed at incorporating the desirable characteristics of promising chemotypes chosen from wild populations into the progeny by repeated breeding and selection cycles until satisfactory levels of distinctiveness, stability and uniformity have been achieved. This has been done with a fair degree of success for a number of economically important members of temperate and Mediterranean Labiatae and, to a lesser extent, of Compositae. New cultivars with desired oil composition have been developed for a number of crops including *Lavandula, Mentha, Melissa, Thymus* among Labiatae, and *Achillea* and *Matricaria* among Compositae. A detailed treatment of this work is outside the scope of this chapter. The principles underlying the breeding and selection of medicinal and aromatic plants have been discussed by Tétényi (1991) and Putievsky (1993). The results obtained in recent breeding programmes are summarised in an excellent recent review by Franz (1993).

Plant improvement in tropical spice crops has traditionally been by cloning of selections. Many of the crops involved are vegetatively propagated and some are self-incompatible and can only be bred with difficulty. In most cases, the main criteria for selection have been yield and disease resistance. Although the variations in the chemical compositions of essential oils in some cases (e.g. cinnamon, see Rao *et al.*, 1988) can be as wide as discussed above for species of Labiatae, producers of spices are probably not under the same pressure from consumers as, for example, growers of lavender aimed at the perfumery industry. Extensive literature has been published from India on the improvement of black pepper, ginger, turmeric, cardamom, coriander, cumin and other spices and condiments. Irradiation and colchicine treatment of propagating material have been attempted for some crops with a view to obtaining mutants with improved characters. Recently published status reports include Edison *et al.*(1991) and Rethinam *et al.* (1994). During the past 10-15 years, there has been a significant shift in the nature of plant improvement work towards the use of *in vitro* techniques (Babu *et al.*, 1993).

IN VITRO culture techniques

Unlike in traditional plant improvement work involving breeding and selection, as far as *in vitro* culture studies are concerned, aromatic plants have received their fair share of attention from research workers around the world. Over a thousand papers reporting experimental work involving *in vitro* culture of aromatic plants other than citrus crops have been published during the past two decades. A similar number of papers reporting results of *in vitro* studies on citrus crops have also appeared during the same period. With a limited number of exceptions, most of the commercially important cultivated aromatic plants have been the subject of *in vitro* studies carried out with different objectives.

Most but not all *in vitro* culture studies are either aimed at or have relevance to plant improvement. The range of objectives pursued and applications explored in tissue and cell culture work have expanded very rapidly. These can be considered under the following headings: (1) micropropagation, (2) production of virus-free stock, (3) preservation of germplasm; (4) early testing of selections, (5) search for somaclonal variation, (6) *in vitro* mutagenesis, (7) haploidy and related applications; (8) somatic hybridization, (9) *in vitro* grafting, (10) *in vitro* pollination and fertilization, (11) 'hairy root' culture and production of transgenic plants, and (12) *in vitro* production and biotransformation of secondary metabolites. All but the last application listed represent tools used in the conservation, multiplication or improvement of plant material.

An excellent account of recent *in vitro* culture work on a large number of species of aromatic plants is given in many individual chapters in the book series edited by Bajaj (1988, 1989, 1991, 1993a, 1993b, 1994a, 1994b). Other recent reviews include Smith and Drew (1990), Babu *et al.* (1993) and Krikorian (1994). Basic information on *in vitro* culture procedures can be found in Kyte (1987), Pierik (1987), Zryd (1988), Torres (1989), Vidalie (1989), Debergh and Zimmerman (1990), Stanford and Warren (1991), Bonga and Aderkas (1992), George (1993), Razdan (1993), Razdan (1993), Vasil and Thorpe (1994) and many other books.

By far the widest application of *in vitro* techniques, providing most of the practical benefits from them so far, has been for micropropagation. For the nurseryman, the main attraction of micropropagation has been the ability to produce large numbers of healthy plants genetically identical to the parent plant within a short period of time. Other advantages include the possibility of propagating subjects which are difficult to root by conventional methods, the ability to produce plants at any time during the year and the improved vigour and precocity observed following field planting in some species compared with conventional propagating materials. The research effort which has gone into overcoming technical difficulties encountered at various stages of culturing and subculturing specific types of herbaceous and woody plants until the fully rooted plantlet is obtained and during its acclimatization following transfer to non-sterile environment has been so extensive that standard procedures have now been established for the routine micropropagation of a very large number of species. Techniques are now readily available for the micropropagation of many cultivated species of aromatic plants.

Micropropagation has facilitated the production of disease-free planting stock which is much more difficult to achieve using conventional methods of propagation. *In vitro* culture has also proved valuable in the conservation of genetic resources, especially in saving endangered species from extinction, and has enabled the germplasm of economically important species and their parent lines collected from the wild to be held in gene banks. In many species, *in vitro* culture has enabled selection tests to be carried out much earlier than by conventional procedures.

In some cases, plants raised *in vitro* exhibit growth characters different from that expected for the species under normal conditions. These changes, which may sometimes be desirable and advantageous, become visible either at some stage during the *in vitro* culture or during subsequent growth in the field following transplantation. Desirable somaclonal variations induced by tissue culture have been observed and taken advantage of in plant improvement work in a very large number of species. Examples of aromatic plants in which desirable somaclonal variations have been reported include *Mentha arvensis* (Kukreja *et al.*, 1991), *Cymbopogon winterianus* (Mathur *et al.*, 1988), *Artemisia annua* (Elhag *et al.*, 1992), lavender (Panizza *et al.*, 1990), rose (Arene *et al.*, 1993), sandalwood (Rao *et al.*, 1994), parsley (Watin and Bigot, 1989), fennel (Hunault and Desmaret, 1990), cardamom (Reghunath and Priyadarshan, 1993) and *Citrus* spp.(Gritter, 1986).

Inducing mutations by exposing seeds or other propagating material to ionising radiations or by treating them with colchicine or other mutagenic agents are techniques which have been used for many decades. In more recent experiments, plants growing *in vitro* have been subjected to such treatments because of the obvious advantages offered by the culture medium for the introduction of such chemicals and the sterile environment in which the effects on the treated plant can be monitored. Examples of aromatic plants which have been the subject of colchicine treatment studies include *Artemisia annua* (Woerdenbag *et al.*, 1993), fennel (Hunault, 1987), rose (Roberts *et al.*, 1990), *Pelargonium roseum* (Tokumasu and Kato, 1979), peppermint (Bricout *et al.*, 1978)and lavandin (Maia *et al.*, 1973). Tetraploidy has been induced in roses by exposure to gamma radiation (Kaicker, 1992). Lavender (Tsvetkov, 1977) and jasmin (Devaiah and Srivastava, 1989) are among other species which have been the subject of studies involving exposure to gamma radiation.

Another important advantage of *in vitro* culture techniques to the plant breeder is the ease with which haploid plants can be raised by anther culture. By chromosome doubling (either spontaneous or induced by treatment with anti-microtubule agents such as colchicine, amiprofos-methyl and oryzalin), it is possible to produce homozygous male and female dihaploid plants which can then be used in breeding work. Examples of aromatic plants which have been the subject of such work include *Pelargonium* (Bennici, 1974) and several species of *Citrus* (e.g. Germana *et al.*, 1994).

Another area in which significant advances have been made during the past two decades is somatic hybridization by fusion of protoplasts (and cybridization by fusion of cytoplasts) isolated from different plants. Somatic hybrids have been produced not only between related species of plants but even between plants belonging to distant genera. In many cases, the regenerated plants have successfully been grown in soil, including in the field for several years. Among essential oil yielding plants, substantial progress has been made in this field with citrus crops. Somatic hybrids have been produced among a large number of *Citrus* species (see Grosser and Gmitter, 1990 for a review) and between species of *Citrus* and other genera from Rutaceae (*Microcitrus, Citropsis, Fortunella, Poncirus, Murraya*). Many such plants resulting from somatic hybridization which are yet to be named, have successfully been grown in the field until flowering and fruiting.

In vitro grafting is a technique used in many tree crops, including a wide range of citrus crops, mainly for obtaining virus-free grafted plants. Techniques involving *in vitro* pollination or culture of ovules isolated from flowers after different times from pollination followed by embryo culture for plant regeneration have successfully been developed in a number of species including roses and citrus crops. Using these techniques, it is possible to overcome the difficulties involved in producing hybrid plants by conventional breeding techniques, e.g. hip abscission which commonly occurs in roses after fertilisation (Gudin, 1993).

Finally, advanced genetic engineering techniques are being developed for the introduction of foreign genes into cultivated crops with a view to conferring on them desirable attributes such as pest and disease resistance and better quality of harvested produce. In the case of essential oil yielding plants, an important objective of such studies has been the manipulation of biosynthetic pathways leading to the accumulation of target compounds. Examples of aromatic plants which have been the subject of such studies include parsley (e.g. Staiger *et al.*, 1990), coriander (Cahoon *et al.*, 1994), rose (Firoozabady *et al.*, 1994), edelweiss (*Leontopodium alpinum*) (Hook, 1004), ginseng (e.g. Inomata *et al.*, 1993; Yoshikawa *et al.* 1993; Asada *et al.*, 1993), *Artemisia absinthium* (Kennedy *et al.*, 1993), *Artemisia annua* (Jaziri *et al.*, 1995) *Ruta graveolens* (Babucke and Eilert, 1995; Junghanns *et al.*, 1995), *Valeriana officinalis* (Granicher *et al.*, 1992, 1995), *Mentha* x *piperita* (Spencer *et al.* 1990, 1993), *Pelargonium* sp.(Guiltinan and Chang, 1993), *Chamaemelum nobile* (Fauconnier *et al.*, 1993) and several citrus crops (e.g. Kobayashi and Uchimiya, 1989; Hidaka *et al.*, 1990; Moore *et al.*,1992; Pena *et al.*, 1995). A large number of these studies have been based on genetic transformation mediated by the crown gall forming bacterium *Agrobacterium tumefaciens* or the 'hairy root' inducing bacterium *Agrobacterium rhizogenes*. The major part of this work has been aimed at *in vitro* production and biotransformation of secondary metabolites.

4. AGRONOMY OF SELECTED CROPS

The main aspects of cultivation of a selected range of commercially important aromatic crops are briefly described below. The crops chosen include some of the major spice crops yielding oleoresins and others which are sources of important volatile oils used in perfumery. Both herbaceous and woody species are represented. The agrotechnology of some of them which can only be grown in the tropics (pepper, ginger, turmeric) has largely been developed in the main growing regions of the tropics. Others (mint, vetiver) are adapted to a wider range of agroclimatic conditions and are therefore grown in tropical, subtropical and temperate regions.

PEPPER (*Piper nigrum*)

Black and white peppers from *Piper nigrum* are the most important of all spices traded on the world markets. Black pepper is obtained by drying the whole fruit picked while it is still green whereas white pepper is produced by removing the mesocarp of the mature berry before drying. Other pepper products include variously processed (pickled, cured, dehydrated, freeze-dried and frozen) green berries, pepper oleoresin obtained by extraction of black pepper with organic solvents and pepper oil obtained by steam distillation.

Piper nigrum is a native of south-western India, but is now cultivated in many tropical countries. The main producing countries are India, Indonesia, Malaysia, Sri Lanka and Brazil.

The plant

Piper nigrum (Piperaceae) is a perennial woody climber which can grow to heights of 10m or more. The base of the vine is woody and 4-6 cm in diameter and the climbing branches have internodes 5-12 cm long. Fruiting branches originate from the axillary buds of the climbing branches. In addition to the main stem, the vines also produce stolons or 'runners' originating from the base. Both terminal shoots and runners can be used for propagation. The root system consists of (a) the main adventitious roots with absorbing function at the base of the plant and (b) the climbing roots from the nodes of the climbing branches which cling to the support.

The spikes appear on the opposite side of the leaves on the fruiting branches. The flowers can be unisexual or bisexual, but most cultivated varieties are bisexual and self-fertile. Each spike produces 50-60 berries, 4-6 mm in diameter, which are green, turning deep red when fully ripe.

Many cultivars adapted to local conditions are in use in all the growing countries. Most are hermaphrodite clones selected from the wild over the centuries; some are selections from open-pollinated progeny of established varieties. Only very few cultivars are the result of breeding work.

Soil and climate

In its natural habitat, pepper thrives on well-drained red lateritic soils, although with adequate soil management, and especially ample drainage, it can be grown on a wider range of soil types. Pepper is essentially a crop of the humid tropics. It can be grown successfully between 20^0 N and 20^0 S and at altitudes ranging from sea level to 1500 m. The crop tolerates temperatures between 10^0 and 40^0C. Semi-shaded conditions provide the ideal microclimate. When grown on slopy sites, care should therefore be taken to avoid direct exposure to solar radiation during summer.

Systems of cultivation

These include (a) monocropping, (b) mixed cropping, and (c) companion cropping. As pepper is a climbing vine, provision of a suitable living or non-living support is an important aspect of cultivation. In a monocropping (i.e. entire plantation exclusively planted with pepper) or a mixed cropping (i.e. interspersed with other crops) system, it is usual to start by planting the support trees which also serve as shade trees at the required spacing and getting these trees established before planting the vines. The support trees themselves are raised as seedlings or cuttings, or by planting tree trunks, depending on the species available locally. Tree species used for this purpose in different parts of the world include *Erythrina indica, E. subumbrans, E. variegata, Garunga pinnata* and *Grevillea robusta, Shorea obtusa, Xylia xylocarpa, Acacia mangium, Lucaena* spp., *Gliricidia* sp., *Inga* sp., *Gleditsia* sp., *Pithecellobium* sp., *Cyanodaphne cuneata* and others.

As a companion crop, on the other hand, the vine is planted at the foot of another established tree crop such as coconut, arecanut, jackfruit (*Artocarpus heterophyllus*) or mango. In many such plantations, many other trees, not all of which are of obvious economic importance, commonly grow among the trees of the crop planted. These trees can also serve as support and shade trees for planting pepper.

Propagation

Except in connection with breeding work, pepper is always propagated vegetatively by cuttings. Cuttings are raised either from the terminal shoots or the stolons. When terminal shoots are used, the terminal bud is cut off from the selected stem and the leaves growing on 4-5 nodes below the third node from the apex are removed, About ten days later, when a new bud begins to grow from the node nearest to the point of decapitation, the shoot is cut below the lowest defoliated node. The cutting with the aerial roots still intact, is planted either in the nursery or directly at its final destination, with the defoliated nodes being buried 10-15 cm deep. The shoots originating from the exposed nodes with intact leaves will grow into climbing shoots.

When stolons are used for propagation, the strong and healthy runners selected are kept raised above ground level to prevent them from premature rooting. This is usually done by coiling them around wooden pegs. During the cropping season, the runners are severed from the parent vine and, after trimming the leaves, cuttings carrying 3-4 nodes are planted either in nursery beds or in plastic bags containing a rooting medium. Adequate shading and watering is essential in both cases. The cuttings raised by either method will be ready for transplanting in 8-10 weeks.

A more recent method of propagation involves planting rooted cuttings in the nursery, allowing the shoots to grow horizontally while maintaining them above ground level and layering at each node using plastic bags containing appropriate rooting media.

Micropropagation techniques are currently being developed at several centres around the world. Although these attempts have been successful on a laboratory scale, multiplication rates achieved so far have been too slow for the technique to be used for propagation on a commercial scale.

Planting and aftercare

As a monocrop, the recommended spacing is 2.5m x 2.5m, giving about 1600 vines per hectare. As a companion crop, the spacing is determined by the established tree crop. Two or three rooted cuttings are planted at the base of the support tree in semicircular pits, about 30 cm deep, which are refilled with top soil. The young plants should be protected from direct sunlight by shading and until the climbing roots are able to provide the anchorage required, the vines should be tied to the support. In monocropping systems, the total height is restricted to 4-6 m. As the vine grows up the support, pruning of the climbing branches is sometimes practised in order to encourage the growth of lateral fruiting branches, although the benefits of pruning have not been conclusively established in pruning trials.

Fertilizer requirement

When grown as a monocrop on lateritic soils, the recommended fertilizer dose is 100 g N + 40 g P_2O_5 + 140 g K_20 per vine per annum, applied in two split doses during the year, from the third year onwards. Only a third of this dose is applied during the year of planting and twice this amount in the second year. In addition, application of farmyard manure every year and lime (500 g per vine) every other year is also recommended. Weeding and mulching should also be regularly carried out.

When grown as a companion crop, the above fertilizer doses should be modified to meet the requirements of the tree crop as well. Similarly, although in a monocropping system pepper is usually grown as a rainfed crop, when grown as a companion crop, it benefits from the irrigation provided to tree crop, e.g. arecanut.

Harvesting and yields

The vines start yielding berries 2-3 years after planting and have an average productive life span of 15-18 years, after which the yields decline. The berries are ready to pick approximately 6 months after flowering, when a few berries on the spike turn red. The spikes are picked individually by hand using ladders at weekly or fortnightly intervals over a harvesting period lasting up to 3 months. The berries are detached from the spikes by rubbing between hands or by trampling before further processing.

An important factor determining the quality of the final product is the stage of maturity at harvest. The volatile oil content of the berries reaches its maximum level at 4-5 months after pollination and then declines. On the other hand, the piperine content continues to rise even after the oil content has peaked. Berries intended for direct use as spice, where the aroma and flavour qualities are important, should therefore be picked earlier than those for oleoresin production where the pungency level is more important.

Widely different levels of yield have been reported from different countries depending on the level of input of resources and of management. These range from 200-300 kg/ha in India to 2500 kg/ha in Sri Lanka, 3500 kg/ha in Brazil and up to 15000 kg/ha in Sarawak, Malaysia.

Approximately 35-40 kg of black pepper and 25-30 kg of white pepper of commerce are obtained from 100 kg of freshly picked berries.

Pests and diseases

Up to twenty species of insect pests have been known to affect the performance of pepper plantations. The most important among these are the pepper weevil, *Lophobaris serratipes*, in Malaysia and the pollu beetle, *Longitarsus nigripennis*, in India, both of which cause extensive damage by boring holes into the berries. Other insect pests include the shoot borer, *Cydia hemidoxa*, the gall-forming thrip, *Liothrips karnyi*, and a number of species of mealy bugs. Serious damage can also result from the slow decline caused by the nematode pests, *Radopholus similis* and *Meloidogyne incognita*.

The most serious among fungal diseases is foot rot caused by *Phytophthora palmivora*. Control measures include provision of adequate drainage and use of copper-based fungicides. Other diseases of fungal origin include white foot rot caused by *Fomes lignosus*, red root rot (*Ganoderma lucidum*), fungal pollu (*Colletotrichum* spp.) and die-back resulting from several fungal pathogens.

Postharvest treatment and handling

Sun-drying is the conventional method of drying the harvested pepper in preparation for the market. In India, the berries separated from the spikes are spread on bamboo mats, concrete floors or other suitable flat surfaces and exposed to the sun. In order to accelerate the drying process and to facilitate uniform drying, it is important to stir and turn over the material periodically. This is especially important during the early phases of drying when the humidity level in the microenvironment between the berries can be high. In some other countries, the common practice to dry the harvested spikes directly in the sun for a few days before separating the berries. The recommended final moisture content of the dried product is 10-11%.

An important limitation to sundrying is its dependence on weather conditions prevailing during the period immediately following harvest. The total drying time can therefore vary between 3 and 12 days. A number of mechanical dryers in which the entire drying process is complete in a few hours have been designed. These are generally equipped with devices for generating hot air using an external fuel source and fans for circulating the hot air produced through an enclosed chamber. In these dryers, the berries are exposed to circulating hot air. In addition to achieving faster drying regardless of weather conditions, mechanical dryers also offer the advantages of better quality of the final product and minimising the need for further cleaning, as they can also be equipped for winnowing.

The dried pepper is cleaned to remove any extraneous matter, dirt, grit, stones, crop debris, etc. On small farms, the cleaning is usually done manually by winnowing to remove lighter contaminants and hand-picking the heavier ones. In large-scale processing houses, cleaning and grading is done mechanically using pneumatic and magnetic separators, winnowing machines and sieves. Bulk packaging is usually done in double-lined jute sacks and retail packaging in high-density polyethylene bags. In major exporting countries, large-scale storage facilities are available in warehouses equipped for fumigation and proper protection of the produce awaiting shipment.

GINGER (*Zingiber officinale*)

Ginger of commerce, marketed fresh, dried, candied or preserved, is derived from the rhizomes of *Zingiber officinale*. The crop has been cultivated in Asia since ancient times. In addition to being an important spice crop, ginger is also known for its medicinal value and is widely used in traditional medicine in several countries. The volatile oil obtained by steam distillation of ginger rhizomes and the oleoresin obtained by solvent extraction are important flavouring agents used in the food industry.

Zingiber officinale is a plant of South-East Asian origin. India is the largest producing and exporting country, accounting for about half the total world production. Other important ginger-producing countries are Jamaica, Sierra Leone, Nigeria, China and Japan. Substantial quantities are also produced in Australia, Thailand and other South-East Asian countries.

The plant

Zingiber officinale (Zingiberaceae) is a herbaceous perennial bearing thick, scaly rhizomes. The erect pseudostem, which grows to a height of up to 1m, is formed of long, concentrically arranged leaf sheaths. The leaf lamina, which grows approximately at right angles to the pseudostem, is up to 30 cm long and 2-3 cm wide. The inflorescence consists of cone-like spikes typical of Zingiberaceae.

Only a limited number of cultivars have been developed in the main growing countries. Most are clonal selections adapted to local conditions.

Soil and climate

Ginger is mainly grown in the warm and humid regions of the tropics, at altitudes ranging from sea level up to 1500 m, although it has a wider geoclimatic tolerance than most other spice crops. In areas with adequate and well-distributed rainfall, it is usually grown as a rainfed crop. In drier regions, it can be grown successfully as an irrigated crop.

The crop is particularly sensitive to waterlogging. Provided drainage is adequate, it can be grown successfully on a wide range of soil types ranging in texture from clays to sandy loams. The crop performs particularly well on humus-rich virgin forest soils.

Propagation

The established method is vegetative propagation using portions of rhizomes known as seed pieces or setts. Well-developed, undamaged, disease-free rhizomes collected from the previous crop are treated with fungicides and stored in pits lined with saw dust or sand and maintained under shade until they are required for planting. The rhizomes are cut into small pieces, 3-4 cm long, ensuring that each piece has at least one 'eye' or growing point. Optimum spacing and seed rates reported in the literature vary widely between locations depending on the climate and soil conditions in the area concerned. Whereas the recommended seed rate in the main growing region of southern India is 1200-1400 kg/ha (and, in some planting systems, as low as 600 kg/ha), at higher altitudes in the Himalayan regions, seed rates of up to 3000 kg/ha have been recommended.

Significant advances have been made in recent years in propagating ginger by *in vitro* culture techniques. Standard protocols for micropropagation which enable rapid clonal multiplication have been developed. *In vitro* culture enables the production of disease-free planting material and also offers the possibility of developing new lines by taking advantage of somaclonal variation. In the not too distant future, therefore, micropropagation may well become the standard method for propagating ginger.

Planting and aftercare

Thorough preparation of land by repeated ploughing and harrowing so as to obtain a fine tilth is essential in order to enable strong and healthy growth of rhizomes. The optimum spacing is 25-45 cm between rows and 15-20 cm between plants. The setts are planted 5-10 cm deep and this is followed by light irrigation if required. Mulching is recommended to conserve moisture and to minimise weed growth. Mechanical or chemical weed control is essential to produce a good crop of ginger. In areas of low or uneven rainfall, irrigation at weekly or fortnightly intervals will also be necessary. The first shoots emerge from the ground approximately 2 weeks after planting.

Fertilizer requirements

Ginger is a heavy feeder and has a relatively high fertilizer requirement. In soils low in organic matter content, application of 25-30 tonnes of cattle manure or compost per hectare at the time of preparing the bed for planting is recommended. Inorganic fertilizers are applied at a rate of 75 kg N + 50 kg P_2O_5 + 50 kg K_2O per hectare in three split doses during the first 4 months of cultivation.

Crop rotation and intercropping

Being an exhausting crop, it is not desirable to grow ginger on the same site repeatedly year after year. The common practice is to grow ginger as part of a 3- to 4-yr rotation involving cereal crops, legumes, short-season vegetables and fallowing. Ginger is sometimes grown as an intercrop on raised beds prepared between rows of trees in plantations of tree crops such as coconut and arecanut.

Harvesting and yields

Ginger is harvested between 7 and 10 months from planting depending on the end use. For preserves and pickles, rhizomes are harvested while they are tender whereas for dried ginger, harvesting can be left until much later although the fibre content increases rapidly during the final stages of maturity. Senescence of aerial parts is a good indicator of the stage of maturity of the rhizomes. Harvesting is usually done manually by digging out the rhizomes carefully with a spade or fork without damage or injury. Mechanical diggers are also used in some countries. After removing any adhering soil, the shoots and the fibrous roots, the rhizomes are washed.

Yields ranging from 7-10 tonnes of fresh rhizomes per hectare under average conditions of cultivation to 40 tonnes/ha under intensive management using high-yielding cultivars have been reported. Depending on the cultivar, the dry ginger yield can vary between 12 and 25% and the crude fibre content from 2 to 10% or more.

Pests and diseases

The main diseases affecting ginger are rhizome rot caused by various species of *Fusarium*, bacterial wilt caused by *Pseudomonas solanacearum*, and leaf spots caused by *Phyllosticta zingiberi* and *Colletotrichum zingiberis*. Preventive measures include treating the setts with fungicides and antibiotics, and drenching the soil and spraying the crop with copper fungicides.

The main insect pests are the shoot borer, *Dichocrocis punctiferalis* and the rhizome scale, *Aspidiotus hartii*. Nematodes causing damage to rhizomes include *Meloidogyne incognita* and *Rotylenchus similis*. Furthermore, the spice beetle, *Stegobium paniceum*, can cause serious damage to dried ginger stored in warehouses.

Postharvest treatment and handling

The shelf life of rhizomes harvested for the fresh market can be increased by cold storage. When stored at 2-5°C and 90% relative humidity, the rhizomes stay fresh for up to four months.

Dried ginger is traded on the international markets in a variety of forms. The most basic among these are the peeled (or scraped) and unpeeled (or unscraped) forms. Peeled ginger is prepared by scraping off the brown corky skin with the sharp edge of a bamboo splinter without damaging the immediately underlying tissue which has the largest concentration of volatile oil. The rhizomes are then washed and dried in the sun for 7-10 days with periodic turning over. The unpeeled form, on the other hand, is produced by sun-drying thoroughly washed rhizomes without removing the skin. The moisture content of the dried product is usually in the 7-12% range.

The more important among other forms are 'bleached ginger' prepared by treating peeled rhizomes with lime and/or sulphur dioxide before drying and 'black ginger' obtained by dipping the rhizomes in boiling water for a few minutes before drying. Different degrees of breaking, dividing and slicing of the rhizome (harvested as a clump) before it is dried lead to the various shapes and sizes of the dried ginger sold on the market. The most important determinants of aroma quality and pungency of the final product are cultivar and stage of maturity at harvest.

Packaging for export is commonly done using double-lined jute sacks which can carry 50-75 kg of the dried product. The packed product often requires long-term storage before shipment. Good hygiene and measures against storage pests are essential during this period to avoid spoilage and deterioration.

TURMERIC (*Curcuma longa*)

Turmeric, the dried rhizome of *Curcuma longa*, is an important spice crop of India, Sri Lanka, Indonesia and other parts of South-East Asia. In addition to being widely used as a culinary spice in these countries, turmeric has long been used in traditional medicine. Its antioxidant and antiseptic properties are being increasingly recognized world-wide. Because of its strong yellow colour, which easily turns red in the presence of lime or other alkalis, turmeric also has important applications as a dyestuff. On extraction with solvents, turmeric yields an oleoresin which is used in the food industry.

Curcuma longa was first brought into cultivation in southern or South-East Asia. India is the largest producer and exporter of turmeric in the world. The crop is also commonly cultivated in Sri Lanka, Bangladesh, Pakistan, China, Indonesia, Jamaica and some other countries.

The plant

Curcuma longa (Zingiberaceae) is an erect herbaceous perennial, which grows to a height of about 1m. The leaf sheaths form a pseudostem, but unlike ginger, has a distinctive, thin petiole so that the leaf lamina is at a distance from the pseudostem. The leaves are up to 30 cm long and 6-10 cm wide. The rhizomes, with a characteristic odour and taste, develop as a dense clump, and are covered with scales, but are bright orange in colour inside. The inflorescence is a spike, 10-12 cm in length and 5-6 cm in diameter.

There are approximately 30 recognized cultivars. These are either high-yielding lines selected from germplasm collections or clonal selections from other cultivars already grown.

Soil and climate

Turmeric performs well in the hot and humid tropical regions, where it is usually grown as a rainfed annual crop although it can be cultivated other areas with adequate rainfall or irrigation. It can be grown from sea level to altitudes of up to 1500 m in areas where the temperature during the growing season remains in the 15-35^{0}C range.

Turmeric thrives on fertile soils with a loose texture. Well-drained alluvial sandy loams are ideal for growing turmeric. Sites with heavy clays or gravel should be avoided. The crop is very sensitive to waterlogging. It is therefore essential to provide adequate drainage.

Propagation

Although significant advances have been made in recent years in the micropropagation of turmeric in the laboratory, conventional vegetative propagation using parts of rhizomes from the previous crop still remains the only method used at the field level.

Fully mature, well-developed, disease-free rhizomes selected from the previous harvest are treated with fungicides and stored in a cool and dry place for use as planting material. The rhizome has a certain dormancy period and should therefore be stored for 2-3 months before planting. Although both main rhizomes and fingers can be used for propagation, setts prepared from the main rhizomes usually give better germination and higher yields. Setts for planting are prepared by cutting the rhizomes into 20-g pieces containing one or two buds. The recommended seed rate is 2.5 tonnes per hectare.

Micropropagation techniques have now reached an advanced stage of development. Protocols are now available for the routine and rapid multiplication of plantlets *in vitro*. These techniques have also enabled the selection of lines with high curcumin contents. In countries with adequate technical resources and know-how, micropropagation is likely to become the standard procedure for propagating turmeric on a commercial scale in the near future.

Planting and aftercare

As with other rhizome crops, thorough preparation of land so as to obtain a fine tilth is essential for growing a good crop of turmeric. Although planting can be done on flat beds, the preferred method is to prepare ridges and furrows. The setts are then placed in the furrows and covered with the loose soil from the ridges so that the setts are about 8-10 cm below the soil surface. The recommended spacing is 45-60 cm between rows and 25 cm between plants.

Mulching with green leaves and manual or chemical weed control are essential to obtain a good crop. In dry areas, irrigation will be necessary at weekly to fortnightly intervals. The first shoots emerge after about two weeks from planting. After further growth of shoots for about 8 weeks, earthing up may be necessary.

Fertilizer requirements

Turmeric requires heavy manuring. Incorporation of 40 tonnes of compost or cattle manure per hectare at the time of ploughing and land preparation followed by application of fertilizers at a rate of 30 kg N + 30 kg P_2O_5 + 60 kg K_2O per hectare under rainfed conditions, and twice this amount of N and 50% more K under irrigated conditions, in 3 split doses, is recommended.

Crop rotation and intercropping

Turmeric is often grown as an intercrop between rows of trees in coconut or other tree plantations. It is also commonly grown as one of several herbaceous species included in mixed cropping or agroforestry systems. In wetlands, it s grown in rotation with rice, sugarcane, banana and vegetables. In other situations, it is grown in rotation with millets, pulses, tuber crops and short-season vegetables.

Harvesting and yield

The crop is ready for harvest 7-9 months from planting when the lower leaves turn yellow and shrivel off. The clumps of rhizomes are dug out with a spade taking care to avoid any damage, and lifted intact with the dry shoots. After cutting off the tops, the loose earth adhering to the rhizomes is shaken off and the rhizomes are washed. The main rhizomes, primaries and secondaries ('fingers') are separated in order to facilitate grading of the final product.

Yields reported for fresh rhizomes are in the 10-20 tonnes/ha range under average conditions although it has been shown that yields of up to 45 tonnes/ha can be achieved with some high-yielding selections. Weight of dried turmeric is approximately a fifth of its fresh weight.

Pests and diseases

The most serious diseases of turmeric are rhizome rot caused by *Pythium graminicolum*, leaf blotch caused by *Taphrina maculans* and leaf spot caused by *Colletotrichum capsici*. The main insect pests are the shoot borer, *Dichocrocis punctiferalis*, which attacks the pseudostems and rhizomes, the leaf-roller, *Udaspes folus*, and the scale insect, *Aspidiotus hartii*. The dried turmeric is also susceptible to the attack of stored products pests.

Postharvest treatment and handling

Postharvest processing for obtaining the dry turmeric for marketing should be carried out within a week of harvest. This involves boiling the fresh rhizomes with the minimum volume of water required to soak them. The main rhizomes and the 'fingers' are cured separately because they require different lengths of cooking time.

The washed and cleaned rhizomes are placed in a suitable container, water is added until the water level is no more than a few centimetres above the rhizomes and then boiled for 45-60 minutes, until the rhizomes are slightly tender to touch. At this stage, frothing occurs and dense fumes with the characteristic aroma of turmeric appear. After draining the boiling liquid, the rhizomes are cooled and spread in the drying yard and exposed to the sun for 10-15 days, turning them occasionally. The dried product should have a moisture content of 8-10%.

To prepare the material for marketing, the dried rhizomes are then 'polished' by removing the outer skin, the rootlets and any loose debris, until the product looks bright yellow and attractive. This can be done either manually or using a polishing drum.

Packaging of dry turmeric is usually done in doubled-lined jute sacks. While being stored in warehouses, the packaged product requires protection against stored products pests. This is commonly done using fumigants, but in this case it is essential to ensure that the fumigation is carried out under controlled conditions, following the recommended procedure and doses, so that no residues are left on the product.

CINNAMON (*Cinnamomum zeylanicum*)

The spice cinnamon is the dried bark of *Cinnamomum zeylanicum* (syn. *C. verum*). In addition to its culinary uses in Asian and European recipes, it has important applications in medicine. Volatile oils obtained by steam distillation of stem bark, root bark, twigs and leaves are used in perfumery and in the food industry. These oils are also known for their antifungal and antimicrobial properties. The bark also yields oleoresins used in food flavouring.

Aromatic oils and oleoresins of commercial importance are also extracted from a number of other species of *Cinnamomum*. The most well-known among these are *C. cassia, C. burmannii, C. loureirii* and *C. camphora*.

Cinnamomum zeylanicum is a native of Sri Lanka which is still the largest producer and exporter of cinnamon. It also grows in the Seychelles, Madagascar and other Indian Ocean islands, India and South East Asia. Much of the literature published on cinnamon originates from India and Sri Lanka.

The plant

C. zeylanicum (Lauraceae) is a perennial evergreen tree which can grow to a height of up to 15 m in the wild. In cultivation, however, the trees are coppiced and trimmed down to maintain a bushy growth no taller than 2-3 m. In the wild state, the trunk can grow up to 50 cm in diameter. The stem and older branches are covered with a brown bark whereas younger twigs have a green bark The petioles, which are 1-2 cm long, and the leaf lamina, 10-15 cm long and 5-10 cm wide, are highly aromatic. Inflorescences are axillary and terminal panicles, consisting of clusters of very small flowers. The fruit is a fleshy, 1-2 cm long, ovoid drupe, black in colour when ripe.

Although it is known that "varieties" with local names and known characteristics exist in growing areas, there appears to be virtually no published information on them. There is some recently published information from India, however, on plant improvement work currently under way, mainly involving germplasm collection and *in vitro* culture.

Soil and climate

Cinnamon is a tropical crop, requiring a warm and humid climate. It is usually grown as a rainfed crop in areas with an annual rainfall of 250 cm or more and mean temperatures of 25-30^{0}C. The crop performs well at altitudes of less than 500 m although in the wild they can be seen in forests at higher altitudes.

The quality of bark is known to be influenced by the soil on which the tree is grown. Trees growing on fine sandy soils with a good supply of organic matter are known to produce a good quality bark. The tree grows well on red lateritic soils, but as an introduced crop, it has been grown successfully on a wider range of soil types. In the wild state, the trees can be seen on a wide range of soils, including on sites of low fertility which are unsuitable for the cultivation of most crops.

Propagation

Cinnamon has traditionally been propagated both by seeds and by vegetative means using rooted cuttings. *In vitro* propagation techniques enabling rapid multiplication of desirable genotypes are now available (Rai *et al.*, 1987), although not in use on a commercial scale.

As the essential oil composition is known to vary significantly between trees (Rao *et al.*,1988), in order to minimize genetic variability, it is desirable to adopt vegetative propagation rather than using seeds. Furthermore, trees raised by cuttings or by micropropagation grow much faster and become ready for harvesting earlier than those from seedlings.

For raising seedlings, seeds from freshly collected ripe fruit are used after removing the mesocarp and washing. The seeds are sown in rows 30 cm apart, at a depth of 2-3 cm, in a well-prepared nursery bed. Germination takes about 10-12 days. Irrigation and partial shading of seedlings may be required in hot and dry weather. Weed growth also should be kept under check. The seedlings should be ready for transplanting 9-12 months from sowing.

An alternative practice is to raise seedlings initially at closer spacing on nursery beds in groups of 15-20 by sowing seeds in 10-cm holes. The clumps of seedlings are transplanted into pots or plastic bags containing a suitable growing medium when they are 3-4 months old and reach a height of about 15 cm. These seedlings will be ready for transplanting into the field 4-5 months later. Instead of transplanting, the seeds may be sown *in situ*. In this case, several seeds are sown in each planting hole, about 2 m apart.

Vegetative propagation may be by means of cuttings, layering or dividing old rootstocks. Air-layering of 1-yr-old shoots using rooting hormones is probably the most effective method. The recommended procedure is to remove a ring of bark, 2 cm long, from the selected twig, to apply a paste containing 1 000 p.p.m. IBA + 2 500 p.p.m. NAA and to cover the girdled portion with sphagnum moss or suitable rooting medium held together by tying a plastic sheet around. The layers will be ready for separating from the mother tree and transplanting 2-3 months later.

Planting and aftercare

Planting is done at a spacing of approximately 2m between trees. The traditional practice is to plant 15-20 seedlings in a single 30-cm-diameter hole. However, recent work from India recommends the use of no more than 3 seedlings per hole and a wider spacing between stands. Aftercare mainly consists of weeding and removing any dead or damaged branches.

After two or three years of growth, the plants are coppiced by cutting the stems at a height of no more than 10 cm from the ground level and earthing up to encourage new shoot growth. Harvesting of stems for bark removal commences when the new shoots have grown for a further two years.

Fertilizer requirements

Little information is available on the nutritional requirement of cinnamon. Fertilizer recommendations given in a small number of recent papers appear to be inconsistent and speculative rather than being based on the results of experimental work. The crop is usually grown without applying fertilizers.

Mixed cropping and intercropping

Cinnamon is sometimes grown as a component of agroforestry systems involving other plantation crops (e.g. rubber) and forest trees. In other situations, it is grown in combination with one or more other crops such as coconut, arecanut, cloves, nutmeg, cocoa, pepper, banana, pineapple and ginger in multitier cropping systems.

Harvesting and yield

Harvesting is usually done during the rainy season. Well-developed shoots, 2-4 cm in diameter are cut and bundled up after removing the leaves and smaller branches. The leaves may be saved for distillation if required.

Average yield of quills from the first harvest is of the order of 50-60 kg/ha. The yield increases during subsequent years to reach a maximum of around 200 kg/ha. A gradual decrease in annual yield is commonly observed after about 10 years.

Pests and diseases

Seedlings are susceptible to the leafspot fungus, *Glomerella cingulata*, which, in severe cases, can cause partial drying up of plants. Young trees occasionally suffer from stripe canker caused by *Phytophthora cinnamomi* infections on trunks and branches, resulting in bark loss. Insect pests reported include *Chilasa clytia, Sorolopha archimedias, Acrocercops telestis, Popillia complanata, Singhala helleri, Trioza cinnamomi, Graphium sarpedon, Orthaga vitialis* and others.

Postharvest handling

The outer bark is first removed from freshly harvested stems using a curved knife and the surface of the inner bark is cleaned up. The inner bark is then carefully dislodged from the wood using a stripping knife after ringing the stem and making longitudinal incisions to give quills of required size. The quills are then rolled by hand and dried in the shade.

CORNMINT (*Mentha arvensis* var. *piperascens*)

Mint oils are among the most important essential oils derived from cultivated plants which are traded on the world markets. Both in terms of volume and cash value of the commodity traded, cornmint is second only to orange (Lawrence, 1993).

The genus *Mentha* includes some 25 species and hybrids of Mediterranean and Eurasian origin, several of which are now distributed widely throughout the temperate and subtropical regions and have also been introduced into many tropical regions. Among the cultivated species, the most important are (i) cornmint or Japanese mint, *Mentha arvensis* var. *piperascens* (ii) peppermint, *M.* x *piperita* (=*M.* x *spicata* x *M. aquatica*), (iii) spearmint, *M.* x *spicata* (=*M. longifolia* x *M. suaveolens*), (iv) bergamot or lemon mint, *M. citrata*, and (v) pennyroyal, *M. pulegium*.

Mint oils have wide-spread uses in virtually every area of application of essential oils (see Introduction). Mints are therefore among the most extensively studied groups of aromatic plants. The commercial importance and geographic spread of cornmint, peppermint and spearmint are reflected in the volume of literature published on them, which is second only to that on citrus crops. Every aspect of agrotechnology of mint crops has been studied in great detail. Space does not permit an in-depth treatment of these developments. Instead, cultivation details are presented at a very basic level, concentrating on cornmint, *M. arvensis* var. *piperascens*, which is better adapted to subtropical and tropical conditions than other mints. The plant is of Eurasian origin, and grows wild in China and Japan. It is cultivated on a commercial scale in Japan, China, Thailand and other parts of south-east Asia, Western Australia, India, some parts of Eastern Europe, Brazil, Argentina and other Latin American countries.

The plant

Mentha arvensis var. *piperascens* (Labiatae) is a perennial sub-shrub which grows to a height of 70-80 cm in cultivation. Stems are green or purple in colour, quadrangular in cross-section and bear many branches. Leaves are opposite, lanceolate, and up to 10 cm long and 3-4 cm wide when fully grown. The plant multiplies itself by producing numerous horizontally spreading, branched underground stolons.

Improved cultivars produced by breeding and selection are in use in many of the growing countries. Superior, high-yielding somaclones developed *in vitro* are also in cultivation on a field scale.

Soil and climate

Cornmint has a wide climatic tolerance and can be grown successfully under warm temperate, sub-tropical and tropical conditions. Highest dry matter and oil yields are obtained at a day/night temperature combination of $30^0/18^0$C (Duriyaprapan *et al.*, 1986).

Deep, fertile loams or clay-loams with a pH of 6-7.5, a good supply of organic matter and a good water holding capacity are ideal for the cultivation of cornmint. The plant is sensitive to waterlogging. Only sites with adequate drainage should therefore be chosen.

Propagation

Cornmint is always propagated vegetatively. The most common practice is to use stolons obtained from the previous crop. Other propagating material used include suckers and rooted cuttings. However, plant establishment is faster and growth and yields are higher when stolons are used for propagation.

Strong and healthy stolons should be selected from previous year's crop. Stolons obtained from plants more than a year old have been found to be less satisfactory. Sites affected by nematode infestation should be avoided for collection of propagating material.

Planting and aftercare

The land should be well prepared before planting. Freshly collected stolons are divided into 10-12 cm lengths and planted by placing them horizontally at a depth of 8-10 cm, allowing a spacing of 10 cm within the row and 45-60 cm between rows. This amounts to a seed rate of 300-400 kg of stolons/ha. If the soil remains dry, a light irrigation is recommended soon after planting. New shoots emerge from the stolons within 2 weeks of planting.

Aftercare mainly consists of weeding either manually or using recommended herbicides. Irrigation may be necessary in dry areas. Mulching has also been found to be beneficial.

Fertilizer requirements

The nutrition of mints has been studied extensively. Cornmint responds well to N fertilization. Under average conditions, application of 150 kg N + 60 kg P_2O_5 + 40 kg K_2O per hectare per year is recommended, P and K being applied before planting and N in 2-3 split doses (at planting and during regrowth following each harvest).

Harvesting and yield

The highest oil content has been reported to be either at the pre-flowering stage or at the 50% full-bloom stage. The crop becomes ready for the first harvest 100-120 days from planting. Harvesting is done either mechanically or manually by cutting the herbage 8-10 cm from ground level. The second harvest is taken 80-90 days after the first. Depending on the time of planting and location, a third harvest is obtainable 80-90 days later in some areas.

Cumulative annual yields (from all harvests) reported for fresh herbage under well managed conditions are in the region of 40-50 tonnes/ha. The corresponding oil yields under the same conditions are 160-200 kg/ha.

Unlike peppermint which only requires replanting every 3-4 years, cornmint is usually replanted every year.

Pests and diseases

A large number of insect and nematode pests of mints have been reported from around the world. Insect pests reported specifically on cornmint (many of which are also pests of other mints) include *Aphis affinis, Cacoecia epicyrta, Helicoverpa armigera, Raphidopalpa foveicollis, Spilosoma obliqua, Syngamia abruptalis,* and *Thysanoplusia orichalcea.* Nematode pests which can cause serious damage include *Meloidogyne incognita, M. javanica, Pratylenchus curvitatus* and *P. penetrans.*

Fungal pathogens include *Corynespora cassiicola, Curvularia lunata, Erysiphe cichoracearum, Fusarium oxysporum, Macrophomina phaseolina, Marasmiellus epochnous, Peronospora menthae, Puccinia menthae, Rhizoctonia solani, Thielaviopsis basicola, Verticillium dahliae* and others. Mints are also susceptible to virus and mycoplasma diseases in some parts of the world.

Postharvest handling

The harvested herbage is allowed to wilt for a few hours in the field before being gathered for transport to the distillery.

PATCHOULI (*Pogostemon cablin*)

The volatile oil distilled from the leaves of *Pogostemon cablin* has a wide range of uses in perfumery and cosmetics, in the food, drink and confectionary industries, and in medicine. In terms of volume traded on the world markets, patchouli oil is among the top 15 essential oils. The oil is also known to have antimicrobial and insect-repellent properties. Patchouli is thought to be of South-East Asian origin. The main producing countries are Indonesia, Malaysia and China.

The plant

Pogostemon cablin (Labiatae) is a herbaceous perennial plant, about 1m tall when fully grown, with many branches and aromatic leaves 5-10 cm long and 3-8 cm wide, on petioles up to 8 cm long. Inflorescence consists of terminal and axillary spikes carrying small bisexual flowers.

Cultivars referred to in the literature carry the names of countries or regions from which the plant material was introduced. Both oil content and composition vary according to cultivar.

Soil and climate

Patchouli is a hardy plant which grows in the wild on a wide range of soils in many locations. A warm and humid climate is particularly favourable to its growth. The crop performs best on slightly acidic, well-drained loamy soils with a high organic matter content.

Propagation

Patchouli can be propagated by seeds or vegetatively using cuttings. As with most members of Labiatae, plants raised from seeds show a high degree of variability in morphology and, more importantly, in oil content and composition. The preferred method of propagation is therefore using cuttings. *In vitro* propagation techniques for mass production of virus-free plants are now available (Kukreja *et al.*, 1990), although not in widespread use.

Rooted cuttings are raised either in nursery beds or in plastic bags or other suitable containers. As the crop is particularly susceptible to nematode attack, it is important to adopt appropriate measures before planting to reduce the incidence in all cases. In plastic bags and containers, the growing medium should be sterilized by steam treatment and the nursery bed should be subjected to fumigation or treatment with nematicides.

As the plants are also susceptible to yellow mosaic virus, cuttings should be taken from healthy, virus-free plants. Stem cuttings with 3-4 nodes and the terminal bud intact give best results. Treatment of cuttings with IBA (500-2000 p.p.m.) or NAA (500-1000 p.p.m.) has been found to give 100% rooting and plant survival (Selvarajan and Rao, 1982).

The nursery beds should be well prepared to obtain a fine tilth. Cuttings are planted at a spacing of 10 cm x 10 cm. Shading and irrigation should be provided if necessary. The rooted cuttings should be ready for transplanting in 5-6 weeks.

Planting and aftercare

The soil should be thoroughly prepared by ploughing and harrowing and treated with nematicides. The rooted cuttings are transplanted in rows at a distance of 60 cm between plants and 90 cm between rows. Planting is sometimes done on ridges, 20-25 cm high, prepared previously.

Both foliage and oil yields are higher when the crop is grown under partially shaded conditions (Radhakrishnan *et al.*, 1991). When patchouli is grown as a monocrop, it is usual therefore to plant *Glyricidia*, *Erythrina* or other locally available trees as shade trees.

Weeding is the main operation to be carried out during the growing season. Irrigation may also be necessary during dry periods.

Fertilizer requirements

Patchouli is a soil-exhausting crop and therefore requires a high initial level of soil fertility and responds well to fertilizer application, especially to nitrogenous fertilizers. Under average conditions, the application of a basal dressing of 25 kg N + 50 kg P_2O_5 + 50 kg K_2O per hectare followed by 25 kg N at bimonthly intervals is recommended.

Mixed cropping and intercropping

Patchouli is often profitably grown as an intercrop in plantations of tree crops such as oil palm, rubber, coconut and arecanut, or in mixed cropping systems which also include other crops.

Harvesting and yield

Harvesting of foliage commences 4-6 months after planting. The first harvest yields the largest amount of foliage. Subsequent harvests can be done at intervals of 3-4 months. Lengths of shoots carrying 3-5 pairs of mature leaves are collected. In order to ensure a satisfactory regrowth of the plant after harvest, it is important that an adequate length of shoot is left unharvested.

The crop can be harvested for 2-3 years from planting. Oil yield and quality decline after this period and the crop should therefore be replanted. In some countries, patchouli is grown as an annual crop.

Widely different yields have been reported in the literature. The actual yields obtained under field conditions in most cases appear to be in the range 1-4 tonnes of air-dry foliage per hectare per year although potential yields approaching 7 tonnes are said to be achievable. Oil yields reported are mostly in the range 100-200 kg/ha.

E. PESTS AND DISEASES

Patchouli is susceptible to a wide range of pests and diseases. As mentioned previously, the most serious pests are root nematodes which can cause serious crop losses. The most commonly reported species of nematodes are *Pratylenchus brachyurus, Meloidogyne incognita and M. hapla* Other nematode pests include various species of *Scutellonema, Rotylenchulus, Helicotylenchus, Hemicriconemoides* and *Xiphinema*. Many insect pests are also reported to cause significant crop damage. These include *Psara stultalis, Oxya chinensis, Aularches miliaris, Graphium* sp.,*Pachypeltis* sp.and *Myzus persicae*.

Diseases caused by viruses (yellow mosaic virus, mild mosaic virus and mottle virus) transmitted by flies (*Bemesia tabaci*) and sap-feeding insects can also cause significant damage to crops. Patchouli is also susceptible to leaf blight caused by pathogenic fungi (*Cercospora* sp., *Alternaria alternata*).

F. POSTHARVEST TREATMENT AND HANDLING

The oil from the tissues is not easily expelled unless the harvested material is subjected to pretreatments such as scalding or drying in order to facilitate the rupture of cell walls during distillation. The harvested foliage should therefore be properly cured before distillation. This is usually done by drying it in the shade. The leaves are spread out in thin layers in a well-aerated shed, turning them occasionally to achieve uniform drying and to prevent fermentation and mould growth. The drying process takes about 3 days, at the end which the cured material has a stronger patchouli aroma than the fresh leaves. Unless it is to be distilled immediately, the cured material is compressed and packed into bales for storage or for export.

CITRONELLA (*Cymbopogon winterianus*)

Among the various cultivated aromatic grasses of the genus *Cymbopogon*, the most important are *C. winterianus* (Java citronella), *C. nardus* (Ceylon citronella), *C. flexuosus* (East Indian lemongrass), *C. citratus* (West Indian lemongrass) and *C. martinii* (palmarosa). Other cultivated species include *C. jwarancusa, C. pendulus* and jamrosa (a hybrid between *C. nardus* and *C. jwarancusa*). Additionally, many other aromatic species of *Cymbopogon* grow in the wild.

Because of the large differences in the composition of volatile oils they contain, these various species yield oils of different aroma characteristics with different applications in perfumery and cosmetics, and as flavouring agents. Although there are significant differences between the species in the biosynthetic pathways leading to the accumulation of volatile oils, they share some important common physiological features as far as their primary metabolism is concerned. They therefore exhibit many similarities in their agronomic behaviour.

Being C_4 plants, all species of *Cymbopogon* are fast-growing crops with a high photosynthetic efficiency. Their photosynthesis is characterised by a low (0-5^0 l/l) CO_2 compensation point, the lack of light saturation of CO_2 uptake at high photon flux densities, a high temperature optimum (35^0C) for net photosynthesis, high rates of net photosynthesis (55-67 mg CO_2 dm^{-2} leaf area h^{-1}), and little or no response of net photosynthesis to atmospheric levels of oxygen. In the tropics where temperature and irradiance are non-limiting, it should therefore be possible to obtain high dry matter yields by proper management.

Among all the cultivated species of *Cymbopogon*, commercially the most important is *C. winterianus* (Java citronella). The cultivation of this species is therefore described below.

Cymbopogon winterianus (known as 'Maha Pengiri' in Sri Lanka) is the result of selection from *C. nardus* which is native to Sri Lanka. It is now widely cultivated in many tropical and subtropical regions around the world.

The plant

Cymbopogon winterianus (Gramineae) is a herbaceous perennial grass which grows in tufts with profuse tillering and a dense, fibrous, adventitious root system. The plants can be up to 2 m tall and the leaves are about a metre in length and 1.5 cm in width. The inflorescence consists of a panicle which can be up to 30 cm in length.

Numerous improved cultivars adapted to local conditions are available in most countries where citronella is grown. *In vitro* techniques have been developed for micropropagation as well as for plant improvement work based on selection of somaclonal variants with desirable traits.

Soil and climate

Citronella can be grown under a wide range of situations in the tropics and the subtropics. The crop requires plenty of sunshine and performs well in areas with an annual rainfall of 200-250 cm where it can be grown as a rainfed crop. High atmospheric humidity is more favourable to crop growth than high soil moisture. Citronella can be grown at altitudes of up to 1500 metres.

The crop is sensitive to waterlogging. Provided there is adequate drainage, it can be grown on a wide range of soils. However, sandy loams allowing free drainage and easy root penetration are particularly favourable.

Propagation

Citronella is usually propagated vegetatively using slips obtained by dividing healthy clumps dug out from a previous crop. This should be done as close to the planting time as possible so that the plants are not subjected to any significant water stress before planting. Each slip consists of 2-3 strong and healthy plants. In order to facilitate planting, it is common practice to trim the extremities of shoots and roots.

Planting and aftercare

The slips are planted with the base of the stem positioned at a depth of 8-10 cm. Numerous spacing trials carried out at different locations in India have shown that the spacing required for obtaining the maximum dry matter and oil yields per hectare varies, depending on the level of soil fertility, between 60 cm x 30 cm and 90 cm x 90 cm. For ease of management, however, the generally recommended spacing is 60 cm x 60 cm on sites of average soil fertility and 90 cm x 90 cm in very fertile soils.

Aftercare mainly consists of weeding and, in dry areas, irrigation. Weeding is done 3-4 times a year, either mechanically or using herbicides. The most convenient period for weeding is within 2-3 weeks of each harvest. Competition from weeds can be particularly severe during the first year, before the crop is fully established. Mulching with the spent grass obtained as distillation waste material has been found to be beneficial. This practice should be recommended as it also represents a convenient method of recycling wastes.

Fertilizer requirements

Although fertilizer requirements will vary according to the level of soil fertility, the results of most fertilizer trials reported in the literature suggest that the crop responds well to N application, particularly during the early years. In many cases, both herbage yield and oil yield increased with increasing N up to 400 kg ha^{-1} yr^{-1}applied in up to 6 split doses. Under average conditions, the recommended practice is to apply 40 kg each of P_2O_5 and K_2O per hectare each year in a single application and 100-200 kg of N ha^{-1} yr^{-1} in four equal split doses.

Harvesting and yield

Depending the extent of vegetative growth observed, the first harvesting can be done 5-6 months from planting and subsequent harvests at 3-4 monthly intervals. The growth rate itself being dependent on the cultivar and environmental factors, the number of harvests per year will vary from 3 to 4. As there are large differences in the oil content and composition between different plant parts, only the leaf blades are used for distillation. The oil content of the leaf blade is about 3-4 times higher than that of the leaf sheath, the condensed stem or the inflorescence. The leaf blades are therefore selectively harvested, leaving all other plant parts behind.

Fresh herbage yields of up to 25 tonnes/ha and oil yields of up to 250 kg/ha can be obtained under favourable conditions.

Highest oil yields per hectare are obtained during the second and third years of cropping. Thereafter, the yields decline and it is not economical to maintain a plantation more than 4 years old. At this stage, the plants should be dug out of the soil and the site cultivated with other crops such as legumes and vegetables for a few years before replanting with citronella.

Pests and diseases

Several species of insects are reported in the literature as pests of citronella. The most serious among these are *Chilo infuscatellus* and *Microtermes obesi.* Diseases reported include collar rot and wilt caused by *Fusarium moniliforme*, and leaf blight and leaf spot caused by *Curvularia andropogonis*.

Postharvest handling

The fresh leaves are distilled immediately after harvest. It has been reported (Nandi and Chatterjee, 1991) that oil yield in the herbage increases during the first 24 h following harvest and decreases thereafter. However, it is not clear whether this is due to continuing biosynthesis and accumulation of oil after harvest or due to loss of moisture from the harvested foliage during this period.

VETIVER (*Vetiveria zizanioides*)

The volatile oil distilled from the roots of vetiver is extensively used in perfumery and cosmetics. The oil is also known for its antifungal, antibacterial and insect-repellent properties. The dried roots are used for making screens, mats, fans, baskets and other household articles and also have applications in traditional medicine.

Other traditional uses of the grass include thatching, mattress stuffing, making brooms and baskets, as bedding material for livestock, in paper manufacture and as a fodder crop. Because of its deep root system with the ability to hold the soil particles together and to prevent surface runoff, vetiver grass is grown for erosion control on slopy terrain in many parts of the world.

In India, Sri Lanka and South-East Asia, the roots have been used since ancient times. The plant grows wild in many parts of the world. For soil conservation purposes, it has been introduced to many areas susceptible to erosion. According to a recent report (Smyle and Magrath, 1993), *Vetiveria zizanioides* has been found in the flora of 86 countries. However, the oil is usually derived from plants cultivated for the purpose. Haiti, Reunion and Indonesia are the main producing countries.

The plant

Vetiveria zizanioides (Gramineae) is a densely tufted, narrow-leaved, perennial grass which grows to a height of up to 2 m. The inflorescence is a prominent panicle with numerous slender racemes. The dense lacework of spongy aromatic roots are concentrated in the top 20-30 cm of soil, but individual roots can reach depths of up to 100 cm in the soil profile.

Many improved cultivars are available in countries where vetiver is grown. Most are hybrid clones. Breeding and selection work has been mainly aimed at producing cultivars with higher oil content. *In vitro* propagation techniques have been developed but are not in wide-spread use.

Soil and climate

Vetiver has a wide climatic tolerance and, for soil conservation, has successfully been grown with little attention under a variety of geoclimatic conditions. It grows wild on waterlogged soils in many tropical regions. Being a C_4 plant, it thrives in warm and humid tropical regions, but has been grown in colder climates where vegetative growth occurs only during periods when the temperature remains above 15^0C. The crop performs best in areas with an annual rainfall of 100-200 cm and average daily temperature of $25\text{-}30^0$ C.

Although oil content of roots is known to be influenced by soil type, very little information is reported in the literature on soil and fertilizer requirements. Luxuriant vegetative growth with moderate oil content is attainable when cultivated on well drained sandy loam soils or on peat soils. However, highest oil yields are obtained from crops grown on red lateritic soils.

Propagation

Although plant multiplication in nature occurs both vegetatively and by seed dispersal, under cultivated conditions it is usually propagated using slips obtained from the previous crop. Other methods of propagation include: (a) ratooning, where the plants are cut back close to ground and allowed to regrow; (b) using rooted cuttings raised by planting stem cuttings with 2 nodes each in nursery beds or in plastic bags after treatment with rooting hormones; (c) using branches produced at the nodes when the culms are cut back to a height of 30-40 cm from the ground.

For large scale multiplication for commercial planting or in connection with soil conservation work, slips containing 2-3 tillers are planted 15-20 cm apart in well prepared nursery beds. While weeding will be essential on most sites, irrigation and fertilization are also sometimes practised. Widely different growth rates have been reported from different parts of the world. In the tropics, after 5 months in the nursery, multiplication rates of up to 20 tillers per tiller planted can be obtained. If these plants are not lifted for transplanting within 3-4 months, they are cut back to a height of 40 cm to encourage new tiller production.

Planting

Slips prepared by dividing the clumps to 3-4 cm diameter at the base and trimmed to a height of 20 cm are planted 8-10 cm deep. The recommended spacing under tropical conditions is 60 cm x 30 cm. Wider spacing is used when mechanically planted, e.g. in southern United States.

Fertilizer requirements

As mentioned earlier, very little is known about the nutritional requirement of vetiver or on the effect of nutrition on oil yield and composition. Work reported from India some 20 years ago showed that application of P and K fertilizers increased the oil content of roots without increasing root yield.

Harvesting and yield

Time from planting to harvest varies between locations depending on the amount of growth made under the geoclimatic conditions prevailing in the site. In the tropics, the optimum stage of growth for harvesting the roots is reached 15-18 months from planting. Oil quality is reported to be the best at this stage when a large percentage of roots are thick, fleshy and carry fine rootlets. On further maturity, the roots become brittle and both oil content and quality deteriorate. For harvesting, the shoots are first cut or mowed at a height of 15-20 cm above ground and the clumps are uprooted from a minimum depth of 50 cm.

Although it has been known for a long time that both the content and quality of volatile oil distilled from vetiver roots are greatly influenced by climate, soil, nutrition, plant age at harvest and other factors, very little research effort has gone into elucidating the mechanisms involved at a plant physiological level. Factors determining the biosynthetic pathways of aroma compounds remain virtually unexplored.

While roots from plants growing wild in a variety of situations are unsuitable for oil extraction, even for roots from cultivated crops the oil contents reported in the literature vary as widely as from 0.1% to 4.0%. Average oil yields appear to be in the range 20-25 kg/ha although yields of up to 70 kg/ha have been obtained in some cases.

Pests and diseases

The most commonly reported fungal pathogens are *Curvularia trifolii, Nigrospora* sp. and *Fusarium* sp. Bacterial blight caused by *Xanthomonas* sp. is also reported from some areas. No serious arthropod pests are reported.

Postharvest handling

The harvested roots are thoroughly washed and dried in the shade for 2-3 days. They can then be stored in a cool shed if required awaiting distillation. The aroma quality is said to improve during the initial few weeks of storage. The dried roots require soaking in water overnight prior to distillation.

5. ORGANIC FARMING

During the past two decades, there has been increasing concern world-wide over environmental issues such as pollution of land, air and water, loss of biodiversity and damage to ecosystems resulting from intensive, chemical-based agricultural activities and unrestrained use of non-renewable resources. There has also been an increasing recognition of the potential risks to human health from pesticide residues in food and water. This has led to a noticeable shift in emphasis in world agriculture away from intensive farming systems based on expensive, potentially harmful inputs, towards organic farming based on environmentally sound principles and practices. In many countries, the consumer demand for organically grown produce has exceeded the rate of supply, with the result that the prices for such produce command a significant premium.

Definition

Although organic farming has been defined in several ways, the main elements of most definitions which can be found in the literature on this subject are essentially the same. The US Department of Agriculture has adopted the following definition (USDA, 1980):

"Organic farming is a production system which avoids or largely excludes the use of synthetically compounded fertilizers, pesticides, growth regulators, and livestock feed additives. To the maximum extent feasible, organic farming systems rely upon crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic wastes, mechanical cultivation, mineral-bearing rocks, and aspects of biological pest control, to maintain soil productivity and tilth, to supply plant nutrients, and to control insects, weeds and other pests."

Main features of organic farming systems

Unlike the conventional approach which aims to dominate and suppress many natural processes by mechanical, physical or chemical means, organic farming aims to work in harmony with natural biological interactions. In organic farming, the farmer aims to integrate the biological interactions between the crop and its living environment consisting of soil flora and fauna, pests, predators and weeds into a profitable agricultural system which yields produce of optimum quality in a sustainable way. Such farming practices ensure minimum damage to the environment and minimum use of non-renewal resources.

The key characteristics of organic farming include: (a) safeguarding the long-term fertility of soils by maintaining organic matter levels, promoting the growth of soil flora and fauna, and careful tillage aimed at soil conservation; (b) supplying nutrients in relatively insoluble organically bound forms as manures, composts and crop residues, the nutrients being made available to the crop by the action of soil micro-organisms; (c) use of leguminous plants in crop rotations or in mixed cropping systems so as to take advantage of biological nitrogen fixation at least partially to meet the N requirement of the crop; (d) use of appropriate cultivation techniques, employing tools and implements of the right type and size, causing minimum damage to soil structure and least disturbance to organisms living in the soil; (e) adopting pest, disease and weed control measures which rely on the use of resistant varieties, crop rotations, predators and other natural enemies; and (f) ensuring that the farming activities do not have any damaging consequences on the wider environment, e.g. air and water quality, conservation of wildlife and natural habitats.

In organic farming, the use of inorganic fertilizers and agrochemical pesticides, including all herbicides, is prohibited. IFOAM (see below) standards require that a conventionally farmed site must undergo a minimum conversion period of two years in order to qualify for the organic status.

Use of certain plant-derived pest control products (e.g. pyrethrum and derris insecticides) which work by contact action and break down within 48 hours without leaving any residues, is allowed. Similarly, use of biological control agents is also permitted.

Certification and legislation

The organic movements operating on a voluntary basis in many countries have exercised self-regulation and set guidelines which their members have followed for many years. In Europe, France was the first country to introduce the official label AB (*Agriculture Biologique*) for organic produce. Organic standards were officially ratified by a law passed in 1980. Since then a series of further legislative measures have been introduced in France which now define precisely the criteria to be met in order to qualify for the 'AB' label. The standards are set and regularly reviewed by the Comité National d'Homologation which has representatives of farmers, consumers and the government among its members. The Committee puts forward recommendations for approval by the Ministry of Agriculture.

Britain followed France in national certification of organic produce. In 1988, the UK Ministry of Agriculture, Fisheries and Food appointed a Board called the United Kingdom Register of Organic Food Standards (UKROFS), which was run by the Food From Britain organization. UKROFS, with the help of its expert committees, formed a UK standard for organic food production and processing (UKROFS, 1993). It also created a certification system to ensure that rules are obeyed. UKROFS inspectors check a random sample of 10% of the approved producers at least once a year.

The EC Regulation

The EC Council Regulation No. 2092/91 on Organic Food (EC,1991a) came into force on 23 July 1992. From this date, no produce could be marketed as 'organic' in any of the member states unless it has been properly inspected and certified by the Designated Inspection Authority (DIA) of the country concerned. Each EC country has an appointed DIA which implements and polices the scheme either alone or in collaboration with approved organic certifying bodies. The DIA in the UK is UKROFS. The 'organic'-labelled produce is subject to inspection by the DIA at all stages including growing the crop, processing, preserving, packaging and importing.

Imports of organic produce into the EC can only be from countries on the EC's approved list. Those on the list must follow the EC scheme's organic production guidelines and recommendations and should have the necessary inspection measures in place. Any produce entering the EC must be accompanied by an inspection certificate issued by the designated inspecting authorities of the exporting country concerned.

Legislation in other countries

Similar legislation is also either being prepared or already in force in USA, Canada and Australia.

International certification

Most national certification bodies are non-governmental organizations which have been setting standards and certifying producers and processors in their own countries. Their umbrella organization is the International Federation of Organic Agriculture Movements (IFOAM) which has its headquarters at Tholey-Theley in Germany. IFOAM has its member organizations in over 60 countries including all the major national European organic certifying associations.

IFOAM has put forward a set of minimum production standards for the benefit of all its members. These standards (IFOAM, 1992), which were adopted by the IFOAM General Assembly held at São Paulo, Brazil in 1992, cannot be used on their own but provide a framework within which national organizations have to develop their own standards, in accordance with local conditions. They cover both crop production and animal husbandry, and include requirements with regard to animal breeding and feeding, veterinary care and animal welfare, food storage, transport, processing and labelling, and the inspection and certification of production processes.

Proposals for international organic standards are also being developed by the Food and Agriculture Organization of the United Nations as part of its *Codex Alimentarius* (FAO, 1991).

6. CODES OF GOOD AGRICULTURAL PRACTICE

The environmental impact of intensive agriculture is now widely recognized. In many industrialized countries, intensive farming has been a major source of pollution of both surface and groundwater. The nutrient discharges from excessive use of nitrogen and phosphorus fertilizers have resulted in lake eutrophication, damage to ecosystems and pollution of drinking water. The erosion of top soil resulting from the use of heavy machinery has led to long-term damage to soil fertility. Farm effluents often cause serious pollution of soil, water and air. The large amounts of plant protection chemicals used have not only caused pollution and pesticide residue problems, but also have led to resistance in pests and pathogenic organisms against which these chemicals have now become ineffective.

In order to protect the environment, legislative and other measures have been introduced in many countries. For example, over the past two decades, the European Community Action Programmes have led to the introduction of some 200 pieces of legislation covering pollution of atmosphere, water and soil, waste management, safeguards in relation to chemicals and biotechnology, product standards, environmental impact assessments and protection of nature.

The EC Fifth Programme of Policy and Action in relation to Environment and Sustainable Development (COM/92/23 final, EC, 1992) outlines the main priority areas for action up to the year 2000. These include reduction of nutrient loss to water, particularly nitrate and phosphate, and reduction of ammonia emissions, in addition to pesticide content and other measures. It also includes economic measures to be used to protect the environment and training of farmers.

Codes of Good Agricultural Practice have been developed in many countries in order to provide a framework for the reorientation of agriculture towards a more sustainable basis and to help the farming community to minimise environmental damage caused by farming operations. In most cases, these codes are complementary to other guidelines, recommendations and legislative measures relating to Health and Safety matters, toxic residues in food and water, and the like.

The details contained in codes of good agricultural practice will, of course, vary from country to country and from region to region, depending on the climate, soil, the range of farming activities, the sources of pollution and the type of environmental degradation in each case. No single set of codes can therefore be devised which will be universally applicable. Some aspects of EC legislation and the codes of good agricultural practice devised by the UK Ministry of Agriculture, Fisheries and Food to deal with problems specific to the UK are discussed below as examples.

With the specific objective of reducing nitrate pollution of water, the European Community Nitrate Directive (EEC/91/676, EC, 1991b) made it mandatory for all Member States of the Community to establish codes of good agricultural practice before the end of 1993 and to set up training programmes for farmers to help them implement these codes. The Directive further specifies, in Annex II of Article 4:

"a) A code or codes of good agricultural practice with the objective of reducing pollution by nitrates and taking account of conditions in the different regions of the Community, should contain provisions covering the following items, in so far as they are relevant: (1) periods when the land application of fertilizer is inappropriate; (2) the land application of fertilizer to steeply sloping ground; (3) the land application of fertilizer to water-saturated, flooded, frozen or snow-covered ground; (4) the conditions for land application of fertilizer near watercourses; (5) the capacity and construction of storage vessels for livestock manures, including measures to prevent water pollution by run-off and seepage into the groundwater and surface water of liquids containing livestock manures and effluents from stored plant materials such as silage; (6) procedures for the land application, including rate and uniformity of spreading, of both chemical fertilizer and livestock manure, that will maintain nutrient losses to water at an acceptable level".

"b) Member States may also include in their code(s) of good agricultural practice the following items: (7) land use management, including the use of crop rotation systems and the proportion of the land area devoted to permanent crops relative to annual tillage crops; (8) the maintenance of a minimum quantity of vegetation cover during (rainy) periods that will take up the nitrogen from the soil that could otherwise cause nitrate pollution of water; (9) the establishment of fertilizer plans on a farm-by-farm basis and the keeping of records of fertilizer use; (10) the prevention of water pollution from run-off and the downward movement beyond the reach of crop roots in irrigation systems".

As practical guides to help farmers and growers to avoid causing long-term damage to soils and pollution of water and air, the UK Ministry of Agriculture, Fisheries and Food has drawn up the following sets of codes:

(i) The Code of Good Agricultural Practice for the Protection of Soil (MAFF, 1993) describes the main risks of causing irreversible physical, chemical or biological changes to soils which would reduce their ability to support living organisms. The code provides guidance on protecting the current condition of the soil by preventative action. It also includes remedial actions to cope with contamination incidents.

The introductory chapter briefly describes the scope of various EC directives and UK laws relating to the protection soil. The section on soil fertility deals with chemical and biological processes which affect soil fertility, including the acidification of soils, maintaining the soil's nutrient reserves and its organic matter content. The ways of avoiding physical degradation due to soil compaction, erosion or loss of topsoil are explained in the next section. The following section describes the risks associated with the chemical contamination of soils and the recommended measures to avoid such contamination arising (a) directly as a result of applying materials such as industrial wastes, sewage sludge, pesticides, fertilizers or contaminated irrigated water, or (b) indirectly by atmospheric deposition such as lead from car exhaust fumes. The final section gives practical advice on the restoration of sites disturbed by mineral extraction, laying pipelines and other civil engineering works.

(ii) The Code of Good Agricultural Practice for the Protection of Water (MAFF, 1991) describes the risks of causing water pollution from various agricultural sources and provides practical guidance on ways of minimising such risks.

The introductory chapter lists the laws controlling water pollution and briefly describes the various point sources and diffuse sources of water pollution. The next two chapters give very detailed guidelines on the storage and application to land of livestock wastes and other organic wastes. The design and construction of different types of safe stores for slurries are described at length. The good practices to minimise water pollution from silage effluents, fertilizers, fuel oil, sheep dips, pesticides and the disposal of animal carcasses are explained in the next few chapters. Ways of minimising loss of nitrogen from farmland to surface water and groundwater by adopting appropriate crop management practices and other means are explained in the final chapter.

The chapters dealing with fertilizers and pesticides give advice on the storage and handling of these chemicals. The types of tanks and containers to be used and the precautions to be taken for the disposal of containers, contaminated materials etc. are discussed in detail. Some specific examples of good practices relating to the application of fertilizers and pesticides are given below:

Paragraph 187: "Take special care when applying any inorganic fertilizer on fields where there is a risk of runoff to surface water. The risk is greatest when the field is waterlogged or frozen hard. Do not apply nitrogen fertilizer between 1 September and 1 February unless a crop needs it during this time".

Paragraph 188: "Avoid applying fertilizer to a watercourse. Full width distributors will not generally cause any problems if you use them carefully. Spinning disc and oscillating spout machines are more difficult to operate so that the full application rate is spread right up to the edge of the field. This can cause some fertilizer to go into a watercourse. You can adjust some newer machines by fitting headland discs or tilting the tractor linkage to avoid this happening. Otherwise the machine should be driven further away from the watercourse, leaving an area next to the water where the application rate is lower. Use liquid fertilizer applicators in a way that avoids the wind blowing droplets into watercourses. Keep machinery that spreads fertilizer in good condition and adjust it following the maker's instructions".

Paragraph 225: "Never apply pesticides where they could drift onto water unless they are specifically approved to be used in or near water. Ask the National Rivers Authority if you want to use a pesticide in or near water, and get their agreement if you want to use a herbicide to control weeds in water".

Paragraph 226: "To make the danger of pesticides drifting into water as low as possible use the right spraying techniques (for example to produce the correct droplet size, and to avoid unnecessary spraying of field margins and boundary areas close to watercourses). Also take into account the weather conditions, in particular wind speed and direction. These points are covered in the Pesticide Code (Paragraph 216)".

(iii) The Code of Good Agricultural Practice for the Protection of Air (MAFF, 1992) is a practical guide to farmers to avoid causing air pollution from odours, ammonia and smoke, or from greenhouse gases which cause global warming.

The introductory chapter lists the air pollution problems arising from agricultural activities and draws attention to the laws controlling air pollution and planning consents. The next chapter outlines the causes of odours and explains how they are measured. Good agricultural practices for minimising odour nuisances from (a) housed cattle, pigs and poultry, (b) storing and handling slurry and manure, and (c) spreading of livestock wastes on agricultural land are described in the following three chapters. Methods of treating livestock wastes so as to further reduce odour emissions during storage and spreading and advice on siting of livestock buildings, manure and slurry stores are given in the next two chapters. Good practices for reducing smoke pollution from burning plastic materials, tyres and rubber, waste oils, animal carcasses, fuels produced on the farm and other waste materials are outlined in the next few chapters. The final chapter describes ways of reducing emissions of greenhouse gases (carbon dioxide, methane, nitrous oxide and chlorofluorocarbons).

Here are some examples of good agricultural practices aimed at reducing odour emissions during spreading of manure and slurry:

Paragraph 86: "Use a weather forecast to help choose suitable conditions for spreading (paragraph 83). Check wind direction in relation to nearby houses before spreading. Avoid spreading in fields close to and upwind of houses unless slurry is band spread, injected or has been treated to effectively reduce its odour".

Paragraph 87: "Avoid applying more than 50 m^3/ha (4500 gallons to the acre), or 50 tonnes/ha (20 tonnes per acre) at one time if odour could be a problem. Always apply slurry and manure in a way that avoids the risk of causing water pollution".

Paragraph 88: "On bare land, lightly cultivate the land after surface spreading to mix in the material as soon as possible".

Paragraph 89: "When spreading slurry, use a spreader which is suitable for the location of the fields it will be used in". Avoid over-filling the tankers or spreaders. This may be an offence under the Highways Act, 1980. Clean the outside of spreading machinery regularly".

Paragraph 90: "Unless slurry is band spread, injected or has been treated to reduce odour effectively, avoid spreading at weekends, bank holidays or in the evening".

In addition to the above codes of good agricultural practice, detailed guidance on the safe handling of pesticides is given in the Code of Practice for the Safe Use of Pesticides on Farms and Holdings (MAFF/HSE, 1990) and a number of other related publications.

As mentioned earlier, the above examples from the UK are intended to illustrate how detailed codes of good agricultural practices can be drawn up to help farmers reduce pollution arising from agricultural activities specific to a given country or region.

7. CONCLUSION

In this paper, it has only been possible to highlight a few selected topics of current interest relating to the agrotechnology of aromatic plants and to give a brief description of the cultivation of a very limited number of commercially important crops chosen as examples. A large volume of literature has been published over the past few decades on the botany, agronomy, chemical composition and uses of aromatic plants. Both cultivated and wild species have been the subject of these studies.

Detailed information on aromatic plants covering all the above topics and many other aspects which are not dealt with in this paper can be found in a number of electronic databases. A list of databases from which such information can be obtained is given in Appendix 2.

Annex 1. A list of cultivated plants yielding aroma substances

Species	Family	Habit	Plant part(s) used as source(s) of essential oil
Abelmoschus moschatus	Malvaceae	Annual herb	Seeds
Abies alba	Pinaceae	Perennial tree	Leaves
Abies balsamea	Pinaceae	Perennial tree	Leaves
Acacia dealbata	Leguminosae	Perennial tree	Flowers
Acacia farnesiana	Leguminosae	Perennial tree	Flowers
Achillea millefolium	Compositae	Perennial herb	Flowers
Acinos alpinus	Labiatae	Perennial herb	Leaves
Acinos suaveolens	Labiatae	Perennial herb	Leaves
Acorus calamus	Araceae	Perennial herb	Rhizomes
Aegle marmelos	Rutaceae	Perennial tree	Fruit rind
Aeolanthus gamwelliae	Labiatae	Perennial herb	Aerial parts
Agathosma betulina	Rutaceae	Perennial shrub	Leaves
Agathosma crenulata	Rutaceae	Perennial shrub	Leaves
Allium cepa	Liliaceae	Biennial herb	Bulbs
Allium porrum	Liliaceae	Biennial herb	Leaves
Aloysia triphylla	Verbenaceae	Perennial shrub	Leaves
Alpinia galanga	Zingiberaceae	Perennial herb	Rhizomes, Leaves
Alpinia officinarum	Zingiberaceae	Perennial herb	Rhizomes
Alpinia zerumbet	Zingiberaceae	Perennial herb	Rhizomes, Flowers
Amomum subulatum	Zingiberaceae	Perennial herb	Seeds
Anethum graveolens	Umbelliferae	Annual herb	Seeds
Anethum sowa	Umbelliferae	Annual herb	Seeds
Angelica archangelica	Umbelliferae	Biennial herb	Roots, Seeds
Aniba canelilla	Lauraceae	Perennial tree	Bark
Anthriscus cerefolium	Umbelliferae	Annual herb	Aerial parts
Apium graveolens	Umbelliferae	Biennial herb	Seeds
Artemisia absinthium	Compositae	Perennial herb	Aerial parts
Artemisia annua	Compositae	Annual herb	Aerial parts
Artemisia dracunculus	Compositae	Perennial herb	Aerial parts
Artemisia herba-alba	Compositae	Perennial shrub	Aerial parts
Artemisia maritima	Compositae	Perennial shrub	Aerial parts
Artemisia mutellina	Compositae	Perennial herb	Aerial parts
Artemisia pallens	Compositae	Annual herb	Aerial parts
Artemisia vulgaris	Compositae	Perennial herb	Aerial parts
Boenninghausenia albiflora	Rutaceae	Perennial shrub	Roots, leaves
Boronia megastigma	Rutaceae	Perennial shrub	Flowers
Boswellia serrata	Burseraceae	Perennial tree	Secretion from bark
Bunium persicum	Umbelliferae	Annual herb	Seeds
Bursera penicillata	Burseraceae	Perennial tree	Fruit husks, seeds, wood
Calamintha sylvatica	Labiatae	Perennial herb	Aerial parts
Cananga odorata	Annonaceae	Perennial tree	Flowers
Canarium luzonicum	Burseraceae	Perennial tree	Secretion from bark
Capsicum annuum	Solanaceae	Annual shrub	Fruits
Capsicum frutescens	Solanaceae	Annual shrub	Fruits
Carum carvi	Umbelliferae	Biennial herb	Seeds
Caryopteris incana	Verbenaceae	Perennial shrub	Leaves
Cedrus deodara	Pinaceae	Perennial tree	Wood

Chamaecyparis obtusa	Cupressaceae	Perennial tree	Leaves, bark
Chamaemelum nobile	Compositae	Perennial herb	Flowers
Chamomilla suaveolens	Compositae	Annual herb	Aerial parts
Cinnamomum aromaticum	Lauraceae	Perennial tree	Leaves, twigs
Cinnamomum burmanii	Lauraceae	Perennial tree	Leaves, bark
Cinnamomum camphora	Lauraceae	Perennial tree	Leaves, wood
Cinnamomum tamala	Lauraceae	Perennial tree	Leaves, bark
Cinnamomum zeylanicum	Lauraceae	Perennial tree	Leaves, bark
Cistus ladanifer	Cistaceae	Perennial shrub	Aerial parts
Citrus aurantifolia	Rutaceae	Perennial tree	Fruit rind
Citrus aurantium	Rutaceae	Perennial tree	Flowers, Fruit rind, Leaves, Twigs
Citrus bergamia	Rutaceae	Perennial tree	Fruit rind
Citrus limon	Rutaceae	Perennial tree	Fruit rind
Citrus meyeri	Rutaceae	Perennial tree	Fruit rind
Citrus reticulata	Rutaceae	Perennial tree	Fruit rind
Citrus sinensis	Rutaceae	Perennial tree	Fruit rind, flowers, leaves
Other *Citrus* spp.	Rutaceae	Perennial tree	Flowers, fruit rind
Clausena anisata	Rutaceae	Perennial tree	Leaves
Convallaria majalis	Liliaceae	Perennial herb	Flowers
Conyza canadensis	Compositae	Annual herb	Aerial parts
Coriandrum sativum	Umbelliferae	Annual herb	Fruits
Cuminum cyminum	Umbelliferae	Annual herb	Fruits
Cupressus sempervirens	Cupressaceae	Perennial tree	Leaves, bark
Curcuma longa	Zingiberaceae	Perennial herb	Rhizomes
Cymbopogon citratus	Gramineae	Perennial herb	Leaves
Cymbopogon flexuosus	Gramineae	Perennial herb	Leaves
Cymbopogon jwarancusa	Gramineae	Perennial herb	Leaves
Cymbopogon martinii	Gramineae	Perennial herb	Leaves
Cymbopogon nardus	Gramineae	Perennial herb	Leaves
Cymboposon pendulus	Gramineae	Perennial herb	Leaves
Cymbopogon winterianus	Gramineae	Perennial herb	Leaves
Cyperus scariosus	Cyperaceae	Perennial herb	Rhizomes
Daucus carota	Umbelliferae	Biennial herb	Seeds
Dianthus caryophyllus	Caryophyllaceae	Perennial herb	Flowers
Dipteryx odorata	Leguminosae	Perennial tree	Seeds
Elettaria cardamomum	Zingiberaceae	Perennial herb	Fruits
Elsholtzia ciliata	Labiatae	Annual herb	Aerial parts
Eriocephalus punctulatus	Compositae	Perennial shrub	Leaves
Eucalyptus citriodora	Myrtaceae	Perennial tree	Leaves
Eucalyptus globulus	Myrtaceae	Perennial tree	Leaves
Other *Eucalyptus* spp.	Myrtaceae	Perennial tree	Leaves, seeds
Ferula assa-foetida	Umbelliferae	Perennial herb	Root exudate
Foeniculum vulgare	Umbelliferae	Biennial herb	Seeds
Geranium macrorrhizum	Geraniaceae	Perennial herb	Leaves, roots
Hedeoma pulegioides	Labiatae	Perennial shrub	Aerial parts
Helichrysum italicum	Compositae	Perennial herb	Flowers
Hyacinthus orientalis	Liliaceae	Perennial herb	Flowers
Hyssopus officinalis	Labiatae	Perennial shrub	Aerial parts
Illicium verum	Illiciaceae	Perennial tree	Fruits
Inula helenium	Compositae	Perennial herb	Rhizomes
Iris germanica	Iridaceae	Perennial herb	Rhizomes
Iris pallida	Iridaceae	Perennial herb	Rhizomes
Jasminum auriculatum	Oleaceae	Perennial shrub	Flowers
Jasminum grandiflorum	Oleaceae	Perennial shrub	Flowers

Jasminum officinale	Oleaceae	Perennial shrub	Flowers
Jasminum sambac	Oleaceae	Perennial shrub	Flowers
Other *Jasminum* spp.	Oleaceae	Perennial shrub	Flowers
Juniperus communis	Cupressaceae	Perennial shrub	Fruits
Juniperus virginiana	Cupressaceae	Perennial tree	Heartwood, leaves
Laurus nobilis	Lauraceae	Perennial tree	Leaves
Lavandula angustifolia	Labiatae	Perennial shrub	Aerial parts
Lavandula x intermedia	Labiatae	Perennial shrub	Aerial parts
Other *Lavandula* spp.	Labiatae	Perennial shrub	Aerial parts
Levisticum officinale	Umbelliferae	Perennial herb	Aerial parts
Lindera benzoin	Lauraceae	Perennial shrub	Leaves, twigs
Liquidambar styraciflua	Hamamelidaceae	Perennial tree	Secretion from bark
Litsea cubeba	Lauraceae	Perennial tree	Fruits
Lonicera japonica	Caprifoliaceae	Perennial shrub	Flowers
Luvunga scandens	Rutaceae	Perennial shrub	Fruits
Matricaria chamomilla	Compositae	Annual herb	Flowers
Melaleuca alternifolia	Myrtaceae	Perennial tree	Leaves
Melissa officinalis	Labiatae	Perennial herb	Aerial parts
Mentha arvensis	Labiatae	Perennial herb	Aerial parts
Mentha citrata	Labiatae	Perennial herb	Aerial parts
Mentha x *piperita*	Labiatae	Perennial herb	Aerial parts
Mentha pulegium	Labiatae	Perennial herb	Aerial parts
Mentha x *spicata*	Labiatae	Perennial herb	Aerial parts
Other *Mentha* spp.	Labiatae	Perennial herb	Aerial parts
Michelia champaca	Magnoliaceae	Perennial tree	Flowers
Monarda citriodora	Labiatae	Annual herb	Aerial parts
Monarda didyma	Labiatae	Perennial herb	Aerial parts
Monarda russeliana	Labiatae	Perennial herb	Aerial parts
Monodora myristica	Annonaceae	Perennial tree	Fruits, seeds
Myristica fragrans	Myristicaceae	Perennial tree	Aril, seeds
Myroxylon balsamum	Leguminosae	Perennial tree	Secretion from bark
Myrtus communis	Myrtaceae	Perennial shrub	Leaves, flowers, fruits
Narcissus jonquilla	Liliaceae	Annual herb	Flowers
Narcissus poeticus	Liliaceae	Annual herb	Flowers
Nardostachys chinensis	Valerianaceae	Perennial herb	Roots, rhizomes
Nardostachys jatamansi	Valerianaceae	Perennial herb	Roots, rhizomes
Nepeta cataria	Labiatae	Perennial shrub	Aerial parts
Ocimum basilicum	Labiatae	Annual herb	Aerial parts
Ocimum gratissimum	Labiatae	Perennial shrub	Aerial parts
Ocimum sanctum	Labiatae	Perennial shrub	Aerial parts
Ocotea pretiosa	Lauraceae	Perennial tree	Leaves, bark
Ocotea quixos	Lauraceae	Perennial tree	Fruit calyx
Origanum majorana	Labiatae	Perennial shrub	Aerial parts
Origanum onites	Labiatae	Perennial shrub	Aerial parts
Origanum smyrnaeum	Labiatae	Perennial shrub	Aerial parts
Origanum vulgare	Labiatae	Perennial shrub	Aerial parts
Osmanthus fragrans	Oleaceae	Perennial shrub	Flowers
Panax pseudoginseng	Araliaceae	Perennial herb	Roots
Panax quinquefolius	Araliaceae	Perennial herb	Roots
Pelargonium graveolens	Geraniaceae	Perennial shrub	Leaves
Pelargonium roseum	Geraniaceae	Perennial herb	Leaves
Perilla frutescens	Labiatae	Annual herb	Aerial parts
Petroselinum crispum	Umbelliferae	Biennial herb	Aerial parts

Peumus boldus	Monimiaceae	Perennial tree	Leaves
Pimenta dioica	Myrtaceae	Perennial tree	Fruits, Leaves
Pimenta racemosa	Myrtaceae	Perennial tree	Leaves
Pimpinella anisum	Umbelliferae	Annual herb	Fruits
Pinus spp.	Pinaceae	Perennial tree	Heartwood
Piper betle	Piperaceae	Perennial shrub	Leaves
Piper nigrum	Piperaceae	Perennial shrub	Fruits
Pistacia lentiscus	Anacardiaceae	Perennial tree	Leaves, secretion from bark
Pogostemon cablin	Labiatae	Perennial herb	Leaves
Polianthes tuberosa	Agavaceae	Perennial herb	Flowers
Pteronia incana	Compositae	Perennial shrub	Leaves
Ribes nigrum	Grossulariaceae	Perennial shrub	Flower buds
Rosa damascena	Rosaceae	Perennial shrub	Flowers
Rosa centifolia	Rosaceae	Perennial shrub	Flowers
Other *Rosa* spp.	Rosaceae	Perennial shrubs	Flowers
Rosmarinus officinalis	Labiatae	Perennial shrub	Aerial parts
Ruta graveolens	Rutaceae	Perennial shrub	Leaves, flowers, fruits
Ruta montana	Rutaceae	Perennial shrub	Leaves, flowers, fruits
Salvia lavandulifolia	Labiatae	Perennial herb	Aerial parts
Salvia officinalis	Labiatae	Perennial shrub	Aerial parts
Salvia sclarea	Labiatae	Perennial herb	Aerial parts
Santalum album	Santalaceae	Perennial tree	Heartwood
Santolina chamaecyparissus	Compositae	Perennial shrub	Leaves
Sassafras albidum	Lauraceae	Perennial tree	Root bark
Satureja hortensis	Labiatae	Annual herb	Aerial parts
Satureja montana	Labiatae	Perennial shrub	Aerial parts
Saussurea lappa	Compositae	Perennial herb	Roots
Skimmia laureola	Rutaceae	Perennial shrub	Leaves
Spartium junceum	Leguminosae	Perennial shrub	Flowers
Styrax benzoin	Styracaceae	Perennial tree	Secretion from bark
Syringa vulgaris	Oleaceae	Perennial tree	Flowers
Syzygium aromaticum	Myrtaceae	Perennial tree	Flower buds
Syzygium cumini	Myrtaceae	Perennial tree	Leaves
Tagetes erecta	Compositae	Annual herb	Flowers
Tagetes glandulifera	Compositae	Annual herb	Flowers
Tagetes minuta	Compositae	Annual herb	Flowers
Tagetes patula	Compositae	Annual herb	Flowers
Tanacetum balsamita	Compositae	Perennial herb	Aerial parts
Tanacetum vulgare	Compositae	Perennial herb	Aerial parts
Thuja occidentalis	Cupressaceae	Perennial tree	Leaves
Thymus capitatus	Labiatae	Perennial shrub	Aerial parts
Thymus satureioides	Labiatae	Perennial shrub	Aerial parts
Thymus serpyllum	Labiatae	Perennial shrub	Aerial parts
Thymus vulgaris	Labiatae	Perennial shrub	Aerial parts
Thymus zygis	Labiatae	Perennial shrub	Aerial parts
Trachyspermum ammi	Umbelliferae	Annual herb	Fruits
Tropaeolum minus	Tropaeolaceae	Annual herb	Flowers
Valeriana officinalis	Valerianaceae	Perennial herb	Roots, rhizomes
Vanilla planifolia	Orchidaceae	Perennial herb	Fruits
Vetiveria zizanioides	Gramineae	Perennial herb	Roots
Viola odorata	Violaceae	Perennial herb	Leaves
Zingiber officinale	Zingiberaceae	Perennial herb	Rhizomes

Annex 2. A LIST OF ELECTRONIC DATABASES CONTAINING INFORMATION ON AROMATIC PLANTS

Name of database	Publisher	Type of information (secondary or tertiary)*	Subject areas covered
AGRIS	FAO, Rome, Italy	S	Multidisciplinary
AGRICOLA	NAL-USDA, Beltsville, MD, USA	S	Multidisciplinary
AHEAD	Publication co-ordinated by PID-CSIR, New Delhi, India	S and T	Asian Health and environment
APINMAP	Network of 14 participating organisations, with HQ in the Philippines	T	Specific to medicinal and aromatic plants
BACIS	BACIS, Huizen, Netherlands	T	Specific to essential oils, flavours and fragrances
BIOLOGICALLY ACTIVE PHYTOCHEMICALS	CRC Press, Press, Boca Raton, FL, USA	T	Phytochemistry
BIOSIS PREVIEWS	BIOSIS, Philadelphia, PA, USA	S	Biology and medicine
CA SEARCH	Chemical Abstracts Service, Columbus, OH, USA	S	Chemistry
CAB ABSTRACTS	CAB INTERNATIONAL, Wallingford, UK	S	Multidisciplinary
CHINESE MEDICINAL PLANTS	Computing Centre, Academic Sinica, Beijing, China	T	Phytochemistry and biomedicine
DICTIONARY OF NATURAL PRODUCTS	Chapman and Hall, London, UK	T	Natural products chemistry
DROGENANALYSE	Deutscher Apotheker Verlag, Stuttgart, Germany	T	Botany, phytochemisty

EMBASE	Elsevier Science, Amsterdam, Netherlands	S	Biomedicine
FLAVOUR AND FRAGRANCE MATERIALS	Allured Publishing Corporation, Carol Sream, IL, USA	T	Chemistry
FLOTURK	TBAM, Eskisehir, Turkey	T	Botany, agronomy, chemistry, pharmacology of Turkish Flora
GREEN MEDICINE	Journal of Chinese Medicine, Hove, Sussex, UK	T	Botany, pharmacology
HERBALIST	Hopikins Technology, Hopkins, MN, USA	T	Botany, herbal medicine
INMEDPLAN	Foundation for the Revitalisation of Local Health Traditions, Bangalore, India	T	Botany, chemistry, pharmacology plants used in Indian traditional medicine
MAPI	CIMAP, Lucknow, India	S and T	Botany, chemistry, agronomy of aromatic plants
MAPIS	PID-CSIR, New Delhi, India	S	Multidisciplinary and specific to medicinal and aromatic plants
MEDICINAL PLANTS OF MALTA	University of Malta, Msida, Malta	T	Botany
MEDICINAL PLANTS OF PAPUA NEW GUINEA	Wau Ecology Institue, Wau, Papua New Guinea	T	Botany, phytochemistry, ethnopharmacology
MEDLINE	National Library of Medicine, Bethesda, MD, USA.	S	Biomedicine
NAPRALERT	University of Illinois, Chicago, USA	S and T	Botany, phytochemistry, ethnopharmacology

NATTS	CDRI, Lucknow, India	T	Botany, Chemistry and pharmacology of Indian medicinal plants
PASCAL	INIST, Nancy, France	S	Multidisciplinary
PHYTOCHEMICAL CONSTITUENTS OF GRAS HERBS AND OTHER ECONOMIC PLANTS	CRC Press, Boca Raton, FL, USA.	T	Phytochemistry
PLANTES MEDICINALES	Algovision, Paris	T	Botany, pharmacology
POISONOUS PLANTS IN BRITAIN AND IRELAND	HMSO, London	T	Botany, chemistry, toxicology
SAPRIS	PROSEA, Wageningen, Netherlands	S and T	Economic botany
SEPASAL	Royal Botanic Gardens, Kew, Richmond, UK.	T	Economic botany of plants of arid lands
TRADIMED	Natural Products Research Institute, Seoul National University, Korea Republic.	T	Botany, phytochemistry, pharmacology4

*** S = secondary information source, i.e. reproduced or summarised from original source without additional interpretation or comments, largely bibliographic information with or without abstracts, indexed for easy retrieval.**

T = tertiary information source, i.e. interpreted information gathered from many sources, processed and presented in a "digested" form by the compiler, with own comments in most cases.

REFERENCES

1. Arene, L., Pellegrino, C., Gudin, S., (1993). *A comparison of the somaclonal variation level of Rosa hybrida L. cv. Meirutral plants regenerated from callus or direct induction from different vegetative and embryonic tissues*. Euphytica 71, 83-90.

2. Asada, Y., Saito, H., Yoshikawa, T., Sakamoto, K., Furuya, T., (1993). *Biotransformation of 18-beta-glycyrrhetinic acid by ginseng hairy root culture.* Phytochemistry, 34, 1049-1052.

3. Babu, K.N., Lukose, R., Ravindran, P.N., (1993). *Tissue culture of tropical spices*. In: Vidhyasekaran, P., (Ed) Genetic Engineering, Molecular Biology and Tissue Culture for Crop Pest and Disease Management. Daya Publishing House, Delhi, India. pp. 257-267.

4. Babu, K.N., Rema, J., Ravindran, P.N., Peter, K.V., (1993). *Biotechnology in spices*. Indian Horticulture 38, 46-50.

5. Babucke, S., Eilert, U., (1992). *Secondary metabolite production by hairy root cultures of Ruta graveolens*. Planta Medica 58, A604-A605.

6. Bajaj, Y.P.S., (1988) (Ed). *Biotechnology in Agriculture and Forestry* **4**. Medicinal and Aromatic Plants I. Springer-Verlag, Berlin, Germany. xix + 550 pp.

7. Bajaj, Y.P.S., (1989) (Ed). *Biotechnology in Agriculture and Forestry* **7**. Medicinal and Aromatic Plants II. Springer-Verlag, Berlin, Germany. xix + 545 pp.

8. Bajaj, Y.P.S., (1991) (Ed). *Biotechnology in Agriculture and Forestry* **15**. Medicinal and Aromatic Plants III. Springer-Verlag, Berlin, Germany. xx + 502 pp.

9. Bajaj, Y.P.S., (1993a) (Ed). *Biotechnology in Agriculture and Forestry* **21**. Medicinal and Aromatic Plants IV. Springer-Verlag, Berlin, Germany. xxi + 443 pp.

10. Bajaj, Y.P.S., (1993b) (Ed). *Biotechnology in Agriculture and Forestry* 24. Medicinal and Aromatic Plants V. Springer-Verlag, Berlin, Germany. xxi + 391 pp.

11. Bajaj, Y.P.S., (1984a) (Ed). *Biotechnology in Agriculture and Forestry* 26. Medicinal and Aromatic Plants VI. Springer-Verlag, Berlin, Germany. xviii + 426 pp.

12. Bajaj, Y.P.S., (1984b) (Ed). *Biotechnology in Agriculture and Forestry* 28. Medicinal and Aromatic Plants VII. Springer-Verlag, Berlin, Germany. xviii + 473 pp.

13. Bennici, A., (1974). *Cytological analysis of roots, shoots and plants regenerated from suspension and solid in vitro cultures of haploid Pelargonium*. Zeitschrift fur Pflanzenzuchtung 72, 199-205.

14. Bonga, J.M., von Aderkas, P., (1992). *In vitro Culture of Trees*. Forestry Sciences Vol. 38. Kluwer Academic Publishers, Dordrect, Netherlands. xii + 236 pp.

15. Bricout, J., Garcia-Rodriguez, M-J., Paupardin, C., (1978). *Effect of colchicine on essential oil synthesis in Mentha piperita tissues grown in vitro. (Action de la colchicine sur la synthèse d'huile essentielle par des tissus de Mentha piperita cultivés in vitro)*. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences D 286, 1585-1588.

16. Cahoon, E.B., Dormann, P., Ohlrogge, J.B., (1994). *Petroselinic acid biosynthesis and production in transgenic plants*. Progress in Lipid Research 33, 155-163.

17. Debergh, P.C., Zimmerman, R.H., (1990) (Eds). *Micropropagation: Technology and Application.* Kluwer Academic Publishers, Dordrecht, Netherlands. vi. + 484 pp.

18. Devaiah, K.A., Srivastava, M.C., (1989). *Sensitiveness of jasmine cuttings to gamma radiation.* Herba Hungarica 28, 11-14.

19. Duriyaprapan, S., Britten, E.J., Basford, K.E., (1986). *The effect of temperature on growth, oil yield and oil quality of Japanese mint.* Annals of Botany 58, 729-736.

20. EC (1991a). Council Regulation (EEC) No. 2092/91 of 24 June 1991 on *organic production of agricultural products and indications referring thereto on agricultural products and feedingstuffs.* Official Journal of the European Communities 91 (L198), 1-15.

21. EC (1991b). *Protection of waters against pollution by nitrates from agriculture.* Council Directive EEC/91/676. Official Journal of the European Communities 91 (L375), 31 December 1991.

22. EC (1992). COM/92/23. *Towards Sustainability. A European Community Programme of Policy and Action in relation to the Environment and Sustainable Development* (*EC Fifth Environment Programme*). Office for Official Publications of the European Communities, Luxembourg.

23. Edison, S., Johny, A.K., Babu, K.N., Ramadasan, A., (1991). *Spice Varieties. A Compendium of Morphological and Agronomic Characters of Improved Varieties of Spices in India.* National Research Centre for Spices, Calicut, India. 68 pp.

24. Elhag, H.M., El-Domiaty, M.M., El-Feraly, F.S., Mossa, J.S., El-Olemy, M.M., (1992). *Selection and micropropagation of high artemisinin producing clones of Artemisia annua L.* Phytotherapy Research 6, 20-24.

25. Fauconnier, M.L., Jaziri, M., Marlier, M., Roggemans, J., Wathelet, J.P., Lognay, G., Severin, M., Homes, J., Shimomura, K., (1993). *Essential oil production by Anthemis nobilis L. tissue culture.* Journal of Plant Physiology 141, 759-761.

26. FAO (1991). *Draft Guidelines for the Production, Processing, Labelling and Marketing of Organic/Biodynamic Foods.* Codex Alimentarius Commission, Food and Agriculture Organization of the United Nations, Rome.

27. Firoozabady, E., Moy, Y., Courtney-Gutterson, N., Robinson, K., (1994). *Regeneration of transgenic rose (Rosa hybrida) plants from embryogenic tissue.* Bio/Technology 12, 609-613.

28. Franz, Ch., (1993) *Genetics.* In Hay, R.K.M.; Waterman, P.G. (Eds) Volatile Oil Crops. Longman Scientific and Technical, Harlow, UK. pp. 137-176.

29. George, E.F., (1993). *Plant Propagation by Tissue Culture. Part 1.* The Technology. Exegetics Limited, Westbury, UK. viii + 574 pp.

30. Germana, M.A., Wang, Y.Y., Barbagallo, M.G., Iannolino, G., Crescimanno, F.G., (1994). *Recovery of haploid and diploid plantlets from anther culture of Citrus clementina Hort. ex Tan. and Citrus reticulata Blanco.* Journal of Horticultural Science 69, 473-480.

31. Granicher, F., Christen, P., Kamalaprija, P., Burger, U., (1995). *An iridoid diester from Valeriana officinalis var. sambucifolia hairy roots.* Phytochemistry 38, 103-105.

32. Granicher, F., Christen, P., Kamalaprija, P., Burger, U., (1995). *An iridoid diester from Valeriana officinalis var. sambucifolia hairy roots.* Phytochemistry 38, 103-105.

33. Granicher, F., Christen, P., Kapetanidis, I., (1992). *Production of valepotriates by hairy root cultures of Valeriana officinalis var. sambucifolia.* Planta Medica 58, A614.

34. Gritter, F.G., Jr., (1986). *Citrus embryogenesis in vitro: culture initiation, plant regeneration, and phenotypic characterization [Abstract]*. Dissertation Abstracts International, B-Sciences and Engineering 47, 1357B. p.169.

35. Grosser, J.W., Gmitter, F.G. Jr., (1990). *Protoplast fusion and citrus improvement.* Plant Breeding Reviews 8, 339-374.

36. Gudin, S., (1993). *Embryo rescue in Rosa hybrida L.* Euphytica 72, 205-212.

37. Guiltinan, M.J., Chang, C., (1993). *Biotechnological approaches for the improvement of pelargoniums*. In: Craig, R. (Ed) Proceedings of the Third International Geranium Conference, Odense, Denmark, 31 August to 4 September 1992. Ball Publishing, Batavia, IL, USA. pp. 61-64.

38. Hidaka, T., Omura, M., Ugaki, M., Tomiyama, M., Kato, A., Ohshima, M., Motoyoshi, F., (1990). *Agrobacterium-mediated transformation and regeneration of Citrus spp. from suspension cells.* Japanese Journal of Breeding. 40, 199-207.

39. Hook, I., (1994). *Secondary metabolites in hairy root cultures of Leontopodium alpinum Cass. (edelweiss).* Plant Cell, Tissue and Organ Culture 38, 321-326.

40. Hunault, G., Desmaret, P., (1990). *Field assessment of somaclonal variation during somatic embryogenesis in bitter fennel.* Bulletin de la Société Botanique de France, Actualités Botaniques 137, 45-49.

41. Hunault, G., (1987). *Cytogenetic status of fennel plants (Foeniculum vulgare Miller) produced by somatic embryogenesis from polyploid cell suspensions. (Etat cytogénétique des plantes de fenouil (Foeniculum vulgare Miller) obtenues par embryogenèse somatique à partir de suspensions cellulaires polyploïdes.* Revue de Cytologie et de Biologie Végétales- leBotaniste 10, 155-172.

42. IFOAM (1992). *Basic Standards of Organic Agriculture and Food Processing.* International Federation of Organic Agriculture Movements, Tholey-Theley, Germany. 24 pp.

43. Inomata, S., Yokoyama, M., Gozu, Y., Shimizu, T., Yanagi, M., (1993). *Growth pattern and ginsenoside production of Agrobacterium-transformed Panax ginseng roots.* Plant Cell Reports 12, 681-686.

44. Jaziri, M., Shimomura, K., Yoshimatsu, K., Fauconnier, M.L., Marlier, M., Homes, J., (1995). *Establishment of normal and transformed root cultures of Artemisia annua L. for artemisinin production.* Journal of Plant Physiology 145, 175-177.

45. Junghanns, K.T., Kneusel, R.E., Baumert, A., Maier, W., Groger, D., Matern, U., (1995). *Molecular cloning and heterologous expression of acridone synthase from elicited Ruta graveolens L. cell suspension cultures.* Plant Molecular Biology 27, 681-692.

46. Kaicker, U.S., (1992). *Rose breeding in India and cytology of induced mutants of H. T. cv. 'Folklore'*. Acta Horticulturae No. 320, 105-112.

47. Kennedy, A.I., Deans, S.G., Svoboda, P.K., Gray, A.I., Waterman, P.G., (1993). *Volatile oils from normal and transformed root of Artemisia absinthium.* Phytochemistry 32, 1449-1451.

48. Kobayashi, S., Uchimiya, H., (1989). *Expression and integration of a foreign gene in orange (Citrus sinensis Osb.) protoplasts by direct DNA transfer.* Japanese Journal of Genetics 64, 91-97.

49. Krikorian, A.D., (1994). *In vitro culture of plantation crops.* In: Vasil, I. K., Thorpe, T. A. (Eds) Plant Cell and Tissue Culture. Kluwer Academic Publishers, Dordrecht, Netherlands. pp. 497-537.

50. Kukreja, A.K., Dhawan, O.P., Mathur, A.K., Ahuja, P.S., Mandal, S., (1991). *Screening and evaluation of agronomically useful somaclonal variations in Japanese mint (Mentha arvensis).* Euphytica 53, 183-191.

51. Kukreja, A.K., Mathur, A.K., Zaim, M., (1990). *Mass production of virus-free patchouli plants [Pogostemon cablin (Blanco) Benth.] by in vitro culture.* Tropical Agriculture 67, 101-104.

52. Kyte, L., (1987). *Plants from Test Tubes. An Introduction to Micropropagation.* Timber Press, Portland, Orgeon, USA. 160 pp.

53. Lawrence, B.M., (1993). *A planning scheme to evaluate new aromatic plants for the flavor and fragrance industries.* In Janick, J., Simon,J.E. (Eds) New crops. John Wiley and Sons, Inc., New York, USA. pp. 620-627.

54. MAFF (1991). *Code of Good Agriculturaal Practice for the Protection of Water.* Ministry of Agriculture, Fisheries and Food, London, UK. vi + 80 pp.

55. MAFF (1992). *Code of Good Agriculturaal Practice for the Protection of Air.* Ministry of Agriculture, Fisheries and Food, London, UK. vi + 74 pp.

56. MAFF (1993). *Code of Good Agriculturaal Practice for the Protection of Soil.* Ministry of Agriculture, Fisheries and Food, London, UK. vi + 55 pp.

57. MAFF/HSE (1990). *Code of Practice for the Safe Use of Pesticides on Farms and Holdings.* HMSO, London, UK.

58. Maia, E., Bettachini, B., Beck, D., Venard, P., Maia, N., (1973). *Contribution to the improvement of health in clone Abrial of lavandin. (Contribution à l'amélioration de l'état sanitaire du lavandin, clone 'Abrial').* Annales de Phytopathologie 5, 115-124.

59. Mathur, A.K., Ahuja, P.S., Pandey, B., Kukreja, A.K., Mandal, S., (1988). *Screening and evaluation of somaclonal variations for quantitative and qualitative traits in an aromatic grass, Cymbopogon winterianus Jowitt.* Plant Breeding 101, 321-334.

60. Moore, G.A., Jacono, C.C., Neidigh, J.L., Lawrence, S.D., Cline, K., (1992). *Agrobacterium-mediated transformation of Citrus stem segments and regeneration of transgenic plants.* Plant Cell Reports 11, 238-242.

61. Nandi, R.P., Chatterjee, S.K., (1991). *Improved cultivation and distillation methods followed by citronella plantation of Darjeeling hills.* Indian Perfumer 35, 24-29.

62. Navarro, L., Ortiz, J.M., Juarez, J., (1985). *Aberrant citrus plants obtained by somatic embryogenesis of nucelli cultured in vitro.* HortScience 20, 214-215.

63. Panizza, M., Tognoni, F., Mensuali-Sodi, A., (1990). *In vitro propagation of lavandin: Morphological changes in regenerated plants.* Acta Horticulturae No. 280, 463-466.

64. Pena, L., Cervera, M., Juarez, J., Ortega, C., Pina, J.A., Duran-Vila, N., Navarro, L., (1995). *High-efficiency Agrobacterium-mediated transformation and regeneration of citrus.* Plant Science (Limerick) 104, 183-191.

65. Pierik, R.L.M., (1987). *In vitro Culture of Higher Plants.* Martinus Nijhoff, Dordrecht, Netherlands. v + 344 pp.

66. Putievsky, E., (1993). *Selection and breeding of aromatic plants - Old and new approaches.* Short review. Acta Horticulturae No. 330, 137-139.

67. Radhakrishnan, V.V., Viswanathan, T.V., Sosamma C., Reghunath, B.R., (1991). *Shade tolerance studies on patchouli (Pogostemon patchouli).* South Indian Horticulture. 39, 387-388.

68. Rao, P.S., Bapat, V.A., Mhatre, M., (1984). *Regulatory factors for in vitro multiplication of sandalwood tree (Santalum album Linn.). II. Plant regeneration in nodal and internodal stem explants and occurrence of somaclonal variations in tissue culture raised plants.* Proceedings, Indian National Science Academy, B- Biological Sciences 50, 196-202.

69. Rao, Y.R., Paul, S.C., Dutta, P.K., (1988). *Major constituents of essential oils of Cinnamomum zeylanicum.* Indian Perfumer 32, 86-89.

70. Razdan, M.K., (1993). *An Introduction to Plant Tissue Culture.* Intercept Limited, Andover, UK. 398 pp.

71. Reghunath, B.R., Priyadarshan, P.M., (1993). *Somaclonal variation in cardamom (Elettaria cardamomum Maton) derived from axenic culture of juvenile shoot primordia.* Acta Horticulturae No. 330, 235-242.

72. Roberts, A.V., Lloyd, D., Short, K.C., (1990). *In vitro procedures for the induction of tetraploidy in a diploid rose.* Euphytica 49, 33-38.

73. Rethinam, P., Edison, S., Sadanandan, A.K., Johny, A.K., (1994). *Major achievements of AICRP on spices. Indian Cocoa,* Arecanut and Spices Journal 18, 16-21.

74. Selvarajan, M., Rao, V.N.M., (1982). *Studies on rooting of patchouli cuttings under different environments.* South Indian Horticulture 30, 107-111.

75. Smith, M.K., Drew, R.A., (1990). *Current applications of tissue culture in plant propagation and improvement.* Australian Journal of Plant Physiology 17, 267-289.

76. Smyle, J.W., Magrath, W.B., (1993). *Vetiver grass - a hedge against erosion.* In Ragland, J., Lal, R., (Eds) Technologies for Sustainable Agriculture in the Tropics: Proceedings of two international symposia held in San Antonio, TX and Denver, CO, 1990 and 1991 respectively. American Society of Agronomy, Madison, USA. pp.109-122.

77. Spencer, A., Hamill, J.D., Rhodes, M.J.C., (1993). *In vitro biosynthesis of monoterpenes by Agrobacterium transformed shoot cultures of two Mentha species.* Phytochemistry 32:, 911-919.

78. Spencer, A., Hamill, J.D., Rhodes, M.J.C., (1990). *Production of terpenes by differentiated shoot cultures of Mentha citrata transformed with Agrobacterium tumefaciens T37.* Plant Cell Reports 8, 601-604.

79. Stafford, A., Warren, G., (1991) (Eds). *Plant Cell and Tissue Culture.* Open University Press, Milton Keynes, UK. x + 251 pp.

80. Staiger, D., Kaulen, H., Schell, J., (1990). *A nuclear factor recognizing a positive regulatory upstream element of the Antirrhinum majus chalcone synthase promoter.* Plant Physiology 93, 1347-1353.

81. Tétényi, P., (1991). *Biological preconditions for cultivation and processing of medicinal plants.* In Wijesekera, R.O.B.(Ed) The Medicinal Plant Industry. CRC Press, Boca Raton, USA. pp. 33-41.

82. Tokumasu, S., Kato, M., (1979). *Variation of chromosome numbers and essential oil components of plants derived from anther culture of the diploid and the tetraploid in Pelargonium roseum.* Euphytica 28, 329-338.

83. Torres, K.C., (1989). *Tissue Culture Techniques for Horticultural Crops.* Van Nostrand Reinhold, New York, USA. vii + 285 pp.

84. Tsvetkov, R., (1977). *Radiation mutagenesis in lavender. I. Effect of magnitude and rate of dose on the sensitivity of lavender seeds to gamma irradiation.* Rasteniev"dni-Nauki 14, 34-44.

85. UKROFS (1993). *UKROFS Standards for Organic Food Production.* UK Register of Organic Food Standards, Food From Britain, London, UK.

86. USDA (1980). *Report and Recommendations on Organic Farming.*

87. Vasil, I.K., Thorpe,T.A., (1994) (Eds). *Plant Cell and Tissue Culture.* Kluwer Academic Publishers, Dordrecht, Netherlands. x + 593 pp.

88. Verlet, N., (1993). *Commercial aspects.* In Hay, R.K.M., Waterman, P.G., (Eds) Volatile Oil Crops. Longman Scientific and Technical, Harlow, UK. pp. 137-176.

89. Vidalie, H., (1989) (Ed). *In vitro culture and its Horticultural Applications.* (*La Culture in vitro et ses Applications Horticoles).* Lavoisier, Paris, France. 225 pp.

90. Watin, C., Bigot, C., (1989). *Regeneration of fertile plants from embryogenic calluses of common parsley (Petroselinum hortense Hoffm.). (Régéneration de plantes fertiles à partir de cals embryogènes de persil commun (Petroselinum hortense Hoffm.)).* Comptes Rendus de l'Académie des Sciences.-Séries-3, Sciences de la Vie 309, 653-659.

91. Woerdenbag, H.J., Luers, J.F.J., van Uden, W., Pras, N., Malingre, T.M., Alfermann, A.W., (1993). *Production of the new antimalaria drug artemisinin in shoot cultures of Artemisia annua L.* Plant Cell, Tissue and Organ Culture 32, 247-257.

92. Yoshikawa, T., Asada, Y., Furuya, T., (1993). Continuous production of glycosides by a bioreactor using ginseng hairy root culture. *Applied Microbiology and Biotechnology* **39**, 460-464.

93. Zryd, J.P., (1988) (Ed). *Plant Cell, Tissue and Organ Culture. Theoretical Basis and Practical Applications.* (*Cultures de Cellules, Tissus et Organes Végétaux. Fondements Théoriques et Utilisations Pratiques*) Presses Polytechniques Romandes, Lausanne, Switzerland. xiii + 308 pp.

CHAPTER 3

THE ISOLATION OF AROMATIC MATERIALS FROM NATURAL PLANT PRODUCTS

BRIAN M. LAWRENCE
R.J. Reynolds Tobacco Company
Winston-Salem, NC 27105 USA

1. INTRODUCTION TO NATURAL VOLATILE CONCENTRATES

Before aromatic materials are isolated from natural sources, attention must be paid to the type of material that is to be processed. For example, a large number of different plant organs are processed into aromatic isolates which, in turn, are used as raw materials in the flavor, fragrance, or pharmaceutical industries. The types of materials to be processed can be seen in Table I.

It is well known that when a geranium leaf is lightly touched an odor is emitted because the long stalked oil glands are very fragile. Similarly, the application of a very slight pressure on a peppermint leaf will also cause the release of oil from the ruptured oil gland. In contrast, pine needles or eucalyptus leaves do not release their oils until the epidermis of the leaf is broken. Hence, it is obvious that the types of structures in the plant part, within which the oil is contained, are different depending upon the plant type, and are plant family specific. For example, aromatic members of the Labiatae, Verbenaceae and Geraniaceae all possess protruding glandular hairs within which the oil can be found, while the Umbelliferae possess oil-rich ducts on the fruit and internal secretory cavities known as schizogenous cavities or ducts (cells separated from each other) in their leaves and roots. These schizogenous cavities can also be found in the leaves of the Myrtaceae, Graminae, and Compositae. Other internal secretory structures such as Lysigenous canals (spaces resulting from the dissolution of cells) can be found in other families such as the Rutaceae (Esau 1977). In contrast, resin canals occur throughout the Coniferae while gum canals can be found in the Cistaceae and Burseraceae.

Unfortunately, not enough is known about these oil secretory structures to carefully categorize them, although according to Denny (1991), from a practical standpoint they can be categorized into superficial and subcutaneous oils. Based on the currently known information, it is fairly safe to say that oils of the Labiatae, Verbenaceae and Geraniaceae families are the only superficial oils known; consequently, the others should be considered as subcutaneous oils.

The types of volatile isolates that are obtained commercially are essential oils, concretes, absolutes, pommades, resinoids, spice/herb oleoresins, extracts, infusions, and tinctures. The definitions of these, and a few other important terms can be seen as follows:

Concrete:	An extract of fresh plant parts by the use of a hydrocarbon solvent. It is rich in hydrocarbon soluble material and devoid of water soluble components. It is generally a waxy semi-solid dark colored material free from the original solvent.
Absolute:	A highly concentrated alcoholic extract of a concrete which contains only alcohol soluble materials. Its primary use is in alcoholic perfumes. It contains only a very low level of alcohol.
Pommade:	The product of the enfleurage fat extraction of fresh flowers.
Resinoid:	A solvent extract of a resin-rich containing natural exudate or dried plant material with a hydrocarbon solvent. Resinoids are generally viscous to semi-solid mixtures. They can be considered as being concretes of dried materials.

Extract:	A concentrate of a dried less volatile aromatic plant part obtained by solvent extraction with a polar solvent.
Infusion:	A hot extraction of either a plant part of its exudate with either water or an organic solvent. Infusions are not at all popular because it is difficult to control their chemical composition.
Tincture:	An alcoholic or aqueous alcoholic extract of a natural raw material in which the solvent is left in the extract as a diluent. Tinctures are used both in the fragrance and pharmaceutical industries. The amount of alcohol in the tincture, which ranges from 20-95%, is standardized by the manufacturer.
Spice/Herb Oleoresin:	A solvent extract of a dried spice or herb which is virtually free from the extracting solvent. It is used exclusively by the food and pharmaceutical industries as a replacement of ground spices and spice tinctures.
Essential Oil:	The isolated aromatic portion of a plant that is borne in that plant within distinctive oil cells. In some exceptional cases the essential oil is formed during processing. Most essential oils are isolated by either hydrodistillation or cold pressing.
Extrait:	An alcoholic extract of a pommade produced by enfleurage. It is a tincture of a pommade.
Balsam:	A natural exudate obtained from a shrub or a tree. It is characterized by being rich in benzoic and cinnamic acids and their corresponding esters.
Gum Resin:	A natural exudate obtained from a tree or plant. It is comprised of gums and resins. If the gum resin source also contains an essential oil, it is called an oleo-gum-resin.
Oleoresin:	The natural tree trunk or bark exudate which is extremely rich in an essential oil.

Concretes

As noted earlier, a concrete is the hydrocarbon extract of fresh flowers, herbs, leaves, and the flowering tops of plants. The flowers that must be processed as soon as possible after they have been harvested are:

acacia
boronia
carnation
champaca
false acacia
gardenia
genet
helichrysum
hyacinth
jasmin
jonquil
karokaroundé
lilac
mimosa
narcissus
orange flower
rose
rose-de-mai
tuberose
ylangylang

The reason for such a rapid processing is that the odor of these flowers either changes or evaporates only a few hours after harvesting. The reason for this is that the aroma of flowers is controlled by the inherent cicadian rhythm within the plant. The aromatic components are glycosidically bound and at a specific development stage and a specific time in the 24-hour cycle of the flower, a controlled enzymatic degradation of the bound volatiles, along with other biosynthetic reactions, takes

Table I

Various Natural Materials Used for the Production of Aromatic Products in the Flavor, Fragrance, and Pharmaceutical Industries

Type of Material	Example
Dried Bud	Cloves
Flowers	Jasmin
Herbaceous Plant	Sage
Evergreen Leaves & Twigs	Cedarleaf
Bark	Cinnamon
Wood	Sandalwood
Roots	Angelica
Rhizome	Ginger
Bulb	Onion
Fresh Fruit	Lime
Fresh Fruit Peel	Orange
Dried Fruit	Fennel
Seeds	Cardamom
Grass	Citronella
Lichen	Treemoss
Balsam	Peru Balsam
Resin	Mastic
Oleo-gum Resin	Opopanax
Animal Products	Castoreum

place resulting in a release of volatiles. Harvesting time is synchronized with this release of volatiles ensuring that the volatile concentrate obtained from the flowers is maximized.

Fresh flowers have a water content of 80-90%, fresh leaves 60-80%, and fresh stems 50-65% (Naves and Mazuyer, 1947). As a result, if they were dried before extraction a lot of volatiles would be lost during dehydration. Consequently, the solvent of choice must be non-water miscible, otherwise the water would dissolve in the extracting solvent and change its polarity and its ability to dissolve the flower volatiles.

The solvents used for concrete manufacture are butane, pentane, hexane, petroleum, ether 60-80°C, benzene, toluene, etc., although petroleum ether and hexane are now most commonly used. About 30 years ago, the solvent of choice used to be benzene; however, because of the toxic properties associated with this solvent, its use has almost completely stopped.

In practice, concretes are produced using processing equipment similar to that shown in Figure 1 (Meyer-Warnod, 1984). Initially the fresh flowers are charged into both of the static extraction vessels. These vessels are fitted with numerous perforated trays so that the flowers will not become compressed by their own weight. Each perforated tray has a spacer so their number used and distance between them is predetermined. This set of perforated trays can be within a removable cylindrical basket. In the center of the lower tray there is a column on which the spacers and perforated trays are fitted, while at the top there is a ring or hook so that the entire contents of the extractor can be readily removed by simple leverage, even if there is not a cylindrical mesh basket surrounding the trays.

It should be noted that before changing the extraction vessel, care must be taken to minimize the bruising and damage of the flowers because such damage can result in the release of enzymes in the "flower juice" that will promote reactions that will have a detrimental effect on the aroma quality of the concrete produced from them. The flowers held on the trays within the extractor are immersed in solvent by introducing it in from the bottom of the extraction vessel. This avoids any compression that would damage the flowers if the solvent were added from the top. The static extraction process, which is also known as maceration, takes place at a temperature below that of the boiling point of the solvent, although it is generally above room temperature. The number of washes with solvent, the duration of extraction, and the condition of the flowers and which type of flower, dictates the processing time. According to Meyer-Warnod (1984), this is the "art" of concrete manufacture; however, with experimentation some science can be put into this "art".

In concrete manufacturing it is normal to circulate fresh solvent through a battery of extraction vessels. For each cycle, the solvent becomes more enriched with the flower volatiles until extraction is complete. The number of extraction vessels has to be synchronized with the number of solvent washes, the solvent recovery time, the charging and discharging times. According to Naves and Mazuyer (1947), for three passes of the solvent through the floral material that takes 60 minutes, then nine (9) extraction vessels of 100L capacity are needed. Typical charges for a 100L capacity are jasmin 140 kg., rose-de-mai 150 kg., orange flower 230 kg., lavender 300 kg., and oakmoss 60 kg.

Because of the compactness of the orange flower, it is necessary to split it into pieces so that the solvent can fully penetrate and extract all of the volatiles of the flower. This can be done mechanically by using an apparatus which consists of two channeled cylinders that are turned in opposite directions at the same circumferential speed. The space is regulated such that the petals will not crush but simply break off the flowers. The feed rate of the flowers should be low so that no flowers can accumulate within the cylinders, thereby causing compression and flower damage. Not all extractors of orange flowers bother to separate the flowers. Instead they increase their extraction time to compensate for the increased length of time that it takes for the solvent to penetrate the compact flowers.

The flowers used for extraction must be free from unwanted leaves and stems which would not only lower the yield but would change the character of the concrete. If, for any reason, there is a delay before the flowers can be extracted, they must be spread out as a thin layer in a fresh and moderately

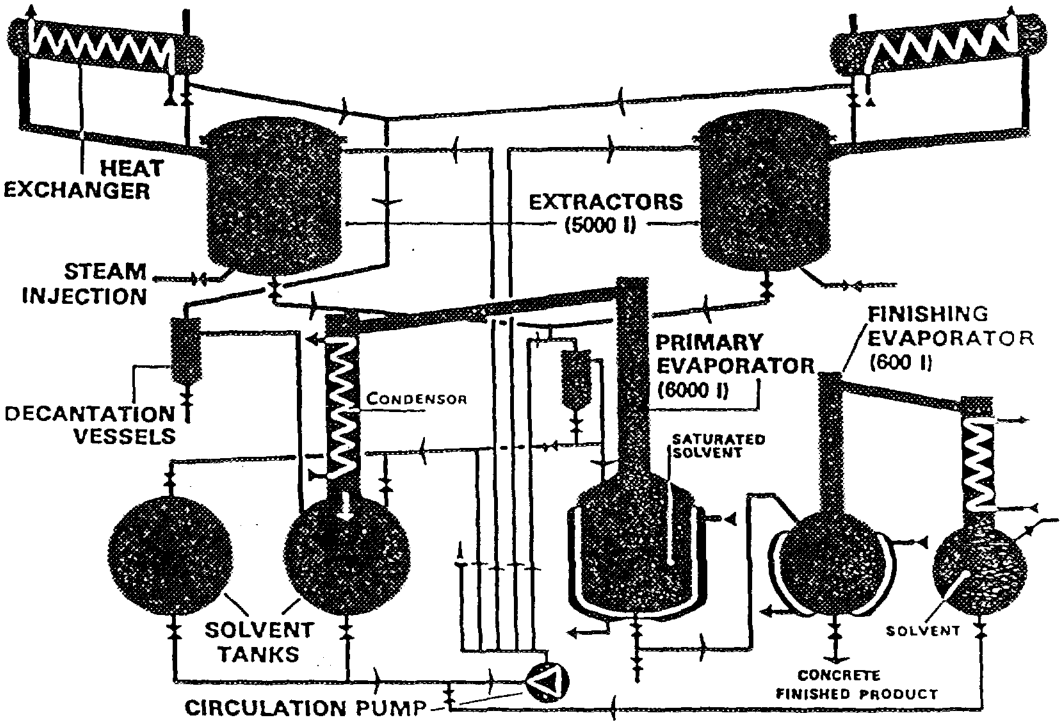


Figure 1: Diagrammatic representation of the concrete extraction process

ventilated area and be moved from time to time to prevent fermentation. There will undoubtedly be some loss in volatiles by evaporation but this is less of a problem than if the flowers became damaged through compression or fermentation. In addition to having less than ideal aroma properties, concretes from bruised, crushed, or fermented flowers have a deeper color than those processed from fresh unblemished flowers.

Materials such as lavandin, lavender and clary sage are chopped into pieces 2-5 cm. in length before extraction, while vetiver root is cut into 1-2 cm. pieces before extraction. For dried materials such as oakmoss or treemoss, it is better to wet the lichens for 24 hours prior to their extraction to allow the cells within the plants to expand, thus allowing better solvent penetration. Violet leaf concrete is produced from fresh chopped leaves, while patchouli concrete is produced from dried partially fermented leaves.

The value of static extraction is that the capital cost of equipment is minimized. Moving or rotating extractors can be and are used commercially to make concretes, although the additional money spent on these extractors is not generally worthwhile unless the volume of flowers to be extracted is so large that the reduction in extraction time becomes an important factor. A description of the types of moving extractors used is outside the scope of this discussion.

Once extraction or maceration is complete, the solvent highly enriched with flower extraction is pumped to the primary evaporator whose heat is maintained at the same temperature as the boiling point of the solvent. In this evaporator the solvent content is reduced to about one-tenth the volume, the recovered solvent being pumped to the solvent tanks to be used again. It is then pumped into the finishing evaporator where the solvent is removed more carefully under high vacuum, again the solvent being returned to the tanks for repeated use. The resultant concrete is either a viscous to solid extract whose odor is very similar but stronger than the material from which is was extracted. In addition to containing the odorous portion of the plant, the concrete also contains all other hydrocarbon soluble compounds such as the waxes, diterpenes, fixed oil, free fatty acids and colors, etc., while all of the water soluble materials are eliminated along with the fiber and cellulose, etc. The approximate yields of concretes produced from various raw materials (Naves and Mazuyer, 1947) can be seen in Tables II and III. As can be seen, the flower concrete yields are almost all less than 1.0%, and for many (2/3), a yield of less than 0.5% is found.

As the resultant concrete is not very soluble in perfume bases, further conversion into an absolute is needed if the material is to be used in a fine fragrance. However, the concrete can be and is used in a number of consumer items particularly the household care products.

Absolutes

As concretes are not widely used in perfumery, they have to be converted into an alcohol soluble volatile concentrate which is known as an absolute, i.e., they have to be extracted with alcohol. The transformation of a concrete into an absolute can be done using the equipment shown in Figure 2.

Initially the concrete is warmed to between 30° - 60°C, mixed with absolute alcohol (ethanol), and strongly agitated to get everything into solution. Once the solution is complete, it is refrigerated to between -5° to -10°C so that the waxes can be removed by precipitation. According to Meyer-Warnod (1984), waxes are not soluble in alcohol at temperatures below -1°C. These waxes are removed by subjecting the cold heterogeneous mixture to cold rotary filtration. In the past this used to be done by hand by filtering the cold mixture through large, thick fluted filter papers containing filter aids to prevent clogging; however, even though filtration could be achieved, it was slow, labor intensive, and resulted in some losses due to evaporation which made it additionally a fire hazard problem. After filter paper filtration, the mixture was re-cooled and vacuum filtered through large cooled buchner filters filled with glass sinters of porosity 3. According to Naves and Mazuyer (1947), the efficiency of separation could be enhanced if some of the alcohol was removed from the alcohol/concrete mixture by vacuum distillation prior to wax precipitation.

Table II

Yield of Flower Concretes Made With Pet Ether 60° - 80°C

Flower	Yield (%)
boronia	ca. 0.40
broom (genet)	0.09 - 0.18
carnation	0.23 - 0.29
cassie	0.50 - 0.70
cassie romaine	0.60 - 0.86
champal	0.16 - 0.20
gardenia	0.04 - 0.05
helichrysum	0.90 - 1.15
hyacinth	0.17 - 0.20
jasmin	0.28 - 0.34
jonquil	0.39 - 0.45
karokaroundé	ca. 0.13
lilac	0.60 - 0.95
mimosa	0.70 - 0.88
narcissus	0.24 - 0.28
orange flower (neroli)	0.24 - 0.27
robinia (false acacia)	0.15 - 0.20
rose	0.22 - 0.25
rose-de-mai	0.24 - 0.27
tuberose	0.08 - 0.11
violet	0.07 - 0.13
ylang ylang	0.80 - 0.95
wisteria	0.60 - 0.95

Table III

Yield of Concretes* From Other Plants

Plant	Yield (%)
black currant buds or cassis	2.20 - 2.90
clary sage	0.60 - 0.80
geranium (benzene)	0.20 - 0.25
labdanum	3.0 - 6.0
lavandin	1.40 - 2.50
lavender	1.50 - 2.20
mignonette or reseda	0.07 - 0.15
oakmoss	1.5 - 3.0
orris	1.0 - 1.9
patchouli (benzene)	4.5 - 5.8
sandalwood (benzene)	6.0 - 8.5
treemoss	2.0 - 4.0
verbena	0.25 - 0.30
vetiver	3.0 - 5.5
violet leaf	0.05 - 0.13
zdravets	0.30 - 0.45

*pet ether concretes unless otherwise noted

As the resultant concrete is not very soluble in perfume bases, further conversion into an absolute is needed if the material is to be used in a fine fragrance. However, the concrete can be and is used in a number of consumer items particularly the household care products.

Absolutes

As concretes are not widely used in perfumery, they have to be converted into an alcohol soluble volatile concentrate which is known as an absolute, i.e., they have to be extracted with alcohol. The transformation of a concrete into an absolute can be done using the equipment shown in Figure 2.

Initially the concrete is warmed to between 30° - 60°C, mixed with absolute alcohol (ethanol), and strongly agitated to get everything into solution. Once the solution is complete, it is refrigerated to between -5° to -10°C so that the waxes can be removed by precipitation. According to Meyer-Warnod (1984), waxes are not soluble in alcohol at temperatures below -1°C. These waxes are removed by subjecting the cold heterogeneous mixture to cold rotary filtration. In the past this used to be done by hand by filtering the cold mixture through large, thick fluted filter papers containing filter aids to prevent clogging; however, even though filtration could be achieved, it was slow, labor intensive, and resulted in some losses due to evaporation which made it additionally a fire hazard problem. After filter paper filtration, the mixture was re-cooled and vacuum filtered through large cooled buchner filters filled with glass sinters of porosity 3. According to Naves and Mazuyer (1947), the efficiency of separation could be enhanced if some of the alcohol was removed from the alcohol/concrete mixture by vacuum distillation prior to wax precipitation.

The filtrate from the rotary filter is pumped into the primary evaporator where it is concentrated to about 10% alcohol. Finally, the concentrated miscella is pumped into an agitated finishing evaporator where the alcohol is carefully removed under high vacuum. This last step is very critical because the product is very costly. As a result, the pot temperature must not be allowed to rise above 40°C; otherwise some of the floral top notes will be lost. The fractionating column used with the finishing evaporator must be highly efficient and the reflux ratio is set quite low so that any losses of floral notes are prevented. Alcohol removal is stopped when the mixture in the pot shows signs of turbidity; a good indication that losses in floral notes could be occurring. Absolutes are not distilled until they are totally free from alcohol because when the alcohol has been reduced to a very low level it becomes enriched with water, thereby offering an azeotropic way for odorous components to be distilled. The alcohol that is removed during this finishing step always seems to acquire an odor-note of the flower despite all precautions. As a consequence, it is reused to make absolutes from the same type. The yields of the commercially more important absolutes (Naves and Mazuyer, 1947) can be seen in Tables IV and V.

Pommades

Pommades are obtained by the process known as enfleurage which is undertaken almost exclusively in Southern France. The enfleurage process is primarily used for producing aromatic materials from flowers which contain minute quantities of aromatic components and are too delicate to withstand other processing conditions, such as exposure to heat or steam. In addition, enfleurage also takes advantage of the phenomenon that certain flowers continue to give off an aroma after they have been picked (enzymatic breakdown of bound glycosides, etc.).

Enfleurage is a cold, fat extraction method. The fat of choice is a neutral, odorless, purified tallow or lard which possesses a certain viscosity. It must be highly absorptive, almost insoluble in cold ethanol and should not become rancid on standing. The fat is spread out on glass plates leaving a clear margin near the edge which are contained in wooden frames. The absorptive surface of the fat is increased by grooves made on the surface with the aid of a wooden spatula. Fresh petals or whole flowers are spread out on the surface of the fat and the frames are stacked in piles (Naves and Mazuyer, 1947). After the perfume oils have been absorbed from the flowers, the spent flowers are removed by hand. Fresh flowers are again spread out on the same fat surfaces. It can take anywhere

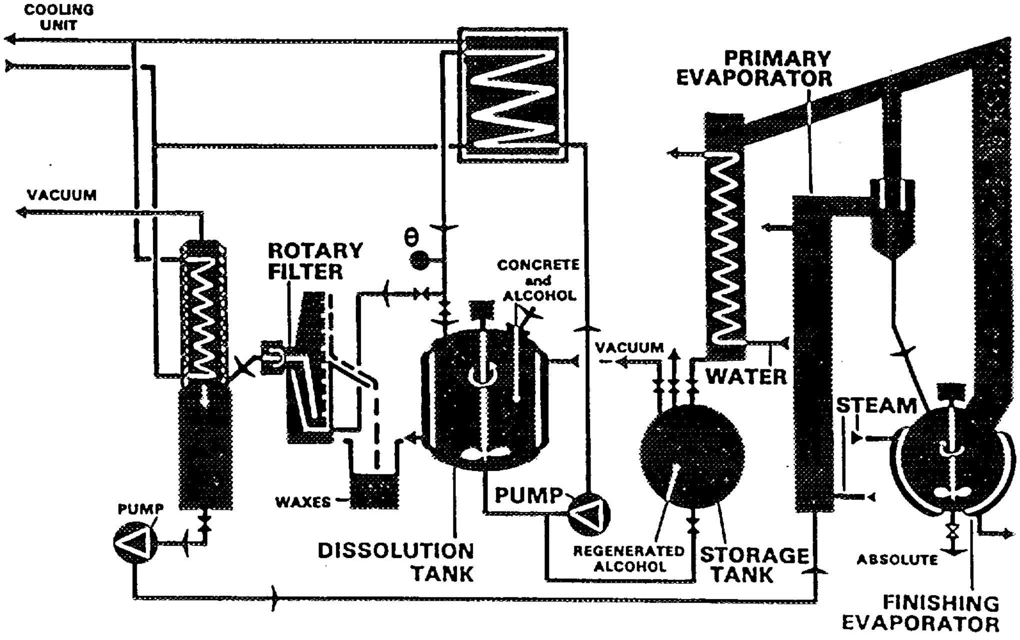


Figure 2. Diagrammatic representation of the process to convert concretes into absolutes

Table IV

Yield of Floral Absolutes from Concrete

Flower	Yield (%)
broom (genet)	30 - 40
carnation	9 - 12
cassie	ca. 33
cassie romaine	ca 33
champac	ca. 50
gardenia	ca. 50
helichrysum	60 - 70
hyacinth	10 - 14
jasmin	45 - 53
jonquil	40 - 55
karokaroundé	60 - 65
lilac	35 - 45
mimosa	20 - 25
narcissus	27 - 32
orange flower (neroli)	36 - 55
robinia (false acacia)	35 - 40
rose	50 - 60
rose-de-mai	55 - 65
tuberose	18 - 23
violet	35 - 40
ylang ylang	75 - 80
wisteria	40 - 50

Table V

Yield of Other Absolutes from Concretes

Plant	Yield (%)
black currant buds (cassis)	ca. .80
clary sage	80 - 85
geranium	70 - 80
labdanum	45 - 65
lavandin	45 - 55
lavender	50 - 60
mignonette (reseda)	30 - 35
oakmoss	35 - 60
orris	50 - 60
treemoss	60 - 70
verbena	50 - 60
violet leaf	35 - 45
zdravets	40 - 45

from 12 - 30 hours for jasmin and 24 - 100 hours for tuberose (Sozio, 1956) for the perfume oils to be absorbed, depending upon which flowers are being extracted. This process is continued until the fat is saturated with the perfume oils. It can take anywhere from 24 - 36 batches before the fat is saturated. The pommade, which is also known as "corps gras" is then ready for cold alcoholic extraction. The advantage of the enfleurage method over the steam extraction methods is that delicate perfume oils removed from flowers are not hydrolyzed by moisture or decomposed by heat, as could be the situation with steam methods of oil isolation.

At one time enfleurage was a very popular method for the removal of delicate flower perfume oils. Because of the economics of producing these perfume oils and the ability to process much larger quantities of flowers by solvent extraction, enfleurage is rarely a technique of choice anymore. Nevertheless, a small quantity of jasmin and tuberose flowers are still treated by enfleurage even in the 1990's. Pommades can also be produced by hot fat extraction (sometimes known as maceration); however, this technique is no longer used. It has been completely replaced by solvent extraction.

Resinoids

A resinoid is an extract of a natural resinous material with a hydrocarbon solvent. Raw materials that are extracted as resinoids are asafoetida, galbanum, labdanum, mastic, myrrh, olibanum, opopanax, styrax, Tolu balsam, etc. They are generally produced in equipment similar to that shown in Figure 3. The raw material and solvent are charged into the extraction vessel and the mixture is rapidly agitated. Once the raw material has been extracted, it is pumped into the concentrator where the solvent is removed. The resultant resinoid is discharged while warm because, at room temperature, it is somewhere between a very viscous liquid and a solid. The most frequently used solvent for the production of resinoids is petroleum ether (60°-80°C). Resinoids can be thought of as being concretes of dried organic material such as balsams, gum resins, or oleo-gum-resins. Resinoid extraction can be thought of as being a process similar to percolation.

Extracts

In contrast to a resinoid, which is produced from a resin or resin-rich plant material, an extract is produced from resin-free dried plant material. The equipment used to make extracts can be the same as that used for resinoid manufacture or the dried plant material can be placed in a cloth or muslin bag that has ample space for the swelling that will take place on contact with the polar solvent. This bag is placed in a stainless steel vessel containing a solvent such as aqueous ethanol (40%v/v or 60% v/v) ready for extraction. Maceration extraction takes 5-8 days during which time the solvent diffuses throughout the plant material causing the cells to soften and expand. Generally, the mixture is subjected to some type of stirring or agitation. During maceration extraction the aroma-bearing organic components, such as the essential oils and the non-water soluble resins, will dissolve in the ethanolic portion of the solvent system while the sugars, bitter materials, colors, etc. will dissolve in the water.

The menstruum (extracting solvent) is drawn off and the marc (plant material) is pressed to recover the rest of the maceration extract. To ensure that all of the extract is removed, the marc is washed with a fresh quantity of the menstruum after which it is pressed again and the extracts are combined. The extract is either centrifuged, filtered, or left to stand for a few days so that the final maceration extract is a clear solution. Some manufacturers adjust the alcohol content of the extract (or the active solvent content) to a standard level. Another name for the maceration aqueous alcoholic extract is a tincture, a product which, at one time, was very popular with pharmacists. Extracts can be and are made with solvents other than alcohol, aqueous alcohol, methanol, propanol or isopropanol. Solvents such as propylene glycol, glycerin and aqueous solutions of both single or combined solvents are popular for the extraction of materials which are not rich in volatile components. Some of the materials extracted this way are alfalfa leaves, black walnut hulls, carob bean hulls, chicory, damiana, chestnut leaves, fenugreek seed, kelp, kola nuts, lovage root, maté leaves, mountain maple, oak chips, rye, dried tamarind fruit, valerian root, and wild cherry bark.

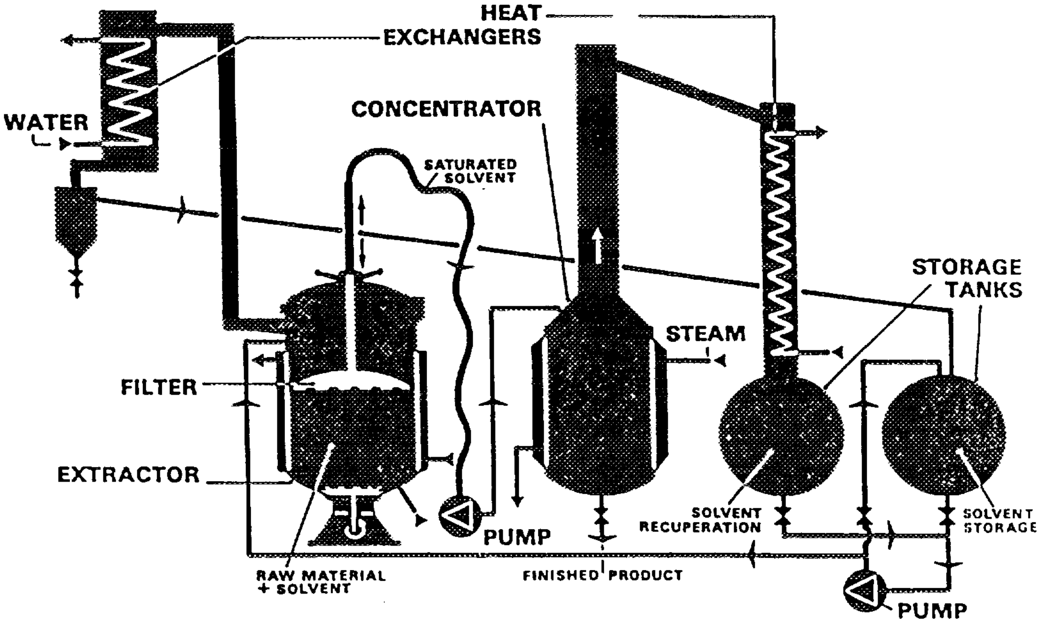


Figure 3. Diagrammatic representation of the resinoid extraction process.

Large quantities of vanilla bean extract are manufactured using alcohol as the solvent. It is generally produced by a percolation process in which chopped beans are placed on a series of perforated trays that are placed inside a stainless steel extraction vessel. The menstruum is sprayed on the top trays and allowed to percolate throughout the vessel. With the aid of a recirculating pump, the menstruum is cycled through the marc. According to Merory (1968), to prepare 100 U.S. gallons (379L) of a two-fold vanilla extract (2-fold = 26.7 ounces of beans per U.S. gallon), the beans (166.9 lb.), which contain ca. 30% H_2O, are first moistened with a small amount of the first menstruum (62 gallons of 65% aqueous alcohol), comminuted and placed on the stainless steel trays. The trays are then placed in a covered stainless extraction vessel and the menstruum is circulated twice a day for 8-10 days.

Once the first extraction step is complete, the beans are drained (2 days) from which ca. 152L of extract can be collected. A second menstruum comprising 22 gallons (83.4L) of warm (60°C) water is added to the extractor and it is circulated through the vanilla beans twice daily for 3 days. On the fourth day, draining is commenced from which ca. 20 gallons (70L) are collected. This process of water extraction is repeated once more with a further 20 gallons of warm water. Finally, the beans are washed with 5 gallon proportions of a 20 gallon aliquot every 30 minutes until 20 gallons have been collected. A combination of all of the combined extracts will result in the 100 gallons of 2-fold vanilla bean extract in 35% aqueous alcohol.

After aging of between 3-6 months, the extract is centrifuged or filtered. During the aging process the flavor will change to the mellow note normally associated with vanilla bean extract. As can be seen, there is a lot of art associated with the manufacture of vanilla bean extracts; nevertheless, since the advent of modern analytical separation and spectroscopic techniques, a lot of science has been put into the art.

Co$_2$ Extracts

All dry plant materials can be readily extracted with liquid CO_2 under pressure. CO_2 behaves similarly to all other extraction solvents except that it can be used as a subcritical liquid or as a supercritical liquid. To understand the difference between these two, reference must made to a phase diagram (Figure 4). In this graph it can be seen that in the shaded area CO_2 is a liquid, while above the critical point, it cannot be changed from a gaseous fluid to a liquid no matter what pressure is exerted. This is the supercritical phase, whereas the other liquid area is the subcritical phase.

Carbon dioxide is a popular choice of solvent for liquid or superfluid extractions because of its following criteria:

1. odorless, colorless, tasteless and non-toxic
2. non-combustible
3. inexpensive and readily available
4. easily removed leaving no solvent residue
5. because of low viscosity it can readily penetrate comminuted dry plant material
6. by varying the temperature and pressure it can be used in a more selective manner.

According to Moyler (1991), the isolation of volatile concentrates of dried aromatic plants under subcritical conditions is a commercially viable alternative to the traditional steam distillation because the operating conditions of 50-80 bar pressure and 0 to 10°C are quite readily maintained in moderate pressure equipment. A schematic representation of the extraction process can be seen in Figure 5. The vessels and piping have to be made of heavy gauge stainless steel and every vessel, pipe and weld has to be both pressure tested and x-ray evaluated. According to Moyler, for a single vessel capacity of 500-600 kg. the equipment value shown in the schematic diagram is ca. 4.5 - 5.0 million dollars. As a result, the cost of equipment is prohibitive except for large flavor, fragrance, beer, or pharmaceutical manufacturers. The reason beer is mentioned is that almost all of the hop extracts used around the world are produced by CO_2 extraction (Harold and Clarke, 1979), because during solvent

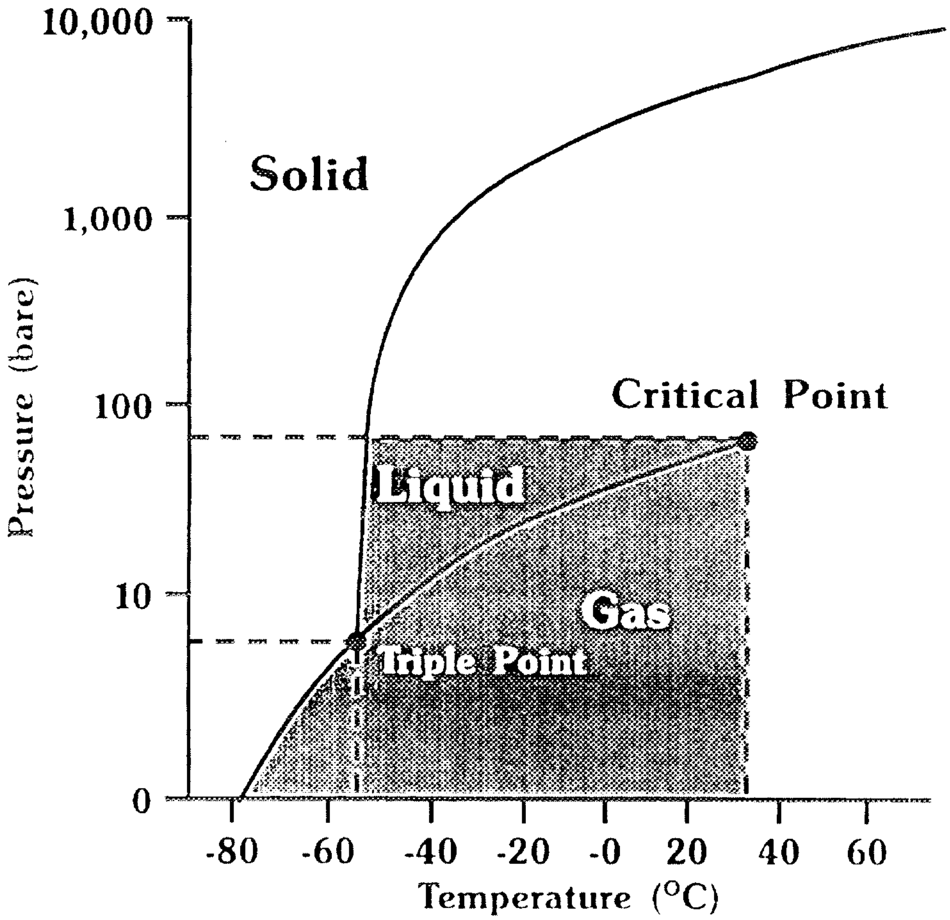


Figure 4. Graphical representation of phase changes for CO_2

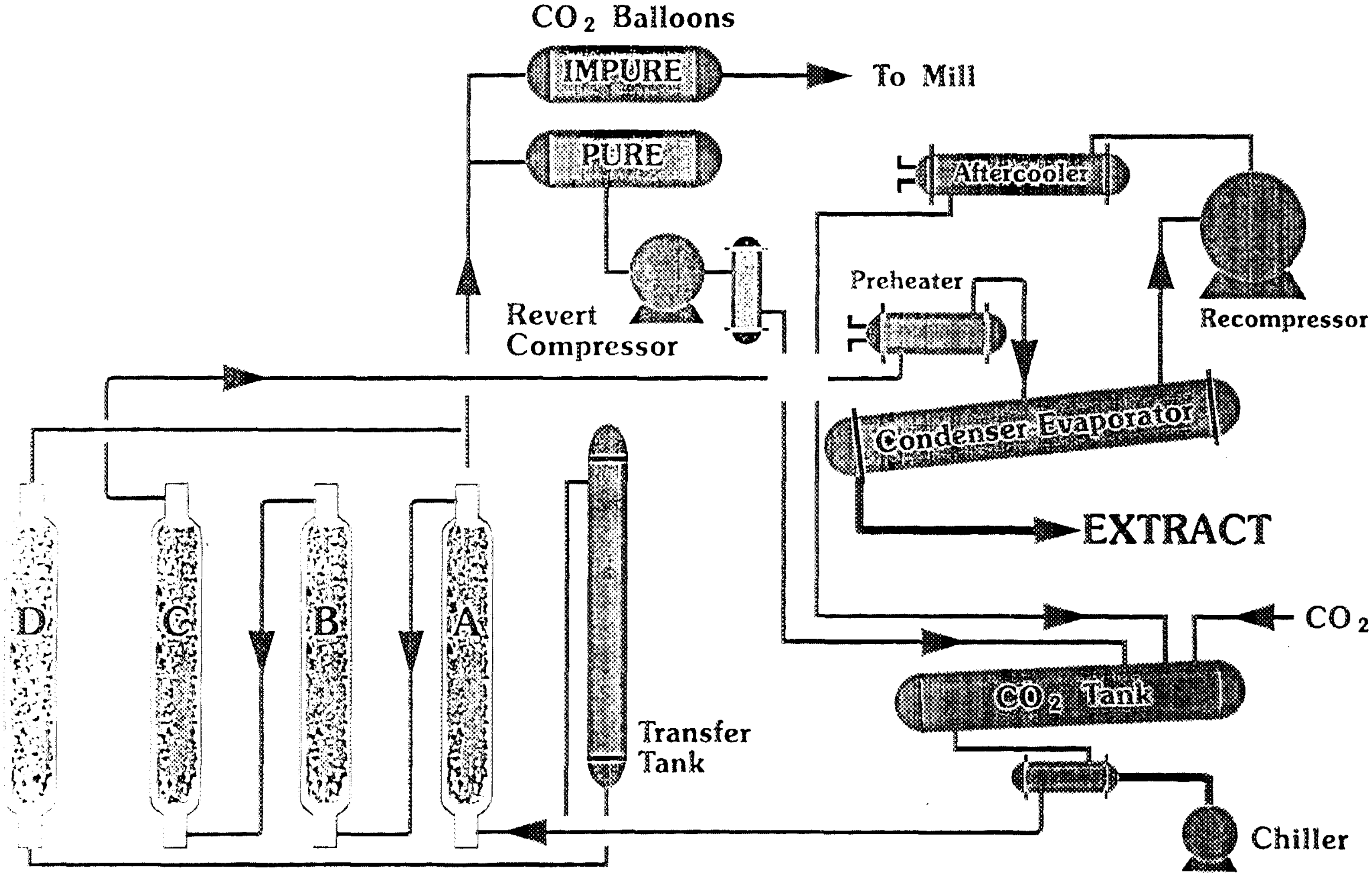


Figure 5. Schematic representation of a commercial CO_2 extraction process

removal after conventional solvent extraction, the important bitter tasting α- and β- acids degrade lowering the value of the produced extract. The fact that liquid CO_2 extraction of hops is the preferred method of hop extract production has led to the proliferation of sub and supercritical CO_2 extraction systems. These systems have excess capacity that has led to the production of volatile concentrates from other dry plant materials in a cost effective way. This could only be done if CO_2 extracts of other plant products did not carry any, or carried only a limited amount of capital expenditures amortized against their sales price, otherwise the cost of these materials would be prohibitive.

The merits of a sub or supercritical CO_2 extract are that they possess aroma profiles almost identical to the raw material from which they were extracted. During steam distillation or solvent removal from an extract, the mixture is subjected to necessary heat treatment that can and does cause irreversible hydrolytic, isomerization and polymerization reactions. In addition, the top notes of the oil or extract are either lost or confounded by sugar degradation reaction products, Maillard reaction products or other degradations that are responsible for the "still notes" or off notes associated especially with oils produced by hydrodistillation.

Liquid CO_2 is a good solvent for compounds whose mol. wt. is below 250. This includes compounds such as the hydrocarbons and oxygenated monoterpene compounds found in essential oils. The solubility for compounds whose mol. wt. is between 250 and 400 in liquid CO_2 is, at best extremely poor, while compounds with molecular weights in excess of 400 are almost insoluble in liquid CO_2. This means that liquid CO_2 extracts will not contain waxes, polyphenols, carbohydrates, carotenoids, chlorophylls, etc. Subcritical CO_2 extracts of plants that are rich in oxygenated sesquiterpenes and sesquiterpene lactones will contain less of the desired constituents than supercritical fluid (SCF) CO_2 extracts because of the increased diffusibility of the fluid. However, an SCF can be further fractionated during production whereas a subcritical CO_2 extract cannot.

The capital expenses for the construction of an SCF factory is almost prohibitive unless the production of a single product such as SCF hop extract can carry the majority of the costs, because for equipment of the same capacity as the subcritical liquid CO_2 equipment described earlier, the costs are in excess of seven million dollars.

In summary, the use of CO_2 extraction as a method to produce extracts of aromatic plants is of interest, but unless the company has a large cash reserve, the investment costs are prohibitive. Finally, it should be mentioned that SCF extraction has been used in citrus oil deterpenation; however, this will be discussed in Chapter 3.

Microwave Extracts

In 1991, Paré et al. performed successful extractions of mint, cedar leaves, and garlic using microwaves and microwave transparent solvents. Solvents which are transparent to microwave energy allow all of the energy to be absorbed by the plant material. The sudden increase in temperature causes the cell walls of the essential oil glands to rupture and release their oil to the surrounding solvent. Recently, Chen and Spiro (1995) examined the kinetics of this process. They found that when the oil glands were subjected to severe thermal stress, as experienced with microwaves, they not only ruptured but totally disintegrated into aggregates of powdery fragments; however, this process was solvent dependent. The authors concluded that fast extraction rates were found when the leaf:hexane ratio was optimized, and the mixture was subjected to high microwave power. Furthermore, they stated that microwaves are definitely advantageous for rupturing oil glands; however, they should only be used to rupture the oil glands. Even though patents have been issued for this process, it has yet to become fully commercialized.

Infusion

Infusions are hot water extracts of dried plant material. Brewed tea can be thought of as an example of a tea infusion. Sometimes an infusion of a dried material can yield a solution that is particularly rich in solids. As a result, this can be either drum dried or spray dried using its own solids as the carrier for the volatiles. An example of this type of material is licorice powder. Infusions are generally made by processes similar to maceration extraction, although hot water percolation is also an option.

Tincture

Tinctures are defined as aqueous alcoholic extracts of dried plant material. They are produced by using equipment similar to that used in resinoid manufacture, percolation extraction or static extraction. The alcohol content of tincture is adjusted by the manufacturer and is usually 20-60%, although some 95% aqueous alcoholic tinctures are known.

Spice/Herb Oleoresins

The term oleoresin is used for the natural exudate which is rich in essential oils; however, it is also used in the seasonings, flavor, and food industries to describe the solvent extractables of spices and herbs from which the solvent has been removed. Spice/herb oleoresins contain, not only the essential oil of the original plant material, but also the fixed or vegetable oil, the color, and the active principles, although their composition is solvent specific. The process of spice/herb oleoresin manufacture can be thought of as following the flow outlined schematically in Figure 6.

Initially the herb or spice is ground so that the penetration of solvent is rapid and complete. The type of equipment used for grinding and the mesh size (fineness of grind), which is a critical part of oleoresin manufacture, is raw material specific, and according to the major commercial manufacturers, proprietary information (Lupina, 1995). Nevertheless, the optimum mesh size requirements for maximum oleoresin yield for each specific spice and herb can be readily determined experimentally.

Any detrimental effect of grinding on the aroma and flavor attributes of raw herb or spice must be kept to a bare minimum; otherwise, the value of the resultant oleoresin will be decreased. Because heat is generated, during grinding, the use of cryogenic grinding processes are recommended to ensure that the loss of volatiles is minimized. Sometimes this just means the use of a mixture of dry ice (solid CO_2) in combination with the spice during the processing through a hammermill. For spices such as nutmeg, cardamon, clove and black pepper, dry ice grinding will suffice; whereas, for aromatic seed spices such as anise, caraway, coriander, dill, fennel, parsley, etc., the use of liquid nitrogen cooling during their grinding through a roller mill is recommended.

The extraction process can be either that of a single stage or a double stage extraction. Whether extraction can be completed in one stage or two stages is dependent on whether the manufacturer has steam distillation equipment to isolate the oil prior to extraction. The solvents used for oleoresin manufacture are controlled by the food laws of the country within which the end product oleoresin will be used (Moyler, 1991, Hainrihar, 1991) They are further controlled by the setting of permitted residual levels (Table VI). In the United States, the permitted solvents are acetone, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and trichloroethylene; however, the use of chlorinated solvents is currently under review by a combined industry-federal government study group. Solvents chosen for oleoresin extraction should meet the following requirements (Pagington, 1983):

- should be acceptable as food solvent
- should not leave any harmful residue in food product can be removed to a very low level without harming the oleoresin such as vacuum distillation, azeotropic distillation, or both

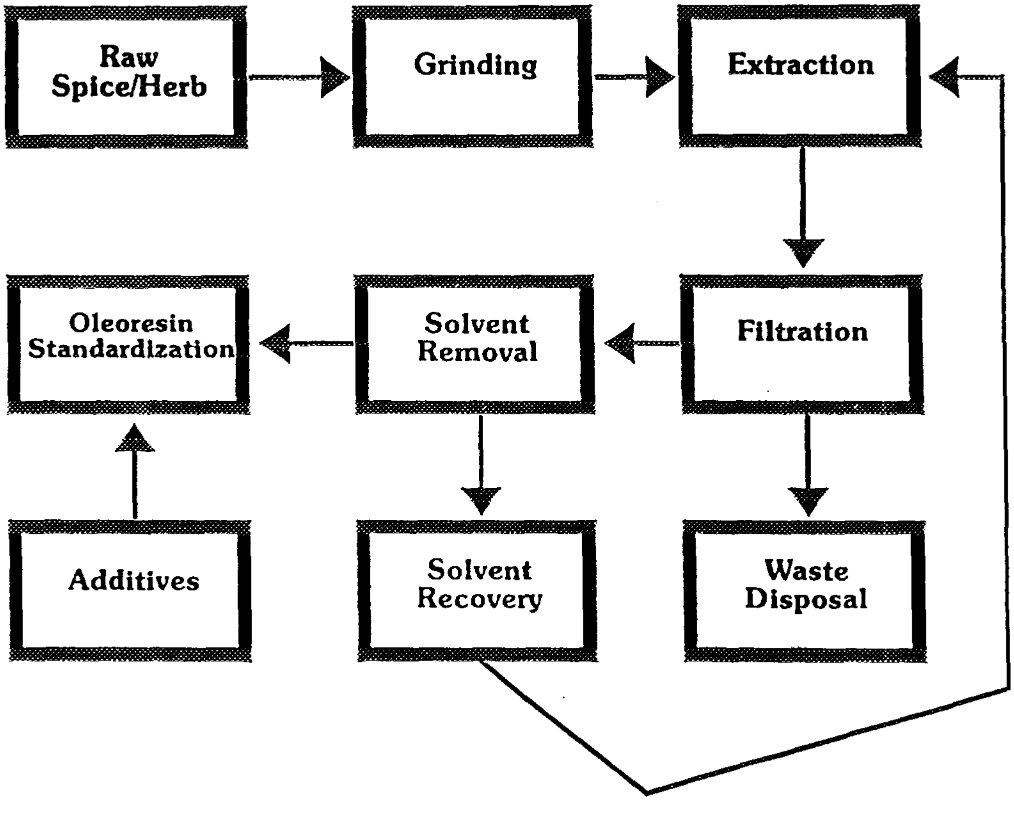


Figure 6. Flow diagram of the single stage oleoresin manufacturing process

- should readily dissolve all of the flavor, aroma, and active principles of the spice
- should be inexpensive
- should be readily recoverable

In the single stage extraction, the oleoresin is extracted from the spice with the selected solvent, the mixture is separated and the solvent is carefully removed leaving behind the oleoresin. In contrast, for double stage extraction (Figure 7) the spice is initially steam distilled to remove the essential oil, dried, and then extracted as in the single stage process. After removal of the solvent (a process where less care is needed because the volatile portion of the spice has already been removed), the non-volatile portion of the oleoresin is combined with the essential oil to form a more standardized product. In either case, the extraction process is the same. The ground spice is placed in a basket, the mesh size of which will not allow the ground spice to be lost, and the basket is placed in a steam jacketed reaction vessel which is sealed. Under strict temperature control, which is maintained by the steam jacket around the reaction vessel, the spice is washed with a known amount of solvent for a known period of time to remove all of the essential oil and desirable non-volatile components.

The extraction can either be a batch process (as already described) or it can be a continuous process. In a continuous process there must be an air lock as the ground material is introduced into the extractor. It is conveyed via some process through the various solvent wash stages after which the solvent is removed once the composition of extractables in the miscella has reached saturation point. The marc (ground spice) is conveyed out of the extraction vessel via another air lock after which it is desolventized.

The solvent plus all of the extractables (known as the miscella) are subjected to a careful solvent removal process so that the final oleoresin will contain no more than the permissible solvent levels as defined by the food laws of the country within which the oleoresin will be sold. For most oleoresins, the miscella is subjected to vacuum fractional distillation. Initially, the low boiling solvent is easily removed with a relatively fast reflux ratio. As the solvent level gets below 1%, the vacuum is increased to prevent the pot temperature from rising. In addition, the reflux ratio is slowed down to increase the efficiency of the fractional distillation. It is not always possible to prevent the removal of some of the low boiling compounds, and as a result, further fractional distillation of certain fractions is necessary to isolate the essential oil components from the solvent. These so-called light fractions are blended back into the oleoresin at the standardization stage.

Standardization, which can entail the addition of some essential oil or even some vegetable oil, is a necessary step in recreating a concentrate with the flavor and aroma profile of the original spice or herb. Any additives to the oleoresin must be declared on its label. Because oleoresins are very viscous liquids, the addition of these additives is best done in the order of their decreasing viscosities with the recovered volatiles being added last. Mixing is achieved with the use of either a homogenizer, a high shear action stirrer, or a colloid mill. As mentioned earlier, oleoresin extraction can either be done on a batch or as a continuous process. For black pepper oleoresin it is more common to use the two stage process as it is easier to produce this way than by the single stage process, because black pepper is rich in monoterpene hydrocarbons and the alkaloid piperine. Finally, it is of interest to note that a unique single stage extraction system known as a Nauta extractor (see Figure 8) has been successfully used for the manufacture of a ginger oleoresin.

Table VI

Allowable Solvents in Foodstuffs

Solvent	Permitted Residue Level E U	 USA	 Japan
propane/butane	not regulated	-	T.U.Q.
ethanol	not regulated	T.U.Q.	T.U.Q.
ethyl acetate	not regulated	-	T.U.Q.
carbon dioxide	T.U.Q.	T.U.Q.	T.U.Q.
acetone	<50 ppm	30 ppm	T.U.Q.
methanol	not regulated	50 ppm	-
hexane	1 ppm	25 ppm	1 ppm
ethylene dichloride	under review	30 ppm	-
methylene chloride	0.1 ppm	30 ppm	-
tricholorethylene	-	30 ppm	-
isopropanol	under review	30 ppm	-
propylene glycol	not regulated	not regulated	not regulated
glycerol	not regulated	not regulated	not regulated

T.U.Q. = Technically Unavoidable Quantities

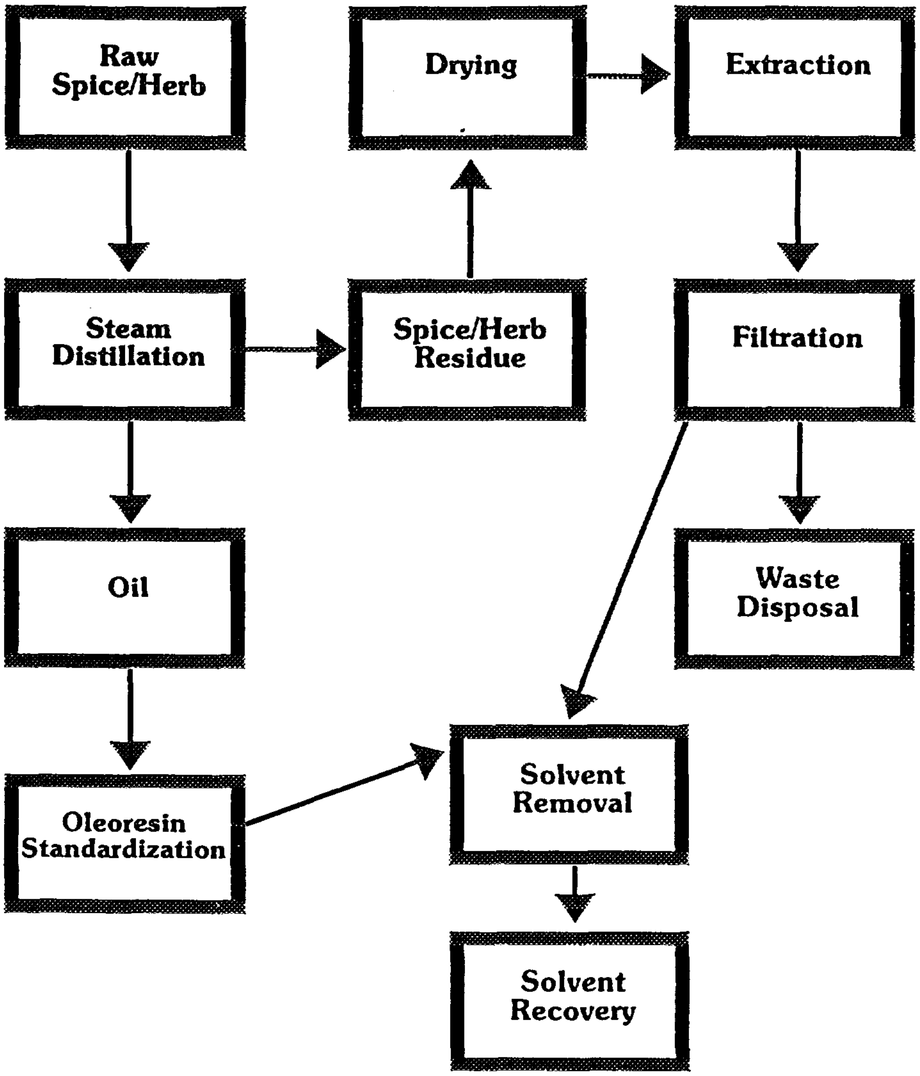


Figure 7. Flow diagram of the double stage oleoresin manufacturing process

Essential Oils

As mentioned earlier, essential oils are mainly produced by some hydrodistillation process except for most of the citrus oils which are produced by expression. The details of essential oil production will be discussed in the next section.

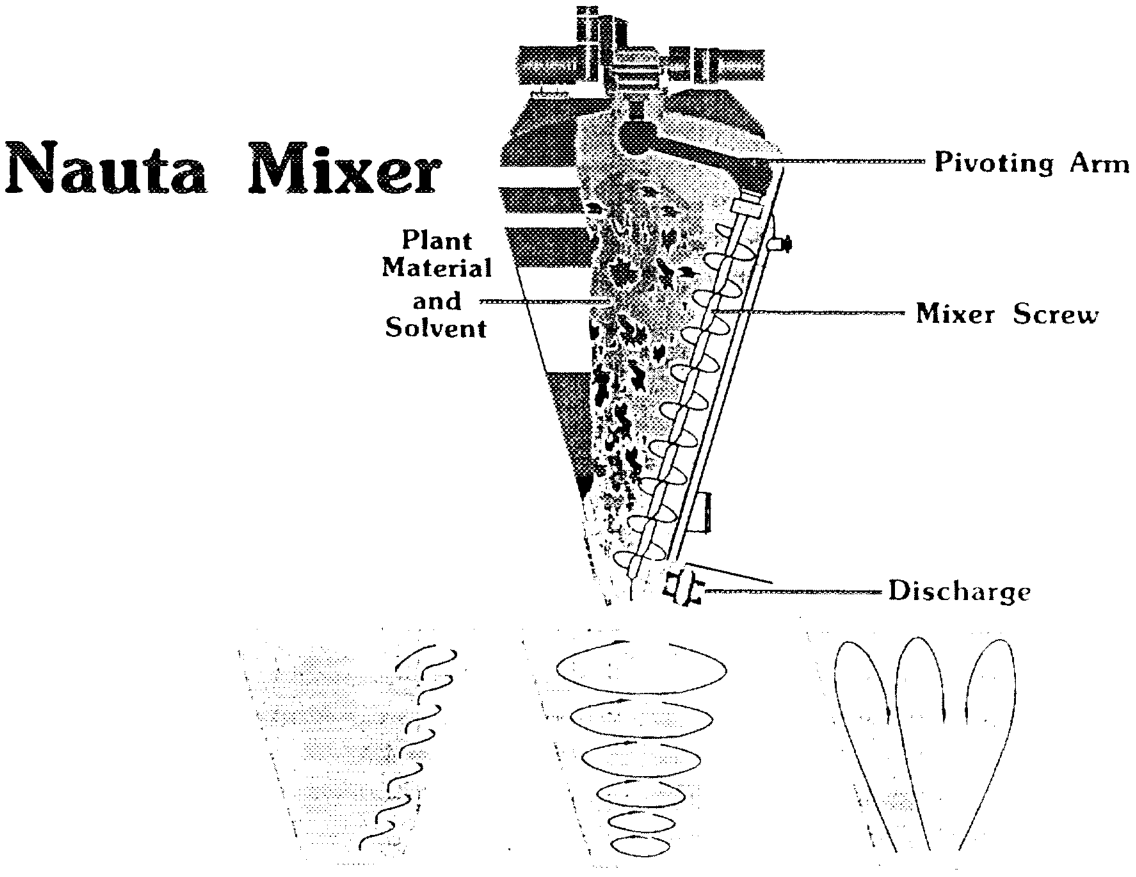


Figure 8. Diagrammatic representation of a Nauta mixer and its mixing action

2. ESSENTIAL OIL PRODUCTION

The main methods used to obtain essential oils from plant material are: water distillation, steam and water distillation, steam distillation, maceration distillation, empyreumatic (or destructive) distillation, and expression. With the exception of the latter process, all others need heat to release the oil. Each process will be discussed separately, although for water distillation, and steam and water distillation, some of the discussion will be directed towards the production of oils in various parts of the world, so that some of the currently used equipment can be surveyed. Under the discussion on steam distillation, processes such as mobile distillation, continuous distillation and hydrodiffusion will also be treated separately.

Water Distillation

The principle of water distillation is to boil a suspension of an aromatic plant material and water so that its vapors can be condensed. The oil, which is immiscible with the water, is then separated. Most water distillations are performed in rural areas where no access to a steam boiler is possible, or if a satellite boiler is available it complicates the process of oil production. In water distillation the plant material is always in direct contact with water. An extremely important factor is that in stills where the water is boiled by direct contact with the fire, the water present in the still must always be more than enough to last throughout the distillation, otherwise the plant material can overheat and char. When this happens, depending upon the plant material charge, off-notes are formed from Maillard reactions, Strecker degradations and eventually the pyrolytic degradation of the material. As a result, the oil can assume a variety of off-notes, generally known as still notes.

The plant material in the still must be kept agitated as the water boils, otherwise agglomerations of dense material will settle on the bottom of the still and become thermally degraded. A way to avoid this is to ensure that the charge of plant material is readily dispersed into the water such as might be found in powdered material. Certain plant materials like cinnamon bark are very rich in mucilage. As a result, if the cinnamon bark is powdered such that the charge can readily disperse in the water, as the temperature of the water increases, the mucilage will be leached from the ground cinnamon bark. This greatly increases the viscosity of the water/charge mixture, thereby allowing it to char. Consequently, before any field distillations are done, a small scale lab water distillation in glassware should be performed to observe whether any changes take place during the distillation process. Also, from this lab trial an accurate oil yield can be obtained from a known weight of plant material. The lab equipment recommended for any trial distillations is the modified Clevenger system which can be seen in Figure 9.

Water distilled oils are commonly darker in color and have much stronger still notes than oils produced by other methods. If the oil contains higher boiling components (phenols, alkenyl phenol ethers, or sesquiterpenes), the distillation time is increased over oils that contain only monoterpene hydrocarbons and oxygenated constituents. It is generally not possible to put enough water in the still and have it last for the whole distillation cycle. To compensate for this, a cohobation tube is placed in the side of the still and water is constantly returned to the pot. An example of cohobation can be seen in Figure 10 in which the CISIRILL "Manakoka" still is shown.

As mentioned above, oils produced by water distillation tend to be of lower quality than oils produced by other methods for the following reasons:

- The oil components like esters are sensitive to hydrolysis while others like acyclic monoterpene hydrocarbons or aldehydes are susceptible to polymerization (the pH of the water is often times reduced during distillation so that hydrolytic reactions can be facilitated).
- Oxygenated components such as phenols have a tendency to partially dissolve in the still water so their complete removal by distillation is not possible.

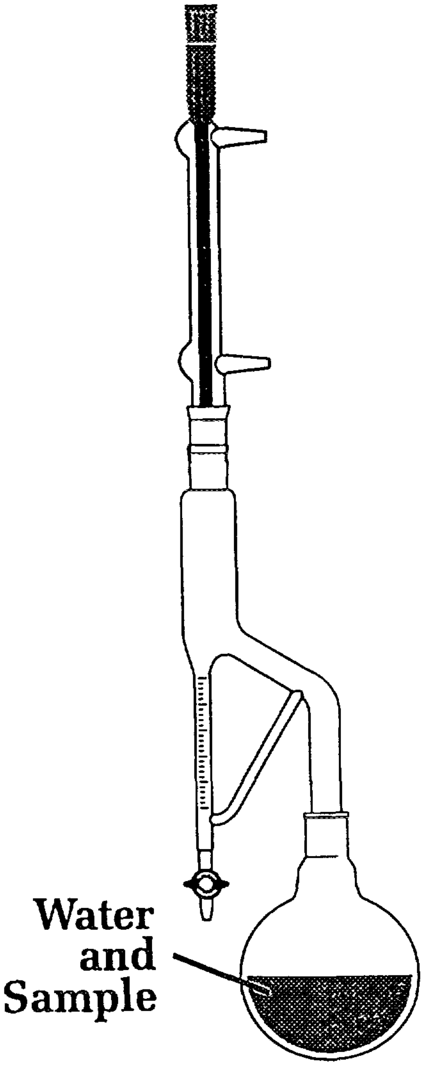


Figure 9: Diagrammatic representation of a Clevenger-type lab-scale hydrodistillation

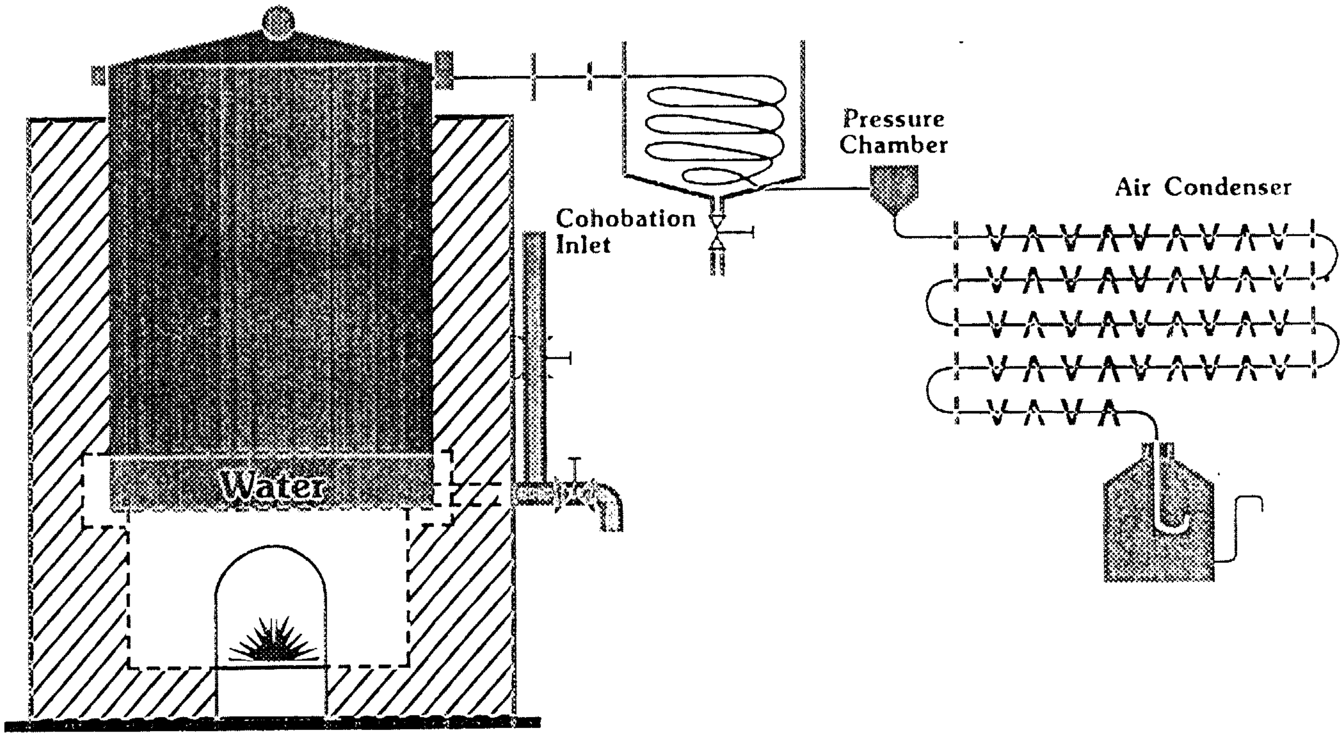


Figure 10: Diagrammatic representation of the CISIRILL 'Manakoka still showing cohobationand an air condenser
(Ceylon Institute for Scientific and Industrial Research, Sri Lanka)

- As water distillations tend to be small (being operated by one or two people), it takes a long time to accumulate much oil, so good quality oil is often times mixed with bad quality oil.
- The distillation process is treated as an art by the local distillers and few often try to optimise both their yield or quality
- The distillation process is treated as an art by the local distillers and few often try to optimise both their oil yield or quality.
- Water distillation is a slower process than either steam and water or steam distillation; consequently, it is less energy efficient.

An advantage of water distillation is that for plant material that has a tendency to agglomerate or to agglutinate into an impenetrable mass when steam is passed through it (like rose petals), water distillation is reputed to be the preferred method of oil isolation (Kahol). In 1992, Baser described that in rural Turkey, 10 kg of rose flowers and 60L water are charged into a 300L copper or galvanized steel direct fired still fitted with a removable spherical head that is connected to a pipe which leads through a pool of water (the condenser) and then into a 9L glass collection flask. He further stated, however, that the industrial production of rose oil in Turkey is by steam distillation.

Perhaps it can be concluded then that the only advantage of water distillation is that the cost of process equipment is extremely low, the design of the stills, condensers and collection flasks are simple, and the need for electricity is not prohibitive.

Water distillation is still used around the world to produce a few essential oils. An example of its use can be seen in Figure 11.

Steam and Water Distillation

In steam distillation, the steam can either be generated in a satellite boiler or within the still, although separated from the plant material. Steam and water distillation is the latter of these processes. Like water distillation, it is a widely used process in rural areas as it does not require a great deal more capital expenditure than water distillation. Also, the design of equipment that is used is generally very similar to that used in water distillation, only the plant material is supported above the boiling water on a perforated grid as shown in Figure 12. In fact, it has become the traditional progression from water distillation to steam and water distillation.

It follows that once the rural distiller has produced a few batches of oil by water distillation, there is a realization that the quality of oil that was produced is not very good because of its still notes. As a result, some modifications are made. Using the same still, a perforated grill, or plate is fashioned so that the plant material is raised above the water. This reduces the capacity of the still but affords a better quality of oil. If the amount of water will not last long enough to allow the completion of distillation, a cohobation tube is attached and condensate water is added back to the still manually, thereby ensuring that the water, which is being used as the steam source, will never run out. It is also is believed that this will, to some extent, control the loss of dissolved oxygenated constituents in the condensate water because the re-use of the condensate water will allow it to become saturated with dissolved constituents, after which no more will dissolve in it.

Cohobation

Cohobation is a procedure that can only be used for water distillation or steam and water distillation. it uses the practice of returning the distillate water to the still after the oil has been separated from it so that it can be re-boiled. The principle behind it is to minimize the losses of oxygenated components, particularly phenols which will dissolve to some extent in the distillate water. For most oils this level of oil loss through solution in water is less than 0.2%, whereas for phenol-rich oils the amount of oil dissolves in the distillate water is between 0.2 - 0.7%. As this material is being constantly re-vaporized, condensed and re-vaporized again, any dissolved oxygenated constituents will promote hydrolysis and degradation of itself or other constituents of the oil. Similarly, if an oxygenated is constantly brought in contact with a direct heat source or side of a still, which is considerably hotter than

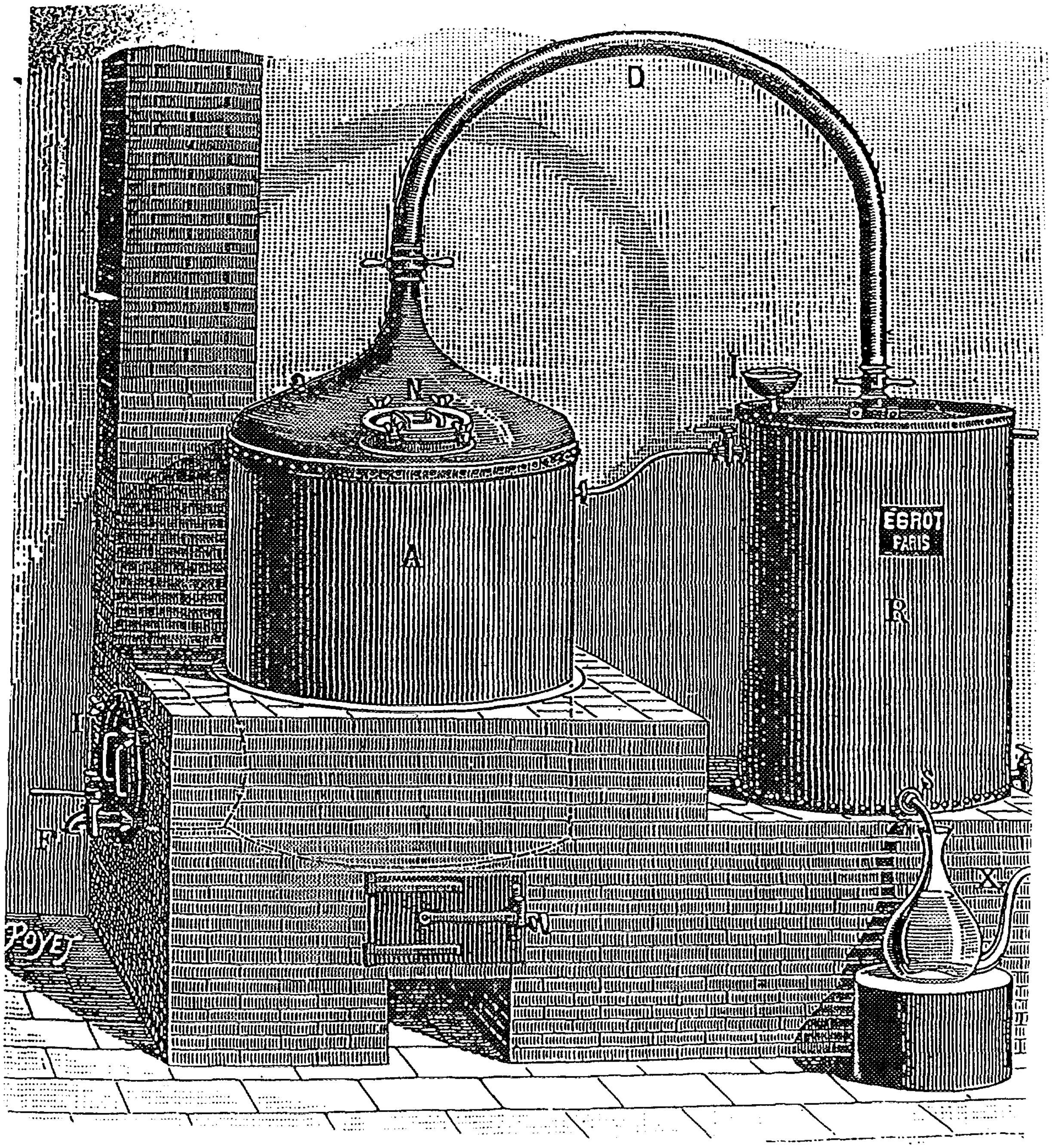


Figure 11: Reproduction of a sketch of water distillation performed in France at the turn of the century

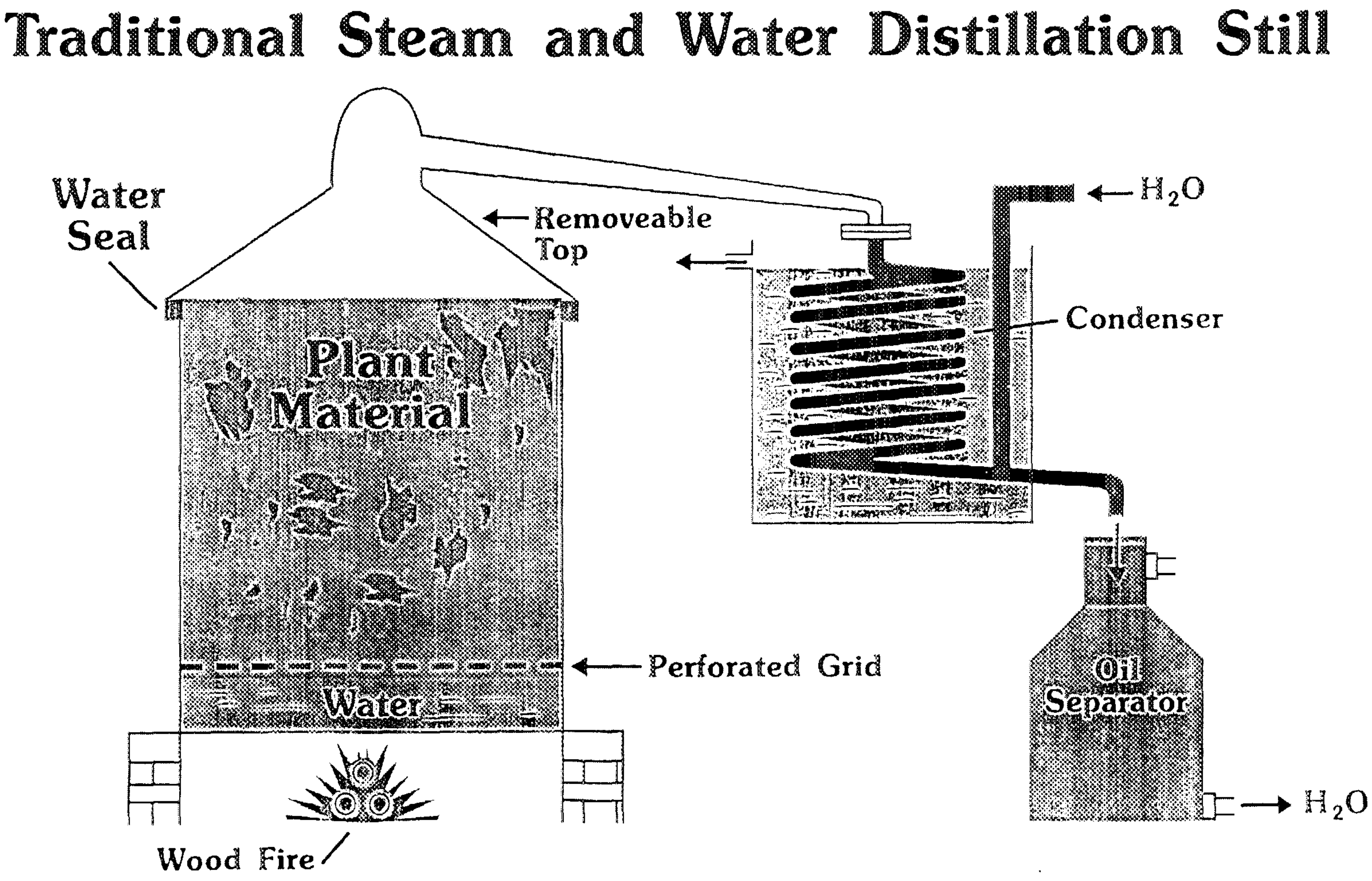


Figure 12: Diagrammatic representation of a steam and water distillation system

considerably hotter than 100^0 C, then the chances of degradation are enhanced. As a result, the practice of cohobation is not recommended unless the temperatures to which the oxygenated constituents dissolved in the distillate exposed are maintained no higher than 100^0 C.

In steam and water distillation, the plant material cannot be in direct contact with the fire source beneath the still; however, the walls of the still are good conductors of heat so that still notes can also be obtained from the thermal degradation reactions of plant material that is touching the sides of the still. As the steam in the steam and water distillation process is wet, a major drawback in this type of distillation is that it will make the plant material quite wet. This will slow down distillation as the steam has to vaporize the water to allow it to condense further up the still, etc.

There is one way to prevent the lower plant material resting on the grid from becoming waterlogged. It requires the use of a baffle to prevent the water from boiling too vigorously, resulting in direct hot water contact on the plant material. An example of how this can be prevented can be seen in Figure 13.

In addition to the fact that the wetness of the lower plant material slows down the distillation, an accumulation of water within the lower part of the still can cause it to reflux. This means that in the lower portion of the still the water will constantly boil and condense which will not only be detrimental to the steam passage through the charge, but it will also dissolve some of the oil constituents. According to Denny (1991), this refluxing process, which can also be a problem in steam distillation as well as steam and water distillation, can be controlled by the installation of a reflux control as shown in Figure 14.

The advantages of steam and water distillation over water distillation are as follows:

a. Higher oil yield

b. Oil components of the oil are less susceptible to hydrolysis and polymerization (the control of wetness on the bottom of the still affects hydrolysis, whereas the thermal conductivity of the still walls affects polymerization)

c. If refluxing is controlled, then the loss of polar compounds is minimized

d. Oil quality produced by steam and water distillation is more reproducible

e. Steam and water distillation is a faster process than water distillation so it is more energy efficient

Many oils are currently produced by steam and water distillation. The examples of Lemongrass production in Bhutan using a rural steam and water distillation system can be seen in Figures 15A and 15B.

Steam Distillation

As the name suggests, steam distillation is the process of distilling plant material with steam generated outside of the still in a satellite steam generator generally referred to as a boiler. As in steam and water distillation, the plant material is supported on a perforated grid above the steam inlet. A real advantage in the satellite steam generation is that the amount of steam can be readily controlled. An example of a modern steam distillation unit can be seen in Figure 16. Because steam is generated in a satellite boiler, the heat contact of the plant material will be no higher than 100°C. Consequently, the amount of heat with which the plant material will come in contact is acceptable and should not cause any thermal degradation of it. The process of steam distillation is the most widely accepted process for the production of essential oils on the large scale. Throughout the flavor and fragrance supply business it is a standard practice.

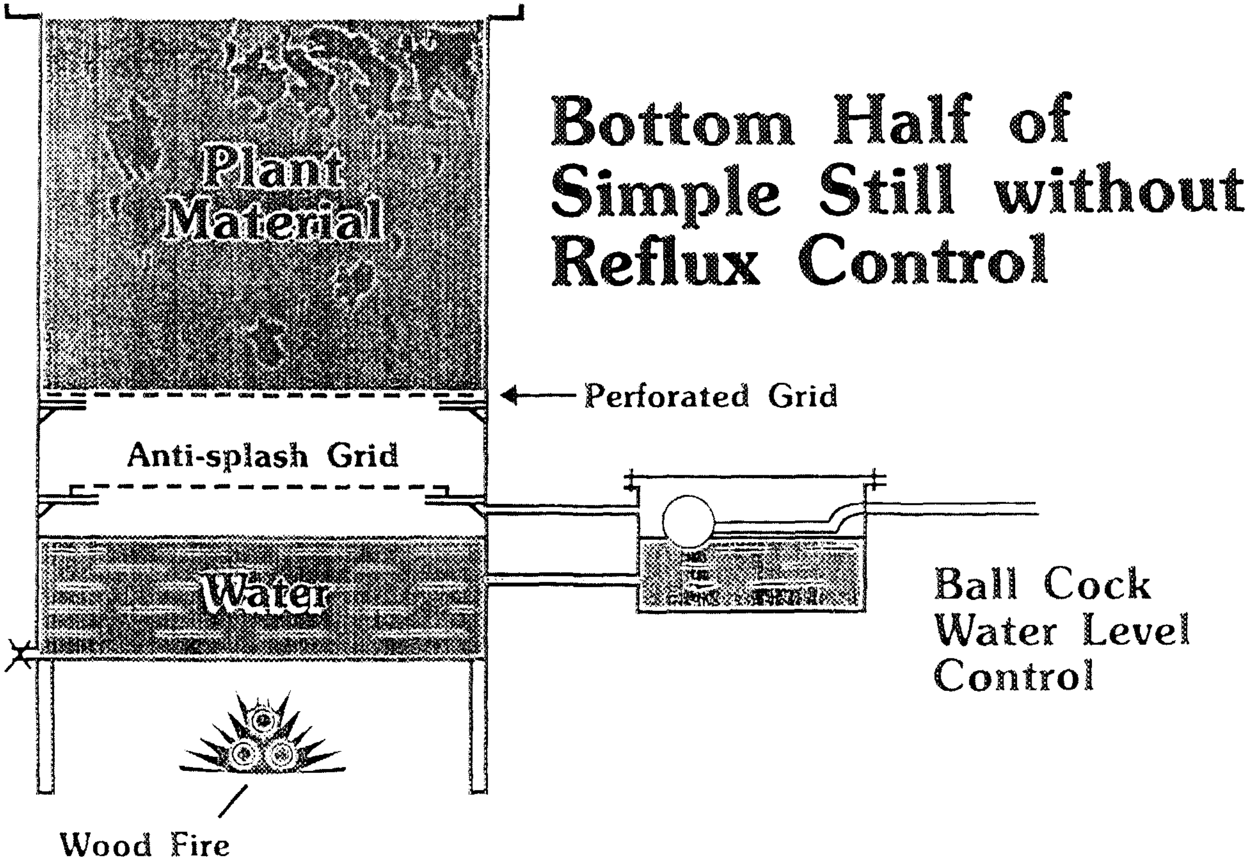


Figure 13: Diagrammatic representation of a baffle system to prevent direct water contact with the plant material on the perforated grid

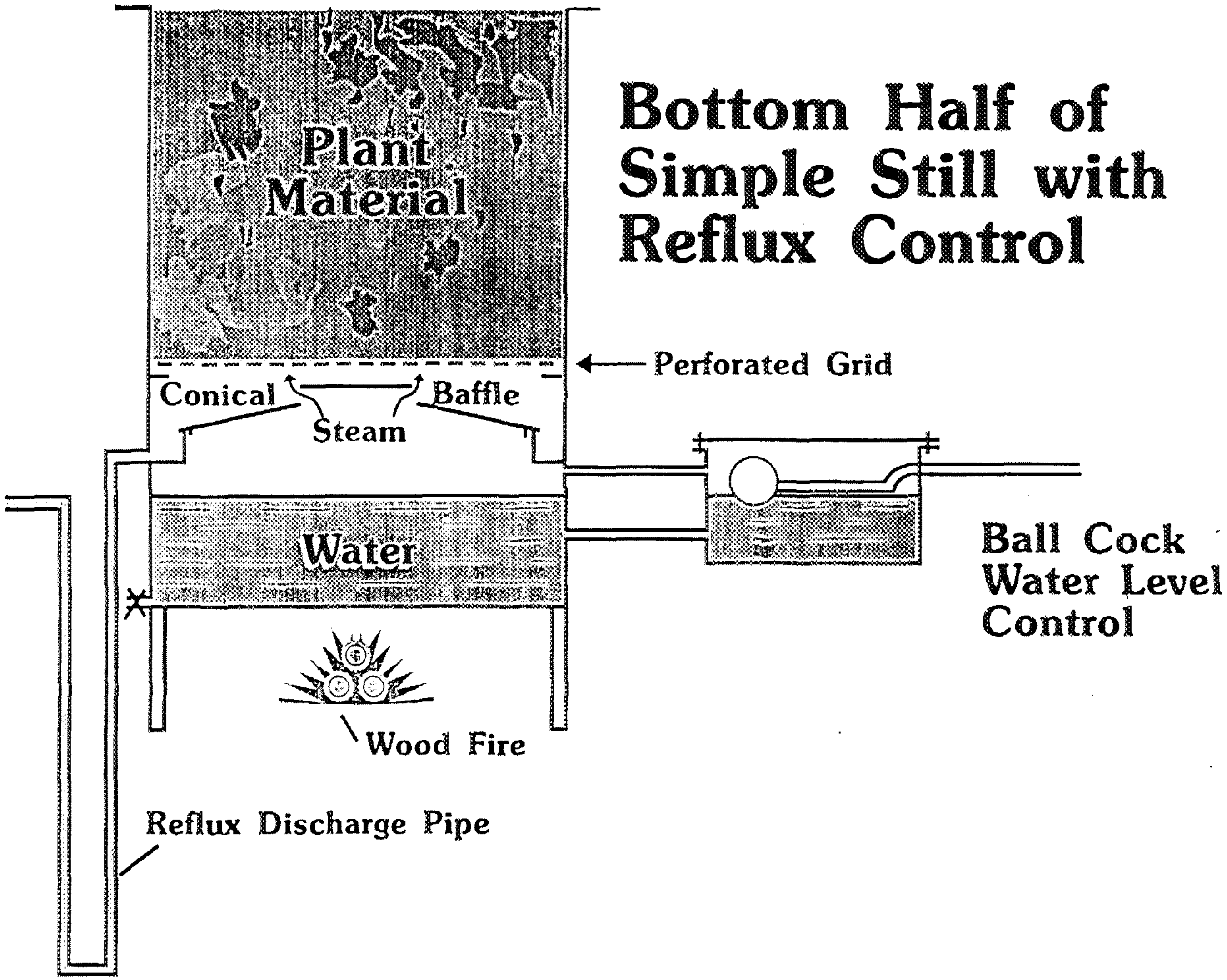


Figure 14. Diagrammatic representation of a reflux control system to prevent water build-up in the lower part of the still

Figure 15A: Photograph of rural lemongrass distillation in Bhutan

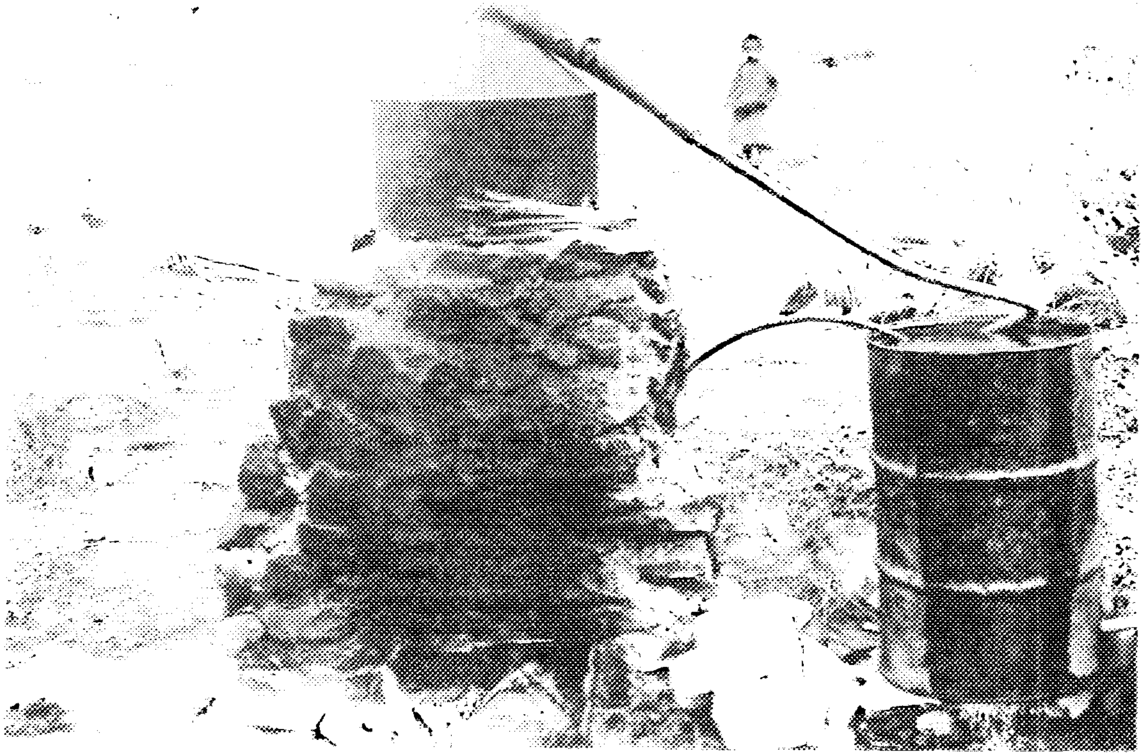


Figure 15B: Photograph of rural Lemongrass distillation in Bhutan

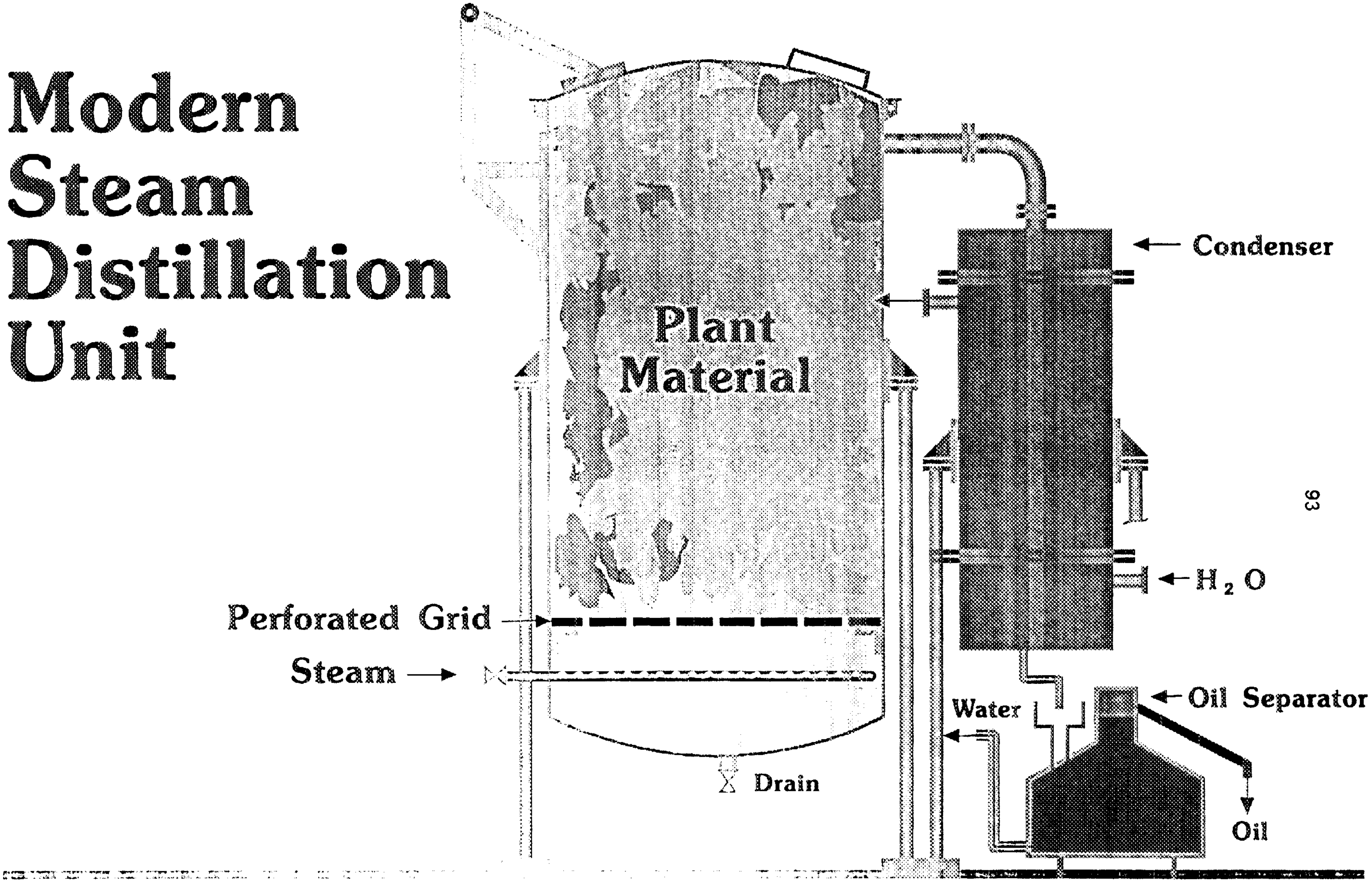


Figure 16: Diagrammatic representation of a modern steam distillation unit

An obvious drawback to steam distillation is the much higher capital expenditure needed to build such a facility. In some situations, such as the large scale production of low cost oils such as rosemary, Chinese cedarwood, lemongrass, litsea cubeba, spike lavender, eucalyptus, lemongrass, citronella, cornmint, etc., the world market prices of the oils are barely high enough to allow them to be produced by steam distillation without amortizing the capital expenditure required to build the facility over a period of 10 years or more. To further discuss steam distillation, it is more prudent to examine the process by describing the parameters for distillery design which will be covered in the next section.

Mobile Distillation

The only difference between steam distillation and mobile distillation is that the plant material is harvested in the field where it is wilted prior to being chopped and transferred directly into a trailer. Within this trailer or tub (as it is commonly called), a steam inlet system has been installed so that once it is full of chopped wilted plant material, it is taken to the distillery where it is connected to the condenser and steam inlet system, thereby becoming the mobile still. In most mobile units the condenser systems are generally of the coiled tube type held within a tank of water. Oils are collected in an oil separator that was designed a number of years ago by the Chemical Engineering Department of Oregon State University.

Mobile distillation evolved around the weakest link in the whole distillation system, namely the time and labor required to load cut herbs into a cart, transport it to the still, unload the cart, and fill the still. To simplify this, some bright entrepreneurs had the idea to put the still on wheels, load it in the field, and transport it to the distillery. This resulted in reducing the time for the whole process, from chopping the wilted plant material to disposing of the spent material, from 6-8 hours to less than 3 hours. In addition, the size of the labor force was reduced by at least 50%.

Rather than spend any time discussing this process in detail, a schematic representation of the process can be seen in Figure 17.

This mobile process is currently used for the manufacture of peppermint, both spearmint oils, clary sage, and dill oil in the United States. In addition, it is being used for some eucalyptus oil production in Australia, peppermint oil production, and some lavandin oil production in both Bulgaria and France.

A few years ago this author reported on the peppermint and spearmint oil industry in the United States (Lawrence, 1992). Within this treatise the cost of establishing a 4-tub distillery was discussed, a summary of which can be seen as follows:

	<u>USDollars</u>
6 x 16 ft. portable tubs	60,000
Building to house distillery	16,000
4 condensers and tanks	8,000
700 horsepower boiler	90,000
4 receiving cans	2,400
Miscellaneous (pipes, fuel tank, valves, hoses)	6,000
Labor (one season)	4,000
Well and pump	4,000
Redistillation equipment	10,000
Overhead (permits, taxes, etc.)	2,000
Interest	<u>20,000</u>
	218,000

It should be noted that this distillery can support a 400-acre planting of either peppermint or spearmint.

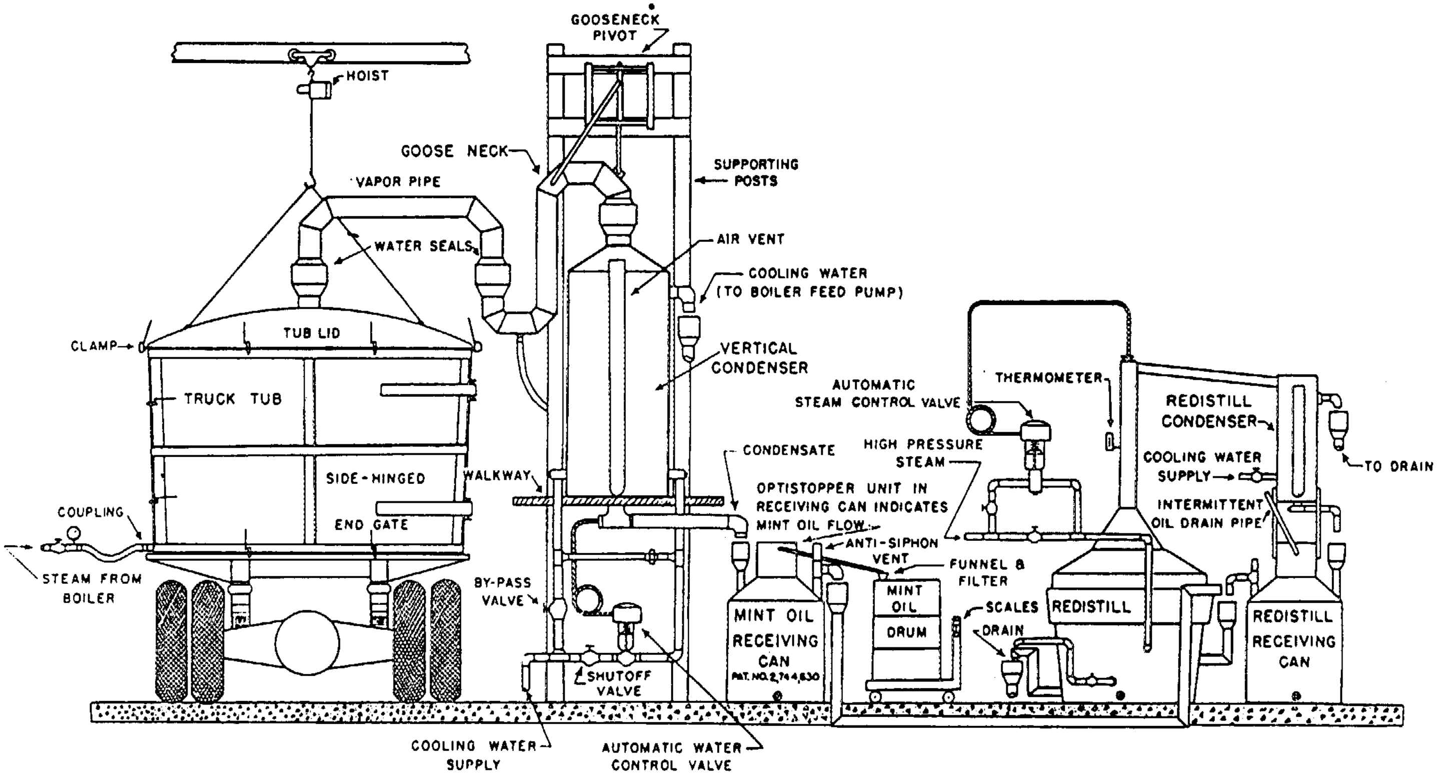


Figure 17: Diagrammatic representation of mobile distillation unit

Continuous Distillation:

The use of a continuous process to steam distill essential oils has been in production in the former Soviet Union for more than 20 years. More recently a process for the manufacture of Texas cedarwood has been described by Bouchard et al. (1986), and Bouchard and Serth (1991). This unique process commences with pulverizing cedar logs into powder that has a mesh size of ca. 0.25 mm. The hot powdered wood (up to 80°C from the friction of grinding) is pneumatically conveyed into a distillation cell within which it is brought in contact with a superheated steam in a 1:1 ratio. The oil is removed from the powdered wood by a simple desorption or heat stripping process, which can be thought of as partial pressure distillation. As long as the conditions in the still are manipulated so that the oil cannot exist in liquid form at that temperature and pressure, it will vaporize.

Bouchard and Serth (1991) reported that, although the residence time in the partial pressure distillation cell was only 25-30 seconds, the entire pneumatic apparatus, which is comprised of ca. half a mile of 16 gauge mild steel, is housed in an insulated unit (plenum) that is 2.5m tall, 1.2m wide, and 9m long. It is reported that the continuous distillation apparatus has a nominal capacity of processing 12 metric tonnes of cedar logs per day. A schematic representation of this continuous process can be seen in Figure 18.

In 1990, Carle and Fiedler described the use of a cylindrical continuous distillation system that is produced commercially under the name Padovan. According to Arnaudo (1991), the plant material is fed through a distillation chamber via an Archimedian screw with the steam passage countercurrent to the material flow. The still is ca. 6m in height with a diameter of 1.6m. Once the steam is enriched with oil vapor, it is sent to the condenser which is of the vertical multiple tube type. The hot vapors reach the condenser by passing over the external surface of the Archimedean screw to heat the plant material before is it contacted by the steam. This process, which is best used for powder materials, is currently being used in France for fennel oil production.

Another continuous distillation system described by Arnaudo is known as the DCF process. It is a process that has already been used for sometime to recover alcohol from the residue left over after grapes are fermented. According to Arnaudo, it is a modular system containing an endless screw in which the 5m long modules are mounted in series while plant material is fed from module to module in a cascade fashion. The configuration of the screws allows the plant material being conveyed to form plugs at regular intervals, thus preventing any escape of steam. The process, which does not use a large amount of steam, is favored for use with plants containing superficial oils or low boiling subcutaneous oils such as peppermint, roman chamomile and tarragon.

One final continuous distillation process (known as the Biolandes process) has been described by Arnaudo (1991) and Vacchiano (1992); a schematic representation which can be seen in Figures 19 and 20. It consists of a high capacity hopper within which plant material can be crushed and pulverized. From this, the mass is conveyed to the stills by endless screws which are made up of 7.5 cu. m distillation cylinders, and as it is conveyed through the cylinders, steam is passed through the plant material countercurrent to the mass flow. Usually, the mixture of oil and water vapors are condensed and the latent heat of condensation is lost. In the Biolandes process, an aerothermic radiator, which possesses a high thermal transfer capacity, is ventilated with a known quantity of air so that the 100°C steam is converted into water at close to 100°C. The hot air that is produced is used to dry most of the exhausted plant material rapidly so that it can be conveyed to the boiler for use as boiler fuel. By carrying out this heat exchange process, the cold water requirement of the condensing system is drastically reduced. According to Arnaudo, a system comprising of two 7.5 m stills will process 3.0 tonnes of pine needles, 1.5 tonnes of juniper branches or 250 kg. of cistus branches per hour. He believes that this Biolandes process fulfills the following objectives:

1. Short time period processing of large quantity of plant material
2. Reduced energy needs by becoming boiler energy self-sufficient
3. Reduced water reauirement
4. Reduced disposal costs for spent plant material

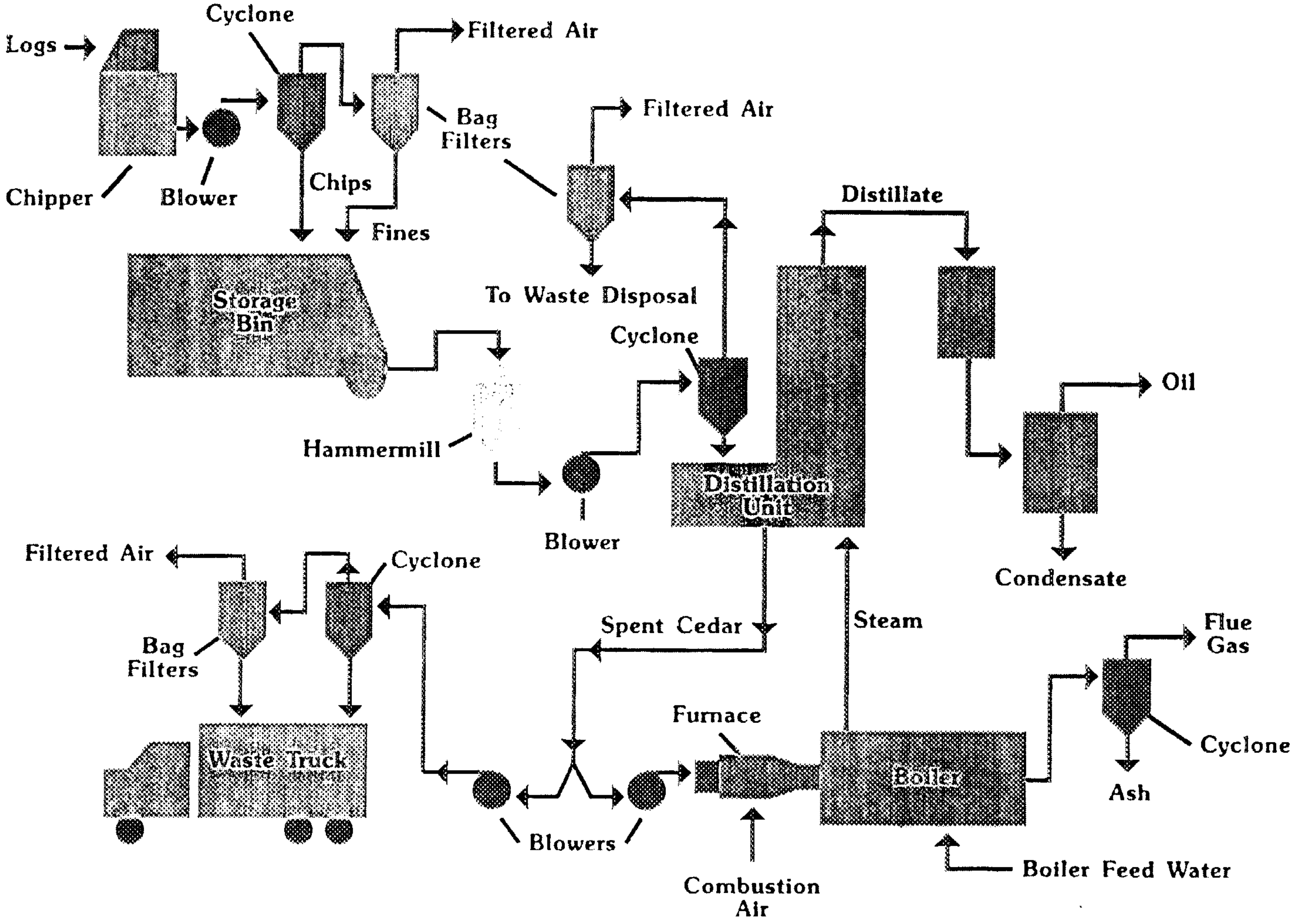


Figure 18: **Schematic representation of a continuous steam distillation of cedarwood**
(Permission to use this diagram was given by G. Bouchard)

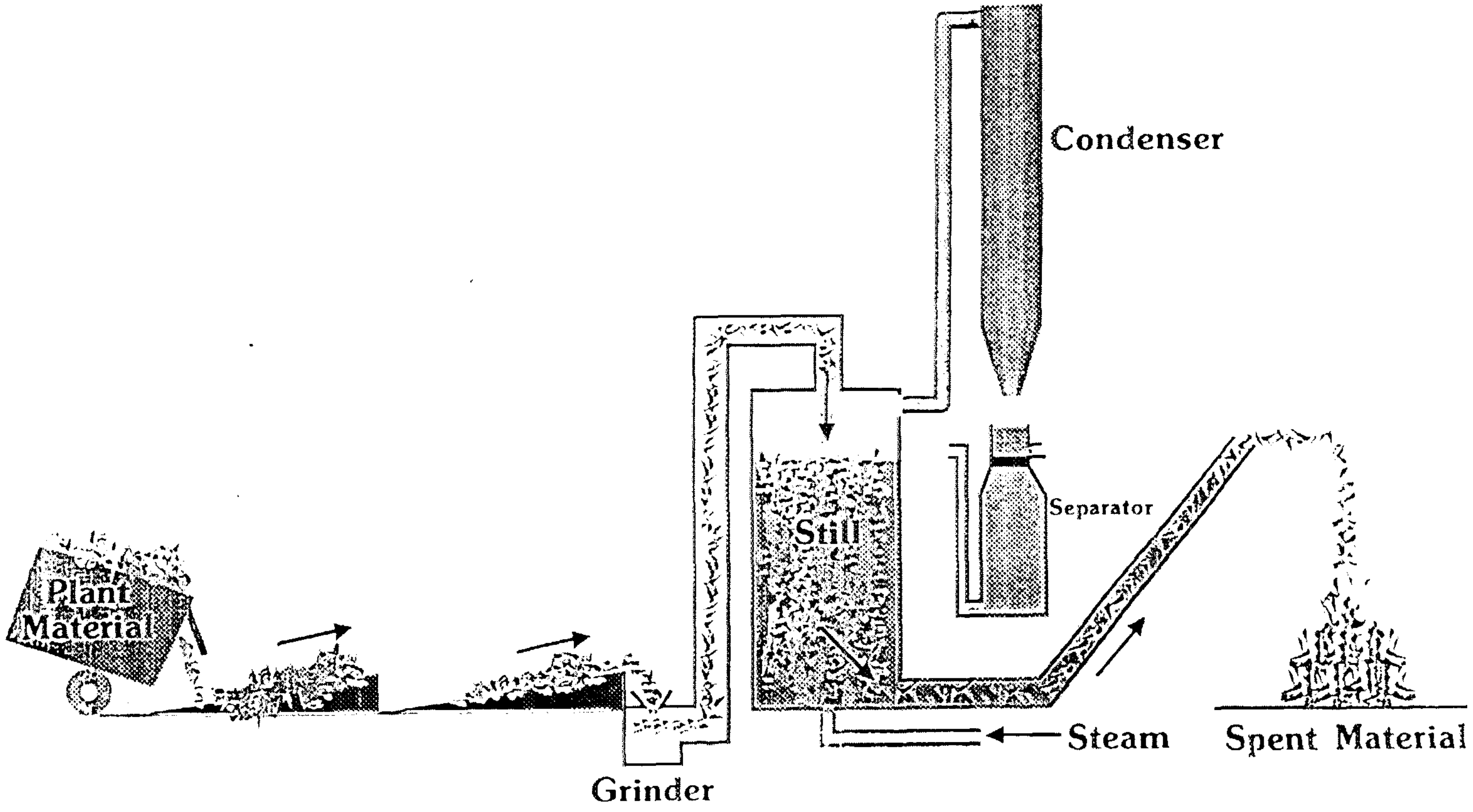


Figure 19: **Diagrammatic representation of Biolandes continuous steam distillation process.**
(Permission to use this diagram was given by J.F. Arnaudo)

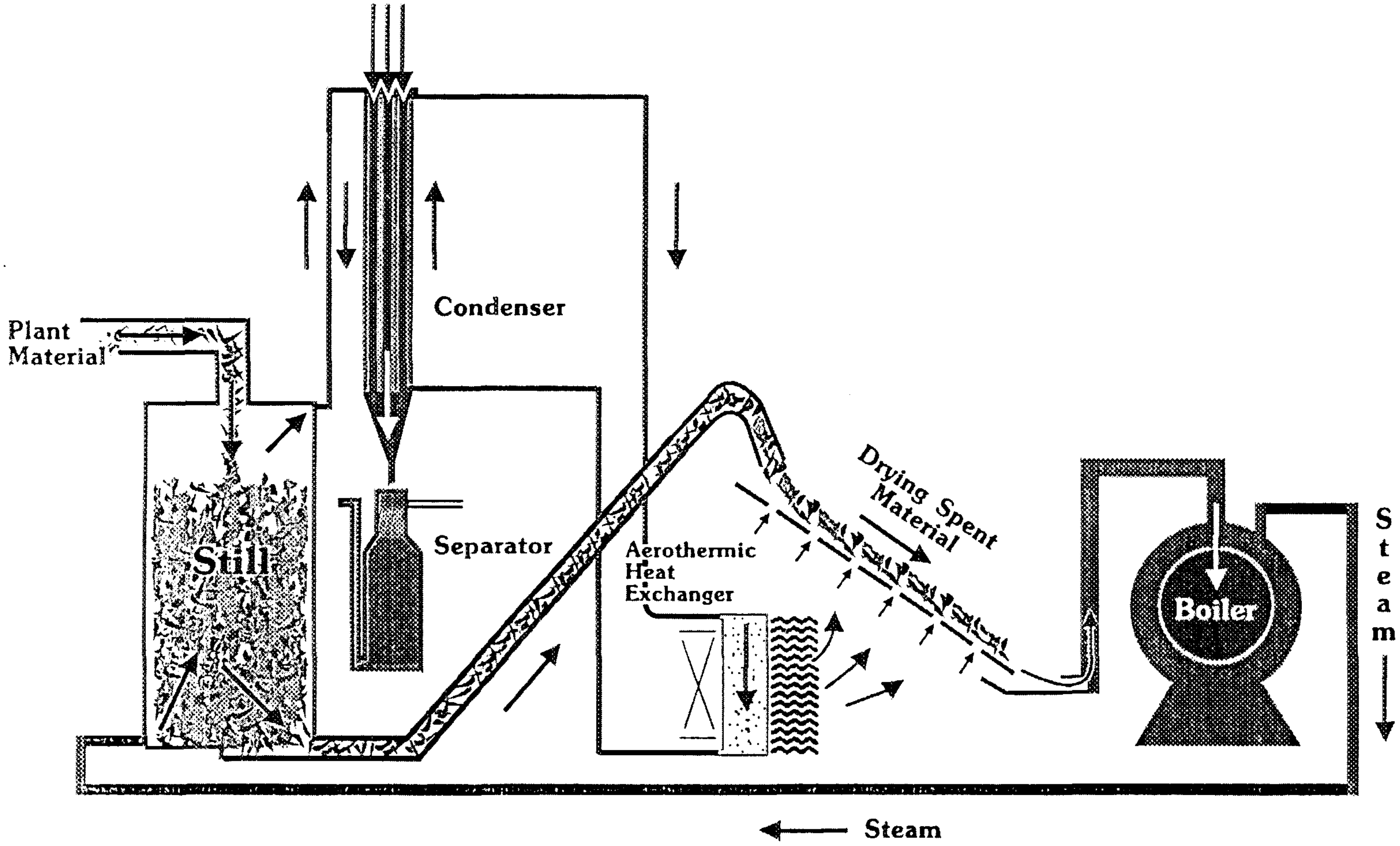


Figure 20: **Diagrammatic representation of the heat exchange system in the Biolandes continuous steam distillation process**
(Permission to use this diagram was given by J.F.Arnaudo)

5. Reduced labor costs because process is automated

6. Improved quality and consistency of oil because process is very reproducible (computer controlled)

The major drawback in establishing continuous distillation is the high capital expenditure for equipment in comparison with that for the conventional equipment; nevertheless, for industrial application it is certainly a very efficient and cost effective way to proceed.Hydrodiffusion

Hydrodiffusion

In 1983, Legast and Peyron first described the technique of oil isolation which was called hydrodiffusion. It was a revelation to see a new steam distillation process that attracted the attention of many scientists. Unlike traditional steam distillation, hydrodiffusion works off the diffusion principle of allowing steam to enter the top of the plant charge and diffuse its way through the charge by gravity. According to Legast and Peyron, the process uses the principle of osmotic pressure to diffuse oil from the oil glands, regardless of whether the glands are surface, subcutaneous or present in fat-rich ducts, onto the surface of the plant material by the condensed steam.

The hydrodiffuser is quite different to the traditional still as it is described by the authors as having a "parallelepipedical" shape which can be seen in Figure 21. The comminuted plant materials placed into the basket within the still walls. The system is connected and low pressure steam is passed into the plant material from a satellite steam generator. The condenser, which is directly under the basket within the still, is of the tube type. The oil and water are collected below the condenser in a typical oil separator. Once distillation is complete, the moveable wall of the still is opened and the charge within the basket is conveyed sideways to discharge the plant material (as shown in Figure 22).

According to Legast and Peyron, and Pellecuer et al. (1983), the value of hydrodiffusion can be summarized as follows:

1. Easy to use, especially the process of charging and discharging the still
2. Can be readily used with well-comminuted plant material
3. Only uses low pressure wet steam
4. Oil yield is higher than that obtained with traditional steam distillation
5. Distillation time is shorter, therefore, it is less costly to run because it uses less steam
6. Hydrolysis of oil components is reduced because of shortened distillation times and the lack of "refluxing" within the still oil produced will always be free from burned notes
7. Physico-chemical parameters of oils produced generally meet national or international specifications.

In 1990, Boelens et al. compared the oil yields and distillation times for a few different plants distilled both by hydrodistillation and hydrodiffusion (see Table VII). From this data it would appear that hydrodiffusion is the most efficient process to use; however, it should be remembered that the hydrodiffusion distillations were performed using a 5OL pilot scale still under ideal conditions, whereas the hydrodistillations were commercial scale steam distillations. Unfortunately, this higher oil yield and reduced steam use that is one of the key promotional factors for the hydrodiffusion system has not been borne out of experience in use of the technique (Dann 1990, Simon et al. 1987).

Simon et al. found that cedarleaf oils produced by hydrodiffusion had an average yield of 0.39%, whereas on steam distillation of a similar charge of similar comminuted cedarleaf, the average yield was 0.68%. Furthermore, they found that the chemical composition of oils produced by hydrodiffusion varied more widely than oils produced by the traditional steam distillation. Simon et al. did, however, point out that the oils produced by hydrodiffusion had a better aroma than those produced by the conventional steam distillation procedures.

Table VII

Comparison Between Hydrodiffusion and Hydrodistillation

Plant Material	Hydrodiffusion		Hydrodistillation	
	Time (h)	% Yield	Time (h)	% Yield
cistus leaves	8	0.15	16	0.05
lavender	0.5	0.73	1	0.75
lavandin	0.5	1.7	1	1.4
cumin	4	5.0	12	3.7
caraway	4	3.6	10	4.5

The fact that the steam and any condensed water flow in a downward motion in a hydrodiffusion process will definitely result in the water dissolving certain non-volatile or less volatile materials such as lipids, chlorophyll, fatty acids, coumarins, and psoralens, etc., resulting in oils which contain low levels of components not found in steam distilled oils. Although the lack of burned notes or "still notes" is a positive attribute for hydrodiffused oils, the occurrence of trace components such as eluted chlorophyll could promote oxidation in the oil and thereby have a detrimental effect on the shelf life. Similarly any eluted fatty acids could promote hydrolytic or trans-esterification reactions, which would also have a detrimental effect on the oil. Finally, any eluted coumarins and psoralens could act as skin sensitizers (a problem for perfume use) and add to the biological activity of the oil. Nevertheless, it is quite possible that hydrodiffusion could well be of value in processing very dry materials such as comminuted seeds/fruits (Denny, 1991).

Empyreumatic Distillation

As the name suggests, this empyreumatic distillation process, which is only used to produce birch tar oil and cade oil, is a thermal degradation process. According to Guenther (1952), for the production of cade oil the trunk wood, branches, and roots of Juniperus oxycedrus L. are cut into pieces which are stacked on a concave slab from the center of which a pipe leads down to a collector. An iron pot just above the stacked wood is filled with coal and burned so that it glows red. As a result of this heat, the wood releases its essential oil which, because of the extreme heat, thermally decomposes to some extent. As the wood chars readily, it releases its own pyroligneous substances which combine with the oil to form a viscous homogenous dark brown liquid which possesses a strong smoky odor.

Recently, it was shown by Chalchat et al. (1990) that many sesquiterpene hydrocarbons survived the drastic distillation conditions. As a result, the amount of oil decomposition was not as great as once thought, except that certain sesquiterpene hydrocarbon rearrangements did occur. Finally, it is of interest to note that French cade oil is produced from Juniperus oxycedrus whereas Spanish cade oil is produced from J. phoenicea L. or J. sabina L.

Maceration Distillation

Certain plants require maceration in warm water prior to the release of their essential oil because their volatile components are glycosidically bound. These include bitter almond kernels, onion, garlic, mustard seed, wintergreen leaves, and sweet birch bark and leaves. A summary of the enzymatic reactions (Guenther 1952, Block 1985) needed to release these "bound" oils can be seen in Table VIII.

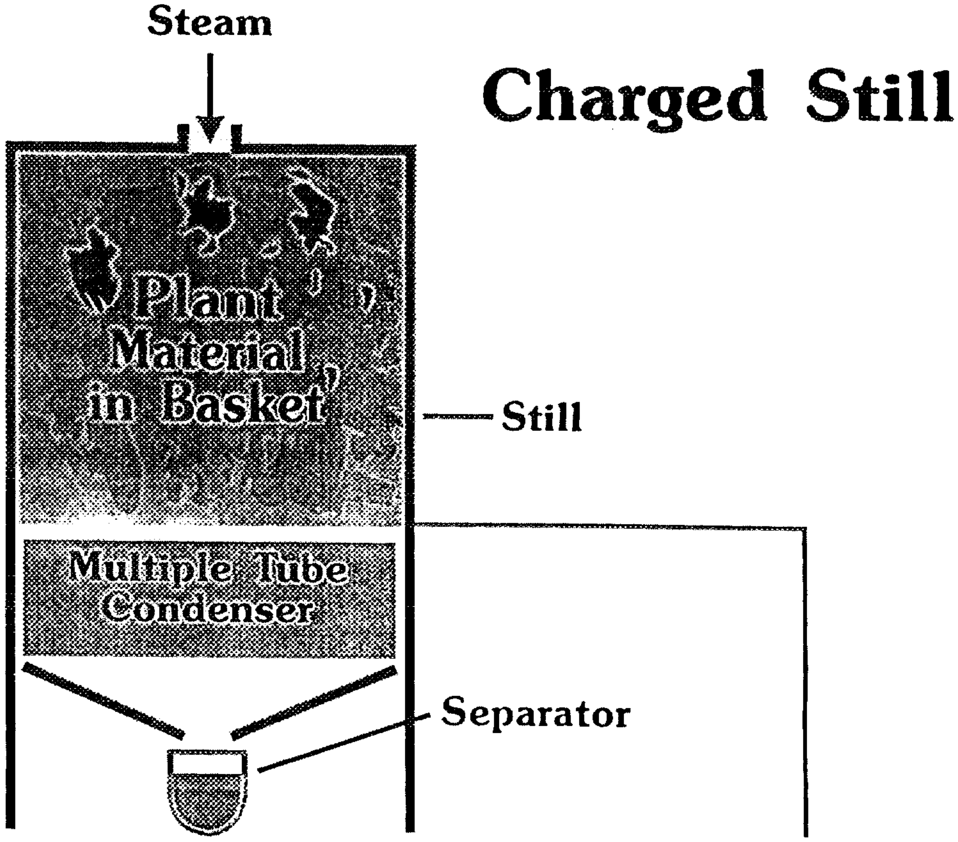


Figure 21: Diagrammatic representation of the hydrodiffusion distillation process

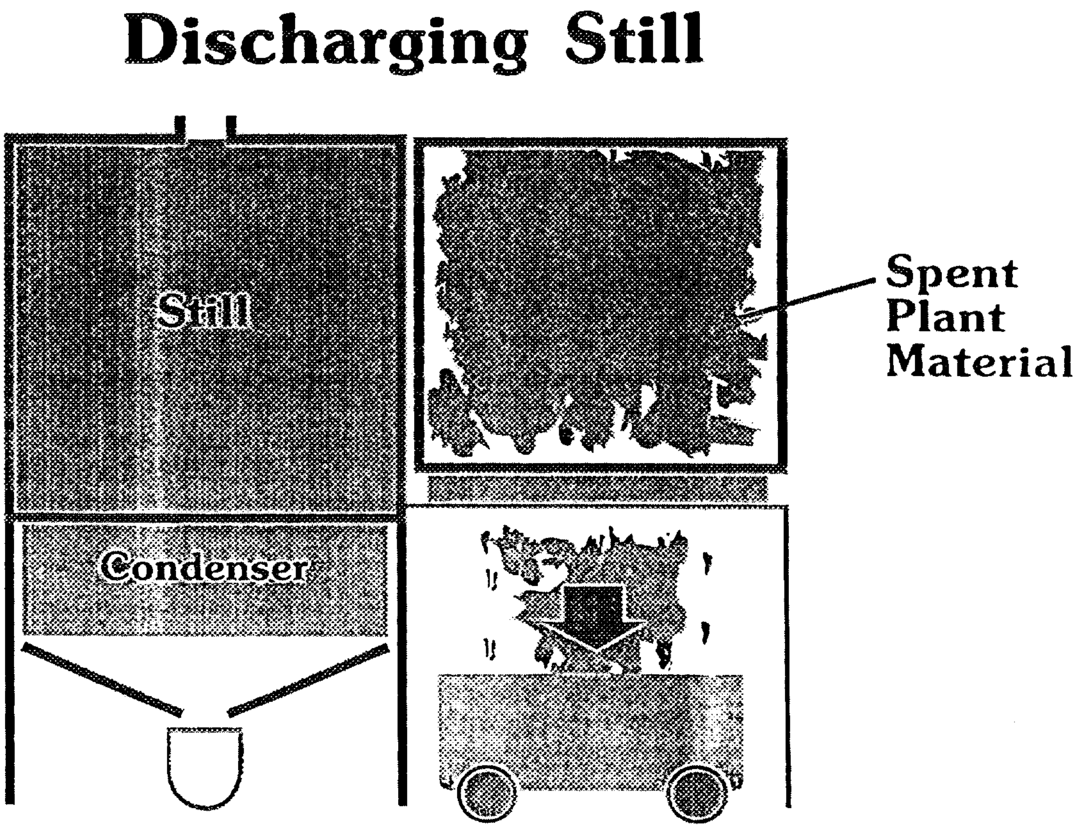


Figure 22: Diagrammatic representation of the discharge of spent plant material in the the hydrodiffusion process

A number of years ago, the production of wintergreen oil was a small cottage scale industry. There were small stills all over the Appalachians in the southeastern USA producing wintergreen oil, while in eastern Pennsylvania and South Carolina, birch oil was being produced (Lawrence 1979). Because of the great difficulty and cost of collecting wintergreen (Gaultheria procumbens), a plant which grows along the ground, production of oil in the United States has been discontinued. An oil of wintergreen is being produced in China, although to date little is known about the botanical origin of the oil other than it is supposed to come from a Gaultheria species. This author has no experience in the production of the other oils produced by maceration so they will be left out of this discussion.

Expression

Expression or cold pressing, as it is also known, is confined to citrus oils. It is a description of any physical process in which the essential oil glands in the peel are crushed or broken to release the oil. One method that was practiced many years ago , particularly in Sicily (Spugna method), commenced with halving the citrus fruit followed by pulp removal with the aid of a sharpened spoon-knife (known as a rastrello). The oil was removed from the peel, either by pressing the peel against a hard object of baked clay (known as a concolina) which is placed under a large natural sponge, or by bending the peel into the sponge, thereby releasing the oil. The oil emulsion, which was absorbed by the sponge, was removed by squeezing it into the concolina or some other container. It is reported that the oil produced this way contains more of the fruit odor character than oil produced by any other method.

A second method known as equelling (or the scodella method), uses a shallow bowl of copper (or sometimes brass) with a hollow central tube making the whole equelling tool similar in shape to a shallow funnel. The bowl is equipped with brass points with blunt ends across which the whole citrus fruit is rolled by hand with some pressure until all of the oil glands have been burst. The oil and aqueous cell contents are allowed to dribble down the hollow tube into a container from which the oil is separated by decantation. Obviously, hand pressing is impractical because it is an extremely slow process. For example, on the average only 2-4 lbs. of oil per day can be produced by a single person using one of these hand methods. As a result, over the years a number of machines have been designed to either crush the peel of a citrus fruit or crush the whole fruit and then separate the oil from the juice.

Today there are four major processes that are used to isolate citrus oils. These are Pellatrice, Sfumatrice, FMC extractor, and the Brown Peel Shaver. A summary of each of these processes will be discussed separately. Other processes known as Pipkin roll press, screw press, Fraser brace excoriator, AMC scarifier, etc. (Guenther, 1949) have been used in the past but are rarely used today. The majority of orange oil produced is with the FMC in line extractor. In North and South America the FMC process and the Brown process are by far the most widely used, whereas in Italy the use of Pellatrice and Sfumutrice processes are more widely used.

Pellatrice Process

In the Pellatrice process, citrus fruits are fed from a hopper into the abrasive shell of the machine. The fruits are rotated against the abrasive shell by a slow moving Archimedian screw whose surface rasps the fruit surfaces causing some of the essential oil cavities on the peel to burst and release their oil water emulsion (Figure 23). This screw further transports the fruit into a hopper in which rollers covered with abrasive spikes burst the remaining oil cavities. The oil and water emulsion is washed away from the fruit by a fine spray of water. The emulsion next passes through a separator where any solids are removed, after which it passes through two centrifugal separators working in series to yield the pure oil (Arnaudo, 1991). Most bergamot oil and some lemon oil is produced this way in Italy.

Table VIII

Summary of the Oils Produced by Enzyme Release

Plant	Precursor		Enzyme	Aromatic Product
Wintergreen	gaultherin (ormootropiside)	+	primeverosidase + H_2O	methyl salicylate + primeverose
Bitter Almond	amygdalin (mandelonitrile gentiobioside	+	emulsin + H_2O	benzaldehyde + glucose + HCN
Brown Mustard	sinigrin (potassium myronate)	+	myrosinase + H_2O	allyl isthiocyanate + glucose + $KHSO_4$
Onion	onion alliin (mixed s-alkyl cysteine sulphoxide)	+	allinase	dipropyl disulphide + propionaldehyde (major)
Garlic	garlic alliin (s-allyl cysteine sulphoxide)	+	allinase	diallyl disulphide (major)

Sfumatrice Process

Initially the fruits are cut into halves, after which they are subjected to a rotating separator where the pulp and juice are removed from the peel. The peels are then conveyed into a lime bath where they remain for 24 hours (Arnaudo, 1991) to enable the peels to harden. In addition, the lime will neutralize the peel, thereby preventing any acid catalyzed hydrolyses or decomposition of oil components. The Sfumatrice equipment consists of a metallic chain that is drawn by two horizontal ribbed rollers. The peels are conveyed through these rollers during which time they are pressed and bent to release their oil (Figure 24). As in Pellatrice, the oil is washed away from the Sfumatrice rollers by fine sprays of water. Again the oil is initially passed through a separator prior to being sent to two centrifuges in series, so that purified oil can be produced. At one time Sfumatrice was the most popular process for citrus oil isolation in Italy; however, today the Pellatrice method appears more popular.

FMC Juice Extractor

The FMC extractor allows for the simultaneous isolation of both the oil and the juice. Initially the fruit is sized after which each fruit is processed by being positioned on the lower half of a fixed cup in which metallic fingers are interlocked as the top cup forces down on the bottom cup crushing the fruit. The cups containing the fruit are mobile and are actuated by the vertical reciprocating action of the metallic fingers. As the upper cup moves down on the lower cup holding the fruit, a citrus peel disc is cut from the bottom of the fruit so that the juice exits the fruit from this hole that is pierced by a tube which cores the fruit. As the metallic fingers press the fruit, the oil is released and washed away from the crushing fruit by strong water jets that are emitted from the upper mobile cups (see Figure 25). The oil is, therefore, ingeniously kept separate from the juice. Finally, the oil is isolated by the use of centrifugal separators. As mentioned earlier, a majority of the orange oil produced in the world is produced by the FMC extractor.

Brown Peel Shaver

Initially the fruit is fed into the extractor where it settles between pairs of rollers. The fruit moves in a single layer across a bed of rollers covered with needle-like projections mounted in a shallow tank all moving in the same direction. The rollers alternate between high and moderate speed. The entire surface of the fruit is punctured because the rollers not only rotates it vertically but also rotates it horizontally. The released oil water emulsion is washed away from the fruit by a countercurrent flow of water. After passing through a solids separator the oil is removed from the water by centrifugal action.

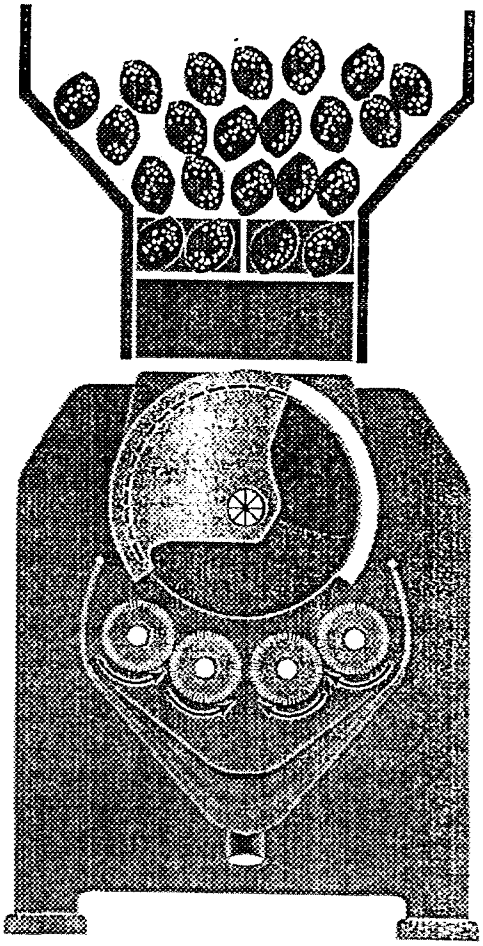


Figure 23: Diagrammatic representation of Pellatrice Citrus Oil expression process

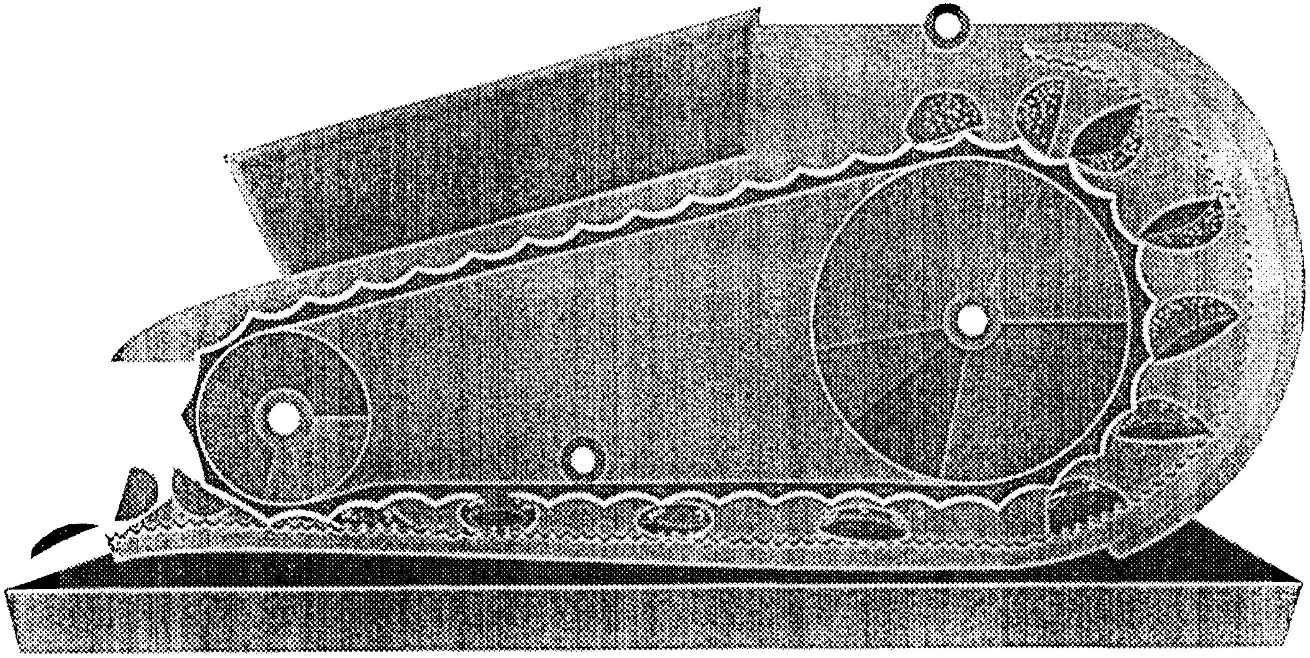


Figure 24: Diagrammatic representation of the Sufmatrice Citrus Oil expresssion process

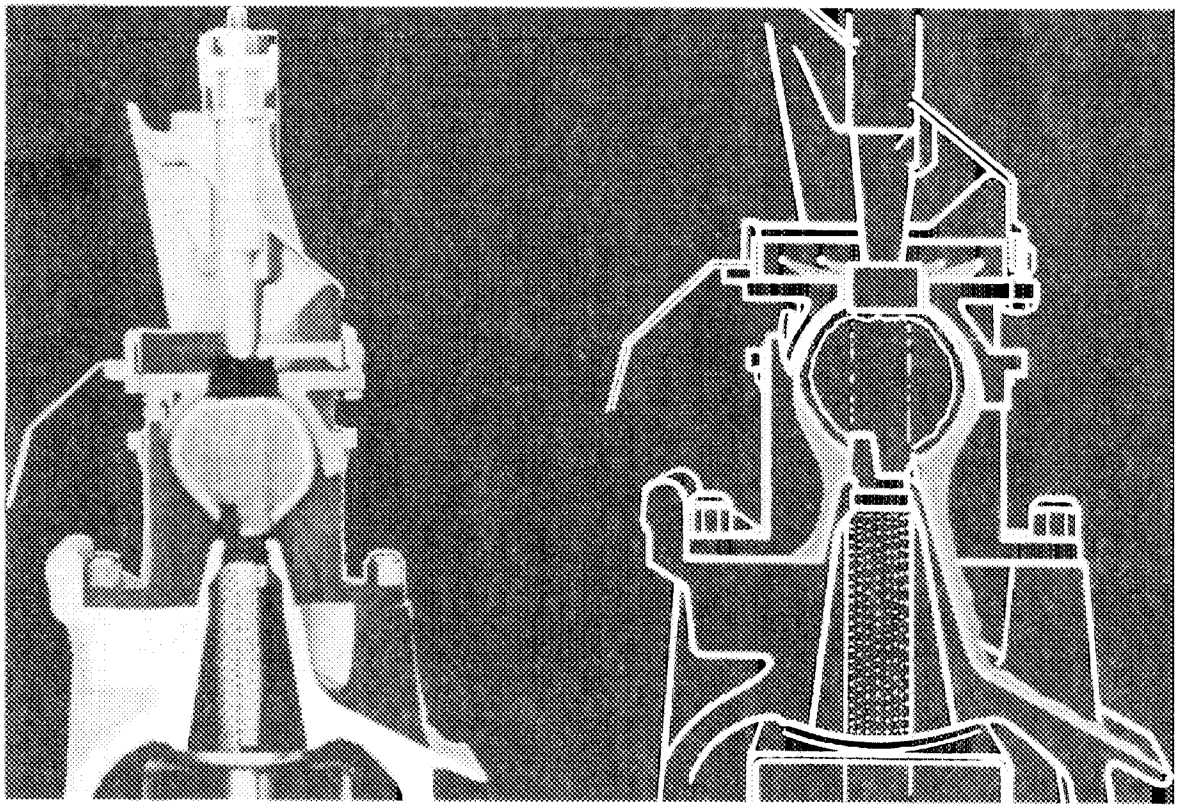


Figure 25: Photograph of the oil expression process used in the FMC juice extractor process (Permission to use this copyrighted photograph was given by Jose Flores, FMC)

3. EXTRACTION OF NATURAL ISOLATES

Natural isolates can be obtained from essential oils by fractional distillation. Compounds such as β-caryophyllene and eugenol are isolated from clove leaf oil for further processing into other compounds requiring the same or similar base skeletons. Compounds such as caryophyllene oxide and caryophyllene alcohol are produced from β-caryophyllene, while eugenol acetate, methyl eugenol, and other ethers, as well as isoeugenol and isoeugenol esters and ethers, are produced from eugenol that has been converted to isoeugenol.

Other components that are isolated from essential oils by fractional distillation are linalool, geraniol, neral/geranial, methyl chavicol, anethole, etc. In addition, one of the most important sources of monoterpenoid components is turpentine oil from which a wide variety of compounds are obtained by fractional distillation for sale as is or as starting materials for a variety of syntheses. Finally, menthol, an important flavor additive, is isolated from cornmint oil by freeze crystallization.

Biotechnology

Over the past few years the requirement for natural components for use in flavors and, to a lesser extent in fragrances, has dramatically increased. This has been driven by the consumer product marketing departments who want to claim "natural" on their product; a claim which has important consumer appeal. To satisfy the need for both more and a wider range of natural materials than can be obtained from essential oils, fermentation and other enzymatic reactions of natural substrates have been used to a wide extent to fill the void. This latter area of work has become known as biotechnology; an all encompassing word that covers everything from gene transferred protoplast fusion to fermentation and the reactions of microorganisms (Gatfield, 1988).

As the actual production of natural materials from enzymatic reactions is outside of the scope of this treatise, the reviews of Drawert and Berger (1981), Jourdain et al. (1985), Gatfield (1986), Armstong and Brown (1994) and Seitz (1994) adequately summarize the work in this field.

Another area of biotechnology that is of interest to the producer of isolates from natural materials is the use of tissue or callus culture of plant fragments, to produce a component of the oil or the full oil itself in a sterile environment such as in a fermentation chamber. Although work on this topic has been underway for many years (Suga and Hirata 1990), to date nobody has successfully produced a flavor or fragrance raw material in a cost effective way to allow the process to become commercialized. In addition to the article by Suga and Hirata, other reviews by Whitaker et al. (1986), Collin (1988), and Sahai (1994) have summarized the current state of the art.

Deterpenation

For the most part essential oils can be thought of as being complex mixtures of naturally occurring polar and non-polar compounds. The polar compounds are alcohols, esters, ketones, aldehydes, oxides, ethers, lactones, etc. of the monoterpene (C_{10} compounds), sesquiterpene (C_{15} compounds), aromatic (compounds possessing an aromatic ring), and aliphatic classes; whereas, the non-polar compounds are hydrocarbons of the same above-listed classes. The proportion of polar and non-polar compounds in an essential oil can vary considerably depending upon the botanical origin of the oil.

Within essential oils, even though the non-polar compounds may be present in the largest proportions, they along with some nitrogen containing compounds, are mainly responsible for the characteristic aroma associated with the essential oil. In addition, the polar compounds, which are mainly oxygenated, are, with the exception of some aldehydes, much more soluble in alcohol (ethanol) and its aqueous solutions. On the other hand, the hydrocarbons or non-polar compounds are by their nature unsaturated and, therefore, they tend to polymerize (resinify) or oxidize on exposure to air and light. This deterioration of the non-polar constituents results sometimes in a very pronounced off-odor that can completely mask the original odor of the oil. The resulting oil will also have a lower solubility in alcohol and its aqueous solutions.

To make the oil more soluble in aqueous media the hydrocarbons have to be removed. This process is known as deterpenation, or the end products of it are known as terpeneless oils. Deterpenation is a somewhat loose term as it can mean:

(a) some or all of the monoterpene hydrocarbons have been removed
(b) the monoterpene and sesquiterpene hydrocarbons have been removed (sometimes this oil is called a "sesquiterpeneless" oil)
(c) all or nearly all of the hydrocarbons or non-polar compounds have been removed.

If a citrus oil has a portion of its hydrocarbons removed, it is referred to as a folded oil. This folding process is carried out to improve the stability of the oil by making it less susceptible to oxidation, to improve the solubility of the oil in polar solvents such as low-proof alcohol, food solvents, or aqueous solutions, to concentrate the "aroma" properties of the oil, and to reduce the storage and transportation costs. The folding of a citrus oil depends on the level of oxygenated constituents in the oil; however, it can be determined in a similar way as in the following example:

100 lbs. orange oil contains 96% monoterpene hydrocarbons (MTHC)
If MTHC removed we can get:

50 lb. oil	50 lb. MTHC	=	2 fold
10 lb. oil	90 lb MTHC	=	10 fold
5 lb. oil	95 lb. MTHC	=	20 fold
1 lb. oil	99 lb. MTHC*	=	100 fold
2 lb. oil	98 lb. MTHC*	=	50 fold
4 lb. oil	96 lb. MTHC	=	25 fold

* not possible because the oil contains only 96% MTHC; therefore, the maximum concentration for this orange oil is 25 fold.

The commercial processes used to remove hydrocarbons from an oil have a direct relationship as to which non-polar compounds are removed. The processes that are used commercially are as follows:

Fractional distillation under vacuum extraction:

(a) single solvent
(b) two-phase solvent system
(c) supercritical CO_2

Solid phase carrier extraction:

(a) adsorption chromatography
(b) polymeric adsorbent
(c) poroplast extraction

Combination processes

Other processes

Fractional Distillation

High vacuum fractional distillation practiced in a batch-wise process is still the most frequently used method for deterpenifying an oil. The process is, however, quite challenging for the chemical engineer because in theory, separation of one or more compounds from a mixture is only optimized if the vapor/liquid ratio is kept in equilibrium and in batch distillation this is not possible (Ellis and Freshwater, 1954). During distillation the composition of the mixture in the pot (kettle) and the vapor/liquid mixture in the column are in a state of flux, constantly changing with time. As a result, there are three major parameters that are varying, e.g.

(a) the unit charge composition
(b) the physical distribution of the charge during distillation
(c) the time that it takes to achieve equilibration in the system before any product has been withdrawn.

Typically the chemical engineer has to obtain vapor/liquid equilibrium data from which the number of theoretical plates necessary to obtain the required separation can be calculated. From this information, the column dimensions, the pot size and the optimum reflux ratio to produce product at a desired rate whose specifications meet those of the desired commercial product (Ellis and Freshwater, 1955) can be obtained. A typical design for equipment can be seen in Figure 26.

For deterpenation, batch fractional distillation is preferable to continuous distillation because, in addition to it being much less capital intensive, the throughput of oil is probably not large enough to warrant such a complexity. Also, it is flexible in that a number of different fractionations can be performed on the same equipment. Little or no changes are needed when changing product mixes to be fractionated because, although the pot size column dimensions and packing are fixed, the reflux ratio (the amount removed vs. the amount retained in the column) and throughput can be easily varied.

In vacuum fractional distillation (fractionation), the vacuum determines the boiling point of the mixture, i.e., the higher the vacuum the lower the boiling point. Also, as the pot temperature is lowered there is a corresponding decrease in off-notes or burnt notes of the distillate. Typically, a vacuum of 1mm of Hg or less is used for deterpenation.

As a rule of thumb, the column should have an internal diameter of greater than 3 inches. Also, for efficient packings one can expect 1 HETP for every 7 inches of column height. Hence, for a 3 inch column, which is 14 feet in height about 20 theoretical plates would be expected. If an accurate measure of the number of theoretical plates is needed, then this can be calculated by use of the Fenske formula (Eckert. 1979):

$$\frac{X_A}{Y_A} = \alpha^{(n-1)} \frac{X_B}{Y_B}$$

Where X_A = mole fraction of the more volatile component at still head.

X_B = mole fraction of same components in still pot.
Y_A = mole fraction of least volatile component at still head.
Y_B = mole fraction of same component in still pot.
α = relative volatility (ratio of vapor pressures at boiling point)
n = number of theoretical plants

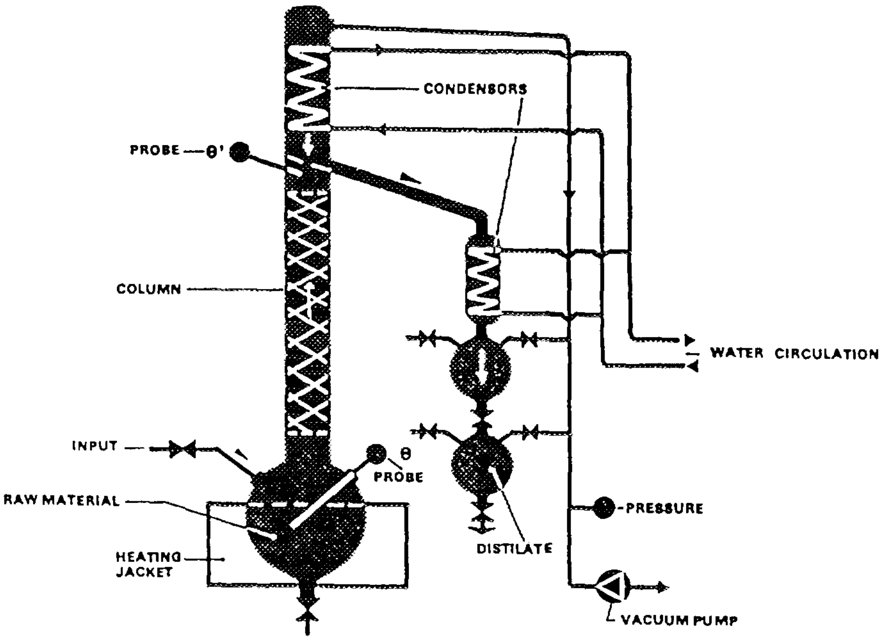


Figure 26: Diagrammatic representation of fractional distillation process

For vacuum fractionation, packed columns are recommended because they are more efficient than the fixed plate columns and offer a great more versatility. According to Ellis and Freshwater (1955), the growing demand for more highly purified products has led to the development of a large number of column packings. A list (Eckert 1979) of the most well-known ones can be seen as follows:

High efficiency high pressure drop (HEHPD)
High efficiency low pressure drop (HELPD)
Rachig rings
Goodloe
Berl saddles Hyperfil
Pall rings Neo-Kloss
Intralox saddles Koch-Sulzer

For deterpenation, the recommended column packing is Koch-Sulzer. This is a cylindrical or cylinder segmented section of exact column dimensions made out of parallel corrugated strips of stainless steel woven wire fabric. The packing comes in sections which are about 6.7 inches thick and are stacked on top of each other to fill the column.

The Koch-Sulzer column is extremely efficient with an extremely low pressure drop. The value of the low pressure drop is that the higher the pressure drop the higher the pot temperature. As the pot temperature for deterpenation needs to be as low as possible, then low pressure drop column packings are a must.

A survey of the literature reveals that many authors (Naves 1947, Jacobs 1952, L'Eplattenier 1952, Broderick 1955, Fleisher 1994) do not favor fractional distillation as the best method to deterpenify an oil even though it is the most widely practiced commercial technique. According to Fleisher, it is the most commonly used method for deterpenifying or folding a citrus oil because it is the process with the lowest cost, and because the odor profiles of oils produced this way are well accepted in the flavor and fragrance industry.

Because the vapor pressures of the monoterpene hydrocarbons are very close, it is not possible through simple fractionation to completely deterpenify a citrus oil; however, it is possible to fold the oil or reduce the hydrocarbon content substantially. Similarly, because of the similarity in boiling points of oxygenated monoterpenes and sesquiterpene hydrocarbons, it would not be possible to design a column that could remove the monoterpene and sesquiterpene hydrocarbons from a citrus oil and not remove any of the aliphatic and monoterpene oxygenated compounds. Therefore, in summary, it can be stated that fractional distillation is a useful technique to reduce the level of monoterpene hydrocarbons in an oil.

Rotating or static thin film evaporators (Tateo, 1990) have also been used to reduce the monoterpene hydrocarbon content of an oil. A few years ago, Vora et al. (1983) was able to readily produce a 10-fold and a 25-fold orange oil using a wiped film evaporator. The thin film evaporators have the advantage of subjecting the oil to a low residence time into the heating zone, thereby, minimizing any thermal degradation reactions.

Extraction

(a) **Single solvent extraction**

It is well known that mono- and sesquiterpenes are much less soluble in alcohol than are the oxygenated constituents; with the solubility of these latter compounds increasing in the following order: ether and oxides < esters < ketones < aldehydes < alcohols. As a result, alcohol washing can be used to remove the oxygenated compounds of an oil. The process of removing the alcohol is, however, more difficult requiring a series of brine washings followed by a careful fractional distillation to remove all of the alcohol. The main advantage of this method is that the resultant oil is free of both mono- and sesquiterpene hydrocarbons. The process is quite inefficient, however, requiring a lot of art to control it. Also, the use of alcohol to deterpenify oils such as citrus oils, where aldehydes are the important aroma components, is not recommended. This is because of the ease of acetal formation resulting in a change in odor and composition of the terpeneless oil.

(b) **Two-Phase Solvent System**

In 1937, Dijck and Ruys described the removal of terpenes from an essential oil by the use of a binary two-phase solvent system in a countercurrent fashion. The solvents chosen for this process were pentane and alcohol. A schematic representation of this system can be seen in Figure 27. The oil is introduced into the center of a tube, within which can be found pairs of inert mesh spacers, between which can be found individual stirrers that are connected to and rotated by a central motor. The apparatus is constructed so that the tube is on a slight incline, thereby allowing the alcohol to flow in a countercurrent way with gravity while the pentane is introduced under pressure. During extraction, the hydrocarbons of the oil dissolve in the pentane and the oxygenated compounds dissolve in the alcohol. Each fraction must then be concentrated to remove the solvent. This is done by fractional distillation. The oils produced this way are free from monoterpene and sesquiterpene hydrocarbons. In their original report, Dijck and Ruys showed that this technique could be used to successfully deterpenify lemon, orange, and ginger oil (two oils rich in monoterpene hydrocarbons, and one rich in sesquiterpene hydrocarbons).

The technique of deterpenifying an essential oil, in particular a citrus oil by the use of a two-phase solvent extraction, has been perfected commercially, and certain manufacturers sell terpeneless oils produced this way (L'Eplattenier 1952, von Campe 1990). In these processes, and that of Dijck and Ruys, it is important to ensure the following:

1. The two solvents may not be mutually soluble so the extraction is specifically two-phase
2. For good extraction the phases must be very well mixed
3. Solvents must have different polarities so that non-polar compounds dissolve in non-polar phase and vice versa
4. If emulsions are formed they should be readily broken
5. Extraction should take place in a countercurrent fashion
6. Extraction should take place at the lowest temperature possible
7. During extraction, oxygen and air must be completely eliminated to prevent oxidation.

The use of aqueous alcohol as an extraction solvent system to deterpenify citrus oils has been discussed by Littlejohn (1954) and later by Arctander (1960); a description of Arctander's recommended method for oil deterpenification will be presented as a mixed process. However, in 1986, Owusu-Yaw et al. mixed orange oil with either 60% or 70% aqueous alcohol at the oil to alcohol ratios of 1:3, 1:7 and 1:15.

The mixture was allowed to continuously stir at 2°C for 6 days. Each mixture was then placed in a separatory funnel and the aqueous alcohol plus oxygenated orange oil constituents were separated from the insoluble mono- and sesquiterpene hydrocarbons. A survey of the results revealed that the best deterpenified oil was obtained by using oil:alcohol ratio of 1:7 and 70% aqueous alcohol. Although the 1:7 level achieved the highest monoterpene hydrocarbon reduction, it also had the highest total aldehyde content.

The technique of Owusu et al. has been put into commercial practice by Moyler and Stephens (1992). They indicated that the density of the two phases used in this countercurrent extraction of citrus oils using aqueous alcohol was sufficient to enable simple rapid phase separation. They achieved this by ensuring that the density differential between their two phases was less than 0.04 g/mL. Using the apparatus that can be seen schematically represented in Figure 28, the authors were able to reduce the limonene content of orange oil from 95% to less than 0.5%.

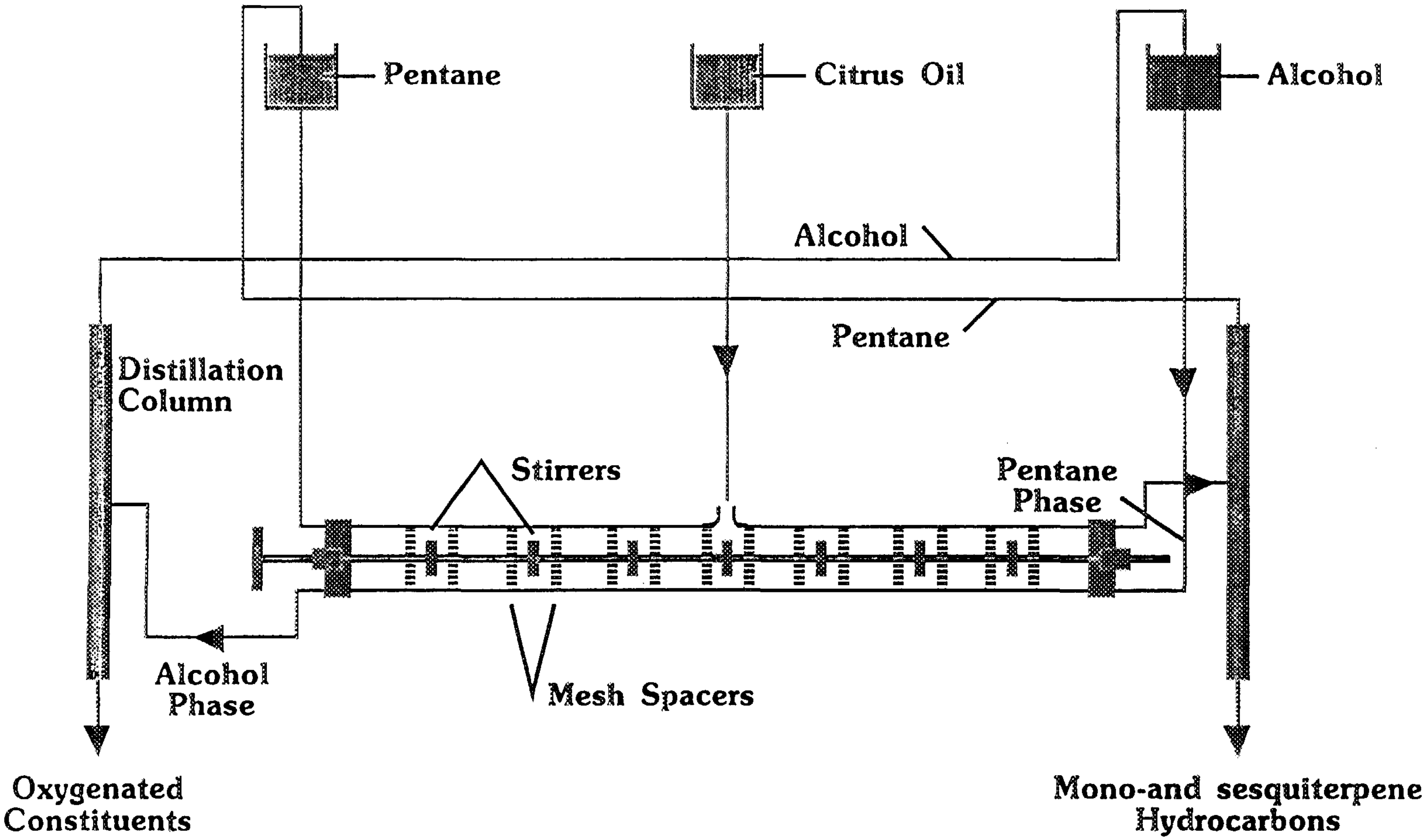


Figure 27: Diagrammatic representation of a two-phase solvent system countercurrent deterpenation of citrus oil.

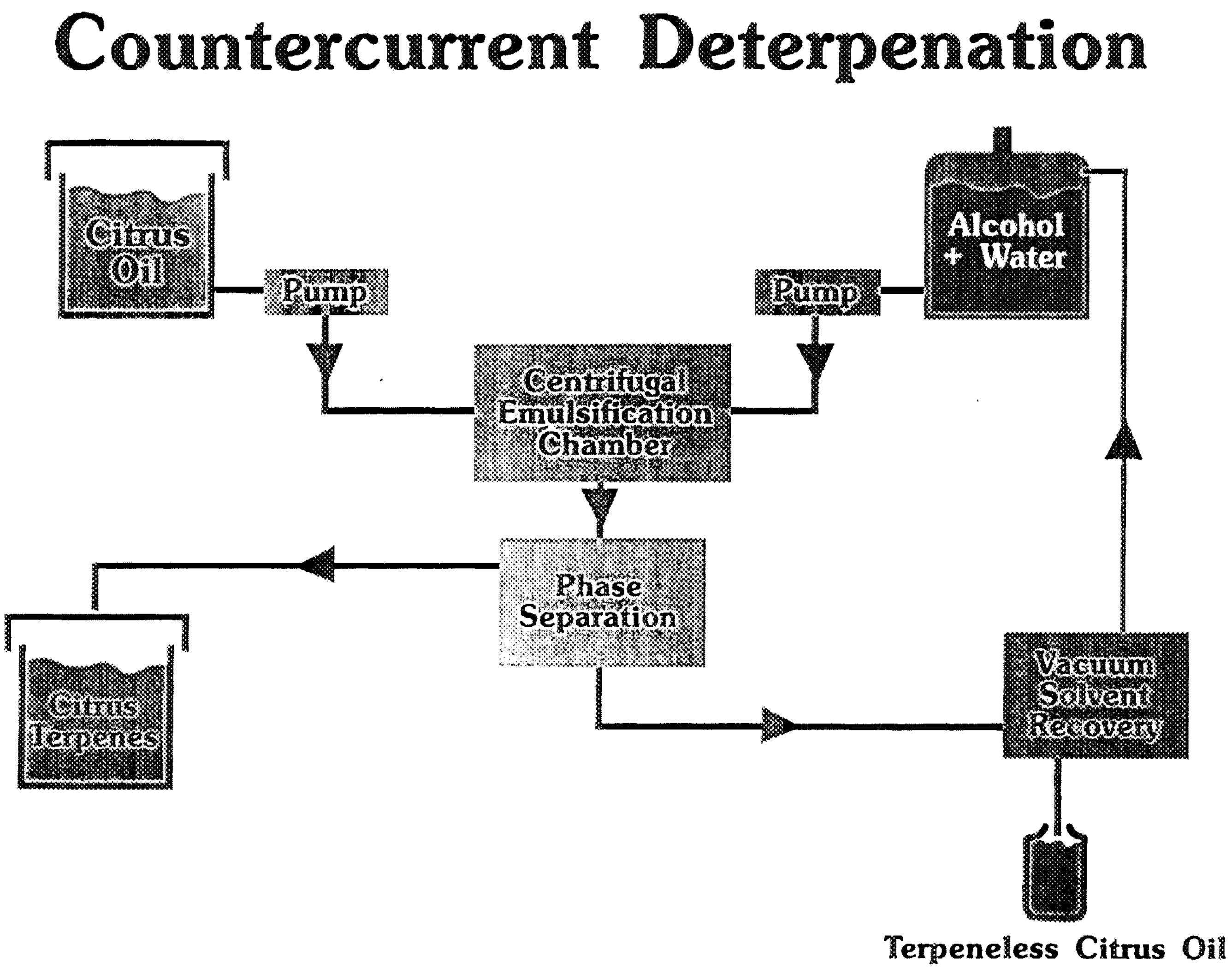


Figure 28: Diagrammatic representation of a second two-phase solvent system counter current deterpenation of citrus oil using aqueous ethanol as one of the solvents
(Permission to use this diagram was given by D.A.Moyler)

(c) **Supercritical CO_2 Extraction**

In 1984, Gerard first described the use of liquid CO_2 as an extraction solvent for the deterpenification of citrus oils. Temelli et al. (1988) used supercritical fluid (SCF) CO_2 to concentrate the oxygenated constituents of a sample of orange oil; however, they believed, at that time, that the process was not advanced enough for large scale commercialization. More recently Barth et al. (1994) used SCF to stepwise remove the hydrocarbons, waxes, coumarins, and psoralens from lemon oil that were coated on a column of kiesel gel adsorbent. To achieve these results, the authors performed the desorption process by, in a stepwise way, increasing the density of the CO_2 used to elute the various fractions. Using a similar approach, Dugo et al. (1995) used SCF CO_2 to elute the hydrocarbons from a silica gel column to which either lemon or orange oil was added. The silica gel retained the oxygenated constituents while the hydrocarbons could be eluted at low temperature and low pressure. Elution of the oxygenated constituents could be achieved by increasing the temperature and pressure of the CO_2.

The value of this SCF CO_2 process is that CO_2 is a readily available non-flammable, easily removable, non-toxic solvent. As the process is operated a low temperature, the resultant terpeneless oils are free from process induced chemical changes other than hydrocarbon removal.

Solid Phase Carrier Extraction

(a) **Absorption Chromatography**

In 1952 Kirchner and Miller showed that by using silica gel and a non-polar eluent, the hydrocarbons of an oil could be easily separated from the oxygenated constituents. Since then numerous scientists (Braverman and Solomiansky 1957, Shankaranayarana et al. 1977, Ferrer and Matthew 1987, Tzamtzis et al. 1990) have discussed the merits of the technique as a commercially viable process. The latter authors showed that, using the silica gel procedure, any amount of deterpenation and recovery of terpeneless oil could be achieved. In addition, they presented an empirical equation connecting the feed rate of oil, the degree of deterpenation, and the recovery of terpeneless oil for orange and lemon oil where the amount of deterpenation exceeded 50%. Unfortunately, they did not discuss the commercial practicality of the process.

In 1990, Tzamtzis et al. examined a modification of adsorption chromatography on which the column separation was performed under constant low pressure. They found that the amount of oil to adsorbent ratio was a necessary parameter that had to be monitored because he used a fixed eluent flow under pressure. For example, for orange oil (which is low in oxygenated constituents), an oil to adsorbent ratio of 12:1 could be used, whereas with oils like lavandin whose oxygenated constituents are in excess of 80%, an oil to adsorbent ratio of 0.8:1 was the maximum that could be used. Even though the authors showed that this was a successful laboratory practice, it is doubtful whether it would be successfully scaled up to commercial scale.

(b) **Polymeric adsorbent**

In 1981, Tateo was able to demonstrate that an aqueous ethanolic solution of lemon oil could have the hydrocarbons removed from it, if it was passed through a column of food grade Kastel S 112 (a non-polar co-polfrom a lab to a pilot scale process with great success. He further noted that the process could be readily set up continuously and that production times were reduced from normal countercurrent extractions of aqueous alcohol solutions of citrus oils.

(c) Poroplast Extraction

This technique is based on the concept of liquid-liquid extraction where one of the solvents is held on a non-adsorptive stationary phase (Fleisher 1994). A teflon "sponge" is used as the stationary phase and is initially coated with the citrus oil. The oil is eluted with aqueous alcohol which pushes the oil through the column distributing the hydrophilic compounds onto the hydrophilic repellent teflon in a layer of ca 1 micron in thickness. Coincidentally, the oxygenated compounds are dissolved in the aqueous ethanol leaving the hydrocarbons behind on the column. A schematic representation of this process, which is in commercial production, can be seen in Figure 29. Initially, the first extraction (poroplast) column is charged with an oil after which it is displaced with aqueous ethanol. This process is carefully cycled so that the amount of aqueous alcohol just displaces the oil and vice versa. No emulsions are formed because separation takes place at the interface. The liquid phases possess specific gravities that are very similar (difference 0.005 g/mL) and are readily separated after elution from the column.

After reduction of the alcohol in the aqueous alcohol phase to ca. 30%, the recovered alcohol is returned to the process. The aqueous dispersion of oil is then passed through the second extraction (poroplast) column which has been previously charged with a low boiling polar solvent such as Freon or methylene chloride. The oxygenated constituents are then dissolved out of the aqueous phase at the interface. Once the polar solvent phase has been eluted from the aqueous phase, which has become exhausted of the oxygenated constituents, it is removed by vacuum distillation leaving behind the deterpenified oil. This process is used for the commercial production of terpeneless citrus oils.

Combined Processes

In 1960, Arctander noted that citrus oils could be readily deterpenifed by using a combination of fractional distillation and selective solvent extraction. In the process he recommended that the oil be first subjected to fractional distillation in an all-glass apparatus under vacuum ensuring that the pot temperature did not exceed 50°C. When ca. two-thirds of the hydrocarbons were removed, distillation should be stopped and the reduced volume of oil should be extracted with a solution of aqueous alcohol (50-60%). On completion of extraction, Arctander recommended that the alcoholic extracts should be combined and evaporated under vacuum. Finally, the terpeneless oil should be obtained by fractional distillation of the mixture under high vacuum (<0.1 mm Hg).

In 1975, Rockland and Debenedict recommended that a terpeneless oil could be readily prepared by a combination of molecular distillation of the crude oil (to remove any non-volatile constituents) and adsorption chromatography over neutral alumna. After elution with pentane to remove the hydrocarbons and elution with ethanol to remove the oxygenated constituents, the fractions have to be concentrated by vacuum fractional distillation. To this author's knowledge, this process was never made commercial.

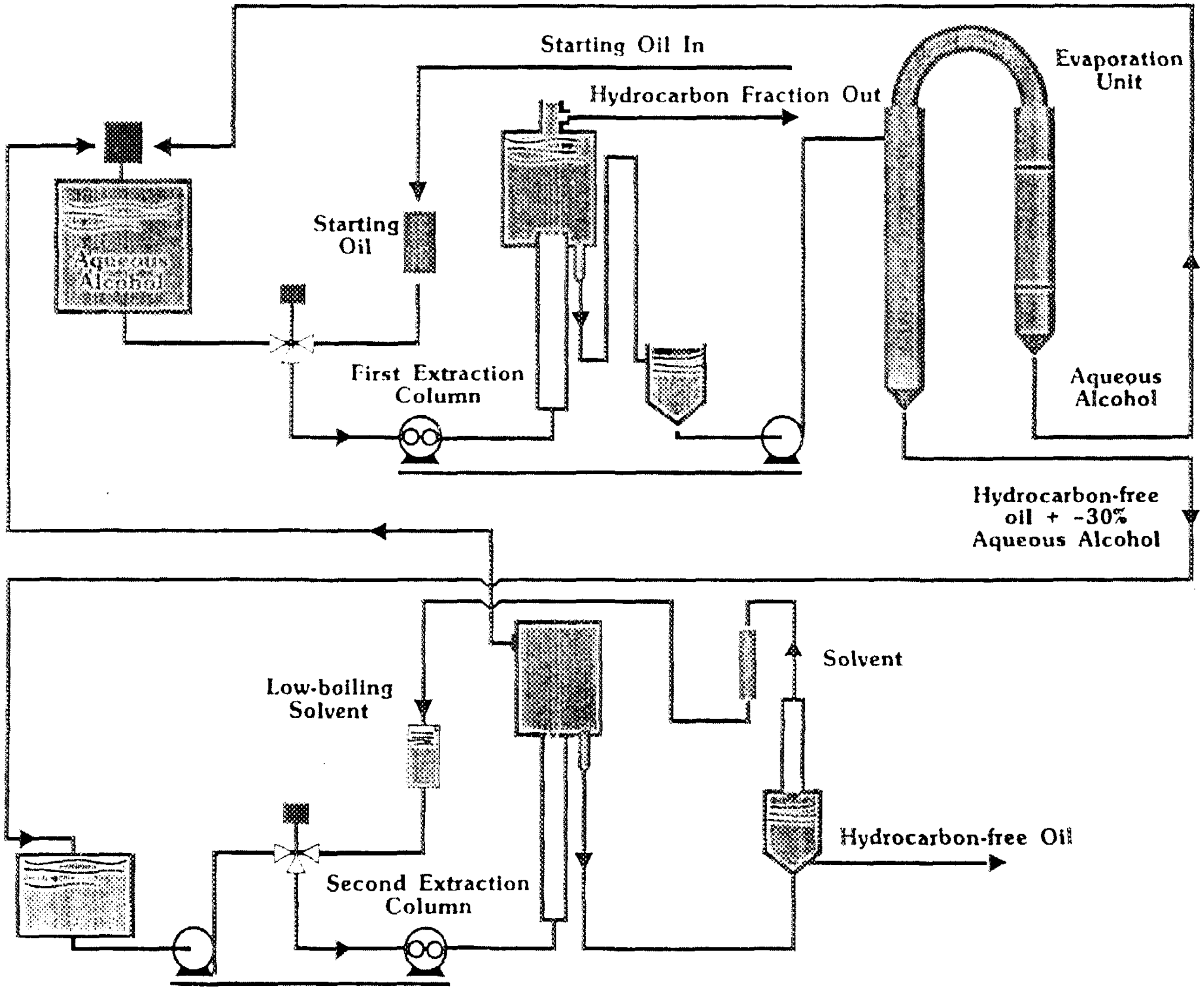


Figure 29: Diagrammatic representation of poroplast extraction deterpenation of citrus oil
(Permission to use this diagram was given by D.A.Moyler)

Other Processes

In 1966 Norman and Craft showed that citrus oils could be deterpenified by two-phase solvent extraction using dimethyl sulphoxide (DMSO) and pentane. Although the procedure worked well in the laboratory environment, it is unlikely to be scaled up to a commercial process because of the difficulties in completely removing DMSO from the deterpenified oil.

In 1992, Brose et al. used the high selectivity of cyclodextrin (CD) to preferentially bind the oxygenated components of a citrus oil at low temperature (oxygenated constituents bind to CD better at lower temperatures). In practice, the oil was circulated on one side of a hydrophilic membrane while a cold aqueous CD solution was circulated on the other side of the membrane. The oxygenated constituents of the orange oil diffused across the membrane and preferentially formed CD inclusion complexes. Heat was applied to the aqueous CD solution and it was pumped through a second hydrophilic membrane. As the temperature of the solution increased, the oxygenated constituents were released from the CD and passed through the second membrane into a solution of hot orange oil, resulting in an orange oil with an enriched level of oxygenated constituents. According to Brose et al., they were able to produce 5-fold orange oil on the pilot scale basis using this procedure.

4. PROCESS OF DISTILLATION

Historically, the first record of distillation took place in the Indus Valley in what is now known as Pakistan (Rovesti, 1977) around 5000 years ago (ca. 3000 B.C.). This pre-dates the previously believed origin of distillation which was attributed to the Arabs only ca. 1000 years ago. According to Rovesti, he stated that, had the Indus Civilization not perished tragically, they would have been able to pass on their knowledge of distillation rather than mankind having to a wait an additional 4000 years until the Arabs re-invented it. Since the invention of distillation, many centuries have past, although if the range of site distillations are examined, the number of modern refinements have not been that many. A survey of water, steam and water and steam distillation has been the subject of earlier discussion (section 2).

Distillation Fundamentals

Before discussing the salient points about the design of steam distillation equipment, it is important to understand the basic principles associated with steam distillation. An excellent discussion of these are given in a recent publication of Denny (1991), a review of which will be given in this discussion.

At this point, it is prudent to discuss the interrelationships between the First and Second Laws of Thermodynamics, Latent Heat and Vapor Pressure.

1st Law of Thermodynamics:

Energy (heat) cannot be either created or destroyed in a system of constant mass.

2nd Law of Thermodynamics:

Heat cannot be transferred from a cooler system to a hotter system by any self sustaining, continuous process.

Latent Heat:

The change from liquid to vapor state uses energy which is supplied in the form of heat. If the heat is not replaced the body of liquid will cool, i.e., Latent Heat of Vaporization. The converse is also true.

Vapor Pressure:

Within a temperature controlled, constant volume, closed system the molecules in the vapor phase of a liquid will reach an equilibrium with those in the liquid phase, i.e., the Vapor Pressure. If the temperature is raised, the number of molecules in vapor phase will increase, consequently the Vapor Pressure will increase. At equilibrium the vapor is saturated. If volume of a closed system is increased while the temperature is unchanged, there will be an increase in the number of molecules in vapor state, a slight cooling because of latent heat of vaporization and a corresponding reduction in Vapor Pressure.

Consider a situation where the liquid is completely evaporated and more heat is applied. The vapor molecules will reach a point where they can no longer attain equilibrium. The vapor increases in temperature becoming higher than that of the same saturated vapor exerting the same pressure, i.e., it is superheated. Hence, it can be considered that superheated steam has a temperature higher than that of boiling water (100°C at 760mm). When superheated steam comes in contact with any moisture it will immediately evaporate the water to try and reach saturation. As a result, there will be a corresponding reduction in temperature. In an open system, the vapor molecules above the liquid are constantly evaporating, and relatively few will condense and return to the body of liquid. As a result, vapor-liquid equilibrium can never be reached.

As this liquid evaporates, the rate of which depends upon its vapor pressure, it will cool. The evaporation rate will only be kept constant if the temperature and pressure remain constant. If the liquid is heated the vapor pressure will rise and the liquid will evaporate to a point when the vapor pressure becomes equal to the atmospheric pressure. At this point, the evaporation has become vigorous and the temperature of the liquid will not rise anymore. This temperature is known as its boiling point or atmospheric boiling point. If the atmospheric pressure was reduced, as might happen on the top of a mountain, then the boiling point of the liquid would decrease accordingly. If the liquid under discussion were water, then it would boil below 100°C at a reduced pressure. Hence, it can be said that the vapor pressure of water is the pressure that is exerted by saturated steam.

According to Denny, the most important feature of steam used in steam distillation, which he calls "rate of displacement", is the rate of steam generation and the amount of distillate attained per unit cross sectional area of the still (per sq. m). As a rule of thumb, condensate flow rates of 2L/min/sq. m indicate that "refluxing" is occurring in the still from water being deposited in combination with plant juices (readily seen by seeing dark colored water accumulated at the bottom of the still). Denny notes that oil losses of up to 30% can be obtained from "refluxing". Before discussing the distillation process any further, some other fundamental parameters need to be discussed. As stated before, heat is energy and energy is needed to perform mechanical work. Heat is measured in calories, i.e., it takes one calorie to raise one gram of water 1°C. A more practical measure of heat is the British Thermal Unit (BTU), whereby it takes 1 BTU to raise one pound of water 1°F. Using this information, the normally accepted fact that boilers produce 1.6 kg of steam at 100°C per kilowatt per hour can be understood as follows:

Mechanical Equivalent of Heat:

to raise 1g H_2O by 1°C requires 1 calorie

to raise 1 lb. H_2O by 1°F requires 1 BTU

For practical purposes 1 cal = 4.2 joules (1k cal = 4.2 k joule)
and a machine which operates at 1 watt is consuming 1 joule/sec

To make this of practical value:

If it takes 540 k cal to vaporize 1 kg H_2O in 1 second, a boiler needed to do this is operating at:

$$4.2 \times 540 = 2268 \text{ k watts}$$

If run for 1 hour then 3600 kg H_2O will be vaporized

$$\frac{3600}{2268} = \underline{1.59 \text{ kg/hr}}$$

Boilers generally produce 1.6 kg steam at 100°C per kilowatt per hour.

For all compounds able to exist in vapor form, there is a critical temperature above which no matter what pressure is exerted the vapor cannot be liquified (see Figure 4). The laws that describe the behavior of ideal gases can be used to approximately describe the behavior of vapors. They are:

Boyle's Law:	The volume of a gas of constant mass is inversely proportional to the pressure of the gas at constant temperature.

Charles' Law: The volume of a gas of constant mass is proportional to its absolute temperature under constant pressure.

Avagadro's Law: Equal volumes of ideal gases contain equal numbers of molecules under equal conditions of temperature and pressure.

Absolute temperature = °K = °C + 273

1 mole gas at 273°K occupies 22.4L at 1 absolute atmosphere of pressure.

The latent heat of vaporization of oil components can be calculated from the following equation:

$$L = \frac{-2.3R\,d(\text{Log } p)}{d\,(1/T)}$$

L = molar latent heat in cals/gram mole

R = gas constant (1.987 cals/K/mole)

T = absolute temperature °K

p = vapor pressure exerted by linalool

Using the data for linalool:

°C	°K	1/T	p(mm Hg)	Log
79.8	352.8	2.84 x 10-3	10	1.000
93.8	366.8	2.73 x 10-3	20	1.3010
109.9	382.9	2.61 x 10-3	40	1.6020

Rearranging the formula we have:

$$\frac{d\,(\text{Log } p)}{d\,(1/T)} = \frac{-(1.6020 - 1.000)}{(2.84 - 2.61) \times 10^{-3}} = -2617$$

$$L = -2.3 \times 1.987 \times -2617 = 11959.95 \text{ cal.}$$

Since mol. wt. of linalool is 154, its latent heat is $\frac{11959.95}{154}$ = 77.66 cals per gram

The boiling point of most essential oil components exceeds that of water; however, a mixture of two immiscible liquids will boil at a temperature when the sum of their vapor pressures equals that of the surrounding pressure. For example, at 99.5°C linalool has a vapor pressure of 27.8mm, while at the same temperature water will have a vapor pressure of 732.2mm, so the mixture will exist at vapor pressure of 732.2 + 27.8 or 760mm.

If a sample of linalool is boiled, the temperature of the liquid will be ca. 198°C. Similarly if water is boiled, its temperature will be 100°C. When the two immiscible liquids are brought together, and once their vapors have reached saturation, the temperature will immediately drop to 99.5°C, which is the temperature at which the sum of the two vapor pressures equals 760mm (atmospheric pressure). This is the reason why high boiling components of essential oils can be isolated from plant material by steam distillation at temperatures well below the boiling points of their individual constituents. These components will boil while giving up energy in the form of latent heat of vaporization, so to maintain their boiling, energy (in the form of heat) has to be supplied externally.

From previous discussions (assume X grams linalool and Y grams water in mixture)

$$\text{no. of moles linalool} = \frac{X}{\text{mol wt. linalool (154)}}$$

$$\text{no. of moles of water} = \frac{Y}{\text{mol wt. } H_2O \text{ (18)}}$$

The mole fraction of linalool in the mixture is:

$$\frac{\text{no. of moles of linalool}}{\text{no. of moles of linalool + no. of moles of water}}$$

Dalton's Law of Partial Pressures states that the total pressure of a mixture is equal to the sum of the partial pressures of the components making up that mixture, i.e., $P_{total} = P_A + P_B + P_c + P_n$

As linalool and water are immiscible liquids

$$P_{total} = P_{linalool} + P_{water}$$

$$\text{Then } P_{linalool} = \frac{P_{total} \text{ x wt. in grams of linalool}}{\text{mol. wt. of linalool x total no. moles in mixture}}$$

$$\text{wt. of linalool} = \frac{P_{linalool} \text{ x mol wt linalool x total no moles in mixture}}{P_{total}}$$

$$\text{wt. of water} = \frac{P_{water} \text{ x mol wt. water x total no. of moles in mixture}}{P_{total}}$$

It follows then in a mixture of water and linalool saturated vapor above the mixture is:

$$\frac{\text{wt. of linalool}}{\text{wt. of water}} = \frac{\text{Vapor pressure of linalool x mol wt. of linalool}}{\text{Vapor pressure of water x mol wt. of water}}$$

Considering the linalool-rich oil example:

C	Vapor Pressure linalool mm Hg	Vapor Pressure H_2O mm Hg	Total Vapor Pressure mm Hg	Vapor Pressure Ratio water:linalool	Mass (distillate) Ratio water:linalool
66.5	5	200.5	205.5	1:40.1	1:4.69
79.8	10	354.0	364.0	1:35.4	1:4.14
93.8	20	598.5	618.5	1:29.9	1:3.49
99.5	27.8	732.2	760.0	1:26.3	1:3.07

or for every 3.07 lbs. of water condensed in the distillate, there should be 1 lb. of a linalool-rich oil assuming that the still is running at maximum efficiency.

Oil Isolation Fundamentals

According to Denny, for the oil to change from the liquid phase to the vapor phase, it must receive latent heat such as, within a field still, could only come from condensing steam. Consequently, the temperature of the steam within the still must be higher than the temperature at which the oil boils in the presence of water on the surface of the charge of plant material, otherwise there would not be a temperature gradient to take the latent heat from the condensing steam to vaporize the oil droplet (2nd Law Thermodynamics).

This follows Fourier's Law that states that heat is conducted from one surface to another surface at a rate proportional to their contact area. This difference in temperature is the temperature gradient noted above. Obviously the thermal conductivity of a material is a critical factor in the heat transfer, although for this discussion it is not a pertinent point.

On the surface of the charge, the concentration of the vapor of the oil at its evaporation point, and the proportion of the total ambient pressure it exerts will be at its maximum, which is appropriate to saturation at the prevailing temperature. Referring back to the temperature of boiling a mixture of two immiscible liquids (one being water), the boiling temperature will be lower than the boiling point of the two liquids individually. In fact, it will be the lowest that can produce a boiling liquid (mixture of oil and water) under the prevailing pressure.

During distillation within a still the oil vapor is dispersed throughout the steam vapor. As a result, the concentration of oil vapor:water vapor is reduced so that the proportion of the total vapor pressure that the oil exerts is much less because in this situation it is present in a less than saturated condition. Consequently, the share of pressure that the steam must exert within the still is even greater than that which it exerted at the surface of the charge. The temperature gradient between the vapor space in the still and the evaporation point on the charge is the principle of the distillation process. The magnitude of the transference of latent heat from the condensing steam to the evaporating oil governs the rate at which the oil will evaporate from the charge surface. In practice, to isolate any oil by steam distillation under controlled conditions, there is a point above which the oil to water vapor concentration cannot be surpassed. According to Denny, any further enrichment of the mix in oil vapor would result in a reduction in temperature and a reduction of the gradient, i.e., less heat would get to the oil, the rate of evaporation would be reduced until a new balance was reached, after which the optimized rate of oil isolation would be restored. Denny referred to this automatic control system as feedback. Furthermore, he noted that several factors could have an affect on the temperature gradient and correspondingly the ratio of oil to water in the condensate.

The factors that affect temperature gradient at which feedback controls the oil:water ratio in the condensate are:

1. The amount of steam required to pass through a charge depends on the amount of oil to be isolated (the oil yield) not on the mass of the charge
2. The varied action of different charge surfaces with the wetness fraction carried by saturated steam (the ability of the charge to remove the saturation from the steam)
3. Steam cloud particles (in saturated steam) can roll up coatings of oxygenated compounds when they impinge on the surface of the charge by hydrophilic bonding
4. The steam can preferentially solubilize to a limited extent the more polar oxygenated compounds.

In the introduction to this discussion, the types of oil glands that can be found in aromatic plants were discussed briefly; however, they were categorized using Denny's descriptions as being superficial oils and subcutaneous oils.

A good rule of thumb to determine whether the oil is a superficial oil or not is to take 3 still charges of equal size and steam distill them from the same still; however, different steam rates should be used so that the distillate rates should differ. If the oil isolation time is inversely proportional to the distillate flow rate, then the charge is a plant which has a superficial oil, whereas if it is not, it is a plant which has a subcutaneous oil.

During distillation of a superficial oil, the steam will rise through the still moistening the leaves of the charge by condensing on them and raising their temperature correspondingly because of the latent heat given off during condensation. Throughout the charge numerous oil patches will appear on the surface as a result of gland rupturing around these mini-puddles of oil (oil droplet); there will be an oil water interface. As the temperature around this interface increases to ca. 98°C, the additive effect of the vapor pressures of the two immiscible liquids at the interface will cause them to boil. As a result, oil saturated vapor will now rise from all of the interface points or from the entire periphery of the oil droplets such that the vapor above the remaining droplets will be saturated with oil vapor or a state of equilibrium between the oil droplet and its vapor will exist (Figure 30). Thus, a situation exists where the temperature and vapor pressure of the oil droplet and its vapor are equal. At this point the oil can evaporate from the surface. Because of this it cannot receive any latent heat of condensation of steam unless this mini-equilibrium is broken, as might be caused if a new puddle of oil rose to the surface; however, with superficial oils this situation cannot exist. Nevertheless, saturated steam vapor will readily condense on existing mini-puddles of water on the surface of the leaves that have direct contact with oil droplets releasing their latent heat to vaporize oil with which the puddle has contact.

Distillation time can be thought of as being the time it takes to evaporate the surface oil droplets which is inversely proportioned to the speed of steam in the still; where the speed of steam in the still depends on the oil vaporization rate, and the rate at which the steam can reach the oil droplet surface to replace where saturated steam has already condensed and released its latent heat to vaporize the oil. Obviously the size of the oil droplet on the leaf surface is one of the most critical factors in steam distillation because the time it takes to completely evaporate it is inversely proportional to the steam flow rate.

Using the example quoted by Denny, consider that within a still the charge has hypothetical layers of 1cm thick as shown in Figure 31. Also, assume that it takes 7 seconds for the charge to be raised to the temperature where the oil will evaporate from it. Consequently, after 7 seconds the oil will evaporate from the first layer of charge and condense on the second layer. After 14 seconds the deposited oil and the existing oil will have been evaporated and condensed onto the third layer. This situation will be repeated continuously as time goes on so that the amount of oil condensing on each layer will increase proportionally depending on the number of layers below it in the charge.

As more layers release their oil, the size of the oil droplets are likely to increase the further up the still they are. Consequently, the top layers of the still will take the most time to distill. Hence, for determining the isolation time only the time to exhaust the oil from the plant material at the top of the still needs to be considered.

An important point to note is that the charge surface must be sufficiently absorptive to be able to accept all of the condensed oil on it without becoming saturated. For some herbs like lavender or lavandin this is not a problem, because they are highly absorptive because of their widespread pubescence, whereas for others, the partial wilting in the field or prior to distillation allows them to form a more absorptive surface.

Unfortunately, the time that it takes to distill a herb charge cannot be calculated from theory. It must be determined by running some trial distillations. These distillations must be carried out on the same still and should be separate distillations of the similar charges (i.e., similar density and moisture content plant material) that must have different heights in the still (obviously they will have different masses) and must be packed to approximately the same packing density. Using a fixed steam flow, the rates at which oil and water are condensing at all stages of the distillation must be determined. This is easily done by placing a plastic bucket under the exit of the condenser and collecting the volume of oil and water every two minutes. Using a plastic graduated cylinder, the oil to water quantity must be recorded for each of the 2 minute samples. This is done until no more oil is distilling over. From the data, the amount of water and the amount of oil obtained for the full distillation can be easily determined. If the total amount of water collected, except for the water in the first bucket (when flow from the condenser was not normal), divided by the time it took to collect the water, gives the average flow rate of water for the complete distillation. The time that it took to do this is then the distillation time for that charge.

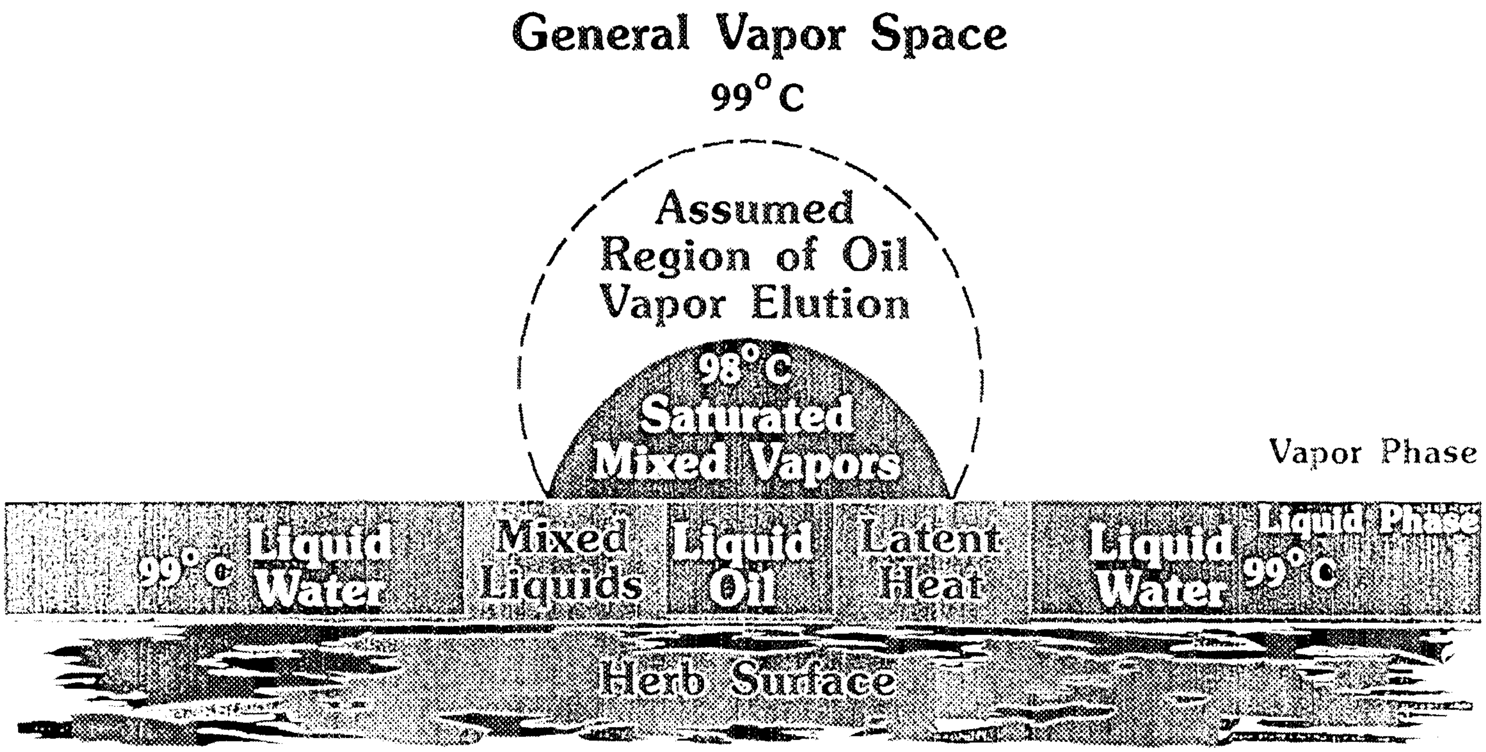


Figure 30: Diagrammatic representation of oil droplet interface with water on charge surface

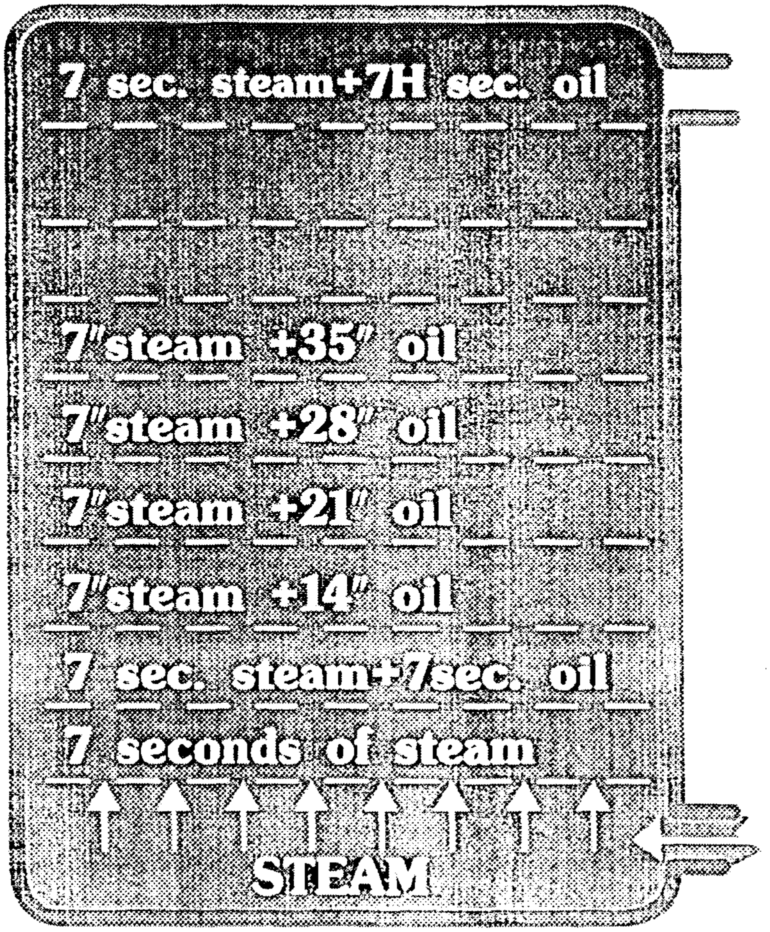


Figure 31: Hypothetical schematic representation of steam/oil movement during steam dilstillation

A reference to the schematic representation in Figure 31 shows that the charge height is the important factor in distillation, not the cross sectional area of the charge, because exhausting the oil from the top of the charge is critical for determining distillation time.

Further discussion on the optimum time for distillation can be found in the publication by Denny (1991).

Distillery Design

To design a distillery, a number of parameters must be taken into consideration, such as:

- Site Choice
- Distilation Charge
- Still
- Boiler
- Condenser
- Oil Separator
- Storage
- Quality Control

Site Choice

A site that is chosen on which a steam distillery will be constructed must have the following requirements:

- Ample availability of water
- Availability of energy sources, such as electricity, boiler fuel, etc.
- Easy access by road, preferably on higher ground than the surroundings
- Availability of skilled and unskilled labor
- Close proximity to the plant material that will be distilled
- Availability of a machine shop that can fabricate still parts out of stainless steel and make repairs
- No governmental or environmental restrictions on zoning distillery and waste water discharge.

These parameters are fairly obvious so only a couple will be the subject of discussion. The main advantage of locating a still site on ground that is higher than its surroundings is that the charging and discharging of the still can be made easier if the natural incline of the land is used to the benefit of these two processes. Also, it is easier to drain water from an elevated site. In some still sites that have been visited by this author, the still is dug into the side of a hill so that the earth surrounding it can be used as insulation; however, this is more common in steam and water distillation sites than steam distillation sites. Although there is some value to this use of the natural incline of the land, depending upon the design, it can be somewhat detrimental when repairs have to be made to the walls of the still or the stills have to be discharged manually.

In developing countries, the location of a still site in a rural area near the plant material is generally not a problem; whereas, in many developed countries the need to get the land zoned for industrial use, permitted for having a still on the site, and either approved for distillation waste water run off or additionally permitted for a water recovery still is a necessity before a distillery can be set up.

Distillation Charge

The amount of plant material to be distilled during a single cycle of a still is determined by the following:

- size of the still
- oil content of the plant material
- What plant material is going to be distilled
- amount of plant material available on a daily basis and the frequency of receiving and the state of the plant material (wet, wilted, or dry)
- distance the plant material has to be moved from harvesting site to the distillery and how it will be moved
- required pre-treatment of plant material prior to distillation
- time taken to charge and discharge the still
- storage capacity for plant material prior to distillation (in case of inclement weather at distillation time)
- waste disposal or post treatment of plant material after distillation
- whether distillation will be performed on a 24-hour basis

The site of the still will be controlled by a number of factors relating to the plant material, although the major factor will be the steam capacity of the satellite boiler. Obviously, the type of plant material, its state, its oil content, what pre-treatment is needed, etc. are critical factors that will assist in determining what should be the optimum charge size and correspondingly the still size.

Still

As mentioned above, the most critical parameter in the design of the still, or number of stills to be serviced, is the steam capacity of the boiler. If the amount of steam necessary to displace the oil, and the oil content is known, then the size of the boiler should be able to be determined.

Plants are generally harvested over a short period of time when they have reached their maximum oil content so the amount of plant material that can be delivered to a distillery is determinable. For most herbaceous materials containing superficial oils, a higher oil yield will be obtained if the plant material is chopped into 2-5cm pieces before it is distilled; however, this is not necessary as this will take time, will require more labor charges, and for the incremental increase in oil yield it may not be cost effective.

The still size can be designed, taking into consideration as to whether the oils present in the plant material are superficial or subcutaneous oils and whether they are mainly monoterpenoid in nature or whether they are rich in sesquiterpenes, phenols, or alkenyl phenol ethers. As a rule of thumb, if run under optimized conditions, steam distillation of 200-300 kg of plant material containing monoterpenoid-rich superficial oils will take between 15-20 minutes, whereas for the same quantity of plant material containing monoterpene-rich subcutaneous oils the time will be increased. For the high boiling oils the time taken to isolate them will be directly proportional to the amount and pressure of the steam as well as the height of the charge.

The still should be stainless steel in design. It should be easy to charge and discharge (Figure 32). The use of a basket within the still within which the plant material is placed, which is either connectable to a motor driven pulley or some mechanical or counterweighted pulley so that the plant material can be easily discharged from the still, is important if the downtime of the still is to be minimized. Similarly, the top of the still should be counter weighted so that it can easily be removed. It should either be hinged so that full removal is not necessary (it need only be opened just greater than 90°), or it should contain a hook or an eye on the top so that it can be elevated by a pulley system and moved horizontally away from the still body so that the basket containing the plant material can be removed.

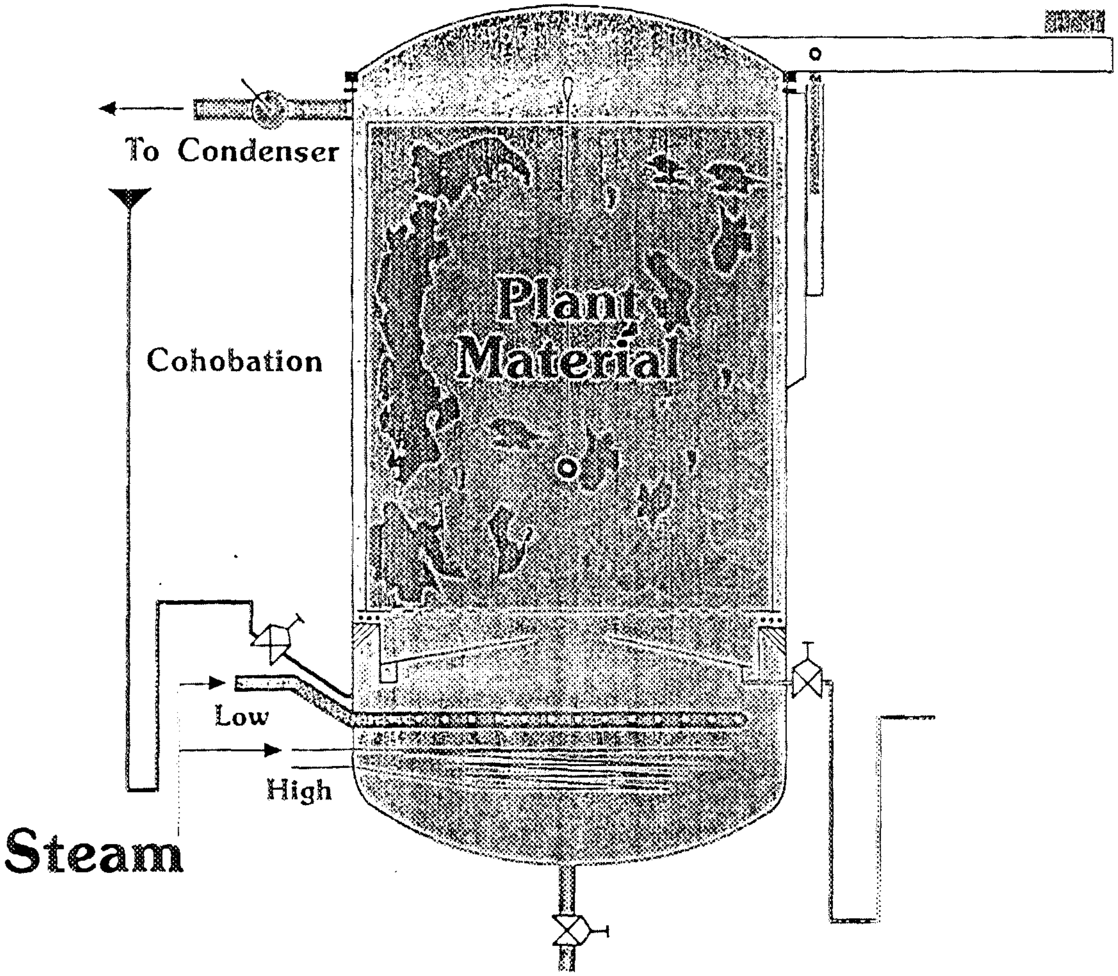


Figure 32: Diagrammatic representation of a general purpose still showing pulley connector for easy discharge

The most practical design for the top of the still is either flat or a slight bulge. The use of the so-called goose neck tops are unnecessary with modern condensers. The top of the still containing the vapor tube should be connected to the condenser either by expansion tubing or preferably by a gasket bolted with flat face connectors. The vapor tube does not need support because it is an integral part of the still top. The flanged still top is either held to the flanged top of the still body by either slip-hole washered bolts or by C- or G-clamps. At some distilleries this author has seen, water seals are used to hold the top onto the still body; however, they are not favored because the amount of water depth needs to be at least 12 inches to prevent any vapor loss, and this is a waste of stainless steel.

Boiler

There are numerous types of boilers that can be used as steam generators, some which are heated vats of water that generate saturated steam, while others are industrial boilers that can be made of varying capacity and sophistication. According to Denny, boilers in field distillation units that he has visited generally have a working pressure of 8 atmosphere abs. while the stills are operating at atmospheric pressure, although theoretically, for each kilowatt of capacity, a boiler should produce 1.59 kg H_2O/hour.

The type of boiler used for the distillation is outside of the scope of this discussion, although an obvious pre-requisite is that it should produce enough water to more than adequately remove the oil from the still charge that it is servicing.

A way to determine how much steam is being generated from a homemade boiler or a commercially produced boiler is to measure it. This can be done simply by taking an oil drum containing some water that has been tared and then run the steam line into the drum under the water to condense the steam for a set time period. Taking care not to get scalded by the steam or hot water, the weight of steam per unit time can be measured. From this crude determination the approximate capacity of the boiler can be determined.

Condenser

As the name suggests the responsibility of the condenser is to change the phase of oil and water vapor back into liquid so that as immiscible liquids they can be separated. Some people refer to a condenser as a heat exchanger, which of course it is.

Usually a condenser is a tube or a series of tubes that are water cooled (below the temperature of water vapor) so that the vapor emerging from the vapor tube will rapidly change into the liquid phase. The reason water is used to cool the condenser is because it is readily available and it absorbs the latent heat of condensation away from the hot vapors allowing more of them to condense. Before discussing the tubular condensers, it is worth mentioning the air condensers have been used in cinnamon leaf distillation as shown in Figure 11. In this situation, the long tube with multiple fins along its length was designed to dissipate the heat of the oil and water vapors enough to condense them. It was designed to be used in areas where the accessibility to water was limited. It does work, albeit not very efficiently, and it is extremely cumbersome.

The two main types of condensers are the multiple tube type or the coiled tube type. In the past almost all condensers were designed as coiled tubular condensers which were placed into some type of tank into which water was slowly added from the bottom to exit at the top. These tubular condensers were set lower than the still tops so that no condensation would occur above the still and cause any run back into the top of the still. Because the coiled tubular condenser was narrower than the vapor tube exiting the still top, there was a back pressure build-up in the condenser. This back pressure build-up was the reason why goose neck still heads (tops) were designed so that there was a pressure equalizer before the hot vapors entered the tubular condenser.

Since the multiple tube condensers, sometimes known as shell and tube condensers, have been designed there has been no need to build in the pressure equalizing still head. This has led to the design of flat or slightly curved still tops are the normally designed still tops with the multiple tube condensers. The main differences between the two condensers can be summarized as follows:

Coiled Tubular Condenser	Multiple Tube Condenser
Easy to make	Difficult to make (requires shop manufacture
Made of variety of materials All metal types used	Stainless steel
Poor heat transfer	Good heat transfer
High pressure build-up during distillation	No pressure build-up during distillation
Needs tank of water with sparse use of running water	Needs running water
Non-economical use of water	Economical use of water

The temperature of the water in the condenser has a direct relationship on the ability of the condenser to dissipate the heat from the oil and water vapor, thereby allowing rapid condensation. However, it does not necessarily follow that condensed oil will separate more easily from a cold condensate water.

The amount of heat that is removed from the hot oil and water vapor/condensate within the condenser is expressed as:

$$Q = U \times A \times \Delta T$$

Where Q is measured in BTU/hr.

U =Condenser coefficient express in BTU/hr/sq ft/°F

A =Internal surface of coiled tubular condenser or external surface of multiple tube condenser that comes in contact with hot vapors/condensate (heat transfer area)

ΔT =Logarithmic mean difference between average temperature of coolant water and that of hot vapors/condensate.

Obviously with a coiled tubular condenser the value for A is lower, ΔT is smaller and so the U is smaller in comparison with the multiple tube condenser. Finally, schematic representation of the multiple tube condensers can be seen in Figure 33.

Oil Separator

Once the oil and water vapors have condensed, the oil droplets must have time to coalesce. This is done in the oil separator, a vessel which is also known as an oil receiving can or even a florentine flask. Oils separate from water according to their density because they are immiscible or only sparingly soluble. If their density is less than 1.00, then they will float and are called "lighter than water" oils, whereas if their density is greater than 1.00, then they will sink and are referred to as heavier than water" oils. Most of the oils from herbaceous plants and leaves are lighter than oils,while only a few

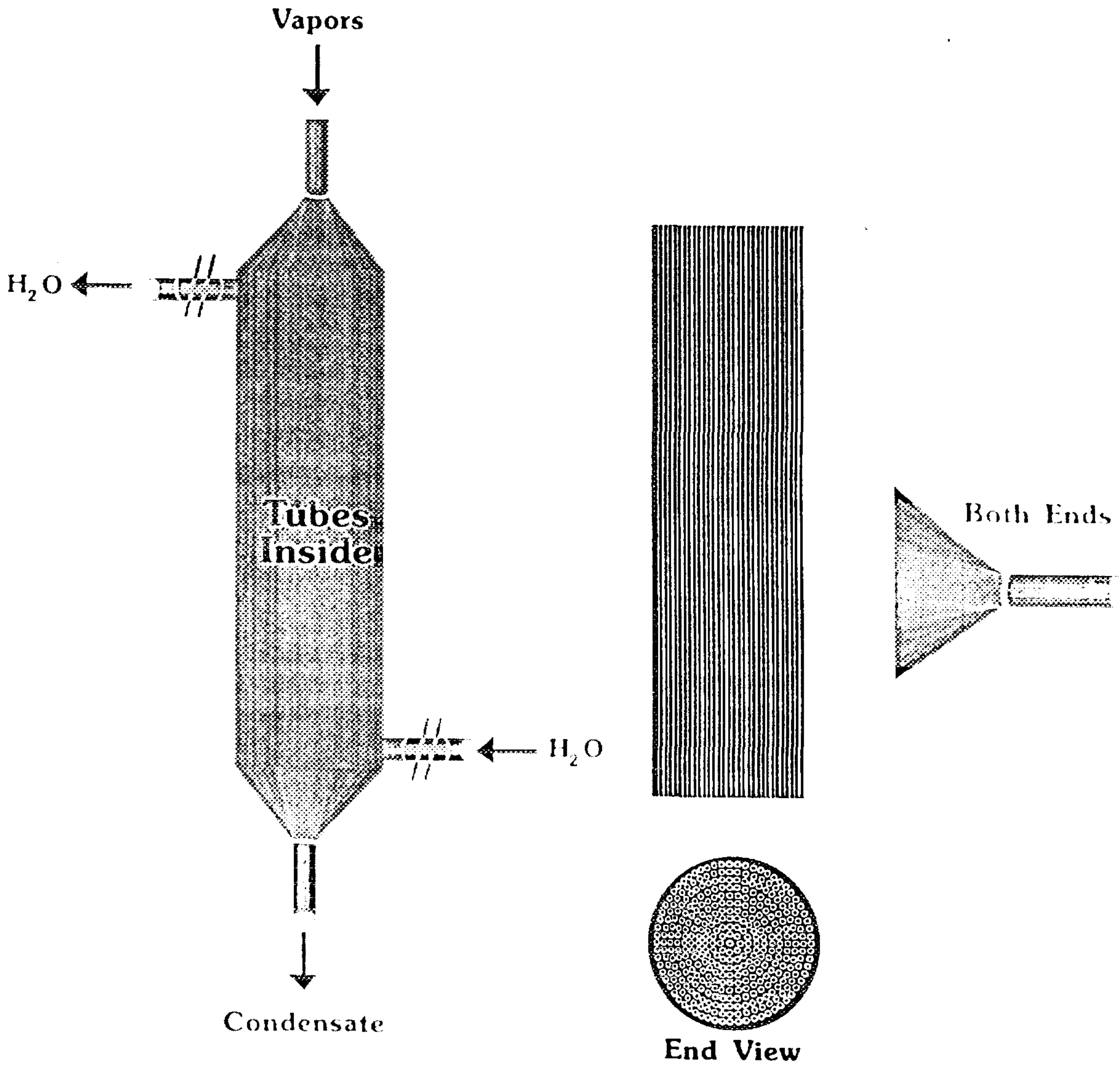


Figure 33: Diagrammatic of representation of a multiple tube condenser.

of the wood and root oils are heavier than water oils. Because of this difference, different oil separators have to be used for the two types of oils.

Examples of some of the different separators used can be seen in Figure 34. The most important factor of an oil separator is that it must be large enough to give the coalescing oil particles time to form distinct oil droplets that readily separate from the oil. This author recommends that it be large enough to take the equivalent of more than 4 minutes of distillate flow without overflowing. For oils that are lighter than water, the inlet for the water/oil emulsion should reach down towards the bottom of the receiver, after which it should curve so that the oil/water emulsion mixture enters the water in the separator with a slight swirling action. If the inlet tube is made so that it can be raised, then the same separator can be used to collect both oils, otherwise, a specific separator has to be made for the heavier than water oils.

Separation of oils whose density is very close to water or if one of the major components of the oil has a density greater than 1.00, while all of the others have a density less than 1.00, is more difficult. To complete separations of such oils the temperature of the condensate must be increased so that the temperature in the oil separator is sufficiently high for separation. According to Denny (1991), and Porter and Lammerink (1994), as the temperature in the separator increases, the density of the oil (and its components) decreases. This decrease in oil density is far greater for the oil than it is for water, so separation of hard to separate oils can be achieved if temperatures are of 50°C or more. To separate the oil from water, the temperature in the separator must be maintained to give sufficient time for the oil droplets to either rise or sink. The increase in temperature will reduce the density of the oil. It will also cause the viscosity of condensate water to decrease more significantly than that of the oil, thereby removing another barrier that could prevent minute oil particles from coalescing into droplets which will readily separate from water.

Earlier, the rural distillation of rose oil was discussed. During this distillation, the oil content is so low the oil will not separate in the all-glass receiver (Baser 1992). Consequently, the rose water has to be bulked and redistilled to yield the true distilled rose oil.

Finally, it should be noted that the use of scim separation or cascading florentine flasks as seen in Figure 34 are not recommended as oil separators. It defeats the purpose to put the effort into isolating an oil by distillation if the recovery process is less than adequate.

Storage

Once an oil has been separated, it should be filtered to remove any minute particles or debris that have unobtrusively found their way into the oil. To prevent the oil from any untoward aging reactions, it is a good practice to remove some of the dissolved water. This can be done either by storing the oil in a cold environment so that the water will come out of the solution and be separated or more preferably it should be filtered through a bed of anhydrous sodium sulfate.

To remove any residual still notes from the oil plus any dissolved air (oxygen), the oil should be ebulated with a stream of nitrogen from a nitrogen cylinder. This is best done when the oil has been transferred into a tared, clean, epoxy-lined oil drum approved for oil storage. If it is not possible to ebulate the oil, then the drum of oil should be placed in a well ventilated area with the bung removed and a piece of clean cloth covering the bung hole to allow the oil to "breathe". After 24 hours the drum should be topped up with oil so that no air exists in the drum. Prior to closing the drum, a sample (up to 0.5 kg) should be taken. After closing the drum the weight should be recorded after which it is ready to be sold.

Quality Control

It is the responsibility of the distiller to produce an oil that meets the international standards of odor, color, physio-chemical characteristics, chemical composition and overall quality at a competitive

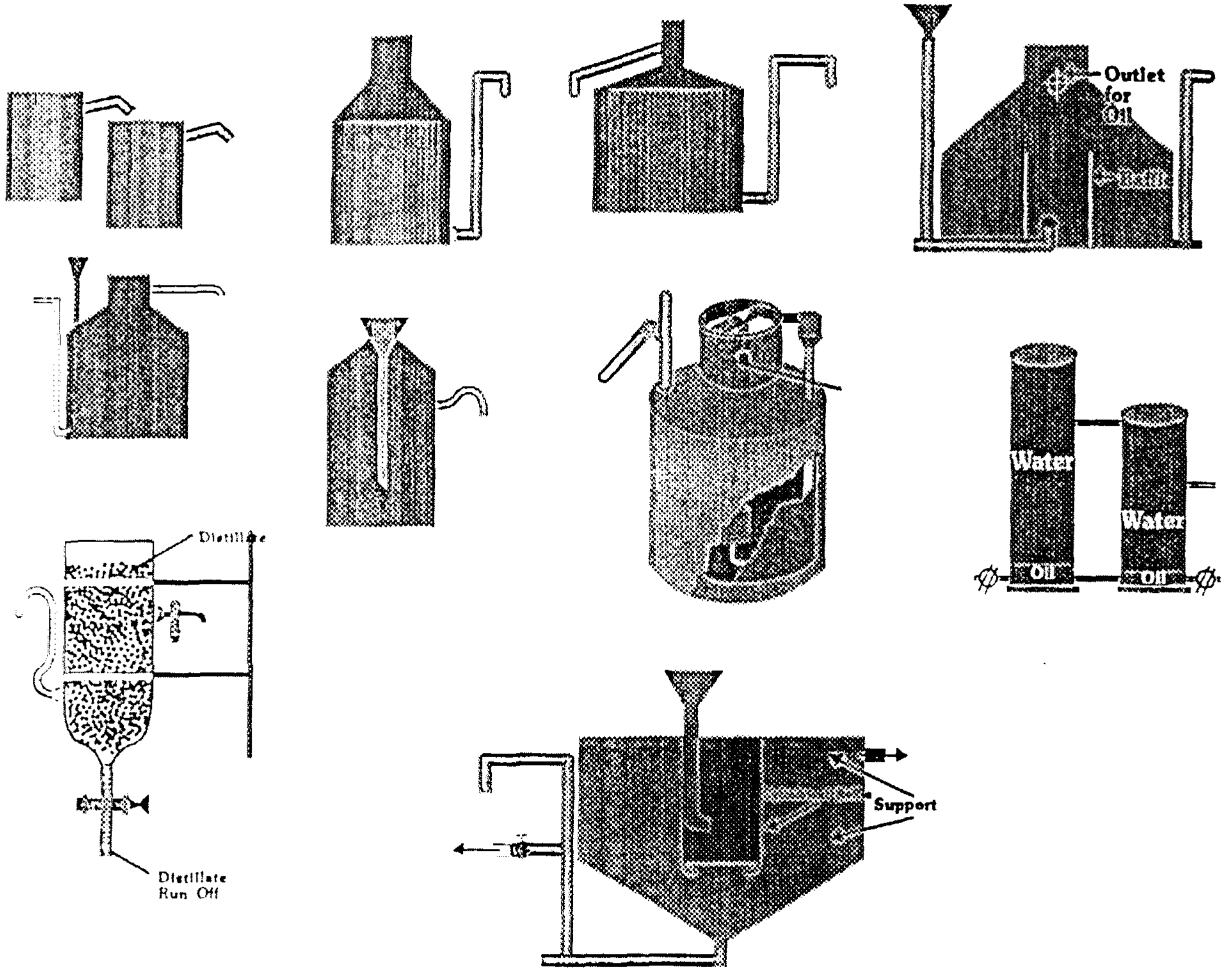


Figure 34: Diagrammatic representation of a number of different oil separators. All separators are for lighter-than-water oil except two at bottom right that are for heavier-than-water oil

price. An oil which meets the standards or conforms to the recognized specifications of the specific oil, can be thought of as possessing a fitness for use in whatever industry it is needed.

It is useful for a new distiller to obtain a sample of a good quality oil so that a comparison can be made between freshly distilled oils and those which are acceptable in commerce. In addition to comparing the color and odor of the oil, it should meet the physio-chemical specifications such as specific gravity (generally at 25°C) or density, which is a measure of wt/mL; refractive index (generally at 20°C), which is a measure of the velocity of light in air versus that of the oil, and is expressed as the ratio of sine of the angle of incidence of refraction for a light ray passing through the oil; and optical rotation (generally at 20°C) which is the ability of the oil to rotate the plane of polarized light either to the left (-) or right (+), and is expressed as the angle (no. of °) of this rotation. In addition, specific tests such as solubility in alcohol, acid value, percent alcohols, ester value, phenols, aldehydes and ketones, assay for major constituent, etc. are also well-used analytical tests to determine oil quality. Over the past 20 years more stringent analyses such as GC profile, infra-red spectrum, and GC//MS analysis have become routine procedures for assessing the quality of oils.

In conclusion, it should be emphasized that the distiller should become familiar with these analyses by seeking the assistance of a government sponsored laboratory or the chemistry department of a local university who can help the distiller ensure that the oils produced meet the necessary international specifications, thereby ensuring product acceptance.

Pilot Scale Still

The definition of pilot scale stills means different things to different people. It has the same set of requirements for the full scale still unless it is completely unitized as is the situation with the still that was designed by the Tropical Products Institute (now known as the Natural Resources Institute) in London. This unit, which is both self sufficient and totally portable, requires only a source of water and fuel oil as it has its own electricity generator that is used to power the steam generator and water pumps. Recently, the power and steam generators along with the full electrical system have been used in conjunction with another still in Bolivia to successfully produce cornmint oil.

A number of years ago, this author had a 25 gallon pilot still designed for him, a summary of which can be seen in Figures 35-37. Unfortunately, the multiple tube condenser is not shown. Also the florentine flask used for the oil separator was not acceptable. The only value in it was that it was made of glass and could easily accept the distillate volume of 2-4 mins.

More recently, this author had a totally portable small still designed that could be easily dismantled and transported to a new location. This still, which was designed for use in Bhutan for screening the lemongrass populations, is known as the Yak Pak. The reason for this name is that once dismantled the two still body parts could be transported in very hilly terrain by a Yak (one of the beasts of burden in Bhutan). The upper cylinder and lower cylinder parts of the still could be strapped onto the yak, one on each side, within which the various parts of the steam generator, condenser collector and all connecting tubes could fit. The final weight of the full system was 109 kg.

A schematic representation of the Yak Pak can be seen in Figure 38. Obviously, 205L stainless steel drums can be used as substitutes for the two cylinders (upper and lower). To support the plant material in the still, a stainless steel perforated plate is used supported by tubular legs that have been welded to the plate. The perforated plate is free standing and loose enough to support the plant material and be removed with ease.

The multiple tube condenser contains the 19 tubes arranged in a hexagon pattern. To make the coolant water flow across the outside of the tubes, 3 baffles are equipositioned around the tubes before the end plate is welded to the tube ends. At the other end of the condenser a conical tube is used to join the two endplates to a short piece of pipe which is threaded at the end. This pipe can then be screwed to the threaded tube that is welded to the top plate of the upper cylinder. During set-up, a girdle clamp secures the condenser to the top plate of the still.

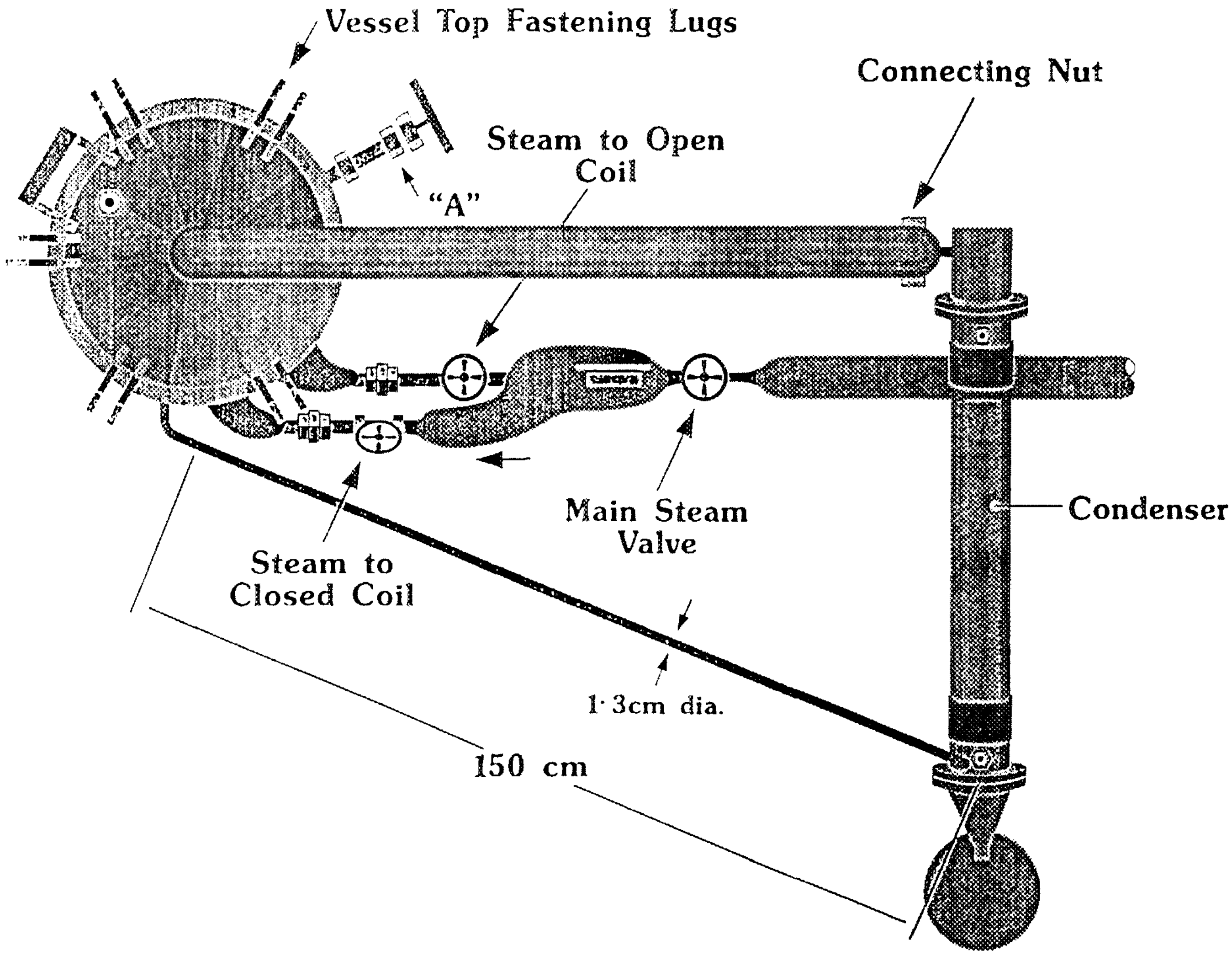


Figure 35: Diagrammatic top view of pilot scale distillation unit

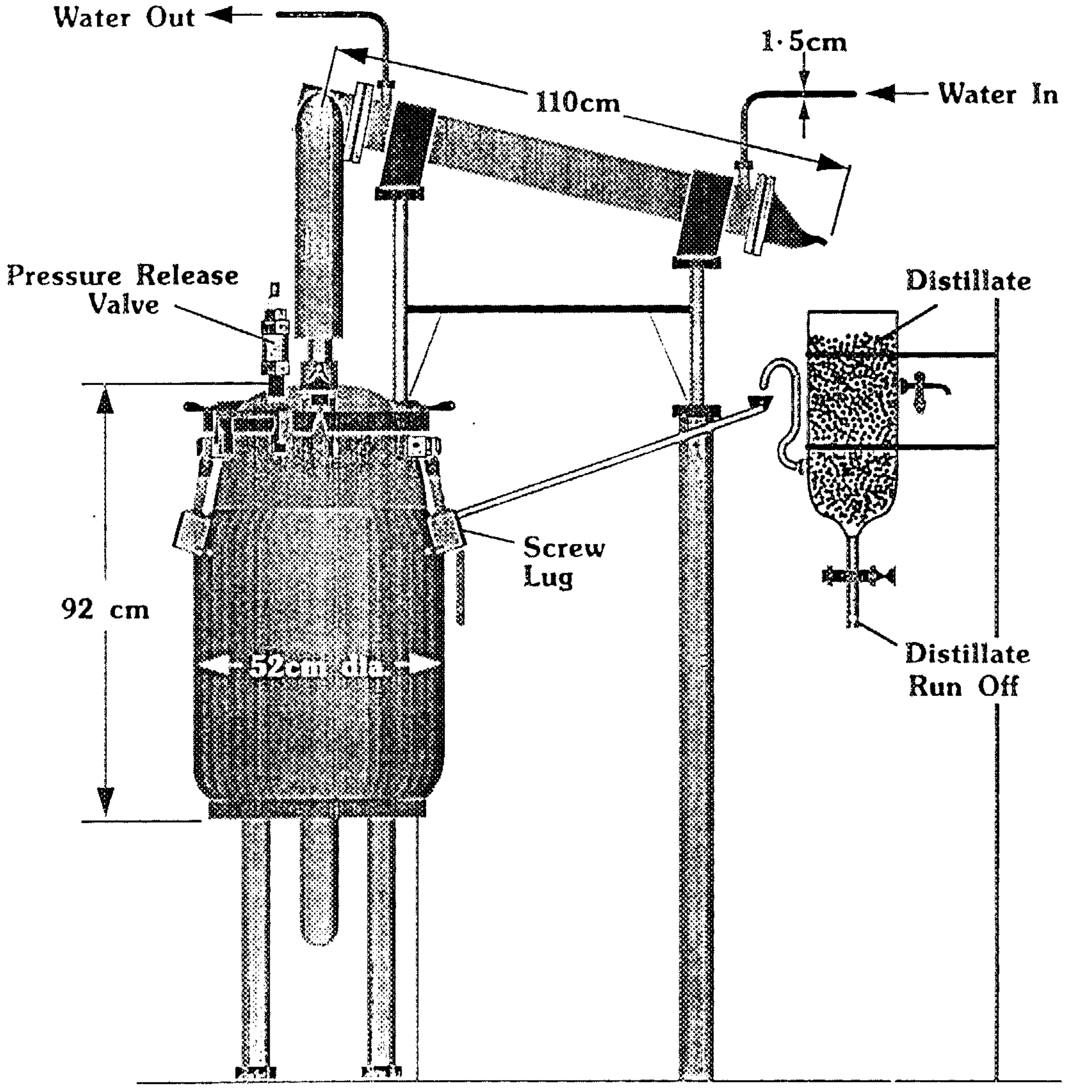


Figure 36: Diagrammatic side view of pilot scale distillation unit showing condenser

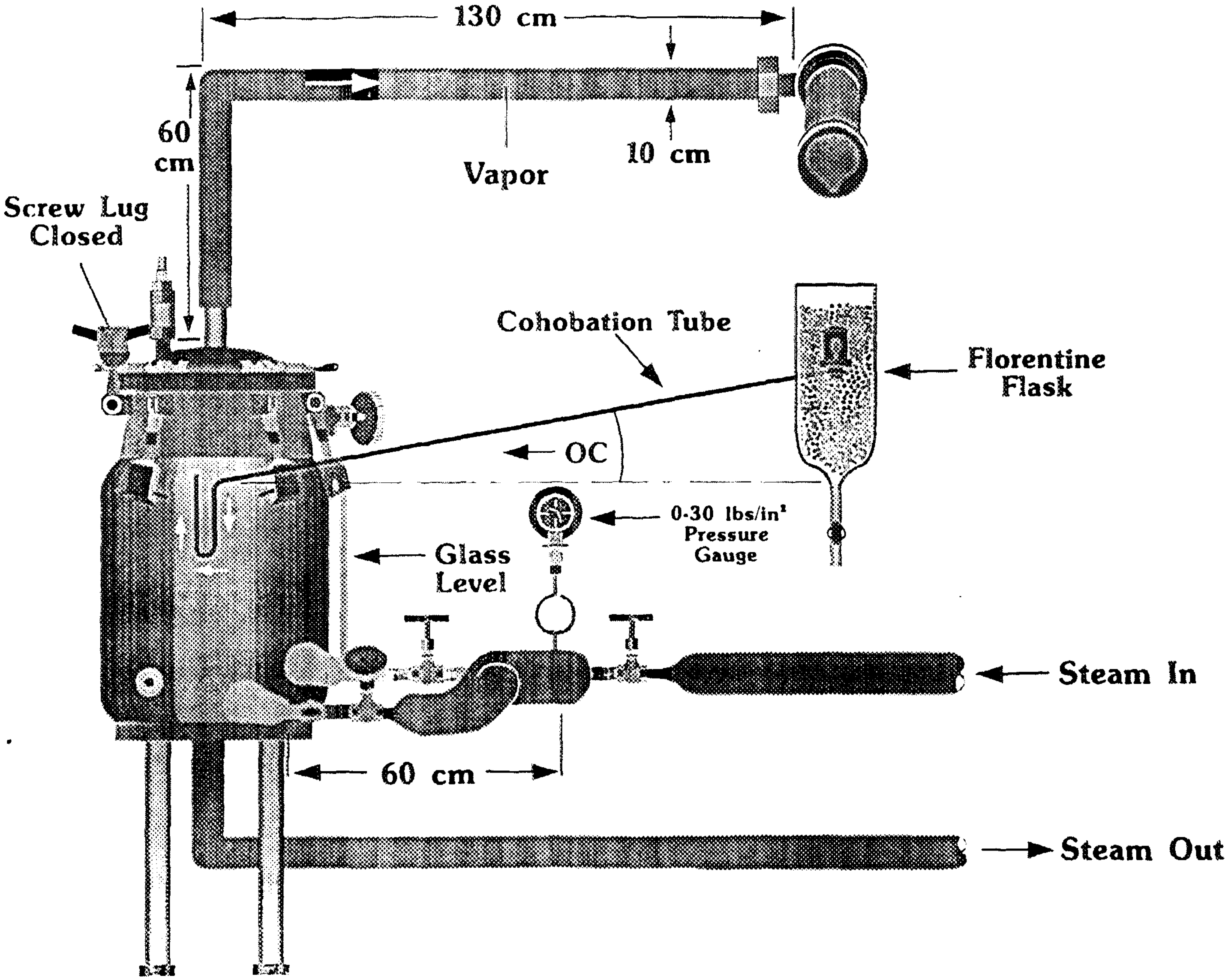


Figure 37: Diagrammatic side view of pilot scale still showing steam inlet

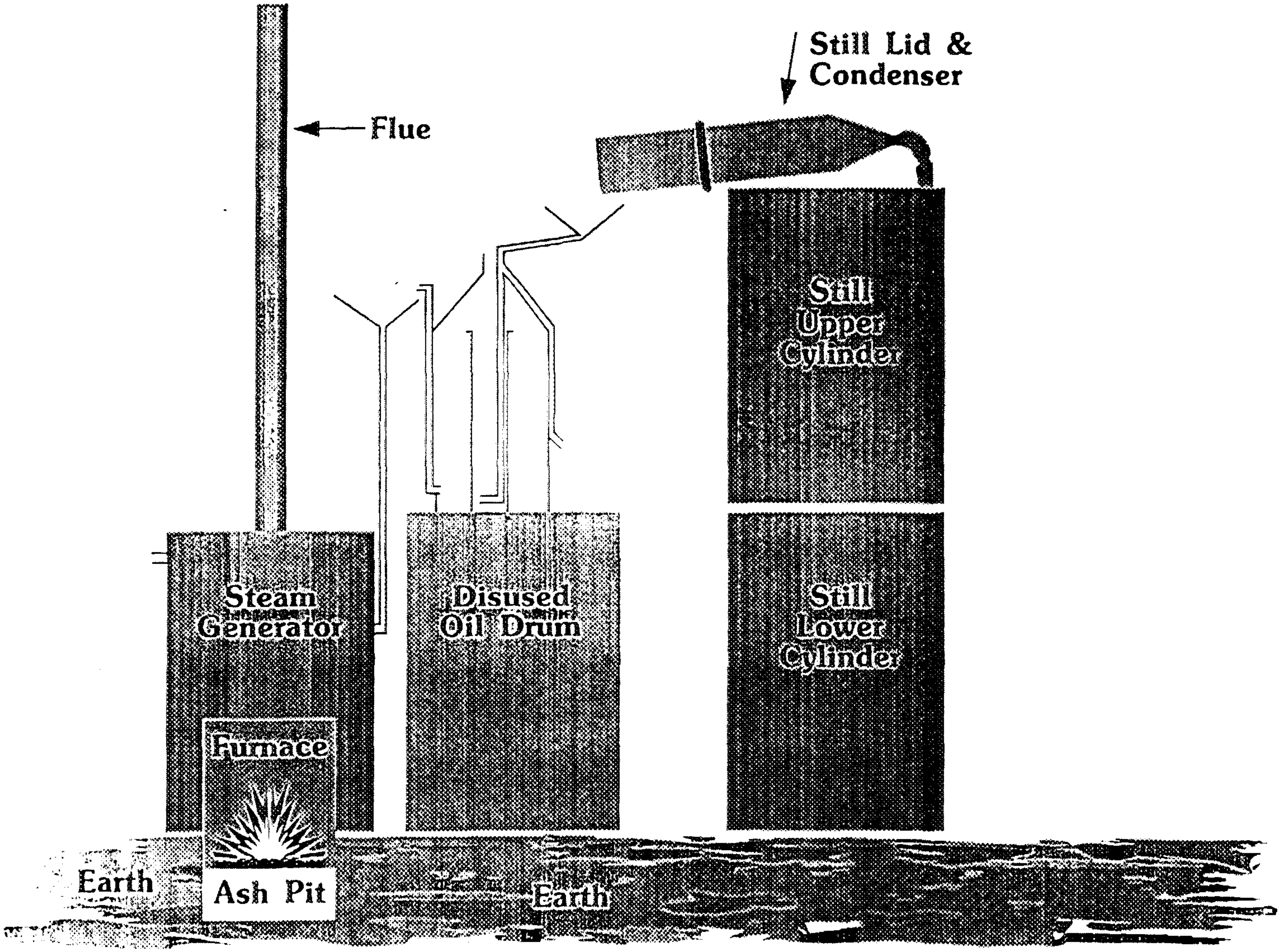


Figure 38. A diagrammatic representation of the Yak Pak pilot scale still

The separator is one of typical design as can be seen in Figure 38. The design of the steam generator can be seen in Figure 39. The end plates and fire box walls need to be heat resistant. Similarly the fire tubes should also be made of heat resistant stainless steel. The fire bars can be made of cast iron, whereas the tank base should be stainless steel. All other sheet metal should be stainless steel, as should the pipes and fittings. Finally, the fire box door should be standard pivoting on rings welded to the door and turning on pegs welded to the box frame. When being used, if the pressure within the generator builds up to a force exceeding 1.35 psi, it will release pressure by overflowing through the standpipe.

After dismantling the Yak Pak, the various parts will fit into the two cylinders or drums as noted earlier. The only requirement is that the two cylinders be closed by strapping the various drum end pieces to each end of each drum. This is favored over the use of clamps to secure the ends, as during transportation they could vibrate loose which could potentially be problematical.

The only drawback of the Yak Pak, which can be seen in Figures 39-41, is that a reservoir of water must be located somehow above the condenser so that water can be piped through it to dissipate the latent heat of condensation generated by the hot oil and water vapor. This can be readily done by using plastic tubing, pipe connectors, and an elevated drum of water than can be regularly refilled. Using the Yak Pak, ca. 1 tonne of plant material like lemongrass could be processed in an 8-hour period assuming that the steam generator is kept well stoked.

5. CONCLUSIONS

It is hoped that the processes that have and are still being used to isolate aromatic materials from natural products of plant origin have been adequately described.

It is perhaps pertinent to conclude with some comments that were made a few years ago on the future of natural products in the flavor and fragrance industries. It is believed the pressures that essential oil producers and users will face are:

- World population will put pressure on land for food production and more rapid cash return
- Reduced government funding for research on essential oils will have to be provided by other sources, otherwise the production side of essential oils will suffer
- The number of scientists familiar with all facets of essential oils is diminishing, i.e., chemists are unfamiliar with plant physiology, botany, and agronomy and vice versa
- Oil adulteration is self-defeating the use of essential oils
- Synthetic replacements could replace certain essential oils
- New toxicological findings may cause raw material use modifications
- There are no nationally or internationally funded genepool reserves of oil-rich species
- Essential oils could become luxury items unless there is a closer working relationship between the user and the producer.

Finally, it is the belief of this author that, in spite of the pressures put on essential oil production and use, essential oils will continue to be important ingredients of food, pharmaceutical and fragrance preparations, because there will be a greater cooperation between the producers and users of essential oils over the next century, even though there may be changes in their areas of production.

Acknowledgments

This author would like to acknowledge the assistance of Mr. E. F. K. (Tim) Denny for the design of the Yak Pak and his input into developing an understanding of steam distillation that is very well described in his book entitled "Field Distillation of Herbaceous Oils" 2nd Edition 1991. The diagrams in the following figures Fig.13 (page 90), Fig.14 (page 91), Fig.30 (page 129), Fig.32 (page 133) and Fig.34 (page 138) are derived from the originals in that book. Acknowledgment is also extended to Mr. Stan Allured, from Allured Publishing for allowing me to use some diagrams that appeared in Perfumer and Flavorist, to Jean-François Arnaudo, from Biolandes and to Jose Flores, from FMC for their assistance. Finally, acknowledgment is extended to Gretchen Braswell, from RJR Tobacco Company for her beautiful artwork, and to Ann Niten, RJR Tobacco Company who typed this report in an extremely short timeframe.

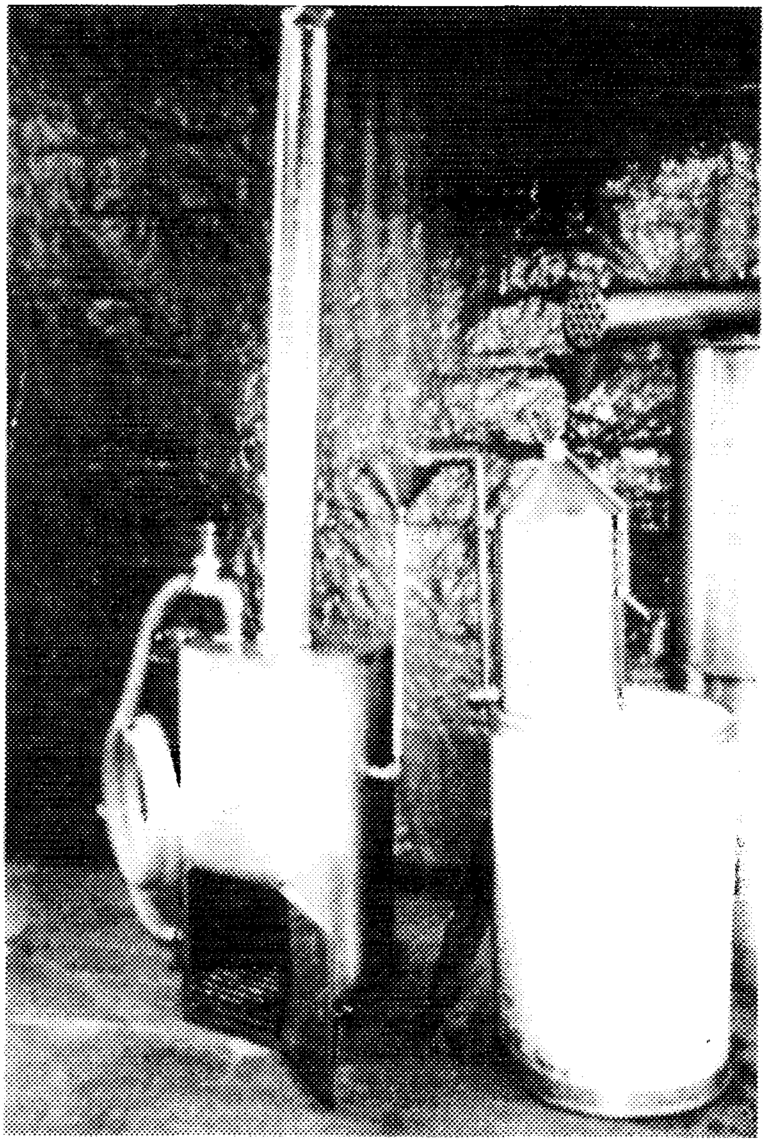


Figure 39. Photograph of Yak Pak showing the steam generator

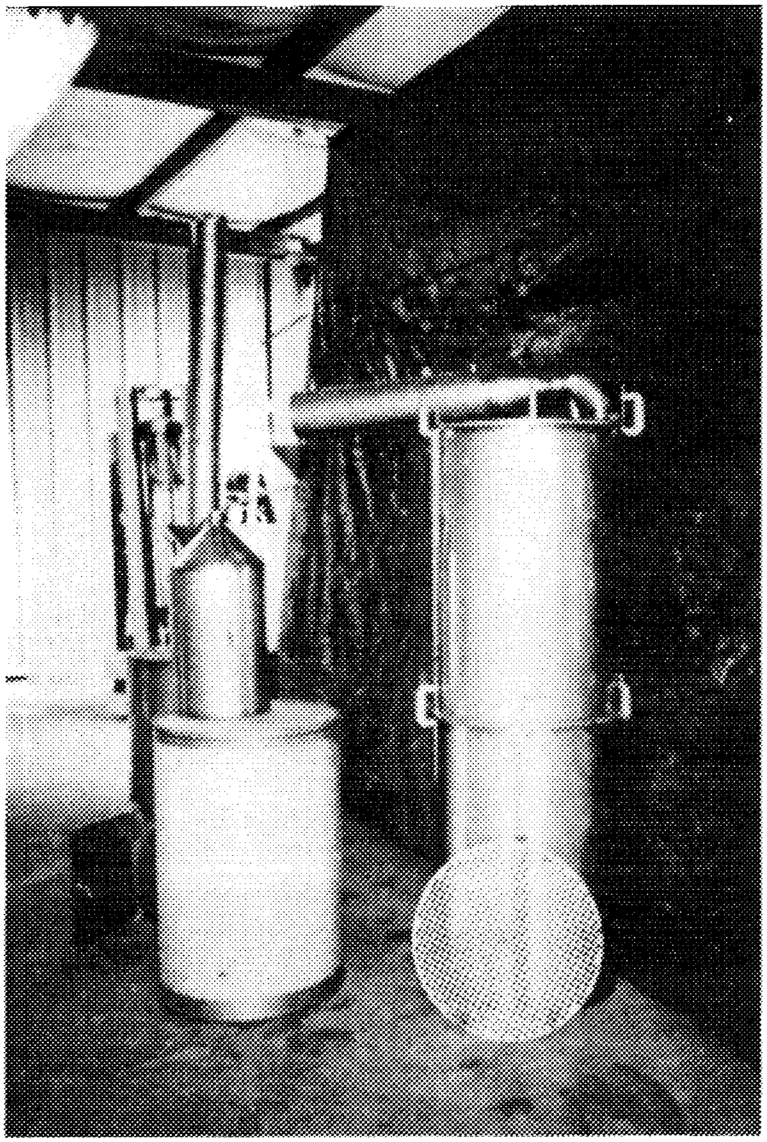


Figure 40. Photograph of Yak Pak showing the still top condenser and perforated plate

Figure 41. Photograph of Yak Pak showing the end of the condenser and the separator

REFERENCES

Part 1.

1. Naves, Y.R. and Mazuyer, G. , (1947). *Natural Perfume Materials.* Reinhold Publ. Corp., New York.

2. Sozio, H., (1956). *Essential Oils. Products of Enfleurage*. Perfum. Essent. Oil Rec., 47, 160-162.

3. Merory, J. , (1968). *Food Flavorings. Composition, Manufacture and Use*. AVI Publ. Co., Westport, Connecticut.

4. Esau, K. , (1977). *Secretory Structures*. Chapter 13 In: Anatomy of Seed Plants. 2nd Edn., J. Wiley & Sons, New York (1977).

5. Harold, F.V. and Clarke, B.J. , (1979). *Liquid CO2 Hop Extraction - The Commercial Reality.* The Brewers Digest, (Sept.), 45-58.

6. Pagington, J.S., (1983). *A Review of Oleoresin Black Pepper and Its Extraction Solvents.* Perfum. Flav., 8, (4), 29-36.

7. Meyer-Warnod, B., (1984). *Natural Essential Oils. Extraction Processes and Application to Some Major Oils.* Perfum. Flav., 94, (2), 93-104.

8. Denny, E.F.K. (1991). *Field Distillation for Herbaceous Oils.* 2nd Edn., 265 p., Denny-McKenzie Assoc., Lilydale, Tasmania.

9. Moyler, D.A. , (1991). *Oleoresins, Tinctures, and Extracts.* In: Food Flavorings. Edit., P. Ashust, pp. 54-86, AVI Van Nostrand Reinhold.

10. Hainrihar, G. , (1991). *Spice Oleoresins.* International Food Ingredients, (4), 52-57.

11. Paré, J.R.J., Sigouin, M. and Lapointe, J. , (1991). *Microwave-assisted Natural Product Extraction.* U.S. Patent 5,002,784, March 26.

12. Lupina, T. , (1995). Kalsec, Personal Communication.

13. Chen, S-C. and Spiro, M. , *Kinetics of Microwave Extraction of Rosemary Leaves in Hexane, Ethanol, and a Hexane + Ethanol Mixture.* Flav. Fragr J., 10, 101-112.

Part 2.

1. Kahol, A.P. , (no date). *Distillation Technology. In: Practical Manual on: The Essential Oils Industry.* Edit., R.O.B Wijesekera, UNIDO, Vienna.

2. Guenther, E. , (1949). *The Essential Oils.* Vol. 3, D. van Nostrand, Princeton, N.J.

3. Guenther, E. , (1952). *The Essential Oils.* Vol. 6, D. van Nostrand, Princeton, N.J.

4. Lawrence, B.M., (1979). *Commercial Production of Non-Citrus Essential Oils in North America.* Perfum. Flav., 3 (6), 21-33.

5. Legast E. and Peyron, L., (1983). *Hydrodiffusion Industrial Technology to Produce Essential Oils.* Proceedings of 9th International Congress of Essential Oils, Tech. Book. No. 1, pp. 69-73, Singapore.

6. Pellecuer, J. , De Lauzun, V., Attisso, M., De Bouchberg, M. Simeon., Jacob M. and Iderne, M., (1983). *A Study of Producing and of the Quality of Essential Oils Obtained by a New Process of Extraction: Hydrodiffusion.* Proceedings IXth International Congress of Essential Oils. Tech. Book 5, pp. 115-120, Singapore.

7. Block, E. (1985). *The Chemistry of Garlic and Onions.* Scientific American, (March), 114-120.

8. Boucard, G.R., Boucard, V.C. , Serth, R.W. and Ainsworth, B.S. (1986). *Pilot Plant for Continuous Distillation of Essential Oil-bearing Materials.* AICHE. Symp. Ser., (82), 193-200.

9. Simon, D.Z., Beliveau, J. and Aube, G., (1987). *Cedarleaf Oil (Thuja occidentalis L.) Extracted by Hydrodiffusion and Steam Distillation. A Comparison of Oils Produced by Both Processes.* Intern. J. Crude Drug Res., 25, 4-6.

10. Boelens, M.H. , Valverde, F., Sequerios L. and Jimenez, R. (1990). *Ten Years of Hydrodiffusion Oils.* Perfum. Flav., 15 (5), 11-14.

11. Carle, R. and Fiedler, G. , (1990). *Über ein kontinuierliches Verfahren zur Gewinnung ätherischer Öle.* Die Pharmazent. Indust., 52, 1142-1146.

12. Chalchat, J.C., Garry, R.P., Michet, A. and Peyron, L. (1990). *Chemical Composition of Natural and Empyreumatic Oils and Extracts from Juniperus oxycedrus and Juniperus phoenicea wood.* J. Essent. Oil. Res., 2, 231-236.

13. Dann, A.E., (1990). Personal Communcation.

14. Arnaudo, J. F., (1991). *Le Gout du Naturel*. Booklet, Biolandes Arômes Laboratories, Mougins Cedex, France .

15. Boucard, G.R. and Serth, R.W., (1991). *A Continuous Steam Stripping Process for the Distillation of Essential Oils.* Perfum. Flav., 16 (2), 1-8.

16. Denny, E.F.K., (1991). *Field Distillation for Herbaceous Oils.* 2nd Edn., 265 p., Denny-McKenzie Assoc., Lilydale, Tasmania.

17. Lawrence, B. M. (1992). *The Spearmint and Peppermint Industry of North America.* In: Compte Rendu des 3ème Recontres Techniques et Economiques Plantes Aromatiques et Medicinales. Edit., Verlet, N. , pp. 59-90, Centre Formation Professionele et Promotion Agricole (C.F.P.P.A), Nyons .

18. Vacchiano, C., (1992). *Distillation and Extraction: Evolution to Biolandes Process.* Indian Perfum., 36, (2), 129-133.

19. Baser, K.H.C. (1992). *Turkish Rose Oil.* Perfum. Flav., 17 (3), 45-52.

Part 3.

1. van Dijck ,W.J.D. and Ruys, A.H. , (1937). *Terpeneless Oils. A New Method of Production.* Perfum. Essent. Oil Rec., 22, 91-94.

2. Naves, Y.R. (1947). *Terpeneless and Sesquiterpeneless Oils.* Mfg. Chemist Mfg. Perfumer, 18, (4) 172-176.

3. L'Eplattenier, J.J., (1952). *Naardenised Oils. Their Significance and Utilization Perfumery.* Soap Perfum. Cosmet., 25, 383-386.

4. Jacobs, M.B. (1952). *Terpeneless Oils Preparation.* Amer. Perfum., 59, 121-122.

5. Jacobs, M.B. (1952). *Preparation of Terpeneless Oils.* Amer. Perfum., 59, 293-294.

6. Kirchner, J.G. and Miller, J.M., (1952). *Preparation of terpeneless essential oils.* Ind. Eng. Chem., 44, 318-321.

7. Littlejohn, W.J., (1954). *Terpeneless and Sesquiterpeneless Essential Oils: Their Characteristics, Advantages, and Mode of Employment.* Perfum. Essent. Oil Rec., 45, 117-121. Ellis, S.R.M. and Freshwater, D.C., (1954). Distillation Part I. Equilibrium Data. Perfum. Essent. Oil Rec., 45, 271-286.

8. Ellis S. and Freshwater, D.C. (1954). *Distillation Part III. Vapour-Liquid Equlibria in Non-Ideal Systems.* Perfum. Essent. Oil Rec., 45, 308-311.

9. Ellis, S.R.M. and Freshwater, D.C. (1954). *Distillation Part V. Plate-type Column Design and Performance.* Perfum. Essent. Oil Rec., 45, 418-423.

10. Ellis, S.R.M. and Freshwater, D.C., (1955). *Distillation Part VI. HETP Data in Packed Distillation Columns.* Perfum. Essent. Oil Rec., 46, 26-28.

11. Ellis, S.R.M. and Freshwater, D.C. , (1955). *Distillation Part IX. The Separation of Terpene Fractions.* Perfum. Essent. Oil Rec., 46, 128-131 (1955).

12. Ellis, S.R.M. and Freshwater, D.C., (1955). *Distillation Part X. The Separation of Terpene Fractions (continued).* Perfum. Essent. Oil Rec., 46, 161-166.

13. Broderick, J.J., (1955). *Terpeneless Oils.* Amer. Perfum. Essent. Oil Rev., 65, 19-23.

14. Braverman, J.B.S. and Solomiansky, L., (1957). *Separation of Terpeneless Essential Oils by Chromatographic Method.* Perfum. Essent. Oil Rec., 48, 284-289.

15. Arctander, S., (1960). *Perfume and Flavor Materials of Natural Origin.* Publ. by author Elizabeth, N.J. (avail. Allured Publ., Carol Stream, USA).

16. Norman, S. and Craft, C.C., (1966). *Deterpenation of Citrus Essential Oils by Solvent Position with Dimethyl Sulfoxide*. J. Food Sci., 31, 529-533.

17. Rockland, L.B. and Debenedict, C.R. , (1975). *Production of Terpeneless Essential Oils*. U.S. Patent No. 3,867,262, Feb. 18.

18. Shankaranayarana, M.B., Anandaramar, S. and Natarajan, C.P., (1977) *Studies on the Chromatographic Deterpenation of Lemon and Lime Oils*. J.Food Sci.,Technol.,14,120-125

19. Eckert, J.S., (1979). *Design of Packed Columns. In: Handbook of Separation Techniques for Chemical Engineers*. Edit., P. A. Schweitzer, pp. 1-221, McGraw-Hill Book Co., New York.

20. Drawert, F. and Berger, R., (1981). *Possibilities of the Biotechnological Production of Aroma Substances by Plant Tissue Culture*. In: Flavor '81. Edit., P. Schreier, pp. 509-527 Walter de Gruyter, Berlin.

21. Tateo, F., (1981). *Use of Synthetic Polymer Absorbents for Processing and Recovering Essential Citrus Fruit Oils*. In: Flavor '81. Edit., P. Schreier, pp. 671-682, Walter de Gruyter, Berlin.

22. Vora, J.D., Matthews, R.F., Crandall, P.G. and Cook, R. , (1983). *Preparation and Chemical Composition of Orange Oil Concentrates*. J. Food Sci., 48, 1197-1199.

23. Gerard, D., (1984). *Continuous Removal of Terpenes from Essential Oils by Counter Current Extraction with Compressed Carbon Dioxide*. Chem. Ing. Tech., 56, 794-795.

24. Jourdain, N., Goli, T., Jallageas, J.C., Crouzet, C., Ghommidh, Ch., Navarro, J.M. and Crouzet, J. (1985). *Aroma Components Production by Immobilized Microbial Cells. In: Topics in Flavor Research*. Edits., Berger, R.G., Nitz S. and Schreier, P., Eichorn, H., Marzling-Hangenham, Germany .

25. Gatfield, I.L., (1986). *Generation of Flavor and Aroma Components by Microbial Fermentation and Enzyme Engineering Technology*. In: Biogeneration of Aromas. Edits., T Parliment and R. Croteau, ACS Symp. Series 317, Amer. Chem. Soc., Washington, DC.

26. Owusu-Yaw, J. , Matthews, R.F. and West, P.F., (1986). *Alcohol Deterpenation of Orange Oil*. J. Food Sci., 51, 1180-1182 (1986).

27. Whitaker, R.J., Hobbib, G.C. and Steward, L.A. (1986). *Production of Secondary Metabolites in Plant Cell Cultures. In*: Biogeneration of Aromas. Edits., T. H. Parliment and R. Croteau, ACS Symp. Series 317, Amer. Chem. Soc., Washington, DC.

28. Anon., (1987). *Biolandes unité d'hydro-distillatin en continu*. Parfums Cosmet. Arômes, No. 73, 63-66.

29. Ferrer, O.J. and Matthews, R.F., (1987). *Terpene Reduction in Cold-Pressed Orange Oil by Frontal Analysis-Displacement Adsorption Chromatography*. J. Food Sci., 52, 801-805.

30. Collin, H.C., (1988). *Flavors, Phytochemical in Plant Cell Cultures*. Cell Cutl. Somatic Cell Genet. Plants, (5), 569-585.

31. Gatfield, I.L., (1988). *Production of Flavor and Aroma Compounds by Biotechnology*. Food Technol., (Oct.), 110-169.

32. Temelli, F., Chen, C.S. and Braddock, R.J. (1988). *Supercritical Fluid Extraction in Citrus Oil Processing*. Food Technol., (June), 145-150.

33. SugaT. and Hirata T., (1990). *Biotransformation of Exogenous Substrates by Plant Cell Cultures*. Phytochemistry, 29, 2393-2406.

34. Tateo, F., (1990). Production of Concentrated Orange Oils Using Thin Film Evaporator.J. Essent. Oil Res., 2, 7-13.

35. Tzantzis, N.E., Liodakis, S. E. and Parissakis, G.K., (1990). *Deterpenation of Essential Oils by Preparative Liquid Chromatography*. Rivista Ital. EPPOS, No. 1, 23-31.

36. von Campe, G., (1990). *Terpene-free Citrus Oils*. Dragoco Rept., (1), 3-17.

37. Brose, D.J., Chidlaw, M.P., LaChapelle, E.D. and van Eikeren, P. (1992). *Fractionation of Citrus Oils Using Membrane Mediated Cyclodextrin Extraction*. Proceedings of 6th International Cyclodextrin Symposium, Paris, pp. 573-578.

38. Moyler, D.A. and Stephens, M.A., (1992) 37-38.. *Counter Current Deterpenation of Cold Pressed Sweet Orange Peel Oil*. Perfum. Flav., 17 (2),

39. Armstrong, D.W. and Brown, L.A., (1994). *Aliphatic, Aromatic and Lactone Compounds*. In: Bioprocess Production of Flavor, Fragrance and Color Ingredients. Edit., A. Gabelman, pp. 41-94, John Wiley & Sons, Inc., New York.

40. Barth, D, Chouctii, D., Della Porta, G., Reverchon E. and Perrut, M. (1994). *Desorption of Lemon Peel Oil by Supercritical Carbon Dioxide: Deterpenation and Psoralens Elimination*. J. Supercritical Fluids, 7, 177-183.

41. Fleisher, A., (1994). *Citrus Hydrocarbon-free Essential Oils*. Perfum. Flav., 19 (1), 11-15.

42. Sahai, O., (1994). *Plant Tissue Culture. In: Bioprocess Production of Flavor, Fragrance and Color Ingredients*. Edit., A. Gabelman, pp. 239-275, John Wiley & Sons, Inc., New York.

43. Seitz, E.W., (1994). *Fermentation Production of Pyrazines and Terpenoids for Flavors and Fragrances*. In: Bioprocess Production of Flavor, Fragrance and Color Ingredients. Edit., A. Gabelman, pp. 95-134, John Wiley & Sons, New York.

44. Dugo, P., Mordello, L., Bartle, K.D., Clifford, A.A., Breen, D.G.P.A. and Dugo, G., (1995). *Deterpenation of Sweet Orange and Lemon Essential Oils with Supercritical Carbon Dioxide Using Silica Gel as an Adsorbent*. Flav. Fragr., J., 10, 51-58.

Part 4.

1. Rovesti, P., (1977). *Distillation is 5000 Years Old.* Dragoco Rept., (3), 59-62.

2. Denny, E.F.K. (1991). *Field Distillation for Herbaceous Oils.* 2nd Edn., 265 p.,Denny-McKenzie Assoc., Lilydale, Tasmania.

3. Porter, N.G. and Lammerink, J.P., (1994). *Effect of Temperature on the Relative Densities of Essential Oils and Water.* J. Essent. Oil. Res., 6, 269-277.

4. Baser, K.H.C., (1992). *Turkish Rose Oil.* Perfum. Flav., 17 (3), 45-52.

CHAPTER 4

ANALYSIS AND QUALITY ASSESSMENT OF ESSENTIAL OILS

K. HUSNU CAN BASER
Anadolu University Medicinal and Aromatic Plant and Drug Research Centre
Eskişehir, Turkey

1. INTRODUCTION

Essential oils, also known as essences or volatile oils, are complex mixtures of volatile substances biosynthesized by living organisms. They can be liberated from the host organism by distillation, expression or extraction with a suitable solvent. They are used in flavour, fragrance, food, cosmetics, perfumery, toiletry and pharmaceutical industries.

Constituents of essential oils include hydrocarbons and their oxygenated derivatives which comprise alcohols, acids, esters, aldehydes, ketones, amines, sulphur compounds, etc. Mono-, sesqui- and even diterpenes constitute the composition of a majority of essential oils. In addition, phenylpropanoids, fatty acids and their esters are also encountered in a number of essential oils.

Essential oils are called oils due to their liquid nature at room temperature. They should not be confused with fixed oils which are composed of a naturally occurring mixture of lipids and are not volatile. Therefore, essential oils differ entirely in both chemical and physical properties from fixed oils.

Essential oils are derived mainly from aromatic plants. A few of them are obtained from animal sources, *e.g.* musk, civet, etc. or produced by microorganisms. Mosses, liverworts, seaweeds and fungi have also been shown to contain essential oils. In plants, essential oil is contained in oil cells, secretion ducts or cavities, or in glandular hairs. In some cases, they are bound with sugars in the form of glycosides. In such cases, they must be liberated by hydrolysis of the glycosidic bond. They are frequently associated with other substance such as gums and/or resins. Such natural combinations are called *oleogummiresina* or *oleoresina*. Essential oils can be freed from such products by distillation.

Essential oils are obtained by distillation *e.g.*, water (or hydro) distillation, steam distillation, water and steam distillation, hydrodiffusion, expression (applied mainly in the production of Citrus oils), and extraction. When non-polar solvents are used in the extraction of fresh plant material the final product is called a *concrete*, which can be re-extracted with ethanol to obtain an *absolute*. When dried plant material is used, the final product is called an *oleoresin*. A special technique used for the extraction of fragrance components from fresh flowers with fats of very high purity is called *enfleurage*. The fat containing the oils , also called a *pommade*, is later extracted with ethanol to obtain an *absolute*.

Such great variations encountered in the quality of essential oils have necessitated the establishment of quality criteria. Therefore, standards and specifications have been drawn up by international and national standards authorities, pharmacopoeias, codices, etc. If a standard does not exist for a given oil, the produce should comply with the buyer's specifications.

Commercial essential oils are required to comply with sets of standards. The standards are numerical values with lower and/or upper limits. Oils whose numerical values fall within the accepted range can find their way into the market. Such standards and specifications are set out in monographs published by standards organizations such as International Standards Organization (ISO), British Standards (BS), Essential Oil Association of USA (EOA), American Society for Testing and materials (ASTM), Turkish Standards Institute (TSE), Indian Standards (IS), German DIN Standards, French AFNOR Standards, etc., or in pharmacopoeias or codices such as British Pharmacopoeia (BP), US Pharmacopoeia (USP), European Pharmacopoeia (EP), Deutsche Arzneibuch (DAB), Codex Alimentarius Commission, Food Chemicals Codex (FCC), etc.(3-5). Numbers of ISO standards for some essential oils are given in Annex 1. Full list of standards on essential oils can be obtained from the ISO.

2. QUANTITATIVE DETERMINATION

The determination of essential oil content in an aromatic plant is carried out using a Clevenger type glass apparatus. This apparatus is considered official in many pharmacopoeias, *e.g.* European Pharmacopoeia (EP), British Pharmacopoeia (BP). It consists of a 2 L flask in which plant material and water are placed the flask sits in a heating mantle, connected to a condenser, a graduated tube, a return tube which returns the overflowing distillate water back to the flask. It is a close-circuit continuous distillation apparatus which is run for *ca.*3 hours for each distillation. There are two different designs where the return tube may be either on top or at the bottom of the graduated tube to be used according to the density of the oil in water. In the case of low essential oil yielding plants a known amount of xylene is placed in the graduated tube over water. Since essential oils are not miscible with water but soluble in organic solvent, increase in the volume of xylene indicates the amount of oil in the plant material. Fig.1 illustrates the Volumetric Essential Oil Determination Apparatus for lighter than water oils.

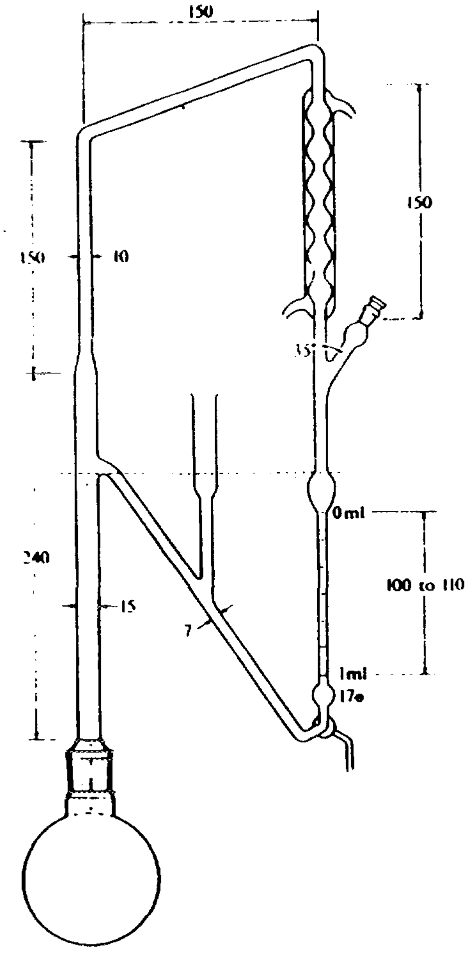


Figure 1. Volumetric Essential Oil Content Determination Apparatus

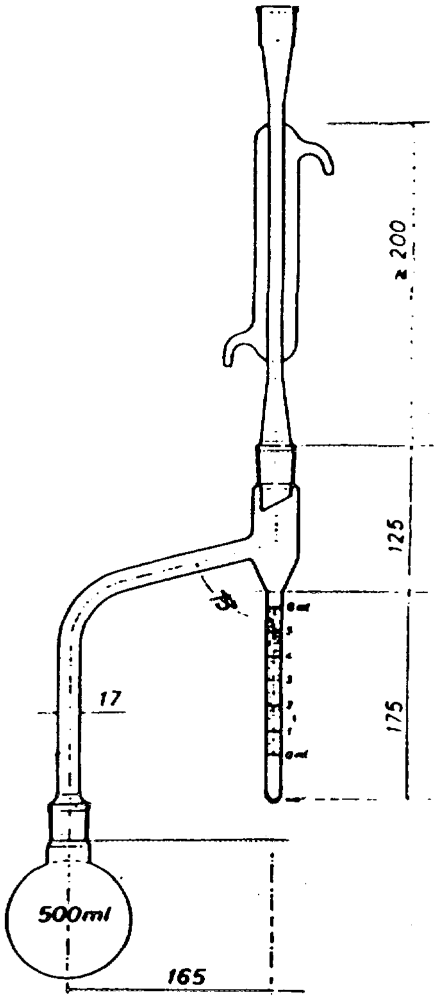


Figure 2. Moisture Content Determination Apparatus

Essential oil determination results should always be expressed on moisture-free basis. Therefore, alongside essential oil determination, moisture content determination of the same plant material should also be carried out. Volumetric moisture content determination technique is based on distillation of the sample with an immiscible solvent (*e.g.* xylene or toluene) presaturated with water. Moisture in the plant material is co-distilled with the volatile solvent and the distillate is collected in a graduated tube just below the condenser assembly. The water distilled accumulates at the bottom of the graduated tube and the immiscible solvent is continuously returned to the distillation flask. Volumetric moisture content determination apparatus is shown in Figure 2.

The following techniques are used mainly for research purposes or for trapping the odoriferous compounds in little amounts of the test material.

Likens-Nickerson Simultaneous Distillation Extraction (SDE) Apparatus

This apparatus permits a many thousandfold concentration of volatiles from aqueous media in one step using a closed-circuit distillation system. A major advantage of this technique is the use of small amount of material.

The material is distilled with water. The distillate comes into contact with an immiscible solvent and is continuously extracted. The whole process ends in 1 hour. If a suitable internal standard is added to the solvent prior to distillation, then quantitative measurement of ppm amounts of essential oils can be possible. There are two versions of this apparatus. One is for high density solvents such as dichloromethane and the other one is suitable for use with lighter than water solvents (1).

Head Space (HS) Trapping

Steam or water distilled essential oils do not necessarily represent the true composition of the actual fragrance of an aromatic plant. Essential oils are mostly artefacts formed during distillation. Since they have been around for a long time, they are the oils found and accepted in commerce. Head space (HS) technique aims at capturing the real odoriferous compounds of a plant material. It can be applied to live or cut flowers and to anything that smells. The technique consists of trapping the odour on an adsorbent (*e.g.*, active charcoal, Tenax, Porosil, etc.) which is either thermally desorbed or more suitably eluted with the minimum amount of a suitable solvent prior to analysis by GC or GC/MS. HS technique is mainly used for research purposes to analyze and reconstitute odours. It is a powerful tool for innovative perfumers (2).

3. QUALITY ASSESSMENT TECHNIQUES

Methods for quality assessment of essential oils can be classified under the following headings:

1. Sensory evaluations
2. Physical tests
3. Chemical tests
4. Instrumental techniques

Quality judgement of an essential oil should be based on the combined data obtained by the above analyses (3).

1. ***Sensory evaluations*** can be carried out only by expert noses. Such an ability can be gained after years-long tedious but systematic olfactive training. To an experienced nose, the evaporation pattern of an essential oil smeared on a smelling strip, over a period of time, gives information about its source, age, main components and even its authenticity. Smelling must be carried out at intervals

immediately after dipping to 1, 2 and 6 hours, and after standing overnight over a period not less than 18 hours. Comparison with an authentic sample of acceptable quality will help the assessor to make a correct judgement. As obvious, sensory evaluations are subjective and may vary from person to person. Therefore, such assessments are, in general, realized by a panel of experts and in all cases their assessments should be verified and documented by experimental proof.

2. ***Physical tests***: The following physical tests are conducted:

a) Moisture content
b) Specific gravity
c) Optical rotation
d) Refractive index
e) Residue on evaporation
f) Freezing or congealing point
g) Solubility in dilute alcohol

a) Moisture content determination of an essential oil can be determined by Karl-Fischer titration, gas chromatographic, spectroscopic or electrometric methods. Drying of an essential oil is accomplished by the addition of or filtering through desiccating agents such as anhydrous sodium sulphate. A simple way to check the presence of moisture in an essential oil is carried out by mixing 0.5 ml essential oil with 1 ml carbondisulphide. A clear solution indicates the absence of moisture.

b) The specific gravity (d_{20}^{20}) of an oil is the weight of a given volume of the oil at a specified temperature compared with the weight of an equal volume of water at the same temperature, all weighings being taken in air. A pycnometer is used for this determination.

c) The optical rotation ($[\alpha]_D^{20}$) of an oil is the angle through which the plane of polarization is rotated when polarized light passes through it. Results are expressed as dextrorotatory (+) or levorotatory (-) according to whether the plane of polarization is rotated clockwise or anticlockwise, respectively, as determined by viewing towards the light source. Unless otherwise specified, the optical rotation is measured at the wavelength of the sodium D line (589.3 nm) at a temperature of 19.5° to 20.5° in a layer 1 dm thick. Polarimeters are used for the determination of optical activity. This technique provides useful data on the optical purity of the oil.

d) The refractive index ($[n]_D^{20}$) of an oil with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the oil. It varies with the wavelength of the light used in its measurement. Abbe type refractometers are widely used for the determination of the refractive index.

e) The residue on evaporation is the percentage by weight of the oil that remains after evaporation when determined in a heat-resistant glass evaporating basin.

f) The freezing or congealing point is the highest temperature observed during the solidification of a supercooled liquid.

g) Solubility in dilute alcohol: All essential oils are soluble in absolute alcohol and many are soluble in dilute alcohol. It is, therefore, possible to determine the number of volumes of dilute alcohol required for the complete solubility of one volume of oil. Sometimes, however, the solutions obtained are not clear, then, they are defined as "opalescent".

3. *Chemical tests*:

Chemical tests include determination of aldehydes, acid value, ester value (for calculation of esters and combined alcohols), ester value after acetylation (for calculation of free alcohols), carbonyl value, phenol content, etc.

Determination of the following constituents are specifically mentioned in some monographs for quality assessment of the below mentioned essential oils: anethole in anise and star anise oils; carvone in caraway, dill and spearmint oils; cineole in cardamom, rosemary and sage oils; linalool in coriander and basil oils.

Acid value is a numerical value equivalent to the number of milligrams of potassium hydroxide required to neutralize the free acids present in 1 g of the oil.

Carbonyl value is numerically equivalent to the number of milligrams of potassium hydroxide that is equivalent to the amount of hydroxylamine required to oximate the carbonyl compounds present in 1 g of the oil.

Ester value is the number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of the esters present in 1 g of the oil.

Ester value after acetylation is numerically equivalent to the number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of 1 g of the acetylated oil.

4. *Instrumental techniques*:

These include chromatographic and spectroscopic techniques or their combinations (often described as *hyphenated techniques*) such as the following:

Chromatographic techniques:

Column Chromatography (CC)
Thin Layer Chromatography (TLC)
Gas-Liquid Chromatography (GLC or GC)
High Pressure Liquid Chromatography (HPLC)
Medium Pressure Liquid Chromatography (MPLC)
Supercritical Fluid Chromatography (SFE)
Size Exclusion Chromatography (SEC)

Spectrophotometric techniques:

Ultraviolet and Visible Spectrophotometry (UV/VIS)
Infrared Spectrophotometry (IR)

Spectroscopic techniques:

Mass Spectrometry (MS)
Nuclear Magnetic Resonance Spectroscopy (NMR)

- ^{13}C-NMR Spectroscopy
- Site-Specific Natural Isotope Fractionation NMR (SNIF-NMR)

Combined techniques:

Gas Chromatography/Mass Spectrometry (GC/MS)
Liquid Chromatography/Mass Spectrometry (LC/MS)
Gas Chromatography/Fourier-Transform Infrared Spectrophotometry (GC/FT-IR)
Gas Chromatography/Fourier-Transform Infrared Spectrophotometry/Mass Spectrometry (GC/FT-IR/MS)
Gas Chromatography/Atomic Emission Detector (GC/AED)
Gas Chromatography/Isotope Ratio Mass Spectrometry (GC/IRMS)
Multidimensional Gas Chromatography (MDGC)

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was once used for essential oil analysis. However, this technique is not, nowadays, used for qualitative or quantitative purposes due to poor separation power for complex mixtures such as essential oils. Nevertheless, it can still be useful for the evaluation of simple mixtures (6).

Liquid Chromatography (LC) Techniques

Liquid Chromatography techniques have always been used in essential oil analysis mainly for the prefractionation of essential oils. For the separation of terpene hydrocarbons from oxygenated hydrocarbons simple column chromatography (CC) with normal phases is used. Fractions are eluted first with n-hexane or pentane, followed by diethylether.

High Pressure Liquid Chromatography (HPLC), Medium Pressure Liquid Chromatography (MPLC), Size Exclusion Chromatography (SEC) (= Gel Permeation Chromatography) and Supercritical Fluid Chromatography (SFC) techniques are used for prefractionation of complex mixtures of essential oils prior to gas chromatographic analysis.

Gas Chromatography (GC) is the most important technique for the separation of essential oil constituents. Thanks to the advances in technology there is, now, a wide range of column packing materials and detectors. With the advent of fused-silica capillary columns, separation, identification and quantification of numerous constituents in essential oils have become possible without peak distortion even after long elution times (1,7,8).

Gas chromatography (GC) is based on separation of volatile components of a liquid or gas mixture which is volatilized in a column packed with a liquid phase adsorbed as a thin film on a support (stationary phase). Mobile phase is nitrogen or helium gas which carry volatile compounds through the column. The column is placed in an oven where isocratic and temperature programmed conditions can be achieved. The components of a mixture are separated due to differences in the partition coefficients of the said components between the gas phase and the stationary liquid phase. Compounds which have less affinity to the stationary phase are eluted first from the column.

In routine capillary GC analysis, 1µl of a 10% solution of essential oil in n-hexane or diethylether is injected by a microsyringe. Split ratio is normally decided after experimentation, but the normal practice is 1:60 - 1:40 . That is, one part of 60-40 goes directly into the column for analysis, the rest is discarded in order not to overload the column.

Before the advent of capillary columns, metal or glass columns packed with solid support materials coated with a liquid stationary phase were in use. Due to much higher separation power in a shorter analysis time, nowadays, capillary columns are preferred. These are long (20-60 m) glass or fused silica columns with small diameter. The most popular types of capillary columns are the following: wall-coated open tubular (WCOT) where the liquid stationary phase is directly deposited on inside the column wall; porous layer open tubular (PLOT) and support-coated open tubular (SCOT) where the liquid stationary phase is coated on neutral porous support particles. WCOT columns are preferably used for essential oil analysis. The narrow bore columns of 0.25-0.32 mm i.d. with film thickness of around 0.2-0.5µm are widely used. On the other hand, wide bore columns of 0.5 mm i.d. with 0.3-0.5 µm film thickness are preferred for splitless injections.

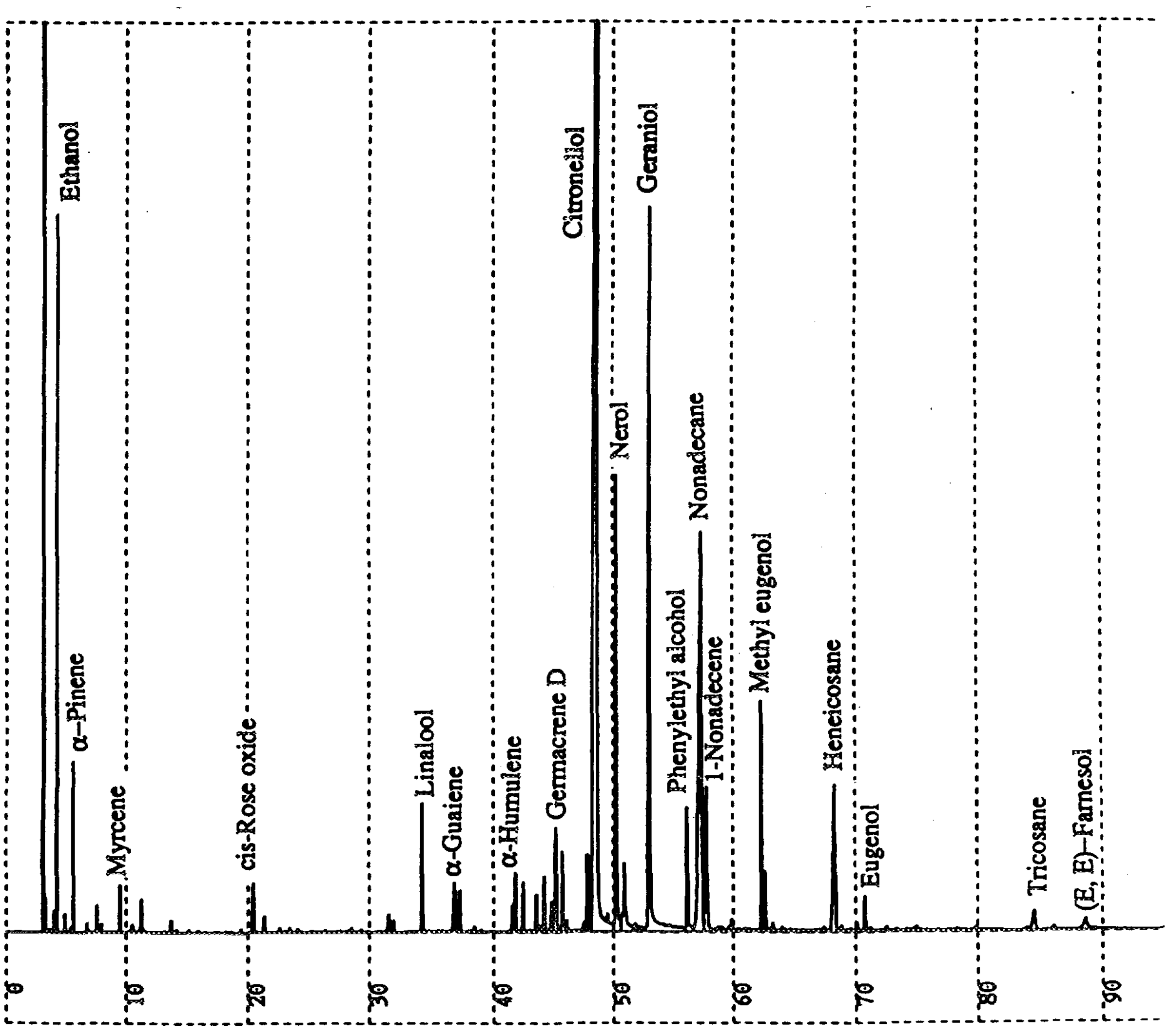


Figure 3. Gas Chromatogram of Turkish Rose Oil.
Chromatograph : Shimadzu GC-9A with C-R4A Integrator. *Column*: 50 m x 0.25 mm i.d. Thermon-600T. *Temperature programme* : 70°C for 10 min, and programmed to 180°C at a rate of 2°C/min then kept at 180°C for 30 min. Injection and detector (FID) temp. 250°C.

In capillary columns gums or gum-like phases are preferred. Apolar silicone phases such as SE-30, SE-52, SE-54, OV-1, OV-73, OV-101, DB-1 and DB-5 have become quite popular for essential oil analysis. Polyethylene glycol (PEG), Polypropylene glycol (PPG), Carbowax phases and their nitroterephthalic esters (FFAP phases) are also used in essential oil analysis. PEG 20M, Carbowax 20M are polar phases.

The eluted components, then, go to the detector. The most commonly used detectors are Thermal Conductivity Detector (TCD), Flame Ionization Detector (FID), Electron Capture Detector (ECD), Nitrogen Phosphorus Detector (NPD), Flame Photometric Detector (FPD), and more recently Atomic Emission Detector (AED), Infrared Spectrophotometer (IR), Ion Trap Detector (ITD), Mass Spectrometry (MS), etc. The detected components are processed by an integrator and appear on chart papers as a series of peaks on a time scale. The chart is, then, called a gas chromatogram. The integrators calculate the retention time (R_T), as well as the relative percentages of each peak.

Essential oils are complex mixtures of volatile compounds whose separation depends upon the skill of the analyst to optimize the conditions of analysis, such as the selection of column type, stationary phase, temperature programming parameters and other operational conditions. Once these conditions are set, then, a well resolved chromatogram can be obtained (Fig 3). Each peak on the chromatogram represents at least one constituent and each constituent can be identified by measuring its position relative to a standard peak of a known substance (relative R_T), or its retention time or retention index (See 5.4.2.2.5) can be compared with those of known compounds chromatographed under identical operational conditions. This exercise gives a strong indication on the identity of major peaks. However, it does not provide enough proof for their absolute identification. A measure of certainty can be achieved by the co-injection of an authentic sample of the suspected constituent with the essential oil. This technique is called "peak enriching" or "spiking". Any enhancement of the size of a peak is seen as proof of its identity with the spiked-compound.

Gas chromatography can be safely and routinely used with essential oils of known composition whose constituents have already been elucidated by hyphenated techniques such as GC/MS, GC/FT-IR, NMR, etc. The use of GC alone, for the analysis of new or uncommon essential oils is recommended only for reference information leading to tentative identification of the major constituents. This information also requires support by sensory evaluation.

Quantitative determination of the separated constituents can be achieved by gas chromatography. In modern systems, analyte quantitation is performed by a computer-based data processor called "integrator". It has been shown that, for a given compound, quite different quantitative results can be obtained using different integrators and detectors (23). Therefore, prior standardization or validation of the GC equipment is necessary to achieve reliable and reproducible results. Accurate quantitative results can be obtained only by the use of an internal standard or calibration using reference.

Odour evaluation of the eluted components from a gas chromatography column is possible through introduction of a "sniffing port" fixed to the end of the column *via* an all-glass splitting device. This allows the analyst to sniff the compound eluted while it simultaneously appears as a peak on the integrator/recorder. The wide bore columns are preferred for this exercise due to increased sample capacity which allows sample introduction without splitting.

Lawrence regularly reviews the literature for the composition of essential oils. These are very useful information sources for essential oil analysts (9).

Atomic Emission Detector (AED)

Atomic Emission Detector(AED)coupled to a gas chromatograph produces a series of heteroatom-specific chromatograms in a single run, each indicating peaks of compounds possessing a certain element such as nitrogen, sulfur, chlorine, etc. Eventually by comparing these chromatograms it is possible to ascertain which elements are present in each peak (or each compound) of an essential oil (10).

Mass Spectrometry (MS)

Gas Chromatography/Mass Spectrometry (GC/MS) is a sophisticated technique whereby MS acts as a detector for the compounds separated by a GC. The isolated compounds are illustrated in the form of a series of peaks in a Total Ion Chromatogram (TIC). Modern GC/MS equipment have computerized libraries and advanced search systems. Mass spectrum of each compound detected can be instantaneously taken and searched through thousands of mass spectra contained in computerized libraries for identification (Fig. 3). Mass spectral data alone cannot always be sufficient for correct identification and retention data should also be available for comparison. If a compound shows identical mass spectrum and retention data with a known compound then, they are considered identical. However, in cases of doubt, co-injection with a standard sample of the predicted compound, or running the same oil in a column with different polarity should be applied. The ideal solution to the problem could be the isolation of the compound in sufficient quantity to take a NMR measurement or to complement the analysis with FT-IR data. In case of new compounds, analysis of fragmentation data gives a clue to its chemical structure (11-15).

Ion Trap Detector (ITD)

Ion Trap Detector (ITD) is a table top mass spectrometer with a mass scanning range of 10 to 650 amu. It differs from quadruple mass spectrometry in some technical aspects. However, ion trap spectra are quite comparable to quadruple mass spectra. Intensities of some ions may appear different but the overall spectra are, in most cases, superimposable with those of the quadruple mass spectra. A GC/ITD database of essential oil components is available (16).

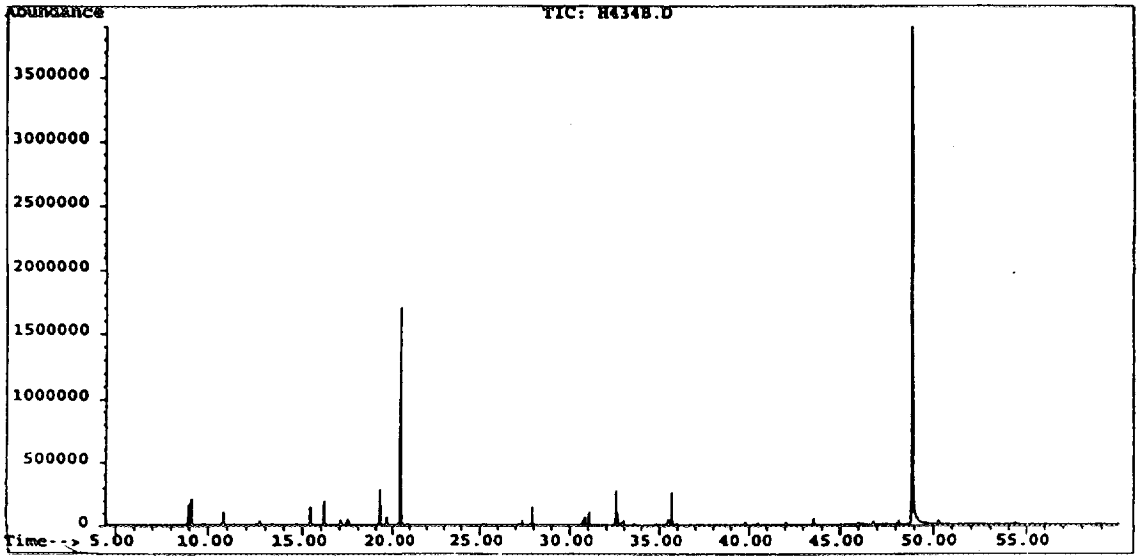
GC/MS Libraries

Several commercial mass spectral libraries for essential oil analysis are now available for use with GC/MS systems. Useful ones available on diskette include Wiley/NBS Mass Spectral Database (130.000 spectra), Identification of Essential Oil Components by Mass Spectrometry by R.P.Adams [LIBR(TP)] (1200 spectra), EPA/NIH Mass Spectral Database (ca.40.000 spectra), TNO-Compilation of Mass Spectra of Volatile Compounds in Food (ca. 1600 spectra), Compilation of Mass Spectral Data. They are also available in printed form together with the following: The Eight Peak Index of Mass Spectra (ca.30.000 spectral data), Spectral Atlas of Terpenes and Related Compounds, Analysis of Essential Oils by GC/MS, Qualitative Analysis of Flavor and Fragrance Volatiles by GC/MS, Archives of Mass Spectral Data, Monoterpenes: IR, MS, ^{1}H-NMR, ^{13}CNMR Spectra, and Kovats Indices, Identification of Essential Oils by Ion Trap Mass Spectrometry by R.P.Adams (1, 17-22). A few of the above collection includes retention index data of each compound.

Retention Index

In liquid chromatography, the time elapsing between the injection of a sample and recording of the peak maximum is defined as Retention time (R_T). Ideally, same operational conditions should result in the production of same retention time for a given solute. However, since R_T of a solute varies with temperature and with flow rate, it is difficult to maintain constant conditions throughout an analysis.The need to express analytical results in a more uniform and reproducible way has resulted in the development of retention indices (1).

```
File         : C:\UCUCUYAG\H434B.D
Operator     : Betul Demircakmak
Acquired     : 17 Feb 95  10:52 am using AcqMethod IWAX-1
Instrument   :   GCD
Sample Name: H434B- Origanum acutidens
Misc Info    : Verim: % 1.36
Vial Number: 1
```



```
Library Searched : C:\DATABASE\TBAM.L
Quality          : 97
ID               : Carvacrol
```

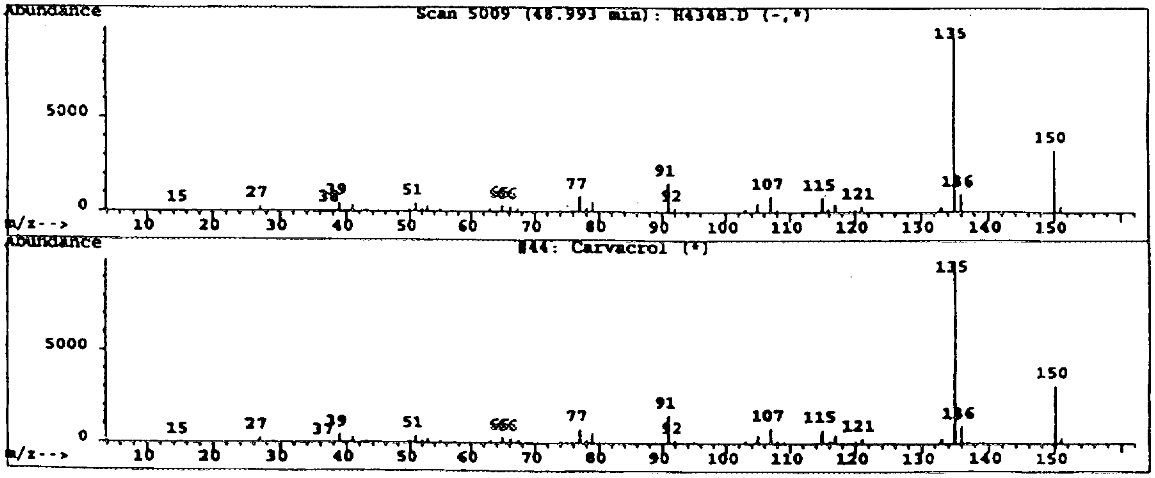


Figure 4. Total Ion Chromatogram and Identification of Carvacrol in *Origanum* Oil by GC/MS

Retention indices express the retention information of a given solute relative to the retention data of a reference solute which normally is applied in a homologous series. n-Paraffin hydrocarbons are used as homologous series in Kovats Retention Index System (KI).

$$KI = 100N + 100n \frac{\log t'_{R(A)} - \log t'_{R(N)}}{\log t'_{R(N+n)} - \log t'_{R(N)}}$$

where $t'_{R(N)}$ and $t'_{R(N+n)}$ are the adjusted retention times of n-paraffin hydrocarbons of carbon number N and N+n, respectively, eluting before and after Solute A with the adjusted retention time $t'_{R(A)}$. In practice, the adjusted R_T is obtained by subtracting the actual R_T of a solute from that of air or a low boiling point solvent. KI can be measured on a polar [KIP] (*e.g.*, Carbowax 20M), or a non-polar [KIA] (*e.g.*, OV-101, DB-1) stationary phases. Theoretically, isothermal conditions should be maintained to calculate the Kovats Indices. In practice, however, temperature programming can also be used.

Ethyl Ester Index System (EI) has been developed for use mainly with polar stationary phases. Homologous series used here are ethyl ester standards starting from ethyl formate to ethyl tetradecanoate.

Retention indices are useful in determining the elution order of solutes from polar or non-polar columns. Published retention indices can be safely used for identification provided retention data from a column of same polarity are compared.

A GC/MS analyst should follow the following recommendations:

- The MS and GC retention data should be strictly identical to those of reference sample present in computerized libraries. Additional use of IR data is preferable.
- The libraries should be developed by the analyst from genuine samples. The samples could either be isolated or synthesized by the analyst, obtained from reliable sources or identified in mixtures with known composition (23,24).

Wide variations in relative percentage values of compounds by using a range of GC integrators have been demonstrated. Therefore, reliable and reproducible results can be obtained using the same GC and GC/MS systems under identical operational conditions. It should be borne in mind that some compounds decompose in polar columns and are never detected. Exogenous contaminations due to plastic materials (*e.g.*, phthalates) and synthetic antioxidants present in some solvents (*e.g.*, butylated hydroxytoluene= BHT in diethylether) may lead to their identification as genuine constituents of essential oils.

However sophisticated and advanced the analytical equipment may be, efficient and reliable analytical work depends upon the skill and the degree of experience of the analyst. It is the responsible analyst's duty to avoid contaminating or polluting the literature.

Gas Chromatography/ Chemical Ionization Mass Spectrometry (GC/CI-MS)

This technique is used as complementary to the Electron Impact (EI) Mass Spectrometry Technique. It is a soft ionization process, in which, a limited amount of energy is transferred to the sample derived ion. Therefore, fewer fragment ions are generated, however, molecular weight of the compound can be determined more readily. Chemical ionization, in short, consists of the creation of sample derived ions by ion-molecule reaction of reactant ions with neutral sample molecules. Reactant gases used are methane, isobutane and ammonia. Chemical ionization can be applied both to positive and negative ions. Typical and simple fragmentation patterns of compounds in CI-MS help their identification more readily than EI-MS. Several manipulations of this technique have been described (1,25).

Fourier-Transform Infrared Spectrophotometer (FT-IR)

In this technique, a gas chromatograph is coupled with a Fourier Transform Infrared Spectrophotometer (FT-IR) which acts as a detector. Vapour phase spectra are taken using "light-pipe" as an interface which is operated in transmission mode. Several interferograms collected are transformed into "real time IR spectra". This allows creation of chromatograms which are quite similar to Total Ion Chromatograms (TIC) in a GC/MS. The sensitivity of this type of interface ranges from 10-20 nanograms for strong IR absorbers and up to several hundred nanograms for weak IR absorbers. Since vapour-phase spectra are quite different from condense-phase spectra, conventional IR libraries cannot be used for identification in GC/FT-IR systems. However, vapour-phase IR libraries such as Sadtler IRVAP Library are available.

Recently, interfaces such as matrix isolation and cryodeposition, as well as direct deposition techniques have been introduced. The latter is considered to have higher sensitivity similar to that of a mass detector.

GC/FT-IR is a useful analytical tool in the analysis of volatile mixtures such as essential oils. The IR data is plotted as total response chromatograms and as a series of selected wavelength chromatograms, each reflecting different functional groups such as carbonyl, aldehyde, ketone, hydroxyl, ether, ester, nitro, amine, aromatic, aliphatic, etc. that could be present in a given compound. GC/FT-IR technique is considered useful especially for the identification of isomers which have identical mass spectra (1,23).

Multidimensional Gas Chromatography (MDGC)

This technique is useful for separating a mixture into its components using two columns with different polarities. It can also be seen as a GC-GC system. The first column does a prefractionation of the substance of interest. Through column switching devices, the selected compound is diverted into the second column (so called "heart cutting"). If the second column is a chiral column, the compound can be separated into its enantiomers. Otherwise, MDGC allows the analyst to concentrate and purify selected compounds contained in an essential oil. When coupled with a mass spectrometer and/or an infrared spectrophotometer on-line spectral analysis of the compounds can be carried out. In reparative systems, isolation of such compounds is also possible for off-line analyses (1,10,25).

Gas Chromatography/Isotope Ratio Mass Spectrometry (GC/IRMS)

This technique is a powerful tool to detect adulteration caused by the addition of synthetic racemic compounds into essential oils. In this technique, an Isotope Ratio Mass Spectrometer (IRMS) is used as a detector for the compounds separated by GC. It is particularly useful when utilized with a Multidimensional Gas Chromatography (MDGC) system. The IRMS is capable of measuring the ratios of stable carbon isotopes $^{13}C/^{12}C$. The ratio of a sample (expressed as $\delta^{13}C$ value) is deviation of ratio in parts per thousand from that of $CaCO_3$ in a fossil from Pee Dee Belemnite (PDB) in South Carolina.

$$\delta\,^{13}C = [\,(^{13}C/^{12}C \text{ sample} : {}^{13}C/^{12}C \text{ PDB}) - 1] \times 1000\ [‰]$$

In GC/IRMS system, compounds eluted from a GC column enter the combustion interphase where they are broken down into CO_2 and H_2O on contact with CuO at 800°C. Water is continuously removed from the system and $^{13}C/^{12}C$ ratios of the liberated CO_2 are measured.

Each component of an essential oil shows a different isotope ratio due to its generation through distinct biosynthetic routes. Differences in $\delta^{13}C$ values observed between natural and synthetic isomers enable the analyst to detect adulteration. However, the carbon isotope ratio does not help differentiating the natural or synthetic nature of the same type of enantiomer. In such cases, hydrogen isotope ratio

(δ^2H) is used. It is based on deviation of ratios in parts per thousand of both the sample and the standard (V-SMOW= Vienna Standard Mean Ocean Water). For δ^2H value, a calculation similar to that mentioned above for $\delta^{13}C$ value is used (23,25).

This technique has recently been used to determine $\delta^{13}C$ and δD values of dried plant material and essential oil fractions of *Chamomilla recutita* and *Vanillosmopsis erythropappa*, and of their isolated components. The values obtained for (-)-α-bisabolol from both taxa were different to enable the analyst to detect and differentiate the origin of (-)-α-bisabolol (26). The same technique has also been used to differentiate natural and synthetic citral, linalool, menthol, anethole and vanillin (28).

Site-specific Natural Isotope Fractionation deuterium Nuclear Magnetic Resonance Spectroscopy (SNIF-NMR)

The principle behind the SNIF-NMR technique is that the internal distribution of deuterium isotopes within natural molecules is usually not homogeneous. This inhomogeneity arises from isotope effects in the primary and secondary metabolisms. Moreover, the total deuterium content in natural compounds depends on various factors such as the habitat and the geography of the producing plant, the deuterium content of local rainwater, etc. The SNIF-NMR technique measures the $^2H/^1H$ ratio in selected compounds in an oil or an extract. It is particularly useful in detecting artificially deuterium-enriched compounds. The technique was primarily used to detect illegal addition of sucrose or invert sugar in wine production. It is still used as an official technique in EC countries.

SNIF-NMR has been successfully used for origin-specific analysis of (-)-α-bisabolol in chamomile oil and in the wood of *Vanillosmopsis erythropappa* (Compositae). $^2H/^1H$ ratios for the olefinic protons of (-)-α-bisabolol originating from the two oils and from three chamomile preparations were calculated by comparing the individual ^2H NMR integrals of the olefinic resonances with that of the internal standard (benzene). After calculation of the corresponding δD values, it has become apparent that δD values for (-)-α-bisabolols from chamomile and vanillosmopsis were significantly different. This helped in detecting the indecent addition of (-)-α-bisabolol from vanillosmopsis to chamomile (27).

^{13}C- Nuclear Magnetic Resonance Spectroscopic (^{13}C-NMR) Analysis of Essential Oils

^{13}C-NMR Spectroscopy technique enables one to analyze an essential oil sample without prior separation into its components. A further advantage of the technique is that it gives direct information about molecular structure and functional groups of individual components. For routine analysis of essential oils, proton broad-band decoupling technique is employed. In special cases, techniques like off-resonance decoupling, gated decoupling, selective excitation, etc. can be applied (8, 29).

For qualitative ^{13}C-NMR analysis of essential oils, ^{13}C-NMR spectrum of the oil as well as those of its components should be compared. It is recommended to use the same experimental conditions throughout the experiment. With careful analysis of the spectrum, all the main components of an oil can be identified and even quantified. Fig.5 gives a comparative view of the ^{13}C NMR spectra of *Origanum majorana* oil with 75% carvacrol and pure carvacrol.

Enantioselective Analysis of Essential Oils

Many aroma chemicals are chiral compounds *i.e.*, they contain one or more asymmetric carbon atoms and exhibit optical activity. Such components of natural origin generally have a characteristic distribution of enantiomers that is attributable to stereoselectively controlled biogenetic formation mechanisms. Most compounds in essential oils occur as enantiomeric mixtures typical of a certain plant. Thus, their pattern may serve as fingerprint to prove the authenticity of a certain oil. Enantiomers differ not only in their qualitative organoleptic properties but also exhibit differences in their odour threshold values. Therefore, differentiation of enantiomers is important to find out the odour quality of an oil, an extract or an aromatic chemical. It is also a very useful tool to detect adulteration. High ratio of stereospecificity is achieved in enzyme-catalyzed reactions. Therefore, high enantiomeric purity is

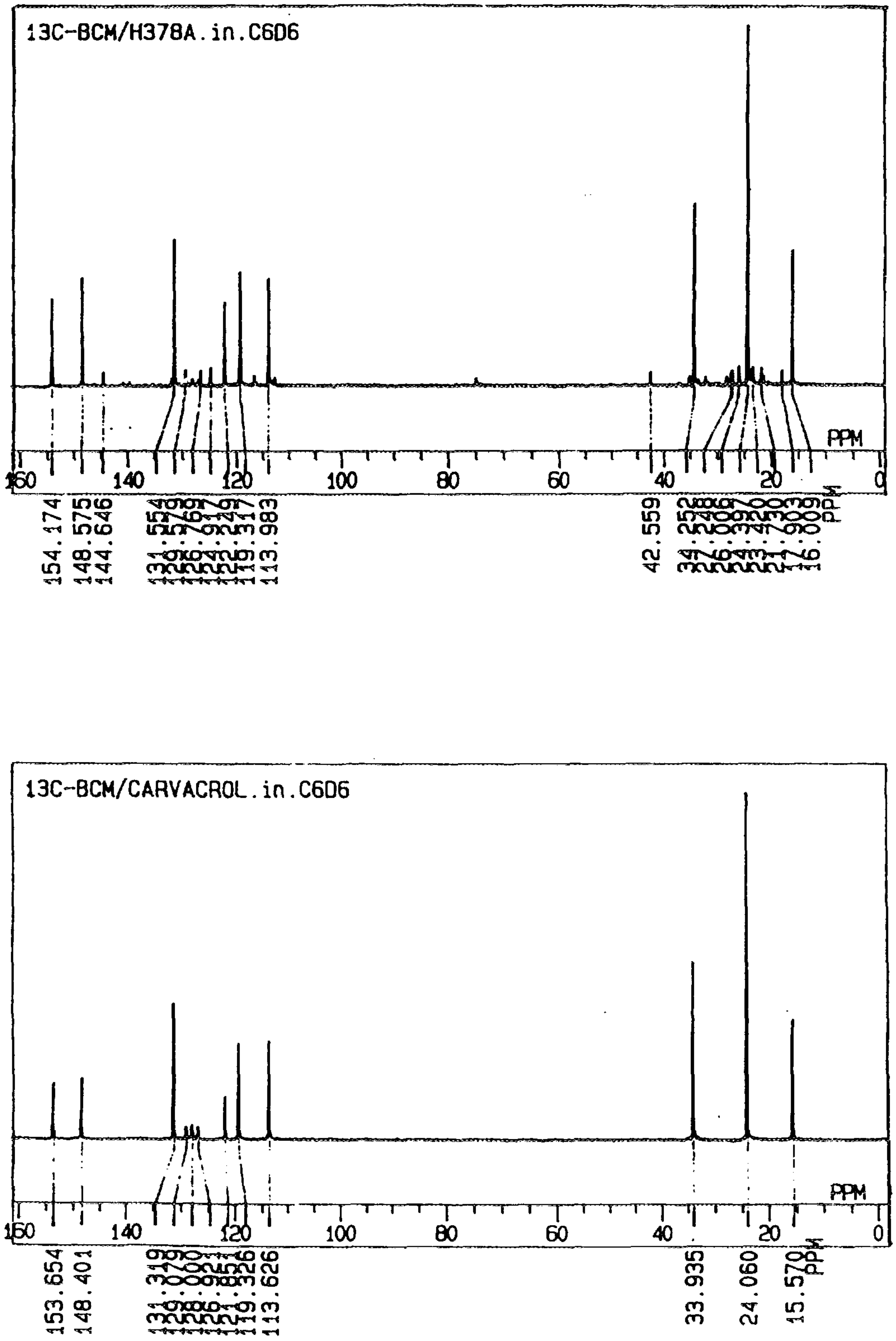


Figure 5. ^{13}C-NMR spectra of *Origanum majorana* oil (top) and Carvacrol (bottom). Carvacrol content in the oil is 75%.

expected in chiral natural products. On the other hand, achiral chemical synthesis lead to the production of racemic compounds. Therefore, if the enantiomeric ratios of chiral compounds in an essential oil is known, then, adulteration with racemic compounds can be detected.

Capillary gas chromatography using modified cyclodextrins as chiral stationary phases has been used as a method of choice for the separation of enantiomers. Fused silica capillary columns coated with several alkyl or acyl α-, β- and γ-cyclodextrin derivatives are suitable for most aromatic chemicals. In order to separate compounds from complex mixtures of essential oils prior to chirospecific analysis, reparative capillary gas chromatography (off-line method) or multidimensional gas chromatography including heart-cutting techniques from an achiral preseparation column on to a chiral main column (on-line coupling) are used (30).

4. CONFORMITY TO SPECIFICATIONS

Essential oils are complex mixtures and their quality assessment require multifarious analyses. The first and the simplest analysis is carried out by sensory organs, that is the smell, colour and viscosity mean a lot to an experienced perfumer. Instrumental techniques developed in recent decades have revolutionized the advancements in the science and trade of essential oils for detecting adulteration, and for elucidating the structures of many hitherto unknown aroma chemicals occurring in essential oils. Such compounds have also been a model for new families of flavour and fragrance chemicals. It has also made quantification of even less than *part per billion (pbb)* amounts of essential oil constituents possible. With such a degree of sophistication, it is now a reality to assure the quality of an essential oil in almost absolute terms.

For known essential oils of commerce, specifications have been drawn and published by authoritative organizations, the most important for essential oils being TC54 Committee of the International Standards Organization (ISO). Essential oil and aroma chemical monographs published by the Essential Oil Association of USA have now become almost obsolete but can still be safely used for referencing. 9 volume set of Flavour and Fragrance Ingredient Data Sheet Set compiled by the Flavour and Extract Manufacturers' Association (FEMA), The Research Institute for Fragrance Materials (RIFM) and the Fragrance Materials Association of the United States (FMA) contain safety information on over 1500 raw materials used in the fragrance and flavour industries. International Fragrance Association (IFRA) and International Organization of the Flavour Industry (IOFI) publish safety and use limits data on essential oils and aroma chemicals in their regularly updated "Code of Practice". FEMA produces FEMA GRAS List (GRAS stands for *Generally Recognized As Safe*). Of the 1783 substances listed in the list ca. 1400 have been identified in nature. The remaining substances have not yet been identified in nature but their chemical structures suggest that they will probably be identified as natural constituents. Pharmacopoeias and Food Chemicals Codex provide monographs for essential oils used in food and pharmaceuticals. International Federation of Essential Oils and Aroma Trades (IFEAT) has published a guideline for classification and labelling of essential oils for transport and handling. Flash points of essential oils are also indicated in the guideline (31). An essential oil is expected to conform to such specifications in order to fetch the market value and be appreciated by the buyers.

5. ADULTERATION

Due to the highly complex nature of essential oils it is almost impossible to entirely replicate them synthetically. High market value of most oils, *e.g.* rose oil, agarwood oil, etc., may lead to frequent cases of deliberate adulteration. For the production of such fake oils, cheap aroma chemicals are used for reconstitution or a poor quality oil is spiked with a synthetic form of the desired aroma chemical. Sometimes, the natural aroma chemical is isolated from an oil to be replaced by its synthetic racemate. The above examples are cases of deliberate adulteration.

Patchouli oil is sometimes falsified with gurjun balsam. GC analysis can detect this adulteration easily. The abnormal presence of α-gurjunene and alloaromadendrene in patchouli oil is enough proof for its adulteration with gurjun balsam. Origin of an oil is important in order to assess its quality. There are three distinct sources of geranium essential oil: geranium Bourbon (from Reunion), Egyptian geranium and Chinese geranium. The Egyptian geranium oil contains 10-epi-γ-eudesmol (3-5%) which is completely absent from the other two. Geranium Bourbon oil can be differentiated from Chinese geranium oil by the presence of higher amount of geranyl formate (6.3% as against 1.5% in Chinese oil) (1).

In most cases, the oil produced by a decent producer in good faith may not conform to desired specifications due to the presence of undesired compounds or to the lack or lesser amounts of the desired components. This may be caused due to the use of wrong or inferior quality plant materials in distillation, or to the utilization of an inappropriate production technique.

6. ENRICHMENT

When processing wild plants for essential oil production, it may not always be possible to obtain a standard essential oil. Some corrective measures are then deemed necessary to achieve the production of an essential oil of acceptable quality. An essential oil may contain lesser amounts of a certain component or high amounts of an undesired component. In order to counteract such situations the following measures can be taken: All batches of essential oils are kept separate, and after the analysis of each batch the oils can be blended in such a way to ensure conformity of the final product to specifications, or the inferior oil can be rectified or fractionated by fractional distillation to remove unwanted component(s). This also results in an increase in the amount of the desired components.

Chemical processes are not recommended for the rectification operations since they may lead to the production of many isomers, racemates, oxidation or hydrolysis products.

Enrichment of an essential oil by the addition of a synthetic racemate or enantiomer, or of the same compound from another source can, now, be detected resulting in rejection of the oil so produced.

Deterpenation is a usual practice for some oils (*e.g.*, citrus oils) and some oils are found in the market as fractions (*e.g.*, ylang-ylang oil). In such cases special specifications should be applied for their quality assessment.

Essential oils are utilized either as such in blending flavour and fragrance compositions or as sources of aroma chemicals and cut-fractions. Therefore, some buyers may impose their own specifications on producers. They may demand an oil with a higher percentage of a certain constituent. In such a case, the producer may resort to the above mentioned manipulations. Such extra processes lead to an increase in the cost of the essential oil.

The recently expanding aroma therapy market requires essential oils from organically grown plants, or from wild growing plants collected from areas away from inhabited or cultivated land so that no pesticide, herbicide or synthetic fertilizers have played a part in their natural life.

7. PACKAGING AND STORAGE OF ESSENTIAL OILS

Essential oils should be stored in proper containers which do not cause any change in the composition of the oil even after long storage periods.

Bulk containers (200 L) or small scale containers (25-100 L) are used for the shipment and storage of essential oils. They should be fabricated from inert materials such as galvanized, tinned or enamelled iron, aluminum, stainless steel or glass with appropriate closures which should not be made of plastic or rubber. Rose oil is shipped in 1-2 L tinned-steel canisters called "kumkuma". Italian bergamot oil is shipped in aluminium canisters of 20 kg.

ISO (R210) recommends the use of following containers for packing essential oils:

1. Metallic containers (such as casks, barrels, drums, cans) which should be sound, new or in good condition, clean and dry and made of the following materials:

   a. for edible essential oils: only adequately tinned or enamelled iron, or iron coated internally with a resistant lacquer or enamel or aluminium.
   b. for other essential oils: adequately tinned, galvanized or enamelled iron, or iron coated internally with a resistant lacquer or enamel paint, copper or tinned copper, tin plate or aluminium.

2. Glass containers of any capacity or shape provided that they are protected against breakage and light.

3. Plastic containers or containers lined inside with plastic material, provided that they conform to the requirements set out in respect of metal containers mentioned above should be inert towards the essential oils for which they are to be used.

Closures may be of cork, glass, aluminium, tin or any other suitable material which has no effect on the product. Containers should be provided with a tamper-proof seal on the closure.

Phenol-containing essential oils were found to react rapidly with zinc, while oils containing monoterpenes have been reported to react strongly with aluminium. It is recommended not to use aluminium for long storage of essential oils.

The containers should be well filled so that derivatization due to the presence of oxygen in the headspace can be avoided Filling should comply with international agreements on the carriage of dangerous products and the headspace should be between 5-10% of the volume of the containers, depending on the method of transport adopted. ISO recommendation R211 describes labelling and marking of essential oils.

Small containers should be made of amberglass to minimize the effects of light. Essential oils should always be stored in a cool place (32,33).

8. TOTAL QUALITY MANAGEMENT - The Ultimate Goal in Quality Control

ISO 9000 series of international standards includes requirements for quality systems which can be used to achieve common interpretation, development, implementation and application of quality management and quality assurance. ISO 9000 series quality system standards require development and implementation of a documented quality system. Further, these standards also require the preparation of a quality manual.

Nowadays, more and more manufacturers are striving for a certificate from a quality management (QM) system in order to prove that their company meets the international standards ISO 9000 to 9004 and 14000.

Companies which possess this certificate are considered as qualified suppliers and their products are more readily accepted by their clients. Companies which do not have a certified QM system have to go through extensive qualification procedures. In order to check the quality efficiency of a potential supplier which does not possess such a certificate, several batches of samples are required and references and supplier assessment data are deemed necessary. All these raise the cost of supplier qualification significantly. Another advantage of certification is that it gives the certified companies a more competitive position in the market.

Total Quality Management (TQM) system has quickly surpassed the former quality concepts such as *Quality Control* which only checks the quality of the final product, and *Quality Management (QM)* which controls the quality of the product and the process, and documents every step in a systematic way. The TQM philosophy can be summarized as follows: Customer orientation, process control and constant improvement of quality.

ISO 9001 requires the top management of a company to adopt a quality policy and train the staff for its implementation.

The basic philosophy of a total quality management is to check the quality of a product at every stage of its manufacture starting from the quality of raw materials to every step in processing until the production of the final product designed to meet complete satisfaction of the customer. It is essential to document the results in order to take corrective measures when required.

The ISO 9000 series help companies to create a company-specific total quality management system. Such companies enjoy the following benefits: increase in customer satisfaction, reduction in complaints, improvement of product quality, better competition with rival companies. Furthermore, processes are optimized, responsibilities in the companies are clearly defined, communication within the company is better coordinated and optimized, employees are motivated towards working for improvements in the company leading to auto-control and better management of the company (34,35).

Annex 1. **A Selected List of Standards issued by the International Standards Organization (ISO) on Essential Oils** *(Full list can be obtained from ISO).*

Techniques

Reference	Title
ISO/R 210	Essential oils - Packing
ISO/R 211	Essential oils - Labelling and Marking Containers
ISO 212	Essential oils - Sampling
ISO 279	Essential Oils - Determination of relative density at 20°C (Reference method)
ISO 280	Essential oils - Determination of refractive index
ISO 365	Essential oils - Preparation of test sample
ISO 592	Essential oils - Determination of optical rotation
ISO 709	Essential oils - Determination of ester value
ISO 875	Essential oils - Evaluation of miscibility in ethanol
ISO 1041	Essential oils - Determination of freezing point
ISO 1202	Essential oils - Determination of 1,8-cineole content
ISO 1241	Essential oils - Determination of ester value after acetylation and evaluation of free alcohols and total alcohols content
ISO 1242	Essential oils - Determination of the acid value
ISO 1271	Essential oils - Determination of carbonyl value, Free hydroxylamine method
ISO 1272	Essential oils - Determination of phenols content
ISO 1279	Essential oils - Determination of carbonyl value - Hydroxylammonium chloride method
ISO 3218	Essential oils - Principles of nomenclature
ISO 3793	Essential oils - Estimation of primary and secondary free alcohols content by acetylation in pyridine
ISO 3794	Essential oils (containing tertiary alcohols) - Estimation of free alcohols content by determination of ester value after acetylation
ISO 4096	Essential oils (containing tertiary alcohols) - Evaluation of free alcohols content by determination of ester value after cold formylation
ISO 4715	Essential oils - Quantitative evaluation of residue on evaporation
ISO 4735	Citrus oils - Determination of CD value by ultraviolet spectrophotometric analysis
ISO 5991	Essential oils - Determination of residue from distillation under reduced pressure
ISO 7660	Essential oils - Determination of ester value of oils containing difficult-to-saponify esters

Monographs

Reference	**Title [Botanical name] (Plant part used)**
ISO 590	Oil of sassafras, Brazil [*Ocotea pretiosa* (C.G.Nees) Mez.] (Every part)
ISO 770	Oil of *Eucalyptus globulus* Labill. (Leaves, terminal branches)
ISO 855	Oil of lemon, Italy , obtained by expression [*Citrus limon* (L.) N.L.Burman] (Pericarp)
ISO 856	Oil of peppermint, France, Italy, U.K. and U.S.A.[*Mentha x piperita* L.] (Whole aerial plant)
ISO 1342	Oil of rosemary [*Rosmarinus officinalis* L.] (Leaves, flowering tops)
ISO 3033	Oil of spearmint [*Mentha spicata* L.] (Whole aerial plant)
ISO 3043	Oil of pimento berry [*Pimenta dioica* (L.) Merrill] (Fruits)
ISO 3044	Oil of *Eucalyptus citriodora* W.J.Hooker (Leaves, terminal branches)
ISO 3045	Oil of bay [*Pimenta racemosa* (P.Miller) J.W.Moore] (Leaves)
ISO 3054	Oil of lavandin abrialis [*Lavandula angustiolia* P.Miller x *Lavandula latifolia*
ISO 8902	(L.fil.) Medikus] (Flowering tops)
ISO 3061	Oil of black pepper [*Piper nigrum* L.] (Fruits)
ISO 3063	Oil of ylang-ylang [*Cananga odorata* (Lamark) J.D.Hooker] (Flowers)
ISO 3140	Oil of sweet orange [*Citrus sinensis* (L.) Persoon, CH] (Pericarp)
ISO 3141	Oil of clove leaf [*Syzygium aromaticum* (L.) Merrill et Perry] (Leaves)
ISO 3142	Oil of clove bud [*Syzygium aromaticum* (L.) Merrill et Perry] (Buds)

ISO 3143	Oil of Clove stems [*Syzygium aromaticum* (L.) Merrill et Perry] (Stems)
ISO 3215	Oils of nutmeg [*Myristica fragrans* Houttuyn] (Seeds)
ISO 3216	Oil of cassia [*Cinnamomum cassia* (C.G.Nees) ex Blume] (Bark)
ISO 3217	Oil of lemongrass [*Cymbopogon citratus* (A.P.de Candolle) Stapf] (Whole aerial plant)
ISO 3475	Oil of aniseed [*Pimpinella anisum* L.] (Fruits)
ISO 3515	Oil of French lavender [*Lavandula angustifolia* P.Miller] (Flowering tops)
ISO 3516	Oil of coriander [*Coriandrum sativum* L.] (Fruits)
ISO 3517	Oil of Neroli [*Citrus aurantium* L. subsp. *aurantium*] (Flowers)
ISO 3518	Oil of sandalwood [*Santalum album* L.] (Wood)
ISO 3520	Oil of bergamot, Italy [*Citrus bergamia* (Risso) Risso et Poiteau] (Pericarp)
ISO 3523	Oil of Cananga [*Cananga odorata* (Lamarck) J.D.Hooker et Thomson] (Flowers)
ISO 3524	Oil of Cinnamon leaf [*Cinnamomum zeylanicum* Blume] (Leaves)
ISO 3525	Oil of Amyris [*Amyris balsamifera* L.] (Wood)
ISO 3527	Oil of parsley fruit [*Petroselinum sativum* Hoffman] (Fruits)
ISO 3528	Oil of mandarin [*Citrus reticulata* Blanco] (Pericarp)
ISO 3714	Oil of pennyroyal [*Mentha pulegium* L.] (Whole aerial plant)
ISO 3756	Oil of cubeb [*Piper cubeba* L.fils] (Fruits)
ISO 3757	Oil of patchouli [*Pogostemon cablin* (Blanco) Bentham] (Leaves)
ISO 3760	Oil of celery seed [*Apium graveolens* L.] (Fruits)
ISO 3761	Oil of rosewood, Brazil [*Aniba rosaeodora* A.Ducke var *amazonica* or *A.parviflora* (Meissner) Mez.] (Wood)
ISO 3809	Oil of lime, Mexico [*Citrus aurantiifolia* (Christmann)Swingle] obtained by mechanical means (Pericarp)
ISO 3848	Oil of Java citronella [*Cymbopogon winterianus* Jowitt] (Whole aerial plant)
ISO 3849	Oil of Ceylon citronella [*Cymbopogon nardus* (L.) Rendle] (Whole aerial plant)
ISO 4716	Oil of Vetiver [*Vetiveria zizanioides* (L.) Nash] (Roots)
ISO 4718	Oil of lemongrass [*Cymbopogon flexuosus* (Nees ex Steudel) W.Watson] (Whole aerial plant)
ISO 4719	Oil of spike lavender [*Lavandula latifolia* Medikus] (Flowering tops)
ISO 4724	Oil of Cedarwood, Virginia [*Juniperus virginiana* L.] (Wood)
ISO 4725	Oil of Cedarwood, Texas [*Juniperus mexicana* Schiede] (Wood)
ISO 4727	Oil of palmarosa [*Cymbopogon martinii* (Roxburgh) W.Watson var.*motia*] (Whole aerial plant)
ISO 4728	Oil of Spanish wild thyme [*Thymus mastichiana* L.] (Flowering tops)
ISO 4729	Oil of pimento leaf [*Pimenta dioica* (L.)Merrill] (Leaves)
ISO 4730	Oil of tea tree [*Melaleuca alternifolia* (Maiden et Betche) Cheel] (Leaves, terminal twigs)
ISO 4731	Oil of geranium [*Pelargonium graveolens* L'Heritier ex Aiton] (Herbaceous parts)
ISO 4733	Oil of cardamom [*Elettaria cardamomum* (L.) Maton var. *minuscula* Burkill] (Fruits)
ISO 4734	Oil of mace [*Myristica fragrans* Houttuyn] (Arils)
ISO 8896	Oil of caraway [*Carum carvi* L.] (Fruits)
ISO 8897	Oil of juniper [*Juniperus communis* L.] (Fruits)
ISO 8898	Oil of mandarin petitgrain [*Citrus reticulata* Blanco] (leaves, twigs)
ISO 8899	Oil of lemon petitgrain [*Citrus limon* (L.) N.L.Burman] (Leaves, twigs)
ISO 8900	Oil of bergamot petitgrain [*Citrus bergamia* (Risso) Risso et Boiteau] (Leaves, twigs)
ISO 8901	Oil of bitter orange petitgrain [*Citrus aurantium* L. subsp. *aurantium*] (Leaves, twigs)
ISO 9301	Oil of Cumin [*Cuminum cyminum* L.] (Fruits)
ISO 9776	Oil of Japanese mint [*Mentha arvensis* L.] (Whole aerial plant)
ISO 9841	Oil of Hyssop [*Hyssopus officinalis* L.] (Flowering tops and leaves)
ISO 9842	Oil of Rose [*Rosa damascena* P.Miller] (Flowers)
ISO/DIS 9843	Oil of cedarwood, China [*Cupressus funebris* Endlicher] (Twigs and leaves)
ISO 9844	Oil of bitter orange [*Citrus aurantium* L. subsp. *aurantium*] (Pericarp)
ISO 9909	Oil of Dalmatian sage [*Salvia officinalis* L.] (Flowering tops)
ISO 10115	Oil of Tarragon [*Artemisia dracunculus* L.] (Tops)

REFERENCES

1. Sandra, P. and Bicchi, C. (Eds.), (1987), *Capillary Gas Chromatography in EssentialOil Analysis*. Huethig .

2. Brunke, E.-J., Hammerschmidt, F.-J., and Schmaus G., (1992), *The headspace analysis of flower fragrances*. Dragoco Rep., (1) 3-31.

3. Wijesekera, R.O.B., (Ed.), (1990), *Practical Manual on the Essential Oil Industry*. UNIDO.

4. *Martindale: The Extra Pharmacopoeia*, (1993), 30th Edn., The Pharmaceutical Press, London

5. *Food Chemicals Codex*, (1981), 3rd Edn., National Academic Press, Washington,D.C.

6. Wagner, H. , Bladt, S. and Zgainski, E.M., (1984), *Plant Drug Analysis*. Springer, N.Y .

7. Srinivas, S.R., (1986), *Atlas of Essential Oils*. Anadams, New York.

8. Formacek, V. and Kubeczka, K.-H., (1982), *Essential Oil Analysis by Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy*. John Wiley & Sons, New York .

9. Lawrence, Brian M., *1976-1978* (1979), *1979-1980*(1981), *1981-1987* (1989),*1988-1991* (1993)., *Essential Oils*: Allured, IL (USA).

10. Leibrand, R.J., Cambia, B.D. and Free, M., (1993), *Use of multispectral analysis in the characterization of a perfume*. *In*: H. Woidich and G. Buchbauer (Eds.), Proceedings 12th International Congress of Flavours, Fragrances and Essential Oils, 4-8 October 1992, Post Congress Volume, Fachzeitschriften Verlags, Vienna, pp. 145-155.

11. Masada, Y., (1976), *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*. John Wiley & Sons, New York.

12. Yukawa, Y. and Ito, S. (Eds.), (1973), *Spectral Atlas of Terpenes and the Related Compounds*. Hirokawa Publ. Co., Tokyo.

13. Jennings, W. and Shibamoto, T. , (1980), *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography. Academic Press*. New York.

14. Swigar, A.A. and Silverstein, R.M., (1981), *Monoterpenes: Infrred, Mass, ^{1}H NMR and ^{13}C NMR Spectra, and Kovats Indices*. Aldrich Chem. Co., Milwaukee.

15. Ardley, R.E., Allan, A.R., Bal, T.S. , Joyce, J.R. and Moffat, A.C., (1985),*Pharmaceutical Mass Spectra*. The Pharmaceutical Press, London.

16. Adams, R.P., (1989), *Identification of Essential Oils by Ion Trap Mass Spectroscopy*. Academic Press, San Diago, CA .

17. Adams, R.P., (1995), *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Allured, IL .

18. McLafferty, F.W. and Stauffer, D.B. , (1988), *Wiley-NBS Registry of Mass Spectral Data*. 7 volume set, J.Wiley & Sons, New York .

19. Ardley, R.E., Brown, C., Allen, A.R., Bal, T.S. and Moffat, A.C., (1983), *An Eight Peak Index of Mass Spectra of Compounds of Forensic Interest*. The Forensic Science Soc., Scottish Acad. Press.

20. The Mass Spectrometry Centre, (1986), *An Eight Peak Index of Mass Spectra*, 7 volume set, Royal Society of Chemistry, Nottingham, UK.

21. Pfleger, K., Maurer, H.H. and Weber, A., (1992), *Mass Spectral and Gas Chromatographic Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*. 2nd Edn., VCH, Weinheim .

22. ten Noever de Brauw, M.C., Bouwman, J., Tas, A.C., Gramberg, L.G. and La Vos, G.F. (1979-1988), *Compilation of Mass Spectra of Volatile Compounds in Food*. TNO-CIVO, The Netherlands, Vols 1-16.

23. Joulain, D., (1994), *Modern analysis methodologies: use and abuse*. Perf.Flav., 19, 5-17.

24. Joulain, D., *Investigating new essential oils: Rationale, results and limitations. In*: K.H.C.Bafler (Ed.), Proceedings-13th International Congress of Flavours, Fragrances and Essential Oils, 15-19 October 1995, Vol.2, AREP, Istanbul (1995), pp. 55-66.

25. Carle, R. (Ed.), (1993), *Aetherische Öle - Anspruch und Wirklichkeit*. Wiss.Verl.

26. Carle, R., Fleischhauer, I., Beyer, J. and Reinhard, E. , (1990), *Studies on the origin of (-) bisabolol and chamazulene in chamomile preparations: Part I. Investigations by isotope ratio mass spectrometry (IRMS)*. Planta Med., 56, 456-460.

27. Carle, R., Beyer, J., Cheminat, C. and Krempp, E., (1992), *^{2}H-NMR Determination of site-specific natural isotope fractionation in (-)-bisabolols.* Phytochemistry 31(1) 171-174.

28. Teisseire, P.J., (1994), *Chemistry of Fragrant Substances*. VCH Publishers, New York.

29. Kubeczka, K.-H., (1982), *Aetherische Öle: Analytik, Physiologie, Zusammensetzung*. Thieme verlag, Stuttgart.

30. Werkhoff, P., Brennecke, S., Bretschneider, W., Güntert, M., Hopp, R. and Surburg, H., (1993), *Chirospecific analysis of essential oils, fragrance and flavour research: A review. In*: H.Woidich and G.Buchbauer (Eds.), Proceedings 12th International Congress of Flavours, Fragrances and Essential Oils, 4-8 October 1992, Fachzeitschriften Verlags, Vienna, pp. 64-128.

31. Protzen, K.-D. (Ed.), (1989), *Guideline for Classification and Labelling of Essential Oils for Transport and Handling*. IFEAT, London.

32. Verghese, J., (1986), *On Essential Oils*, Synthite Ltd., India.

33. Anon., (1986., *Essential Oils and Oleoresins: A Study of Selected Producers and Major Markets*. ITC-UNCTAD/GATT, Geneva.

34. Anon., (1991), *Quality Control for the Food Industry - An Introductory Handbook*. ITC-UNCTAD/GATT, Geneva .

35. Wolf, G., (1994), *The Certificate: The Entrance to Total Quality Management*. Dragoco Rep., (1) 24-31 .

Annex II:

USEFUL ADDRESSES

1. **ISO (International Standards Organisation)/Central Secretariat**
1, Rue de Varemte
CH-1211 Geneve, Switzerland

2. **ISO TC-54 Technical Committee**
c/o AENOR Ms Carmen Tapia, Secretary
Fernandez de la Hoz 52
28010 Madrid, Espana
tel. +34-1-3104551
fax. +34-1-3104976

3. **IFEAT (International Federation of Essential Oils and Aroma Trade)**
Ms Jackie Clarke, Secretary
Federation House, 6 Catherine Street,
London WC2B 5JJ, England
tel. +44-171-8362460
fax. +44-171-8360580

4. **IFRA (International Fragrance Association)**
IOFI (International Organisation of Flavour Industry)
8, rue Charles Humbert
CH-1205 Geneve, Switzerland
tel. +41-22-3213548
fax. +41-22-7811860

5. **FEMA (Flavour and Extract Manufacturers' Associations of the United States)**
FMA (Fragrance Materials Association of the United States)
1620 I Street, N.W.
Washington, D.C. 200006, USA
tel. +1-203-2935800

CHAPTER 5

FORMULATION AND EVALUATION OF FRAGRANCE FOR PERFUMERY COSMETICS AND RELATED PRODUCTS.

WLADYSLAW S. BRUD
"POLLENA-AROMA" Warsaw, Poland

1. INTRODUCTION

Art of perfumery is one of most fascinating but also very difficult professions. As it is well known, sense of smell is the most sensitive and selective of human senses. From the primitive beginning human being used his nose as a source of most important informations on food sources, danger, strangers, etc. It was also a very old custom to change the original natural human body odour to mask it in case of danger or to improve it for the benefit of other members of the group or sex partner. Of course easiest available were odoriferous plant materials and some animal excretions. These primitive behaviours with all development of technology and culture have not changed very much.

Contemporary women and men isolated themselves from the odoriferous environment and in fact for most people odours of the surrounding world hardly exist. Although human odour receptors detect all the surrounding odours we do not use conscious perception of them. They create some kind of background smell which is there as street noise or for many people music used just for isolation from other sounds, not necessary for pleasure of listening to it. It shall be noted here that although we can switch off our conscious perception of odours or sound they affect our mind and body. Odour compounds can also affect our reactions in subthreshold concentrations i.e. in quantities which are to low to be detected by nose.

But as in ancient times people follow the example of our ancestors and try to change the odour of their bodies and environment for nearly the same reasons as primitive humanoids. We use air fresheners or aromatherapy burners to create "good" (or rather commonly accepted in our society) room odour. We wear perfumes, use soaps and shampoos to change odour of our body or breath aiming to become similar to other people of our social group (tribe). applying some minor differences to save our individuality.

In fact there is not much difference in behaviour between the Borneo primitive tribe woman who rubs her body with fragrant herbs and paints her face with vegetable or mineral colours and the Paris haute couture lady who takes a foam bath in fragrant oils, drops most fashionable perfumes on her body and applies up to date make up on her face to be unique but equal part of her tribe.

What is considered a contemporary fragrance industry started at the end of the XIX century when big fragrance houses emerged. But history of perfumery or rather perfume as we understand today an alcoholic solution of fragrance materials started in 1367 when "Queen of Hungary Water" was created and introduced in Europe by the Polish born Queen of Hungary Elisabeth. It was based on rosemary, thyme and some other herbs and was known and used as a perfume and medicine until the end of last century. The next milestone in history of contemporary fragrances was "Aqua mirabilis" later developed as "Eau de Cologne" by Jean-Antoine Farina in 1690 existing to our times in hundreds of modifications and remaining most popular men's daily fragrance.

For centuries fragrances were used by royal courts, rich people and were prepared and distributed by perfumers who created compounds, produced solutions in alcohol, fat or oil, and distributed to customers. It was in the XVIII century when the revolution in perfumery happened. Three different but strictly connected phenomena appeared on the perfumery field. First was formation of professional perfumery houses, which manufactured and distributed ready made perfumes making these available on the market and attracting more users. At the same time fragrance raw materials producers and compounders started their activities producing essential oils and creating fragrance compounds used by perfumery houses. And finalty organic chemistry emerged with the first synthetic organic products proving that nature can be imitated by chemistry.

First perfumery houses started in France and in England. These were Houbigant in Paris (1775), Atkinsons (1790) and House of Yardley (1790) in London. In 1828 Pierre Francois Pascal Guerlain opened his company in Paris. This date is considered as the beginning of modern perfumery.

First companies which specialised in the manufacture of fragrance raw materials and compounding were established in Grasse in France (where tradition of perfume production existed) Germany, Great Britain, and USA. First document concerning Grasse position in perfumery was issued in 1190 by Philippe-August specifying privileges and conditions given to the perfumers profession. This was recognised by Catherine de Medici who, coming to France in the XVI century, established her perfumers in Grasse. First companies which were registered in Grasse were Antoine Chiris in 1768 and Cavallier Freres in 1784. Few years later in 1798 Dodge and Olcott started in New York. None of them exists nowadays. Among the biggest compounders operating now in the international markets, the oldest is Givaudan-Roure (with Roure established in 1820), followed by Bush Boake Allen with (Stafford Allen est. 1833) and Haarman-Reimer est. in Holzminden in 1874. Others are Firmenich (1895 Geneve), Naarden now Quest (1905 Bussum, Holland), Dragoco (1919 Holzminden, Germany), Takasago (1920 Tokyo), and the International Flavours and Fragrances (IFF, 1959 New York).

First book on natural fragrance raw materials and fragrance compounding "Traite des Odeurs" was published in France in 1797 by M. Dehean.

2. RAW MATERIALS FOR FRAGRANCE DEVELOPMENT

Contemporary fragrance industry has in its files few thousands of individual fragrance raw materials of different origin and practically unlimited number of so called specialities which are mixtures of the individual materials with specific odour. Individual raw materials are either natural or synthetic. Natural materials are produced either from plant or animal sources. It shall be emphasized that due to activities of animal protection and wildlife organisations, fragrance materials obtained from animals are very rarely used and in most cases are replaced by synthetic chemicals. Rather for historical knowledge of perfumery than for practical use some of them will be mentioned below.

Majority of the natural fragrance raw materials are obtained from plants by different methods. General back to nature and ecological trends as well as growing demand for good odours, not only in perfumery and cosmetic products but also in everyday life have created a growing demand for natural raw materials, especially because there is no way to make real a 100 % copy of a natural fragrance with synthetic materials. Furthermore, environmental limitations in industrialised countries and cost of waste treatment have increased the total cost of production of many aroma chemicals to such a level that products obtained from plants, being usually of better quality than chemicals are very often cheaper. Additionally, beyond great value as natural fragrance plant materials for perfumery products many of them have healing properties and are used as such both in pure therapy (like Aromatherapy) or their beneficial properties are used in cosmetic products or so called parapharmaceuticals.

Aroma chemicals i.e. synthetically obtained fragrance components appeared in the XIX century. First of them was vanillin prepared by Bucholtz in 1816. Benzyl aldehyde was prepared by Liebig in 1831. First company to start regular production of aroma chemicals was Haarman-Reimer with vanillin (1874), coumarin (1876), anisaldehyde (1877), and heliotropin (1878). Between the end of the XIXth century and the beginning of XXth hundreds of new chemicals were introduced which were used in fragrances creating unlimited possibilities for perfumers. Numerous chemists and most modern laboratories work on the synthesis of new aroma chemicals, isolates, natural essential oils, new methods of purification, testing and safety.

Several international and national organisations, manufacturers and users worked together to establish and introduce common rules and good manufacturing practices. IFRA (International Fragrance Research Association - Geneva, see Literature cit.13) and IFEAT (International Federation of Essential Oils and Aroma Trade - London) are leading international cooperation with IOFI (International Organisation of Flavour Industry - Geneva) and many national organisations - strongest in USA, France, UK, Germany and Japan. IFRA provides worldwide recognised GUIDELINES which give information on safety of use of fragrance raw materials. IFEAT publications and annual conferences give informations in essential oils trade and production. TC-54 Technical Committee of the International Standard Organisation is permanently working on standards for Essential Oils. Every three years a big International Congress of Flavours, Fragrances and Essential Oils is organised and numerous international and national conferences on essential oils (e.g. IFEAT Annual Conference), aroma chemicals, perfumery etc. are available worldwide to discuss all aspects of the industry.

Natural fragrance raw materials

As said above natural fragrance raw materials of animal origin are very rarely used in common fragrance practice due both to limitations based on animal protection hobby and very high prices. Best known product of animal origin is Musk which is obtained from preputial follicle (musk pod) removed from the abdomen of male deer (*Moschus moschiferus*) and few other species of this kind living in the Himalayan mountains. The pod is removed without harming the animal and the product used in fragrance industry is an alcoholic tincture of grains (seeds) extracted from the pod. It has an extremely powerful long lasting odour and is an excellent fixative.

Similar in use, properties and form is Civet (sometimes called "Musk Civette") obtained from the paste-like secretion taken from a pouch under the tails of civet cats (*Viverra civetta* or *V. rasse* or *V. tangalunga)* living in regions of Ethiopia, Indonesia, Burma and Thailand.

Still in use is Castoreum (called also Beaver Oil or Beaver Musk) a product obtained from the secretions of beavers (*Castor fiber*) living in Canada, north parts of Russia and Poland. It is also used in the form of very diluted tinctures.

Extremely rare and practically nonexistent in use is Ambergris (Amber) obtained as a tincture from excretions of sperm whale (*Physeter macrocephalus*) which can be found in lumps floating mainly in the Indian Ocean. Its odour is similar to Labdanum, very strong and is used only as diluted tinctures.

All above products in their original form or freshly made tincture are usually unpleasant in odour and can be used in very low concentrations only. If used at all, they can be found in most expensive perfumes and in most cases fragrance houses and compounders will not use them. Samples of these products as well as of materials for their production can be seen in some perfumery museums.

Totally different situation exists in materials produced from plants. These are readily available (although some of them are limited either due to big demand or high production cost) and their production and consumption are growing worldwide. These materials create very interesting sources of added value for developing countries as most of essential oils bearing plants grow in developing countries.

Odoriferous raw materials can be produced from thousands of plants although only limited number of them is commonly used in perfumery and even less are cultivated for the purpose. Odoriferous substances can be isolated from all parts of the plant including flowers, seeds, fruits, leaves, branches, roots, rhizomes, bark, needles, wood, excretions or the whole plant. Very often products obtained from different parts of the plant are totally different e.g. oils from orange tree which gives "orange oil" from the pericarp of the fruit, "petitgrain oil" from the leaves and "neroli oil" from the flowers, are totally different in odour.

According do ISO DRAFT STANDARD No 9235.2, natural fragrance materials from plants can be divided into the following categories according to the method of production:

Essential oils obtained from natural plant raw materials:

- by distillation with water or steam (most of the oils - e.g. lavender, rose, rosemary, citronella, sandalwood, vetiver, mint, ylang-ylang, cinnamon bark, neroli, geranium and many others)
- by mechanical expression (mainly from epicarp of citrus fruits - e.g. lemon, orange, grapefruit, bergamot)
- by dry distillation (very few products - e.g.birch tree bark oil) .

The essential oils when separated from water by physical means can be further processed (by distillation or fractional distillation) to give **Rectified essential oils** (e.g. mint oils), **Terpeneless, Sesquiterpeneless**, or "X-less" essential oils, (e.g. terpeneless citrus oils, bergaptene free bergamot oil) **"Folded"** (concentrated) essential oils (2,3 - fold orange oil), and **Isolates** (e.g. citral ex *Litsea cubeba*) and side products of above processes i.e. Aromatic waters, (e.g.rose water, lavender water) **Terpenes**, (e.g. lemon terpenes) and **Residues**.

Extraction products can also be classified according to the solvent and the method of extraction **Tinctures** (obtained by maceration of raw material in ethanol e.g Tonka Tincture), **Concretes** (obtained by treating natural raw material with a non-aqueous, non-polar solvent and its evaporation e.g. rose, oak moss), **Pomades** (obtained by diffusion of odoriferous constituents of the flowers with cold or hot fats e.g. tuberose), **Resinoids** (obtained by treating dried natural raw material, usually resins, with a solvent e.g. Labdanum, Olibanum, Myrrh) and **Absolutes** (obtained from concretes, pomades or resinoids by extraction with ethanol and its evaporation from the extract e.g. rose, lavender, violet leaf, jasmine).

Sources of most important essential oils and other perfumery natural raw materials as described above are listed in ISO DRAFT STANDARD No.4720.3 "Nomenclature" which gives the name of the plant in English and French, Latin name of species, and the parts of the plant used for production of the material.

Apart from their unique odour value which in practice cannot be replaced by synthetics, natural fragrance raw materials are more and more important in various uses where no synthetics are allowed. Although not that important in fragrance compounding, it is a crucial factor in flavours where natural origin is a basic criteria for the material to be used in food products. Numerous problems appeared when such criteria were introduced as very often it is difficult to recognise individual chemical compounds obtained from natural sources from its synthetic equivalent. "Nature identical" description was introduced for synthetic products identical with those obtained from natural raw materials. Most sophisticated methods are used to verify origin of the material including most modern isotope tests and chiral analysis which for time being can prove the origin of the product correctly.

As said above natural fragrance raw materials after decades of their replacement with synthetic chemicals are becoming more important. Their use in perfumery and the demand and production are expanding..

List of basic natural raw materials necessary for the starting stage of a creative perfumery laboratory is given in Annex 1.

Aroma chemicals

As said above aroma chemicals entered fragrance industry at the end of the XIX century and for many decades were and in many areas of fragrance industry are most important part of the compounding work. Basic book on aroma chemicals by S.Arctander lists ca 3000 different chemicals. The current number of individual products known for their good odour may be 4000-5000 but in practical use no more than 1000 are used.

Aroma chemicals belong to many chemical groups including hydrocarbons, terpenes, alcohols, esters, ketones, aldehydes, and cyclic compounds, also containing nitrogen, sulphur, and chlorine. There are few theories trying to explain the relation between odour type and strength and structure of the chemicals. None of them proved to be good enough to explain all phenomena which appear in this area. There are differences in odour of isomers even optical. Very similar (chemically) products have totally different odours while very different structures smell nearly the same. In practice there is not enough knowledge to synthesize an aroma chemical with a specified odour although according to some structural data, type of odour can be predicted. Thus only few chemicals synthesised in laboratories enter production and are used in compounding. On the other hand continuous research works on toxicity and other adverse properties of fragrance raw materials limit the use or eliminates certain chemicals forcing additional research on replacements (e.g. nitromusks).

Selection of aroma chemicals to be used by a creative laboratory and compounding company depends on many parameters - technical, economical, perfumers practice etc. It is usual that some commodities are readily available in all stores while some specialities or rare or expensive chemicals are used only occasionally. It is also common that aroma chemicals produced inside the company are preferred in the compounds offered to customers with priority given to specialties. Some of these specialties are not available on the market creating a special advantage to the producer in his compounds. Some important specialties are transformed into bases or "hearts" i.e. mixtures with specific odour which can be used in creative work for development of requested fragrances. Aroma chemical mixtures very often with some natural ingredients are offered as replacements for natural scarce or expensive products i.e. so called reconstituted essential oils or synthetic replacements for animal products (musks, amber, civette, castoreum). Thus the number of components (oils, chemicals, bases, specialties etc.) is unique to each company and only the best perfumers and computers in the company will know the full list of materials used.

There are several computerised systems used in fragrance industry covering a large although never the full range of fragrance and flavour raw materials. Such an annual list is published as a book and on computer discs by S.Allured in USA.

List of synthetic raw materials necessary in the starting stage of a creative perfumery laboratory is given in Annex 2 .

3. FRAGRANCE CHARACTERISTICS

Sense of smell and odour descriptions are based on associations i.e. there are no objective descriptions of odours as there are in colours or sound. In describing odours one usually uses the words "like" i.e. "like rose", "like jasmine", or just adjectives like "fatty", "green", "sweet", "warm", "fruity", "floral" etc. For general descriptions, very often the terminology taken from music is used. "Notes", "accords","harmony", "tone" are terms used to describe individual odours or their combinations.

Several attempts had been made in fragrance research in odour description and odour classification. Linnaus who started classified seven basic odours in his "Odores Medicamentorum" (1752):

Aromatic
Fragrant
Musk-like
Garlic-like
Goat-like
Repulsive (foul)
Nauseating

There were and are many other classifications including Amoore attempt to find primary odours and correlate them to molecule shapes of fragrant compounds. The following odour types were described as primary:

Etheral
Camphoraceous
Musky
Floral
Minty
Pungent
Putrid

Brud Odour Profiles applied to simple description of aroma chemicals, The following odour descriptions were used and odour standards selected by nearly 100 perfumers from all over the world:

Odour description	**Odour Standard**
Green	Cis-3-hexen-1-ol, Galbanum res.
Fruity	Undecalactone
Floral	Rose oil, Jasmine absolute
Fatty-Aldehydic	Lauric aldehyde
Herbal	Rosemary oil
Animal-Musky	Musk Tonquin
Amber	Ambergris tincture, Labdanum res.
Woody	Cedarwood oil, Sandalwood oil
Spicy	Clove bud oil, Eugenol
Balsamic	Benzoin Siam
Earthy-Fungoid	HADMA, PADMA
Chemical	Diphenyl ether, Bromostyrene

Many other methods were elaborated and published including a variety of odour profiles, hedonic systems, computerised programmes based on big panels work, up to multidimensional spherical models of odours. Most of these very complicated methods, created with special computer programs, were of pure scientific interest, without much practical use. For routine work many fragrance compounding companies use their own systems of odour descriptions and classification for internal use by their perfumers.

These systems apply both to raw materials; natural and synthetic, and fragrance compounds and semiproducts (specialities and bases). There are also few published classifications of known perfumes which can be used for the classification of fragrance compounds and market products. One of them is very clear and useful "The Genealogy of Fragrances" elaborated and published by Haarman-Reimer for feminine and masculine fragrances giving a classification of odours in characteristic groups and placing all popular perfumes in the groups.
The following odour types have been established:

FEMININE

- Floral:
  - green
  - fruity
  - fresh
  - floral
  - aldehydic
  - sweet
    - sweet
- Oriental:
  - sweet
  - spicy
- Chypre:
  - fruity
  - floral-animalic
  - floral
  - green

MASCULINE

- Lavender:
  - fresh
  - spicy
- Fougere:
  - fresh
  - floral
  - woody
- Oriental:
  - spicy
  - sweet
- Chypre:
  - woody
  - leathery
  - coniferous
  - green
- Citrus:
  - floral
  - fantasy
  - fresh
  - green

Examples of all known perfumes are given with the date of introduction and manufacturer. Characteristics of each odour group are also presented. Few other companies have published similar classifications with somewhat a different approach.

For beginners in fragrance compounding the most practical is the Brud Odour Profile system for aroma chemicals and specialties, common literature description for essential oils and the Haarman and Reimer system for classification of fragrance compounds created by each laboratory on selected market standards.

4. SENSORY EVALUATION

Evaluation of fragrances is closely combined with odour descriptions used and the general classification and evaluation systems in the company. However when starting a fragrance laboratory one shall consider two ways of evaluation of fragrance compounds. First of them, a comparative one is used for the comparison of one fragrance or raw material with another one - standard or target sample. The second which can be considered objective or hedonic is based on ranking the sample (or samples) with the aim of selecting the best one of the group or degree of approval for a specific purpose.

There are many more or less complicated methods to achieve the purpose of odour evaluation. All of them are based on panel work. People for the panel are selected according to their odour discrimination, ability and odour memory (see section VII).

For small laboratories and beginners a simple method elaborated by author of this paper (see Literature cit.10) is the most convenient and easy to apply with freshly trained staff.

Evaluation of raw materials odour quality, and fragrance compound odour in comparison with target sample (market product, customers sample etc) can be performed by a so called triangle method and 8-point scale of similarity. The samples shall be evaluated by 5-9 members panel selected and trained for the purpose. The triangle method is used to answer the basic question "Is the sample evaluated identical with standard or target sample or different?". In the performance of this method paper smelling strips are dipped in the two compared samples. Each panellist is presented with three coded strips. Two of them were dipped in one of the samples (target or evaluated) one in the other. The panellists shall specify which strip has different odour or find no difference. There is simple mathematical version for the calculation of the results. All details are given in the paper mentioned above (Literature pos.10).

Triangle odour evaluation test (Sample vs Standard or target product)

For each test, we need 6-9 panellists (preferably 7). Each of them is presented with coded paper strips dipped in appropriate products. There are two ways of preparation of the test according to the number of tests to be made and working time available for test preparation. The simple way is presentation of strips marked A,B and C to all panellists in the same order, e.g., each blotter represents the same material.

Sample Marking for Test made by 7 Panellists

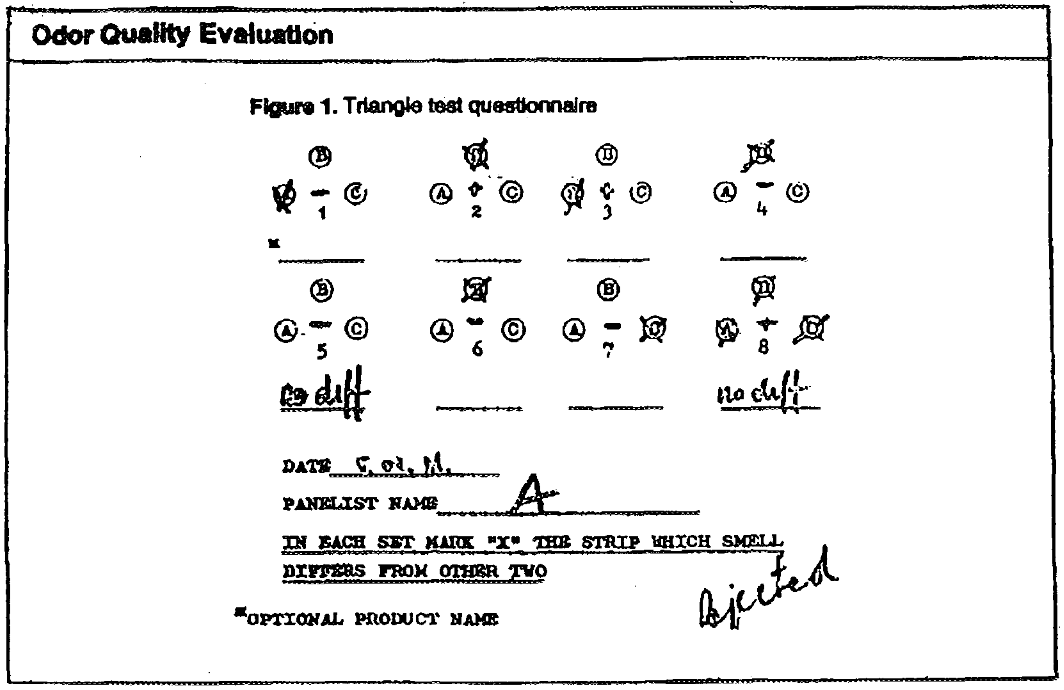
I. For each product 1 set of paper strips
Marking: 1A, 1B, 1C, Where 1 = Sample Number

II. For each product 7 sets of paper strips dipped in samples with the same sequence.
Marking: As above multiplied 7 times

III. For each product 7 sets of paper strips dipped in samples with random sequence.
Marking: 1/1A, 1/1B, 1/1C, 1/2A, 1/2B, 1/2C, etc.
Where 1/= Sample number and /1,2,3, ... = set of paper strips number

More complicated in preparation and calculation of results, but more objective (especially if panel is working in the same room) is the presentation of strips dipped for each sample in different sequences so that each set of three strips represents a different combination. For very simple and quick work, if more samples are to be evaluated, only one set of strips is prepared for each product and panellists smell them in turn. Panellists are requested to specify which of three strips has a different odour from two of the others (or which two strips are the same). With the questionnaire shown in figure 1, a quick record of results is collected.

One very important point should be mentioned here. The whole idea presented in this paper, which allows us to obtain reliable results with a minimum number of highly-qualified and expensive staff who should be more effectively used for creative work, requires one very efficient and responsible person, a panel secretary. This person should prepare all samples from production, suppliers, purchase, or others, code strips, organize panel meetings, keep all records and confidential documentation, and deliver results to appropriate departments in the company. Panellists should not know the sources of evaluated samples to avoid any preferences which may affect results. Therefore, the panel secretary should be the most confidential person available.



Odor Quality Evaluation

Figure 1. Triangle test questionnaire

*

no diff

no diff

DATE 5. 02. 91.

PANELIST NAME A

IN EACH SET MARK "X" THE STRIP WHICH SMELL DIFFERS FROM OTHER TWO

*OPTIONAL PRODUCT NAME

Rejected

Figure 1. Triangle test questionnaire

A final conclusion from results obtained from the panel should be made by the panel secretary. According to literature sources on statistical data evaluation, there is a direct relation between the number of panellists (or tested sample sets) and the minimum number (or percentage) of correct answers for a reliable result. These data are shown in Table II.

Table II. Number or Percentage of Correct Answers in Triangle. Test as Minimum for Reliable Result

Number of Samples Panellists (a)	Minimum Correct Answers (b)	True Percentage Correct Answers T (c)
5	4	min. 70
6	5	min. 70
7	5	min. 60
8	6	min. 60
9	6	min. 50
10	7	min. 50
20	11	min. 33
50	24	min. 22
100	43	min. 15

a) For one panellist and one product number of samples.
For group of panellists their number for each product.

b) At significance level 0.05.

c) As above according to formula for true percent good answers after correction for guessing $T=1.5\ (P-33.3)$, where P=percent correct answers in the test.

Very often in creative perfumery but also in evaluation of ingredients it is necessary to evaluate the difference between two samples which might not be identical but are similar enough to replace each other. For that purpose an 8-point scale differentiation test has been elaborated with following description of marks;

1 - totally different
2 - different with some note of standard
3 - different top or base note
4 - distinct different note
5 - weak stable different note
6 - weak passing different note
7 - just noticeable difference
8 - no difference, identical with standard

With trained panel and proper calculation of mean values, the average result of a minimum of 5.5 may allow one to accept the product. This particular method is especially useful when samples of natural raw materials are tested which due to sources, season, harvesting and method of production always show small differences in odour. The same happens with fragrance compounds. Production with use of always the same raw materials and exactly the same formula may give minute differences which result from the summing up of unnoticeable differences in raw materials. Thus a simple routine method is necessary to evaluate the level of difference and allow the management to make proper decisions. It shall be emphasized that a trained perfumer with experience can take such a decision without the time consuming panel evaluation.

There is no universal method of quality evaluation of new fragrances. Experience of the perfumer, market knowledge achieved by the marketing group and perfumers, customers demands, price limits and many other factors influence the creative and marketing ability of the company which will express itself in the proper selection of compounds to be presented to potential customers. In everyday practice a good fragrance is the one which is accepted and purchased by a customer.

5. FRAGRANCE DEVELOPMENT AND FORMULATION

Good fragrance formula is the aim and the dream of every perfumer and fragrance compounding company management. It is the most valuable asset of the company and the collection of the formulas shall be the most carefully guarded treasure of manufacturer.

The following factors shall be considered in the development and formulation of a fragrance;

1. Type of odour
2. Product for which the fragrance will be used
3. Price limit given by the customer or market research
4. Raw materials available and the raw material policy in the company
5. Manufacturing cost (raw material cost limit)

There are two general approaches to the creation of new fragrances. First is sometimes called "open offer" and is the creation of a fragrance or group of fragrances for general offer to different customers (or one customer) based on general market trends, fragrance fashion or in case of compounders producing their own raw materials fragrances based on some new, interesting note coming from a captive raw material unknown to other suppliers. Such an offer consists of types of odours selected by perfumers and marketing groups according to criteria developed for a purpose, with prices given on similar or different levels and compounds elaborated for one group of products (i.e. soaps, or perfumes or shampoos) or for different products. The idea of presentation may vary according to the potential customer and can consist of e.g. one type of fragrance elaborated for different products (fragrance line) or several types of odours for one product type (product line) The collection can be presented to one customer as a presentation of the company possibilities (new customer, new market) or to several customers, also already known as a presentation of new achievements of the compounder.

The second way of creation is development of a new (or new version) fragrance according to the specified demand of the customer. In this case compounder is invited for briefing with potential customer and detail parameters of fragrance are described including odour type, price limit, product details, fragrance content in the product (also fragrance cost per product volume, especially in bulk products like detergents) and so on. In this case perfumers are somehow limited and the task may be more difficult, but considering that competition usually is involved it is may be very stimulating. Usually perfumers work in regular contacts with the customer and it is common case that the sample of unperfumed product for which fragrance is elaborated is delivered by the customer for testing. Fragrance performance in the product, stability, odour strength etc are tested by the application laboratory of the compounding company before presentation of the fragrance to the customer.

Of course in case of "open offer" all fragrances shall be tested in standard products before presentation but this shall be routine procedure with every fragrance created and selected for possible presentation to customers (see section VI).

There is one more way of fragrance creation which is the imitation of a perfume. It is not much appreciated within a branch but very common. Once a new product with new fragrance type is successfully launched on the market, numerous followers appear with similarly smelling products. There is of course the fashion factor in this case but usually followers go after the new market trend and the best example was famous "green apple" odour which flooded world markets with thousands of different products from washing powders to perfumes with the same or similar odours based on fruity green note of apple. It is often the case that a fragrance compounding company is requested to imitate a specific perfume either from a market product or competitors products at a lower price. Although it can not be considered "good manufacturing practice" it happens very often and from the perfumer point of view it is very difficult, time consuming and an expensive procedure which involves a lot of instrumental analysis. Therefore it is common practice that market success of new fragrance stimulates perfumers to imitate it immediately just "in case".

At the process of fragrance development it is important for creative perfumers to have basic knowledge of market products for which fragrance compounds are used and some important properties of components of these products in order to predict the behaviour of the fragrance in the product and possible interactions of fragrance ingredients with the product. This knowledge saves lot of analytical and testing work.

Although creative perfumers use to protest, as most artists do they shall be familiar with prices of raw materials and the production cost. Approval of the perfume by the customer is always based on the evaluation of fragrance quality vs. its price. Thus any formula when elaborated shall be constantly calculated according to the company rules and the customer or market limits. Permanent monitoring of raw materials prices and market trends shall be introduced in the company and the whole system shall be computerised.

Fragrance formulas when ready and approved shall be stored in a confidential way either in computer system or in files. It is however advisable that one set of approved formulas is saved in a file and kept in safe. As said at the beginning of this chapter formulas and perfumers constitute the value of the compounding company.

One more aspect of creative perfumery in a compounding company, that is very often underestimated is the role of marketing and personal with wide knowledge of markets, customers, products and trends. It is often the case that customers with a product idea will ask a compounder to select the perfume and in many cases to cooperate in the creation of the product itself. In all cases very close cooperation between marketing people, perfumers and chemists is a basis for good results.

Formulation of fragrance with use of base and blocks

In most fragrance houses perfumers work with hearts or bases i.e. ready made mixtures with specific odour characteristics, products compatibility and price. Such a systems facilitates work of perfumers and also by using mixtures with confidential formulae make any misuse of the formulae by unauthorized personnel very difficult.

Preparation of simple, inexpensive rose fragrance is presented below with use of Rose Bulgare base and development of top, middle and fixative parts of the final mixture. The basic part of the compound is "BASE OF ROSE" type "Bulgarose". This formulation with mainly aroma chemicals will give the final compound characteristic rosy odour. The following components will be used:

BASE OF ROSE
(accord of Bulgarose)

Citronellol
Geraniol
Nerol

Phenylethyl Alcohol
Methyleugenol
Rose oxide
Beta-Damascenone

Next step will consist of creation and addition of top a note i.e. mixture which will give first characteristic odour of the products when it is unpacked or opened. The top note will consist of following components.

TOP NOTES

Citronellyl formate
Decyl acetate
Rhodinol
Linalool
Alkohol C9
Benzyl acetate
Aldehyde C11

Middle note mixture will be added to enhance the base and form specificity of the fragrance created. The following constituents will form this part of the formula.

MIDDLE NOTES

Cinnamyl acetate
Heliotropin
Phenylethyl isobutyrate
Ethyl cinnamate
Geranium oil (Bourbon)
Ylang-ylang oil
Cedryl acetate

Components of Basic note will fix the fragrance and keep its odour in time and harmonized. These are mostly low boiling chemicals or resinoids:

BASIC NOTES (Fixatives)

Civet
Phenylacetic aldehyde
Phenylacetic phenylacetate
Hydroxycitronellal
Amber
Benzoe Siam resinoid
Musk Ketone or Galaxolide
Tonka resinoid
Vanillin
Styrax resinoid

Thus the final mixture will consist of following ingredients:

ROSE FRAGRANCE

citronellol
geraniol
nerol
phenylethyl alcohol
methyleugenole
rose oxide
beta-damascenone
citronellyl formate
rhodinol
decyl acetate
linalool
benzyl acetate
alcohol C9
aldehyde C11
cinnamyl acetate
heliotropin
phenylethyl isobutyrate
ethyl cinnamate
geranium oil
ylang-ylang oil
cedryl acetate
civet
phenylacetic aldehyde
phenylethyl phenylacetate
hydroxycitronellal
benzoe rez.
musk ketone
galaxolide
tonka rez.
vanillin
styrax rez.
dipropylene glycol

As said in section 5 it is very practical to elaborate few variations of the same perfume to allow the potential customer to make his choice from these few. It can be done either by modification of the final formula by addition of some components or changing contents of the formula or by using different proportions of the "blocks"in the final formula. In the example above the following two variations of the rose fragrance can be prepared by using different proportions of the four parts. This will make the following two formulae:

BASE OF ROSE
(accord of Bulgarose)

Citronellol	25
Geraniol	15
Nerol	5
Methyleugenol	3
Phenylethyl alcohol	20
Beta-Damascenone	0.5
Rose oxide	1

A-50%, B-60%

TOP NOTES

Citronellyl formate	2
Decyl acetate	3
Rhodinol	8
Linalool	15
Alkohol C-9	0.5
Benzyl acetate	8
Aldehyde C11	1

A-20%, B-37.5%

MIDDLE NOTES

Cinnamyl acetate	2
Heliotropin	3.5
Phenylethyl isobutyrate	2
Ethyl cinnamate	3
Geranium oil (Bourbon)	6
Ylang-ylang oil	2
Cedryl acetate	4

A-20%, B-20.5%

BASIC NOTES (Fixative)

Civet Z/10%	0.5
Phenylacetic aldehyde	1
Phenylacetic phenylacetate	4
Hydroxycitronellal	3
Amber synth.liq.	0.5
Benzoe Siam resinoid	2
Musk Ketone or Galaxolide	4
Tonka resinoid	0.2
Vanilin 10%	0.5
Styrax resinoid	0.5
Dipropylene glycol	6

A-20%, B-21.2%

By using appropriate quantities of the mixtures two different rose compounds A and B can be obtained (A & B % denotes parts by weight).

This very basic example shows possibilities of fragrance creative work and unlimited number of fragrance creations. It also shows knowledge is indispensable to work as creative perfumer.

6. APPLICATIONS

Number of products for which fragrance compounds are used or can be used is practically unlimited. Very often customer demands or imaginations of creative marketers form very unusual but very exciting tasks for perfumers. Of course the basic area of application of fragrances are cosmetics and toiletries, washing and cleansing products, and majority of household chemicals. But there are many other products which for many reasons need fragrance compounds e.g. many fabrics or fabric products from clothing to carpets are perfumed to mask their original often unpleasant odour. Various plastics especially those which imitate natural materials (leather imitations, plastic flowers and plants, synthetic furs) are perfumed with appropriate fragrance which reminds one of the odour of the natural material. Many home used products like furniture, wallpapers, paints etc are also perfumed to cover unpleasant odour of solvents, glues and other components. There is a substantial consumption of fragrances for air fresheners used in toilets and bathrooms but also in airplanes, public places, offices (also in air condition systems) and home. As a new application, mood modifying odours are used based on methods of olfaction psychology and/or aromatherapy.

The last one is however limited to natural essential oils and is a part of the so called alternative or natural medicine. Although relatively new it is expanding very fast and its discoveries are also used in cosmetic and pharmaceutical industries where good odour and healing properties are combined in the products.

In all cases of use of fragrance compounds, they shall be tested in the final product and in most cases it shall be done by the producer of fragrance compounds. An application laboratory has to be set up by a compounding company to test the performance of fragrance in the products.

There are two different ways of testing the fragrances in the final product. First of them is applied when "open offer" is elaborated and either customer or exact product or both are known. Of course as said before different formulas of fragrances (also for those with the same odour type) are prepared for different groups of products. A floral jasmine fantasy bouquet for Eau de Toilette will have a different formula from the same odour compound made for a beauty cream and still different if made for a soap. When the exact product for which samples are created is unknown or when for one reason or another the customer is not willing to supply unperfumed sample, the compounds shall be tested in typical products prepared according to a few standard formulas (e.g. washing powder, two or three types of cleansing products, soap, shampoo, two or three types of cosmetic creams, lotions, liquids, lipstick, and few others typical for the market). Fragrance stability shall be tested by shelf test (storage in standard condition) and at elevated temperatures (40oC for 14 days), for uv and day light resistance and in potential usage conditions (e.g. in hot water, in washing machine, on fabric, on hairs, foamed).

As said in Section V if the compound is prepared for a defined product and a customer it is most desirable to carry on the stability and performance tests in the product, which in unperfumed form shall be requested from the customer.

Behaviour of the compound in the product shall be evaluated by the same or in bigger companies by a special panel which shall compare odour of product sample after the test with freshly made sample of product with the compound and with the fragrance compound itself. The 8-point scale differentiation test as described in Chapter IV shall be used for difference evaluation and the same criteria for similarity applied. As said above the comparison shall be made also to odour remaining after common usage of the product e.g on hairs after shampooing and drying, on fabric after machine washing, on the skin for deodorants and other body care products, in the bathroom for air fresheners and so on. Appropriate equipment and laboratory rooms are necessary for such application tests as well as trained panellists. Sometimes consumer tests are arranged with the cooperation of the customer.

As mentioned before it is important that perfumers shall have the basic knowledge of chemistry of fragrance raw materials and the most common cosmetic ingredients. There are many interactions between fragrance raw materials which in most cases are active chemicals. Similarly interactions can occur with active components of products to be perfumed both chemical and physical changing the composition of the fragrance or affecting the quality of the product. For example "maturation" of a perfume or toilet water will include several chemical reactions between the fragrance components and water and ethanol which are commonly used as the solvents. Most of body care cosmetics contain active agents or plant extracts with their own odour and also active chemicals which will react with fragrance components. Fragrance can also affect the product. Soaps can be discoloured when exposed to light. In some low alcoholic liquids, fragrance can affect the emulsion quality or transparency of the liquid. Therefore an experienced chemist with good knowledge of the variety of market products, their contents and properties is a "must" for a good compounding company.

7. TRAINING REQUIREMENTS

Practically speaking there is no formal education of perfumers at universities or colleges. Business of Perfumery Course at Plymouth University in U.K. educates people working in fragrance production and trade industry but does not give enough knowledge and practice as required to be a creative perfumer. David Williams Correspondence Course in Perfumery supported by IFEAT provided also by the Plymouth University gives excellent but only basic education for beginners. There are no places for training of perfumers except internal courses or special education systems inside fragrance companies. These are hardly available for strangers and are usually organised for employees. Thus good trained perfumers are very expensive and are in high demand and many of them move from one company to another. At the moment there is one training place for perfumers available to UNIDO fellows at POLLENA-AROMA Warsaw Poland and a general course organised by UNIDO at the Anadolu University in Turkey.

There are also no handbooks for self-education as in many other professions. Methods and systems of training are as confidential as fragrance formulas. There is some literature in professional journals e.g. two papers on "Company training of perfumers" published by the author (see Literature cit.12). This however will give only basic information for starters, describing methods of training to be used in a company by experienced perfumers to educate their trainees.

Generally speaking there are no rules on profession or education of candidates for education in perfumery. University degree in chemistry, biochemistry, pharmacy or related sciences will be an advantage giving knowledge of materials and their properties. Main advantage of potential candidates to be perfumers is personality, imagination, sense of arts (an artistic hobby is good sign), wide interests, good appearance and easy contacts with people (perfumers very often work with customers and promote their creations).

It is also common practice that potential perfumers are selected from new employees of the company after testing newcomers for their ability to differentiate odours and odour memory. Very simple test for odour differentiation ability is described in the author's paper (Literature ref.10). Odour memory test is made by perfumers in a company and in its simplest form consists of 20-50 samples of diluted odoriferous chemicals or mixtures which shall be recognised or associated by a candidate with specific products. For example diluted lemon oil (and/or limonene) shall be associated with lemon fruit or lemon juice, dichlorobenzene with disinfectant or toilet freshener, diethyl phtalate with paints or solvents, some pyrazines with burnt food, cis-hexenol with green grass, coumarin with hay etc. These basic tests can be followed by testing the sense of smell sensivity, fragrance imagination and few others.

Selection of Panellists for Odour Evaluation Board

The best panel can be selected from non-professional company employees previously tested for ability in odour differentiation. According to the size of the company and number of samples to be tested, the number of panellists can vary, but most useful is to have about twenty selected and trained people from whom 6-9 are called at random for each session. This system minimizes the routine boredom which affects results from people permanently smelling samples in a control department. For purposes of simple differential evaluation, only one testing method is necessary. A few tests can easily show the ability of the panellist to differentiate two samples. The set of samples in Table I can be recommended.

Table I. Mixtures for Testing of Panellists

Standard "Y"	Standard "X"	
Sandalwood oil	Sandal wood oil	8 parts
	Cedarwood oil virg	1 part
Pine needle oil	Pine needle oil	6 parts
	Terpineol	1 part
Bergamot oil	Bergamot oil	10 parts
	Terpinyl acetate	1 part
Alphaamylcinnamic aldehyde	Alphaamylcinnamic aldehyde	35 parts
	Benzaldehyde	1 part

The test is the simple triangle method. The subject is presented with eight sets of three samples for each of four combinations of materials.

Example Combinations of Sample X and Standard Y

	A	B	C
1.	X	X	Y
2.	X	Y	X
3.	Y	X	X
4.	X	Y	Y
5.	Y	X	Y
6.	Y	Y	X
7.	X	X	X
8.	Y	Y	Y

After evaluation, about thirty minutes for each set, the panellist should specify which of the three samples in each set is different or state "no difference". A minimum of seven correct out of eight answers are necessary to accept the person for the panel.

Course of training is thus different for each company and usually apart from some general lectures, literature study and group seminars, the training is based on individual work with the senior perfumer. Usually education of the perfumer takes 4-5 years. First selection is made after 3-6 months to eliminate people who show no progress. Second selection is made after the first or second year. Most creative candidates are then trained individually to be creative perfumers while others will get training for odour evaluation boards, application laboratories, or marketing and development specialists as well as sales people who in fragrance compounding company should have a wide knowledge of perfumery.

8. POTENTIAL IN DEVELOPING COUNTRIES FOR PRODUCTION OF AROMA CHEMICALS

Production of aroma chemicals is the synthesis of fragrant chemical substances from simple raw materials available from the chemical and petroleum industries. It is also the chemical transformation of materials obtained from plant origin, usually of "isolates" i.e. individual chemical substances separated from e.g.essential oils. Both ways can lead to the same products.

For example ionones can be synthesized either from citral produced from isoprene or acetone as starting materials (obtained from the petroleum industry) or from citral produced in complicated multistep synthesis from turpentine which is obtained as side product in paper industry but can also be distilled from wood, or from citral obtained by the fractional distillation from the essential oil of Litsea cubeba. The first and second processes are chemically complicated, need expensive and sophisticated equipment thus investment resources and of course highly qualified chemists. Furthermore economical analysis show that such a production is economical on a very large scale only and in practice it is carried out only by few producers - usually big multinational companies.

There are few aroma chemicals which can be manufactured in simple units with raw materials available from small chemical industries or as side products in other industries. It is also possible to produce some aroma chemicals from semiproducts or intermediates available from the fragrance industry. This is acceptable economically because of very cheap working power and low custom duties.

Third group of aroma chemicals which are available for production in developing countries are those which create high pollution of water or air and are transferred from developed countries due to high cost of environment protection. This in fact is export of pollution. But here again if valuable products are considered they are transferred to daughter companies of multinationals situated in the countries where pollution control in not so strict.

Procedures and technologies for most of above mentioned chemicals which can be started in developing countries are available in the literature and patents and are collected either in basic books like P.Z.Bedoukian's handbook on aroma chemicals or annual literature reviews made by the same author for the "Perfumer and Flavourist" journal, also available in book form (see Literature cit. 3, 11).

Most interesting and easiest for relatively cheap development are aroma chemicals obtained from single isolates rectified or separated by chemical methods from the essential oils and other natural sources. These are usually available in developing countries and when used in synthesis can form added value products very useful for the local production of fragrance compounds and for export. Ionones and methyl ionones produced from citral distilled from Litsea cubeba, isoegenol from clove oil, heliotropin from sassafras oil are just few examples of possible processes. These shall be developed on the basis of availability of natural raw materials which can give isolates for further synthesis and economical calculation of production cost and market demand. It shall be noted here that there is a growing concern on wide use of pure synthetic chemicals and products based on natural raw materials are considered more interesting both as safer but also richer in their fragrance bouquet.

Annex 1. Basic natural fragrance raw materials in creative perfumery laboratory

- Ambrette seed extract
- Anis oil
- Armoise oil
- Badian oil
- Basil oil
- Benzoin Siam resinoid
- Bergamot oil
- Bois de Rose oil
- Calamus oil
- Chamomile oil
- Camphor oil
- Cananga oil
- Caraway oil
- Cardamon oil
- Carrot seed oil
- Castoreum
- Cedarwood oil
- Celery seed oil
- Citronella oil
- Clary sage oil
- Clove bud oil
- Coriander oil
- Costus root extract
- Eucalyptus oil
- Fir balsam
- Galbanum resinoid
- Geranium oil
- Ginger oil
- Grapefruit oil
- Jasmine absolute
- Juniper berry oil
- Labdanum resinoid
- Lavandin oil
- Lavender oil
- Lemon oil
- Lemon terpenes
- Lemongrass oil
- Mandarine oil
- Myrrh resinoid
- Neroli oil
- Nutmeg oil
- Oakmoss absolute
- Olibanum resinoid
- Opoponax resinoid
- Orange oil
- Orange terpenes
- Palmarosa oil
- Patchouli oil
- Pepper oil
- Peppermint oil
- Petitgrain oil
- Piment oil
- Pine needle oil
- Rose oil
- Rosemary oil
- Sandalwood oil
- Spearmint oil
- Styrax resinoid
- Tagetes oil
- Thyme oil
- Tuberose absolute
- Vetiver oil
- Ylang-ylang oil

Annex 2. Basic aromatic chemicals for creative perfumery laboratory

Acetophenone
Alcohol C-8 (octyl)
Alcohol C-10 (decyl)
Alcohol C-11 (undecylenic)
Alcohol C-12 (lauric)
Aldehyde C-7 (heptanal, oenathic aldehyde)
Aldehyde C-10 (decyl)
Aldehyde C-11 (undecylenic)
Aldehyde C-12 (lauric)
Aldehyde C-12 MNA (methyl nonyl acetic aldehyde)
Aldehyde C-14 (peach, gamma-undecalactone)
Aldehyde C-16 (strawberry, ethyl methyl phenyl glicydate)
Aldehyde C-18 (coconut, gamma-nonalactone)
Aldehyde C-20 (raspberry, ethyl p-methyl beta-phenyl glicydate)
Allyl amyl glycolate
Allyl caproate
Allyl heptylate
Allyl phenoxy acetate
Amber synthetic
Amyl acetate
Amyl benzoate
Amyl butyrate
alpha-Amylcinnamic aldehyde
Amyl cinnamate
Amyl phenylacetate
Amyl propionate
Amyl salicylate
Benzaldehyde
Benzophenone
Benzyl acetate
Benzyl alcohol
Benzyl benzoate
Benzyl cinnamate
Benzyl salicylate
Butyl benzoate
p-tert-Butylcyclohexanyl acetate (Bergarom)
o-tert-Butylcyclohexanyl acetate (Pomarom)
Cedryl acetate
Cibet synthetic
Cinnamic acid
Cinnamic alcohol
Cinnamic aldehyde
Cinnamyl propionate
Citral
Citronellal
Citronellol
Citronellyl acetate
Citronellyl butyrate
Citronellyl propionate
Coumarin
p-Cresyl acetate
p-Cresyl methyl ether
Cyclamen aldehyde
Damascone-alpha

Damascone-beta
delta-Decalactone
Diethyl phtalate
Dihydroisojasmone
Dihydromircenol
Dimethylbenzyl carbinol
Dimethylbenzylcarbinyl acetate
Dimethyloctanol
Dipentene
Diphenylether
Diphenylmethane
Diphenyloxide
Dipropylene glycol (solvent)
Ethyl alcohol (solvent)
Ethyl butyrate
Ethyl cinnamate
Ethylphenyl acetate
Ethyl propionate
Ethylvanilin
Eugenol
Floralozone
Galaxolide
Geraniol
Geranyl acetate
Geranyl butyrate
Geranyl formate
Geranyl propionate
Hedione
Heliotropin
Herbaflorat
cis-Hexenol
cis-Hexenyl acetate
Hexylcinnamic acid
alpha-Hexylcinnamic alcohol
alpha-Hexylcinnamic aldehyde
Hexyl salicylate
Hydroxycitronellal
Indole
Indolene
Ionone 100%
Ionone-alpha
Ionone-beta
Iso-bornyl acetate
Iso-butyl benzoate
Iso-butyl salicylate
Iso-eugenol
Isolongifolanone
Iso-nonyl acetae
Iso-pulegol
Jasmalactone
Lilial
Limonene
Linalool
Linalyl acetate
Lyral
Maltol
Menthol
Mentone
Methyl acetophenone
Methyl anthranilate
Methyl benzoate
Methyl cinnamate
Methyl ionone
Methylphenyl acetate
Methyl salicylate
Musk ketone
Musk T
Musk xylol
Myrac aldehyde
Myrcene
Nerol
Nerolin Bromelia
Nerolin Yara-yara
Nonadienol
Phenylacetic acid
Phenylacetaldehyde 50%
Phenylethyl acetate
Phenylethyl alcohol
Phenylethyl butyrate
Phenylethylmethyl ether
Phenylethylphenyl acetate
Phenylethyl propionate
Pyralone
Rhodinol
Rose oxide
Sandela
Styralyl acetate
Tangerinol
Terpineol
Terpinyl acetate
Tonalide
Trichlorphenylmethylcarbinyl acetate (Rosarom)
Vanilin
Vertacetal
Verotyl
Vetiveryl acetate

REFERENCES

1. ALLURED, (1995), *"Fragrance and Flavour Materials 1995"*.
2. Wells, F.V., Billot M., (1981), *"Perfumery Technology"*, J. WILEY .
3. Bedoukian, P.Z., (1986), *"Perfumery and Flavour Synthetics"*, ALLURED.
4. Dorland, W.E., Rogers, Jr. J.A., (1977), *"The Fragrance and Flavour Industry"*, DORLAND.
5. Groom, N., (1992) , *"The Perfume Handbook"*, CHAPMAN & HALL.
6. Arctander, S., (1969), *"Perfume and Flavour Chemicals"*, ARCTANDER.
7. Harper, R., Bate-Smith, E.C., Land, D.G., (1968), *"Odour Description and Odour Classification"*, CHURCHILL .
8. Amoore, J.E. ,(1970), *"Molecular Basis of Odour"*, C.C.THOMAS .
9. Brud, W.S., (1980), *"Words Versus Odours-How Perfumers Communicate"*, Perfumer and Flavourist, 11, (4), 27.
10. Brud, W.S., (1983), *"Simple Method of Odour Quality Evaluation of Essential Oils and Other Fragrant Substances"*, Perfumer and Flavourist, 8,(4),47.
11. Bedoukian, P.Z., (1982), *"Perfumery and flavouring materials"*, ALLURED.
12. Brud, W.S., et al., (1988), *"Company Training of Perfumers"* Part. I Perfumer and Flavourist, 12, (6), 18(1987), Part. II, ibid., 13, (6), 23.
13. International Fragrance Association Guidelines.
14. Arctander, S. , (1960), *"Perfume and Flavour Materials of Natural Origin"*, ELIZABETH.

USEFUL ADDRESSES

1. **POLLENA-AROMA Ltd.**
Dr. W.S. Brud, President
Klasykow 10,
o3-115 Warszawa, Poland
Tel: +48-22-114270, +48-22-114221
Fax: +48-22-199228

2. **ALLURED PUBLISHING (Perfumer & Flavourist, Journal of Essential Oils Research, Books)**
Stanley E. Allured, Publisher
362, South Schmale Road
Coral Stream, Illinois 60188, USA
Tel: +1-708-6532155
Fax: +1-708-6532192

3. **PLYMOUTH BUSINESS SCHOOL (BA Business of Perfumery, Perfumery Education Centre)**
Course Leader: Tony Curtis PhD
University of Plymouth
Drake Circus
Plymouth PL4 8AA, United Kingdom
Tel: +44-752-232807

4. **BOELENS AROMA CHEMICAL INFORMATION CENTRE**
Mr. Mans H. Boelens
Groen van Prinstererlaan 21
1272 GB Huizen, the Netherlands
Tel./Fax: +31-35-5253558

CHAPTER 6

COMMERCIALIZATION OF ESSENTIAL OILS AND AROMA CHEMICALS

N. VERLET
CERDEPPAM - Ministry of Agriculture
BP 36 26110 Nyons France

1. INTRODUCTION

The present pattern of production and marketing of essential oils is characterized by a competitive supply side, with more and more producers, from developing countries as well as from industrialized countries. The demand situation is complex ; over the last 20 years, there has been a significant increase in industrial turnover, and an expansion in the range of uses of essential oils, thereby strengthening their position in the food flavors and cosmetic industries. However, at the same time, synthetic substitutes have continued to pose a serious threat to essential oils, especially in the perfumery sector. During this period, there has been a regular decrease in the mean price of essential oils.

In this context, any new producer has to carefully analyze the market, the price trends and the situation of the world production. This approach is unfortunately often difficult due to the lack of available economic information.

2. STRUCTURE OF THE INDUSTRY

The commercial chain from the producer to the end user of essential oils and aroma chemicals is complex, and it is difficult to know who has the determinant role in the demand side. Therefore, it is very important to clearly identify each level of the structure of the industry, the organization of economic activities and their respective influence on the commercialization (Verlet 1994).

Production of raw materials

The geography of essential oil crops is very diverse, and few countries nowadays are not involved in their production. Each species includes ecotypes which have become adapted to different environments. This spread in activity is also the consequence of the decline in world price of most commodities; many development programs on alternative crops such as aromatic plants have been initiated throughout the world. This geographical diversity is matched by the great heterogeneity of production and exploitation systems : based on harvest of wild plants, small-scale enterprises using family manual labor at varying levels of mechanization, and large enterprises fully mechanized based on capital investment more than labor.

Extraction facilities

The extraction of essential oils involves different types of equipment, according to the capacity and the technique (steam distillation, hydrodiffusion, solvent extraction...). The extraction equipment can be monitored by the grower or trader, or be part of the industrial process. In the first case, primary processing is in the hand of the producer himself, or of a group (co-operative). It is, in most cases, small or medium size steam distillation units, even if some growers have set up more sophisticated extraction plants. Nevertheless, solvent extraction tends to be restricted to major firms because of the required investment and level of technology. Extraction facilities can also be controlled by companies that buy raw plant material from the producers, meet the cost of distillation, and then sell the essential oils. This practice is relatively uncommon in industrialized countries where distillation is normally carried out by the raw material producers. It is more common in developing countries where the individual raw material producer lacks the capital to invest in distillation plants. In this case, the producer is directly dependent on the distillator and can not market or store his own oil.

The perfume and flavor industry is also involved in extraction, but mostly for high value essential oils, or for extraction where specific technology is required. The industry have often invested in extraction plants in the production areas, establishing agreements with local partners. Dried material is also processed in factories in consuming countries, when added value by specific extraction covers transportation cost.

Traders and brokers

Some important industrial users have their own purchasing department, and major oil producers may be tempted to sell directly to industrial users, in order to get a better price for their oils. Nevertheless, traders and brokers still have important functions in the essential oil sector :

- purchase of oils throughout the world or from specific geographic areas,
- analysis and quality control,
- rectification of the oil to fit the commercial standards,
- blend,
- sale to users.

Different types of traders can be distinguished. Enterprises based in producing countries are mainly involved in the sale and export of local products : they usually deal in large quantities of few commodities produced locally. Enterprises based in consuming countries are concerned with imports and supply of domestic market : they handle a large variety of oils. Lastly, some merchant houses are specialized in international trade of large volume commodities.

Perfumery and flavor industries.

The role of this sector is to bring together natural and synthetic substances, to refine and mix them, and to produce a blend ("composition"), which meet the requirements of the end users. Such enterprises rarely sell a product to the consumer. The perfumery and flavor industry is equipped with sophisticated extraction plants, and produce ""oleoresins, "absolues", from locally grown or imported raw material. The industry also buys essential oils and extracts from competitors, traders and producers. The more specific role of this sector is to carry out specialized operations on natural extracts (extraction of natural isolates, purification, decolorization....). The mixing of these extracts and synthetic materials is carried out by the perfumery and flavor industry creating "aromatic and fragrance compositions, which are incorporated into the commercial products by the industrial end users. Since the technology involved in food flavors is very similar to that of perfumery, most enterprises are engaged in the two activities.

User industries

This is where the volatile oils (usually mixed with many other natural and synthetic compounds) are incorporated into the final product destined to the consumer. Industries using flavors are found mainly in the food stuff sector (beverage and dairy products are the largest users). The tobacco industry is also a large consumer of flavor compounds. Toothpastes and pharmaceuticals are also consumers of essential oils for flavoring purposes. The users of perfumes include manufacturers of washing powders and detergents, air fresheners, and fine perfumes. The cosmetic industry is also an important user of natural compounds, either for their fragrance and for their therapeutic values as well. Essential oils and their derivatives are used in the pharmaceutical industry. The traditional medicine in developing countries, the expanding aromatherapy in industrialized countries are mostly bases on the use of essential oils and medicinal extracts.

Final consumer

Demand depends ultimately on consumer behavior. The strong demand for natural products during the last decade was in favor of the essential oils market. Nevertheless, the essential oil content in a final product is not always specified, and in some cases does not have any influence on the consumer choice (e.g. "haut-de-gamme perfumes"). In other case, the essential oil content is known to the final consumer, and the natural origin is a strong element in marketing (e.g. natural food additives, aromatherapy, cosmetics). Standards and regulations are fundamental in this case.

3. WORLD PRODUCTION AND DEMAND

World production

The estimation of the world production is a particularly difficult task to achieve. Very few statistics are available in most of the producing countries, especially in developing countries which are the leading producing countries. Therefore, a global overview of the world production requires direct investigation through buyers, producers, administrations, scientists... Most of the work in this respect has been done by B. Lawrence (1984, 1988). The following figures are mainly based on Lawrence, ITC (1988), updated by Verlet (1992). Even if changes have occurred, especially in term of value, regarding the price changes in the trade of many essential oils experienced in 1994 (increase in price for eucalyptus, mint, citronella oils), these data are still useful to compare the respective importance of essential oil production and market.

Estimation of the world production in term of volume

The annual world production of volatile oils is estimated at 45 000 to 50 000 tonnes. The products derived from turpentine oil are not included in this review (the world production of turpentine oil is estimated to be a little more than 300 000 T, from which 100 000 T are used as a starting material for the synthesis of perfumery compounds - Joulain 1995a). Fifteen products, each contributing at least 500 T of this total account for nearly 90 % of total production. They are :

- major essential oils used in the flavor industry (mints, cassia and citrus)
- oils used in large volumes in perfumery for detergents, freshners...(lavandin)
- oils used as starting material for hemisynthesis of extraction of natural isolates (eucalyptus, *Litsea cubeba*, citronella, sassafras).

The price of these oils are low (often less than 10 $ / Kg).

Essential oils varrying in annual production vary from 500 T to 100 T are mostly used in the fine fragrances (lavender, petitgrain, vetiver, geranium), or in specific flavors (anise, fennel). Many other essential oils are produced in a volume less than 100 T. They may be very high value essential oils (jasmine, rose, sandalwood) or specialty crops (cabreuva, tarragon....).

Estimation in term of value

The table 1 gives a rough estimation of the essential oils value. The world production was estimated to be 700 millions US$ in 1989 (Verlet 1993). It is probably more correct to estimate the world production in 1995 at more than 1 Billion US $. The 18 most important species represent nearly 75 % of the total value, but this concentration is less marked than for tonnage because of the trade in small volumes of products of high unit value. Classification according to total value brings together high volume cops such as citrus, with high unit value crops such as rose, jasmine and vetiver.

Value > 10 000 000 US$			
Peppermint	Orange	Sandalwood	Cedarwood
Cornmint	Litsea cubeba	Citronella	Lime
Rose	Eucalyptus globulus	Vetiver	Bergamot
Spearmint	Jasmine	Patchouli	Geranium
Lemon		Lavandin	
Value > 1 000 000 US $			
Coriander	Sassafras	Fennel	Basil
Lavender	Star anise	Marjoram	Cedar leaf
Ylang Ylang	Nutmeg	Cinnamon bark	Pimento leaf
Clove leaf	Tangerine	Sage	Cananga
Bois de rose	Cascarilla	Camphor	Garlic
Anise seed	Lemongrass	Celery	Juniper
Petitgrain	Neroli	Onion	Amyris
Camomile	Eucalyptus citriodora	Copaiba	Lovage
Clary sage	Roman camomile	Clove	Thyme
Mandarin	Dill	Grapefruit	Bitter orange
Cinnamon	Ginger	Olibanum	Tea tree
Rosemary	Palmarosa	Tarragon	Pine
Value > 100 000 US$			
Calamus	Parsley	Laurel leaf	Bergamot mint
Cumin	Peru balsam	Pimento bay	Ho
Artemisia sp.	Cajeput	Carrot	Hyssop
Valerian	Angelica	Caraway	Tagete
Spike lavender	Cardamom	Cypress	Spanish sage
Styrax	Gaïac	Myrtle	Elemi
Bay	Oregano	Cabreuva	Pennyroyal
Birch tar	Fir	Buchu	Myrrh
Pepper	Hop	Galbanum	Rue
Davana	Parsley herb		

Table 1 : Estimates of world production
Sources : B.LAWRENCE, N. VERLET, I.T.C.

Trends in production

Long term trends in the production sector can be pointed out, according to the end use of the essential oils.

- A steady increase in the principal essential oils used in the food flavors and additives, such as cassia, citrus and mint. Some countries have strongly developed the production to supply this increasing market, such as India and China for *Mentha arvensis* (table 2).

Mentha arvensis Origin	1990	1994
China	2000	3000
India	2000	3000
North Korea	200	300
Paraguay	800	60
Taiwan	50	50
Vietnam	20	50
Argentina	10	20
Brazil	10	<5
Pakistan	7	10
Japan	1.5	1.5
Nepal	1.0	2.0

Table 2 : Mentha arvensis oil produces worldwide
Source : D. JOULAIN

- A strong increase in the production of oleoresins, falling off slightly after 1980. This market has benefited countries which have been able to set up modern extraction plants. This increasing market is shown on the table 3, representing the imports of oleoresins by the U.S.A..

	Paprika	Pepper	Others
1980	128	91	115
1981	279	105	78
1982	105	146	97
1983	188	123	94
1984	251	110	88
1985	334	158	147
1986	347	180	163
1987	368	218	175
1988	338	219	216
1989	351	131	455
1990	433	190	403
1991	363	272	449
1992	449	255	465
1993	290	286	375
1994	48	266	495

Table 3 : Oleoresins imports into U.S.A. (Tonnes)
Source : U.S.D.A. Circular series

- A contrasting pattern for plants produced for perfumery. This sector has severely suffered from competition with synthetics and the development of the "mass market perfumery". A relative stability can be observed for essential oils such as lavender, ylang ylang, geranium. The table 4 shows that, despite a stability of the global production, the competition between producers has been very sharp. Algeria and La Reunion have declined to the benefit of Egypt and China. Some other oils have experienced a fall in production, suffering from irregularities (vetiver, bergamot).

	1850	1880	1910	1925	1935	1950	1978	1984	1991
GRASSE	Intro			2	Disap				
REUNION		Intro	62	172	100	45	62	29	25
ALGERIA	Intro		33	120	35	10	10	Disap	
MOROCCO				Intro	1	6	25	15	6
EGYPT					Intro	1	110	40	40
CHINA						Intro	30	50	120
INDIA				Intro				5	15
U.S.S.R.				Intro	20	50	50	50	50
KENYA				Intro	6	2	Disap		
ISRAEL							Intro	2	1

Table 4 : World geranium oil production from1850 to 1991
Sources : GUENTHER, ARCTANDER, BERTHIER, CAHEB, LAWRENCE, VERLET

Note : Intro = Introduction
Disap = Disappearance

- Important variations in the availability of essential oils used as starting point for hemisynthesis. Thus lemongrass, which was an important source of citral, has undergone a abrupt decline, being replaced by synthetic citral or extract from *Litsea cubeba* (table 5). In this sector, the production cost is of high importance. According to progress achieved on cultivation practices, genetic improvement, chemistry, major change may occur in the production of essential oils of this category.

Oil Type	1970	1984	1990	1994
Lemongrass	2000	310	300	< 300
Litsea Cubeba	50	900	1050	1400

Table 5 : Natural sources for citral
Source : D. JOULAIN

- Increasing difficulties in the development and marketing of new products involving non-traditional oils. The 50s and 60s saw the introduction of many new species, but a few have been successful. It seems that since 1980 such enterprises have not been able to expand, even though markets for small quantities could provide appreciable income for groups of growers.

World demand

The industrial sector experienced a very substantial expansion, on a world scale during this last decade, which was most marked for the flavor industry. The annual growth of the industry is around 3-5 %. The strong expansion is based on changes on life-style in the industrialized countries. The perfumery and cosmetics industries have responded to the success of health products, whereas flavors have become essential to more sophisticated food products and the associated food-processing industries. In developed countries, which represent 70 % of the world market, it seems unlikely that such rate of increase can be maintained. The scope for expansion in the use of flavors is more important. The fact that more than 70 % of production essential oils, aromachemicals and perfume compositions is consumed by only 15 % of the world population, shows that there remains a considerable potential for growth in developing countries. Development of the market depends largely on economic development, and growth in South-East Asia has opened up new markets for flavor and perfume industries.

The total sales value of flavors and fragrances industry in 1993 is estimated to be US $ 9 Billions. *More than half of the market is in the hand of the six major companies* : I.F.F., Givaudan-Roure, Quest, Haarman & Reimer, Firmenich, Takasago.

Other important companies in this field are Bush, Boak & Allen, Dragoco, Tastemaker, Systems Bio Industry, Robertet, Mane, Charabot.

Trends in perfumery

The figure 1 shows the geographical distribution of the fragrances sales, and figure 2 the distribution by industrial sector.

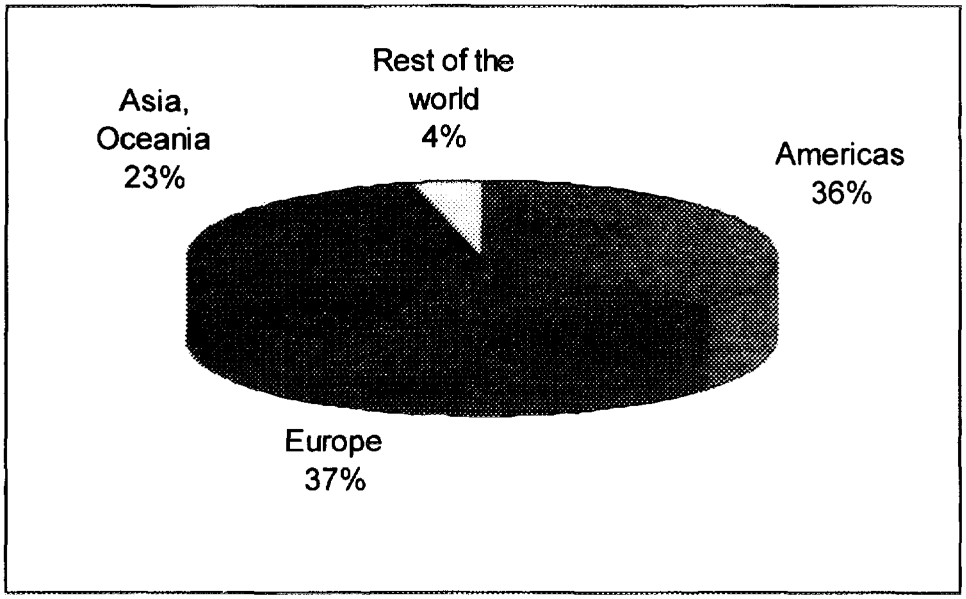


Figure 1. Geographical distribution of the fragrance sales
Source : D.PROTZEN from H & R

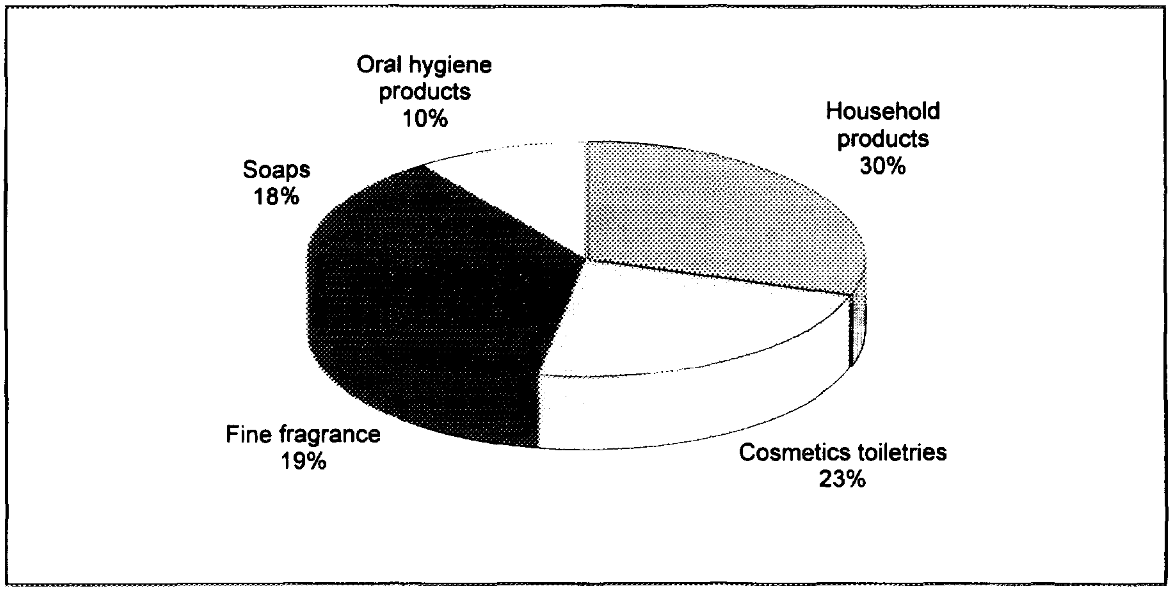


Figure 2 : Distribution of fragarance sales by industrial sector
Source : D. PROTZEN from H & R

Household products, soaps and oral hygiene products represents nearly 60 % of the total sales. It is easy to substitute natural essential oils used in these sectors. The strong growth of the market for these products was mainly based on synthetic molecules. Nevertheless, essential oils have maintained their global position. Relative costs of production predominate in the competition between different sources of compounds for perfumery (natural or synthetic). Some essential oils are still produced at a competitive price, versus synthetic. The natural origin of the fragrance may be of importance for the marketing of detergent and fresheners in a near future. Fresheners based on natural essential oils (pine, eucalyptus, lavandin, lemongrass, citrus...) are more and more successful. It is possible that detergent as well may use the "nature" as a marketing strategy, associating the fragrance with the antibacteriological properties of the essential oils, well known nowadays by the consumers. Therefore, there are some interesting development possibilities in this field, but restricted to low price essential oils.

High value essential oils are, by contrast, experiencing difficult times. Their place are now largely occupied by sophisticated synthetic molecules which have caused an upheaval in the modern perfumery. During the last decade, the perfume industry had to reduce the cost of the fragrance composition. In 1986, it was estimated that 9 out 10 women in the US and France were wearing a perfume (Calame 1995). This means that the market is now more or less saturated. The average life of a perfume is limited, much more money is needed for advertisement. This reduces the possibility to use a large amount of expensive essential oils in the composition. In this respect, it is unlikely that this end-use of essential oil will increase in the future. On the other hand, only few new synthetic chemical compounds were introduced during the last 10 years. This is largely due to cost reasons. Toxicity tests are expensive, and one must be convinced of the improvement in odor type or performance over existing products to invest in the marketing of a new molecule. If essential oils benefit from technical progress, leading to reduction in production costs, and if supply can be made reliable, there is still a future in the fine perfumery sector.

Trends in flavors

The figure 3 shows the importance of the flavors in Europe, which remains from far the leading world market. The use of flavors by sector is described on table 6. Since 1960, these sectors had experienced a continuous growth, stimulating the flavor market. In the same time, consumer's demand for natural flavors was maintained. This combination of factors has sustained the demand for the important essential oils (mints, citrus, cassia...) used in the main sectors. The legislation plays an important role. Even if reversion to synthetic substitutes would result in lower prices, when the natural status of the aroma is clearly defined by the legislation and clearly mentioned to the consumers, the natural essential oils will still be used in most cases. This does not mean that the future of essential oils currently used by the flavor industry is secure and stable. The search is on for new sources of natural flavors and for improvements in existing products by different means, such as development of new methods of extraction and concentration. The trend is towards more sophisticated uses of natural compounds. A natural flavor will be in the future a combination of natural isolates, extracted from various sources (extraction from different plants and provided by microbial route such as fermentation or bioconversion), more than the extraction of a single plant.

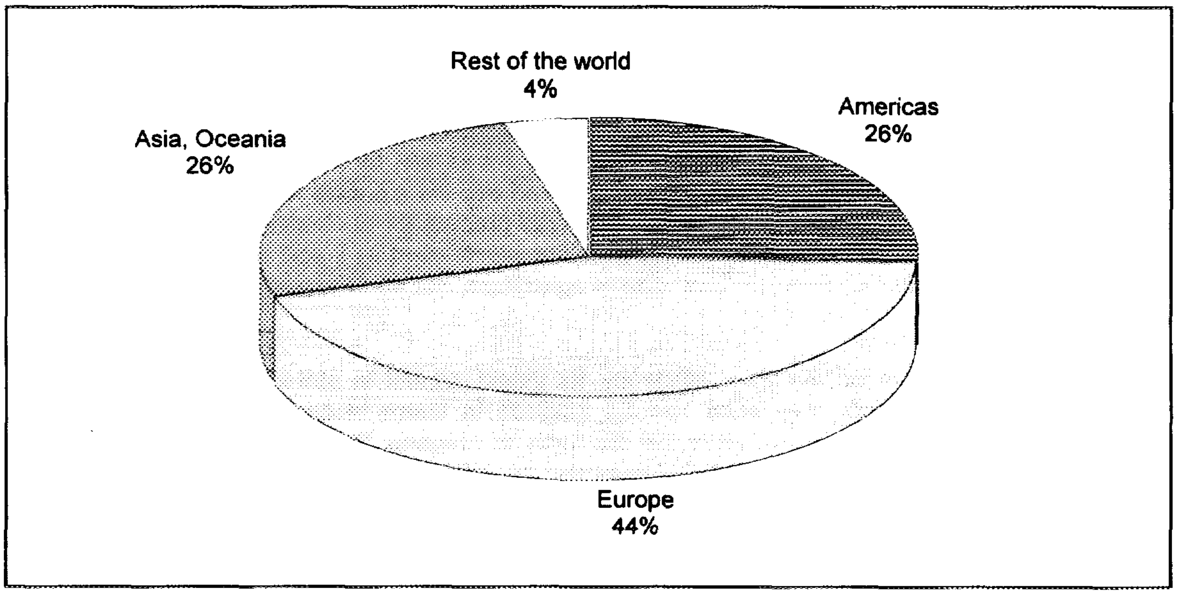


Figure 3. Geagraphical distribution of Flavors sales
Source : D. PROTZEN from H & R

Soft drinks	34%
Confectioneries	13%
Dairy products	12%
Savoury foods	9%
Bakery products	8%
Tobacco	7%
Alcoholilc beverages	5%
Animal feed	3%
Pharmaceuticals	2%
Others	7%

Table 6. Breakdown of flavors uses by sector
Source : J. HARIEL

New uses for essential oils

Aromatherapy and "phyto cosmetics" are one of the fastest growing niche markets in North America (Puhorit 1995). The American consumers are getting more aware of the side affects of the synthetics, and are turning towards products using essential oils in hope of getting safer and milder products. The market of cosmetics where essential oils are used in insignificantly small quantities has been established for a long time in Europe and America. There is, since few years, a tendency for new phytocosmetics (including shampoos, soaps....), formulated to maximize the benefits of natural active ingredients, and containing a significant quantity of essential oil (more than 1%). This trend has already had a positive impact on the essential oil market.

Many new uses are currently investigated by different research programs throughout the world (Palevitch 1995). Large amount of pesticides are used worldwide for crop protection. Increased awareness of the risks to human health, water pollution and wild life, coupled with the problem of pest resistance has revived the interest in use of plant derived-product for controlling pest. Plant derived substance, such as pyrethrins, rotenone, and nicotine are widely used as insect repellents. It is highly probable that essential oils can serve as pesticide in agriculture, but no commercial product has been marketed yet.

Essential oils have proved their efficiency for insect control in stored food products. The traditional synthetic fumigants may pose serious hazards to warm blooded animals and to the environment. The efficiency of several essential oils such as oregano, thyme, dill, citrus leads to the conclusion that essential oils could be exploited as potent insecticides in stored food products in a near future.

The effect of essential oils on potato sprouting is also well established and must be considered as a potential future market. The herbicidal effect of essential oils have been also studied. This would be a considerable market for essential oils. There is a growing need for more environmentally friendly herbicides. Several essential oils have shown inhibitory effect on germination, and an herbicidal effect on weeds. Nevertheless, easy alteration of chemical structure of essential oils and their high volatility remain major difficulties to solve, before commercial use in the field.

4. PRODUCTION IN DEVELOPING COUNTRIES

The world production

Developing countries have a dominant position in the world production, but the competition with industrialized countries and countries from the ex-Comecon remains very strong. The present international situation is complex. Success depends upon an optimal combination of several factors : climate, efficiency of research, capacity to invest and innovate, the existence of economic structure ensuring low costs of production, established commercial relations with the industrial sector. Industrialized countries remain in a dominant position where high yields and full mechanization make cultivation competitive with countries based on low labor cost.

The breakdown of the world production, by economic system, confirm the dominant position of the developing countries (55 %), followed by industrialized countries (35 %), and ex Comecon (10 %).

Main producers in developing countries

The figure 4 shows that four countries among developing countries are benefiting from a leading position (Brazil, Indonesia, India and China), the last two showing the greatest capacity to adapt to the market. In particular, China has been the major player in the 80'. Based on a strong state development policy, China became within few years a major producer for many important essential oils (*Mentha* *arvensis*, cedarwood, eucalyptus, *Litsea cubeba*, star anise, geranium...), gaining a large market share with very low prices. Times are changing since 1994 : liberalization in the production sector has made farmers more reluctant to produce essential oils at a very low profit. China has now the strategy to become a producer of aroma chemicals and of natural components, rather than a producer of raw essential oils. This have been facilitated by joint venture with American and European perfumery firms.

The main producers have based their development and export potential on a reliable domestic market, and have common characteristics :

- Large population supporting a genuine internal demand for essential oils locally produced. Such internal market, in spite of low income per inhabitant, have favoured the development of networks of production, transformation and commercialization.
- A strong position for several oils. It is not unusual for one of these countries to control more than half of the world market (e.g. Indonesia for clove, cananga and patchouli, China for geranium, Litsea, India for some spice oleoresins).

Alongside production for the internal market, specialized businesses dealing in essential oils for export have successfully developed, through entrepreneurs who have the capacity to finance a development program (private sector in India, Brazil and Indonesia, state investment in China).

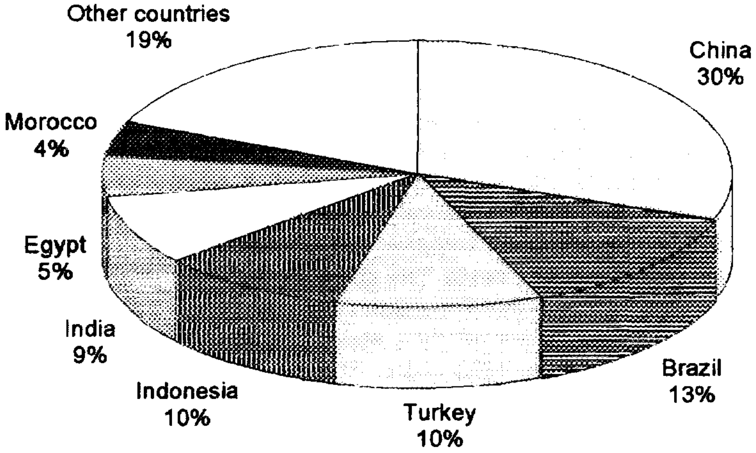


Figure 4 : Breakdown of the production among Developing countries
Source : B. LAWRENCE; N. VERLET; I.T.C.

Export oriented developing countries

Some countries are essentially orientated toward exports. Less populous, they cannot rely on a stable domestic market. Therefore, they are directly exposed to the hazards of the world market. However, in these countries, it is possible to find optimal combination of factors for the production of a specific essential oil (favourable climate, low cost of production, weak competition from other crops, few other sources of export...). Some countries have secured a long-term place in the world market. Included in this group are Haiti (vetiver), Comoros (ylang ylang), Paraguay (Petitgrain, cardamom),

New producers

The revivals of interest in natural product has brought essential oils to the forefront of the international trade. Numerous programs for the development of essential oils have been planned, but progress has been very variable. The poorest countries where economic resources are often restricted to primary raw material, are strongly affected by the decline in world prices. The development of production in these countries is normally initiated by international aid organizations. Recent candidate countries include Bolivia, Columbia, Malawi, Nepal, whereas Madagascar and Vietnam are attempting to reestablish activities in this sector. In spite of some undeniable successes, it should be emphasized that this group of countries occupies only a tiny fraction of the market, and outcome of such development projects remains to be seen. The presence of local entrepreneurs willing to develop profitable enterprises is one of the key for the success. A realistic marketing approach, with a good knowledge of the market and the competitors is essential. This will be discussed in §9 on the future trends and opportunities for developing countries.

5. SOURCE OF ECONOMIC INFORMATION

The lack of economic information is one of the major problems that new producers have to face. Developing countries have to be able to answer important questions to monitor essential oils production and marketing strategies :

- How to make a choice among hundreds of essential oils ?
- How get information on prices, market potential ?
- What volume can be produced ?
- What are the long term trends ?

Unfortunately, very few economic studies have been published on essential oils. Biology, agronomy, chemistry are much more covered by international journals than economics. Data bases such AGRICOLA, CAB abstracts, Chemical abstracts are sources of useful technical information ; but very few economic information may be obtained from these databases. Two reasons explain this situation. Numerous species are involved in essential oils production, trading and industrial uses. Each of these oils have specific characteristics : botanical variety, area of production, price and market trends. In addition, a good knowledge of the market requires to the collection of information all along the essential oil industry, including data in many fields. The second reason is the economic significance of many essential oils. We mentioned in § 3 that the value of most of essential oils are below 1 million US$. Due to the complexity of the collection of data above mentioned, the compilation of a global survey is very costly in comparison of the total value. At least but not last, most of the economic knowledge is possessed by people involved in trade or in industry, and they do no have any time or any advantage to publish economic data which are firstly collected for their own use.

Nevertheless, some economic data are useful and available.

General survey

The main studies on the markets of essential oils were published by the United Nations International Trade Centre (Palais des Nations, 1211 Geneva 10, Switzerland). In its most recent published studies, the I..T.C. has considered the market for more than 50 different oils with an analysis of the situation in the principal producing and consuming countries (I.T.C. 1974, I.T.C. 1986). Unfortunately, despite many requests from developing countries, these markets studies have not been updated, due to drastic financial restrictions applied to the U.N. organizations. I.T.C. has updated only some of the most important potential markets for essential oils and the compilation of trade statistics.

The Natural Resources Institute (Chatham, Kent ME4 4TB, United Kingdom), has been also in the past a very useful source of economic studies for developing countries. They were complementary to the global overview of I.T.C. with more specific analysis, presenting a complete analysis of the production and marketing of certain important essential oils and derivatives : mint oils and menthol (1979), selected herbaceous essential oils (1979), ginger (1982), patchouli and vetiver (1982), lemongrass, citronella and eucalyptus (1983), lime lemon and orange (1983), eucalyptus (1992). Unfortunately, the Natural Resources Institute has also suffered from budget reductions. This explains why few new marketing studies have been published recently. The privatization of the N.R.I. (which was the technical arm of the British Overseas Department) is presently under discussion.

Trade statistics

Imports and exports statistics remain the only source of information on the quantity exchanged worldwide, with mention of the mean price and the origin of the oils. Most of the countries are currently using the HTS classification for trade statistics. It is possible to have a correct view of the world market through statistics available from the main markets (E.U., U.S.A., Japan, Switzerland). Trade statistics are also of particular interest to compare long trends evolution. They present some limits : only the most important oils are identified (citronella, orange...), many others are mixed under a same number (clove, niaouli, ylang), and all the minor essential oils are covered under "other essential oils".

Information on prices

Information on prices are very important, and may be available from brokers prices list. Brokers usually take a small margin on high volume essential oils (2% - 5 %), so these lists give a good idea of the producer price. Many brokers publish a price list, and we have selected here only few of them who cover a large range of essential oils from different origins:

Georges Uhe (weekly)
12 route 17 North
POB 970 Paramus, New Jersey 07653 USA
U.S.A.

L.A. Champon (Weekly)
100 Metro Park South
Laurence Harbor N.J.
U.S.A.

Fuerst Day Lawson (monthly)
St Clare House 30 33 minories
London EC3N 1LN
U.K.

Adrian S.A. (monthly)
15, rue de Cassis
13008 Marseille
France

Periodicals publish also prices for selected essential oils, based from brokers price lists :

Chemical Marketing Reporter (weekly)
80, Broad street
New York 100004 2203
U.S.A.

Public Ledger Commodity week (weekly)
Pioneer House, 44 48 Clerckenwell Road
London EC1M 5PS
U.K.

More generally technical journals may be source of valuable economic informaticn :

Perfumer and Flavourist (U.S.A)
Drug and Cosmetic industry (U.S.A.)
Parfums, cosmétiques, arômes (France)
Arômes et additifs (France)
Cosmetic World News (U.K.)
Soap, Perfumery and Cosmetics (U.K.)
Eurocosmetics (Germany)
Indian Perfumer (India)
Current Research on Medicinal and Aromatic Plants (India)
Rivista Italiana Eppos (Italy).

At last, many congresses are organized throughout the world, and are also a very valuable source of contacts and economic information.

6. INTERNATIONAL TRADE

Estimation of the world trade

The estimates of the international trade have been obtained by compilation of all available customs statistics world-wide. Mixtures of perfumery substances, solutions in alcohol have been excluded. The foreign trade from countries which do not provide any statistics (such as China or Egypt), have been reconstructed from existing statistics. Consequently, exchanges between countries which do not provide any statistics are impossible to estimate, although it can be assumed that the quantities involved are rather small.

The total quantity of the international trade is estimated to be 100 000 T, and a value of 1 billion $US. The breakdown of the main importers (table 7) shows that the importers are mainly situated in industrialized countries. The European Union is from far the main importer. The E.U., U..S.A., Japan, Switzerland and Canada account for more than 75 % of the world total (Hong Kong acts principally as a re-exporter of essential oils from China. Nevertheless, the imports of developing countries are far from negligible. This has to be taken into account because development strategies in developing countries may be based on import substitution by domestic production.

The exports (table 8) shows the good position of developing countries (particularly China, Brazil, Indonesia and India). E.U. and U.S.A. appear on a good position as well, partly based on re-exports of essential oils, but also on export of domestic production (mint, citrus, cedarwood for the U.S.A., lavender, bergamot, thyme and various minor essential oils for the U.E.).

It is important to notice that the world trade has experienced a regular increase in tonnage, associated with an equally regular decrease in the mean price of the oils.

E.U.	42.8 %
U.S.A.	17.3 %
Japan	7.9 %
Hong Kong	5.9 %
Switzerland	5.0 %
Canada	2.8 %
Brazil	1.6 %
Philipines	1.3 %
South Korea	1.2 %
Singapore	1.2 %
Mexico	1.0 %
India	1.0 %
Thailand	0.9 %
Venezuela	0.8 %
Malaysia	0.6 %
Taiwan	0.5 %
Austria	0.5 %
Australia	0.5 %
Indonesia	0.5 %

Table 7. Breakdown of the main importers of essential oils (value)
Source : Modified from trade statistics

China	18.6%
U.E.	16.3 %
U.S.A.	16.1%
Hong Kong	6.2 %
Brazil	18.6.8 %
Indonesia	4.4 %
India	3.2 %
Switzerland	2.2 %
Argentina	2.1 %
Paraguay	1.5 %
Singapore	1.5 %
Thailand	1.4 %
Haiti	1.3 %
Japan	1.2 %
Turkey	1.1 %
Morocco	1.1 %
ex USSR	1.0 %
Mexico	1.0 %
Canada	0.9 %

Table 8 Breakdown of the main exporters of essential oils (value)
Source : Modified from trade statistics

Data on the main markets

American and European statistics are easily available, giving a good approach of the market. The global 1994 imports of the U.S.A.are summarized on table 9

	Kilograms	**1,000 dollars**	**Dollars/ Kilogram**
Anise	70.399	562	7,9
Bergamot	37.970	1.607	42,3
Caraway	5.546	206	37,2
Cassia	473.738	17.571	37
Cedarwood	557.895	2.977	5,3
Citronella	626.107	4.767	7,6
Citrus, others	205.115	2.863	13,9
Clove	463.017	1.916	4,1
Cornmint	258.096	1.614	6,2
Eucalyptus	465.252	1.393	2,9
Geranium	82.707	4.710	56,9
Grapefruit	272.261	2.599	9,5
Jasmine	10.329	2.087	202,1
Lavender	339.621	4.982	14,6
Lemon	1.368.513	22.918	16,7
Lemongrass	79.963	662	8,2
Bois de rose	31.265	740	23,6
Lime	864.563	15.175	17,5
Mint	76.858	834	10,8
Nutmeg	181.242	1.767	9,7
Onion garlic	117.799	3.358	28,5
Orange	14.880.881	23.525	1,5
Orris	8.867	2.339	263,8
Patchouli	454.918	8.999	19,7
Peppermint	305.417	5.622	18,4
Petitgrain	94.528	2.808	29,7
Pine	40.326	148	3,7
Rose	5.443	6.713	1.233,4
Rosemary	58.576	962	16,4
Sandalwood	26.398	3.669	139
Sassafras	242.350	912	3,7
Spearmint	426.144	5.184	12,1
Vetiver	51.997	2.506	48,2
Ylang ylang	30.556	2.415	79
Other oils	2.221.913	45.620	20,5
Total	**25.436.570**	**206.749**	

Table 9 : Imports of essential oils in the United States
Source : U.S.D.A.

The American essential oils trade figures are edited by the United States Department of Agriculture (Room 4644-S, Washington DC 20250 - 1000, or by internet jgoldich@ag.go). Eurostat, the European statistics may be obtained from "Office des publication officielles des Communautés européennes, 2 rue Mercier, L-2985 Luxembourg.

Regulatory requirements for exports

Legislation plays an important role in the essential oils and aroma chemicals industry. The main aspects of the regulation concerns safety for the consumers. This increasing demand for safety has been complicated by the fact that some consumer groups ask for products that are not tested on animals. Recently, the 6th amendment to the EC Cosmetics Directive has been issued which will forbid to sell cosmetic products after January 1, 1998 containing ingredients of combination of ingredients that were tested on animals (Calame 1995). The environmental awareness is also increasing. Biodegradability and volatility of organic compounds have become an important issue. Legislation is going furthereven : in the US, the banning of perfumes in public places has been already discussed. In some communities in California, the wearing of perfumes has effectively been banned, because it was said that it could cause allergies to other people present !

Regulation is particularly important in the case of new essential oils. In certain countries, current regulations require the notification of all new natural and synthetic products, which are subject to commercial transactions including handling, shipment and final uses. These regulations concern among other things the toxicological data, some of which relates to environment. For example, in the North America, Australia and the E.U., strict regulation limits the uncontrolled proliferation of new products. In Europe, the notification of a new substance is regulated by the seventh amendment of the 67/548/EEC guideline on dangerous substances. In the United States, only the essential oil found on the GRAS list can be used in food applications (Joulain 1995).

Some regulatory requirements concern drug control (e.g. sassafras oil). An increasing attention has to be given to packaging and labelling of essential oils and aroma chemicals. Essential oils are considered as "dangerous substances", according to their flammability. Within the E.U., a 1979 Council Directive (79/831/EEC) details "laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances. In this respect, it is very useful to refer to the guide line set up by K.D. Protzen from P. Kaders GmbH, published by IFEAT (Guide line for classification and labeling of essential oils for transport and handling. IFEAT, Federation House, 6 Catherine Street, London WC2B 5JJ, U.K.

7. PRICES

Factors influencing prices

In a system of free market, the equilibrium price of a commodity is determined by the forces of supply and demand for this product on the market. The equilibrium price is achieved when the consumer demand for a commodity over a certain period is exactly equivalent to the amount offered for sale by the producers. Any change in the quantities of supply or demand that are not concomitant will cause a disequilibrium between supply and demand, and subsequently a change in the equilibrium price. The equilibrium price will vary with time ; it plays an important part in the decisions made by consumers and producers.

The global supply of essential oils is a function of the profitability of the crop to the grower. This profitability depends on yields and market price, as well as production costs. Yield and costs vary with the geographic area (depending on local conditions : climate, degree of mechanization), and with time (changes in level of productivity). If the level of profitability increases, new growers will take their chance to the extent that only the most competitive growers can survive. Similarly, international prices will align themselves with the lowest production costs.

On the producers' side, various kinds of changes can take place, influencing prices :

- climatic hazards,
- sudden changes in production costs,

- competition from other products,
- effect of subsidised production on the market,
- political interference.

On the demand side, other factors may influence the market :

- consumption trends,
- development of new products,
- legislation,
- technological innovations.

Competition "natural versus synthetics"

The price of chemicals is another key factor influencing price of essential oils and natural isolates. The relationship between synthetic and natural materials has depended, historically on scientific and technological progress. At the beginning of the century, the scene was dominated by natural products. The first synthetic flavours appeared in 1850 (amyl acetate). Vanillin was synthesized in 1876 and was one of the first to be developed as an industrial product. Chemistry made rapid progresses in the identification and the synthesis of fragrant molecules. Some important chemicals became indispensable to the modern perfumery. In the 60', synthetic citral, geraniol, nerol and linalool appeared to be economically viable alternatives to lemongrass, citronella and bois de rose oils.

The creativity of synthetic chemistry is far from exhausted, since techniques derived from the headspace concept have led to the reconstruction of the fragrance of the living flower, which can not be reproduced by an essential oil or natural extract. One of the advantage of a new chemical is that it is possible to patent it, which is not possible with a natural molecule. In addition, a new molecule stimulates creation. Many new perfumes were based on new chemicals, and often patented "captive" molecules. Synthetic chemicals will continue to play a major role in the fine perfumery in the future, but the introduction of new molecules is very costly for safety reasons.

In reality, natural products have globally maintained their place in the market, under competition from synthetics. In fact, the two types of products have proved to be complementary, because the development of synthetics has led to a strong increase in the world market for flavours and perfumes, which could not have been sustained from natural sources alone, because of their high price. Nevertheless, competition from synthetics has to be taken into account in the production plan, as they can deeply influence price of natural molecules.

Examples of price trends.

The price of essential oils often shows a cyclic evolution. This succession of phases is more or less pronounced according to the individual essence. They are particularly pronounced for citronella oil (figure 5).

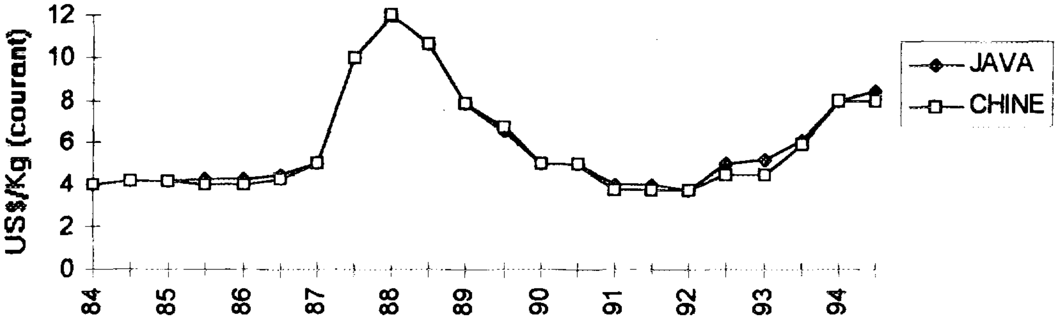


Figure 5. Trends of the New-York spot price of citronella oil

Often, these cycles affect an essential oil and its close substitutes, as lemongrass, *Litsea cubeba* and natural citral (figure 6). The essential oils produced as a by-product of a main crop are much less affected to fluctuation in price. The producer may easily adapt the volume of production according to the need of production.

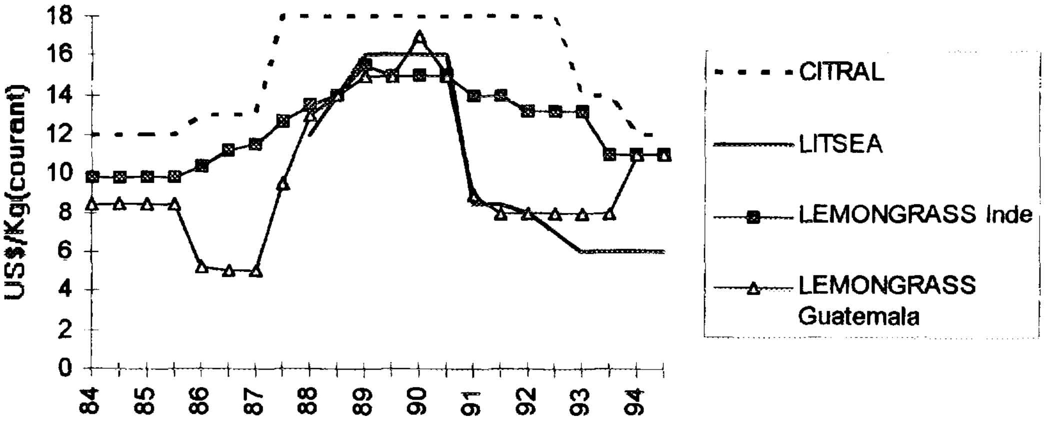


Figure 6 : New-York spot prices of citral and alternative sources

The figure 7 shows the relative stability of the cedarwood oil, while cedar is mainly planted for the timber production. The essential oil industry uses a small part of the available material.

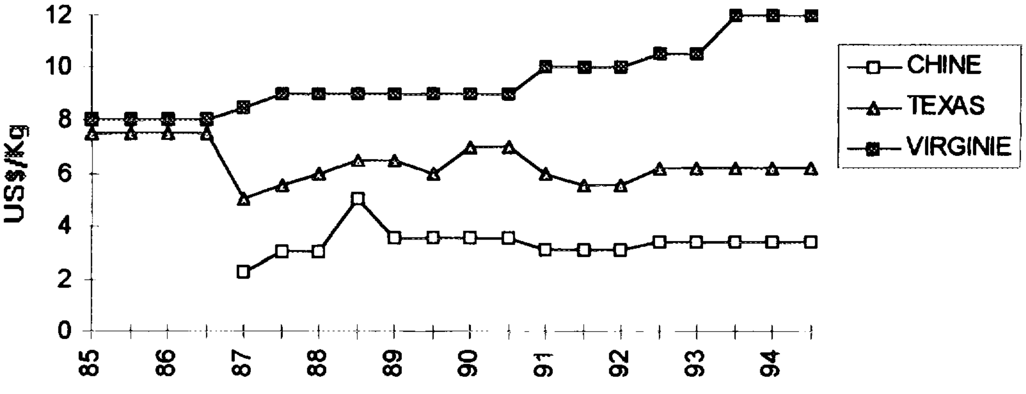


Figure 7 : New-York spot prices of cedarwood oil

8. FLUCTUATION OF MARKETS

Short and medium term fluctuation

The raw material market has always fluctuated. Price variation is an integral part of the market and is at the basis of forward markets, intensifying speculation. For main products such as coffee or cocoa, price variation considerably influences the budgets of developing countries where exports are largely based on these raw materials. Most producers are powerless in the face of these price fluctuations that affect their income.

Most essential oils also undergo considerable price fluctuations resulting from periods of shortage or surplus. These fluctuations in turn have an adverse effect on the industrial users when it is difficult to find a substitute for the natural product. They can severely affect producers who have invested and are therefore particularly sensitive to income variations.

Naturally, the essential oil sector is accustomed to these variations. Producers, traders and industrialists try to anticipate future market developments and adapt their strategies to them. However, the complexity of this specific market makes forecasts difficult and often there is not enough information available. Furthermore, over the last ten years the constant decrease in profit margins for most essential oils and their compositions - especially in the perfumery sector - have made the professional operators even more sensitive to price fluctuations: for example, they try to reduce the stocks that are actually one of the most efficient ways for economic operators to arm themselves against risks of shortages, but at a cost.

The figures in the above paragraph have clearly shown the cyclic fluctuation of the essential oils prices. Different types of cycles may be indentified :

Short term fluctuation.

Within a period shorter than the production cycle (less than a year), the volume of oil offered is influenced by climatic factors, and not dependent upon world prices. Production forecasts can create pressure on prices, aggravated by speculative stockpiles. Such short term sensitivity is especially marked where one country dominates the production and the user does not have access to alternative products.

Medium term cycles

For periods longer than a year, production decisions are sensitive to prices. However, any significant variation in the volume of supply could take several years to implement. Some species do not begin to yield until several years after planting. Thus, production reacts slowly to variation in prices, but also over-reacts : a rise in price provides evidence of pressure on supplies, but is not an accurate guide to the degree of shortage or over-production; in this way, the world price can be artificially inflated by speculation. When a large proportion of trade takes place under fixed-priced contracts, the current disequilibrium between supply and demand refers only to the open market, resulting in further pressure on prices, even though the transactions involved represent only a small fraction of the total trade. Thus, the world price acts as the principal signal to each grower in the planning of this pattern of production and anticipated profit. Variation of the volume of production arise out of the sum of these individual decisions, but without any reference to the real extent of shortage or over-production.

Consumption also reacts slowly to changes in price. To a certain extent, a manufacturer can encourage or discourage consumption of a given essence according to its price. He will investigate the possibility of substituting the natural product with another more reliable essence, or with a synthetic product, but substitution of this kind carries a cost, and the decision will not be made unless the pressure on prices persists. However, once the decision has been made, the user will hesitate to revert to the natural product when it returns to its initial price. After a period of elevated prices, there follows a brutal recession since new crops, encouraged by high prices, flood the market and at the same time strategies to produce substitutes will reduce the demand of the natural product.

Consequences of market instability

Economic research on commodities has dealt with this topic extensively. The problem of price fluctuation and regulation mechanisms is an important issue for our sector (Verlet 1995). However, it is even more important for large-scale markets such as raw materials, minerals or agricultural products (copper, tin, coffee, cocoa, etc.) that many developing countries depend on for most of their export revenues. In the past a number of large-scale measures have been implemented to try to stabilise prices and income (international agreements, stabex, etc.) and numerous studies have attempted to assess both the interest and effectiveness of such measures. To give a rough summary, the theoretical results of market regulation are the following:

- net increased profit for the community ;
- a gain in one side of the market (seller's or buyer's) to the detriment of the other, due to the instability caused by fluctuations in supply and demand ;
- price stabilisation does not eliminate unstable income for producers.

In the essential oil sector, there are two approaches to the problem of price regulation:

Price instability is inherent in the primary product market and all barriers to free trade are detrimental to the market and to the consumer in the medium-term.

Backers of liberalism, who are against all attempts at price regulation, put forward arguments that are mainly based on two principles:

- free price fixing guarantees improved productivity and development of the economic sector as a whole
- stabilisation is costly and cannot be maintained in the medium term.

Therefore, to conclude along these lines, it is preferable to let free competition and "natural" adjustment mechanisms run their own course.

Price instability is a genuine problem as it is a prime factor in the reduction of the natural product market

Price instability of essential oils can be considered as unfavourable to the natural product market. In periods of sharp price rises users will turn to substitute products, in particular products of synthesis with much more stable prices. This substitution is only partly reversible. There is no guarantee that users will return to the natural product when the price has returned to the original level. Furthermore, when a new composition is created, the persons in charge of purchasing will advise the creator not to use products that are unreliable. Therefore, price instability puts natural products at a disadvantage for use in current creations and restricts the introduction of new essential oils or plant molecules.

Forecast and modelisation

Which ever approach one may take to instability, all participants are trying to forecast market development. Econometric models are used to anticipate future development. These models usually consist of a first approach aiming at representing market movement in a general manner. Quantitative data that have been recorded over a long period is used as a basis for quantifying a model according to the explicative variables. In this manner, explicative and predictive models have been developed for tea, coffee and rubber, for example. Much still has to be done in the area of market analysis ; very few works have been applied in the essential oils sector : a research model conducted on the price of American mint (Matzat, 1980), and an application conducted on the market for jasmine from Grasse (Verlet, 1994).

The mint model was developed on the basis of an intuition that the price of mint oil is a function of domestic demand, foreign demand, yield, harvested surface area and stock variation.

The author has analysed a set of data collected since 1945 and conducts research on the most significant parameters according to simple techniques of the lesser square (OLS). This approach leads to the final model through a selection of the most significant variables.

After testing several models, Matzat developed the following function for the price of "Oregon" mint essential oil:

$$FP_t = f(YE_{t-1}, TR, QD_t, PDI_t, ASL_t, FP_{t-1}, AH_{t-1})$$

where:

FP_t = Price of essential oil in year t (on the producer's side)

YE_{t-1} = Mean yield of domestic crops

TR = Time trend

QD_t = Domestic consumption for year t

PDI_t = Estimated mean income for households in year t

ASL = Total pre-harvest amount of stocks in year t

FP_{t-1} = Price of "Oregon" mint oil for year t-1

AHt-1 = Total harvested surface area in USA in year t-1

The forecast equation (function log/log) resembles the following:

$$\begin{aligned} FP_t = \; & 25{,}29 - 0{,}589YE_{t-1} + 24{,}13TR - 14{,}40QD_t - 1{,}76PDI_t \\ & - 0{,}315ASLt + 0{,}537FP_{t-1} + 0{,}116AH_{t-1} \end{aligned}$$

with $R^2 = 0{,}81$.

The results of the model's forecasts are displayed in figure 8.

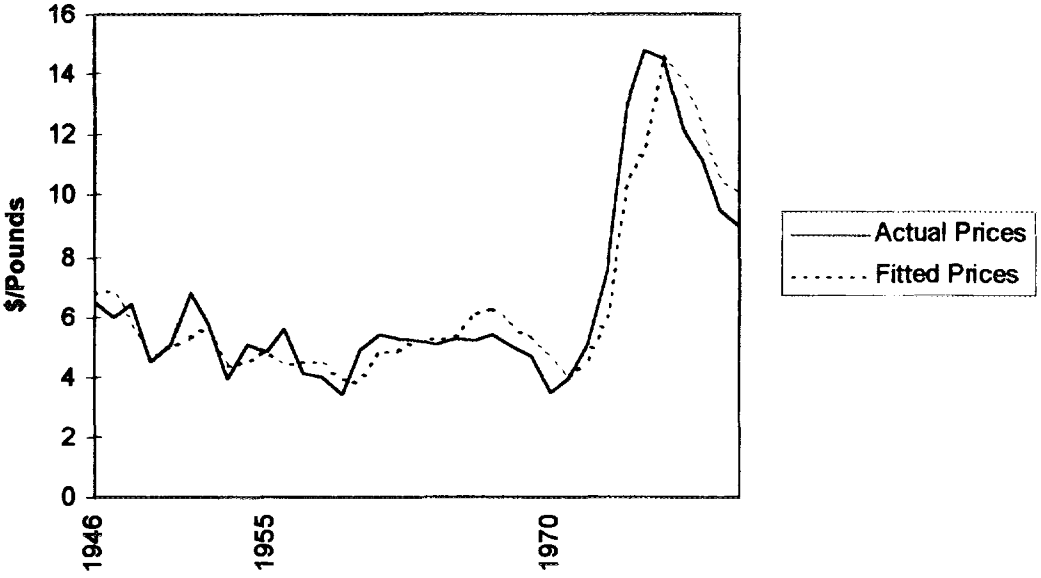


Figure 8 : Forecast prices and real prices, 1946 - 1980
Source: MATZAT 1980

Several comments arise from this figure:

- The error between forecast and real price is not very considerable ($0,90 in mean absolute value). Nevertheless this error is more significant when price fluctuation is high. In 1973 it amounted to more than $3, which represents an error of 100%. These are the very circumstances where a reliable forecast model would be useful...
- The model does not sufficiently highlight the changes in trend since it only took 4 of the 14 inflexion points into account for the considered period.
- There is a discrepancy of nearly one year in the forecasts. It is possible that the rapid increase inprices in 1975 is partly responsible for this, but this does not correct the introduced factor (time trend) to take long term trends into account. Without doubt, it is necessary to have recourse to more sophisticated methods than OLS in order to

The data available for mint is not perfect, but there is none available for most other essential oils. Lavandin, for example, has some aspects in common with mint, but the cultivated surface is not recorded annually in a systematic study. It would be difficult to assess the stocks, and foreign demand is even harder to evaluate since the statistics on compositions include an unknown share of synthetic products.

Elements for developing a model for the jasmine from Grasse

Statistics are available on Grasse jasmine concerning the production and production price of the flower. The complexity of the market makes it impossible to develop a sophisticated model, but it is still useful to consider the mechanisms that have caused the current situation for Grasse jasmine. A theoretical representation is a good means to attempt to measure the entirety of the explicating parameters. Figure 9 displays the regular decline in the flower harvest in Grasse, which tallies with the real needs of the industrial users for this product. This decline does not mean that perfumers have stopped using jasmine, but is the consequence of jasmine imports from Egypt in the 1950s, then of Indian jasmine in the 1980s, both at a lower price than Grasse jasmine. This type of development is typical for essential oils in perfumery, where a source dominating the market is faced with competition from new producers with advantages in terms of production cost. The development of the Bourbon geranium market, for example, clearly displays the transition from a cyclical type of development - corresponding to periods of adapting to economic crises - to a exponential type of development: a reaction to a situation of structural crisis (Verlet 1992).

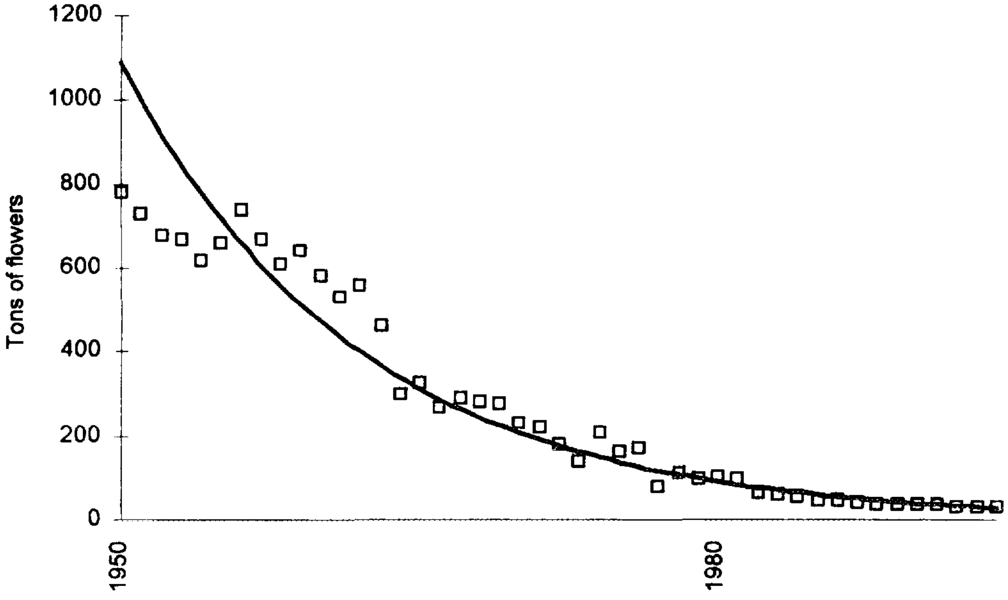


Figure 9: Market loss of Grasse jasmine
Source: VERLET, modified from L.PEYRON, ONIPPAM

Price development of Grasse jasmine is relatively predictable in so far as it is highly correlated with labour costs and standing crop-related charges. The model therefore concerns development of demand illustrated by the curve comparable to an exponential function starting in the early 1960s. Decrease in demand is due to two factors:

- Grasse jasmine not being used in new compositions
- Substitution of Grasse jasmine in "active" compositions.

The effect of "non-renewal"

The decision to use a certain natural product in a new perfume is reached by a company after much discussion. Even if a particular quality appeals to the perfumer, price criteria and security of supply are crucial for the ultimate choice. A price difference that is too high in comparison to another source, or a loss of confidence in the perenniality of the traditional supply source, quickly result in the industrialist changing the origin of his supplies. It is assumed in this model that Grasse jasmine was no longer used in new compositions soon after alternative sources became readily available. This applies especially to the introduction of Egyptian jasmine in the 1950s. This is a realistic hypothesis when one knows the decision-making procedures governing the choice of the raw materials and their origins.

The normal life span of a perfume around average length t is taken, as well as a normal supply of the quantity of Grasse jasmine contained in the perfumes, taking their renewal into account. The market loss of Grasse jasmine, as a result of the disappearance of perfumes and lack of new compositions containing it, corresponds to the following:

$$\exp(-t/T)$$

The effects of substitution

In certain cases attempts have been made to substitute Grasse jasmine in perfumes that are still on the market. To achieve this, research has to be conducted, as well as changes in the composition to guarantee that the original olfactive note is reproduced on the basis of jasmine from a different source and with substantial differences in quality. In order to make a decision on substituting Grasse jasmine in an active composition, the manufacturer of the composition will compare two amounts:

- The global cost of the substitution (research, modifying the manufacturing procedures...), represented by P_D
- The intended gain from this substitution which equals :

$$[P_G(t) - P_M(t)]\, qT$$

where

$P_G(t)$ is the price of Grasse jasmine

$P_M(t)$ is the price of jasmine from the substituting source

q is the quantity of jasmine used in the composition

T is the forecast life span of the perfume.

Therefore, the effect of substitution equals:

$$\exp(-\alpha\, [P_D(t) - P_M(t)]\, qT / P_D)$$

According to the composition of the perfumes depending on the quantity q of Grasse jasmine used in the original composition there will be a double relation:

$$\int_0^\infty n\, p(q,0) = N$$

$$\int_0^\infty n\, p(q,0) = Q(0)$$

where

N is the total number of perfumes containing Grasse jasmine during time period t=0
Q(0) is the total quantity of Grasse jasmine used during time period t=0

p is a whole number positive or null which depends on the criteria of distribution of Grasse jasmine in each perfume.

The cumulated effect of non-renewal and substitution can be represented as follows:

$$Q(t) / Q(0) = \exp(-t/T)\, D$$

where

$$D = [\, 1 + Q(0)\, [\, P_G(t) - P_M(t)\,]\, T \;/\; N\, P_D (\, P + 1\,)\,]^{p+2}$$

These first results presented on jasmine can be followed up by integrating a certain number of other parameters, in particular a decline in the mean life span of perfumes. The main interest of these research studies lies in determining the type of situation which corresponds to a specific model. Moreover, it seems that the results obtained can be extrapolated for most essential oils used in fine perfumery. It can be used for anticipation, as it illustrates in a reproducible manner (which has been confirmed for lavender and geranium) the course of the loss of market for a source faced with external competition by the two types of mechanisms described. There is a time lapse between the moment when the conditions of a loss of market with an exponential quality are reunited, and the perception of this crisis "in the field". Then it is often too late to react when the effects of the market loss are perceptible by anyone. The interest in this type of study lies in clearly identifying the market dynamics and long-term implications so that the natural product can be maintained in industrial uses.

9. FUTURE TRENDS AND OPPORTUNITIES FOR DEVELOPING COUNTRIES

Competition with ex-COMECON and industrialized countries

The importance of the essential oils production in Developing countries has been pointed out in the §4. Nevertheless, it is important to realize that industrialized countries have established intensive production practices in the field of essential oils, and have remained in a dominant position for several species. Because of the recession affecting major crops, many co-operatives or individual producers have undertaken diversification projects involving essential oil production.

The countries from the former COMECON were traditional producers of essential oils, with two objectives : satisfying the internal market, and export. The priority given to meeting internal demands and the poor quality of the oils have limited their situation to be in a dominant position on the international markets. They have been strong producers of coriander, lavender, clary sage and rose oils. However, oils originating in eastern countries have frequently caused instability in the market because of irregularity in supply and currency deals. The production of essential oils by the countries of Eastern Europe has been strongly affected by the policies of economic planning, and the present state of disorganization is still having a negative effect on the production level and irregularities in supply.

It is possible to identify three types of situations regarding international competition :

Essential oils for which developing countries are in a dominant position :

- Species sensitive to environmental factors, such as tropical plants (spices, ginger, cananga, vetiver....), even if the climate is not a real protection to competition.

- Trees in the wild which can abundantly be found in developing countries (cinnamon, camphor, sandalwood....)
- Wild plants which could be easily cultivated in industrialized countries, but for which the wild harvesting remains more profitable than the cultivation (Artemisia sp., rosemary....).
- Crops for which the cultivation and harvest is more profitable in developing countries (jasmine, tuberose, basil, Mentha arvensis...).

Essential oils dominated by industrialized countries :

- Species sensitive to environmental factors, better adapted to temperate climates and which are rather rare (peppermint, lavandin and few herbaceous essential oils).
- Plants benefiting from competitive production cost, when large scale intensive cultivation and full mechanization is more competitive than low labor cost.
- Spontaneous trees for which the harvest is mechanized (coniferus sp.).
- Small crops benefiting from the support of the perfumery and aroma industry, looking for reliable supply, new crops supported by high investment in research.

Essential oils for which the competition between the developing countries and industrialized countries is fierce, with an uncertain outcome :

- Large tree plantations for which essential oil is considered as a by-product (orange, lemon).
- Crops for which wild harvesting and intensive mechanization are able to provide similar economic results (eucalyptus).
- Cultivation in both systems, obtained at a lower price from developing countries but with a better quality and more regular supply in industrialized countries.

Marketing strategies

Production of existing essential oils

Some countries were able to adopt a policy of mass production, aiming to establish a dominant position on the world market, accepting the lowest possible price to enter the market. If this strategy is not based on real production cost, it is difficult to be successful in the long term, producers will be discouraged by the too low price offered for the production. More promising are production programmes based on local advantage (climate, competitive labor cost..), with the help of traders and industrial users in order to ensure a secure supply and homogeneous quality. Success tends to require the existence of local investors, who can organize the production, provide the capital for equipment, and act as reliable partners for the customers abroad.

A new producer in developing country has to take into account the market situation, in order to conclude if the development of an essential oil industry could be a viable commercial opportunity. B. Lawrence (1994) suggests a list of questions that each producer intending to market existing essential oils should consider:

- How many tonnes of oil are available annually ?
- Is there a geographic origin or environmental influence on the oil composition and yield ?
- Will the price of the oil be stable for the next 12 months ?
- Are socio-economic conditions stable in the oil producing area ?
- What new uses can be found for this oil ?

Answers to these question, involving an in depth analysis of the supply and demand trends based at least on the past 10 years, must be done before any development programme for existing essential oils.

Production of new essential oils

A recent paper, D. Joulain (1995) has clearly identify the interest and limitation for the investigation in new essential oils. We have already mentioned the possible new uses for essential oils, particularly regarding the biocide properties. Consumers are becoming more demanding for plant-derived ingredients which are considered environmentally friendly because of their biodegradability and renewability. For this reason, research on new essential oils, or new uses for existing essential oils is still a very promising area. However, tempting the introduction of new species for developing countries, there are many difficulties to introduce successfully a new essential oil on the market. It is common that a sample could catch the attention of a perfumer, flavorist, but if it is a new product, this interest can be destroyed by an insufficient yield and a limited availability of the plant material. The manufacturer who would like to include this new essential oil in one of his formulations will not have any guarantee that the quantities available will fit the growing demand.

We have already mentioned the strict regulatory requirements for the introduction of new oils, which is a real barrier to the development and utiliszation of new oils. In all cases, a very close relationship with the industry is needed.

Production of essential oils for natural isolates

This is a much more easy way for developing countries to find new markets. There is a increasing need in the flavor industry for natural isolates that could be substitutes for chemicals. There are not in this case any barriers from the regulation as far as these molecules are already used in the flavor industry or in the cosmetic industry. There is a lack of natural source for several fragrances of flavors, and some molecules could be profitably extracted from essential oils even if they are present in small quantities. An investigation of the native species in developing countries could lead to potential new markets, but only with the support of a clear economic analysis of the production cost and the price of the existing source.

CONCLUSIONS

The essential oils producers must take up the challenge posed by the changes taking place in the industrial sector, and manage those relationships, both competitive and complementary which link essential oils to their substitutes (from chemical synthesis and biotechnology). At the same time, the producer may benefit from the reviving interest for natural flavors, search for new active ingredients, but has to face with the global decrease in the mean price of essential oils and from a strong competition from developing and industrialized countries.

For a new producer, the success will depend on its level of competitiveness in relation to competing countries already well established in the market. Therefore, a clear analysis of the production and the market potential has to be established, including a global view of the long term trends and the factors influencing the price formation.

Stable relations with buyers and industry, local "entrepreneurs ", growers motivated in relation to technical innovation, well-supported research and assistance from the development organizations are also necessary for success.

BIBLIOGRAPHY AND REFERENCES

1. Calabre, S., (1990a). *L'évolution des prix des produits de base.* Economica, Paris.

2. Calabre, S., (1990b). *Les marchés internationaux des matières premières.* Economica, Paris.

3. Calame, J.P., (1994). *Trends in perfumery. 4th International Meeting on medicinal and aromatic plants,* edited by Verlet, N., Nyons.

4. Caves, R., Jones, R., (1981). *Economie internationale : le commerce.* Armand colin, Paris.

5. Chalmin, P., (1985). *Négociants et chargeurs : la saga du négoce international des matières* 6 . International Trade Centre (1986) *Market for selected oils ans oleoresins.* UNCTAD GATT Genève.

6. Joulain, D., (1994). *Successes and failure of essential oils, an historical review, 4th International Meeting on medicinal and aromatic plants*, edited by N. Verlet. Nyons

7. Joulain, D., (1995). *Investigating new essential oils : rationale, results and limitations. XIII ICEOFF Congress,* edited by H. Can Baser, Istanbul.

8. Landing, J.E., (1989). *American essence : a history of the peppermint and spearmint industry in the United States.* A.M. Tood Foundation, Kalamazoo, Michigan.

9. Lawrence, B., (1984). *A review of the world production of essential oils.* Perfumer & Flavorist 3: 21-33.

10. Madzat, J., (1980). *The peppermint Industry : situation and outlook with emphasis on Oregon.* Oregon State University.

11. Peyron, (1988). *Techniques classiques actuelles de fabrication des matières premières naturelles. aromatiques.* Compte rendu du séminaire de formation CPCIA/ENSIA "Les arômes alimentaires.

12. Peyron, L., (1989). *Un siècle de production de plantes à parfum dans le Sud-Est de la France.* Parfums, Cosmétiques, Arômes, n°89.

13. Pindyck, R., Rubinfeld, D., (1981). *Econometric models and economic forecasts.* Mc Graw Hill, New York.

14. Rozat, J.P., Näf, F., (1995). *General ideas about the flavor and fragrance industry with regards to the use of essential oils and aroma chemicals.* XIII ICEOFF Congress, edited by H. Can Baser, Istanbul.

15. Verlet, N., (1991). *La production et le marché mondial des huiles essentielles. 10èmes journées nternationales des huiles essentielles.* Digne-les-Bains.

16. Verlet, N., (1992). *The essential oils economy.* World Congress of Medicinal and Aromatic Plants and Human Welfare. Maastricht.

17. Verlet, N., (1992). *Facteurs intervenant dans la formation des prix des huiles essentielles.* 11èmes journées internationales des huiles essentielles. Digne-les-Bains.

18. Verlet, N., (1992). *Compte rendu des Troisièmes Rencontres Techniques et Economiques plantes aromatiques et médicinales.* Nyons.

19. Verlet, N., (1993). *Commercial aspect of essential oils. Volatile oils crops*, Waterman & Hay Editors, Longman, London.

20. Verlet, N., (1994). *Les huiles essentielles françaises d'Outre-mer.* Congrès de l'IFEAT, Avignon.

21. Verlet, N., (1994). *Le jasmin de Grasse : un exemple de modélisation du marché des huiles essentielles.* 13èmes Journées internationales des huiles essentielles. Dignes les Bains.