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Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities

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Abstract: Lactic acid bacteria (LAB) are the most important bacteria used in food fermentations. Apart from general demands for starter cultures from the view of safety, technological effectiveness and economics, numerous specific aspects have to be considered when selecting strains for the different food fermentations. Therefore selection criteria for LAB depend on the type and the desired characteristics of the final product, the desired metabolic activities, the characteristics of the raw materials and the applied technology. Special selection criteria for LAB for use in the fermentation of sausages and vegetables as well as for the malolactic fermentation in wine are discussed.

Key words: Starter cultures; Selection criteria; Fermented sausage; Fermented vegetables; Malolactic fermentation

Introduction

Fermented foods

Fermented foods are defined as palatable products which are prepared from raw or heated raw materials and which acquire their characteristic properties by a process in which microorganisms are involved. In certain cases the endogenous enzymes of the raw material play a decisive role [1].

The origin of fermented foods seems to be from the Orient and dates back to prehistoric times. In the beginning, fermentation processes, e.g. alcoholic, acetic acid and lactic acid fermenta-

tion, were mainly used to preserve foods of animal and plant origin. As a result of the development of efficient heat sterilizing and refrigeration systems, fermentation processes lost their importance as preservation methods in the industrialized countries.

However, fermentations are more than preservation methods. Through the ages, people learned more and more to control these processes and fermented foods became an independent class of foodstuffs. In Germany, approximately 25% of the consumed victuals are fermented products [2]. As regards taste, aroma, visual appearance, texture, consistency, shelf life and safety, these different products possess characteristic properties compared to the raw materials or to other similar foods. The acidification of minced meat during the production of dry sausages, for instance, can be attained by the addition of glu-

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cono-delta-lactone or by fermentation. In both cases shelf life, safety and sliceability of the sausage will be achieved, but the original taste and aroma can only be obtained by fermentation.

In general, all classes of microorganisms are used for food fermentations, but in Europe bacteria and yeasts are more common than moulds. Among the bacteria, the lactic acid bacteria (LAB) are the most important. A compilation of those foods which are produced by a process involving LAB is given in Table 1. It can be seen that some processes are solely lactic acid fermentations, whilst others are combined fermentations.

Starter cultures: from ancient to modern times

Natural fermentations are spontaneous processes caused by microorganisms derived from the raw materials or the environment. Formerly, people were ignorant concerning the nature of the expiring processes. But already in the earliest times, artisanal and religious practices were established which were very similar to the starter cultures applied today.

In the 1st century A.D., Plinius the Elder described the preservation of white cabbage in special earthen vessels, which were used only for

Table 1

Fermented foods of the European market which are produced by a process involving lactic acid fermentation

Foodstuff	Raw material	Microorganisms used besides LAB ^a			
		BAC	YEA	MOU	STA
Sauerkraut	White cabbage				+
Various vegetables	Various vegetables				+
Olives	Green and black				+
Vegetable juices	Various vegetables				++
Soy products (yoghurt- or cheese-like)	Soy protein				+++
Soy sauce	Soybeans		×	×	
Sour dough	Wheaten flour rye flour		×		++
Kwass	Malt, bread		×		
Cacao		ACB ^b	×		
Coffee			×		
Wine	Grapes		×		+++
Beer (sour wort/ Berliner Weisse)	Malt		×		+++
Dry sausage	Meat from various animals	MIC ^c			^d
Curdled milk	Milk				+++
Yoghurt	Milk				+++
Butter	Cream				+++
Cheese	Milk	PRO ^c			+++
Mouldy cheese	Milk			×	+++

^a BAC, bacteria; YEA, yeasts; MOU, moulds; STA, use of starter cultures. +, rarely; ++, often; +++, always.

^b Acetic acid bacteria.

^c Micrococci and staphylococci.

^d Depends to the country of production.

^e Propionibacteria.

this purpose. To date, we are certain that under the conditions described by Plinius, the cabbage was fermented to sauerkraut by microorganisms, which were located in the pores of the vessels and which proceeded from a former fermentation.

Even in these days another primitive form of starter application can be observed during preparation of several fermented beverages in some parts of Africa. During a religious ceremony preceding the fermentation, the medicine man dips cult objects into the liquid, which again contain microorganisms from a previous batch.

Since the time of Pasteur it has been known that the natural principle of fermentation depends on a multitude of different microorganisms. On the basis of this knowledge the idea was born to search for microorganisms especially suitable for particular food fermentations. Christian Hansen was the first to isolate and propagate special yeasts to be used in breweries. The first starter culture was born.

From a modern point of view, starter cultures are defined as preparations which contain living

microorganisms which are applied with the intention of making use of their microbial metabolism [1].

Generally, the preparations used as starters today can be classified within three categories: first, 'undefined cultures' which in the dairy industry are called 'mixed-strain cultures'. These starters are based on the use of fermenting substrate, taken from a selected process that resulted in good-quality end products. These starters are also propagated and distributed commercially [1]. The two other categories of starters are represented by 'single-strain cultures' and 'multiple-strain cultures', which contain one or more defined strains, respectively.

In addition to true starter cultures, so-called 'back slopping' is practised in fermentation technology. This process is based on the continuous inoculation by foods from a previous batch, and is industrially applied to date.

Table 2 gives an overview of the types of starter cultures used in the various fields of application today.

Development of starter cultures

General aspects

General requirements for starter cultures as regards safety, technological effectiveness and economics are summarized in Table 3. The particular topics are self-evident and only the question of biogenic amine formation needs to be discussed in more detail.

Another general aspect deals with several potential health and nutritional benefits derived from some species of LAB, e.g. improved nutritional value of food, control of intestinal infections, improved digestion of lactose, control of some types of cancer and control of serum cholesterol levels [3–6]. However, in the field of fermentations the discussion of which now follows, nutritional and health aspects are confined to special applications such as the production of so-called L(+)-products or the destruction of the cyanoglycosides in cassava fermentation (see below).

Table 2
Types of starter cultures used in various fields of food fermentation

Foodstuff	Single strain cultures	Multiple strain cultures	Mixed strain cultures	Back-slopping
Sauerkraut	+	–	–	+ ^a
Various vegetables	+	–	–	–
Vegetable juices	+	–	–	–
Soy-products	+	–	–	–
Sour dough	+	+	+	+
Wine	+	+	–	–
Dry sausage	+	+	–	+
Dairy products	+	+	+	(–)

–, not used; +, applied.

^a Brine from a previous fermentation.

Table 3

General criteria for starter cultures after [2]

1	Safety
1.1	Starter organisms are not in possession of any pathogen or toxic activity
1.2	The preparations are free from hygienic precarious infections or substances
2	Technological effectiveness
2.1	Starter organisms dominate over the spontaneous microflora
2.2	The microorganisms perform the required metabolic activity
2.3	The preparations are free from technological precarious infections or substances
3	Economical aspects
3.1	The propagation must be feasible from the economical point of view
3.2	The starter culture can be preserved by freezing or freeze-drying with little practical loss of activity
3.3	The important properties are stable under defined storage conditions for several months
3.4	The handling of the starter culture must be as easy as possible

Biogenic amines

Biogenic amines have been defined as biologically active aliphatic, aromatic or heterocyclic organic bases of low molecular mass, which can be formed and degraded during the metabolism of man, animals, plants and microorganisms. Biogenic amines are in general either psychoactive or vasoactive substances and are thereby involved in many critical physiological functions in man and animals.

The intestinal tract of mammals possesses an efficient detoxification system in which the enzymes monoamine oxidase (MAO) and diamine oxidase (DAO) play an important role. Therefore the consumption of foods containing small amounts of biogenic amines is not hazardous, unless the detoxification system is inhibited or genetically deficient. However, the oral intake of high amounts may have toxicological effects. Possible symptoms are nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, a bright red rash, oral burning and hypotension [7].

In most foods, the majority of biogenic amines are formed by decarboxylation of the corresponding amino acid through substrate-specific enzymes derived from microorganisms [8].

Prerequisites for a considerable formation of biogenic amines in foods are [3]: (i) The availability of free amino acids, which may occur in the food itself, but also be liberated from proteins as a result of proteolytic activity. (ii) The presence of decarboxylase-positive microorganisms. Among others, genera of Enterobacteriaceae and Bacillaceae as well as species of *Lactobacillus*, *Pediococcus* and *Streptococcus* are reported to be capable of decarboxylating one or more amino acids. (iii) Conditions that allow bacterial growth, decarboxylase synthesis and decarboxylase activity. Considering these requirements, the occurrence of biogenic amines must be expected in all fermented products, especially in those which are fermented spontaneously by an undefined microflora. The main amines found in higher concentrations in foods are histamine, tyramine, putrescine and cadaverine. Fig. 1 shows examples of the biogenic amines and their precursors which were detected in lactic acid-fermented vegetables. The highest amounts reported in sauerkraut are 104 ppm of histamine, 192 ppm of tyramine, 311 ppm of cadaverine and 550 ppm of putrescine [8].

The kinetics of the formation of biogenic amines in spontaneously fermented sauerkraut (25-kg batches) are illustrated in Fig. 2A. Unfortunately, the fermentation was not accompanied by a detailed microbiological analysis. Details do reveal, however, that the initial stage was dominated by *Leuconostoc mesenteroides* and that a strong propagation of *Pediococcus* species was observed parallel to the major formation of histamine. In a following batch the cabbage was inoculated with 2×10^6 cfu g⁻¹. *Lb. plantarum* (commercial starter culture). The effect of inoculation on the formation of biogenic amines can be seen from Fig. 2B. Within the initial stage, the amount of histamine, tyramine and cadaverine remained at the level of the spontaneously fermented batch, but the amount of putrescine was significantly reduced. Despite the inoculation with *Lb. plantarum*, *L. mesenteroides* dominated the

initial phase of fermentation, but growth of *Pediococcus* species was inhibited [14].

From this example it can be concluded that it is strongly recommended to select LAB which fail to produce biogenic amines [3].

Special fermented foods

During the selection of starter cultures for special food commodities, numerous specific aspects have to be considered, including the characteristics of the raw materials, the applied technol-

ogy, the desired metabolic activities, and the desired characteristics of the final product. These special selection criteria shall be discussed below.

Meat products

The production of fermented sausages or raw, dry sausages has a long tradition and originates from Mediterranean countries at the time of the Romans [10]. Although more than 700 000 tonnes of fermented sausages are produced and consumed each year in Europe [11], the application of LAB or other microorganisms in other fields of

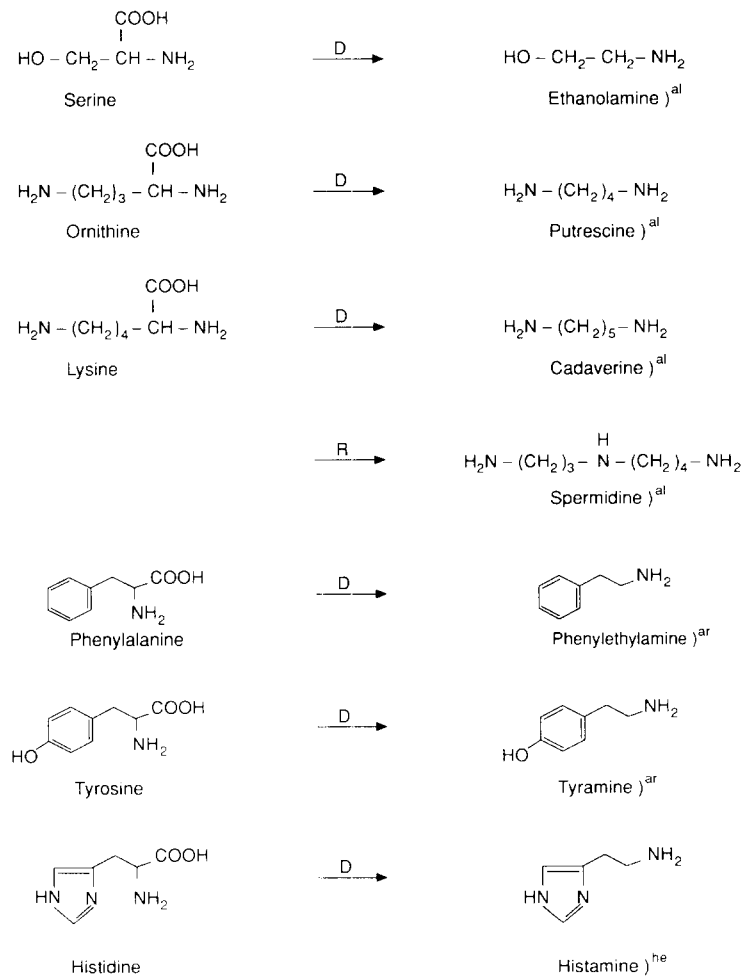


Fig. 1. Biogenic amines and their precursors which were detected in lactic acid fermented vegetables [9]. (D) Decarboxylation. (R) Spermidine is formed by a reaction of putrescine with a propylamine residue which descends from methionine. ^{al}), aliphatic; ^{ar}), aromatic; ^{hc}), heterocyclic.

Table 4

Application of LAB in meat products [12]

Field	Status	Effect of LAB
Production of fermented sausages	In general practical use	Sensory quality; Preservation; Hygienic stability
Production of cooked meat products	In practical use	Sensory quality
Production of smoked ham	Pilot studies (in use)	Standardizing meat quality (counteract DFD condition); Hygienic safety
Extension of shelf life of fresh meat and meat products	Pilot studies	Prevent growth of a spoilage flora
Improving the hygienic status of meat products and of the health of consumers	Research	Inhibition of food pathogens, exerting probiotic effects

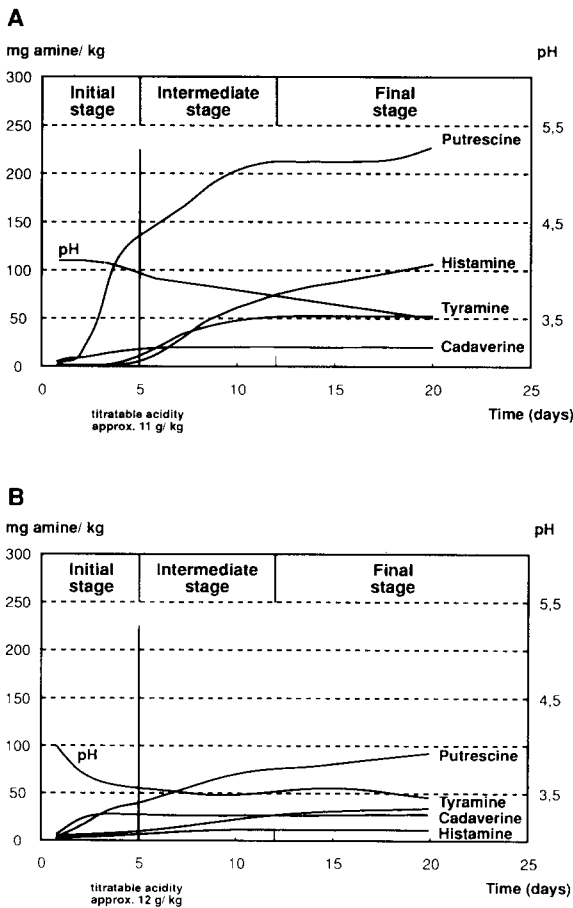


Fig. 2. Formation of biogenic amines during fermentation of sauerkraut [14]. (A) spontaneous fermentation; (B) fermentation after inoculation of *Lactobacillus plantarum*.

meat processing has remained a topic only of minor importance to date. Table 4 gives a survey of possible applications of LAB which are under development in the laboratory or pilot studies.

Fermented sausage

Fermented sausage is defined as comminuted fat and meat, mixed with salt, curing agents, sugar and spices, stuffed into casings, and subjected to a fermentation process carried out by microorganisms. Most fermented sausages are

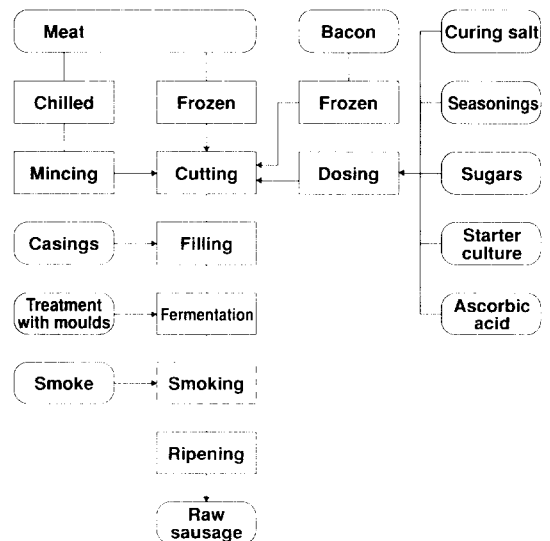


Fig. 3. Flow sheet of raw sausage manufacture.

dried and can be stored with little or no refrigeration. A flow sheet of the general course of the manufacturing process is given in Fig. 3.

The main objectives of the fermentation and ripening process are: (i) formation of the typical red colour (nitrosomyoglobin); (ii) formation of the characteristic fermentation flavour, (iii) development of firmness and sliceability; (iv) inhibition of pathogenic and spoilage bacteria; (v) shelf life.

These goals may be attained by a complex interaction of microbiological, chemical and physical reactions. The spontaneous fermentation of sausages is characterized by the participation of LAB, catalase-positive cocci, yeasts and moulds. However, the most important microorganisms are the bacteria. Figure 4 presents a general overview of the interactions influenced by the activity of these organisms (see below).

These relations, considered most of the commercially available starter cultures are mixtures of LAB and catalase-positive cocci (Staphylococci, micrococci or a blend of both species). Yeasts are sometimes present in those cultures in order to produce sausage with the so-called Ital-

ian taste. Moulds are only used at the surface of mould-ripened sausages which are predominantly produced in Italy; Spain, France and Switzerland, whereas most of the sausages produced in Germany, Belgium or the Scandinavian countries are smoked [11]. The same proportions can be found regarding the general use of starter cultures. Whereas starter cultures are very common in the northern European countries, most of the sausages are fermented spontaneously in the south.

Given the investigations of the COST group (COST = Cooperation in Science and Technology; Concerted Action of the EEC, 1985–1989), it can be concluded that better aroma, longer shelf life, stronger antagonism against pathogens, faster colour formation, and greater handling convenience are the generally desired goals of using a better starter [11].

Given this framework, it is possible to define selection criteria for LAB for use in sausage fermentation (Table 5).

The most important task of the LAB is the formation of lactic acid from added carbohy-

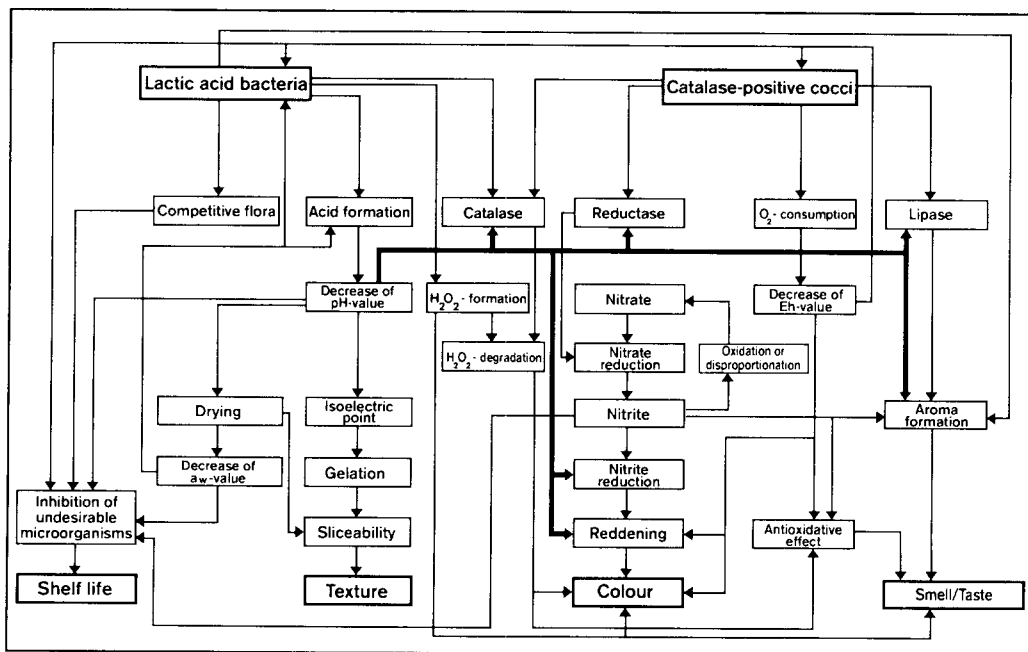


Fig. 4. Interactions during the fermentation of sausages caused by the action of LAB and catalase-positive cocci.

Table 5

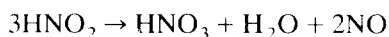
Selection criteria for lactic acid bacteria to be used in the production of fermented sausage

- * Fast production of lactic acid
- * Growth rate at different temperatures
- * Homofermentative species
- * Persistence over the whole fermentation and ripening process
- * Nitrate reduction
- * Catalase positive
- * Lactose negative
- * Formation of flavour
- * No formation of peroxide
- * No formation of biogenic amines
- * No formation of ropy slime
- * Tolerance or even synergy to other microbial components of the starter
- * Antagonism against pathogens
- * Antagonism against technologically undesirable microorganisms
- * Factors to improve the nutritional value of the sausages
- * Economical factors

drates. Figure 4 shows that the resulting decrease in pH causes various effects and interactions. The most important effects are:

(i) Coagulation of the meat proteins, whereby the sausage becomes sliceable.

(ii) All reactions necessary for colour formation are improved at low pH. The most important step is the formation of nitric oxide according to the reaction:



The nitric oxide reacts with myoglobin to nitrosomyoglobin, the substance causing the cured meat colour.

(iii) Improvement of the stability. The encased mixture of meat, fat and additives is highly perishable since the conditions are optimal for bacterial growth. The substrate is characterized by a rich content of nutrients, growth factors and minerals, a water activity of $a_w = 0.96\text{--}0.97$ and a pH of 5.6–5.9. Although added nitrate or nitrite are effective as a preservative, the stability should be reached by decreasing pH, water activity and redox potential in a relatively short time.

To ensure these requirements, both the growth rate and the acid formation under the conditions

in the fermenting substrate are important selection criteria for LAB. Although the rate and the amount of acid formation can be influenced by the temperature as well as the nature and quantity of added sugars, there is a need for species with different growth and acidification patterns. This demand depends on the various types of fermented sausage (approximately 330 different types are produced in Germany alone), the alternative use of nitrate or nitrite and on the applied technology. In the United States, for instance, summer sausage is fermented at high temperatures up to 40°C and the sausages are ready within 48 h. In contrast, in Hungary, raw sausage is traditionally fermented at temperatures below 10°C until the water activity has reached a value between 0.93 and 0.92 [16].

The immediate and fast acid formation at the beginning of the fermentation is an essential requirement but excessive acid formation, often associated with colour defects and sometimes with gas formation, is one of the most important problems in sausage fermentation. Therefore, the experts consulted by the COST-group stated that there is only a marginal need for strains with increased rate of acid production compared to the starters available today [11].

Heterofermentative LAB are not suitable as starters for sausage fermentation because higher concentrations of acetic acid result in a pungent off-flavour. In addition, the formation of higher amounts of carbon dioxide leads to the development of holes of different sizes. Therefore, it has been proposed to use the rate of CO₂ formation from glucose as an important negative selection criterion [17].

Nitrate is used as a curing agent in the production of long-fermented sausage. To enable the reddening process it is indispensable to reduce nitrate to nitrite. Under the conditions in the fermenting sausage, this reaction is only possible by a nitrate reductase, which normally derives from the Micrococceae (see Fig. 4). Since the disproportioning of nitrite shown above leads to a certain amount of nitrate, this enzyme is even necessary in sausages that are manufactured with nitrite.

Nitrate reductases and even heme-dependent

and heme-independent nitrite reductases are reported to be present in LAB strains involved in meat fermentation. Whereas the reduction of nitrite by the heme-dependent nitrite reductase led to ammonia, the heme-independent enzyme released dinitrogenic oxide (N_2O) and nitric oxide (NO). Test fermentations using multiple-strain cultures of *Lb. pentosus*, exhibiting nitrate reductase activity, and strains of *Lb. sake* and *Lb. farciminis*, exhibiting nitrite reductase activities, effected the reduction of nitrate and nitrite, even when nitrate was used as a curing agent. However, the reaction was very slow and the sausages failed the criteria of colour and flavour [18]. Curing in the absence of cocci is only conceivable when LAB are available that possess significantly higher nitrate reductase activities.

The majority of lactobacilli are capable of forming hydrogen peroxide by oxidizing lactate. In certain food fermentations this activity may result in the inhibition of undesirable microorganisms. However, in the case of meat products, peroxides lead to discolouration since the substance attacks the heme pigments. *Lb. sake* and *Lb. curvatus* strains were found to be the dominating flora in spontaneously fermented sausages, and many strains of these species showed a rapid hydrogen peroxide formation [19]. In meat fermentation, LAB are preferred which possess none or only little H_2O_2 -forming activity.

Another way to stabilize and protect the colour is to destroy the peroxide formed by a catalase normally derived from the Micrococcaceae. Since the occurrence of catalase is known in LAB, e.g. in strains of *Lb. sake*, screening for catalase-positive strains, is desirable.

Given the demand of flavour formation, it has to be stated that only little is known about compounds and biochemical activities in products with poor and excellent flavour. Lipolysis and proteolysis are considered to play a key role in the development of aroma, but the importance of lipolytic and proteolytic activities from LAB have not yet been studied in fermenting sausage.

Bacteriophages have not been found to be a problem in sausage fermentation. This may be due to the fact that the absence of liquid will preclude its dissemination. However, phages at-

tacking *Lb. plantarum* have been isolated which caused a delay of 1 or 2 days in a sausage fermentation experiment [20,21].

The search for starters possessing strongly antagonistic effects against food-poisoning microorganisms is of special interest. Those properties were found in strains of *Lb. plantarum*, *Lb. curvatus* and *Lb. sake*, e.g. against *Listeria monocytogenes* [22] or *Staphylococcus aureus* [18]. However, it has to be pointed out that the effects are restricted to a very limited number of microorganisms. Furthermore, it was observed that strains of *Lb. curvatus* and *Lb. sake* inhibit not only food-poisoning microorganisms but also strains that are often used in starter preparations, for example against *Micrococcus varians*. In the latter case, nitrate reduction and the reddening reaction were suppressed when such a combination was used [18]. Further information on the genetics of bacteriocins produced by LAB can be found in the contribution of T.R. Klaenhammer elsewhere in this issue of FEMS Microbiology Reviews.

Some strains of *Lb. sake* [23] are capable of forming a ropy slime. A rate of up to 10^7 cfu g^{-1} for those microorganisms in the fermenting sausages does not seem to have any negative effect on the maturation or the colour and consistency of the sausages. However it is assumed that the processing facilities at the butchers can be open to contamination through sausages containing those microorganisms. This may entail problems by 'cross-contamination', e.g. with cooked meat products, if these are manufactured in the same rooms or sliced using the same machines. Therefore, it may be beneficial to use starters which fail to produce ropy slime.

Vegetable fermentation

It is generally agreed among food scientists that fermented plant products belong to the "Food of the future". A world-wide increasing interest can be observed especially for lactic acid fermented fruit and vegetable products. An up-to-date review concerning the situation of these products in Europe was prepared by the COST group [24]. From this paper it follows that a total

Table 6

Lactic acid fermented vegetables available in the European market

Artichoke	Melons
Capers	Olives
Carrots	Red beets
Cauliflower	Red cabbage
Celery	Sauerkraut (white cabbage)
Cucumbers	Silver-skinned onions
Eggplants	(Levant garlic)
Fungi (not specified)	Swedes (<i>Brassica napus</i>)
Green beans	Tomato shaped paprika
Green tomatoes (unripe fruits of <i>Lycopersicon lycopersicum</i>)	(green and red)
Green paprika	Turnips (<i>Brassica rapa</i>)
Lupinus beans	Waxy paprika
	Whole cabbage (white cabbage)

of 21 different fermented vegetables (Table 6), an unspecified number of variably composed vegetable blends and several fermented vegetable juices are available in the market. At present, only three products are of real economical importance: olives, sauerkraut and cucumbers. The reported production figures for 1985 were 510 000, 220 000 and 45 000 tons, respectively [24].

A highly simplified flow sheet for manufacturing lactic acid-fermented vegetables is given in Fig. 5. The methods vary among vegetable or fruit commodities, depending on the properties of the commodity and the desired characteristics in the final product. Given the fact that the majority of the vegetables are fermented by the naturally occurring lactic flora, it is surprising which methods of treatment are used prior to fermentation.

Fresh vegetables contain a numerous and varied epiphytic microflora and an extremely small population of LAB. For instance, the analysis of 30 different samples of white cabbage from four growing seasons showed that the flora is dominated by aerobic bacteria and yeasts, while LAB normally present only between 0.15 and 1.5% of the total counts [15]. It is certain that the natural flora is affected by methods of pretreatment like trimming, peeling or blanching, but this does not seem to have any significant effect on the fermentation processes.

The final product can either be distributed as a fresh product, packaged or unpackaged, or as a pasteurized product in cans or jars.

When vegetables are distributed as fermented and unpasteurized products, it is important that all fermentable sugars have been removed during the lactic acid fermentation. Otherwise, a secondary fermentation by yeasts can occur which results in gaseous spoilage, brine turbidity [25] and probably in an alcoholic fermentation. In the case of cucumber fermentation particularly, the CO₂ formation may result in bloater formation [26].

To date, pure lactic starter cultures are not very common in European vegetable fermentation, although preparations are available on the market. The major exceptions are the production

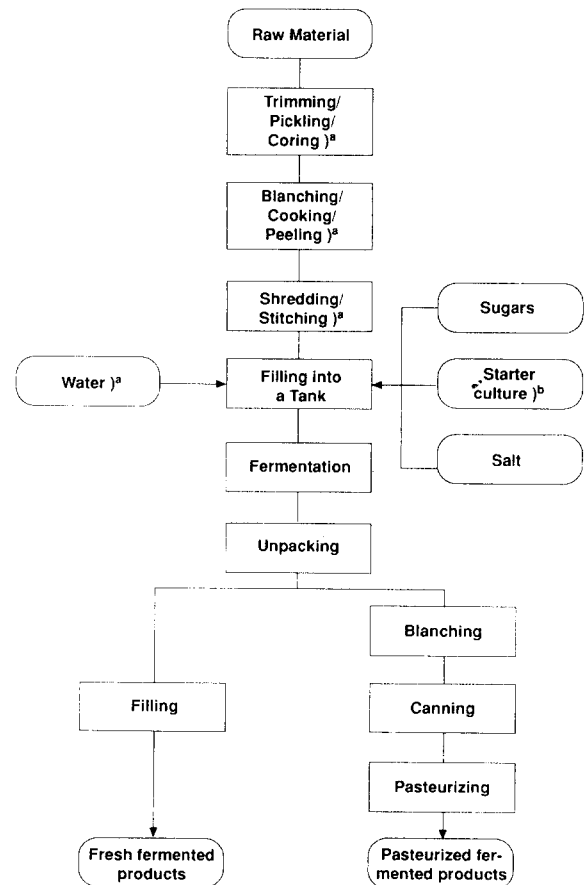


Fig. 5. Flow sheet of the production of fermented vegetables. ^a) depending on the species of vegetables; ^b) see text.

Table 7

Lactic acid bacteria described to be used as starter cultures for the fermentation of vegetable juices [28]

<i>Lactobacillus acidophilus</i>
<i>Lactobacillus bavaricus</i>
<i>Lactobacillus bifidus</i>
<i>Lactobacillus brevis</i>
<i>Lactobacillus casei</i>
<i>Lactobacillus delbrueckii</i>
<i>Lactobacillus helveticus</i>
<i>Lactobacillus plantarum</i>
<i>Lactobacillus salivarius</i>
<i>Lactobacillus xylosum</i>
<i>Lactococcus lactis</i>
<i>Leuconostoc mesenteroides</i>

of so-called L(+)-products, which are characterized by large amounts of L(+)-lactic acid produced by LAB and the manufacture of fermented vegetable juices, except sauerkraut juice. The latter are predominantly fermented by using starter

cultures according to the 'Lactoferment-process' [27]. LAB recommended for juice fermentation are summarized in Table 7.

It is supposed that in the future modern consumer demand and economical requirements will necessitate the development of controlled procedures also in vegetable fermentation in order to provide safe products of a consistently high quality. Besides further advances in technology, the use of starter cultures will be a practical approach to meet these demands, but because of the modest margins of vegetables and vegetable products it is an open question whether the application of starters is economically acceptable.

Starter cultures applied in vegetable fermentation must possess appropriate and specific attributes depending on the properties of the fermented commodity and on the characteristics desired in the final product. Selection criteria for starters to be used for the fermentation of sauerkraut, cucumbers, olives and vegetable juices are summarized in Table 8.

Table 8

Selection criteria for lactic acid bacteria to be used for vegetable and vegetable juice fermentation (after [11,29,30])

Criteria	Sauerkraut	Cucumbers	Olives	Vegetable juices ^a
Technologically relevant criteria				
Rapid and predominant growth	++	++	++	+
Homofermentative metabolism	-	++	++	++
Salt tolerance	+	++	++	0
Acid production and tolerance	++	++	++	+
Inability to metabolize organic acids	++	++	++	+
Growth at low temperatures	++	++	++	0
Few growth factors required	0	0	+	0
Tolerance of phenolic glycosides	0	0	++	0
Formation of dextrans	-	-	-	-
Pectinolytic activities	-	-	-	-
Formation of bacteriocins	+	+	+	0
Bacteriophage resistance	0	+	+	++
Sensorially relevant criteria				
Heterofermentative	++	-	-	-
Formation of flavour precursors	++	++	+	0
Nutritionally advantageous criteria				
Reduction of nitrate and nitrite	+	0	0	++
Formation of L(+)-lactate	+	+	0	++
Formation of biogenic amines	-	-	-	-

++ , important; + , advantageous; 0 , not relevant; - , detrimental.

^a Except sauerkraut juice.

Unfortunately, the fermentation of vegetables is difficult to control, primarily because of their shape, the large number of naturally occurring microorganisms and the variability in nutrient content.

Heat treatment or sterilization by other methods prior to fermentation is impossible in most cases, so that pure culture fermentation can only be achieved in juice production. This means that selected LAB must grow rapidly and be highly competitive in the environmental conditions under which the product is kept. These conditions vary among different vegetables and affect above all the content of sodium chloride (approximately 0.6–2.0% for sauerkraut [24], 5–10% in the brine of cucumbers [31]), fermentation temperature (approx. 5–20°C for sauerkraut, 25–35°C for vegetable juices [28]), pH value upon starting fermentation (approx. pH 5.9–6.5 for cabbage, maximally pH 8.5 for olives) and the redox potential. Finally, however, growth of organisms other than those inoculated may occur, which may have an influence on the properties of the final product.

As an example, Fig. 6 shows the pH values of sauerkraut fermented and stored in small pouches (content approx. 560 g) following a method described earlier [32]. The fermentation was started at 19°C and after 7 days the products were cooled to 4°C and stored. Every point on the curves in Fig. 6 is the mean value of 4–10 different samples and the vertical lines represent the maximum and minimum values. The samples in Fig. 6A were spontaneously fermented and those in Fig. 6B were inoculated with 10^7 cfu g^{-1} *Leuconostoc mesenteroides*. It can be seen that compared to the spontaneous fermentation the use of starter cultures resulted in mild products of high uniformity, but during storage the inoculated organisms were overgrown mainly by *Lb. plantarum*, which resulted in a further decrease of pH and the uniformity between the different samples assimilated to those of the spontaneously fermented products [15,32]. To date, growth of *Lb. plantarum* or other LAB can only be prevented by pasteurizing the fermented sauerkraut at a previously defined degree of acidity.

Several plants possess naturally occurring undesirable substances or toxins which can be re-

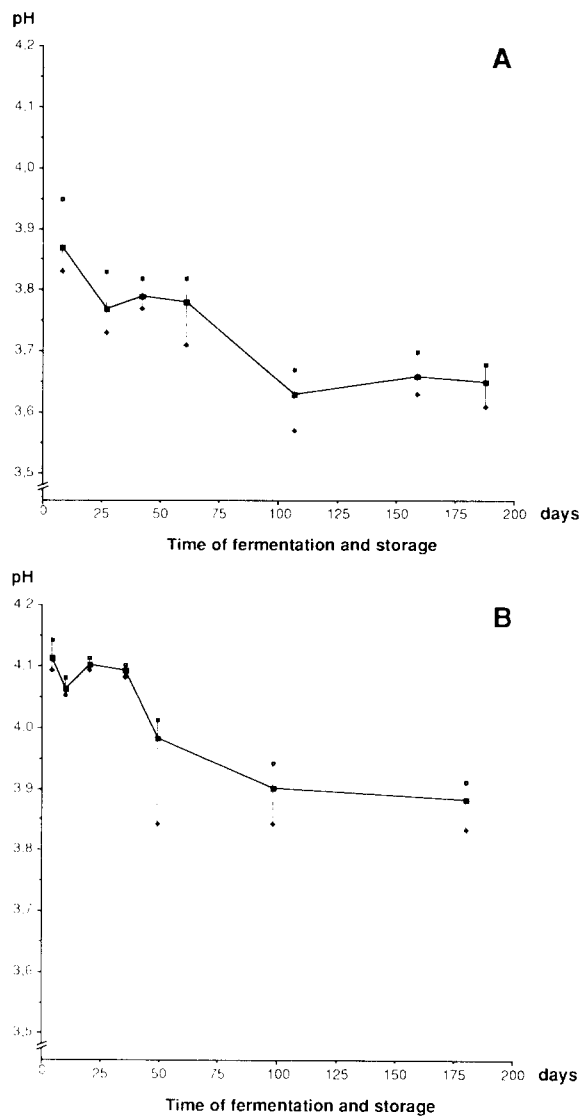


Fig. 6. Fermentation of sauerkraut in small pouches. (A) spontaneous fermentation; (B) fermentation after inoculation of *Leuconostoc mesenteroides*.

moved or destroyed by microorganisms during fermentation processes.

For instance, roots of cassava (*Manihot esculenta* Crantz) contain large amounts of cyanoglycosides, yet cassava represents the staple food of over 500 million people in developing countries. Because of the high level of toxicity, most processes for preparing traditional cassava-based foods include more or less intensive stages of

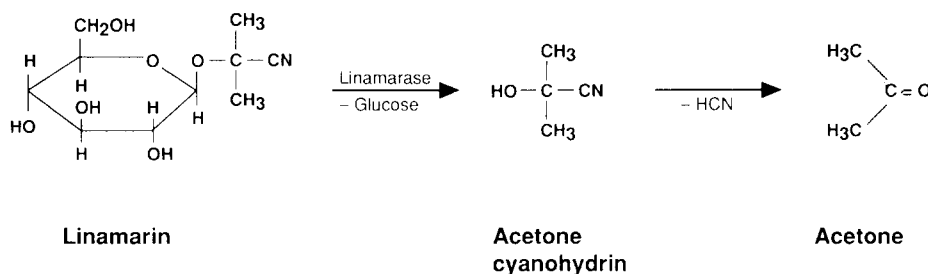


Fig. 7. Hydrolysis of linamarin.

fermentation to eliminate these substances [34,35], but additionally for starch breakdown, acidification and flavour development [33]. The most important cyanoglycoside is linamarin (96% of the total content) which can be destroyed in the presence of linamarase, released from the plant cells when the structure is damaged. Linamarase is a β -glycosidase which catalyses the hydrolysis of linamarin into glucose and acetone cyanohydrin (Fig. 7). It would appear that the amount of plant-origin enzyme or the acid pH conditions caused by the fermentation do not permit the complete breakdown of linamarin. Therefore, ten lactic acid bacteria were screened for linamarase activity after culture on MRS cellobiose. Six of the tested strains displayed linamarase activity, including strains of *Lb. plantarum*, *Streptococcus lactis*, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus*. The strongest linamarase activity was measured in *Lb. plantarum*, especially in strain A6 which was isolated from retted cassava. In order to determine the most suitable carbon substrate for maximum linamarase production, it was found that linamarase seems to be a constitutive enzyme in *Lb. plantarum* A6 and that the amount of enzyme can be increased by induction (Table 9) [35].

Furthermore, useful reductions in oligosaccharide and phytate concentrations were observed in fermenting peas, lentils and chick peas with a mixture of two strains of *Lb. acidophilus*. Strains of *Lb. buchneri* and *Lb. fermenti* were useful as well, but they adversely affected contents of lysine, methionine and riboflavin [36]. In an additional investigation, growth of *Lb. acidophilus* and *Lb. buchneri* was related to decreases in

phytate content of fermenting lupins (*Lupinus albus* cv. Multolupa) [37].

The softening of fermenting or fermented vegetables is one of the most important economical problems of the pickle manufacturers. The softening process can be caused by a multitude of factors, depending on the raw material as well as on the applied technology. Up till now it is not clear whether strains of LAB are significantly involved in these softening processes. Indeed, the presence of a pectinesterase and an endopolygalacturonase activity was found to be present in a strain of *Lb. plantarum* [38,39], but to date this has not been confirmed by other researchers.

Given the assumption that selected strains of *Leuconostoc mesenteroides* are suitable to improve product quality and uniformity of sauerkraut [15,41], research was undertaken to find bacteriocin-producing strains with a selective advantage over competing but sensitive strains. Since these investigations failed, a new approach

Table 9

Linamarase activities of *Lactobacillus plantarum* A6 cultured on different carbon sources [35]

Carbon source	Linamarase (U (g dry biomass) ⁻¹)
Cellobiose	35.5
Melibiose	12.1
Lactose	6.2
Starch	5.6
Sucrose	3.4
Maltose	1.5
Glucose	1.5

was developed using a paired starter-culture system, consisting of a nisin-resistant *L. mesenteroides* strain and a nisin-producing *Lactococcus lactis* strain, both of which were isolated from commercial sauerkraut fermentations [40]. Whether the presence of nisin can actually promote and extend the dominance of heterofermentative *L. mesenteroides* in a natural fermentation has still to be proven [41].

The production of table olives, particularly of green table olives, involves a lye treatment in order to destroy a part of the extremely bitter-tasting oleuropein, followed by a washing step to remove excessive lye (for technological details see [42,43]). The lye treatment leads to a relatively high starting pH, a loss in nutrients and formation of some antimicrobial substances derived from oleuropein. In addition, the fermentation is influenced by the more or less high content of phenolic glycosides, particularly in black olives.

To date, bacteriophages have not been found to be a problem in vegetable fermentation. This may be due to the fact that pure-culture fermentations have only seldomly been used. Thus, in the event that a starter is infected with phage, strains of the naturally occurring lactic flora will take over and carry out the fermentation. However, when pure cultures are used extensively for vegetable fermentations, bacteriophage infections could become a problem, especially in cases where large pieces, like cucumbers, are fermented in a brine. However, in the fermentation of products like sauerkraut or silages, the absence of liquid will preclude the dissemination of the phage.

Malolactic fermentation in wine

Red and white wines are commonly manufactured by alcoholic fermentation of musts prepared from grapes of defined varieties of *Vitis vinifera*. In the cooler winegrowing regions, maturation of the grapes occurs very late in the last weeks of September to October, resulting in wines with high acidity and low pH. In cooler years, these wines are often tart; therefore acid reduction by chemical or biotechnological means is a common procedure in wine technology. The latter method, known as malolactic fermentation

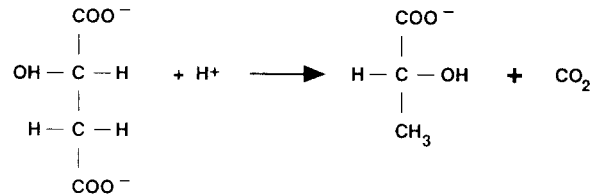


Fig. 8. Transformation of malic acid to lactic acid and carbon dioxide.

(MLF), was discovered at the end of the 19th century by Muller-Thurgau. He described the transformation of L-malic acid to L-lactic acid and carbon dioxide by LAB (Fig. 8). Malolactic fermentation occurs naturally towards the end of or after alcoholic fermentation. It may be delayed or be absent due to inappropriate pH, temperature or SO₂ and alcohol content, or even by phage [44].

Since the beginning of the 1980s, commercial starter cultures for the induction of MLF have been available, consisting of strains of *Lb. plantarum*, *Lb. hilgardii* and *Leuconostoc oenos* as single- or multiple-strain preparations [1]. Malolactic fermentation is more commonly used in red wines but recently also increasingly in white wines [45].

The advantages of inducing malolactic fermentation by inoculation include the better control over time of onset and rate of completion of MLF as well as over the strain of LAB that carries out the process [44]. The latter point is significant because malolactic fermentation is not only a process of acid reduction. Depending on the LAB involved, the following advantages could be obtained:

- (i) contribution to the biological stability [46];
- (ii) flavour modification;
- (iii) contribution to complexity (structure, body);
- (iv) decrease in organic N-compounds, resulting in better storage stability [1];
- (v) decrease in aldehydes and ketones, permitting a limited application of SO₂ [47].

To meet all the different above-mentioned requirements, three groups of selection criteria were defined to develop or improve starter cultures for

Table 10

Selection criteria for lactic acid bacteria for induction malolactic fermentation in wine ^a

1st order criteria

- * resistant to low pH
- * resistant to ethanol
- * tolerant to low temperatures
- * limited metabolism of hexoses and pentoses

2nd order criteria

- * count of living organisms after propagation in a standardized medium
- * time for propagation in a standardized medium
- * yield by propagation in a standardized medium
- * kinetics of survival in a standardized wine
- * kinetics of malate degradation in a tartaric buffer (pH 4.5) and in a standardized wine

3rd order criteria

- * limited interactions with yeasts of the alcoholic fermentation
- * limited interactions with other lactic acid bacteria
- * phage resistance
- * resistant to SO₂
- * resistant to pesticides
- * no formation of biogenic amines
- * citrate metabolism under aerobic and anaerobic conditions
- * potential to form diacetyl and acetoin
- * limited potential to form volatile acids from hexoses and pentoses
- * probably no formation of acetic acid
- * limited metabolism of organic acids of the wine (e.g. succinate)
- * no glycerine degradation
- * sensorial alterations of the wine

^a Krieger, S.A., personal communication.

the induction of malolactic fermentation in wine (Table 10).

Today, active starter cultures are available. Most of them consist of strains of LAB, preferably *Leuconostoc oenos*, with high malolactic activity and high tolerance to low pH and ethanol. The wine conditions necessary to induce a MLF are summarized in Table 11.

The cultures are offered as fresh, frozen or freeze-dried preparations, but from a commercial point of view freeze-dried cultures are definitely preferred. This is due to the fact that fresh starter cultures have to be produced and sold directly in the winegrowing regions. In the case of frozen starters, long-distance distribution is very difficult because the maintenance of the specified temperatures is hard to guarantee.

Especially for smaller wineries, easy handling of the starter cultures is essential because there are no microbiologists present to perform preculturing or reactivation. However, still the direct inoculation of the cultures into wine can fail due to a sharp decrease in the number of viable organisms, mainly as freeze-dried preparations of *Leuconostoc oenos* strains [45]. Studies were performed to obtain a better adaptation of the bacteria to the hostile wine environment. The results led to the suggestion to preculture the malolactic starter cultures, as according to the procedure given in Fig. 9. The procedure described is specific for the reactivation of Vino-starter cultures

Table 11

Conditions necessary to induce malolactic fermentation in wine

Wine type	Condition	Limiting conditions	Normal conditions	Ideal conditions
White wine	Temperature	< 15°C	16–18°C	> 18°C
	pH	< 3.1	3.2	> 3.2
	Alcohol	13.5%	12.5–13.5%	< 12.5%
	Total SO ₂	> 30 ppm	15–30 ppm	No SO ₂
	Inoculation	During active yeast fermentation	At the end of alcoholic fermentation	After yeast fermentation when wine is still turbid
Red wine	Temperature	< 15°C	16–18°C	> 18°C
	pH	< 3.1	3.1	> 3.1
	Alcohol	14%	13–14%	< 13%
	Total SO ₂	> 30 ppm	15–30 ppm	No SO ₂

(Condimenta, Stuttgart) which contain $2-5 \times 10^{11}$ cfu g^{-1} . During reactivation, the bacterial culture increases from approximately 5×10^{10} to 5×10^{11} cfu ml^{-1} . This means an inoculation of the wine with 5×10^7 cfu ml^{-1} . The main component of the reactivation mixture is a specially selected yeast hydrolysate.

The development of malolactic starters which can be applied without any pretreatment is one of the most important requirements for comprehensive acceptance in the wineries.

Production of starter cultures

To meet the requirements of the modern food industry, starter cultures are produced in high-engineering biotechnical plants under strictly defined conditions. Figure 10 gives a general overview of the production of freeze-dried starter cultures for sausage fermentation, but because of the complexity it is impossible to discuss the process here in detail. The propagation of the microorganisms may occur by traditional batch

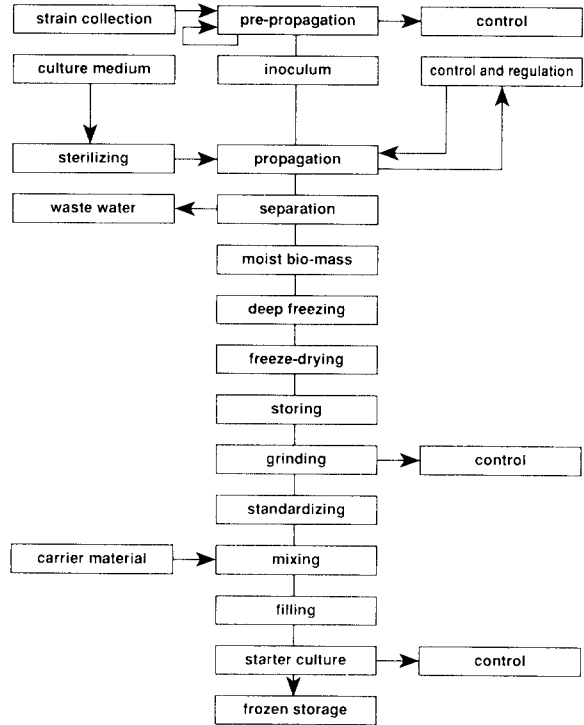


Fig. 10. Large-scale production of starter cultures.

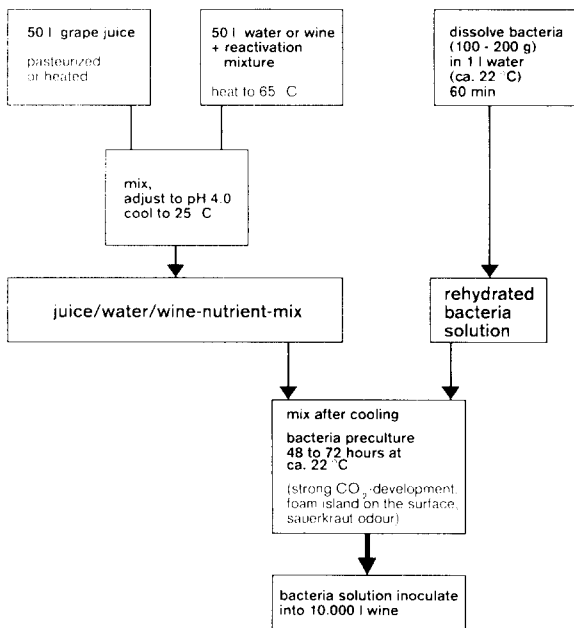


Fig. 9. Reactivation of freeze-dried starter cultures for the induction of malolactic fermentation.

fermentation or by semi-continuous or continuous fermentation with or without cell recycle. However, the method of propagation exerts an influence on the productivity of the installed fermenter capacity, the counts of surviving microorganisms after separation and lyophilisation as well as on the physiological activities of the produced cells. For detailed information see Metz [50].

Freeze drying is a very expensive step in the production of starter cultures, but in the fields of application discussed here, freeze-dried starters are undoubtedly preferred. Frozen-starter preparations are on the market, but the argued advantage of faster activation in the fermenting substrate has no practical relevance. The most important advantages of the freeze-dried starter preparations are the excellent storage stability and the easy handling during storage, distribution and application. Desirable storage conditions for freeze-dried starters are freezing temperatures below $-15^{\circ}C$ and the absence of oxygen and humidity. Under these conditions, the optimal activity of the starter culture will be guaranteed

for 8 months (Gewürzmüller, Stuttgart). Higher temperatures of up to 20°C for a few days and following a repeated storage at freezing temperatures has no significant influence on the stability, so that freeze-dried starter cultures may be distributed without cooling.

Concluding remarks

Lactic acid-fermented foods have proven their wholesome value for thousands of years and are accepted without restrictions by the consumer. The LAB involved possess numerous metabolic properties which are responsible for their successful application as starter cultures in various fields of food production.

Whereas the use of starter cultures in vegetable fermentation until now is limited to very special applications, the controlled induction of MLF in wine is more and more accepted even in the more conservative winegrowing regions. In sausage fermentation, there is no doubt that the use of starter cultures ensures that the aims of the fermentation process are achieved more safely than in the traditional fermentation by the natural microflora.

Despite all the efforts and progress in the field of application discussed here, however, we are far removed from having a complete understanding of the interrelationships between microbiology, technology and internal and external factors influencing the fermentation processes. For further selection and improvement of starter cultures we need better knowledge of the ecological factors in the fermenting substrates, an improved understanding of the role of microorganisms in the formation of aroma, flavour, texture and appearance of the foods, and, last but not least, about the genetics of LAB involved in these fermentations.

Most of the physiological properties which hitherto are recognized to be essential for the different food fermentations are present in the various species of lactobacilli. Therefore, it is a task to combine or enhance the desired properties. This can be achieved by using multiple-strain cultures or by the construction of new strains

using recombinant DNA technology. Several of the desired properties are plasmid-encoded traits, which facilitates genetic manipulation. However, the use of genetically modified microorganisms in food production is a controversial issue in public opinion. In the fields of application discussed here this situation is of special interest, because normally the final products will contain living microorganisms. Regardless of the legal implications at this time in Germany, it is inconceivable to apply genetically modified microorganisms in food production. Given this situation and knowing there is a pool of naturally available strains of LAB, even experts ask the question whether genetic engineering is required for improving starter cultures within the next years [51].

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