Food Fermentations

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Fermentation: Definition and types

Fermentation of foods is one of the means of preserving the foods for longer periods. It is one of the food processing methods in which the conversion of carbohydrates to alcohols, organic acids, and carbon dioxide takes place. This process takes place under anaerobic conditions using bacteria, molds or yeasts, or a combination of these. Food fermentation usually results in a desirable end product. Fermentation provides mechanisms where by fresh commodities can be acidified without seriously impairing the nutritional and physical properties. It generally produces acidic foods that are incapable of supporting the growth of pathogens. It creates products with unique and distinct flavor. Fermentation can be done when the food material is available in plenty and packing can be done at later date, thus it extends the processing season. Unlike other food processing operations, it requires very less energy. Lactic acid fermentation is considered as the simple and valuable biotechnological process to keep and/or enhance the safety, nutritional, sensory and shelf life properties of various food commodities. Spontaneous fermentation is carried out by back sloping. Industrial fermentation is done by using starter cultures. Fermented foods contain high nutritive value and develop a diversity of flavours, aroma, and texture in food substrates. Some of the examples of fermented foods have been shown in Table 5.1.

This unit is divided into

1. Classification of fermented foods
2. Dairy Fermentations
3. Types of starter cultures
4. Probiotics
5. Non – dairy Fermented foods

1. Classification of fermented foods

Fermented foods are classified in different ways, based on the substrate being fermented or the kind of microorganisms involved or based on the function of the food. The four main fermentation processes include acetic, lactic, alkali and alcoholic fermentation. In acetic acid fermentation, the end product is acetic acid produced by A*cetobacter* species (ex: vinegar). Lactic acid fermentation results in the fermentation of milk, vegetables, cereals and meat by lactic acid bacteria. Alkali fermentation results due to the action of bacteria mainly *Bacillus* spp., on seeds and fish, etc. Alcohol fermentation is carried out mainly by yeasts with ethanol as an end product (ex. beer and wines).

Several microorganisms are involved in the food fermentations**.** Some of the examples are given in the Table (Table 5.2).

2. **Dairy Fermentations**

Fermented milks or fermented dairy products are produced with the fermentation of milk by lactic acid bacteria, yeast or molds or in combination of these. These cultures provide good body and texture, taste, flavour and aroma to these products. Cheese, dahi, yoghurt, kefir, kumis are some of the examples to fermented dairy products. The major variation among these products is the milk source and type of culture used for fermentation. The metabolites produced by starter cultures prevent the growth of undesirable, spoilage and harmful pathogenic microbes.

The main advantages of fermented dairy products include

- Better digestibility and absorption of milk proteins and fat
- Better nutritional quality and flavour
- Reduction of lactose content
- Enhanced retention of minerals such as calcium
- Enhanced content of certain B-vitamins
- Production of other metabolites, like flavor compounds

Dairy fermented foods can be classified based on its acid and alcohol content. These are acid-alcohol type (ex: kumis, also known as milk wine, and kefir), high acid type (ex: Bulgarian sour milk), medium acid type (ex: acidophilus milk, yoghurt) and low acid type (ex: dahi, cultured butter milk).

It is a routine practice even at homes to add previous day's curd or butter milk to the freshly boiled cooled milk to prepare curd. This is known as back slopping. However, for large scale production, microbial cultures in pure form are used. These are known as starter cultures.

Dairy starter cultures are defined as carefully selected microorganisms that are intentionally added to milk to initiate and accomplish the fermentation. Fermentation brings specific changes in the body, appearance, flavour and textural characteristics of the desired end product like dahi, yoghurt, cheese, etc. The bacterial cultures like lactococci, lactobacilli, streptococci, pediococci, propionic acid bacteria, leuconostocs, yeasts like *Saccharomyces*, *Torula* and certain species of some molds like *Penicillium*, etc., are used as starter cultures for the preparation of fermented dairy products.

The primary role of dairy starter cultures is to develop acidity which is mainly lactic acid. It is generated by the metabolic action of starters on the lactose present in the milk. Milk proteins get coagulated in the presence of lactic acid. This results in curd formation. The secondary function of acid development is expulsion of moisture, development of pleasant flavour and texture formation. Growth of undesirable microbes is prevented in the fermented products.

Many heterofermentative lactic cultures, especially *Leuconostoc* and *Lc. lactis* ssp. *diacetylactics* can metabolize citrate present in the milk and produce flavour compounds like diacetyl and acetoin. Starter cultures also have proteolytic activity. This helps in flavour enhancement and texture improvement. The lipolytic activity results in the formation of fatty acids from the milk fat. It imparts good flavour to the products. Some of the starters also produce short chain peptides, known as bacteriocins.Bacteriocinshave antimicrobial activity against pathogens and are related closely to lactic acid bacteria. Ex: nisin, acidophilin, pediocin, etc.

3. Types of starter cultures

Dairy starter cultures are of three types. These are bacteria, molds and yeasts. The bacterial starters can further be classified based on their shape, type of acids produced, temperature requirement for the growth, ability to produce one or more compounds from glucose, etc.

Based on the requirement of growth temperature lactic starters can be classified as mesophilic starters and thermophilic starters. Mesophlic starters include, *Lactococcus lactis* sub sp. *lactis*, *Lc. lactis* sub sp. *cremoris*, *Lc. lactis* sub sp. *diacetylactis*, *Leuconostoc mesenteroides, Leu. dextranicum, Leu. citrovorum,* etc. Thermophilic starters include *Streptococcus thermophilus*, *Lactobacillus plantarum*, *Lb. bulgaricus*, *Lb. brevis*, *Lb. acidophilus*, etc.

Based on the ability to produce one or more metabolites by using the lactose, dairy starters can be classified as homofermentative and heterofermentative starters. Homofermenters produce only lactic acid from the sugar through hexose diphosphate pathway (Ex: *Lactococcus lactis* sub sp. *lactis*, *Lc. lactis* sub sp. *cremoris*, *Lb. plantarum*). Heterofermenters produce ethanol, CO_2, acetic acid, and lactic acid via hexose monophosphate pathway (Ex: *Lc. lactis* sub sp. *diacetylactis*, *Leuconostoc mesenteroides*).

The molds used as starter cultures can be classified as white molds, blue molds and others. The molds used in cheese preparation like *Penicillium camemberti*, *P. candidum* are examples to white molds. *P. roqueforti* is known as blue mold. *Saccharomyces kefir*, otherwise known as *Saccharomyces lactis* or *Torula kefir* or *Candida kefir* is used in the preparation of kefir, an acid-alcohol type of fermented dairy product.

Based on their performance, dairy starter cultures can also be classified as technical and therapeutic starters. Technical starters are good fermenting cultures. They help to produce palatable products. However, they do not resist acid environment and stomach bile. Therapeutic starters are resistant to high acid levels and tolerant to bile. They survive passage through the stomach and establish themselves in the small intestine. They can influence health and help in longevity of the consumers.

Good starter must produce sufficient acid. It must continue acid production over the entire range of temperature. It should be resistant to antibiotics & bacteriophages. It should be active in the presence of residual chemicals/sanitizers/ detergents. It must not produce undesirable body characteristics, flavour and aroma. In mixed cultures, the associative action must be quite stable, even after repeated subcultures.

4. **Probiotics**

Probiotics are defined as "live microorganisms which when ingested in sufficient numbers provide health benefit to the consumer beyond basic inherent nutrition". The foods containing these live organisms are called probiotic foods. The minimum number of probiotic microbes to give health benefits is said to be one million live cells per gram of the product. Some of the examples to probiotics are *Lb. acidophilus*, *Lb. rhamnosus, Lb. reuteri*, *Lb. casei*, *Lb. plantarum*, *Bifidoabcterium bifidum*, *Bif. longum*, *Saccharmyces boulardi*, etc. The term probiotics consist of two Greek words, i.e., *Pro* meaning 'For' and *Biotics* meaning 'Life'.

Characteristic features of probiotics include

- The probiotic organism should be able to survive upper digestive tract and reach the intestine.
- It should be able to resist the acid and other digestive chemicals such as bile salt, pancreatic juices, etc. in the stomach.
- It should remain viable in food during storage for longer periods.
- It should be able to inhibit pathogens.
- It should be able to adhere to the intestinal tract.
- It should be easy to produce in large volumes at the industrial level.

Some of the health benefits attributed to probiotics are

- Reducing lactose intolerance by predigesting the lactose content in milk using beta-galctosidase.
- Preventing diarrhea by maintaining the balance in gastrointestinal flora.
- Controlling intestinal pathogens like *E. coli*, *Staph. aureus, Salmonella, Clostridium, Listeria*, etc., by competing for nutrients and space and producing antimicrobial compounds which can kill these pathogens.
- Control of serum cholesterol level by inhibiting the enzyme hydroxy methyl glutaryl COA reductase.
- Improved immunomodulation by producing specific antibodies.
- Anticarcinogenic and antimutagenic effect by inhibiting the procarcinogens or mutagens.
- Vitamin synthesis like B_1, B_6 B_{12}, etc.

5. **Non –dairy Fermented foods**

Sauerkraut

It is a fermented food produced due to the action of lactic cultures on cabbage. It is popular in Europe and Oriental countries. Various steps involved in the production of sauerkraut are shown in Fig 5.1.

Microbiology of sauerkraut fermentation

Exclusion of air and judicious use of salt and temperature leads to prolific growth of lactic acid bacteria (LAB). Anaerobiosis restricts growth of spoilage flora. The lactic count increases from 1% to 90% in 2 days at 21°C. The pH drops from 6.2 to 4.5. This inhibits undesirable microbes. Because of the short lag period and generation time, *Leuconostoc mesenteroides* dominates early phase. It initiates acid production. *Leuconostoc* activity decreases with the increase in lactic acid production. Then *Lactobacillus plantarum* and *Pediococcus cerevisiae* start dominating. High level of salt encourages the preferential development of these two organisms leading to high sour flavour. Initial fermentation above 30°C suppresses the growth of *Leuconostoc*. This results in loss of quality with regard to flavour, colour and texture.

The addition of salt to cabbage extracts nutrients from cabbage, which are vital for the establishment of proper microflora. It inhibits the growth of undesirable microorganisms. Salt maintains structural integrity of the shreds and promotes flavour enhancement in the finished product.

Cereal based fermented foods

Classification of cereal based fermented foods

Four basic technologies can be identified for the fermentation of cereals, namely malting, koji, dough fermentation and use of hydrolytic enzymes. Fermentation of cereals significantly improves the protein quality as well as the level of lysine in maize, millet, sorghum, and other cereals. Natural fermentation of cereals leads to a decrease in the level of carbohydrates, some non-digestible polysaccharides and oligosaccharides. Availability of B group vitamins is improved. The antinutritional factors like phytate are reduced. Fermentation increases the amount of soluble iron, zinc and calcium several folds. During cereal fermentations several volatile compounds are formed. These volatile compounds contribute to a complex blend of flavours in the products. Cereals are deficient in lysine, but are rich in cysteine and methionine. Legumes, on the other hand, are rich in lysine but deficient in sulphur containing amino acids. Thus, by combining cereal with legumes, the overall protein quality is improved.

Soy sauce

It is a well-known Oriental fermented cereal based product. It is being used for centuries in East Asian countries. It is a dark coloured salty sauce made by fermenting boiled soybeans and roasted wheat grains. There are two stages in soy sauce fermentation. The first stage is a low salt solid state fermentation carried out for 48 h by *Aspergillus oryzae.* The second stage is a high salt liquid fermentation mediated by lactic acid bacteria and yeast, which may take a few months to three years. The detailed production process is shown in Fig 5.2.

Tempeh

Tempeh is a fermented product of soybean. It originated in Indonesia. It is a solid cake like product with a pleasant taste. It is generally sliced and deep fried after dipping in a salt solution. Production method of tempeh is shown in Fig. 5. 3. In properly fermented tempeh, the mycelial mass tightly binds the soybeans.

Primary benefits of soybean fermentation are the improvement of organoleptic quality and nutritional value. Raw soybeans are bitter in taste. Processing steps of the tempeh fermentation, like soaking, leaching and enzymatic modification result in the removal of the beany flavour. Fermentation results in the formation of delicious new flavours and aroma, creating a unique texture and appearance, besides enhancing the nutritional value and digestibility. Proteases, lipases, carbohydrases and phytases are produced. This results in decrease of anti-nutritional factors. During fermentation flatulence causing raffinose and stachyose are removed mainly by soaking and cooking of soybeans. Several tempeh-forming *Rhizopus* spp. can utilize these as their sole source of carbon and energy. Dietary fibre increases from 3.7 to 5.8%. The content of some B group vitamins, especially riboflavin, niacin, vitamin B6, and vitamin B12, is increased. Carotenoids are formed in small amounts.

Miso

Miso is a fermented soybean paste and a good source of protein. Soybeans, rice, salt and water are the main ingredients for miso preparation. As shown in the figure (Fig 5.4), Koji production and subsequent fermentation with high salt and mashing are the major steps in miso production. Addition of salt arrests the growth of mold. On an average, miso contains 50-60% solids, 3-6% fat, 10-14% protein, 3% fibre and 8-14% salt.

Vinegar

It is a commonly used food preservative. It is produced by converting ethanol by the action of acetic acid bacteria. In the first step, fruit juices, hydrolysed cereals, starches or barely malt are fermented anaerobically by yeasts like *Saccharomyces* sp. It ferments the sugars into alcohol. In the second step, this alcohol is converted into vinegar by acetic acid bacteria through a highly aerobic fermentation. It contains around 4-5% acetic acid. The process details are provided in Fig 5.5.

Vinegar can be produced in three methods. They are natural spontaneous fermentation, the quick vinegar method and the Orleans process. In the first method, sugar palm or coconut sap is allowed to ferment naturally till it becomes sour and it is packed in coke bottles. In quick method (Germen method), a tank filled with beechwood shavings is kept in upright position and acetic acid bacteria are added to it. The alcohol solution is allowed to trickle down over it and oxygen is allowed to enter the tank. Oxidation of alcohol results in vinegar. Orleans process is originated in France. In this method, high grade vinegar is used to start the fermentation in wooden barrels of 200 L capacity. Wine is added at weekly intervals. After four weeks, vinegar is collected from the barrel. It is a continuous method. A gelatinous pellicle forms over the surface of the liquid due to the production of exopolysaccharides (cellulose) by the acetic acid bacteria.

Dhokla (Indian traditional food)

Dhokla is prepared using rice, Bengal gram and black gram dhal at 1.2: 1.8: 1.0 ratio. These are washed, soaked for 8 h and ground into a paste. Curd is added as a source of lactic cultures. Fermentation is allowed to continue for 16-18 h and dispensed into suitable shapes and steam cooked. More details of dhokla preparation are shown in Fig. 5.6.

In conclusion, fermented foods are being prepared from centuries .They can extend the shelf life of the foods and improve the texture and flavor of the foods. The quality of the fermented foods is enhanced by fermenting the foods by using starter cultures. Some of the lactic cultures provide health benefits. Such cultures are known as probiotics. Fermented foods can be prepared using milk, vegetables and cereals. Cereal based foods are fermented in four different ways such as malting, koji, use of hydrolytic enzymes and dough fermentation. Fermentation of cereals reduces the antinutritional factors in the foods, besides providing better nutrition to the consumers.