APPLICATIONS OF BIOTECHNOLOGY TO TRADITIONAL FERMENTED FOODS

Report of an Ad Hoc Panel of the
Board on Science and Technology
for International Development

Office of International Affairs
National Research Council

NATIONAL ACADEMY PRESS
Washington, D.C. 1992

Contents

4

Lesser-Known Fermented Plant Foods

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In many parts of the world, fermented foods form an important part of the diet. These foods are made from plant and animal materials in which bacteria, yeasts, and molds play an important role by modifying the material physically, nutritionally, and organoleptically.

Fermented plant foods may be classified into groups as (a) those made from cereal grains (maize, sorghum, millet, rice, wheat), such as *pozol* (Mexico), *kenkey, ogi,* and *injera* (Africa); (b) those made from pulses, nuts, and other seeds, such as *ontjom* (Indonesia) and *dawadawa* (Savannah Africa); (c) those from tubers (cassava, aroids, potatoes), such as *gari* (Africa) and *farinha puba* (Brazil, Peru, and Ecuador); (d) those from fruits and vegetables, such as *gundruk* (Nepal) and *kimchi* (Korea, East Asia); and (e) beverages derived from tree saps, such as *nipa* wine (Far East) and *pulque* (Mexico).

Most traditional fermented plant foods are prepared by processes of solid-substrate fermentation in which the substrate is allowed to ferment either spontaneously (usually African or Latin American processes) or by adding a microbial inoculum (Far East, South Asia, and Southeast Asia).

Cereal grains account for more than 60 percent of food materials used in the preparation of indigenous fermented foods in Africa. Although maize is a comparatively well-researched crop, no significant research has been done on some of the important traditional crops, such as sorghum and millet (1). *Tef* (*Eragrostis tef*), a staple food grain of Ethiopian subsistence farmers, is still relatively less well known.

Many indigenous fermented foods, some of which long predate recognition of the existence of microorganisms, are eaten in various parts of the world. Increasing interest in this field is reflected in the range of publications (2-10). This paper presents information on some of the lesser-known fermented plant foods that are still produced and marketed on a small scale and that serve as a staple diet for millions of people in developing countries.

REGIONAL PERSPECTIVES

Cereals are major staples in many developing countries, and the fermentation of cereal grains to prepare a variety of foods has a long history. Fermented products from maize are usually found in Africa and Central and South America and those from sorghum (guinea corn) and millet in Africa and South Asia. Food fermentations based on rice are practiced in India, China, Southeast Asia, and the Far East, while those from wheat are particularly important in the Middle East, Turkey, and the Far East (11).

Fermented foods from tubers are usually found in Africa, among the Andean Indians and in the South Pacific, and the process of detoxification of the tuber before fermentation is still carried out by soaking in water.

Chica, an alcoholic beverage made from maize in Peru since pre-Hispanic times, also is produced from potato, *oca* (*Oxalis tuberosa*), *arracacha* (*Arracacia xanthorrhiza*), *maca* (*Lepidium evenii*), and other Incan crops that science has almost totally neglected. Although cassava and sweet potatoes provide nourishment for more than 500 million people, only a small proportion of this highly perishable staple crop is used in food fermentations in Africa and Latin America.

Legumes account for a substantial amount of food protein intake in developing countries. Of the total world production of over 58 million metric tons in 1990, developing countries produced 62 percent, together with 54 percent of world nut production (12). Fermented products from legumes are not as popular in Africa or Latin America as in the Far East and South and Southeast Asia, where soybean, for instance, is used extensively in the production of fermented products such as soy sauce, *miso*, and *tempe*, and black gram dhal for the production of *idli* and *dosa*. Fermented seed products, however, are often used as condiments in Savannah Africa.

In the tropics, highly perishable foods such as fruits and vegetables may be preserved as fermented products. Some fermented vegetables provide vitamins, particularly during long cold months in the northern parts of East Asia, and others are consumed as part of traditional family life in Southeast Asia. In Mexico refreshing beverages are prepared from a variety of fruits, including pineapples, apples, and oranges.

PRODUCTS FROM CEREAL GRAINS

Ahai

Ahai is a sweet, malty-tasting beverage brewed from maize in Southern Ghana and is usually served as a welcome drink and at outdoor ceremonies, wakes, and funerals. Whitby (13) has reported that the traditional method of preparing *ahai* is much the same as for *pito*, an acid-alcohol beer brewed from sorghum or millet in West Africa, except that *ahai* is not boiled again after fermentation. So far, no studies have been made on the microbiological, biochemical, and nutritional changes that take place during *ahai* production.

Ting

Ting is a staple food for a large proportion of the population of Botswana. It is prepared from maize by natural fermentation. In other regions it is prepared from sorghum or millet. Moss et al. (14) made an extensive study of *ting* fermentation and noted that the success of the fermentation depends on a number of factors, among which temperature is very important.

The microbiology of *ting* fermentation is well documented, but further studies need to be carried out, particularly on the nutritional value. *Ting* may be similar, nutritionally, to other acid-fermented cereal gruels like *kenkey* (West Africa), *kisra* (Sudan), and *pozol* (Mexico).

Maasa

Maasa is a snack food made from millet or sorghum and is very popular in Savannah Africa, particularly during Ramadan. The method of preparation of *maasa* has been reported (9), but there is no information on the microbiology and biochemistry of this fermented product.

There are hundreds of fermented products from cereal grains in the tropical regions of the world that require extensive studies on methods of preparation and biochemical, microbial, and nutritional changes. These include the West African *fura* or *fula, jamin-bang* of the Kaingang Indians of Brazil, and the Maori's *kaanja-kopuwai*, a process of fermenting maize in water prior to eating. The Maoris claim *kaanja-kopuwai* is health giving, and many of the older people attribute their age to this part of their diet.

PRODUCTS FROM ROOT TUBERS

Farinha puba

Farinha puba is a coarse flour made from cassava and is found in the Amazonian regions of Brazil, Peru, and Ecuador. Woolfe and Woolfe (15) presented an outline on the preparation of *Farinha puba*, which is also known as *farinha de mandioca* in Brazil. They noted that the technology was exported to West Africa in the nineteenth century and presumably adapted locally to give the *gari* process. *Gari*, a popular West African staple food that is also eaten in other tropical African countries, is prepared by fermenting cassava; details of improved methods of production are given by Steinkraus et al. (6).

The processes involved in the production of *farinha puba* and *gari* are similar, but unlike *gari* very little information has been published on the methods of production and on the microbiology, nutritional values, and toxicological problems of *farinha puba*. It has been reported that cassava fermentation as practiced in Africa, Asia, and Latin America (16) is an unreliable detoxification method, and the process further reduces the already low protein content. Other studies have shown that cassava fermentation for *gari* production does not totally eliminate the cyanide content but reduces it by at least 65 percent (17,18).

Fatalities from cassava poisoning appear to be rare, but long-term toxic effects, (e.g., goiter and cretinism) in cassava-consuming populations may be more serious, especially in the Amazon, where the pressed-out juices are used for making soups and stews (15). In Africa the pressed-out juice is often used for the production of cassava starch for laundry purposes. The use of pure microbial cultures under controlled fermentation conditions might bring about not only complete hydrolysis of the poisonous glycoside but also an enhanced fermentation process.

Kokonte

Kokonte, another important cassava-based staple, is eaten by millions of people in Savannah Africa. Like many other fermented foods, *kokonte* (Ghana) is known by various names such as *ilafun* (Nigeria) and *icingwadal* (East Africa). The method of preparation of *kokonte* has been reported, but further studies need to be done, particularly on microflora and production of mycotoxins during fermentation (19,20).

Masato (masata)

Masato, or cassava beer, is an alcoholic beverage produced from cassava in the Amazon. It has an alcohol content of 6 to 12 percent by volume and is offered to guests as a sign of hospitality. It is considered an offense to refuse a drink (15). In Brazil it is called *kaschiri* and in Mozambique *masata*. Preparation of *masato* is similar to that of *chica* by the Andean Indians. As a first step of fermentation, cassava is chewed and spat out by women. In Mozambique women chew the yucca plant to produce a similar product.

So far, no scientific account of the *masato* fermentation process has been published. Studies on improving the traditional methods of production are necessary to save this ancient art of the Andean Indians from extinction.

Chuno

Chuno is a food product from potato prepared by the inhabitants of the high Andes of Peru, Chile, Ecuador, Colombia, and Bolivia. An outline of the method of production has been reported, but the microorganisms involved in the fermentation are still not known (9).

The Incan anu (*Tropaecolum tuberssum*) is a tuber that must be fermented before being eaten baked, fried, or added to stew (21). The crop is cultivated in Colombia, Peru, and Bolivia and is also grown as a flowering ornament in Britain and the United States. The fermentation involved during "curing" has not been reported.

PRODUCTS FROM LEGUMES, PULSES, AND OTHER SEEDS

In Savannah Africa, fermented products from legumes and other seeds are important food condiments and are generally strong smelling. Quite often seeds that are used for fermentation are inedible in their raw unfermented state. Fermentation of the West and Central African *iru* or *dawadawa* is similar to the Japanese *natto*, and there is adequate literature on the preparation, biochemistry, microbiology, and industrialization of *iru*. Other indigenous products that are receiving some attention include *ugba* (African oil bean seed), *ogiri* (seeds of watermelon), *ogiri-igbo* (castor oil seed), and *ogiri-nwan* (fluted pumpkin beans).

Lupins (*Lupinus mutabilis*), which are native to the Andes, contain bitter alkaloids and can cause toxicity problems. Lupin seeds are debittered by soaking them in running water, a process similar to the Maoris' process for corn fermentation and the Ichunol methods of Peru and Bolivia. So far, no report has been published on the debittering of lupins by fermentation, but the soaking may involve some fermentation.

Kenima is a Nepalese fermented product from legumes. There is no published information on the method of preparation, microbiology, and nutritional value.

PRODUCTS FROM FRUITS AND VEGETABLES

Colonche is a sweet fizzy beverage produced in Mexico by fermenting the juice of tunas (fruits of the prickly pear cacti, mainly *Opuntia* species). *Tepache* is also a refreshing beverage prepared originally from maize but from various fruits and is consumed throughout Mexico.

Although some studies have been made on these products (22), it appears that more work is needed, particularly on the biochemical and nutritional changes that take place during the preparations.

The Nepalese pickle or *gundruk* is a fermented dried vegetable served as a side dish with the main meal and is also used as an appetizer in the bland starchy diet. Several hundred tons of *gundruk* is produced annually, and production is still at the household level. Dietz (23) reported on the method of preparation and the role of *gundruk* in the diet of Nepalese people. It has been found that a disadvantage of the traditional process is loss of 90 percent of the carotenoids. Improved methods and further studies might help reduce vitamin loss.

COMMERCIALIZATION

To industrialize some of these fermented plant foods from traditional processes, extensive studies must be made to determine the essential microorganisms, optimum fermentation conditions, biochemical changes, nutritional profile, and possible toxicological problems associated with certain plant materials or the fermented product itself.

Commercial or large-scale processes for indigenous fermented foods need to be adapted to specific local circumstances. Advantages of industrialization include a product with an extended shelf life, maximum utilization of raw materials, production of important by-products, and bioenrichment or fortification of a product for specific consumers such as special diets, weaning foods and exclusion of or reduction in the

levels of mycotoxins. Mycotoxins appear to be a major problem in some fermented products, particularly those of cereal and root tuber origin.

Studies in Japan on *okara*, a by-product of the tofu industry, have shown that fermenting it with *tempe* fungus could result in a product that is useful as a high-fiber, low-energy food material (24).

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5

Lactic Acid Fermentations

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Lactic acid bacteria perform an essential role in the preservation and production of wholesome foods. The lactic acid fermentations are generally inexpensive, and often little or no heat is required in their preparation, making them fuel efficient as well. Foods fermented with lactic acid play an important role in feeding the world's population on every continent.

Lactic acid bacteria perform this essential function in preserving and producing a wide range of foods: fermented fresh vegetables such as cabbage (sauerkraut, Korean *kimchi*); cucumbers (pickles); fermented cereal yogurt (Nigerian *ogi*, Kenyan *uji*); sourdough bread and bread-like products made without wheat or rye flours (Indian *idli*, Philippine *puto*); fermented milks (yogurts and cheeses); fermented milk-wheat mixtures (Egyptian *kishk*, Greek *trahanas*); protein-rich vegetable protein meat substitutes (Indonesian *tempe*); amino acid/peptide meat-flavored sauces and pastes produced by fermentation of cereals and legumes (Japanese *miso*, Chinese soy sauce); fermented cereal-fish-shrimp mixtures (Philippine *balao balao* and *burong dalag*); and fermented meats (e.g., salami).

Lactic acid bacteria are generally fastidious on artificial media, but they grow readily in most food substrates and lower the pH rapidly to a point where competing organisms are no longer able to grow. *Leuconostoc*s and lactic streptococci generally lower the pH to about 4.0 to 4.5, and some of the lactobacilli and pedicocci to about pH 3.5, before inhibiting their own growth.

In addition to producing lactic acid, lactobacilli also have the ability to produce hydrogen peroxide through oxidation of reduced nicotinamide adenine dinucleotide (NADH) by flavin nucleotide, which reacts rapidly with gaseous oxygen. Flavoproteins, such as glucose oxidase, also generate hydrogen peroxide and produce an antibiotic effect on other organisms that might cause food spoilage; the lactobacilli themselves are relatively resistant to hydrogen peroxide.

Streptococcus lactis produces the polypeptide antibiotic nisin, active against gram-positive organisms, including *S. cremoris*, which in turn produces the antibiotic diplococcin, active against gram-positive organisms such as *S. lactis*. Thus, these two organisms compete in the fermentation of milk products while inhibiting growth of other gram-positive bacteria.

Carbon dioxide produced by heterofermentative lactobacilli also has a preservative effect in foods, resulting, among others, from its flushing action and leading to anaerobiosis if the substrate is properly protected.

Brining and lactic acid fermentation continue to be highly desirable methods of processing and preserving vegetables because they are of low cost, have low energy requirements for both processing and preparing foods for consumption, and yield highly acceptable and diversified flavors. Depending on the salt concentration, salting directs the subsequent course of the fermentation, limiting the amount of pectinolytic and proteolytic hydrolysis that occurs, thereby controlling softening and preventing putrefaction. Lactic acid fermentations have other distinct advantages in that the foods become resistant to microbial spoilage and toxin development. Acid fermentations also modify the flavor of the original ingredients and often improve nutritive value.

Because canned or frozen foods are mostly unavailable or too expensive for hundreds of millions of the world's economically deprived and hungry people, acid fermentation combined with salting remains one of the most practical methods of preservation, often enhancing the organoleptic and nutritional qualities of fresh vegetables, cereal gruels, and milk-cereal mixtures.

SAUERKRAUT

Lactic acid fermentation of cabbage and other vegetables is a common way of preserving fresh vegetables in the western world, China, and Korea (where *kimchi* is a staple in the diet). It is a simple way of preserving food: the raw vegetable is sliced or shredded, and approximately 2 percent salt is added. The salt extracts liquid from the vegetable, serving as a substrate for the growth of lactic acid bacteria. Anaerobic conditions should be maintained, insofar as possible, to prevent the growth of microorganisms that might cause spoilage.

The sequence of organisms that develop in a typical sauerkraut fermentation is as follows: *Leuconostoc mesenteroides* initiates the growth in the shredded cabbage over a wide range of temperatures and salt concentrations. It produces carbon dioxide and lactic and acetic acids, which quickly lower the pH, thereby inhibiting development of undesirable microorganisms that might destroy crispness. The carbon dioxide produced replaces the air and facilitates the anaerobiosis

required for the fermentation. The fermentation is completed in sequence by *Lactobacillus brevis* and *Lb. plantarum*. *Lb. plantarum* is responsible for the high acidity. If the fermentation temperature or salt concentration is high, *Pecicoccus cerevisiae* develops and contributes to acid production.

As would be expected, the rate of completion of the fermentation depends on the temperature and salt concentration. At 7.5°C fermentation is very slow: under these circumstances, *L. mesenteroides* grows slowly, attaining an acidity of 0.4 percent in about 10 days and an acidity of 0.8 to 0.9 percent in a month. Lactobacilli and pediococci cannot grow well at this temperature, and the fermentation may not be completed for 6 months. At 18°C a total acidity (as lactic acid) of 1.7 to 2.3 percent will be reached, with an acetic to lactic acid ratio of 1:4, in about 20 days. At 32°C a similar activity will be reached in 8 to 10 days, with most of the acid being lactic acid produced by the homofermentative bacteria *Lb. plantarum* and *P. cerevesiae*.

Increasing the salt concentration to 3.5 percent results in 90 percent inhibition of growth and acid production for both *L. mesènteroides* and *Lb. brevis*. The ratio of nonvolatile to volatile acid produced has a marked effect on flavor, *Lb. brevis* producing a harsh, vinegar-like flavor and *L. mesenteroides* a mild, pleasantly aromatic flavor. The homofermenters *Lb. plantarum* and *P. cerevesiae* yield unacceptable products.

KOREAN *KIMCHI*

Korean *kimchi* differs from sauerkraut in two respects: it has, optimally, much less acid and it is carbonated. Chinese cabbage and radish are the major substrates; garlic, green onion, ginger, leaf mustard, hot pepper, parsley, and carrot are minor ingredients.

Kimchi is available year-round, is served three times daily, and is a diet staple along with cooked rice and certain side dishes. It accounts for about an eighth of the total daily food intake of an adult. Its popularity is largely due to its carbonation derived from fermentation with natural microflora.

Salting of the cabbage can be done at 5 to 7 percent salinity for 12 hours or 15 percent salinity for 3 to 7 hours, followed by rinsing and draining. Optimum salt concentration during *kimchi* fermentation is approximately 3 percent. Lower temperatures (about 10°C) are preferred to temperatures above 20°C. Optimum acidity of *kimchi* is 0.4 to 0.8 percent lactic acid with a pH between 4.2 and 4.5; higher acidity makes it unacceptable. Organisms isolated from *kimchi* include *L. mesenteroides*, *S. faecilis*, *Lb. brevis*, *Lb. plantarum*, and *P. cerevesiae*.

PICKLED VEGETABLES

Pickling of cucumbers and other vegetables is widely practiced today. Although a variety of techniques are used, placing cucumbers in a 5 percent salt brine is a satisfactory method. The cucumbers absorb salt until there is an equilibrium between the salt in the cucumbers and the brine. Acidity reaches 0.6 to 1.0 (as lactic acid) with a pH of 3.4 to 3.6 in about 2 weeks, depending on the temperature.

In Malaysia the most common vegetables pickled are cucumbers, ginger, onion, leek, chili, bamboo shoots, and leafy tropical vegetables like mustard leaves. Young unripe fruits commonly pickled include mangoes, papaya, pineapple, and lime. In Egypt carrots, cucumbers, turnips, cauliflower, green and black olives, onions, and hot and sweet peppers are among the vegetables pickled. They are used as appetizers and served with practically every meal.

INDIAN *IDLI* AND *DOSA*

Indian *idli* is a small, white, acidic, leavened, steam-cooked cake made by lactic fermentation of a thick batter made from polished rice and dehulled black gram dhal, a pulse (*Phaseolus mungo*). The cakes are soft, moist, and spongy and have a pleasant sour flavor. *Dosa*, a closely related product, is made from the same ingredients, both finely ground. The batter is generally thinner, and *dosa* is fried like a pancake.

Idli fermentation is a process by which leavened bread-like products can be made from cereals other than wheat or rye and without yeast. The initial step in the fermentation is to wash both rice and black gram dhal. They are then soaked for 5 to 10 hours and drained. The coarsely ground rice and black gram are then combined with water and 1 percent salt to make a thick batter. The batter is fermented in a warm place (30 to 32°C) overnight, during which time acidification and leavening occur. The batter is then placed in small cups and steamed or fried as a pancake. The proportions of rice to black gram vary from 4:1 to 1:4, depending on the relative cost on the market.

Idli and *dosa* are both products of natural lactic acid fermentation. *L. mensenteroides* and *S. faecalis* develop during soaking, then continue to multiply following grinding. Each eventually reaches more than 1 × 10^9 cells per gram, 11 to 13 hours after formation of the batter. These two species predominate until 23 hours following batter formation. Practically all batters would be steamed by then. If a batter is further incubated, the lactobacilli and streptococci decrease in numbers and *P. cerevisiae* develops. *L. mesenteroides* is the microorganism essential for leavening of the batter and, along with *S. faecalis*, is also responsible for acid production. Both functions are essential for producing a satisfactory *idli*.

In *idli* made with a 1:1 ratio of black gram to rice, batter volume increased about 47 percent 12 to 15 hours after incubation at 30°C. The pH fell to 4.5 and total acidity rose to 2.8 percent (as lactic acid). Using a 1:2 ratio of black gram to rice, batter volume increased 113 percent and acidity rose to 2.2 percent in 20 hours at 29°C. Reducing sugars (as glucose) showed a steady decrease from 3.3 milligrams per gram of dry ingredients to 0.8 milligrams per gram in 20 hours, reflecting their utilization for acid and gas production. Soluble solids increased, whereas soluble nitrogen decreased. Flatulence-causing oligosaccharides, such as stachyose and raffinose, are completely hydrolyzed.

A 60 percent increase in methionine has been reported during fermentation. The increase would be of considerable nutritional importance if true, but the results conflict with earlier findings. Thiamine and riboflavin increases during fermentation and phytate phosphorous decreases have also been reported.

PHILIPPINE *PUTO*

Philippine *puto* is a leavened steamed rice cake made from year-old rice grains that are soaked, ground with water, and allowed to undergo a natural acid and gas fermentation. Part of the acid is neutralized with sodium hydroxide during the last stage of fermentation. *Puto* is closely related to Indian *idli*, except that it contains no legume.

SOURDOUGH BREADS AND RELATED FERMENTATIONS

There is a close relationship between yeasts and lactic acid bacteria in sourdough breads, soy sauce, *miso*, and *kefir*. Sourdough leaven contains both yeasts and lactobacilli. The method of preparing such leavens is ancient. Wheat, rye, or other cereal grain flour is mixed with water and incubated for a few days in a warm place. Initially, a wide range of microorganisms develop, but eventually the lactic acid bacteria predominate because of their acid production. Yeasts also can survive, because they tolerate acid well. More flour is added to make a dough. This dough is then subdivided and used to make a batch of bread, while the rest of the dough is kept for future bread making. Wherever sourdough leavens have been studied, the organisms found have been similar.

The essential microorganisms in sourdough are a *Lactobacillus* sp. and a yeast, *Torulopsis holmii. Saccharomyces inusitatus* also has been isolated and identified in sourdough leaven. The lactobacillus species has a preference for maltose and uses the maltose phosphorylase pathway to metabolize the sugar, whereas *T. holmii* grows on glucose but not on maltose, so that both develop in a dough where the amylases hydrolyze starch to maltose.

The basic biochemical changes that occur in sourdough bread fermentation are (1) acidification of the dough with lactic and acetic acids produced by the lactobacilli and (2) leavening of the dough with carbon dioxide produced by the yeast and the lactobacilli. Typical flavor and aroma development can be traced to biochemical activities of both lactobacilli and yeasts. The chewy characteristic of sourdough bread may be due to the production of bacterial polysaccharides by the lactobacilli.

NIGERIAN *OGI* (KENYAN *UJI*)

Nigerian *ogi* is a smooth-textured, sour porridge with a flavor resembling that of yogurt. It is made by lactic acid fermentation of corn, sorghum, or millet. Soybeans may be added to improve nutritive value. *Ogi* has a solids content of about 8 percent. The cooked gel-like porridge is known as "pap."

The first step in the fermentation is steeping of the cleaned grain for 1 to 3 days. During this time the desirable microorganisms develop and are selected. The grain is then ground with water and filtered to remove coarse particles. After steeping, the pH should be 4.3. Optimum pH for *ogi* is 3.6 to 3.7. The concentration of lactic acids may reach 0.65 percent and that of acetic acid 0.11 percent during fermentation. If the pH falls to 3.5, it is less acceptable.

Ogi is a naturally fermented product. A wide variety of molds, yeasts, and bacteria are present initially. *Lb. plantarum* appears to be the essential microorganism in the fermentation. Following depletion of the fermentable sugars, it is able to utilize dextrins from the corn. *Saccharomyces cerevisiae* and *Candida mycoderma* contribute to the pleasant flavor.

NIGERIAN *GARI*

Nigerian *gari* is a granular starchy food made from cassava (*Manihot utilissima* or *M. esculenta*) by lactic acid fermentation of the grated pulp, followed by dry-heat treatment to gelatinize and semidextrinize the starch, which is followed by drying. Cassava tubers are washed, peeled, and grated. An inoculum of 3-day-old cassava juice or fermented

mash liquor is added. The pulp is placed in a cloth bag, excess water is squeezed out, and the pulp undergoes an anaerobic acid fermentation for 12 to 96 hours. Optimum temperature is 35°C. When the pH of the mash reaches 4.0, with about 0.85 percent total acid (as lactic acid), the *gari* has the desired sour flavor and a characteristic aroma. In village processes, further moisture may be removed, and the pulp is then toasted (semidextrinized) in shallow iron pots and dried to less than 20 percent moisture. Village-processed *gari* has a carbohydrate content of about 82 percent with 0.9 percent protein. Lactic, acetic, propionic, succinic, and pyruvic acids have been identified in *gari*, with aldehydes and esters providing the aroma.

For consumption the *gari* is added to boiling water, in which it increases in volume by 300 percent to yield a semisolid plastic dough. The stiff porridge is rolled into a ball (10 to 30 grams wet weight) with the fingers and dipped into stew.

PHILIPPINE *BALAO BALAO*

Balao balao is a lactic acid fermented rice-shrimp mixture, generally prepared by blending cooked rice, whole raw shrimp, and solar salt and then allowing the mixture to ferment for several days or weeks, depending on the salt content. The chitinous shell becomes soft, and when the fermented product is cooked, the whole shrimp can be eaten.

With a salt concentration of 3 percent added to the rice-shrimp mixture, the pH falls to an organoleptically desirable value of 4.08, with titratable acidity reaching 1.32 percent acid (as lactic acid) in 4 days.

Balao balao made with 3 percent salt is best in color, odor, flavor, texture, and general acceptability and is the least salty. *Balao balao* offers a basic method of preservation for cereal-shrimp-fish mixtures. When properly packed to exclude air, sufficient acid is produced to preserve the products without resorting to high-temperature cooking.

MEXICAN *PULQUE*

Pulque is a white, acidic, alcoholic beverage made by fermentation of juice of *Agave* species, mainly *A. atrovirens* or *A. americana*, the century plants. It has been a national Mexican drink since the time of the Aztecs. *Pulque* plays an important role in the nutrition of low-income people in the semiarid regions of Mexico. The essential microorganisms in the *pulque* fermentation are *Lb. plantarum*, a heterofermentative *Leuconostoc*, *Sac. cerevisiae*, and *Zymomonas mobilis*.

The heterofermentative *Leuconostoc* plays the essential role of producing dextrans, which contribute a characteristic viscosity to *pulque* and also increase the acidity of the agave juice very rapidly, inhibiting growth of other less desirable bacteria. *Lb. plantarum* contributes to the final acidity of *pulque*. *Sac. cerevisiae* appears to be a major producer of ethanol, but *Z. mobilis* is considered to be the most important ethanol producer in *pulque*. Under anaerobic conditions, *Zymomonas* transforms 45 percent of the glucose to ethanol and carbon dioxide. It also produces some acetic acid, acetylmethylcarbinol, and some slime gums, which may contribute to the viscous nature of traditional *pulque*.

Soluble solids in the fresh agave juice decrease from 25-30 percent to 6.0 percent in *pulque*. The pH falls from 7.4 to 3.5-4.0. Total acid increases from 0.03 percent to 0.4-0.7 percent (as lactic acid). Sucrose decreases from 18.6 percent to less than 1 percent. Ethanol increases from 0 percent to 4-6 percent (v/v). The B vitamins are present in nutritionally important quantities, with ranges reported as follows (in milligrams per 100 grams): thiamine, 5 to 29; niacin, 54 to 515; riboflavin, 18 to 33; pantothenic acid, 60 to 335; p-aminobenzoic acid, 10 to 12; pyridoxine, 14 to 23; and biotin, 9 to 32.

EGYPTIAN *KISHK*, GREEK *TRAHANAS*, AND TURKISH *TARHANAS*

Egyptian *kishk*, Greek *trahanas*, and Turkish *tarhanas* are mixtures of sheep's milk yogurts and parboiled wheat. Tomato, tomato paste, or onion are sometimes added. In all cases the milk or buttermilk undergoes a typical lactic acid fermentation in which the pH ranges from 3.5 to 3.8 and titratable acidity is 1.3 to 1.8 percent (as lactic acid). Proportions of wheat to yogurt range from 2:1 to 1:3. The wheat is parboiled at some stage in the process. In its simplest form the wheat is added directly to the yogurt and the mixture is boiled until the wheat has absorbed the free moisture. The mixture is cooled and formed into biscuits that are sun dried. If the wheat is ground prior to mixing with the yogurt, the fines are discarded because they harden the final product.

In Egypt the principal microorganisms reported in *kishk* are the heterofermentative *Lb. brevis* and the homofermentative *Lb. casei* and *Lb. plantarum*. In Cyprus sheep's milk yogurt contains principally *S. thermophilus* and *Lb. bulgaricus*. Dried *kishk* and *trahanas* are not hygroscopic and can be stored in open jars for several years without deterioration. They also are well balanced nutritionally.

OTHER FOODS

Lactic acid fermentation also plays an essential role in the production of Indonesian *tempe*, a vegetable (soybean) protein meat substitute the texture of which is provided by mycelium of *Rhizopus oligosporus*, which overgrows and knits the soaked, partially cooked cotyledons into compact cakes that can be sliced thinly and deep fried or cut into chunks and used in soups in place of meat. The essential part played by lactobacilli occurs during the initial soaking when the pH falls from about 6.5 to between 4.5 and 5.0. The lower pH facilitates growth of the mold and prevents development of undesirable bacteria that might spoil the *tempe*.

In Chinese soy sauce (Japanese *shoyu*) and Japanese *miso* and related meat-flavored, amino acid peptide sauces and pastes, the essential microorganism for amylolytic, proteolytic hydrolysis of the soybean-wheat or soybean-rice or barley substrates is *Aspergillus oryzae*. Following overgrowth of the substrate by the mold, the *koji* is subsequently allowed to ferment in approximately 19 percent salt brine for the sauces and 6 to 13 percent salt for the pastes. Lactobacilli grow and lower the pH to about 4.5, which then allows the osmophilic yeast *Sac. rouxii* to grow and produce some ethanol. The ethanol combines with organic acid in the substrate, producing esters that contribute to the agreeable flavor and aroma.

Given the fact that these acid fermentation techniques are simple, effective, and inexpensive, their application in developing countries should be encouraged.

6
Mixed-Culture Fermentations

Clifford W. Hesseltine

Mixed-culture fermentations are those in which the inoculum always consists of two or more organisms. Mixed cultures can consist of known species to the exclusion of all others, or they may be composed of mixtures of unknown species. The mixed cultures may be all of one microbial group—all bacteria—or they may consist of a mixture of organisms of fungi and bacteria or fungi and yeasts or other combinations in which the components are quite unrelated. All of these combinations are encountered in Oriental food fermentations.

The earliest studies of microorganisms were those made on mixed cultures by van Leeuwenhoek in 1684. Micheli, working with fungi in 1718, reported his observations on the germination of mold spores on cut surfaces of melons and quinces. In 1875 Brefeld obtained pure-culture of fungi, and in 1878 Koch obtained pure cultures of pathogenic bacteria. The objective of both Brefeld's and Koch's studies was to identify pathogenic microorganisms. They wanted to prove what organism was responsible for a particular disease. Thus, part of Koch's fame rests on his discovery of the cause of tuberculosis.

An early paper on mixed-culture food fermentation was an address by Macfadyen (1) at the Institute of Brewing, in London, in 1903 entitled, "The Symbiotic Fermentations," in which he referred to mixed-culture fermentations as "mixed infections." Probably this expression reflected his being a member of the Jenner Institute of Preventive Medicine. About half of his lecture was devoted to mixed-culture fermentations of the Orient. Among those described were Chinese yeast, *koji*, Tonkin yeast, and *ragi*.

Mixed cultures are the rule in nature; therefore, one would expect this condition to be the rule in fermented foods of relatively ancient origin. Soil, for example, is a mixed-organism environment with protozoa, bacteria, fungi, and algae growing in various numbers and kinds, depending on the nutrients available, the temperature, and the

pH of the soil. Soil microorganisms relate to each other—some as parasites on others, some forming substances essential to others for growth, and some having no effect on each other.

ADVANTAGES

Mixed-culture fermentations offer a number of advantages over conventional single-culture fermentations:

- Product yield may be higher. Yogurt is made by the fermentation of milk with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Driessen (2) demonstrated that when these species were grown separately, 24 mmol and 20 mmol, respectively, of acid were produced; together, with the same amount of inoculum, a yield of 74 mmol was obtained. The number of *S. thermophilus* cells increased from 500×10^6 per milliliter to 880×10^6 per milliliter with *L. bulgaricus*.
- The growth rate may be higher. In a mixed culture one microorganism may produce needed growth factors or essential growth compounds such as carbon or nitrogen sources beneficial to a second microorganism. It may alter the pH of the medium, thereby improving the activity of one or more enzymes. Even the temperature may be elevated and promote growth of a second microbe.
- Mixed cultures are able to bring about multistep transformations that would be impossible for a single microorganism. Examples are the *miso* and *shoyu* fermentations in which *Aspergillus oryzae* strains are used to make *koji*. Koji produces amylases and proteases, which break down the starch in rice and proteins in soybeans. In the *miso* and *shoyu* fermentations, these compounds are then acted on by lactic acid bacteria and yeast to produce flavor compounds and alcohol.
- In some mixed cultures a remarkably stable association of microorganisms may occur. Even when a mixture of cultures is prepared by untrained individuals working under unsanitary conditions, such as in *ragi*, mixtures of the same fungi, yeasts, and bacteria remain together even after years of subculture. Probably the steps in making the starter were established by trial and error, and the process conditions were such that this mixture could compete against all contaminants.
- Compounds made by a mixture of microorganisms often complement each other and work to the exclusion of unwanted microorganisms. For example, in some food fermentations yeast will produce alcohol and lactic acid bacteria will produce lactic acid and other organic acids and change the environment from aerobic to anaerobic. Inhibiting compounds are thus formed, the pH is lowered, and anaerobic conditions are developed that exclude most undesirable molds and bacteria.

• Mixed cultures permit better utilization of the substrate. The substrate for fermented food is always a complex mixture of carbohydrates, proteins, and fats. Mixed cultures possess a wider range of enzymes and are able to attack a greater variety of compounds. Likewise, with proper strain selection they are better able to change or destroy toxic or noxious compounds that may be in the fermentation substrate.

• Mixed cultures can be maintained indefinitely by unskilled people with a minimum of training. If the environmental conditions can be maintained (i.e., temperature, mass of fermenting substrate, length of fermentation, and kind of substrate), it is easy to maintain a mixed-culture inoculum indefinitely and to carry out repeated successful fermentations.

• Mixed cultures offer more protection against contamination. In mixed-culture fermentations phage infections are reduced. In pure-culture commercial fermentations involving bacteria and actinomycetes, invariably an epidemic of phage infections occurs, and the infection can completely shut down production. Since mixed cultures have a wider genetic base of resistance to phage, failures do not occur, often because if one strain is wiped out, a second or third phage-resistant strain in the inoculum will take over and continue the fermentation. In such processes, especially with a heavy inoculum of selected strains, contamination does not occur even when the fermentations are carried out in open pans or tanks.

• Mixed-culture fermentations enable the utilization of cheap and impure substrates. In any practical fermentation the cheapest substrate is always used, and this will often be a mixture of several materials. For example, in the processing of biomass, a mixed culture is desirable that attacks not only the cellulose but also starch and sugar. Cellulolytic fungi along with starch- and sugar-utilizing yeasts would give a more efficient process, producing more product in a shorter time.

• Mixed cultures can provide necessary nutrients for optimal performance. Many microorganisms, such as the cheese bacteria, which might be suitable for production of a fermentation product, require growth factors to achieve optimum growth rates. To add the proper vitamins to production adds complications and expense to the process. Thus, the addition of a symbiotic species that supplies the growth factors is a definite advantage.

DISADVANTAGES

Mixed-culture fermentations also have some disadvantages.

• Scientific study of mixed cultures is difficult. Obviously, it is more difficult to study the fermentation if more than one microorganism is involved. That is why most biochemical studies are conducted as single-culture fermentations because one variable is eliminated.

• Defining the product and the microorganisms employed becomes more involved in patent and regulatory procedures.

• Contamination of the fermentation is more difficult to detect and control.

• When two or three pure cultures are mixed together, it requires more time and space to produce several sets of inocula rather than just one.

• One of the worst problems in mixed-culture fermentation is the control of the optimum balance among the microorganisms involved. This can, however, be overcome if the behavior of the microorganisms is understood and this information is applied to their control.

The balance of organisms brings up the problem of the storage and maintenance of the cultures. Lyophilization presents difficulties because in the freeze-drying process the killing of different strains' cells will be unequal. It is also difficult, if not impossible, to grow a mixed culture from liquid medium in contrast to typical fermentations on solid mediums, without the culture undergoing radical shifts in population numbers. According to Harrison (3), the best way to preserve mixed cultures is to store the whole liquid culture in liquid nitrogen below $-80^{\circ}C$. The culture, when removed from the frozen state, should be started in a small amount of the production medium and checked for the desired fermentation product and the normal fermentation time. Subcultures of this initial fermentation, if it is satisfactory, may then be used to start production fermentations.

FUTURE

Mixed-culture fermentations will continue to be used in traditional processes such as soybean and dairy fermentations. As noted above, the extensive uses of mixed-culture fermentations for dairy and meat products are well known as to the type of cultures used and the fermentation process. However, there are a large number of food fermentations based on plant substrates such as rice, wheat, corn, soybeans, and peanuts in which mixed cultures of microorganisms are used and will continue to be used

One example of the complex sequential interaction of two fermentations, and which employs fungi, yeast, and bacteria, is the manufacture of *miso*. This Oriental food fermentation product is based on the fermentation of soybeans, rice, and salt to make a paste-like fermented food. *Miso* is used as a flavoring agent and as a base for *miso* soup. There are many types of *miso*, ranging from a yellow sweet *miso* (prepared by a quick fermentation) to a dark, highly flavored *miso*. The type depends on the amount of salt, the ratio of cereals to soybeans, and the duration of the fermentation.

The *miso* fermentation begins with the molding of sterile, moist, cooked rice that is inoculated with dry spores of *Aspergillus oryzae* and *A. soyae*. The inoculum consists of several mold strains combined, with each strain producing a desired enzyme(s). The molded rice is called *koji* and is made to produce enzymes to act on the soybean proteins, fats, and carbohydrates in the subsequent fermentation.

After the rice is thoroughly molded, which is accomplished by breaking the *koji* and mixing, the *koji* is harvested before mold sporulation starts, usually in 1 or 2 days. The *koji* is mixed with salt and soaked and steamed soybeans. This mixture is inoculated with a new set of microorganisms, and the four ingredients are now mashed and mixed. After the production of *koji* with molds, the paste is placed in large concrete or wooden tanks for the second fermentation. The inoculum consists of osmophilic yeasts *Saccharomyces rouxii* and *Candida versatilis* and one or more strains of lactic acid bacteria, typically *Pediococcus pentosaceus* and *P. halophilus* (4). Conditions in the fermentation tanks are anaerobic or nearly so, with the temperature maintained at 30°C. The fermentation is allowed to proceed for varying lengths of time, depending on the type of *miso* desired, but it is typically 1 to 3 months. The fermenting mash is usually mixed several times, and liquid forms on the top of the fermenting mash.

The initial inoculum is about 10^5 microorganisms per gram. Typically, 3,300 kg of *miso* with a moisture level of 48 percent is obtained when 1,000 kg of soybeans, 600 kg of rice, and 430 kg of salt are used. When the second fermentation is completed, aging is allowed to take place. A number of other mixed-culture fermentations are similar to the *miso* process, including *shoyu* (soy sauce) and *sake* (rice wine).

A legitimate question can be asked as to the future prospects for the use of mixed cultures in food fermentations. What will be the effect of genetic engineering on the use of mixed cultures? Would engineered organisms be able to compete in mixed culture? Many laboratories are busy introducing new desirable genetic material into a second organism. The characteristics being transferred may come from such diverse organisms as mammals and bacteria and may be transferred from animals to bacteria. In general, the objective of this work involves introduction of one desirable character, not a number. For instance, strains of *Escherichia coli* have been engineered to produce insulin. However, I suspect that it may be a long time, if ever, before a single organism can produce the multitude of flavors found in foods such as cheeses, soy sauce, *miso*, and other fermented foods used primarily as condiments. The reason for this is the fact that a flavoring agent

such as *shoyu* contains literally hundreds of compounds produced by the microorganisms, products from the action of enzymes on the substrate, and compounds formed by the nonenzymatic interactions of the products with the original substrate compounds.

To put such a combination of genes for all these flavors into one microorganism would, at present, be almost impossible. Second, the cost of producing the food, which is relatively inexpensive as now produced, would become economically prohibitive. The use of mixed cultures in making fermented foods from milk, meat, cereals, and legumes will continue to be the direction in the future.

Harrison (3), in his summary of the future prospects of mixed-culture fermentations, very succinctly concluded as follows:

> No claim for novelty can be made for mixed cultures: They form the basis of the most ancient fermentation processes. With the exploitation of monocultures having been pushed to its limits it is perhaps time to reappraise the potential of mixed culture systems. They provide a means of combining the genetic properties of species without the expense and dangers inherent in genetic engineering which, in general terms, aims at the same effect.

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25

Biotechnology for Production of Fruits, Wines, and Alcohol

J. Maud Kordylas

Fermentation is biotechnology in which desirable microorganisms are used in the production of value-added products of commercial importance. Fermentation occurs in nature in any sugar-containing mash from fruit, berries, honey, or sap tapped from palms. If left exposed in a warm atmosphere, airborne yeasts act on the sugar to convert it into alcohol and carbon dioxide. The making of wines and beers uses this biotechnology under controlled conditions. Alcoholic beverages have been produced for centuries in various societies. They are often central to the most valued personal and social ceremonies of both modern and less literate societies. In such traditional ceremonies as childnaming, marriage feasts, and funerals, alcoholic beverages are often present. In Africa, maize, millet, bananas, honey, palm and bamboo saps, and many fruits are used to ferment nutrient beers and wines. The best known being kaffir beer and palm wines.

Industrial fermentation processes are conducted with selected microorganisms under specified conditions with carefully adjusted nutrient concentrations. The products of fermentation are many: alcohol, glycerol, and carbon dioxide are obtained from yeast fermentation of various sugars. Butyl alcohol, acetone, lactic acid, monosodium glutamate, and acetic acid are products of bacteria action; citric acid, gluconic acid, antibiotics, vitamin B_{12}, and riboflavin are some of the products obtained from mold fermentation.

YEASTS

Yeasts, the main microorganisms involved in alcoholic fermentation, are found throughout the world. More than 8,000 strains of this vegetative microorganism have been classified. About 9 to 10 pure

strains, with their subclassifications, are used for the fermentation of grain mashes. These belong to the type *Saccharomyces cerevisiae*. Each strain has its own characteristics and imparts its special properties to a distillate when used in fermentation. A limited number of yeasts in the classification *Saccharomyces ellipsoides* are used in the fermentation of wines from which brandy is distilled. The strains used in the fermentation of grain mashes are also used in the fermentation of rum from sugarcane extracts and in beer production. Since yeasts function best in slightly acid medium, the mash, juice, sap, or extract prepared for fermentation must be checked for adequate acidity. If acidity is insufficient, acid or acid-bearing material are added. For distilled liquors, fermentation is carried out at 24° to 29°C for 48 to 96 hours, when the mash or must is ready for distillation. The alcohol content of the fermented must is about 7 to 9 percent.

RAW MATERIALS

Cereals and Starchy Roots

For most distilled liquors, the raw material used is a natural sugar as found in honey, ripe fruit, sugarcane juice, palm sap, beet root, milk, or a substance of amylaceous (starchy) nature that can be easily converted into simple sugars using enzymes present in cereals or through the addition of suitable malted cereal. Maize or corn is the most important grain used as fermentable starchy cereal. Starchy roots and tubers are also used. Industrial production of alcohol from cassava in Brazil has been described by De Menezee (1). The alcohol produced is concentrated in a second distillation column to 97.2 percent and is further dried to 99.9 percent and blended with gasoline for energy purposes.

Malt is important in distilled liquor. In addition to converting starches from other carbohydrates to sugars, malt contains soluble proteins that contribute flavor to the distillate obtained from the fermentation of grain malt mixtures.

Sugarcane

Grown throughout the tropics and semitropics, sugarcane and its products, including cane juices, molasses, and sugar are used to make rum and an alcohol derived from rum. Pressed juice from sugarcane can be used as the base raw material for fermentation, or the juice can be concentrated for sugar production with the molasses residue from sugar crystallization used as a base for alcohol fermentation. Molasses contains about 35 percent sucrose and 15 percent reducing sugars. This gives molasses its principal value as an industrial raw material for fermentation to produce rum. Two or 3 liters of molasses produces 1 liter of rum. Acetone and butanol also are produced from molasses by fermentation with *Clostridium* bacteria. Food yeast *Torulopsis utilis*, is prepared from molasses, as are baker's and brewer's yeasts (2).

Coconut Palm

The coconut palm finds many uses on the tropical islands of the Pacific. Toddy is produced by tapping the unopened flower spathe of the coconut palm. The spathe is bruised slightly by gentle tapping with a small mallet and is tied tightly with fiber to prevent it from opening. It is bent over gradually to allow the toddy to flow into a receptacle. About 5 centimeters is cut from the tip of the spathe after about 3 weeks. Thereafter, a thin slice is shaved off once or twice a day and the exuding sap is collected. Palms are tapped for 8 months of the year and rested for 4 months. The average daily yield per palm is about 2 liters. The yield per spathe varies from 15 to 80 liters, and an average palm can yield 270 liters during 8 months of tapping. The fresh sweet toddy contains 15 to 20 percent total solids, of which 12 to 17.5 percent is sucrose.

Toddy ferments rapidly due to naturally occurring yeasts. Fermented toddy contains about 6 percent alcohol. After 24 hours the toddy contains 4 to 5 percent acetic acid and is unpalatable as a beverage. It can be used for the production of vinegar. Fermented toddy can be distilled to produce arrack. Freshly fermented toddy is used instead of yeast in bread making. Constant tapping of coconut palms for toddy eliminates the nut crop. In 1952 in wine distilleries in Sri Lanka, over 49 million liters of toddy was fermented to give 4.5 million proof liters of arrack (2).

Oil Palm

By tapping the male inflorescence of the oil palm, a sweet sap is obtained. The leaf subtending the immature male inflorescence is removed to provide access, the inflorescence is excised, and thin slices are cut once or twice daily. The exuding sap is funneled into a calabash or a bottle. The fresh sap contains 15 percent sugar. Tapping is done daily for 2 to 3 months, yielding about 3.5 liters of sap per day. The sap ferments by the action of bacteria and natural yeast to produce a beverage with a milky flocculent appearance and a slight sulfurous odor known as palm wine. Palm wine is produced and marketed in considerable quantities in Nigeria.

The sap may be boiled to produce dark-colored sticky sugar or jaggery, which does not keep well. About 9 liters of juice produces 1 kilogram of jaggery. The fermented sap also yields yeasts and vinegar. A mean annual yield of 4,000 liters of sap per hectare of 150 palms has been recorded in eastern Nigeria. This was estimated to have a value more than double that of oil and kernels from similar palms. Tapping, however, reduces the fruit yield. Sap can also be obtained by tapping the crown of the tree laterally or by felling the palm and drilling a hole through the growing point. Both these methods are very wasteful since they kill the plant. The Palmyra palm yields about 2 liters of palm sap per day. Large palms with several tapped inflorescences give as much as 20 liters per day. A single palm of this type is estimated to produce 12,000 liters of sap during its tapping life.

Fruits

Grapes are the most common fruit used as raw material for alcoholic fermentation. They are used in distilled liquor to make brandy. Historically, wine is the product of fermentation of grape species *Vitis vinifera*. The high sugar content of most *V. vinifera* varieties at maturity is the major factor in their selection for use in much of the world's wine production. Their natural sugar content provides the necessary material for fermentation. It is sufficient to produce a wine with an alcohol content of 10 percent or higher. Wines containing less alcohol are unstable because of their sensitivity to bacterial spoilage. The grape's moderate acidity when ripe is also favorable to wine making. The fruit has an acidity of less than 1 percent, calculated as tartaric acid, the main acid in grapes, with a pH of 3.1 to 3.7. The flavor of grapes varies from neutral to strongly aromatic, and the pigment pattern of the skin varies from light greenish-yellow to russet, pink, red, reddish violet, or blue-black. Grapes also contain tannins needed to give bite and taste in the flavor of wines and to protect them from bacteria and possible ill effects if overexposed to the air.

Other fruits can be used to produce wine. When fruits other than grapes are used, the name of the fruit is included, as in papaya or pineapple wine. Apples and citrus fruits with sufficient fermentable sugars are crushed, and the fermentable juices are either pressed out for fermentation or the entire mass is fermented. Tropical fruits such as guava, mangos, pineapple, pawpaw, ripe banana, ripe plantain, tangerine, and cashew fruit also contain fermentable sugars with levels varying from 10 to 20 percent. Overripe plantain pulp was reported to contain 16 to 17 percent fermentable sugar, with the skin containing as much as 30 percent (3).

The tropical climate prevailing in Africa is ideal for the growth and multiplication of microorganisms. The environment is abundant in biomass and in raw materials, which are high in starches and sugars and can be used for fermentation. The available literature is sufficient in information on conditions and control measures required for optimum microbial activity in the various microbial processes. Convincing research results are also available to support utilization of microorganisms in the production of high-quality products of commercial importance. What is lacking, however, is organization of the available information to enable selection of appropriate microbial processes that can be put together to form an integrated system to harness desirable microorganisms as a labor force for industrial exploitation. Below an account is given of an attempt to organize four microbial processes into a production system to produce fruits, wines, and alcohol in an experimental project.

INTEGRATED PRODUCTION SYSTEM

An experimental project was established aimed at providing adequate conditions and control measures in four separate biological subsettings to produce quality products through the action of microorganisms. An attempt was then made to synchronize the activities of the subsettings into an integrated system for the production of fruits, wines, and alcohol with jam production as an integral part of the production system.

The four biotechnological subsettings used were: a compost pile, stimulated microbiological activity in the soil for release of nutrients, yeast activity in extracted fruit juices for the production of wines, and yeast activity in juice extracted from pineapple by-products for the production of alcohol.

Composting

In 1984 a two-compartment wooden structure measuring 2 × 1 × 1 meters was constructed to hold two piles of composting material. Cut grass, straw, dried leaves, and other high-carbon organic wastes were collected from the neighborhood. They were layered with chicken manure to provide a nitrogen source to form compost piles within the compartments. Kitchen waste and, later, wastes from fruit processing were also added to the piles. The piles were kept sufficiently moist by sprinkling with water. To encourage optimum microbiological activity, the piles were aerated by constant turning. Observation of heat generation and the rates at which the piles were digested were used to indicate effective microbial activity. The lack of offensive odor from

the piles was considered a sign of adequate control conditions within the piles.

Microbial Activity in Soil

The compost obtained was used to prepare selected sites in a backyard plot measuring 9 × 20 meters that was originally filled with clay soil. The clay soil was removed, and mixed with compost. The mixture was placed into the holes to form raised beds for planting. Two guava seedlings obtained from the research station at Njombe were added to other fruit seedlings nursed in pots. These were transplanted into the prepared sites. As more compost was made available, more fruit seedlings were transplanted into position. By mid-1986 the backyard plot was planted with the following fruit trees: six soursops, five guavas, three pawpaw, eight carambola bushes, one mango, and one avocado pear. The fruit trees were interplanted with plantains, cocoyam, pepper, and a few winged bean plants to form a multistory system as usually obtained in traditional cropping systems in Africa.

Sufficient compost was applied regularly to the soil to encourage microorganisms and other soil dwellers to function and to enhance mycorrhizal fungi association with root hairs, to provide nourishment and protection and for the well-being of the plants. The compost was applied by removing the topsoil around the plant to expose the roots. Two to three loads of compost were distributed evenly around the roots and were covered with the topsoil. Fallen leaves around the yard were raked and used as mulch to cover the top of the disturbed soil to prevent it from eroding away during heavy rains. The leaf mulch was also used to protect the soil surface from the pounding rains. It also kept the soil cool during the dry season and helped to conserve soil moisture when the plants are irrigated. To encourage microbial activity in the soil, no inorganic fertilizer was applied and no pesticides were sprayed anywhere in the yard.

The fertility of the soil around the growing plants was regularly monitored using a two-prong fertilizer analyzer that indicated whether the soil had sufficient nitrogen, potassium, and phosphorus. Where a deficiency was indicated, more compost was applied to the soil. The method of removing the topsoil to apply compost aerated the soil. During the rainy season the edges of the soil around the raised beds were lifted slightly with a fork to allow air in without disturbing the soil. The improvement in soil fertility over the years, the physical appearance of the growing trees, the lack of disease, and later the fruit yield were used as parameters to indicate optimum conditions in the soil that promoted microbial activity. Fruit harvests were recorded daily.

Wine from Fruit Juices

Extracted juices from pawpaw and carambola harvested from the backyard and juice extracted from pineapples obtained from the local market were used to carry out wine-making experiments. The pulp remaining after juice extraction from fruits was used to make jam.

To prevent the growth of undesirable microorganisms, the juice extracts were pasteurized. All utensils, tools, and equipment that came into contact with the wine in making, were sterilized and rinsed thoroughly. No chemicals were used in the preparation of the must. Sufficient amounts of yeast nutrients were added for yeast growth. The pH of the must was adjusted and sufficient sugar was added where needed to produce 11 percent alcohol in the finished wine. A small amount of tannin solution was added to provide bite and flavor to the finished wine. The yeasts used for the first experiments were activated according to the manufacturer's directions. Thereafter, pawpaw, pineapple, and carambola wine yeasts were reserved from wines made. These were kept under refrigeration and used for subsequent wine production. All the wine-making stages—first and second fermentations, raking, storage and aging—were carried out in an air-conditioned room so that constant temperatures could be maintained. Finished wines were bottled, pasteurized, cooled, and corked for storage to age in the bottles.

Alcohol Production from Pineapple

The preparation of pineapples usually produced about 40 to 50 percent waste materials. This was made up of the top crown, the fibrous outside skin, the seeded inner cover, and the hard central core. The crown and the fibrous skin were added to the compost pile. The seeded cover and the central core were crushed and kept frozen until needed for juice extraction for fermentation. The sugar level of the pasteurized juice was checked and sufficient amounts of granulated sugar were added to produce about 12 percent alcohol in the fermented must. The pH of the preparation was also adjusted. The fermented must was then distilled. The temperature of the distillation was carefully controlled so that a high concentration of alcohol could be obtained from one distillation. The bulk of the alcohol collected was over 90 percent concentration. This alcohol was used in experiments with fruits to make aperitif drinks and liquors.

INTEGRATION

The activities of the four microbial processes were synchronized and integrated into an interdependent production system where the subprocesses provided support for each other. The composting setup received wastes from fruit processing. The compost was used to enrich the soil in which the fruit trees were planted. Harvested fruits provided juice extracts for wine making, and by-products from fruit processing provided raw materials for alcohol production. Jams were produced from fruit pulp and were marketed to provide financial support for needed research and to purchase equipment.

RESULTS AND DISCUSSION

Composting

It took about 12 months of composting to arrive at the number of turnings needed, and the correct ratios of high-carbon materials to nitrogenous material required to prepare a compost pile without an ammonia odor. When the correct proportions were used, the compost was completed within 3 weeks during the hot dry weather, and in 4 to 5 weeks during the cool rainy season. Sufficient heat was generated to sterilize the compost, and no odor was detected.

Soil and Fruit Production

It took 2 to 3 years of regular application of compost for the clay in the planted sites to change into dark fluffy soil. Earthworms were seen in the soil after 3 to 4 applications of compost. During the first 3 years the growing plants were constantly affected by plant diseases. The infections diminished, however, as the soil fertility improved. None of the infections were serious enough to require action. The attacks increased during the dry season and again toward the end of the rains, especially during periods when the rains were long and heavy.

Table 1 shows guava, soursop, and carambola yields over the years. After their first bearings, most of the trees lost their seasonality and continued to flower, set fruit, mature, and ripen fruit as long as the weather and soil conditions remained favorable. The rains usually started in March/April and enhanced fruit yield. Thereafter, fruit yields were affected by how heavy the rainy season was and how long it lasted. Flowering and fruit settings were greatly diminished in the guava and the soursop during heavy rains. They were, however, resumed as soon as there was a break in the rains. The next harvests were delayed if the rains were heavy and lasted for a long time. The

TABLE 1 Fruit Yields (kilograms), 1986–1991

Year	Guava				Soursop				Carambola			
	Jan.-June	July-Dec.	Total	Ave./Tree	Jan.-June	July-Dec.	Total	Ave./Tree	Jan.-June	July-Dec.	Total	Ave./Tree
1986	7.2(2)	41.9(2)	49.0(2)	24.50	—	—	—	—	—	—	—	—
1987	54.8(2)	76.4(2)	131.2(2)	65.6	163.2(4)	9.9(2)	173.0(4)	43.3	—	0.4(1)	0.4(1)	4.0
1988	86.4(3)	131.1(3)	217.5(3)	72.5	132.4(4)	19.2(4)	151.5(4)	37.9	17.0(6)	46.6(6)	63.6(6)	10.6
1989	109.9(3)	98.5(4)	208.3(4)	52.1	294.1(6)	105.6(5)	394.0(6)	66.2	86.2(8)	135.3(8)	221.5(8)	27.7
1990	165.5(5)	129.5(5)	295.0(5)	59.0	195.7(6)	92.6(5)	286.3(6)	47.7	143.5(8)	135.7(8)	279.0(8)	34.9
1991		116.6(5)			341.2(6)				154.9(8)			

( ), number of trees bearing fruit.

carambola somehow continued to flower and set fruit during the rainy season as long as there was periodic sunlight.

Quality was high in guavas and soursop harvested at the beginning of the rains. The fruits were large and well formed and had good flavor. Most of the fruits harvested at the ends of the dry and rainy seasons were smaller, malformed, or diseased. This may be due to the effects of too little or too much water on the health of the plants. Too little water may have affected the activities of microorganisms in the soil, and too much water may have reduced air supply to microorganisms in the soil and leaching of nutrients from the soil. Diminished microbial activity may have affected the well-being of the plants. These assumptions might, however, need to be confirmed through controlled experiments.

The 180-square meter backyard plot yielded sufficient quantities of fruits—guava, soursop, and carambola—to provide raw materials for processing to make jams available on the local market throughout 1989 and thereafter. Carambola yields were also sufficient for wine making. The amount of pawpaw harvested from the backyard was not sufficient, however, for both jam production and wine making. More pawpaw was therefore purchased from the local market to supplement the amount harvested. The quantity of mango obtained from the one mango tree was also not sufficient to keep up with the demand for mango jam on the market. More was obtained from the local market.

Table 2 shows total yields for guava, soursop, carambola, and pawpaw harvested from 1986 to 1990. Although two of the four pawpaw trees died, total yields of fruits from the backyard continued to increase over the years. Yields from crops interplanted among the fruit trees, including pepper, cocoyam, plantain, and winged beans, and from the one avocado tree that started bearing fruit in 1990, when added to those obtained from trees in Table 2, provided an overall yield of over 1 ton from the backyard plot in 1989 and again in 1990.

Wine Production

Wine of acceptable quality were produced from pawpaw, pineapple, and carambola. The wines made were either dry, semidry, or sweet.

TABLE 2 Fruit Yields (kilograms), 1986–1990

Fruit	1986	1987	1988	1989	1990
Guava	49.0	131.2	217.5	208.3	295.0
Soursop	—	173.0	151.5	397.0	286.3
Carambola	—	0.4	63.6	221.5	279.0
Pawpaw	—	28.3	100.9	72.6	40.0
Total	49.0	332.9	533.5	899.4	900.3

Although no controlled organoleptic assessment was organized to evaluate the acceptability of the wines, reactions from random individuals who tasted the wines were favorable. Marketing trials will be conducted.

Alcohol Production

Juice extracted from the crushed pineapple core and the inner seeded cover contained sufficient sugar to produce 6.5 to 7 percent alcohol after fermentation. With the addition of extra sugar, however, the alcohol content was increased to 10 percent. A total of 25 liters of over 90 percent concentration alcohol was distilled from 200 liters of discarded wines and 100 liters of fermented pineapple waste extract. Portions of the alcohol were used to carry out experiments to produce aperitif drinks with guava, pineapple, passion fruit, carambola, and ginger. The experiments are still in progress.

BIOTECHNOLOGY PRODUCTION SYSTEM

The integrated bioechnology research and development system is shown in Figure 1. The broken-line arrows indicate units not yet included but for which information has been collected to enable their future integration into the system. The chickens are needed to produce manure for the composting process, with meat and eggs as additional marketable products. Wastewater from fruit processing would be recycled to provide water for irrigation and for composting to economize on the use of potable water for those processes.

From the data collected and from experience gained through the project, the integrated biotechnology production system has many advantages:

- It is environmentally sound: Wastes generated from fruit processing and from the backyard plot are recycled through the composting process to produce organic fertilizer.
- Labor requirements have not been excessive: Once the necessary conditions are met and controls applied for microorganisms to grow and multiply, the productive processes for wine and alcohol production, for composting, and for nutrient release for plant nourishment are carried out with little or no supervision.
- Energy requirements are low: Apart from the energy needed for production of jams and for pasteurization and to run the small-scale equipment used in processing, the integrated production system needs limited amounts of energy input to function. The microbial processes generate their own energy. The need for air conditioning to maintain

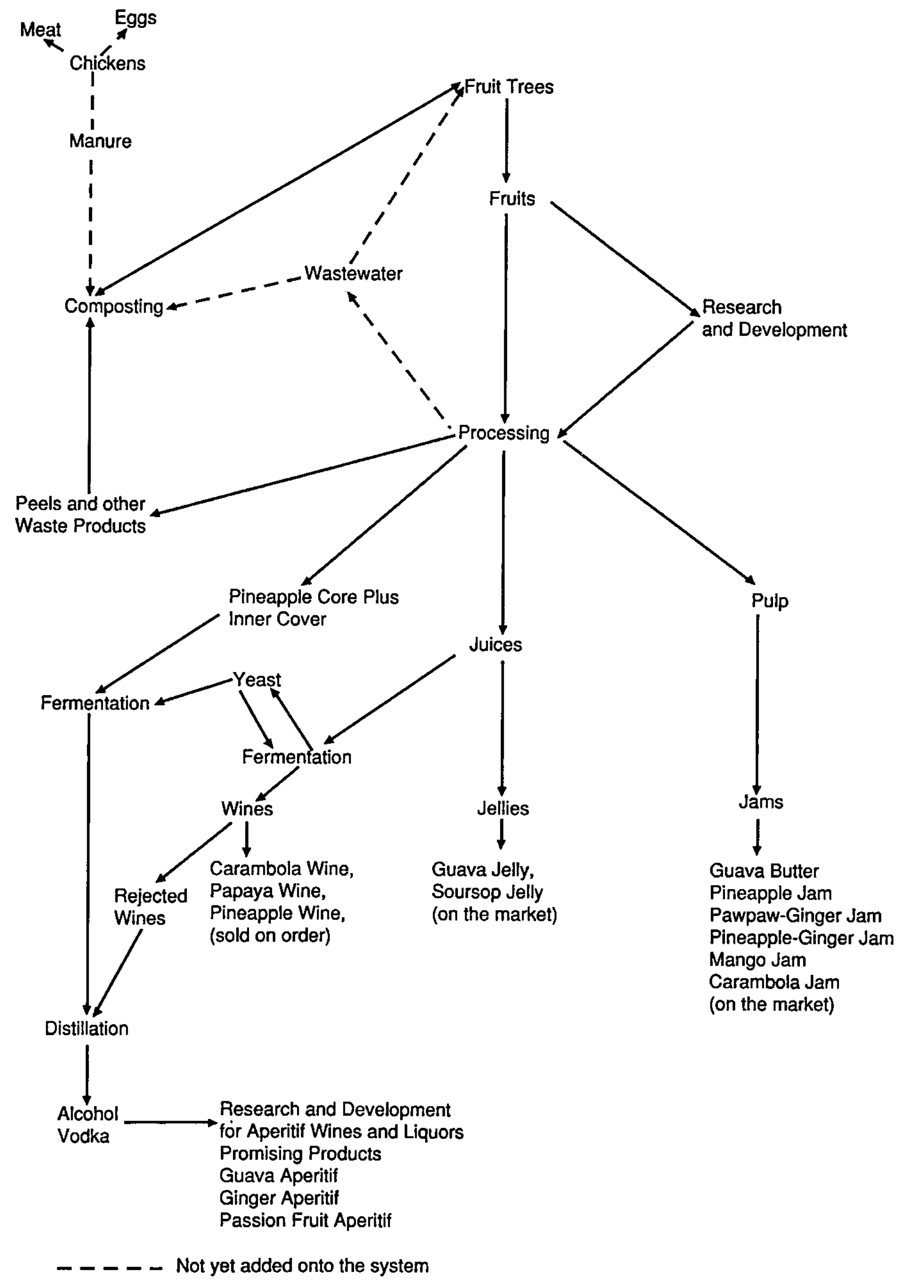

FIGURE 1 An integrated research and development system using biotechnology in the production of fruits, wines, and alcohol.

constant environmental temperatures will likely add to the energy costs.

- The system is sustainable: The interdependency of the microbial subprocesses provides sustainable support to each other with limited input required from outside. Funds generated from the sale of products (jams, wines, apertif drinks) are used to support needed research and to purchase equipment and supplementary produce required to sustain the production of marketable products.
- Only practical research is undertaken: Experiments carried out are those needed to solve immediate problems arising from the production system. These are carried out either to improve the quality of a product, to formulate new products from raw materials or by-products generated within the system, or to enhance marketability of a product.
- Realistic data is collected for feasibility reports. Production and trial marketing of products from the system have enabled real data to be collected. These are being used to evaluate the system economically and to produce a feasibility report based on actual figures to make decisions on establishing an industry based on the prototype research and development unit.
- Valuable experience has been gained: The project has provided valuable experience in the management of a small enterprise.

CONCLUSIONS AND RECOMMENDATIONS

A good number of efficient microbial processes are available. Sufficient knowledge has been accumulated and information provided on their management and control. If properly selected, synchronized, and integrated, the activities of microorganisms from such processes may be harnessed and used. Their exploitation may be a more promising alternative to large-scale industrial technologies imported from developed countries, which developing countries in Africa cannot afford, sustain, or manage.

The priority for research is, therefore, on selecting the right types of microbial processes that can be put together to form sustainable productive systems, with research trials carried out on prototypes to determine the most economically viable combinations to be adopted for commercial exploitation.

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