Food Technologies: Fermentation

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FOOD TECHNOLOGIES

Fermentation

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Glossary

Back-slopping A method to initiate fermentation by using a small quantity of previously fermented products as a starter.

Fermentation (1) Anaerobic metabolism for energy generation and (2) any microbiological process used to achieve desirable food product properties.

Food environment A combination of intrinsic (chemical composition) and extrinsic factors (external factors, such as temperature, relative humidity, and microbial contaminations) that influence the speed of biological and chemical reactions in the food.

Natural enrichment A fermentation starter with often complex and unknown microbiological composition, obtained by repeated natural fermentation with back-slopping.

Natural fermentation Fermentation in which no starter is added, but which relies on creating a favorable food environment to allow extrinsic microorganisms to multiply and establish desirable food properties.

Operation Technical actions, including physical (mixing, separating, and size reduction) and thermal (heating and cooling) treatments.

Process An organized assembly of operations and ingredients, aimed at producing a final product from its ingredient raw materials.

Starter An additive containing high numbers of living microorganisms used to initiate fermentation; starters may contain single or mixed pure-culture microbial strains, or may be natural enrichments.

Introduction

Fermentation (from *fermentare*, causing to rise) in the strict sense, refers to the anaerobic metabolism and generation of energy. This takes place, among others, in alcoholic fermentations caused by fermenting yeasts and in lactic acid fermentations caused by lactic acid bacteria (LAB). In the wider sense, the word 'fermentation' is used to refer to biological processes resulting in desirable modifications of ingredients. It also refers to the aerobic growth and metabolic activity of acetic acid bacteria in the oxidative production ('fermentation') of acetic acid, of bacilli in alkaline fermentations, and of filamentous fungi (molds) in food processing and enzyme production; the word fermentation is even used for some processes that are enzymatic rather than microbial, for example, the fermentation of tea leaves and the fermentation of certain Oriental fish sauces. In this article, the word 'fermentation' will be used in its broader sense.

A food manufacturing process is defined as the organized use of ingredients, equipments, and operations aimed at achieving a final product. The fermentation itself is only a part of the process, starting from the ingredients to the final product.

Ingredients for fermented foods may include practically all types of primary agricultural food items ranging from plant-derived foods (cereals, pulses, starchy roots and tubers, fruits, vegetables, herbs, and spices) to animal-derived products (meats, poultry, eggs, fish, and shellfish), but they always include water in order to enable biological activity and, thus, fermentation. Essential ingredients for fermentations are the microorganisms; these will be mentioned below.

Equipments used for fermentation may be simple or more sophisticated depending on the socioeconomic context. Equipment is needed to prepare ingredients by grading, removing unwanted parts, mincing, mixing, heating, cooling, etc. In addition, containers for soaking, cooking, and the actual fermentation are required. Other equipments may be needed to finish and distribute the final products to the consumer market.

Process operations can be categorized in groups, including physical and thermal operations. Physical operations include activities such as (1) grading, for example, sorting by color or size; (2) size reduction by grinding; (3) separation of unwanted parts by sieving; and (4) mixing, for example, by stirring. Thermal operations refer to all phenomena involving heat transfer, mainly heating and cooling. Heating may be (1) dry heating by contact with hot air or with hot surfaces as in roasting or frying; heating can also be (2) in wet conditions, such as steaming, immersion in hot water, or boiling under atmospheric or higher pressure. Cooling may be important at various stages in the process, for example, to adjust the temperature of previously heated ingredients to enable the optimum activity of added microorganisms for fermentation. Cooling may also be used to arrest fermentations at a level of

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optimum food composition reflected, for example, in the most attractive taste. Finally, cooling helps to lengthen the shelf life of foods.

Microorganisms are the driving force of fermentation. They need to be present in adequately large numbers and to be metabolically active, so as to exert their impact in changing the composition of the ingredients toward the desirable characteristics of the expected outcome (the fermented food). In principle, there are two approaches to ensure the presence of active microorganisms.

The first approach is called 'natural' or 'uncontrolled' fermentation; in principle, no microorganisms are added to the ingredients. Instead, the process conditions allow the survival and selective outgrowth of desirable microorganisms that were present in low numbers in one or more of the ingredients. This principle entails that – to allow microbial survival – ingredients are not heated before fermentation. In addition, process conditions may include the exclusion of air in sealed containers in order to favor the dominance of (facultative) anaerobic microorganisms. Also, certain ingredients, such as herbs, spices, and salt, may be used to favor desirable microbes, while restricting the development of potentially undesirable microorganisms or enzymatic activities.

The second approach is called 'starter-mediated' fermentation; in principle, a highly concentrated dose of desirable fermentation microorganisms is added to the ingredients, which may have been previously freed of potential competitive microbiota, and the fermentation is conducted under conditions that favor the added starter. Starters exist in various types, which could be categorized as (1) natural enrichments, (2) traditional multistrain starters, and (3) single or mixed pure-culture starters.

Natural enrichments are accumulations of groups of microorganisms that can be present in equipment, for instance, in porous textures of natural fermentation vessels, such as utensils, calabashes, leather bags, clay pots, etc. Accumulations may also be present at the processing site, for example, the ceiling, walls, and other surfaces of incubation rooms. It is well known that certain traditional Belgian beers can be made successfully only in specific brew houses, where the 'house flora' plays an essential role in the natural fermentation.

Traditional multistrain starters used for fermentation are concentrates of a mixed microbial flora in liquid or dry form. These starters are made with simple technologies without the use of selected pure cultures. In many oriental rice fermentations, such starters are used to initiate the enzymatic degradation of rice starch and the fermentation of the resulting sugars. The dry starters are sold as tablets in the open market; these have a shelf life of at least 6 months in ambient conditions.

Single or mixed pure-culture starters consist of laboratory-controlled pure strains of bacteria or fungi, which have been selected or created for their desirable fermentation outcomes. By combining diverse strains in one mixed culture, the objective is to enhance fermentation outcomes, such as combined acidification and flavor formation. The shelf life of pure-culture starters can be prolonged by freeze-drying (lyophilization) or by freezing. Usually, the viability of the strains needs to be supported by cryoprotectants, such as skimmed milk powder or glycerol.

Types of Food Fermentations

The worldwide diversity of fermented foods is amazing. Several authors observed similarities across national and continental borders and proposed categories of foods and beverages, according to the microorganisms that are responsible for the fermentation, and the kind of biochemical changes that take place, which are of relevance to the characteristic product qualities. In this article, five main categories of fermented foods will be discussed, namely (1) those acidified by the formation of organic acids, (2) alcoholic beverages, (3) those alkalized by the formation of ammonia from amino acids, (4) predigested food caused by the activity of filamentous fungi and their enzymes, and (5) those having undergone enzymatic processes in high-salt conditions.

Acidified by the Formation of Organic Acids

The major organic acids formed in fermented foods are lactic acid, acetic acid, and propionic acid. The most important microorganisms responsible for lactic acid production are LAB, although certain food-grade molds can also form lactic acid. Lactic acid is mainly produced from pyruvate, formed through glycolysis (degradation of monosaccharides). Although homofermentative LAB produce lactic acid as their only fermentation product, heterofermentative LAB may form acetic acid, ethanol, and CO_2 besides lactic acid. Lactic acid results in a decrease of pH and acidic taste; it can also affect the physical properties of food macromolecules, such as starch, and thereby influence food texture. The increased acidity may also have beneficial effects on nutritional aspects, for example, acidity increases the solubility of minerals, thereby facilitating their assimilation in the human gastrointestinal tract.

Thus, although the formation of acetic acid is mostly caused by acetic acid bacteria, it may also be formed by heterofermentative LAB. The major substrate for acetic acid production by acetic acid bacteria is ethanol, which is oxidized by acetic acid bacteria in a strictly aerobic, exothermic reaction. However, acetic acid is produced in a fermentative way by heterofermentative LAB. Acetic acid, like lactic acid, results in a pH decrease and acid taste. The internationally best-known acetic acid product is vinegar, which in principle is a watery solution of approximately 4% w/v of acetic acid. Vinegar is widely used as a natural food preservative and flavoring ingredient.

Propionic acid is formed as a result of the metabolism of lactic acid by propionic acid bacteria. Simultaneously with the formation of propionic acid, acetic acid and CO_2 gas are produced. This could lead to the characteristic taste and texture of certain Swiss cheeses.

Table 1 shows several examples of fermented foods in which organic acids dominate the quality of the product. Organic acids can be formed in a wide variety of food ingredients, such as cereals, vegetables, and dairy products. Combinations with animal protein are also possible, such as meat and fish products. However, in the latter cases, often an additional source of fermentable sugars is required for the LAB. This can be achieved by the incorporation of sugar or

Table 1 Fermented foods dominated by acidification

Name of food	Origin	Main ingredients	Predominant microbiota	Typical metabolites	Typical pH values
Pozol	Mexico	Maize	LAB (Lp, Lb, and S) and Y (C and T)	Lactic acid and vitamin B	3.8–4.0
Kenkey	Ghana	Maize	LAB (Lf) and Y (Ck and Sc)	Lactic acid, acetic acid, acetoin, and 2,3-butanediol	3.8–4.2
Sauerkraut	Europe	White cabbage and salt 1.5%	LAB (Lm, Lb, and Lp)	Lactic acid, acetic acid, and ethanol	3.8
Kimchi	Korea	Chinese cabbage, garlic, onion, ginger, chilli, and salt 3%	LAB (Lm, Wc, Ls, and Lc)	Lactic acid, acetic acid, and CO_2	3.9
Lassi	India	Cow/buffalo milk	LAB (Bb, Lec, Led, Lecr, St, La, and Pfs) and Y (Sc)	Lactic acid, acetic acid, ethyl alcohol, and CO_2	4–4.5
Yogurt	Europe	Cow milk	LAB (Lbg and St)	Lactic acid and acetaldehyde	4–4.5
Sik-hae	Korea	Fish, millet, and 6–7% salt	LAB (Lm and Lp)	Lactic acid and peptides	<5.0

Abbreviations: Bb, *Bifidobacterium bifidum*; C, *Candida* spp.; Ck, *Candida krusei*; La, *Lactobacillus acidophilus*; Lb, *Lactobacillus brevis*; Lc, *Lactobacillus curvatus*; Lf, *Lactobacillus fermentum*; Lm, *Leuconostoc mesenteroides*; Lp, *Lactobacillus plantarum*; Ls, *Lactobacillus saké*; Lbg, *Lactobacillus bulgaricus*; Lec, *Leuconostoc citrovorum*; Led, *Leuconostoc dextranicum*; Lecr, *Leuconostoc cremoris*; Pfs, *Propionibacterium freudenreichii* spp. *shermanii*; S, *Streptococcus* spp.; Sc, *Saccharomyces cerevisiae*; St, *Streptococcus thermophilus*; T, *Trichosporon* spp.; Wc, *Weissella confusa*; Y, yeast.

cereals, such as in the Korean product *sik-hae* in which millet provides the carbohydrates.

Pozol and *kenkey* are both made from whole-grain maize, which is processed by soaking in water, grinding, and cooking. The natural fermentation is uncontrolled, and the microbiota consists of mainly LAB, yeasts, and some minority organisms. LAB and yeasts can form rather stable communities, each group relying on metabolic outcomes of the other. In that sense, this kind of microbiota is similar to the 'sourdough fermentation communities' in which protocooperative growth of yeasts and LAB has been documented. When the fermentation is considered as completed – this depends on producers and on consumer demand – *pozol* balls are sold in the market, to be diluted with water or milk to make a beverage. *Kenkey* is wrapped in plant leaves and steam cooked, resulting in a compact sliceable mass, which is eaten with breakfast.

Sauerkraut and *kimchi* are occidental and oriental counterparts in the sense that both are made from cabbage and salt, and fermented predominantly by successive LAB without the use of added microbial starter. In *sauerkraut* making, the cabbage leaves are shredded, that is, cut to thin strips of approximately 1.5 mm width, which causes extensive damage to the plant cells. The addition of salt causes an osmotic gradient, which causes the expulsion of plant juice. Soon the cabbage is covered by its own juice, which helps to create anaerobic conditions, containing fermentable sugars and some allyl thiocyanates that all have a favorable effect on LAB. In *kimchi*, the cabbage is first wilted in salty water and then stuffed with a pasty mixture containing garlic, onion, chilli fermented fish, etc. The cabbage is left intact, and the stuffed cabbages are arranged in a fermentation vessel, which is hermetically closed. *Kimchi* is typically consumed in the raw (uncooked) state, in contrast to *sauerkraut*, which may be consumed raw or cooked.

Lassi and *yogurt* are also fermented dairy counterparts from India and Europe. *Lassi* is made by mesophilic LAB in milk from cows or buffaloes to which some salt may have been added. *Yogurt* is more solid and is obtained by cooking cow's milk and inoculating with thermophilic LAB.

Alcoholic Fermentation

Alcoholic fermentation refers to the production of ethanol. In foods, ethanol can be produced by several groups of microorganisms, including filamentous fungi, yeasts, and heterofermentative LAB.

Of these, yeasts are the major type of microorganisms that are used to produce alcoholic beverages and other alcohol-containing products, such as bread dough. In principle, two types of yeasts, that is, the oxidative and the fermenting yeasts, can be distinguished. Although the former group depends on aerobic metabolism to generate energy and thus does not produce ethanol, the latter group can derive energy under anaerobic conditions (the true fermentation) by reducing pyruvate that was formed from monosaccharides through the Embden–Meyerhof glycolysis pathway. Among these yeasts, the species *Saccharomyces cerevisiae* is the most important from an economic point of view. It is used in the fermentation of beers, wines, and other alcoholic beverages. Some major alcoholic fermented products are summarized in Table 2. *Saccharomyces cerevisiae* also plays an important role in the maturation (fermentation) of leavened bread dough. In dough, the main function of yeast is to produce CO_2 gas to extend the dough volume. As a favorable spin-off, yeast enzymes contribute to the bread dough texture by increasing its extensibility. In addition, the ethanol formed can react with acids to form esters, which contribute to the flavor of bread crumb.

As can be seen in Table 2, different principles of processing are in use to obtain alcoholic beverages. The simplest way is to harvest ripe fruit or palm sap, both of which contain high concentrations (10–30%) of mono- and disaccharides (glucose, fructose, and sucrose) that can be fermented directly by yeasts. The fruits are pressed, and the fresh juice is left to ferment naturally, that is, no yeast is added. The wide range of wines that are known worldwide are all made according to this simple principle. When all sugars have been assimilated, the fermentation will stop. Usually, grape wines will contain 10–14% v/v of ethanol.

Making of beer is more complicated. Because the main ingredient for beer is a cereal grain (barley, wheat, sorghum,

Table 2 Alcoholic fermentations

Name of product	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical alcohol content (% v/v)*	*Remarks*[a]
Beer	Europe	Barley and hops	Y (Sc)	4–8	1
Grape wine	Europe	Grapes	Y (Sc)	10–15	3
Sake	Japan	Rice	M (Ao) and Y (Ha and Ss)	15–16	2
Bai-jiu	China	Sorghum	M (R) and Y (Ef)	38–45	2 and 4
Palm wines	Africa–Asia	Palm sap	Y (Sc and Sp) and LAB (Lm and Lp)	2–4	3

[a]1, Brewed from starch with malt (germinated barley); 2, brewed from starch with fungal enzyme starter; 3, direct fermentation of fruit sugars; 4, distilled.
Abbreviations: Ao, *Aspergillus oryzae*; Ef, *Endomycopsis fibuligera*; Ha, *Hansenula anomala*; Lm, *Leuconostoc mesenteroides*; Lp, *Lactobacillus plantarum*; M, molds; R, *Rhizopus* spp.; Sc, *Saccharomyces cerevisiae*; Sp, *Saccharomyces pombe*; Ss, *Saccharomyces saké*; Y, yeast.

Table 3 Alkaline-fermented food products

Name of food	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical pH values*	*Remarks*
Kinema	Nepal–India	Soybeans	B (Bs) and LAB (Ef)	7–8.5	Curry base
Dawadawa and *Soumbala*	Nigeria and Burkina Faso	Locust bean	B (Bs)	7–8	Soup flavoring (condiment)
Tayohounta	Bénin	Baobab seed kernels	B (Bs, Bl, and Bt)	7–8	Soup flavoring
Iru	Bénin	Locust bean	B	7–8	Soup flavoring
Natto	Japan	Soybeans	B (Bn and Bs)	7–8	Poly-DL-glutamic acid causes elastic texture

Abbreviations: B, bacteria; Bl, *Bacillus licheniformis*; Bn, *Bacillus natto*; Bs, *Bacillus subtilis*; Bt, *Bacillus thermoamylovorans*; Ef, *Enterococcus faecalis*.

maize, or millet), the availability of freely available fermentable sugars is low. These will first have to be released by the degradation of starch, the bulk polysaccharide in cereal endosperm. It is interesting to note that worldwide, two fundamentally different strategies have evolved to achieve this aim.

The first strategy is to use starch-degrading enzymes, which are formed during the germination of cereal seeds. Germinated, and then dried, seed is called malt; it contains α-amylase (starch-liquefying enzyme), β-amylase (enzyme releasing maltose from starch and its dextrins), proteases, phosphatases, and other enzymes that are essential for the release of nutrients for the young growing seedling. The word 'brewing' refers to the transformation of starch into fermentable sugars, in this case mainly maltose. After brewing, the resulting sugar solution ('wort') is boiled with hops (providing desired bitter flavor) and inoculated with selected strains of brewer's yeast, usually *S. cerevisiae*. Two fermentation stages include the primary fermentation and the maturation, after which beer is bottled and pasteurized.

The second strategy to degrade cereal starch has evolved in Asia. Instead of using malt enzymes, fungal enzymes are used. In this principle, cereal grains are first soaked and steam cooked, either as whole kernels or as flour, to hydrate and gelatinize the starch. After cooling down, the cooked starch is inoculated with amylolytic starters consisting of mixed populations of starch-degrading molds and yeasts. During the first aerobic incubation stage, the growth of mycelial fungi is favored. These are producers of amyloglucosidase, an enzyme that splits single glucose molecules from starch and dextrin molecules. As a result, a strong accumulation of glucose (approximately 25% w/v) can result. As soon as fermentable sugars are available, the yeasts will start to assimilate them and grow. At the time when the starchy mass starts to exude liquid, the fermentation conditions are changed to favor anaerobic conditions in order to enhance the alcoholic yeast fermentation.

The fermented products resulting from both strategies can be distilled to obtain a variety of strong liquors, which can be matured subsequently. Worldwide, a diversity of strong liquors is known, including whisky and *bai-jiu* (see Table 2).

Alkaline Fermentation

The characteristic feature of alkaline fermentations is the increase of pH during fermentation, to values of approximately 7–8. This is usually caused by a decrease of organic acids in combination with the accumulation of NH_3, which tends to increase the pH. The kinds of products that undergo alkaline fermentation are mainly leguminous seeds with a relatively high protein content, such as soybeans, locust beans, baobab seed kernels, etc. To bring about the above-mentioned changes, the fermentation must be dominated by microorganisms that produce proteolytic and other lytic enzymes, in addition to enzymes acting on peptides and amino acids, such as various peptidases, deaminases, transaminases, etc. Although these kinds of enzymes are found in a range of microorganisms, the major microorganisms that have impact are filamentous fungi and *Bacillus* spp. Among the filamentous fungi, *Aspergillus* spp., for example, *Aspergillus oryzae*, as well as Phycomycetes such as *Rhizopus*, *Mucor*, and *Actinomucor* spp. are associated with alkaline fermentations. However, by far, the most important are the *Bacillus* spp. such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus*, etc. Some examples of alkaline-fermented foods are summarized in Table 3.

The main use of alkaline-fermented foods is as a flavoring agent. It is remarkable how very similar processing approaches have evolved as traditional fermentations in the Orient and in Africa. The common features are the use of plant seeds with a relatively high protein content (40–45% dm), a prolonged boiling of dehulled seeds in water, and a fermentation of 2–3 days at warm (30–35° C) temperatures. Prolonged cooking will not only kill all vegetative microbial cells, but also activate the heat-resistant *Bacillus* endospores to germinate. As a result, *Bacillus* spp. will germinate and already start growing during the cooling down phase; their high numbers and the metabolites formed restrict the growth of postprocessing contaminating microorganisms.

Fungal Fermentation

Fungi, especially filamentous fungi (molds), were mentioned already in the previous section. Although thousands of mold species are known, only a small number of species have a long history of safe use in (traditional) food fermentations. The most important are summarized in Table 4 along with examples of the foods and other products in which they are a functional part of the fermentation. There are some specific reasons for the use of molds, such as (1) they provide texture by forming a dense and edible mycelium, (2) they produce desirable color and flavor compounds, and (3) they are strong producers of enzymes that cause desirable modifications of the food ingredients. The latter aspect can have profound impact on the nutritional and health beneficial food properties.

Tou-shi is a fermented paste of black soybeans; the fermentation is dominated by *Aspergillus* and *Mucor* spp., which cause the partial degradation of soy protein. It is a type of product that could also be listed under alkaline-fermented foods, but the latter are dominated by *Bacillus* spp. The result is a typical zesty flavor, which makes *tou-shi* an appreciated ingredient in various Chinese dishes.

Angkak or *red kojic rice* is made by fermenting steamed whole-rice kernels with *Monascus* molds. These produce secondary metabolites, including pigments, which are used as bio-colorants in food, for example, *tou-fu-ru* and beverages. Recently, *angkak* has attracted much attention because it also contains antibiotic-like compounds, such as monacolin K, which have been associated with health benefits.

Oncom is a by-product of small-scale pressing of peanut oil. After pressing, the residue or press cake can be valorized by fungal fermentation, to provide texture and flavor to a meat replacer in Indonesia. Two different types of *oncom* are known. Red *oncom* is fermented with *Neurospora intermedia* and black *oncom* with *Rhizopus* spp.

Tapé ketan, known in Indonesian and Malaysian cuisine, is a delicious fermented snack made from glutinous rice. After steaming the rice, it is inoculated with ragi tape, an amylolytic starter (see section Alcoholic Fermentation), and incubated overnight. This will result in a partial liquefaction of the rice making it juicy, accumulation of glucose, and the production of traces of alcohol and lactic acid, all of which combine to give a sweet and sour taste with a tickle on the tongue.

Kochujang has some similarity with *tou-shi*. After fermentation, it is pounded into a paste. This is used as a side dish as well as an ingredient for *kimchi* (see Table 1).

Enzymatic Brine or Salty Fermentations

Brine is a solution of salt in water. The salt is usually NaCl, but other trace ions may be present, such as K^+, Mg^{2+}, SO_4^{2-}, CO_3^-, etc. The salinity or salt concentration depends on the product and processor's preferences. Probably, the historic reason for applying salt is its preservative effect. As it turned out, certain microbial species can grow (albeit slowly) and thus find a niche in brines. Such halophilic and halotolerant species may contribute to the flavor of salt-preserved foods. Another effect of salts is that they increase the ionic strength and improve the dissolution of, for example, enzymes. The latter effect plays a crucial role in many of the popular Asian fungal-fermented foods, such as soy sauce and fish sauces. Table 5 provides a summary of major brine fermentations.

Soy sauce is made in a two-stage process, starting with 3–5 days of fungal solid-state '*koji*' fermentation to favor the formation of fungal lytic enzymes. The molded mass is fermented in brine to be degraded to water-soluble components (amino acids, peptides, etc); during this '*moromi*' stage that may take several months; halophilic LAB and halotolerant yeasts grow slowly and contribute to flavor formation.

Tou-fu-ru is a flavorsome spreadable paste, which is a popular addition to the Chinese rice breakfast. It is made from soybeans by first producing soy milk, which is coagulated into *tofu*. *Tofu* is inoculated with a pure culture of *Actinomucor elegans*, which will cover pieces of *tofu* with its mycelium. The molded pieces ('*pehtze*') are matured in a vessel with brine containing salt, rice wine, and natural flavorings and pigments. Maturation may take 6 months and results in the accumulation of glutamic acid (taste enhancer) and the softening of the texture.

Salami and many other types of raw fermented sausages evolved from the need to preserve meat for the army. This was

Table 4 Fungal-fermented foods

Name of food	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical pH values*	*Remarks*
Tou-shi	China	Soybeans	M (Ao and M)	8	Condiment
Angkak red rice	China	Rice	M (Mp)	8	Biocolorant
Oncom	Indonesia	Peanut press cake	M (Ni)	6–7	Side dish
Tapé Ketan	Indonesia	Glutinous rice	M (Ar) and Y (Ef and Hb)	5	Snack
Kochujang	Korea	Soybeans	M (M and R)	6–7	Side dish

Abbreviations: Ao, *Aspergillus oryzae*; Ar, *Amylomyces rouxii*; Ef, *Endomycopsis fibuligera*; Hb, *Hyphopichia burtonii*; M, molds; Mp, *Monascus purpureus*; Ni, *Neurospora intermedia*; R, *Rhizopus* spp; Y, yeast.

Table 5 Brine food fermentations

Name of food	*Origin*	*Main ingredients*	*Predominant microbiota*	*Typical pH values*	*Remarks*
Soy sauce	East Asia	Soybeans and wheat	M (Ao and As), Y (Zr and H), and LAB (Th)	5–6	Condiment
Tou-fu-ru	China	Soybeans	M (Ae and M)	6–8	Soy paste
Salami sausage	Europe	Lean meat and fat (lard)	LAB (Lc, Lp, and Ls) and B (Sca and Sx)	5–6	Protein food
Cucumber pickles	Europe	Cucumbers and salt brine	LAB (Lp) and Y (Sc and Sr)	4	Side dish
Olives	Europe	Olives and salt brine	LAB (P, L, Lp, and Ld)	4–8	Side dish

Abbreviations: Ae, *Actinomucor elegans*; Ao, *Aspergillus oryzae*;; As, *Aspergillus sojae*; B, bacteria; H, *Hansenula* spp.; L, *Leuconostoc* spp.; Lc, *Lactobacillus curvatus*; Ld, *Lactobacillus delbrückii*; Lp, *Lactobacillus plantarum*; Ls, *Lactobacillus saké*; M, mold; P, *Pediococcus* spp.; Sca, *Staphylococcus carnosus*; Sr, *Saccharomyces rosei*; Sx, *Staphylococcus xylosus*; Th, *Tetragenococcus halophila*; Y, yeast; Zr, *Zygosaccharomyces rouxii.*

achieved by a combination of salting and drying. A good way to prepare a homogenous product that can be dried efficiently is to mince red muscle meat and lard (fat) with herbs, spices, starter microorganisms, and some salt, and stuffing the resulting 'dough' into pork intestines (natural casing). At present, China is the largest producer of natural casings. Nowadays, many meat processors use artificial casings made of edible fiber; these are more evenly shaped. The stuffed fresh sausages are first incubated in a warm brine bath or temperature-controlled ripening chamber to allow a rapid acidification (pH <5.5 within 12 h) by LAB, which is followed by a gradual drying process to achieve the required moisture content.

Cucumber pickles and fermented olives are preserved in brine for several months, during which an exchange of salt, sugars, and moisture will take place. At intervals, the brine concentration is adjusted. The combination of temperature, salt, fruit sugars, and anaerobic situation favors the dominance of LAB and some yeasts.

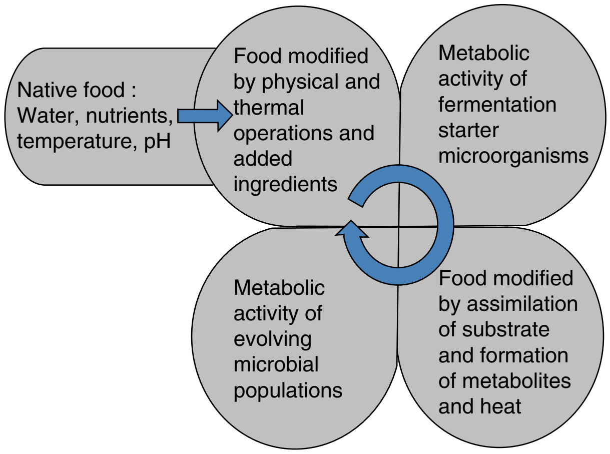


Figure 1 The impact of fermentation on the food environment.

Antimicrobial Principles Formed During Fermentation, Contributing to Food Safety

In fermented foods, the presence of high numbers of microorganisms has a profound impact on the food environment, that is, the chemical and physical conditions that may influence the opportunities for survival and growth of minority microorganisms. The latter could include contaminants that could cause food spoilage, or that could be of pathogenic character.

Figure 1 explains the impact of fermentation on the food environment. It shows that, in principle, fermentations are dynamic and that environmental conditions keep changing. Table 6 summarizes the major microbial metabolites that restrict the growth of pathogenic contaminants. In addition to the accumulation of metabolites, a depletion of available substrates (sugars and amino acids) occurs, which causes intercellular competition. This results in a gradual termination of the fermentation and a competition with spoilage-causing microorganisms.

In acidified foods, the impact of the undissociated organic acids has been well documented. Other articles in this book deal with organic acids, bacteriocins, and reuterin, and the reader is referred to them.

The inhibitory effect of alcohols, ethanol in particular, is due to an increased permeability of the plasmalemma resulting in the decrease of pH gradient and of membrane potential. Although C_2H_5OH is the most abundant alcohol, the C_6 alcohols have the highest impact on the plasmalemma.

Carbon dioxide is formed as a result of alcoholic and heterofermentative lactic acid fermentations, and decarboxylation reactions. In aqueous conditions, it is in equilibrium with HCO_3^-, which has an antimicrobial effect at pH ≤ 6 as its undissociated acid. However, at those pH ranges, the solubility of CO_2 is low, and in fermented foods, this substance has little antimicrobial impact. However, in closed packages, CO_2 in gas form has a significant antimicrobial effect when present at $>10\%$ of the gas atmosphere.

Although diacetyl has a broad antimicrobial activity, it is of little preservative importance because of its flavor, which has a low threshold value, thus making applications at higher concentrations unacceptable to the consumer.

Hydrogen peroxide is produced by microorganisms, but it is also broken down by catalase. Because of its unstable presence, there is little evidence that it has antimicrobial impact in fermented foods.

Ammonia when formed at significant levels in alkaline-fermented foods may also have an antimicrobial impact because it increases the pH to suboptimal levels. However, little evidence about the effect of ammonia has been published.

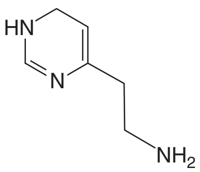
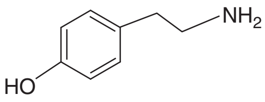
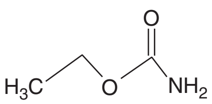
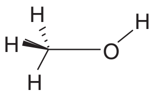
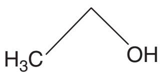
One other important effect of fermentation is the assimilation, by the functional starter microbiota, of substrates (see Figure 1), which thereby causes a competition with contaminant microorganisms. A well-documented example is

Table 6 Antimicrobial fermentation metabolites

Name	*Sensitive microbiota*	*MIC or pH levels*	*Remarks*
Lactic acid	All microorganisms	pH dependent; pK_a 5.2	Causes acidic taste
Acetic acid	All microorganisms	pH dependent; pK_a 4.75	Causes acidic taste
Ethanol	All microorganisms	At concentrations approximately $\geq$10%	Only in suitable products
CO_2	Most microorganisms	Aqueous at pH $\geq$6 Gas: at 20–50%	Can cause textural defects (bloating)
Diacetyl	Yeasts, Gram-negative bacteria Non-LAB, Gram-positive bacteria	At 200 ppm At 300 ppm	Butter flavor threshold: 2–4 ppm
Hydrogen peroxide	All microorganisms	500 ppm	Restricted use
Reuterin	Many bacteria and fungi		FDA approved
Bacteriocins, i.e., nisin	Gram-positive bacteria	50–100 ppm	Generally recognized as safe

Abbreviation: FDA, Food and Drug Administration.

Table 7 Hazardous compounds potentially encountered in fermented foods and beverages

Compounds	*Structure*	*Accepted level*	*Toxic effect*
Biogenic amines			
Histamine		100 ppm	Skin flushing, headache, abdominal cramps, and nausea
Tyramine		100–800 ppm	Tyramine pressor response (increased systolic blood pressure)
Ethyl carbamides			
Ethyl carbamate		Wines $<$15 ppb Strong liquor $<$125 ppb	Not acutely toxic, but potentially mildly carcinogenic
Alcohols			
Methyl alcohol			Highly toxic; depression, headache, and dizziness; and at 10 ml permanent blindness
Ethyl alcohol		40% strong liquor 10–15% wines to be consumed with moderation	At $>$0.1% blood alcohol content: nausea, vomiting, and intoxication; chronic: liver damage
Higher alcohols (propanol, butanol, pentanol, etc.)		Level of toxicity similar to ethyl alcohol	Headache, nausea, and hangover effect

the role of fermentative yeasts in cucumber pickle fermentation. The yeasts 'scavenge' all remaining fermentable sugars and thereby prevent growth of spoilage microorganisms.

Potential Hazardous Effects Related to Substandard Processing

Although food fermentation has a positive image because of its association with attractive sensory aspects, like all processes it also has its limitations. In this section, some of these will be shortly discussed.

Biogenic Amines

Biogenic amines (Table 7) are formed from amino acids by decarboxylation, or by amination and transamination of aldehydes and ketones. Because of the structure of their precursor amino acids, they can have either aliphatic, aromatic, or heterocyclic chemical structures. Although some play a role in the physiology of the living cell, the formation of biogenic amines during fermentation is of most concern here. Some biogenic amines, such as histamine and tyramine, are mildly toxic. A total level of approximately 1000 ppm is associated with toxicity, and in good manufacturing practice, 100 ppm histamine, or a total of biogenic amines of 200 ppm are regarded as acceptable, no-effect levels. Prerequisites for the formation of biogenic amines include the presence of free amino acids (such as in meat, fish, cheese, and also in wine, beer, and cabbages), the ability to decarboxylate them, that is, decarboxylases (Enterobacteriaceae, Enterococci, and some heterofermentative LAB are the most relevant), and suitable conditions for the growth and metabolic activities of these bacteria (low pH and high NaCl concentrations are associated with higher levels of biogenic amines). Preventive measures include the pasteurization of cheese milk and good hygienic practice, including the use of starters selected for low decarboxylase activity.

Ethyl Carbamate

Ethyl carbamate (EC; Table 7) is formed from ethanol with urea or carbamic acid. It is found in several fermented foods, especially those in which yeasts and LAB have been active and some ethanol was formed. Urea and carbamic acid can be formed from the deamination of arginine, a pathway found in, for example, LAB. This pathway serves to generate energy and can be instrumental in the homeostasis of intracellular pH levels. The reaction leading to the formation of EC is favored by increased temperatures, and this is reflected in the higher EC levels in products such as distilled liquors (saké and whisky) and roasted products (toasted bread). Although in some countries maximum tolerated levels of EC are enforced, its toxicity is quite low compared with the health risks of the corresponding consumption of strong alcoholic liquors.

Alcohols

Methyl alcohol is highly toxic. It is formed by the enzymatic degradation of pectic polysaccharides, mainly originating from stone fruits, such as cherries, apples, plums, and pears. In pectins, the polygalacturonate backbone is methylated, and during processing, the endogenous enzymes, such as pectin methyl esterase, will release methanol from pectins. In regular fruit wines, the levels of methanol are too low to be of any concern. However, for a number of popular liquors distilled from these fermented fruits, such as kirsch, apple jack, slivovitch, and Williams, it may have safety consequences.

Although enjoyed by many, ethyl alcohol, formed by alcoholic fermentation through reduction of pyruvate, is also toxic. Excessive use leads to intoxications and if chronic, to liver cirrhosis.

Higher alcohols, such as propanols, butanols, pentanols, and their branched structures, are formed by the deamination and reduction of amino acids in yeast cells. Fermented products containing appreciable levels of free amino acids include beers from barley, maize, and other cereals in which proteins have been degraded. If such brews are used as an ingredient for distilling of, for example, whisky, their concentration may increase as a result of the distillation process. Although modest levels of higher alcohols contribute to the flavor of whiskies, in other distilled products, such as vodka, they are associated with unwanted off-flavors. In principle, the toxicity of higher alcohols is similar to that of ethyl alcohol, whereas in some reports they have been associated with the 'hangover effect.'

Hazards of Microbiological Nature and Control Measures

Inadequate processing, such as lack of pasteurization of vulnerable ingredients, poor hygienic practices, and use of low-grade ingredients, may result in fermented products that are contaminated with microbial toxins, or in which pathogenic microorganisms survive and may be the cause of foodborne infections.

Because the respective compounds and microorganisms are discussed in detail in other articles, a short summary of hazards and measures to control them will be presented here.

Raw milk, fish, and meat are known carriers of pathogenic bacteria, parasites, and viruses. Although some traditional fermented products are claimed to be of better quality when prepared from raw ingredients, experience has shown that it is wise to preheat (pasteurize) such ingredients before or after fermentation.

Poor hygiene may lead to postprocessing contamination, mostly with pathogenic and/or spoilage-causing bacteria. Although some fermented products have inherent antimicrobial properties (especially the low-pH products), many other products offer good chances for survival. In traditional fermented raw milk, the levels of Enterobacteriaceae are reduced only if pH values lower than 4.5 are obtained. Even despite this acid-sensitivity of most Enterobacteriaceae, it must be noted that some strains of *Escherichia coli*, for example, *E. coli* O157, as well as viruses are not adequately affected by acidity. Well known are the outbreaks caused by *E. coli* contaminated Brie cheese, among others. Also, several fatal cases of infections with *Listeria monocytogenes* and *E. coli* have been associated with the consumption of similar raw-milk white-mold ripened cheeses, such as Vacherin Mont d'Or. Pasteurization of cheese milk and strict hygiene are therefore control measures to minimize these hazards.

In addition to raw milk, other uncooked ingredients may cause safety problems. For instance, the custom of fermenting raw pork meat in South East Asia (e.g., Thailand and Vietnam) has caused infections with parasites, such as *Trichinella*. Such parasites are not or hardly killed by acid or salt: The only adequate way to eradicate them is heating. Therefore, the fermented meat must be cooked well before consumption. In a similar way, the consumption of raw (uncooked) fishery products may lead to foodborne infections with *Vibrio parahaemolyticus*, a typical seafood-associated pathogen.

Another hazard associated with raw (uncooked) water, shellfish, vegetables, etc. are viruses, such as norovirus and rotavirus. Cooking food before consumption is the only safe method to inactivate them.

Table 8 Major food safety hazards in fermented foods and beverages, with control measures

Hazards	*Control measures*
Chemical hazards	
Biogenic amines	Control lysine decarboxylase activity by, for example, blanching and avoidance of Enterobacteriaceae by GMP and hygiene
Ethyl carbamate	No measures
Methyl alcohol	Optimize separation of distilling units to avoid methyl alcohol collection from fermented stone fruits
Higher (fusel) alcohols	Optimize temperatures and free amino acid content in alcoholic fermentation to reduce formation, and in distilling optimize separation capacity of column
Microbiological hazards	
Botulin	Prevent outgrowth of *C. botulinum* spores
Staphylococcal enterotoxins	Prevent growth of *S. aureus*
Mycotoxins	Prevent growth of mycotoxigenic fungi and remove molded ingredients
Enterobacteriaceae (*E. coli*, *Salmonella*, *Shigella*, etc.)	Pasteurization; pH <4.4; $a_w<0.95$
L. monocytogenes	Pasteurization; pH <4.4; $a_w<0.92$
V. parahaemolyticus	Pasteurization; pH <4.8; $a_w<0.94$
S. aureus	Pasteurization; pH <4.0; $a_w<0.83$
C. botulinum	Botulinum 12D cook; pH <4.5 and/or apply sodium nitrite to avoid germination of surviving spores
Viruses	Cooking
Parasites	Freezing (not always adequate) and cooking

Abbreviations: a_w, water activity; GMP, good manufacturing practice.

In addition to the heat treatment of raw (uncooked) ingredients, some other process factors can contribute to the safety of fermented foods, such as:

- rapid (within 12 h) acidification to pH <4.5 by LAB – sometimes supported by acidulants, such as glucono-δ-lactone – supports their competition with other microbiota. In particular, the growth or survival of most Enterobacteriaceae is minimized. *Staphylococcus aureus* may still grow, although due to competition pressure from starter microbiota, it does not produce its enterotoxins;
- salt (NaCl) and nitrite used in meat curing are essential, not only for taste reasons but also to especially avoid the germination, outgrowth, and botulin formation by *Clostridium botulinum*;
- reducing the water activity (a_w) by dehydration, or by addition of sugar, salt, or other solutes; and
- hermetic sealing to prevent oxygen entry will reduce oxidative quality defects, but especially it will prevent mold growth and associated risks of mycotoxin formation in products that are cured or stored for prolonged periods of time.

Of special concern is the hazard of mycotoxins, mostly because these occur at low concentrations and lead to chronic mycotoxicoses only after long-term ingestion.

Mycotoxins may occur in fermented foods if the ingredients used were contaminated before processing. Most mycotoxins (in particular aflatoxins and ochratoxins) cannot sufficiently be degraded or detoxified during heating and fermentations to expect safe food from unsafe ingredients. However, patulin (frequently found in apple juice) has been shown to be degraded by lactic fermentation involving *Lactobacillus plantarum*.

With regard to the quality of starters used for fermentation, it has been observed that in some traditional mold-fermented products, such as sausages and country-cured hams, some strains of, for example, *Penicillium roqueforti* and *Penicillium camemberti* used as starters in the process, were actually able to produce mycotoxins when grown in the laboratory as pure cultures. Although these are very different cultivation conditions than in, for example, meat or cheese, it is an indication that much care should be taken with the selection of starter strains. Therefore, food legislation should require the use of selected starter strains selected for their inability to form mycotoxins or other toxic substances.

In Table 8, an overview is provided of the major hazards mentioned and measures to control them. Which measures will be feasible will depend on the type of food and the market preferences.

In conclusion, fermentation has much to offer as a technology that renders foods attractive, digestible, and safe. However, there remain several aspects that require research for further development, and the technology has its limitations. Research is required in order to (1) develop starter microorganisms that are both safe and beneficial for human health, (2) selection of starter microorganisms that can kill or compete with disease-causing microbes, and (3) adaptation of scientific knowledge to application in regions or situations that lack infrastructure in order to harness fermentation as a beneficial technology to public health.

See also: Disciplines Associated with Food Safety: Food Microbiology; Food Virology; Parasitology. Food Technologies: Biopreservation. Foodborne Diseases: Overview of Emerging Food Technologies. Mycotoxins: Mycotoxins – General. Processing Contaminants: Biogenic Amines

Further Reading

Adams MR and Nout MJR (eds.) (2001) *Fermentation and Food Safety.* Gaithersburg, MD: Aspen Publishers.

Motarjemi Y (2001) An introduction to the Hazard Analysis and Critical Control Point (HACCP) System and its application to fermented foods. In: Adams MR and Nout MJR (eds.) *Fermentation and Food Safety*, pp. 53–70. Gaithersburg, MD: Aspen Publishers, Inc.
Nout MJR and Motarjemi Y (1997) Assessment of fermentation as a household technology for improving food safety: A joint FAO/WHO workshop. *Food Control* 8: 221–226.
Nout MJR and Rombouts FM (2000) Fermented and acidified plant foods. In: Lund BM, Baird-Parker TC, and Gould GW (eds.) *The Microbiological Safety and Quality of Food*, pp. 685–737. Gaithersburg, MD: Aspen Publishers.
Nout MJR, Sarkar PK, and Beuchat LR (2007) Indigenous fermented foods. In: Doyle MP and Beuchat LR (eds.) *Food Microbiology: Fundamentals and Frontiers*, pp. 817–835. Washington, DC: ASM Press.
Parkouda C, Nielsen DS, Azokpota P, *et al.* (2009) The microbiology of alkaline-fermentation of indigenous seeds used as food condiments in Africa and Asia. *Critical Reviews in Microbiology* 35: 139–156.

Relevant Websites

http://www.foodmicrobiology.wur.nl/
Laboratory of Food Microbiology, Wageningen University.
www.ronfostec.nl
Rob Nout Food Fermentation Science and Technology Consultancies.
http://www.taylorandfrancis.com/books/series/fermented_foods_and_beverages_series_CRCFERFOOBEV/
Taylor & Francis: Fermented Foods and Beverages Series.