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RESEARCH ARTICLE

REDUCING THE DRUDGERY INVOLVED IN THE FERMENTATION OF MAIZE (*ZEA MAYS*) GRUEL (OGI)

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ABSTRACT

Ogi, a popular fermented cereal-based foods consumed as weaning foods by infants and as dietary staples for adults in west Africa is usually processed by natural fermentation, which takes up to 48h to produce. This is the first report of producing the maize gruel (ogi) in less than 5h, by pre-treatment of the maize grains before fermentation. Pre-treatment involved particle size reduction of the maize grains, hydrolysis of starch (steeping of maize grains) and addition of fermentation starter, with short term low heat exposure (at 45° to 60°C; for one to 3h) to encourage the growth of fermenting microbes (1° fermentation). The desired product was obtained after blending, sieving and stabilizing the fermented maize gruel for 1 to 2h at ambient temperature (2° fermentation). For consumption, the gruel was stirred with an appropriate quantity of boiling water into a consistent gel paste, ogi. The microbial load of the different combinations of pre-treated maize gruels increased appreciably, as the inoculum size of the fermentation starter increased and fermentation progressed. The incidence of different strains of *Lactobacillus fermentum* was very prominent in all the samples. Other species of the genus Lactobacillus, such as *L. plantarum* and *L. lactis* were also isolated from the maize gruel samples, even after the pre-treatment of the maize grains.

INTRODUCTION

Stiles (1996) and Ogunbanwo *et al*. (2004) have estimated that 25% of the European diet and 60% of the diet in many developing countries consist of fermented foods. Many microorganisms play very important role during fermentation by modifying the substrates physically, nutritionally and organoleptically (Steinkraus, 1997). During the process, these microbes could also produce a wide range of metabolic end-products that function as preservatives, texturizers, stabilizers, flavouring and colouring agents, make some of these fermented foods more acceptable (Harlander, 1992; Basillico *et al.,* 2008). Naturally, lactic acid bacteria (LAB) are mainly responsible for such fermentations (Oyewole, 1992; Magnusson and Schnurer, 2001; Adebayo *et al*., 2013). The LABs equally play fundamental roles in the microbial ecology (Adebayo and Aderiye, 2007), synthesizing a variety of antimicrobial compounds (Aderiye and Ajibade, 2007; Adebayo and Aderiye, 2010a; b; Aderiye and David, 2013) such as organic acids, hydrogen peroxide, diacetyl and bacteriocins (Lindgren and Dobroqosz, 1990; Adebayo and Aderiye, 2011). Their ability to produce antimicrobial substances against other competing microbiota ensures their predominance and food safety (Adebayo *et al*., 2014a; b). The majority of Nigerian fermented food products such as ogi (from maize, sorghum, and millet), gari and fufu (from cassava) are through lactic acid fermentation (Oyewole, 1992). Aderiye and Laleye (2003) in their study on the relevance of fermented foods in the south-western Nigeria reported that at least 69.2% of the adults consumed gari, ogi, iru, elubo and fufu. They concluded that the ease of food preparation, price of food and the length of storage contribute to the frequency of consumption of these fermented foods. Ogi is a fermented maize gruel consumed by over 25 million people especially in southwest Nigeria and other parts of West Africa. Fermentation of maize to produce ogi usually takes 24h or more through chance inoculation. The traditional process of retting the maize grains (chance inoculation) to produce ogi usually takes between 36 and 48hours, with the appearance of LABs after 12h (Adebayo and Aderiye, 2007). During the fermentation, microbial activity reached its peak (1.63 x 10^7 CFU/g) on the third day with about 20.3% reduction in the total aerobic plate count (Adebayo *et al.*, 2013). However, the LABs constituted between 7 to 48.5% of the total bacteria cells. Meanwhile, the cells of *Lactobacillus fermentum* were frequently isolated shortly (1h) after retting the substrate in water and still found after 72h (Adebayo and Aderiye, 2007). Furthermore, most of the LAB cells (84.6%) especially those of *L. bulgaricus* and *Leuconostoc mesenteroides* were isolated as from the 48th hour of fermentation. Recently from our laboratory, it was revealed that when the maize grains were pre-treated before fermentation, the period required producing *ogi* reduced

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drastically, within 12h (unpublished report). Further work reported here has shown that the product can be obtained within 3-5h.

MATERIALS AND METHODS

1. Source of raw materials

i. Maize grains: freshly shelled dry maize grains (average moisture content of 10 ± 1.5%) were sorted and partially milled in a grindery with a pulverizing machine (Triton, Kent England), winnowed and later passed through a series of laboratory test sieves (Endecotts Ltd., London England). The sieved maize grain particle size used in this study was between 1.40 and 3.35 mm.
ii. Community ogi: freshly prepared community ogi prepared by chance inoculation of maize grains (subsequently referred to as the fermentation starter) was purchased from the main market at Ado-Ekiti and transported under ice packs to the laboratory of the Department of Microbiology, Ekiti State University, Ado-Ekiti.

2. Determination of water absorption capacity of maize grain particles

Ten gram of partially milled maize grains was soaked in 50mL water in a 100mL beaker at 30°, 45° and 60°C for 5min and later agitated in a shaker water bath at 100rpm at the respective temperatures for 20 min. The volume of water absorbed by the grain particles was determined and the water absorption rate calculated over 25min.

3. Fermentation: Primary (1°) and Secondary (2°) fermentations

A known quantity (50g) of the maize particles was soaked in 250mL distilled water and inoculated with varying amounts of community ogi (as fermentation starter). From the foregoing, a combination of different maize grain particle sizes, inoculated with various amounts of fermentation starter (community ogi) continuously stirred and fermented at different temperatures (45°, 50° and 60°C), as exemplified in Table 1, for an initial period of one to 3h (1° fermentation). (This to soften the grains (hydrolyze the starch) and encourage the growth of fermenting microbes). Later, the resulting primary ferment was homogenized and allowed to stabilize for another 1 to 2h at ambient temperature (25° ±1°C) (2° fermentation) (Fig. 1). Following the secondary fermentation, the liquor was filtered through a 1µm sieve. The filtride was de-watered and either made into molds ready for packaging and storage or prepared for consumption as described below.

4. Preparation and Sensory evaluation of *ogi samples*

4.1. Preparation of ogi for consumption

In the preparation of *ogi* for sensory evaluation, a known quantity of the fermented maizegrain gruel (mold) was mixed with an appropriate amount of water and later boiled waterwas added with continuous stirring until a consistent gel paste of desired thickness is achieved.

4.2 Sensory Evaluation Ogi samples were subjected to sensory evaluation at different sessions by a panel of 20 people each comprising male and female staff and students of Ekiti State University, Ado-Ekiti. The community ogi served as the control sample. A written consent was signed by all the participants. The selection criterion of panel members was based on the non existence of allergic reactions to ogi, regular consumption of ogi and ogi production. Freshly prepared ogi samples (50mL) were served at room temperature in clear plastic cups. Each panelist was given a clean tablespoon and water to rinse the mouth at interval of testing the ogi meal, to avoid any carry over effect. Sets of three digits random numbers was assigned to each sample.

All sensory analyses were carried out in individual booths Aderiye and Ogunjobi, 1998). A prepared comparison rating, Hedonic scale of 7 points (7 – best, 6 – better, 5 – good, 4 – average, 3 – poor, 2 – very poor, 1 – bad for consistency, odour and texture while 7 – enjoyed, 6 – preferred, 5 – good, 4 – average, 3 – fair, 2 – disliked, 1 – very disliked was respectively rated for taste and general acceptability. The order of presentation was balanced and randomized to eliminate contrast effect and positional bias. The sensory scores for ogi samples were subjected to analysis of variance (ANOVA) to determine whether there were statistically significant preferences in sensory attributes and Duncan′s multiple range test was used to determine which of the samples were significantly preferred (Larmond, 1977; Rao *et al*., 2009).

5. Isolation and Enumeration of microorganisms from fermented maize gruel "ogi" After fermentation, one gram was aseptically withdrawn into a sterile test tube containing 9.0mL of distilled water, (pH 7.0) to provide 10^{-1} dilution, ten-fold dilutions were later made (Adebayo and Aderiye, 2010). Dilutions were prepared for the isolation using the spread plate method on MRS Agar (de Mann Rogosa-Sharpe (MRS) agar (Oxoid, UK). The MRSA culture plates were incubated anaerobically at 30°C for 72 h and the number of the colony forming units were determined. Also the incidence of LAB colonies was monitored throughout the experiment.

6. Proximate Analyses

The ash and moisture contents were determined using the air-oven and dry ashing methods respectively as described by the Association of Official Analytical Chemists (AOAC, 2000). Determination of the fat content was performed following the Soxtec method previously described by Noureddini and Byun (2010), the total nitrogen content of the samples was evaluated according to the method described by Ng *et al*. (2008). Meanwhile, the total carbohydrate content (%) in the samples was calculated by difference.

7. Mineral Analyses The method described by the Association of Official Analytical Chemists (AOAC, 2005) was used for mineral analyses. Two grams (2 g) of each of the samples was digested with concentrated nitric acid and hydrogen peroxide, filtered and the filtrate in a 5mL volumetric flask was loaded to Atomic Absorption Spectrophotometer, (model 703 Perkin Elmes, Norwalk, CT, USA). Calcium (Ca), magnesium (Mg) and iron (Fe) were determined at wavelengths 317.9 nm, 285.2 nm, 259.9 nm and 324.7 nm respectively. Potassium (K) was determined using the flame emission photometer (Sherwood

Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) with KCl as the standard (AOAC, 2005). All the values were expressed in mg/100g.

8. Determination of Hydrogen Ion Concentration (pH)

Two gram (2g) of each sample was homogenized with 20mL of distilled water and stirred thoroughly to form slurry. The digital pH meter (Crison Basic model 20) which was standardized with buffer 7.0 was used to monitor the pH values of the samples.

9. Enzyme Assays

Amylase activity was assayed using the method of Berfeld *et al*. (1955) and lipolytic activity was determined by colorimetric method based on the activity in cleavage of p-nitrophenylpalmitate (p-NPP) at pH 8.0 as described by Lotrakul and Dharmsthiti (1997). Protease activity was determined by the method of Anson (1938) while Glucanase was assayed for by incubating 500µL of 5.0% Laminarin in 50mM acetate buffer pH 4.8 with 200µL maize gruel solution at 45°C for 30 min and the amount of reducing sugars determined with DNSA as described by Miller (1959). Xylanase activity was evaluated as described by Bailey *et al*. (1992) using 1% birch wood xylan as substrate. The method of Nelson (1944) measuring the amount of reducing sugars released from sucrose was employed in the determination of sucrase activity. Maltase activity was determined by using p-nitrophenyl- α -D-glucoside as substrate according to the method of Eugene and Charles (1981).

10. Molecular Characterization of Bacterial Isolates

The characterization of the fermenting organisms was carried out using molecular techniques as described by Pitcher *et al.* (1989); Mozioglu *et al.* (2014); Aderiye and Oluwole (2014) and Kogno *et al*. (2017).

10.1. Extraction of DNA

For extracting genomic DNA, bacterial strain was incubated in 5mL of medium as mentioned above and bacterial cells were collected from 1mL of the culture by centrifugation at 13,500 rpm for 3 min (Aderiye and Oluwole, 2014). The pellet was resuspended in 200µL of STET buffer (0.1 M NaCl, 10mM Tris-Cl (pH 8.0), 1mM EDTA (pH 8.0) and 5% (v/v) Tween 20), from which the genomic DNA was extracted with a guanidium thiocyanate based method (Pitcher *et al.,* 1989). Finally the DNA pellet was re-suspended in 200µL of 10mM Tris-Cl (pH 8.0), 1mM EDTA (TE) buffer and 5µL of the extract was run on 1% (w/v) Agarose gel to detect the presence of DNA.

10.2. Polymerase Chain Reaction (PCR)

About 2.5ng of bacterial genomic DNA was added to a 50µL PCR mix which contained 1 X Hot start reaction buffer, 0.25 mM dNTPs, 0.01 M (each) primers 27F and 1525R, and 2.5 U Hot start polymerase (Jenabioscience). Thermal cycling was done in a Veriti thermal cycler (Applied Biosystems, USA) and cycling conditions were at 95ºC for 3 min followed with 45cycles at 95ºC for 30sec, at 45ºC for 1 min, 72ºC for 1 min 30 sec with ramp from 45ºC to 72ºC set at 40%. Subsequently, the reaction was held at 72ºC for 10min after which it was held at 4ºC till terminated. PCR products were resolved on 1% (w/v) agarose gel stained with ethidium bromide and viewed on a transilluminator (Mozioglu *et al.,* 2014).

10.3. Sequencing of amplified 16S rRNA gene

The PCR products were purified using Montage PCR Clean up kit (Millipore). The purified PCR products of approximately 1,500bp were sequenced using 4 primers (27F - AGA GTT TGA TCM TGG CTC AG, 1492R - TAC GGY TAC CTT GTT ACG ACT T, 518F - CCA GCA GCC GCG GTA ATA CG and 800R - TAC CAG GGT ATC TAA TCC). Sequencing was performed using Big Dye terminator cycle sequencing kit v.3.1 (Applied BioSystems, USA) and sequencing products were resolved on an Applied Biosystems model 3130XL automated DNA sequencing system (Applied BioSystems, USA) at the Macrogen, Inc., Seoul, Korea.

10.4. Bioinformatics analysis

Sequences were analyzed using the BLAST (Basic Local Alignment Search Tool) bioinformatics program on the NCBI (National Center for Biotechnology Information) website. BLAST was done to identify the 16S rRNA sequences in Genbank most similar to the query sequence.

11. Statistical Analysis The mean and standard error of means (SEM) of the triplicate analyses were calculated. The data were analyzed using the SPSS version 16.0. The analysis of variance (ANOVA) was performed to determine the significant differences between the means, while the means were separated using the new Duncan Multiple Range Test at $p<0.05$.

RESULTS

Water absorption capacity and water absorption rate of maize grain particles

As the particle size of the maize granules increased, water absorption capacity reduced irrespective of the extent of heat applied. For example, maize grains of ≤ 1.40mm particle size absorbed 1.35, 1.65 and 1.25mL water at the rate of 0.054, 0.066 and 0.050mL/min at 30°, 45° and 60°C respectively. Meanwhile, the grains with particle size greater than 3.35mm when soaked, absorbed water at the rate of 0.042, 0.026 and 0.022mL/min at the same respective temperatures (Table 1). Percentage reduction in water absorption rate ranged between 70.37% in ≤ 1.40mm and ≤ 2.36mm particles at 30°C, 39.39% between ≤ 1.40mm and ≥ 3.35mm particles at 45°C and 61.11% between ≤ 3.35 and grain particle sizes ≥ 3.35 mm at 60°C.

pH of maize grain particles Table 1 also revealed that with increased heat treatment (30° to 60°C), the pH values of the partially milled maize grains reduced. At 30° and 45°C, the pH values recorded for the grain particles of size ≤ 3.35mm were relatively lower (6.63; 6.31) than those obtained from maize grains with particle size of ≤ 1.40mm (6.88, 6.69) retted in water for 25min at the respective temperatures. Comparatively, the pH values obtained from the different particle sizes at 60°C

were relatively lower (6.21 to 6.40) than those investigated at 30° and 45°C.

Palatability attributes of highly rated (accepted) fermented maize gruel

Of over 60 fermented maize gruel samples subjected to sensory evaluation by a mixed panel of ogi producers (manufacturers), habitual ogi consumers and casual ogi consumers, only four of these samples (with particle size ≤ 1.40mm) subjected to pre-treatment at 60°C, described and coded as D60.1, C90.1, E90.3 and E120.3 (Table 2) were highly acceptable when compared to the community ogi (control) (Table 3). The ogi sample, from maize grain particles (size ≤ 1.40mm) inoculated with 200mg.g-1 of fermentation starter incubated for 5h (E120.3) was rated very highly (generally accepted) and significantly comparable to the control (ogi producers, 7.80 ± 0.13: 7.90 ± 0.10; habitual ogi consumers, 7.30 ± 0.21: 7.60 ± 0.16 and casual ogi consumers, 7.10 ± 0.10: 7.20 ± 0.13) (Table 3). When the textural attribute of these samples was considered the E120.3 sample was rated better by ogi producers (7.60 ± 0.16) and the casual ogi consumers (7.70 ± 0.15) than all the other samples including the control. Subsequently, these samples were subjected to physicochemical, enzymatic, mineral and microbial analyses. Furthermore, there was a significant difference between samples D60.1 and E120.3 while samples C90.1, E90.3 and the control showed no significant difference in the texture. For taste, sample D60.1 differed significantly from the control but samples E120.3, C90.1 and E90.3 exhibited no significant difference. For smoothness, the sample containing 8g of fermentation starter, homogenized and placed in water bath at 60°C for 60min and later fermented for 1hr (D60. 1) at 25°C differed significantly in smoothness from the other samples (C90.1, E90.3, E120.3 and the control). For general acceptability of ogi, sample D60.1 differed significantly from E120.3, C90.1 and E90.3 samples. The odour rating for D60.1 and E120.3 samples was the same. There was no significant difference between samples C90.1 and E90.3 but these differed significantly from the control. Samples C90.1 and E90.3 are not significantly different from the control.

Proximate component of fermented maize gruel

The proximate components of the preferred *ogi* samples and the community *ogi* (control) are shown in Table 4. E120.3 sample was rated better than the control in the crude fat (8.07 ± 0.03; 7.57 ± 0.38%), crude fibre (5.37 ± 0.43; 4.83 ± 0.49), ash (3.96 ± 0.03; 3.50 ± 0.11%) and protein (21.73 ± 0.55; 20.50 ± 1.09%) contents. The carbohydrate (excluding the fibre) and the moisture contents constitute over 63% of the components in each of the samples, exhibiting a ratio of 3: 2 in E120.3 and 2: 1 in E90.3. Of all the samples, the moisture and the protein contents of C90.1 and E120.3 were the highest (25.60 ± 1.67 and 21.73 ± 0.55% respectively). Incidentally, the fibre content of E120.3 (5.37 ± 0.43%) was relatively higher than the E90.3 sample. Like the fibre, the ash component of the fermented maize gruel samples was very low (ranging between 3.30 ± 0.40% in C90.1 and 4.33 ± 0.49% in D60.1). For each of the experimental samples, there was significant difference in the value obtained for carbohydrate, fat, ash, fibre and protein when compared to that of the control.

Mineral component of fermented maize gruel

Potassium and magnesium were the most prominent minerals in all the fermented maize gruel samples, constituting between 53.3 % and 42.1% respectively in E120.3 and E90.1 samples (Table 5). There was no significant difference among the samples when Mg^{2+}, K and Fe^{3+} were considered. Meanwhile, there was significant difference in the calcium content of C90.1 and E90.1 (2.85 ± 0.14 and 2.93 ± 0.14 mg/10g respectively) when compared to the community ogi with Ca^{2+} concentration of 3.96 ± 0.14 mg/10g and E120.3 with 3.95 ± 0.28mg Ca/10g ogi.

Enzymatic activity (mmol/min/mL) in fermented maize gruel

The activity of the enzymes evaluated in the fermented maize gruel (ogi) samples is shown in Table 6.

Table 1. Water absorption capacity, water absorption rate and pH of partially milled grains at different temperatures

Particle size (mm)	30°C			45°C			60°C		
	WAC (mL)	WAR (mL/min)	pH	WAC (mL)	WAR (mL/min)	pH	WAC (mL)	WAR (mL/min)	pH
≤ 1.40	1.35	0.054	6.88	1.65	0.066	6.69	1.25	0.050	6.40
≤ 2.36	0.95	0.038	6.86	1.45	0.058	6.52	1.05	0.042	6.21
≤ 3.35	1.20	0.048	6.63	0.90	0.036	6.31	0.90	0.036	6.32
≥ 3.35	1.05	0.042	6.71	0.65	0.026	6.60	0.55	0.022	6.34

WAC: Water absorption capacity
WAR: Water absorption rate

Table 2. Composition of pre-treated maize grain particles for production of ogi

SampleTreatment of maize grains*

Code
D60.1: Fifty gram of partially milled maize was inoculated with one hundred and sixty (160) milligram of community ogi in a flask in shaker water bath at 60°C for 60min, sieved and allowed to stabilize at ambient temperature for 1h.
C90.1: Fifty gram of partially milled maize was inoculated with one hundred and twenty (120) milligram of community ogi in a flask in shaker water bath at 60°C for 90min, sieved and allowed to stabilize at ambient temperature for 1h.
E90.3: Fifty gram of partially milled maize was inoculated with two hundred (200) milligram of community ogi in a flask in shaker water bath at 60°C for 90min, sieved and allowed to stabilize at ambient temperature for 3h.
E120.3: Fifty gram of partially milled maize was inoculated with two hundred (200) milligram of community ogi in a flask in shaker water bath at 60°C for 120min, sieved and allowed to stabilize at ambient temperature for 3h.
Control: Community ogi (fermentation starter)

•Maize grain particles and fermentation starter Community ogi in 250mL distilled water

Table 3. Sensory evaluation of fermented maize gruel

Sample	*Panelist	Texture (feel)	Taste	Consistency (smoothness)	Odour	General acceptability
	OP	7.00 ± 0.21[a]	6.70 ±.0.34[a]	6.70 ± 0.15[a]	7.00 ± 0.21[a]	6.90 ± 0.23[a]
D60.1	HOC	6.50 ± 0.22[a]	6.60 ± 0.34[a]	6.40 ± 0.16[a]	6.90 ± 0.18[a]	6.50 ± 0.31[a]
	COC	6.80 ± 0.20[a]	7.13 ± 0.18[a]	6.80 ± 0.20[a]	6.90 ± 0.18[a]	7.00 ± 0.00[a]
	OP	7.60 ± 0.16[b]	7.20 ± 0.13[ab]	7.50 ± 0.22[b]	6.90 ± 0.23[a]	7.80 ± 0.13[b]
	HOC	6.90 ± 0.10[ab]	7.10 ± 0.50[a]	7.10 ± 0.31[ab]	7.30 ± 0.21[ab]	7.00 ± 0.30[ab]
E120.3	COC	7.70 ± 0.15[b]	7.10 ± 0.10[a]	7.10 ± 0.18[ab]	7.10 ± 0.10[a]	7.20 ± 0.13[ab]
	OP	7.40 ± 0.16[ab]	7.20 ± 0.25[ab]	7.50 ± 0.17[b]	7.40 ± 0.16[ab]	7.80 ± 0.13[b]
	HOC	7. 10 ± 0.18[ab]	7.20 ± 0.29[a]	6.60 ± 0.16[a]	7.20 ± 0.29[a]	7.40 ± 0.22[b]
C90.1	COC	7.40 ± 0.22[ab]	7.20 ± 0.20[a]	7.60 ± 0.16[b]	7.10 ± 0.18[a]	7.50 ± 0.22[b]
	OP	7.50 ± 0.17[ab]	7.20 ±.0.20[ab]	7.60 ± 0.16[b]	7.40 ± 0.16[ab]	7.50 ± 0.17[b]
	HOC	6.50 ± 0.31[a]	6.80 ± 0.25[a]	6.80 ± 0.21[ab]	7.10 ± 0.23[ab]	7.10 ± 0.18[ab]
E90.3	COC	7.10 ± 0.23[ab]	7.00 ± 0.21[a]	7.50 ± 0.17[b]	6.80 ± 0.25[a]	7.30 ± 0.15[ab]
	OP	7.50 ± 0.17[ab]	7.70 ± 0.15[b]	7.50 ± 0.17[b]	7.60 ± 0.16[b]	7.90 ± 0.10[b]
Control	HOC	7.20 ± 0.13[b]	7.40 ± 0.16 [a]	7.40 ± 0.27[b]	7.60 ± 0.16[b]	7.20 ± 0.20[ab]
	COC	7.20 ± 0.20[ab]	7.30 ± 0.15[a]	7.20 ± 0.13[ab]	7.20 ± 0.13[a]	7.30 ± 0.15[ab]

*: OP: Ogi producers; HOC: Habitual ogi consumers; COC: Casual ogi consumers
Values with the same superscript letter (s) along the same column are not significantly different (P<0.05).

Table 4. Proximate composition of the maize gruel samples

Sample	Proximate composition (%)					
	Moisture	CHO	Fat	Ash	Fibre	Protein
D60.1	21.80±0.52[b]	40.90±0.52[b]	8.83±1.06[a]	4.33±0.49[a]	4.60±0.12[b]	15.47±1.53[b]
E120.3	25.47±1.18[a]	39.40±1.04[b]	8.07±0.03[b]	3.96±0.03[b]	5.37±0.43[a]	21.73±0.55[a]
C90.1	25.60±1.67[a]	41.20±2.25[ab]	7.60±0.98[c]	3.30±0.40[c]	4.00±0.28[c]	18.30±2.79[ab]
E90.3	21.30±0.52[b]	43.30±1.04[a]	8.60±0.46[a]	4.00±0.23[ab]	5.20±0.46[a]	17.63±0.43[b]
Control	23.23±1.07[ab]	40.43±0.33[b]	7.57±0.38[c]	3.50±0.11[c]	4.83±0.49[ab]	20.50±1.09[a]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter (s) along the same column are not significantly different (P<0.05).

Table 5. Mineral component (mg/10g) of fermented maize gruel

Mineral Component	Fermented maize gruel (ogi) sample				
	Control	C90.1	D60.1	E90.1	E120.3
Calcium (Ca)	3.96 ± 0.14[a]	2.85 ± 0.14[b]	3.97 ± 0.22[a]	2.93 ± 0.14[b]	3.95 ± 0.28[a]
Magnesium (Mg)	42.37 ± 0.29[a]	42.40 ± 0.28[a]	42.39 ± 0.13[a]	42.38 ± 0.22[a]	42.36 ± 0.17[a]
Potassium (K)	52.25 ± 0.00[a]	53.28 ± 0.14[a]	53.27 ± 0.23[a]	53.41 ± 0.16[a]	52.97 ± 0.28[a]
Iron (Fe)	1.20 ± 0.14[a]	1.23 ± 0.18[a]	1.22 ± 0.15[a]	1.25 ± 0.22[a]	1.24 ± 0.14[a]

Table 6. Enzymatic Activity (mmol/min/mL) in Fermented Maize Gruel 'ogi' samples

Samples	Enzyme activity (mmol/min/mL)						
	Amylase Activity	Sucrase activity	Maltase activity	Glucanase activity	Xylanase activity	Protease activity	Lipase activity
D60.1	41.16±1.17[b]	12.15±1.13[bc]	29.69±0.54[d]	0.81±0.06[c]	0.13±0.06[b]	5.13±0.03[bc]	0.67±0.03[a]
E120.3	47.77±2.34[c]	17.54±1.69[d]	28.34±1.10[d]	0.40±0.03[b]	0.04±0.03[a]	4.58±0.06[ab]	1.02±0.03[c]
C90.1	48.72±1.13[c]	9.45±0.51[b]	22.94±0.51[b]	0.13±0.02[a]	0.27±0.02[c]	4.80±0.52[b]	0.80±0.02[b]
E90.3	31.04±1.13[a]	13.46±1.11[c]	25.64±1.20[c]	1.08±0.05[d]	0.40±0.05[d]	5.16±0.55[bc]	1.16±0.03[cd]
Control	29.55±1.13[a]	8.10±0.53[a]	17.54±0.06[a]	0.40±0.01[b]	0.13±0.01[b]	4.04±0.55[a]	0.93±0.06[c]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter (s) along the same column are not significantly different (P ≤ 0.05).

Table 7. Lactic acid bacteria count (log_{10} CFU/g) of fermented maize gruel

Sample	Fermentation Process (in minutes)								
	First fermentation at 60°C					Second fermentation at 25°C			
	Initial	30	60	90	After washing & sieving	30	60	180	210
D60. 1	4.85	4.94	4.995		4.97	5.05	5.06	-	-
C90.1	4.74	4.85	4.92	5.01	4.94	4.97	4.98	-	-
E90.3	4.91	4.94	4.99	5.13	5.06	5.12	5.12	5.13	-
E120.3	4.94	4.99	5.06	5.12	5.15[a], 5.06	5.10	5.14	5.14	5.15
Control	5.00	5.14	-	-	-	-	-	-	-

[a] Microbial load of E120.3 after 2h fermentation at 60°C

Table 8. Lactic acid bacteria associated with fermented maize grain 'ogi' samples

S/N	Lactic Acid Bacteria strain	Ogi sample				
		Fermentation starter	D60.1	C90.1	E90.3	E120.3
1	*L. acidophilus*			+		+
2	*L. brevis*	+				+
3	*L. casei*			+	+	
4	*L. cellobiosus*		+			
5	*L. fermentum* CP011536.1	+				+
6	*L. fermentum* JQ669802.1	+				+
7	*L. fermentum* KU851162.1			+		
8	*L. fermentum* KT633926.1					+
9	*L. fermentum* NC_010610.1					+
10	*L. fermentum* NR_104927.1	+				+
11	*L. helviticus*				+	
12	*L. lactis* KM822772.1	+	+	+		+
13	*L. mesenteroides*	+				+
14	*L. plantarum* KJ003857.1		+	+	+	

Meanwhile, amylase was the most active enzyme noticed, with its activity ranging between 31.04 ± 1.13mmol/min/mL in E90.3 and 48.72 ± 1.13mmol/min/mL in C90.1 ogi samples. Maltase and sucrase activities were also prominent in E120.3 (28.34 ± 1.10 and 17.54 ± 1.69mmol/min/mL respectively). There is no significant difference in amylase activity for samples C90.1, E90.3 and the control. Similarly, there was no significant difference in the protease activity in all the samples. However, there were variations in the sucrase, maltase, glucanase, xylanase and lipase activities in the samples. Xylanase activity was extremely low (0.04 ± 0.03 to 0.40 ± 0.05mmol/min/mL).

Monitoring the growth of Lactic acid bacteria in fermented maize gruel

The growth of lactic acid bacteria (LAB) in maize gruel during fermentation was monitored (Table 7). The community ogi (fermentation starter) had a load of $\log_{10}$ 5.0 CFU/g. The initial inoculum size of the experimental maize gruel ranged from $\log_{10}$ 4.74 CFU/g in 50g maize grain particles inoculated with 6g fermentation starter and parboiled for $1^1/_2$h (C90.1) to $\log_{10}$ 4.94 CFU/g in E120.3 sample (50g maize grain particle mixed with 10g fermentation starter). There was an increase in the LAB cells in all the samples and the control when continuously stirred and fermented at 60^{o}C for various periods. Even 30min after the introduction of the community *ogi,* there was a percentage increase of 13.2, 24, 29 and 36.5 in the microbial cells in E120.3, D60.1, C90.1 and the control respectively. The microbial load had almost doubled (1: 1.88) in C90.1 after 90min. The growth rate of the LAB cells in E90.1 sample was the highest (0.06CFU/min) while the rate was lower in the other samples (D60.1, 0.048 CFU/min; E120.3, 0.049 CFU/min; C90.1, 0.054 CFU/min; and E90.3, 0.06 CFU/min).

Following fermentation at 60^{o}C for various periods, each maize gruel sample was washed and sieved before the second fermentation at ambient temperature 25^o ± 2^{o}C. The microbial load at this point had reduced by 0.07, 15.5 and 16% in D60.1, C90.1 and E90.3 samples respectively. However, a slight increase was observed in the concentration of microbial cells in sample E120.3. Correspondingly, the growth rate (ranging between 0.003 and 0.012 CFU/min) of the LAB cells in these fermented maize gruel samples was very low after washing and sieving.

Lactic acid bacteria associated with fermented maize gruel

The lactic acid bacteria isolates obtained from the different ogi samples including the fermentation starter (community ogi) are shown in Table 8. The cells of *Lactobacillus lactis, L. fermentum* NR_104927.1, *L. brevis*, *L. fermentum* JQ669802.1, *L. mesenteroides, L. fermentum* CP011536.1 were isolated from the community *ogi* (fermentation starter). Except for *L. fermentum* NC_010610.1 and *L. fermentum* KT633926.1, similar types of LAB strains found in the community *ogi* were also isolated from E120.3. The different strains of *L. fermentum* (CP011536.1 with 99% identity, JQ669802.1 with 74% identity, KT633926.1 with 88% identity, NC_010610.1 with 99% identity and NR_104927.1 with 84% identity) obtained from the fermentation starter and E120.3 *ogi* sample constitute about 50% and 62.5% of the LAB cells. This group of organisms found among the consortium of fermenting microbes was presumed to have been responsible for ogi production.

DISCUSSION

In our previous work, it was observed that the occurrence and growth of the chance inoculants reached the peak (1.63 x 10^7 CFU/g) when the maize grain was fermented for 72h in the traditional method of producing *ogi* (Adebayo and Aderiye, 2007). The concentration of lactic acid bacteria (LAB) cells was reported to constitute between 7 and 48.5% of the total bacteria load within 24- 48h. Olsen *et al.* (1995) also reported a reduction in the total bacteria load of steeped maize from 10^6 to 10^2 CFU/g after 24h fermentation which was attributed to an interaction between the microflora of the indigenous fermented foods and the total bacteria counts of the non-lactics. With the benefit of this hindsight, a novel fermentation process was developed to sidetrack the conundrum experienced among the fermenting microorganisms in the early stages of fermentation by the introduction of a fermentation starter (a consortium of fermenters from previous food product). The moisture content of stored maize grain used in this study was between 9.5 and 10.25%. In this state, the constituents of the maize grain are not readily available to the microorganisms and the endogenous enzymes are inactive (Hammes *et al.*, 2005). Fermentation was therefore enabled under the influence of improved technological procedures including comminuting of the maize grains, addition of water and parboiling to ensure controlled management of microorganisms and enzyme activities. The particle size reduction of the maize grain was very advantageous to the fermentation process. The addition of water affected the ecological factors considerably, increasing the water activity (through water absorption), reducing the redox potential and resulting in a drop in the pH (by respiration and fermentation) (Hammes and Ganzle, 1998). With smaller particle size, the maize granules (for example, particles < 1.40mm) absorbed more water even at higher temperatures, which may be as a result of the larger surface area and the heat applied during parboiling (Mustapha, 1979). During retting, it was observed that the starch granules took up water and swelled. Subsequently, the maize ingredients became easily accessible to microbial activity due to endogenous hydrolytic activities such as amylolysis and proteolysis (Davey *et al*., 2002). Also, the physiological activities of deliberately added microorganisms will cause a continuous change of the ecological state in the grain matrix. The starch subsequently underwent gelatinization. It is expected that the shearing effect of stirring will remove the gelatinized outer layer and these long chained starch molecules dissolved into solution (Davey *et al*., 2002). Eventually, hydrolysis will occur and encourage the growth of fermenting organisms. At this stage, amylase cleaves randomly the inner part of the starch molecule as it is an endo type enzyme. Dextrin would be formed in the beginning, and as the process continues the maltoses will accumulate (Kolusheva and Marinova, 2007).

The mineral content of the grains is generally sufficient for microbial growth but differs in various fractions obtained after particle size reduction (milling) (Betschart, 1988). Meanwhile, in this study, magnesium and potassium were very essential for microbial activities. Blandino *et al.* (2003) retorted that the type of bacteria flora developed in any fermented foods depends on the water activity, pH, salt concentration,

temperature, nutritional and composition of the matrix. The ash content is generally recognized as a measure of quality for the assessment of the functional properties of foods (Hofman *et al.,* 2002). All the *ogi* samples contained low levels of ash, between 3.30 and 4.33%. This translates to high potassium (52.25 to 53.41mg/10g) and magnesium (42.36 to 42.4mg/10g) contents, followed by calcium and iron. Potassium is an electrolyte that counteracts the effects of sodium, helping to maintain a healthy blood pressure. It is also important for maintaining acid-base balance. Potassium's primary functions in the body include regulating fluid balance and controlling the electrical activity of the heart and other muscles (He and MacGregor, 2008). Man requires at least 100 milligrams of potassium daily which *ogi* can provide if consumed sufficiently to support key bodily processes. Magnesium has a medium level bioavailability; it is predominantly absorbed by the small intestine, with the efficiency of absorption depending on the amount of magnesium in the diet, the health of the gastrointestinal tract, the overall magnesium status of a person, and their diet as a whole. Ogi can conveniently restore and maintain deteriorating human health status as reported by Aderiye and Ajibade (2007), possibly due to the presence of magnesium. Ogi with 2.85 to 3.97mg Ca/10g can be considered an appropriate dietary source of calcium to maintain the biological role of nerve transmission, muscle contraction, glandular secretion as well as mediating vascular contraction and vasodilation (Straub, 2007). Nearly all living beings, from single-celled bacteria to humans, require iron. The human body typically contains 2 to 5 grams of iron, depending on the height, weight and muscle mass. The body efficiently recycles iron with daily loss usually summing up to only 1 to 2 milligrams. The RDA recommended for iron (8 milligrams, of iron daily) by the Institute of Medicine (2001) is greater than the amount lost daily because the body typically absorbs only 10 to 15 percent of the iron contained in food. The amount of iron found in the fermented maize gruel (1.20–1.24 mg/10g) may be high enough as reported by Aggett (2012) to suggest *ogi* as a good source of dietary iron to overcome nutritional deficiency of iron.

The concentration of free total sugars in cereal grains range between 0.5 and 3%, with sucrose as the major compound representing about 50% of the sugars (Shelton and Lee, 2000). With the activities of maltase present in the endosperm, maltose generation in the gruel proceeds efficiently after the addition of water to grain particles. The cooperation of enzymes such as amylolytic enzymes and the reductases provides microbes with the ability to vigorously metabolize carbohydrates (Lee *et al.*, 2016). Sugars are generally consumed as carbon sources, yielding energy for proliferation and growth via carbohydrate metabolic pathways. Polysaccharides must be degraded by microbial enzymes such as amylase or glycosidase before being absorbed (Gänzle, 2014). Our result showed that amylase activities were higher than other enzyme activities. Amylase was reported to decompose starch into disaccharides and oligosaccharides, which helped in the production of monosaccharides in carbohydrate microbial metabolism (Baek *et al.,* 2010). Enzymes produced by microorganisms inoculated as starter affect the repertoire of metabolites found in *koji* fermentations (Kum *et al*., 2015). This relationship means that the microbial fermenters may also have decisive effects, as in ogi, on the favour and nutritional value of the product. Nevertheless, in terms of natural product stability, high moisture content tends to promote microbial contamination and chemical degradation (Hussain *et al.,* 2009), as it provides a medium for many reactions to occur. The low moisture contents observed in ogi samples showed that the fermented maize gruel samples can be stored for long time. For instance, the low moisture content in the food samples did not facilitate the growth and multiplication of microorganisms and the activities of these microorganisms are the major factors responsible for the food spoilage (Aderiye and Laleye, 2003). It is evident that fermentation increased the protein content of the fermented food products (15.47-21.73%) compared to its value of 11.2% in raw maize grains (Adebayo and Aderiye, 2007). This increase was attributed to microbial activities, which involved using crude fiber and carbohydrate as sources of energy and synthesizing structural proteins that are integral part of the microbial cells (Nwokoro and Chukwu, 2012). This present result agrees with the findings of Inyang and Zakari (2008) who reported on the increase in protein content of fermented food products. The crude fibre content of the samples ranged between 4.0 ± 0.28% and 5.2 ± 0.43%. Nutritional study has shown that adequate fiber intake render some health benefits like lowering the serum cholesterol level, risk of coronary heart diseases, hypertension, constipation and diabetes (Ishida *et al.*, 2000).

Adebayo and Aderiye (2007) reported that the cells of *Lactobacillus fermentum* were frequently isolated from the maize grains shortly (1h) after retting the substrate under water and throughout the 3-4 day natural fermentation. In this study, there was an increase in the LAB cells in all the samples and the control when continuously stirred and fermented at 60^{o}C for various periods, with the growth rate as high as 0.06CFU/min. Thirty (30) minutes after the introduction of the community *ogi* (fermentation starter), there was a percentage increase of 24 to 36.5 in the microbial cells in all the samples. The microbial load had almost doubled (1: 1.88) in C90.1 after 90min. It is obvious that the incidence of *Lactobacillus casei* (C90.1; E90.3), *L. cellobiosus* (D60.1), *L. fermentum* KU851162.1 (C90.1), KT633926.1 (E120.3), NC_0106100.1 (E120.3), *L. helviticus* (E90.3), and *L. plantarum* KJ003857.1 (D60.1; C90.1; E90.3) in the respective fermented maize gruel samples did not emanate from the fermentation starter. The occurrence of *Lactobacillus casei* (C90.1; E90.3), *L. cellobiosus* (D60.1), *L. helviticus* and *L. plantarum* KJ003857.1 (D60.1; C90.1; E90.3) may have originated from the maize grains while the isolation of *L. fermentum* KU851162.1 (C90.1), KT633926.1 (E120.3), NC_0106100.1 (E120.3) could possibly has arisen from the genetic modification of other strains of *L. fermentum* in the fermentation starter (community ogi), due to heat treatment.

Conclusion

The traditional production of ogi depends on the preference of the individual (manufacturer/consumer) such as taste (sourness or sweetness), smoothness (texture and/or viscosity). The experimental ogi samples have given expression to the variations preferred by different processors and compared favourably to the community ogi. These 'novel' bioproducts and the bioprocess are therefore recommended for industrial

production and commercialization of ogi. The automation of this bioprocess is in progress.

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