Consortium for Educational Communication

Module on

# FERMENTED FOODS AND THEIR SIGNIFICANCE

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Fermented foods play a major role in diets worldwide. Fermented foods are foods that have been subjected to the action of microorganisms or enzymes. Fermented foods originated several thousand years ago, in different parts of the world, when microorganisms were introduced incidentally into local foods. The microorganisms caused changes which helped to preserve the foods or improved their appearance or flavor or functional value. The fermented foods can be divided into various categories on the basis of the substrates:

a) Fermented milk products.

b) Fermented vegetable and fruit products

c) Fermented legume foods

d) Fermented cereal foods

e) Fermented cereal-legume foods

f) Fermented meat and fish products.

## a) Fermented Milk products

Fermented milk products refer to those which have been made by employing selected starter cultures to develop the characteristic flavor and /or body and texture. A lactic starter is a basic starter culture with widespread use in the dairy industry.

### Yoghurt

Yoghurt is made from whole or partly defatted milk containing small amounts of skim milk powder or concentrate. The fat content of yoghurt may vary from 0 to 5% and solids content from 9 to 20%.

Yoghurt starter for the lactic fermentation is a mixed culture of *Str. thermophilus* and *Lact. bulgaricus* in a 1:1 ratio. *Leuconostoc strains, Str. diacetilactis and L. acidophilus* are also added to improve the flavor of the yoghurt.

The product is prepared by heating the milk to 85-88°C (185-190F) for 30 to 45minutes and cooled to 43°C (110F). The yoghurt starter is now added at a level of around 2% by volume and incubated at 45°C for 3-5h, followed by cooling to 5°C. Initially *Str. thermophilus* grows rapidly to attain acidities of 0.85 to 0.95%, the increased acidity then slows the growth of *Str. thermophilus* and promotes *L. bulgaricus* which increases acidity up to 1.20-1.50%. Freshly produced yoghurt typically contains around $10^9$ cfu/g, but during storage numbers may decrease to $10^6$ cfu/g, especially when stored at 5°C for up to 60 days.

**Receiving whole/defatted milk**

**Addition of skim milk powder concentrate**

↓

**Filtration/Clarification (35-40°C)**

↓

**Pre-heating the mix (60°C)**

↓

**Homogenization (2500 psi)**

↓

**Pasteurization (85°C/30min.)**

↓

**Cooling (43C)**

↓

**Inoculation (2%)**

↓

**Packaging**

↓

**Incubation (41-42°C/3hrs) (0.75%T.A.)**

↓

**Cooling and storage (5-7°C)**

**Fig.1: Flow diagram of Yoghurt production**

***Kefir***

Kefir is one of the oldest cultured milk products. It originates from the Caucasus region. It is a self carbonated beverage, containing 1% lactic acid and 1% alcohol. The raw material is milk from goat, sheep or cow. Kefir should be viscous and homogenous, and have a shiny surface. The taste should be fresh and acid, with a slight flavor of yeast. The pH of the product is usually 4.3 – 4.4. A special culture, known as Kefir grain, is used for the production of Kefir. The grains consist of proteins, polysaccharides and a mixture of several types of micro-organisms, such as *Lactobacilus bulgaricus, L. lactis, L. kefir, L. fermentum, L. brevis, L. plantarum, str. Lactis* and lactose fermenting yeasts such as *Geotricum candidum, Candida kefir, and S. cerevisiae.* The yeasts represent about 5–10% of the total microflora.  During the fermentation process, the lactic-acid bacteria produce lactic acid, whereas the lactose-fermenting yeast cells produce alcohol and carbon dioxide. Some breakdown of protein also takes place in the yeast metabolism, from which Kefir derives its special yeast aroma.

The product is prepared by standardization of milk to a fat content of 2.5 to 3.5%, the milk is then homogenised at about 65 –70°C and 17.5 – 20 MPa. The standardized milk is heated at 90 – 95°C for 5 minutes. Following heat treatment, the milk is cooled to inoculation temperature, usually about 23°C, after which 2 – 3% starter is added, incubated at 30-35°C for 8-12h and then cooled to 5-8°C and packaged.

**Whole milk**
↓
**Homogenization (2500psi)**
↓
**Pasteurization (90-95°C/ 3-10min).**
↓
**Addition of kefir grains (2-3%)**
↓
**Fermentation for 8-12 hr (30-35°C)**
↓
**Slow cooling (8°C/10-12h)**
↓
**Kefir**

**Fig. 2: Flow diagram of Kefir production.**

***Buttermilk***

Buttermilk is a by-product of butter production from sweet or fermented cream. The fat content is about 0.5%, and it contains a lot of membrane material including lecithin. The shelf life is short, as the taste of the buttermilk changes fairly quickly because of oxidation of the membrane material content. Whey separation is common in buttermilk from fermented cream, and product defects are therefore difficult to prevent.

***Cultured buttermilk***

Fermented buttermilk is manufactured on many markets in order to overcome problems such as off-flavors and short shelf life. The raw material can be sweet buttermilk from the manufacture of butter based on sweet cream, skim milk or low-fat milk. This is obtained by inoculation and incubation of pasteurized skim milk with lactic starter. The product is prepared by heating the skim milk at 82-88°C (180-190°F) for 30 minutes and then cooled to 22°C (72°F) until coagulation occurs and an acidity between 0.80-0.85% is reached. The curd is then broken up by slow agitation to give a smooth consistency and cooled to 5- 10°C. It is then packaged and stored at 5-10°C until distribution. Acidity at packaging should be between 0.85- 0.90%.

**Receiving skim milk**
↓
**Filtration**
↓
**Pasteurization (82-88°C/30 min.)**
↓
**Cooling (22°C)**
↓
**Inoculation (1%)**
↓
**Incubation (21-22°C)**
↓
**Coagulation (12-16hrs) (0.80-0.85% T.A.)**
↓
**Break-up of coagulum**
↓
**Cooling (5-10°C)**
↓
**Packaging and storage (5-10°C)**

**Fig. 3: Flow diagram of Cultured butter milk production**

**Acidophilus milk**

This type of fermented milk is produced by inoculation of *Lactobacillus acidophilus* into sterile skim milk, whole, or partly defatted milk. The product is obtained by heating the fresh milk to 115°C (240°F) for 15 minutes to obtain sterile milk. The milk is then cooled to 38-40°C (100-104°F) and inoculated with 3-5% inoculums (mother starter). The inoculated milk is mixed thoroughly and incubated at 38-40°C (100-104°F) until the milk coagulates. The coagulum is slowly broken up and cooled to 10°C (50°F). The milk is stirred until smooth, packaged and stored at 5°C (40°F) until used.

**Receiving milk (skim/whole/defatted)**
↓
**Filtration/Clarification (35-40°C)**
↓
**Homogenization**
↓
**Sterilization (115°C/15min.)**
↓
**Cooling (38-40°C)**
↓
**Inoculation (3-5%)**
↓
**Incubation (38-40°C/12-16hrs)**
↓
**Coagulation**
↓
**Break-up of coagulum**
↓
**Cooling (10°C)**
↓
**Packaging and storage (5°C)**

**Fig. 4: Flow diagram of Acidophilus milk production**

### Kumiss

This is a lactic acid –alcohol fermented milk prepared from mare's or cow's milk, originated in Russia. The culture consists of *Lactobacillus acidophilus, L. bulgaricus and S. cerevisae.* The first incubation period is followed by the addition of yeast and a second incubation period. The finished product may contain up to 2.5% alcohol.

**Whole milk**
↓
**Homogenization (2500 psi)**
↓
**Pasteurization (85-95°C/3-10min.)**
↓
**Inoculation**
↓
**Fermentation (8-12h)**
↓
**Kumiss**

**Fig. 5: Flow diagram of kumiss production**

### b. Fermented Fruit and Vegetable products

The fermentation of vegetables and fruits is a complex network of interactive microbiological, biochemical and enzymatic reactions.

Lactic acid fermentation is a valuable tool for the production of a wide range of fruit and vegetable products.

**Sauerkraut**

Sauerkraut is a fermented product of fresh cabbage. *Sauerkraut* is a German term meaning “sour cabbage”, this food has became popular in the United States and other European countries. The starter for sauerkraut production is usually the normal mixed flora of cabbage. The major end products of this fermentation are lactic acid and to lesser amounts other acids. After removal of the outer leaves, green leaves and the core, the cabbage is washed and sliced into shreds as fine as 2.5 cm of thickness. This mass is placed in a fermenter in 2 kg layers alternating with salt layers, the first and last layers being salt. The salt added is 2.25-2.5% of the weight of the cabbage to enhance the release of tissue fluids during fermentation. In order to create anaerobic conditions the layers are pressed mechanically, to remove the cabbage juice which contains fermentable sugars and other nutrients required for microbial activity. Anaerobic conditions promote the growth of lactic acid bacteria.

The sequences of lactic acid bacteria carry out fermentation; gas-forming microorganisms *(Leuconostoc mesenteroides*) initiate acid production. When an acidity of 0.25-0.3% is reached (calculated as lactic acid), the growth of cocci begins to slow and they gradually die. However, their enzymes are released by autolysis and continue to be active in the fermentation process. By the time the acidity has reached 0.7-1.0% most of the cocci have disappeared. However, *Lactobacillus plantrum* and *Lactobacillus brevis* are active and increase the acidity to 1.5- 2.0%, despite the inhibitory effect of the salt and low temperature. Finally, *Lactobacillus sake* and *lactobacillus curvatus* complete the fermentation and produce an acidity of 1.7-2.3% with lactic acid at 1-1.5%. The time required for fermentation is 1-2 months and the optimum temperature has been reported to be 18.3$^{o}$C.

**Fresh cabbage**
↓
**Cleaning and washing**
↓
**Cutting and shredding**
↓ ← **2.25-2.5% salt added**
**Fermentation (1-2 months/ lactic acid 1-1.5%)**
**(By natural microflora)**
↓
**Storage at room temperature**

**Fig.6: Flow diagram of Sauerkraut production.**

**Pickles**

Pickles from vegetables and fruits like mango, gooseberry and lemons and vegetables like cucumber, carrot, cabbage, bitter gourd, beans, chillies, pepper, jackfruit, garlic, ginger, brinjal, and onion are popular throughout the world. There are two chief types of fermented pickles-salt or salt-stock pickles and dill pickles. The salt pickles are prepared for use in making special products such as sour, sweet-sour and mixed pickles and relishes. Dill pickles are so named because they are flavored by addition of dill herb. The spices in the pickles aid digestion by stimulating the flow of gastric juices. Salting or curing is done by introducing 10-15% dry salt or brine to the raw materials which contain plenty of water. The weak brine that is formed contains nutrients like proteins, carbohydrates and minerals and this helps in active fermentation through lactic acid fermentation through lactic cultures, which come as surface contamination with raw materials. The organisms involved are salt resistant lactic acid bacteria, belonging initially to heterofermentative *Leuconostoc spp.* and *lactobacillus brevis* which are generally replaced by homofermentative *Lactobacillus plantarum* and *Pediococcus spp*. the coliform bacteria, particularly *Enterobacter* and *Klebsiella spp*. are also involved. As acids are produced and pH falls down, yeasts become more important. Along with lactic acid small quantities of acetic acid, alcohol and sometimes hydrogen and carbon dioxide are also produced during fermentation. There is a considerable degree of change in the physical appearance of the fruit or the vegetable during salting or curing process.

**Fresh vegetable/fruit**
↓
**Cleaning, washing and grading**
**Dill herb (optional)** → ↓ ← **10-15% salt added (NaCl)**
**Slicing**
↓
**Fermentation process (2-4 weeks)**
**(By natural microflora)**
↓
**Packaging in glass jars**
↓
**Storage at room temperature**

**Fig.7: General flow chart of Pickle production.**

### Fermentation of Olives

Olives (*Olea europaea*) to be fermented (Spanish, Greek, Sicilian) are done so by the natural flora of green olives, which consists of a variety of bacteria, yeasts and molds. The olive fermentation is preceded by a treatment of green olives with 1.6 to 2.0% lye (NaOH), depending on type of olive, at 21 to 24$^{o}$C for 4 to 7h to remove oleuropein, a bitter glucoside, which is degraded into glucose and aglycone. Following the complete removal of lye by soaking and washing, the green olives are submerged in a 10% NaCl solution for lactic fermentation. After 6 to 7 months, the olives have the particular characteristics of the end product. Inoculation with *L. plantarum* may be necessary because of destruction of organisms during lye treatment. The fermentation may take as long as 6 to 10 months and the final product have a pH of 3.8 to 4.0 following up to 1% lactic acid production.

**Green olives**
↓
**Cleaning, washing and grading**
↓
**Lye treatment (1.6-2.0% NaOH/21-24oC/4-7 h)**
↓
**Submerged in 10% NaCl solution**
↓ ← **starter addition (*L. plantarum*)**
**Fermentation (6-7 months)**
↓
**Fermented olive (1% lactic acid)**

**Fig.8: Flow diagram of fermented Olives**

**c. Fermented legume products**

Fermentation improves digestibility of legumes for humans. The process can detoxify legumes by reducing haemaglutinin- capable of agglutinating red blood cells, phytate- ability to form insoluble complexes with minerals at physiological pH, oligosaccharides, and trypsin inhibitors- interfere with protein digestion. Other attributes are improved such as, flavor, appearance, and reduced cooking time.

**Soy sauce**

Soy sauce or shoyu is the chief Oriental fermented food and is brown, salty, tangy sauce. It is produced in a two-stage manner. The first stage, the koji, consists of inoculating either soyabeans or a mixture of beans and wheat flour with *Aspergillus oryzae or A. soyae* and allowing them to stand for 3 days at 30°C. This results in the production of large amounts of fermentable sugars, peptides and amino acids. The second stage, the moromi, consists of adding the fungal-covered product to 18% NaCl and incubating at room temperatures for at least a year. The liquid obtained at this time is soy sauce. During the incubation lactic acid bacteria, *L. delbrueckii* in particular and yeasts such as *Zygosaccharomyces rouxii* carry out an anaerobic fermentation of the koji hydrolysate. Pure cultures of *A. oryzae* for the koji and *L. delbrueckii* and *Z. rouxii* for the moromi stages have been shown to produce good quality soy sauce.

**Soybeans**
↓
**Soaked and pressure cooked**
**Mixed with wheat flour → ↓ ← A. oryzae/A. soyae**
**Incubation (3 days/30°C)**
↓
**Salt added (18% NaCl)**
↓
**Incubated in deep tanks (12 months/room temp.)**
↓
**Filtration**
↓
**Liquid collected**
↓
**Pasteurized and filtered again**
↓
**Soy sauce**

**Fig.9: Flow diagram of Soy sauce addition.**

**Tofu**

Soybean cheese or tou-fu-ju or tofu, is a chineese fermented food made by soaking soybeans, grinding them to a paste, and then filtering them through linen. The protein in the filtrate is curdled by means of a magnesium or calcium salt, after which the curd is pressed into blocks. The blocks arranged on trays are held in a fermentation chamber for a month at 14°C, during which period white molds, probably *Mucor spp*., developed. Final ripening takes place in brine or in a special wine.

**Soybeans**
↓
**Soaking & grinding with water**
↓
**Filtering through linen**
↓ ← **Mg or Ca salt addition.**
**Filtrate coagulating**
↓
**Pressed in mould**
↓
**Fermenting (1month/14°C)**
↓
**Ripening in brine or wine**
↓
**Tofu**

**Fig.10: Flow diagram of Tofu production.**

**Tempeh**

Temph is a fermented soybean product, originated in Indonesia. Tempeh is made by fermenting dehulled soybean with a mold *Rhizopus*, the mycelia bind the soybean cotyledons together in a cake like product. The preparation of soybean for fermentation consists of soaking soybeans overnight in order to remove the seed coats or hulls. After dehulling the beans are boiled for 30 minutes, drained, cooled and inoculated with spores of *Rhizopus oligosporus* or small pieces of tempeh from a previous fermentation batch are incorporated as starters and incubated. The incubated beans are mixed, packed in petridishes and placed in incubator at 30-31°C for about 20h until the beans are covered with white mycelium and bound together as cake. The cake is sliced or thin dipped in salt solution and deep fat fried in coconut oil or cut into pieces and used in soups.

**Soybeans**
↓
**Dehulled**
↓
**Soaked (12-14hrs)**
↓
**Boiled**
↓
**Excess water drained**
↓ ← **Starter added and mixed**
**Surface drying**
↓
**Packaged**
↓
**Fermentation (Room temperature/24-48hrs)**
↓
**Fresh tempeh cake**
↓
**Sliced**
↓
**Dipped in salt solution**
↓
**Deep fried**

**Fig. 11: Flow diagram of Tempeh production**

### Natto

Natto is a fermented soybean food in Japan. It is made from whole soybeans by fermentation with a bacterium, *Bacillus subtilis.* The process is simple and quick, similar to that of tempeh fermentation, except that a different microorganism is used. Natto has a characteristic strong odor, flavor, and slimy appearance.

**Soybeans**
↓
**Wash and soak**
↓
**Steamed**
↓ ← **Starter added (*B. subtilis*)**
**Excess water drained**
↓
**Packaged**

**Incubation (16-18hr/48-50°C)**

↓

**Maturation (8hr/3-10°C)**

↓

**Natto**

**Fig.12: Flow diagram of Natto production.**

## FERMENTED CEREAL-BASED FOODS

Cereals are important substrates for fermented foods in all parts of the world and are staples in the Indian subcontinent, in Asia, and in Africa. Fermentation causes changes in food quality indices including texture, flavor, appearance, nutrition and safety.

### Breads

Sourdough bread such as San Francisco bread is produced by fermentation of dough. Historically the starter for sourdough bread consists of the natural flora of bakers barm (sour ferment or mother sponge, with a portion of each inoculated dough saved as starter for the next batch). The barm generally consists a mixture of yeast and lactic acid bacteria. The yeast has been identified as *Saccharomyces cereviseae*. The souring is caused by the acids produced by the bacterium, while the yeast is responsible for the leavening action through the production of $Co_2$ and also produces some desirable flavoring substances. The pH of these sourdoughs ranges from 3.8 to 4.5. Both acetic and lactic acids are produced in addition to ethanol, with the former accounting for 20-30% of the total acidity.

**Select ingredients**
↓ ← **(wheat flour, water, salt and yeast)**
**Mixing**
↓
**Dividing**
↓
**Proving**
↓
**Moulding**
↓
**Final proving**
↓
**Baking in oven**
↓
**Depanning**
↓
**Cooling**
↓
**Slicing and packaging**

**Fig.13: General flow diagram of Bread production.**

**Banku**

Banku is prepared from maize and/or from a mixture of maize and cassava. The preparation of banku involves steeping the raw material (maize or a mixture of maize and cassava) in water for 24 hrs followed by wet milling and fermentation for 3 days. The dough is then mixed with water at a ratio of 4 parts dough to 2 parts water; or 4 parts dough to 1 part cassava and 2 parts water. Continuous stirring and kneading of the fermented dough is required to attain an appropriate consistency during subsequent cooking. Microbiological studies of the fermentation process revealed that the predominant microorganisms involved were lactic acid bacteria and molds.

**Corn**
↓
**Clean**
↓
**Steep for 24 hr**
↓
**Wet mill**
↓

**Ferment for 3 days**

↓

**Dough formation**

↓

**Stirr and knead**

↓

**Cook**

↓

**Banku**

**Fig.14: Flow diagram of Banku production**

### Ogi

Ogi is a porridge prepared from fermented maize, sorghum or millet in West Africa. The traditional preparation of ogi involves soaking of corn kernels in water for 1 to 3 days followed by wet milling and sieving to remove bran, hulls and germ. The pomace is retained on the sieve and later discarded as animal feed while the filtrate is fermented (for 2-3 days) to yield ogi, which is sour, white starchy sediment. The lactic acid bacterium *Lactobacillus plantarum*, the aerobic bacteria *Corynebacterium* and *Aerobacter*, the yeasts *Candida mycoderma*, *Saccharomyces cerevisiae* and *Rhodotorula* and molds *Cephalosporium*, *Fusarium*, *Aspergillus* and *Penicillium* are the major organisms responsible for the fermentation and nutritional improvement of ogi. *Corynebacterium* hydrolysed corn starch to organic acids while *S. cereviseae* and *Candida mycoderma* contributed to flavour development.

**Corn**

↓

**Clean for 2 to 3 days**

↓

**Wet mill**

↓

**Sieve and discard pomace**

↓

**Ferment filtrate and allow to sediment**

**for 1-3 days**
↓
**Ogi**

**Figure 15: Flow diagram of Ogi production**

**Mahewu**

This is a fermented maize meal commonly consumed as a staple among black South Africans. It is traditionally prepared by adding one part of maize meal to 9 parts of boiling water. The suspension is cooked for 10 minutes, allowed to cool and then transferred to a fermentation container. At this stage, wheat flour (about 5% of the maize meal used) is added to serve as a source of inoculum. Fermentation occurs in a warm sunny place within 24 hrs. *Streptococcus lactis* is the main fermenting organism in traditionally prepared mahewu.

**Maize meal**
↓
**Mix in warm water to give 8% solids content**
↓
**Cook at 121°C for 15 minutes**
↓
**Cool**
↓
**Inoculate**
**(5% wheat flour or an adapted pure culture of *Lactobacillus delbrueckii*)**
↓
**Incubate at 30-50°C for wheat innoculum, or at 45°C for *L. delbrueckii* innoculum**
↓
**Ferment for 36 hrs with mixing only at the beginning of fermentation**
↓
**Heat for 10-15 mins under pressure (7 psi)**
↓
**Spray or drum dry**
↓
**Mahewu**

**Fig. 16: Flow diagram of mahewu production.**

**Fermented cereal legume foods**

This is the most dominant group of fermented foods next to dairy products. The addition of legume gives higher protein content and provides a better balanced ratio of amino acids, overcoming the danger of lysine deficiency from cereals alone or sulphur containing amino acid (methionine) deficiency from legume alone.

**Idli**

Idli is a fermented bred type product common in South India, particularly Tamil Nadu. It is made from rice and black gram. The method of idli preparation consists of washing and soaking rice and black gram in the proportion of 2:1 for 2-4h at ambient temperatures and grinding them separately. They are then mixed together with water to give a smooth and uniform batter. The batter is then allowed to ferment for 18-30h by means of natural microflora or by inoculating with fermented batter from previous batch after the addition of approximately 1% salt. The fermented and raised product is cooked by steaming for 10-20 minutes and served hot to give a fine textural idli ready for consumption with chutney or curry or jam. During the fermentation, the initial pH of around 6.0 falls to the values of 4.3-5.3 with 2.5% lactic acid, based on dry grain weight. Most of the organisms consisted of gram-positive cocci or short rods, with *Leuconostoc mesenteriodes* being most abundant spp., followed by *Enterobacter faecalis*. Other probable fermenters include *L. delbrueckii, L. fermentum, L. lactis, Streptococcus faecalis* and *P. cerevisiae,* while the yeast flora generally involved in fermentation includes *Torulopsis candida, Trichosporon pullulans, Candida cacaoi, Debaryomyces tamari, Rhodotorula graminis, Candida fragicola* and *Hansenula anomala.*

**Black gram dhal** | **White rice**
↓ | ↓
**Washing and soaking (2-4h)** | **Washing and soaking (2-4h)**
↓ | ↓
**Fine grinding** | **Fine grinding**

↓

**Mixed with water**

↓

**Batter**

↓

**Salt addition (1% NaCl V/V)**

↓ ← **Previous batter**

**Incubation (18-30 h)**

↓

**Steaming (10-20 min.)**

↓

**Idli**

**Fig.18: Flow diagram of Idli production.**

**Dosa**

Dosa is a thin, crisp, fried, pancake like staple food of South India, eaten mainly as a breakfast food. It is prepared by soaking 1-3 parts of rice and 1 part of black gram dal or yellow lenticels in water for 4-6h at ambient temperature, grinding to a fine paste by adding 2-2.5 parts (w/w) water and mixing the two together to make a free running batter. The batter mixture is then allowed to ferment over night for 12-24h after the addition of about 1% salt, by means of the natural microflora or by inoculating with the fermented batter of the previous batch which serves as the starter. About 60-80ml of the batter is then spread on a hot greasy griddle for a few minutes where it assumes the form of a crisp pancake. It is then rolled and served with coconut chutney or lenticel-vegetable curry.

*L. mesenteroides* is the most common bacterium followed by *S. faecalis, L. fermentum, L. delbrucaekii, B. subtilis* and *Enterobacter spp*., while *Sacharomyces cerevisiae* is the most predominant yeast involved followed by *Debaryomyces hansenii, Hansenula anomala, Candida kefyr* and *C. krousci*. The dosa fermentation indicates that the pH declines from 5.36-4.14, while the total acidity increases from 0.40-1.10.

**Black gram dhal** → **Washing and soaking** → **Fine grinding**

**rice** → **Washing and soaking** → **Fine grinding**

**Combine slurries into a thick batter**
↓ ← **Mix well**
**Salt addition (1% V/V)**
↓ ← **Previous batter**
**Incubation (12-24 h)**
↓
**Spread on hot greasy griddle (few min.)**
↓
**Rolled**
↓
**Dosa**

**Fig.19: Flow diagram of Dosa production.**

**Miso**

Miso is the most popular fermented food in Japan and is prepared by mixing or grinding steamed or cooked soyabeans with koji and salt and allowing fermentation to take place usually over 4-12 months. White or sweet miso may be fermented for only a one week. Similar products are also made and consumed in other parts of the orient and each nation has its own name for product like chiang in China, tauco in Indonesia, doenjang in Korea and tao-chi in Thialand. The koji for miso is a culture of *Aspergillus oryzae* grown at about 35$^{o}$C on a steamed polished rice mash in shallow trays until the grains are completely covered but the mold has not sporulated. The koji is mixed with a mash of crushed, steamed soyabeans, salt is added and the fermentation is allowed to proceed for a week at 28°C and then for two months at 35°C, after which the mixture is ripened for several weeks at room temperature. Involved in the main fermentation are the enzymes of the koji, yeasts (*Saccharomyces rouxii and Zygosaccharomyces spp.),* lactic acid bacteria and *bacilli*. The final product is ground into a paste to be used in combination with other foods.

**Polished rice**
↓
**Soaked and Cooked**
↓ ← ***A. oryzae***
**Incubated**
↓
**Koji**

**Whole soy beans**
↓
**Soaked**
↓
**Cooked**

↓
**LAB → Mixed ← Salt**
↓
**Fermented (4-12 months)**
↓
**Aged**
↓
**Mashed**
↓
**Miso**

**Fig.20: Flow diagram of Miso production.**

**Dhokla**

This is a fermented food commonly used in Gujrat, prepared from a batter made of coarsely ground rice and bengalgram (Cicer arietinum) dal powder in the proportion of 3:1, mixed with one part of curd and adequate water and fermented overnight at room temperature. Green chillies and salt are added and the batter is poured on greased plates and steamed in a suitable pan for 20min.

After cooling, the dhokla is cut into squares seasoned with coriander leaves and green chillies, mustard and asafetida. Bacteria belonging to *L. fermentum, L. lactis, L. delbrueckii, Leuconostoc mesenteroids* and yeast belonging to *Hansenula silvicola* from fermenting batter are generally associated with dhokla fermentation. As the fermentation progresses, the pH of the batter falls from 5.25-4.0 and that of cooked product from 5.5-4.5.

**Black gram dhal** → **Fine grinding**

**rice** → **Fine grinding**

↓

**Mixed**

↓ ← **Curd & water**

**Fermentation (over night/room temp.)**

↓ ← **salt added**

**Addition of green chillies**

↓

**Steamed (20 min.)**

↓

**cooling**

↓

**Cutting into squares**

↓

**Seasoned**

↓

**Dhokla**

**Fig.21: Flow diagram of Dhokla production.**

**e. Fermented meat and fish products**

Meat and fish being highly perishable, their preservation through fermentation has been practiced since ancient times. Among the various fermented products, meat sausages and fish sauces and pastes are the most widely known and consumed fermented meat and fish products.

**Fermented meat sausages**

Fermented sausages are classified as semidry or dry sausages. They are generally prepared by mincing and mixing lean and fat with additives (e.g., nitrite, nitrate, NaCl, ascorbate) and seasonings (e.g., sugar, garlic, pepper). After stuffing, the products are fermented and air dried. Fermentation and drying, which constitute ripening, play an important role in the development of the characteristics of the final products.

For preparation of sausages, lean meat is used while source of meat varies as per consumers choice in a particular market i.e., pork, beef or poultry meat are used in different types of preparations. The meat is chilled or partially frozen followed by comminution in a meat grinder or cutter. At this stage the additives including microbial starters are added for mixing. Widely used additives are salt (2-4%) containing sodium nitrite ($NaNo_2$) added as curing salt, glucose (0.5-1%), sodium ascorbate or ascorbic acid (0.5-1%) and spices. The use of nitrite is considered essential because of its antimicrobial, colour forming, antioxidant and flavouring properties. Both ascorbate and ascorbic acid are used to improve the stability of the nitrosylated pigment. Ground pepper (0.2-0.3%), paprika and garlic has been shown to be effective antioxidant comparable to ascorbate. Additional additives sometimes used for Northern type sausages are phosphates (0.5%) to improve stability against oxidation, glucono- -lactone (Gdl, 0.5%) to ascertain fast chemical acidulation, manganese sulfate (~50ppm) as cofactor for lactic acid bacteria, which may also accelerate fermentation. The starter cultures are added at the concentration of $10^6$ Cfu/g e.g., *Lactobacillus sakei, L. curvatus, L.*

*plantarum, Pediococcus acidilactici, P. pentosaceus, Staphylococcus chrysogenum, P. nalviogense*; yeasts such as, *Debaryomyces hansenii* which is a predominant yeast in fermented meats and is sold as starter and molds such as *Penicillium nalgiovense, P. chrysogenes* and *P. camenberti*. The mixed batter is immediately stuffed under vacuum into natural (i.e., gut), semisynthetic (collagen) or synthetic casings that are permeable to water and air and both ends are clipped. The sausages are then hung in racks and placed in natural or mostly air conditioned fermentation chambers at high relative humidity. The temperature-RH-time combinations during fermentation differ between semidry (27-41°C/ 90%/ 15-20h) and dry (Northern) sausages (20-30°C/ 58-95%/ 18-48h). The drying temperature-RH-time combinations also differ between semidry (10°C/68-72%/ 2-3 days) and dry (12-15°C/75-80%/1-3 weeks) sausages. At the end of the fermented period, dry (Northern) type sausages are subjected to smoking. Smoke contributes to antimicrobial and antioxidant effects, besides generating specific flavour and colour components. In case of semidry sausages, the final pH ranged from 4.4-5 and final water activity is 0.93-0.98, where as in case of dry sausages final pH ranged from 4.6-5.1 and water activity from 0.92-0.94.

**Meat and fat selection (4°C)**

↓

**Comminution**

↓ ← **addition of starter, spices, salt, $NaNo_2$, sodium ascorbate.**

**Mixing and Stuffing (Natural or synthetic casings)**

↓

**Fermentation**

↓

**Drying (semidry sausage-27-41°C/15-20 h/90% RH, Dry sausage-20-30°C/18-48 h/58-95% RH)**

↓

**Smoking (12-22°C/10-45 h)**

↓

**Packaging (for retail distribution).**

**Fig. 22: Flow chart for sausage preparation.**

## Fermented fish products

### Fish Sauces

Fish sauce is a brown liquid with a characteristic meaty flavour and aroma. It is mainly used as a condiment to flavour rice and other cereal dishes. Fish sauces are known by various names such as *ngapi* (Burma), *nuoc-mam* (Cambodia & Vietnam), *nam-pla* (Laos & Thiland), *Ketjap-ikan* (Indonesia), *balao-balao* (Philippines) and so on. LAB are found as the dominant microorganisms in fermented fish products.The production of some of these sauces begins with the addition of salt to uneviscerated fish at a ratio of approximately 1:3, salt to fish. The salted fish are then transferred to fermentation tanks and built into the ground or placed in earthenware pots and buried in the ground. The tanks or pots are filled and sealed off for at least 6 months to allow the fish to liquefy. The liquid is collected, filtered, and transferred to earthenware containers and ripened in the sun for 1-3 months. The finished product is clear dark-brown in colour with distinct aroma and flavour. The pH from start to finished product ranged from 6.2 to 6.6 with NaCl content 30-33% over the 12 months period. Members of *Streptococci, Micrococci* and *Staphylococci* along with bacillus spp., were apparently involved in the development of flavour and aroma of these products. The safety of products of this type is due to the 30-33% NaCl.

### Fish Pastes

Fish pastes are eaten almost everywhere in Southeast Asia generally as a condiment for rice dishes. They are known by various names such as, *mam-tom* of China, *mam-ruoc* of Cambodia, *bladchan* of Indonesia, *shiokara* of Japan, *bagoong* of Philippines, and *nam-pla* of Thailand. The production of some of these pastes begins with the fishes are first cleaned, the mixed with 20-25% salt. The mixture is then allowed to ferment for several days. In this type of fermented fish most of the protein break down is accomplished by the fish enzymes. It is eaten either as a sauce or as a main dish after it is sautéed with garlic and onion.

**Fish (fresh or dry)**
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**Cleaning of fresh or dry fish**
↓
**Addition of salt in large containers**
↓
**Thick fish paste product**
↓
**Maceration, mixing and dilution in water**
↓
**Addition of ground spices, cereal adjuncts or Fruit and filling in containers**
↓
**Fermentation at room temperature**
↓
**Ready-to-serve sauce (2 weeks to 1 year)**

**Fig. 23: The overall processing of fish paste and sauce**

**Significance of fermented foods**

**Safety and Preservation**

As one of the oldest forms of food preservation, fermentation has played a key role in enabling people to survive periods of food shortage. Lactic acid bacteria alone or in combination with yeasts, molds and other bacteria, play a critical role in safety and preservation of foods through production of antimicrobial metabolic products such as lactic acid, acetic acid, formic acid, free fatty acids, ethanol, ammonia, hydrogen peroxide, carbon dioxide, diacetyl, acetoin, 2, 3-butanediol, acetaldehyde, benzoate, D-amino acids, bacteriolytic enzymes, bacteriocins ( e.g., nisin, lactocins, sakacin A, pediocin PA-1, carnobacteriocins, helveticin and caseicin) and antibiotics. Fermentation minimizes the amount of less desirable or toxic components in some foods for example phytate content in bread and lectins in legumes are lowered due to the fermentation process.

**Nutritional significance of fermented foods**

Fermented foods are the major group of "functional foods", which provide extra benefits to our diet beyond those expected from the major nutrients present. The fermentation process increases the protein efficiency ratio (PER) of some foods. During fermentation certain micro-organisms produce vitamins and thus increase the value of water-soluble vitamins, such as riboflavin, folic acid, thiamine and nicotinic acid, depending on the food type. Fermentation can enhance the nutritional value of foods, especially of plant materials, involves enzymatic splitting of cellulose, hemicelluloses, and related polymers that are not digestible by humans into simpler sugars and sugar derivatives. People who cannot tolerate milk find that fermented milks are more acceptable, because microorganisms break down the lactose due to the secretion of enzymes such as, lactase or β-galactosidase. The fermentation process also might improve your ability to absorb minerals, such as zinc and iron.

**Health benefits of fermented foods**

One of the reasons for the increasing interest in fermented foods is its ability to promote the functions of the human digestive system in a number of positive ways. This particular contribution is called probiotic effect. Studies on supplementation of food formulations with *Lb. acidophilus* showed that the serum cholesterol in humans was reduced. Apart from this, there are interesting data on anticarcinogenic effect of fermented foods showing potential role of *Lactobacilli* in reducing or eliminating procarcinogens and carcinogens in the alimentary canal. The enzymes β-glucuronidase, azoreductase and nitroreductase which are present in the intestinal canal, are known to convert procarcinogens to carcinogens. Oral administration of *Lb rhamnosus, L. acidophilus and B. longum* was shown to lower the faecal concentration of β-glucuronidase in humans implying a decrease in the conversion of procarcinogens to cancinogens. Fermented milk containing *Lactobacillus acidophilus* given together with fried meat patties significantly lowered the excretion of mutagenic substances compared to ordinary fermented milk with *Lactococcus* fed together with fried meat patties. The process of fermentation of foods is also reported to reduce the mutagenicity of foods by degrading the mutagenic substances during the process. Lactic acid bacteria isolated from dadih, traditional Indonesian fermented milk, were found to be able to bind mutagens and inhibit mutagenic nitrosamines. The presence of acid, especially acetic or lactic acid was found to lower the glycemic index in breads to a significant level. Some lactic acid bacteria which are present in fermented milk products are found to play an important role in the immune system of the host after colonisation in the gut. Oral administration of *Lactobacillus casei, L. acidophilus* caused an improvement of the function of the peritoneal macrophages and increased the production of IgA.

**Sensory attributes of fermented foods**

The sensory characteristics of fermented foods are achieved by the interaction of microbial, physical and biochemical reactions. During the fermentation process, acidification produces reactions and changes that ensure the development of colour, texture and flavor specific to the fermented foods.

Fermentation improves the texture of some products. In milk products changes in texture are due to lactic acid, which causes a reduction in electrical charge on the casein micelles. They coagulate at the isoelectric point to form characteristic flocks. The acidification process also improves the sliceability of the meat products. The characteristic flavor of fermented products such as, milk products and meat sausages are due to breakdown of carbohydrates, lipids and proteins through the action of microbial enzymes. The fermentation process release compounds of low molecular weight such as diacetyl, acetoin, butanediol, acetaldehyde, ethanol, acetic acid, 2, 3-methylbutanol, 2, 3-methylbutanal, 2, 3-methylentanoic acid, dimethyldisulfide and benzeneacetaldehyde which impart flavor to the fermented food products.