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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
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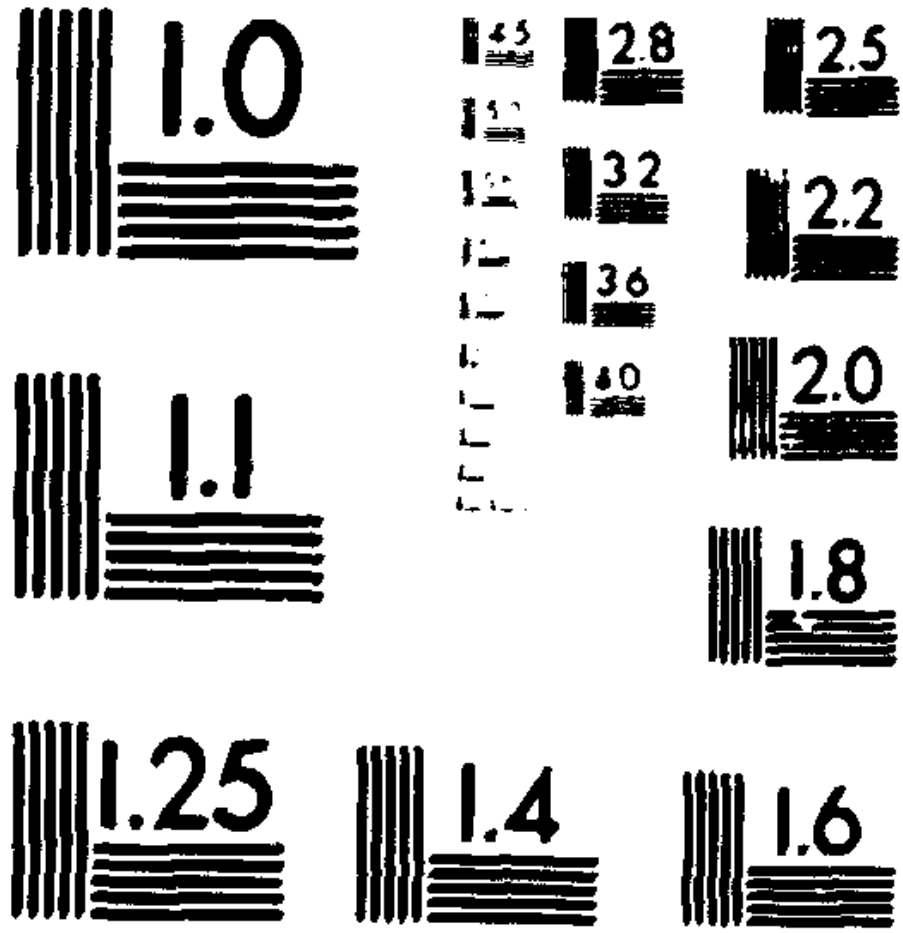

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Application of Biotechnology and Genetic Engineering to African Fermented Food Processes

by
Dr. Keith H. Steinkraus
Professor Microbiology and Food Science
Institute of Food Science
Cornell University
Geneva/Ithaca, New York

3015

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from: E. Kain

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Introduction

The total arable land available for crop production on earth cannot be increased significantly and there is a continuous loss of cropland as a result of desertification particularly serious in Africa. With world population expected to increase by 50% by the year 2000, it is going to be very difficult to maintain per capita consumption of food at its present level. There is a possibility the per capita food consumption in Africa south of the Sahara may actually decrease. What factors can be used to counteract such undesirable decreases in food production and consumption? Much more effort should be applied to breeding crop plants with improved natural resistance to insects and plant diseases and with improved nutritional value. Also, there should be more emphasis on use of natural insect pathogens for insect control. Secondly, new growth hormones which increase the growth rate of young animals or increase the production of milk, the production of vaccines and the use of antibiotics for both controlling animal diseases and also fostering rapid growth should be expanded. Genetic engineering will likely provide the tools needed to accomplish these changes. At least 25% and perhaps as much as 60% of the food produced is lost to insect, rodent and microbial spoilage. At least a portion of these losses can be avoided by proper harvesting and processing of the crops. Fermentation widely practiced in Africa can lead the way to expanded food supplies in the form of Single Cell Protein (SCP) grown on inedible substrates, Microbial Biomass Protein (MBP) grown on edible substrates and serve as a means of processing and preserving the food supply. Fermented foods such as Nigerian ogi, Kenyan uji, South African mahewu, Nigerian gari, Ghanian kenkey and sorghum (Bantu) beer are important parts of the diet. Most of these fermentations can be upgraded and expanded.

Genetic Engineering

Fossilized microorganisms have been found in rocks 3.5 billion years old; and it is likely that all life on earth evolved from microorganisms. Certainly all life is heavily dependent upon microorganisms which continuously recycle carbon, nitrogen, oxygen and sulfur in the environment.

The bacteria, yeasts, molds, algae, protozoa and viruses were poorly characterized up to a hundred years ago; but since then, scientists have been vigorously studying both the beneficial affects produced by microorganisms and the harmful affects such as animal and plant diseases, food spoilage, etc. Recently these studies reached an explosive stage when molecular biologists and microbial geneticists discovered the biochemical basis for genetic control in all cells-deoxyribonucleic acid (DNA). DNA directs the synthesis of all proteins (and therefore all enzymes) and therefore, it controls physical structure, growth and reproduction. More recently scientists have succeeded in synthesizing DNA molecules in the laboratory and also have devised means whereby these coded sequences of nucleotides can be combined with DNA in the cells and thereby cause the cells to produce specific products useful to man and with value on the marketplace. Splicing genes into single-celled microorganisms is called recombinant DNA or genetic engineering. Because of the potential

commercial as well as scientific value of genetic engineering, commercial firms have been established some of which are joint ventures with leading universities.

The developed world believes that genetic engineering is going to lead to a new multi-billion dollar business over the coming years. This suggests that the developing nations also must keep up with this new technology or they may lose some of the potential benefits.

How can genetic engineering and biotechnology become useful to the developing nations in Africa? UNIDO has been very active in looking for ways and means these advances can be made available. Genetic engineering offers means of increasing food production by modifying the genetic make-up of plants, incorporation of nitrogen-fixing genes into cereal crops making them less dependent upon fertilizer, improving resistance of plants to insects making the farmer less dependent upon imported, dangerous pesticides and influencing the nutritional value of foods by incorporating synthetic DNA that codes for production of limiting essential amino acids such as lysine and methionine. Genetic engineering also holds out some hope for "man-made" organisms with enhanced capacity to hydrolyze both lignin and cellulose which would, in turn, unlock the huge quantities of glucose presently "locked" into cellulose for use in producing fermented products of value to man.

Genetic engineering is a highly sophisticated relatively expensive science involving close collaboration among microbiologists, biochemists, geneticists and molecular biologists and requiring the most advanced computerized analytical equipment and a wide variety of relatively expensive chemicals and biochemicals.

Genetic engineering to date has dealt primarily with production of proteins (polypeptides) which include certain hormones, enzymes, antibodies and vaccines. A good example is insulin required for maintenance of health in diabetics. Insulin can be extracted from human or animal pancreases; chemically synthesized; produced by pancreas cells in tissue culture or produced by microbial fermentation using genetically modified cells. To determine the best industrial process it is necessary to analyze the cost of raw materials, cost of recovery and purification, cost of labor and equipment and the suitability of the insulin produced for injection into human patients. Fermentation methods at present yield about 1 gram from 2000 liters while 16 grams of animal pancreas will yield the same amount. Genetically synthesized insulin still must be approved by the United States Food and Drug Administration before it can be used to treat humans. Clinical trials proving its safety and efficacy will be required.

Other genetically engineered products being intensively investigated include: human growth hormone used to prevent bone decalcification, parathyroid hormone also of use in osteoporosis, antigens for vaccine production, interferones for use in treatment of viral disease and cancer and antibodies useful in treating a variety of diseases.

The food industry is the oldest user of biotechnology. There are an estimated 700 companies throughout the world using fermentation technics to

produce a variety of products. Originally molds, yeasts and bacteria were used in fermentations. In recent years, cells of both plants and animals have been utilized growing them in tissue culture. Under controlled conditions, large quantities of cells with uniform characteristics can be produced. Until recently, the sequence of manufacturing a specific product was to search for an organism that produced it. This was the case with many of the antibiotics-penicillin and streptomycin for example. With "genetic engineering", cells can be "tailored" to produce compounds beyond their natural capability. For example Escherichia coli, a common intestinal microorganism can be coded with synthetic DNA to produce insulin, interferon, human growth hormone, proteins with increased content of essential amino acids and enzymes of value both scientifically and commercially.

The genes for nitrogen fixation have been transferred into yeasts thus opening up the possibility of producing nitrogen fertilizers by fermentation. However, it should be noted also that traditional fermented foods such as Indonesian tempeh have been shown to contain a natural nitrogen fixer-Klebsiella pneumoniae. Such organisms should be studied further to see if they can be produced efficiently by fermentation and yield fertilizer nitrogen as well as other products. For example, K. pneumoniae also produces vitamin B-12.

Thus far, non-protein antibiotics cannot be produced through genetic engineering. However, technics involving mutation of the genetic structure of the original producing microorganisms have resulted in vast increases in antibiotic production.

It is obvious that microorganisms and fermentation technics are essential elements needed in utilizing genetic engineering and biotechnology. Thus, a country's indigenous microbial flora and its present fermentation technics can serve as a foundation for further development.

In order to participate in these new developments, each country must plan to send selected young scientists to Genetic Engineering Centers for advanced training in the technics involved.

The first step in each country or region toward application of biotechnology and genetic engineering is to re-assess the total availability and prospective cost of raw materials such as hydrocarbons, flare gas, lignocellulosic wastes including bagasse, straw, human and animal wastes, etc., by-products of the food processing industry such as molasses, coconut water, cassava starch and cassava processing waste. Much of this information may already be available in the Ministry of Agriculture. Raw materials currently used for food should not be included as there are already shortages of food and animal feeds in many areas. The availability of low-cost carbon substrates either hydrocarbon or carbohydrate will determine the possibility of producing SCP within the country. Replacement of even a portion of the cereal grains/legumes fed to animals with SCP can increase the availability of cereal grains/legumes for human consumption and, in turn, the SCP can enable the country to expand its animal production as a source of meat in the diet.

The availability of human and animal wastes determines the possibility of producing cooking fuel by means of biogas generators. Biogas generators which have proven to be valuable in both India and China can yield cooking fuel, which is generally in short supply, while still preserving much of the value of the waste for use as fertilizer.

Surplus, low-cost molasses or cassava starch can be fermented to produce alcohol, SCP or other products of value on the national or export market. And, in fact, if ethanol is the principal product, the residual yeast cells become a valuable product as either a human food or animal feed ingredient.

Genetic engineering always involves a fermentation in which the "engineered" microorganism is cultured and produces the desired product. Thus, the African food fermentations, particularly those that have already been industrialized (Nigerian Ogi, Nigerian Gari, South African Mahewu and Bantu Beer) constitute a base upon which genetic engineering processes can be built.

The essential microorganisms in each indigenous food fermentation should be identified. The biochemical, nutritional and organoleptic changes that occur in the substrate through microbial action should also be characterized.

Microorganisms with an unusual ability to produce organic acids such as lactic, citric or acetic; essential amino acids such as lysine or methionine; vitamins such as thiamine, riboflavin or vitamin B-12 or enzymes such as amylase, protease, lipase or pectinase may serve as a base for industrial production of products saleable locally or on the export market. As soon as an organism with particular ability to produce a product with market value is identified, it becomes possible to increase the efficiency of production by selection of mutants.

Having isolated, identified and studied the essential microorganisms in your traditional fermentations and studied their products, growth conditions and enzymology, it is a small step to optimizing each fermentation to produce high quality fermented foods efficiently. With these data, you can go rather rapidly to pilot plant (small factory) processes and then onto industrial production which can expand production to levels required for your large cities.

Food Fermentations

Fermentation is one of the oldest and remains one of the most economical methods for processing and preserving food for man. Yeast breads made from wheat or rye flours are a staple in the diets of the developed world. The Indians have developed a fermentation process (Indian Idli/Dosai) whereby a bread texture can be produced from rice and legumes. it requires no eggs or milk and the process is likely to be widely used in the years to come. It is basically a lactic acid fermentation; Leuconostoc mesenteroides is the essential microorganism.

Most humans like meat flavors but millions of people cannot afford to purchase much meat for consumption. The Oriental soy sauce/shoyu fermentation provides a means whereby meat flavors can be developed from cereal/legume substrates. The essential microorganisms are Aspergillus oryzae which provides the amylolytic and proteolytic enzymes for hydrolysis of the starches to sugars and hydrolysis of the proteins to amino acids and peptides; a Lactobacillus sp. that produces lactic acid and Saccharomyces rouxii that produces alcohol during a high salt brine fermentation. In ancient times, maize was used as a substrate for meat-flavored sauces. Coconut also is an excellent substrate for production of meat flavors. Thus, production of meat flavored sauces is easily within the capability of Africa using locally grown substrates.

It is worth noting that it was the soy sauce/miso fermentation that led to development of the internationally prominent and lucrative enzyme industry today. It also led to the huge mono-sodium glutamate (MSG) and nucleotide flavor enhancing industries.

Meat textures are also highly prized by much of mankind. The developed Western world has spent hundreds of millions of dollars developing meat textures in vegetable protein substrates. The basic processes are extrusion of cereal/legume protein isolates through platinum dies to produce fibers that can then be processed and formulated into meat substitutes or extrusion of cereal/legume doughs to produce "chewey" nuggets. Rank, Hovis MacDougall in England has developed a meat substitute by growing an edible mold on low-cost starch in large tanks. This is actually a type of SCP. The mold mycelium is recovered by filtration, formulated with meat flavors and fats and the products are used by the consumer in place of meat in the diet. The mold mycelium provides the meat-like texture.

However, centuries ago, the Indonesians discovered a process by which they grow mold mycelium on a soybean substrate. The mold knits the dehulled partially cooked soybeans into a tight cake producing a meat-like texture which allows the cake to be sliced thin or cut into chunks so that it can be utilized as a meat analogue in the diet. The Indonesian "tempeh" process is low cost, village or cottage techology. "Tempeh" contains as much as or more protein than meat, and it adds desired texture to the Indonesian diet. The price is within the purchasing power of the masses. Since, the fermentation decreases the cooking time for the soybeans from approximately 6 hours to 5 minutes deep fat frying or 10 minutes boiling, "tempeh" is one of the world's first quick cooking foods. And, if the above attributes are not enough, the "tempeh" fermentation also introduces vitamin B-12 into the fermented product. Vitamin B-12 is generally derived from animal products such as milk or meat. Vegetarians are in danger of developing pernicious anemia because of insufficient vitamin B-12 in their diets. Tempeh as produced indigenously contains enough vitamin B-12 to satisfy the need of the vitamin if the consumer obtains his daily requirements of protein from this source.

The Indonesians centuries ago also demonstrated how to double the protein content of rice, selectively increasing the content of the limiting amino acid lysine by 15% and increasing the thiamin content 3 times. They

accomplished this in the "Tape Ketan" fermentation. The increase in protein content is due in large part to the utilization of some of the starch during the growth of the fermenting mold and yeast. In a similar fermentation, "tape ketella", the protein content of cassava (manioc) is increased 2 to 4 times for the nutritional benefit of the consumers. A similar fermentation could be utilized to improve the nutritive protein content of African cassava.

Those involved in research on indigenous fermented foods recognize that we have only investigated the surface of a gold-mine of knowledge available on other indigenous fermented foods used daily in many relatively remote parts of the world. To complete our scientific knowledge requires that we bring all these fermentations to light, determine the essential microorganisms involved, study the biochemical changes that occur in the proteins, lipids, vitamins, and other components in the substrates, determine the flavors and textures produced and how they can be controlled, and finally give the world a broader view of how microorganisms can be grown on edible substrates and contribute more to the total proteins and nutrients available for man in the future.

Okafor (1981) suggested a series of steps for improvement of fermented foods in Africa south of the Sahara. The steps or stages were as follows: (1) Isolation and identification of the microorganisms involved (2) Determination of the role played by each of the microorganisms (3) Selection and genetic improvement of the essential microorganism(s) (4) Improvement in processing control of the fermentation (5) Improvement of the raw substrates (6) Laboratory production of the fermented food (7) Pilot plant scale-up of the laboratory process and (8) Industrial production of the fermented foods.

Four African fermented foods have been industrialized: Kaffir corn (sorghum) beer; Nigerian ogi, Nigerian gari and South African Mahewu (refer to appendix). Nigerian ogi is a smooth-textured, sour porridge with a flavor resembling yogurt made by fermentation of corn, sorghum or millet. Ogi is a natural fermentation and a wide variety of microorganisms-molds, yeasts and bacteria are initially present. The essential microorganisms appears to be Lactobacillus plantarum (Banigo and Muller, 1972). L. plantarum is able to use dextrins after the initial sugars are fermented. Aerobacter cloacae has been isolated and may be responsible in part for increases in the content of riboflavin and niacin in ogi. Corynebacterium sp. is reported to be able to hydrolyze starch and produce organic acids. Saccharomyces cerevisiae and Candida mycoderma contribute to the flavor (Akinrele, 1970). Banigo et al (1974) suggested use of a mixed inoculum containing Lactobacillus plantarum, Streptococcus lactis and Saccharomyces rouxii. Ogi is obviously a complex fermentation. The essential microorganism involved have not as yet completely characterized.

Nigerian gari is a granular starchy food made from cassava (Manihot esculenta) by acid fermentation of the grated pulp of the tuber followed by a dry-heat treatment (garification) which gelatinizes semi-dextrinizes and dehydrates the pulp. Gari is of unusual interest because it is made from cassava-a major source of food for the world's poor. Its protein content generally is less than 1% and it cannot, by itself, provide sufficient

protein for adequate nutrition. For consumption, gari is added to boiling water to produce a semi-solid, plastic dough. During cooking, the volume increases 300%. This places gari in the position of being starch with unusual functional characteristics. It may very well have value as an ingredient in other foods.

Collard and Levi (1959) reported that Corynebacterium sp. and Geotrichum candidum were the important microorganisms in the fermentation. Okafor (1977) isolated and enumerated five genera in the gari fermentation. These were Leuconostoc, Alcaligines, Corynebacterium, Lactobacillus and Candida. Only Leuconostoc, Candida and Alcaligines were present in significant numbers and the Alcaligines died out after the second day. Leuconostoc and Candida appeared to be the essential microorganisms in the gari fermentation. These studies reveal how difficult it is to identify the essential microorganisms in mixed natural fermentations. Often there is a sequence of essential microorganisms as was shown years ago for sauerkraut (Pederson and Albury, 1969).

There are many other fermentations in Africa. Some of these have complex fermentations. One example is kenkey-a staple food in Ghana. It is a fermented maize dough-ball. Maize kernels are washed, soaked in water 12 to 48 hours, ground finely, moistened and packed tightly in drums and fermented anaerobically for 1 to 3 days. Then part of the fermenting mash is cooked and mixed with the remaining dough, shaped into dough balls about 9 cm in diameter or cylinders, wrapped in dry plantain leaves and cooked in boiling water (Christian, 1966; Nyako, 1977). The kenkey fermentation is completely uncontrolled. Initially the maize kernels contain a variety of molds--Aspergillus, Penicillium and Rhizopus. These along with a gram-negative, catalase-positive coccus dominate the initial fermentation. The fungi die out. The gram-negative coccus also dies out after about 2 days. Gram-positive, catalase-negative acid producing cocci (probably Leuconostoc) appear after about 9 hours and reach peak population in 24 to 36 hours. Lactobacillus brevis and other Lactobacilli and Acetobacter are present. Yeasts including Saccharomyces appear on the surface of the dough-ball after 1 day. By 4 days there is a thick slimy layer of yeasts on the surface of the dough-ball. It is in this surface layer that the typical kenkey flavor (diacetyl and acetic acid along with butyric acid) is concentrated. Further study will be required before the essential microorganisms for the kenkey fermentation will be identified and characterized. But, among those essential microorganisms may be some that have unique qualities well worth capitalizing through biotechnology.

If the steps suggested by Okafor (1981) are followed, indigenous fermentations such as kenkey can be vastly improved. The quality and nutritive value of the kenkey will be better. Fermentation time will likely be shortened. Studies of the synthetic capabilities of the essential microorganisms may reveal some with unusual ability to produce enzymes, essential amino acids or vitamins or antibiotics or other products with potential commercial value. Studies of this type also stimulate microbiological research. All of these things lay the ground work for future genetic engineering studies.

As soon as laboratory studies are begun on an indigenous fermentation, the opportunity arises to apply micro-electronic and micro-processing controls. The simplest, of course, is measurement of pH, followed by control of pH in the fermenting substrate. Temperature also can be easily monitored and controlled. If aeration is important, oxygen monitoring electrodes can be utilized to advantage. As a fermentation moves into the pilot plant stage, then micro-electronic control becomes essential at each step. Depending upon the type of fermentation, it is sometimes desirable to monitor the oxygen concentration and the CO_2 concentration as well as pH, temperature, humidity (of solid state fermentations) etc. And in the industrial production, electronic controls become very valuable. In modern, large scale production of SCP, almost every step in production can be monitored on a central panel. Such processing is a marvel of modern-day microbiology. When a country has achieved the capability of producing SCP in large aerated, temperature, pH, oxygen controlled fermentors, it is ready for application of modern genetic engineering and biotechnology.

In conclusion, genetic engineering and biotechnology are developing extremely rapidly. An international link is necessary if the developing nations are to participate in and benefit from the new developments. UNIDO is already deeply involved in bringing these developments to the developing world. At the same time, it is essential that the African nations assess their present resources in the fermentation area. Africa has a long history of producing fermented foods. The microorganisms involved and the technology involved particularly in those fermentation which have already been industrialized can be used to good advantage in applying the new genetic engineering and biotechnology. Application of the new engineering will be greatly facilitated if African scientists can be sent to the International Centre for Genetic Engineering and Biotechnology for training in the specific technics involved.

Summary

Genetic engineering and biotechnology, coupled with rapid developments in micro processors and computers, are presently creating a revolution in agriculture, food production, food processing and preservation, in the health sciences and in scientific research. Microorganisms, microelectronics and microprocessors are at the heart of these new developments. The nations of Africa can benefit greatly if efficient means can be developed for absorbing and putting to active use this new technology. Among the new technologies, the production of single cell or microbial protein (SCP) has the possibility of significantly contributing to the supplies of both animal feeds and human foods. SCP can be grown on petroleum derivatives, methanol or methane, flare gas, surplus starch, surplus molasses, lignocellulosic wastes and even carbon monoxide. Production of SCP does not require arable land. It requires a low cost source of carbon for energy and inorganic nitrogen for synthesis of protein. At present the cost of petroleum has made production of SCP on that substrate uneconomical except in places where oil is being dewaxed or flare gas is being burned. However, as SCP production expands, especially if lignocellulosic substrates can be efficiently utilized, it can provide a

major feed-stuff for animals thus releasing vast quantities of cereal grains and legumes presently fed to animals for food for humans.

SCP is generally microbial protein produced on inedible substrates. Africa is rich in fermented foods which might be described as the production of SCP or microbial biomass protein (MBP) on edible substrates. These indigenous fermented foods need far more study delineating the essential microorganisms, the microbial, biochemical and nutritive changes wrought by the microorganisms and how the fermentations can be conducted at optimum efficiency maintaining highest organoleptic and nutritional quality while preserving the foods and also maintaining a low cost to the consumer. Of particular importance in this regard are foods such as gari based upon cassava. The food has an attractive flavor and sufficient calories but it is extremely low in protein. By growing the proper microorganisms on the cassava, it is possible to substantially raise the protein content vastly improving it as a source of essential nutrients, particularly protein for the consumer.

Genetic engineering offers potential rewards throughout the food production system. It offers means whereby nitrogen-fixing genes may be transferred to plants which do not ordinarily fix nitrogen, for example maize, thus decreasing the need for expensive fertilizer. Plant breeding can lead to varieties with higher protein contents improving the nutritional value of the food, and to more disease and insect resistance thus decreasing the need for expensive pesticides. Where legumes are grown, there should be a reliable source of low-cost Rhizobia for use in inoculating the seeds at time of planting. It is likely that nitrogen-fixing organisms can be introduced to the root systems of sorghum and millets thus decreasing the need for chemical fertilizers. The nitrogen-fixing microorganisms both symbiotic and non-symbiotic (free-living) should be studied in each country. They can decrease the need and expense of chemical fertilizers. The indigenous microorganisms in African soils, in African root systems, in the nodules on legumes, and in African fermented foods constitute a valuable largely untapped resource. Who knows how many new and valuable antibiotics may be produced by these strains? How many are nitrogen-fixing? How many produce unusual amounts of essential amino acids such as lysine or methionine? How many might be useful for production of vitamins or enzymes? Strains that have adapted to a semi-arid environment may be considerably different than those found in the humid tropics. And how many might prove to be of unusual value in future genetic engineering developments? Wherever and whenever possible, African indigenous microbial strains should be characterized and identified and tied into international culture collections such as the World Federation of Culture Collection (WFCC), and the World Data Centre (Microbial Resource Center (MIRCEN), University of Queensland, Brisbane, Australia.

Africa also is rich in lignocellulosic residues such as bagasse, coconut, cassava, pineapple, coffee, sisal, and food processing by-products such as molasses and others that might be converted to useful, valuable products. For example, the production of methane as a cooking fuel (biogas) from human and animal waste is well established in India and China. It can just as well serve as a source of fuel in Africa. The residue following generation of methane is still valuable as a fertilizer

and the initial digestion can destroy pathogens and parasites thus decreasing the danger of using human waste for fertilizer. Surpluses of cassava or molasses can be readily converted to ethyl alcohol or other valuable products such as SCP. Lignocellulosic waste such as straw can be readily converted to edible mushrooms as has been amply demonstrated in Southeast Asia. The spent beds then serve as excellent soil conditioners and add nitrogen to the soil.

Recommendations

1. The ministry of Agriculture and the Ministry of Health in each country should designate one or more scientists to serve as coordinators of furtner developments in Genetic Engineering and Biotechnology. To be most effective, the two appointees should work closely together coordinating efforts directed toward development and use of genetic engineering in the agriculture/food production and processing area and in the medical/health area.

2. International Centre for Genetic Engineering and Biotechnology. Each country should, insofar as practicable, send one or more, established microbiologists geneticists, biochemists or molecular biologists to the new International Centre for advanced training.

3. Each country should send selected students to university centers such as Cornell University, Stanford University or Massachusetts Institute of Technology (MIT) for advanced training in biochemistry, genetics, molecular biology and microbiology as related to Genetic Engineering and Biotechnology. These students upon their return can serve as the nucleus for a Genetic Engineering and Biotechnology Center within the country.

4. The indigenous food fermentations in each country should be studied thoroughly to determine the essential microorganisms or sequences of microorganisms and the function of each organism in the fermentation. The organisms should be identified and tied into World Federation of Culture Collections (WFCC) and the World Data Centre, University of Queensland, Brisbane, Australia.

5. Each indigenous food fermentation should be studied from the bioengineering viewpoint with the objective of modernizing, expanding and finally industrializing production. This will insure efficient production of high quality product with optimum nutritional value for distribution in the urban centres.

6. If not already established, each Ministry of Agriculture should establish a Division of Symbiotic and Non-Symbiotic Nitrogen-Fixing Microorganisms. This Division should isolate and identify the strains and their relative efficiencies in fixing nitrogen. Strains of Rhizobium related to each of the legumes grown within the country should be cultured and preserved in viable form and distributed to farmers thus decreasing the need for nitrogen fertilizer and insuring increased yields of the legumes.

7. Within the Ministry of Agriculture of each country, there should be a division devoted to more efficient utilization of agriculture and other food processing wastes, or surplus by-products such as straw, molasses, coconut water, cassava processing wastes, etc. This Division should also supervise the installation of biogas generators utilizing human, animal and other wastes.

8. It is recommended that the countries of Africa establish ties if they have not already done so with the UNEP/UNESCO/ICRO Microbiological Resource Centers (MIRCENS) as follows:

   1. Professor S. O. Keya, Rhizobium MIRCEN, Departments of Soil Science and Botany, University of Nairobi, P. O. Box 30197, Nairobi, Kenya.

   2. Professor A. Y. Gibriel, Biotechnology MIRCEN, Ain Shams University, Faculty of Agriculture, Shobra-Khaima, Cairo, Arab Republic of Egypt.

   3. Professor V. B. D. Skerman, World Data Centre MIRCEN, Department of Microbiology, University of Queensland, St Lucia, Brisbane, Queensland 4067, Australia.

   4. Professor C. G. Heden, Biotechnology MIRCEN, Department of Bacteriology, Karolinska Institutet, Fack, S-104 01 Stockholm, Sweden.

   5. Professor P. Atthasampunna, Fermentation, Food and Waste Recycling MIRCEN, Thailand Institute of Scientific and Technological Research, 196 Phahonyothin Road, Bangken, Bangkok 9, Thailand.

   6. Professor C. A. Rolz, Biotechnology MIRCEN, Applied Research Division, Central American Research Institute for Industry (ICAITI), Ave. La Reforma 4-47 Zone 10, Apartado Postal 1552, Guatemala.

The MIRCENS are a world-wide programme for preserving microbial gene pools and making them accessible to the developing countries.

They are designed to:

a) provide the infrastructure for a world network which would incorporate regional and interregional co-operating laboratories geared to the management, distribution and utilization of the microbial gene pool;

b) reinforce efforts relating to the conservation of microorganisms, with emphasis on Rhizobium gene pools, in developing countries, with an agrarian base;

c) foster the development of new inexpensive technologies native to the region;

d) promote the applications of microbiology in order to strengthen rural economies; and

e) serve as focal centres for the training of manpower and the diffusion of microbiological knowledge.

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APPENDIX – Steinkraus

Kaffir corn (Sorghum) beer also called Bantu beer of South Africa is an example of a primitive beer that is still produced as a household fermentation and also is produced in high volumes, an estimated 1 billion liters/year in municipal plants (Hesseltine, 1979; Steinkraus, 1979). Kaffir beer is an alcoholic, effervescent, pinkish brown beverage with a sour yogurt-like flavor and the consistency of a thin gruel. It is opaque because of its content of undigested starch granules, yeasts and other microorganisms. It is not hopped or pasteurized and is consumed while still actively fermenting. The essential steps in kaffir beer brewing are malting, mashing, souring, boiling, conversion, straining and alcoholic fermentation. In the indigenous process, kaffir beer is made in 115-180 liter batches in large pots. Sorghum, maize or millet grains or combinations are malted by soaking in water for 1 or 2 days, draining and allowing the seed to germinate 5-7 days until it has a distinct plumule. The sprouted grain is then sun-dried and allowed to mature for several months. It is then pulverized and slurried to form a thin gruel, boiled and cooled and a small amount of fresh uncooked malt is added as a source of amylases and also yeasts for the subsequent fermentation. About equal quantitites of malted and unmalted grains are mashed in cold and boiling water and the two mashes are combined to yield a mixture and temperature favorable to saccharification, souring and yeast fermentation. The mixture is incubated the first day. The second day it is boiled and cooled. The third and fourth days more uncooked malt is added; the fifth day the brew is strained through a coarse basket to remove husks. The beer is then consumed (Platt, 1964).

In the indigenous process, saccharification, souring and alcoholic fermentation proceed more or less simultaneously without the addition of pure cultures. In the industrialized process, (Novellie, 1968), there are two distinct fermentations: The first is saccharification accompanied by lactic acid souring. The second is the alcoholic fermentation. Souring is achieved by holding the mixture of sorghum malt and water at 48 to 50°C for 8 to 16 hours until the proper acidity, pH 3.0-3.3 with a total acidity of 0.3-1.6% (average 0.8%) as lactic acid is attained. This "sour" is about 1/3rd of the final beer volume. The souring step controls the course of the remaining fermentation including mashing, body and alcoholic content of the beer (Novellie, 1966a, 1966b). Although pure culture inoculation of lactic acid bacteria is not used, 10% of each batch of sour is used to inoculate the next batch (van der Walt, 1956). The soured malt mixture is pumped to the cooker and diluted with 2 volumes of water. An adjunct, usually maize grits, is added and the whole mash is boiled 2 hours. The thick cooked mash is cooled to 60°C, conversion malt is added and the mixture is held for 1-1/2 to 2 hours. The sweetened mash, now thinner, is cooled to 30°C and inoculated with a top-termenting strain of Saccharomyces cerevisiae. The yeast is obtained as dry yeast which is produced locally and is slurried before pitching. No yeast is recovered as it is consumed as part of the beer. The pitched mash is passed through coarse strainers, either screw presses or basket centrifuges, to remove husks. The wort is then fermented 8 to 24 hours. Fermentation continues in the packages in which it is distributed. These are unique in that they allow escape of excess gas.

Large amounts of kaffir beer are piped directly to beer parlors where it is sold as draught beer.

The municipal breweries produce about a billion liters of sorghum (kaffir) beer each year (Novellie, 1976). An equal amount may still be produced by indigenous processes in the home. Draught sorghum beer sells for the equivalent of 8 cents (U.S.)/liter. A liter of sorghum beer in cardboard cartons lined with polyethylene sells for the equivalent of 12 cents, probably the cheapest industrially produced beer in the world (Novellie, 1976).

Starch is a very important component in kaffir beer which must contain both gelatinized and ungelatinized starch to be acceptable in texture. The gelatinized starch helps keep the ungelatinized starch in suspension, makes the beer creamy and adds body (Novellie, 1966b; Novellie and Schutte, 1961).

Novellie (1966c) reports that the content of thiamine, riboflavin and niacin in kaffir beers has tended to decrease in recent years. This may be due in part to a decrease in the proportion of sorghum to maize. Traditional kaffir brewing may use 4.9 parts sorghum to 1 part maize while municipal breweries may use 1.2 parts sorghum or less to 1 part maize. This represents a serious loss of nutrients which has occurred with modernization in an important food staple in South Africa. It would be even more serious nutritionally if attempts were made to produce clear beers as are used in the Western World.

The most important processing changes that have occurred in the industrialization of kaffir beer are the more careful malting of the grain which is thoroughly precleaned, washed and watered during malting (Novellie, 1962a, 1962b, 1962c). Division of the process into two distinct step, i.e., souring and alcoholic fermentation makes it possible to control both steps better. Souring is carried out at 48-50°C, optimum for thermophilic lactobacilli which then complete the souring in from 8 to 16 hours. Inoculation of each new batch with "sour" from a previous batch also helps control this step in the process. Souring is carried to the desired pH 3.0-3.3. Amylolytic conversion is conducted at 60°C which is favorable for conversion of the starch to produce the desired viscosity and sugars used by the yeast for alcohol production. A selected strain of Saccharomyces cerevisiae is inoculated into the mash at a temperature of 30°C favorable for the alcoholic fermentation. All these modifications of the traditional processing are desirable. They could not be used in the indigenous processing of kaffir beer. Unfortunately the use of less sorghum in the industrialized process has resulted in a decrease in the nutritive value of the product. This should be corrected if possible.

Traditional and industrialized meyhods for manufacturing Nigerian Ogi are compared in Figures 1 and 2. Traditional ogi preparation is a batch process carried out on a small scale 2 or 3 times a week. The cleaned maize kernels are steeped in pots for 1 to 3 days. During this time, the desirable microorganisms responsible for souring develop. The grain is then wet-ground with a stone slab or mortar and pestle. In the improved process, the grinding is done more efficiently by hammermills. The ground material

is slurried with water and passed through a fine wire sieve (aperture 300-880 microns). The unfiltered coarse material is washed with several lots of water. Alternatively the slurry may be washed through a cloth filter tied over a pot. The filtered slurry settles and ferments for 1 or 2 days at ambient temperature. The fermented sediment is ogi which is boiled either in water or in the ogi water (supernatant) to give ogi porridge (pap). The uncooked ogi is sold wrapped in leaves after removal of excess water. Shelf life is less than 30 hours unless refrigerated. In the industrialized process (Figure 2) the maize is dry milled to a fine flour and subsequent inoculation of a flour/water slurry with a mixture of lactobacilli and yeast. This gives a more reliable fermentation. A further improvement is the manufacture of soy-ogi. Maize is cleaned, soaked, wet-milled and sieved in the traditional manner. Soybeans are similarly cleaned, dehulled, cooked, wet-milled and sieved through a vibroscreen (72 mesh). The two slurries are mixed, fermented, sweetened and spray dried using a Niro Atomiser. The dried product is flavored, enriched with vitamins and minerals and packaged in polyethylene bags for sale. Addition of soy improves the protein content and the nutritive value very much.

FIGURE 1

TRADITIONAL NIGERIAN OGI MANUFACTURE (Banigo et al, 1974)

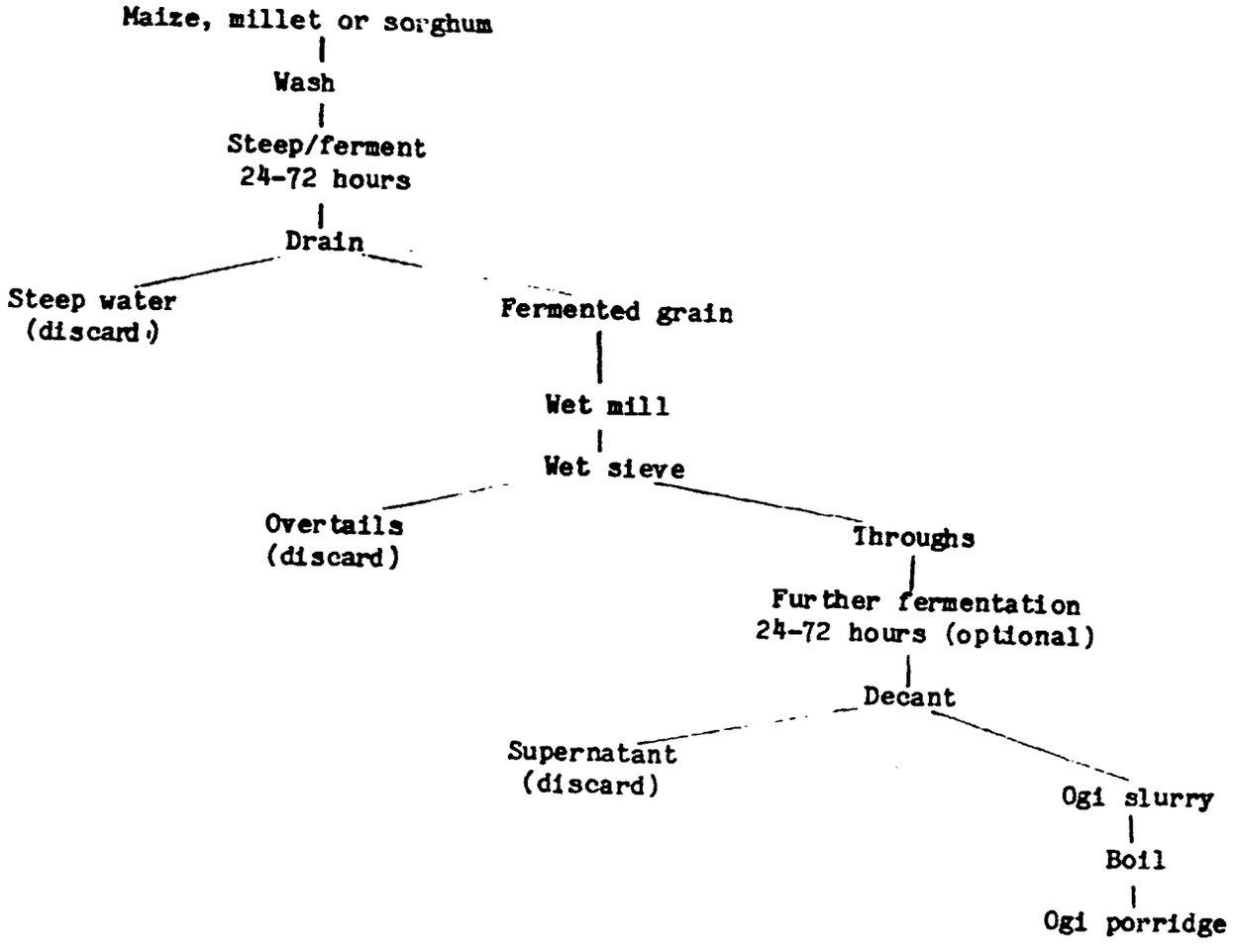
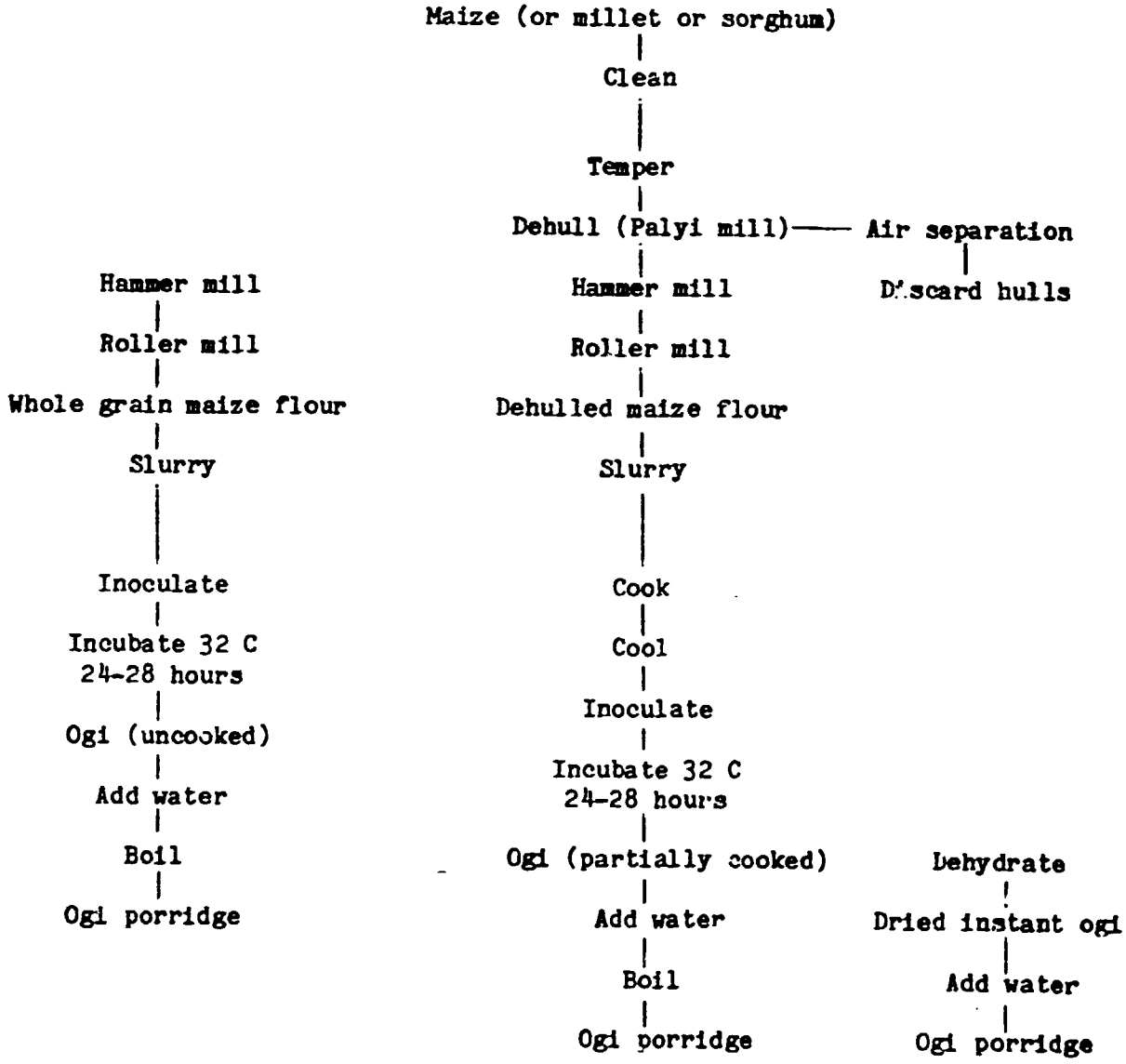


FIGURE 2

IMPROVED NIGERIAN OGI MANUFACTURE (Banigo et al, 1974)



Nigerian Gari fermentation by traditional and pilot plant processes are compared in Figures 3 and 4.

Gari is a granular starchy food made from cassava (Manihot utilissima, M. esculenta) by fermenting the grated pulp, followed by semi-dextrinising, drying, and grading. A related product, cassava flour or lafun, is made by soaking whole tubers in water for a few days, peeling, cutting, drying to 13% moisture content, grinding, and sieving.

Gari is primarily consumed in the form of a meal called eba. This is prepared by soaking gari in boiling water to swell the starch and by working the mixture in a wooden mortar and pestle into a semi-solid, plastic dough. Boiled yams may be added to the dough to enhance the flavor. The stiff porridge is rolled into a ball of about 10-30 gm wet weight with the fingers and is dipped into a stew containing vegetables, palm oil, and meat or fish. The amount of nourishment obtained depends upon the quality of the stew. Gari is the staple diet of the majority of low-income persons, who consume it regularly (two or more times daily). It is estimated that 90% (over 30 million) of the Nigerians living in the southern states consume gari regularly at least once or twice daily. It contributes up to 60% of the total calorie intake in Western Africa (Jones, 1959), the rest being derived from other sources like yams, rice, and maize. An average adult consumes 300 g of gari in a meal.

The utensils required for household scale production are a knife to peel off the outer layers, a grater to reduce the roots to fine particles, a bag to squeeze out liquid from the grated pulp, and a pot to fry the partially dried pulp. The major substrate for gari production is the enlarged root of the cassava plant. The central inner fleshy region of the cassava root is the portion which is eaten. The two outer coverings, the brown, external paper-like skin and the inner leathery whitish covering are removed with a resultant loss of 30% of the total solids by weight. The central fibrous region is grated along with the fleshy portion.

Traditionally, gari is made in the villages by women in the home, from cassava roots bought or grown locally, using a time consuming, unhygienic process (Figure 3). Roots not used 48 hours after harvesting are no longer suitable for gari processing due to biodeterioration.

The roots are peeled with sharp kitchen knives to remove the inner cortex, which may develop a mauve color. Peeled roots are grated into a fine pulp using aluminum sheets perforated with nails and fixed on wooden frames. Sometimes grating is done in a central place in the village by a pulping machine. Grated pulp is placed in Hessian sacks, which are tied up and compressed with heavy stones or wood. The sacks are left outside for up to 4 days to allow the mash to drain and ferment.

The fermented pulp is semi-dry (about 60% moisture) and harsh. Using sieves locally fabricated from palm fronds, coarse fibers are removed and discarded; the finer grains are then toasted in shallow iron pots heated to about 120 C on an open fire. A piece of calabash is used to turn the toasting pulp to prevent sticking. This temperature is sufficient to semi-dextrinize the starch and to dry the mash to about 20% moisture. People in

some parts of the country prefer yellow gari. This is made by adding a small amount of palm oil during the toasting process. After the gari is cooked, it is sieved again and stored in open enamel basins to await sale to middlemen.

Because of the increasing tendency of both husband and wife to work, the difficulty of collecting and transporting sufficient cassava root to meet the demands of a rapidly growing urban community and subsequent escalation in prices, it became increasingly apparent that the whole production system must be modernized. The Federal Institute of Industrial Research, Oshodi, Lagos, (FIIRO) pioneered research in the fermentation of cassava with subsequent development of a pilot gari processing plant, which was a model for larger plnts in other parts of the country. The Projects Development Agency (PRODA), Enugu, Anambra State, Nigeria also developed a pilot plant. Basically, the method adopted in the gari processing pilot plant is an upgraded village method (Figure 4) (Akinrele et al, 1971).

(1) Root preparation

The bitter variety of cassava (which contains more than 100 mg hydrogen cyanide per kg. of pulp) is the substrate. One-to two-year-old cassava is preferred. Within 48 hours after harvesting, the roots are processed by removing the ends and chopping the remainder into short pieces (about 15-20 cm long) with sharp knives. The roots are then fed into a peeler.

(2) Peeling

The cassava peeler is a rotating concrete mixer-like eccentric drum with an abrasive lining. By means of a large feed chute with a sliding gate at the bottom, chopped cassava is fed into the peeler. A megator water pump provides the required water pressure for the peeler. Peeling is accomplished within 3 minutes through the combined action of the abrasive lining and the cassava roots rubbing one another as the drum revolves at 40 r.p.m. The water washes the peel away from the roots. Peeling loss based on the weight of roots is 25-30% but can be as high as 40% if the process is unduly prolonged. Peeled roots are discharged onto wheeled inspection trays by gravity where incompletely peeled roots are finished by hand.

(3) Grating

The peeled roots are fed into a grating machine (Type TM-3 manufactured by Robert Friess K-G, Landmaschinenfabrik, Stuttgart) with revolving blades of 2.5 cm impact cross section. The resulting mash when dewatered to about 50% moisture content, should have at least 70% of its weight retained on a 0.058 cm aperture sieve mesh but should pass through a 0.25 cm aperture. Cassava liquor from a 3-day old fermented mash is premixed with the grated pulp at the same time in an Adelphi Mixer (Preston, England) at the rate of 1 liter of liquor to 45 g of pulp. inoculating the pulp in this way reduces the normal fermentation time from 4 days to 24 hours.

(4) Fermentation

The fermentation of cassava is one of the most important steps in gari preparation. The grated pulp is transferred to a cylindrical silo made of fiberglass with a smooth inner surface of bonded plastic. A conical bottom with an adjustable gate facilitates withdrawal of mash for process control and for degassing.

The fermentation is anaerobic. The 3-day-old cassava juice used for seeding contains microorganisms in their early stationary phase. When the pH of the mash reaches 4.0 ± 0.15 with about 0.85% total acid (as lactic), the desired sour flavor and characteristic aroma will be attained.

(5) Dewatering

The fermented mash is transferred by hand to a 53 cm basket centrifuge (Type 86, Broadbent Huddersfield, England) which reduces the moisture to form a cake of 47-50% moisture. Alternatively, the pulp is placed in nylon bags and dewatered in a hydraulic press (Newell Dunford, England). Experience has shown that the basket centrifuge is not very efficient. About 50 kg mash might require 10 to 15 minutes to process, depending on the age of the cassava. Generally, cassava older than 18 months is difficult to free of water. A continuous screw press might reduce handling and operating costs.

(6) Granulating

The filtered cake is disintegrated in a continuous sieve type granulator (Jackson and Crockette No. 4, Kek manufacture) with a BS 10 sieve to remove the trash. The trash is collected separately, dried, and sold along with the sun-dried peels as animal feed-stuffs.

(7) Garifying

"Garification" involves toasting the cake in a rotary kiln (Newell Dunford design) externally heated by a jacket of hot air. The cassava mash is partially gelatinized when the core temperature in the kiln has reached 250-280 C. The "garifier" is a stainless steel tube with a rotary rake which dislodges the gelatinizing bed of cassava pulp from the garifier wall to prvent sticking and burning. The gelatinization process requires high heat and low mass transfer. The garification stage is critical for proper swelling of the gari. The moisture of the pulp is about 40% and the gelatinizing temperature (80 C) should be attained within 15 minutes before surface drying of the gari particles occurs.

(8) Drying

The gelatinized mash falls via a vibrator into a directly fired louvre dryer, 1 m long x 0.78 m wide. The drying requires low heat transfer and high mass transfer, the opposite of the garifier. The hardened cake has a moisture of 8% when cool.

(9) Milling and Packaging

Cool gari is fed into a disc mill (Bentall Corn Mill), ground and subsequently sieved through BS. No. 14. The fines (flour) going through BS. No. 18 are packaged separately as gari flour, which is usually eaten by blending into cooked kidney beans in palm oil stew.

Control of Processes

The most important stages of gari production are fermentation and toasting. These process stages must be controlled in order to obtain an acceptable product. Iron metal discolors the pulp, therefore, plastic, stainless steel, or aluminum equipment is necessary.

A natural fermentation takes at least 4 days to reach the requisite acidity (0.85% as lactic acid) but the time is reduced to 24 hours when an inoculum of 3-day-old fermented mash liquor is used. The process proceeds best at a temperature of about 35°C; sunlight and frequent mixing of the pulp accelerate the fermentation (Akinrele, 1963). Cassava produces its own liquor during fermentation, therefore no water should be added. Where ambient temperature is outside the range of 25-35 C or where fermentation tanks are very large there should be temperature control of the mash. Allowance should also be made for degassing the mash. Most of the gasses HCN, H_2, and CO_2 are believed to escape through the conical spout from which some of the juice continuously drains. There should be adequate ventilation in the building to prevent cyanide poisoning.

The garification and drying stages determine the swelling capacity, as well as the shelf life. Gari should expand in cold water to at least 300% of its original volume.

FIGURE 3

TRADITIONAL PRODUCTION OF NIGERIAN GARI (Okafor, 1977)

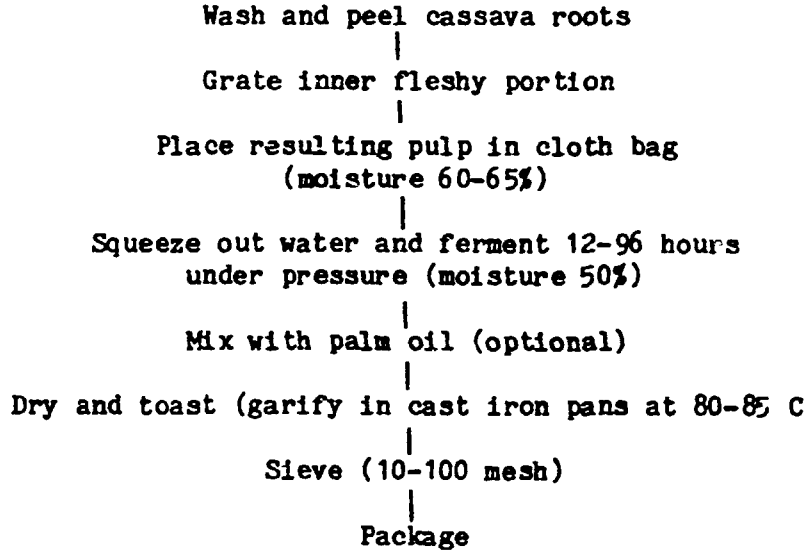
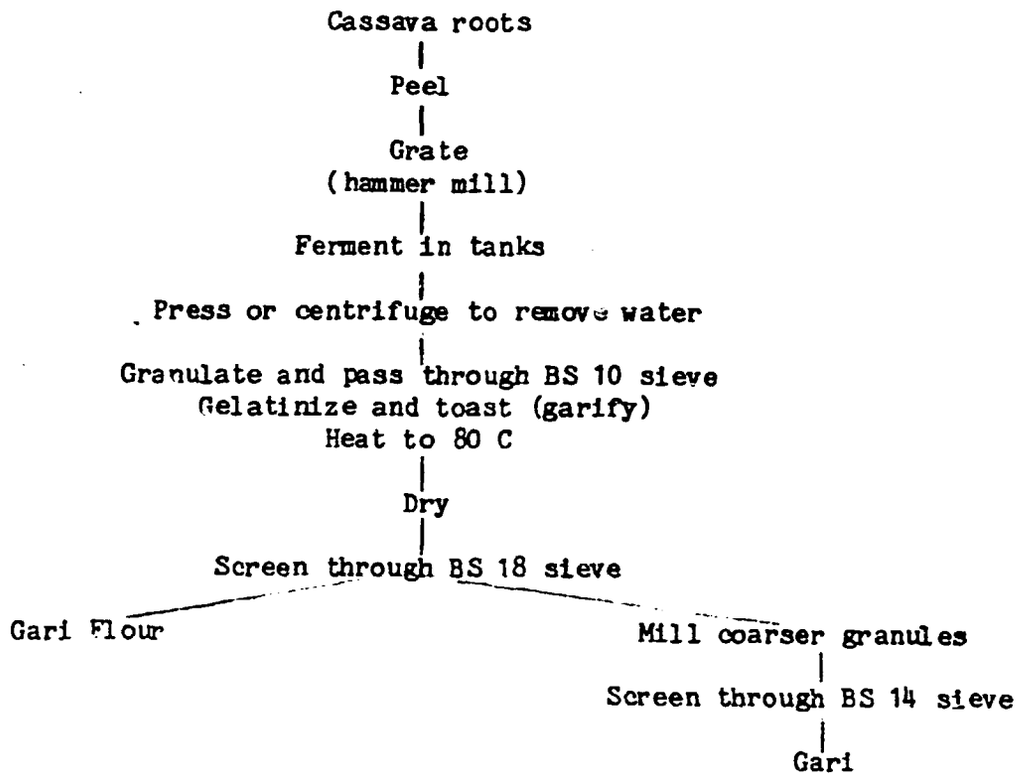


FIGURE 4

NIGERIAN GARI PILOT PLANT PROCESS (Akinrele et al, 1971)



Mahewu (Magou) is a traditional, sour, non-alcoholic maize beverage popular among the Bantu people of South Africa. It is made by traditional, spontaneous fermentation in the villages. It is also produced on a large scale by industrial concerns and mining companies for consumption by their laborers. As consumed, Mahewu contains about 8 to 10% solids and has a pH of about 3.5 with a titrateable acidity of 0.4 to 0.5% (lactic acid). Reduction of its bulk by producing it in concentrated form or as a dry powder has the advantage of easier distribution of marketing.

The traditional, spontaneous Mahewu is made by mixing maize meal and water in a ratio of approximately 450 g maize to 3.8 liters water (8 to 10% solids), boiling until the porridge is cooked (approximately 1 1/2 hours), cooling, and adding a small quantity of wheat flour or meal (about 5% of the weight of maize meal). The wheat flour/meal serves as source of inoculum and source of growth factors for the spontaneous fermentation. It is the major difference separating Ogi and Mahewu which are otherwise similar products. Following inoculation, the Mahewu is incubated in a warm place for about 36 hours at which time the desirable sour flavor has developed.

Schweigart et al (1960) described an improved method for producing Mahewu under controlled conditions (Figure 5). Wheat flour was added to the dilute maize porridge as a source of growth factors but the mixture was then inoculated with either Lactobacillus delbruckii or Lactobacillus bulgaricus and incubated at 45 C to insure a rapid and uniform fermentation.

Hesseltine (1979) described recent industrial production of Mahewu in South Africa. Coarsely ground white maize is used as substrate. The inoculum consists of a mixture of pure cultures which have been isolated from traditional Mahewu and cultured on a coarsely ground whole wheat flour. The maize meal slurry with about 9% solids is cooked by boiling for 1 hour and holding for an additional 45 minutes. The thick maize slurry is cooled to 47-52 C and inoculated with the starter. The fermentation then proceeds in 1000 gallon tanks in which temperature is not controlled for about 20 to 24 hours during which time the pH falls to between 3.65 and 3.95. The fermented Mahewu is mixed with defatted soybean meal, sugar, whey or buttermilk powder and yeast. The additives are incorporated to improve the nutritional value. The mixture is then spray-dried to a moisture level of 3.5 to 4% and has a keeping quality of at least 1 year. It is prepared for consumption by mixing the dried powder with water (about 9% solids).

Production of Concentrated Mahewu (25 Percent Solids)

If the mahewu has to be distributed over long distances, it is advantageous to reduce the water content by preparing a more concentrated form. This concentrated mahewu can then be adjusted to the normal solids content at the place where it is to be consumed merely by mixing it with the requisite amount of water. Alternatively, mahewu can be dried to a powder (Van der Merre, Schweigart and Cachi, 1964; Schweigart et al, 1960).

Several conventional drying methods can be used for mahewu, but only two of these appear to be practicable for large-scale production, namely spray drying as used for milk or drum drying as used for the drying of mashed potatoes and similar pastes. Drying in circulating hot air tray driers has the disadvantage that the layer of mahewu must be broken by mechanical means during the process, drying is slow and the mahewu becomes brown even if the temperature is kept at 50 C.

No technical problems arise in the spray or the roller drying of mahewu (8 percent solids) if it is homogenous. This condition can be easily achieved by passing it through a colloid mill.

Schweigart et al (1960) used "Niro" laboratory spray drier in drying experiments; intake air temperature was 190 C to 210 C and exhaust temperature was 90 C to 110 C. The capacity of this drier was approximately 3 pints per hour for drying mahewu of 8 percent solids.

In experiments with roller driers, a small, single drum drier with a diameter of 12 inches was used.

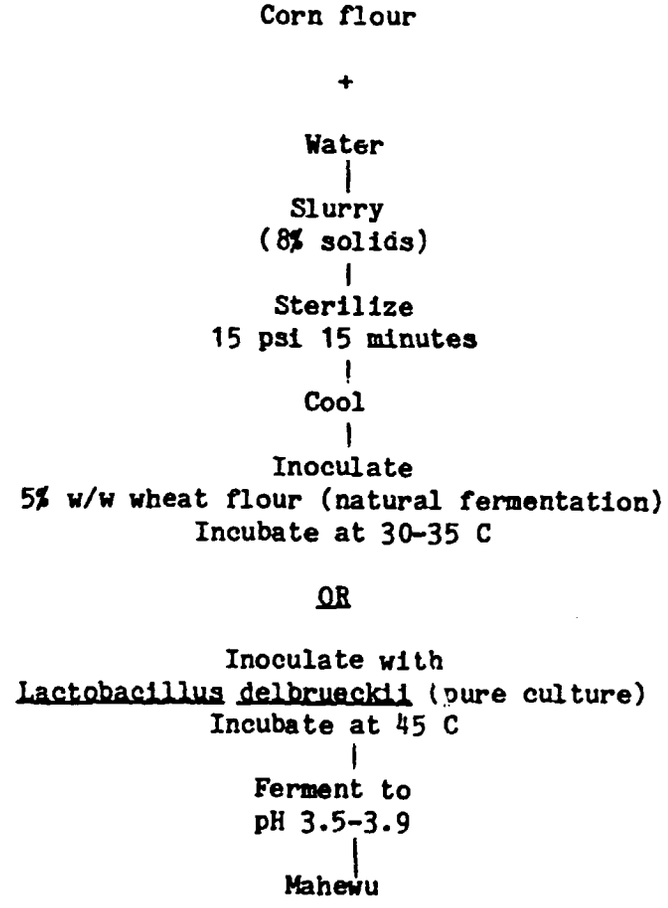
The drum dipped into a vat and revolved at a speed of 1.2 r.p.m. With a steam pressure of 30 lbs psi, capacity of the drum drier for a 8 percent solids mahewu was 12 to 13 pints per hour.

Because the mahewu has a high degree of acidity it can only be dried in an apparatus made of acid resistant, non-corrodible material. Owing to the high viscosity of concentrated mahewu, 25 percent solids was the highest concentration which could be dried on the laboratory spray drier. It is probable that this will also apply to large factory spray driers. Ordinary double drum driers such as those used for milk powder cannot be used on a highly concentrated mahewu. On a laboratory roller drier the maximum concentration was 9 percent solids. Mahewu powder prepared from spontaneously soured mahewu has an unsatisfactory flavor and the shelf life of the powder is very limited (2 days).

In the spray drying process, where the temperature of the product does not exceed 45 C, enzymatic changes such as fat-splitting can take place and lead to rancidity of the product. Apparently enzymatic action is inhibited by the use of a buffer as the buffered mahewus do not become rancid after one-years storage.

FIGURE 5

INDUSTRIAL PRODUCTION OF MAHEWU (Schweigart et al, 1960)



Comparisons of the traditional methods of producing ogi, Gari, Mahewu and Kaffir beer with their industrialized processes give some idea of the problems involved. What types of questions must be answered if other fermented food processes are to be industrialized?

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