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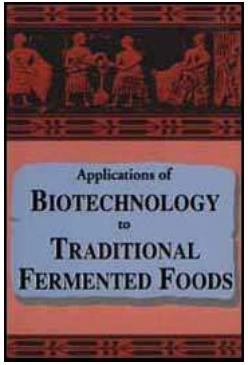

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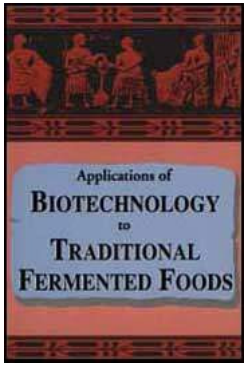
Applications of Biotechnology to Traditional Fermented Foods (BOSTID, 1992, 188 p.)



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

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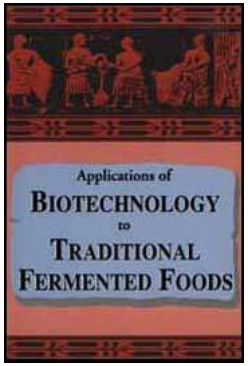
VII. Commercialization
24 Commercialization of Fermented Foods in Sub-Saharan



Applications of Biotechnology to Traditional Fermented Foods (BOSTID, 1992, 188 p.)

VII. Commercialization

24 Commercialization of Fermented Foods in Sub-Saharan Africa

Nduka Okafor

Fermented foods form an important part of the diets of people throughout the world, and the people of sub-Saharan Africa are no exception. In many parts of the world, as urbanization increases, the preparation of fermented foods moves from the small-scale household level to large-scale operations. Under these new conditions the foods are prepared with better scientific knowledge. For this reason large scale factory procedures may differ from traditional approaches. For example, cheese that used to be produced with protease present in rennet may now be produced with protease produced by fungi.

With this in mind, a review was carried out in 1981 (1) to learn the extent to which some important fermented foods of sub-Saharan Africa had progressed toward

commercialization. The stage that each food had attained was measured on a scale of 8, as shown in Table 1.

The purpose of this paper is to indicate to what extent various sub-Saharan fermented foods have progressed in the past decade toward being industrialized and to examine the role, if any, that modern techniques of biotechnology, particularly genetic engineering, have played in commercialization.

INDUSTRIALIZATION OF FERMENTED FOODS

Table 1 lists the fermented foods about which information is available, including those reviewed earlier (1). A review of the extent of progress toward industrialization of alcoholic beverages of sub-Saharan Africa was recently published (2) and is incorporated here into Table 1.

The following conclusions can be drawn:

· In 1981 the following foods had been produced on an industrial or semiindustrial scale: ogi, garri, palm wine, mahewu, and sorghum (kaffir) beer.

Two new products are now being produced on an industrial or semiindustrial scale. The first is a Nigerian condiment known as dawa-dawa. It is being produced under the trade name of Dadwa by the firm of Cadburys in Nigeria from Parkia seeds as in the traditional fermentation. The second is a Zimbabwean fermented milk product known as Lacto. It is similar to the traditional fermented milk of Zimbabwe (3).

· The organisms involved in the fermentation of several foods that were unknown in 1981 have now been identified. They are foo-foo (4), kokonte (5), ugba (ukpaka) (6), and ogili (7,8).

The case of dawa-dawa is interesting. In 1981 the organisms involved were unknown; in 1991 not only are they known (9), but the food itself has been commercialized.

· Some foods not previously recorded have been added: tej from Ethiopia (10); nono, a milk-based product from Nigeria; and Zimbabwean fermented milk (3).

TABLE 1 Fermented Foods of Africa South of the Sahara

Food	Region	Processing	Level of Advance	1981 and 1991	Microorganisms
CASSAVA-BASED					
Garri	West Africa; Zaire	Pulp fermented	1,4,6,7	8	
Foo-foo (4)	Nigeria	Whole roots fermented	0	1	Cornebacterium Bacillus
					Lactic acid bacteria
Chikwangue	Zaire	Whole roots fermented	0		
Lafun	Nigeria	Flour from chips	0		
Kokonte	Ghana	Flour from chips	0		
Cingwada	East, Central & South Africa	Flour from chips	0		
CEREAL-BASED NON-ALCOHOLIC					
Ogi	Nigeria, Benin	Fermented ground	1,2,4,6	8	
	Republic	cereal		7,(8?)	

Koko (aflate) (5)	Ghana	Fermented ground	1	1	Lactic acid bacteria
		cereal			
Mahewu (Mogow)	South Africa	Fermented ground cereal	1,2,4,5, 6,7,8		
Injera(10) ceae,	Ethiopia	Fermented ground	1,2	1,2	Entero bacteria
		cereal			Lactic acid bacteria
MILK-BASED					
Ayib (16)	Ethiopia	Cheese-like	-	1,2	Lactic acid bacteria and yeasts
Nono	Nigeria	Fermented milk		-	Lactobacillus bulgaricus
					L plantanum,
					L. helveticus
					Streptococcus cremoris
Fermented milk (3)	Zimbabwe	Fermented mlik	-	1,2	Lactococcus spp.
"Lacto" (3)	Zimbabwe	Fermented milk	-	8	Lactococcus spp.
ALCOHOLIC					
Burukutu/Pito	West Africa	Fermentation of malted sorghum	1,2		
Sorghum (KaHir)	South Africa	Fermentation of	1,2,4,5,		

beer		malted sorghum	6, 7, 8		
Merissa (2)	Sudan	Fermentation of malted sorghum		0	
Bussa (2)	Kenya	Fermentation of malted sorghum		1,2	
PALM-BASED					
Palm wines	East, West, Central and South Africa	Spontaneous fermentation of palm sap	1,2,7		
MISCELLANEOUS					
Iru (dawadawa) (10)	Nigeria	Fermented seeds of Parkia	0	8	Lactic acid bacteria
Ogili (17)	Nigeria	Fermented seeds of castor oil	0	1,2	Lactic acid bacteria
Ugha (Ukraka) (6)	Nigeria	Fermented seeds of oil-bean	0	1,2	Bacillus
Fura (Ghussab)	Mali	Millet and cheese	0		
Asami	East, South Africa	Central Fermented milk	0-		

Key:
1 = Organisms isolated
2 = Role(s) of organism(s) determined
3 = Selection and genetic improvement of organisms
5 - Improvement in raw material used
6 = Laboratory simulation of fermented food production

7 = Pilot plant production
8 = Industrial plant production

DISCUSSION AND CONCLUSIONS

As can be seen, very little has changed in the progress of the fermented foods of Africa toward industrial production. The 1990s are the era of biotechnology, especially genetic engineering. Fermented foods are brought about by microorganisms, and one would expect that these organisms would be subjected to the technology of gene cloning to improve their activity in the fermentation of foods.

For example, the fermentation of most carbohydrate foods such as cassava or maize is brought about by lactic acid bacteria. One would therefore have expected that these organisms would be targeted for improvement by gene cloning. Only one example of the advantage of the use of this technique will be given.

In garri fermentation lactic acid bacteria play an important part in producing the flavor of the food (11). Yet these organisms cannot split starch. If the amylase gene can be cloned into a lactic acid bacterium involved in garri fermentation, it is conceivable that fermentation will occur faster. If the gene for linamarase production can also be simultaneously cloned, then not only will the fermentation be faster but detoxification also will occur (12).

The only work having any relationship to gene cloning in organisms involved in fermentation was the isolation of plasmids from cassava fermenting organisms by Nwankwo et al. (13). They found that they could not transfer the plasmids to E. cold and there the work ended.

The lack of ability to exploit this new technique in an area of vital importance to Africa south of the Sahara is a clear example of (an almost?) missed opportunity in an age when

seemingly everyone is cloning a gene from one source or another. Nevertheless, there have been some developments in other directions. For example, Ofuya and Nnajiofor (14) have developed a starter culture for garri that should prove useful in the commercialization of the food. Also, Ofuya and Fiito (15) have developed a rapid method for assessing the quality of garri based on an iodine reaction.

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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 B12, 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 I liter of rum. Acetone and butanol also are produced from molasses by fermentation with Clostridium bacteria. Food yeast Torulopsis utitis, 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 I 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 x 1 x 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 x 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 carambola somehow continued to flower and set fruit during the rainy season as long as there was periodic sunlight.

TABLE 1: Fruit Yields (kilograms), 1986-1991

	Guava				Soursop				Caram		
Year	Jan.-June	July-Dec.	Total	Ave./Tree	Jan.-June	July-Dec.	Total	Ave./Tree	Jan-June	July-Dec.	T
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
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)	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)	2
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)	2
1991		116.6(5)			341.2(6)				154.9(8)		

( ), number of trees bearing fruit.

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

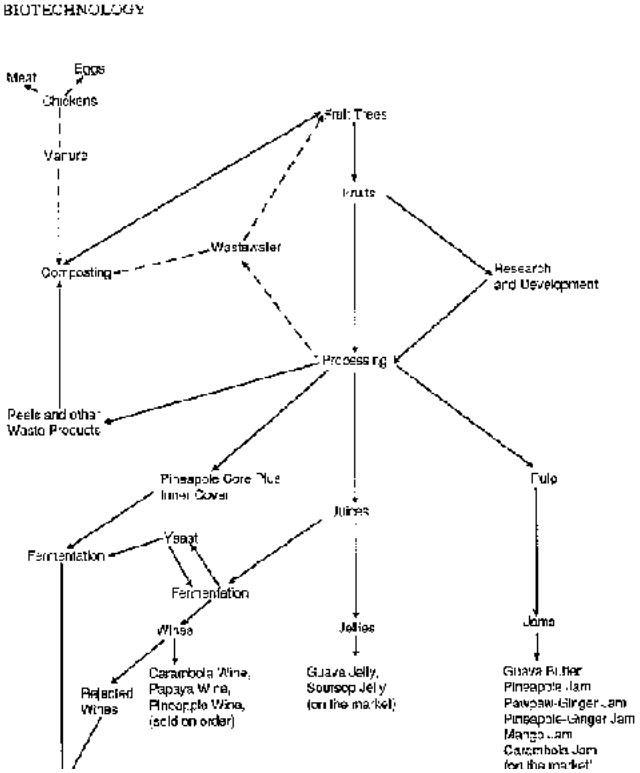
The integrated bioechnology research and development system is shown in Figure 1. The broken-line arrows indicate units not yet included but for which information has been collected to enable their future integration into the system. The chickens are needed to produce manure for the composting process, 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.

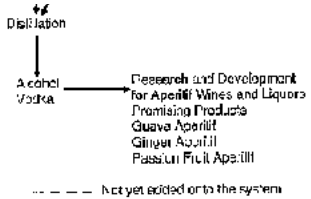
From the data collected and from experience gained through the project, the integrated biotechnology production system has many advantages:

· 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 constant environmental temperatures will likely add to the energy costs.





FIGURE

· 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 byproducts 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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26 Future Directions

Leslie Fook-Min Yong

The preparation of fermented foods predates the recorded history of Man. Early humans used observation of the apparent effects of microbial alteration of food characteristics to develop processes for food fermentation. The resultant fermented products normally have

a different texture and flavor compared to the unfermented starting materials, thus making them more palatable and digestible and prolonging their shelf life. Technical progress was initially slow, as reflected in the long fermentation periods required; it was incremental to the technical know-how and basic scientific information then available. It is probably fair to say that in the very early days brew-masters were more artisans than technologists. With the rapid advancement in understanding of the basic sciences of microbiology and biochemistry, coupled with the introduction of new equipment, the developed nations have forged ahead in improving the safety and efficiency of the bioprocesses used to manufacture traditional fermented foods, such as cheese fermentation.

"OLD" AND "NEW" BIOTECHNOLOGY

With the rapid progress in the biological sciences, both basic and applied aspects, it has been possible to gain a better understanding of the mystery that has surrounded fermentation processes. The types of microorganisms involved has been isolated and identified, and the physiology and metabolism of these organisms have been studied. Hence, traditional fermented foods can now be made better, faster, and more economically. The application of available knowledge to improve traditional food fermentations in developed countries has far outpaced that in developing countries.

In this paper I draw on my experience working with soy sauce fermentation and then proceed to discuss the production of flavor and fragrance materials by microbial fermentation. Experience gained from this traditional fermented condiment has enabled me to develop novel bioprocesses for the production of aroma chemicals.

The terms "old biotechnology" and "new biotechnology" have been used - "old" to mean the undirected manipulation of microorganisms and plants, such as by mutagenesis and selection of the better strains. In this old biotechnology I would like, for convenience, to

include directed control of the physical and chemical environments of the fermentation process, which could result in better performance of the useful microbes.

Though mutation increases the ability to select better strains, there can, of course, be little directed alteration of genetic material. The new biotechnology, such as recombinant DNA techniques, overcomes this problem. The new biotechnology can, of course, be of tremendous help in producing superstrains of microbes that could enable acceleration of fermentation processes, provide more efficient utilization of raw materials, and produce better-quality products. How best can developing nations apply these biotechnologies to traditional fermented foods? Should it be application of the "old" before the "new," "new" without the "old," or "old" and "new" simultaneously?

In their enthusiasm to promote the new biotechnology for traditional fermented food applications, scientists from developed countries should not forget the different environments that exist in developed and developing countries. In developed countries the old biotechnology is already well understood and practiced efficiently in fermented food industries. Developing countries may need to acquire a better understanding of the old biotechnology before efficiently absorbing and implementing the new biotechnology to its fullest.

APPLICATION OF BIOTECHNOLOGY

Preparation of traditional fermented foods is more complex and time consuming than that involved in the production of single chemical substances. For example, in soy sauce fermentation more than one type of microorganism is involved, whereas in citric acid fermentation only one species of fungus is normally used. How can developing countries apply new knowledge in the old and new biotechnologies to their own complex traditional food fermentations?

Take soy sauce fermentation as an example of a traditional fermentation process

conducted in a developed country, such as Japan compared with that in a country like Malaysia. The technology in use in Japan is sophisticated, very advanced, and highly productive and mechanized. The microbes used have been selected over the years for their performance in producing a better-quality product. The cottage industry soy sauce fermentation in Malaysia is highly labor intensive and usually relies on "natural" inoculation of raw materials using unwashed trays for previous fermentations rather than using a separately prepared inoculum of Aspergillus oryzae.

The equipment used in Japan to conduct the fermentation is state of-the-art machinery with microprocessor or computer control to provide the optimum conditions for microbial growth and activity. The microorganisms used have been manipulated by mutagenesis to give better performance, such as better enzymatic activity to give better hydrolysis of proteinaceous matter in defatted soybean meal as well as better flavor production. In comparison the average process used in Malaysia could be considered primitive.

This disparity is attributable to a better understanding of the theoretical and practical bases of soy sauce fermentation by scientists and technicians in Japan's soy sauce factories. The old biotechnology involved in this type of traditional fermentation is well understood in Japan, and the Japanese are now able to make better use of the new biotechnology - such as the directed alteration of genetic material of the mold (Aspergillus oryzue), yeast (Saccharomyces rouxii), and bacteria (Pediococci) used in soy sauce fermentation so as to improve their fermentative qualities.

Necessary Prerequisites

For developing countries to make full use of the available biotechnologies in their traditional food fermentations, an understanding and acquisition of expertise in the following areas are essential.

Art of fermentation

A clear understanding by the master brewer of every step used in the fermentation is needed. This is the art of fermentation. Although the master brewers might not have scientific backgrounds, they could normally ensure a proper fermentation as a result of years of experience. Without a knowledge of the art of traditional food fermentation, a scientist cannot provide a scientific explanation for the process and proceed to provide assistance in improvement of the process.

Microbiology

It is essential to know which microorganisms involved in food fermentations are useful and how the physiology and metabolism of these microbes are affected by the physical and chemical environments of fermentations, as well as how their microbial activities in turn affect the fermentation processes. Microorganisms normally break down carbohydrates, proteins, and lipids present in the raw materials to be fermented by releasing enzymes into the medium. As the raw materials are hydrolyzed, the environment is changed, as sometimes reflected by a drop in pH value. Moreover, the breakdown products such as peptides and amino acids can be further converted into smaller volatile molecules that are odoriferous and hence improve the flavor characteristics of the fermented foods.

Upstream and downstream processing

Normally raw materials are pretreated before fermentation. It is important to comprehend how such pretreatment could affect the fermentation process. In soy sauce fermentation, whole soybeans are steamed to make the soy protein more easily hydrolyzable by the proteases of Aspergillus oryzae. In so doing, too much moisture is introduced and wheat flour must be added to lower the moisture content to a level that does not favor early bacterial growth and hence prevents spoilage of the fermentation.

Downstream processing does not affect the bioprocess involved. However, it could alter

the normal organoleptic properties of the product, especially when downstream processing involves heating, such as in the pasteurization of soy sauce. Heating causes a change in the flavor of soy sauce due to nonenzymic browning reactions, which could result in the production of pyrazine compounds.

Biochemistry

An understanding of the biochemical activities of the microbes actively participating in the fermentation could help to explain the change in the texture of the raw material as well as the origin of flavoring substances often present in fermented foods. Flavor and texture are important properties of fermented foods. Elucidation of flavor production in such fermentations could result in the development of processes for producing of flavoring materials by fermentation, as in the production of cheese flavors by Penicillium roquefortii.

Fermentation equipment and techniques

Practical experience in the use of both solid-state and submerged culture fermentation equipment is very useful. Normal training includes submerged culture bioreactors but not solid-state fermenters. It is useful to know both types of fermentations because traditional food fermentations often involve solid state fermentation. In soy sauce fermentation an initial solid-state fermentation is followed by a submerged fermentation step. Systems that measure and control pH, dissolved oxygen, temperature, and moisture help to make these bioprocesses more efficient and reduce the time required for production of a quality product.

CONCLUSIONS

For developing countries, future directions in applying biotechnology to traditional fermented foods should be: (1) training of a pool of technicians in the art and science of

traditional food fermentations and (2) investigations by local scientists into the scientific basis of indigenous food fermentations.

Theoretical basic science education, such as the microbiology and biochemistry of food fermentations, could be taught in schools; so could the use of modern bioreactor systems. However, the application of such biotechnological knowledge to actual commercial fermentations can come about only after a practical experience in a fermented food factory for a period of time. The approach to be taken in applying biotechnology to traditional food fermentations should be that of finding solutions to existing bioprocessing problems and not trying to find problems with newly acquired biotechniques.

Only after the old biotechniques of fermentation have been successfully used can industries in developing countries look forward to using the new biotechniques of recombinant DNA to improve the genetic constitution of the microorganisms involved.

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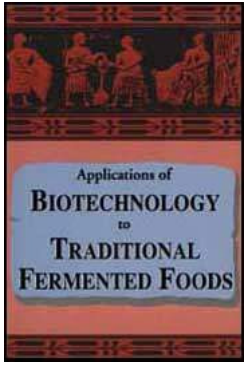

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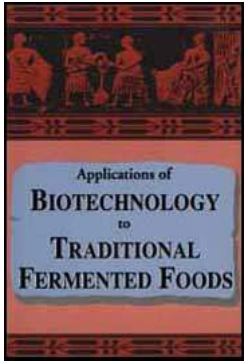

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The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competence and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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This report has been prepared by an ad hoc advisory panel of the Advisory Committee on Technology Innovation, Board on Science and Technology for International Development, Office of International Affairs, National Research Council. Staff support was funded by the Office of the Science Advisor, Agency for International Development, under Grant No. DAN-5538-G-00-1023-00, Amendments 27 and 29.

Library of Congress Catalog Card Number: 91-68331 ISBN 0-309-04685-8 S526 Printed in the United States of America

COVER DESIGN by DAVID BENNETT

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Applications of Biotechnology to Traditional Fermented Foods (BOSTID, 1992, 188 p.)

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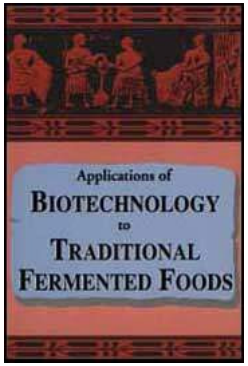

Preface

The purpose of this report is to create greater awareness of the opportunities to reduce hunger and improve nutrition in developing countries through the application of biotechnology to widely practiced methods of food preparation and preservation. The report discusses opportunities for the application of biotechnology to traditional fermented foods. Scientists from developed and developing countries describe their research in this field and provide their recommendations on priorities for future research.

Preparation of this report was coordinated by the Board on Science and Technology for International Development in response to a request from the U.S. Agency for International Development.

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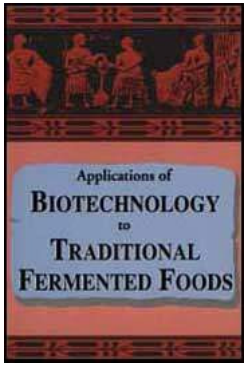
Applications of Biotechnology to Traditional Fermented Foods



Applications of Biotechnology to Traditional Fermented Foods (BOSTID, 1992, 188 p.)

I. Research priorities

Research Priorities in Traditional Fermented Foods

The Advisory Panel

Biotechnology has been described as the application of scientific and engineering principles to the processing of materials for the provision of goods and services through the use of biological systems and agents. In a very real sense, biotechnology originated with traditional food fermentations in developing countries. Over the generations, this pioneering practice has been expanded and improved so that microorganisms and other biological agents have found use in many other areas. Recent developments in genetics, enzymology, recombinant technology, and fermentation technology have led to advances in biotechnology far beyond the original traditional scope.

In many developing countries, village-art methods and age-old techniques are still used

for food processing. Developing countries appear to be neglecting the advances in biotechnology. But they cannot continue to depend on historic methods for food processing. Increasing populations, drought and other natural disasters, and inadequate food production dictate that better options for food processing be adopted. Biotechnology offers this opportunity.

Current food biotechnological research in developing countries seems largely limited to the identification of microorganisms for starter culture development. There is little research involving gene manipulation and there are few centers of operational biotechnological research. The reasons for this are obvious. Biotechnological research is capital intensive, usually in scarce foreign exchange. Also, biotechnology requires the use of sophisticated equipment and reagents backed with a consistent energy and water supply, which are often not available in developing countries. A crucial part or essential chemical - which should be no more than a telephone call away, and can be obtained, at most, overnight in industrialized countries - cannot be obtained in months or even years. Or, just when all the necessary personnel and materials are available, the electricity is cut off.

To meet the current and future challenges in developing countries, it is important that these countries develop the capabilities to benefit from biotechnological developments. Developing countries will need to acquire expertise in biotechnology through education and training. The infrastructure and equipment required for biotechnological research will need to be established. Scientists of the developing world will need to collaborate with laboratories in advanced countries in order to benefit from their knowledge and to obtain infrastructural support and funding. It is through these strategies that the earliest application of biotechnology can be enhanced through help from its heirs.

PRIORITIES

The recommended research priorities encompass four broad categories: (1) improving understanding of the fermentation processes; (2) refining of the processes; (3) increasing the utilization of the processes; and (4) developing local capabilities. In this research, special emphasis should be given to fermented products that serve as major sources of nourishment for large populations (cassava, for example), processes that reduce food loss, foods that may alleviate starvation in famine or drought, and foods for weaning and young children.

IMPROVING THE KNOWLEDGE BASE

For fermented products like cheese, bread, beer, and wine, scientific and technological knowledge of the processes is well developed. However, for traditional fermented products, this knowledge is poor. Many indigenous fermented foods are produced by spontaneous or natural fermentation, but specific microorganisms predominate. Isolation and characterization of predominant organisms is essential.

Information should be collected on all traditional fermented foods and it must be thorough. No food should be excluded because it is not important or well known. A thorough microbiological, nutritional, and technical investigation should be carried out on each of the processes. The various microorganisms involved in each fermentation should be isolated, characterized, studied, and preserved. The biotechnological worth of each organism should be determined. Isolation should not be confined to the dominant organisms because other microbes found in lower numbers might have an important function in the process. The role of each organism should be identified.

Much basic research is needed to determine the scientific and technological factors in the preparation of these traditional products. Since the qualities of fermented foods are largely controlled by the participating microorganisms, understanding their role is vital.

IMPROVING THE TECHNOLOGY

In food fermentations, raw materials are converted to products through the use of biocatalysts. Each member of this equation is important. For widely used plant substrates, for example, breeding to reduce toxic or antinutritional components, or to increase protein or vitamin content, would be useful. Alternatively or additionally, it would be valuable to identify microorganisms that can synthesize important ingredients (e.g., essential amino acids, vitamins) for populations where malnutrition is a problem. Some additional desirable traits for these microorganisms are: an ability to produce flavor components that which favor consumption of these foods in traditional and new markets; the capability to break down antinutritional factors (i.e., physic acid) present in some substrates; the production of enzymes to utilize recalcitrant wastes as substrates; the inability to synthesize toxins and other undesirable secondary products; and thermotolerance and osmotolerance, which are important characteristics in solid substrate fermentation processes.

For lactic acid bacteria used in food fermentations, physiological characteristics of acid stability, bile stability, adherence to human intestinal cells, colonization of the human intestinal tract, and antagonism to pathogenic bacteria and cariogenic bacteria (oral health) are all desirable.

The safety and shelf life of fermented products may also be improved through the development of organisms that produce alcohols, antibiotics, or other substances that can inhibit the growth of undesirable organisms.

The art of traditional processes needs to be transformed into a technology to incorporate objective methods of process control and optimization, and to standardize quality of the end products without losing their desirable attributes. Fermentations can only be optimized when conditions like time, temperature, pH, substrate pretreatment, inoculum-substrate ratio, and so forth, are controlled. Because of the surface:volume relationships, the scale-up of solid state fermentations is particularly difficult. These solid state

reactions can be valuable in reducing raw material losses.

The equipment needed for the improvement of some traditional processes can be a challenge in itself. Fermentations carried out in vessels with unusual surface characteristics such as charred wood, semi-porous clay, gourds, or the like, are difficult to replicate.

Research is also needed on the implementation of continuous fermentations using bioreactors with immobilized enzymes and cells. Research on the development of bioreactors with improved performance is required.

IMPROVING UTILIZATION

The introduction of new processes or products should take into account the sensory requirements of target social groups. Thus, the elucidation of the microbial origin of flavors in fermented foods and the relationship between microflora and the organoleptic properties of the product are imperative. Flavor and color must be generated to meet local population preferences.

The use of alternative plant materials such as triticale, oca, amaranth, and achira, which have been successfully grown in some developing countries, should be examined as substrates for fermentations. Puto is a fermented rice cake in the Philippines. In a taste test, puto in which cassava was substituted for half of the rice was preferred over pure rice puto. Acha (Digitaria exilis), a West African cereal crop also known as "fonio," and ensete (E,zsete ventricos'´m) are being tested as alternative substrates for food fermentations. A major drawback of ensat is its low protein content (1.5 percent) compared with other cereals; a plus is that it contains twice as much methionine as maize and wheat. Acha is being examined for the production of traditional porridge, beer, pasta, and even bread. Studies of these lessknown fermented products could lead to processes with minimum production cost and maximum substrate utilization, resulting in products

with improved nutritional value, extended shelf life, improved quality, and a better spectrum of essential nutrients. Inclusion of soy or other vegetable proteins could also enhance the nutritive value of many low protein foods.

The ability to use alternative substrates could also reduce problems of sporadic nonavailability of traditional starting materials. Acceptability of new products or improvement of traditional ones could be improved through the distribution of starter cultures. Some cultures are difficult to maintain in dehydrated form, and this is an important area for research. Acceptability of fermented products based on alternative raw materials may hinge on using familiar processing steps such as roasting or germination.

Research on fermentations that use wastes as raw materials has several possible benefits. The use of agroindustrial residues and other wastes to produce fermented foods and feeds can optimize indigenous resources, increase the availability of nutritious products, and reduce pollution problems.

Research is also needed on improving the economics of fermentation processes. Reducing the time necessary to pretreat raw materials or the processing time can be valuable. It would be helpful, for example, to reduce the boiling time (6 to 8 hours) of sesame seed before fermentation. Reducing fermentation time can optimize equipment use.

DEVELOPING LOCAL CAPABILITIES

Biotechnology is possible only within an infrastructure of supply companies that can provide specialized equipment and reagents. In addition, there must be a constant source of electricity for continuing experiments, and often for the air conditioning necessary for the growth of specific organisms. Developing local or regional production of commonly used enzymes would help.

Training in basic microbiology, biochemical engineering, and the new techniques of

molecular biology for personnel of less developed countries is one of the key components in improving traditional fermentation processes. In addition, developing country scientists would also benefit from opportunities for regional and international collaboration. This kind of information sharing could be facilitated through periodic seminars and workshops, through joint research programs, and through the establishment of computer networks. Each of these interactions could include scientists from industrialized countries. Centers of excellence, specializing in regionally important areas, could be established for the mutual benefit of cooperating institutions.

For large-scale fermentations, developing countries should give higher priority to industrializing appropriate indigenous processes, rather than importing the technology of the industrialized world. This imported technology often relies on imported crops or crops not well suited to the climate or soils of the country.

In modernizing the production of traditional fermented foods at the village level, appropriate and affordable technology should be emphasized. Process changes should take into account the role of the poor who originated and preserved the processes and how they will benefit from the modifications.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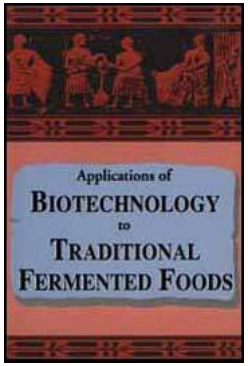

Applications of Biotechnology to Traditional Fermented Foods (BOSTID, 1992, 188 p.)

II. Overview

1 Upgrading Traditional Biotechnological Processes

M. J. R. Nout

TRADITIONAL FOOD FERMENTATION

The general aims of food technology are to exploit natural food resources as efficiently and profitably as possible. Adequate and economically sound processing, prolongation of shelf life by preservation and optimization of storage and handling, improvement of safety and nutritive value, adequate and appropriate packaging, and maximum consumer appeal are key prerequisites to achieving these aims.

Fermentation is one of the oldest methods of food processing. The history of fermented foods has early records in Southeast Asia, where China is regarded as the cradle of mold-

fermented foods, and in Africa where the Egyptians developed the concept of the combined brewery-bakery. The early Egyptian beers were probably quite similar to some of the traditional opaque sorghum, maize, or millet beers found in various African countries today (1).

In technologically developed regions, the crafts of baking, brewing, wine making, and dairying have evolved into the large-scale industrial production of fermented consumer goods, including cheeses, cultured milks, pickles, wines, beers, spirits, fermented meat products, and soy sauces.

The introduction of such foreign "high-tech" fermented products to tropical countries by early travelers, clergymen, and colonists was followed by an accelerated demand during the early postindependence period. Their high price ensured status, and their refined quality guaranteed continued and increasing consumption.

In contrast, many of the traditional indigenous foods lack this image; some may even be regarded as backward or poor people's food. Factors contributing to such lack of appeal include inadequate grading and cleaning of raw materials, crude handling and processing techniques, and insufficient product protection due to lack of packaging. Such unhygienic practices are easily translated into a fear of food-borne diseases. From a nutritionist's point of view, many traditional starchy staples are deficient in energy, protein, and vitamins. Variable sensory characteristics (quality) and lack of durability (shelf life) reduce convenience to the consumer: time needs to be spent selecting products of adequate quality, whereas perishable products require frequent purchasing and result in increased wastage. In addition, ungraded heterogenous products, inconvenient unpacked bulk foods, or unattractive presentation inhibit consumers to develop regular purchasing attitudes.

The contrast outlined here serves as a general guideline to the major targets for upgrading

the present status of traditional indigenous fermented foods. The latter are part of the regional cultural heritage; they are well known and accepted by consumers and consequently provide an appropriate basis for development of a local food industry, which not only preserves the agricultural produce but also stimulates and supports agroindustrial development.

DECENTRALIZED SMALL-SCALE PROCESSES

In most African countries, 70 percent or more of the population lives in rural areas. However, if the present trend in urbanization continues (urban growth rates of 5 to 10 percent annually), 50 percent of the African population will be living in cities by the year 2000. Governments become increasingly aware that rural industrialization is a worthwhile investment because it creates job opportunities, improves agricultural productivity, and helps to check urbanization. But even at the present urbanization rate, a rapidly increasing low-income population will be located in urban areas. The resultant uncoupling in place and time of primary production and food consumption necessitates the manufacture of wholesome, low-cost, nutritious products that can withstand low-hygiene handling.

Agro-allied industries are closely linked to regions of primary production, and it is particularly in the field of food processing, with low-cost perishable raw materials, that establishment of a rural network of small-scale processing facilities is most appropriate. Home- or village-scale enterprises require only modest capital investment, which should be made available on a "soft loan" basis. Against this background, some basic process improvements that increase the appeal of traditional fermented foods and that can be carried out by simple means will be outlined (2).

BASIC PROCESSING OPERATIONS

In food manufacturing several operations are required to prepare raw materials, handle and process them into products, and finally prepare the finished product for distribution

and sale by preservation and/or packaging. One might think of sorting, grading, cleaning, disinfection, grinding, or packaging. The establishment and success of some indigenous enterprises in Nigeria and Kenya show that the appeal and marketability of such products as beans, peas, gari, and spices, formerly sold in bulk, increase significantly when they have "only" been sorted, cleaned, graded, sometimes ground, labeled, and packaged in simple polythene bags.

NUTRITIVE VALUE

The nutritive value of traditional fermented foods needs improvement. The energy density of starch-based porridges is inadequate, particularly when used for weaning purposes. Root crop- or cereal-derived products have rather low protein contents, and the quality of their protein is limited by the amount of lysine present. Various antinutritional factors, including polyphenols, physic acid, trypsin inhibitors, and lectins, are present in legumes and cereals.

Composite products (legume additions to starchy staples) offer an opportunity to improve protein quantity and quality. Combinations of simple unit operations, including roasting, germination, and fermentation, afford increased energy density in porridges and reduce antinutritional factors considerably (3).

STABILIZATION OF NATURAL FERMENTATIONS BY INOCULUM ENRICHMENT

Most traditional fermented products result from natural fermentations carried out under nonsterile conditions. The environment resulting from the chemical composition of the raw materials, fermentation temperature, absence or presence of oxygen, and additives such as salt and spices causes a gradual selection of microorganisms responsible for the desired product characteristics.

The main advantage of natural fermentation processes is that they are fitting to the rural

situation, since they were in fact created by it. Also, the consumer safety of several African fermented foods is improved by lactic acid fermentation, which creates an environment that is unfavorable to pathogenic Enterobacteriaceae and Bacillaceae.

In addition, the variety of microorganisms present in a fermented food can create rich and full flavors that are hard to imitate when using pure starter cultures under aseptic conditions.

However, natural fermentation processes tend to be difficult control if carried out at a larger scale; moreover, the presence of a significant accompanying microflora can accelerate spoilage once the fermentation is completed. Particularly with increased holding periods between product fermentation and consumption when catering for urban markets, uncontrolled fermentations under variable conditions will cause unacceptable wastage by premature spoilage.

Techniques to stabilize fermentations operating under nonsterile conditions would therefore be appropriate in the control of natural fermentations. For this purpose the use of pure culture starters, obtained either by laboratory selection procedures or genetic engineering, offers no realistic solutions because they are expensive and require sterile processing conditions. A more feasible approach is to exploit the ecological principle of inoculum enrichment by natural selection. This can be achieved by the sourdough process, in which some portion of one batch of fermented dough is used to inoculate another batch. This practice is also referred to as "back-slopping" or inoculum enrichment. The resulting starters are active and should not be stored but used in a continuous manner.

Sourdoughs from commercial sources, having been maintained by daily or weekly transfers during 2 or more years, contain only two or three microbial species, although they are exposed to a wide variety of potential competitors and spoilage-causing microorganisms each time the sourdough is mixed with fresh flour for a transfer. It can

take as long as 10 weeks of regular transfers before a sourdough population becomes stabilized. Such populations could contain a yeast, Saccharomyces exiguous, and one or two Lactobacillus species, namely Lb. brevis var. linderi II and Lb. sanfrancisco. Although the mechanism of the stable coexistence of sourdough populations is not yet fully understood, lack of competition for the same substrate might play an important role. Other factors besides substrate competition, such as antimicrobial substances produced by lactic acid bacteria, might play an important role in the stability of such stable populations, obtained by "back-slopping" (4).

Similar experiments in the field of tempe manufacture showed that the first stage of the tempe process - soaking of soybeans - can be rendered more predictable in terms of acidification of the beans, by simple inoculum enrichment. Depending on soaking temperatures, stable soaking water populations were obtained after 30 to 60 daily transfers, containing Leuconostoc spp. at 14° and 19°C, yeasts and Lactobacillus spp. at 25 C, Lactobacillus spp. at 30 C, or Pediococcus and Streptococcus spp. at 37° and 45°C. Tempe made with well-acidified beans contained fewer undesirable microorganisms and was more attractive (5).

Based on the same principle of inoculum enrichment, the intrinsic microbiological safety of composite meals of cereals and legumes can be improved significantly by lactic fermentation (6). This offers interesting possibilities in the manufacture of food for vulnerable consumer groups, such as infants, malnourished patients, and the elderly (7).

Although development of such gradually evolved and stable fermentation starters will be an attractive proposition for use in small-scale fermentations under nonsterile conditions, they will not be the most appropriate in all cases. This is exemplified by the sauerkraut (lactic acid fermented cabbage) fermentation, during which flavor development is determined by a succession of Leuconostoc and Lactobacillus species occurring during the course of the fermentation. Practical experience in the sauerkraut industry in the

Netherlands has shown that carryover of previous sauerkraut into a fresh batch of cabbage will cause a rapid domination of homofermentative Lactobacillus spp., which should normally only dominate during the final stage of fermentation. The result is an excessively sour-tasting product that lacks the flavor otherwise produced by the heterofermentative Leuconostoc and Lactobacillus spp.

In the exercise of upgrading traditional food fermentation techniques, it would therefore be worthwhile to investigate the effect of inoculum enrichment on product characteristics and consumer acceptance.

MULTISTRAIN DEHYDRATED STARTER

A different tool to stabilize fermentations under nonsterile conditions is the use of multistrain dehydrated starters, which can be stored at ambient temperatures, enabling more flexibility. Such homemade starters are widely used in several Asian food fermentations. Examples are the manufacture of tempe (mainly from soybeans) and tape (from glutinous rice or cassava). Indonesian traditional tempe starters (usar) are essentially molded hibiscus leaves that carry a multitude of molds, dominated by Rhizopus spp., including the Rh. oryzae and Rh. microsporus varieties. Instead of using usar, Indonesian tempe production is increasingly carried out with factory-prepared "pure" starters consisting of granulated cassava or soybean fiber carrying a mixed population of Rhizopus species (5). These starters are more homogenous and their dosage is convenient, but because they are manufactured under nonsterile conditions, some are heavily contaminated with spoilage-causing bacteria and yeasts. This requires quality monitoring of the inoculum and of the fermentation process in which it is used.

Other examples of durable home-prepared starter materials used in Asian food fermentations are Indonesian ragi and Vietnamese men tablets (8). Depending on their specific purpose, these dehydrated tablets, prepared from fermented rice flour, contain

mixed populations of yeasts, molds, and bacteria. Ragi tablets can be stored up to 6 months and constitute a convenient starter material for application in home and small-scale industrial fermentations of rice or cassava, for example.

Especially in the fermentation of neutral pH, protein-rich substrates, such as legumes, one should be extremely careful with the use of substandard inoculum. If the process lacks factors that control microbial development, pathogens may survive or produce toxins in such products. Tempe manufacture is a good example of a process with intrinsic safety. The preliminary soaking of the beans results in an acidification that inhibits the multiplication of bacterial contaminants during the mold fermentation stage. Also, antimicrobial substances of Rhizopus oligosporus would play a protective role against outgrowth of several genera of microorganisms. Moreover, near-anaerobic conditions and microbial competition during the fermentation stage, and the usual cooking or frying of tempe prior to consumption, strongly reduce the chances of food-borne illness (5).

Nevertheless, the introduction of fermentation processes in regions where they are not traditionally mastered requires adequate guidance, supervised processing, and monitoring of product safety.

ENZYME PRODUCTION BY KOJI TECHNIQUE

Not only microorganisms but also enzymes play an important role in the manufacture of traditional fermentation processes. In cassava processing the naturally occurring enzyme linamarase is able to degrade potentially toxic cyanogenic glycosides (e.g., linamarin). This enzymatic detoxification has always been an integral part of traditional cassava fermentations, such as in gari and lafun. Under certain conditions the detoxification of linamarin is accelerated by linamarase addition (9). It is conceivable that there will be commercial applications for the enzymatic process of linamarin decomposition, which could be used to detoxify cassava without having to ferment it; the result would be a

neutral and bland-flavored product.

Enzyme sources for African traditional beer brewing are mostly germinated sorghum and millet varieties, whereas sorghum and millet malts possess adequate diastatic power with alfa-amylase, resulting in poor conversion of dextrins into maltose (10). The availability of cheap technical-grade beta-amylase preparations could lead to the development of novel brewing processes utilizing home-grown starch sources instead of imported barley malt.

In East Asia, koji is used as a source of enzymes in the manufacture of soy sauce and rice wine. Koji is obtained by solid-substrate fermentation of cereals or soybeans with fungi (e.g., Aspergillus oryzae and Asp. soyae). Depending on the particular substrate to be degraded, selected strains of molds are used, often as mixed cultures. Their enzymes include amylases, proteases, and cellulolytic enzymes. During fermentation the enzymes are accumulated into the koji. The enzymes produced are subsequently extracted from the koji using brine solutions. Koji fermentations are carried out in East Asia at a small home scale, as well as in the large-scale industrial manufacture of soy sauce and rice wine (11). Although mycotoxin-producing molds such as Aspergillus flavus and Asp. parasitious occur in koji as natural contaminations, they have not been observed to produce aflatoxins under the given conditions.

The principle of fungal solid-substrate fermentation may be used to prepare enzyme concentrations for conversion of starch, detoxification of cyanogenic glycosides, and other applications.

DRY MATTER BALANCE

Food fermentation is advantageously used for food preservation and to obtain desirable flavor and digestibility. However, some processes are rather wasteful. For instance, prolonged soaking and microbial respiration of organic matter may lead to considerable losses of valuable raw material dry matter. Examples can be found in the traditional

process of ogi manufacture (fermented maize cake) and the tempe process, during which up to 30 percent of the raw material may be lost by leaching during soaking steps. Encouraging research has been carried out by Banigo et al. (12) in the field of Nigerian ogi manufacture, aimed at reducing these raw material losses by omitting soaking stages. It would certainly be worthwhile to investigate dry matter balances of traditional fermentations with a view to reducing losses of raw material by implementing "dry" instead of "wet" processing.

IMPLEMENTATION

No matter how much research is carried out on improved traditional processes or novel products, the ultimate aim is implementation.

Unfortunately, a wide gap exists between research data published in scientific journals and the practice of food processing. Much attention should be given to the extent of usefulness of new products to the end user. To this effect, not only should the sensory, nutritional, and other quality characteristics of newly developed products or processes be taken into account, but they should also be integrated with sound price calculations, market surveys, and extension efforts. Only a competitive process has good chances of being implemented.

In conclusion, the importance of a business-oriented approach and close contact between researchers and food processors, working together toward mutual benefit, must be stressed.

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2 Genetic Improvement of Microbial Starter Cultures

Susan K. Harlander

Fermentation has been used for preserving food for hundreds of years and virtually every culture has, as part of its diet, a variety of fermented milk, meat, vegetable, fruit, or cereal products. Microorganisms, including bacteria, yeasts, and mold, produce a wide range of metabolic end products that function as preservatives, texturizers, stabilizers, and flavoring and coloring agents. Several traditional and nontraditional methods have been used to improve metabolic properties of food fermentation microorganisms. These include mutation and selection techniques; the use of natural gene transfer methods such as transduction, conjugation and transformation; and, more recently, genetic engineering. These techniques will be briefly reviewed with emphasis on the advantages and disadvantages of each method for genetic improvement of microorganisms used in food fermentations.

TRADITIONAL GENETIC IMPROVEMENT STRATEGIES

Mutation and Selection

In nature, mutations (changes in the chromosome of an organism) occur spontaneously at very low rates (one mutational event in every 10e6 to 10e7 cells per generation. These mutations occur at random throughout the chromosome, and a spontaneous mutation in a metabolic pathway of interest for food fermentations would be an extremely rare event.

The mutation rate can be dramatically increased by exposure of microorganisms to mutagenic agents, such as ultraviolet light or various chemicals, which induce changes in the deoxyribonucleic acid (DNA) of host cells. Mutation rates can be increased to one mutational event in every 10e1 or 10e2 cells per generation for auxotrophic mutants, and one in 10e3 to 10e5 for the isolation of improved secondary metabolite producers. A method of selection is critical for effective screening of mutants as several thousand individual isolates may need to be evaluated to find one strain with improved activity in the property of interest.

Mutation and selection techniques have been used to improve the metabolic properties of microbial starter cultures used for food fermentations; however, there are severe limitations with this method. Mutagenic agents cause random mutations, thus specificity and precision are not possible. Potentially deleterious undetected mutations can occur, since selection systems may be geared for only the mutation of interest. Additionally, traditional mutation procedures are extremely costly and time-consuming and there is no opportunity to expand the gene pool. In spite of these limitations, mutation and selection techniques have been used extensively to improve industrially important microorganisms and, in some cases, yields of greater than 100-times the normal production level of bacterial secondary metabolites have been achieved.

Natural Gene Transfer Methods

The discovery of natural gene transfer systems in bacteria has greatly facilitated the understanding of the genetics of microbial starter cultures and in some cases has been used for strain improvement. Genetic exchange in bacteria can occur naturally by three different mechanisms: transduction, conjugation, and transformation.

Transduction

Transduction involves genetic exchange mediated by a bacterial virus (bacteriophage).

The bacteriophage acquires a portion of the chromosome or plasmid from the host strains and transfers it to a recipient during subsequent viral infection. Although transduction has been exploited for the development of a highly efficient gene transfer system in the gram-negative organism Escherichia coli, it has not been used extensively for improving microorganisms used in food fermentations. In general, transduction efficiencies are low and gene transfer is not always possible between unrelated strains, limiting the usefulness of the technique for strain improvement. In addition, bacteriophage have not been isolated and are not well characterized for most strains.

Conjugation

Conjugation, or bacterial mating, is a natural gene transfer system that requires close physical contact between donors and recipients and is responsible for the dissemination of plasmids in nature. Numerous genera of bacteria harbor plasmid DNA. In most cases, these plasmids are cryptic (the functions encoded are not known), but in some cases important metabolic traits are encoded by plasmid DNA. If these plasmids are also self-transmissible or mobilizable, they can be transferred to recipient strains. Once introduced into a new strain, the properties encoded by the plasmid can be expressed in the recipient. The lactic acid bacteria naturally contain from one to more than ten distinct plasmids, and metabolically important traits, including lactose-fermenting ability, bacteriophage resistance, and bacteriocin production, have been linked to plasmid DNA. Conjugation has been used to transfer these plasmids into recipient strains for the construction of genetically improved commercial dairy starter cultures.

There are some limitations in the application of conjugation for strain improvement. To exploit the use of conjugative improvement requires an understanding of plasmid biology and, in many cases, few conjugative plasmids encoding genes of interest have been identified or sufficiently characterized. Conjugation efficiencies vary widely and not all strains are able to serve as recipients for conjugation. Moreover, there is no opportunity

to expand the gene pool beyond those plasmids already present in the species.

Transformation

Certain microorganisms are able to take up naked DNA present in the surrounding medium. This process is called transformation and this gene transfer process is limited to strains that are naturally competent. Competence-dependent transformation is limited to a few, primarily pathogenic, genera, and has not been used extensively for genetic improvement of microbial starter cultures. For many species of bacteria, the thick peptidoglycan layer present in gram-positive cell walls is considered a potential barrier to DNA uptake. Methods have been developed for enzymatic removal of the cell wall to create protoplasts. In the presence of polyethylene glycol, DNA uptake by protoplasts is facilitated. If maintained under osmotically stabilized conditions, transformed protoplasts regenerate cell walls and express the transformed DNA. Protoplast transformation procedures have been developed for some of the lactic acid bacteria; however, the procedures are tedious and time-consuming, and frequently parameters must be optimized for each strain. Transformation efficiencies are often low and highly variable, limiting the application of the technique for strain improvement.

Electroporation

The above mentioned gene transfer systems have become less popular since the advent of electroporation, a technique involving the application of high-voltage electric pulses of short duration to induce the formation of transient pores in cell walls and membranes. Under appropriate conditions, DNA present in the surrounding medium may enter through the pores. Electroporation is the method of choice for strains that are recalcitrant to other gene transfer techniques; although optimization of several parameters (e.g., cell preparation conditions, voltage and duration of the pulse, regeneration conditions, etc.) is still required.

GENETIC ENGINEERING

Genetic engineering provides an alternative method for improving microbial starter cultures. This rapidly expanding area of technology provides methods for the isolation and transfer of single genes in a precise, controllable, and expedient manner. Genes that code for specific desirable traits can be derived from virtually any living organism (plant, animal, microbe, or virus). Genetic engineering is revolutionizing the science of strain improvement and is destined to have a major impact on the food fermentation industry.

Although much of the microbial genetic engineering research since the advent of recombinant DNA technology in the early 1970s has focused on the gram-negative bacterium Escherichia coli, significant progress has been made with the lactic acid bacteria and yeast. Appropriate hosts have been identified, multifunctional cloning vectors have been constructed, and reliable, high-efficiency gene transfer procedures have been developed. Further, the structural and functional properties, as well as the expression in host strains, of several important genes have been reported. Engineered bacteria, yeast, and molds could also be used for the production of other products, including food additives and ingredients, processing aids such as enzymes, and pharmaceuticals.

Prerequisites

Metabolism and Biochemistry of the Host

A necessary prerequisite for the application of genetic engineering to any microorganism is a fundamental understanding of the metabolism and biochemistry of the strain of interest. Although for hundreds of years the metabolic potential of microbial starter cultures has been exploited, in many cases little is known about specific metabolic pathways, the regulation of metabolism, or structural and functional relationships of critical genes involved in metabolism. This information is essential for the design of genetic improvement strategies, as it provides the rationale for selection of desirable

gene(s) and assures that once inserted into a new host, the gene(s) will be appropriately expressed and regulated as predicted.

Transformable Hosts

Plasmid-free, genetically characterized and highly transformable hosts, coupled with multifunctional expression vectors, provide the necessary tools for transfer, maintenance, and optimal expression of cloned DNA in microbial starter cultures. Many microbial starter cultures harbor plasmid DNA, and although most plasmids remain cryptic, resident plasmids interfere with identification of plasmid-containing transformants. Use of plasmid-free hosts also eliminates plasmid incompatibility problems and the possibility of cointegrate formation between transforming and endogenous plasmids. It is important to note that plasmid-free strains are used for the development of model systems; however, ultimately it will be necessary to engineer commercial strains.

Vector Systems

A vector can be defined as a vehicle for transferring DNA from one strain to another. Plasmids are frequently used for this purpose because they are small autonomously replicating circular DNA forms that are stable and relatively easy to isolate, characterize, and manipulate in the laboratory. Native plasmids do not naturally possess all of the desirable features of a vector (e.g., multiple cloning sites, selectable marker(s), ability to replicate in several hosts, and so forth). Therefore, genetic engineering is frequently used to construct multifunctional cloning vectors. Although antibiotic resistance markers greatly facilitate genetic engineering in microbial systems, vectors derived solely from food-grade organisms may be critical in obtaining regulatory approval for use of the organisms, as antibiotic resistance determinants may not be acceptable in food systems.

An alternative vector strategy involves the development of linear fragments of DNA that are capable of integrating into the host chromosome via homologous recombination.

Although transformation frequencies are very low, the advantage of the integrative vector is that transformed genetic information is targeted to the chromosome where it will be more stably maintained. Insertion sequences (IS elements) naturally present in the chromosome that can transpose chromosomal DNA to plasmids could be used as an alternative strategy for developing integrative vectors for some strains of lactic acid bacteria.

Efficient Gene Transfer Systems

Once gene(s) have been identified and cloned into the appropriate vector in the test tube, they must be introduced into a viable host. Since the recombinant DNA is a naked DNA molecule, gene transfer systems based on protoplast transformation and electroporation are most applicable in genetic engineering experiments. High transformation efficiencies (greater than 104 to 105 transformants per kilogram of DNA) greatly facilitate screening and identification of appropriate transformants. Electroporation is the transformation procedure of choice for most microbial strains.

Expression Systems

Transfer of structural genes to a new host using genetic engineering does not guarantee that the genes will be expressed. To optimize expression of cloned genes, efficient promoters, ribosome-binding sites, and terminators must be isolated, characterized, and cloned along with the gene(s) of interest. Identification of signal sequences essential for secretion of proteins outside the cell may be useful for situations where microbial starter cultures are used to produce high-value food ingredients and processing aids. Secretion into the medium greatly facilitates purification of such substances.

Properties of Interest

Several properties could be enhanced using genetic engineering. For example, bacteriocins

are natural proteins produced by certain bacteria that inhibit the growth of other often closely related bacteria. In some cases, these antimicrobial agents are antagonistic to pathogens and spoilage organisms commonly found as contaminants in fermented foods. Transfer of bacteriocin production to microbial starter cultures could improve the safety of fermented products.

Acid production is one of the primary functions of lactobacilli during fermentation. Increasing the number of copies of the genes that code for the enzymes involved in acid production might increase the rate of acid production, ensuring that the starter will dominate the fermentation and rapidly destroy less-aciduric competitors.

Certain enzymes are critical for proper development of flavor and texture of fermented foods. For example, lactococcal proteases slowly released within the curd are responsible for the tart flavor and crumbly texture of aged cheddar cheese. Cloning of additional copies of specific proteases involved in ripening could greatly accelerate the process.

An engineered Saccharomyces cerevisiae (baker's yeast), which is more efficient in leavening of bread, has been approved for use in the United Kingdom and is the first strain to attain regulatory approval. This strain produces elevated levels of two enzymes, maltose permease and maltase, involved in starch degradation.

Limitations

There are a number of issues that must be resolved before genetically engineered starter cultures could be used in food. Engineered strains will need to be approved for use by appropriate regulatory agencies. To date, no engineered organisms have been approved in the United States, and specific criteria for approval have not been established by the Food and Drug Administration.

The public must be assured that the products of biotechnology are safe for consumption.

If consumers have the perception that the products are not safe, the technology will not be utilized. Although genetic engineering is probably safer and more precise than strain-improvement methods used in the past, most U.S. consumers are not aware of the role of bacteria in fermented foods and do not have a fundamental understanding of recombinant DNA technology, and they may be unwilling to accept the technology. This may be less of a problem in developing countries where improved microbial starter cultures could provide significantly safer and more nutritious foods with longer shelf life and higher quality.

Another limitation is that genetic improvement of microbial starter cultures requires sophisticated equipment and expensive biological materials that may not be available in developing countries. Where equipment and materials are available in industrialized countries, there may be little incentive for researchers to improve strains that would probably not be used in their own countries.

Genetic improvement of microbial starter cultures is most appropriate for those fermentations that rely solely or primarily on one microorganism. In many cases, our knowledge about the fermentation is limited, making selection of the target strain very difficult. Since many food fermentation processes are complex and involve several microorganisms, genetic improvement of just one of the organisms may not improve the overall product.

3 Sudan's Fermented Food Heritage

Hamid A. Dirar

If we accept the idea that Africa is the birthplace of Man, it would seem logical that the first human or humanoid to consume a fermented food would have lived there. That fermented product could have been a piece of meat or some kind of berry picked up or stored by a hunter-gatherer. Later, and after those early men, or rather women, developed

the taste for such goods they began to intentionally store fresh food items to undergo spontaneous fermentation.

Should this be the case, one would expect to find in Africa today a diverse array of fermented food products. Unfortunately, we know very little about African fermented foods because no genuine attempt has been made by any African scientist to document all the fermented foods of his or her country.

For at least one African country, the Sudan, I set out 6 years ago to collect, confirm, reconfirm, sift, and classify information on all fermented foods in the country. The major source of information was the elderly rural women of Sudan. The list of fermented foods and beverages, which now includes 60 different items, will make the basis for a book that should be ready for publication within a year. In the following sections I discuss some of the important aspects that came out of this personal initiative, which was not in any way sponsored by any agency, except perhaps some help from Band Aid of Britain.

FERMENTED FOODS

The Sudanese seem to bring just about anything edible or barely edible to the forge of the microbe, to the extent that one could confidently say: food in Sudan is fermented. The raw materials to be fermented include the better-known products such as sorghum, millet, milk, fish, and meat. Also, a number of unorthodox raw materials are fermented: bones, hides, skins, hooves, gall bladder, fat, intestines, caterpillars, locusts, frogs, and cow urine.

The bulk of these foods is poured into the bowl of sorghum porridge' being either a sorghum (or millet) staple or its sauce and relish. The few remaining ones are alcoholic or nonalcoholic beverages, the most important of which are prepared from sorghum. In other words, every fermented food item orbits around the sorghum grain.

Sorghum-Based Foods

Sorghum fermented foods and drinks are the most sophisticated and are prepared by the most complicated procedures. Compared with similar sorghum products of Africa and indeed of the whole world, the Sudan's sorghum products stand out as unique in many respects:

· The Sudan seems to have the greatest number of fermented sorghum products. There are about 30 such products that are basically different from one another.

· There is a wide use of sorghum malt in the preparation of food and drink. Throughout Africa sorghum malt is more commonly used in the preparation of beers. In Sudan, however, while malt is used in three major beer types, it is also used to make some seven solid food products. This situation does not seem to hold true for other African countries, judging by the literature.

· The making of bread-type foods from sorghum is not common in Africa. The Sudan, however, has about 12 sorghum breads (discs, sheets, flakes). Close scrutiny of these breads reveals an influence from the Middle East; some of these breads carry names and are prepared by methods used for similar products in the Arab World.

· A comparison of the procedures followed in the preparation of some sorghum food products in Sudan with procedures for making similar products in other African countries suggests that the art of making these products traveled from Sudan to West Africa and perhaps to East Africa, too. In some cases the product travelled carrying the same Arabic-Sudanese name.

This suggests that sorghum food culture is more ancient than in other areas of Africa, and this food evidence may be taken to strengthen previous hypotheses that the origin of sorghum domestication is somewhere in northeast Africa.

Dairy Products

The most common fermented milk product of Sudan is rob. Milk is fermented overnight, and the resulting sour milk is churned to give butter; the remaining buttermilk is rob. The principal aim behind rob production is the need to facilitate the extraction of butter from the milk. The butter (furssah) is later boiled to give butter oil or ghee, which can be stored for use in the lean season. Rob production is in the hands of animal-owning nomadic tribes, and the bulk of it is produced during the rainy season (July-October). Huge amounts of rob are thrown away during this season as useless after the butter has been removed. Some women, however, allow the souring process to proceed further after butter extraction until the curd is separated from the whey. They then collect the curd and sun dry it to give a kind of granular cheese called kush-kush that is turned into sauce for sorghum porridge in later months.

Another kind of sour milk is fermented camel milk, called gariss.

This is probably the only fermented food product invented by men. Gariss is prepared by camel boys who depend on it as their major nourishment when they roam with their herds into remote areas. The milk is fermented in a skin bag hitched to the saddle of a camel that is allowed to go about its business as usual - grazing, sleeping, walking, trotting, etc. This product, unlike rob, is fermented for consumption and no butter is removed from it.

A third indigenous dairy product is biruni, also called leben-gedim, which is a fermented unchurned milk ripened for up to 10 years! A related product, but not ripened, is mish, which is made by prolonged fermentation to the extent that maggots thrive in it. The product is consumed whole, with the maggots included. These two products are closely related to Egyptian mish (1).

Dairy products that have entered the Sudan from Egypt within the last century are jibnabeida (white cheese), zabadi (yogurt), and black cumin-flavored mish. These

products are strictly confined to urban communities, where the Egyptian influence is more strongly felt.

Fish Products

Southeast Asia takes all the fame in the literature concerning the production of fermented fish products. But if one sorts out all the various products of that corner of the world carrying a confusing array of names, one finds that the products boil down to four major categories: sauces, pastes, dried fish, and whole salted fish. These four types of fermented fish products are also found in the Sudan, only they are all prepared from freshwater Nile fish. This situation has not been reported for other African or Arab countries. The Sudanese fish products include kejeik (large sun-dried split fish); fessiekh (salted fermented whole tiger fish); mindeshi (pounded small fish paste, fermented, and may be dried later); and terkin or meluha (fermented fish sauce or paste - not dried).

Meat Products

While some urban people in Sudan make very thin strips of red beef and dry them in the sun to give shermout, the traditional rural product is a truly fermented one. Thick strips of fat-bearing meat are hung on a rope indoors and left to undergo fermentation and slow drying to give a proteolytic product, shermout.

The Sudanese also ferment the sheath of fat surrounding the stomach to give the strongest-smelling product of all, miriss. Others ferment the small intestines to give musran. The clean small intestines may also first be sun dried together with strips of the lungs, heart, kidneys, liver, etc., and then all pounded together and mixed with some potash and molded into a fist-sized ball and allowed to slowly ferment and dry, to give twini-digla. The large intestine is cleaned and stuffed with fat and hung to ferment and dry for a month, to give the sausage called skin.

Beirta is prepared from he-goat meat. Small pieces of muscle meat, lungs, kidneys, liver, heart, etc., are mixed with milk and salt, packed into a clay pot, and allowed to undergo some sort of pickling, presumably.

Um-tibey is best prepared from gazelle's meat. The rumen is carefully emptied and then stuffed with the vertebrae of the neck, cut-up heart, kidneys, liver, etc. The rumen is next tied and hung high to undergo fermentation. The whole thing may then be cooked by burying it in hot ashes and embers.

Fresh bones may be fermented in a number of ways. The large bones, with pieces of attached meat and tendons, may simply be thrown on a thatched roof to ferment slowly for weeks or even months to give the product called adum (bone). The meshy ball bone endings of the ball and socket joints may be pounded fresh and fermented into a paste called dodery. The vertebrae of the backbone may be chopped into smaller pieces that are sun dried, pounded with stones, mixed with a little water and salt, molded into a ball, and allowed to ferment and dry to give kaidu-digla (bone ball).

The fresh hide, skin, or hoof may be buried in mud or moist ash to undergo fermentation. The fermented product can then be cut into strips or pieces and sun dried and stored. The gall bladder is removed full with its gall juice. Some sorghum flour or grains are added to the juice to absorb it and then hung to undergo slow drying. The product, itaga, is later pounded into a sort of spice usually consumed with fatty meat dishes.

Vegetable Products

A number of fermented vegetable products are produced in rural Sudan. Interestingly, these products can be grouped into either meat substitutes or sour milk (rob) substitutes, the two major flavors of sauces in the country. Kawal (2,3) is the major meat substitute. It is a strong-smelling product derived by fermentation of the pounded green leaves of the wild legume Cassia obtusifolia, which grows during the rainy season. The product is used

in the preparation of sauces to completely replace meat or for use as a meat extender. Its protein is of high quality, rich in the sulfur amino acids. Furundu, a similar meat substitute, is prepared from the seeds of red sorrel Hibiscus sabdariffa. Sigda is another meat substitute and is prepared by fermentation of sesame oilseed presscake. All these products are dried after fermentation in the form of hard, irregular, small balls and may keep for a year or so. Other ill-defined but related products are kerjigil (from a mixture of pumpkins, sesame, and cowpea) and teshnuti (from okra seeds).

Sour milk (rob) substitutes are made from oil-bearing seeds in a manner analogous to the use of soybeans to give dairy product analogs. Rob-heb is made from the seeds of the watermelon. Rob-ful is made from peanuts. In either case the seeds are pounded into a paste that is allowed to undergo a souring fermentation. When mixed with water and turned into sauce the product has the color (off white) and taste (sour) of the sour milk sauce called mulah-rob. A related product is um-zummatah, obtained by the souring fermentation of watermelon juice. The same name is sometimes given to the sour steep water, also called mayat-aish, of fermented whole sorghum or millet grain.

Alcoholic Products

Opaque beers are commonly brewed in Africa but procedures vary. The brewing of merissa in Sudan is probably the most complicated and advanced of all (4,5). The unique features of this brewing method include the use of only a small amount (5 percent) of sorghum malt as an enzyme preparation, rather than a substrate. Malt constitutes 25 to 100 percent of the substrate in the brewing of most African and European beers. Another unique feature is the use of a caramelized sorghum product, called surij, in the process. Third, there is a special starter activation step during the process that is lacking from other African brewing procedures. Also, the brewer women seem to be aware of the properties of enzymes and microbes as well as those of the acids produced during fermentation. This explains the unique treatment of the substrate, where parts of it are half cooked, others

fully cooked, and yet others overcooked to meet enzyme requirements for a mixture of raw and gelatinized starch and to effect sterilization of products when needed. The merissa process has been well recognized as a complex process that deserves further investigation.

Clear beers are not common in Africa, and the literature gives reports only on otika of Nigeria and amgba of Cameroon (6,7). The Sudan has a clear sorghum (or millet) beer called assaliya (or um-bilbil). A look at the production of these three beers reveals that the assaliya process, involving some 40 steps, is far more complicated than the otika or amgba procedures, which involve fewer than 20 steps. It is suggested that the art of brewing clear beers traveled to West Africa from Sudan. Amgba of Cameroon is even called bilbil.

In Sudan there are perhaps 30 to 50 opaque beer types with different but related brewing methods. The area seems to be a center of diversity of sorghum beers, and perhaps the art of brewing of opaque beers traveled to East Africa from this region.

The traditional wines of Sudan are the date wines. The palm wine of West Africa is not known in Sudan - nor is lagmi, the wine obtained by fermentation of the sap of the date palm as practiced in northwest Africa. Only the fruit of the date palm is fermented in the Sudan, and the bulk of wines thus made are produced and consumed in the Northern Province where most of the date palms exist. At least 10 different date wines are produced, the most important of which are sherbot, nebit, and dakkai (8).

In the Southern Sudan a kind of mead is produced by fermentation of diluted wild bee's honey. The product, called duma, is primed by a specially prepared starter culture called duma-grains (iyal-duma).

FERMENTED FOODS AND SURVIVAL STRATEGIES

A careful examination of fermented food products of Sudan would immediately suggest a close link between food fermentation and food shortage in this part of the world. First, about 80 percent of these foods, particularly the marginal ones using bones, intestines, fat, etc., are found in western Sudan in the Kordofan and Darfur regions, the traditional famine areas. Second, most of the foods are preserved by both fermentation and drying, which means that they are intended for long storage and that food shortages or even famine are anticipated. In other words, the inventors of such foods have the experience of repeated famines.

Further, practically all fermented sauce ingredients are produced during the late months of the rainy season, which shows that, unless a person secures all of his or her food requirements from this short season, he or she will probably suffer greatly in the remaining 9 months of the year. The harsh environment has actually dictated the need to ferment and dry anything that might prevent starvation. To live on the edge of the desert must have been a great force in sharpening the sense for survival and creativity.

The strong link between many fermented foods and food shortages is also revealed by the fact that if a family became rich it would drop a number of fermented foods from its menu, not because of social pressure but because there was no longer any need for them now that ample supplies of meat, milk, poultry, etc., were available. Poor people who ferment bones, hides, locusts, etc., do so not because they relish these foods but because it is part of the coping strategy they follow to deal with the vagaries of a capricious environment.

The first victims of any famine are the children, among whom death exacts a great toll. Babies and children die in the laps of women more than they do in the laps of men. Maternal compassion must be the greatest impetus behind the rural woman's desperate attempts to save her child that propel her to look for an insect, a piece of hide, a frog, or a bone as savior. Many fermented foods are thus famine foods, and rural women must be credited with their invention. These women must have saved thousands of children from

certain death during famines. Their vital role must be recognized and hailed.

BIOTECHNOLOGY AND FERMENTED FOODS

This relationship has not been discussed widely in the literature. One can imagine, however, that biotechnology can be of help in the improvement of fermented foods at three levels:

· Raw materials. Fermented foods are produced from either animal or plant starting materials, and the availability of these substrates will of course aid in the production of fermented foods. Biotechnological methods to improve animal and plant production have been dealt with by experts in those fields on many occasions.

Only a special reminder should be made not to neglect certain wild plants and marginalized crops - the so-called lost crops of Africa (e.g., sorrel and okra). Attempts to restore the forest cover should give some attention to trees that bear fruits used during famines or even trees that host caterpillars.

· Fermentation engineering. Recent developments in biotechnology have given rise to great innovations in bioreactor designs. Most of these designs deal with liquid reaction media, but it should not be forgotten that a great number of fermented foods are produced through a solid-substrate fermentation in which the fermenting paste is frequently hand mixed. Bioreactors to simulate such a process are needed for the modernization of such traditional fermented foods.

· Microbiology and enzymology. There are many opportunities for biotechnological innovations in the microbiology of fermented foods.

First, all the microorganisms involved should be isolated, characterized, and preserved as a germplasm collection. Second, the metabolic role of each of the strains involved should

be clearly identified, and their full potential, even in other fields of biotechnology, should be studied. The powerful technique of monoclonal antibodies for the characterization of different strains of the same species can be of great help in this area.

Many of these organisms have the enzyme complement to produce vitamins and amino acids in fermented foods. This potential can be improved through the technique of recombinant DNA technology to produce strains that are capable of producing and releasing the required amino acid or vitamin into the food.

To avoid food losses due to spoilage-causing organisms and to avoid possible development of food-poisoning microbes, it is possible to genetically engineer a strain required for a process as a pure culture. Such a strain may bring about all the changes required in the food and grow at a convenient temperature.

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4 Lesser-Known Fermented Plant Foods

Kofi E. Aidoo

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) end 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 tiny fermentation and noted that the success of the fermentation depends on a number of factors, among which temperature is very important.

The microbiology of tiny 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

Kohonte, 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 lupine 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

Keith H. Steinkraus

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. Leuconostocs 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. mesenteroides 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 I 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 x 109 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 I 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 I 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. cased 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 oryzue. Following overgrowth of the substrate by the mold, the koVi 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. rouxli 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 Leenwenhoek 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 pureculture 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 mixedculture 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 hulgaricus. 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 x 106 per milliliter to 880 x 106 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 oryzue strains are used to make koVi. 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 mixedculture 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 pureculture 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 phageresistant 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°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 Aspergill´'s oryzue and A. soyue. The inoculum consists of several mold strains combined, with each strain producing a desired enzyme(s). The molded rice is called l~oji 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 I or 2 days. The Hot 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 hoji with molds, the paste is placed in large concrete or wooden tanks for the second fermentation. The inoculum consists of osmophilic yeasts Saccharomyces rouxli and Ca'~dida 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 105 microorganisms pergram. 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 cold 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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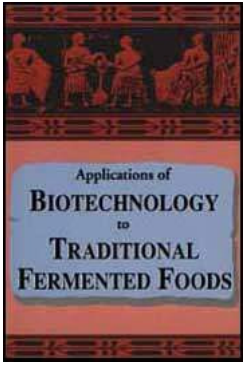

Applications of Biotechnology to Traditional Fermented Foods (BOSTID, 1992, 188 p.)

III. Milk derivatives

7 Fermented Milks Past, Present, and Future

M. Kroger, J. A. Kurmann, and J. L. Rasic

Milk is the most important foodstuff for a mammal and has always been the first food of the newborn. One could argue that the deliberate souring or fermentation of milk was one of the key achievements that nurtured mankind to grow and develop into a productive and preeminent species. Had fermented milk been considered spoiled and inedible and thus not have entered the human diet in the thousands of years to come, human development would have taken an entirely different course. Although there is no perfect food, milk is the most nearly perfect food known.

At some stage in the course of human evolution it was recognized that the milk of other mammals was equally satisfying in meeting physiological demands for moisture, energy, and nutrients. Milk from eight species of domesticated mammals (cow, buffalo, sheep, goat, horse, camel, yak, and zebu) has been used to make traditional fermented milk products throughout the world.

From a biological standpoint, fermented milks are characterized by the accumulation of microbial metabolic products. It was realized very early that such microbial metabolites as lactic acid, ethyl alcohol, and dozens of other chemicals collectively called flavor substances, were not altogether unpleasant and even contributed to overall preservative action.

CLASSIFICATIONS

Despite the long historical record and worldwide distribution of fermented milks, few people know more than five or 10 of the several hundred specific products that could be described. Even current food science and dairy technology textbooks fail to do the subject justice.

For example, the latest (fourth) edition of Food Microbiology (1) covers fermented dairy products in only two pages. The textbook used in the Pennsylvania State University dairy technology course is The Science of Providing Milk for Mar' (2). Cultured and acidified milk products occupy 10 pages, and cultured buttermilk, sour cream, yogurt, acidophilus milk, and ymer and lactofil are given only subchapter status. Koumiss and kefir are merely mentioned as being popular in Eastern Europe. Cheese and Fermented Milk Foods (3) is somewhat more comprehensive, but it deals mainly with practical concerns and primarily with cheese.

By far the best compilations on fermented milks have been and are being published as documents of the International Dairy Federation (4,5). One chapter of the latter lists some

80 fermented milks, including both traditional and nontraditional products. A soon-to-be-published encyclopedia of fermented fresh milk products (6) describes some 200 traditional fermented milks and several hundred nontraditional ones.

Traditional and Nontraditional

The most fundamental division of fermented milk products is into traditional and nontraditional types. Traditional fermented milk products have a long history and are known and made all over the world whenever milk animals were kept. Their production was a crude art. It was not until the days of Pasteur - about 100 years ago - that the microbiology underlying fermentations was revealed. In contrast, nontraditional fermented milk products are recently developed. They are based on known scientific principles; their microbial cultures are known; and their quality can be optimized. This is not the case with traditional products made with ill-defined, empirical cultures where you have to take what you get out of the fermentation. Yogurt is both a traditional and a nontraditional product - the latter being represented by ever-changing varieties.

Medium and Procedure

Classification by technology differentiates between fermented milks and fermented products not based directly on milk. It is obvious that products other than fresh milk can serve as the fermentation medium or substrate, such as cream, whey, buttermilk, and dry milk solids It is also possible to further manipulate or change the curd recovered after coagulation.

Further Processing

Neither law nor taboo forbids experimentation with fermented milks. Numerous products are known that are mixtures of milk and other foodstuffs and that have been subjected to fermentation. These include fermented milk-vegetable products, fermented milk-meat

extract mixtures, and fermented milk-fishmeal hydrolyzate mixtures. Consequently, we find societies that have utilized specific plants, meat extracts, or fishmeal hydrolyzates to enhance their nutritional status and the flavor and variety of their cuisine.

Pharmaceutical preparations are unique in that they emphasize microorganisms only instead of milk nutrients or product flavor. The subject of probiotics (a word coined in 1974) will undoubtedly emerge as a major field of study. We see it in animal science now where some work is being done to get specific bacteria implanted or colonized in the gastrointestinal tract of animals, obviously in the interest of animal health and improvement of farm animal food production. So-called health food stores make available preparations that provide people with specific doses of bacteria, such as Lactobacillus acidophilus, commonly found in some fermented milk products. The subjects of health and probiotics, as well as myth and faddism, are beyond the scope of this paper.

End Uses

Traditionally, fermented milk products have been consumed as beverages, as meal components, or as ingredients in cookery. As social patterns have changed, however, meal eaters have become snackers and grazers. Furthermore, food technologists and food innovators have created a multitude of new products for the shelves of modern supermarkets. Most of the developments have been in the dessert and confectionery category.

Microbial Actions

Homemade fermented milk products, especially in nomadic or village environments, are still occasionally made by spontaneous fermentation, but most likely they are made by the use of an empirical culture. In other words, the inoculum is obtained from a previous production and its microbial identity is unknown.

The bacteria utilized are either mesophiles or thermophiles, terms indicating optimum bacterial growth temperatures, roughly 70°C and 100°F (22° and 38°C), respectively. More specific and important is the bacterial species present. A fermented milk is mainly characterized by its sensory properties, and the sensory properties, such as taste, odor, and viscosity, are the direct results of specific bacterial action. The current names of microorganisms recognized in fermented milks are listed in Table 1.

TABLE 1 Current Names of Microorganisms In Fermented Milks

	Number of Former
Current Name	**Designations and Synonyms**
Genus Lactobacillus	
L. delbrueckii	8
L. delbrueckii subsp. lactis	10
L. delbrueckii subsp. bulgaricus	8
L. acidophilus	2
L. helveticus	7
L. casei	6
L. brevis	29
L. fermentum	10
L. kefir	4
Genus Leuconostoc	
L. mesenteroides	2
L. mesenteroides subsp. dextranicum	6
L. mesenteroides subsp . cremoris	3

L. lactis	1
Genus Pediococcus	
P. pentosaceous	3
P. acidilactici	1
Genus Propionibacterium	
P. freudenreichii subsp. shermanii	
P. freudenreichii subsp. freudenreichii	
Genus Streptococcus	
S. lactis	1
S. lactis subsp. diacetylactis	1
S. lactis subsp. cremoris	3
S. thermophilus	1
Genus Bifidobacterium	
B. bifidum	13
B. longum(1)	1
B. infantis	3
B. breve	2
Genus Acetobacter	
A. aceti	11
Yeasts	
Torulaspora delbrueckii	1
Kluyveromyces marxianus subsp. marxianus	3
Kluyveromyces marxianus subsp. bulgaricus	1
Candida kefyr	3

Candida kefyr	0
Saccharomyces cerevisiae	0

(1)In an earlier edition of Bergey's Manual, B. longum was listed as having two subspecies: B. longum subsp. longum and B. longum subsp. animalist The latter was translocated in the new Bergey's into two species: B. animalis and B. pseudolongum.

With regard to bacterial species, a number of products have evolved that are now characterized by the presence of specific organisms. Modern yogurt is now defined by the regulations of many governments to be made from and to contain only Lactobacillus bulgaricus and Streptococcus thermophilus. But there are no hard-and-fast rules, and, theoretically, any combination of organisms could be utilized to make a fermented milk product. The ultimate test is palatability. Frankly, there is still much confusion over the microbial identity of most of the known traditional fermented milk products in the world. Some have never been studied in depth. Some are very variable from batch to batch. Only yogurt has been given a proper definition by regulatory authorities in some countries. All other products are only loosely defined.

RESEARCH

Milk has always turned sour, but at some point in human history artisans deliberately caused milk to coagulate. However, the scientific principles behind the phenomenon of milk fermentation have remained unrevealed until recent decades.

We had to wait for the pioneers in microbiology to lead the way. Louis Pasteur (1822-1895) studied alcohol fermentation; Heinrich Anton DeBary (1831-1888) studied the infection of plants by fungi; and Robert Koch (1843-1910) studied human disease caused by bacteria. It was Elie Metchnikoff (1845-1916) who, while working at the Pasteur Institute in Paris, moved milk fermentations and the unheard-of subject of probiotics into the limelight. In 1908 he shared the Nobel Prize in Physiology and Medicine. Metchnikoff

developed a theory that lactic acid bacteria in the digestive tract could, by preventing putrefaction, prolong life. His book, The Prolongation of Life (7), was translated into English in 1907 (reviewed in Harper's Weekly, February 8, 1908) and received much exposure worldwide. In a way it made Metchnikoff the godfather to everyone who, to this day, believes in the therapeutic value of fermented milk.

World War I put a damper on this type of human diet/health preoccupation. In the United States, it was 1921 before an American figure emerged who should be given much more credit, Leo Frederick Rettger. Rettger was a professor of bacteriology at Yale for most of his career. Two of his publications are A Treatise on the Transformation of the Intestinal Flora with Special Reference to the Implantation of Bacillus Acidophilus (8) and Lactolbacillus Acidophilus and Its Therapeutic Application (9).

On the practical front at that time, A. D. Burke, head of the Dairy Department of Alabama Polytechnic Institute, published Practical Manufacture of Cultured Milks and Kindred Products (10). Burke's book is, according to the subtitle, "a complete and practical treatise on the manufacture of commercial cultured buttermilks of all types - lactic, Bulgarian, acidophilus, kefir, kumiss, yogurt." It is also a practical treatise on commercial casein, cottage cheese, cream cheese, and commercial sour cream, with information on dried, condensed, and fruit-flavored buttermilk.

Then came World War II, and until about 1950 very little research and development was seen on fermented milks. Since then increasing attention has been paid to fermented milk products worldwide. The American Cultured Dairy Products Institute was created in the United States in 1965. Several good books have been published, and scientific publications on the subject are proliferating. Manufacturers, researchers, and the public are experimenting with cultured dairy products in North America - and not only with yogurt but with other products as well. Kefir has been available in Los Angeles for more than a decade. In 1985 a New Jersey corporation began producing kefir for the East Coast, and in

1987 several major grocery chains began selling leben.

The future of fermented milk in North America and elsewhere will undoubtedly be exciting and complex.

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8 Lactobacillus GG Fermented Whey and Human Health

Seppo Salminen and Kari Salminen

Traditionally, whey has been a troublesome waste product at cheese factories. New uses have now been developed for cheese whey to utilize the whey nutrients, including protein and carbohydrates.

Fermented milk products have been reported to have an important role in the treatment of infant diarrhea in malnourished children (1,2). More recently, Isolauri and co-workers (3) have shown in a double-blind controlled trial that Lactobacillus GG bacteria promote recovery from acute diarrhea in children. These results suggest that whey-based products may be used in this application.

A process for manufacturing a fermented flavored whey drink has been developed that combines the nutritional properties of whey and the health benefits of Lactobacillus strain GG. The objective has been to improve the utilization of whey through use of a scientifically selected Lactobacillus strain with proven health benefits. For this purpose, demineralized lactose-hydrolyzed whey concentrate has been fermented with Lactobacillus GG. Whey and lactic acid bacteria have thus been combined to provide a wholesome and nutritious beverage.

WHEY HYDROLYSIS PROCESS

Important steps in whey processing are the hydrolysis of lactose and demineralization to remove excess salt. A continuous whey hydrolysis process has been developed using immobilized beta-galactosidase enzyme. This process is more economical than batch hydrolysis. Lactose hydrolysis is important for lactose-intolerant populations and for malnourished children. Malnourished children may experience worsening of acute diarrhea when lactose is given during treatment (1). Salt removal can be completed using an ion exchange process. After concentration to 60 percent dry matter, a hydrolyzed demineralized whey syrup is obtained that has a good shelf life and a pleasant rich taste.

LACTOBACILLUS GG

Lactobacillus cased strain GG (Lactobacillus GG) is a new Lactobacillus strain that is of human origin and has been shown to colonize the intestinal tract (4). This strain was originally isolated from a healthy human volunteer based on its ability to tolerate acid and bile, to produce an antimicrobial substance, and to adhere to human intestinal cells (5,6). It is among the first strains with clinically proven health benefits in various intestinal disorders in adults, children, and infants. The most important evidence of its health benefits comes from studies of infant diarrhea. Isolauri and co-workers (3) published the first study on infant rotavirus diarrhea in which the duration of diarrhea was reduced by 50 percent through the use of either freeze-dried Lactobacillus GG or Lactobacillus GG fermented milk products.

LACTOBACILLUS GG FERMENTED WHEY DRINK

A new fermented flavored whey drink has been manufactured from demineralized lactose-hydrolyzed whey concentrate using Lactobacillus GG. It is a low-lactose product that contains no fat and is lightly sweetened with fructose. It has special sensory characteristics - smooth texture, mild acidity, and the rich taste from whey. Fruit juices or fruit flavoring have been used to modify the flavor to appeal to different people.

Fermentation of whey may also influence lactose content when suitable bacteria are used. Additionally, whey proteins may undergo slight changes to ease their digestibility. The end product may offer alternatives for people not currently attracted to fermented milks.

CONCLUSIONS

This development in whey processing offers new alternatives for utilizing cheese by-products and applies new technologies to nutritionally important products. Combining whey processing with lactobacilli that have been obtained using new selection methods may prove to be beneficial to human health in many intestinal imbalances. It may also offer possibilities in utilizing new technologies in food production in different cultures and in providing nutritionally attractive foods from low-value by-products.

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9 The Microbiology Ethiopian Ayib

Mogessie Ashenafi

In Ethiopia, smallholder milk processing is based on sour milk resulting from high ambient temperatures, while meeting consumers' preferences and improving keeping quality (1). Ayib, a traditional Ethiopian cottage cheese, is a popular milk product consumed by the various ethnic groups of the country. It is made from sour milk after the butter is removed by churning. Traditional ayib making has been described by O'Mahony (1). Milk for churning is accumulated in a clay pot over several days. This is kept in a warm place (about 30°C) for 24 to 48 hours to sour spontaneously. Churning of the sour milk is carried out by slowly shaking the contents of the pot until the butter is separated. The butter is then removed from the churn and kneaded with water. The casein and some of the unrecovered fat in skim milk can be heat precipitated to a cottage cheese known as ayib. The defatted milk is heated to about 50°C until a distinct curd forms. It is then allowed to cool gradually, and the curd is ladled out or filtered through a muslin cloth. Temperature can be varied between 40° and 70°C without markedly affecting product composition and yield. Heat treatment does not appear to affect yield but gives the product a cooked flavor.

AYIB CHARACTERISTICS

Ayib comprises about 79 percent water, 15 percent protein, 2 percent fat, 1 percent ash, and 3 percent soluble milk constituents. The yield should be about 1 kilograms of ayib from 8 liters of milk (1).

The safety of cheese with respect to food-borne diseases is of great concern around the world. This is especially true in developing countries, where production of milk and various dairy products often takes place under unsanitary conditions. Since there was no published information on the microbiology of milk and milk products in Ethiopia, a study was carried out in our laboratory to evaluate the microbiological quality of ayib as available to the consumer (2). One hundred samples of ayib were purchased at the Awassa market over 10 weeks. Since Awassa is an open-air market, ayib was generally handled at ambient temperatures (about 25° to 27°C during the study period). Samples were microbiologically analyzed within two hours of purchase.

Standard microbiological procedures were followed to determine the counts of aerobic mesophilic microorganisms, psychrotrophs, yeasts and molds, coliforms, bacterial spores, enterococci, Bacillus cereus, Listeria monocytogenes, staphylococci, and lactic acid bacteria. The pH of the samples was also measured.

Ayib samples showed high numbers of mesophilic bacteria, enterococci, and yeasts (Table 1). More than 90 percent of the samples had aerobic mesophilic counts of 10e8 cfu/g (colony forming units) or higher; more than 75 percent of the samples had yeast counts of 10e7 cfu/g or higher, and over 85 percent contained enterococci in numbers of 10e7 cfu/g or higher. The majority of the samples had mold and lactic acid bacteria counts of 10e5 cfu/g or higher, spore-formers of about 104, and psychrotrophs of about 10e6 cfu/g (Table 1).

Over 32 percent had coliform counts of more than 10e2/g, and about 27 percent contained

fecal coliform loads of more than 10e2/g. Listeria spp. were not detected from the samples. B. cereus and S. aureus were isolated in 63 percent and 23 percent of the samples, respectively, but at very low numbers (10e2 to 10e3 cfu/g). About 40 percent of the ayib samples had pH values of less than 3.7, and 60 percent had values of 3.7 to 4.6.

Most of the production of milk and various milk products in Ethiopia is generally a household process that usually takes place under unsanitary conditions. However, despite its high moisture content, the low pH of ayib may prevent the further proliferation of various microorganisms. Yeasts, which can grow at lower pH values, may affect the flavor and keeping quality of ayib. In another study (3), proteolytic yeasts made up 47 percent of the total yeast isolates and all isolates showed lipolytic activities. Since traditional ayib making involves removal of fat from the sour milk, ayib contains only about 1 percent fat, and thus the lipolytic isolates may not play an important role in affecting the flavor or keeping quality of ayib.

TABLE 1 Frequency Distribution (Percent) of Aerobic Mesophilic Organisms, Yeasts, and Enterococci in Ayib Samples

	(a)cfu/g			
Microorganisms	$<10^7$	10^7	10^8	$>10^8$
Aerobic mesophils	-	8	65	27
Yeasts	25	70	5	-
Enterococci	13	45	37	5

(a)Colony forming units

Although proteolytic yeasts are important in cheese types that require ripening, their presence in a fresh product such as ayib is undesirable.

The findings in the previous studies indicated that ayib purchased from local markets was highly contaminated with various microorganisms. It was not known, however, whether these microorganisms were survivors of the heat treatment process or were postheating contaminants.

MODIFIED PROCESS

Another study was therefore conducted to determine the effect of cooking temperatures used in various parts of Ethiopia on the microbiological quality of the finished product and to recommend cooking temperatures that can decrease or destroy most microorganisms (4). Ayib was made in the laboratory using traditional methods. Pooled raw milk was allowed to sour naturally at room temperature. After removal of the fat by churning, the casein in the sour skimmed milk was heat precipitated at 40°, 50°, 60°, and 70°C in a water bath, and the curd was recovered by filtering through sterile cheese cloth.

Microbial analysis of raw milk, sour milk, and ayib indicated that heat treatment of the curd was effective at higher temperatures (Table 2). At these temperatures the time required for casein precipitation was also low. Heating the curd at 70°C for 55 minutes at pH 4 destroyed most of the microorganisms. The low pH also inhibited the proliferation of most surviving microorganisms. The high degree of contamination of market ayib could be due to either low curd cooking temperatures or addition of various plant materials to the finished product to give it desirable flavor, the packaging of ayib with Musa leaves, or other unhygienic handling practices. Thus, heat treatment of curd at 70°C and an appropriate handling of the product could result in a less contaminated and safer ayib.

TABLE 2 Frequency Distribution (Percent) of Lactic Acid Bacteria, Bacterial Spores, Molds, and Psychrotrophs in Ayib Samples

		(a)cfu/g		
Microorganisms	10^3	10^4	10^5	10^6

Microorganisms	10^5	10^4	10^5	10^6
Lactic acid bacteria	14	33	13	40
Bacterial spores	4	68	24	4
Molds	12	25	35	28
Psychrotrophs	-	13	20	67

(a)Colony forming units

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10 Moroccan Traditional Fermented Dairy Products

Abed Hamama

In Morocco 20 to 30 percent of all milk produced is still processed by private individuals. These dairy shops and farmers manufacture traditional Moroccan dairy products such as

lben and raib (fermented milks), zabda (farm butter), and jben (fresh cheese). These products are made from raw milk, and their physical properties are similar to those of commercially produced buttermilk, yogurt, butter, and fresh cheese. Although they are usually made from cow's milk, milk from sheep, goats, and camels also can be used. These products are very popular in Morocco mainly because of their refreshing qualities.

Basically, all these traditional dairy products are prepared by simply allowing the raw milk to ferment spontaneously at room temperature (15° to 25°C) for 1 to 3 days depending on the season. The coagulated milk is called raib. It can be consumed as such or churned in a clay jar to separate the liquid phase (lben) from fat (zabda). Jben is prepared by placing the coagulated milk in a cloth at room temperature and draining the whey. Salt is added to jben made in northern Morocco.

COMPOSITION AND MICROBIOLOGICAL CHARACTERISTICS

The Moroccan traditional fermented dairy products have been investigated (1-4) for their composition (Table 1) and their microbiology (Table 2). Data in these tables are average results only. In fact, a high level of variability for all the parameters was seen among samples of the same product. This heterogeneity is a consequence of the lack of standard procedures for preparation of these products.

Despite the acidic nature of these products (pH 4.0 to 4.5), they showed high counts of indicator microorganisms (e.g., coliforms, enterococci). This probably reflects poor hygienic conditions in the preparation of these products and/or poor bacteriological quality of the raw milk used for their manufacture.

TABLE 1 Average Physical-Chemical Composition of Moroccan Traditional Dairy Products

Composition	Lben(4)	Jben(3)	Raib(3)	Zabda(1)
pH	4.25	4.1	4.2	4.5

% lactic acid	0.81	1.04	0.62	0.77
% total solids	6.5	37.5	10.7	76.7
% fat	0.9	16.47	2.22	73.7
% protein	2.5	15.8	3.1	1.8
% lactose	2.7	4.1	4.2	1.2
% chlorides	0.17	0.5	0.17	ND
% ash	ND	1.26	0.54	ND

ND, not determined.

In addition to the indicator microorganisms, pathogens such as Salmonella sp., Yersinia enterocolitica, Listeria monocytogenes, and enterotoxigenic Staphylococcus aureus have been recovered mainly from samples of lben and jben. Although there are no epidemiological reports of outbreaks linking Moroccan traditional dairy products with diseases caused by these pathogens, their presence in these products indicates potential health hazards for consumers. Therefore, there is need to implement corrective procedures to eliminate or reduce this risk. This can be achieved by the use of heat-treated milk instead of raw milk and through the use of selected starter cultures for preparation of these products.

OBJECTIVES

The application of modern technology to Moroccan traditional dairy products aims to assure the following:

· Large-scale production of these products year-round by replacing raw milk with dry milk and butter oil. This will solve the problem of seasonality in Moroccan milk production.

· Production of dairy products with standardized chemical and microbiological composition so that their quality can be more easily controlled and standards for each product can be established.

TABLE 2 Average Microbiological Counts of Moroccan Traditional Dairy

Products (cfu/g or ml)

Microorganism	Lben(4)	Jben(3)	Raib(3)	Zabda(2)
Streptococci	7.6×10^8	5.1×10^8	1.4×10^8	5.0×10^6
Lactobacilli	1.0×10^3	3.2×10^8	2.6×10^6	2.4×10^5
Leuconostocs	1.7×10^5	2.6×10^8	2.8×10^6	1.8×10^4
T. coliforms	5.0×10^4	4.3×10^5	1.7×10^5	6.5×10^4
F. coliforms	1.0×10^3	2.7×10^4	4.2×10^3	2.1×10^4
Enterococci	1.0×10^5	2.4×10^5	2.2×10^4	8.6×10^4
Fungi	8.5×10^2	2.3×10^6	2.3×10^4	ND
Total flora	2.9×10^9	8.2×10^8	3.5×10^8	4.6×10^7

ND: not determined.

· Elimination of massive contamination of these products and reduction of health hazards associated with these contaminations by using heat-treated milk and improving the sanitation and fermentation conditions.

· Adoption of simple and standardized processes for the preparation of these products that could be easily applied in the dairy industry.

PRELIMINARY STUDY

Preparation of traditional dairy products using improved technological processes requires, for each type of product, determination of the characteristics that constitute an excellent-quality product. For this purpose, samples of each product were evaluated by a gustatory panel. The best products were then analyzed to determine their physical characteristics, chemical composition, and microbiological profiles. The objective of the study was to assess the censorial and compositional parameters (e.g., acidity, total solids) that the improved product should have to be acceptable to consumers.

Selection of Starters

Microbiological analysis of the different traditional fermented dairy products showed that an important proportion of their microflora was represented by lactic acid bacteria. Lactic streptococci were predominant in lben, raib, and zabda, while streptococci, lactobacilli, and leuconostocs were found in jben at almost the same average levels (10e8 cfu/g or ml) (colony forming units). From each product isolates from the predominant lactic flora were identified using biochemical tests. The principal species found in lben, raib, and zabda were Streptococcus lactis, and S. diacetylactis, while S. lactis, Lactobacillus cased casei, and Leuconostoc lactis were the main species recovered in jben.

Owing to the nature of traditional Moroccan dairy products (fresh fermented products), the major criterion considered for selection of lactic starters was their acid production ability at different incubation temperatures. Production by lactic strains of certain substances contributing to the overall aroma of these products also was taken into account. Thus, several lactic strains were retained to be used for preparating improved products.

Manufacture of Traditional Dairy Products from Heat-Treated Milk

To prepare each type of product, a simple and economically feasible technology, which industrial dairy plants could easily adopt, was used.

The improved processes proposed for use with raib (fermented milk) and jben (fresh cheese) are as follows:

· Manufacture of raib:

Reconstitution of dry milk to 90 percent water and 10 percent solids.

Pasteurization at 63°C for 30 minutes.

Addition of calcium chloride and storage at 7°C for 10 hours.

Addition of fresh pasteurized milk (60 percent of the total volume).

Inoculation (S. lactis, S. diacetylactis @ 3.0 percent).

Distribution into plastic containers and incubation at 30°C for 3 to 4 hours.

Refrigeration at 4° to 6°C.

· Manufacture of jben:

Reconstitution and pasteurization of powdered milk.

Addition of calcium chloride and storage at 7°C for 10 hours.

Addition of fresh pasteurized milk (60 percent of the total volume).

Inoculation (S. lactis, S. diacetylactis, L. cased cased @ 3.0 percent).

Storage of inoculated milk at 20° to 25°C until 0.25 percent lactic acid is formed.

Addition of rennet (5 to 10 milliliters/100 liters).

Fermentation at 20° to 25°C until 0.60 percent lactic acid is formed.

Curd cutting and whey draining.

Unmolding when titratable acidity reaches 0.9 percent lactic acid and total solids content reaches 28 to 30 percent.

Cutting of cheese into suitable pieces (150 grams/piece).

Surface dry salting, if desired (I percent salt) and wrapping.

RESULTS

This study is still in progress. The final results regarding censorial quality, chemical composition, and microbiological quality of traditional dairy products made with the improved technology are not yet available. Nonetheless, preliminary data obtained for raib and jben are very encouraging:

· Sensorial quality: Laboratory samples of improved raib and jben gave similar or even higher sensory scores than market samples. The characteristics considered in this evaluation are mainly acidity, texture, and aroma.

· Chemical composition; Because standard procedures were used for making raib and jben, the samples obtained had uniform compositions. This information will be useful in establishing standards for these products.

· Microbiological quality: The use of heat-treated milk in the manufacture of raib and jben had a profound effect on the microbiological quality of the products. The improved products were free from pathogens such as S. aureus, Salmonella, L. monocytogenis, and Y. enterocolitica. They were either free of or contained very few coliforms (<10 cfu/g). Their microbiological quality was substantially improved compared with currently marketed traditional products.

CONCLUSIONS

Although data on all traditional dairy products are not yet available, information on the quality of laboratory-made raib and jben indicates that the use of modern technology in their manufacture has enhanced their bacteriological quality and reduced the risks of dairy-borne infections. This new technology has also begun to establish standards for these products.

In addition, the manufacture of traditional dairy products at an industrial scale will increase the production of these products and assure better distribution and marketing.

On the other hand, the use of dry milk, which is more economical than raw milk for preparing products such as raib and jben, has the advantage of being available any time of the year. This is very important in Morocco, where seasonal variabilities in milk production are a major problem for the dairy industry. Furthermore, the availability of dairy products that are rich in nutrients (e.g., proteins, fat) at a modest price and throughout the year will contribute to reduced malnutrition especially among children in rural areas.

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11 Fermented Milk Products in Zimbabwe

Sara Feresu

Fermentation is the oldest means of preserving milk (1). Originally, unpasteurized milk was left to ferment naturally, and fermentation involved microorganisms present in the raw milk and surrounding air. With the development of modern technologies, specific lactic-acid-producing microorganisms are now introduced to carry out fermentations under controlled conditions. In this way fermented products of superior nutritional, physical, chemical, and sanitary qualities are produced.

In Zimbabwe one finds the modern fermented products such as yogurt and different types of cheese. The rural population, however, still ferment their milk traditionally. Fresh unpasteurized cow's milk is allowed to stand, at ambient temperature, in an earthenware pot loosely covered by a plate. This allows microorganisms inherent in the milk, from the pot, and from the surrounding air to ferment the milk. Fermentation takes 1 to 2 days depending on the ambient temperature (20 to 25°C). The fermented milk is not refrigerated and has an estimated shelf life of 3 days at ambient temperature.

In response to the urban population's desire for fermented milk, the Zimbabwe Dairy

Marketing Board produces a fermented milk called Lacto on an industrial scale. Milk is standardized, pasteurized at 92°C for 20 minutes, cooled to 22°C, and inoculated with 1.2 percent of an imported mesophilic starter culture, similar to that used to produce "filmjolk," a Scandinavian fermented milk. The milk is immediately packaged into sachets, left to ferment at ambient temperature for 18 hours, and stored at 5°C ready for the market. The shelf life of refrigerated Lacto is 7 days.

Our studies have compared traditionally fermented milk with Lacto. We included traditionally fermented pasteurized milk, since substitution of unpasteurized with pasteurized milk might be an alternative for upgrading hygienic standards. The initial study was concerned with the effects of pasteurization and of the container used during fermentation on the total microbial cell counts, the counts of lactic acid bacteria, the amount of lactic acid produced, and the acceptability of the fermented milk by a panel (2).

We have characterized 10 predominant lactic acid bacterial isolates from traditionally fermented milk and four isolates from Lacto (3). We have also carried out studies to determine the fate of pathogenic and nonpathogenic Escherichia cold during fermentation of Lacto and traditionally fermented pasteurized and unpasteurized milk. The survival of E. cold was also tracked during storage of the fermented products at ambient (20°C) and refrigeration temperatures (5°C) for 4 days (4), since it is possible that pathogenic bacteria may gain access to these products before, during, and after fermentation. In the case of traditionally fermented milk, coliform contamination from cattle dung or from the milker's hands is possible. Contamination with coliforms during Lacto production can occur through bulk starter cultures and from inadequately sanitized equipment.

TRADITIONALLY FERMENTED MILK AND LACTO

In an earlier study (2) unpasteurized milk and pasteurized milk were fermented in clean nonsterile earthenware pots and sterile glass containers. At the same time, Lacto was

fermented in plastic sachets and sterile glass containers. Bacterial counts and lactic acid levels were determined. The acceptability of the fermented milks was ranked by 11 panelists. Comparisons of all parameters were made after 24 and 48 hours of fermentation, when Lacto and traditionally fermented milk are likely to be consumed.

The numbers of lactic acid bacteria, lactic acid production, and acceptability were always higher for unpasteurized than pasteurized traditionally fermented milk irrespective of the container used.

Earthenware pots are better containers for traditional fermentation of milk. This is because earthenware pots have micropores in their walls, which, if not sterilized, may harbor lactic acid bacteria from the previous fermentation, which then act as inocula for the next fermentation. Our results suggest that earthenware pots are good containers to ferment milk in and may still have a place in milk fermentation in the home.

Unpasteurized milk fermented traditionally in either container was significantly more acceptable to the panel than Lacto, although the products were similar in all the other parameters assessed. It was therefore impossible to explain the differences in the acceptability of traditionally fermented milk and Lacto on the basis of this work. We suggested that the differences were probably due to the types of microorganisms involved in the fermentation of the two milk products rather than pasteurization or the container used for fermentation. Thus, we set out to isolate and characterize the lactic acid bacteria in traditionally fermented milk and Lacto.

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA

From the previous study (3), 10 predominant morphologically different lactic acid bacteria colony types from plates inoculated with traditionally fermented milk and four morphologically different types of colonies from Lacto plates were selected and isolated into pure culture. The isolates were identified using numerical taxonomic techniques and

reference strains. The isolates and reference strains were examined for 32 characteristics. Data were analyzed using the simple matching coefficient, and clustering was by unweighted pair group average linkage (5).

All the isolates from traditionally fermented milk belonged to the genus Lactobacillus. Seven of the isolates could be identified as belonging to L. helveticus, L. plantarum, L. delbrueckii subspecies lactis (two isolates), L. cased subsp. cased (two isolates) and L. cased subsp. pseudoplantarum. Three of the isolates could only be identified as either betabacteria or streptobacteria. The four isolates from Lacto were identified as Lactococcus lactis. They could not, however, be identified to subspecies level.

From this study we concluded that the differences in acceptability of traditionally fermented milk and Lacto are probably due to differences in the biochemical pathways and resulting types and levels of end products produced by the different bacteria responsible for fermentation of the two products. We suggested that more work should be done to determine the particular flavors and aroma present in traditionally fermented milk that are absent in Lacto and to determine whether any of our isolates are responsible for producing these desired properties.

E. COLI STRAINS IN LACTO AND TRADITIONALLY FERMENTED MILK

In another study (4) the growth and survival of pathogenic and nonpathogenic strains of E. cold were determined in traditionally fermented pasteurized and unpasteurized milk and Lacto. Unpasteurized and pasteurized milk and freshly inoculated Lacto, together with sterile control milk, were each inoculated with two strains of pathogenic and one strain of nonpathogenic E. cold to give approximately 103 cells/ milliliter. All the milk treatments were left to ferment at ambient temperature (20°C) for 24 hours. One set of the fermented products was stored at ambient temperature, and the other set was refrigerated (5°C) for another 96 hours. Samples were taken at 24-hour intervals and tested for numbers of E.

coli, pH, and percentage of lactic acid.

Lacto inhibited all three E. cold strains. Two strains (one pathogenic and one nonpathogenic) could not be recovered, and the third (pathogenic) survived only in very low numbers after 24 hours of storage of Lacto at both 20° and 5°C.

All three E. cold strains survived and multiplied to maximum cell numbers in the range 107 to 109/milliliter during traditional fermentation of unpasteurized milk. Cell numbers decreased to 103 to 10e6 and 10e2 to 10e5 during storage of the fermented product at 20° and 5°C, respectively. These results indicated that traditional methods of fermenting milk in Zimbabwe pose a potential health hazard because, if milk is contaminated during milking or fermentation, E. coli, and possibly other enteric pathogens, are able to multiply to infective doses and retain relatively high numbers during storage of the product at both refrigeration and ambient temperatures. The results also indicated that more than acid production alone is involved in the fate of E. cold during fermentation and storage of Lacto and traditionally fermented unpasteurized milk since more E. cold survived in unpasteurized fermented milk despite similar final lactic acid and pH levels of both milk products. We suggested that, since in our earlier studies we found that different lactic acid bacteria were responsible for fermentation of the two milk products, it is likely that these organisms produce different types and quantities of other inhibitory products (antibiotics, volatile acids, hydrogen peroxide) during fermentation.

Higher maximum numbers, 109 to 10'° of the three strains of E. coli, were attained during traditional fermentation of pasteurized milk. The numbers decreased to 10e5 to 10e3 and 10e4 to 10e7 during storage of the fermented product at 20° and 5°C, respectively. Under our experimental conditions there appeared to be more danger in traditionally fermenting pasteurized milk than unpasteurized milk; since less acid was produced, more E. cold multiplied and survived during fermentation and during storage of the pasteurized fermented milk. The practical relevance of this result should be interpreted with caution,

since pasteurization also removes milk-borne organisms such as E. cold and Salmonella spp. and since it is unlikely that airborne recontamination of the milk by E. cold would result in initial numbers as high as 103 cells/milliliter. Thus, use of pasteurized milk in practice may not be as inappropriate as it might appear in theory.

Generally, fewer E. cold survived when the fermented milk products were stored at refrigeration than at ambient temperature. However, most people in rural areas of Zimbabwe do not have access to refrigerators.

CONCLUSIONS

We are currently determining the amounts of some B vitamins and of aroma and flavor compounds in traditionally fermented unpasteurized milk and Lacto. Preliminary results indicate that traditionally fermented milk contains more thiamine, riboflavin, pyridoxine, and folic acid than Lacto. Again, traditionally fermented unpasteurized mill: is performing better than Lacto.

From the work we have done so far there are two options to follow in our future studies. We know that traditionally fermented milk has similar amounts of lactic acid and a pH level similar to that of Lacto and that it might also have higher amounts of some B vitamins; however, it is not hygienically acceptable. We know some of the lactic acid strains involved in the fermentation, but we also know that in a situation where raw milk is used and fermentation is carried out under conditions where asepsis is not observed, other microorganisms, in addition to lactic acid bacteria, contribute to the production of aroma and flavor compounds. Supposing we were to develop a starter culture based mainly on members of the genus Lactobacilllus, it is debatable whether we would have the same organoleptic properties in a traditionally fermented pasteurized milk as found in traditionally fermented unpasteurized milk. If we developed and sold this starter culture for home use in fermentation of boiled milk, it is also unlikely that poor rural people would

adopt such a fermentation since it has an added cost when compared with traditional fermentation.

Alternatively, we could incorporate some isolates from traditionally fermented milk into the Lacto starter culture and see whether the organoleptic properties of Lacto can be improved. Such a product would have to taste much better than traditionally fermented unpasteurized milk so as to entice rural populations to abandon traditional fermentation and adopt Lacto. Educational programs would have to be instituted for the public to appreciate the wisdom of spending money on buying Lacto, a hygienically safer product. At present, it is unlikely that Lacto will replace traditionally fermented milk in the foreseeable future.

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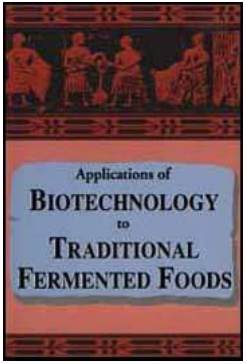

Applications of Biotechnology to Traditional Fermented Foods (BOSTID, 1992, 188 p.)

IV. Plant derivatives

12 Cassava Processing in Africa

Olusola B. Oyewole

Cassava is an important food crop in the tropics and many countries in Africa. The crop contributes significantly to the diets of over 800 million people, with per capita consumption averaging 102 kilograms per year. In some areas of Africa it constitutes over 50 percent of the daily diets of the people.

Traditionally, cassava is processed before consumption. Processing is necessary for

several reasons. First, it serves as a means of removing or reducing the potentially toxic cyanogenic glucosides present in fresh cassava. Second, it serves as a means of preservation. Third, processing yields products that have different characteristics, which creates variety in cassava diets.

The objective of this paper is to detail the strategy and program being followed in our laboratory to utilize the knowledge of biotechnology to improve the processing of cassava in Africa.

TRADITIONAL PROCESSING

Processing of cassava for food involves combinations of fermentation, drying, and cooking. Fermentation is an important method common in most processings. While there are many fermentation techniques for cassava, they can be broadly categorized into solid-state fermentation and submerged fermentation. Solid-state fermentation, typified by gari production, uses grated or sliced cassava pieces that are allowed to ferment while exposed to the natural atmosphere or pressed in a bag. Submerged fermentation involves the soaking of whole peeled, cut and peeled, or unpeeled cassava roots in water for various periods, as typified by the production of fufu and lafun in Nigeria. Traditionally, cassava is fermented for 4 to 6 days in order to effect sufficient detoxification of the roots.

(The support of the International Foundation for Science, Stockholm, Sweden, is gratefully acknowledged.)

Some processors, out of economic pressure, ferment cassava for less than 2 days. Some cases of food poisioning have been attributed to this practice. Application of biotechnology to traditional cassava processing has prospects for producing safe and well-detoxified products.

RESEARCH APPROACH

Our approach on cassava processing research is divided into three areas:

1. Investigating the science of the traditional submerged fermentation of cassava to fufu and lafun production;

2. Optimization of the processing through process controls; and

3. Improvement of traditional processing through application or biotechnological techniques.

The microorganisms involved in the submerged fermentation process have been isolated and found to include Bacillus subtilis, Klebsiella sp., Candida tropicalis, C. krusei, and a wide spectrum of lactic acid bacteria, major among which are Lactobacillus plantarum and Leuconostoc mesenteroides. A microbial succession trend was found with the starch degrading Bacillus subtilis, giving way to the lactic acid bacteria and yeasts that dominate the latter part of the fermentation. The pH and titratable acidity of the fermenting cassava roots increased from 6.3+0.2, 0.08+0.03 percent to 4.0+0.3, 0.36+0.05 percent, respectively, after the 96-hour fermentation period. Organic acids produced during fermentation include lactic, acetic, propanoic, and butanoic acids, among others, and these are believed to contribute to the characteristic flavor of fermented cassava products. Fermentation causes release of some bound minerals, including calcium and magnesium. The most important contribution of fermentation is the release from the plant tissues of the enzyme linamarase, which is involved in the breakdown of the linamarin and lotaustralin (cyanogenic glucosides) of cassava, which releases hydrogen cyanide and thus detoxifies the product.

PROCESS OPTIMIZATION

Processing conditions for optimizing the fermentation process have been investigated in our laboratory. We found that the temperature range of 30° to 35°C, with a soaking period

of 48 to 60 hours, is best for submerged processing. The size to which the roots are cut, peeling or nonpeeling of roots before processing, changing or nonchanging of fermentation water at intervals during processing, and the age of roots all affect the characteristics of the final product. In addition, the protein contents of products can be improved by cofermentation with legumes such as soybeans and cowpea.

BIOTECHNOLOGICAL INVESTIGATIONS

The overall goal of our biotechnological investigations is to develop an appropriate starter culture for cassava processing that will effectively produce linamarase enzymes for detoxifying cassava, break down starch to the simple sugars needed for acid production, improve the protein content of the products, reduce processing time, and yield products with stable desired qualities. The following summarizes our current findings:

· The microorganisms involved in fermentation have been characterized.

· Characterized isolates were used as single and multiple starter cultures for cassava fermentation. This has made it possible to understand the roles of each of the microorganisms implicated in the natural fermentation process. Bacillus subtilis and Klebsiella spp. contribute significantly to the rotting of cassava roots. In addition, B. subtilis produces amylase enzymes that are necessary for the breakdown of starch to sugars, which are needed for the growth of other fermenting microorganisms, including the tactics. Yeasts play a major role in odor development and, where high yeast biomass is encouraged, protein-enriched products are not. Lactic acid bacteria convert cassava sugars to lactic and other acids that contribute to the flavor in addition to having preservative effects.

· Appropriate starters have been developed that can produce amylase and linamarase enzymes necessary for starch breakdown and cyanogenic glucoside hydrolysis; two major biochemical processes needed in cassava processing. For this, the lactic acid bacteria

were investigated since they were the predominant microbial group present at the beginning of fermentation and which persist and survive the acidic conditions that prevail in cassava fermentation. To date, we have found strains of Lactobacillus plantarum that are capable of producing amylase and linamarin. The linamarase produced has been purified, and it exhibits optimal activity at pH 5 to 8 and temperature of 30° to 40°C. Prospects for cassava processing using a selected single culture with properties for starch hydrolysis, cyanide detoxification, and acid production have thus evolved.

· To initiate genetic manipulation of cassava lactic acid bacteria, the plasmid profiles of the lactobacilli isolated from cassava were studied. The presence of plasmids among cassava lactobacilli has been confirmed. Further research is needed to investigate the correlation between possession of plasmids and linamarase production in order to establish prospects for genetic manipulation.

FUTURE RESEARCH

Beyond the selection of appropriate starter cultures for cassava fermentation, it will be necessary to improve the starter culture. Genetic manipulation of the starter culture offers the best hope for improved cassava processing, with higher economic returns and improved stable qualities.

Cassava processing could also be enhanced by using biotechnological principles to modify structural and processing characteristics of cassava cultivars to meet specific product requirements.

The linamarase elaborated by cassava plant tissues and fermenting microorganisms has been found to be unstable under high acidic conditions characteristic of the latter part of natural fermentation. Techniques for increasing the stability of linamarase enzyme to acidic conditions could be investigated.

The usefulness of cassava fermenting microorganisms could be further investigated for the production of other economically viable products such as acidulants and antimicrobial agents.

A biotechnological approach could be investigated for the treatment of odorous fermented cassava water and cassava root peels.

13 Improving the Nutritional Quality of Ogi and Gari

T. G. Sokari

Ogi is a blancmange-like product processed by fermenting the slurry from wet-milled maize (or sorghum or millet). Used as both a weaning food for infants and as a breakfast food by adults, ogi is one of the most important food items in Nigeria. Yet it is nutritionally inferior to maize, which is deficient in certain essential amino acids, because of the maize-milling process that is an integral part of ogi production.

Cassava, another very important food crop, has the problem of possible nutritional complications because it contains the cyanogenic glucosides linamarin and lotanstralin. Although the cyanogens in cassava are hydrolyzed to hydrogen cyanide during processing by the endogenous enzyme linamarase (1,2), not all processes are equally effective. It has even been suggested that traditional processing techniques are unlikely to remove all the cyanide from cassava (3,4).

In view of this, studies were undertaken to increase the protein content of ogi relatively inexpensively and to develop a technique for processing cassava into gari that would eliminate cyanogens from the product or reduce them to innocuous levels.

PROCESSING OF OGI

The traditional technique for processing maize into ogi is summarized in Figure 1. Also shown in Figure I is an alternative to this procedure; a 20-minute boiling step is substituted for the normal 24- to 28-hour steeping of maize prior to wet milling (5). Cowpea can be combined with maize to increase the protein content of ogi.

Substituting 20 minutes of boiling for the traditional 24 to 28 hours of steeping prior to wet milling maize reduced processing time from 72-76 hours to about 24 hours.

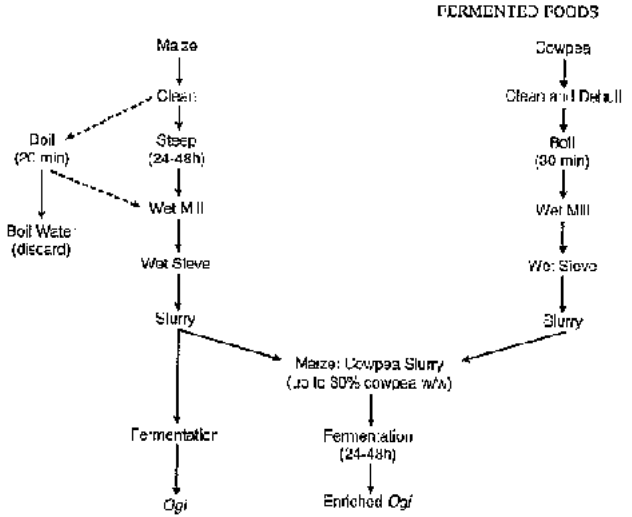

FIGURE 1 Processing of maize into ogi including cowpea protein enrichment; dotted lines show a bypass by which processing time can be reduced.

There was, however, no significant difference (p> 0.05) in the aroma, color, taste, and overall acceptability between the products obtained by the short-time processing and

traditional processing (5). The same was also true for unenriched and protein-enriched ogi except for color (6).

CYANIDE REDUCTION DURING CASSAVA PROCESSING

Two foods processed from cassava (gari and ijapu) were studied. Adding water to grated cassava at the 75 percent (v/w) level and heating at 50°C for 6 hours resulted in linamarin reduction of >99 percent (Figure 2). The pH of the mash fell from 6.4 to 6.3 during the period (7). After dewatering, the mash was adjusted to a pH below 4 by equilibrating with a 3-day fermented cassava liquor (40 percent, v/w) at 50°C for 12 to 18 hours. The equilibrated mash was then dewatered and toasted (Figure 3). A panel of tasters who were familiar with gari but otherwise untrained could not differentiate between the product and traditionally processed gari. Both sets of products were equally acceptable to the panel.

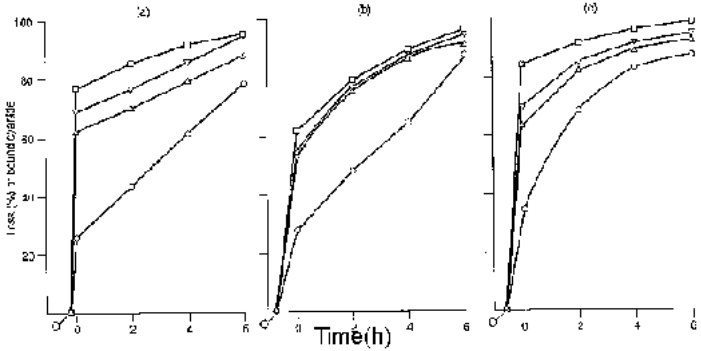

Figure 2 Effect of added water at 0% - i.e., control o-o, 25% D-D, 50% v-v, 75% - levels on linamarin hydrolysis in grated cassava at (a) 30°C, (b) 40°C, (c) 50°C.

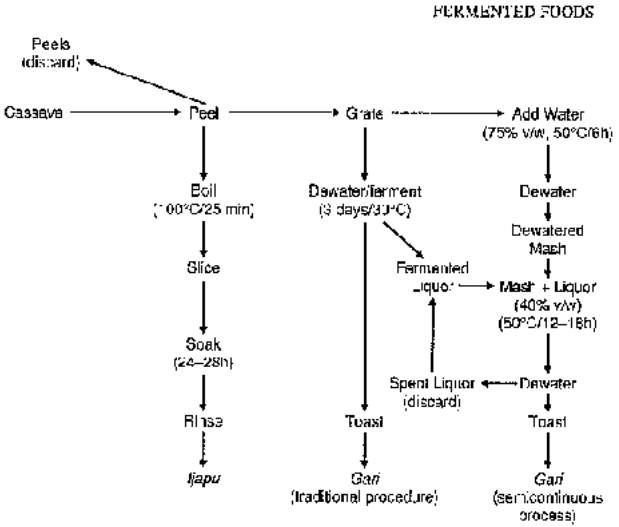

FIGURE FIGURE 3 Processing of ijapu and gari from cassava.

The modified procedure for processing cassava into gari reduced the processing time from >96 hours to about 24 hours. Cyanogens were not detectable in the product by the method of Cooke (8,9).

The study of the traditional production of ijapu (Figure 3) was intended to aid in understanding the loss of cyanogens during cassava processing. About 54 percent of the cyanogens in raw cassava were lost after boiling peeled cassava, but a substantial proportion remained in the water (Table 1). After slicing the boiled cassava and steeping the slices, a substantial proportion of the cyanogens was again lost.

TABLE 1 Cyanide Content of Cassava During Processing Into Ijapu

Cyanide Content (ppm)						
Material analyzed	pH	Total	Free	HCN	Bound	Cyano
Unprocessed peeled cassava	6.3	76.1+15.3	5.5+2.2	2.9+0.4	70.6	2.6
Boiled cassava	6.0	35.1+8.7	2.4+1.2	2.0+0.7	32.7	0.4
		(53.9)			(53.7)	
Boil water	6.2	12.8+2.1	1.1+0.2	0.5+0.2	11.6	0.5
"Ijapu"						
(after 24 hours steeping)	ND	11.8+1.4	4.2+1.4	3.1+1.4	7.6	
		(84.5)			(89.2)	
Steep water	4.0	16.2+3.9	5.7+3.4	5.6+3.4 7.8	0.1	

Note: Cyano, Cyanohydrin; numbers in parenthesis, percent loss; ND, not determined

Much of the loss could, however, be accounted for in the steep water, and the proportion lost depended on the duration of steeping and the cassava: water ratio (Tables I and 2).

ROLE OF FERMENTATION

The reduction of >99 percent in the linamarin content of grated cassava within 6 hours of adding water, with little or no change in the pH of the mash, would imply that fermentation had nothing to do with the detoxication. Linamarin breakdown is essentially a hydrolytic process catalyzed by the endogenous enzyme linamarase (1,2). The results of the present study indicate that the addition of water aids in the hydrolytic process. Apparently not all of the water normally in raw cassava tuber is available for hydrolysis.

During the boiling of cassava for processing into ijapu, linamarase would be inactivated. Yet a substantial proportion of the linamarin in cassava was still lost, appearing to a large

extent in the water used for boiling and for steeping (Tables 1 and 2). This would suggest that leaching could be an important factor in cyanide loss during cassava processing. This would be true not only during the boiling and steeping of cassava for ijapu production but also during the dewatering of grated cassava for gari production.

CONCLUSION

Cassava detoxication during processing is essentially an enzymic hydrolysis of cyanogens in cassava (1,2,8). Fermentation has little role

TABLE 2 Effect of Sliced Boiled Cassava: Steep Water Ratio on Cassava Detoxification

Ratio

(w/v)	Material analyzed	pH	Total	Free	HCN	Bound	Cyano
1:1	Unboiled cassava	6.4	90.2	4.5	3.0	85.7	1.5
	Boiled cassava(a)	ND	51.7	1.5	0.5	50.3	1.0
		(42.7)				(41.3)	
	Boil water	6.1	20.2	0.5	0.3	19.7	0.2
	Sliced, boiled	ND	5.6	1.1	0.6	4.5	0.6
	cassava(b)	(93.8)				(94.7)	
	Steep water	4.3	25.5	5.7	4.5	19.7	1.3
1:2	Sliced boiled	ND	5.7	1.7	0.6	4.0	1.2
	cassava(b)	(93.7)				(95.3)	
	Steep water	4.3	21.3	1.9	1.4	19.4	0.5
1:3	Sliced, boiled	ND	4.2	1.6	0.5	2.6	1.0

	cassava(b)	(95.3)				(97.0)	
	Steep water	4.2	11.5	2.1	1.1	9.4	1.0

(a) Prior to slicing and soaking in water.
(b) After 24 hours soaking; other notes as in Table 1.

in this process and may even be antagonistic to it (10). Leaching is another important process for cyanogen reduction during cassava processing. Although fermentation does not aid in cassava detoxication during processing, it is important in flavor development (11,12) and preservation of the product.

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14 Solid-State Fermentation of Manioc to Increase Protein Content

Nguyen Ngoc Thao and Nguyen Hoai Huong

Manioc (cassava) is grown extensively in Vietnam and other tropical countries for its high yields in infertile soil. Although manioc is high in carbohydrates, its use is limited by its

low protein content (1 to 4 percent). Manioc has been used at levels of 10 to 15 percent in poultry feed and 35 to 50 percent in pig feed. Powdered dried fish debris (gills, scale, tail, etc., from the fish processing industry), oil cake (from coconut or peanut oil production), or soybean flour have been used to raise protein levels in such feeds, but these products raise the price of feed significantly.

To upgrade the protein content in manioc, yeast cells or fungi can be inoculated in a manioc-containing medium along with nutrients containing nitrogen, phosphorus, and potassium. The use of mycelial fungi has the following advantages:

· The protein content in fermented product can increase to 30 percent.

· Fungal protein can be substituted completely for animal protein.

· The product has a low nucleic acid content.

· The product contains a favorable spectrum of amino acids.

Solid-state fermentation and liquid-state fermentation are two methods used for cultivation of fungus. Liquid-state fermentation processes are well developed in industrialized countries but are not suitable for rural farms in developing countries. Solid-state fermentation is a simple process that does not require modern equipment, power supply, or sterile conditions. In addition, the capital investment is low, permitting countryside operation and the use of available manual labor.

Many studies of solid-state fermentation of manioc have been conducted. The cultivation of Aspergillus niger in a manioc medium at 35° to 40°C for 30 hours has resulted in protein content increases of 5 to 18 percent; carbohydrate content decreased from 65 to 28 percent (1). The protein from this fermentation can be competitive with soybean protein.

In addition to A. niger, other fungi such as A. awamori, A. hennebegii, A. fugamitus, Rhizopus chinensis, and Sephalo sporium lichlorniae can be grown in acid medium at high temperatures. The protein content of the fermented product can reach 48 percent.

In Vietnam, A. niger and A. hennebegii were cultivated on a maltolized-manioc medium or a mixture of manioc and rice flour. This research comes from the demand of the husbandry industry and is designed to develop a fermentation process for on-farm use.

MATERIALS

Dried manioc pieces were ground to the size of 5 to 10 mm. Spores of A. niger were cultivated by surface fermentation on a medium containing rice hulls, rice bran, or manioc flour as carbohydrate, and urea (2 percent), ammonium sulfate (8 percent), and potassium phosphate (4 percent) at pH 4.5. Spores were collected after 7 days of cultivation.

METHODS

After a defined period of fermentation, the product was dried at 65° to 70°C, ground, and analyzed. The moisture content was determined by drying at 105°C to constant weight. The protein content was determined by precipitating with a solution of CaSO4 (6 percent) and NaOH (1.25 percent); the precipitate was analyzed by the Kjeldahl method. The starch content was determined by hydrolyzing the preparation with HCl and using the Bertrand method. The reduced glucose content was determined by the Bertrand method.

Table I shows that A. niger could not grow in medium containing urea as the only nitrogen at a concentration of 4.5 percent because of the resultant alkalinity. With (NH4)2SO4 as the N source, the pH was maintained at 4 to 5 during the fermentation. The maximum protein content was attained in medium containing urea (4 percent) and ammonium sulfate (5.8 percent). The content of protein can reach 17 percent in comparison with the one cultivated in only urea-containing medium. However, 1.55 percent N protein was

achieved in the culture medium containing 3.1 percent N with the transformation efficiency of 49 percent.

TABLE 1 Effect of Nitrogen Sources on Protein Formation

N Protein

Percent Percent N Protein

N in Culture in Fermented in Fermented

N°	Source	Medium	Product	Preparation
1 Urea	2.0	0.93	5.2	0.83
	3.3	1.54	8.0	1.28
	4.0	1.86	8.2	1.3
	4.5	2.10	no growth	--
	5.0	2.33	no growth	--
2 Ammonium sulfate				
(AS)	7.4	1.54	4.4	0.70
Urea 2% + AS 10%		3.05	7.2	152
Urea 3.3% + AS 4%		3.10	9.36	1 49
Urea 4.0% + AS 5.8%		3.1	9.7	1.55
Urea 4.5% + AS 7.4%		3.1	no growth	

The transformation efficiency was 70 percent in medium containing only urea (4 percent).

The P and K elements (Table 2) were added to the medium containing urea 3.3 percent and ammonium sulfate 4.4 percent (1-5) or urea 3.3 percent (6-9), respectively. The results suggested that the P and K sources had no clear effect on protein formation.

In Table 3, the effect of humidity on the protein content is shown. Table 4 illustrates the effect of sterilizing conditions on the yield of protein. Table 5 shows the effect of the amount of inoculum culture on protein synthesis.

RESULTS

Manioc flour cannot be used as a carbohydrate source in the culture medium because it agglomerates and excludes air necessary for the growth of the fungal mycelium.

Manioc pieces of 0.5 to 1.0 centimeters are best for this solid fermentation method. The protein content of fermented preparation decreased 50 percent when using manioc pieces that were 1.0 to 2.0 centimeters in size.

The analysis of a fermented preparation after 2 days of fermentation, drying at 65° to 70°C, and grinding is shown in Table 6.

TABLE 2 Effects of Nutrients on Biosynthesis of Protein (The P and K elements were from chemical fertilizers)

Chemical Fertilizer	P Percent + K Percent	Protein Percent
1	3.3 + 1.0	10.21
2	2.3 + 0.5	11.34
3	2.3 + 1.5	10.41
4	4.3 + 0.5	11.46

4	4.3 + 0.5	11.46
5	4.3 + 0.5	9.3
6	3.3 + 1.0	10.0
7	2.3 + 0.5	10.0
8	1.3 + 0.5	11.25
9	0.3 + 0.5	9.62

TABLE 3 Effect of Initial Humidity on Protein Content

	Humidity of	**Protein**	
N°	**Culture Medium(a)**	**Percent**	**Notes**
1	45	7.9	The change of humidity from
2	50	9.36	60 to 70 percent occurred
3	55	9.64	depending on the atmospheric
4	60	11.08	temperature and humidity.
5	65	11.23	
6	70	11.37	
7	75	Poor growth	

a The medium for this experiment contained urea (4 percent), P (1.3 percent), and K (0.5 percent).

TABLE 4 Effect of Sterilization Conditions on Protein Production

N°	**Temperature °C**	**Time Minutes**	**Percent Protein**	**Notes**
1	100	45	8.6	The culture medium

2	100	90	7.35	can be sterilized
3	120	30	7.6	at 100°C in
4	120	45	7.50	45 minutes.
5	120	60	6.30	

TABLE 5 Effect of the Amount of Inoculum Culture on Protein Synthesis

	Percent of Inoculum	**Percent**	**Notes**
N°	**Culture**	**Protein**	
1	0.5	6.0	The maximum percent of
2	1.0	8.6	protein was achieved in 2 percent
3	1.5	10.0	of inoculum culture. There was
4	2.0	12.5	formation of black spore in
5	3.0	12.0	fermented preparation when using more than 2 percent of inoculum culture.

TABLE 6 Product Analysis

			Fermented
		Manioc Pieces	Preparation,
N°	Index	Culture-Medium, Percent	Percent
1	Protein	1.5-2.0	10.0-13.3
	Starch	33	11

	Starch	95	11
	Reduce sugar	4.4	8.11
	Total sugar	8.4	13.00

CONCLUSION

This solid-state fermentation method can be used to upgrade by six to seven times the protein content in manioc pieces. The resulting fermented product contains 10 to 13 percent protein, which is suitable for use as a feed additive.

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15 Leaf and Seed Fermentations of Western Sudan

David B. Harper and M. A. Collins

Kawal, sigda, and furundu are fermented foodstuffs indigenous to the Kordofan and Darfur provinces of Western Sudan. All are produced by solid state fermentation of readily available plant materials of little or no economic value which, though unpalatable in its natural state (and indeed toxic in the case of kawal), contain protein rich in sulphur amino acids. In each case, fermentation yields a product that is not only organoleptically acceptable but also sufficiently highly regarded nutritionally by the local people to be employed as a meat substitute. As the sun-dried food can be stored indefinitely without deterioration, these fermentation processes represent a food preservation technique particularly well suited to the climate and conditions of this part of Africa. Biochemical and microbiological aspects of these fermentations and their nutritional implications have been investigated by Dirar (1), Dirar et al.(2), and Elfaki et al.(3).

PREPARATION

Kawal is prepared from the fresh leaves of a wild and reputedly toxic legume, Cassia obtusifolia, which are pounded to a paste and packed into an earthenware zeer buried to the neck in the ground in a shaded location. A layer of green sorghum leaves is placed on the surface of the paste and the zeer fitted with a lid that is sealed with mud. At intervals of 3 days the vessel is opened, the sorghum leaves removed, and the paste remixed thoroughly by hand. The repacked paste is covered with fresh sorghum leaves and the zeer resealed. After 11 to 15 days, the strongly smelling black mass is removed, molded into small balls, and dried in the sun for 5 days. The dried kawal is usually consumed in a stew with onions, okra, or other local vegetables.

The seedcake remaining after oil extraction from Sesame indicum seed is the raw material for the sigda fermentation. The bitter, indigestible seedcake made from nondecorticated seed is often used only as animal feed. In the traditional sigda process the seedcake is ground to a paste with warm water. Kambo, a local form of potash from the dried leachate of the ash of the central stems of the sorghum seed head, is frequently, but not invariably, added (3 to 20 g/kg). The mixture is packed in an earthenware vessel sealed with a cotton cloth and a close-fitting lid to minimize access of air. The fermentation lasts 3 to 7 days at=30 C° with occasional remixing, addition of water if necessary, and resealing of the container, after which the product is molded into small balls and sun-dried. Like kawal, sigda is usually consumed in a vegetable stew. A similar fermented food, furundu, is prepared from the crushed seeds of karkade (Hibiscus sabdariffa) by a process almost identical to that employed for sigda.

MICROBIOLOGY

The microflora of C. obtusifolia leaves (the substrate for the kawal fermentation) was dominated by four bacterial species, Bacillus subtilis, Lactobacillus plantarum,

Propionibacterum sp., and Staphylococcus sciuri, and two yeasts, Candida krusei and Saccharomyces sp. Although the relative proportions of these organisms changed, all persisted in detectable numbers throughout fermentation. The principal species present during fermentation were B. subtilis and Propionibacterium sp., the other organisms comprising a comparatively small proportion of the population. No marked interspecial successional pattern occurred during fermentation.

The microflora of unfermented sesame seedcake was dominated by two bacterial species, Pediococcus sp. and Streptococcus sp., and two yeasts, Saccharomyces sp. and Candida sp. Pediococcus sp. was eliminated after the second day of fermentation, and the occurrence of the two yeasts was confined to the first half of the fermentation period. However, the homofermentative lactic acid bacterium Streptococcus sp. dominated the microflora throughout most of the fermentation. Additionally, the yeasts Debaryomyces sp. and Torulopsis sp. appeared in low numbers late in fermentation.

No detailed examination of the microflora during fermentation of furundu has been attempted, but the principal organism present in the final product was identified as a Bacillus sp.

PROTEIN CONTENT AND QUALITY

The crude protein content decreased only slightly, if at all, during fermentation of each substrate, indicating little loss of nitrogen during the process (Table 1). It is clear that the high sulphur amino acid content of all the fermentation substrates is largely retained in the fermented products, which compare favorably with the FAO reference protein in this respect (Table 2). The branched chain amino acids valine, leucine, and isoleucine also tend to be at a higher level in the protein of sigda and furundu than in the protein of their respective substrates. The other noteworthy feature is the markedly enhanced concentration of alanine in sigda and, to a lesser extent, in furundu, compared with the

unfermented substrate. This increase is probably attributable to the transamination of pyruvate formed by oxidation of the lactic acid produced in the fermentation. Significantly, alanine concentration did not rise during the kawal fermentation where lactic acid production is negligible.

The overall protein quality of each of the fermented foods is determined by the content of lysine, which is limiting in the raw material for both the sigda and furundu fermentations and does not increase appreciably during fermentation. Nevertheless, the proteins of kawal and furundu, with chemical scores of 73 and 80, are of surprisingly good quality, whereas that of sigda, with a chemical score of 33, is no poorer nutritionally than the protein of the local staple cereal, sorghum.

MINERAL, CRUDE FIBRE, AND OIL CONTENT

Ash content of all fermented foods showed a substantial increase on that of the unfermented substrate, which, in part, reflects the mineral contribution made by clay scraped from the interior of the fermentation vessel during preparation but also, in the case of sigda and particularly furundu, the liberal addition of kambo (Table 1). The latter consists largely of potassium bicarbonate with smaller quantities of potassium chloride, silicate, and sulphate. C. obtusifolia leaves display an unusually high calcium content, which is believed to be critical in determining the course of fermentation (see below). Oil and crude fibre contents of the fermented foods was not significantly different from that of the unfermented substrates, suggesting that participation of these fractions in the fermentation process is unlikely.

CHANGES DURING FERMENTATION

The dominant role of lactic acid and the marked decrease noted in pH during the sigda fermentation contrast strongly with the high concentrations of volatile fatty acids (VFA) and minimal pH change observed in the kawal fermentation (Table 3 and Figure 1).

TABLE 1 Composition of Field Collected Kawal, Sigda, and Furundu Compared With That of Their Fermentation Substrates on a Dry Weight Basis

			Crude	Crude									
	Ash	protein	Oil	fibre	KNa	Ca	Mg	P	S	Fe	Zn	Mn	Cu
	%	%	%	%	%%	%	%	%	%	mg kg^{-1},	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Leaves of C. obtusifolia	12.6	24.3	2.5	13.5	ND(a)	ND(a)3.85	0.30	0.26	ND	534	32	75	ND
Kawa/	19.6	26.2	3.8	12.1	2.5ND(a)	4.13	0.42	0.28	0.52	82	84	112	11
Sesame seedcake(b)	14.0	45.6	14.4	7.4	1.04<0.01	1.87	0.66	1.12	0.74	708117	68	38	
Sigda	18.2	43.8	16.9	8.2	1.830.67	2.25	0.66	1.11	0.75	509	127	83	34
Karkade seed	6.2	32.6	21.1	25.1	1.27<0.01	0.31	0.43	0.62	0.36	313	90	118	18
Furundu	22.8	26.5	23.3	26.5	5.650.08	0.58	0.69	1.08	0.63	347	116	122	21

(a) ND Not detemmined

(b)Assara extracted

TABLE 2 Amino Acid Composition of Kawal, Sigda, and Furundu and Their Fermentation Substrates Compared With the FAO Reference Protein

Amino add concentration (g 16 g^{-1} N)

										NH_2	NH_2									
	Asp	**Thr**	**Ser**	**Glu**	**Pro**	**Gly**	**Ala**	**Val**	**Cys**	**Met**	**ILeu**	**Leu**	**Tyr**	**Phe**	**His**	**Lys**	**Arg**	**Orn**	**But**	**B**
Leaves of c	12.1	6.2	4.6	13.6	7.7	6.7	7.5	7.5	1.4	2.1	6.0	10.4	5.3	6.8	3.3	7.7	7.2	<0.1	<0.1	2
obtusifolia																				
Kawal	7.7	3.3	2.8	8.2	4.2	5.0	6.8	6.4	1.2	1.5	5.1	8.3	3,5	5.4	2.0	4.0	4.0	0.1	0.7	4
Sesame	7.8	3.1	3.7	20.9	3.9	5.4	4.7	6.3	1.6	2.4	3.2	6.6	3.2	4.2	2.4	2.0	12.8	<0.1	<0.1	<
seedcake																				
Sigda	7.7	2.6	3.1	20.1	4.2	6.0	9.9	6.0	2.1	2.5	4.6	8.0	2.8	4.5	2.0	1.9	10.4	<0.1	1.2	4
Karkade seed	11.0	3.2	4.8	24.3	4.1	5.4	4.4	4.5	2.2	3.1	3.4	6.8	3.2	4.8	2.4	4.2	13.0	<0.1	<0.1	<
Furundu	10.4	3.5	3.5	20.2	4.5	5,9	6.2	5.3	1.9	2.63.6	7.1	2.6	4.3	2.0	4.4	7.7	0.9	<0.1	0.2	
FAO reference			4.0						5.0	3.5	4.0	7.0	6.0			5.5				
protein																				

TABLE 3 Lactic Acid and Volatile Fatty Acid Content of Field Collected Kawal, Sigda, and Furundu (Mean and Range in g 100 g^{-1} Dry Matter)

Acid	**Kawal**	**Sigda**	**Furundu**
Lactic	0.21	3.07	0.50
	(0.03-0.51)	(2.85 3.35)	(0.03-1.67)
Acetic	5.08	1.10 1.59	
	(2.12-6.75)	(1.00-1.19)	(1.22-2.05)
Propionic	0.90	0.04 0.09	

Propionic	0.90	0.04-0.09	
	(0.51-1.59)	(0.03-0.05)	(0.02-0.25)
Isobutyric	0.24	<0.01	<0.01
	(0.04-0.38)		
n-Butyric	2.94	0.08	0.24
	(1.18-4.73)	(0.02-0.15)	(0,05-0.73)
Isovaleric	0.22	0.02	0.17
	(0.06-0.60)	(0.01-0.03)	(0.02-0.35)
n-Valeric	0.18	<0.01	0.01
	(0.01-0.61)	(0.01-0.02)	
Total VFA	9.56	1.24	2.17
	(4.70-12.1)	(1.12-1.38)	(1.65-2.63)

Thus, by the eleventh day of the latter fermentation, VFA - mainly n-butyric (8 percent), acetic (5 percent) and n-propionic (9 percent) - comprised 15 percent of the fermentation mixture. However, the pH had not changed by more than 0.5 unit from the initial value. On the other hand, by the fifth day of the sigda fermentation, when a total acid concentration of 6 percent had been attained, the pH of the fermentation mixture had fallen to 4.0 from an initial value of about 6.0. This difference in the course of fermentation is almost certainly attributable to the stronger buffering capacity of the substrate of the kawal fermentation, C. obtusifolia leaves, which possess approximately double the calcium content of sesame seedcake. Conditions in kawal do not, therefore, favor the selection of acidoduric lactic acid bacteria.

In addition to these bacteria, the two yeast species present in unfermented sesame seedcake proliferated during the initial period of fermentation. Concomitantly, starch

levels were observed to fall rapidly from 2 percent in the unfermented substrate to zero after the first two days of fermentation. As the only amylolytic organisms present, the yeasts presumably were responsible for degradation of starch, rendering it available to the lactic acid bacteria. The poorly fermentative yeasts Torulopsis sp. and Debaryomyces sp. isolated in the final stages of the fermentation can utilize lactic acid aerobically and may cause the decline in concentration of the compound during this period. The addition of kambo did not appear to have any significant effect on the course of the sigda fermentation, and it was concluded that this supplementation was probably practiced mainly on organoleptic grounds.

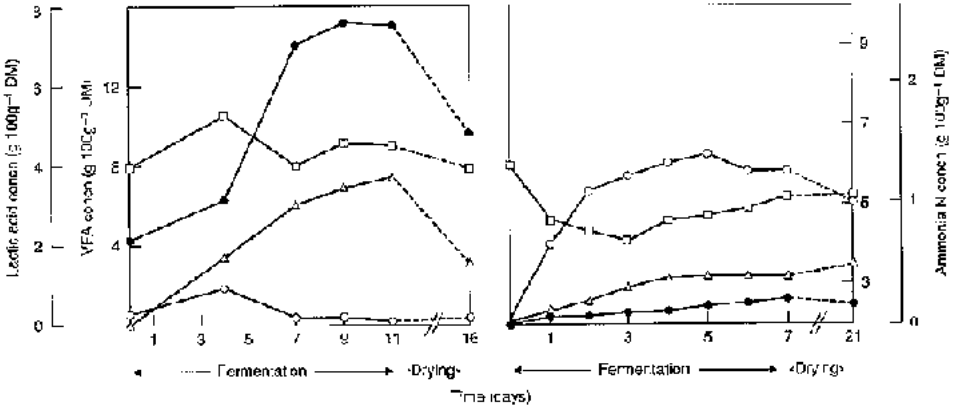

FIGURE 1 Changes in pH, VFA, lactic acid, ammonia-N concentrations during fermentation of (a) kawal and (b) sigda.

--, pH; -·-,VFA concentration; -O-, Am, lactic acid concentration; Am, -D- ammonia-N concentration

The VFA, primarily n-butyric and acetic acids, which are the principal products of the

kawal fermentation, are characteristic of clostridial fermentation of plant material as is the accumulation of ammonia nitrogen. However, all attempts to isolate clostridial species from the fermentation mixture were unsuccessful, indicating that the microbial origin of VFA must be sought elsewhere. The formation of acetic acid can probably be ascribed to the heterofermentative B. subtilis, the co-dominant microorganism, whereas propionicacid is probably an end product of anaerobic fermentation by propio'Zi~acte ~ium sp. for which lactate is a preferred substrate. Utilization of lactate in this way could explain the low level of lactate in Karl, despite the substantial population of Lactoloacill~´s plantar ´´m. The microbial pathway leading to formation of n-butyric acid is difficult to define, although its production may be characteristic of fermentation by this type of mixed culture as a whole rather than that by any single microorganism. n-Propanol (2.3 percent), n-butanol (01 percent), and ethanol (0.1 percent) were also detected in Karl toward the end of fermentation, though all were lost from the product during the drying phase. Formation of such alcohols is probably due to anaerobic fermentation of carbohydrate by the yeast species present.

The identification of Bacillus sp. as the principal microorganism in furundu when considered in the context of a final pH of 6.2 and the presence of both VFA and lactic acid in the fermentation mixture suggest that the furundu fermentation may be intermediate in character between those of sigda and kawal. Further investigation of thefuru,~du fermentation would be most instructive in this respect.

CONCLUSIONS

The sigda and furundu fermentations appear quite unlike the traditional oilseed fermentations practiced in Nigeria and elsewhere in West Africa where foods such as ogili and ogiri are fermented from castor oil seed (Ricinus communis) and melon seed (Citrullus vulgaris). There are even variations of the fermentation which use sesame seed and karkade seed known as ogiri-sara and red sorrel, respectively. During these West

African fermentations, the pH increases to over 9.0 and ammonia production is frequently observed in the later stages. The fermentations are dominated by Bacillus s p., frequently Bacillus s´~btilis, an organism associated with spoilt sigda in Sudan. The principal reason for the difference would appear to be in the preparation of the seeds prior to fermentation, which in West Africa involves boiling for several hours in water until soft. Such pretreatment may alter the course of fermentation by two mechanisms - first, by rendering protein and polysaccharide more available for degradative attack by microorganisms, and second, by effectively eliminating much of the heat-sensitive indigenous microflora. The removal of amylolytic yeasts may well favor the selection of amylase-producing bacteria such as Bacillus sp. rather than lactic acid bacteria incapable of utilizing starch.

The three fermentations studied appear to afford a route by which unpalatable plant material or oilseed cake of little economic value can be converted into acceptable meaty-tasting food that is particularly rich in sulphur amino acids, which tend to be deficient in diets where access to meat or fish is limited. Phytic acid present in seeds can frequently hinder absorption of minerals in the gastrointestinal tract. As fermentation of plant products has been shown to reduce physic acid levels substantially, it is likely that the bioavailability of minerals in both sesame and karkade seed is increased in sigda and furundu.

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16 Continuous Production of Soy Sauce in a Bioreactor

Takashi Hamada, Yaichi Fukushima, and Hiroshi Motai

Soy sauce is a traditional all-purpose seasoning with a salty taste and sharp Havor. In the conventional method of brewing soy sauce (Figure 1), cooked soybeans and roasted wheat are mixed with spores of Aspergillus species and fermented in solid culture for 2 days to produce koji. The koji is then mixed with brine to make moromi, the mash that ferments to produce soy sauce. Over time the soybeans and wheat are hydrolyzed by enzymes such as proteinases, peptidases, and amylases. During the first stage of moromi fermentation, Pediococcus halophilus grows and produces lactic acid, which lowers the pH. Accompanying the decrease in pH, vigorous alcohol fermentation by Zygosaccharomy-ces rouxii occurs. As a result, 2 to 3 percent ethanol and many kinds of aroma components are produced by this yeast. At the same time, phenolic compounds such as 4-ethylguaiacol (4EG) and 4-ethylphenol, which add characteristic aroma to soy sauce, are produced by other types of yeasts such as Candida versatilis and Candida etchellsii.

It takes over 6 months for the entire fermentation and aging of the morommi mash. Therefore, shortening this period is important and new processes for soy sauce brewing are desirable. This paper describes the continuous production of soy sauce in a bioreactor system, which consists of reactors containing immobilized glutaminase and immobilized cells of P. halophilus, Z. rouxii, and C. versatilis.

MANUFACTURING PROCESSES

The processes for soy sauce production using the conventional and bioreactor methods

are shown in Figure 1. The bioreactor method differs from the conventional one in the following ways: (a) proteases from continuous submerged culture are used (1), (b) fermentation is carried out in the liquid state, and (c) the fermentation period is considerably shorter. It takes several months for the conventional fermentation but only about 2 days for the bioreactor method.

In the bioreactor method, raw liquid was successively passed through, first, a glutaminase reactor to increase glutamic acid; second, a P. halophilus reactor to carry out lactic acid fermentation; and, third, a Z. rouxii reactor to carry out alcohol fermentation and a C. versatilis reactor to produce phenolic compounds such as 4-ethylguaiacol. Two reactors containing immobilized yeast cells were set in parallel, and the flow rate of the feed solution to the Z. rouxii and C. versatilis reactors was set in a ratio of 10 to 1. Carrier, packed gel volume, and operating conditions such as residence time, temperature, and aeration in each reactor are shown in Table 1.

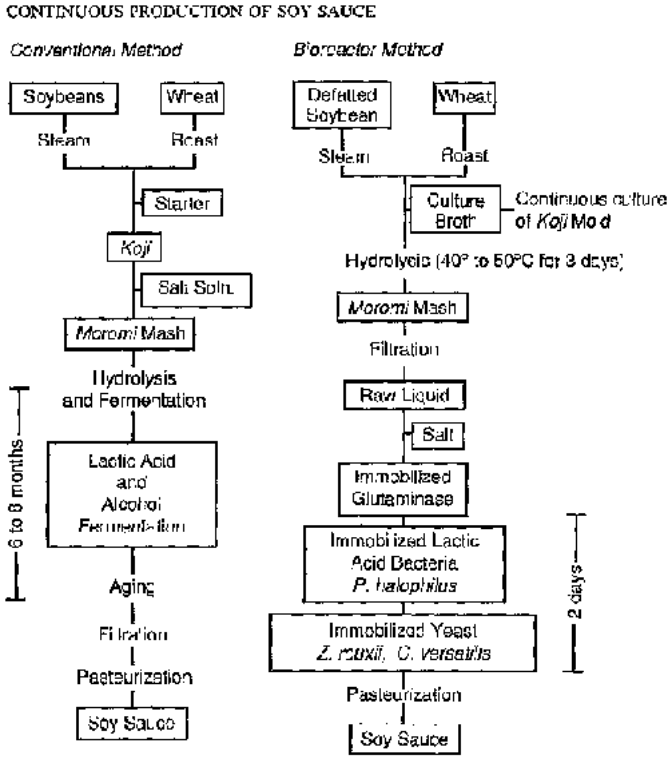

FIGURE 1 Manufacturing processes for soy sauce by conventional and bioreactor methods.

TABLE 1 Conditions of Fermentation in Each Reactor

		Column	Packed Gel			
		Volume	Volume	Residence		

Reactor Carrier		(L)	(L)	Time,hours	Temperature, °C	Aeration,wm
Glutaminase	Chitopearl	1.8	0.6	0.7	40	--
P. halophilus	AS	7.5	5.0	61	27	--
Z. rouxli	Al	27.0	8.0	25.5	27	0.005
C. versatilis	Al	1.0	0.2	10.7	27	0.08

AS, Alginate-colloidal silica. Al, Alginpte.

CONTINUOUS FERMENTATION

A profile of continuous fermentation by immobilized cells of P. halophilus, Z. round, and C. r~ersatilis is shown in Figure 2. The fermentation continued for over 100 days without any microbial contamination. A consistent increased level of glutamic acid (in the range of 0.3 to 0.4 percent) was found in the effluent from glutaminase reactor, with a residence time of 0.7 hours. Lactic acid was produced by immobilized cells of P. halophilus in quantities of 0.7 to 1.0 percent at a residence time of about 6 hours, and consequently the pH declined to 4.9 to 5.0, similar to that of conventionally brewed soy sauce. Ethanol was produced constantly by immobilized cells of Z. rotlxti in quantities of 2.5 to 2.7 percent at a residence time of about 26 hours. This is the standard ethanol content in soy sauce. About 10 ppm (parts per million) of 4-ethylguaiacol was produced by immobilized cells of C. versatilis at a residence time of about 10 hours, and the final 4ethylguaiacol content after mixing the two fermented liquids from the reactors of Z. rouxii and C. versatilis was about 1 ppm, which is the optimum concentration in conventional soy sauce. The total residence time for lactic acid and alcohol fermentation was about 30 hours in this system. This was considerably shorter than the conventional fermentation period of 3 to 4 months required to produce the same amounts of lactic acid and ethanol.

High numbers of viable cells were present in the gel and liquid in each reactor. The

number was 10- to 100-fold higher in moromi mash. The shortening of the fermentation period in the bioreactor method is possibly due to the high density of immobilized cells in the gel and free cells in the liquid.

The main chemical components of the fermented liquid from the bioreactors were examined, including lactic acid, glucose, ethanol, and nitrogenous compounds.

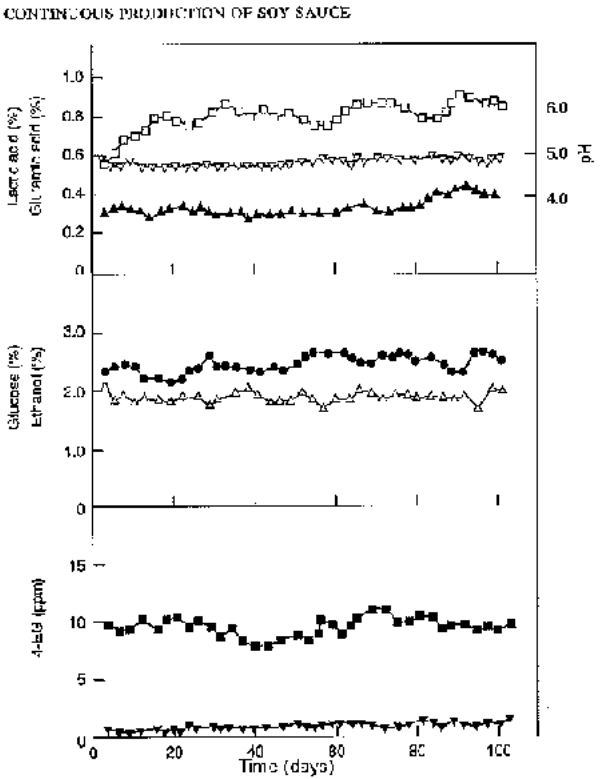

FIGURE 2 Profile of continuous fermentation of soy sauce by a bioreactor system. (), lactic acid; (a), glutamic acid (values indicate the increase in the amount of glutamic acid); (v) pH; (•), ethanol; (D), glucose; 4-KG after passing through the C. versatilis reactor; 4-KG in the final product.

PROPERTIES

The organic acids and aroma components in the bioreactor soy sauce were examined. The proportions of organic acids except citric acid were not much different between the bioreactor soy sauce and the conventional one, although the former was a little lower in acetic acid and succinic acid. It appears that the high residual content of citric acid in the bioreactor soy sauce arises from the inability of P. halophilus to utilize citric acid. Aroma components present in both the bioreactor and conventional soy sauces were not qualitatively different. However, the former was higher in isoamyl alcohol and acetoin and lower in isobutyl alcohol, ethyl lactate, 4-hydroxy-2(orS)-ethyl-5(or2)-methyl3(2H)-furanone, and 4-hydroxy-5-methyl-3(2H)-furanone.

To evaluate the aesthetic qualities of the bioreactor-produced soy sauce, sensory tests were carried out. For example' the intensity of the alcoholic, fresh, sweet, acid, and sharp odors as well as the special lZiga (baking aroma) and bushoshu (foul fermented aroma) were compared between the bioreactor and conventional soy sauces. The odors are important for the quality of soy sauce. Although the bioreactor soy sauce was a little weaker in aroma and fresh odor than the conventional soy sauce, the quality of the former was generally judged to be similar to that of the latter.

The total time required for the production of soy sauce by the bioreactor system, including enzymatic hydrolysis of the raw materials, fermentation with immobilized whole cells, and the refining process, is only about 2 weeks (2). This is considerably shorter than the 6 months with the conventional method of soy sauce brewing consisting of koji making, fermentation and aging of morons, and refining.

From these results we conclude that the quality of the bioreactor soy sauce was very similar to that of the conventional soy sauce from both chemical and sensory evaluations and that the bioreactor system is practical for the production of soy sauce.

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