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Fermentations in World Food Processing

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Introduction (Steinkraus 1995, 1996a, 1997)

Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases, lipases hydrolyze the polysaccharides, proteins and lipids to nontoxic products with flavors, aromas and textures pleasant and attractive to the human consumer. If the products of enzyme activities have unpleasant odors or undesirable, unattractive flavors or the products are toxic or disease producing, the foods are described as spoiled.

Fermentation plays at least five roles in food processing:

(1) Enrichment of the human dietary through development of a wide diversity of flavors, aromas and textures in food;

(2) Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid, alkaline fermentations and high salt fermentations;

(3) Enrichment of food substrates biologically with vitamins, protein, essential amino acids and essential fatty acids;

(4) Detoxification during food fermentation processing and

(5) a decrease in cooking times and fuel requirements.

Classification of Food Fermentations (Steinkraus 1995, 1997)

Food fermentations can be classified in a number of ways (Dirar 1993): by categories (Yokotsuka 1982)— (1) alcoholic beverages fermented by yeasts; (2) vinegars fermented with *Acetobacter*; (3) milks fermented with lactobacilli; (4) pickles fermented with lactobacilli; (5) fish or meat fermented with lactobacilli; and, (6) plant proteins fermented with molds with or without lactobacilli and yeasts; by classes (Campbell-Platt 1987) (1) beverages; (2) cereal products; (3) dairy products; (4) fish products; (5) fruit and vegetable products; (6) legumes; and, (7) meat products; by commodity (Odunfa 1988) (1) fermented starchy roots; (2) fermented cereals; (3) alcoholic beverages; (4) fermented vegetable proteins; and, (5) fermented animal protein; by commodity (Kuboye 1985) (1) cassava based; (2) cereal; (3) legumes; and, (4) beverages. Dirar (1993) states that the Sudanese traditionally classify their foods, not on the basis of microorganisms or commodity but on a functional basis: (1) Kissar (staples)-porridges and breads such as aceda and kissra; (2) Milhat (sauces and relishes for the staples); (3) marayiss (30 types of opaque beer, clear beer, date wines and meads and other alcoholic drinks); and, (4) Akil-munasabat (food for special occasions). Steinkraus (1983a; 1996) classified fermentations according to the following categories that will serve as the basis for this paper:

1. Fermentations producing textured vegetable protein meat substitutes in legume/cereal mixtures. Examples are Indonesian tempe and ontjom.

2. High salt/savory meat-flavored/amino acid/peptide sauce and paste fermentations. Examples are Chinese soy sauce, Japanese shoyu and Japanese miso, Indonesian kecap, Malaysian kicap, Korean kanjang, Taiwanese inyu, Philippine taosi, Indonesian tauco, Korean doenjang/kochujang, fish sauces: Vietnamese nuocmam, Philippine patis, Malaysian budu, fish pastes: Philippine bagoong, Malaysian belachan, Vietnamese mam, Cambodian prahoc, Indonesian trassi and Korean jeotkal. These are predominately Oriental fermentations but the use of these products is becoming established in the United States.

3. Lactic acid fermentations. Examples of vegetable lactic acid fermentations are: sauerkraut, cucumber pickles, olives in the Western world; Egyptian pickled vegetables in the middle East; Indian pickled vegetables and Korean kim-chi, Thai pak-sian -dong, Chinese hum-choy, Malaysian pickled vegetables and Malaysian tempoyak. Lactic acid fermented milks include: yogurts in the Western world, Russian kefir, Middle-East yogurts, liban (Iraq), Indian dahi, Egyptian laban rayab, laban zeer, Malaysian tairu (soybean milk). Lactic acid fermented cheeses in the Western world and Chinese sufu/tofu-ru. Lactic acid fermented yogurt/wheat mixtures: Egyptian kishk, Greek trahanas, Turkish tarhanas. Lactic acid fermented cereals and tubers (cassava): Mexican pozol, Ghanian kenkey, Nigerian gari; boiled rice/raw shrimp/raw fish mixtures: Philippine balao balao, burong dalag; lactic fermented/leavened breads: sourdough breads in the Western world; Indian idli, dhokla, khaman, Sri-lankan hoppers; Ethiopian enjera, Sudanese kisra and Philippine puto; Western fermented sausages and Thai nham (fermented fresh pork).

4. Alcoholic fermentations. Examples are grape wines, Mexican pulque, honey wines, South American Indian chicha and beers in the Western World; wines and Egyptian bouza in the Middle East; Palm and Jackfruit wines in India, Indian rice beer, Indian madhu, Indian ruhi; in Africa, Ethiopian tej, Kenyan muratina, palm wines, Kenyan urwaga, Kaffir/bantu beers, Nigerian pito, Ethiopian talla, Kenyan busaa, Zambian maize beer; in the Far East, sugar cane wines, palm wines, Japanese sake, Indonesian tape, Malaysian tapuy, Chinese lao-chao, Thai rice wine, Indonesian brem, Philippine tapuy.

5. Acetic acid/vinegar fermentations. Examples are apple cider and wine vinegars in the West; palm wine vinegars in Africa and the Far East, coconut water vinegar in the Philippines; tea fungus/ Kombucha in Europe, Manchuria, Indonesia, Japan and recently in the United States; Philippine nata de pina and nata de coco.

6. Alkaline fermentations. Examples are Nigerian dawadawa, Ivory Coast soumbara, African iru, ogiri, Indian kenima, Japanese natto, Thai thua-nao.

7. Leavened breads. Examples are Western yeast and sourdough breads; Middle East breads.

8. Flat unleavened breads. The above classes of fermented foods are found around the world. The lines between the various classifications are not always distinct. Tempe in class 1 involves a lactic acid fermentation during soaking of the soybeans. Yeast (alcoholic)/lactobacilli (lactic acid) interactions are rather frequent-for example in sourdough breads, in primitive beers and wines and in Chinese soy sauce/Japanese shoyu/Japanese miso fermentations (Wood 1985). Nevertheless, Steinkraus (1983a, 1996) has found the above classification useful and a way of predicting what microorganisms may be involved and what chemical, physical and nutritive changes may occur in new unfamiliar fermented foods. The classification also relates well to safety factors found in fermented foods.

Fermented foods were originally household and expanded to cottage industry as consumer demand required. Some food fermentations such as Japanese shoyu, miso and sake, South African maize/sorghum beers, South African mageu/mahewu Nigerian ogi and gari have been industrialized (Steinkaus 1989).

Evolution of Indigenous Fermented Foods (Steinkraus 1996a)

Philosophy includes theory or investigation of the principles or laws that regulate the universe and underlie all knowledge and reality. Archaeology is the scientific study of the life and culture of ancient peoples. Anthropology is the study of races, physical and mental characteristics, distribution, customs, social relationships, and so on. When we start to study man's foods, we become involved in all the above. In fact, when we study fermented foods, we are studying the most intimate relationships among man (men/women-humans), microbes and foods. There is a never-ending struggle between man and microbes to see which will be first to consume the available food supplies.

Religion was an attempt by humans to explain the unexplainable origin of the universe, the earth and man long before there was a scientific method or the means to study these difficult problems and no concept of, for example, microorganisms, knowledge of which we obtained only about 300 years ago when Leeuwenhoek discovered tiny animacules under his primitive lenses and only a little more than a hundred years ago when Pasteur demonstrated the role of microorganisms in fermentation and Koch showed that microbes cause disease. And it is only in the last 50 years that knowledge of the role polymeric deoxyribonucleic acid (DNA) plays in all forms of life was discovered.

According to present scientific thought, the earth is about 4.5 billion years old. The first forms of life to appear or evolve on earth were microorganisms. Fossil organisms have been found in earth rocks 3.3 to 3.5 billion years old (Schopf and Packer 1987). Since then and still today, microorganisms have had and have the principal task of recycling organic matter in the environment. As such they are absolutely essential to the health of the earth, whereas, humans are nonessential polluters who may eventually make the earth uninhabitable.

Whether it was by chance or by design, it was extraordinarily fortunate that the earth was originally colonized by microorganisms which are capable of recycling organic matter including dead bodies, and so on. Without them, the earth would be a gigantic, permanent waste dump. The earth was inhabited by microorganisms for probably a billion years before other forms of life evolved.

The next forms of life to evolve, according to present scientific thought, were plants that serve as a basis for man's food. For at least a billion years before man arrived, plants were producing food consisting of leaves, stems, seeds, nuts, berries, fruits, tubers, and so on So when humans were created or evolved on earth, the basis for their foods was already present and productive.

Both plants and animals evolved where the microbes were ready, willing and able to recycle all organic matter. Plants and animals had to evolve into and survive in a microbial environment. They had to develop ways of resisting microbial invasion and consumption. Plants did this, in part, by having a lignocellulosic body very resistant to microbial breakdown.

Until recently, we might have accepted the hypothesis that microorganisms, insects, animals, plants, humans were all created or evolved independently. There was no good reason to believe that all forms of life are closely interrelated. This changed when Watson and Crick unraveled the structure of DNA, the basis of the genetic code demonstrating that it is based upon a 4 molecule alphabet that controls the structure and function of all forms of life including microorganisms, plants and all animals including man.

Early plant evolution was essential as plants not only provide the basis for food for animals and man but they were principally responsible for the development of the oxygen atmosphere necessary for man and animals.

Plants also introduced a very effective way of transforming the sun's radiation into food materials such as sugars, starches and cellulose through the green pigment chlorophyll. Plants and plant structures such as leaves, stems, roots and seeds all of which serve as food for microorganisms and animals including ourselves are literally sun's energy, radiation converted to matter.

Humans also had to evolve from the sea of microorganisms. They had to develop internal and exterior systems of protection against invasion by microorganisms. Then, as now some microorganisms could invade the live animal or human and cause disease. Animals including humans evolved with a "normal" flora of microorganisms that live in the skin, mouth, throat, intestinal tract, vagina, and so on. The normal flora protects us against invasion by other microorganisms which might cause disease. Nowhere is this more pronounced or more evident than in the human infant.

Within the uterus, the infant is essentially sterile but it can be readily invaded by wide variety of microorganisms in the birth environment. The fact is, however, that, if the infant is breast-fed, as nature intends, its intestinal tract becomes colonized by a particular species of bacteria, *Bifidobacterium bifidus* which produces lactic acid and protects the infant against both intestinal and respiratory diseases. The skin of the human also is fairly resistant to invasion by undesirable microorganisms because of its "normal" flora of cocci. Occasionally cocci may invade the skin and cause infection but their role as "protectorants" is of much greater importance.

Early man very likely consumed fruits, leaves, berries, seeds, nuts probably tubers foraging from place to place as apes do today. Their bodily wastes, as well as their bodies at death, were recycled by microorganisms. There was a relatively large potential food supply and relatively few humans. Excess food supplies, fruits, berries, fell on the ground and the seeds either germinated or the carbohydrates, proteins, fats, and so on, were consumed by microorganisms using enzymes that converted fermentable carbohydrates to alcohol or acids and finally to water and carbon dioxide. Seeds and nuts and other protein-containing components were converted to their essential amino acids, peptides and finally to ammonia and water and a wide variety of chemical products. All of these reactions occurred (as recycling) for a billion years before man arrived or evolved on earth.

As population increased it became desirable for humans to collect fruits, berries, nuts, seeds, leaves as a store of food to tide humans over during periods of bad weather (winter, for example) when fresh food was not readily available.

Insects, rodents, birds and most types of animals were already well-established on earth when man arrived. Food consumed by insects or other animals is not available to man unless the insects and animals are, in turn, consumed as food.

Foods invaded by bacteria producing toxins or by fungi producing mycotoxins are dangerous to man. If the products of invasion are ill-smelling, off-flavored or toxic, human consumers try to avoid them and the foods are described as spoiled. If the microbial products are pleasantly flavored, have attractive aromas and textures and are nontoxic, the human consumer accepts them and they are designated as fermented foods.

Certain flavors such as sweet, sour, alcoholic and meat-like (savory) appeal to large numbers of humans. Milks sour naturally. Fruit and berry juices rapidly become alcoholic. Over many centuries, people have developed tastes for such products that continue on in modern man. Some anthropologists have suggested that it was stimulation and desire for alcohol that motivated man to settle down and become agriculturists (Braidwood 1953; Katz and Voight 1987).

Safety of Fermented Foods (Steinkraus 1995, 1997)

Fermented foods generally have a very good safety record even in the developing world where the foods are manufactured by people without training in microbiology or chemistry in unhygienic, contaminated environments. They are consumed by hundreds of millions of people every day in both the developed and the developing world. And they have an excellent safety record. What is there about fermented foods that contributes to safety?

While fermented foods are themselves generally safe, it should be noted that fermented foods by themselves do not solve the problems of contaminated drinking water, environments heavily contaminated with human waste, improper personal hygiene in food handlers, flies carrying disease organisms, unfermented foods carrying food poisoning or human pathogens and unfermented foods, even when cooked if handled or stored improperly. Also improperly fermented foods can be unsafe. However, application of the principles that lead to the safety of fermented foods could lead to an improvement in the overall quality and the nutritional value of the food supply, reduction of nutritional diseases and greater resistance to intestinal and other diseases in infants.

Principles Behind Safety of Fermented Food Processes

The safety of food fermentation processes is related to several principles.

The first is that food substrates overgrown with desirable, edible microorganisms become resistant to invasion by spoilage, toxic or food poisoning microorganisms. Other, less desirable (possibly disease producing) organisms find it difficult to compete. An example is Indonesian tempe (Steinkraus 1996). The substrate is soaked, dehulled, partially cooked soybeans. During the initial soaking, the soybeans undergo an acid fermentation that lowers the pH to 5.0 or below, a pH inhibitory to many organisms but highly acceptable to the mold. Following cooking the soy cotyledons are surface dried which inhibits growth of bacteria that might spoil the product. The essential microorganism is *Rhizopus oligosporus,* or related *Rhizopus* species, which knit the cotyledons into a compact cake that can be sliced or cut into cubes and used in recipes as a protein-rich meat-substitute. These molds have the ability to grow very rapidly at relatively high temperatures, that is, 40 to 42 °C too high for many bacteria and molds. The mold utilizes the available oxygen and produces CO_2 which inhibits some potential spoilage organisms. The mold also produces quantities of spore dust that permeate the environment and contribute to inoculation of the desired microorganisms. The combination of a relatively low pH, no free water and a high temperature in the fermenting bean mass enables *Rhizopus oligosporus* to overgrow the soybeans in 18 h. Organisms that might otherwise spoil the product are unable to compete with the mold. In addition, the mold also produces some antibiotic activity that inhibits other organisms that might invade and spoil the product (Wang and others 1969). The principle is that, as soon as the substrate is overgrown by the desired organism (s), it is resistant to invasion by other microorganisms. An additional safety factor is that the raw tempe is sliced or cut into cubes and cooked before consumption which destroys vegetative microorganisms that are present.

Soybean tempe (tempe kedelee) has an excellent record of safety in Indonesia and Malaysia where the product is fermented as above and has been used for centuries. In temperate countries including the United States, soybeans do not undergo natural lactic acid fermentation during soaking. The pH remains closer to 6.0 a level that permits growth of contaminating bacteria. It has been shown (Tanaka and others 1985) that unacidified soybeans can be invaded by a variety of food poisoning organisms including *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum*. This can be prevented by artificially acidifying the soybeans or inoculating the soak water with *Lactobacillus plantarum*; but American producers have often been careless and used unacidified beans or insufficiently acidified soybeans for tempe production. In some cases this has led to spoilage and could lead to food poisoning.

While soybean tempe has an excellent safety record, there is a type of tempe that has a reputation for causing sickness and even death in the consumer. This is tempe bongkrek made from the coconut residue from coconut milk production. Again, if the substrate is properly acidified, it is generally safe and the mold overgrows the substrate knitting it into a compact cake. But, if the substrate is not sufficiently acidified and/or, if the fungal inoculum is insufficient, a bacterium *Burkholdaria* (*Pseudomonas*) *cocovenenans* can grow in the coconut residue producing two toxic compounds-bongkrek acid, the most lethal, and toxoflavin (Van Veen 1967; Steinkraus 1996). These toxic compounds are inhibitory to the mold and it does not overgrow the substrate properly. Bongkrek acid is colorless and it can be lethal to the consumer. Toxoflavin is yellow and so, if tempe bongkrek has a yellow color or if the mold has not overgrown the substrate properly, it should not be consumed Yet, quite a number of Indonesians in central Java die every year from consumption of improperly fermented tempe bongkrek. This is an example of a fermented food that can and does become toxic but it is amply documented and there is no good excuse for people consuming it and becoming ill or dying.

It has been a mystery why bongkrek poison appears to develop only in coconut tempeh fermented in Java. Part of the answer has been discovered by Garcia, Hotchkiss and Steinkraus (1999). Forty and fifty percent coconut fat concentrations in the substrate (coconut presscake or the residue from coconut milk production) supports production of 1.4 mg/g bongkrek acid while less than 10% coconut fat while, supporting growth of the bacterium, yields no bongkrek acid. Oleic acid was most stimulatory in production of bongkrek acid (2.62 mg/g dry substrate). Lauric, myristic and palmitic acids also stimulated production of bongkrek acid but at lower levels.

A valid concern is mycotoxins that are present in many cereal grain and legume substrates before fermentation. Aren't these dangerous to the consumer of fermented foods? Certainly they are a problem; however, it is not the fermentation that produces mycotoxins. They are produced when the cereal grains or legumes are improperly harvested or stored. It has been found that in the tempe fermentation, mycotoxin levels are reduced. Van Veen and others (1970) reported that the ontjom mold, *Neurospora* and the tempe mold *Rhizopus oligosporus* could decrease the aflatoxin content of peanut presscake 50% and 70% respectively during fermentation. And during soaking and cooking of raw substrates before fermentation, many potential toxins such as trypsin inhibitor, phytate and hemagglutinin are destroyed. So, in general, fermentation tends to detoxify the substrates.

Sudigbia and Sumantri (1990) developed an infant formula containing tempe as 39.6% of its weight. Other major ingredients were wheat flour 31.6%, sugar 23.3% and vegetable oil 2.9%. Infants suffering from acute diarrhea and consuming the formula recovered more rapidly than infants not receiving the formula and also gained weight rapidly. It would appear that inclusion of tempe in infant formulas on a broader scale would not only decrease the overall incidence of diarrhea but also improve infant/child growth rates and nutrition.

Lactic acid fermentations

A second principle is that fermentations involving production of lactic acid are generally safe. Lactic acid fermentations include those in which the fermentable sugars are converted to lactic acid by lactic acid organisms such as *Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, Pediocccus cerevisiae, Streptococcus thermophilus, Streptococcus lactis, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus citrovorum, Bifidobacterium bifidus*, and so on.

This single category is responsible for processing and preserving vast quantities of human food and insuring its safety. We are all aware of the excellent safety record of sour milks/yogurts, cheeses, pickles, and so on. In addition the lactic acid fermentations provide the consumer with a wide variety of flavors, aromas and textures to enrich the human diet.

The most ancient lactic fermentation, likely, is fermented/sour milk. Raw, unpasteurized milk will rapidly sour because the lactic acid bacteria present in the milk ferment milk sugar, lactose to lactic acid. In the presence of acid and a low pH, other microorganisms that might invade the milk and transmit disease-producing organisms are less able to do so.

If the whey is allowed to escape or evaporate, the residual curd becomes a primitive cheese. Fermented cheeses and milks are an extensive topic on their own (Kosikowski 1977; Wood, 1998). The readers are referred to Dr. Kosikowski's classic book "Cheese and Fermented Milk Foods" for details.

Vegetable foods and vegetable/fish/shrimp mixtures are preserved around the world by lactic acid fermentation (Steinkraus 1983a,c; 1996). The classic lactic acid/vegetable fermentation is sauerkraut (Pederson and Albury 1969; Pederson 1979). Fresh cabbage is shredded and 2.25% salt is added. There is a sequence of lactic acid bacteria that develop. First, *Leuconostoc mesenteroides* grows producing lactic acid, acetic acid and CO_2 which flushes out any residual oxygen making the fermentation anaerobic. Then *Lactobacillus brevis* grows producing more acid. Finally *Lactobacillus plantarum* grows producing still more lactic acid and lowering the pH to below 4.0. At this pH and under anaerobic conditions, the cabbage or other vegetables will be preserved for long periods of time.

Korean kimchi is a fermentation similar to sauerkraut but it includes not only Chinese cabbage but radishes, red pepper, ginger and garlic. It is less acid than sauerkraut and is consumed while still carbonated. It is a staple and makes a major contribution to the Korean diet in which consumption of 100 g or more/capita/day is not uncommon (Mheen and others 1977; Steinkraus 1983 a,c). Kimchi is still a household fermentation in Korea although it can be purchased commercially.

Pickled vegetables, cucumbers, radishes, carrots and very nearly all vegetables and even some green fruits such as olives, papaya and mango are acid fermented in the presence of salt around the world. Mukerjee (1987) demonstrated that Indian farmers can safely preserve their surplus vegetables by lactic acid fermentation on the farm. This improves the supply and availability of vegetable foods throughout the year and improves the nutrition of the Indian population.

Pit fermentations

Lactic acid fermentations include the "pit" fermentations in the South Pacific Islands. They have been used for centuries by the Polynesians to store and preserve breadfruit, taro, banana and cassava tubers (Steinkraus 1986; Aalbersberg and others 1988). The fermented pastes or whole fruits, sometimes punctured, are placed in leaf-lined pits. The pits are covered with leaves and the pits are sealed. It has recently been found that pit fermentations are lactic acid fermentations (Aalbersberg and others 1988) The low pH and anaerobic conditions account for the stability of the foods. An abandoned pit estimated to be about 300 years old contained breadfruit still in edible condition.

In Ethiopia, pulp of the false banana (*Ensete ventricosum*) is also fermented in pits (Steinkraus 1983a). It undergoes lactic acid fermentation and is preserved until the pit is opened. Then the mash is used to prepare a flat bread kocho-a staple in the diet of millions of Ethiopians.

Lactic acid fermented rice/shrimp/fish mixtures

Philippine balao balao is a lactic acid fermented rice/shrimp mixture prepared by mixing boiled rice, raw shrimp and solar salt (about 3% w/w), packing in an anaerobic container and allowing the mixture to ferment over several days or weeks (Arroya and others 1977); Steinkraus 1883a,c). The chitinous shell of the shrimp becomes soft and when the product is cooked, the whole shrimp can be eaten. The process provides a method of preserving raw shrimp or pieces of raw fish (burong dalog) (Orillo and Pederson 1968). The products are well-preserved by the low pH and anaerobiosis until the containers are opened. Then they must be cooked and consumed.

Yogurt/cereal mixtures

Another household lactic acid fermentation of considerable nutritional importance includes Egyptian kishk, Greek trahanas and Turkish tarhanas. These products are basically parboiled wheat/yogurt mixtures that combine the high nutritional value of wheat and milks while attaining excellent keeping qualities. The processes are rather simple. Milk is fermented to yogurt and the yogurt and wheat are mixed and boiled together until the mixture is highly viscous. The mixture is than allowed to cool, formed into biscuits by hand and sun-dried. Trahanas can be stored on the kitchen shelf for years and used as a base for highly nutritional soups. In the Egyptian kishk process, tomatoes, onions and other vegetables are sometimes combined with the yogurt and wheat in the biscuits (Abd-el-Malek and Demerdash 1977; Steinkraus 1983a;1996).

Cereal/legume sour gruels/porridges/beverages

Cereal/legume sour gruels/porridges/beverages include Nigerian ogi, Kenyan uji, South African mahewu/magou, and Malaysian soybean milk yogurt (tairu). In Nigerian ogi, maize, millet or sorghum grains are washed and steeped for 24 to 72 h during which time they undergo lactic acid fermentation. They are drained, wet milled and finally wet-sieved to yield a fine, smooth slurry with about 8% solids content. The boiled slurry called "pap", is a porridge. Pap is a very important traditional food for weaning infants and a major breakfast food for adults. Infants 9 months old are introduced to ogi by feeding once a day as a supplement to breast milk (Steinkraus 1983a; Banigo 1969; Banigo and Muller 1972). Unfortunately the nutritional value of ogi is poor. It is vastly improved by the addition of soybean to produce a soy-ogi (Akinrele and others 1970).

Kenyan uji is a related product except that the grains are ground to a flour before mixing with water and fermenting. The initial slurry is 30% solids. This is fermented for 2 to 5 days yielding 0.3 to 0.5% lactic acid. The slurry is then diluted to 10% solids and boiled. It is then diluted to 4 to 5% solids and 6% sucrose is added for consumption (Dirar 1993; Steinkraus 1983a; Gatumbi and Muriru 1987; Mbugua 1981)).

South African mageu/mahewu/magou is a traditional sour, non-alcoholic maize beverage popular among the Bantu people of Africa (Holzapfel 1989). Corn flour is slurried with water (8 to 10% solids), boiled, cooled and inoculated with 5% w/w wheat flour (household fermentation) which serves as a source of microorganisms and incubated at ambient temperature or the boiled slurry is inoculated with *Lactobacillus delbrueckii* (industrial process) and incubated at 45 C. The slurries are fermented to a pH of 3.5 to 3.9 and then are ready for consumption.

Lorri (1993) and Svanberg and others (1992) reported that lactic acid-fermented gruels inhibited the proliferation of Gram-negative pathogenic bacteria including toxicogenic *Escherichia coli, Campylobacter jejuni, Shigella flexneri* and *Salmonella typhimurium*. The mean number of diarrhea episodes in preschool children over a 9 month period was 2.1 per child using fermented gruels compared with 3.5 per child using non-fermented gruels. Lorri also reported that using a natural lactic acid culture and flour of germinated seeds (power flour), it was possible to prepare liquid cereal gruels from maize, white sorghum, bulrush millet and finger millet with a 30 to 35% flour concentration. The energy density of such a lactic acid-fermented gruel was about 1.2 kcal/g, as compared with 0.4 kcal/g in a non-fermented gruel prepared to the same consistency. This is a 3-fold increase in energy density. Also, the in vitro protein digestibility of high-tannin cereal varieties was significantly increased from a range of 32 to 40% before fermentation to a range of 41 to 60% after lactic acid-fermentation. Lactic acid fermentation of non-tannin cereals with added flour of germinated sorghum grain or wheat phytase increased iron solubility from about 4% to 9% and 50% respectively. Thus lactic acid fermentation has very profound effects on the nutritive value of cereal gruels for feeding infants and children in the developing world.

Ingestion of foods containing live lactic acid bacteria is likely to improve the resistance of the gastrointestinal tracts of infants and children to invasion by organisms causing diarrhea.

Cereal/legume steamed breads and pancakes

Indian idli, a sour, steamed bread, and dosa, a pancake are examples of a household fermentation that could be useful around the world. Polished rice and black gram dahl in various proportions, that is, 3:1 to 1:3 are soaked by the housewife separately during the day. In the evening, the rice and black gram are ground in a mortar and pestle with added water to yield a batter with the desired consistency. The batter is thick enough to require the use of the hand and forearm to mix it properly. A small quantity of salt is added. The batter ferments overnight during which time *Leuconostoc mesenteroides* and *Streptococcus faecalis*, naturally present on the grains/legumes/utensils grow rapidly outnumbering the initial contaminants and dominating the fermentation. The organisms produce lactic acid (total acidity as lactic can reach above 1.0%) and carbon dioxide that makes the batter anaerobic and leavens the product. In the morning, the batter is steamed to produce small white muffins or fried as a pancake. Soybean cotyledons, green gram, Bengal gram can be substituted for the black gram. Wheat, maize or kodri can be substituted for the rice to yield Indian dhokla (Ramakrishnan 1979a, 1979b; Steinkraus 1983a; 1996). A closely related fermentation is Ethiopian enjera that yields the large pancake that serves as the center of the meal.

Alcoholic fermentations

A third principle contributing to food safety is that fermentations involving production of ethanol are generally safe foods and beverages (Steinkraus 1979). These include wines, beers, Indonesian tape ketan/tape ketella, Chinese lao-chao, South African kaffir/sorghum beer and Mexican pulque. These are generally yeast fermentations but they also involve yeast-like molds such as *Amylomyces rouxii* and mold-like yeasts such as *Endomycopsis* and sometimes bacteria such *Zymomonas mobilis*. The substrates include diluted honey, sugarcane juice, palm sap, fruit juices, germinated cereal grains or hydrolyzed starch all of which contain fermentable sugars that are rapidly converted to ethanol in natural fermentations by yeasts in the environment. Nearly equal weights of ethanol and carbon dioxide are produced and the CO2 flushes out residual oxygen and maintains the fermentation anaerobic. The yeasts multiply and ferment rapidly and other microorganisms most of which are aerobic cannot compete. The ethanol is germicidal and, as long as the fermented product remains anaerobic, the product is reasonably stable and preserved.

With starchy substrates such as cereal grains, it is necessary to convert some of the starch to fermentable sugar. This is done in a variety of ways, that is, chewing the grains to introduce ptyalin (Andes region of South America) where maize is a staple or germination (malting) of barley or the grains themselves in most of the world where beers are produced. In parts of Africa, a young lady cannot get married until she is capable of making Bantu beer for her husband.

In Asia, there are at least two additional ways of fermenting starchy rice to alcoholic foods. The first is the use of a mold such as *Amylomyces rouxii* which produces amylases converting starch to sugars and a yeast such as *Endomycopsis fibuliger* which converts the glucose/maltose to ethanol. The sweet sour/ alcoholic product of rice fermentation is called tape ketan in Indonesia. It is consumed as a dessert. When cassava is used as substrate, the product is called tape ketella (Merican and Yeoh 1989; Steinkraus 1983a). This process can also be used to produce rice wines, Chinese lao-chao and Malaysian tapuy/tapai.

Another method is the Japanese koji process used to ferment rice to rice wine (sake) (Yoshizawa and Ishikawa 1989; Steinkraus 1979b; 1983a). In this process, boiled rice is overgrown with an amylolytic mold *Aspergillus oryzae* for about 3 days at 30 C. The mold-covered rice called a "koji" is then inoculated with a culture of the yeast *Saccharomyces cerevisiae* and water is added. Saccharification by the mold amylases and alcoholic fermentation by the yeast proceed simultaneously. The result of slow fermentation is high yeast populations and ethanol contents as high as 23% v/v.

Acetic acid/vinegar fermentation

If the products of alcoholic fermentation are not kept anaerobic, bacteria belonging to genus *Acetobacter* present in the environment oxidize portions of the ethanol to acetic acid/vinegar (Conner and Allgier 1976; Steinkraus 1983a;1996).

Fermentations involving production of acetic acid yield foods or condiments that are generally safe. Acetic acid also is bacteriostatic to bactericidal depending upon the concentration. In most cases, palm wines and kaffir beers contain not only ethanol but acetic acid. Acetic acid is even more preservative than ethanol. Vinegar is a highly acceptable condiment used in pickling and preserving cucumbers and other vegetables. The alcoholic and acetic acid fermentations can be used to insure safety in other foods.

Over the last few years, the American public has become aware of a fermented acetic acid beverage called kombucha or tea fungus. Actually kombucha has been known and produced for a long period of time historically in China, Russia and Germany (Steinkraus and others 1996; Greenwalt and others 1998; 2000). The fermentation involves sweetened (5 to 15% sugar) tea infusion and a microbial mat containing *Acetobacter* and a variety of yeasts that grows on the surface of the substrate producing primarily acetic and gluconic acids in approximately equal quantities (above 3% total). pH is about 2.5. While various health-promoting qualities have been ascribed to kombucha, the fact appears to be that the acetic acid is the primary microbial inhibitor and, if it is consumed in too large quantities, it can cause indigestion and even death.

Leavened Bread Fermentation

Yeast breads are closely related to the alcoholic fermentation and have an excellent reputation for safety. Ethanol is a minor product in bread because of the short fermentation time; but carbon dioxide produced by the yeasts leavens the bread producing anaerobic conditions and baking produces a dry surface resistant to invasion by organisms in the environment. Baking also destroys many of the microorganisms in the bread itself.

Yeast breads are made by fermentation of wheat and rye flour doughs with yeasts, generally *Saccharomyces cerevisiae* (Sugihara 1985). Sour dough breads are fermented with lactic acid bacteria and yeasts; Indian idli/dosa-rice/legume mixtures are fermented with *Leuconostoc mesenteroides* and *Streptococcus faecalis* and sometimes added yeasts (see lactic acid fermentations above).

Alkaline Fermentations

Fermentations involving highly alkaline fermentations are generally safe. Africa has a number of very important foods/condiments that are not only used to flavor soups and stews but also serve as low-cost sources of protein in the diet (Odunfa 1988). Among these are Nigerian dawadawa, Ivory cost soumbara and West African iru made by fermentation of soaked, cooked locust bean *Parkia biglobosa* seeds with bacteria belonging to genus *Bacillus*, typically *Bacillus subtilis*. Nigerian ogiri made by fermentation of melon seed (*Citrullus vulgaris*); Nigerian ugba made by fermentation of the oil bean (*Pentacletha macrophylla*); Sierra Leone ogiri-saro made by fermentation of sesame seed (*Sesamum indicum*); Nigerian ogiri-igbo made by fermentation of castor bean (*Ricinus communis*) seeds and Nigerian ogir-nwan made by fermentation of the fluted pumpkin bean (*Telfaria occidentale*) seeds. Soybeans can be substituted for locust beans. (Steinkraus 1991).

This group also includes Japanese natto, Thai thua-nao and Indian kenima all based upon soybean.

The essential microorganisms are *Bacillus subtilis* and related bacilli. The organisms are very proteolytic and the proteins are hydrolyzed to peptides and amino acids. Ammonia is released and the pH rapidly reaches as high as 8.0 or higher. The combination of high pH and free ammonia along with very rapid growth of the essential microorganisms at relatively high temperatures-above 40 °C (recall principle 1) make it very difficult for other microorganisms that might spoil the product to grow. Thus, the products are quite stable and well-preserved especially when dried. They are safe foods even though they may be manufactured in an unhygienic environment.

These are all household fermentations that depend upon *Bacillus subtilis* spores in the environment. No deliberate inoculum is needed. The seeds are soaked and the seed-coats are removed. The seeds are then cooked and drained. Shallow pots or pans are used for the fermentation. The pans are sources of the required spores, which germinate and overgrow the seeds forming a sticky mucilaginous gum on the surfaces of the beans/seeds.

They are very nutritious and a source of low-cost nitrogen for the diet.

Interestingly, the Malaysians ferment locust beans by a similar alkaline process to yield garlic flavored products. The Japanese ferment soybeans with *Bacillus subtilis* after soaking and cooking to yield a protein-rich food called natto. The Thais also ferment soybeans by a similar process to produce a product called thua-nao consumed in Northern Thailand as a substitute for fish sauce. The Indians also ferment soybeans by similar processes to produce kenima. Thus, these alkaline fermentations involving bacilli fermenting protein rich beans and seeds are of considerable importance in widely separated parts of the world. They are all household fermentations but Japanese natto has been commercialized (Steinkraus 1991).

High Salt Savory Flavored Amino/Peptide Sauces and Pastes

Addition of salt in ranges from 13% w/v or higher to protein-rich substrates results in a controlled protein hydrolysis that prevents putrefaction, prevents development of food poisonings such as botulism and yields meaty, savory, amino acid/peptide sauces and pastes that provide very important condiments particularly for those unable to afford much meat in their diets.

Meaty/savory flavored amino acid/peptide sauces and pastes include Chinese soy sauce/Japanese shoyu/Japanese miso/Indonesian kecap/ Taiwan inyu/Korean kan-jang/Philippine taosi made by fermentation of soybeans or soybean/rice-barley mixtures with *Aspergillus oryzae* and fish sauces and pastes made by fermentation of small fish and shrimp using principally the proteolytic gut enzymes of the fish and shrimp (Fukashima 1989; Ebine 1989; Steinkraus 1983a,b; 1989).

The ancient discovery of how to transform bland vegetable protein into meat-flavored amino acid/peptide sauces and pastes was an outstanding human accomplishment. Like many discoveries, it was probably initially accidental. The earliest substrates were likely meat or fish mixed with a millet koji (Fukashima 1989).

In the most primitive process known today, soybeans are soaked and thoroughly cooked (Shurtleff and Aoyagi 1976). They are mashed, formed into a ball, covered with straw and hung to ferment under the rafters of the house. In 30 days they are completely overgrown with the mold *Aspergillus oryzae*. Packed into a strong salt brine (about 18% w/v) the beans are hydrolyzed by the proteases, lipases and amylases in the mold yielding an amino acid/peptide liquid sauce with a meaty flavor and a similar meat-flavored paste/residue-a primitive miso. One can only guess what a dramatic effect this meat-flavored sauce and paste had on consumers of the typical bland rice diet in the Orient. The process not only enriches the flavor but it also contains the essential amino acids in the soybean. Because of the relatively high lysine content of the soybean, soy sauce/miso is an excellent adjunct to the nutritional quality of rice. Soy sauce started as an Oriental food; but, in recent years it has been adopted by the West, particularly the Americans.

In the presence of high salt concentration (above 13% w/v and generally in the range of 20 to 23%) fish gut enzymes will hydrolyze fish proteins to yield amino acid/peptide, meaty flavored fish sauces and pastes similar to soy sauces/pastes. Halophilic pediococci contribute to the typical desirable fish sauce flavor/aroma. Both soy sauce and fish sauce fermentations are carried out at the household level as well as by large commercial manufacturers.

In summary, food fermentations that improve food safety are as follows:

1. Food substrates rapidly overgrown by edible microorganisms leading to desirable flavors, aromas and free of toxins are resistant to development of spoilage, food poisoning or toxin-producing organisms.
2. Food fermentations involving lactic acid production
3. Food fermentations involving ethanol production
4. Food fermentations involving acetic acid production
5. Food fermentations involving highly alkaline conditions with liberation of free ammonia
6. Food fermentations carried out in the presence of high salt concentrations (above 13% w/w) are generally safe.

Nutritional Aspects of Fermented Foods

"Nutrition" or "nutritional" is the supplying of calories/energy. Protein, essential amino acids/peptides, essential fatty acids and vitamins and mineral requirements to satisfy metabolic needs of the consumer (Steinkraus 1994, 1995, 1997).

Two major food problems exist in the world. Starvation (or under-nutrition where there is insufficient food or insufficient economic means to provide the necessary food) and obesity (or over-consumption of food in the wealthy, developed world). There is outright starvation and death in countries such as Ethiopia, Sudan, Somalia and Bangladesh due to poverty, drought, environmental disasters and war combined with lack of economic means to purchase food.

There are a number of nutritional diseases in the developing world today. Kwashiorkor, the result of protein deficiencies, and marasmus, caused by a combination of protein and calorie deficiencies, are found in large numbers of children between the ages of 1 and 3 in the developing world. The immune systems of the children are impaired on restricted protein diets. Such children often develop diarrhea and the mothers place them on restricted diets such as rice broth. Since the children are already deficient in protein, they quickly develop kwashiorkor with tissue edema, bloated abdomens, susceptibility to infection and changes in hair pigments. Kwashiorkor can develop in children consuming sufficient calories if their protein is deficient. On the other hand marasmus is the result of both calorie and protein deficiencies in children under 1 years of age due to early cessation of breast-feeding and the attempted replacement with artificial milks with very dilute protein contents (Jelliffe 1968). The principal features of marasmus are growth retardation, muscle wasting and loss of subcutaneous fat. Both kwashiokor and marasmus can result in mental retardation if the child survives.

Other nutritional diseases common in the developing world are xerophthalmia, childhood blindness due to vitamin A deficiency; beri-beri due to thiamine deficiency including infantile beri-beri where sudden death may occur from heart failure in children being nursed by mothers deficient in thiamine; pellagra due to niacin deficiency, riboflavin deficiency; rickets cause by vitamin D deficiency; anemia due to vitamin B-12 deficiency or insufficient iron in the diet (Jelliffe 1968).

The nutritional impact of fermented foods on nutritional diseases can be direct or indirect. Food fermentations that raise the protein content or improve the balance of essential amino acids or their availability will have a direct curative effect. Similarly fermentations that increase the content or availability of vitamins such as thiamine, riboflavin, niacin or folic acid can have profound direct effects on the health of the consumers of such foods. This is particularly true of people subsisting largely on maize where niacin or nicotinic acid is limited and pellagra is incipient and in people subsisting principally on polished rice which contains limited amounts of thiamine and beri-beri is incipient (Jelliffe 1968). Biological enrichment of foods via fermentation can prevent this.

On the other hand, fermentation does not generally increase calories unless they convert substrates unsatisfactory for humans to human quality foods such as mushrooms produced on straw or waste paper or converting peanut and coconut presscakes to edible foods such as Indonesian ontjom (oncom).

While the Western world can afford to enrich its foods with synthetic vitamins, the developing world must rely upon biological enrichment for its vitamins and essential amino acids. The affluent Western world cans and freezes much of its food but the developing world must rely upon fermentation, salting and solar dehydration to preserve and process its foods at costs within the means of the average consumer. All consumers today have a considerable portion of their nutritional needs met through fermented foods and beverages. This is likely to expand in the 21st century when world population reaches 8 to 12 billion (Steinkraus 1994).

Biological Enrichment by Fermentation (Steinkraus,1998)

Enrichment with protein

In the Indonesian tape ketan fermentation referred to earlier, rice starch is hydrolyzed to maltose and glucose and fermented to ethyl alcohol. The loss of starch solids results in a doubling of the protein content (from about 8% to 16% in rice) on a dry solids basis (Cronk and others 1977). Thus, this process provides a means by which the protein content of high starch substrates can be increased for the benefit of consumers needing higher protein intakes. This is particularly important for people consuming principally cassava which has a protein content of about 1% (wet basis). If the tape ketella fermentation is applied to cassava as it is in Indonesia, the protein content can be increased to at least 3% (wet basis) - a very significant improvement in nutrition to the consumer. In addition, the flavor of the cassava becomes sweet/sour and alcoholic, flavors consumers may prefer to the bland starting substrate.

Bio-enrichment with essential amino acids

The Indonesian tape ketan/tape ketella fermentation not only enriches the substrate with protein, the microorganisms also selectively enrich the rice substrate with lysine, the first essential limiting amino acid in rice (Cronk and others 1977). This improves the protein quality.

Several researchers have reported from 10.6 to 60.0% increases in methionine during the Indian idli fermentation. (Rao 1961; Rajalakshmi and Vanaja 1967; Steinkraus and others 1967). An increase in methionine, a limiting essential amino acid in legumes greatly improves the protein value.

Bio-enrichment with vitamins

In the wealthy, Western world, nutrients particularly vitamins are added to selected, formulated, manufactured foods as a public health measure. Examples are addition of vitamin D to milk, vitamin A and D to milk and butter and riboflavin to bread. Fruit juices may be fortified with ascorbic acid (vitamin C). While enrichment/fortification are within the means of the Western World, they are far beyond the means of the developing world. Thus, much of the world must depend upon biological enrichment via fermentation to enrich their foods (Steinkraus 1985).

Highly polished white rice is deficient in vitamin B-1 (thiamine). Consumers subsisting principally on polished rice are in danger of developing beri-beri, a disease characterized by muscular weakness and polyneuritis leading eventually to paralysis and heart failure (Robinson 1972). Infants being nursed by mothers suffering from thiamine deficiency may develop infantile beri-beri in which sudden death occurs at about 3 months of age due to cardiac failure (Jelliffe 1968). The microorganisms involved in the tape ketan fermentation synthesize thiamine and restore the thiamine content to the level found in unpolished rice (Cronk and others 1977) This can be a very significant contribution to the nutrition of rice eating people.

Tempe is a protein-rich meat substitute in Indonesia made by overgrowing soaked, dehulled, partially cooked soybeans with *Rhizopus oligosporus* or related molds (Steinkraus 1983a). The mold knits the cotyledons into a firm cake that can be sliced and cooked or used in place of meat in the diet. During the fermentation, proteins are partially hydrolyzed, the lipids are hydrolyzed to their constituent fatty acids, stachyose (a tetrasaccharide indigestible in humans) is decreased decreasing flatulence in the consumer, riboflavin nearly doubles, niacin increases sevenfold and vitamin B-12 usually lacking in vegetarian foods is synthesized by a bacterium that grows along with the essential mold (Liem and others 1977; Steinkraus 1983a, 1985). The tempe process can be used to introduce texture into many legume/cereal mixtures yielding products with decreased cooking times and improved digestibility. The bacterium responsible for the vitamin B-12 production is a nonpathogenic strain of *Klebsiella pneumoniae*. When inoculated into the Indian idli fermentation, it also produces vitamin B-12.

Mexican pulque is the oldest alcoholic beverage on the American continent. It is produced by fermentation of juices of the cactus plant (*Agave*) (Steinkraus 1983a, 1979b). Pulque is rich in thiamine, riboflavin, niacin, panthothenic acid, p-amino benzoic acid, pyridoxine and biotin (Sanchez-Marroquin 1977). Pulque is of particular importance in the diets of the low-income children of Mexico.

Kaffir beer is an alcoholic beverage with a pleasantly sour taste and the consistency of a thin gruel (Steinkraus 1983a, 1989). It is a traditional beverage of the Bantu people of South Africa. Alcohol content ranges from 1 to 8% v/v. Kaffir beer is generally made from kaffircorn (*Sorghum caffrorum*) malt and unmalted kaffircorn meal. Maize or millet may be substituted for kaffircorn. During the fermentation, thiamine remains about the same, but riboflavin more than doubles and niacin/nicotinic acid nearly doubles, which is very significant in people consuming principally maize. Consumers of usual amounts of kaffir beer are not in danger of developing pellagra.

Palm sap is a sweet, clear, colorless liquid containing about 10 to 12% fermentable sugar and neutral in reaction (Okafor 1975); Steinkraus 1979b). Palm wine is a heavy, milk-white opalescent suspension of live yeasts and bacteria with a sweet taste and vigorous effervescence. Palm wines are consumed throughout the tropics. Palm wine contains as much as 83 mg ascorbic acid/liter (Bassir 1968). Thiamine increased from 25 ug to 150 ug/liter, riboflavin increased from 35 to 50 ug/liter and pyridoxine increased from 4 to 18 ug/liter during fermentation. Surprisingly, palm wine contains considerable amounts of vitamin B-12, 190 to 280 pg/ml (Van Pee and Swings, 1971). Palm toddies play an important role in nutrition among the economically disadvantaged in the tropics. They are the cheapest sources of B vitamins.

Reduction of toxins

During soaking and hydration that raw substrates undergo in various fermentation processes and the usual cooking, many potential toxins such as trypsin inhibitor, phytate and hemagglutinin and cyanogens in cassava are reduced or destroyed. Even aflatoxin, frequently found in peanut and cereal grains substrates is reduced in the Indonesian ontjom fermentation (Steinkraus, 1983a). Van Veen and others (1968) found that the ontjom mold, Neurospora and the tempe mold *Rhizopus oligosporus* could decrease the aflatoxin content of peanut presscake 50% and 70% respectively during fermentation.

Reduction of cooking times

Economy of fuel requirements is very important in the developing world where housewives may spend hours every day collecting enough leaves, twigs, wood and dried dung to cook the day's food. Lactic-acid fermented foods generally require little, if any, heat in their fermentation and can be consumed without cooking. Examples are pickled vegetables, sauerkraut, kimchi. Indonesian tempe fermentation converts soybeans that would require as much as 5 to 6 h cooking to a product that can be cooked in soup with 5 to 10 min boiling (Steinkraus 1983a).

Summary

Fermented foods provide and preserve vast quantities of nutritious foods in a wide diversity of flavors, aromas, and textures which enrich the human diet. Fermented foods have been with us since humans arrived on earth. They will be with us far into the future, as they are the source of alcoholic foods, beverages, vinegar, pickled vegetables, sausages, cheeses, yogurts, vegetable protein amino acid/peptide sauces and pastes with meat-like flavors, and leavened and sourdough breads. All consumers today have a considerable portion of their nutritional needs met through fermented foods and beverages. This is likely to expand in the 21st century, when world population reaches 8 to 12 billion.

References

Aalbersberg WGL, Lovelace CEA, Madheji K, Parkinson SV. 1988. Davuke, the traditional Fijian method of pit preservation of staple carbohydrate foods. Ecol Food and Nutr. 21:173-180.

Abd-el-Malek T, Demerdash M. 1977. Microbiology of kishk. Symposium on Indigenous Fermented Foods (SIFF), Global Impacts of Applied Microbiology (GIAMV) Nov 21-27, 1977. Bangkok Thailand

Akinrele IA, Adeyinka O, Edwards CCA, Olatunji FO, Dina JA, Koleoso OA. 1970. The development and production of soy ogi. FIIR Research report nr 42. Fed Ministry of Industries. Lagos, Nigeria.

Arora DK, Mukerji KG, Marth EH.1991. Handbook of Applied Mycology. Vol.3. Foods and Feeds. New York, NY: Marcel Dekker

Arroyo PT, Ludovico-Pelago LA, Solidum HT, Chiu YN, Lero M, Alcantara EE. 1977. Studies on rice-shrimp fermentation: balao balao. Phil J Food Sci and Tech 2:106-125.

Augustin MA,Ghazali HN 1984. Proc. Regional Seminar-Workshop on Biotechnology in Industrial Development. Mar 27-30, 1984. Kuala Lumpur, Malaysia. University Pertanian Malaysia and UNESCO. 308 p.

Banigo EOI. 1969. An investigation into the fermentation and enrichment of ogi. Ph.D. thesis. University of Leeds. Leeds, England.

Banigo EOI, Muller HG. 1972. Manufacture of ogi (a Nigerian fermented cereal porridge): Comparative evaluation of corn, sorghum and millet. Can Inst Food Sci Tech J. 5:217-221.

Braidwood EJ. 1953. Symposium: Did man once live by beer alone? Am Anthro 55:515-525.

Campbell-Platt G. 1987. Fermented Foods of the World: a Dictionary and guide. London, UK: Butterworths. 290 p.

Central Food Technological Research Institute. 1986. Traditional Foods. Central Food Technological Research Institute. Mysore, India.

Conner HA, Allgier FJ. 1976. Vinegar: Its history and development. Adv Applied Micro. 20:81-133.

Cronk TC, Steinkraus KH, Hackler LR, Mattick LR. 1977. Indonexian tape ketan fermentation. Appl Environ Micr 33:1967-1073.

DaSilva EJ, Dommergues YR, Nyns EJ, Ratledge C.1987. Microbial Technology in the Developing World. Oxford Science Publications.

Dirar HA. 1994. The Indigenous Fermented Foods of the Sudan. Wallingford, Oxon, UK. CAB Int. 552 p.

Ebine H. 1989. Industrialization of Japanese Miso Fermentation. In: Steinkraus KH,

editor. Industrialization of Indigenous Fermented Foods. New York, NY: Marcel Dekker. P 89-126.
Fukashima D. 1989. Industrialization of Fermented Soy Sauce Production Centering around Japanese Shoyu. In: Steinkraus, H. editor. Industrialization of Indigenous Fermented Foods. New York, NY: Marcel Dekker. P 1-88.
Garcia RA, Hotchkiss JH, Steinkraus KH. 1999. The effect of lipids on bongkrekic (bongkrek) acid toxin production by *burkholderia cocovenenans* in coconut media. Food additives and contaminants 16:63-69.
Gatumbi RW, Muriru N. 1983. Kenyan uji. In: Steinkraus KH, editor. Handbook of Indigenous Fermented Foods. New York, NY: Marcel Dekker. P 198-203.
Greenwalt CJ, Ledford RA, Steinkraus KH. 1998. Determination and characterization of the antimicrobial activity of the fermented tea kombucha. Lebensm.-Wiss u. Technol. 31:291-296.1998.
Greenwalt CJ, Ledford RA, Steinkraus KH. 2000. Kombucha, the fermented tea: microbiology, composition, and claimed health effects. J Food Protect 63:976-981.
Hattori T, Ishida Y, Maruy Y, Morita RV, Uchida A. 1989. Recent Advances in Microbial Ecology. Sendai, Japan: Japan Scientific Societies Press. 704 p.
Hermana M, Mahmud KMS, Karyadi D. 1990. 2nd Asian Symposium on Non-salted Soybean Fermentation. Feb 13-15, 1990. Nutrition Research and Development Centre. Bogor, Indonesia. 116 p.
Hesseltine CW, Wang HL 1986. Indigenous Fermented Foods of Non-Western Origin. Berlin, Germany: Cramer. 351 p.
Holzapfel WH. 1989. Industrialization of Mageu Fermentation. In: Steinkraus KH, editor. Industrialization of Indigenous Fermented Foods. New York, NY: Marcel Dekker. P 289-328.
International Foundation for Science (IFS) 1985. Development of Indigenous Fermented Foods and Food Technology in Africa. Proc. IFS/UNU Workshop. October 1985. Douala, Cameroun. International Foundation for Science (IFS). Stockholm, Sweden. P 34-70.
Jelliffe DB. 1968. Infant Nutrition in the Tropics. World Health Organization (WHO). Geneva, Switzerland. 335 p.
Katz SH, Voight MM. 1987. Bread and beer. Expedition. 28:23-24.
Kosikowski, FV. 1977. Cheese and Fermented Milk Foods. 2nd Edition. Brooktondale, NY: F.V. Kosikowski and Assoc.
Kuboye AO. 1985. Traditional Femented Foods and Beverages of Nigeria. In: Development of Indigenous Fermented Foods and Food Technology in Africa. Proc. IFS/UNUWorkshop. Douala, Cameroun. October 1985. Stockholm, Sweden: International Foundation for Science (IFS). P 224-237.
Lappe P, Ulloa M. 1989. Estudios Etnicos, Microbianos y Quimicos del Tesgunio Tarahumara. Universidad Nacional Autonoma de Mexico.
Liem IT, Steinkraus KH, Cronk TC. 1977. Production of vitamin B-12 in tempe, a fermented soybean food. Appl and Environ Micro. 34:773-776.
Lorri WSM. 1993. Nutritional and microbiological evaluation of fermented cereal weaning foods. PhD thesis. Dept. of Food Science. Chalmers University of Technology. Goteborg, Sweden.
Mbugua SK. 1981. Microbiological and biochemical aspects of uji (an East-African Sour Cereal Porridge) fermentation and its enhancement through applicatuon of lactic acid bacteria. PhD thesis. Cornell University. Ithaca, New York.
Merican Z, Yeoh QL, Idrus AZ. 1987. Malaysian Fermented Foods. Asean Protein Project Occasional Paper No.10. MARDI, Serdang, Malaysia.
Merican Z, Yeoh QL. 1989. Tapai Processing in Malaysia. In: Steinkraus KH, editor. Industrialization of Indigenous Fermented Foods. New York, NY: Marcel Dekker. P 169-190.
Mheen TI, Lee KH, Chang CK, Lee SR, Park KI, Kwon TW. 1983. Korean Kimchi. In: Steinkraus KH, editor. Handbook of Indigenous Fermented Foods. New York, NY: Marcel Dekker. P 114-118.
Mukerjee S. 1987. Introduction of the sauerkraut fermentation at the farm and village level in India. In: Proc. Symposium on Low-cost Preservation of Vegetables. Mar 28, 1987. Calcutta, India: Jadavpur University.
Odunfa SA. 1988. Review: African fermented foods: from art to science. Mircen J 4:255-273.
Okafor N. 1975. Microbiology of Nigerian palm wine with particular reference to bacteria. J Appl Bact 38:81-88.
Orillo CA, Pederson CS. 1968. Lactic acid bacterial fermentation of burong dalag. Appl Micro 16:1669-1681.
Paredes-Lopez O, Harry GI. 1988. Food Biotechnology Review: Traditional Solid-State Fermentations of Plant Raw Materials - Application, Nutritional Significance, and Future Prospects. CRC Crit Rev in Food Sci and Nutr. 27:159-187.
Pederson CS. 1979. Microbiology of Food Fermentations. 2nd Edition. Westport, CT: Avi Publishing. 283 p.
Pederson CS, Albury MN. 1969. The sauerkraut fermentation. N.Y.S, Ag. Exp. Station Bull 824. Geneva, NY.
Platt BS. 1964. Biological ennoblement: Improvement of the nutritive value of foods and dietary regimens by biological agencies. Food Tech. 18:662-670.
Rajalakshmi R, Vanaja K. 1967. Chemical and Biological Evolution of the Effects of Fermentation on the Nutritive Value of Foods Prepared from Rice and Gram. Brit J Nut 21:46-473.
Ramakrishnan CV. 1979a. Terminal report of American PL 480-project Nr GF-IN-491. Study of Indian Fermented foods from legumes and production of similar fermented foods from U.S. soybean. Biochemistry Department, Baroda University, Baroda, India.
Ramakrishnan CV. 1979b. Studies on Indian fermented foods. Baroda J Nut 6:1-57.
Rao MVR. 1961. Some observations on fermented foods. In: Meeting the Needs of Infants and Children. National Academy of Sciences, National Research Council. Publication Nr 843. P 291-293.
Robinson CH. 1972. Normal and Therapeutic Nutrition. New York, NY: Macmillan. 753 p.
Sanchez-Marroquin A. 1983. Mexican Pulque. In: Steinkraus KH, editor. Handbook of Indigenous Fermented Foods. New York, N.Y. Marcel Dekker. P 328-336.
Schopf JW, Packer BM. 1987. Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warraweena group. Australia. Science. 237:70-73.
Shurtleff W, Aoyagi A. 1976. The Book of Miso. Kanagawa, Japan: Autumn Press. 255 p.
Steinkraus, KH, Van Veen AG, Thiebeau, DB. 1967. Studies on Idli-an Indian Fermented Black Gram-rice food. Food Tech. 21:110-113.
Steinkraus KH. 1978. Contributions of Asian Fermented Foods to International Food Science and technology. In: Stanton WR, DaSilva EJ, editors. GIAM 5. State of the Art: GIAM and its Relevance to developing countries. UNEP/UNESCO/ICRO Panel on Microbiology Secretariat, Kuala Lumpur, Malaysia. P 173-179.
Steinkraus KH. 1979b. Nutritionally significant indigenous fermented foods involving and alcoholic fermentation. In: Gastineau CF, Darby WJ, Turner TB, editors. Fermented Food Beverages in Nutrition. New York, NY: Academic Press. P 36-50.
Steinkraus KH. 1980. Introduction: Food from Microbes. Bioscience. 30:384-386.
Steinkraus KH. 1983a. Handbook of Indigenous Fermented Foods. New York, NY: Marcel Dekker. 671 pages
Steinkraus KH. 1983b. Fermented foods, feeds and beverages. Biotech Adv. 1:31-46.
Steinkraus KH. 1983c. Lactic acid fermentation in the production of foods from vegetables, cereals and legumes. Antonie van Leeuwenhoek. 49:337-348.
Steinkraus KH. 1983d. Progress in the preservation of food through fermentation. In: Shemilt LW, editor. Chemistry and World Food Supplies: the New Frontiers CHEMRAWN II.
Steinkraus KH. 1985a. Bio-enrichment: Production of vitamins in fermented foods. In: Wood BJB, editor. Microbiology of Fermented Foods. London, UK: Elsevier Applied Science Publisher. P 323-343.
Steinkraus KH. 1985b. Production of Vitamin B-12 in Tempe. In: Proc. Asian Sympoium on Non-Salted Soybean Fermentation. July 15-17, 1985. Tsukuba Science City, Ibaraki, Japan. P 205-208.
Steinkraus, KH. 1985c. Potential of African Indigenous Fermented Foods. In: Development of Indigenous Fermented Foods and Food Technology in Africa. Stockholm, Sweden: International Foundation for Science (IFS). P 34-70.
Steinkraus KH. 1986. Fermented foods, feeds, and beverages. Biotech Adv. 4:219-243.
Steinkraus KH. 1988. Microbial interaction in fermented foods. In: Hattori T and others, editors. Recent Advances in Microbial Ecology. Tokyo, Japan: Japan Scientific Society Press.
Steinkraus, KH. 1988. Contributions of Indigenous Fermented Foods in the World's Dietary. In: Moyal MF, editor. Diet and Life Style. London, UK: John Libbey.
Steinkraus KH. 1989. Industrialization of Indigenous Fermented Foods. New York, NY: Marcel Dekker. 439 p.
Steinkraus KH. 1989. Microbial Interaction in Fermented Foods. In: Hattori T and others, editors. Recent Advances in Microbial Ecology. Tokyo. Japan: Japan Scientific Society Press. P 547-552.
Steinkraus KH. 1991. African alkaline fermented foods and their relation to similar foods in other parts of the world. In: Westby A, Reilly PJA, editors. Traditional African Foods—Quality and Nutrition. Stockholm, Sweden: International Foundation for Science. P 87-92.
Steinkraus KH. 1992. Lactic Acid Fermentations. In: Applications of Biotechnology to Traditional Fermented Foods. National Research Council. Washington, DC: National Academy Press. P 43-51.
Steinkaus KH 1993. Comparison of Femented Foods of East and West. In: Lee CH, Steinkraus, KH, Reilly PJA, editors. Fish Fermentation Technology. Tokyo, Japan: United Nations University Press. P 1-10.
Steinkraus KH. 1994. Nutritional significance of fermented foods. Food Res Int. 27:259-267.
Steinkraus KH. 1995. Classification of Household Fermentation Techniques. Background Paper for WHO/FAO Workshop on Assessment of Fermentation as Household Technology for Improving Food Safety. Dec. 11-15, 1995. Dept. of Health. Pretoria, South Africa.
Steinkraus KH. 1996. Handbook of Indigenous Fermented Foods. 2nd Edition Revised and Enlarged. New York, NY: Marcel Dekker. 776 p.
Steinkraus KH. 1996a. Introduction to Indigenous fermented Foods. In: Steinkraus KH, editor. Handbook of Indigenous Fermented Foods. 2nd edition. New York, NY: Marcel Dekker. pages 1-5.
Steinkraus KH. 1997. Classification of fermented foods: worldwide review of household fermentation techniques. Food Control. 8:311-317.
Steinkraus KH, Shapiro KB, Hotchkiss JH, Mortlock RP. 1996. Investigations into the antibiotic activity of tea fungus/kombucha beverage. Acta Biotch 16:199-205.
Steinkraus KH. 1998. Bio-enrichment: production of vitamins in fermented foods. In: Wood BJB, editor. Microbiology of Fermented Foods. Vol. 2. 2nd Edition. London: Blackie Academic. P 603-621.
Suigbia I, Sumantri A. 1990. The Use of Tempe in Medical Practice. In: Hermana Mien KMS, Karyadi D, editors. Proceedings Second Asian Symposium on Non-Salted Soybean Fermentation. Jakarta, Indonesia. Feb. 13-15, 1990. Bogor, Indonesia: Nutrition Research and Development Center. P 105-109.
Sugihara TF. 1985. Microbiology of Breadmaking. In: Wood BJB edtor. Microbiology of Fermented Foods. Vol. 1. London, UK: Elsevier Applied Science Publishers. P 249-262.
Svanberg U, Sjogren E, Lorri W, Svennerholm A-M, Kaijser B. 1992. Inhibited growth of common enteropathogenic bacteria in lactic-fermented cereal gruels. World J of Micro and Biotech. 8:601-606.
Tanaka N, Kovats SK, Guggisberg JA, Meske LM, Doyle MP. 1985. Evaluation of the microbiological safety of tempeh made from unacidified soybeans. J Food Prot 48:438-441.
Ulloa M, Herrera T, Lappe P. 1987. Fermentaciones Tradicionales Indigenas de Mexico. Mexico, D.F. Instituto Nacional Indigenista. P 1-77.
Van Pee W, Swings JJ. 1971. Chemical and microbiologial studies of Congolese palm wines (Elaeis guineensis). East Afr Agr For J 36: 311-314.
Van Veen AG. 1967. The bongkrek toxins. In: Mateles RI, Wogan GN, editors. Bio-

chemistry of some food-borne microbial toxins. Cambridge, MA: MIT press.
Van Veen AG, Graham DCW, Steinkraus KH. 1968. Fermented Peanut Presscake. Cerea Sci Today. 13:96-99
Van Veen AG, Steinkraus KH. 1970. Nutritive value and wholesomeness of fermented foods. Agr and Food Chem. 18:576-578.
Wang HL, Ruttle DI, Hesseltine CW. 1969. Antibiotic compound from a soybean product fermented by Rhizopus oligosporus. Proc Soc Exp Biol Med 131:579-583.
Westby A, Reilly PJA. 1991. Traditional African Foods Quality and Nutrition. Stockholm, Sweden. International Foundation for Science (IFS) 197 p.
Wongkhalaung C, Boonyaratanakornkit M. 1986. Fermented Foods in Thailand and Similar Products in ASEAN and Elsewhere. Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand.
Wood, BJB. 1985. Microbiology of Fermented Foods. London, UK: Elsevier Applied Science Publishers, Vol. 1, P 1-371; Vol. 2, P 1-292.
Wood, BJB. 1998. Microbiology of Fermented Foods 2nd Edition. London, UK: Blackie Academic. Vols. 1 and 2.
Yanagida F, Takai Y, Homma S, Abdo Y. 1987. Traditional Foods and Their Processing in Asia. Tokyo, Japan: NODAI Research Institute. Tokyo University of Agriculture.
Yokotsuka T. 1982. Traditional Fermented Soybean Foods. In: Rose AH, editor. Fermented Foods. London, UK: Academic Press.
Yoshizawa K, Ishikawa T. 1989. Industrialization of Sake Manufacture. In: Steinkraus KH, editor. Industrialization of Indigenous Fermented Foods. New York, NY: Marcel Dekker. P 127-168.