FERMENTATION OF HIGH QUALITY CASSAVA FLOUR INTO AGBELIMA, AN INDIGENOUS FERMENTED CASSAVA DOUGH

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ABSTRACT

High quality cassava flour produced by the dehydration of unfermented de-watered grated cassava is currently being promoted nationwide for use as composite flour in the bakery industry. This work was carried out to ferment such high quality flour into agbelima, a fermented cassava meal. *Lactobacillus plantarum* was isolated as the dominant lactic acid bacteria in both unfermented flour and agbelima. *L. plantarum* isolates were successfully used as a single starter culture for fermenting cassava dough, reconstituted from unfermented cassava flour into agbelima within 72h. Spontaneous fermentation of the reconstituted cassava flour also produced agbelima even without the use of a starter culture. Addition of glucose to reconstituted cassava flour increased the rate of fermentation even without glucose fermentation occurred because the flour contained enough fermentable sugars to support the growth of the lactic acid bacteria leading to acidification of the dough. Banku prepared from both reconstituted cassava flour inoculated with *L. plantarum* as starter culture and the spontaneously fermented reconstituted cassava flour were preferred to reconstituted agbelima flour and a market sample of agbelima. There was no difference in the overall acceptability between the reconstituted cassava flour fermented spontaneously or with *L. plantarum* as starter culture. Four enteric pathogens *Salmonella typhimurium* 9, *Shigella disenteriae* 2357T, *Echerichia coli* D2188, *Vibrio cholerae* C-230 inoculated into fermenting cassava mash died off by 48h of fermentation. High quality cassava flour fermented for 72h into agbelima will be free of enteric pathogens studied, which are among the major food- contaminating organisms. Agbelima can therefore be safely prepared from unfermented cassava flour simply by reconstituting it with water into a dough and fermenting spontaneously for 72h.

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1. INTRODUCTION

Fermented foods mainly cereals and root and tubers are consumed extensively in Ghana. Most of these indigenous fermented foods are fermented spontaneously with the notable exception of agbelima and pito in which fermentsation is initiated by the introduction of an inoculum. Cassava and maize are agreeably the most extensively processed and fermented food crops.

Cassava is currently one of the most widely cultivated root crops in Ghana and serves as a principal source of energy in the diet. The important role of cassava in agricultural production and the potential of the crop as a non-traditional export crop has been recognized by the government of Ghana. For this reason, the government has initiated a cassava improvement and utilization program and farmers are being encouraged to go into cassava production. This will lead to a glut if existing market for cassava is not expanded because freshly harvested cassava roots are highly perishable.

With the exception of cold storage various methods available for the storage of fresh cassava tubers are of limited usefulness since they extend its shelf life for only a couple of days or weeks. One solution to this is the processing of cassava into shelf-stable products. Such traditional cassava processed products are mainly fermented. The three major types being agbelima, gari and kokonte. Processing of cassava into these fermented foods is one of the major rural or informal food industries in Ghana, serving as a means of livelihood for a number of rural women. It also plays an important role in the food supply system by preventing the post-harvest loss of the highly perishable crop. In Ghana and Togo the most important traditionally fermented cassava product in terms of production and consumption appears to be agbelima, a fermented cassava meal. Traditionally, agbelima is produced by fermenting grated cassava to which a cassava inoculum called *kudeme* has been added. Even though the fermentation of cassava into agbelima is carried out by grating the inoculum *(kudeme)* together with peeled cassava, it is possible to ferment the dough through the use of starter culture rather the traditional inoculum. A starter culture is normally a pure culture and is added to the substrate at a high concentration with the aim of a rapid and complete fermentation (Brock & Madigan, 1991). The use of a starter culture in the fermentation of foods avoids the inoculation of unwanted organisms into the substrate, ensuring its safety. This prevents the product from developing undesirable organoleptic properties, since the inoculum is known.

Research work carried out in recent years have elucidated the role played by lactic acid bacteria, *Bacillus* species and yeasts in the fermentation of cassava into agbelima and the development of the characteristic odour of the fermented dough. *Lactobacillus plantarum* has been isolated as the dominant lactic acid bacteria responsible for souring/acidification of cassava dough during agbelima fermentation (Amoa-Awua et al., 1996).

In recent years the Ministry of Food and Agriculture in collaboration with the CSIR-FRI and other relevant institutions have embarked on an extensive campaign to promote the processing of cassava into high quality unfermented flour which is shelf stable and can be used in the baking industry. This nationwide campaign is being carried out under the IFAD funded Roots and Tuber Improvement Programme and it is envisaged that in the foreseeable future there could be a glut of high quality unfermented cassava flour on the market. Under such circumstances technologies should be available to farmers to convert the cassava flour into the stable traditional cassava products which are eaten as staple foods i.e agbelima, gari and kokonte. Availability of such technologies will also enable farmers to preserve their cassava harvest by processing into shelf stable flours which can later be fermented into the stable dishes.

The aim of this work was therefore to develop a process for fermenting high quality unfermented cassava flour into the traditional fermented cassava meal, agbelima.

Objectives:

1. To isolate strains of the dominant lactic acid bacteria in agbelima, *L. plantaarum* and develop it into a starter culture
2. To use the starter culture to ferment cassava flour into agbelima
3. To determine chemical and the organoleptic properties of the agbelima prepared from high quality cassava flour and assess its acceptability by a taste panel.

2. LITERATURE REVIEW

2.1 Botany and classification

Cassava is a perennial shrub of the family Euphorbiaceae and is known by many different names. In Brazil, it is known as Manioca, Yuca in Spanish-speaking America, in Anglophone Asia as Tapioca, and in the rest of English-speaking world as Cassava. Many cultivars are known but little is known about their wild ancestors. Two supposedly wild forms Manihot saxicola (Lanj) and Manihot melanobasis (Muell. Arg) are so similar to cassava that it is difficult to separate them. However, the roots of these species show only rudimentary thickening and neither of them is as easily propagated by cutting as is cassava. This species and others may represent the ancestral pool from which our present day cassava have been selected (Dosoo and Amoa – Awua, 1992).

2.2 The importance of cassava in Ghana

Cassava has taken a central stage in food production in Ghana because it is now considered as a food security crop with a potential for industrial processing. The importance of cassava in the world food supply is due to its durability as a plant and also due to it being a cheap and excellent source of dietary carbohydrate. The drought-tolerant cassava often referred to as an excellent famine reserve crop, is undemanding as a crop and able to grow under a variety of climatic and soil conditions including low fertility and high acidity. Once established it has no critical period when lack of rain cause crop failure and is well adapted to marginal soils on which other crops suffer. It is high yielding, has high return of food per unit of energy input and its harvesting which is flexible can be staggered for up to 3 yrs. It is also resistant to locust attack (Cooke and Coursey 1981; De Bruijn and Fresco 1989). The drought-tolerance of cassava gives it a special advantage as a food crop in an era where persistent drought has taken a toll on food production in several parts of Africa.

Cassava is currently the most widely cultivated root crop in Ghana. It is grown in almost all regions of the country with the exception of Upper East and West. Its total annual production is about 7.17 million tonnes, contributing about 22% dietary energy intake and serves as one of the cheapest sources of calories for human consumption (Ministry of food and agriculture, Ghana, 1995). It is a major source of carbohydrates for both human and livestock. It is therefore

processed into a variety of forms e.g. cassava flour and chips, agbelima, akyeke, fufu, kokonte, gari, tapioca and starch.

2.3 Problems associated with cassava

In spite of its importance as a major food crop, cassava has three major limitations. It is a highly perishable root crop, which has a natural storage life of only about 2-3 days after harvesting. Physiological deterioration is followed within 5-7 days by microbial deterioration and root tissue softening caused by activities of a complex of fungi followed by Pythium, Mucor, Rhizopus and Penicillium as well as some bacteria such as Bacillus and Xanthomonas species (Booth, 1976).

Another major limitation to cassava utilization is its low protein content of about 1-3%. The presence of cyanogenic glucoside, linamarin and totaustralin, which impart toxicity to the tuber presents the third limitation to its importance as a food crop (Breeching et al, 1994). Environmental factors have effect on root toxicity; drought, low soil fertility and especially potassium deficiency have been seen to increase the root cyanogenisis (Dosoo and Amoa – Awua, 1992).

2.4 Cassava processing in Ghana

The limitations of cassava, high perishability, low protein content, and the presence of cyanogenic glucosides can be overcome by processing (Hahn, 1982; Oyewole and Aibor, 1992). The process of fermentation is used in many parts of the world as a means for improving its preservation and sensory characteristics. In Ghana cassava processing is usually carried out by rural women and it forms one of the major informal industries in the country. The methods used include peeling, grinding, milling, grating, fermenting, drying washing, steaming, pounding, sieving, packaging and mixing in cold or hot water. Specific combinations of these steps lead to a variety of cassava products such as gari, kokonte, and agbelima.

Three major types of products are processed from cassava in Ghana, agbelima, gari and kokonte and they are all produced through semi-solid or solid state fermentations. The processing procedure for the production of agbelima, gari and kokonte are shown in Fig.1 The procedure for

the production of gari and kokonte will be described briefly but more detailed information will be given on agbelima.

2.4.1 Gari

The traditional method for the processing of cassava into gari begins with the peeling of the cassava tubers. The tubers are washed and then grated and packed into woven polythene sacks and allowed to ferment spontaneously for 2 to 4 days. During fermentation weights are placed on top of the bags to slowly dewater the mash. The fermented dewatered mash is sieved to break up the cake and fibre strands removed. The granulated dough is roasted in an open pan. During roasting the granules gelatinise and are cooked into gari (Jones 1959; Amoa-Awua and Dzokoto 1991). Gari is produced in a slightly different form in some South American countries like Brazil where it is known as "farihna de manioca" (Jones 1959; Doku 1969; Amoa-Awua and Dzokoto 1991).

2.4.2 Kokonte

Kokonte, a fermented cassava flour, is traditionally produced by washing peeled cassava tubers and cutting them into slices of about 5 to 10 cm by 2 to 3 cm. The chips are sun-dried for a period of 7 to 14 days or more depending on the suitability of the weather and the thickness of the slices. During sun drying the cassava chips ferment and the white cream coloured cassava can be seen to be covered with moulds especially on prolonged sun drying due to intermittent rain. In the wet season the chips may be dried near the cooking fire in the kitchen, the heat and smoke rendering them dark brown or black. Dried kokonte pieces are milled or pounded into flour and cooked into a stiff dough mixed with water (Doku 1969; Aidoo 1986; Amoa-Awua and Dzokoto 1991).
In the Upper East Region of Ghana where higher cyanide containing cassava varieties are used, kokonte is produced by first submerging peeled cassava chunks in water for a few days to ferment and detoxify before they are sundried and milled into flour.

2.4.3 Agbelima

Agbelima, a moderately high moisture off-white fermented cassava meal is extensively consumed along the coastal regions and southern Ghana as a whole. Peeled washed cassava tubers are grated

together with a traditional cassava dough inoculum called kudeme in a ratio of about 50:1 in a motor driven cassava grater. The milled cassava is packed into polythene sacks and left for 2 to 3 days to ferment whilst weights are placed on top of the sacks to partially dewater the mash during fermentation (Dovlo 1975; Amoa-Awua and Jakobsen 1995). The fermented meal, agbelima, is sold in an active state of fermentation in the local markets and is cooked mixed with fermented maize meal in varying proportions into banku or akple which are stiff porridges eaten with a stew.

2.4.4 Cassava Dough Inoculum, Kudeme

Several different types of traditional cassava dough inoculum called kudeme, all produced from cassava are used to ferment agbelima. According to traditional cassava processors, the sole aim of using inoculum to ferment cassava mash into agbelima is to break down the texture of the mash into agbelima in order to obtain fermented dough with a smooth texture. Several types of traditional cassava dough inocula are used to ferment agbelima and some of the common methods are;

1. Small chunks of peeled cassava are partially roasted over an open fire, wrapped in a piece of cloth, placed in a basket and left in a warm place for 2-4 days to ferment into inoculum.
2. Small chunks of peeled cassava are blanched by boiling for 5 minutes, wrapped in a piece of cloth, placed in a basket and allowed to ferment in a warm place for a bout 2-4 days.
3 Small chunks of peeled cassava are exposed to the sun for about 30 minutes, wrapped in a piece of cloth, placed in a basket and left in a warm place to ferment for 2-4 days.
4 Split, peeled cassava tubers are partially sun-dried for about 8 hours and stuck under a thatch roof for 2-4 days to ferment.
5 Small chunks of peeled cassava are roasted in a pan and stuck under a thatch roof for 2-4 days.
6 Small unpeeled cassava tubers are tightly packed into an earthenware and covered with plastic sheets and left for about 7 days.

Split cassava pieces are sun-dried for 3-5 days and used as inoculum (Dovlo 1975; Dziedzoave, 1989; Budu, 1990; Sefa –Dedeh, 1995; Amoa-Awua et al. 1995).

In La Cote d'Ivoire a similar traditional inoculum is prepared by boiling cassava tubers for 15-20 min wrapping the boiled cassava with a plastic sheet, jute bag or plantain leaves and allowing it to ferment for 3 days for the roots to soften. The inoculum is used to ferment cassava dough during

preparation of indigenous products such as attieke and placali in order to obtain smooth- textured dough with characteristic flavour (Aboua, 1995).

2.5 Fermentation of cassava into agbelima

The fermentation of cassava dough into agbelima has been studied in detail (Amoa-Awua and Jakobsen 1995; Amoa-Awua 1996; Amoa-Awua et al. 1996; Amoa-Awua et al.1997) and the biochemical and microbiological changes which occur during fermentation have been summarised in Fig 2. Four objectives are accomplished during the fermentation

1. the coarse texture of grated cassava is broken down into a smooth textured dough
2. acidification of the meal occurs leading to a souring of the product
3. there is enhanced detoxification of cassava
4. the dough develops a characteristic flavour

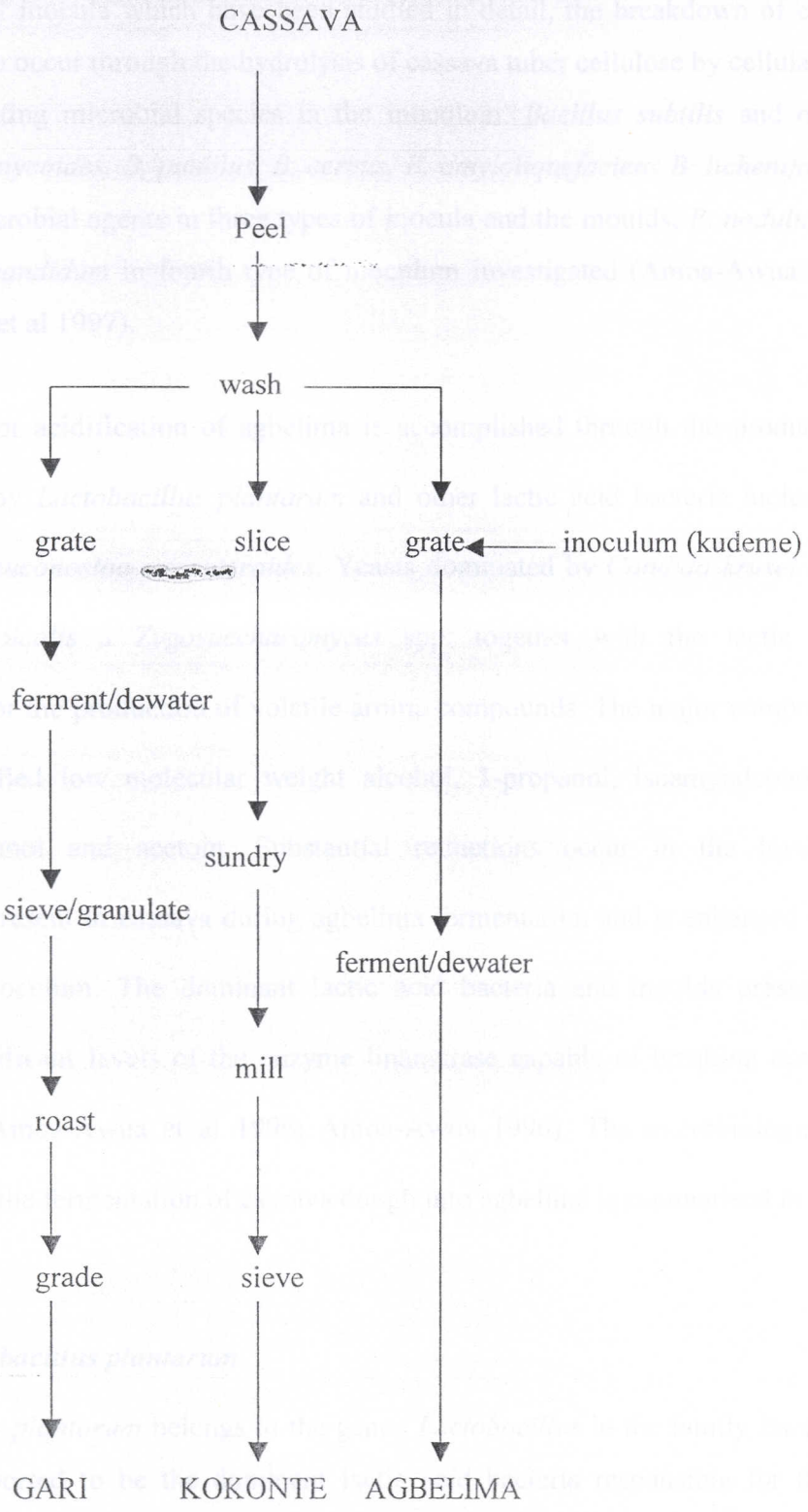


Fig 1. Traditional processing of cassava into gari, kokonte and agbelima in Ghana

In all types of inocula which have been studied in detail, the breakdown of cassava texture has been shown to occur through the hydrolysis of cassava tuber cellulose by cellulases produced by some dominating microbial species in the inoculum. *Bacillus subtilis* and other *Bacillus* spp. including *B. mycoides, B. pumilus, B. cereus, B. amyloliquefaciens B. licheniformis* are the tissue degrading microbial agents in three types of inocula and the moulds, *P. nodulum, P. citrinum* and *Geotrichum candidum* in fourth type of inoculum investigated (Amoa-Awua & Jakobsen 1995; Amoa-Awua et al 1997).

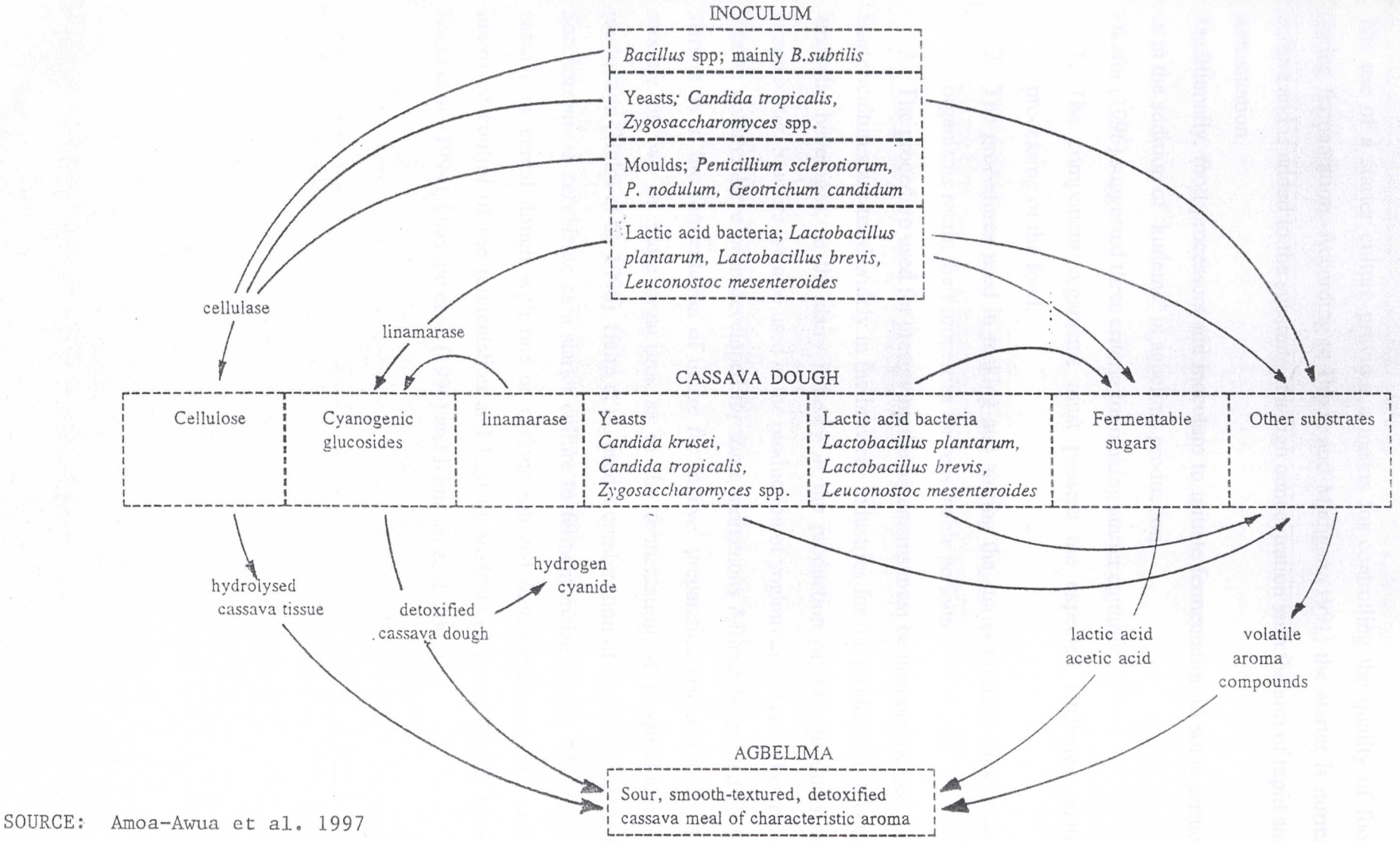
The souring or acidification of agbelima is accomplished through the production of lactic and acetic acids by *Lactobacillus plantarum* and other lactic acid bacteria including *Lactobacillus* brevis and *Leuconostoc mesenteroides*. Yeasts dominated by *Candida krusei* and also including *Candida tropicalis* a *Zygosaccharomyces* spp. together with the lactic acid bacteria are responsible for the production of volatile aroma compounds. The major compounds produced are a non identified low molecular weight alcohol, 1-propanol, isoamylalcohol, ethylacetate, 3-methyl-1-butanol and acetoin. Substantial reductions occur in the levels of cyanogenic compounds present in cassava during agbelima fermentation and is enhanced through the use of traditional inoculum. The dominant lactic acid bacteria and moulds present in agbelima all produce significant levels of the enzyme linamarase capable of breaking down the cyanogenic glucosides (Amoa-Awua et al 1996; Amoa-Awua 1996). The microbiological changes which occur during the fermentation of cassava dough into agbelima is summarised in fig. 3.

2.6 *Lactobacillus plantarum*

Lactobacillus plantarum belongs to the genus *Lactobacillus* in the family *Lactobacteriaceae* and has been reported to be the dominant lactic acid bacteria responsible for the acidification of agbelima during fermentation (Amoa-Awua et al. 1996). It dominates lactic acid bacteria population of the inoculum, 'kudeme' and also all stages of the fermentation process. *Lactobacilli* are identified as gram-positive, catalase-negative, oxidase-negative, fermentative, non-motile regular rods, which grow both aerobically and anaerobically. They commonly occur in dairy

products, silage, sauerkraut, pickled vegetables, cow dung, sour dough; human mouth, intestinal tract, stool and sewage (Kandler andWeiss,1986).

Among the lactic acid bacteria, there has been much interest in *L plantarum* due to its potential application as starter culture for the fermentation of vegetables, meat and fish products (Mackay and Baldwin, 1990). *L. plantarum* is one of the widely used *lactobacilli* in food fermentation and several studies have identified it in significant numbers in several indigenous fermented African foods (Okafor 1977; Ngaba and Lee 1979; Oyewole and Odunfa 1990). A microbiological study of the fermentation of sorghum for ogi production showed that the main microorganisms involved were Lactobacillus spp. particularly *L plantarum* and two yeasts, *Candida krusei* and *Debaryomyces hansenii* (Odunfa and Adeyele, 1985). In the production of Uji, a sour cereal gruel of East Africa, *L plantarum* was found to be mainly responsible for the souring of the Uji, although early activities of heterofermentative strains, *L fermentum, L cellobiosus, L buchneri* during the fermentation is evident (Mbugua, 1981).



SOURCE: Amoa-Awua et al. 1997

Fig. 2 Fermentation of cassava into gbelima

2.7 Starter cultures

The use of a starter culture provides a means for controlling the quality of food products during fermentation. According to Brock and Madigan (1991) the starter is normally a pure culture and is added to the substrate at a high concentration with the aim of rapid and complete fermentation.

Traditionally, food processors add inoculum to initiate fermentation in some fermented foods as in the addition of 'kudeme' in agbelima production.

Okafor (1996) suggested three criteria for making starter cultures:

1. The component organisms must possess the expected attributes relating to the processing of the food.
2. The procedures used in making and storing the starter cultures must ensure that the organisms retain their properties for reasonable periods.
3. The procedure used for preserving the organisms must be unsophisticated.

Starter cultures are used widely in the brewery industries for the production of beer and other alcoholic beverages; in the dairy industry in the production of milk products and ripened cream butter. Starters are also used in the production of yoghurt and cheese (Petersson, 1988).

Starter cultures have been developed for some indigenous African fermented foods as single starters as in the fermentation of maize for 'mawe' preparation (Hounhouigan, 1994) or a mixture of two or more organisms as in the fermentation of maize dough for kenkey production (Halm et al. 1996). Halm et al. used a combination of *L. fermentum* and the yeast, *Saccharomyces cerevisiae* as a starter culture to ferment maize dough. Mass inoculation of cassava or cereal dough with one or various selected *lactobacillus* strains would provide improved control of the fermentation and lead to products of better quality (Sanni (1993), Sanni et al. (1994), Olasupo et al. (1996) and Kimaryo et al. 2000).

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Agbelima

Agbelima from which initial isolates were obtained was produced by a traditional cassava processor at Medie near Pokuase in the Greater Accra Region.

3.1.2 Cassava flour

Cassava flour used for this work was produced at the Cassava Processing Demonstration Unit of the CSIR-Food Research Institute at Pokuase near Accra.

3.2 Method

3.2.1 Sampling

Fermented cassava dough samples (agbelima) were aseptically taken from the interior of the traditionally produced fermenting (24hr) dough into sterile sampling bags, placed in an ice-chest and taken immediately to the laboratory for analysis.

3.3 Processing

3.3.1 Production of cassava flour

Cassava tubers were peeled and washed twice in sinks under running water till very clean. They were grated in a grating machine and then pressed to de-water in a manual screw-press. After pressing it was grated again to obtain a finer texture. It was then dried, by spreading on aluminium sheets in a mechanical dryer at 60°C for 7 h. The dried product was milled into powder, sieved and then packaged. Cassava flour produced this way has a shelf life of about one year. The process for cassava flour production is shown in the flow diagram below (Fig. 4).

3.3.2 Fermentation of cassava flour into agbelima

After all glassware and apparatus were sterilized, 50ml of sterile distilled water was added to 50g of flour in conical flasks. Two batches were prepared; to one batch 0.5g of glucose was added. This was to make initial simple sugar available for fermentation to begin. The two batches were then inoculated with a drop of 48 h- culture of *Lactobacillus plantarum* isolated from fermented cassava dough (agbelima). A control was set up where the dough was not

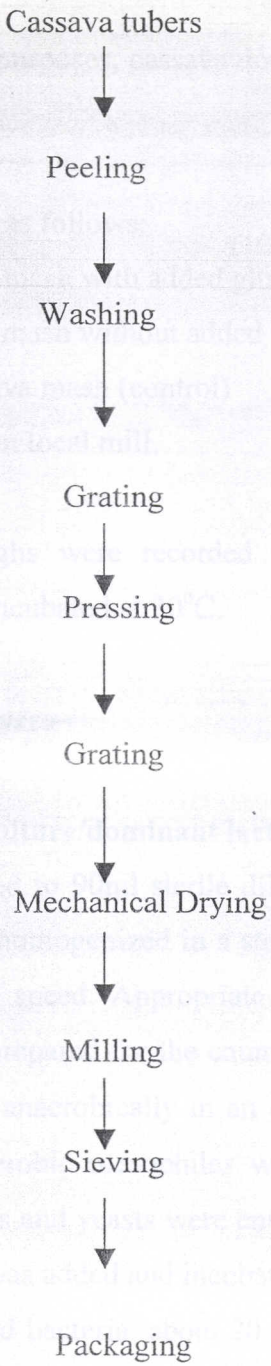


Fig. 3 Flow diagram for the preparation of cassava flour

inoculated. For comparative purposes, cassava dough obtained from a cassava mill at Madina was included in the set-up.

The whole set-up was as follows:

A. Inoculated cassava mash with added glucose

B. Inoculated cassava mash without added glucose

C. Uninoculated cassava mash (control)

D. Cassava dough from local mill.

The initial pH of the doughs were recorded and repeated at 24-h intervals. For each fermentation the dough was incubated at 30°C.

3.3 Microbiological analyses

3.3.1 Isolation of starter culture/dominant lactic acid bacteria

10g of the sample were added to 90ml sterile diluent containing 0.1% peptone, 0.8% NaCl, with pH adjusted to 7.2 and homogenized in a stomacher (Lab Blender, Model 4001, Seward Medical) for 30 s at normal speed. Appropriate 10-fold dilutions were made. From these dilutions, MRS plates were prepared for the enumeration and isolation of the dominant lactic acid bacteria, and incubated anaerobically in an anaerobic jar with anaerocult A (Merck) at 30°C for 3 d. Similarly aerobic mesophiles were enumerated on Plate Count Agar and incubated aerobically. Moulds and yeasts were enumerated on Malt Agar to which 100mg per 1000ml of chloramphenicol was added and incubated at 30°C for 7 d.

For the isolation of lactic acid bacteria, about 20 colonies from a segment of a suitable MRS plate were taken into MRS broth and streaked continually on MRS agar till pure colonies were obtained.

3.3.2 Identification of lactic acid bacteria

Lactic acid bacteria on MRS agar were identified by colony and cell morphology, Gram reaction and catalase production. *Lactobacillus* spp were recognized as Gram positive, oxidase negative, catalase negative rods. Species identification of the dominant *Lactobacilli* were carried out by examining the utilization of sugars by representative isolates.

Gram reaction was carried out according to a modification of the method of Lillie (1928) by Parry et al (1983). For catalase production, a loopful of culture was mixed into a drop of 3%

hydrogen peroxide on a microscope slide and observed for gas bubbles to indicate production of catalase.

3.3.3 Tests for utilization of sugars by the *Lactobacillus* spp.

A 10% solution of each of the sugars was prepared and sterile-filtered separately into sterile bottles. 0.5 ml of each sugar was added to 16 different tubes containing 4.5 ml of modified MRS broth containing chlorophenol red as indicator. The sugars tested were Amygdalin Arabinose, Cellobiose, Fructose, Galactose, Glucose, Lactose, Maltose, Manitol , Melezitose Melibiose,Raffinol, Salicin, Sorbitol, Sucrose, and Trehalose. The tubes were then inoculated with pure single colonies of the isolates. The tubes were incubated for up to 7 d. A change in colour of the media from red to yellow was recorded as positive. The results obtained were then compared with the carbohydrate fermentation pattern of the different species of *Lactobacillus* in the Bergey's Manual.

3.4 Chemical analyses

The cassava flour was analysed for moisture, reducing sugars, sucrose and total sugars. For the fermented cassava dough (agbelima), only the moisture content was determined.

3.4.1 Determination of moisture content of agbelima and cassava flour

Moisture was determined according to AOAC 925·10 (1990) for the determination of moisture in flour.

3.4.2 Determination of Total sugars, Reducing sugars and Sucrose.

The percentage of total sugars, reducing sugars and sucrose in the cassava flour was determined according to Lane and Eynon (1970).

Reducing sugars (before inversion)

To obtain reducing sugars, about 10-20g of cassava flour sample was weighed into 250 ml of volumetric flask. 200ml distilled water was added. 5ml each of potassium ferrocyanide and zinc acetate was added as cleaning agents, and left for 10min.The level was then made to 250ml with distilled water and filtered. The filtrate was heated to boil. The boiling filtrate was then titrated against 10ml A+B Fehling's (in equal amount) reagent. The solution turned brick red at the end of the titration. About 2 drops of methylene blue indicator was added which changed the solution to blue.

Titration continued till a brick red colouration was achieved again. The factor for this titre volume achieved at this stage was read from a conversion table(Invert sugar table for 10ml Fehling's solutin.

Calculation:

% reducing sugars =

$$\frac{\text{Factor x volume x diluting factor}}{1000 \text{ x Weight of sample}}$$

Reducing sugars (after inversion)

To obtain the % sucrose in the cassava flour,20ml of the filtrate was placed in a 100ml volumetric flask. 10ml of HCl (diluted with equal volume of water). The solution was kept in 68-70°C waterbath and 3 drops of 1% phenolphthalein indicator added to it. The solution was then cooled to room temperature and 40% NaOH added dropwise till the solution changed to a slightly pink colour. This solution was then made up to 100ml with distilled water and shaken to mix. This was titrated against Fehling's solution A+B as before. The factor for this titre volume was obtained from the conversion table

Calculation:

% sucrose = difference between values obtained after inversion and before inversion x 0.95%

To obtain Total sugars:

% total sugars = %Reducing sugar + % Sucrose

3.4.3 Determination of pH

To determine the pH about 10 ml aliquots of the samples were taken into plastic cups and the pH measured using a pH meter (PHM 92, LAB pH meter).

3.4.4 Determination of titrable acidity

To determine titrable acidity, 10 g of dough was dissolved in 200 ml of distilled water. 100 ml of the filtrated was titrated against 0.1 N NaOH with 1% phenolphthalein. 1 ml of 0.1N NaOH was taken as equivalent to 9.008×10^{-3}.

3.5 Organoleptic assessment

Banku was prepared from four different samples of cassava dough, each mixed with fermented maize meal and assessed by a taste panel.

Sample A: inoculated cassava mash

Sample B: control (uninoculated mash)

Sample C: Reconstituted agbelima flour produced commercially by CSIR-Food Research Institute

Sample D: market sample of cassava dough

Fermented maize meal was prepared in the test kitchen to guarantee its gumminess and safety for use as banku ingredient. The banku was prepared by mixing maize and cassava dough in the ratio of 1:2. A measured volume of water and salt were added to the mixture and cooked. The following parameters were assessed by 15 trained panellists and the results analysed using the Hedonic Scale.

a. Appearance
b. Smoothness
c. Biting feel
d. Aroma
e. Taste
f. Overall acceptability.

3.6 Antimicrobial studies

3.6.1 Survival of enteric pathogens in fermenting cassava mash

The ability or inability of four different pathogens to survive in fermenting cassava mash was studied. This was to determine whether the final product obtained from the fermentation of cassava flour into agbelima would be microbiologically safe for consumption in case of contamination during processing.

The four pathogens studied were

- *Salmonella* typhimurium 9
- *Shigella dysenteriae* 2357T
- *Escherichia coli* D2188
- *Vibrio cholerae* C-230

Pure cultures of each pathogen were plated out on Nutrient Agar in duplicate and incubated at 37°C for 24h. 9 ml of salt peptone solution was poured on one Nutrient Agar plate with visible growth and the entire growth on the plate washed. The colonies were removed using a sterile inoculation needle and these were harvested and transferred unto the second plate
Three different cassava mash substrates were prepared as follows: to each 1kg of flour, 1 litre of sterile distilled water was added to make a mash. The different substrates were then treated variously and identified as follows

A. Cassava mash to which 0.5% of glucose and a starter culture (*L. plantarum*) had been added
B. Cassava mash to which only a starter culture (*L. plantarum*) had been added
C. Control; cassava mash without glucose or a starter culture.

1.5ml of cell suspension of pathogens were added to each cassava mash. The mixture was stirred thoroughly. Samples were taken immediately after mixing (0h) and after 4, 8, 24 and 48h. The population of surviving pathogens were enumerated by spread plate on Statens Serum Institut Agar (SSI). 10-fold dilutions of each sample were prepared using SPS. 0.1 ml of each serial dilution was inoculated onto SSI Agar by spread plate and incubated at 37°C for 24h. The plates were examined under a colony counter and the colonies of each of the pathogens counted separately.

Salmonella typhimurium 9 appeared as black colonies.

Vibrio cholerae C-230 appeared as cream colonies.

Echerichia coli D2188 appeared as bright red colonies.

Shigella dysenteriae 2357T appeared as dark reddish brown colonies.

4. RESULTS AND DISCUSSIONS

4.1 Microbial population of agbelima and high quality cassava flour (HQCF)

The microbial population of agbelima from which the starter culture was isolated and the unfermented cassava flour which was used to produce agbelima in this work are shown in Table 1. Surprisingly the cassava flour which had been produced by dehydrating pressed grated cassava at about 65°C was not sterile but rather contained a higher population of aerobic mesophiles than the traditional agbelima sample. These results show that both the aerobic mesophiles and the lactic acid bacteria were able to survive the dehydration process of heating at a temperature of about 65°C for 10 hours. The higher count of the aerobic mesophiles in the cassava flour may be explained by the fact that the results are not expressed in dry weight basis and whereas the flour had a moisture content of 9.9%, the agbelima sample had a moisture content of 50.5% (Table 4). In the MRS population a lower count was observed in the dry cassava flour than in the wet agbelima sample and this is because the MRS population will be composed of mostly lactic acid bacteria and one will not expect them to be as durable as the aerobic mesophiles to the dehydration process. The presence of the microorganisms in the cassava flour also suggest that microbial growth might actually have occurred during drying since the product had not been previously fermented. The microbial population before dehydration would be minimal since the freshly harvested tubers would be sterile before peeling. However, some contamination would occur during peeling and especially grating but it is doubtful if these alone could account for the level of counts observed without further growth during dehydration.

The population of aerobic mesophiles in the agbelima sample obtained from the traditional processor, 4.3 x 10^8 cfu/g included Gram positive, catalase negative cocci and rods as well as Gram positive, catalase negative cocci and rods. The dominating species were Gram positive, catalase positive rods and they mostly bore phase bright spores and were tentatively identified as *Bacillus* spp.

The yeasts and a few moulds were found in the agbelima on the MA plates but no counts were obtained in the dehydrated cassava flour.

The population on MRS agar consisted mainly of Gram-positive, catalase-negative rods. These isolates were also found to be oxidase-negative, non-sporing and were recognized as *Lactobacilli.*

Table 1: Microbial population (cfu/g) of agbelima and high quality cassava flour

Media	Agbelima from traditional processors (cfu/g)	High quality cassava flour (HQCF) (cfu/g)
PCA	4.3×10^8	1.42×10^{10}
MRS	5.5×10^9	7.3×10^6
MA	1.02×10^7	0

The dominance of lactobacilli found in this work is in agreement with Amoa-Awua et al (1996) who reported high occurrence of lactic acid bacteria in fermenting cassava dough at all stages of the fermentation process. Amoa-Awua et al, (1996) also isolated Streptococci and other cocci belonging to the lactic acid bacteria group during initial stages of fermentation of cassava dough. These were, however, not found in the already fermented agbelima sample analysed as expressed since the sample was at an advanced stage of fermentation (> 72hrs).

Lactobacilli were identified to the species level by their sugar utilization patterns. *Lactobacilli* which fermented all the 16 sugars tested except Arabinose were identified as *Lactobacillus plantarum* (Table 2). Isolates identified as *Lactobacillus fermentum* fermented Cellobiose, Fructose, Galactose, Glucose, Lactose, Maltose, Melibiose, Raffinose, Sucrose and Trehalose. Those identified as *Lactobacillus brevis* fermented Arabinose, Fructose, Glucose, Lactose, Maltose, Melibiose and Raffinose. *Lactobacillus salivarius* was identified as having the ability to ferment Fructose, Galactose, Glucose, Lactose, Maltose, Mannitol, Melibiose, Raffinose, Sorbitol, Sucrose and Trehalose.

Table 2: Carbohydrate fermentation patterns of the *Lactobacilli* isolated from agbelima

Sugars / Isolates	Amygdalin	Arabinose	Cellobiose	Fructose	Galactose	Glucose	Lactose	Maltose	Mannitol	Melezitose	Melibiose	Raffinose	Salicin	Sorbitol	Sucrose	Trehalose
L. plantarum	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L. fermentum	-	-	+	+	+	+	+	+	-	-	+	+	-	-	+	+
L. brevis	-	+	-	+	-	+	+	+	-	-	+	+	-	-	-	-
L. salivarius	-	-	-	+	+	+	+	+	+	-	+	+	-	+	+	+

The dominant *Lactobacillus* species identified in the agbelima sample was *L. plantarum* which occurred at a level of 10^{10} cfu/g followed by *L. brevis and L. fermentum* which were present at levels of 10^{9} cfu/g and *L. salivarius* which occurred at a level of 10^{7} (Table 3).

L plantarum was selected as the starter culture for the fermentation of the cassava flour since it was the dominant lactic acid bacteria in the agbelima sample analysed. This was also in agreement with work of Amoa-Awua et al (1996) who found *L. plantarum* to be the dominant lactic acid bacteria in all the samples of cassava dough they analysed at various stages of fermentation. Okafor and Uzuebu (1987) also reported that out of several lactic acid bacteria isolated from spontaneously fermenting cassava mash it was only *L. plantarum* and *L. mesenteroides* which were present in large numbers throughout 96 h fermentation. Ngaba and Lee (1979) also isolated *L. plantarum* and *L. mesenteroides* in addition to Streptococcus spp. as the dominant lactic acid bacteria in spontaneously fermenting cassava dough.

L. plantarum has been reported to have a wide distribution in a variety of foods and is also fastidious. It is therefore a good choice as a starter culture. Randler and Weiss (1986) suggested that the wide distribution of *L plantarum* which has been isolated from most traditional habitats of lactobacilli such as vegetables and other plant materials, fish, meat and various indigenous foods as well as from human and animal mucus membranes, might partly be due to the fact that it is less fastidious than most of the other lactobacilli in its requirements for amino acids and vitamins.

Lactic acid bacteria are known to play an important role in the souring of the dough during cassava fermentation into agbelima through the production of lactic acids. They are also known to be involved in the hydrolysis of cyanogenic glucosides during detoxification of cassava (Amoa-Awua and Jakobsen 1995; Amoa-Awua et al 1996). The cellulolytic activity of yeasts and moulds as well as *Bacillus* spp in making the final texture of agbelima smooth, as reported by Amoa- Awua and Jakobsen, 1995 and Amoa- Awua et al., 1997 was not required since the present work aimed at producing agbelima from cassava flour, which is already smooth, hence no cellulolytic microorganims were isolated from the PCA and MA plates and incorporated into the starter culture.

There are reports of the contribution of yeasts in the development of the aroma of agbelima but this is only secondary compared to the role played by lactic acid bacteria.

The sour taste of agbelima has been attributed to the production of acids during fermentation. Since the lactic and acetic acids present in agbelima are produced by lactic acid bacteria, these microorganisms are directly responsible for the development of the dominant aroma of agbelima (Amoa-Awua and Jakobsen, 1996). This supports the successful use of a single starter culture, *L. plantarum* to ferment cassava flour into agbelima in this study. No yeasts were incorporated into the starter culture in the present work but this will be investigated in future work.

Table 3: Composition and population of *Lactobacillus* spp isolated from agbelima

Isolate	Population in (cfu/g)
L. plantarum	$2x10^{10}$
L. fermentum	$3x10^{9}$
L. brevis	$2x10^{9}$
L. salivarius	$9x10^{7}$

4.2 Composition of High Quality Cassava Flour

Results of some chemical analyses of cassava flour and agbelima carried out are as shown in Table 4. The results show that the cassava flour contained some amount of sugars including sucrose which the starter, *L. plantarum* is able to utilize as a carbon source for growth as seen from the carbohydrate fermentation pattern of *L. plantarum* in Table 2. Agbelima from the market contained about 50% moisture, about 10 times that of the dehydrated cassava flour. The amount of moisture in the agbelima sample from the market was important in reconstituting the High Quality Cassava Flour (HQCF).

Table 4: The content of simple sugars in cassava flour

	Cassava flour	Agbelima
% Moisture	9.9	50.5
% Reducing sugars	0.4	n.d
% Sucrose	0.4	n.d
% Total sugars	0.7	n.d

n.d – not determined

4.3 Fermentation of mash prepared from High Quality Cassava Flour

The pH values obtained from the fermentation of the mash prepared from cassava flour and inoculated with *L. plantarum* as well as that obtained from freshly milled cassava mash (obtained from a cassava processing mill at Madina) were interpreted as a direct indication of the rate of fermentation of the different samples. This is because the main products of the lactic acid fermentation of agbelima are lactic and acetic acids resulting in the acidification of the products. The fermentation process led to a drop in pH for all the substrates (Table 5). The initial pH value of the freshly prepared cassava mash was the highest at 6.16 but dropped to the lowest value of 3.78 in 72 hours. pH values for the other substrates ranged from an initial of 5.62 - 5.71and a final of 4.32 - 4.40 at the end of fermentation. All these results confirm the fermentation of cassava into agbelima to be an acidification process. The fastest rate of fermentation was obtained in the cassava dough containing the traditional inoculum, kudeme which according to Amoa-Awua et al (1996; 1997) contains lactic acid bacteria at a level of 10^9 cfu/g and yeasts at levels of 10^5 cfu/g. Thus this sample containing a high level of mixed population of both lactic acid bacteria and yeasts fermented at a faster rate than the samples inoculated with a single strain of lactic acid bacteria at a level of 10^{10} cfu/g. Yeasts are reported to have synergistic and stimulating effect on the growth of lactic acid bacteria and this may as well have contributed to the faster fermentation rate in the traditional sample.

The addition of glucose to reconstituted cassava flour before inoculation with *L. plantarum* as a starter culture increased the rate of fermentation and after 24h the pH obtained was 4.69 as

compared to 5.1 in the sample without added glucose. The glucose served as a ready source of fermentable carbohydrate for the starter culture, hence the faster rate of fermentation observed. Addition of glucose had no marked effect on the fermentation process. Fermentation occurred even in the reconstituted HQCF flour to which no additional carbon source or starter culture was added showing that the flour contained enough sugars to support growth of fermentative flora which were already present in the flour.

Table 5: pH values obtained from fermentation of cassava mash prepared from High Quality Cassava Flour (HQCF) and inoculated with *L. plantarum*

Samples	PH readings			
	0h	24h	48h	72h
Reconstituted HQCF with added glucose and inoculated with *L. plantarum*	5.62	4.69	4.49	4.32
Reconstituted HQCF without added glucose and inoculated with L. *plantarum*	5.71	5.10	4.51	4.36
Reconstituted HQCF only (control)	5.66	5.11	4.55	4.40
Traditional agbelima	6.16	4.53	4.03	3.78

To determine the microflora responsible for the spontaneous fermentation of reconstituted cassava flour sample, the control sample and the fresh flour were cultured. *L plantarum* was isolated as the dominant organism in both samples at levels of 10^8 and 10^6 for the control and the flour respectively. Other lactic acid bacteria found were similar to those previously isolated from agbelima. *Bacillus* spp were also isolated at levels of 10^6 and 10^4 for the control and flour respectively. No yeasts or moulds were isolated from both samples when they were culture on MA.

As mentioned earlier the lactic acid bacteria were expected to be eliminated or their population reduced drastically during the processing of the flour, especially during drying (at 65°C) of the grated meal, however this appears not to have been the case. Possibly some level of *L plantarum* was also re-introduced from the grinding mill and handling of the flour. The flor used in the present work had been freshly produced, however, it not expected that during storage of the flour the lactic acid bacteria will be able to survive for long in the flour of a moisture content of less than 10%.

4.4 Titrable acidity

Results of titrable acidity, which is a measure of the free lactic acid produced during fermentation, are as shown in Table 6. The Freshly milled cassava (uninoculated) had the highest initial titrable acidity value of 0.31% before fermentation and a final value of 0.75% at the end of fermentation. Results for the other substrates range from 0.11% at the beginning to 0.56% at the end of fermentation.

Table 6: Percentage titrable acidity of cassava mash prepared from High Quality Cassava Flour (HQCF) during fermentation into agbelima

Time / Samples	0 hr	24 hr	48hr	72hr
Reconstituted HQCF with added glucose and inoculated with *L plantarum*	0.13	0.22	0.39	0.56
Reconstituted HQCF without added glucose and inoculated with *L. plantarum*	0.11	0.22	0.34	0.37
Reconstituted HQCF only (control)	0.23	0.27	0.39	0.41
Traditional agbelima	0.31	0.51	0.62	0.75

The tirable acidity values indicate a periodic increase in the amount of lactic acid produced during the fermentation. In all the samples the amount of acid produced increased with time. This production of the acid led to the drop in pH. These results also confirm that the fermentation of cassava flour into agbelima is an acidification process as reported by Amoa-Awua et al (1996) in their study on lactic acid fermentation of cassava into agbelima.

4.5 Sensory evaluation of banku prepared from fermented cassava mash

The four samples A, B, C and D assessed by the taste panel for Appearance, Smoothness, Aroma, Taste, Biting feel and Overall acceptability were

Sample A: Cassava mash prepared from High Quality Cassava Flour and inoculated with L. plantarum

Sample B: Uninoculated cassava mash prepared from High Quality Cassava Flour (control)

Sample C: Reconstituted agbelima flour produced commercially by CSIR-Food Research Institute Sample D: A market sample of cassava dough (agbelima).

The four samples analysed were similar in appearance, although the control and the reconstituted agbelima flour were slightly darker. There were no differences detected in the smoothness of the four samples. Surprisingly, the aroma and taste of the inoculated cassava mash and the control, all prepared from cassava flour were preferred to the traditional agbelika and the reconstituted agbelima flour. The inoculated cassava mash and the control scored mean values of 7.90±1.1 and 7.86±0.61 respectively whilst reconstituted agbelima flour and market sample of agbelima scored 7.4±1.4 and 7.6±0.74 respectively.

The market sample of agbelima had the most biting feel, with a mean score of 6.93±1.53. Assessing the overall acceptability, it was found that the inoculated cassava mash and the control were more acceptable than the market sample and the reconstituted agbelima flour. The mean scores for the parameters analysed and their interpretations are shown in Tables 7.

Results of the sensory evaluation of on the appearance of the four banku samples indicated that the inoculated cassava flour scored the same as the market sample. Banku prepared from the control and reconstituted agbelima flour samples were found to be slightly darker in appearance. There was no difference in the smoothness of the four samples but the aroma of the banku prepared from the inoculated cassava mash and the control were preferred to that prepared from reconstituted agbelima

Table 7: Sensory evaluation of banku prepared from fermented cassava mash

(mean sensory scores)

Parameters / Sample	Appearance	Smoothness	Aroma	Taste	Biting feel	Overall acceptability
Sample A Reconstituted HQCF inoculated with L. plantarum	8.2±0.59 Like very much	7.9±0.59 Like very much	7.9±1.1 Like very much	7.87±0.92 Like very much	7.73±0.88 Like very much	7.93±0.79 Like very much
Sample B Reconstituted HQCF only (Control)	8±0.65 Like very much	8±0.65 Like very much	7.86±0.61 Like very much	7.87±0.83 Like very much	7.73±1.09 Like very much	7.86±0.74 Like very much
Sample C Reconstituted agbelima flour	7.93±0.7 Like very much	8.06±0.59 Like very much	7.4±1.4 Like moderately	7.5±1.4 Like moderately	7.6±1.24 Like moderately	7.46±1.45 Like moderately
Sample D Market cassava dough	8.2±0.67 Like very much	7.6±0.89 Like moderately	7.46±0.74 Like moderately	7.53±0.83 Like moderately	6.93±1.53 Like slightly	7.53±1.12 Like moderately

HQCF- High Quality Cassava Flour.

flour and the market cassava dough sample. The taste of banku prepared from the inoculated cassava mash and control were preferred to that prepared from reconstituted agbelima flour and the market cassava dough, although the biting feel was more pronounced in the latter.
In all, banku from the inoculated cassava mash and the control were more acceptable than that from the reconstituted agbelima flour and the market cassava dough. Scores for appearance, smoothness, aroma, taste and biting feel in banku prepared from either the inoculated mash or the un-inoculated control were very close indeed. Since both were equally acceptable, agbelima can be prepared from the flour by reconstituting it with equal amount of water and fermenting it either spontaneously or by the inclusion of a starter culture.

4.6 Survival of enteric pathogens in agbelima prepared from High Quality cassava Flour

Survival of four enteric pathogens (*Salmonella typhumurium* 9, *Vibrio cholerae* C-230, *Escherichia coli* D 2188 and *Shigella dysenteriae* 2357T) in three different types of fermenting cassava mash from HQCF indicated by their counts at various stages of fermentation are shown in Figs. 4, 5 and 6. In the sample with added glucosse (Fig.4), *Salmonella typhumurium* 9 and Vibrio cholerae C-230 showed slight drop in log units within the 8h of incubation. E. coli however showed a slight initial increase in log units while Shigella had more than 1log unit within the first 8h. In the other samples without added glucose (Figs. 4 and 5) there were drops in log units for the four pathogens within the first 8h. Mante (2000) reported that the possible main factor that influenced the survival of the different pathogens he studied was low pH. In the present study the added glucose probably contributed to the initial increase in numbers of the *Salmonella* and *E. coli*. Thereafter, there was a general trend of considerable drops in numbers of all the pathogens after 8 h, until they died out completely in all samples analysed, conforming to Mante's work.

Vibrio cholerae died out fastest (by 8 h) than the other three pathogens whilst E. coli persisted longest (up to 24 h). Obviously V. cholerae was least able to survive in the increasing acidic environment created by the fermentation process. This is supported by Mante (2000) who reported that Vibrio cholerae C-230 was the most sensitive, as they did not survive after 30min of inoculation into cassava dough –agbelima as well as cooked cassava dough.

Salmonella and *E. coli* had more ability to withstand the fermenting environment in all three samples for a while but disappeared by 48 h (Figs 4, 5 and 6). A few studies have shown E.

coli to be tolerant to acidic conditions with fermented foods where the organisms survived for at least 24 h. In a study by Feresu and Nyati (1990) E coli strains were detected 24h after inoculation in broth traditional and commercial fermented milk, but there was a decrease in numbers. In a study of rice based weaning food, in which E. coli ATCC 25922 was inoculated inhibition occurred only when a high initial level of lactic acid bacteria were present in comparison with a low level of the pathogen. The pathogen was unable to grow when added to the 24h prefermented weaning food (Yusof et al., 1993). Kingamkono et al., (1994) observed that Enterotoxigenic E. coli ETEC 28662, reduced to undetectable limits after 32h in a cereal gruel containing lactic acid bacteria starter cultures.

Mante (2000) reported that all five enteric pathogens inoculated into fermenting agbelima died off within 24 hours. In the present work the pathogens died out within 48h as observed in the samples

inoculated with the starter culture, L. plantarum (Figs. 4 and 5). In the uninoculated control the pathogens died by 24 h. The addition of the starter culture on the survival of the pathogens in the fermenting cassava mash might have an effect, which needs further investigation.

Fig. 4 Survival rate of pathogens in fermenting cassava mash (with added glucose)

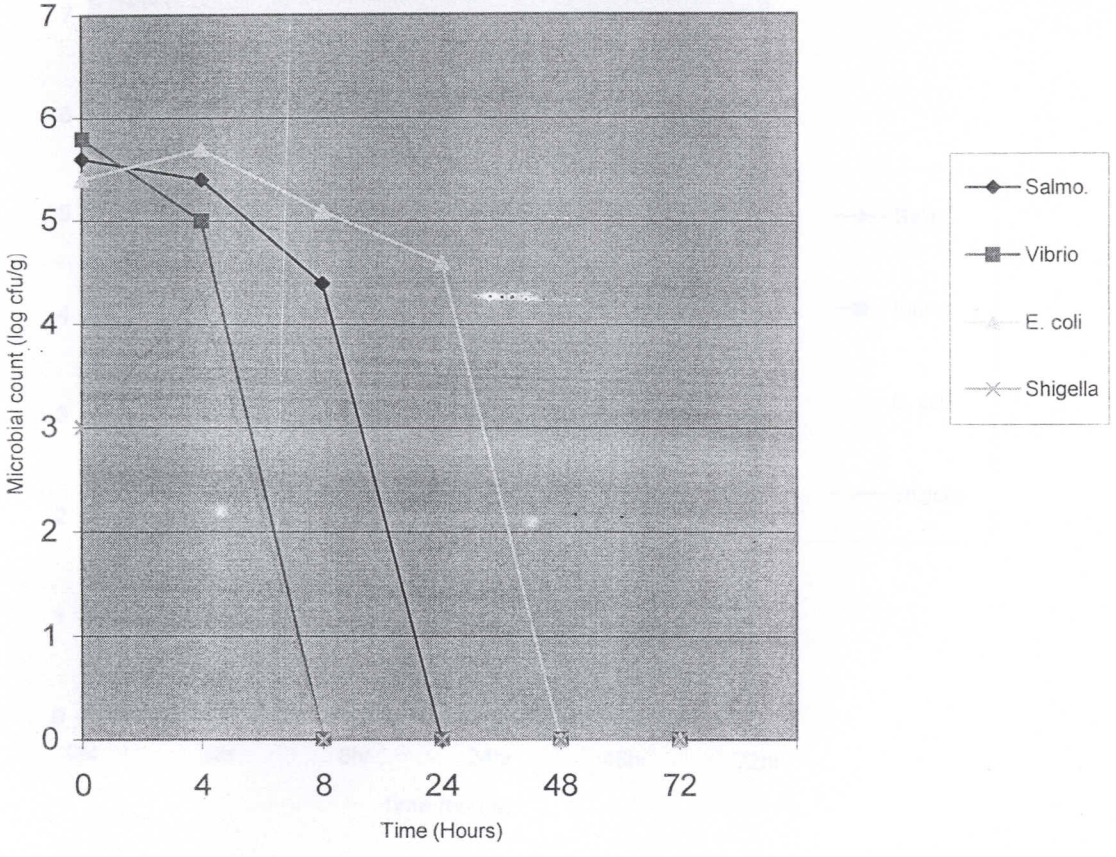


Fig. 5 Survival rate of pathogens in fermenting cassava mash (without added glucose)

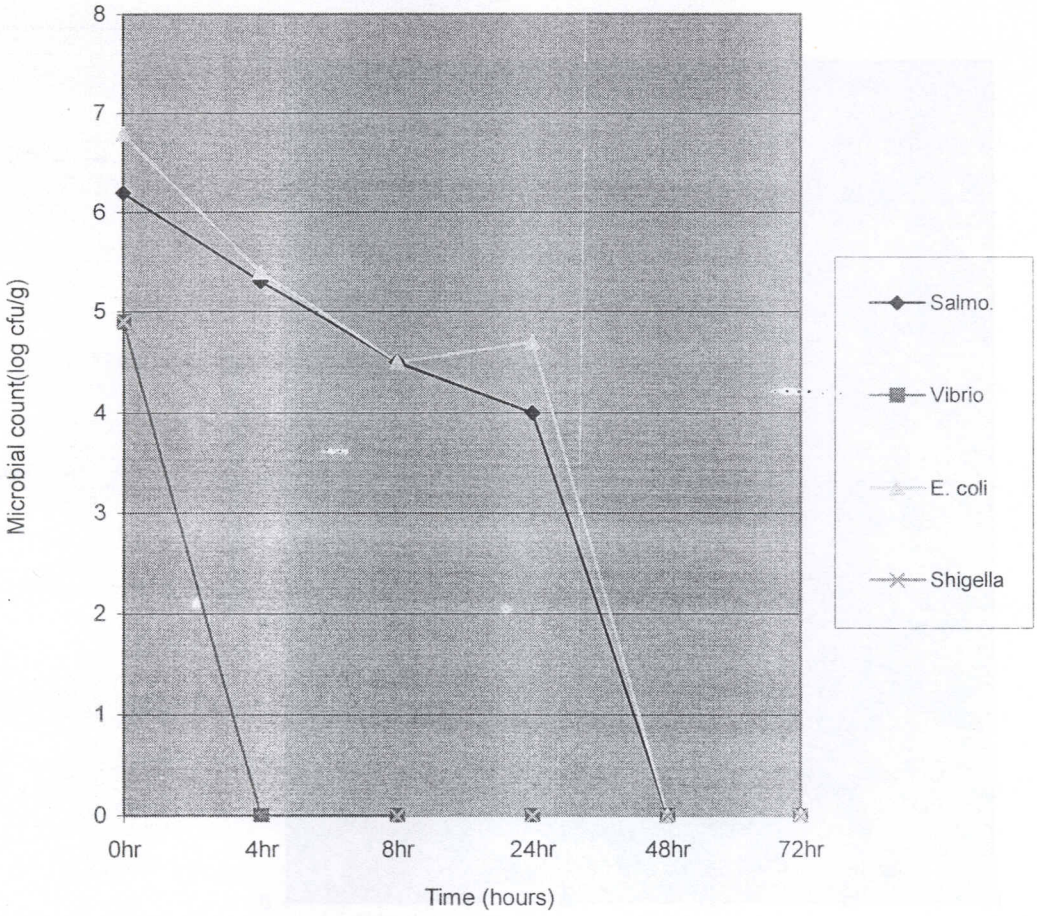
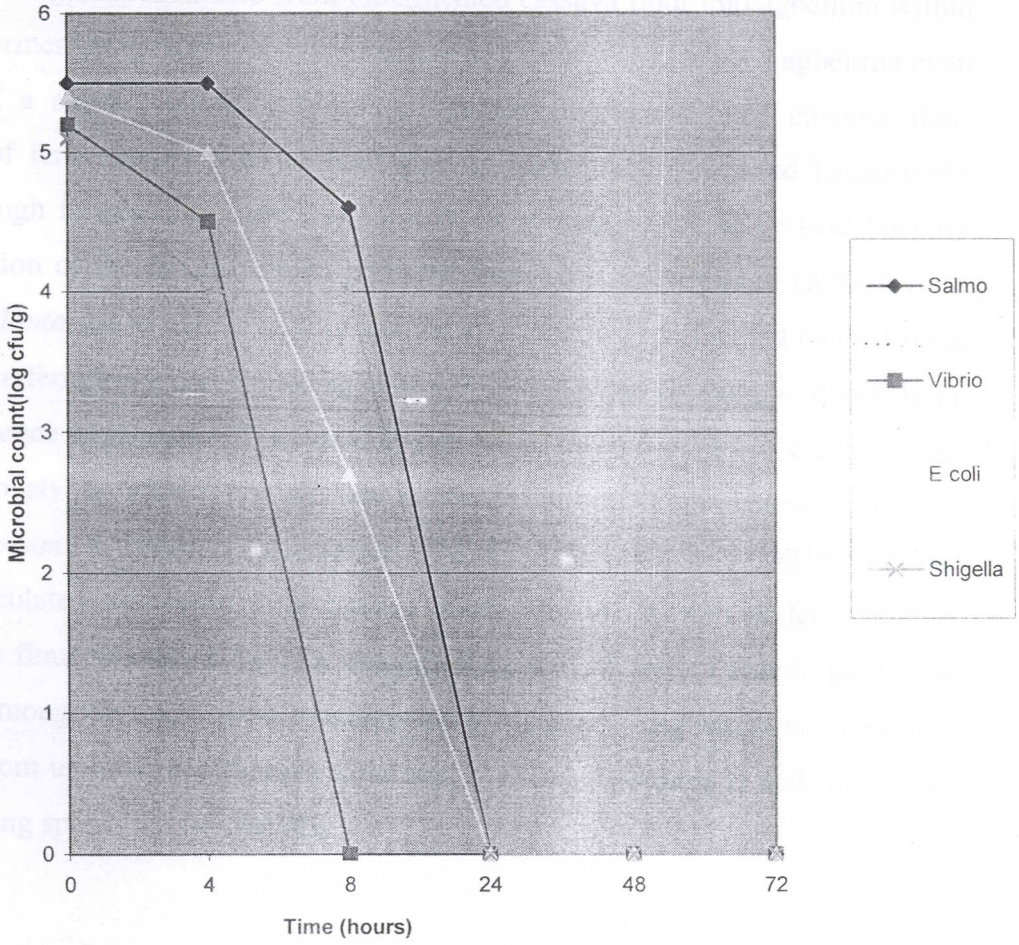


Fig. 6 Survival rate of pathogens in fermenting cassava mash(control)



5. CONCLUSIONS

Lactobacillus plantarum was isolated as the dominant lactic acid bacteria in both unfermented flour and agbelima. *L. plantarum* isolates were successfully used as a single starter culture for fermenting cassava dough, reconstituted from unfermented cassava flour into agbelima within 72h. Spontaneous fermentation of the reconstituted cassava flour also produced agbelima even without the use of a starter culture. Addition of glucose to reconstituted cassava flour increased the rate of fermentation even without glucose fermentation occurred because the flour contained enough fermentable sugars to support the growth of the lactic acid bacteria leading to acidification of the dough. Banku prepared from both reconstituted cassava flour inoculated with *L. plantarum* as starter culture and the spontaneously fermented reconstituted cassava flour were preferred to reconstituted agbelima flour and a market sample of agbelima. There was no difference in the overall acceptability between the reconstituted cassava flour fermented spontaneously or with *L. plantarum* as starter culture. Four enteric pathogens *Salmonella typhimurium* 9, *Shigella disenteriae* 2357T, *Echerichia coli* D2188, *Vibrio cholerae* C-230 inoculated into fermenting cassava mash died off by 48h of fermentation. High quality cassava flour fermented for 72h into agbelima will be free of enteric pathogens studied, which are among the major food- contaminating organisms. Agbelima can therefore be safely prepared from unfermented cassava flour simply by reconstituting it with water into a dough and fermenting spontaneously for 72h.

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