**Paper No.: 02**

**Paper Title: The Principles of the Food Processing & Preservation**

**Module No. : 21**

**Module Title: Food Fermentation**

## 21.0 Introduction

Fermentation could be described as a process in which microorganisms change the sensory (flavor, odor, etc.) and functional properties of a food to produce an end product that is desirable to the consumer.

## 21.1 History

Humans are unable to survive without food and drink; therefore, the supply of these essentials has had a major impact on the development of the human species and continues to do so even today. The rapidly increasing world population necessitates that the amount of food wasted due to spoilage is kept to a minimum. Food production is only one part of the process to ensure continuous, diverse, safe, food supplies to meet the consumer demands. Food must also be stored and preserved to achieve this objective. The requirement to store and preserve foods has long been recognized, from the time well before there was any knowledge of microbiology.

Fermentation, along with salting, cooking, smoking, and sun drying, is one of the earliest ancient traditions developed by cultures all around the world to extend the possible storage time of foods. Before the initiation of preservation technology, humans frequently had to choose between starvation and eating spoilt foods and then suffer the possible consequences of this. For thousands of years, raw animal and plant ingredients have been fermented. Fermented fruits were probably among the first fermented foods eaten. The methods for fermentations were developed by trial and error and from the experiences of many generations.

## 21.2 Common Fermented Foods

A selection of the most common fermented foods that have wide geographical distributions are shown in Table.1. The key type of microorganisms associated with these foods is also included.

**Table 1: Examples of the More Common Fermented Foods**

| Food | Principal Ingredient | Key Microorganisms |
|---|---|---|
| Wine | Grapes | Yeasts |
| Beer | Barley | Yeasts |
| Cider | Apples | Yeasts |
| Sake | Rice | Molds |
| Bread | Wheat | Yeasts |
| Yogurt | Milk | LAB (lactic acid bacteria) |
| Cheese | Milk | LAB |
| Buttermilk | Milk | LAB |
| Kefir | Milk | LAB + yeasts |
| Vinegar | Grapes | Yeasts followed by *Acetobacter* or *Gluconobacter* |
| Tempeh | Soybeans | Molds |
| Soy sauce | Soybeans | Molds+LAB+yeasts |
| Pickled cucumbers | Cucumbers | LAB + yeasts |
| Sauerkraut | Cabbage | LAB |

## 21.3 Fermentation as a Preservation Method

As new preservation techniques have been developed, the importance of fermentation processes for food preservation has declined. Yet fermentation can be effective at extending the shelf life of foods and can often be carried out with relatively inexpensive, basic equipment. Therefore, it remains a very appropriate method for use in developing countries and rural communities with limited facilities. In addition, the non-dependence of fermentation on the use of chemical additives to the food appeals to the ñmore awareò consumer market. The chemical composition of most foods is relatively stable; therefore, generally preservation is based on eliminating microorganisms or controlling their growth and the overall composition of the microflora. To reduce or prevent microbial spoilage of food, four basic principles can be applied:

1. Minimize the level of microbial contamination onto the food, particularly from ñhigh-riskò sources (asepsis)
2. Inhibit the growth of the contaminating microflora
3. Kill the contaminating microorganisms
4. Remove the contaminating microorganisms

Fermentations use a combination of the first three principles. Fermentations should not be expected to sterilize substandard raw products, but rather should use high-quality substrates. Microorganisms can improve their own competitiveness by changing the environment so that it becomes inhibitory or lethal to other organisms while stimulating their own growth, and this selection is the basis for preservation by fermentation. A number of different bacteriocidal and bacteriostatic factors that can be produced by lactic acid bacteria (LAB) are shown in Table. 2. Fermentation improves the safety of foods by decreasing the risks of pathogens and toxins achieving the infective or toxigenic level, and extends the shelf life by inhibiting the growth of spoilage agents, which cause the sensory changes that make the food unacceptable to the consumer.

**Table 2: Factors Produced by the Metabolic Activity of Microorganisms That Can Contribute to the Increased Stability and Safety of Fermented Foods**

- Low pH
- Organic acids, e.g., lactic acid, acetic acid, and formic acid
- Low redox potential
- Nutrient depletion
- Accumulation of inhibitors, e.g., toxins, bacteriocins, antibiotics, lactococcins, nisin, natamycin, hydrogen peroxide
- Ethanol
- Diacetyl
- Carbon dioxide

**21.4 Microbial Contamination of Foods**

Foods are derived from other living organisms and during their development and preparation they are continuously exposed to microbial contamination. The resultant contaminating microflora can have different effects on the food. These include negative effects such as spoilage, where the food becomes unfit for human consumption or health risks when infectious or toxigenic microorganisms are present. Negligible effects on the food occur when the microflora does not cause disease or any detectable changes in the food. However, benefits can also be reaped from the action of the microorganisms when their activity brings about improvements in the appeal of the food. In developed countries, the improved appeal is the major reason for microbial fermentations of foods continuing today.

The nutrient content and intrinsic properties of many raw foods make them ideal environments for microbial replication. The rate at which the microorganisms grow depends not only on the intrinsic properties of the food (pH, redox potential, water activity, etc.) but also on the conditions under which it is being stored, the extrinsic factors, for example, temperature. Therefore, many raw food types need to be consumed soon after production to be of high nutritional value. Without preservation measures, delays lead to the nutrients being degraded and utilized by the contaminating microflora.

A major consideration needs to be that under ideal conditions microorganisms can grow very rapidly, being able to double in number in a short period of time. It must also be noted that there is a variation in the optimum environmental conditions for different types and species of microorganisms, for example, microorganisms can be categorized into broad groups such as aerobes and anaerobes depending on their tolerance and use of oxygen and psychrophiles, mesophiles, and thermophiles based on the temperature range optimum for their growth. In addition, the biochemical activity of different microorganisms varies and may change in response to fluctuations in environmental factors, leading to a range of metabolic end products (Table.3). By manipulating the environmental conditions, it is possible to select for specific kinds of microorganisms that impart a particular taste, odor, texture, or appearance to the food. This is the basis of fermentation.

**Table 3: Examples of Microbial Metabolic End Products Used in Fermented Foods**

| Metabolic End Product | Example of Uses |
|---|---|
| Carbon dioxide | Leavening bread |
| Ethanol | Alcoholic beverages |
| Acetic acid | Vinegar |
| Lactic acid | Fermented vegetables |
| Flavor compound - Diacetyl | Dairy products |
| Flavor compound - Acetaldehyde | Yogurt |

## 21.5 Benefits of Fermented Foods

Microorganisms *per se* can be used as food sources, but in many instances it is their effects on other food sources that are of major interest. The acceptability of a food to the consumer is based mainly on its sensory properties. The sought-after sensory properties of fermented foods are brought about by the biochemical activity of microorganisms. Fermented foods were developed simultaneously by many cultures for two main reasons: (i) to preserve harvested or slaughtered products, which were abundant at certain times and scarce at others and (ii) to improve the sensory properties of an abundant or unappealing produce. However, a range of benefits can be obtained from food fermentations, some of which are shown in Table.4. Consequently, fermented foods and drinks still retain an important role in the human diet. Fermentation has low energy demands and can often be carried out without sophisticated technology and designated plants. The simple techniques mean that the procedures can often be carried out in the home. Also, a number of studies have shown that consumers regard fermented food products as healthy and natural, increasing consumer demands and their profitability.

**Table 4: Potential Benefits of Fermented Foods**

| Increased | Lowered |
|---|---|
| Safety | Toxicity |
| Health benefits | Cooking time |
| Retail value | Production costs |
| Nutritional value | Equipment needs |
| Digestibility | Levels of anti-nutritional factors |
| Suitability for subsequent processing | |
| • Sensory properties | |

- Ease of storage and transportation
- Shelf life

## 21.6 Microorganisms Used in Food Fermentations

A variety of groups of microorganisms are frequently used in fermented foods. The principal groups are shown in Table.5.

**Table 5: Principal Groups of Microorganisms Used for Food Fermentations**

| Microbial Group | Product |
|---|---|
| Lactic acid bacteria (LAB) | Lactic acid |
| Acetic acid bacteria | Acetic acid |
| Yeasts | Alcohol and carbondioxide |
| Molds | Enzymes |

### 21.6.1 Lactic Acid Bacteria

LAB perform an essential role in the preservation and production of wholesome foods. Examples of lactic acid fermentations include (a) fermented vegetables such as sauerkraut, pickled cucumbers, radishes, carrots, and olives; (b) fermented milks such as yogurt, kefir, and cheeses; (c) fermented/ leavened breads such as sourdough breads; and (d) fermented sausages (Table.1). LAB have been grouped together as they possess a range of common properties, and all produce lactic acid that can kill or inhibit many other microorganisms. The primary use of lactic acid in the food industry is as a preservative, an acidulant, or a dough conditioner. LAB are subdivided based on their products from glucose fermentation. Homofermenters produce lactic acid as the major or sole product from glucose, while heterofermenters produce equimolar amounts of lactate, carbon dioxide, and ethanol. Heterofermenters have an important role in producing aroma components such as acetaldehydes and diacetyl. LAB have a range of methods for outcompeting other microorganisms. Their most effective mechanism is to grow readily in most foods, producing acid, which lowers the pH rapidly to a point where other competing organisms can no longer grow. Lactobacilli also have the ability to produce hydrogen peroxide, which is inhibitory to spoilage organisms, while lactobacilli are relatively resistant to hydrogen peroxide. The role of hydrogen peroxide as a preservative is likely to be minor, especially when compared with acid production. Carbon dioxide produced by heterofermenters also has a preservative effect, resulting partially from its contribution to anaerobiosis.

Consumers are taking a greater interest in the quality of foods and are creating a demand for chemicalfree, ñnatural healthò foods. This has stimulated extensive research into the applications of LAB for both the control of pathogenic and spoilage microorganisms and also for health promotion. A range of potential health benefits has been associated with the consumption of LAB. Some benefits are as a consequence of their growth and activity during food fermentations, and some from the resultant colonization of the gastrointestinal tract (Table 6).

**Table 6: Potential Health Benefits from Lactic Acid Bacteria**

**Benefits**

*From foods*

- Improved nutritional value, e.g., production of vitamins or essential amino acids
- Reduced toxicity, e.g., by degradation of noxious compounds
- Increased digestibility and assimilability of nutrients

*From colonization*

- Control of intestinal infections
- Improved digestion of lactose

- Inhibition of tumor growth
- Lowering of serum cholesterol levels
- Immune stimulation

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**21.6.2 Acetic Acid Bacteria**

A second group of bacteria with importance in food fermentations are the acetic acid producers. Acetic acid is one of the oldest chemicals known; it is named after the Latin word for vinegar ñacetum.ò The acetic acid bacteria are acid tolerant, grow well at pH levels below pH 5.0, are Gram-negative, motile rods, and are obligate aerobes. They derive energy from the oxidation of ethanol to acetic acid following the reaction shown below.

$$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O$$

They are found in nature where ethanol is produced from the fermentation of carbohydrates by yeasts, such as in plant nectars and damaged fruits. Other good sources are alcoholic beverages like fresh cider and unpasteurized beer. In liquids, they grow as a surface film because of their demand for oxygen. The acetic acid bacteria consist of two genera, *Acetobacter* and *Gluconobacter*. *Acetobacter* can eventually oxidize acetic acid to carbon dioxide and water using Krebs cycle enzymes referred to as overoxidation. This is not the case with *Gluconobacter*. The most desirable action of acetic acid bacteria is in the production of vinegar. The same reaction can also occur in wines, when oxygen is available, and here the oxidation of alcohol to acetic acid is an undesirable change, giving the wine a vinegary off-taste.

**21.6.3 Yeasts**

Yeasts are widely distributed in natural habitats that are nutritionally rich and high in carbohydrates, such as fruits and plant nectars. Yeasts are rarely toxic or pathogenic and are generally acceptable to consumers. After extensive study, yeasts have been classified into about 500 species. However, only a small number are regularly used to make alcoholic beverages. *Saccharomyces cerevisiae* is the most frequently used and many variants are available. *Saccharomyces cerevisiae* ferments glucose but does not ferment lactose or starch directly. Yeasts are used to produce ethanol, CO2, flavor, and aroma. The reaction can be represented by the following equation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

$$\text{yeast} + \text{glucose} \rightarrow \text{ethyl alcohol and carbon dioxide}$$

Other metabolic products include minor amounts of ethyl acetate, fusel alcohols (pentanol, isopentanol, and isobutanol), sulfur compounds, and leakage of amino acids and nucleotides that can all contribute to the sensory changes induced by yeasts.

**21.6.4 Molds**

The majority of fungal species have filamentous hyphae and are referred to as molds. They are grouped into four main classes based on the physiology and production methods of their spores. Molds are aerobic and have the greatest array of enzymes. Some molds are used in the food industry to produce specific enzymes such as amylases for use in bread making. They are relatively tolerant to extreme environments and are able to colonize and grow on most foods. Molds are important to the food industry, both as spoilers and preservers of foods and in particular in fermentations for flavor development. Certain molds produce antibiotics, while mycotoxin production by others is an emerging cause of concern in the food industry. The *Aspergillus* species are often responsible for undesirable changes in foods, although some species such as *A. oryzae* are used in fermentations of soybeans to make miso and soy sauce. *Mucor* and *Rhizopus* are also used in some traditional food fermentations. *Rhizopus oligosporus* is considered essential in the production of tempeh from soybeans. Molds from the genus *Penicillium* are associated with the ripening and distinctive flavor of cheeses.

### 21.6.5 Starter Cultures
Fermented foods may be produced by the action of fermentative microorganisms naturally found on the raw materials or in the production environment. However, to improve reliability ñstarter culturesò are frequently used. Starter cultures may be pure or mixed cultures. Using mixed starter cultures can reduce the risks of bacteriophage infection and improve the quality of the foods when the organisms are mutually beneficial. Food fermentations frequently involve a complex succession of microorganisms induced by dynamic environmental conditions. Fermentative microorganisms must be safe to eat even in high numbers and must produce substantial amounts of the desired end product(s). For practical reasons, the organisms should be easy to handle and should grow well, enabling them to outcompete undesirable microorganisms. The organism also needs to be genetically stable with consistent performance both during and between food batches. In many traditional fermentations, the natural micro flora were used for the fermentation. Natural fermentations have a degree of unpredictability, which may be unsatisfactory when a process is industrialized. Starter cultures are increasingly used to improve not only the reliability, but also the reproducibility and the rate at which the fermentation is initiated. Failed, poor-quality, or unsafe products lead to loss of customers and revenue, therefore their incidence must be minimized. The composition of starter cultures is based on knowledge of food-grade microbial genetics, metabolism, and physiology as well as their interactions with foods. Starter cultures are now developed mainly by design rather than by screening. The overall objective is to exploit the properties of the starter cultures to ensure reproducible standards of safety and quality.

## 21.7 Classification of Fermented Products
Fermented foods are classified in a number of different ways. They may be grouped based on the microorganisms, the biochemistry, or on the product type. Campbell-Platt (1987) identified seven groups for classification, namely, (1) beverages, (2) cereal products, (3) dairy products, (4) fish products, (5) fruit and vegetable products, (6) legumes, and (7) meat products [25], whereas Steinkraus (1997) classified fermentations according to the type of fermentation.

### 21.7.1 Fermented Products
#### 21.7.1.1 Alcoholic Beverages
Throughout history alcoholic beverages have had a place in most cultures. They require the alcoholic fermentation of fruits or other high-sugar materials by yeasts. The alcohol content of the beverage acts as a preservative and many of these products have long shelf lives. Over the years, brewing yeasts have evolved by selection and mutations, and have been developed by genetic engineering. Major advances have been made in improving the characteristics of the fermentation strains driven by the high revenue associated with the alcoholic beverage industry.

**Beer**

Beer is produced by the fermentation of partially germinated cereal grains, referred to as malt, by yeasts. Beers have a final ethanol content of about 3%ï 8%; a huge variety of beers exists and they include ales, lagers, and stouts. Both lagers and ales can be either light or dark in appearance. Ale is produced using *Saccharomyces cerevisiae*, a top fermenter yeast, whereas lagers are produced using pure cultures of *Saccharomyces carlsbergensi*, a bottom fermenter yeast. Ales are produced using warm fermentation temperatures, 12°Cï 18°C and lager fermentation temperature is generally cold, 8°Cï 12°C [12]. Most beer produced is of the lager variety.

**Wine**

Wine can be produced from any fruit juice with sufficient levels of fermentable sugars, in most cases wine is a beverage obtained by full or partial alcoholic fermentation of fresh, crushed grapes or grape juice (must), with an aging process. Wine-type grapes from cultivars of *Vitis vinifera* vines are most commonly used to produce wines.

### 21.7.1.2. Distilled Spirits

The fermented products discussed above can only produce a maximum alcohol content of about 17%. Concentrations in excess of this inhibit the metabolism of the yeasts. To obtain higher alcohol concentrations, the fermented product must be subsequently distilled. Whiskey, gin, vodka, rum, and liqueurs are examples of distilled spirits. Although the process for producing most products of these types is quite similar to that for beers, the content of alcohol in the final products is considerably higher.

### 21.7.1.3 Lactic Acid Products

**Dairy Products**

***Yogurt:*** Yogurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Streptococcus thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus.* Yogurt is prepared using either whole or skim milk, where the nonfat milk solids are increased to 12%ï 15% by concentrating the milk, or adding powdered skim milk or condensed milk. The concentrated milk is pasteurized at 82°Cï 93°C for 30ï 60 min and cooled to the starter incubation temperature of 40°Cï 45°C. Yogurt starter is then added at a level of around 2% by volume and incubated for 3ï 5 h, or until the titratable acidity of the final product reaches 0.85%ï 0.90% or a pH of 4.4ï 4.6. The yogurt is then cooled to 5°C to inhibit further acid production. The type of yogurt starter used can change the physical characteristics of the final yogurt product.

***Cheese:*** Cheese is a concentrated milk product obtained after coagulation and whey separation of milk, cream or partially skimmed milk, buttermilk, or a mixture of these products. Cheese may be consumed fresh or after ripening. Cheese is commonly made from cow, ewe, goat, or buffalo milk. The majority of cheeses are made from pasteurized milk. The use of sub-pasteurization heat treatment of milk or thermization is also practiced to limit heat-induced changes in milk without compromising microbiological safety. 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.

### 21.7.1.4 Fermented Vegetables

A large number of vegetables are preserved by lactic acid fermentation around the world. The most important commercially fermented vegetables are cabbage (sauerkraut), cucumbers, and olives. Others include carrots, cauliflower, celery, okra, onions, and peppers. Typically, these fermentations do not involve the use of starter cultures and rely on the natural flora. Brine solutions are prepared in the fermentation of sauerkraut, pickles, and olives. The concentration of salt in the brine ranges from 2.25% for sauerkraut to 10% for olives. The fermentation yields lactic acid as the major product. The salt extracts liquid from the vegetable, which serves as a substrate for the growth of LAB. Growth of undesirable spoilage microorganisms is restricted by the salt. Aerobic conditions should be maintained during fermentation to allow naturally occurring microorganisms to grow and produce enough lactic acid, and to prevent growth of spoilage microorganisms. Olives receive a special treatment before brining in that green olives are treated with a 1.25%ï 2% lye solution (sodium hydroxide), usually at 21°Cï 25°C for 4ï 7 h. This treatment is necessary to remove some of the oleuropein, a bitter compound in olives. In some countries, the fermentation of cucumbers is controlled by the addition of acetic acid to prevent growth of spoilage microorganisms, buffered with sodium acetate or sodium hydroxide, and inoculated with *Lb. plantarum* alone or in association with *Pediococcus cerevisiae*.

### 21.7.1.5 Fermented Animal Products

The primary reason for developing methods to ferment meats and fish was to extend the shelf life of these highly prized, perishable foods. Gram-positive micrococci have an important role in these fermentations. Several products became popular, including fermented sausages, fish sauces, and fish pastes. Many of the traditional fermentation methods are still used although the primary reason for their use is no longer preservation, but because the products are popular for their enhanced flavors.

***Fermented Sausages:*** A variety of procedures for producing stable, fermented meat sausages have developed around the world. In general, preservation of the meat is achieved by adding salts and the generation of lactic acid by bacteria, which leads to a rapid fall in the pH. Micrococci, staphylococci, and yeasts are responsible for the development of color, taste, and flavor during the fermentation. In addition to fermentation, sausage processing may also include curing, smoking, drying, and aging to improve both the flavor and the shelf life. In addition to the major inhibitory factors of low-water activity achieved by the addition of salt, and in some cases drying and the accumulation of lactic acid, a ñhurdle effectò is created by a combination of other inhibitors, contributing to the preservation of sausages.

Fermented sausages are prepared by mixing ground meat with various combinations of spices, flavorings, salt, sugar, additives, and frequently, starter cultures. Common additives include acidulant, ascorbic acid, and colorings. Pork, beef, mutton, or turkey meat can be used, but to achieve good sensory properties and safety, the meat must be fresh and of high quality. The meat is generally used raw, with no heat processing, as this can damage the texture of the sausage product. Frequently fermented sausages are eaten without any cooking stage.

***Fermented Fish:*** Fermentation of fish is most common in southeast Asia where fish is a major component of the human diet. The carbohydrate content of fish is low, usually less than 1%; therefore, for lactic fermentation an additional source of carbohydrate is required. Ingredients such as rice and garlic may be added as carbohydrate sources; the carbohydrate reserve in garlic is inulin. The higher the level of supplemented carbohydrate, the faster the fermentation. The product is often ready after only a few weeks, making the process much more efficient. The supplementation of carbohydrates enables the fish to ferment and an acidic, stable product to be made. The most common products from fish are produced by microbial fermentation and by the degrading activity of autolytic fish enzymes. These products are known as fish sauces and fish pastes.

### 21.7.1.6 Combined Fermentations

The release of carbon dioxide by microorganisms has two major roles in food fermentations: (i) it can act as a leavening agent and (ii) it can be used for carbonation of beverages. One of the most common uses of carbon dioxide is to leaven dough during bread making.

#### Bread

The use of yeasts to produce bread dates back thousands of years. Breads have relatively short shelf lives; therefore, the primary reason for their production was not preservation, but to improve the digestibility and eating appeal of grains. To make leavened bread, flour produced from grains, such as wheat, that contain gluten proteins are used. When the bread is kneaded, the gluten forms a matrix that makes the dough elastic and extensible. These properties enable the dough to stretch and retain sufficient amounts of gas to produce loaves with good volume and a fine, soft open structure. The gas is a combination of air incorporated during mixing and kneading and CO2 produced from the fermentation of sugars by yeasts. The yeasts contribute to the flavor and provide an appealing aroma. When the loaves are baked the proteins are denatured, fixing the structure, and the low levels of ethanol produced by the yeasts evaporate.

**Sourdough**

Sourdough bakery products have an extended shelf life. The fermentation combines the metabolic activity of LAB for souring and yeasts for leavening. Methods for their fermentation date back thousands of years. The sourness of the product depends on many factors, including fermentation temperature and time, type of grain, and the strains of yeast and LAB. The complexity of the bread flavor is based on the lactic and acetic acids produced by LAB and flavor compounds formed by the activity of endogenous cereal enzymes, microbial metabolism, and the baking process. The metabolism of LAB and yeasts also provides a range of desirable aroma products.

**Vinegar**

Vinegar is one of the oldest known culinary products. It is thought that it was discovered by accident from spoilt wine, in fact it is named after the French term ñvin aigreò meaning sour wine. Vinegar is classified as a condiment that contains a minimum of 4% w/v (40 g/L) acetic acid and has a pH value between 2.0 and 3.5. The strength of vinegars may also be quoted in grains, with 10 grains being equivalent to a concentration of 1% acetic acid. Higher strength vinegars may be used for pickling; spirit vinegar is made from an alcoholic solution that has been distilled. Although vinegar has been produced for thousands of years, it remains very popular; it is estimated that the annual worldwide production of vinegars is around 2000 million liters. Vinegar is one of the great successes of the preservation industry, although acetic acid has numerous applications in the food industry. The shelf life of a wide range of foods is extended by storing the product submerged in vinegar; this includes pickled vegetables such as gherkins, olives, and onions.

Vinegars are produced from a two-stage fermentation: initially an anaerobic, alcoholic fermentation of sugars by yeasts, followed by oxidation of the ethanol to acetic acid by bacteria; this second reaction is known as acetification. Acetification is also a common cause of spoilage of alcoholic beverages. Acetification can be described by the equation:

$$C_2H_5OH + O_2 \rightleftharpoons CH_3COOH + H_2O$$

**Kefir**

Kefir is produced by an acid/alcohol fermentation of pasteurized milk with a mixture of LAB, yeasts, and other bacteria. The final product is acidic, slightly alcoholic, liquid to semiliquid, and effervescent, and is consumed as a beverage. Kefir grains are used to inoculate the milk. Kefir grains comprise proteins, polysaccharides, and a mixture of microorganisms, mainly lactose-fermenting yeasts, and aroma bacteria and LAB. The yeasts consist mainly of *Candida kefir* and *Saccharomyces kefir,* while the LAB comprise mainly of *Lactobacillus kefir*, *Leuconostoc* species, and *L. lactis.* The yeasts are responsible for the production of ethanol and carbon dioxide from lactose, the lactococci produce lactate from lactose, and the lactobacilli and *Leuconostoc* species are responsible for the production of lactate, acetate/ethanol, and carbon dioxide. Kefir fermentation requires a moderate room temperature (17°Cï 23°C). The final composition of kefir includes 0.8% lactic acid, 1%ï 3% alcohol, diacetyl, and acetaldehyde.

**21.7.1.7 Oriental Fermented Products**

The production of soy sauce, miso, and saki involves koji fermentation. Koji comprises soybeans or grains on which molds grow to produce enzymes such as proteases, lipases, and amylases. The fungal enzymes produced digest proteins, carbohydrates, and lipids into nutrients that are used by microorganisms in subsequent fermentations. Koji is produced in many varieties depending on the products to be manufactured. Koji differs in terms of the molds, the substrate, the method of preparation, and the stage of harvest.

**Soy Sauce**

Soy sauce is a dark brown liquid produced by the fermentation of soybeans and wheat in a salt brine. The manufacture of soy sauce starts with the treatment of raw material. Soybeans or defatted soybean flakes are moistened and cooked. The cooked beans are then mixed with roasted, cracked wheat in varying ratios for each type of soy sauce. The mixture is inoculated with a pure culture of *Aspergillus oryzae* (*A. soyae*). After 3 days of fermentation, 17%ï 19% salt solution is added to the koji to produce a mash called moroni. LAB such as *Pediococcus soyae* or *Lb. delbrueckii* are allowed to grow on the moroni to make it acidic enough to prevent spoilage and to make it acidic in taste. Yeasts such as *Saccharomyces rouxii* and *Torulopsis* sp. grow on the moroni to produce alcohol and help the formation of flavor. The moroni is aged, pressed to produce a liquid, soy sauce, which is then pasteurized.

**Tempeh**

Tempeh is a protein-rich food that is considered one of the worldôs first meat analogs. It is made by growing the mold *R. oligosporus* or related species on soaked, dehulled, partially cooked soybeans, knitting them into a firm cake, which can be sliced and deep-fried or cut into cubes and used in place of meat in soups. Tempeh production is not a means of improving shelf life, but it does improve the acceptability and the nutritional quality of its raw material (soybeans).

**21.8 Conclusion**

Fermentations should not be expected to sterilize substandard raw products, but rather should use high-quality substrates. Microorganisms can improve their own competitiveness by changing the environment so that it becomes inhibitory or lethal to other organisms while stimulating their own growth, and this selection is the basis for preservation by fermentation. Fermentation improves the safety of foods by decreasing the risks of pathogens and toxins achieving the infective or toxigenic level, and extends the shelf life by inhibiting the growth of spoilage agents.