AN ABSTRACT OF THE THESIS OF

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in Food Science and Technology presented on 8/12/77

Title: MICROBIAL CHARACTERIZATION OF CASSAVA (MANIHOT ESCULENTA CRANTZ) FERMENTATION

Abstract approved: _____
Dr. J. S. Lee

Micro-organisms associated with gari, produced in Cameroun, Africa, by the traditional method and that produced in the laboratory, were isolated, identified, and screened for their abilities to produce gari flavor. Gari production, under the controlled conditions was attempted utilizing these isolates and the dairy starter cultures. Some of the volatiles responsible for the gari flavor were isolated by gas chromatography employing the head-space analysis technique and tentatively identified by mass spectral analysis.

Cassava fermentation involved mainly the lactic acid fermentation. Lactobacillus sp., and to a lesser extent, Streptococcus sp. were responsible for the acid production and gari flavor development during this fermentation.

A starter culture that consisted of Lactobacillus plantarum or a combination of L. plantarum and Streptococcus sp. were suitable for use in gari production. L. acidophilus 3532, a widely used dairy starter culture, was as effective as L. plantarum in producing acid but it contributed a foreign (dairy) flavor to the gari.

Yeasts were not found to play a major role in gari production and the molds were detrimental. Potassium sorbate could be used as a mycostat without greatly affecting the acidity development but it had negative influence on the gari flavor production.

In addition to lactic acid, the fermented cassava mash contained acetic acid, 2-methyl-propanol-1, 2-methyl-butanol and n-hexanal as the principal flavor volatiles. Gari in the traditionally served form (fermented, palm oil added and heated) contained n-hexanal, hex-2-enal, heptanal, hepta-2, 4-dienal, 1-pentanol, methoxy-ethanol, 2,5 dimethyl pyrazine, 2-ethyl-3, 5, 6-trimethyl pyrazine and limonene.

Microbial Characterization of Cassava (*Manihot esculenta* Crantz) Fermentation

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented on August 12, 1977

Commencement June 1978

APPROVED:

Professor of Food Science and Technology
in charge of major

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Technology

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Date thesis is presented August 12, 1977

Typed by Lyndalu Sikes for Pierre Rolland Ngaba-Mbiakop

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. J. S. Lee without whom this project would not have materialized.

Special thanks are extended to Dr. M. E. Morgan for his guidance and suggestions, to Dr. Libbey for helping in the mass spectral data analysis, and to Dr. P. Krumperman for his help in aflatoxin determinations.

The author would like to express his special gratitude to his friend Gary Narkaus for mailing the cassava samples from New York and to the group of Camerounian students who took part in the flavor panel.

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INTRODUCTION

Annual production of cassava (Manihot esculenta Crantz) worldwide was estimated at 105 M tons in 1972 (24) with a total production value of approximately one and a half billion dollars (44). It is cultivated mainly in the tropics in the area whose total population is estimated at one billion people (5). Due to its many advantages over other tropical food plants such as drought and insect resistance, highest caloric yield per acre, its ability to grow on poor soils, its semi-perenniality, etc., cassava has been labeled as the "insurance crop for the future" (44).

The most important use of cassava is for human food. At least 300 to 400 million people in the tropics depend largely on cassava as their staple food, and it is estimated that by 1980 its consumption will rise to 71 million tons per year (5). Other uses include animal feeding and in industry.

The utilization of fresh cassava roots is limited due to its rapid post-harvest deterioration and its toxicity (high cyanoglucoside content mainly in the form of linamarin). This can be rectified through fermentation of the grated mash. Fermentation not only

improves keeping quality but also brings about the disintegration of the glucoside which is unstable at low pH (2, 18, 47). Due to improvements in flavor, aroma, appearance and consistency, the fermented product is generally more attractive to the consumer than the raw cassava (29, 65).

Gari, the fermented cassava product, is a straw-colored, dry, granular and gelatinized starch. It is relatively non-toxic and can keep for several months at room temperature, whereas the shelflife of the fresh roots is from 2 to 5 days (9, 10, 11, 12, 31). Gari is a major item in the diet of certain areas of West Africa and probably the main form in which cassava is processed and consumed in Africa (13, 41). In a study he conducted for USAID in 1972, Ayres (5) recommended that research on the methods of rapid detoxification and the uses of starter cultures and controlled fermentation for the production of gari should be initiated. To date little is known about the nature of this fermentation process, which is carried out at village level by the traditional method. This investigation was carried out to:

1. Isolate and identify the microorganisms associated with gari.
2. Screen the isolates for the production of the characteristic gari flavor and aroma.
3. Conduct controlled fermentation experiments.

4. Emulate the dairy starter culture system for gari production.

5. Develop a gari starter culture system.

6. Isolate and identify the organic volatile compounds responsible for the typical flavor and aroma of gari.

LITERATURE REVIEW

Cassava Production

Geographical Distribution

Cassava is a starch-producing root crop cultivated extensively in the tropical regions of Africa, South America and parts of Asia (13, 43, 66). It is generally referred to as the "potato" of the tropics (14). Cassava (English) is also known by many names, such as manioc (French), yuca (Spanish) and mandioca (Portugese). Tapioca refers to the starch powder produced from cassava. The correct scientific name for the commercial species is Manihot esculenta Crantz, whereas in earlier literature the name Manihot utilissima Pohl was widely used (5).

The plant is a perennial woody shrub that reaches the height of 4-15 feet and grows best at temperatures between 26 and 29°C (78 to 85°F).

It is grown between 30° north and south latitudes and at altitudes below 2000 meters (6000 feet). Cool weather retards the growth of cassava and the yield decreases as the temperature decreases. It is extremely susceptible to frost damage, and seldom found beyond 30° latitudes from the equator.

While the plant grows in a few southernmost states of the United States it has little importance as a commercial crop in this country (5).

Production Data

In 1972 the Food and Agriculture Organization of the United Nations (FAO) estimated the world production of cassava at approximately 105 million tons (231 million lbs) of fresh root per year on a total cultivated area of 11 million ha, and Africa accounted for 5 million ha (24). Approximately fifty percent of the root crops produced in the tropics is cassava (9, 31). The average yield of cassava is about 10 tons per ha and this is low. It has been experimentally shown, however, that this yield figure can be improved. Yield of up to 70 tons per ha is not an impossibility because in Malaysia, harvesting of 60 tons per ha is quite common (44). Through improved breeding and proper agricultural practices, productions of 30 to 50 tons per ha can be achieved easily, thus boosting the total world production to between 300 and 500 million tons per year.

Farm prices for the fresh root ranges from 10 to 50 dollars per ton. With a world production figure of 100 million tons per year, this commodity contributes at least one billion to one and a half billion dollars to the areas where it is grown (5).

The true production cost for cassava is difficult to assess because the main input is the family labor and the land. In subsistance farming areas, the land may be communally owned. An approximate production cost of 6 dollars per ton has been estimated (41).

Potential of Cassava as a Staple Crop

Cassava represents an "untapped resource in tropical agriculture" (44). Indeed this plant has many important advantages over other tropical food plants. Heading the list is the high yield in food calories per acre at the minimum production cost. Next, come the good productivity in poor soil and the plant's semi-perennial characteristic. It is also resistant to drought, insect damages (particularly by locusts), and to diseases (17, 26, 59). In terms of yields of starch per acre, cassava is unrivalled. Inexpensive to produce, it has the lower man-day per ton labor requirements than either maize or rice (9, 10, 33, 43).

The wide difference in the yield figures of different countries and that of research stations within a country indicate that with improved production efficiency, the cost could be further lowered, thus making cassava a strongly competitive source of starch.

When other crops fail due to drought, cassava survives. In the tropics where hunger is often widespread and severe drought is

common, cassava could be counted on to provide the subsistence (14). Thus the term "insurance crop" has often been used to describe cassava.

A further attribute of cassava is that it has no particular season for harvest. As far as is known it grows almost indefinitely, increasing its yield with time, hence the farmer can harvest his crop when it is convenient or when he can command higher prices. This adds greater flexibility to a crop program based on cassava (17), even though it can be argued that this causes the land to be occupied by the standing crop unnecessarily (9, 31).

Unfortunately, in spite of such great promises, the world cassave production remains static. Poor agronomic practices such as lack of weed and insect control, depletion of soil, poor or no varietal selection, some diseases especially the African cassava mosaic, and the bacterial blight, have kept the cassava production at the present low level.

In view of the intensive research project currently underway there is hope that with good land management, irrigation, and fertilization, the day is near when yields of 70 to 90 tons per ha will be the rule rather than the exception (5).

Cassava Utilization

Cassava is currently the subject of a worldwide research effort

to develop it as a food, a feed and an industrial commodity (33).

Cassava as Animal Feed

The low yields of most cereal and soybean crops in the tropics constitutes one of the main obstacles in developing an efficient animal production in those areas. Cassava can contribute to the solution of this problem because of its high comparative yield and low production cost. Moreover, the excess from human consumption could be fed to livestock, thus providing meat to supplement diets that often lack animal protein (12).

At present, cassava seldom comprises more than 10% of compound feeds, although on occasion it has been used at levels as high as 40% and in some instances its level has reached 60% (51). Presently there is an intensive effort to develop cassava-containing rations (45).

Cassava is not only being used in animal rations in the tropics but also in Europe. The dried roots of cassava have been used for more than 40 years as the source of starch for European animal feed, particularly after World War II when grain prices rose drastically. Major current users of cassava in animal feed are the compounders of the European Economic Community (EEC), who import most of the cassava from Thailand, where it is seldom fed to either man or animal. Comprehensive discussion on the

production and export of cassava pellets is given by Mathot (39).

A further attribute of cassava is that there is a prospect of utilizing its leaves. Cassava leaf has the protein values between 20 and 30% on a dry matter basis. Thus both the root and the foliage can be processed to dry materials with acceptable storage capabilities, which then can effectively substitute for cereals in animal rations (45).

It is fair to say that there are very strong market prospects for cassava as animal feed in both developed and developing countries. It is projected that the international trade in dried cassava chips or pellets, worth approximately 100 million dollars in 1973, will increase to between 200 to 500 million dollars by 1980 (45).

Cassava as Human Food

The most important use of cassava has to be for human food. About 300 to 400 million people in the tropics depend largely on cassava as the staple food. It is believed that by 1980 the consumption of cassava will rise to about 71 million tons. Cassava is a major component of the diet of many developing countries such as Brazil, Indonesia, Nigeria and countries of Equatorial Africa, where it provides from 25 to 60% of food calories (2, 13, 46, 48, 49). Particularly in the desert polygon of Northeast Brazil, coffee with sugar and cassava have provided the mainstays against starvation

for much of the population during dry years (21, 22). And at one time it was said that the entire population of Nigeria could rely on cassava alone to meet 80% of the 2600 calories per person a day requirement (44). It should be emphasized that cassava contains very little protein and needs supplementation (2, 49).

Lipid-protein isolates from cassava leaves prepared and used for animal rations have the same potential for human use since the leaves are already being used in certain parts of Africa as a vegetable. Also, such an isolate is free of cyanide which is present in toxic concentrations in the fresh leaves (58).

Cassava starch has many applications in foods. Due to its high amylopectin content, it is ideal for pudding and pie fillings and it has many other applications in the confectionery industry. The most promising use of cassava is for the breadmaking where it can partially replace the wheat flour. Cassava flour has been studied as a substitute to wheat flour, both alone and in mixtures with other flours. Cassava bread holds a great promise. In fact, one report claims that a cassava-soy flour bread was superior to common wheat bread when net protein utilization (NPU) and biological value (BV) were measured in nutritional experiments with rats (35).

The form in which cassava is consumed is varied and the traditional processing procedures are labor intensive and may be

very complex. The roots may be boiled, chopped and soaked in fresh water for days, dried and used in cassava soups and porridges, grated and fermented into gari or to the Brazilian counterpart Polvilho Azedo, to impart typical flavor and aroma. Gari will be reviewed more thoroughly in another part of this discussion.

Upgrading of Cassava and Cassava Products

Because of its low protein content, nutritional enrichment of cassava has received a great deal of attention. Some work has been carried out with enriching the traditional cassava products, such as gari, cassava soups, and porridges (4). Gari has been supplemented with coconut to yield 5% protein and further supplemented with 15% orange. The mixture gave a new food suitable for children (40). Araujo Neto (4) studied the enrichment of cassava meal with food yeast and D-L methionine and increased its crude protein value to 12.2%. High protein mixes containing cassava flour are being studied in Brazil. The cassava flours are fortified with fish protein concentrate (FPC), soy protein isolate, torula yeast or caseinate. Cassava macaroni have been made and enriched with cowpeas, soybean meal, peanut meal, or by the addition of the deficient amino acids, methionine, tryptophane, lysine, and vitamins, without affecting the palatability or the texture (4, 21, 22).

Another enrichment process of cassava is fermentation by bacteria and fungi. One method consists in the use of both acid and enzymatic hydrolysis to convert cassava starch into fermentable sugars, thus increasing the microbial growth (59). This process could be incorporated into the making of gari. Also feasible is the use of a direct solid-substrate fermentation and a liquid fermentation using starch degrading fungi with the addition of mineral nitrogen, after which the fungal biomass is recovered (13). These two processes can increase the protein content of cassava from 3 to 6 fold (13).

Other Uses of Cassava

Besides being used as food and feed, cassava has other industrial applications. In the early 1960's cassava starch was competing with corn starch and potato starch in paper and paper box industries. Due to its low amylose and high amylopectin content, cassava starch has high viscosity and great dimentional strength (14). Dextrins made from cassava flour can be used to make adhesives (63). Cassava stalks have also been used to make particle boards.

Tapioca is used to size cotton worsted or spun rayon warp to impart greater tensile strength to the textile (5). Tapioca has also been hydrolyzed to produce glucose (50). A feasibility study for a

cassava-based glucose industry was carried out in Nigeria (19) and the process is now being used commercially in Colombia, India and Singapore (1).

Another potential use of cassava is in the brewing industry where cassava starch has been substituted for malt, and more successfully in place of corn grits, corn flakes and rice (1).

Limiting Factors in Cassava Utilization

Factors limiting the effective utilization of cassava include its poor nutritive balance, its toxicity, and its poor post-harvest stability.

Limiting Nutrients

It is well documented that cassava alone is nutritionally poor (5, 21, 22, 45, 49, 59). In 1968, Wu Lung (69) gave a typical proximate analysis for cassava and gari (fermented cassava meal). The tubers are high in carbohydrates which constitute 33% of the fresh roots, but protein accounts for only 1% of the fresh root. Even that small amount of protein is still deficient in sulfur-containing amino acids, particularly methionine (22, 49).

Because of the low protein content, malnutrition and the related kwashiokor are prevalent in areas where cassava is the major item in the diet (2, 5, 22, 59). Cretinism (45) and hunger

oedema have also been reported in cassava-dependent areas (45).

Several attempts have been and are being made to upgrade the nutritional quality of cassava (13, 14, 34, 56), but in most instances the "upgraded" cassava has yet to leave the laboratory.

Toxicity of Cassava

The toxicity of the fresh cassava root is due to the presence of cyanoglucoside and the occasional presence of mycotoxins, such as aflatoxins.

In 1906, Dunstan et al. first isolated a cyanogenic glucoside from cassava (16). Since then, the presence of hydrocyanic acid in the form of glucosides has been well documented (8, 38, 48, 66, 67). Linamarin [2-(β-D-glucopyranosyloxy) isobutyronitrile] was shown to be the principal glucoside of cassava, while methyllinamarin [2-hydroxy-2-methylbutyronitrile] glucoside occurs to a lesser extent (7). This makes cassava the only plant, besides lima bean (Phaseolus lunatus), with the distinction of containing dangerously toxic amounts of hydrocyanic acid in the harvested product (66). The HCN content of the tubers of commonly grown varieties was said to be between 35 and 50 ppm (66, 67); although values of 50 to 100 and 250 to 350 ppm have been reported for sweet and bitter cultivars respectively. The HCN tolerance level for humans is approximately 180 ppm (61).

Cyanogenic glucosides of cassava have been known to be responsible for both acute and chronic toxicity in humans for years but the chronic studies have only been conducted extensively in the last decade. High cassava consumption has been associated with goitre, cretinism and the tropical ataxic neuropathy (17, 45).

Besides cyanogens, aflatoxins have been reported to be present in cassava, the starchy root being an excellent medium for the growth of Aspergillus flavus (62). In Brazil, the high incidence of black fever in children of the upper Amazon is thought to be associated with high levels of aflatoxins in cassava flour (44).

Post-Harvest Deterioration

Once harvested, cassava keeps very poorly. This rapid post-harvest deterioration usually prevents their storage in the fresh state for more than a few days (31, 45).

Two types of deterioration are involved. The primary deterioration due to decay or rot accompanied by the dark bluish or brownish discoloration renders roots completely unacceptable for any use. The secondary deterioration is caused by wound pathogens such as Lasiodiplodia, Threobromae and Trichoderma (45).

The use of chemicals to prevent root deterioration has been investigated with limited success. This prompted Ingram and Humphries (31) to conclude that knowledge of techniques for

preserving and storing fresh cassava is still rudimentary and few reliable methods exist.

Upon storage, the quality of the roots declines. The roots become dehydrated, hard and fibrous, and the texture becomes uneven (11). A solution to this problem would be to keep the roots in the soil until needed, but this would tie up the land that can be used for other purposes. An estimated 0.75 million ha of land are so tied up in the tropics (11, 31). It appears that the immediate processing is the must for the effective utilization of this crop.

Fermented Cassava Products

These include alcohol, beer, single cell protein (SCP) and gari.

Alcohol and Beer Production from Cassava

Cassava is one of the richest fermentable substrates for alcohol production. The fresh root contains 30% starch and 5% sugars and the dried root, about 80% fermentable substances (53). Several processes have been proposed for the production of alcohol from cassava (60). Until recently these processes were not being used on an industrial scale. However, the current high price of fuel oil has led India and Brazil to reassess such potential processes where the alcohol is viewed as the petroleum substitute (44).

On laboratory scale, it has been claimed that certain varieties of cassava can substitute for malt in beer production, with the resulting product comparing favorably with the conventional beer (53). This claim remains to be proven on a commercial scale. For years, cassava has been used as an adjunct in beer production in the Philippines in place of corn grits, corn flakes and rice (1).

Single Cell Protein (SCP) from Cassava

Single cell protein (SCP) has become a widely accepted term for microbial cell material intended for use as food or feed. As the world population increases faster than the agricultural output, the need for unconventional protein sources becomes more urgent, particularly in countries with limited resources. Raw materials for the production of SCP must be cheap and abundant. Cassava is one of such materials and it has been used to cultivate bacteria, yeast and fungi (13, 34, 59). By fermentation the protein content of cassava can be increased by 3 to 6 fold. This means that 3 to 6 times increase in food value could be attained from the same yield, the same crop on the same acreage (27, 33). In terms of nutritional value, the bacterial SCP is comparable to the fish meal and the yeast SCP is comparable to the soy meal (30).

The enrichment of cassava with SCP has a long list of advantages. For example, cassava dishes can be prepared the same way

using a protein rich product, since little change in taste is attributed to SCP; no expensive washing and separation are required as with other SCP substrates; the process can be adapted to local conditions. It is also economical since the developing countries will not have to import proteins for enrichment. The protein enriched cassava can be fed to animals while it is being tested for its safety to humans.

Recently in Canada, a practical low-cost laboratory process for the production of protein-rich animal feed was developed by using the filamentous fungi to convert cassava starch and inorganic nitrogen into a microbial protein (33, 64).

Minor Fermented Cassava Products

There are several fermented cassava products of regional nature in cassava producing areas. Among them, Polvilho Azedo is the most widely studied. Polvilho Azedo (Acetic starch) is a typical Brazilian product with wide applications in native cookery where it is often irreplaceable due to its peculiar flavor and behavior. Its preparations include: root washing and peeling, cutting, grinding, pressing and sifting under running water, decanting or centrifugation to separate the starch from water, fermentation of the starch slurry followed by sun drying. During fermentation the pH decreases from approximately 6.5 to 4.0 and a strong characteristic aroma develops (42). Although Polvilho Azedo has

some similarities with gari, the African counterpart, they differ in several important ways.

Gari Production

Gari is a fermented granular gelatinized starch preparation from cassava root. It is a major item in the diet of certain areas of West Africa and probably the main form in which cassava is processed and consumed in Africa (13, 41).

Traditional gari processing involves: peeling to remove most of the bitterness since the cyanogenic glucoside is concentrated on the outer layers, grating, packing of the mash into a cloth bag and placing heavy objects on the bags to press out the water. During the time of holding, which varies from 24 to 96 hours, fermentation of the pulp is believed to take place (2, 18, 47). Then the fermented product is fried on a hot plate at moderate temperature with or without palm oil. This step is called garifying and the straw-colored granular product is obtained.

There are numerous ways in which gari is eaten. It can be mixed with hot water to yield a paste which is eaten with vegetable soup or stew (41), or it can be soaked in water and fried in spiced palm oil. Water and sugar can be added to the gari and the mixture eaten with peanuts or coconut.

To date, very limited information exists on the nature of the fermentation process, even though several attempts have been made to characterize the microorganisms involved.

In 1959 Collard and Levi (18) proposed that the gari fermentation was a two-stage process, involving two distinct microorganisms. They proposed that during the first stage, the Corynebacterium sp. ferments starch with the production of acids. When sufficient acid is produced the fungus, Geotrichum candida sp. proliferates and produces a variety of aldehydes and esters responsible for the characteristic taste and aroma of gari. Although these authors acknowledged the possibility of lactic acid bacteria involvement, they did not succeed in isolating them.

In 1964, Akinrile (2) endorsed the two-stage fermentation hypothesis of Collard and Levi without identifying the microbial species involved. He was interested in the biochemical aspects of the fermentation and he found that the optimum temperature for the fermentation was 35^{o}C, the medium was self-sterilizing, the geographic acceptability of gari was influenced by its sourness which was related to the degree of fermentation, and that atmospheric oxygen was presumably inhibitory to the process, and the lowered pH accompanying the fermentation was favorable to the linamarase activity which broke down linamarin.

Recently, Okafor (47) questioned the two-stage fermentation concept put forth by Collard and Levi. According to him, Corynebacterium sp. did not play a significant role during cassava fermentation. He isolated and identified mostly lactic acid bacteria, with Leuconostoc sp. being the most abundant. Alcaligenes sp., Lactobacillus sp. and yeasts were also present.

If little is known about the fermentation process of gari, even less is known about the nature of the gari flavor. The only study of this nature by Akinrile (2) showed that lactic and formic acids were the major products of fermentation, and that gallic acid, p-hydroxybenzoic acid, vanillin and pyrochatechol were the other flavor components of gari.

Development of Modern Dairy Industry

It was known centuries ago that after milk was drawn from the animal, it became sour. The sour milk became more stable against proteolysis and other undesirable changes. Ancient men thus handled the milk so as to encourage souring in order to increase its keeping quality. The large numbers of fermented milk products now available trace their origins to ancient times.

Sometime before the end of the 19th century microbiologists became interested in sour milks. They isolated and studied the organisms of the fermented milks to determine which ones were

important and what changes they caused (25).

During the first half of the 20th century, knowledge of the bacteriology of fermented milk increased to the extent that it was possible to produce some of them under controlled conditions and obtain consistently the desired physical and organoleptic properties (25).

The history of the development of the modern dairy industry, therefore, would serve as a valuable blueprint in our effort to upgrade the gari production method.

MATERIALS AND METHODS

Cassava

Fermented Cassava from Cameroun

Four samples of fermented cassava obtained from different batches in Cameroun were aseptically sealed in sterile screw cap tubes and airmailed to this laboratory. Upon receipt they were labeled A, B, C, and D. One gram of the material was taken from each sample for microbial study and the rest stored in a refrigerator at 5°C.

Cassava Roots from New York

Shipments of 6 to 8 lbs of cassava roots were received regularly from New York and either used immediately or stored at -40°F until needed. The frozen roots were thawed at room temperature for approximately two hours before use.

Gari from Cameroun

A sample of gari, the fermented cassava product that had been dried on hot plate with added palm oil, was bought at a market in Cameroun and shipped to this laboratory. Upon receipt it was put in a sterile beaker, covered with aluminum foil and stored at -40°F.

This served as the positive control.

Isolation of Microorganisms Responsible for Gari Flavor

Microbial Counts--Isolation of Representative Colonies

One g of fermented cassava was weighed aseptically in a plastic dish and transferred into a test tube containing 9 mls of Butterfield's phosphate buffer (37) and mixed well on an SP. deluxe mixer (Scientific Products, McGaw Park, Illinois 60085). One ml was removed from the tube and transferred into another sterile test tube containing 9 ml of buffer and mixed well. This 10-fold dilution was continued to 10^{-6}. Then 0.1 ml from each dilution were spread-plated on 5 different isolation media in order to recover the maximum numbers and varieties of microorganisms. The initial isolation media used were: Brain heart infusion (BHI) agar (DIFCO), as a general purpose medium, L agar (BBL), for the isolation of lactobacilli, KF streptococcus agar (DIfCO) for streptococci, AK agar (BBL) a medium for the cultivation of sporulating micro-organisms, and potato dextrose agar (DIFCO) for yeasts and molds. The above isolation plates were prepared in two sets. One set was incubated at 25°C and another at 37°C for 48 hours to a week. Different colony types from each plate were picked with a sterile loop and streaked on a medium identical to the isolation medium and

incubated at the temperature of isolation. The isolated colonies were purified and then transferred on BHI slants for further study.

Screening Microorganisms for Gari Flavor

Frozen cassava was thawed, washed, peeled, and the root grated on a kitchen grater. Approximately 10 to 15 g each of the cassava mash was placed in test tubes and the tubes autoclaved for 15 minutes at 121.1 ^{o}C (250 ^{o}F). Loopfuls of the microbial isolates from the slants were mixed in 9 mls of sterile distilled water and inoculated into the tubes of sterile cassava mash and mixed well with a sterile spatula. The tubes were incubated at 25 ^{o}C for 24 to 96 hours.

Six Oregon State University students from Cameroun who are familiar with the gari flavor were asked to score the inoculated cassava mashes for the typical gari flavor. Scores ranged from 0 (no gari flavor) to 4 (typical). Off-flavors were noted with a star. The autoclaved non-inoculated cassava mash served as a negative control and the cassava mash inoculated with fermented cassava from Cameroun served as the positive control.

Only those isolates which scored high in the flavor tests were used in further study.

Identification of Typical Gari Flavor Producing Microorganisms

Gram Positive Rods

Nine colonies of Gram positive rods were found to produce gari flavor. They were checked for catalase production. The presence of heat-stable spores was tested with the aged culture by heating at 60°C for 30 minutes and checking for the survivors. Further identification tests were made following the method outlined by Sharpe (55) and Sharpe, Fryer and Smith (56) with slight modifications. For the carbohydrates utilization test a modification of the broth of de Man, Rogosa and Sharpe (Table 1) was utilized. Brom cresol purple was used as the indicator in place of chlorophenol red and a millipore filter type HA, 0.45 μ pore size (Millipore Corporation Bedford, Massachussetts) was used instead of a Seitz filter to filter xylose, arabinose and maltose. Sterile Butterfield's phosphate buffer instead of sterile water was used to wash the cells before inoculation into the fermentation tubes. The washing prodedure followed is described below: each culture was grown in de Man Rogosa and Shape broth for 24 hours. Then some of the culture was transferred aseptically into a round bottom centrifuge tube. Centrifugation was at 2500 G using a Sorvall superspeed angle centrifuge (Ivan Sorvall Inc., Newton, Connecticut) and the supernatant

eliminated. Sterile Butterfield's buffer was added to the tube and centrifuged 2 to 3 more times. Then heavy inocula of the washed cells were transferred into the fermentation tubes.

Table 1. de Man, Rogosa and Sharpe (MRS) broth[a] used to cultivate gram positive rods.

Ingredient	Quantity
Proteose peptone[b]	10.0 g
Yeast extract[b]	10.0 g
Tween 80	1.0 g
Dipotassium phosphate	2.0 g
Sodium acetate trihydrate	5.0 g
Triammonium citrate	2.0 g
Magnesium sulfate · 7 H_2O	0.2 g
Manganese sulfate · 4 H_2O	0.05 g
Carbohydrate	20.0 g
Brom cresol purple[c]	8.0 mls
Distilled water	1000.0 mls

[a] Glucose and meat extract were omitted in broth. For MRS agar, 15 g agar and 10 g meat extract were added.

[b] Difco laboratories

[c] 0.5 g in 100 mls (66.6 mls H_2O + 33.3 ml ethanol)
pH was adjusted to 6.2-6.5 before sterilization at 250°F for 15 minutes.

Gram Positive Cocci

Two Gram positive cocci were found to produce gari flavor. These isolates were checked for catalase and cytochrome oxidase

production. The tests used in their identification were those of Sharpe, Fryer and Smith (56) and the criteria used for their identification were those of Sherman (57).

Yeasts

The four strains of yeast isolated were classified by the step by step method described by Barnett and Pankhurst (6).

Isolation of Microorganisms during Cassava Fermentation in the Laboratory

All instruments used below were sterilized in the autoclave at 121.1°C for 15 minutes. Cassava was peeled, grated with a kitchen grater on a sterile aluminum foil. About 150 g of the mash was transferred aseptically into a sterile beaker and the beaker was closed with sterile foam plugs (dispo plugs, Scientific Products McGaw Park, Illinois) and covered with aluminum foil. All beakers were incubated at 35°C. The mash was mixed at regular intervals with a stirring rod. After 4, 14, and 48 hours, 1 g of the mash was taken aseptically into a test tube containing 9 mls buffer and subsequent dilutions were made by transferring 1 ml of the liquid into 9 mls buffer. Then 0.1 ml from each dilution was spread-plated on BHI agar, L agar and PD agar and the plates incubated both at 25°C and 37°C for up to one week. Plate counts were made and representative colonies were isolated and their identification attempted as

described above.

Controlled Cassava Fermentation

Use of Gari Isolates as Starter Cultures

To determine the effects of the isolated microorganisms on cassava fermentation for gari production, experimental fermentation models were set up. Cassava mash was prepared using aseptic techniques as described above and 200 g of the mash was weighed into a 500 mls beaker. Ten loopful of colonies of pure culture from the slants were mixed with about 10 mls of sterile water. The heavy cell suspension was inoculated into the cassava mash and 100 mls of sterile water was added to the mash. Autoclaved foam plugs were used to close the beaker, and covered with aluminum foil and the cover tied with a rubber band. The beakers were incubated at 35 ^{o}C in an environmental incubator shaker (New Brunswick Scientific Co., Inc., New Brunswick, New Jersey). The beakers thus contained either uninoculated cassava mash or mash inoculated with Lactobacilli plantarum A_8, or Streptococcus sp. D_d or a mixture of L. plantarum and Streptococcus sp. Both organisms were previously identified as typical gari producers. Constant shaking was maintained to keep the mash in agitation. The pH was measured at zero time with a Beckman Zeromatic II pH-meter (Beckman Instruments, Inc., Fullerton, California) and every

2 to 3 hour intervals during the first 16 hours and at longer intervals thereafter. The duration of the fermentation was up to 96 hours. To avoid contamination of the mash the beaker was opened under a laminar flow hood and some of the fermenting mash poured into a 10 ml beaker and the electrode dipped into it.

The control consisted of noninoculated mash. The experiment was terminated when the pH had stabilized to a given value for 24 hours. The fermented mash was examined for the desirable straw color formation and a panel of judges was called in to select those which had the characteristic gari flavor. Any unusual properties such as gassiness, swelling, unnatural odor were also recorded. The judges were asked to score from 0 (no gari flavor) to 4 (excellent gari flavor); the scores were compiled and the data analyzed by analysis of variance.

Use of Dairy Cultures

Three dairy cultures (L. lactis, L. acidophilus N and L. acidophilus 3532) were obtained from the Department of Microbiology, Oregon State University and tested for their effects on cassava fermentation). All tests were done as with the gari isolates. At the end of the fermentation the six-member panel was asked to judge the unmarked flasks containing the different dairy starters for the presence of typical gari flavor.

Use of a Chemical Preservative

Two preservatives commonly used in the food industry were tested for their possible use in controlling the mold growth. One percent solutions of sodium benzoate (Mallinckrodt, St. Louis, Missouri) and potassium sorbate (Sorbistat-K, FCC Chas Pfizer and Co., Inc., New York, New York) were prepared. BHI broth were prepared in 50 mls volumes and distributed into 150 ml beakers. The pH of the broth was adjusted to 7.0, 6.0, 5.0, 4.0, 3.5 and 3.0 with sterile HCl solution. These pH's covered the range of pH's encountered during cassava fermentation. The volume of HCl solution needed to bring about the desired final pH was predetermined in a separate experiment. The above broth solutions were distributed into test tubes and the sodium benzoate and potassium sorbate solutions added to bring their concentration in the range of 0.025 to 0.2%.

Heavy inocula of L. plantarum A_8 and a mold isolated from fresh cassava root were inoculated into the tubes and their growth or absence of growth measured by turbidity.

The one percent solution of potassium sorbate was incorporated into a cassava mash (final concentration 0.1%) which was then inoculated with a mixed culture of gari isolates and the pH change and gari flavor development scored.

Flavor Panel

A final panel was set up to score gari made by different ways. The fermented cassava mashes consisted of: a control, uninoculated mash, and mashes made with the following starter cultures:

1. *L. plantarum* A_8
2. *Streptococcus* sp. Dd
3. *L. plantarum* A_8 and *L. acidophilus* 3532
4. *L. plantarum* A_8 and *Streptococcus* sp. Dd
5. *L. acidophilus* 3532 and *Streptococcus* sp. Dd
6. *L. plantarum* A_8, *L. acidophilus* 3532 and *Streptococcus* sp. Dd
7. *L. plantarum* A_8 and *L. acidophilus* 3532 and 0.1% potassium sorbate.

The flavor was evaluated using a nine point hedonic scale that ranged from one (extremely undesirable) to nine (extremely desirable). The data was then analyzed by analysis of variance. Care was taken to insure objectivity by identifying the fermentation mixtures only by the codes, and always presenting the flasks to the panels in a random order.

Headspace Analysis of Gari Volatiles

Sample Collection

The volatile compounds of the different gari samples were

collected using a variation of the gas-entrainment on-column trapping technique described by Morgan and Day (40) and modified by Heatherbell et al. (28). Our technique was as follows: 6 g of fermented cassava mash or native gari were weighed into a 200 ml screw-capped bottle. Twenty grams of anhydrous sodium sulfate, analytical reagent (Mallinckrodt Chemical Works, St. Louis, Missouri) and 50 ml of distilled water were added to the content of the bottle. The bottle was dropped in a water bath maintained at 60°C. Both the bottle and the water bath contained stirring rods. After 10 minutes and when the entrainment apparatus (Figure 1) was set to go, nitrogen gas was used to purge the sample.

Poropak Column Preparation and Conditioning

Poropak columns (precolumns) were prepared by filling 4 in x 1/8 in i.d. stainless steel tubing with acetone-washed 100-120 mesh Porapak Q (Waters Associates, Inc., Framingham, Mass.). The packed precolumns were conditioned by placing them in a 200°C oven overnight. The ends of each precolumn were labeled either n°1 or n°2.

Entrainment of Volatiles on Precolumns

During entrainment of volatiles (Figure 1) the n°1 end of the precolumn was connected to the headspace bottle. The needle

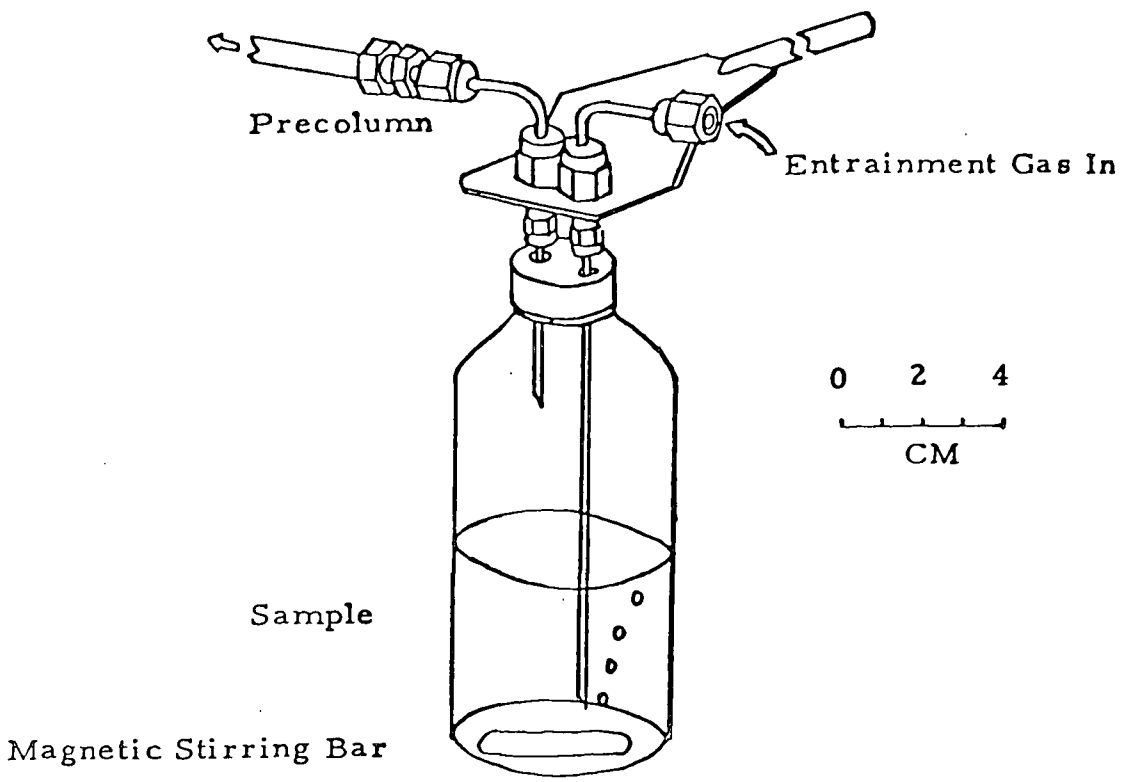

Figure 1. Entrainment (collection) assembly for headspace analysis.

through which gases left the headspace vial was connected to the precolumn by a 4 in x 1/8 in o.d. piece of stainless steel tubing, heated as was the column, at 55°C. The following conditions were followed throughout:

Water bath temperature ca 60°C

Purging nitrogen flow 30 ml/min at 55°C

Entrainment time 35-50 min.

The bottles were purged with a continuous flow of nitrogen gas causing the organic volatiles to be eluted on to the precolumns.

Entrainment of Volatiles on Capillary Trap

Residual water was removed from the precolumn at 55°C with the same flow of nitrogen for about 30 minutes. The precolumn was then reversed and heated to 135°C and the organic compounds eluted with nitrogen at 12 ml per minute, for 45 to 55 minutes. The eluted compounds were collected in a stainless steel capillary trap (27 in x 1/16 in o.d.) that was immersed in a slurry of dry ice. The capillary trap was closed very tightly and kept in a freezer until ready for a gas chromatographic (GC) analysis or a combined gas chromatography mass spectral (GC-MS) analysis, at which time the organic compounds were flushed onto an analytical column in the manner recommended by Scanlan et al. (54).

Gas Chromatographic (GC) Analysis

Gas chromatographs were made of the following samples:

1. Native gari (palm oil added and heated)
2. Raw cassava
3. Non-inoculated fermented cassava mash and fermented mashes made with the following cultures
4. *L. plantarum* A_8
5. *L. acidophilus* 3532
6. *Streptococcus* sp. Dd
7. *L. plantarum* A_8 and *L. acidophilus* 3532
8. *L. plantarum* A_8 and *L. acidophilus* 3532 and *Streptococcus* sp. Dd

Capillary traps containing the volatiles from the above samples were taken from the freezer and attached to the injection port of a F and M research chromatograph model 810. (F and M Scientific Corporation, Avondale, PA). The column used was a 0.030 in i.d. x 500 ft. stainless steel packed with SF-96. The oven temperature was brought to 70°C and the GC was programmed to start at 80°C for 5 minutes, then increase the temperature by 2°C per minute to 160°C.

Combined Gas Chromatography - Mass Spectrometer (GC-MS)

Mass spectral analysis was done of the native gari and of the

L. plantarum inoculated gari whose gas chromatograph showed the most volatiles. The GC-MS system consisted of a SF-96, 500 ft x .030 in i.d. stainless steel column. The GC was a Varian Aerograph 1400 GC (Varian Associates, Instrument Division, Palo Alto, California). The gas flow (helium) was 15 ml per minute. The program used was the same as described above. The MS was a total ionization mass spectrometer mode 1015C (Finnigan Corp., Sunnyvale, CA). The GC-MS system was interphased to a System Industries System 250 data system and the data was collected in the IFSS (integration as a function of signal strength) mode. Volatiles were tentatively identified using their M/e.

RESULTS AND DISCUSSION

Microorganisms Associated with Cassava Fermentation

A total of 29 distinct microbial colony types were isolated from the 4 fermented cassava samples (A, B, C, and D) obtained from Cameroun. The microbial isolates were divided according to their Gram reactions. The sources of these microorganisms, the conditions of their initial isolation, and their relative abundance in the samples, are presented in Tables 2, 3, 4, and 5. From these tables, it can be seen that samples A and B contained most of the Gram positive rods and some Gram positive cocci, whereas samples C and D contained more coccobacilli, cocci, yeasts, and mold-like microorganisms. This extreme variability of the microbial flora of fermented cassava could have been the cause of the conflicting literature on cassava fermentation.

Table 6 shows the flavor test results after these isolates were inoculated into autoclaved cassava mash and tested for the gari flavor production. A noteworthy finding of this study was that all judges found that the mold-like organisms gave the off-flavors, indicating their presence to be incidental rather than being the essential element, as proposed by the two-stage fermentation hypothesis of Collard and Levi (18). Microscopic observations of

Table 2. Gram positive cocci isolated from fermented cassava samples A, C and D.

	Colony Type				
	A_{10}	C_1	C_3	D_c	D_d
Isolation medium	AK[a]	BHI[a]	BHI[a]	KF[a]	BHI[a]
Incubation temperature	37°C	25°C	25°C	37°C	25°C
Total plate count	1.3×10^8	6.4×10^8	6.4×10^8	2.2×10^8	5.4×10^8
Differential count	5.3×10^7	4.8×10^8	2.8×10^8	2.0×10^8	3.3×10^8
Percent of total	41%	75%	44%	90%	61%

[a] BHI = Brain Heart Infusion (Difco),
KF = Streptococcus agar (Difco).
AK = Sporulating agar (BBL)

Table 3. Gram variable coccobacilli isolated from fermented cassava samples C and D.

	Colony Type			
	C_2	D_2	D_f	D_g
Isolation medium	BHI	BHI	LA[a]	BHI
Incubation temperature	25°C	25°C	37°C	37°C
Total plate count	6.4×10^8	5.4×10^8	5.0×10^8	6.0×10^8
Differential count	4.8×10^8	1.9×10^8	2.3×10^8	3.5×10^7
Percent of total	75%	35%	45%	6%

[a] LA = Lactobacilli agar (BBL).

Table 4. Gram positive rods isolated from fermented cassava samples A and B.

	Colony Type						
	A_3	A_4	A_5	A_6	A_7	A_8	A_9
Isolation medium	AK[a]	KF	LA	BHI	LA	KF	AK[a]
Incubation temperature	25°C	25°C	25°C	25°C	37°C	37°C	37°C
Total plate count	1.2×10^8	3.6×10^7	9.0×10^7	5.4×10^7	1.3×10^8	7.0×10^7	1.3×10^8
Differential count	4.3×10^7	3.6×10^7	9.0×10^7	5.4×10^7	4.7×10^7	7.0×10^7	7.3×10^7
Percent of total	37%	100%	100%	100%	36%	100%	56%

	Colony Type					
	B_1	B_2	B_3	B_4	B_7	B_8
Isolation medium	BHI	KF	AK[a]	AK[a]	LA	BHI
Incubation temperature	25°C	25°C	25°C	37°C	37°C	37°C
Total plate count	2.2×10^8	1.5×10^8	2.5×10^8	2.7×10^8	4.2×10^8	2.3×10^8
Differential count	2.2×10^8	1.5×10^8	1.9×10^8	8.6×10^7	3.3×10^8	1.9×10^8
Percent total	100%	100%	76%	32%	78%	82.6%

[a] AK = Sporulating agar (BBL).

Table 5. Yeasts and mold-like organisms isolated from fermented cassava samples C and D.

	Yeasts			Mold-like organisms		
	Colony Type					
	C_a	C_b	C_c	D^I	D^{II}	D^{III}
Isolation medium	PD[a]	PD[a]	PD[a]	PD[a]	PD[a]	PD[a]
Incubation temperature	25°C	25°C	25°C	37°C	37°C	37°C
Total plate count	1.6×10^7	7.6×10^7	1.6×10^7	1.1×10^7	9.3×10^7	1.1×10^7
Differential count	1.8×10^5	2.3×10^7	1.8×10^5	9.5×10^6	3.9×10^7	1.1×10^7
Percent of total	1.1%	30%	1.1%	86%	42%	100%

[a] PD = Potato dextrose agar (Difco).

Table 6. Gari flavor production by microorganisms isolated from fermented cassava (traditional).

Isolates	Description	Judge Number					
		1	2	3	4	5	6
A_{10}	Gram positive cocci	3[a]	4	2	4	2	4
C_1		0	0	1	0	0	0
C_3		0	0	0	0	0	0
D_c		3	3	2	3	3	4
D_d		3	2	4	4	3	2
A_3	Gram positive rods	3	4	3	4	3	2
A_4		3	2	4	3	4	3
A_5		4	4	3	4	4	3
A_6		3	4	3	4	3	3
A_7		*	*	*	*	*	*
A_8		3	4	2	4	3	4
A_9		3	4	3	3	2	4
B_1		0	0	1	0	0	0
B_2		0	0	0	0	0	0
B_3		3	4	4	3	4	3
B_4		0	0	0	0	0	0
B_7		4	4	3	3	4	3
B_8		3	3	4	3	3	4
C_a	Yeasts	0	0	0	0	0	0
C_b		2	2	1	3	2	2
C_c		0	0	0	0	0	0
D^{I}	Streptomycetes	*	*	*	*	*	*
D^{II}		*	*	*	*	*	*
D^{III}		*	*	*	*	*	*
C_2	Gram variable coccobacilli	0	1	0	0	1	0
D_2		1	0	0	0	0	0
D_f		0	0	0	0	0	0
D_g		0	0	0	0	0	0

[a] 0 = neutral, 1 = slight, 2 = moderate, 3 = good, 4 = typical, and * = off flavor.

the wet mounts of these mold-like organisms (coded D^{I}, D^{II} and D^{III}) revealed spore morphologies characteristic of Streptomycetes. The same structures were isolated from raw cassava roots, but were absent in all laboratory-fermented cassava mashes. The presence of these Streptomycetes in native gari could be explained by the fact that during the traditional fermentation process for gari production, the mash is loaded into bags and laid on the ground for the duration of the fermentation. These microorganisms could have contaminated gari from the soil which is their primary habitat (52).

Among the cocci, cultures A_{10}, D_c and D_d contributed moderate to typical gari flavor. Among the Gram positive rods, nine isolates A_3, A_4, A_5, A_6, A_8, A_9, B_3, B_7 and B_8 contributed some flavor to cassava. Among the yeasts, only one colony C_b contributed gari flavor to fermented cassava mash. None of the 4 coccobacilli colonies isolated C_2, D_f and D_g had produced gari flavor (Table 6).

Among the 9 isolates of Gram positive rods screened for contributing gari flavor, 8 of them (A_3, A_4, A_5, A_8, A_9, B_3, B_7, B_8) were short stubby to long thin rods; they were homofermentative, catalase negative non-motile, non-spore forming; they grew at 15°C, produced acid from lactose, sucrose, raffinose and glucose, but did not produce NH_3 from arginine (Table 7). They were thus identified as Lactobacillus plantarum, according to the classification scheme of Sharpe, Fryer and Smith (56). A_6 was a short

Table 7. Morphological and biochemical properties of the Gram positive rods isolated from traditionally fermented cassava.[a]

Code	Morphology	Catalase	Motility	Spore formation	Growth at 15°C	Growth at 45°C	NH_3 from arginine	Litmus milk	Carbohydrate Utilization: Hugh and Leifson	Arabinose	Cellobiose	Glucose	Lactose	Trehalose	Melobiose	Melizitose	Raffinose	Rhamnose	Sucrose	Xylose	.4% teepol	Genus/ Species
A_3	Short stubby rods	-	-	-	+	+		Coagulation acid in upper 1/2 of the tube	A/	SL		A	A		A		A	-	A	-	SL	L. plantarum
A_4	as A_3	-	-	-	SL	+		as A_3	A/ A	SL		A	A		A		A	A	A	-	+	L. plantarum
A_5	as A_3	-	-	-	+	-		as A_3	A/ A	A		A	A		A		A	-	A	-	+	L. plantarum
A_6	Short rods	-	-	-	+	SL	+	as A_3	A/. A	A	-	A/ G	A	SL	A	A	SL			A	+	L. buchneri
A_8	Slender rods in groups	-	-	-	+	+		as A_3	A/ A	A		A	A		A		A	SL	A	-	+	L. plantarum
A_9	Thin long rods	-	-	-	+	+		as A_3	A/ A	A		A	A		A		A	SL	A	A	+	L. plantarum
B_3	Slender long rods	-	-	-	+	+		as A_3	A/ A	A		A	SL		A		A	A	A	A	+	L. plantarum
B_7	as A_8	-	-	-	+	+		as A_3	A/ A	SL		A	A		A		A	A	A	-	+	L. plantarum
B_8	as A_8	-	-	-	+	+		as A_3	A/ A	A		A	A		A		A	SL	A	A	+	L. plantarum

[a] A = acid - = negative reaction no acid produced A/G = acid and gas produced from glucose
+ = positive SL = slight growth, slight acidity A/A = oxidative and fermentative

heterofermentative rod, catalase negative, non-motile, non-spore forming, grew at 15°C and 45°C, produced acid and gas from lactose, sucrose, glucose, raffinose and produced NH_3 from arginine. Thus A_6 had properties identical to those of L. buchneri as described by Sharpe, Fryer and Smith (56).

Two of the Gram positive cocci isolated (D_c, D_d) were catalase negative, homofermentative non-spore formers; they grew at pH 9.6, in the presence of 6.5% sodium chloride but not 0.1% methylene blue, they produced acid from both sorbitol and arabinose; they had almost all the properties of Streptococcus avium described by Sherman (57) except that they did not produce NH_3 from arginine. Thus they were identified as Streptococcus sp. The other Gram positive cocci isolate (A_{10}) was heterofermentative and had properties similar to those of Leuconostoc sp. described by Sharpe et al. (56) except that it produced NH_3 from arginine. Thus it was also classified as Streptococcus sp. All three cocci produced curd when inoculated in reconstituted milk.

The sole yeast isolate found to contribute some gari flavor to cassava mash (C_b) belonged to part C of the Master Key proposed by Barnett and Pankhurst (6). It produced acid from maltose, cellobiose, raffinose, lactose, and ethanol but not erythritol. It was identified as a Brettanomyces sp.

Table 8. Morphological and biochemical properties of Gram positive cocci isolated from traditionally fermented cassava.[a]

Code	Morphology	Haemolysis	Catalase	Spore formation	Growth at 10°	Growth at pH 9.6	Growth in 6.5% NaCl	Growth in .1% methylene blue	Growth at 45°	NH_3 from arginine	.04% tellurite tolerance	Gelatine liquefacation	Starch hydrolysis	Arabinose	Carbohydrate Utilization: Hugh and Leifson	Glucose	Glycerol, anaerobic	Lactose	Maltose	Mannitol	Melobiose	Melizitose	Sorbitol	Trehalose	Genus
A_{10}	Stubby cocci almost like coccobacilli	-	-	-	+	-	+	-	SL	+	+	-	-	A	O/F	A/G	A		A		A	A	SL	SL	Streptococcus sp.
D_c	single, double and chain cocci	-	-	-	-	+	+	-	+	-	+	-	-	A	O/F	A	A	A	A		A	A	A	A	Streptococcus sp.
D_d	Cocci in groups	-	-	-	+	+	+	-	+	-	+	-	-	A	O/F	A	A	A	A		A	A	A	A	Streptococcus sp.

[a] - = negative growth, no acid produced
O/F = oxidative and fermentative
A/G = acid and gas produced
SL = slight growth or slight acidity
+ = positive reaction
A = acid

Organisms Isolated during Laboratory Fermentation of Cassava

Four to fourteen hours after starting cassava fermentation in the laboratory, seven typical microorganisms (a_1, a_2, h, c, b_1, b_2, f) were isolated (Table 9). a_2, h, and f were Gram positive non-spore forming, catalase negative, homofermentative, rods. They produced acid but no gas from xylose, sucrose, cellobiose, arabinose. They had all the characteristics of L. plantarum outlined by Sharpe (55) except that they failed to grow in the presence of 0.4% Teepol. They were classified as Lactobacillus sp. The a_1 and b_1 were Gram positive cocci, cytochrome oxidase and catalase negative, non-spore forming, homofermentative, Hugh-Leifson glucose fermentative, produced slight to strong curd when inoculated into sterile reconstituted milk, produced acid but no gas from xylose, sucrose, cellobiose, arabinose and trehalose. They had properties of Streptococcus sp. according to the scheme of Sherman (57 The isolate c was a Gram negative coccobacillus, catalase positive, non-sporulating, sensitive to 3.0 I.U. of penicillin G, and did not utilize any carbohydrates. These properties were identical to those of Maraxella sp. as described in Bergey's manual of determinative bacteriology (36). The isolate b_2 also was a Gram negative coccobacillus, cytochrome oxidase negative,

Table 9. Morphological and biochemical properties of microorganisms isolated during laboratory fermentation of cassava.[a]

Code	Time of isolation	Morphology	Cytochrome oxidase	Catalase	Sporulation	Growth on Penicillin agar	Growth at 15°C	Growth at 45°C	Curd in Milk	.4% teepol	Hugh and Leifson	Carbohydrate Utilization: Arabinose	Cellobiose	Galactose	Glucose	Mannose	Melibiose	Raffinose	Rhamnose	Sucrose	Trehalose	Xylose	Percentage / isolation medium	Genus/ Species
a_1	4 h	Gram + coccobacilli in chain	-	-	-		+	-	SL		O/F	A	A	A	A	A	-	-	SL	A		A	20% LA	Streptococcus sp.
a_2	4 h	Gram + medium size rods	-	-	-		+	-	+	-	O/F	A	A	SL	A	-	-		-	A		A	80% LA	Lactobacillus sp.
h	4 h	Gram + short rods	-	-	-		-	SL	SL	-	O/F	A		A		A			-	A		A	80% LA	Lactobacillus sp.
c	14 h	Gram - coccobacilli in pockets of 4	-	+	-	-				-	-/-	Carbohydrates not utilized											40% BHI	Moraxella sp.
b_1	14 h	Gram + coccobacilli	-	-	-				+		O/F	A	A		A		A			A		A	30% BHI	Streptococcus sp.
b_2	14 h	Gram - coccobacilli	-	-	-				-		O/F	A	A	A	A	A	-		-			A	30% BHI	Acinetobacter sp.
f	14 h	Gram + short rods	-	-	-				SL	-	O/F		A	SL		-	A	A	-	A		-	20% BHI	Lactobacillus sp.
d_1	48 h	Gram + cocci in doubles + chains	-	+	-		+	+	+		O/F	A	A		A	A	A			A	A	A	20% LA	Streptococcus sp.
d_2	48 h	Gram + slender rods in groups	-	+	-	+	+	-	+	+	O/F						A		A	SL		A	80% LA	L. plantarum

Table 9. Continued

Code	Time of isolation	Morphology	Cytochrome oxidase	Catalase	Sporulation	Growth on Penicillin agar	Growth at 15°C	Growth at 45°C	Curd in Milk	.4% teepol	Hugh and Leifson	Carbohydrate Utilization											Percentage isolation medium	Genus/ Species
												Arabinose	Cellobiose	Galactose	Glucose	Mannose	Melibiose	Raffinose	Rhamnose	Sucrose	Trehalose	Xylose		
e	48 h	Gram + short and stubby rods	-	-	-		-	-	+	-	O/F	-	A	A	A	A	A		-	A		A	40% BHI	Lactobacillus sp.
i	48 h	Gram + thick cocci	-	-	-		-	+	SL		O/F	A	A	A		A	A		SL	A		A	50% BHI	Streptococcus sp.

[a] A = acid
O/F = oxidative and fermentative
SL = slight growth or acidity

+ = positive reaction
- = negative reaction, no acid produced

catalase positive, non-spore forming, resistant to 3.0 I.U. of penicillin G. It produced acid but no gas from cellobiose, arabinose galactose, mannose and did not produce acid from rhamnose and melibiose. It was identified as an Acinetobacter sp. according to the criteria of Lautrop (36).

After 48 hours of fermentation and when the pH had dropped below 4.0, only four types of microorganisms could be isolated and they were L. plantarum d_2, Lactobacillus sp. e. and Streptococcus sp. i and Streptococcus sp. d_1.

During the entire fermentation process, no yeast or mold could be isolated on potato dextrose (PD) agar, BHI or LA.

The cassava fermentation thus appears to follow the typical lactic fermentation process in which a variety of microorganisms are present at the beginning, but as time passes the other species are quickly outgrown by the acid producing species of Streptococcus and Lactobacillus.

Microorganisms Responsible for Gari Flavor Production

Further Screening of Microorganisms for Gari Flavor Production

Table 10 gives the flavor scores of cassava mash fermented with various microorganisms initially isolated from the native

Table 10. Flavor panel scores of cassava mashes inoculated with gari isolates.

Culture code	Judge 1	2	3	4	5	6	Mean[x] scores
A_3	2	3	2	4	3	2	2.67[b]
A_4	2	4	3	3	4	2	3.00[b]
A_5	3	3	2	4	2	4	3.00[b]
A_6	2	2	2	4	2	3	2.50[b]
A_8	4	4	4	4	4	3	3.83[a]
A_9	4	4	2	3	3	4	3.33[a]
B_3	4	3	3	3	4	3	3.33[a]
B_7	3	4	3	4	3	3	3.33[a]
B_8	2	4	4	3	3	3	3.16[b]
A_{10}	2	3	2	2	3	2	2.33[b]
D_c	3	4	3	3	2	2	2.83[b]
D_d	3	4	4	4	4	3	3.67[a]
C_b	*	*	*	*	*	*	*
$A_8 \times D_d$	4	4	4	4	4	4	4.00[a]

x = data was analyzed by analysis of variance. Mean scores with the same superscript letters are not significantly different at the 5-percent level of probability. 4 = Typical odor 3 = Good 2= Moderate, 1=slight and *Unnatural smell.

product and selected for their ability to produce gari flavor. The positive control was the imported fermented cassava. The table shows that the culture A_8 (L. plantarum) produced the most gari-like flavor among the single cultures and that the combination of A_8 and D_d (Streptococcus sp.) produced the most gari flavor.

Acid Production during Cassava Fermentation

Tables 11 and 12, and Figures 2 to 4 show the pH values obtained at various times of cassava fermentation when autoclaved and unautoclaved mashes were inoculated with different starter cultures. With the autoclaved substrate, the lowest pH obtained with either L. plantarum A_8 or Streptococcus sp., or the combination of both cultures, was 3.95, with the mixed culture producing more acidity than either of the organisms alone (Figure 2). L. plantarum was a stronger acid producer than the Streptococcus sp.

In terms of consumer acceptability, fermented cassava mashes with pH of 3.9 or above were found to produce gari with poor taste and flavor (2). Also, autoclaving brought about the gelatinization of the starch, making it unsuitable for certain uses of gari. We used autoclaved cassava mash in order to screen those microorganisms responsible for the gari flavor production. However this would not be the process recommended for the industrial production of gari. The starter cultures, in that event, have to out compete the natural

Table 11. pH of inoculated cassava mashes during fermentation (autoclaved substrate).

Time (hours)	Cultures		
	L. plantarum (A_8)	Streptococcus sp. (Dd)	Misture of A_8 and D_d
0	6.20	6.20	6.20
3	5.80	6.20	5.95
6	5.60	6.00	5.70
10	5.30	5.55	5.20
12	5.20	5.35	4.95
17	5.10	5.20	4.70
24	4.80	5.00	4.30
28	4.65	4.95	4.20
34	4.50	5.00	4.00
47	4.40	4.65	3.90
54	4.30	4.45	3.90
60	4.25	4.35	3.90
67	4.20	4.35	3.90
74	4.15	4.35	3.90
96	4.15	4.35	3.90

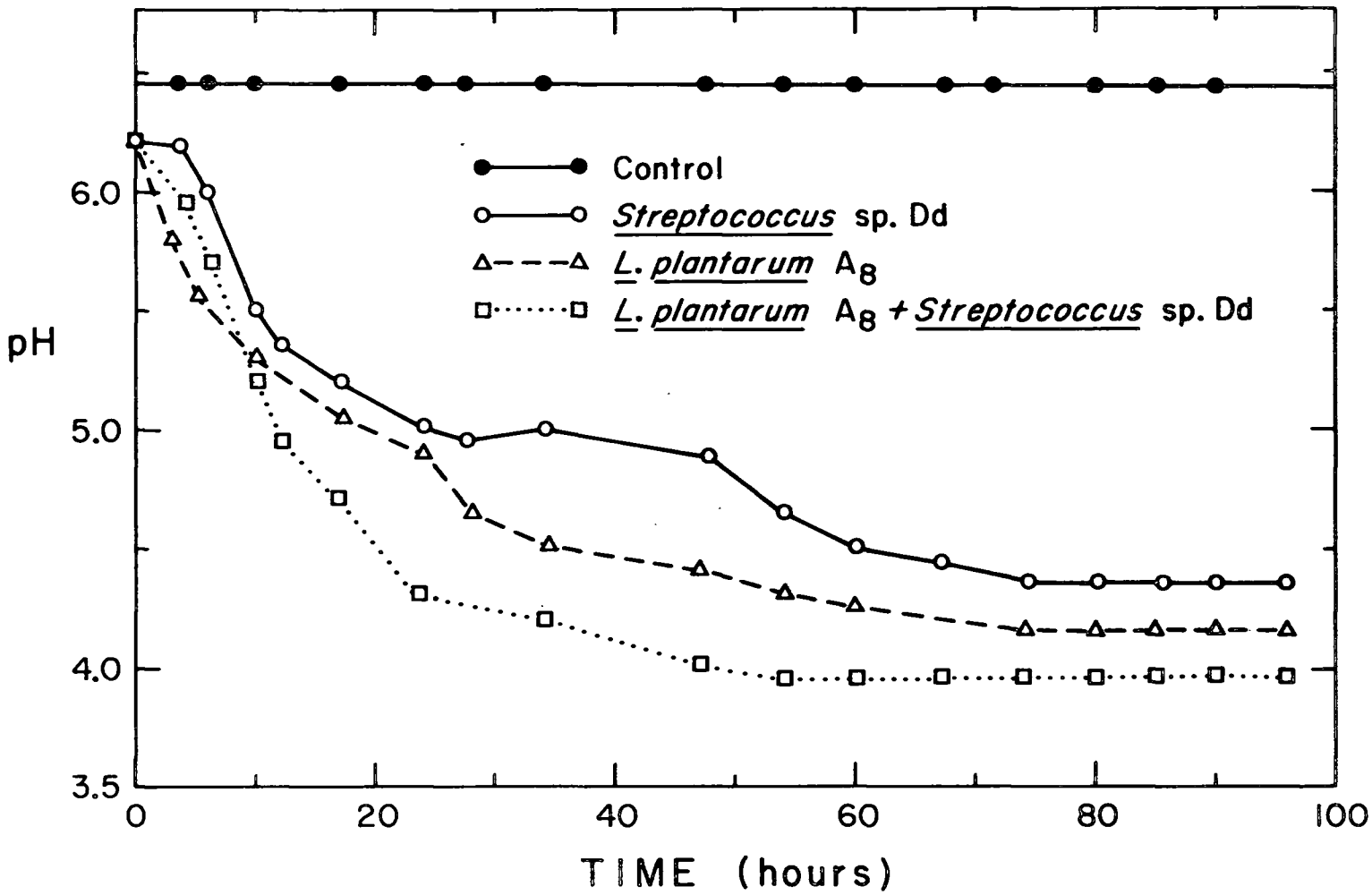

Figure 2. pH changes in autoclaved cassava mash inoculated with microorganisms from native gari.

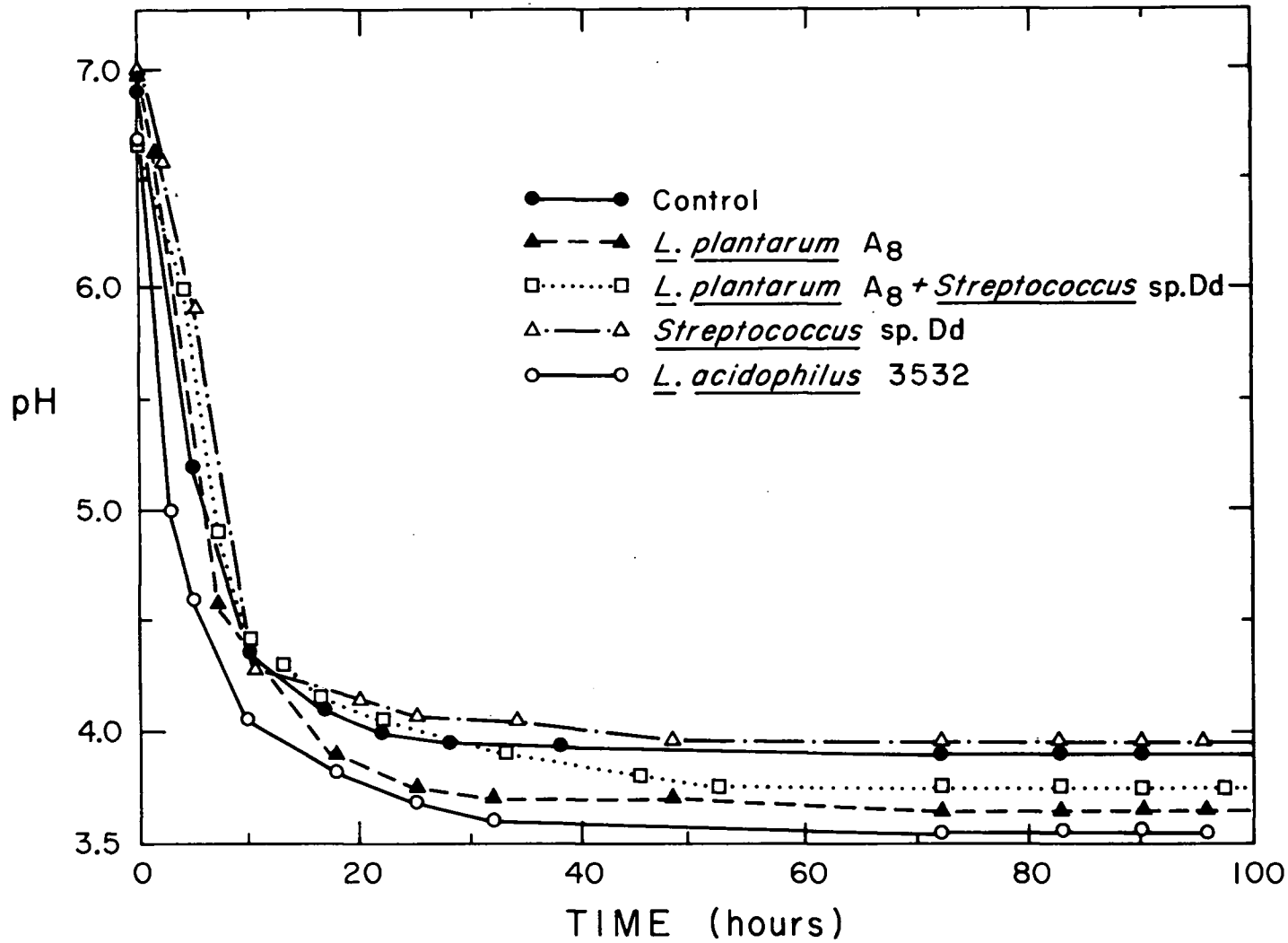

Figure 3. pH changes in non-autoclaved cassava mashes inoculated with lactic acid bacteria.

flora that are present in raw cassava.

When fermentation was carried out with the non-autoclaved mash, both A_8 (L. plantarum) and D_d (Streptococcus sp.) were able to reduce the pH more rapidly than when they were used to inoculate the autoclaved mash. The final pH, after 3 days of fermentation was 3.65 for the former and 3.95 for the latter (Table 12 and Figure 3). Since a fermented mash with a final pH of 3.95 is still undesirable (2) it would seem that Streptococcus sp. alone, could not be used as a starter culture. On the other hand, L. plantarum A_8 could be used in single culture as a starter, and still lower the pH to a value characteristic of a good gari. Figure 3 shows that the mixture of the two cultures in non-autoclaved mash lowered the pH slightly less than L. plantarum A_8 alone (3.75 vs. 3.65). Since a final pH of 3.75 is still acceptable for a good gari, a starter culture system employing the mixture of L. plantarum A_8 and Streptococcus sp. D_d may be used, if such combination would produce a better flavor than that by L. plantarum A_8 alone.

Among the 3 dairy cultures tested for their possible use as "gari starter", two of them, L. lactis and L. acidophilus N were found to contribute objectionable color to the fermenting mash, which turned brownish yellow to orange in contrast to the acceptable white to light yellow color of the traditionally fermented mash. L. acidophilus 3532 did not produce the undesirable color changes. It

lowered pH more rapidly than L. plantarum and produced the lowest pH of 3.55 (Figure 3 and Table 12). It, however, imparted a dairy flavor that is foreign to gari.

The use of a mixture of L. acidophilus 3532 and the gari isolates appears to be feasible. A combination of L. acidophilus 3532 and L. plantarum A_8 appeared to be more effective in lowering the pH in fermenting cassava mash than by L. plantarum alone or a mixture of L. acidophilus 3532 and Streptococcus sp. D_d (Figure 4). The final pH was still 3.55 for the mash cultured with both L. acidophilus 3532 and L. plantarum. When the mash was examined for gari odor, it was found to be reasonably satisfactory (Table 14). A mixture of the 3 organisms L. plantarum, L. acidophilus 3532 and Streptococcus sp., did not produce more acid than when only two of the cultures were used (Table 12).

It was remarkable to observe that the dairy culture could indeed out compete the native isolate of gari during cassava fermentation. This undoubtedly is the result of a careful genetic selection and manipulation received by the former. An equal opportunity should exist for the future development of the desired gari starters.

Effects of Using a Preservative in Cassava Fermentation

Table 13 shows the effects of sodium benzoate and potassium sorbate on the growth of L. plantarum A_8 and the mold-like isolate at

Table 12. pH of inoculated cassava mashes during fermentation (non-autoclaved substrate).

Time (hours)	Cultures[a]								
	C	A_8	D_d	$A_8 + D_d$	3532	A_8 + 3532	D_d + 3532	$A_8 + D_d$ + 3532	A_8 + 3532 + KS
0	6.80	6.80	6.80	6.60	6.70	6.70	6.70	6.70	6.70
3	5.90	6.20	6.70	6.25	5.40	6.20	6.25	5.90	6.20
6	5.20	5.15	5.95	5.5	4.60	5.25	5.50	5.25	5.00
10	4.35	4.30	4.60	4.5	4.05	4.25	4.25	4.20	4.30
12	4.25	4.10	4.25	4.40	4.00	4.10	4.20	4.15	4.25
17	4.20	3.92	4.15	4.15	3.85	3.90	4.00	4.00	4.00
24	3.98	3.75	4.05	4.00	3.70	3.80	3.87	3.85	3.92
28	3.95	3.72	4.05	3.95	3.65	3.80	3.87	3.75	3.92
34	3.92	3.70	4.00	3.90	3.60	3.78	3.87	3.67	3.90
47	3.90	3.70	3.95	3.80	3.55	3.75	3.87	3.80	3.87
54	3.90	3.65	3.95	3.75	3.55	3.75	3.85	3.80	3.87
60	3.90	3.65	3.95	3.75	3.55	3.55	3.80	3.80	3.90
67	3.90	3.65	3.95	3.75	3.55	3.55	3.80	3.80	3.80
74	3.90	3.65	3.95	3.75	3.55	3.55	3.80	3.67	3.90
96	3.90	3.65	3.95	3.75	3.55	3.55	3.80	3.60	3.90

[a] C = control (fermented uninoculated)
A_8 = L. plantarum sp.
D_d = Streptococcus sp.
3532 = L. acidophilus 3532
KS = potassium sorbate (0.1%)

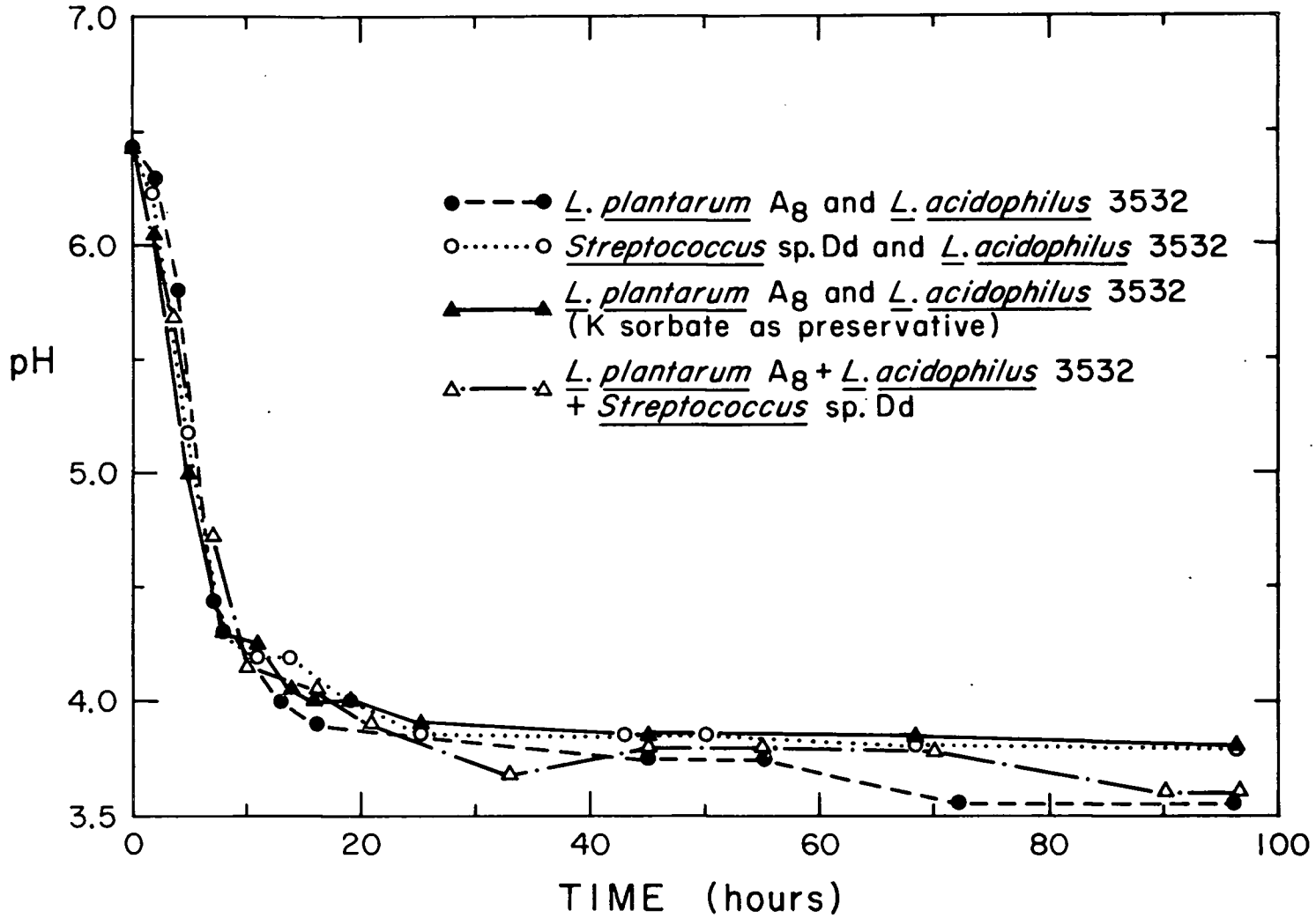

Figure 4. pH changes in non-autoclaved cassava mash inoculated with mixtures of lactic acid bacteria.

Table 13. Antibacterial and antimycotic activities of sodium benzoate and potassium sorbate at various pH.

a. Effect against L. plantarum A_8.

Na Benzoate / Ksorbate	pH 7.0	6.0	5.0	4.0	3.5	3.0
.025%	+ / +	+ / +	+ / +	+ / +	SL / +	- / +
.05%	+ / +	+ / +	+ / +	+ / +	SL / +	- / +
.1%	+ / +	+ / +	+ / +	+ / +	SL / +	- / +
.2%	+ / +	+ / +	+ / +	+ / +	SL / +	- / +

b. Effect against a mold-like isolate.[b]

Na Benzoate / Ksorbate	pH 7.0	6.0	5.0	4.0	3.5	3.0
.025%	+ / +	+ / +	+ / +	+ / -	- / -	- / -
.05%	+ / +	+ / +	+ / +	- / -	- / -	- / -
.1%	+ / +	+ / +	+ / +	- / -	- / -	- / -
.2%	+ / +	+ / +	+ / -	- / -	- / -	- / -

[a] + = growth
SL = slight growth
- = no growth

[b] = Isolated from fresh cassava root.

various pHs. It can be seen that the potassium sorbate was more selective in its action against the mold. L. plantarum A_8 grew in the presence of 0.025% to 0.2% of potassium sorbate between pH 7.0 and 3.0 (Table 13a) which covered the range of pHs normally encountered during the fermentation of cassava. The mold-like isolate did not grow in the presence of 0.2% potassium sorbate at pH 5.0 and at concentrations ranging from 0.25% to 0.2% and the pH between 3.0 and 4.0 (Table 13b). Potassium sorbate, therefore, could be added to cassava mash if mold inhibition is to be desired and used in 0.1% concentration (volume per weight), which is the most widely used concentration of potassium sorbate in acidic foods (15). Although not inhibitory to growth, 0.1% potassium sorbate had retarded the acid production by the lactic acid bacteria. The lowest pH reached by the L. plantarum A_8 and L. acidophilus 3532 mixture in cassava mash was 3.80, compared to 3.55 that was attained in the absence of potassium sorbate (Table 11 and Figure 4).

Flavor Panel

Table 14 gives the scores and means of the final flavor test of the fermented cassava mashes, inoculated with all the cultures and their combinations described above. The six individual judges are identified by the numbers 1 through 6 and their responses in 9 point hedonic scale are presented. This shows that the judges

Table 14. Flavor panel scores of various cultured gari samples.[a]

Judge	Sample Codes 367	814	317	415	512	615	718	987	Sample Preferred
1	9	8	1	3	4	6	2	5	367
2	9	9	7	4	5	2	5	4	814
3	8	9	2	4	5	4	7	5	814
4	9	8	6	4	7	4	5	5	367
5	8	6	2	3	6	5	7	4	367
6	5	8	7	7	4	6	8	3	814
Mean Scores	8.00^{b}	8.00^{b}	4.17^{c}	4.17^{c}	5.17^{c}	4.50^{c}	5.67^{c}	4.33^{c}	

Least Significant Difference (LSD .05) = 2.01

a samples codes

367 = control, traditionally made

814 = L. plantarum cultured gari

512 = L. plantarum + L. acidophilus

615 = L. acidophilus + Streptococcus sp.

415 = L. acidophilus + L. plantarum + Potassium sorbate (0.1%)

987 = Streptococcus sp.

317 = L. plantarum + Streptococcus

718 = L. plantarum + L. acidophilus + Streptococcus sp.

b,c Data was analyzed by analysis of variance. Mean scores with the same superscript letter are not significantly different at the 5 percent confidence limit. LSD is substracted from the highest means and all means greater than the difference are not significantly different from each other.

could not differentiate between the gari made with L. plantarum A_8 starter (No. 814) and the native product (No. 367). The other cultures and their mixtures contributed flavor that were statistically different from the native product but were not different among themselves. However, it is worth noting that products made with L. plantarum A_8 and L. acidophilus 3532 (No. 512) or L. plantarum A_8 L. acidophilus 3532 and Streptococcus sp (No. 718), scored slightly higher than the rest of the samples. This suggests that the possibility of combining various starter cultures to obtain the optimum gari flavor should not be discarded.

It can thus be concluded that L. plantarum A_8 may be used as a starter culture to ferment cassava for gari production and may be able to produce a gari flavor as good as that of a good batch of traditionally made gari. Also when this starter culture was used, the pH drop was more rapid and the final pH reached was lower than that of the gari naturally produced (Figure 3). If L. plantarum A_8 was used as a starter, the fermentation time will be reduced and the product will have more consistent flavor and aroma. The shelf-life of the gari will be increased and it would help eliminate the post-harvest deterioration problems.

No taste test was conducted during this study because we lacked the clearance for such a test. But the taste would be comparable to that of the aroma scores.

Our data strongly suggest that the main microorganisms involved in cassava fermentation are the lactic acid bacteria. The two-stage fermentation hypothesis advanced by Collard and Levi (18) and later endorsed by Akinrele (2) seems untenable. The hypothesis states that during the early stages of cassava fermentation Corynebacterium sp. would hydrolyze starch and produce various organic acids, thus producing conditions favorable for Geotrichum candida sp., the latter in turn produce a variety of aldehydes and esters responsible for the typical gari flavor and aroma. During the course of this study we were unable to isolate Corynebacterium sp. Okafor (47) also reported his failure to isolate Corynebacterium from fermented cassava. The microorganisms he isolated were mainly Leuconostoc sp., Alcaligenes sp., yeasts and Lactobacillus sp. Furthermore Geotrichum candidum is a saprophyte and is commonly found on unclean food processing surfaces (23). possible that the molds found by Collard and Levi might have been such a contaminant.

Molds would be undesirable in gari production as its action on the organic acids tends to raise the pH. Mold contamination of cassava mash therefore must be controlled, not only to produce the desired pH of the gari, but also to guard against the potential danger of mycotoxins. When we inoculated cassava mash with Aspergilus flavus (NRRL 2999), aflatoxins B_1 and G_1 were readily produced.

Our attempt to employ potassium sorbate as a fungicide met with a limited success. Potassium sorbate at 0.1% concentration did not inhibit the growth of our starters L. plantarum A_8 and Streptococcus sp. D_d. It, however, impeded the acid production.

Volatile Flavor Components of Gari

Gas Chromatography

Gas chromatograms of headspace volatiles were determined from fresh raw cassava, naturally fermented cassava, cassava fermented with above starters, and the ready-to-eat gari which was heated with palm oil. A comparison of the chromatograms showed that a distinct change in the types of volatile compounds were noted from raw to fermented cassava. Fermentation seemed to contribute a large portion of highly volatiles compounds not found in the raw cassava. Cassava samples fermented with various starter cultures showed essentially the same patterns, with only minor variations. The L. plantarum A_8 inoculated sample showed the most number of peaks and the L. acidophilus 3532 inoculated sample the least. The ready-to-eat gari (fermented, palm oil added and heated) showed a very clear change of pattern from the other chromatograms. There were fewer peaks of the more volatile compounds, indicating perhaps that the heating process had volatilized many of these compounds.

GC-MS of L. plantarum Inoculated Cassava Mash

Figure 5 shows the total ionization chromatogram of the L. plantarum A_8 fermented cassava mash. One limitation of the column used was the inability to elude more polar compounds such as the formic and lactic acids which are known to exist in such samples (2). Table 15 lists the flavor compounds tentatively identified in this sample. They include some low molecular weight acids and alcohols of probable bacterial origin, such as acetic acid, 2-methyl-propanol-1, and 2-methyl-butanol-1. Hexanal was also found in moderate amounts. In addition, large molecular weight compounds such as ethyl benzene, trimethyl benzene, phenyl-ethanol were also present, although their origin is not known. Styrene was also found but this is probably an environmental contaminant. There were also 2 unknown compounds found in large concentrations.

GC-MS of the Ready to Eat Gari (Fermented Palm Oil Added and Heated

Figure 6 shows the total ionization chromatogram obtained for the ready-to-eat gari made by the traditional method in Africa. Table 16 lists the tentative identities of the organic volatile compounds isolated from this sample. As shown on the gas chromatograms, the low molecular weight acids and alcohols present in the

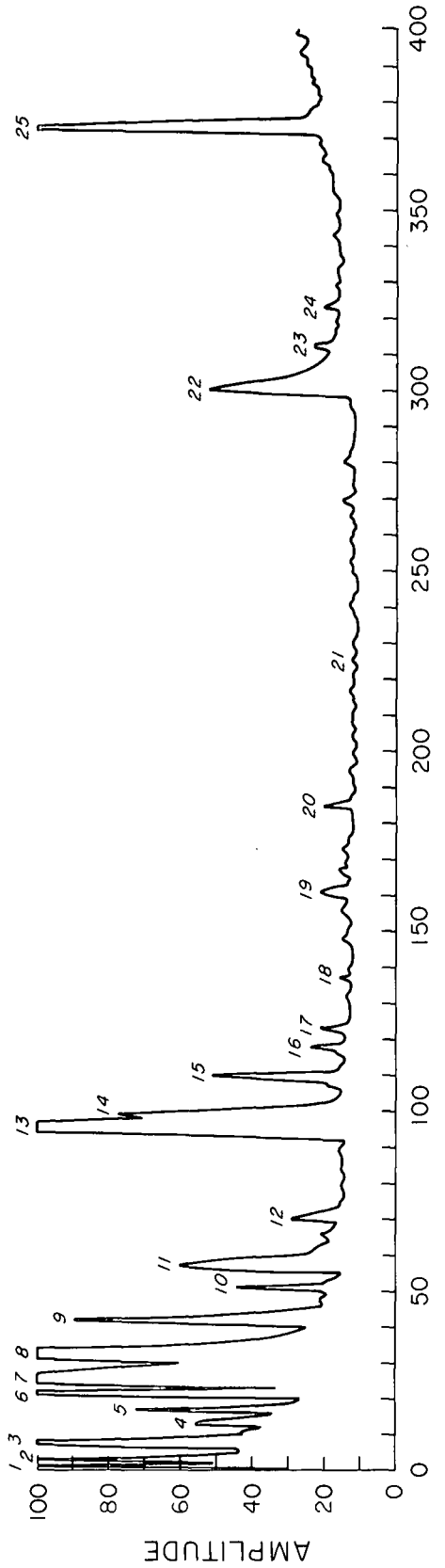

Figure 5. Total ionization chromatogram of L. plantarum A_8 fermented cassava mash.

Table 15. Headspace volatiles[a] identified in cassava mash fermented with the L. plantarum starter.

Peak number	Tentative identity of the volatiles	Concentration[b]	Source and Comments
3	2-methyl-propanol-1[c]	L	Metabolite in large concentration
6	Unknown	L	
7	Acetic acid[c]	L	Metabolite in large concentration
8	2-methyl-butanol-1[c]	L	Metabolite in large concentration
9	Toluene	M	Contaminant
10	Hexanal[c]	M	Metabolite
13	Unknown monoterpene	L	?
15	Ethyl benzene	S	Contaminant?
20	Trimethyl benzene	S	Contaminant?
21	Dichlorobenzene	S	Laboratory Contaminant?
22	Phenyl ethanol	M	?
25	Styrene	L	Column artifact

a = tentative identification

b = concentration: arbitrary peak height

c = may be related to flavor

Scale: L = large, M = medium, S = small

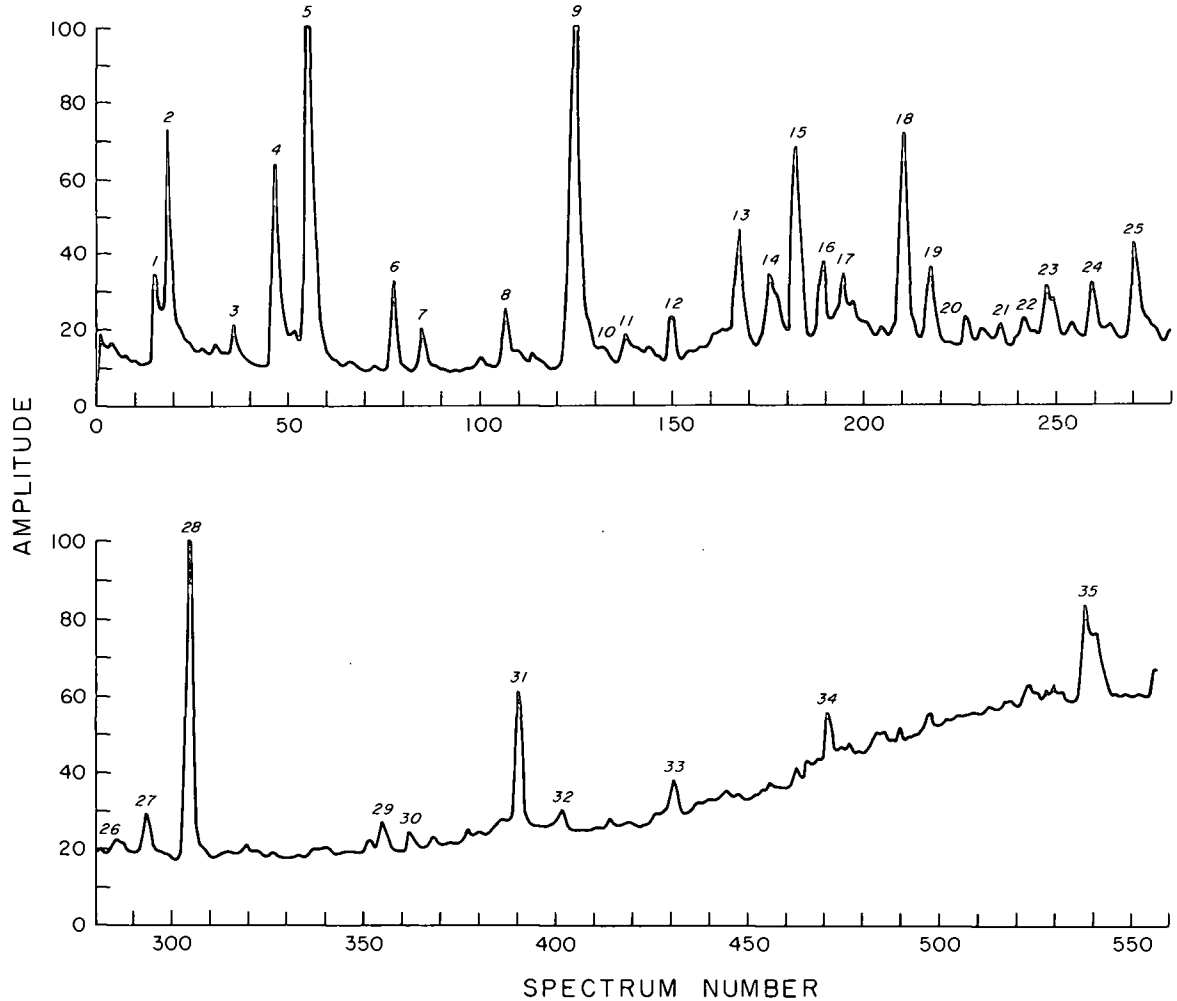

Figure 6. Total ionization chromatogram of the ready-to-eat gari (fermented, palm oil added and heated).

Table 16. Headspace volatiles[a] identified in ready-to-eat gari (fermented palm oil added and heated).

Peak number	Tentative identity of the volatiles	Concentration[b]	Source and Comments
1	methoxyethanol	M	contaminant?
3	Unknown enal	S	autoxidation of palm oil?
4	1-pentanol[c]	L	Metabolite
5	n-hexanal[c]	L	Metabolite. Palm oil
6	Silicone	S	Artifact
7	Hex-2-enal[c]	S	Palm oil
8	Xylene	S	Laboratory
9	Xylene	L	contaminant
10	2, 5-dimethyl pyrazine	S	
13	Heptanal[c]	M	Palm oil
15	Trimethyl benzene	M	?
19	Hepta-2, 4, dienal[c]	M	Palm oil, autoxidation of fatty acid
20	Dichlorobenzene	S	?
23	Limonene	S	?
26	2-ethyl-3,5,6-trimethyl pyrazine[c]	S	Potent compound. May be important to flavor
29	Unknown aromatic (MW 134)	S	?

a = tentative identification

b = concentration: arbitrary peak height scale

c = may be related to gari flavor

L = large M = medium S = small

non-heated sample were no longer detectable and were replaced by aldehydes such as heptanal, hex-2-enal and hepta-2, 4-dienal. Two of these aldehydes, heptanal and hepta-2, 4-dienal have been reported to be present in large quantities in palm oil (20) and their presence in gari is undoubtedly linked to the addition of the palm oil to gari. Hex-2-enal and hexanal were also present. Hex-2-enal might have originated from the autooxidation of aldehydes present in palm oil. Hexanal, however, was also present in the non-heated sample and it cannot be said that it has originated exclusively from the oil, even though it is reported to be present in the palm oil (20). The other volatile compounds found in ready-to-eat gari were: 1-pentanol, methoxyethanol, xylene, 2, 5 dimethyl pyrazine, 2-ethyl-3, 5, 6-trimethyl pyrazine, trimethyl benzene, silicone, dichlorobenzene, limonene and 2 unknown enal and monoterpene. It can be speculated that 2-ethyl-3, 5, 6-tremethyl pyrazine, which has an odor threshold value of 0.4 ppb, could still contribute to gari flavor even though it is present in small amount. Silicone is undoubtedly an artifact from the column, xylene and methoxyethanol are organic solvents that were probably picked up in the laboratory. 1-pentanol is probably a bacterial metabolite. The origin of trimethylbenzene, which was also found in the unheated sample, is not known.

The major events that take place during cassava fermentation for gari production thus appear to be that the lactic acid bacteria grow and produce mainly lactic acid and some other organic acids and alcohols. During frying some of these compounds are volatilized and replaced by aldehydes from the palm oil and the auto-oxidation products of the unsaturated fatty acids of palm oil.

SUMMARY

The microorganisms associated with cassava fermentation and responsible for the gari flavor were isolated, both from the traditionally fermented cassava and from the cassava mash fermented in the laboratory, and identified. Dairy starter cultures were tested for their potential use as gari starters.

The volatile organic compounds that constitute the gari flavor were tentatively identified by GC-MS analysis.

The results and conclusions may be summarized as follows:

1. During the early stage of fermentation, Lactobacilli, Streptococci, Moraxella sp. and Acinetobacter sp. were found in large proportions, but as the fermentation progressed, the Lactobacilli and Streptococci became the predominant microorganisms.
2. Lactobacilli, Streptococci, yeasts and Streptomycetes were isolated from fermented cassava mash received from Africa, but Lactobacilli and Streptococci were clearly the predominant microbial species.
3. The primary role of the bacteria was to produce acid, reducing the pH of the mash from approximately 6.8 to around 3.75.
4. A L. plantarum starter culture could complete the fermentation within 36 hours while the traditional process requires 96 hours.

5. L. plantarum also produced gari of satisfactory flavor.

6. Among the dairy cultures tested, L. acidophilus 3532 could grow on cassava and produce desirable acidity, but it imparted the dairy odor foreign to the gari.

7. Yeasts appeared to play little role in the cassava fermentation process.

8. Molds were undesirable in gari production.

9. Potassium sorbate at 0.1% had little effect on growth of bacteria but impeded the acid production.

10. Headspace volatiles identified in freshly fermented cassava were mostly bacterial metabolites, such as acetic acid, 2-methyl-propanol-1, 2-methyl-butanol-1 and hexanal.

11. In ready to eat gari (fermented, palm oil added and heated) the concentrations of the highly volatile compounds were reduced and compounds that originated from palm oil and its auto-oxidation products began to appear. They were hex-2-enal, heptanal, hepta-2, 4-dienal, and hexanal. Also present were 2, 5 dimethyl pyrazine, 2-ethyl-3, 5, 6-trimethyl pyrazine and limonene.

12. Employing the starter cultures in cassava fermentation for gari production is a feasible and promising concept.

BIBLIOGRAPHY

1. Acena, A. and Puno, G. D. 1957. A study on the use of cassava in the beer industry. Philippine Journal of Agriculture 20(1-2):1-13.

2. Akinrele, I. A. 1964. Fermentation of cassava. Journal of the Science of Food and Agriculture 15:589-594.

3. Anderson, C., Langton, J., Maddix, C., Scrammell, G. W. and Solomons, G. L. 1973. The growth of microfungi on carbohydrates. In: Single-cell protein II, ed. by Tannenbaum, S. R. and Wang, I. C. p. 314-329. M. I. T. Press, Cambridge, Massachusetts.

4. Araujo Neto, J. S. 1974. Food staples as vehicles for protein concentrates. Nutrition Reports International 9(1):85-90.

5. Ayres, J. C. 1972. Manioc: the potential exists for increased use of this tropical plant and its products. Food Technology 26(4)128-135.

6. Barnett, J. A. and Pankurst, R. J. 1974. A New Key to the Yeasts. Elsevier Publishing Co. Inc., New York, New York.

7. Bissett, F. H., Clapp, R. C., Coburn, R. A., Ettinger, M. G. and Long, L. 1969. Cyanogenesis in manioc: concerning lotaustralin. Phytochemistry 8:2235-2247.

8. Bolhus, G. G. 1954. The toxicity of cassava roots. Netherland Journal of Agricultural Science 2(3):176-185.

9. Booth, R. H. 1974. Post-harvest deterioration of tropical root crops: losses and their control. Tropical Science 16: 49-63.

10. Booth, R. H. 1976. Storage of fresh cassava (Manihot esculenta). 1. Post-harvest deterioration and its control. Experimental Agriculture 12:103-111.

11. Booth, R. H. and Coursey, D. G. 1974. Storage of cassava roots and related post-harvest problems. In: Cassava processing and storage: proceedings of an interdisciplinary workshop, Pattaya, Thailand, 17-19 April 1974, ed. by Araullo, E., Nestel, B. L. and Campbell, M. p. 27-42. International Development Research Centre IDRC-031e Ottawa, Canada.

12. Booth, R. H., De Buckle, T. S., Cardenas, O. S., Gomes, G. and Hervas, E. 1976. Changes in quantity of cassava during storage. Journal of Food Technology 11:245-264.

13. Brook, E. J., Stranton, W. R. and Wallbridge, A. 1969. Fermentation methods for protein enrichment of cassava. Biotechnology and Bioengineering 11:1271-1284.

14. Butler, E. J., Brown, E. E. and Davis, L. H. 1971. An economic analysis of the production, consumption, and marketing of cassava (tapioca). Research Bulletin No. 97. College of Agriculture Experiment Station. University of Georgia, Athens, Georgia. 54 p.

15. Chichester, D. F. and Tanner, F. W. 1968. Antimicrobial food additives. In: Handbook of food additives, ed. by Furia, T. E. p. 151-159. The Chemical Rubber Co., Cleveland, Ohio.

16. Clapp, R. C., Bissett, F. H., Coburn, R. A. and Long, L. 1966. Cyanogenesis in manioc: linamarin and isolinamarin. Phytochemistry 5:1323-1326.

17. Cock, J. H. 1974. Agronomic potential for cassave production. In: Cassava processing and storage: Proceedings of an interdisciplinary workshop, Pattaya, Thailand, 14-17 April 1974, ed. by Araullo, E. V., Nestel, B. and Campbell, M. p. 21-26. International Development Research Centre IDRC-031e, Ottawa, Canada.

18. Collard, P. and Levi, S. 1959. A two-stage fermentation of cassava. Nature 183:620-624.

19. Dina, J. A. and Akinrile, I. A. 1970. Economic feasibility study for the establishment of a glucose industry in Nigeria. Quoted in: Nestel, B. L. 1974. Current trends in cassava research, p. 16. International Development Research Centre IDRC-036e. Ottawa, Canada.

20. Dirinck, P., Schreyen, L., DeShoenmacker, L., Wychuyse, F. and Schamp, N. 1977. Volatiles components of crude palm oil. Journal of Food Science. 42(3):645-648.

21. Dutra de Olivera, J. E. and Salata, E. B. Z. M. 1971. Methionine fortified manioc flour to combat protein malnutrition. Nutrition Reports International 3:291-293.

22. Dutra de Olivera, J. E., Salata, E. B. Z. M. and Campos, J. 1973. Manioc flour as a methionine carrier to balance common bean-based diets. Journal of Food Science 38:116-118.

23. Eisenberg, W. V. and Cichowicz, S. M. 1977. Machinery mold. Indicator organism in foods. Food Technology 31(2): 52-60.

24. Food and Agriculture Organization. 1972. Production Yearbook. United Nations, Rome, Italy.

25. Foster, E. M., Nelson, F. E., Speck, M. L., Doetsch, R. N. and Olson, J. C. 1957. Dairy Microbiology. p. 318-333. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

26. Harper, R. S. 1973. Cassava growing in Thailand. World Crops 25(2):94-97.

27. Harris, R. V. 1970. Effect of rhizopus fermentation on the lipid composition of cassave flour. Journal of the Science of Food and Agriculture 21:626-627.

28. Heatherbell, D. A., Wrolstad, R. E. and Libbey, L. M. 1970. Isolation, concentration and analysis of carrot volatiles using on-column trapping and GLC-MS. Paper No. 21, Agricultural and Food Chemistry Division, 160th American Chemical Society National Meeting, September 14-18. Chicago, Illinois.

29. Hesseltine, C. W., Wang, H. L. and Smith. 1967. Traditional fermented foods. Biotechnology and Bioengineering 9(3):275-288.

30. Humphrey, A. E. 1974. Current developments in fermentation Chemical Engineering. December 9, p. 98-112.

31. Ingram, J. S. and Humphris, J. R. O. 1972. Cassava storage: a review. Tropical Science 142:131-148.

32. Johnson, M. J. 1971. Fermentation. Yesterday and tomorrow Chemical Technology 1:338-341.

33. Khor, G. L., Alexander, J. C., Santos-Nunez, J., Reade, A. E. and Gregory, K. F. 1975. High temperature production of protein-enriched feed from cassava by fungi. Applied Microbiology 30(6):897-904.

34. Kilberg, R. 1972. The microbe as a source of food. Annual Review of Microbiology. 26:427-465.

35. Kim, J. C. and De Ruiter, D. 1968. Bread from non-wheat flours. Food Technology 22(7):868-878.

36. Lautrop, H. 1974. Moraxella and Acinetobacter. In: Bergey's manual of determinative bacteriology. Eighth edition, ed. by Buchanan, R. E. and Gibbons, N. E. p. 433-438. The Williams and Wilkins Company, Baltimore, Maryland.

37. Leininger, H. V. 1976. Equipments, media, reagents, routine tests and stains. In: Compendium of methods for the microbiological examination of foods. p. 79-80, ed. by Speck, M. L. American Public Health Association, Washington, D.C.

38. Liener, I. E. 1966. Toxic substances associated with seed proteins. In: World Protein Resources. Advances in Chemistry Series. 57:187-196.

39. Mathot, P. J. 1974. Production and export control in Thailand and the marketing in Europe of tapioca pellets. In: Cassava processing and storage: proceedings of an interdisciplinary workshop, Pattaya, Thailand, 17-19 April 1974, ed. by Araullo, E. V., Nestel, B. L. and Campbell, M. p. 27-42. International Development Research Centre IDRC-031e Ottawa, Canada.

40. Morgan, M. E. and Day, E. A. 1965. Simple on-column trapping procedure for gas chromatographic analyses of flavor volatiles. Journal of Dairy Science 48:1382-1384.

41. Morse, R. E. and Uriah, N. 1974. Protein enriched gari. Canadian Institute of Food Science and Technology Journal 7(2):151-154.

42. Nakamura, I. M. and Park, Y. K. 1975. Some physico-chemical properties of fermented cassava starch ("Polvilho Azedo"). Die Stärke 27(9):295-297.

43. Nestel, B. L. 1973. Current utilization and future potential for cassava. In: chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England 29-30 January 1973, ed. by Nestel, B. L. and MacIntyre, R. p. 11-26. International Development Research Centre IDRC-010e Ottawa, Canada.

44. Nestel, B. L. 1974. Current trends in cassava research. International Development Research Centre IDRC-036e. Ottawa, Canada. 32 p.

45. Nestel, B. L. and Cock, J. 1976. Cassava. The development of an international research network. International Development Research Centre IDRC-059e. Ottawa, Canada. 69 p.

46. Nicol, B. M. 1952. The nutrition of Nigerian peasants, with special reference to the effects of deficiencies of the vitamin B complex, vitamin A and animal protein. British Journal of Nutrition 6(1):34-55.

47. Okafor, N. 1977. Micro-organisms associated with cassava fermentation for gari production. Journal of Applied Bacteriology 42:279-284.

48. Oke, O. L. 1966. Chemical studies on some Nigerian foods: gari. Nature 212:1055-1056.

49. Oke, O. L. 1973. Leaf protein research in Nigeria, a review. Tropical Science 15(2):139-145.

50. Park, Y. K. and Lima, D. C. 1973. Continuous conversion of starch to glucose by an amyloglucosidase-resin complex. Journal of Food Science 38:358-359.

51. Phillips, T. P. 1974. Cassava utilization and potential markets. International Development Research Centre IDRC-020e Ottawa, Canada. 182 p.

52. Pridham, T. C. and Tresner, H. H. 1974. Streptomycetaceae In: Bergey's manual of determinative bacteriology. Eighth edition, ed. by Buchanan, R. E. and Gibbons, N. E. p. 747-845. Williams and Wilkins Co., Baltimore, Maryland.

53. Rajogopal, M. V. 1977. Production of beer from cassava. Journal of Food Science 42(2):532-533.

54. Scanlan, R. A., Arnold, R. G. and Libbey, R. C. 1968. Collecting and transferring packed-column gas chromatographic fractions to capillary columns for fast-scan mass spectral analysis. Journal of Gas Chromatography 6:372-374.

55. Sharpe, M. E. 1962. Taxonomy of the Lactobacilli. Dairy Science Abstracts 24(3):109-118.

56. Sharpe, M. E., Fryer, T. F. and Smith, D. G. 1967. Identification of the lactic acid bacteria. In: Identification methods for microbiologists. Part A., ed. by Gibbs, B. M. and Skinner, F. A. p. 65-79. Academic Press, New York.

57. Sherman, J. M. 1937. The streptococci. Bacteriological Reviews 1:3-97.

58. Smith, R. H. 1966. Lipid-protein isolates. In: World Protein Resources. Advances in Chemistry Series No. 57: 145.

59. Strasser, J. O., Abbot, J. A. and Battey, R. F. 1970. Process enriches cassava with protein. Food Engineering 5:112-116.

60. Teixera, C. G. 1950. Producao de alcool de mandioca. Bragantia 10(10):277-286.

61. Terry, E. and MacIntyre, R. 1975. Summary of general discussion. In: The international exchange and testing of cassava germ plasm in Africa, ed. by Terry, E. and MacIntyre, R. p. 35-36. International Development Research Centre IDRC-063e Ottawa, Canada.

62. Toury, J. and Giorgi, R. 1966. Aflatoxine et fluorescence. Annales de la Nutrition et de l'Alimentation 20:111-118.

63. Treadway, R. H. 1967. Manufacture of potato starch. In: Starch chemistry and technology. 2. Industrial aspects, ed. by Whistler, R. I., Paschall, E. F., Bemiller, J. N., and Roberts, H. J. p. 87. Academic Press, New York.

64. Trevelyan, W. E. 1974. The enrichment of cassava with protein by moist-solids fermentation. Tropical Science 16(4): 179-194.

65. van Veen, A. G. and Steinkraus, K. H. 1970. Nutritive value and wholesomeness of fermented foods. Journal of Agricultural and Food Chemistry 18(4):576-578.

66. Wood, T. 1965. The cyanogenic glucoside content of cassava and cassava products. Journal of the Science of Food and Agriculture 16:300-305.

67. Wood, T. 1966. The isolation, properties and enzymic breakdown of linamarin from cassava. Journal of the Science of Food and Agriculture 17:85-90.

68. Woolen, A. 1972. Innovations from Europe: Cassava root process. Food Engineering 44(11):61-63.

69. Wu Lung, W. T. 1968. Food composition table for use in Africa. p. 34-35. Food and Agriculture Organisation of the United Nations, Nutrition Division, Rome Italy and U. S. Department of Health, Education and Welfare. Public Health Service, Bethesda, Maryland.