Consortium for Educational Communication

Module
on
Role Of Microbes In Foods

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1.TEXT

2.General introduction

Preservation of food includes the use of fermentation of perishable raw materials that has been used by man since the Neolithic period (around 10000 years BCE). The scientific rationale behind fermentation started with the identification of microorganisms in 1665 by Anton Van Leeuwenhoek and Hooke. The role of a sole bacterium, “Bacterium” lactis (Lactococcus lactis), in fermented milk was shown around 1877 by Sir John Lister. Fermentation, from the Latin word fervere, was defined by Louis Pasteur as “La vie sans l’air” (life without air). Fermentation can be defined as the metabolic process of deriving energy from organic compounds by the use of different microorganisms without the involvement of an exogenous oxidizing agent turning out the complex raw materials into simpler products. Since the dawn of civilisation, methods for the fermentation of milk, meat, fruits and vegetables have been described with earliest records dating back to the middle of the 6000 BC. However by the middle of the 19th century a common understanding about the role of microorganisms came into existence that resulted in their efficient and controlled use in fermentation. The microorganisms involved in fermentation of foods from practical point of view are divided into three groups i.e., bacteria, yeasts and molds. Bacteria important in food microbiology may be divided into groups according to the product of fermentation for example lactic acid bacteria, acetic acid bacteria, propionic acid bacteria. Lactic acid bacteria are widely used in dairy industry and acetic acid bacteria in vinegar production. Molds are generally concerned for their use in cheese making while as yeasts are widely used for their ability to ferment sugars to ethanol and CO_2. Milk fermentations must undoubtedly be among the oldest of all fermented foods. Milk obtained from a domesticated cow or camel or goat, some thousands of years ago, would have been fermented within hours by endogenous lactic acid bacteria, creating a yogurt-like product. Milk is particularly suitable as a fermentation substrate owing to its carbohydrate-rich, nutrient-dense composition. Fresh bovine milk contains 5% lactose and 3.3% protein and has a water activity near 1.0 and a pH of 6.6 to 6.7, perfect conditions for most microorganisms. Lactic acid bacteria are saccharolytic and fermentative, and, therefore, are ideally suited for growth in milk.

3.Role of fermentation in food processing

Fermentation plays different roles in food processing.

Major roles considered are:

(1) Preservation of food through formation of inhibitory metabolites such as organic acid (lactic acid, acetic acid, formic acid, propionic acid), ethanol, bacteriocins, etc.,

often in combination with decrease of water activity (by drying or use of salt).

(2) Improving food safety through inhibition of pathogens or removal of toxic compounds.

(3) Improving the nutritional value.

(4) Food fermentation also serves an important purpose that is to enrich the diet through development of a diversity of flavors, aromas, and textures in food substrates and also enriches the food substrates with protein, essential amino acids, and vitamins and subsequently eliminates antinutrients thereby reducing cooking time and the associated use of fuel.

4. Role of microbes in different food products

4.1. Role of bacteria in fermented milk products

4.1.1.Buttermilk as the name suggests, is the milk that remains after cream is churned for the production of butter. The commercial product is usually prepared by inoculating skim milk with a lactic or buttermilk starter culture and holding until souring occurs. The resulting curd is broken up into fine particles by agitation, and this product is termed cultured buttermilk. Cultured sour cream is produced generally by fermenting pasteurized and homogenized light cream with a lactic starter. Among the flavor-producing bacteria used in buttermilk cultures are L. lactis subsp. Lactis (diacetyl-producing strains) and Leuconostoc mesenteroides subsp. cremoris or Leuc. lactis.

4.1.2. Yogurt

Yoghurt is a fermented milk product produced with yogurt starter, which is a mixed culture of S. salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus in a 1:1 ratio. Both these microorganisms are primarily responsible for initial acid production at a higher rate than that produced by either when grown alone.The more acetaldehyde (the chief volatile flavor component of yogurt) is produced by L. delbrueckii subsp. bulgaricus when grown in association with S. salivarius subsp. thermophilus.

4.1.3.Kefir

Kefir is a fermented milk product prepared by the use of kefir grains, which contain one or more bacterial species of the genera Acetobacter, Lactobacillus, Lactococcus, Leuconostoc, and one or more yeast species of the genera Candida, Kluyveromyces, and Saccharomyces. These symbionts are held together by coagulated protein. The important Lactobacillus spp. in kefir are: L. kefiri, L. parakefiri, L. Kefiranofaciens subsp. kefiranofaciens, and L. kefirgranum. The last two are responsible for the production of kefiran (a water-soluble polysaccharide), which accounts for about 24% of kefir grains.

4.1.4.Acidophilus milk

Acidophilus milk is produced by the inoculation of an intestinal implantable strain of L. Acidophilus into sterile skim milk. The inoculum of 1–2% is added, followed by holding the product at 37 ◦ C until a smooth curd develops.

4.1.4.Butter

Butter contains around 15% water, 81% fat, and generally less than 0.5% carbohydrate and protein. The main source of microorganisms for butter is cream. The biota of whole milk may be expected to be found in cream because as the fat droplets rise to the surface of milk, they carry up microorganisms chief ones including the LAB.

4.1.5.Cheeses

Most but not all cheeses result from a lactic fermentation of milk. Milk is prepared and inoculated with an appropriate lactic starter containing the lactic acid bacteria. The starter produces lactic acid, which, with added rennin, gives rise to curd formation. In case of Swiss cheese, a mixed culture of L. delbrueckii subsp. bulgaricus and S. salivarius subsp. thermophilus is usually employed along with a culture of Propionibacterium shermanii or P. freundenreichii added to function during the ripening process, in flavor development and eye formation. The genus Brachybacterium enters the list with two species, B. alimentarium and B. tyrofermentans. Both species have been characterized as important and beneficial components of the surface microbiota of Gruyère and Beaufort cheese. Microbacterium enters the list with one species, M. gubbeenense. M. gubbeenense is a component of the traditional red smear surface culture of surface ripened cheeses. This species is like the two other Brevibacterium species, B. linens and B. casei, that form the components of the red smear ripening microbiota for surface ripened cheeses.

4.1.6.Fermented meat products

Fermented meat sausages are produced generally as dry or semidry products, although some are intermediate. Dry sausages contain 30–40% moisture. In their preparation, curing and seasonings are added to ground meat, followed by its stuffing into casings and incubation for varying periods of time at 80–95◦F. Following incubation Lactobacillus predominate overall, with plantarum being the most commonly isolated species. L. brevis and L. buchneri increase during the six-day incubation period as a result of changes in pH and relative humidity Semidry sausages are prepared in essentially the same way as dry sausages but are subjected to less moisture treatments. Micrococcus is represented with the two species, M. luteus and M. lylae which are used for meat fermentation. Weissella species are also used for fermentation of meat. Among the enterococci, Enterococcus faecalis plays an important role owing to its use in dairy, meat, vegetables fermentation and probiotics Halomonas elongata, a new species of the family Enterobacteriaceae also performs a relevant role in meat fermentation

4.1.6.Fish Products

The fish sauces are described as the products with clear, dark-brown colour with a distinct aroma and flavor. The halophilic aerobic spore formers are the predominant microorganisms of these products. Lower numbers of streptococci, micrococci, and staphylococci are also found, and they, along with the Bacillus spp., are apparently involved in the development of flavor and aroma. The pH from start to finish ranges from 6.2 to 6.6 with the NaCl content around 30% over the 12-month fermentation period. These parameters, along with the relatively high fermentation temperature, result in the growth of halophilic aerobic spore formers as the predominant microorganisms of these products.

4.1.7.Idli

Idli is a fermented bread-type product common in southern India. It is made from rice and black gram mung (urd beans). These two ingredients are soaked in water separately for 3–10 hours and then ground in varying proportions, mixed, and allowed to ferment overnight. The fermented and raised product is cooked by steaming and served hot. It is said to resemble a steamed, sourdough bread. During the fermentation, the initial pH of around 6.0 falls to values of 4.3–5.3.Most of the organisms consist of Gram-positive cocci or short rods, with L. mesenteroides being the single most abundant species, followed by E. faecalis. The leavening action of idli is produced by L. mesenteroides. This is the only known instance of a lactic acid bacterium having this role in a naturally fermented bread. L. mesenteroides reaches its peak at around 24 hours, with E. faecalis becoming active only after about 20 hours. Other probable fermenters include L. delbrueckii subsp. delbrueckii, L. fermentum, and Bacillus spp. Only after idli has fermented for more than 30 hours does P. cerevisiae become active. The product is not fermented generally beyond 24 hours because maximum leavening action occurs at this time and decreases with longer incubations. When idli is allowed to ferment longer, more acidity is produced.

4.1.8.Sauerkraut

Sauerkraut is a fermentation product of fresh cabbage. The starter for sauerkraut production is usually the normal mixed biota of cabbage. The addition of 2.25–2.5% salt restricts the activities of Gram-negative bacteria, while the lactic acid rods and cocci are favored. Leuconostoc mesenteroides, Lactobacillus plantarum, and Leuconostoc fallax are the three most dominant lactics in sauerkraut production, with the two Leuconostoc species having the shorter generation time and the shorter life span. The activities of the cocci usually cease when acid content increases to 0.7–1.0%. The final stages of sauerkraut production are affected by L. Plantarum and L. brevis. P. cerevisiae and E. faecalis also contribute to product development.

Pickles

Pickles are fermentation products of fresh cucumbers, and as is the case for sauerkraut production, the starter culture generally consists of the normal mixed biota of cucumbers. In the natural production of pickles, the following lactic acid bacteria are involved in the process in order of increasing prevalence: L. mesenteroides, E. faecalis, P. cerevisiae, L. brevis, and L. plantarum. of these, the pediococci and L. plantarum are the most involved, with L. brevis being undesirable because of its capacity to produce gas. L. plantarum is the most essential species in pickle production, as it is for sauerkraut. The controlled fermentation method employs acidification with acetic acid, the addition of sodium acetate, and inoculation with P. cerevisiae and L. plantarum, or the latter alone

5.Combined role of Yeasts and Molds.

The barm used for bread making generally contains a mixture of yeasts and lactic acid bacteria. In the case of sourdough bread, the yeast has been identified as Saccharomyces exiguus (Candida holmii and the responsible bacteria are Lactobacillus sanfranciscensis, L. fermentum, L. fructivorans, some L. Brevis strains, and L. pontis. The key bacterium is L. sanfranciscensis, and it preferentially ferments maltose rather than glucose and it requires fresh yeast extractives and unsaturated fatty acids. The souring is caused by acids produced by these bacteria, and the yeast is responsible for the leavening action, although some CO_2 is produced by the bacterial biota. Both acetic and lactic acids are produced, with the former accounting for 20–30% of the total acidity. Lactobacillus paralimentarius is another of the sourdough bacteria. Sourdoughs are placed into three groups and each has its unique fermentation period. Sourdoughs are fermented at 20–30 ^{0}C and the two primary organisms are L. sanfranciscensis and L. pontis. Type I doughs employ baker's yeast as a leavening agent, and the dominant lactics are L. pontis and L. panis. Type III doughs are dried products of traditional fermentations. The fermentation consortium in the traditional wheat product consists of L. sanfranciscensis, L. brevis, L. paralimentarius, and Weissella cibaria. Among other organisms found in some sourdough fermentations are Candida humilis, Dekkera bruxellensis, Saccharomyces cerevisiae, and Saccharomyces uvarum.

5.2. BEER, ALE, WINES, CIDER, AND DISTILLED SPIRITS

5.2 (a) Beer and Ale

Beer and ale are malt beverages produced by brewing. An essential step in the brewing process is the fermentation of carbohydrates to ethanol by fermenting yeasts. The malt is first prepared by allowing barley grains to germinate. The brewing process begins with the mixing of malt, malt adjuncts, hops, and water. The soluble part of the mashed materials is called wort. In some breweries, lactobacilli are introduced into the mash to lower the pH of wort through lactic acid production. The species generally used for this purpose is L. Delbrueckii. Wort and hops are mixed and boiled for 1.5–2.5 hours for the purpose of enzyme inactivation, extraction of soluble hop substances, precipitation of coagulable proteins, concentration, and sterilization. Following the boiling of wort and hops, the wort is separated, cooled, and fermented. The fermentation of the sugar-laden wort is carried out by the inoculation of S. cerevisiae. Ale results from the activities of top-fermenting yeasts, which depress the pH to around 3.8, whereas bottom-fermenting yeasts (S. "carlsbergensis" strains) give rise to lager and other beers with pH values of 4.1–4.2. The top fermentation is complete in 5–7 days and bottom fermentation requires 7–12 days. When lactic acid bacteria are present in beers, the lactobacilli are found more commonly in top fermentations, whereas pediococci are found in bottom fermentations.

5.2 (b) Wines

Wines are normal alcoholic fermentations of sound grapes followed by aging. Wine making begins with the selection of suitable grapes, which are crushed and then treated with a sulfite such as potassium metabisulfite to retard the growth of acetic acid bacteria, wild yeasts, and molds. The pressed juice, called must, is inoculated with a suitable wine strain of S. "ellipsoideus." The fermentation is allowed to continue for 3–5 days at temperatures between 21 ^{0}C and 32 ^{0}C, and good yeast strains may produce up to 14–18% ethanol. The wines are aged for a period upto 4 years. The final sedimentation of yeast cells is accelerated by reducing the temperature of the wine to around 25 ^{0}C and holding for 1–2 weeks. Malic and tartaric acids are two of the predominant organic acids in grape must and wine.

5.2 (c) Cider

Cider is a product that represents a mild fermentation of apple juice by naturally occurring yeasts. In making apple cider, the fruits are selected, washed, and ground into a pulp. The pulp "cheeses" are pressed to release the juice. The juice is strained and clarified called as cider. Six species of Acetobacter are noted that display a preference for sugars to be found early in the cider process, whereas those that are more acid tolerant and capable of oxidizing alcohols appear after the yeasts have converted most of the sugars to ethanol. Zymomonas spp., Gram negative bacteria that ferment glucose to ethanol, have been isolated from ciders, but they are presumed to be present in low numbers. Saccharobacter fermentatus is similar to Zymomonas in that it ferments glucose to ethanol and CO_2. Zymobacter palmae is an ethanol fermentor isolated from palm sap. It produces ethanol from mannitol.

5.2 (d) Distilled Spirits

Distilled spirits are alcoholic products that result from the distillation of yeast fermentations of grain, grain products, molasses, or fruit or fruit products. In its production the fruit mash is generally soured by inoculating with a homolactic such as L. delbrueckii subsp. delbrueckii, which is capable of lowering the pH to around 3.8 in 6–10 hours. The malt enzymes (diastases) convert the starches of the cooked grains to dextrins and sugars, and upon completion of diastatic action and lactic acid production, the mash is heated to destroy all microorganisms. It is then cooled to 24–27 ◦C and inoculated with a suitable strain of S. cerevisiae for the production of ethanol. Upon completion of fermentation, the liquid is distilled to recover the alcohol and other volatiles, and these are handled and stored under special conditions relative to the type of product being made. The fermentation process results in the sap becoming milky white in appearance due to the presence of large numbers of fermenting bacteria and yeasts. This product is unique in that the microorganisms are alive when the wine is consumed. The following genera of bacteria are the most predominant in finished products:

Micrococcus, Leuconostoc, “Streptococcus,” Lactobacillus, and Acetobacter. The predominant yeasts found were Saccharomyces and Candida spp., with the former being more common. The fermentation occurs over a 36- to 48-hour period, during which the pH of sap falls from 7.0 or 7.2. Fermentation products consist of organic acids in addition to ethanol. During the early phases of fermentation, Serratia and Enterobacter spp increase in numbers, followed by lactobacilli and leuconostocs. After 48-hour fermentation, Acetobacter spp. begin to appear. Sake is an alcoholic beverage commonly produced in Japan. The substrate is the starch from steamed rice, and its hydrolysis to sugars is carried out by Aspergillus oryzae to yield the koji. Fermentation is carried out by Saccharomyces sake over periods of 30–40 days, resulting in a product containing 12–15% alcohol and around 0.3% lactic acid.

6.Role of different yeasts in food fermentation.

Fungi can be added as fiber, vitamins and proteins to fermented foods or be consumed as single cell protein (SCP). Aspergillus species for instance Aspergillus oryzae and A. sojae are used in the production of miso and soya sauce fermentations. Aspergillus oryzae and A. niger are also used for production of sake and awamori liquors, respectively Aspergillus acidus is used for fermenting Puerh tea. Rhizopus oligosporus is used in the fermentation process of tempeh. Fusarium domesticum has been used for cheese fermentations (cheese smear). Fusarium solani a type of yeast was isolated from a Vacherin cheese, Fusarium venenatum is being used extensively for mycoprotein production.

7.Role of different Molds in food fermentation

7.1Cheese ripening

Penicillium camemberti is used for all white mold cheeses. Another mold P. caseifulvum grows naturally on the surface of blue mold cheeses and has a valuable aroma. Blue-mold cheeses are always fermented with Penicillium roqueforti, however, P. roqueforti itself can produce the secondary metabolites roquefortine C, mycophenolic acid and andrastin A in pure culture. Mycophenolic acid roquefortine C and andrastin A have been found in blue cheese. Verticillium lecanii strain has been listed as potentially useful for cheese ripening. For blue cheeses such as Roquefort, the curd is inoculated with spores of Penicillium roqueforti, which effect ripening and impart the blue-veined appearance characteristic of this type of cheese. In a similar fashion, either the milk or the surface of Camembert cheese is inoculated with spores of Penicillium camemberti. There are over 400 varieties of cheeses representing fewer than 20 distinct types, and these are grouped or classified according to texture or moisture content, whether ripened or unripened, and if ripened, whether by bacteria or molds. The three textural classes of cheeses are hard, semihard, and soft.. All hard cheeses are ripened by bacteria over periods ranging from 2 to 16 months. Semihard cheeses are ripened by bacteria over periods of 1–8 months. Blue and Roquefort are two examples of semihard cheeses that are mold ripened for 2–12 months. Brie and Camembert are examples of soft mold-ripened cheeses.

7.2 Meat products

The species Penicillium solitum is found on naturally fermented lamb meat and may be used as a starter culture. On the other hand Penicillium nalgiovense and few strains of Penicillium chrysogenum are used especially for mold-fermented salami.

8.Role of molds in the production of various other food products

Aspergillus species, notably *Aspergillus oryzae*, and *A. sojae*, are used to ferment soybean and wheat mixture to make soybean paste and soy sauce. These molds break down the starch in rice, barley, sweet potatoes, etc., by a process called saccharification during the production of sake.