

Predominant Lactic Acid Bacteria Involved in the Traditional Fermentation of *Fufu* and *Ogi*, Two Nigerian **Fermented Food Products**

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ABSTRACT

Traditional methods of preparation were simulated in the laboratory fermentations of cassava and maize to produce *fufu* and *ogi* respectively. Changes in pH, temperature and titratable acidity, as well as the diversity of lactic acid bacteria species were investigated during both fermentations. Lactic acid bacteria strains involved in the fermentation processes were isolated at twelve hourly intervals, characterized and identified using phenotypic and biochemical methods. A rapid decrease in pH, 5.6 to 3.7 in *fufu* and 5.9 to 3.8 in *ogi*, were observed with temperature increasing from 26°C to 30°C and 25°C to 31°C in *fufu* and *ogi* respectively. Most of the lactic acid bacteria strains isolated were homofermentative and heterofermentative *Lactobacillus* species and heterofermentative *Leuconostoc* species. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were the dominant lactic acid bacteria species in *fufu* while *L. cellobiosus*, *L. plantarum* and *Lc. lactis* were dominant in *ogi* fermentation with the final stages populated with *Lactobacillus* species was observed in both cases and is attributable to differential acid tolerance of the two genera. The frequencies of dominance of the strains in *fufu* were *L. plantarum* (56.25%), *Lc. mesenteroides* (18.75%), *L. lactis* (6.25%), *L. coprophillus* (6.25%), *L. acidophilus* (6.25%), *L. acidophilus* (13.33%) and *Lc. paramesenteroides* (13.33%). The dominant strains can serve as potential starter cultures for *fufu* and *ogi* production.

Keywords: Lactic Acid Bacteria; Fermentation; Fufu; Ogi; Cassava; Maize; Fermented Foods

1. Introduction

Fermented foods are consumed throughout the world and traditional fermentation processes such as those involved in the production of fermented dairy products and alcoholic beverages have been performed for thousands of years [1]. These food products result from the activities of microorganisms which modify the flavour and texture and increase long term product stability [2]. Lactic acid bacteria constitute an important group of these organisms and have been associated with production of fermented foods and feeds for many centuries [3]. They are important in the production of many fermented foods such as sauerkraut, silage, sourdough, dry fermented sausages and cheeses [4]. Some of the reasons for their widespread

use are the ability to retard spoilage, preserve food as well as improve flavour and texture of foods. They also play fundamental role in microbial ecology of foods by synthesizing a variety of antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins [5,6]. They are thus able to inhibit many microorganisms including spoilage and pathogenic organisms. They are increasingly being recognised for their health and nutritional benefits hence some strains are used as probiotics [7-10].

Among the many African, traditionally fermented foodstuffs are *fufu* and *ogi*. *Fufu*, a product of an acid-fermented cassava root tuber serves as main course meals in most areas of Nigeria and Africa as a whole [11]. It is prepared by natural fermentation which transforms the cassava root from rapid spoilage after harvest as cassava

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roots are more perishable than other tuber crops such as yam and sweet potato. Cassava (*Manihot esculenta*) is a perennial woody shrub with an edible root which grows in tropical and sub-tropical areas of the world [12]. In Africa and Latin America, it is mostly used for human consumption, while in Asia and parts of Latin America, it is also used commercially for the production of animal feeds and starch-based products [13]. Other products obtained from fermented cassava roots are gari and lafun. Ogi, an acid-fermented cereal gruel, is a major staple sour porridge food widely taken in West Africa. It also serves as the traditional infant weaning food [11]. It is generally obtained by fermenting maize grains (*Zea mays*).

In general, a wide spectrum of microorganisms is involved during fermentation processes but a few types usually determine the quality of the end product [14]. Given adequate environmental conditions, a particular microbial community will determine the quality of a specific food. The origin, development and succession of a particular microbial community in any food are thus governed by its ecological factors, which influence the physiological expression of microbial cells [15]. Thus strategies for food processing and preservation can be developed on the basis of ecological factors associated with specific foods and beverages [16].

Yeasts and wide varieties of bacteria strains were found to be associated with the fermentation of cassava roots and cereal grains where they contribute significantly to starch breakdown, acidification, detoxification and flavour enhancement [17]. Yeasts and lactic acid bacteria are therefore the most common microorganisms in a wide variety of traditional food and beverage fermentations [18]. Lactic acid bacteria are found in various stages of fermentation processes where they are useful in flavour and aroma development. They also inhibit spoilage bacteria and pathogens and confer several intestinal health and other health benefits related to blood cholesterol levels, immune competence and antibiotics [19]. The quality of the final product is a factor of the diversity and composition of microorganisms and their dynamics and frequency of occurrence. The objective of this research was therefore to isolate and characterize predominant lactic acid bacteria species during the course of traditional fufu and ogi fermentations.

2. Materials and Methods

2.1. Collection of Raw Materials

Unbroken maize grains (*Zea mays*) and freshly harvested cassava root tubers (*Manihot esculenta*) were obtained from the Bodija market in Ibadan, Oyo State, Nigeria. They were brought in sterile polythene bags to the laboratory for immediate processing.

2.2. Laboratory Preparation of Fufu and Ogi

The laboratory fermentation of cassava tubers and maize grains to produce *fufu* and *ogi* respectively, were carried out by simulating the traditional methods of processing (**Figures 1** and **2**).

2.3. Physicochemical Analysis

The hydrogen ion concentration (pH) and temperature changes of the fermenting cassava mash and maize grains were measured at twenty four hourly intervals for 72 h using a Pye-Unicam pH meter and Mercury thermometer respectively [20]. The total titratable acid was determined as percentage lactic acid by titrating 25 ml of the samples used for pH determinations against 0.1 N NaOH. The volumes of the 0.1 N NaOH used were noted. Duplicate determinations were made for each analysis [21].

2.4. Microbiological Isolation

Twenty five millilitre portions were aseptically removed at different stages of fermentation processes: raw water used for steeping cassava roots and maize grains; steep



Figure 1. Traditional method of *fufu* processing.



Figure 2. Traditional method of ogi processing.

waters (sampled each day for 72 h); water used for mashing steeped cassava and for milling *ogi*; the steep water used further fermentation of *ogi* and the final products of fermentation. Each sample was homogenized with 200 ml sterile 0.1% peptone water (Oxoid, UK). This was then serially diluted and 0.1 ml from appropriate dilutions were spread plated on MRS agar plates (Oxoid, UK) in duplicates and incubated in Gas Pak jars (GasPak System, BBL) at 30°C for 72 h. Colonies with distinct morphological differences such as colour, size and shapes were randomly picked from MRS agar plates as presumptive lactic acid bacteria isolates and repeatedly streaked on fresh MRS agar plates to purify the isolates. They were then maintained on appropriate slants at 4°C.

2.5. Characterisation and Identification

Each of the lactic acid bacteria isolate was initially examined for colonial and cell morphologies, cell arrangement, spore formation and motility. Only the Gram positive, catalase negative and non spore forming isolates were then characterized by phenotypic and biochemical tests. An overnight culture (inoculums) of each isolate in MRS broth was used for all tests incubated anaerobically (GasPak System, BBL) at 30°C. The lactic acid bacteria isolates were tested for fermentation of the following carbohydrates (Sigma, Germany): D-Glucose, lactose, sucrose, galactose, maltose, mannitol, sorbitol, mannose, L-arabinose, D-xylose, cellobiose, dulcitol, inositol, raffinose, rhamnose, inulin and salicin. Bromocresol purple broth base was used as basal medium. One percent filtersterilized sugar solution using 0.2 µm Millipore filter (Corning) was added aseptically into sterilized bromocresol purple broth base before inoculation with 18 - 24 h old culture of each lactic acid bacteria strain. The results were assessed with reference to an uninoculated control after anaerobic incubation at 30°C for 5 d. Tubes in which bromocresol purple colour changed to yellow indicated utilisation of sugar or acid production. The various lactic acid bacteria strains were then identified by reference to the Bergey's Manual of Systematic Bacteriology [22] and The Genera of Lactic Acid Bacteria [23] based on the results of the various tests. The identity of the lactic acid bacteria isolates were further confirmed by using the API 50 CHL tests and the Computer Program APILAB Plus (BioMerieux, France).

3. Results and Discussion

The pH of the fermenting cassava roots decreased from 5.6 to 3.7 during the 72 h fermenting period. Correspondingly, the temperature increased from 26° C to 30° C. The total titratable acidity (in % lactic acid) increased from 0.07 ± 0.01 to 0.21 ± 0.01 and decreased to 0.09 by

the end of 72 h fermentation period (**Table 1**). The pH of fermenting maize grains dropped from 5.9 to 3.8 by the end of the 72 h fermentation period. Correspondingly, the temperature increased from 25° C to 31° C during the same period while the total titratable acidity increased from 0.13 ± 0.01 to 0.28 ± 0.01 after 24 hours and then decreased to 0.14 by the end of fermentation period (**Table 1**).

During both *fufu* and *ogi* fermentation processes, the temperature of the fermenting materials increased as fermentation progressed. The two processes are thus exothermic in nature and the heat generated might have resulted from the metabolic activities of the fermenting organisms [24]. The fermenting organisms do contribute to acidity attributable to the production of lactic acid and acetic acid during the processes which exerts a depressive effect on the pH of the fermenting materials [23,25].

Despite the unhygienic wet-milling and wet-sieving processes involved in the traditional preparation of both *fufu* and *ogi*, the low pH of the fermented products would make them safe for consumption. Also the acidic fermentation and lactic acid metabolites are responsible for inactivation of enterobacteriaceae including toxin-producing and foodborne infectious pathogens [26-29]. These result in an improvement of the aroma, flavor, texture, safety and shelf life of the food.

A total of 32 lactic acid bacteria strains were isolated from the various phases of the fermentation of cassava for *fufu* production while 14 strains were obtained from fermenting maize steep for *ogi* production. The isolates were subjected to various morphological, physiological and biochemical characterizations. The characteristics exhibited by the isolates were compared with those of standard strains for their identification [22,30]. All the isolates were Gram positive and catalase negative rods or cocci. All produced no endospore and were non motile. They were facultative anaerobes and were fermentative rather than being oxidative in nature (**Table 2**).

Table 3 shows the periodic distribution of dominant lactic acid bacteria strains during fermentation processes of *fufu* and *ogi*. Strains isolated from *fufu* fermentation included *Lactobacillus plantarum*, *L. lactis*, *L. coprophillus*, *L. acidophilus*, *L. brevis* and *Leuconostoc mesenteroides* while *L. plantarum*, *L. cellobiosus*, *L. acidophilus*, *L. lactis* and *Lc. paramesenteroides* were isolated from *ogi* fermentation. Ecological succession patterns in which the *Leuconostoc* species occur mostly at initial stages and the *Lactobacillus* species at later stages were observed in both fermentations. The most frequently isolated LAB species in *fufu* fermentation were *L. plantarum* (18 strains, 56.25%) and *Lc. mesenteroides* (6 strains, 18.75%) while in *ogi*, both *L. cellobiosus* and *Lc. lactis* had the highest percentage occurrence of 26.67%

Cassava (Fufu)				Maize (Ogi)			
Time (h)	Temperature (°C)	pН	Titratable acidity (% lactic acid)	Temperature (°C)	pН	Titratable acidity (% lactic acid)	
0	26	5.6	0.07 ± 0.01	25	5.9	0.13 ± 0.01	
24	28	5.0	0.21 ± 0.01	27	5.2	0.28 ± 0.01	
48	29	4.4	0.13 ± 0.01	29	4.5	0.21	
72	30	3.7	0.09	31	3.8	0.14	

Table 1. pH, temperature and titratable acid changes during the fermentation of cassava and maize for *fufu* and *ogi* production.

Sample source				Fufu					Ogi		
No. of strains	18	2	2	2	2	6	2	4	2	4	2
Gram reaction/ Morphology	GPR	GPR	GPR	GPR	GPR	GPC	GPR	GPR	GPR	GPC	GPC
Catalase	-	-	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+
Voges Proskauer	-	-	-	-	-	-	-	-	-	-	-
Spore stain	-	-	-	-	-	-	-	-	-	-	-
Oxidative/ Fermentative	F	F	F	F	F	F	F	F	F	F	F
Dextran from sucrose	-	-	+	-	-	+	-	-	-	-	-
Fermentation pattern	HMF	HMF	HTF	HTF	HMF	HTF	HMF	HTF	HMF	HMF	HTF
O2 relationship	FA	FA	FA	FA	FA	FA	FA	FA	FA	FA	FA
Growth in 4% NaCl	+	-	-	-	+	-	+	+	-	+	-
Ammonia from arginine	-	+	+	-	-	-	-	-	-	-	-
Fermentation:											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	-
Sucrose	-	+	+	+	+	-	+	-	+	+	-
Galactose	+	+	+	+	+	-	+	+	+	+	+
Maltose	+	+	+	+	+	-	+	+	+	+	+
Mannitol	+	-	-	+	+	-	+	+	+	+	+
Sorbitol	+	-	-	+	+	-	+	+	+	+	+
Mannose	+	-	-	+	+	-	+	+	+	+	-
Arabinose	-	+	+	+	+	-	+	-	+	+	+
Xylose	-	-	-	-	+	-	-	+	-	-	-
Cellobiose	+	-	-	+	-	-	+	-	+	+	-
Dulcitol	+	-	-	-	-	-	+	-	-	-	-
Inositol	+	-	-	-	-	-	-	+	-	-	-
Inulin	+	-	-	+	-	-	+	+	+	+	-
Raffinose	+	+	+	-	-	+	+	-	-	+	+
Rhamnose	+	-	-	+	+	-	-	+	+	-	-
Salicin	+	+	+	+	-	-	+	-	+	+	-
Identification	L. plantarum	L. lactis	L. coprophilus	L. acidophilu	s L. brevis	Lc. nesenteroide	L. s plantarum	L. cellobiosus	L. acidophilı	Lc. ıs lactis po	Lc. aramesenteroides

Table 2. Morphological and biochemical characterisation of isolates.

Period (h)	Fermentation	Dominating strains of LAB
0	Fufu	L. plantarum, L. brevis, L. coprophillus, Lc. mesenteroides
0	Ogi	L. plantarum, L. cellobiosus, Lc lactis, Lc. paramesenteroides
24	Fufu	L. plantarum, Lc. mesenteroides, L. acidophilus, L. lactis, L. brevis, L. coprophillus
24	Ogi	L. plantarum, Lc. lactis, Lc. paramesenteroides, L. acidophilus, L. cellobiosus,
49	Fufu	L. plantarum, L. acidophilus, L. lactis, L. brevis, Lc. mesenteroides
48	Ogi	L. plantarum, L. cellobiosus, L. acidophilus, Lc. lactis
70	Fufu	L. plantarum, L. acidophilus, L. brevis
12	Ogi	L. plantarum, L. cellobiosus,

Table 3. Periodic distribution of dominant LAB strains during the fermentation of cassava and maize for *fufu* and *ogi* production.

followed by *L. plantarum* with occurrence of 20.0% (**Figures 3** and **4**). The frequencies of dominance of the lactic acid bacteria strains in *fufu* fermentation were *L. plantarum* 56.25%, *Lc. mesenteroides* 18.75%, *L. lactis* 6.25%, *L. coprophillus* 6.25%, *L. acidophilus* 6.25% and *L. brevis* 6.25%. In *ogi* fermentation, they were *L. cellobiosus* 26.67%, *Lc. lactis* 26.67%, *L. plantarum* 20.0%, *Lc. paramesenteroides* 13.33% and *L. acidophilus* 13.33%.

Lc. mesenteroides and *L. plantarum* have been reported as the dominant microorganisms implicated in several natural and spontaneous lactic acid fermentation of vegetables [31,32]. *L. plantarum* was also found to dominate the lactic acid bacteria flora in various food fermentation processes for *akamu* [33], *ogi* [34], *boza* [35], *cachaca* [36], *tempoyak* [37], *ting* [38] and *togwa* [39].

Spontaneous fermentation typically results from the competitive activities of different microorganisms whereby strains best adapted and with the highest growth rate will dominate particular stages of the process [38]. Among the bacteria associated with food fermentation, lactic acid bacteria are of predominant importance. Samples from plant materials were reported to show the greatest diversity of lactic acid bacteria with the *Lactobacillus* strains being predominant in food-related ecosystems [40].

Yeasts and lactic acid bacteria are implicated in the fermentation of a wide variety of traditional food and beverage fermentations [14,18,33,39]. While yeasts are known to facilitate alcoholic fermentations, lactic acid bacteria produce lactic acid as part or major by product from the fermentation of carbohydrates [25,41]. *Fufu* and *ogi* are products of acid fermentations by lactic acid bacteria. This group of bacteria includes several genera that differ considerably in morphological, physiological and functional properties. *Lc. mesenteroides* mostly occur at early stages of most vegetable fermentations with *L. plantarum* predominating towards the end of the processes [14]. This ecological succession is attributed to the differences in the acid tolerance of the two genera. It may also be the reason for the disappearance of the entero-



Figure 3. Percentage occurrence of lactic acid bacteria in fufu fermentation.



Figure 4. Percentage occurrence of lactic acid bacteria in *ogi* fermentation.

bacteriaceae which are observed at early periods during fermentation of plant materials [14]. The water used for steeping or surface microflora of raw materials may be sources of such enterobacteriaceae. The methods of preparation, water used for steeping, surface microflora of raw materials, atmosphere where processing takes place and the handlers all affect the types of microorganisms isolated during fermentation processes for *fufu* and *ogi*. The various species of lactic acid bacteria are however better adapted to the substrates and overcome the possible physiological and biochemical hurdles during the phases of each fermentation process. *Fufu* and *ogi*, as at present, are traditionally prepared by spontaneous fermentation relying on the indigenous flora of the raw materials. This leads to a poorly controlled process and variations in the products. For consistency in the quality of these fermented food products, the development of specific starter cultures is important.

4. Conclusion

It is concluded that an association of lactic acid bacteria composed of homofermentative and heterofermentative *Lactobacillus* species and heterofermentative *Leuconostoc* species are involved from the beginning to the end of both cassava and maize fermentations for the production of *fufu* and *ogi* respectively. Factors which influence these food fermentations include temperature, acidity and hydrogen ion concentration (pH). The assessment of the performance of each of the species under controlled fermentation will enable selection of the best strains that can serve as potential starter culture for the products.

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