

Role of Microbes in Food Processing, Fermented Beverages and Fermented Foods

6.1 Introduction

The development of fermented food is one of the oldest technologies known to man since the dawn of civilisation. Methods of fermentation of milk product like yogurt have been described in ancient scripture like the Vedas in India and the Bible. Records of fermentation of meats, vegetables and milk have also been found as early as 6000 BC (Fox et al 1993). With the discovery of microbes by Antoni van Leeuwenhoek and development of the science of microbiology in the 1850s, the biological basis of fermentation was understood for the first time. Adam (1990) defines food fermentation as a form of energy-yielding microbial metabolism in which an organic substrate usually a carbohydrate is incompletely oxidised and an organic carbohydrate acts as an electron acceptor. Thus, by this definition the production of ethanol by yeasts or organic acids by lactic acid bacteria is considered as fermentation. Thus, fermented foods are the foods which are produced or preserved by the action of microorganisms. Originally, food fermentations were carried with the primary purpose to achieve preservation effect for long-term use. With the development of alternative technologies for food preservation and long-term storage, there is no longer pressing need of food to be preserved by fermentation. Fermented foods are currently being manufactured because of their unique flavour, aroma and texture attributed which are generally relished by the con-

sumer. Today, fermented foods are an integral part of our staple diet.

6.2 Fermented Foods

The substrates used for the commercial production of fermented products are milk, vegetables like cabbage and cucumber, meat, oriental fermented foods and bakery products.

6.2.1 Milk Products

The fermented milk products basically comprise of cheese, yogurt and fermented milks. Cheese is a concentrated form of the milk protein casein and milk fat. There are over 400 different varieties of cheese which can be clubbed into 20 distinct types (Jay 1996). Similarly, there exist an extensive list of types of yogurts and fermented milks. The primary requirement for cheese making is milk, and the variation of cheese is based on the quality and type of milk being used. The milk is pasteurised and a starter culture is added. The starter cultures can be mesophilic or thermophilic based on the type of cheese being manufactured (Table 6.1). For cheddar cheese, *Streptomyces lactis* and *Streptococcus cremoris* are generally used which are mesophiles, while *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are used as starter cultures as they can sustain high milk temperatures around 132 °F

Table 6.1 Starter culture for cheese manufacture

Organism type	Organism name	Cheese type
Mesophiles	<i>Lactococcus lactis</i>	Cottage cheese
	<i>Lactococcus cremoris</i>	Cheddar cheese
	<i>Leuconostoc mesenteroides</i> ssp. <i>cremoris</i>	Cream cheese
		Continental varieties
	<i>Lactococcus lactis</i> ssp. <i>lactis</i> biovar <i>diacetylactis</i>	Blue cheese
		Baby Swiss
Thermophiles	<i>Streptococcus thermophilus</i>	Mozzarella
	<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	Grana
	<i>Lactobacillus helveticus</i>	Swiss

which are used for the preparation of Swiss and Italian cheese. The bacteria produce small amount of acid which helps in clotting of milk required for cheese making. Subsequently, the curd is formed which entraps fat and water. A suitable coagulant is then added to the curd which splits the colloidal casein into carbohydrate-rich peptide fraction and insoluble paracasein that precipitates in the presence of calcium ions.

Fungi which are used in producing coagulants for cheese making are *Mucor miehei*, *Mucor pusillus* and *Endothia parasitica*. After the formation of coagulum, it is cut into pieces or cubes so that there is loss of whey (syneresis). The cubes of coagulum/curd are suspended in whey and heated at a given temperature (37–38 °C) for approximately 30 min in the case of cheddar cheese. This process helps in the control of acid production because the starter culture suppresses growth of spoilage bacteria and influences the structure of the curd. Finally, the whey is removed from the coagulum. This is generally carried out by filling the curd/whey mixture to perforated mould (in case of camembert/Brie cheese) where the curd is allowed to press by its own weight. Semi-continuous pressing systems like Casomatic® are used which comprise of cylindrical columns to which whey/curd mix is dispensed, pressing button to pre-press the curd beneath the whey and perforated bands which enable whey drainage followed by curd cutting/moulding system. The curd particles are knot into a cohesive mass depending upon the

temperature, pressure and time. Sodium chloride is applied to the curd in several ways; dry salt may be sprinkled on loose curds as in manufacture of cheddar cheese or rubbed on the cheese surface. Salt contributes to the development of flavour, texture and appearance of the cheese and controls the production of lactic acid. Ripening of the cheese involves the finished cheese to be placed in controlled temperature and relative humidity (4 °C/85 % humidity for cheddar cheese) for 3 months to 1 year depending upon the type of cheese.

Yogurt is the fermented milk product which consists of two lactose-fermenting organisms *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yogurt is generally prepared from standardised whole milk, partially defatted milk, condensed skim milk cream and non-fat dry milk. *L. bulgaricus* and *S. thermophilus* are used in 1:1 ratio as starter culture for yogurt manufacture. *Streptococcus* grows first followed by *Lactobacillus bulgaricus* which provides its aroma and flavour. Attempts have been made to prevent syneresis from yogurt by including a slime-producing strain *Streptococcus filant* or *Streptococcus lactis* var. *hollandicus*.

Kefir is an acid and alcoholic fermented milk which is commonly produced and consumed in Russia. The alcohol content of kefir is approximately 1 %. Koumiss is prepared from the mare's milk and contains alcohol which causes mild intoxication. The alcohol content of koumiss varies from 1 to 2.5 %, while the titratable acidity varies from 0.7 to 1.8 % lactic acid. Mare's milk

is low in casein content and does not curdle like cow's milk; it is a greyish white wholesome drink. The starter culture of koumiss consists of *Lactobacillus bulgaricus* and lactose-fermenting *Torulopsis holmii*.

6.2.2 Fermented Vegetables

Lactic acid fermentation or pickling is one of the important methods of food conservation. The pickled foods generally comprise of cucumbers, olives, various peppers and green tomatoes which serve as appetisers or are consumed as substantial part of the meal. Other vegetables which are less frequently pickled are carrots, cauliflower, celery, okra, onion and sweet and hot peppers.

Sauerkraut is a product which is produced as a result of lactic acid fermentation of the shredded cabbages, its literal meaning in sour cabbage. The concept behind sauerkraut fermentation is initiated by *Leuconostoc mesenteroides* and continued by *Lactobacillus brevis* and *Lactobacillus plantarum*.

6.2.3 Fermented Meat Preparations

Fermented sausages are produced as a result of by lactic fermentation of a mixture of comminuted meat mixed with fat, salt, curing agents (nitrate/nitrite), sugar and spices, and these represent traditional foods of central and southern Europe.

Fermentation temperatures vary according to the individual product, but they are generally less than 22 °C for dry- and mould-ripened sausages and 22–26 °C for semidry varieties. European fermented sausages formulated with nitrite are produced with added starter culture, generally consisting of lactic acid bacteria (lactobacilli and pediococci) and catalase-positive cocci (*S. carnosus*, *Micrococcus varians*). Yeasts and moulds that are available as starters include *Debaryomyces hansenii*, *Candida famata* and *Penicillium nalgiovense* and *P. chrysogenum*, respectively.

6.2.4 Traditional Fermented Food

Soy sauce (or shoyu) is a condiment widely used in Japan for cooking and seasoning of Japanese food. There are five main types of soy sauce in Japan, each with its own distinctive colour, flavour and use. The characteristic aroma and flavour of soy sauce is attributed to the enzymatic activities of yeasts, *Tetragenococcus halophilus* and some *Lactobacillus* species.

'Idli' is a fermented steamed cake of rice and dehulled blackgram dhal produced in India. LAB such as *Lc. mesenteroides*, *Lb. delbrueckii*, *P. cerevisiae*, *E. faecalis* and *L. lactis* are responsible for pH reduction and may increase the thiamine and riboflavin content. Nan is a leavened flat sour-dough bread with a central pocket now prepared worldwide. *Saccharomyces*, yeasts and lactic acid bacteria are basically involved in the fermentation process. Philippines' 'puto' is also a fermented product similar to idli. It consists of a steamed rice cake prepared from year-old rice grains which are soaked, ground with water and allowed to undergo a natural acid and gas fermentation. The acid is partially neutralised with sodium hydroxide and does not contain pulses as in idli.

Tempeh is a soyabean-based fermentation product of Indonesia that contains over 40 % protein. Tempeh is a meat substitute that is used in soups or sliced, salted, deep fired in coconut oil and consumed. Lactic acid bacteria including *Lb. casei* and *Lactococcus* species dominate the fermentation.

'Ogi' is a fine paste-like sour gruel eaten in Nigeria resulting from the submerged fermentation of cereals. It is consumed as a breakfast cereal by adults and is an important traditional weaning food of infants. A starter culture has been used to produce an improved version of ogi called DogiK. The starter strains are lactobacilli isolated from local fermented foods possessing strong antibacterial activity.

6.2.5 Bakery Products

Sourdough breads are made with starters containing yeasts such as *Saccharomyces* spp. and

Torulopsis and homo-fermentative and heterofermentative lactic acid bacteria. Heterofermentative strains such as *Lb. sanfrancisco*, *Lb. brevis* and *Lb. fermentum* are responsible for the characteristic sensory qualities of such breads. More than 20 types of yeast are found in sourdoughs. *S. cerevisiae* is frequently present (or added) due to the use of baker's yeast.

The sourdoughs have been classified into three groups: type I, type II and type III. Type I sourdoughs are traditional doughs maintained by the continuous propagation at ambient temperature (20–30 °C). *Lactobacillus (Lb) sanfranciscensis* and *Lb. pontis* are the dominant LAB in these sourdoughs. Bakers' yeast is used for the leavening of type II sourdoughs. This is essential since type II doughs are a less time-consuming, one-stage fermentation process at temperatures exceeding 30 °C. The dominant strains in industrial processes of type II doughs are mostly *Lb. panis*, *Lb. pontis*, *Lb. reuteri*, *Lb. johnsonii*, *Lb. sanfranciscensis*, *Lb. fermentum*, *Lb. delbrueckii*, *Lb. acidophilus*, *Lactococcus lactis*, *Lb. brevis* and *Lb. amylovorus*. The third type of sourdough is basically consist of dried preparations which are made by traditional sourdough fermentation with subsequent water evaporation by freeze-drying, roller/spray drying or drying in a fluidised bed reactor. The type III sourdoughs are the most convenient way to introduce superior bread taste into modern bakery industry.

6.3 Fermented Beverages

Alcoholic beverages have been produced by humans since thousands of years. These drinks were basically based on fermentation of cereals and fruits by yeasts. However, with the advancement in microbiology and biological sciences, the technologies of production have improved and these have been identified with specific names as wine, beer and whisky.

6.3.1 Wine

Wines are primarily prepared from the fermentation of grapes majorly but can be also produced

by some other fruits. The five basic components of wine making involve harvesting, crushing and pressing (mush formation), fermentation, clarification, aging and bottling. Wines are named after the type of grapes or the geographic area or specific village where they were first produced. Burgundy, Bordeaux, Champagne and Alsace are important wines of France. *Saccharomyces ellipsoideus* is the common yeast used for the preparation of table wines. These have an alcohol content of 10–12 %.

Champagne is a sparkling wine in which the alcohol content reaches up to 20 % as it undergoes double fermentation. They have a natural effervescence; others are made effervescent by bubbling them with carbon dioxide. The starter culture for yeast fermentation is *Saccharomyces bayanus* which is also known as Premier Cuvee. Cognac is the distilled product of wine also known as wine brandy.

6.3.2 Beer

Beer is one of the oldest beverages humans have produced, dating back to at least the fifth millennium BC (prior even to writing), and recorded in the written history of Ancient Egypt and Mesopotamia. Beer is prepared from malted barley and malted wheat. Sometimes a mixture of starch sources can be used, such as rice. The main ingredients of beer are water, malted barley, hops and yeast. Other ingredients, such as flavouring or sources of sugar, are called adjuncts and are commonly used; common adjuncts are corn, rice and sugar. The process of making beer is called brewing. It includes breaking the starch in the grains into a sugary liquid, called wort, and fermenting the sugars in the wort into alcohol and carbon dioxide by yeasts. Beers tend to fall in one of the two large families: ale (using top fermenting yeast) or lager (using the bottom fermenting yeasts). *Saccharomyces cerevisiae* is the strain used for manufacturing ale, while *Saccharomyces uvarum* is known as the lager's yeast. On average, beer's alcohol content is between 4 and 6 % alcohol by volume, although it can be as low as 2 % and as high as 14 % under ordinary circumstances. Lagers are the most commonly consumed

beer in the world. They are of Central European origin, taking their name from the German *lagern* which means 'to store'.

Other fermented beverages include the mead, prepared by fermenting honey; cider, a fermented apple juice while perry being a fermented pear juice.

6.3.3 Whiskey

Whiskey is the name for a broad category of alcoholic beverages distilled from grains that are subsequently aged in oak casks. The grains used to make various types of whiskey include barley, malted barley, rye, malted rye, wheat and maize/corn. The alcoholic content in whiskey ranges from 40 to 50 %. The distillate must age for at least 3 years to be called Scotch whisky.

6.3.4 Kombucha

Kombucha tea is a fermented tea beverage produced by fermenting sugared black tea with tea fungus (*kombucha*). The fermentation of kombucha tea is done by symbiotic association between

acetic acid bacteria and yeasts for a period of 14 days. Kombucha is composed of two portions: a floating cellulose pellicle layer and the sour liquid broth. Kombucha has been consumed in Asia for over two millennia and is a popular beverage among traditional fermented foods across the world.

6.4 Summary

Today, microorganisms play a tremendous role in the development of fermented food products by serving as starter culture as well as maintaining the aroma and nutrition of the product. They have a significant effect on the organoleptic properties of the end product.

Selected Reading

- Adams MR (1990) Topical aspects of fermented foods. *Trends Food Sci Technol* 1:141–144
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- Jay JM (1996) *Modern food microbiology*, 5th edn. Chapman & Hall, New York