14 Microbiology of Fermented Food Production

INTRODUCTION

At the dawn of civilization humans recognized, probably by accident, that under certain circumstances, when raw foods from plant and animal sources were stored for future consumption, they might change to different but desirable products with longer storage stability. The possibility of such an event occurring might have been after they learned to produce more foods than they could consume immediately and thus needed storage. It was probably the period during which they learned agriculture and animal husbandry, as well as making baskets and pottery to store the excess products. On this basis, one can assume that fermented food probably originated ca. 7000–8000 B.C. in the tropical areas of Mesopotamia and the Indus Valley. Subsequently, other civilizations also produced fermented foods from different raw materials, particularly to preserve those that were seasonal and thus available in abundance only for a short harvesting period. Fermented milk products, alcoholic beverages from fruits and cereal grains, and leavened breads became popular among the early civilizations in the Middle East and in the Indus Valley and later among the Egyptians, Greeks, and Romans.[1]

Currently, more than 3500 different fermented foods are consumed by humans worldwide; many are ethnic and produced in small quantities to meet the needs of a group in a particular region. Some are, at present, produced commercially, and only a few are produced by large commercial producers. Production by large producers is now on the rise.

At present, there is interest in consumption of many types of fermented foods other than cheese, bread, pickles, and alcoholic beverages. One reason for this increase is consumer interest in natural and healthy foods, which fermented foods have been thought to satisfy. Even countries in which many types of fermented foods have been consumed for a long time, but mostly produced in small volumes, have started commercially producing some products in large volumes. It is anticipated that in the future, consumption of many fermented foods will increase worldwide.[1]

GENERAL METHOD OF PRODUCTION

The production of a fermented product has two related, yet separate, aspects, one involving the importance of metabolic activities of microorganisms during fermentation and storage of the product and the other involving the parameters used during processing and storage of the product. In this chapter, the microbiological aspects are presented. Processing aspects are generally taught in a food-processing course and are thus covered only in brief here.

Fermentation involves exposing the raw or starting food materials to conditions that favor growth and metabolism of specific and desirable microorganisms. As the desirable microorganisms grow, they utilize some nutrients and produce some end products. These end products, along with the unmetabolized components of the starting materials, constitute the fermented foods having desirable acceptance qualities, many of which are attributed to the metabolic end products.

Raw (or Starting) Materials

A large number of raw materials from plant and animal sources are used to produce fermented foods. These include milk (from cows, buffalo, sheep, goats, and mares), meat (beef, pork, lamb, goat, and fowl), fish (many types), eggs (chicken and duck), vegetables and vegetable juices, many fruits and fruit juices, cereal grains, tubers, lentils, beans, and seeds. Some are used in combination.

Microorganisms Used

Many desirable species and strains of bacteria, yeasts, and molds are associated with fermentation of foods. Depending on a product, fermentation may be achieved by a single predominating species and strain. However, in most fermentations, a mixed population of several bacterial species and strains, or even bacteria and yeasts or bacteria and molds, is involved. When a fermentation process involves a mixed population, the members should not be antagonistic toward one another; rather, they should preferably be synergistic. Maximum growth of a desirable microorganism and optimum fermentation rate are dependent on environmental parameters such as nutrients, temperature of incubation, oxidation–reduction potential, and pH. In the fermentation process, if the different species in a mixed population need different environmental conditions (e.g., temperature of growth), a compromise is made to facilitate growth of all the species at a moderate rate. Depending on a raw or starting material and a specific need, carbohydrates (dextrose in meat fermentation), salts, citrate, and other nutrients are supplemented. In some natural fermentations, several species may be involved for the final desirable characteristics of the product. However, instead of growing at the same time, they appear in sequence, with the consequence that a particular species predominates at a certain stage during fermentation. But analyzing the final product to isolate the species involved in fermentation of such a food does not give the right picture. Instead, samples should be analyzed at intervals to determine predominant types at different times and to know the sequences in their appearance. Finally, some minor flora (secondary flora) can be present in a very low level in the raw material and the final product and might not be detected during regular analysis. However, they may have important contributions for the desirable characteristics, particularly some unique aroma, of the product.

Fermentation Process

Foods can be fermented in three different ways, based on the sources of the desirable microorganisms: natural fermentation, back slopping, and controlled fermentation.

Natural Fermentation

Many raw materials used in fermentation (usually not heat treated) contain both desirable and associated microorganisms. The conditions of incubation are set to favor rapid growth of the desirable types and no or slow growth of the associated (many are undesirable) types. A product produced by natural fermentation can have some desirable aroma resulting from the metabolism of the associated flora. However, because the natural microbial flora in the raw materials may not always be the same, it is difficult to produce a product with consistent characteristics over a long period of time. Also, chances of product failure because of growth of undesirable flora and foodborne diseases by the pathogens are high.

Back Slopping

In this method, some products from a successful fermentation are added to the starting materials, and conditions are set to facilitate the growth of the microorganisms coming from the previous product. This is still practiced in the production of many ethnic products in small volumes. Retention of

TABLE 14.1
Fermented Food Groups and Examples

Food groups	Examples
Dairy products	Cheeses, yogurt, buttermilk, sour cream, dahi, kumiss, kefir, acidophilus milk
Meat products	Salami, pepperoni, chorizo, thüringer, sausage, pickled meat, nahm
Cereal products	Breads, pancake, crackers, pizza, nun, idli, dosa, sour rice, miso
Fruits and vegetable products	Pickled fruits, pickled vegetables, olives, sauerkraut, kimchi, achar
Legume products	Tofu, fermented soymilk, tempe, soy sauce, koji, mizo, natto, papadam
Fish products	Bagoong, fish sauces, pickled fish, tarama, paak, mamoni, izushi
Beverages	Beer, wine, distilled spirits, coffee, cocoa, tea
Starch crop products	Fermented products from potato, cassava, sweet potato, bananas, plantains
Miscellaneous products	Fermented eggs, ghee (from fermented cream), vinegar, red palm oil, bongkrek, dage

Source: Adapted from Campbell-Platt, G., *Fermented Foods of the World*, Butterworths, Boston, 1987.

product characteristics over a long period may be difficult because of changes in microbial types. Chances of product failure and foodborne diseases are also high.

Controlled Fermentation

The starting materials (may be heat treated) are inoculated with a high population (10^6 cells/ml or more) of a pure culture of single or mixed strains or species of microorganisms (starter culture). Incubation conditions are set for the optimum growth of the starter cultures. Large volumes of products can be produced with consistent and predictable characteristics each day. Generally, there is less chance of product failure and foodborne diseases. However, there may be no growth of desirable secondary flora. As a result, a product may not have some delicate flavor characteristics.

As indicated before, worldwide there are more than 3500 types of fermented foods. Different methods have been used to arrange them in several major groups. One such method divides fermented foods in nine groups and is presented in Table 14.1.

As it is beyond the scope of this textbook to describe even a few from each group, only the microbiological criteria of several fermented dairy, meat, and vegetable products are briefly discussed here to understand the methods involved in controlled and natural fermentation. For more detailed information, books on fermentation of different food groups can be consulted, some of which have been used as references in this chapter.

FERMENTED DAIRY PRODUCTS

Fermented dairy products can be broadly divided into two groups: fermented milk products and cheeses. In fermented milk products, all the constituents of the milk are retained in the final products, with the exception of those partially metabolized by the bacteria. In cheeses, a large portion of milk constituents is removed in whey to obtain the final products.

Milk Composition and Quality

The growth of desirable microorganisms and the quality of a fermented dairy product are influenced by the composition and quality of the milk used in a fermentation process. Cow's milk contains approximately 3.2% protein, 4.8% lactose, 3.9% lipids, 0.9% minerals, traces of vitamins, and ca. 87.2% water. Among the proteins, casein in colloidal suspension as calcium caseinate is present in higher amounts than the other two soluble proteins, albumin and globulin. Lactose is the main

carbohydrate and is present in solution, and lipids are dispersed as globules of different sizes in emulsion (fat in water). Minerals are present in solution and as colloid with casein. Water-soluble vitamins are present in aqueous phase, whereas fat-soluble vitamins are present with the lipids. The solid components (ca. 12.8%) are designated as total solids (TS), and TS without lipids is designated as solid-not-fat (SNF; ca. 8.9%). The whey contains principally the water-soluble components, some fat, and water.

The growth of desirable microorganisms can be adversely affected by several components that are either naturally present or have entered in the milk as contaminants. The natural antimicrobials are agglutinins and the lactoperoxidase–isothiocynate system. The agglutinins can induce clumping of starter-culture cells and slow their growth and metabolism. The lactoperoxidase–isothiocynate system can inhibit starter cultures. Antimicrobials can cause problems only when raw milk is used, because both are destroyed by heating milk. Milk can also contain antibiotics, either used in the feed or used to treat animals for some infections, such as mastitis. Their presence can also affect the growth of starter cultures. Some milk can contain heat-stable proteases and lipases produced by some psychrotropic bacteria, such as *Pseudomonas* species, during refrigerated storage of raw milk before pasteurization (see Chapter 21). These enzymes remain stable after heating and can cause product defects (low yield of cheese, proteolysis, and rancidity). Before milk is used for fermentation, these aspects need to be considered.

Fermented Milk Products

Many types of fermented milk products are produced in different parts of the world. A few are produced by controlled fermentation, and the microbial types and their respective contributions are known. In many others, fermented either naturally or by back slopping, the microbial profiles and their contribution are not exactly known. Many types of lactic acid bacteria and some yeasts are found to predominate microbial flora in these products,[3,4] some of which are listed:

1. *Buttermilk.* Made with *Lactococcus* species without or with *Leuconostoc cremoris*; some can have biovar diacetylactis in place of *Leu. cremoris* (such as ymer in Denmark), whereas some can have a ropy variant of *Lactococcus* species (langfil in Norway) or mold (*Geotrichum candidum* in villi in Finland).
2. *Yogurt.* Made with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; some types can also have added *Lab. acidophilus*, *casei*, *rhamnosus*, and *Bifidobacterium* spp.; some may also have *Lactococcus* species and *Lab. plantarum* and lactose-fermentating yeasts (dahi in India).
3. *Acidophilus Milk.* Made with *Lab. acidophilus.*
4. *Bifidus Milk.* Made with *Bifidobacterium* spp.
5. *Yakult.* Made with *Lab. casei*; may contain *Bifidobacterium* spp.
6. *Kefir.* Made from *Lab. kefir* (several species of yeasts along with *Leuconostoc*, *Lactobacillus*, and *Lactococcus* spp.).
7. *Kumiss.* Made from *Lab. delbrueckii* subsp. *bulgaricus* and yeasts.

Among these, cultured buttermilk and yogurt are discussed here.

Microbiology of Cultured Buttermilk Fermentation

It is produced from partially skim milk through controlled fermentation with starter cultures.

Product Characteristics

It should have a pleasant acid taste (from lactic acid) and high degree of aroma (from diacetyl), with slight effervescence (from CO_2). It should have white color with smooth, thick body and should pour easily.

Processing

1. Skim milk ≥ 9% SNF + citrate (0.2%)
2. Heated at 185°F (85°C) for 30 min (kills bacterial cells and phages)
3. Cooled to 72°F (22°C), starter added, agitated for 50 min (incorporates air)
4. Incubated at 72°F (22°C) for 12 h, pH 4.7, acidity 0.9%
5. Gel broken, cooled to 40°F (4.5°C), and salted (package)

Starter (Controlled Fermentation)

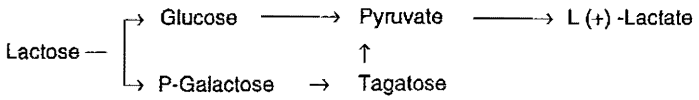
Lac. lactis ssp. *lactis* or *cremoris* is used for acid and *Leu. mesenteroides* ssp. *cremoris* for diacetyl and CO_2. They can be used as direct vat set frozen concentrates. (*Lac. lactis* ssp. *lactis* biovar diacetylactis is generally not used as it may produce too much acetaldehyde, causing green or yogurt flavor defect.)

Growth

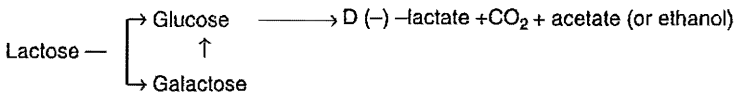
At 72°F, there is balanced growth of the two species, and balanced production of acid, diacetyl, and CO_2. Above 72°F, the growth of *Lactococcus* species is favored, with more acid and less flavor; below 72°F, the growth of *Leuconostoc* species is favored, with less acid and more flavor.

Biochemistry

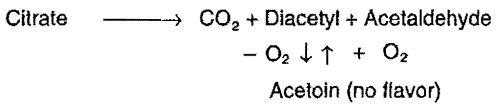
Lactose (transported by PEP-PTS system) is hydrolyzed by P-β-galactosidase in *Lactococcus* spp.:



Lactose hydrolysis by β-galactosidase of *Leuconostoc* sp.:



Citrate metabolism by *Leuconostoc* sp.:



For a desirable flavor, the diacetyl:acetaldehyde ratio should be >3 : 1 to <4.5 : 1.

Genetics

Lactococcus lactis strains should transport and hydrolyze lactose (Lac^+), metabolize P-galactose by the tagatose pathway and galactose by the Leloir pathway, be phage resistant, not produce slime, and not be very proteolytic.

Leuconostoc species should be able to transport and utilize citrate to produce more diacetyl and less acetaldehyde and should ferment lactose, be phage resistant, and not produce slime.

Strains should not produce inhibitory compounds (such as bacteriocins) against each other, but can have antimicrobial activity toward undesirable organisms. Through selection and genetic manipulation, strains that grow rapidly, produce desirable characteristics, and that are resistant to some phages have been developed. Current information on genome sequences of *Lactococcus* and *Leuconostoc* strains and their phages will help develop better strains in the future.

Microbial Problems

Because of too much acetaldehyde production (especially if biovar diacetylactis is used), green (yogurt flavor) may develop. A slimy texture implies contamination with bacteria that produce slime (*Alcaligenes faecalis*) or that starter cultures (some *Lac. lactic* strains) are slime formers (exopolysaccharides). A yeasty flavor implies contamination with lactose-fermentating yeasts, and a cheesy flavor alludes to contamination with proteolytic psychrotrophs (during storage). Proteolysis by proteases of contaminants in starters can also cause development of bitter flavor, especially during storage (also see Chapter 19).

Microbiology of Yogurt Fermentation

Characteristics

Plain yogurt has a semisolid mass due to coagulation of milk (skim, low, or full fat) by starter-culture bacteria. It has a sharp acid taste with a flavor similar to walnuts and a smooth mouth feel. The flavor is due to the combined effects of acetaldehyde, lactate, diacetyl, and acetate, but 90% of the flavor is due to acetaldehyde.

Many types of yogurt are available in the market, for example, plain yogurt, fruit yogurt, flavored and colored yogurt, blended yogurt, sweetened yogurt, heated yogurt, frozen yogurt, dried yogurt, low-lactose yogurt, and carbonated yogurt.[3,5]

Processing

Yogurt is generally fermented in batches, but a continuous method has also been developed. The batch process for a low-fat (2%) plain yogurt is as follows:

1. Homogenized milk (12% TS) + stabilizer (1%). The stabilizer is added to give desired gel structure.
2. Heated to 185°F (85°C) for 30 min, and cooled to 110°F (43.3°C). Heating helps destroy vegetative microbes and slightly destabilize casein for good gel formation.
3. Starter added, incubated at 110°F (29.5°C) to pH 4.8 for ca. 6 h, acidity ca. 0.9%. Starter used as either direct vat set (frozen) or bulk culture (2–3%).
4. Quickly cooled to 85°F in ca. 30 min to slow down further starter growth and acid production, especially by *Lactobacillus* species, agitated, and pumped to filler machine.
5. Packaged in containers, and cooled by forced air to 40°F (4.4°C). Final cooling by forced air results in a rapid drop in temperature to stop the growth of starters.
6. Held for 24 h; pH drops to 4.3.

Starters (Controlled Fermentation)

Frozen concentrates or direct vat set starters can be used. Normally, *Lab delbrueckii* ssp. *bulgaricus* and *Str. thermophilus* are used. Some processors also combine these two with other species, such as *Lab. acidophilus* and *Bifidobacterium* spp., *Lab. rhamnosus*, or *Lab. casei*. However, in general, they do not compete well in growth with the two yogurt starters. Therefore, they are added in high numbers after fermentation and before packaging. They may not survive well when present in yogurt with the regular yogurt starter cultures.

For a good product, the two starter species should be added at a *Streptococcus:Lactobacillus* cell ratio of 1:1; in the final product, the ratio should not exceed 3:2. However, *Lactobacillus* cells are more susceptible to freezing and freeze-drying. In a frozen concentrate starter for use as

DVS, the survivors may not be present in a desired ratio unless they are properly preserved (see Chapter 13).

Growth

For balanced growth of the two species, the fermentation is conducted at ca. 110°F (43.3°C). At this temperature, both acid and flavor compounds are produced at the desired level. If the temperature is raised above 110°F, the *Lactobacillus* sp. predominates, causing more acid and less flavor production; at temperatures below 110°F, growth of *Streptococcus* sp. is favored, forming a product containing less acid and more flavor.

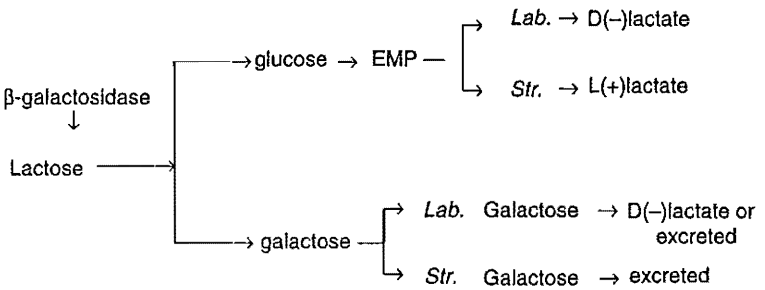
The two species show symbiotic growth while growing together in milk. Initially, *Streptococcus* sp. grows rapidly in the presence of dissolved oxygen and produces formic acid and CO_2. The anaerobic condition, formic acid, and CO_2 stimulate growth of *Lactobacillus* sp., which has good exoproteinase and peptidase systems and produces peptides and amino acids from milk proteins (outside the cells) in the milk. Some of the amino acids, such as glycine, valine, histidine, leucine, and methionine, are necessary for good growth of the *Streptococcus* sp., which lacks proteinase enzymes. *Streptococcus* sp. gets these from the milk and grows rapidly until the pH drops to ca. 5.5, at which time the growth of *Streptococcus* sp. slows down. However, growth of *Lactobacillus* sp. continues fairly rapidly until the temperature is reduced to 85°F, following a drop in pH to 4.8. At 85°F, both grow slowly, but *Streptococcus* sp. has the edge. At 40°F and a pH ca. 4.3, both species stop growing.

The two species also have a synergistic effect on growth rate, rate of acid production, and amounts of acetaldehyde formation when growing together as compared with when growing individually. The species growing separately in milk produce ca. 8–10 ppm acetaldehyde; when grown together, acetaldehyde production increases to a desirable level of 25 ppm or higher.

Biochemistry

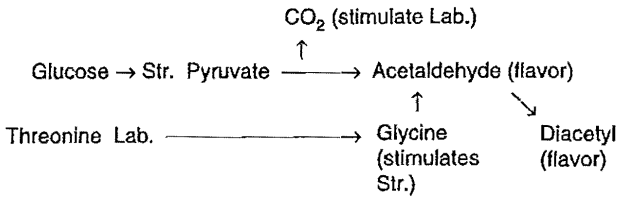
Lactose Metabolism

Both species have a constitutive β-galactosidase system, and lactose (transported by permease systems) is hydrolyzed to glucose and galactose. Both species are homofermentative and produce lactate from glucose by the EMP pathway. *Lab. delbrueckii* ssp. *bulgaricus* strains have enzymes for the Leloir pathway to metabolize galactose, but while actively metabolizing glucose they do not utilize galactose well. Most *Str. thermophilus* strains do not have the enzymes of the Leloir pathway (or have a very weak system) and thus do not metabolize galactose. As a result, galactose is excreted outside, causing its accumulation in yogurt.



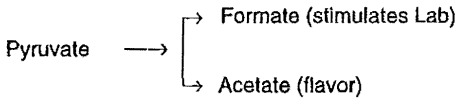
Flavor Production

The major flavor compound in yogurt is acetaldehyde (25 ppm), with some diacetyl (0.5 ppm) and acetate. Acetaldehyde is produced in two ways: from glucose via pyruvate by *Streptococcus* sp. and from threonine (supplied or produced through proteolysis in milk) by *Lactobacillus* sp.



Formate Production

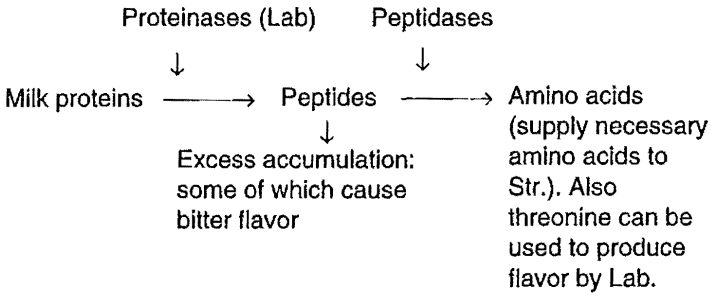
Formate (necessary for *Lactobacillus* growth) is produced by *Str. thermophilus* from pyruvate by the action of formate lyase.



Slime Formation (Glycan)

$\beta-$ galactosidase in some strains of *Str. thermophilus* polymerizes glucose to produce oligosaccharides and glycan, which may give a viscous texture to yogurt.

Proteolysis



Genetics

- *Lac*$^+$ *Phenotype.* In both species, the trait (β-galactosidase and permease) is chromosomally linked, constitutive, and quite stable. Some strains can have strong β-galactosidase activity.
- *Gal*$^+$ *Phenotype. Lab. delbrueckii* ssp. *bulgaricus* is Gal$^+$, but *Str. thermophilus* usually is a Gal$^-$ phenotype. Strains with a good Gal$^+$ phenotype can be developed to reduce galactose accumulation in the product.
- *Pro*$^+$ *Phenotype. Lab. delbrueckii* ssp. *bulgaricus* strains differ in protein hydrolysis ability. Strains with desirable proteolytic activity should be used. Too much proteolysis can adversely affect the texture and enhance development of bitter flavor.
- *Phage Resistance.* Both species have phages; resistant strains need to be developed and used.
- *Symbiotic and Synergistic Relationship.* Strain selection for best combinations is necessary.
- *Antagonistic Effect.* Strains should not produce inhibitory compounds (such as bacteriocins) against each other.
- *Good Survival to Freezing and Drying.* Possible genetic basis needs to be studied to develop resistant strains to produce concentrated starter cultures.

Complete genome sequences of several strains of each species are available now, which will help in the future to develop more desirable strains for use in yogurt production.

Microbial Problems

In plain yogurt, flavor problems can be associated with the concentrations of acetaldehyde. A low concentration gives a chalky and sour flavor, and too much acetaldehyde can give a green flavor. Similarly, too much diacetyl gives a buttery aroma. Too much acid production during storage causes a sour taste. Proteolysis and accumulation of bitter peptides during storage are associated with bitter flavor. Production of exopolysaccharides by the starter can give a viscous and ropy texture (which can be desirable in some situations). Growth of yeasts during storage can also produce a fruity flavor, especially in yogurt containing fruits and nuts. In colored, flavored, and blended yogurt, many of these problems are masked. During long storage, molds can grow on the surface (also see Chapter 19).

CHEESES

Cheeses are made by coagulating the casein in milk with lactic acid produced by lactic acid bacteria, without or with the enzyme rennin, followed by collecting the casein for further processing, which may include ripening.[6] The process was probably accidentally discovered in the Middle East ca. 7000 B.C. from the coagulation of milk stored in a calf stomach by lactic acid produced by lactic acid bacteria (and probably rennin in the stomach). At present, many varieties of cheeses are made worldwide, which probably use more than 20% of the total milk produced. In the United States, the total production of different varieties of cheese in 1982 was 4.4 billion pounds (2 billion Kg) and, in 1987, this increased to 5.3 billion pounds (2.4 billion Kg). Because of the worldwide increase in cheese consumption, cheese production will continue to increase not only in the United States, but also in other countries, especially in dairy-rich countries such as Europe and New Zealand.

Cheese varieties have been grouped in different ways. Examples of several varieties based on starter cultures used and some important secondary flora are listed here.

Unripened Cheese

Soft

- Cottage cheese with starters *Lac. lactis* ssp. *lactis* and *cremoris* and *Leuconostoc mesenteroides* ssp. *cremoris*.
- Mozzarella cheese with starters *Str. thermophilus* and *Lab. delbrueckii* ssp. *bulgaricus*.

Ripened Cheese

Soft

- Brie cheese with starter *Lac. lactis* ssp.; *Penicillium* sp. and yeasts are secondary flora.

Semihard

- Gouda cheese with starters *Lac. lactis* ssp. and *Leuconostoc* spp.; dairy *Propionibacterium* may be secondary flora.
- Blue cheese with starter *Lac. lactis* ssp., *Leuconostoc* spp.; *Penicillium roquefortii*, yeasts, and micrococci are secondary flora.

Hard

- Cheddar cheese with starters *Lac. lactis* ssp.; some lactobacilli and pediococci (and probably enterococci) are secondary (or associative) flora.

- Swiss cheese with starters *Str. thermophilus*, *Lab. helveticus*, and dairy *Propionibacterium* spp.; enterococci can be secondary (or associative) flora.
- Only cottage, Cheddar, Swiss, and blue cheeses are further discussed to understand the microbiological aspects of unripened and ripened cheeses.

Microbiology of Cottage Cheese[6]

Characteristics

Cottage cheese is made from low-fat or skim milk and has a soft texture with ca. 80% moisture. It is unripened and has a buttery aroma due to diacetyl (along with lactic acid and little acetaldehyde).

Processing (from Skim Milk)

1. Pasteurized, cooled to 70°F (22.2°C), starter added, and incubated for 12 h at pH 4.7.
2. Firm curd set, cut in cubes, and cooked at 125°F (51.7°C) for 50 min or more.
3. Whey drained off, stirred to remove more to get dry curd.
4. Salted, creamed, and preservative added.
5. Packaged and refrigerated.

Starters (Controlled Fermentation)

Frozen concentrate for direct vat set can be used. Mixed strains of *Lac. lactis* ssp. *cremoris* and *lactis* are predominantly used for acid. *Leu. mesenteroides* ssp. *cremoris* can be added initially (in which case citrate is added to milk), mainly for diacetyl. *Lac. lactis* ssp. *lactis* biovar diacetylactis can be used for diacetyl, but not inoculated in milk because of formation of too much CO_2 that causes curd particles to float. Instead, it is grown separately in cream, which is then used to cream the dry curd.

Growth, Biochemistry, and Genetics

Growth, biochemistry, and genetics (including current strain improvements) are similar to those described for the microbiology of buttermilk (see Section Microbiology of Cultured Buttermilk Fermentation).

Microbial Problems

- *Slow Growth.* Weak or loss of Lac^+ phenotype, phage attack or less viable cells in frozen concentrate, antagonistic effect among starter strains for reasons described previously.
- *Floatation of Curd.* Caused by too much CO_2 production by flavor-producing starters.
- *Harsh Flavor.* Caused by more acetaldehyde and less diacetyl production from metabolic imbalance and reduced environment.
- *Low Yield.* Caused by partial proteolysis of casein by heat-stable proteinases produced in refrigerated raw milk by psychrotrophs such as *Pseudomonas* spp.
- *Flavor Loss.* Caused by reduction of diacetyl to acetoin in a reduced environment as well as by growth of undesirable bacteria during storage, such as *Pseudomonas* spp.
- *Spoilage.* Because of high moisture and low acid content, spoilage psychrotrophic bacteria (such as *Pseudomonas* spp.), yeasts, and molds can grow during storage. Preservatives such as sorbates can be used to extend shelf life under refrigerated storage (also see Chapter 19).

Microbiology of Cheddar Cheese[6]

Characteristics

Cheddar cheese is made from whole milk, contains less than 39% moisture, 48% fat, is generally orange-yellow in color (due to added color, annatto), and ripened. Smoothness of texture and intensity of characteristic flavor vary with the starters used and the period of ripening. The typical flavor is the result of a delicate balance among flavor components produced during ripening through enzymatic breakdown of carbohydrates, proteins, and lipids in unripened cheddar cheese.

Processing

1. Pasteurized; color (annatto) and starter added.
2. Incubated at 86°F (30°C) for acidity to increase by 0.2% ; rennet added.
3. Incubated for coagulation (ca. 30 min) and cut in cubes.
4. Cooked at 100°F (37.8°C), whey drained, cheddaring to lose whey and curd to mat.
5. Milled, salted, put in form, pressed for 16 h to drain whey, and removed.
6. Dried for 5 days at 50°F (10°C), waxed, vacuum packaged, and cured at 40°F (4.4°C) for 2–12 months.

Starters (Controlled Fermentation)

Starters are selected mixed strains of *Lac. lactis* ssp. *cremoris* or *lactis*. *Leuconostoc*, which may be added for flavor. Starters can be used as frozen concentrates (direct vat set).

Growth

Growth is accomplished by incubating at 86°F (30°C) for mesophilic starters to start early growth and produce lactic acid to a 0.2% level by 60 min. This is important for the coagulation of casein to occur rapidly following the addition of rennin. During curing, cells of the starter (also of secondary flora) slowly die and release intracellular enzymes in the cheese.

Biochemistry

A large number of biochemical reactions occur during the different stages of processing, from the initial incubation to the end of ripening, many not yet properly understood. Some are described here.

Initially, lactose metabolism produces lactic acid as well as some diacetyl, acetate, ethanol, and acetaldehyde, especially under aerobic conditions. Rennin destabilizes casein to produce paracasein that coagulates at low acidity and low temperature. In addition, extracellular proteinases and peptidases of the starter cultures metabolize milk proteins to peptides and amino acids and transport them into the cells. Normally very little, if any, lipolysis occurs due to microbial growth.

During curing, breakdown of remaining lactose in the curd continues. However, a large change occurs in proteins and other nitrogenous compounds. By the actions of rennin (retained in curd) and cellular exo- and endoproteinases and peptidases, peptides of different sizes and amino acids are released. Further breakdown of amino acids produces hydrogen sulfide, methanethiol and related sulfur compounds, amines, and other products. Lipids also undergo lipolysis, releasing fatty acids, including the C4–C8 fatty acids (which are present in milk fat). Other reactions produce lactones, ketones, and thioesters. Some of the reactions are nonenzymatic. The typical Cheddar cheese flavor is the result of a delicate balance among the products produced from carbohydrate, protein, and lipid breakdown during processing and curing. The concentrations of these components change with curing time.

Some secondary microflora that survive heating or gain entrance later in the milk and curd during processing have definite roles in the flavor of Cheddar cheese. These include some enterococci,

lactobacilli, pediococci, micrococci, and some Gram-negative rods. They probably contribute to the typical intense flavor that could be missing in cheese made with defined starter strains only. Some of these flora are known to produce several flavor compounds rather rapidly and at higher concentrations (e.g., volatile fatty acids and H_2S).

Genetics

Phenotypic characteristics, as described before for these species, should be considered. Lac^+ strains capable of producing lactic acid rapidly at the initial stage are preferred. Also, strains with weak proteinases (Pro^+) activity are desirable because they do not cause rapid proteolysis with the accumulation of some peptides and the appearance of bitter flavor in the products. In mixed starters, they should not have an antagonistic effect. Also, the strains should preferably be resistant to multiple phages.

Microbial Problems

Bitter flavor in Cheddar cheese, especially in the aged product, results from the accumulation of bitter peptides that are ca. 1000–12,000 Da and rich in hydrophobic amino acids. Starters capable of hydrolyzing proteins rapidly (fast starters) tend to produce bitter peptides more than slow starters do. Their enzymes hydrolyze proteins quickly, releasing large amounts of peptides that are subsequently hydrolyzed slowly to smaller peptides and amino acid by peptidases, resulting in the accumulation of peptides. Because they are hydrophobic, bitter peptides are generally hydrolyzed slowly, causing them to accumulate. Use of slow starters for protein breakdown at a slow rate or treatment of cheese with peptidase, or both, are effective in reducing bitterness (also see Chapter 19).

Mold growth on the surface or in air pockets inside the cheese can occur after removing the packing material from cheese. The spores are generally present in the raw material or get in the product during processing and before sealing. It is not possible to determine from the colonial morphology whether they are mycotoxin producers. It is better not to consume cheese with heavy growth.

Staphylococcus aureus, following contamination of milk after heating, can grow during processing Cheddar cheese and produce enterotoxins. The toxins remain in the cheese even after the death of cells during curing. Food poisoning can occur from consuming such cheese (even if they are heated in some preparations).

Biological amines (histamine from histidine, and tyramine from tyrosine) can form by decarboxylation (by starter decarboxylases) of some amino acids, especially in cheese ripened for a long time. Allergic reactions can occur from consuming such cheese. Secondary flora may have an important role in such amine formation.

Microbiology of Swiss Cheese[6]

Characteristics

Swiss cheese is made from partially skimmed milk (cow's milk) and coagulated with acid and rennin; it is hard and contains ca. 41% moisture and 43% fat. The cheese should have uniformly distributed medium-sized eyes (openings). It has a sweet taste, due to proline, and a nutty flavor.

Processing

1. Pasteurized, starter added, and incubated at 90°F (32.2°C) for acidity to increase by 0.2%.
2. Rennin added, incubated for firm coagulation, cut in 1/8-in. cubes, and cooked for 1 h at 125°F (51.7°C).

3. Whey removed, curd pressed for 16 h, cut in blocks, and exposed in brine at 55°F (12.8°C) for 1–3 days.
4. Surface dried, vacuum packaged, stored for 7 days at 55°F (12.8°C), and transferred to 75°F (23.9°C) for 1–4 weeks.
5. Cured at 37°F (2.8°C) for 3–9 months.

Starters (Controlled Fermentation)

Starters are *Str. thermophilus* and *Lab. helveticus* as primary for acid and *Propionibacterium* sp. as secondary for eye formation, taste, and flavor.

Growth

Primary starters grow during fermentation, cooking, and processing; growth of *Str. thermophilus* is favored. Propionibacteria grow well during storage at 75°F (23.9°C). During curing, none of the starters grow. The cells slowly die and release intracellular enzymes.

Biochemistry

Lactose is hydrolyzed by β-galactosidase of both lactic acid bacteria and metabolized by the EMP pathway. *Str. thermophilus* produces L(+)-lactic acid and *Lab. helveticus* produces D(−)-lactic acid. Some acetate also forms. Production of large amounts of lactate is very important to facilitate growth of propionibacteria. During storage at 75°F (23.9°C), propionibacteria convert lactate (by lactate dehydrogenase) to pyruvate, which is then converted to propionic acid, acetate, and CO_2. Eye formation occurs from the production of CO_2, and the size and distribution of eyes depends on the rate of CO_2 production. Proteins are hydrolyzed by rennin, intracellular proteinases, and peptidases of starters, especially propionibacteria, resulting in the production of small peptides and amino acids, which impart the nutty flavor and sweet taste (sweet taste is attributed to a relatively high concentration of proline produced by propionibacteria). Very little lipolysis occurs during curing.

Genetics

Rapid production of lactate in large amounts from lactose by lactic acid bacteria and their resistance to phages (some *Lab. helveticus* strains have temperate phages that are activated at high processing temperature, causing starter failure) are important considerations. *Propionibacterium* strains should produce CO_2 at proper rates for desirable numbers and sizes of eye formation (uniform distribution of medium-size eyes preferred).

Microbial Problems

Spores of *Clostridium tyrobutyricum*, present in raw milk or entering as contaminants, can germinate, grow, and cause rancidity and gas blowing in this low-acid cheese. Nisin has been used as a biopreservative to control this problem (also see Chapter 19).

Microbiology of Blue Cheese[6]

Characteristics

Blue cheese is a semihard (46% moisture, 50% fat), mold-ripened cheese made from whole milk (cow's milk). It has a crumbly body, mottled blue color, and sharp lipolytic flavor.

Processing

1. Homogenized, pasteurized, starter added, and incubated at 90°F (32.2°C) for acidity to increase to 0.2%.
2. Rennet added, incubated for firm set, cut, cooked at 100°F (37.8°C), and whey drained.
3. Curd collected in hoop, drained 16 h, and salted in brine for 7 days.
4. Spiked to let air get inside. Mold spores added, stored at 50°F (10°C) in high humidity for 4 weeks.
5. Stored at 40°F (4.4°C) for curing for 3 months.

Starters and Growth (Controlled Fermentation)

Lac. lactis ssp. *cremoris* or *lactis* and *Leu. cremoris* or *lactis* serve as primary starters. *Pen. roquefortii* spores serve as secondary starters. The lactic starters grow until curing, and from lactose, they produce lactate, diacetyl, acetate, CO_2, and acetaldehyde. Mold spores, during storage at 50°F (10°C) in high humidity, germinate quickly, produce mycelia, and spread inside to give the mottled green appearance. Their growth continues during curing. Puncturing the inside of the cheese helps remove CO_2 and let's air in to help the growth of molds.

Biochemistry, Genetics, and Problems

Lactococcus species produce mainly lactic acid from lactose, whereas *Leuconostoc* species produce lactic acid, diacetyl, CO_2, and acetate. Proteolysis is quite limited by the lactic starters. Molds produce extracellular lipases and proteinases and cause lipolysis and proteolysis during curing. Fatty acids are both oxidized and reduced to produce methyl ketone and D-lactone, respectively. These, along with volatile fatty acids, contribute to the sharp flavor of blue cheese.

The desired genotypes of the lactic acid bacteria starters used have been previously described. A white variant of the mold has been isolated and used to produce this cheese without the blue mottled color. *Pen. roquefortii* strains that produce mycotoxins have been identified. Strains that do not produce mycotoxins need to be selected. Ripening for a long time can lead to formation of biologically active amines from some amino acids (e.g., histamine from histidine).

Accelerated Cheese Ripening[7]

During curing of hard and semihard cheeses, milk components are degraded through enzymatic (from starters and secondary flora) and nonenzymatic reactions. This is necessary to develop a desirable flavor of these cheeses. However, because of a long storage time, it is not quite economical. Thus, methods are being studied that will speed up the ripening process. Some of these methods are briefly described.

Curing at High Temperature

Because enzymatic (also nonenzymatic) reactions increase as the temperature is increased, studies were performed to cure cheese above the usual 5–6°C (40°F). Ripening some cheeses at 13–16°C (55–61°F) reduced the curing time by 50% or more. However, at higher temperature, growth of spoilage bacteria has been a problem in some cheeses, and there is some concern about the growth of foodborne pathogens.

Addition of Enzymes

As intracellular enzymes of starters have an important role in curing, enzymes obtained from cell lysates of starter-culture bacteria have been added to increase the rate of curing. In Cheddar cheese, the curing time has been substantially reduced, but has resulted in bitter flavor.

Slurry Method

Cheddar cheese slurry has been prepared by mixing water with cheese to 40% solids (in place of the usual 60%). The slurry is incubated at 30°C for 4–5 days with agitation. This method increases the flavor greatly and the product can be used to make processed cheeses. The major disadvantages are the inability to properly control the enzymatic reactions to produce uniform products and possible growth of spoilage and pathogenic bacteria.

Novel Methods

In recent years, application of a suitable bacteriocin (of lactic acid bacteria) to cheese or exposing cheese to high hydrostatic pressure to lyse the cells of starter cultures is being studied to enhance ripening by the released intracellular enzymes.

FERMENTED MEAT PRODUCTS

Types

Fermented meat products are produced by first mixing meat, fat, salt, sugar, curing agents, and spices; filling the mixture in a casing; and fermenting it either naturally or by adding (during mixing) selected starter-culture bacteria.[3] The acids produced by the starters during fermentation and the curing agents used help control the growth of pathogenic and spoilage bacteria that might be present in the meat. Depending on the type, the fermented products may be dried to reduce A_w or smoked or heated to ensure the safety and shelf life of the products.

Meat fermentation probably originated in the Mediterranean countries and later spread to European countries and North America. In the United States, semidry sausages are most popular, although some dry sausages are also produced. Following fermentation, semidry sausages are heated (also sometimes smoked) before consumption. For dry sausages, following cooking, the products are dried to reduce the A_w. Even now, fairly large amounts of fermented sausages in the United States are produced by natural fermentation, especially those produced by small processors. However, more processors now use selected starter cultures and controlled fermentation. Commercial starter cultures are available as both frozen and freeze-dried concentrates for direct inoculation in the meat mixture.

Semidry and dry sausages include many types, such as pepperoni, Genoa salami, hard salami, summer sausage, beef sticks, beef logs, thuringer, cervelat, and Italian salami. Most are made with beef and pork, but in recent years, some have been made with meat from chicken and turkey. The microbiology of semidry sausages is described here.

Microbiology of Semidry Sausages

Characteristics

Semidry sausages include summer sausage, thuringer, and semidry salami. The average composition is ca. 30% fat, 20% protein, 3% minerals (salts), and 47% water. They have a tangy taste with a desirable flavor imparted by the combined effect of lactate, acetate, and diacetyl, and some breakdown components from proteolysis and lipolysis. The use of spices also contributes to the flavor. Those containing nitrite have a pinkish color in contrast to the grayish color in products without it.

Processing

1. Meat, salts, glucose, cure, spices, and starter mixed uniformly.
2. Stuffed in casings, fermented at 85–110°F (29.4–43.3°C) with 80–90% relative humidity.
3. Incubated until the pH drops to ca. 5.2–4.6, cooked to 140°F (60°C) internal temperature, and cooled to 50°F.

4. Stored at 40–50°F (4.4–10°C) for 3–4 days, vacuum-packaged, and consumed directly.
5. Cures contain nitrite to give a final concentration of ca. 100 ppm. Fermentation can be carried out in a smokehouse. Fermentation time is usually 8–12 h, during which the pH is dropped to desired level.

Starters (Controlled or Natural Fermentation)

In controlled fermentation, frozen or dried concentrates are used directly at 10^{6-7} cells/g mix. Starters should not be mixed with salt, cure, or spices as it can kill injured cells. Instead, they should be thawed and immediately put into the meat. Starters vary, depending on the fermentation temperature and final pH of the product desired. For high temperature and low pH, *Pediococcus acidilactici* strains are preferred; for low temperature and high pH, *Lab. plantarum* strains are preferred. *Ped. pentosaceus* strains can be used under both conditions. Some starters can have both *Pediococcus* and *Lactobacillus* species. In addition, selected *Micrococcus* spp. or *Sta. carnosus* strains are added as secondary flora for their beneficial effects on desired product color.

In naturally fermented sausages, *Lab. sake*, *Lab. curvatus*, and *Leuconostoc* spp. present in raw materials are important starter bacteria, especially when fermentation is set at lower temperatures (60–70°F [15.6–21.1°C]) for several days and the final pH reached is not below 5.0.

Growth

Because the raw meat used may contain pathogens and spoilage bacteria, it is extremely important that starter culture grows rapidly and produces acid in large amounts to reduce pH from the initial 5.7 to ca. 5.3 very quickly to retard their growth. This can be achieved by adding large numbers of active starter cells, adding dextrose to the mix, and setting the temperature of fermentation optimum for the starters used. The optimum growth temperatures for *Ped. acidilactici*, *Ped. pentosaceus*, and *Lab. plantarum* are ca. 40, 35, and 30°C (104, 95, and 86°F), respectively. *Micrococcus* spp. and *Sta. carnosus* grow well at ca. 32.2°C (90°F). Cooking to an internal temperature of 60°C (140°F) kills *Lab. plantarum* and probably *Ped. pentosaceus*, but probably not *Ped. acidilactici*, *Micrococcus*, or *Sta. carnosus*. However, low pH and low A_w prevent their growth in the finished products.

Biochemistry

Both pediococci are homolactic fermentors and metabolize glucose to mainly lactic acid (DL forms), with small amounts of acetate and diacetyl. *Lab. plantarum*, being facultatively heterofermentative, metabolizes glucose to principally lactic acid (DL); however, it can also produce substantial amounts of acetate, ethanol, and diacetyl. Strains of all three species can produce H_2O_2, which can discolor the product by oxidizing myoglobin during fermentation. *Micrococcus* spp. or *Sta. carnosus* have catalase that can destroy H_2O_2. *Micrococcus* spp. or *Sta. carnosus* and some strains of *Lab. plantarum* can also reduce nitrate to nitrite. If nitrate is used in place of nitrite in cure, these bacteria can produce nitrite and help develop the agreeable pinkish color of the product. If the products are cured or stored for long periods of time, some of the intracellular enzymes of the lysed cells of starters are able to cause proteolysis and lipolysis and produce biologically active amines (such as histamine).

Genetics

Rapid acid-producing lactic acid bacterial strains at temperatures of fermentation and non-H_2O_2 producers are desired. Strain selection can also be done for nonproducers of biogenic amines. Strains producing bacteriocins can be used to control pathogens and spoilage bacteria. *Ped. acidilactici* strains that cannot hydrolyze sucrose (Suc^-) can be used to produce sweet and sour products by

supplementing the meat mixture with both glucose and sucrose. Exopolysaccharides-producing strains can improve the texture of products.

Microbial Problems

Slow acid production can be a serious problem if the starters used are not metabolically active or have lower numbers of viable cells or due to other factors such as low glucose and high salts in the mix. Sour or no flavor can occur if the starter, especially *Ped. acidilactici*, grows very rapidly and reduces the pH to below 4.5. Gas formation can occur because of growth of *Leuconostoc* spp. during fermentation and during storage in vacuum packages. *Leuconostoc* spp. are usually present in raw meat. Pathogens, when present in meat, can grow if acid production is slow during fermentation. Acid-resistant pathogens can also survive in the products and cause health hazards. During long storage or curing, biogenic amines can form. Also, mycotoxin-producing molds can grow on the product surface during curing (also see Chapter 19).

FERMENTED VEGETABLE PRODUCTS

Almost all vegetables can be fermented through natural processes, because they harbor many types of lactic acid bacteria. Worldwide, many types of vegetables are fermented, mostly in small volumes. However, some are produced commercially. Vegetable fermentation originated in the early years of human civilization and even now is widely used by many cultures. Examples of some fermented products and vegetables used currently for fermentation are sauerkraut (from cabbage), olives, cucumbers, carrots, celery, beans, peas, corn, okra, tomatoes, cauliflower, peppers, onions, citron, beets, turnips, radishes, chard, Brussels sprouts, and their blends. Most are produced by natural fermentation; however, some, such as cucumbers, are currently produced in limited amounts by controlled fermentation. Production of sauerkraut by natural fermentation is described here as an example.

Microbiology of Sauerkraut[3]

Characteristics

Sauerkraut is produced by fermenting shredded cabbage. The product has a sour taste with a clean acid flavor.

Processing

1. Cabbage cleaned, trimmed, and shredded fine and uniform.
2. Packaged tight to exclude air in vat, and layered with salt (2.25%).
3. Top covered to exclude air, and fermented at 18°C (65°F) for 2 months.

Fine shredding helps the sugars (3–6%) come out of cabbage cells. Tight packaging helps create an anaerobic condition, thus preventing the growth of aerobes. Salt stimulates growth of some lactic acid bacteria, and discourages the growth of some undesirable bacteria and pectinase (in cabbage) action. The top is covered to exclude air and prevent growth of some aerobes. Fermentation at 18°C (65°F) discourages the rapid growth of some undesirable bacteria (facultative anaerobic or anaerobic), but encourages the growth of desirable lactic acid bacteria. Natural inhibitors in cabbage also discourage the growth of undesirable Gram-negative and Gram-positive bacteria.

Starters (Natural) and Growth

The raw material has a large number of undesirable organisms and a small population of lactic acid bacteria (<1%). Among the lactic acid bacteria, most are *Lactococcus* spp. and *Leuconostoc* spp.,

and a small fraction is *Lactobacillus* spp. and *Pediococcus* spp. During fermentation, sequential growth of these lactic acid bacteria occurs. The presence of 2.25% salt, large amounts of fermentable sugars (sucrose, hexoses, pentoses), absence of oxygen, and low fermentation temperature facilitate *Leuconostoc* spp., primarily *Leu. mesenteroides*, to grow rapidly. When the acidity has reached to ca. 1% (as lactic acid), growth of *Leu. mesenteroides* slows down. Then *Lab. brevis* starts growing rapidly until acid production reaches ca. 1.5%. Then *Ped. pentosaceus* takes over and increases the acidity to ca. 1.8%. Finally, *Lab. plantarum* starts growing and brings the acid level to ca. 2%.

Biochemistry

Leuconostoc spp. metabolize sucrose, hexoses, and some pentoses in the raw material to lactate, acetate, ethanol, CO_2, and diacetyl. *Lab. brevis* (obligatory heterofermentative, such as *Leuconostoc* spp.) ferments sucrose, hexoses, and pentoses to products similar to those by *Leuconostoc* spp. *Ped. pentosaceus* metabolizes hexoses to form mainly lactic acid and some pentoses to lactic acid, acetate, and ethanol. *Lab. plantarum* also produces products from sucrose, hexoses, and pentoses similar to those by *Ped. pentosaceus*. *Leuconostoc* spp. produces D(–)-lactate, whereas the other three species produce DL-lactate.

The characteristic flavor of sauerkraut is the result of the combined effects of lactate, acetate, ethanol, CO_2, and diacetyl in proper amounts.

Genetics

If starters are developed in the future for controlled fermentation, some of the characteristics that will be important are rapid acid production, good flavor production, low CO_2 production (to reduce gassy defect), and the ability to produce antimicrobial compounds, especially against pathogens.

Microbial Problems

Off-flavor, soft texture, and discoloration of sauerkraut can occur by growth of molds and yeasts when air is not completely excluded. A slimy texture of sauerkraut can occur due to overgrowth of *Leuconostoc* spp. in the presence of sucrose; they metabolize fructose but synthesize dextrans from glucose (also see Chapter 19).

CONCLUSION

Food fermentation involves use of specific starter cultures in different types of foods as starting materials. The starting materials can be milk, meat, fish, fruits, vegetables, grains, seeds, and others. They are used separately or in combination. Current trend is to use controlled fermentation in place of natural fermentation. Improvement of strains has helped overcome some of the drawbacks of controlled fermentation. Information on genomes will help improve strains in the future.

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QUESTIONS

1. Define and discuss the advantages of different methods used to produce fermented foods.
2. Briefly discuss the factors to be considered while selecting milk to produce fermented dairy products.
3. List the characteristics of buttermilk and describe how they help select specific starter cultures.
4. Describe the symbiotic growth of starter cultures used in the production of yogurt. What genetic improvement will be important in these strains?
5. Most commercial yogurt now contains *Lab. acidophilus* and *Bifidobacterium* with regular yogurt bacteria. However, they are added to yogurt after fermentation. Briefly discuss the reasons.
6. In creamed cottage cheese, cream ripened with diacetyl-producing lactic starter is often used instead of using this starter during fermentation. Discuss the reasons.
7. Discuss the biochemical basis of the typical flavor of Cheddar cheese. What is the basis of bitter flavor formation? How can it be reduced by genetic improvement of the starters?
8. Discuss the role of starter cultures in the development of desired characteristics in Swiss cheese.
9. Describe the role of molds in blue cheese production. What precautions should be taken in selecting the mold strains?
10. Discuss the methods used to accelerate cheese ripening.
11. List the primary and secondary starter cultures used in controlled fermentation of semidry sausages and discuss their specific roles.
12. Describe the sequential growth of lactic acid bacteria during the natural fermentation of sauerkraut.