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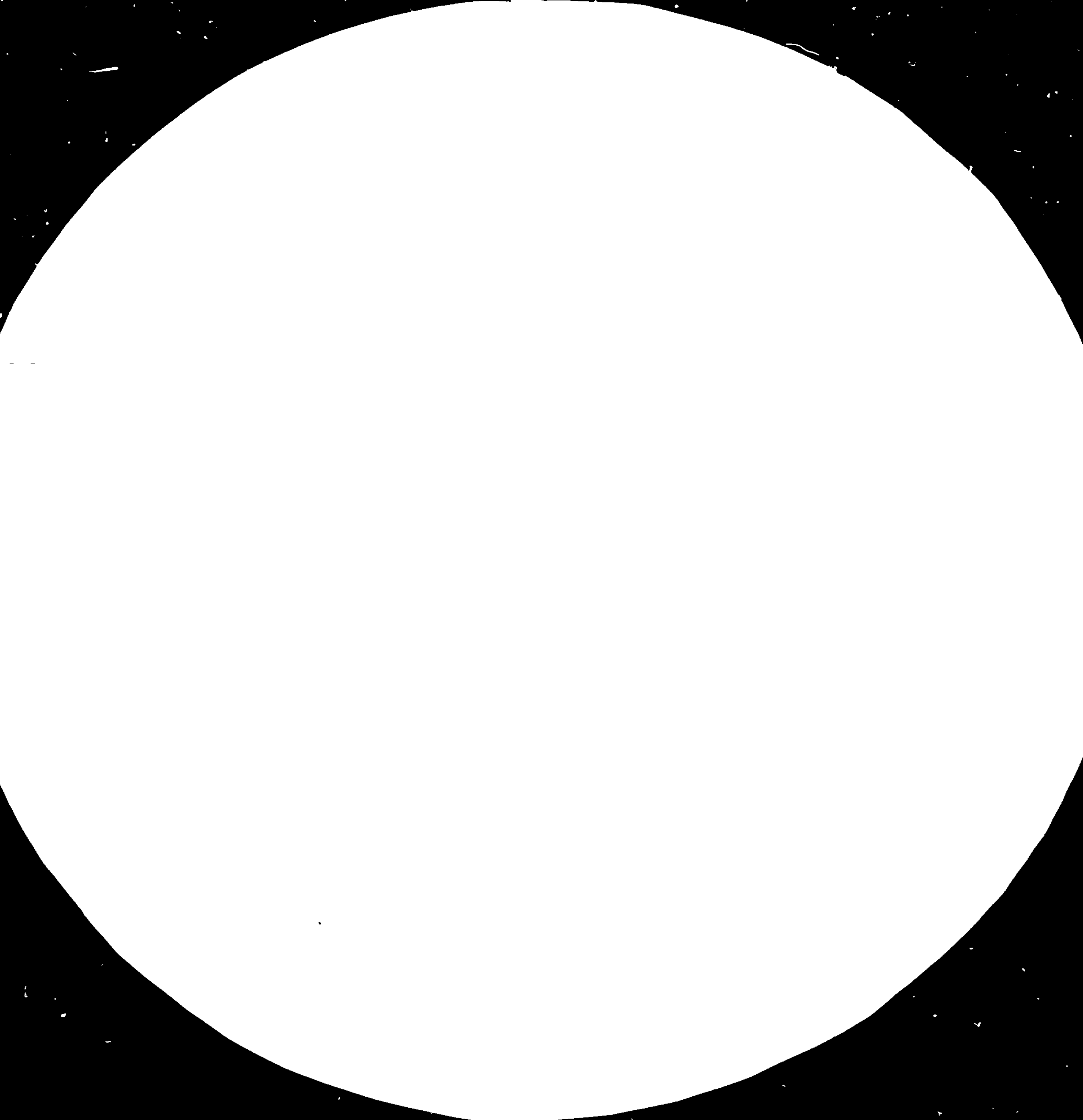

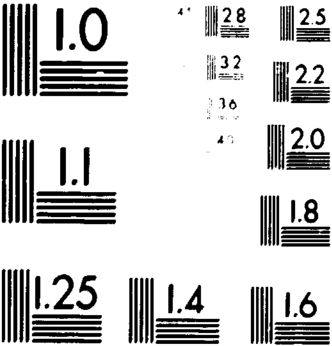

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LIMITED

ID/WG.431/8
1 April 1985

ENGLISH

United Nations Industrial Development Organization

Symposium on the Importance of Lactic Acid Fermentation
in the Food Industry

Mexico City, Mexico, 27 - 29 November 1984

ADVANCES ON THE APPLICATION OF LACTIC ACID FERMENTATION IN THE FOOD INDUSTRY*

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I. BACKGROUND

The food industry requires various starter cultures of lactic acid bacteria for the preservation and processing of products of animal and plant origin. Starter cultures of higher activity and of better quality should promote the manufacture of food of improved quality.

1. Lactic acid bacteria in the dairy industry. The fermentative activity of microbes is extensively utilized in the dairy industry where different starter cultures are used in the production of cheese, butter, sour-milk products, cottage cheese, etc.

2. Application of acid forming microbes in the meat industry. The resulting pH decrease inhibits the propagation of non-desirable bacteria and shortens the drying.

3. Application of lactic acid fermentation for vegetable preservation. The technique is safer on large scale and the quality of the final product could be standardized.

4. Acid flavour and aroma in bread produced by lactic acid bacteria. CITOPAN improves bread quality with another additive which improves leavening - with all the positive effects on product quality.

5. The role of lactic acid bacteria in alcohol containing beverages has been regarded undesirable. Nowadays the bacterial conversion of malic acid to lactic acid and carbondioxide in wine is a natural reduction of acidity and provides an improvement in sensory quality.

II. INTRODUCTION

The fermentative activity of microbes was recognized and utilized alrady in the Neolithic times without having the faintest idea about their existence. Milk was probably one of the first agricultural products and cheese may have been the first fermented foodstuff. By utilizing the spontaneous fermentation procedure various fermented dairy products have been produced around the world.

III. LACTIC ACID BACTERIA IN THE DAIRY INDUSTRY

In the dairy industrial technology different starter cultures of one or more microbe species are used for the production of cheese, butter, sour-milk products, cottage cheese, etc. Starter cultures added to the pasteurized milk or milk products during production basically provide the taste, the consistency and several other characteristics of the milk products. Figure 1 shows the components of starter cultures used in the dairy industry. Starter cultures are significant in controlled fermentation and also in developing flavour and aroma. As the dairy industrial fermentation technology is very thoroughly elaborated and as the basic processing steps are more or less the same in different countries, I would only mention a special, typical Hungarian product: sour cream. The sour milk products and their main characteristics are shown in Figure 2. Sour cream is made by further diluting pasteurized cream of 40% fat content and by souring with lactic acid bacteria.

While most of the fermented dairy products are consumed in natural state, yogurt is often flavoured with fruit or fruit essences. Some exotic dairy products have to be mentioned also such as the Bulgarian milk, kefir and koumis which are popular in Slavic countries, and vilia, which is popular in Finland.

Products of constant quality and of the same quality characteristics can only be produced with starter cultures which are constituted of strain components of known properties thus being suitable to control the production technology and the quality of the products. The cultures applied in the dairy industry may be produced and used in liquid and lyophilic forms. The advantage of the latter is that it can be preserved for a longer time and may be used for bulk culture production and it can also be added directly to the product. All cultures used in the dairy industry are prepared by the Hungarian Dairy Experimental Institute and all plants are supplied centrally. Currently only lyophilized cultures are supplied. The development and the utilization of culture production tends towards concentrated and preserved starter cultures. /BALATONI AND KETTING, 1981/.

IV. APPLICATION OF ACID FORMING MICROBES IN THE MEAT INDUSTRY

The application of microbiological processes in the meat industry is comparatively new and is limited to the starter cultures used for the production of matured meat products. The favourable characteristics of useful bacteria have been utilized for ripening for a long time - let us think here first of all of the different pickling procedures - environmental conditions are experienced which flavour the propagation and metabolism of useful bacteria. During nitrate pickling, the nitrate partly disintegrates into nitrite as a result of the activity of nitrate disintegrating bacteria, while the colour of the product is developed and stabilized. Here the Micrococcus species play the main role. During quick nitrite pickling the microflora participates in the formation of the pickled product flavour. Pickling develops colour, flavour and friability, preserves during and after ripening. The microflora in the pickle also has an aromatic effect. The microflora in pickles consists not only of useful nitrate disintegrating bacteria but also of several other species. These may get into the fresh pickle from the constituents of the pickle or from the environment or from the old pickle by inoculation. The propagation of the microbes starts when meat is put into the pickle; the salt concentration and the temperature of the pickle determine the type of the microorganisms that we propagated.

As the development of the microflora in the pickle the moulding of salami is more or less a spontaneous procedure. Although, the actual role of the white or slightly gray moulding of salami is not fully known, nevertheless, these moulds are to be considered useful microbes. The nice skin is inseparable from the Hungarian salami, and besides the appearance moulds effect the aroma and the water economy, and the rancidity is inhibited by the non-transparent property. Thus, the development of the salami's mould is an important and organic part of the technology of salami manufacturing. Several species take part in the moulding; the consistency of the settling species is determined by the mould infection of the plant atmosphere, while that of the dominant species is influenced by the technological intervention /temperature, humidity,etc./. Moulding is a spontaneous procedure, the synthetic way of moulding by inoculation was not successful neither in Hungary nor abroad. /LŐRINCZ AND LENCSEPETI, 1973/.

Useful microbes as starter cultures have been applied in the meat industry for 30 years, both in Europe and in the United States. Starter cultures in meat products may have the following roles: acid production, nitrate-nitrite disintegration and aroma development. Among these, perhaps the acid production is the most important. The pH decrease inhibits the propagation of the non-desirable bacteria, thus the production becomes technologically more reliable and the product hygienically more safe. The pH decrease increases the water evaporation of the product and so the drying process shortens resulting an economic benefit; the colour stability and consistency are better and the acids take part in the development of the product's characteristic flavour. The nitrate-nitrite disintegrating activity - as mentioned earlier is important for developing and stabilizing the colour and also for decreasing the remaining nitrite-nitrate content. This latter received special importance due to the nitrozamine problem. Aroma is developed not only by the already mentioned acids but also by different carbonil compounds, free fatty acids which come into being in the course of microbe activity. Two types of microbes - acid producing and nitrate-nitrite disintegrating - are used as starter cultures. Acid producing microbes are lacto-bacilli and streptococci /most often Lb. plantarum, str. diacetilactic, Pediococcus cerevisiae/; the nitrate-nitrite disintegrating microbes are cocci /Micrococcus, apathogen Staphylococcus/.

With acid producing cultures products of quick ripening and with nitrate-nitrite disintegrating cultures that of long ripening time may be produced. The mixed cultures /lactobacilli and micrococci/ are suitable for producing products of medium ripening time.

Adding starter cultures to meat products the production becomes not only technologically more reliable and the product hygienically more safe but the application of starter cultures implies also an economic advantage. The new technology saves meat; the work time decreases, the necessary air conditioning plant cuts down the cost of energy; the ripening time of the product is shortened resulting significant capacity growth.

V. APPLICATION OF LACTIC ACID FERMENTATION FOR VEGETABLE PRESERVATION

Fermentation is an ancient food preservational procedure and became a large scale method in our decade. Figure 3. During the fermentation of products of plant origin, the lactic acid fermentation is done by different type of microorganisms forming a population of varied components. Due to the varied and varying character of this natural microflora the lactic acid fermentation is less regulated and does not ensure a constant quality to the final product. Fermentation processes may only be controlled by establishing the suitable environmental factors. Starter cultures eliminate difficulties occurring during spontaneous lactic acid fermentation. The most important products of pickling industry are the sauerkraut, the pickled and leavened cucumber, paprika, tomato and other combined products. The technique of microbiological fermentation protects manyfoldedly against the propagation of microbes that cause deterioration. The indispensable salting when pickling /2-10% NaCl concentration/, is protective itself and at the same time ensures suitable surrounding for the propagation of lactic acid bacteria. These microorganisms are propagated using the sugar content of the medium, acid is formed /pH decreases/, thus these factors increase the stability one by one and together, and the danger of deterioration decreases. Bacteria participating in the acidification and causing the lactic acid fermentation may be listed into three main groups: 1. gas-forming cocci

/e.g. Leuconostoc mesenteroides/, 2. rods not forming gas /e.g. Lactobacillus plantarum/, 3. gas-forming rods /e.g. Lactobacillus pentaaceticus/. Generally pure cultures are applied as the mixed cultures are difficult to handle. In technologies applied presently, gas formation and foaming is eliminated that the propagation of acid-sensitive gas forming bacteria is restrained starting from a given acid concentration.

The quality of the fermented pickles is better than that of the heat treated ones. Applying starter cultures and following the technological parameters, the pickling technique is saferand simpleron large scale level and the quality of the final product is good and steady. /JUHÁSZ-ROMÁN et al,1974/

The various Eastern fermented foods produced from different basic materials of plant origin adding moulds /Rhizopus, sp., Aspergillus oryzae, Monascus purpureus, Mucar sp./ yeasts /Saccharomyces rouxii, Torulopsis sp./ or bacteria /e.g. Pediococcus soyae/ have to be also mentioned.

VI. ACID FLAVOUR AND AROMA IN BREAD PRODUCED BY LACTIC ACID BACTERIA

Bread is our other very important nourishment. The first bread was most probably baked in Egypt, around at the beginning of the ancient history. The first breads were flat and were made without leaven. This type is still made in many parts of the world. Bread is traditionally made world wide with baker's yeast - the suitable strain of Saccharomyces cerevisiae. Figure 4. In Western countries mainly in the

USA bacteria species were used earlier for producing different baking industrial products; in Hungary bread was home made for a long time. Home made bread kept its excellent flavour and arome for some days, it became neither dry nor sticky. When bread baking was industrialized the production time accelerated and the volume increased. Machine technology does not always allow the microbiological changes to take place or to be controlled in the dough. The industrially produced bread usually does not reach the desired level of quality. The quality was greatly improved by CITOPAN, introduced in several plants which makes dough of better quality with additives. CITOPAN is actually a dried leaven active with a souring agent. The essence of another newly introduced and patented procedure is that leaven ensures the propagation of lactic acid bacteria which are responsible for acid production and for the creation of the flavour and aroma constitutents. This is the so-called "thin leavening" procedure which in the technological procedure separates the lactic acid fermentation /leaven making/ producting the acid, flavour and aroma constituents and the yeast leavening of the dough. Lactic acid bacteria are propagated in a separate fermentation step, thus creating favourable leavening conditions. Therefore, fermentation may be optimized, from the point of view of flavour, aroma and acid production. Fermentation may be fully optimized in large scale production. A strain of Lactobacillus brevis is applied in the fermentation.

The new method is comparatively cheap, with smaller alterations it may be used in small and in large scale methods; the procedure significantly improves the quantity and the quality of the products and is at the same time energy and wage saving.

VII. THE ROLE OF LACTIC ACID BACTERIA IN ALCOHOL CONTAINING BEVERAGES

A well-known fermentation procedure is the production of beverages of different alcoholic content, for example from cereals. Archaeological evidence shows that the manufacture of beer was already an advanced art more than 6000 years ago. Figure 5. Here, I would only mention that basically two types of beer-fermentation procedures are applied. During the fermentation of lager-type beers the yeast settles down to the bottom of the fermentation tank /bottom fermentation/. The bottom fermentation yeast was first mentioned in the last decade and named Saccharomyces carlsbergensis. In Britain and in parts of Europe and North America the yeast employed in brewing beer rises to the surface during fermentation /top fermentation/. Top fermentation yeasts are classified as strains of Saccharomyces cerevisiae. Taxonomists consider the two types of yeasts as different strains of the same species, although their traditional name is further used in the brewing industry. /ROSE,1981/

The technology of wine making is much simpler and has hardly changed during the past 5000 years. Figure 6. Until recently the grape juice was allowed to ferment spontaneously by way of microorganisms present on the surface of the grapes. In the later stage of fermentation S.cerevisiae becomes the most important, as the alcohol released from sugar kills the other yeast species. Today specially selected cultures of S.cerevisiae are added to the grape juice./EDELÉNYI,1978/

When speaking of wines, I must say some words of the special Tokay-wine and the so-called Aszu. In the manufacturing, beyond normal fermentation, microbes are also of vital importance. The noble rot of grapes in case of suitable temperature is caused by Botrytis cinerea. The so-called noble rot results in significant water loss, the mycelium weaves through the grape. The metabolism of B.cinerea modifies the increased concentration of the other constituents of grapes, therefore, the vintages and dried noble rotted grapes have different composition than grapes shriveled without moulds. Thus, sugar content and the titrable acid content decreases, while citric acid and gluconic acid content increases. Gluconic acid is characteristic also to wine made of noble rotted grapes as also the higher glycerine content, the grater rate of higher alcohols and the lower amino acid content. The piroracemic acid and 2-keto-glutaric acid content of Sauternes and related wines are higher, the tannic acid and leukoantocianin content is lower than in other wines. The flavour and the aroma of Tokay wines are partly developed by moulds /Cladosporium cellare/ which cover the walls of Tokay wine cellars.

Wines made of grapes with noble rot were always very valuable and popular, several attempts were made for imitation. Neither in vitro, nor in vivo did artificial noble rot meet expectation.

When red wines are manufactured, the natural microorganisms are virtually killed as a result of heat treatment and the developing or the added yeast flora differs from the original one and Saccharomyces species will be dominating.

During the production of the Hungarian red wines the biological malic acid conversion takes an important place. The red wine can be considered as biologically safe, if no sugar or maleic acid occur in it. The maleic acid following the sugars is the second main target of the microorganisms - namely that of the lactic acid bacteria. The maleic acid conversion taking place spontaneously can be either a favourable process resulting in a red wine of very good quality, or may result undesirable changes.

Recently, the control of the biological malolactic fermentation is one of the main concern of the researchers.

The lactic acid bacteria occurring in the wines are shown in <u>Figure 7</u>.

The occurrence of the favourable, homofermentative types is rather low. Out of the homofermentative ones bacteria are the most important which do not attack the tartaric acid and glicerol. These are rather common and they might be dangerous if sugar is present in the wine and the pH is high. The optimal temperature of the malolactic fermentation is 20-25 ^{o}C. Mild aeration might be advantageous, however the oxygen is not the most important factor in the fermentation. The pH seems to be the most important parameter, it is selective to the lactic acid bacteria being present in the wine. The malolactic fermentation starts at pH 3-4, the higher the pH, the faster the process is. Among the bacteria species inducing the favourable biological maleic acid conversion the most frequent is the Leuconostoc oencs, which gives a pleasant flavour to the wine. The species of Lactobacillus and Pediococcus genus cause the spoilage of wines. These species, first of all the Pediococci are sensitive to the pH, and do not multiplicate under pH 3.5. So, as a result of the alcoholic fermentation, the low pH /3.2-3.3 / of the Hungarian wines is favourable for the Leuconostoc. During the malolactic fermentation the pH raises and at a critical level of pH 3.6, the multiplication of Pediococci might start, but having been the Leuconostoc flora already predominant by this time, it generally inhibits the powerful multiplication of the concurrent species. This is the situation with the spontaneous malolactic fermentation. /MÓDOS,1982/

The aim is, that the process should be controllable. The most proper way to do so is the elaboration and application of a suitable starter culture. Research work aiming this has already started in Hungary, however, it is still at the beginning. In some other countries, Leuconostoc starter cultures improving the biological stability of the red wines by the control of the maleic acid conversion have already been on sale.

VIII. CONCLUSIONS

As a summary we may conclude that great part of the products are produced by traditional fermentation procedure. The presently applied technologies have been created experientally during the centuries. Their mutual characteristic is that fermentation was first carried out by a so-called spontaneous microflora and as both the useful and harmful microorganisms are present, the control of the procedure is difficult or not possible at all. Modern food production can only be carried out on large scale level if the procedure - the fermentation is controllable. This is only possible if favourable microbes of known characteristics perform the fermentation . Figure 8.

The recognition that fermented foods have generally higher nutritive value than raw foods has greatly increased the demand towards foods produced by fermentation and the application of starter cultures in certain branches of the industry became general.

Following are the most important advantages of starter cultures:

- more safe and controllable technology,
- increased technology,
- decrease of wastes,
- raw material and work power saving,
- higher biological and nutritive value,
- increased range of the variety of final products.

The requirements of the industry towards starter cultures are rapidly growing. The properties of the available starter cultures are to be improved; starter cultures of higher activity, of better quality should be circulated that promote the manufacture of newer products of improved quality. Therefore, physiological, biochemical and genetical characteristics of starter cultures are to be thoroughly studied and methods aiming at the improvement of properties are to be elaborated.

Figure 1

Starter cultures used in the dairy industry
according to SÁNTA-TURZA,R. /1983/

Type of culture	Components of culture	Taxonomical family	genus
Butter culture	Str. lactis Str. cremoris Str. diacetilactis Leuc.dextranicum	Streptococcaceae Streptococcaceae Streptococcaceae Streptococcaceae	Streptococcus Streptococcus Streptococcus Leuconostoc
Cheese culture	Lb. helveticus	Lactobacillaceae	Lactobacillus
Cheese culture	Lb. casei	Lactobacillaceae	Lactobacillus
Type of culture	Component of culture	Taxonomical family	genus
Cheese culture	Str. thermophilus Lb. helveticus Lb. casei	Streptococcaceae Lactobacillaceae Lactobacillaceae	Streptococcus Lactobacillus Lactobacillus
Cheese culture MK	Str. thermophilus Lb. lactis	Streptococcaceae Lactobacillaceae	Streptococcus Lactobacillus
Yogurt culture	Str. thermophilus Lb. bulgaricus	Streptococcaceae Lactobacillaceae	Streptococcus Lactobacillus
Kefir culture	Str. lactis Str. cremoris Lb. casei Lb. caucasicus Candida pseudotropicalis Kefir bacilus	Streptococcaceae Streptococcaceae Lactobacillaceae Lactobacillaceae Saccharomycetaceae	Streptococcus Streptococcus Lactobacillus Lactobacillus Lactobacillus
Acidophilus culture	Lb. acidophilus	Lactobacillaceae	Lactobacillus
Acidophilus culture taette - with Streptococcus	Lb. acidophilus Str. lactis var. taette	Lactobacillaceae Streptococcacoae	Lactobacillus Streptococcus
Rouge culture	Brevi bacterium linens	Actinomycetes	Arthrobacter
Mould culture	Penicillium roqueforti Penicillium caseicolum	Hyphophialoconidiaceae Hyphophialoconidiaceae	Penicillium Penicillium
Propionic acid culture	Propionibacterium freudenreichii subsp. shermanii	Actynomycetes	Propionibacterium

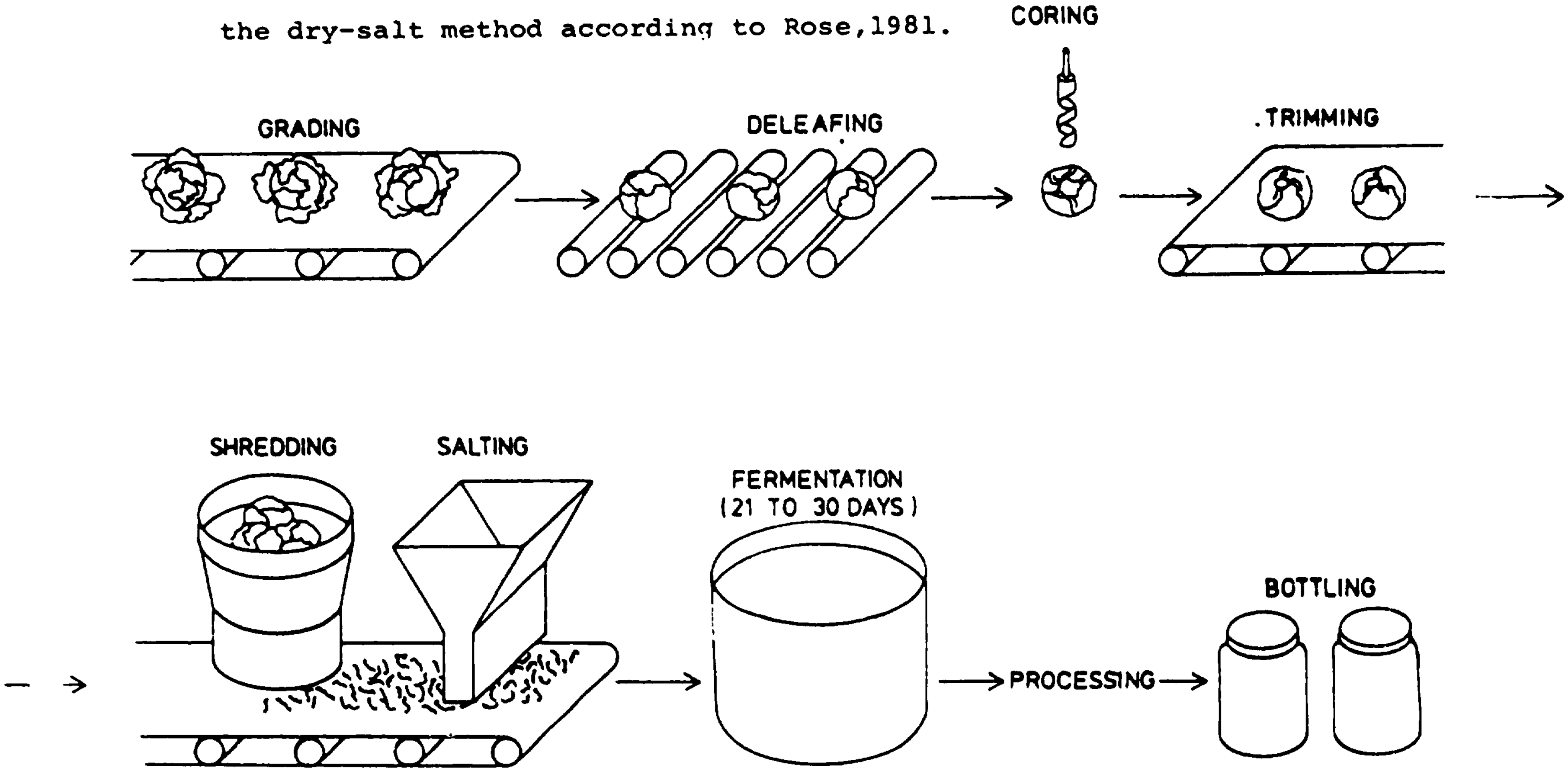
Figure 2

The main characteristics of sour-milk products according to SÁNTA-TURZA,R. /1983/

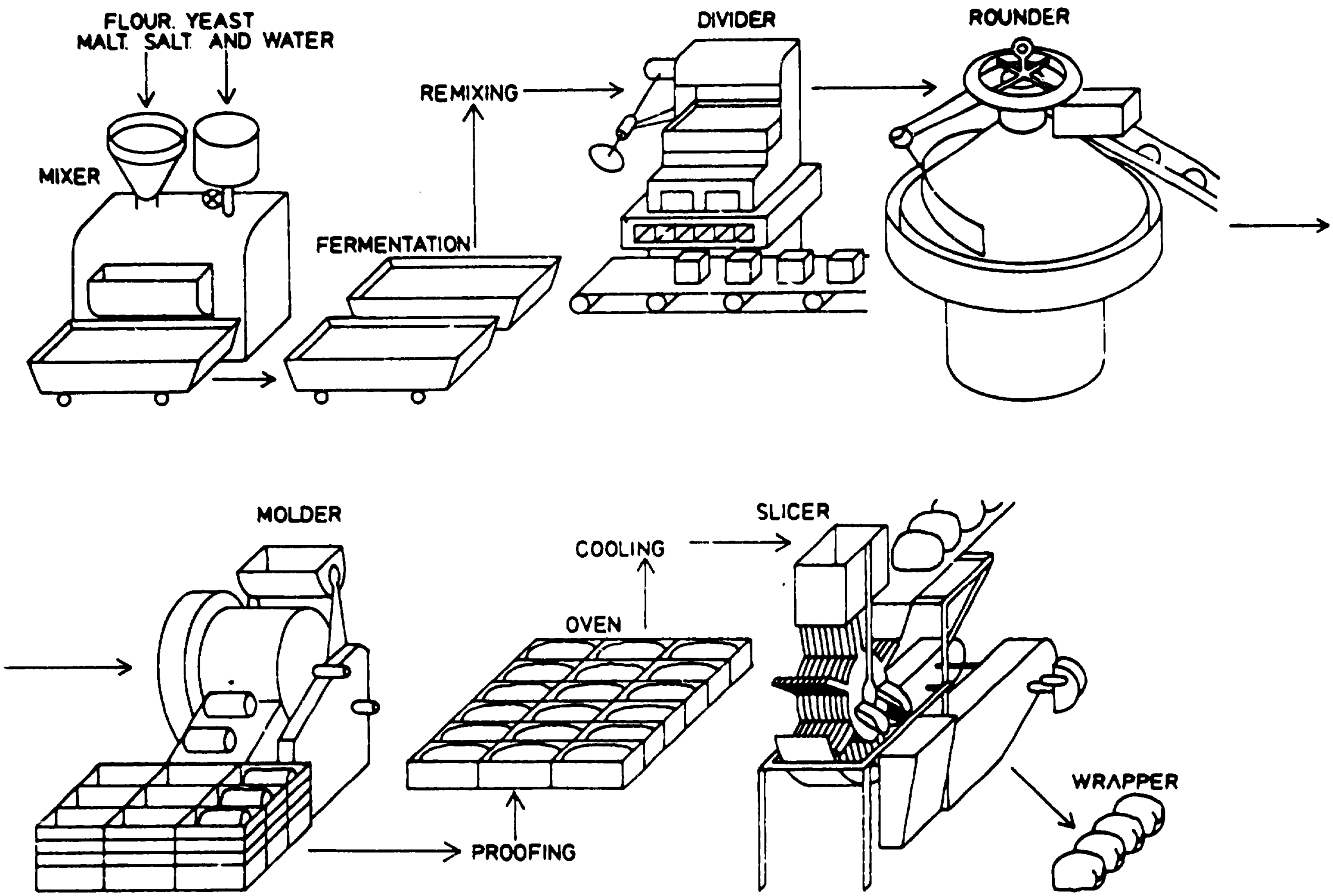
Specification of the product	Applied microbe culture	Main components forming lactic acid flavour
Yogurt and yogurt-type products	Yogurt	Lactic acid, acetaldehyde
Kefir	Kefir culture	Lactic acid, carbonic acid, CO_2, ethyl alcohol
Sour cream	Butter culture	Lactic acid, acetoin, diacetil
Acidophilus milk	Acidophilus culture	Lactic acid
Bioghurt	Acidophilus and Streptococcus	Lactic acid
Coagulated milk	Butter culture	Lactic acid, acetoin, diacetil

Figure 3

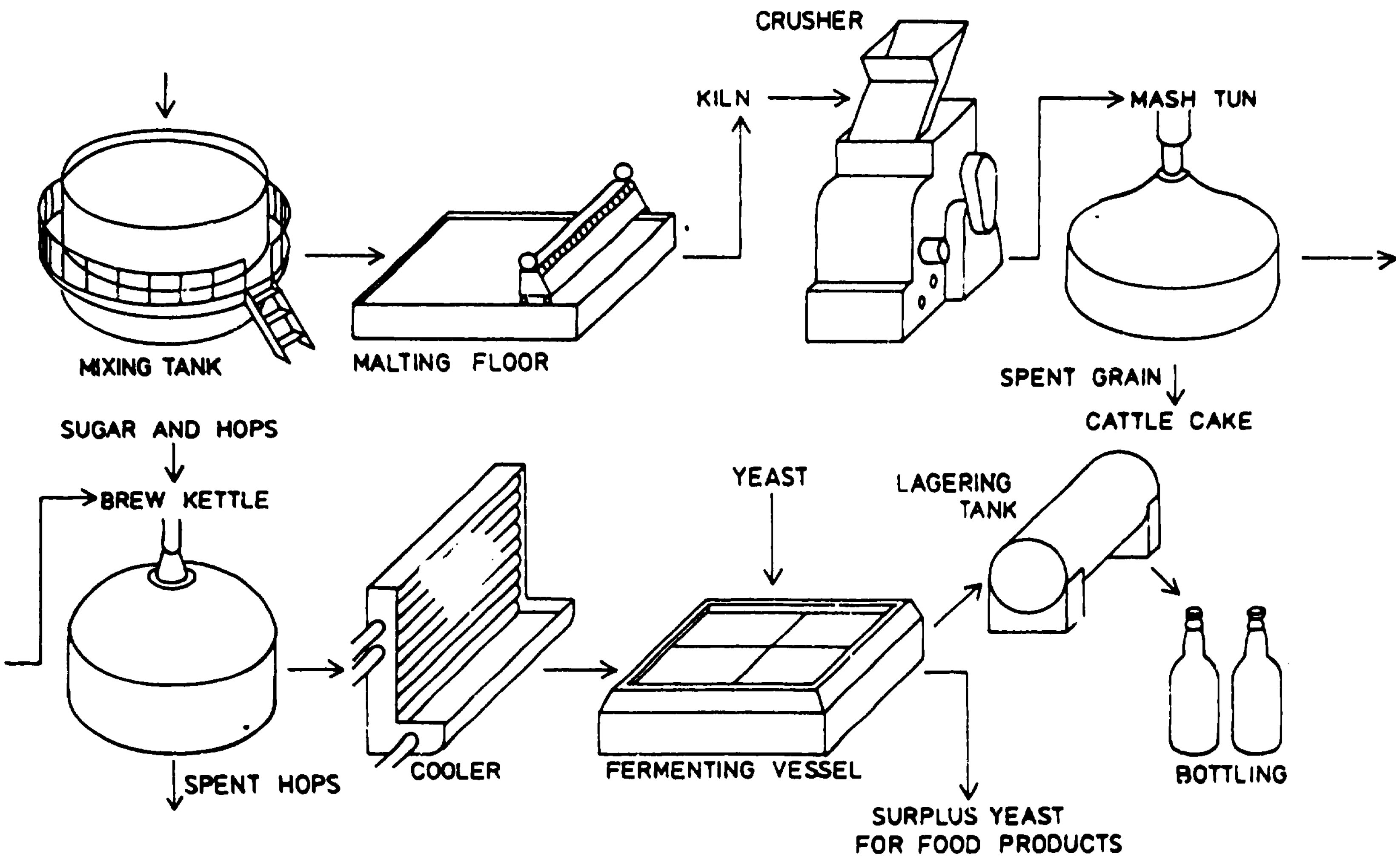
Flow chart of the preservation of cabbageas sauerkraut by the dry-salt method according to Rose,1981.



Flow chart of bread manufacturing according to Rose, 1981. Figure 4



Flow chart of beer production according to Rose,1981. Figure 5



Flow chart of generalized process in wine making according to Rose, 1981.

Figure 6

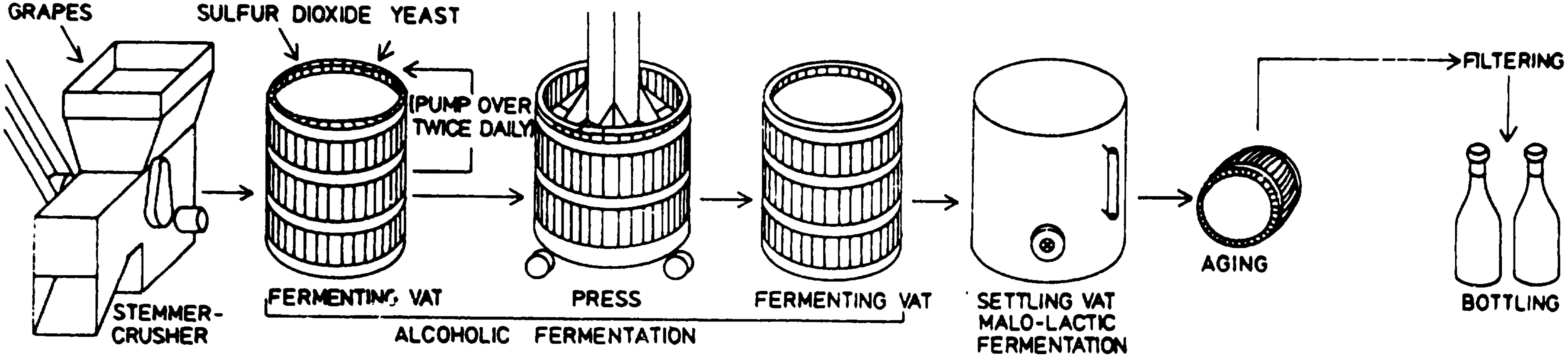


Figure 7

Lactic acid bacteria in wines /Módos,1982/

Cocci

homofermentative:	Pediococcus sp.
heterofermentative:	Leuconostoc gracile ovinos

Bacilli:

homofermentative:	Lactobacillus casei plantarum
heterofermentative:	Lactobacillus hilgard brevis

Figure 3

Production of some fermented foods in Hungary
/on industrial scale/ /Sánta-Turza,R.,1983/

Product		1970	1980	1982
Salami	/1000 t/	3.3	4.8	4.4
Cheese	"	13.3	25.8	31.0
Butter	"	19.4	19.0	21.4
Bread	"	878.5	835.3	794.7
Rolls	/million pieces/	1728.0	2501.1	2593.7
Beer	/million l/	611.5	913.5	934.4
Wine	"	198.9	232.6	210.8
Brandy	"	2.6	4.3	3.4

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