CHAPTER 1

Fermented Foods and Their Production

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FERMENTATION AND FOOD SAFETY

Fermentation

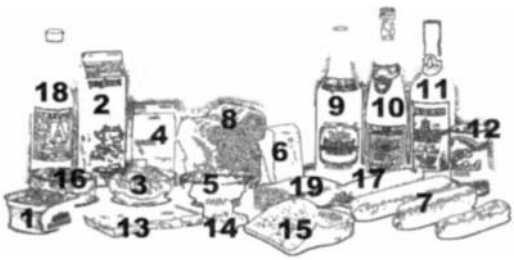
Fermentation is one of the oldest methods of food processing. Bread, beer, wine, and cheese originated long before Christ. Although modern food technology has contributed to the present-day high standard of quality and hygiene of fermented foods, the principles of the age-old processes have hardly changed. In industrialized societies, a variety of fermented foods are very popular with consumers because of their attractive flavor and their nutritional value (Figure 1–1).

In tropical developing regions, fermentation is one of the main options for processing foods. In the absence of facilities for home refrigeration, freezing, or home canning, it serves as an affordable and manageable technique for food preservation. Fermentation can also increase the safety of foods by removing their natural toxic components, or by preventing the growth of disease-causing microbes. It imparts attractive flavor and nutritive value to many products. Fermentation is an attractive technique because it is low cost and low technology and it can be easily carried out at the household level, often in combination with simple methods such as salting, sun drying, or heating (e.g., boiling, steaming, frying).

Contrary to unwanted spoilage or toxin production, fermentation is regarded as a desirable effect of microbial activity in foods. The microbes that may be involved include molds (mycelial fungi), yeasts (unicellular fungi), and bacteria. Examples of food fermentations and the microbes responsible for the desired changes will be presented in this chapter.

In general, the desirable effect of microbial activity may be caused by its biochemical activity. Microbial enzymes breaking down carbohydrates, lipids, proteins, and other food components can improve food digestion in the human gastrointestinal tract and thus increase nutrient uptake. Several bacteria excrete B vitamins into food. As a result of their growth and metabolism, substances of microbial origin are found in the fermented food, including organic acids, alcohols, aldehydes, esters, and many others. These may have a profound effect on the quality of the fermented product. For instance, lactic and acetic acids produced by lactic acid bacteria (LAB) have an inhibitory effect on spoilage bacteria in sourdough bread and yogurt, and the production of ethanol and carbon dioxide determines the acceptability of bread, beer, and wine (ethanol disappears from bread during the baking process).

In addition to enzymes and metabolites, microbial growth causes increased amounts of microbial cell mass. This may be of nutritional and aromatic interest in yeast extract, for instance. The presence of living microbial cells such as in nonpasteurized yogurt may well have advantageous effects on the intestinal microflora and, indirectly, on human health.



Nr.	*Name of product*	*Major ingredient(s)*	*Functional microorganisms*
1	quark	cows' milk	lactic acid bacteria (LAB)
2	yogurt	cows' milk	LAB
3	sauerkraut	white cabbage	LAB
4	cultured milk ("karnemelk")	cows' milk	LAB
5	treated black olives	olives	LAB
6	gouda cheese	cows' milk	LAB
7	raw fermented sausages	pork and/or beef meat	LAB
8	yeast-leavened bread	wheat flour	yeasts (Y)
9	lager beer	barley, hops	Y
10	white wine	grapes	Y
11	sherry	grapes	Y
12	lager beer	barley, hops	Y
13	gruyère cheese	cows' milk	LAB + propionibacteria
14	pumpernickel bread	rye flour	LAB + Y
15	mixed sourdough bread	rye + wheat flour	LAB + Y
16	camembert cheese	cows' milk	LAB + molds
17	raw fermented sausage	pork and/or beef meat	LAB + molds
18	soy sauce	soybeans and wheat	Molds + LAB + Y
19	tempeh	soybeans	LAB + Molds + Y

Figure 1–1 Fermented foods representing different types of fermentations and raw materials

Food Safety

In its widest sense, the safety of food must be achieved through safe production, storage, and handling in order to avoid food-borne illnesses such as food intoxication, infectious diseases, or other detrimental effects. In principle, such illnesses can be caused by agents of biological, chemical, or physical nature, as exemplified in Table 1–1.

In this book, the fermentation of food will be viewed in relation to these safety aspects. The

Table 1–1 Some Examples of Threats to Consumer Safety

Nature	*Type of Causative Agents*	*Specific Examples*	*Illness*
Biological	Pathogenic microorganisms	Bacteria (*Salmonella* spp.)	Enteric infections
		Viruses (Rotavirus)	
		Parasites (*Cryptosporidium* spp.)	
	Toxigenic microorganisms	Mycotoxin-producing fungi (*Aspergillus flavus*)	Liver cancer
		Toxin-forming bacteria (*Clostridium botulinum*)	Respiratory failure
		Biogenic amine-forming bacteria (Enterobacteriaceae)	Hypertension
Chemical	Phytotoxins	Cyanogenic glycosides (linamarin) in cassava tubers	Cyanide intoxication
	Environmental contaminants	Pesticides	Intoxications
		Veterinary drugs	
		Heavy metals	
	Toxic metabolites	Biogenic amines (e.g., in fish)	Hypertension
	Food additives and ingredients	Preservatives	Allergies
		Colorants	
Physical	Foreign matter	Glass	Injuries (cuts, perforations)
		Metal	

book begins with a closer look at fermentation as a food processing technology. Next, the intrinsic safety of fermented foods as well as the principles of the hazard analysis critical control point (HACCP) system are discussed, followed by several examples of hazards that are potential threats to consumer safety.

The use of genetically modified ingredients or microorganisms in food fermentations, as well as the use of microorganisms as probiotics in fermented foods, are aspects that require a systematic and critical assessment of their safety.

In the final synthesizing chapter, the HACCP approach is used to illustrate and compare some of the critical processing steps that affect the safety of fermented foods.

FOOD FERMENTATION COMPONENTS

Food Ingredients

Food ingredients include the raw foods chosen for fermentation which can be of plant or animal origin. These foods contain a variety of nutrients for the consumer but also some of these nutrients will be required for the microbial growth and metabolism during the fermentation process. In order to enable microbial activity, sufficient water must be present. Consequently, water must be added in case the other ingredients are too dry. For several reasons, such as taste or preservation, miscellaneous substances may be added to the ingredient mix. These will be discussed in more detail below.

Food Groups

- **Plant origin:** Fermented foods of plant origin are derived from a variety of raw materials of different chemical composition and biophysical properties. Tuberous roots such as potatoes and cereals and tree crops like breadfruit have a relatively high starch content. Legumes and oil seeds generally have a high protein content. Green vegetables, carrots, beets, tomatoes, olives, cucumbers, okra, and forage crops for animal feed silages have a high moisture content. Fruits contain high concentrations of reducing sugars.

Most fermentative preservations of vegetables and cereals are due to the action of LAB, often in combination with yeasts. But other bacteria, such as *Bacillus* spp., or mycelial fungi, such as *Rhizopus* and *Aspergillus* spp., are equally important in the fermentation of legumes and oil seeds.

- **Animal origin:** Foods of animal origin that are fermented are mainly milk, meat, fish, and seafood. These are all quite perishable, and fermentation has long been an effective method for prolonging the shelf life of these valuable nutrients.

Milk originating from cows, buffaloes, sheep, and, occasionally, other animals has a high moisture content; contains proteins, minerals, and vitamins; and has a neutral pH. A rapid acidification by lactic acid bacterial fermentation to pH values of less than 4.5 strongly inhibits the survival and growth of spoilage-causing or disease-associated bacteria. Milk contains ample amounts of the fermentable carbohydrate lactose, which is an essential ingredient to enable this fermentation. Due to the reduced pH, as well as bacterial glycocalix, the viscosity increases and various liquid fermented milk products are obtained, ranging from fluid cultured milk to stringy viscous Scandinavian milks, mixed sour-alcoholic fizzy fluids, and gel-type products such as yogurt. Milk is also rather voluminous; nomadic tribes developed methods to curdle milk and separate the coagulated casein from the residual liquid (whey). The coagulate represents only approximately 10% of the original milk volume. Fermentation by LAB, and occasional propionibacteria, yeasts, and molds, results in a variety of cheeses.

Meat (cow, pork, goat, etc.) contains very low levels of fermentable carbohydrates, thus posing limitations to lactic acid fermentation. Many of the fermented meats derive their long shelf life from a combination of preservative effects. Acidification by LAB is usually boosted by adding sugar, and added salt, nitrate, or nitrite, as well as some extent of dehydration, are major aspects of the preservation of fermented sausages. It is interesting to note that most fer-

mented sausages have been prepared from raw meat. In order to avoid the risk of raw meat-borne food infections by parasites, it is recommended to freeze the meat prior to processing.

Fish and seafood (e.g., shrimp) pose similar restrictions to bacterial fermentation: They contain only low quantities of fermentable carbohydrates. In practice, this has led to two major types of fish products preserved by fermentation. The first is a mixture of fish and salt that results in liquid protein hydrolysate after several months of fermentation. Halotolerant bacteria and yeasts may play a role in flavor formation, but the high salt content is responsible for the preservation. The second type of fish product is a mixture of fish, little salt, and cooked starchy food (e.g., rice or cassava), the latter providing fermentable carbohydrates; these products are preserved mainly by lactic acid fermentation.

Nutrients Required by Microorganisms

- **Carbon and nitrogen:** Most microorganisms require some form of organic carbon. In natural raw materials, this is found mainly in carbohydrates (e.g., monomers, dimers, etc., and a number of polymeric food-storage and structure-giving polysaccharides such as starch, pectins, hemicellulose, and cellulose). Molds and certain bacteria (e.g., *Bacillus* spp.) are good producers of extracellular carbohydrate-degrading enzymes that can release fermentable mono- and oligomeric substances. Accounts of the response of yeasts to sources of carbon, nitrogen, phosphorus, and sulfur are available.[34,41] Yeasts and LAB are known to be poor converters of polysaccharides and pentoses. Although the presence of chitin and acacia gum was shown to increase the rate of yeast growth and fermentation,[31] these compounds were not metabolized as nutrients.

In addition to fermentable saccharides, other nutrients required for cell growth and metabolism include inorganic (e.g., NH_4^+) or organic sources of nitrogen (e.g., urea, amino acids, and peptides, but rarely extracellular proteins), phosporus (e.g., inorganic phosphate or phosphate esters), and sulfur (e.g., sulfate, sulfite, methionine, glutathione). Fungi usually do not require additional nutrients in food fermentation. Optimum carbon/nitrogen ratios for growth are 10–100. For use in industrial fermentations, it was shown that *R. oryzae* could grow well with only starch and nitrogen salts; the addition of vegetable juice further stimulated growth.[37]

- **Minerals:** Iron, magnesium, potassium, sodium, and calcium are normally required for cell growth.[41] Of additional interest is the effect of Ca^{2+} ions, which was reported to increase the ethanol tolerance of *Saccharomyces cerevisiae*.[24] Manganese plays an important role in LAB; deficiencies may lead to fermentation failures.
- **Vitamins and other growth factors:** The most common growth factors for yeasts are biotin, pantothenic acid, inositol, thiamine, nicotinic acid, and pyridoxine.[41] Riboflavin and folic acid are synthesized by all yeasts, but are required by some bacteria. Fungal requirements for additional vitamins in the food environment seem to be negligible. On the contrary, they may contribute to the nutritive value of fermented foods by vitamin synthesis.

Water

Water is essential for microbial growth and metabolism. The extent to which water is available for biological metabolism is expressed as water activity (A_w) or, occasionally, as water potential.[15] A_w is the more commonly used terminology and is defined by the ratio of equilibrium water vapor pressure of the food and of pure water, at a defined temperature such as 20 °C. A_w ranges from 0 to 1. Most microorganisms require A_w to be greater than 0.70, with optimum greater than 0.99.

Added Food Ingredients

A variety of added ingredients affect the activity of microorganisms and thus can be used to regulate the rate and extent of fermentation. Salt and sugar are well known for their antimicrobial

effect at high concentrations. Salt levels greater than 15% w/w and sugar concentrations greater than 25% w/w reduce the A_w considerably; in addition, NaCl has a specific inhibitory influence caused by Na^+ ions. Herbs, spices, and so forth may contain inhibitory proteins, organic acids, essential oils, pigments, resins, phenolic compounds, caffeine, and so on. On the other hand, some spices are excellent sources of manganese, which is essential for LAB.

Microorganisms

Three groups of microorganisms are used in food fermentation, namely bacteria, yeasts, and molds. Table 1–2 illustrates some prominent species of microorganisms, some of the food fermentations in which they are of importance, and their function.

How do microorganisms enter the fermentation? Different scenarios of increasing complexity can be distinguished,[25] as will be explained in the following sections.

Natural Fermentation in Raw Substrate

Most raw foods of animal or plant origin contain a variety of microorganisms that arrived by chance or that have an ecological association with them, based on preharvest growing conditions. Food processing activities can also add certain microorganisms to food. If food is allowed to ferment without prior heating, most of these microorganisms can multiply. However, their opportunities will be restricted by their ability to grow and compete in the food, and by external conditions. This usually results in a succession of predominant microorganisms, finally stabilizing in a fermented product that contains a mixed microbial population dominated by microorganisms that are particularly suited to the physicochemical conditions prevailing in the final product. This type of fermentation is exemplified by sauerkraut, which is shredded and salted cabbage that is fermented by LAB.[11] Drawbacks of natural fermentations are that they take a relatively long time to complete, and the outcome is always a surprise.

Use of Traditional Mixed Starter Cultures in Raw or Preheated Substrate

The drawbacks of natural fermentation can be reduced when a large quantity of microorganisms that occur in the final product are added. These may be expected to bring about a more rapid domination and a more predictable quality of the fermented product. An example of this approach is the traditional fermentation of sourdough, a mixture of cereal flour (wheat or rye) with water of dough consistency.[12] A small quantity of previously fermented sourdough is mixed into the new dough, and this practice can be successfully carried out for many years to achieve dependable fermentations.

Traditional mixed-culture starters are also applied in the fermentation of precooked ingredients. Examples are the Indonesian fermented soybean food *tempeh* (also known as *tempe*),[18] as well as African alcoholic beverages such as *Ghanaian pito* made from sorghum.[9] For tempeh, a mixed culture of molds (*Rhizopus* and *Mucor* spp.) especially grown for this purpose on plant leaves *(Hibiscus tileaceus)* is used.[27] The fermentation starter for pito is an inoculation belt, a woven rope that is suspended in the fermenting beer and on which the predominating yeasts (*Saccharomyces* spp.) are present as a sediment. The fermentation of a next batch of pito beer is started by immersing the inoculation belt into the sugary liquid made from sorghum, which is a common cereal in tropical climates.

Use of Pure Cultures (Single or Mixed) in Preheated Substrate

With increasing scale of production, more sophisticated technical facilities, and higher investment and operational risks, the use of laboratory-selected and precultured starter cultures becomes a necessity. Because equipment for aseptic processing at a large production scale is extremely expensive, common procedures include pure culture maintenance and propagation under aseptic conditions (e.g., sterile laboratory and pilot fermentors). At production scale, the food ingredient to be fermented is preheated in

Table 1–2 Selected Examples of Microorganisms and their Function in the Fermentation of Foods

Microbial Group	*Species*	*Fermented Foods*	*Importance of Fermentation for Product Characteristics*
Bacteria	Lactic acid bacteria: *Lactobacillus delbrückii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*	Yogurt	Contribute to flavor, shelf life, structure, and consistency by the production of lactic acid, acetaldehyde, diacetyl, and polysaccharides
Yeasts	*Saccharomyces cerevisiae*	Alcoholic beverages (beers, wines)	Produce ethanol, CO_2, and flavor
Molds	*Aspergillus sojae*	Soy sauce	Form proteolytic and saccharolytic enzymes, enabling solubilization and flavor production

order to minimize the level of microbial contamination; subsequently, the pure culture starter (single or mixed cultures) is added and fermentation takes place at the highest practicable and affordable level of hygienic protection.

Use of Pure Cultures in Sterilized Substrate

For the propagation of starter cultures, or in other situations requiring the absolute absence of microbial contamination, food ingredients or otherwise suitable nutrients are sterilized and kept in sterile confinement. Pure cultures of starter microorganisms are added using aseptic techniques. Not only is this an expensive technique, but it is also unnecessary in production-scale food fermentation. Moreover, severe heat treatments required to achieve sterility can harm heat-sensitive nutrients for both the microorganism and the consumer.

Enzymes

The importance of enzymes in fermentation processes lies in their ability to degrade complex substrates. Some examples are in *koji* fermentation, where *A. oryzae* enzymes degrade starch, protein, and cell wall components, and in tempeh production, where *Rhizopus* spp. enzymes degrade cell wall components, protein, lipids, phytic acid, and oligosaccharides. Proteases produced by LAB are important degrading proteins in fermented milk products. Some enzymes involved in the degradation of complex substrates include amylolytic, proteolytic, lipolytic, and cell wall degrading enzymes.

Amylolytic Enzymes

Starch is composed of two polysaccharides, amylose and amylopectin, both consisting of glucose units only (Figure 1–2). *Amylose* is a linear glycan in which the sugar residues are connected by α-1,4 bonds; *amylopectin* is a branched glycan in which glucose residues in the backbone and the side chains are α-1,4 linked. The side chains are attached by α-1,6 linkages.

Starch is degraded by several amylases working simultaneously. α-amylases hydrolyze α-1,4 glucosidic linkages. Iso-amylase is a debranching enzyme; it hydrolyzes α-1,6 glucosidic linkages. Amyloglucosidase liberates single glucose units from the nonreducing end,

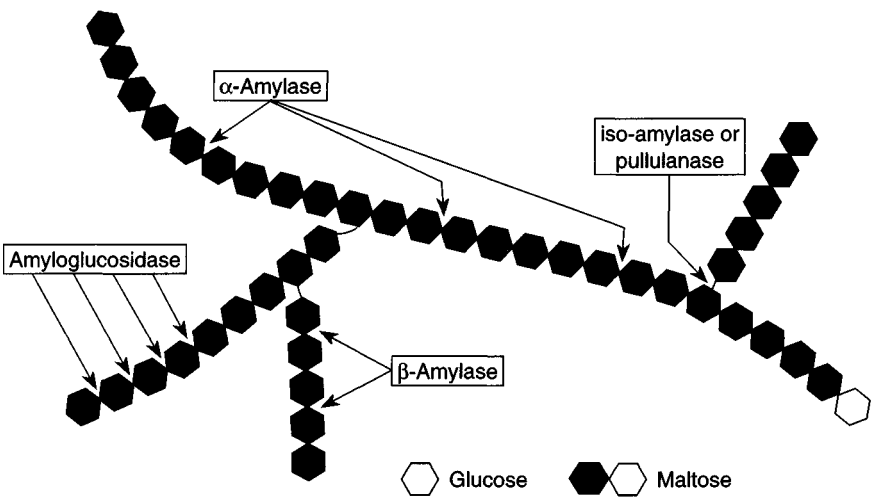


Figure 1–2 Amylopectin molecule and its degradation by several amylases. Adapted with permission from Uhlig, H. (1991). *Enzyme arbeiten für uns. Technische enzyme und ihre Anwendung.* München, Wien: Carl Hanser Verlag.[43]

whereas β-amylase liberates maltose units. These enzymes belong to the class of hydrolases. In industrial-scale production of α-amylases, the enzyme is derived from the pancreatic gland of swine and cattle and from microbial cultures. Microorganisms that produce amylases are *B. subtilis* and *A. oryzae. B. licheniformis* produces a heat-stable amylase that can be used at 100–110 °C. Gelatinization of starch by heating enhances enzyme catalysis. Thus, the swollen gelatinized starch substrate is degraded 300 times faster by bacterial amylase and 10^5 times faster by fungal amylase than the native, unswollen, nongelatinized starch.

Proteases

Proteases are classified according to their source (e.g., animal, plant, microbial), their catalytic action (e.g., endopeptidase or exopeptidase), and the nature of the catalytic site. *Endopeptidases* are the proteases that are used most commonly in food processing, but in some cases, they are accompanied by exopeptidases. Endopeptidases cleave polypeptide chains at particularly susceptible peptide bonds distributed along the chain, whereas *exopeptidases* hydrolyze one amino acid from the end of the chain. The four major classes of endopeptidases are carboxyl proteases (i.e., aspartic protease), cysteine proteases, serine proteases, and metallo proteases (Table 1–3). As the names imply, carboxyl, cysteine, and serine proteases have carboxyl, cysteine, and serine side chains, respectively, as essential parts of the catalytic site. The carboxyl proteases generally have maximum catalytic activity at acid pH. The serine proteases have maximum activity at alkaline pH; the closely related cysteine proteases usually show maximum activity at more neutral pH values. The metallo proteases contain an essential metal atom, usually Zn^{2+}, and have an optimum activity at neutral pH. Ca^{2+} stabilizes these enzymes and strong chelating agents (such as ethylenediaminetetraacetic acid, or EDTA) inhibit them.

Enzymes Degrading Cell Wall Components

In order to understand how enzymes affect plant cell walls, the structure of the walls first must be reviewed. Microscopic investigations have revealed that plant cell walls can be divided into three layers: the middle lamella, primary wall, and secondary wall (Figure 1–3). The *middle lamella* acts as an intercellular binding substance and is mainly composed of pectin. *Secondary cell walls* contain less pectin but contain some lignin. *Primary cell walls* consist of cellulose fibers called microfibrils embedded in a matrix of pectins, hemicelluloses, and proteins (Figures 1–3 and 1–4). Pectin is the major binding component of the cell wall, and its degradation by pectolytic enzymes will cause fruit or vegetables to become soft. This is the first step of enzymatic degradation of the cell wall.

- **Pectolytic enzymes:** The basic structure of pectins is a linear chain of α-linked molecules of pyranosyl D-galacturonic acid. Varying proportions of carboxylic groups can be present as methyl esters. The esterification is usually by methanol, in which case the pectin is said to be methylated. When less than 50% of the carboxyl groups are methylated, the pectin is referred to as *low methoxyl pectin;* when more than 50% of the carboxyl groups are methylated, the pectin is referred to as *high methoxyl pectin.* Pectin situated in the middle lamella is removed from the cell wall relatively easily and is most easily degraded by appropriate enzymes. In contrast, enzymes do not easily degrade the pectin within primary and secondary cell walls. Pectinases are defined and classified on the basis of their action toward the galacturonan part of the pectin molecules. Two main groups are distinguished, pectin esterases and pectin depolymerases.
- **Hemicellulases:** Hemicelluloses are polysaccharides that are extracted from plant cell walls by strong alkali. They are composed of four major substances: arabinans, galactans, xyloglucans, and xylans. As an example, arabinans are degraded by the enzymes, arabinanases. Arabinans are branched polysaccharides with a backbone of α-1,5 linked L-arabinose

Table 1–3 Overview of Proteases

Name	*Type*	*Source*	*pH-Optimum*	*Optimum Stability pH Range*
Proteases of animal origin				
Chymosin	Carboxyl protease	Stomach lining of calves	6.0–7.0	6.5–6.0
Pepsin	Carboxyl protease	Gastric lining of swine or bovine	2.0	
Pancreatic protease*		Pancreas	9.0	3.0–5.0
Proteases of plant origin				
Papain	Cysteine protease	Tropical melon tree (*Carica papaya)*	7.0–8.0	4.5–6.5
Bromelain	Cysteine protease	Pineapple (fruit and stalk)	7.0–8.0	
Ficin	Cysteine protease	Figs (*Ficus carica*)	7.0–8.0	
Bacterial proteases				
Alkaline protease	Serine protease	e.g., *Bacillus subtilis*	7.0–11.0	7.5–9.5
Neutral protease	Metallo protease	e.g., *Bacillus thermoproteolyticus*	6.0–9.0	6.0–8.0
Pronase		*Streptomyces griseus*		
Fungal proteases				
Acid protease	Carboxyl protease	*Aspergillus oryzae*	3.0–4.0	5.0
Neutral protease	Metallo protease	*Aspergillus oryzae*	5.5–7.5	7.0
Alkaline protease	Serine protease	*Aspergillus oryzae*	6.0–9.5	7.0–8.0
Protease	Carboxyl protease	*Mucor pusillus*	3.5–4.5	3.0–6.0
Protease	Carboxyl protease	*Rhizopus chinensis*	5.0	3.8–6.5

*A mixture of trypsin, chymotrypsin, and various peptidases with amylase and lipase as accompanying enzymes.

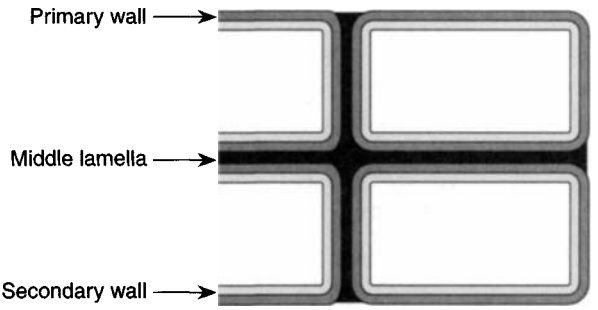


Figure 1–3 Schematic representation of the structure of plant cell walls. Reproduced with permission from Voragen, A. G. J., Van den Broek, L. A. M. (1991). Fruit juices. *Biotechnological Innovations in Food Processing,* pp. 187–210. Edited by Biotol Team. Oxford, UK: Butterworth-Heinemann.[44]

units (Figure 1–5). To approximately every third arabinose molecule, additional arabinose units are attached by α-1,2- or α-1,3 linkages. This can produce complex structures. There are two types of arabinanases: arabinosidase (arabinofuranosidase) and endo-arabinanase. Arabinosidase can be subdivided into two forms, A and B. The B form degrades branched arabinan to a linear chain by splitting off terminal α-1,3 or

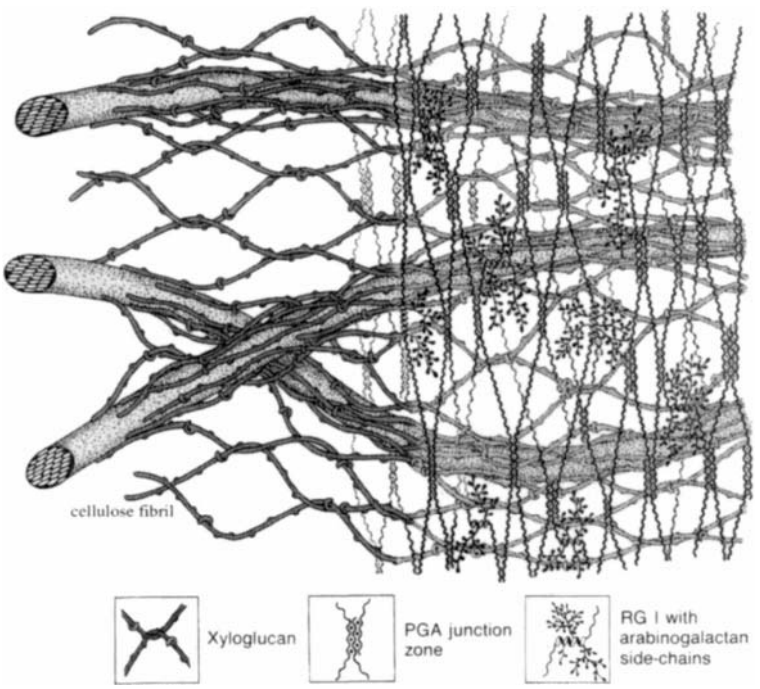


Figure 1–4 Schematic representation of the primary cell wall. PGA and RG are part of pectin. Reproduced with permission from Carpita, N. C., Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal* **3**, 1–30.[7]

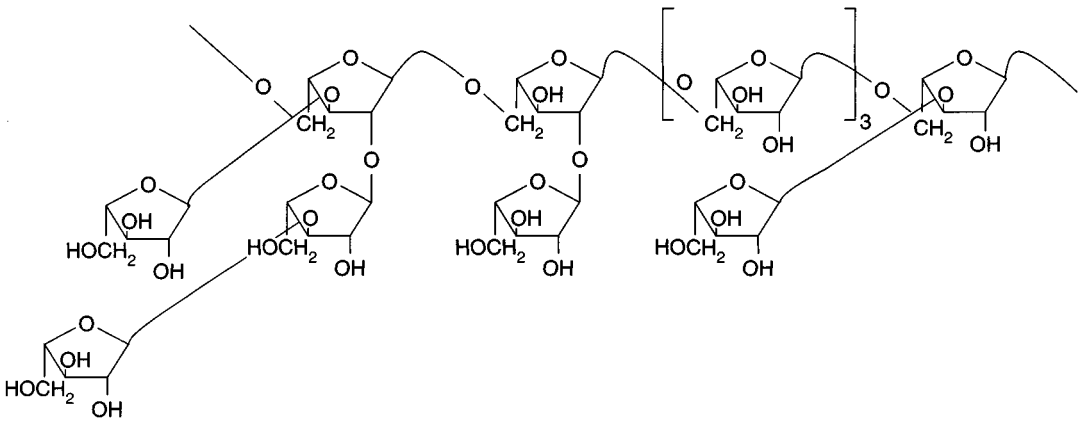


Figure 1–5 The structure of arabinans. Adapted with permission from Beldman, G., Schols, H. A., Pitson, S. M., Searle-van Leeuwen, M. J. F., Voragen, A. G. J. (1997). Arabinans and arabinan degrading enzymes. In *Advances in Macromolecular Carbohydrate Research. Vol. 1,* pp. 1–64. Edited by R.J. Sturgeon. London: JAI Press.[1]

α-1,2 linked side chains. At the same time, this enzyme slowly and sequentially breaks the α-1,5 linkages at the nonreducing end of linear arabinan. Endo-arabinanase hydrolyzes linear arabinan in a random fashion, producing oligomers of shorter lengths. Arabinosidase A degrades the arabinan oligomers to monomers. Arabinanases occur in some plants and microorganisms. Fungal arabinanases have an optimum pH of approximately 4.0; bacterial arabinanases have an optimum pH of approximately 5.0–6.0.

- **Cellulases:** Cellulose is the best known of all plant cell wall polysaccharides. It is particularly abundant in secondary cell walls and accounts for 20–30% of the total dry mass. Cellulose is a linear chain of β-1,4 linked glucose units. In cellulose, these β-1,4 glucan chains aggregate by hydrogen bonds to rigid flat structures called fibrils (Figure 1–4). The degree of polymerization can be very high, up to 14,000 in secondary cell walls. The rigid structure makes cellulose very resistant to degradation by enzymes. Cellulase (often called the cellulase complex) is a multienzyme complex system composed of several enzymes: endo-glucanase, exo-glucanase, cellobiose hydrolase, and cellobiase.

Lipases

Lipases degrade triglycerides. They only act on an aqueous-lipid interface such as a micelle. Generally, the enzymes catalyze the hydrolysis of triglycerides to produce fatty acids and glycerol, but there are also specific enzymes that catalyze the hydrolysis of monoacylglycerides, phospholipids, and esters of sterols. Generally, the mode of action of lipases results in fatty acids being preferentially hydrolyzed from 1- and 3-positions of triglycerides so as to leave 2-substituted monoacylglycerides. Microbial lipases may also catalyze the hydrolysis in all three positions. The composition of the fatty acid (i.e., length, stereoconformation, and degree of saturation) affects the specificity and speed of the lipases.

Phytases

Phytases catalyze the hydrolytic removal of phosphate groups from phytic acid (Figure 1–6), a substance that occurs widely in cereals and legumes and that is known to limit the bioavailability of macro- and micronutrients. The ability to degrade phytate occurs widely among molds *(A. ficuum, R. oligosporus),* yeasts

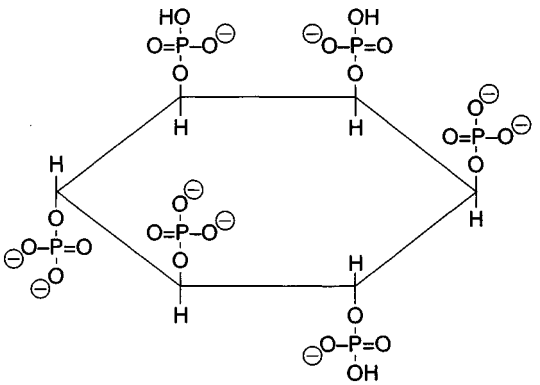


Figure 1–6 Structure of phytic acid

(Candida krusei, Schwanniomyces castelli), and LAB *(Pediococcus pentosaceus, Lactobacillus agilis, Lb. confusus).* Because the optimum pH of most phytases is approximately 5.5, the phytate degradation can be less effective if a lactic acid fermentation has resulted in low pH values.

Tannases

Tannases catalyze the hydrolysis of polyphenols (tannins). Fungal enzymes (e.g., from *A. niger*) are used in the food industry to degrade tannins. Tannin removal results in reduced turbidity in tea-based beverages. It also benefits the nutrient bioavailability in tannin-containing fermented cereal products.[2]

DIVERSITY OF FERMENTED FOODS

There is a wide variety of fermented foods worldwide. Various excellent reference books on fermented foods are available,[5,40,45] as are source books on industrial aspects of food biotechnology, including food fermentation.[23,33,39] Mention was made already of the various food ingredients that can be fermented, as well as the microorganisms and enzymes that are used in fermentations.

In the strict sense, fermentation refers to a form of anaerobic energy metabolism. In the context of fermented foods, however, the microbial growth and metabolism can take place under aerobic conditions as well. For example, mold fermentations require oxygen to facilitate their growth and enzyme production.

An aspect of interest is the physical state under which the fermentation takes place. This can be in the form of liquid in which the microorganisms are suspended while a relatively simple mixing device is used to ensure the homogeneity of microorganisms, substrates, and products. In these liquid fermentations, or LFs, the continuous phase is the liquid, and the control of temperature and levels of dissolved oxygen can be achieved with immersion coolers or heaters, and aeration. Quite a different situation occurs in a heap of cooked soybeans in which homogeneous growth of strictly aerobic molds is required. Whereas the particulate matter (i.e., soybeans) contains sufficient water to allow microbial growth, water is not the continuous phase; gas (i.e., air) is.This physical state is referred to as solid-state fermentation, or SSF. Because gas is a poor conductor of heat and mass, SSFs have a tendency to develop gradients of temperature and gas composition. Control of homogeneity requires more complex measurement and mixing systems as compared to LF. In practice, intermediate situations such as shredded vegetables or particles such as olives, which are fermented while immersed in brine, can be encountered. These can be considered as immersion LF because the continuous phase is liquid but contains immersed particles. Obviously, these do not always behave like LF.

Table 1–4 illustrates selected examples of food fermentations representing different food groups, functional microorganisms and enzymes, oxygen requirements, physical states, and present levels of industrialization. As mentioned earlier, food ingredients of plant as well as animal origin are used to prepare fermented foods. Of the functional microorganisms, LAB play the predominant role in the prolongation of shelf life because of the antimicrobial effect of their acids. In addition, yeasts are often found as minority companions of LAB; they sometimes contribute to shelf life by scavenging residual assimilable carbon sources. This is the case, for example, in fermented pickles. The major func-

Table 1–4 Examples of Food Fermentation Processes

Food Group	*Fermented Food*	*Mode of Consumption*	*Microorganisms and Major Products**	*Enzymic Modifications of The Product*	*Oxygen Requirement*	*Physical State*	*Industrialization†*	*References*
Starchy crops: cassava	Agbéli mawè	Cooked paste	Lactic acid bacteria forming lactic and acetic acid	Degradation of starch and cell walls	Aerobic	Solid	1–2	30
Cereals: wheat and rye	Sourdough	Bread	Lactic acid bacteria and yeasts forming lactic and acetic acid, and carbon dioxide	Degradation of starch and maltose	Anaerobic	Solid	1–4	16
Legumes: soybean	Tempeh	Fried snacks or cooked side dish	Molds forming mycelium and enzymes	Degradation of protein, cell walls, and lipids	Aerobic	Solid	1–3	26, 28
Vegetables: cabbage	Sauerkraut	Vegetable dish or relish	Lactic acid bacteria forming lactic acid		Anaerobic	Immersed in liquid	1–4	3
Fruits: grape	Wine	Alcoholic beverage	Yeasts (and occasionally lactic acid bacteria) forming ethanol and flavor		Anaerobic	Liquid	1–4	10
Meats: pork and beef	Raw dried sausages	Hearty food ingredient or snack	Micrococcaceae, lactic acid bacteria (and occasionally molds) forming organic acids and flavor and stabilizing meat color	Degradation of lipids	Anaerobic	Solid	1–4	36
Milk: cow, buffalo	Yogurt	Breakfast or dessert	Lactic acid bacteria forming lactic acid, acetaldehyde, and diacetyl and providing structure	Degradation of proteins	Anaerobic	Liquid	1–4	42
Milk: cow, sheep, goat	Soft cheeses	Sandwiches and snacks	Lactic acid bacteria and molds forming acidity, release enzymes for ripening and flavor development	Degradation of proteins and lipids	Aerobic	Solid	1–4	8

*Mention is made only of functional groups of microorganisms.
†1, home-produced; 2, village style; 3, national market; 4, international market.

tion of yeasts in yeast-fermented products is their copious production of carbon dioxide, such as gas in bread and beer and ethanol in alcoholic beverages. The functionality of molds is mainly in their production of enzymes that degrade polymeric components such as cell wall polysaccharides, proteins, and lipids. This can have considerable consequences for the texture, flavor, and nutritional value of mold-fermented foods. Depending on the functional microorganisms that should be propagated, the incubation environment (i.e., availability of oxygen for strict aerobes, anaerobic conditions when mold growth is undesirable) and physical state can be selected and optimized. It is of interest to note that, in contrast to predominantly LFs that are common in the pharmaceutical industry, a considerable number of food products are fermented by SSF.

PROCESS UNIT OPERATIONS

The manufacture of fermented foods is organized in a sequence of activities called unit operations.[22] Irrespective of the scale at which the process takes place, the following types of unit operations can be distinguished.

Physical Operations

Transport

Transport is one of the most important unit operations. It has the purpose of transferring ingredients (i.e., transport of mass) to the desired localities and/or equipment. Its aim is also to assist in heating and cooling (i.e., transport of heat). There is a wide variety of materials and methods that can be used for transport. The choice depends on the type of product, scale of production, economic considerations, and local conditions.

Grading and/or Sorting

Grading and/or sorting have the purpose of achieving homogeneity of size, color, maturity, hardness, and so forth. At the same time, items that are spoiled, infected, or otherwise deteriorated are removed. From a food safety point of view, grading and sorting are important tools that can be used to optimize the quality of inputs.

Cleaning and/or Washing

Cleaning and/or washing are carried out to remove dirt, dust, insects, agricultural residues, and so forth. Depending on the type of ingredients, dry or wet cleaning can be chosen. In cereal processing, for example, dry cleaning of wheat prior to flour milling is performed by a combination of aspiration and sieving. But, in maize processing, maize kernels can be washed in water prior to wet milling. Water is becoming ever scarcer and expensive, causing increased interest in dry operations.

Physical Separations

Dehulling, peeling, trimming, and other separations are aimed at obtaining the desired anatomical parts of plant or animal tissue while removing the undesired ones. Examples are the removal of poorly digestible seedcoats from soybeans by dehulling, the removal of the cortex of the cassava root because of its bitter taste and possible toxicity, and the trimming of fat from red meat.

Moisture Adjustment

Moisture adjustment is required to achieve, for example, the desired consistency and edibility of dry seeds and grains. The need for a sufficiently high A_w for microbial metabolism was mentioned earlier. Water can be added as an ingredient and mixed; soaking or steeping in water is also a common method to increase the moisture content of ingredients. Especially during longer periods of soaking at favorable temperatures, microbial activity can take place. Several cereal fermentation processes combine soaking with fermentation.

Size Reduction

Size reduction of particulate matter is required to obtain meal or flour from seeds, to obtain pulp from tubers or fuits, or to prepare a homogeneous slurry of meat and other ingredients for sausage making. Size reduction is performed by cutting, grinding, impact hammering, and so

forth using a wide variety of equipment that has been developed to suit the processing of specific raw materials.

Mixing

Mixing has the purpose of obtaining homogeneity of mass and heat. Mixing of mass is the most common type of mixing and is exemplified by the mixing of ingredients to obtain a homogeneous product. Mixing at a small scale is relatively easy and can be carried out with simple kitchen utensils. The larger the scale of operation, the more complicated mixing becomes. Mixing of dry components can be achieved using specific mixing equipment such as tumblers or augur-type mixers, but it is also feasible to combine mixing with other operations such as grinding or transport. Mixing of liquids is often achieved in stirred tanks. Mixing of wet and dry ingredients can be carried out in kneading machines for doughs or in stirred tanks for less viscous suspensions.

Bioprocessing Operations

For microbial and enzymatic transformations, a first requirement is the presence of the required ingredients, including the desired microorganism(s) and/or enzyme(s), the required substrates and cofactors, and sufficient water. Several of the unit operations mentioned above will be involved to fulfill these requirements.

In order to allow the transformations to take place, incubation under optimum conditions for the correct period of time will be needed. In order to safeguard the constancy of these incubation conditions, unit operations such as mixing, heating, cooling, and transport are needed to ensure even distribution of mass and heat, compensate for heat losses or generated heat, and compensate for deficiencies or overproduction of mass by additions or removal.

Thermal Processing Operations

Heating and cooling are both characterized as the transport of heat. Heat treatments and cooling are of extreme importance in food processing and thus merit specific attention. The primary objective of heating is to render food palatable. It causes the gelatinization of starch, denaturation of protein, and softening of tough tissues, and transforms a number of flavors.

Heat treatments consist of a warming-up phase, a period of holding time, and a cooling-down phase. Temperatures exceeding 70 °C cause enzyme inactivation and kill vegetative microbial cells. The combination of temperature and time determines the lethal effect of heat treatments. In principle, the term *pasteurization* corresponds to mild heat that kills heat-sensitive vegetative cells of bacteria, yeasts, and molds. *Sterilization* signifies killing all living cells, including more heat-resistant spores of bacteria and molds. In food processing practice, sterilization is not always required to ensure long-term shelf life of foods. In this respect, the term *commercial sterility* indicates a situation where some heat-resistant spores may have survived the heat treatment, but the composition of the food prevents their revival during storage.

In view of bioprocessing, the timing of heat treatments is of crucial importance. Fermentation of ingredients without prior heat treatment (e.g., raw cereals, raw meat, etc.) has by definition a mixed character: Microorganisms and enzymes that were present in the ingredient will take part in the fermentation, sometimes as sole actors and sometimes as an accompaniment to added starter microorganisms. At larger scale production, fermentations must be better defined and controlled, and selected or optimized starter cultures must be used. In order to ensure optimum functioning and to remove potentially hazardous microorganisms, the ingredients can be pasteurized or sterilized prior to inoculation and fermentation. In such case, it should be realized that some heat-sensitive growth factors such as enzymes and vitamins may have to be replenished in order to enable effective microbial metabolism.

Cooling in food fermentation can be used as a processing tool. Prior to inoculation, heat-treated ingredients must be cooled to inoculation temperature. During fermentation, the excessive production of metabolic heat must be removed by cooling in order to prevent fermentation fail-

ures. In certain products such as yogurt, the fermentation is halted by cooling in order to prevent excessive acid development. Not all fermented foods have a long shelf life. Refrigerated distribution and storage contribute to shelf life and hygienic safety.

Organization in Flow Diagram

A flow diagram is a pictorial scheme illustrating the sequence in which ingredients and unit operations are combined and providing data regarding processing conditions such as temperature, time, mass, pH, and so forth. Figure 1–7 illustrates this principle.

Appendix 1–A provides flow diagrams for selected food fermentations. For each major food group, two commodities have been selected. These flow diagrams are not intended to provide recipes or exclusive methods of preparing the respective products. Their purpose is to provide an insight into the process conditions and timing, as well as relevant environmental antimicrobial conditions that are of importance in food safety.

PROCESS CONDITIONS

Appendix 1–A aims to provide concrete parameter values (or ranges) affecting microbial growth, metabolism, survival, and death as well as enzymatic activity/stability, that are relevant to food safety. These data can be useful in

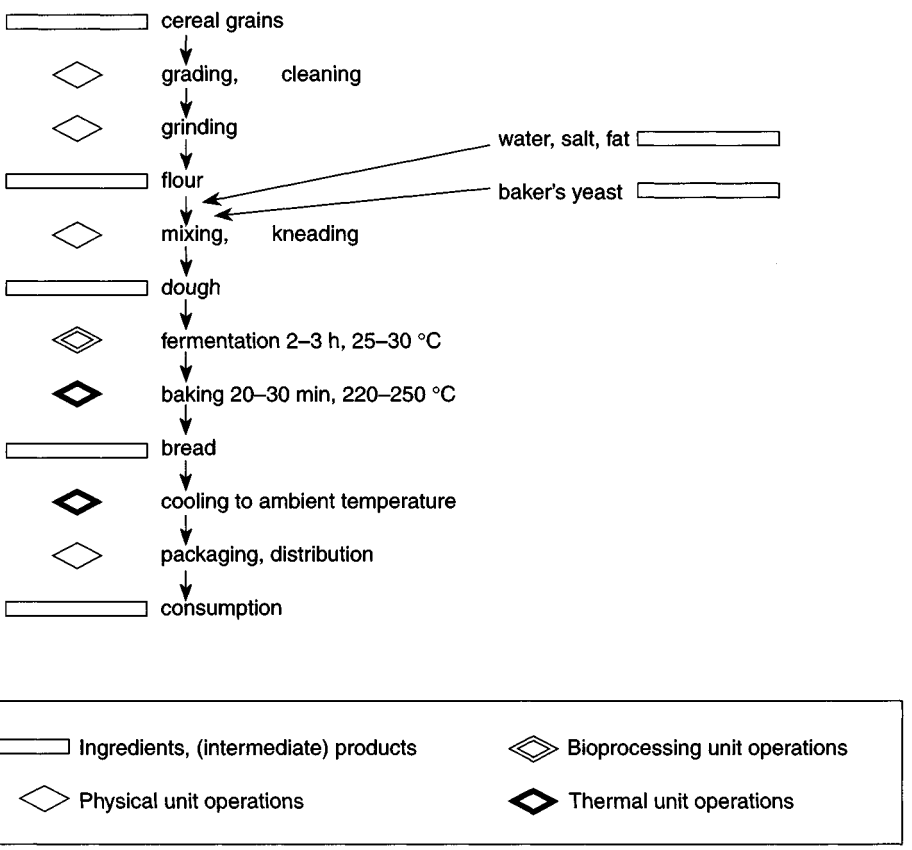


Figure 1–7 Principle of flow diagram (case of bread making)

HACCP approaches. Appendix 1–A provides flow diagrams and process conditions of relevance for the processing of starchy roots and tubers (cassava, Tables 1–A–1 and 1–A–2), cereals (barley, rye, and wheat, Tables 1–A–3 and 1–A–4), legumes (soybeans, Tables 1–A–5 and 1–A–6), vegetables (cabbage, Table 1–A–7 and olives, Table 1–A–8), fruits (grapes, Table 1–A–9 and palm sap, Table 1–A–10), meat (pork, Tables 1–A–11 and 1–A–12), fish (fresh water, Table 1–A–13 and sea water, Table 1–A–14), and milk (yogurt, Table 1–A–15 and cheese, Table 1–A–16). The icons explained in Figure 1–7 are used throughout these flow diagrams to provide a quick overview of the sequence of bio- and thermal unit operations. This is of special importance with regard to safety, as illustrated by the following cases.

- **Case 1:** Is the food heated at all, somewhere during the process? This may be of crucial importance in view of viruses (refer to Chapter 8) and parasites (Chapter 9).
- **Case 2:** If heated, does this take place before or after fermentation? This will have serious implications in view of the activity of endogenous enzymes, which may detoxify endogenous toxic substances (Chapter 4), and in view of naturally occuring pathogens (Chapter 7), as well as the need for added safe starter cultures (Chapters 10 and 11).
- **Case 3:** Is the fermented product usually cooked prior to consumption (e.g., tempeh) or is it eaten uncooked (e.g., most yogurts)? Needless to say, cooking prior to consumption will provide an additional "safety net" to the consumer.

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APPENDIX 1–A

Flow Diagrams for Selected Food Fermentations

Table 1–A–1 Food Group: Starchy Roots and Tubers Product Name: Gari (Fermented and Dried Cassava) Reference: 40

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
cassava tubers	cassava (*Manihot esculenta*)		
wash			
peel (remove cortex)			
grate to coarse pulp			
put in jute or polypropylene bag			
pressurize by weight to enable de-watering			from 65% moisture content to approx 50% moisture content
ferment	natural fermentation dominated by *Lactobacillus plantarum*, *Coryne-bacterium* spp., *Geotrichum candidum*	12–96 h at 25–35 °C	organic acids (lactic, acetic) 0.6–1.2% w/w initial pH 6.2 final pH 4–4.5
break lumps			
garify (toasting for dehydration and starch gelatinization)		15–20 min at 80–85–100 °C	dehydration to approx 8% moisture content
sieve			
package and store			in polythene bags or hermetic tins
reconstitute with cold water or milk			
consume			

Note: Factors contributing to shelf life: Dehydration to about 8% moisture content, combined with hermetic storage allows shelf life of several months.

Other remarks: During this process, the grating facilitates the enzymatic degradation and detoxification of naturally occurring cyanide (mainly in the form of linamarin). HCN (ppm) levels in cassava roots, peeled roots, grated peeled roots, fermented pulp, and finished gari were 306, 184, 104, 52, and 10, respectively.

Table 1–A–2 Food Group: Starchy Roots and Tubers Product Name: Tape Ketella (Fermented Cassava) Reference: 40

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
cassava tubers	cassava (*Manihot esculenta*)		
wash			
peel (remove cortex)			
cut to 5–10 cm pieces			
steam		10–30 min at 80–100 °C	
cool			
inoculate	ragi tapé (inoculum on rice powder) containing *Amylomyces rouxii, Endomyces fibuligera, Endomyces burtonii,* lactic acid bacteria		
ferment		2–3 d at 25–30 °C	ethanol to approx 2% v/v lactic acid 0.1–0.3% final pH approx 5.0
consume			

Note: Factors contributing to shelf life: Tape ketella has no shelf life. It is consumed as a snack or used occasionally as an ingredient in baked goods (cakes).

Table 1–A–3 Food Group: Cereals Product Name: Lager Beer Reference: 35

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
1. Malting:	barley (*Hordeum vulgare*)		
soaking		1–2 d at 10–20 °C with intermittent airing, until moisture content 45–50% w/w	
germination		4–6 d at 15–20 °C	generation of saccharolytic, proteolytic, and other brewing enzymes; partial modification of the barley grain
kilning		at 71–92 °C to reduce moisture content to 4–5% w/w	
2. Mashing:			
coarse milling			
mashing		infusion mashing (30 min–several h at 65 °C) or decoction mashing (2–3 h at increasing temperatures from 35–100 °C)	
filtration	product: sweet wort byproduct: spent grain		
3. Wort boiling:			
hops addition	hops (*Humulus lupulus*)		hops have antimicrobial effects
boiling		1–3 h at 100 °C	
filtration			
cooling		to 2–5 °C	
filtration	product: wort		

continues

Table 1–A–3 continued

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
4. Fermentation:			
pitching	brewers' yeast (*Saccharomyces cerevisiae* or *S. uvarum*) at approx 10^7 yeast cells per ml wort		
primary fermentation		1–2 wks at 10–15 °C	
sedimentation	product: young ("green") beer		
secondary fermentation (lagering)		1–4 wks at 2–6 °C	
filtration	product: mature beer	cooling to 0–2 °C	
5. Bottling			
6. Pasteurization		5 min at 60 °C holding temp	
consume			

Note: Factors contributing to shelf life: Lager beer has no shelf life unless it is kept anaerobic and refrigerated. Bottled lager beer derives its shelf life from pasteurization and the exclusion of air.

Table 1–A–4 Food Group: Cereals Product Name: Wheat Mixed Grain Sourdough Bread Reference: 38

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
mix ingredients for sourdough	rye flour 15 kg seed sour 0.3 kg water 12.0 l		
fermentation of sourdough	yeasts (*Candida krusei, Pichia saitoi, Saccharomyces cerevisiae, Torulopsis holmii*) and lactic acid bacteria (*Lactobacillus sanfranciscensis, Lb. plantarum, Lb. fermentum*)	15–24 h at 23–31 °C	
seed sour			
mixing and kneading of bread dough ingredients	seed sour 27 kg rye flour 15 kg wheat flour 70 kg bakers' yeast 2 kg salt (NaCl) 2 kg water 51 l		
fermentation of bread dough	see above, but dominated by *Saccharomyces cerevisiae* (added bakers' yeast)	1–2 h at 26 °C	pH 4.6–4.7
divide, make-up			
intermediate proof		5 min	
shape and tin			
tin proof		30–60 min at 30–40 °C	
bake		35–40 min; oven temperature 200–250 °C	0.5–0.7% lactic acid in baked product
cool		to ambient temperature	
consume			

Note: Factors contributing to shelf life: Packaging in paper or other bags permitting moisture migration.
Other Remarks: Storage at 20 °C for several days, limited by staling.

Table 1–A–5 Food Group: Legumes Product Name: Tempe Kedele (Soybean Tempeh) Reference: 27

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
soak whole soybeans in water	soybeans (*Glycine max*), lactic acid bacteria (*Lactobacillus plantarum*), yeasts (*Saccharomyces dairensis*), Enterobacteriaceae	6–24 h at 20–30 °C	pH soaking water decreased from 6.5 to 4.1
dehull			
cook		20–40 min at 80–100 °C	ph beans 5.0–6.5
cool			
inoculate with mold starter	*Rhizopus microsporus, R. Oligosporus, R. oryzae, Mucor indicus*		
package	plant leaves (banana) or polyethylene sheets or boxes, with perforation holes		limited perforation allows mycelium growth with very little sporulation
ferment		24–48 h at 25–30 °C external temp; internal temperature may become approx 10 °C higher	initial pH 5.0; final pH 6.5–7.5 NH_3 is formed due to proteolysis
harvest fresh tempeh			
slice or dice			
fry or stew		few min in oil of 180 °C, or 5–10 min in water or sauce of 100 °C	
consume			

Note: Factors contributing to shelf life: Fresh tempeh can be stored refrigerated or frozen.

Table 1–A–6 Food Group: Legumes Product Name: Koikuchi-Shoyu (Soy Sauce) Reference: 13

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
mixing equal quantities of soybeans and wheat	soybeans (cooked, whole) wheat (roasted, crushed)	cooking: 40–45 min at 130 °C or equivalent; roasting: few min at 170–180 °C	
inoculation	koji starter containing *Aspergillus sojae, Asp. oryzae*		
incubation to obtain molded mass = koji		2–5 d at 25–30 °C	
mixing koji and brine to obtain moromi mash	koji brine		NaCL approximately 18% (w/v)
fermentation	*Tetragenococcus halophila, Zygosaccharomyces rouxii*	6–8 months	ethanol 2–3% (v/v), lactic acid 1–2% (w/w), final pH 4.7–4.8
pressing, residue used as animal feed			
obtain raw soy sauce			
pasteurize		70–80 °C	
bottle			
consume			

Note: Factors contributing to shelf life: Combined effect of high salt concentration, mild acidity, and pasteurization.

Table 1–A–7 Food Group: Vegetables Product Name: Sauerkraut (Sour Fermented Cabbage) References: 11, 29

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
white cabbage	*Brassica oleracea*		
harvest, transport			
grading			
coring	byproduct: inner core		
trimming	byproduct: outer leaves		
shredding to 1–2 mm thick			
salting (and homogenous mixing)	add 1.0–3.5 (typically 2.25)% w/w NaCl		
load into fermentation tanks or vats			
cover with sheet			
heading (place weight on cabbage)	using boards and weights or bags filled with water		
fermentation	natural fermentation by microbial succession: *Leuconostoc mesenteroides, Lactobacillus brevis,* dominated by *Lactobacillus plantarum. Lactobacillus bavaricus* occasionally added as a starter culture	3–6 wks at 18–22 °C	at least 1% w/w lactic acid should be formed

Postfermentation Options

1. fresh sauerkraut in bulk	2. fresh packaged sauerkraut		3. fresh packaged long-life sauerkraut		4. canned sauerkraut
	allow secondary fermentation to complete sugar depletion		add preservative (0.1% w/w Na-benzoate)		pasteurize 3 min at 74–82 °C
	fill in plastic pouches		fill in glass or plastic		hotfill in cans

continues

Table 1–A–7 continued

◇ refrigerate and sell shelf life 1–2 wks	◇ refrigerate and sell shelf life > 2 months at < 7 °C; > 3 months under CO_2 modified atmosphere	◇ refrigerate and sell shelf life 8–12 months	◇ refrigerate and sell shelf life 18–30 months
▭ consume	▭ consume	▭ consume	▭ consume

Note: Factors contributing to shelf life: The combination of acidity and depletion of fermentable sugars. In addition, exclusion of air, preservatives, pasteurization, or sterilization, as shown above.

Other remarks: Sauerkraut can be eaten without any heat treatment (e.g., in sandwiches), but it is also popular in cooked dishes with potatoes and meats.

Table 1–A–8 Food Group: Vegetables Product Name: Treated Black Olives in Brine References: 14, 29

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
fresh olives	*Olea europaea sativa* of various stages of maturation (yellowish green to purple)		fruits contain 0.5–1.0% organic acids (citric, oxalic, malic), as well as 1–3% phenolic compounds with antimicrobial effect
wash			
brine	5–7% w/v NaCl		
ferment	natural fermentation; no starter added; lactic acid bacteria (dominated by *Lactobacillus plantarum*) and yeasts (*Pichia membranifaciens, Pichia vinii*)		
lye treatment	1–2% w/v NaOH		
air oxidation (blackening)	purging with air; add iron salts to stabilize black color		
wash, neutralize			pH to 5.8–7.9
storage in brine	2.5–5.0% w/v NaCl		
sort, size			
can	add 1.5% w/v NaCl brine		final NaCl concentration approx 1.5% w/w
sterilize			
consume			

Note: Factors contributing to shelf life: The combination of heat treatment and moderate salt and acidity. The antimicrobial effect of phenolic compounds occurring in fresh olives is not relevant for the shelf life of the finished product because the mentioned substances have been degraded during the lye treatment.

Other remarks: Canned olives are often consumed without prior heating.

Table 1–A–9 Food Group: Fruits Product Name: Red Grape Wine References: 5, 10, 25

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
grapes	*Vitis vinifera*		fermentation until fermentable
destemming			
crushing, sulfiting	SO_2 100–150 mg/l		
	sometimes additional nitrogen sources		
fermentation "on the skins"	starter cultures are occasionally used; otherwise natural fermentation by succession; wild yeasts (*Kloeckera apiculata, Kloeckera apis, Torulopsis stellata, Candida stellata*), followed by *Saccharomyces cerevisiae* (dominating primary fermentation)	1–3 wks at 20–25 °C	sugars less than 0.1%. Final ethanol 11–17% v/v. Acidity: volatile 0.1–0.15% w/v as acetic acid; total acidity 0.5–0.7% w/v as tartaric acid
racking	separate skins ("lees") from young wine		
secondary fermentation	starter cultures used: *Oenococcus oeni*	few days at pH > 3.5 and temp > 15 °C	conversion of malic acid into lactic acid (typically 1.5–3.5 g/l lactic acid)
clarification			
aging	bulk tanks	3 months–2 years	
blending			
filtration			
bottling			
consume			

Note: Factors contributing to shelf life: The combination of ethanol, moderate acidity, and exclusion of air. Residual sulfite may be present, but this is not an essential preservative. Absence of fermentable sugars ("dry wines") contributes to shelf life.

Table 1–A–10 Food Group: Fruit Product Name: Toddy (Palm Wine) Reference: 40

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
collection of palm sap	sap of oilpalm (*Elaeis guineensis*) or coconut palm (*Cocos nucifera*)		
natural fermentation	yeasts (*Saccharomyces cerevisiae, Candida* spp.), lactic acid bacteria (*Lactobacillus* spp., *Leuconostoc* spp.), acetic acid bacteria, *Zymomonas* spp., *Micrococcus* spp.	0–48 h at 25–35 °C	organic acids (lactic, acetic) 0.2–0.4% pH 4.5–3.5 ethanol 4–10%
filtration			
bottle (optional)			
pasteurize (optional)		15 min at 80 °C	
cool (optional)			
consume			

Note: Factors contributing to shelf life: Fresh palm wine has no shelf life except when it is kept refrigerated or bottled and pasteurized.

Table 1–A–11 Food Group: Meat Product Name: Salami (Italian-Style Raw Fermented Sausage) Reference: 6

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
▭ medium chopping ◇ (cuttering)	pure frozen pork or a pork/beef mix, fat, salt (NaCl) 2.5–4.5% w/w $NaNO_2$ 125 ppm pepper, mace, cardamon, garlic totalling 1% w/w		
▭ filling into casings	natural or artificial casings		
◇			
◈ ripening (fermentation)	*Lactobacillus* spp., *Pediococcus acidilactici, Micrococcus* spp., *Debaryomyces hansenii*	15–90 d at 15–25 °C	acidity approx 2.5% w/w as lactic acid, various antimicrobial compounds (e.g., H_2O_2), pH initial 5.8–6.0 to pH final 4.9–6.0
◇ drying		several wks at 12–15 °C to reduce A_w to 0.67–0.92	
▭ consume			

Note: Factors contributing to shelf life: The combination of salt, nitrite, acidity, pH, anaerobic conditions, and reduced water activity. The lower the A_w, the longer the shelf life. This type of sausage is not smoked, in contrast to many other raw fermented sausages.

Other remarks: If no frozen pork was used, it must be heated to 58 °C to kill *Trichinella*. Otherwise, no heat treatment takes place and the product is consumed without prior cooking.

Table 1–A–12 Food Group: Meat Product Name: Lup Cheong (Chinese raw sausage) References: 20, 21

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
coarse chopping	pork meat and fat, sugar, salt		
filling into casings	natural casing (intestine)		
predrying		approx 36 h at 48 °C and 65% RH to A_w 0.9 3 d at 20 °C and 75% RH	final pH 5.9 and A_w 0.8
fermentation	no starter; natural fermentation by lactic acid bacteria, micrococci, etc.		taste preference requires low levels of acidity
cooking			
consume			

Note: Factors contributing to shelf life: The combination of reduced water activity, salt, and moderate acidity.
Other remarks: The product is heated prior to consumption, distinguishing it from the consumption of European-style raw sausage.

Table 1–A–13 Food Group: Fish Product Name: Plaa-Raa (Fish-Rice Paste) Reference: 32

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
small fresh water fish (whole)	gourami (*Trichogastea* spp.), snake-head fish (*Ophicephalus striatus*), catfish (*Clarias* spp., *Heterobagrus* sp.), tawes, bards (*Puntius* spp.), butter catfish (*Ompok* sp.), tilapia (*Tilapia* spp.)		
mix with salt	NaCl		salt 11–24% w/w
ferment	endogenous proteases, *Tetragenococcus halophila, Staphylococcus epidermidis, Micrococcus* spp., *Bacillus* spp.	several days at ambient temperature	
mix with roasted rice	glutinous or normal paddy rice, roast until dark brown, coarse grinding		
pack in earthen or glass jars			
ferment	*Tetragenococcus halophila* and other lactic acid bacteria, *Staphylococcus epidermidis, Micrococcus* spp., *Bacillus* spp.	several days to a year	acidity 0.7–1.9% as lactic acid; final pH varies from 4.1–6.9
consume			

Note: Factors contributing to shelf life: Combined effect of high salt concentration and hermetic storage.

Table 1–A–14 Food Group: Fish Product Name: Nuoc-Mam (Fish Sauce) Reference: 40

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
small sea fish, whole	*Decapterus, Engranlis, Dorosoma, Clupeodes, Stolephorus* spp.		
mix with salt	fish : salt 1:3 to 1:1.5		
transfer to large vessel			
cover with layer of salt			
pressurize by weight			
fermentation	endogenous proteases (halotolerant bacteria: *Paracoccus halodenitrificans, Aerococcus haloviridans* are present; their function is not clear)	few months to 1 year at 20–30 °C	lactic acid approx 1% w/w; final pH 5.7–6.0
drain liquid to obtain first-quality Nuoc-Mam			salt content 24–28% w/v
wash residue with boiling sea water in several stages to obtain second and subsequent qualities			
bottling			
consume			

Note: Factors contributing to shelf life: The combination of high salt content, organic acids, and hermetic bottling ensure a shelf life of several months.

Table 1–A–15 Food Group: Milk Product Name: Stirred Yogurt Reference: 42

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
milk	cow milk		
standardize			
homogenize		55 °C at 20 MPa	
pasteurize (high pasteurization)		5 min at 85 °C	
cool to 30–32 °C			
inoculate	0.025% w/w starter culture containing equal numbers of *Streptococcus salivarius* spp. *thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*		organic acids (lactic, acetic) to approx 0.8–1% w/w as lactic acid; final pH 4.1–4.6
ferment		16–20 h at 32 °C	
stir			
cool to 6 °C			
aseptic packaging			
consume			

Note: Factors contributing to shelf life: The combination of acidity and other antimicrobial compounds produced by lactic acid bacteria, anaerobic conditions in hermetically sealed jar, low storage temperature, and the presence of live (competing) microorganisms.

Other remarks: The product is consumed without prior heating.

Table 1–A–16 Food Group: Milk Product Name: Camembert Surface-Ripened Cheese References: 4, 17, 19

Flow Diagram	*Ingredients and Microorganisms*	*Thermal Data (Time at Temp)*	*Other Conditions of Antimicrobial Relevance (Salt, pH, Preservatives, etc.)*
pasteurization	cow milk 48% fat	20 sec at 70–72 °C	
cooling			
inoculation	mesophilic lactic acid bacteria cheese starter *Lactococcus lactis* spp. *cremoris*, *Lc. lactis* spp. *lactis*, *Leuconostoc mesenteroides* spp. *cremoris*, *Leuc. lactis*		
stir		15–60 min at 34 °C	until 0.2% titratable acidity and pH 4.5–4.6
renetting	rennet 0.01% v/v	2–3 h at 34 °C	
cut, drain whey		24 h at 18 °C	whey pH 5.5
spray-inoculate	spore suspension *Penicillium camemberti*		
superficial drying		5–6 h	
salting	2–3 times by rubbing crystalline salt or in 25% brine	2–3 d at 14 °C 1–1.5 h at 14 °C	
ripening first phase		8 d at 13–16 °C at 95% RH	
ripening second phase		3 wks at 11 °C at 85% RH	pH cheese increases to 6–7
packaging and distribution	aluminium foil, polyethylene film, wooden or cardboard boxes		
consume			

Note: Factors contributing to shelf life: Refrigerated storage at 2–4 °C.