

Full Length Research Paper

Microbiological and physico-chemical changes during fermentation of maize for *masa* production

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Accepted 12 September, 2013

Samples of traditionally fermenting maize meal popularly called *masa* were collected from local producers of *masa* in Ibadan, Oyo State, Nigeria and were analysed for pH, total titratable acidity (TTA), microbiological changes and proximate analysis. The sensory properties (colour, texture, taste, sourness, appearance and general acceptability) were also carried out on a nine point hedonic scale. The values obtained were compared with the starter culture fermenting maize meal prepared in the laboratory. The physico-chemical changes during the traditional and the laboratory prepared fermenting maize meal were observed at 6 h interval during the fermentation process. There was decrease in pH; the pH of the traditional fermenting maize meal ranged between 5.98 ± 0.27 to 3.95 ± 0.05 while the pH of the laboratory prepared fermenting maize meal ranged between 6.4 ± 0.36 to 4.3 ± 0.03 . There was increase in TTA during the fermentation period; in the traditional fermenting maize meal the TTA ranged between 0.144 ± 0.02 to 0.792 ± 0.08 and it ranged between 0.054 ± 0.02 to 0.252 ± 0.00 in the laboratory fermenting maize meal. Microorganisms associated with the traditional fermentation of *masa* were *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus fermentum* and *Saccharomyces cerevisiae*. In all the fermenting maize meal samples collected and the laboratory prepared maize meal samples inoculated with starter organisms, an increase in the microbial load counts (\log_{10} cfu/ml) was observed as the fermentation time progresses, 3.02 to 6.41 (on De Mann Rogosa-Sharpe agar), 2.99 to 3.62 (on malt extract agar), 4.18 to 7.00 (on nutrient agar) during the traditional fermentation, in the laboratory prepared maize meal samples inoculated with starter organisms, the microbial load counts (\log_{10} cfu/ml) ranged between 4.01 to 9.05. The laboratory prepared maize meal samples inoculated with starter organisms only showed minimal changes in their proximate composition as compared with the traditionally fermented samples collected. The statistical analysis of the sensory attributes revealed consumer's acceptance of the product inoculated with the starter organisms.

Key words: Proximate analysis, TTA, physico-chemical changes, *masa*, starter organisms.

INTRODUCTION

Masa is a traditional fermented snack food that is widely prepared and consumed in Northern and some part of Western states in Nigeria. It can be produced from maize (*Zea mays*), millet (*Pennisetum typhoideum*), sorghum

(*Sorghum vulgare*) and rice (*Oryza sativa*). *Masa* serves as breakfast and snacks items for many people (Ayo et al., 2008). Good quality *masa* is round shape, having brown smooth body. *Masa* is eaten both casually and

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ceremonially. It is eaten with granulated sugar or with honey because of its sour tastes due to its high acidity. *Masa* is eaten with honey every Friday after Jumat prayer's by the Yoruba Muslims and it is also eaten on any other days with sugar because of high cost of honey. *Masa* is also produced for consumption a night preceding ceremonies such as funerals, weddings or 40th day after child's birth and are eaten in the following morning. *Masa* is still produced traditionally in the home by the local women and the fermentation is spontaneous and uncontrolled. The fermentation of *masa* is performed by various lactic acid bacteria including *Lactobacillus* sp, *Pediococcus* spp. and yeast *Saccharomyces* sp. (Oyeyiola, 1990).

Cereals are the most important source of the world's food and have significant impact in human diet throughout the world (Adebayo et al., 2010). Cereal grains constitute an important group of substrate for fermented foods in tropical Africa (Odunfa and Oyewole, 1998). The main cereals grown in Nigeria are maize, guinea corn, rice, millet and sorghum. Maize (*Zea mays*) is an important cereal grain in the world and it has a diverse form of utilization including human food uses, animal feeds formulation and as a basic for industrial purpose (Courtois et al., 1991). Maize can be processed into a wide range of foods, snacks and beverages (Afoakwa et al., 2007). Fermentation is widely applied in the processing of cereals for the preparation of a wide variety of dishes in developing countries (Obiri-Danso, 1994). Many African foods are fermented before consumption. Fermented foods constitute a significant component of African diet (Odunfa, 1985). Several maize based fermented products, such as ogi in West Africa, togwa in Tanzania, banku and kenkey in Ghana, mahewu in South Africa, mawe in Benin have been documented (Jepersen et al., 1994). Various workers have carried out studies on fermentation of cereal products (Odunfa, 1985; Adegoke and Babalola, 1988; Sanni et al., 1994; Mbata et al., 2009; Adebayo et al., 2010). However, there is little information on the fermentation of maize grains for *masa* production.

This study was undertaken to investigate the microbiological and physico-chemical changes during the traditional fermentation and starter culture fermentation processes, also presented is the proximate composition and the sensory evaluation of *masa* produced from maize grains.

MATERIALS AND METHODS

Collection of samples

Samples of traditional fermenting white variety of maize meal were collected aseptically from 5 different local household producers of *masa* in Ibadan. During the fermentation process of the samples collected and the laboratory prepared samples inoculated with starter organisms, samples were taken at zero time and 6 h intervals and were analyzed for pH, total titratable acidity (TTA) and

microbial load counts.

Laboratory preparation of *masa*

The traditional method of preparation of *masa* was a slight modification from Oyeyiola (1990). It involved steeping white variety of maize grains in warm water for 4 h and the water was drained off. The maize grains were wet milled, the milled product was mixed with water forming semi solid slurry, and the mash was then covered tightly with tray and allowed to ferment for 24 h at 28 ± 2°C. Onions and salt were added to the fermented maize product and was rolled by hand into balls and fried in hot vegetable oil for 5-8 min.

Determination of pH

The pH of the samples collected (from five different household producers in Ibadan) and the laboratory prepared maize meal inoculated with starter organisms; singly and in combinations were determined according to the method of AOAC (1999). Ten gram of sample was mixed in 100 ml of CO₂ - free distilled water. The mixture was allowed to stand for 15 min shaken at 5 min interval and filtered with Whatman No. 14 filter paper. The pH of the filtrate was measured in duplicate using a pH meter (Model HM-305, Tokyo, Japan).

Determination of total titratable acidity (TTA)

Ten millilitre aliquots of the samples were pipetted and titrated against 0.1 M NaOH solution to phenolphthalein end point and the acidity was calculated as g lactic acid / 100%.

Microbiological analysis

At zero time and 6 h intervals (0, 6, 12, 18 and 24 h), ten gram of each fermenting maize meal was homogenized in 90 ml sterile distilled water for 30 s. The mixture was serially diluted in sterile distilled water by the method of Meynell and Meynell (1970) and from the 10 fold dilutions, colony-forming units (cfu) were determined using pour plate method. Plate counts were carried out using the following media, temperature and incubation periods; De Mann Rogosa-Sharpe (MRS) agar (Oxoid, UK) 37°C, 48 h for LAB; Malt extract agar (MEA) supplemented with streptomycin (30°C), 72 h for yeasts; and Nutrient agar (NA) 37°C, 48 h for total viable counts. Incubation for LAB was done under anaerobic conditions.

Proximate analysis

Samples of the traditional fermented maize meal collected and the laboratory prepared maize meal inoculated with starter organisms were analyzed by the standard procedures as adopted by AOAC (1999) for ash, crude fibre, ether extract, crude protein and the carbohydrate content was estimated by the difference in value obtained when all the chemical composition values were subtracted from 100%.

Sensory evaluation

Sensory evaluation of samples collected from the traditional fermented maize meal and the laboratory prepared maize meal inoculated with starter organisms was carried out to determine their organoleptic characteristics. A twenty member panel of judges

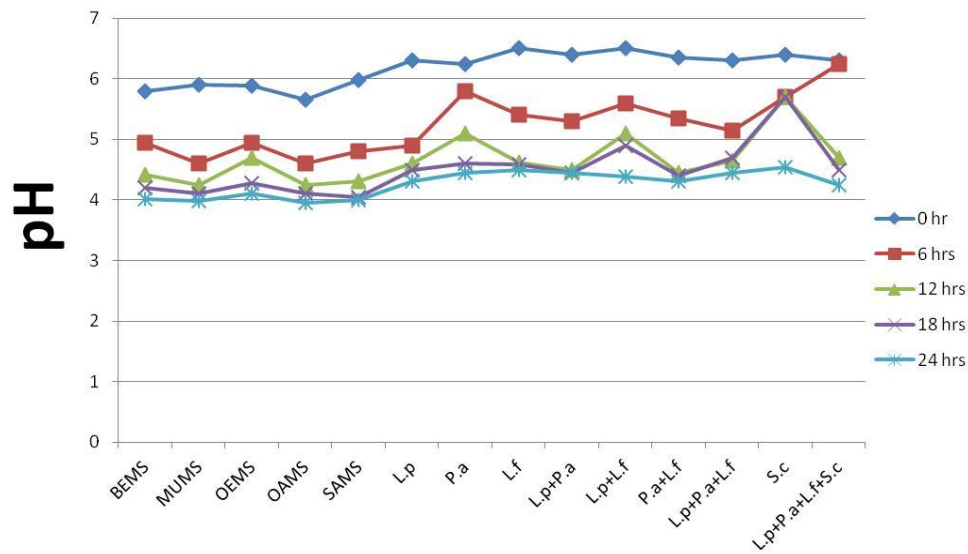


Figure 1. Changes in pH of the traditional fermenting maize meal and the laboratory prepared maize meal inoculated with starter organisms. BEMS: Bere masa sample; MUMS: Muslim masa sample; OEMS: Oje masa sample; OAMS: Oja Oba masa sample; SAMS: Sasa masa sample.

consisting of students and laboratory workers of the University of Ibadan, Department of Microbiology, who are familiar with *masa* was constituted. The panel members were asked to rate the samples for colour, texture, taste, sourness and appearance. The ratings were presented on a 9 - point Hedonic scale ranging from 9 = like extremely to 1 = dislike extremely.

Statistical analysis

Data obtained was subjected to analysis of variance (ANOVA) to determine the least significant differences in their organoleptic attributes at $p \leq 0.05$.

RESULTS AND DISCUSSION

Five samples of fermenting maize meals (*masa*) collected were designated as; BEMS - 'Bere *masa* sample', MUMS - 'Muslim *masa* sample', OEMS - 'Oje *masa* sample', OAMS - 'Oja Oba *masa* sample', and SAMS - 'Sasa *masa* sample' from household producers of *masa* all within Ibadan metropolis. Results obtained in Figure 1 showed the pH of the traditional fermenting maize meal and the laboratory prepared maize meal inoculated with starter organisms. The pH decreased as the fermentation time progresses from 0 - 24 h, the pH of the traditional fermenting maize meal decreased gradually and ranged between 5.98 ± 0.27 (0 h) to 3.95 ± 0.05 (24 h), the pH of the laboratory prepared fermenting maize meal inoculated with single and mixed starter cultures of *L. plantarum*, *P. acidilactici*, *L. fermentum* and *S. cerevisiae* also decreased from 6.40 ± 0.36 (0 h) to 4.30 ± 0.03 (24 h). In the fermentation using *L. plantarum* and *L. fermentum* as single starter, the pH decreased sharply after 0 h from 6.30 ± 0.03 to 4.90 ± 0.00 and 6.25 ± 0.05

to 5.00 ± 0.05 and then decreased gradually after 6 h till the end of the fermentation period. Also in the fermentation using *S. cerevisiae* singly, there was decrease in pH from 6.4 ± 0.03 to 5.7 ± 0.36 at 0 - 6 h but the pH remained constant till 18 h then slightly decrease to 5.4 ± 0.03 at the end of 24 h, the observed decrease in pH agrees with the reports obtained by Afoakwa et al. (2004). This decrease in pH observed is also similar to the reports of Oyeyiola (1990), where he observed that much lactic acid was produced during the fermentation of *masa* which led to a progressive fall in pH and gives the product a sour taste. The low pH obtained after fermentation of the maize meal is important since most bacteria including the pathogenic organisms do not survive in low pH environment and this imparts microbial safety as well as increasing the shelf life of the final product.

Changes in total titratable acidity level in the traditional fermenting maize meal collected and the laboratory prepared maize meal inoculated with starter organisms is shown in Figure 2. The titratable acidity in the five different batches and the starter culture fermenting maize meal used singly and in mixed culture showed a slight increase with time. Increases observed in the titratable acidity is similar with the reports obtained by Afoakwa et al. (2007) and Adesokan et al. (2010).

A total of 84 microbial strains were isolated during the traditional fermentation process and were differentiated on the basis of their cultural, morphological, physiological and biochemical characteristics. Results of the microbial analysis of the traditional fermenting and starter culture fermenting maize meal are presented in Figure 3a, b, c and d. The microorganisms appeared at every stages of fermentation and the cell counts (\log_{10} cfu/ml) increased

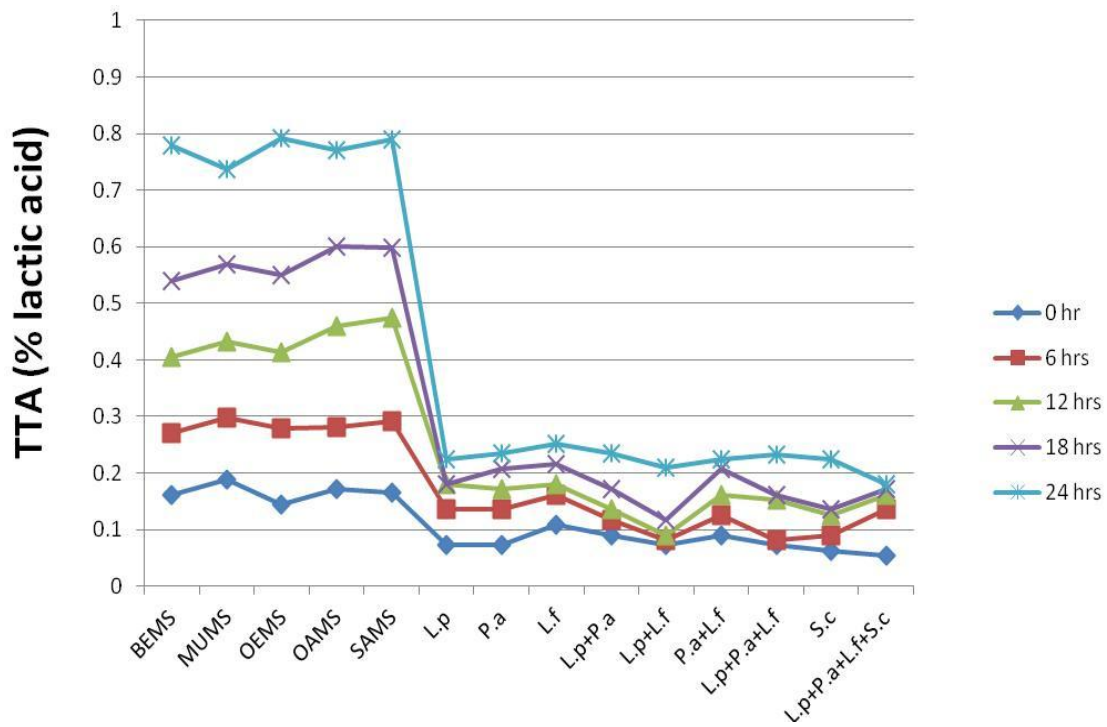


Figure 2. Changes in titratable acidity of the traditional fermenting maize meal and the laboratory prepared maize meal inoculated with starter organisms. BEMS: Bere masa sample; MUMS: Muslim masa sample; OEMS: Oje masa sample; OAMS: Oja Oba masa sample; SAMS: Sasa masa sample.

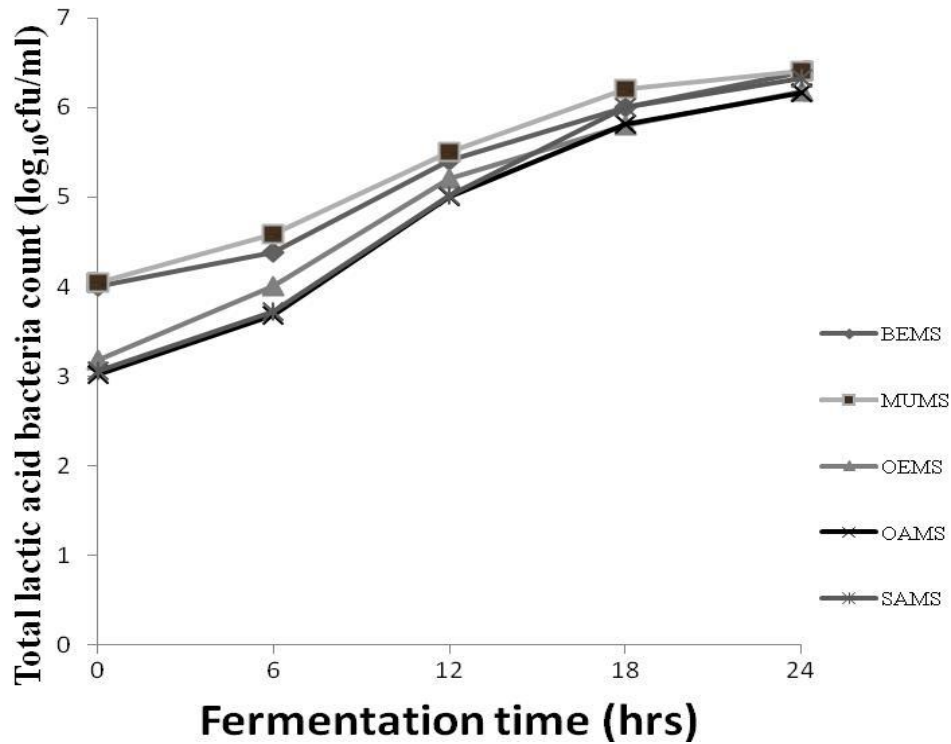


Figure 3a. Total lactic acid bacteria count (\log_{10} cfu/ml) during traditional fermentation of maize meal for masa production. BEMS: Bere masa Sample; MUMS: Muslim masa Sample; OEMS: Oje masa Sample; OAMS: Oja Oba masa Sample; SAMS: Sasa masa Sample.

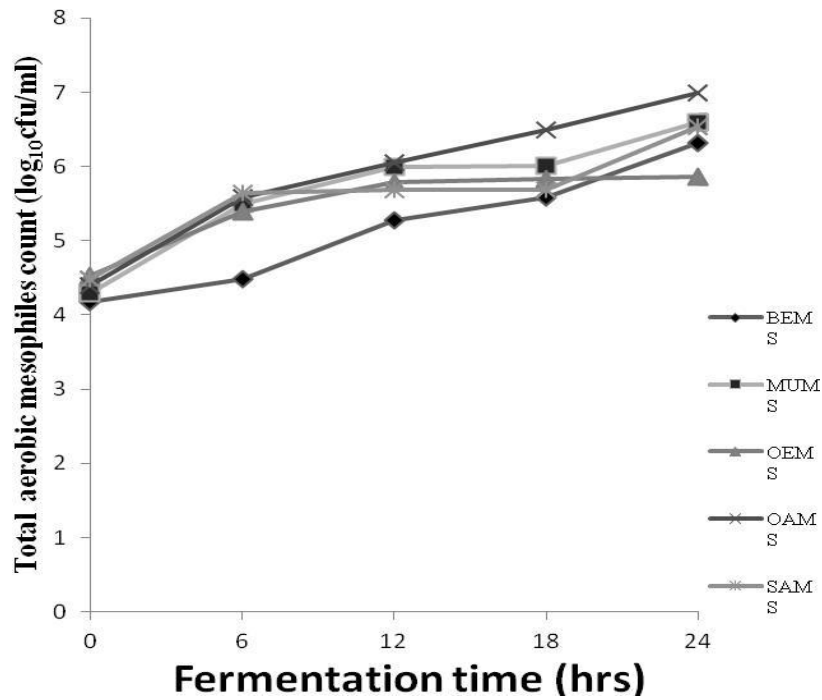


Figure 3b. Total aerobic mesophiles count ($\log_{10}\text{cfu/ml}$) during traditional fermentation of maize meal for *masa* production. BEMS: Bere masa Sample; MUMS: Muslim masa Sample; OEMS: Oje masa Sample; OAMS: Oja Oba masa Sample; SAMS: Sasa masa Sample.

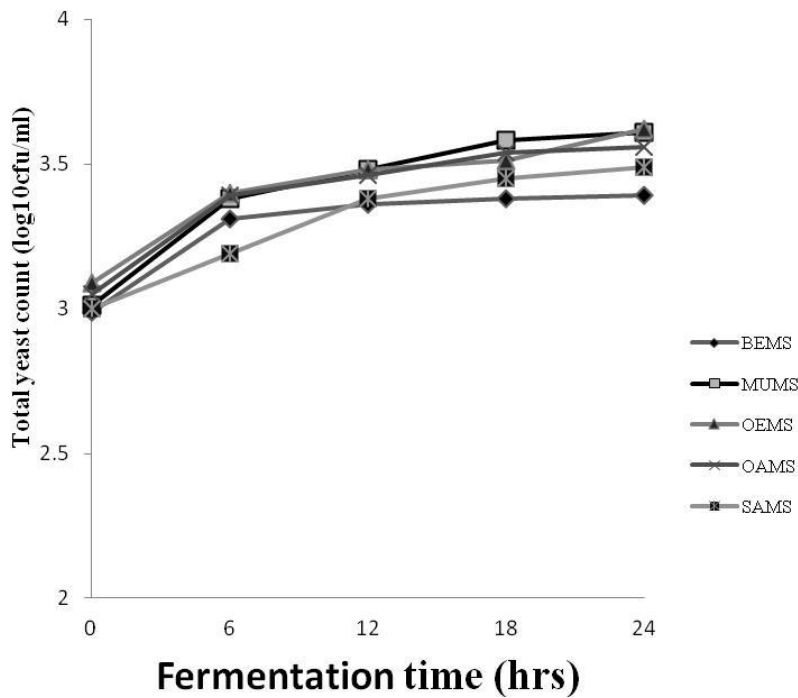


Figure 3c. Total yeast count ($\log_{10}\text{cfu/ml}$) during traditional fermentation of maize meal for *masa* production. BEMS: Bere masa sample; MUMS: Muslim masa sample; OEMS: Oje masa Sample; OAMS: Oja Oba masa sample; SAMS: Sasa masa sample.

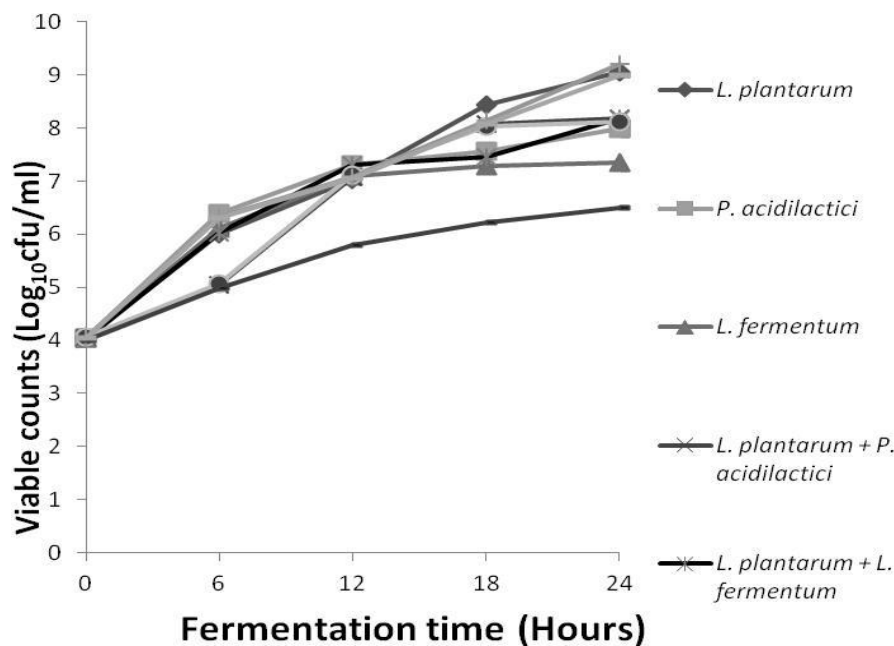


Figure 3d. Changes in viable counts of the organisms inoculated as starter cultures with single and in different combinations ($\log_{10}\text{cfu/ml}$). BEMS: Bere masa sample; MUMS: Muslim masa sample; OEMS: Oje masa sample; OAMS: Oja Oba masa sample; SAMS: Sasa masa sample.

with fermentation time during the traditional fermentation from 0 - 24 h. It ranged from 3.02 (0 h) to 6.41 (24 h) on MRS plates, 2.99 to 3.62 (24 h) on MEA plates and 4.18 (0 h) to 7.00 (24 h) on NA plates respectively. Other researchers have reported similar organisms from fermenting maize dough and gruels. Oyeyiola (1990) identified *L. plantarum*, *P. acidilactici* and *L. fermentum*; as associated microorganisms of fermented maize grains for *masa* production. The dominance of lactic acid bacteria in the traditional fermentation of maize based product was reported by some researchers. Halm et al. (1993) reported obligately heterofermentative lactic acid bacteria as the predominant organisms of fermented maize dough for *kenkey* production. Sanni et al. (1999) also identified *L. plantarum*, *L. brevis*, *L. casei*, *P. pentosaseus*, *P. acidilactici*, and *Lactobacillus* sp. as the organisms that were isolated from spontaneously fermented sour maize meal during the production of sour maize bread using starter culture.

The proximate composition of the fermented maize meal for *masa* production, through traditional fermentation and using different starter cultures was determined as shown in Table 1. There was general decrease in carbohydrate content of all starter cultures fermented maize meal (37.4, 39.54, 38.67, 41.10 and 39.50%) compared to the uncontrolled fermented samples. Fermented maize meal inoculated with combination of *L. plantarum*, *P. acidilactici*, *L. fermentum* and *S. cerevisiae* as starter culture had the highest ash

content (0.60%) and protein content (4.36 %) followed by fermented maize meal inoculated with *L. fermentum* which is 0.59% ash and 4.25% protein, while the least was recorded in fermenting maize meal inoculated singly with *P. acidilactici*. The highest ether extract was obtained in the maize meal fermented with *L. fermentum*, *P. acidilactici*, *L. plantarum* and *S. cerevisiae*. The crude fibre content of traditionally produced *masa* was lower than those of the product inoculated with starter cultures.

The results of the organoleptic assessments of *masa* inoculated with starter cultures of *Lactobacillus* sp., *Pediococcus* sp., *Saccharomyces* and *masa* produced by traditional fermentation are shown in Table 2. There was no significant difference in colour, texture and sourness from *masa* that was produced using starter organisms but were superior to *masa* produced through traditional fermentation. However, a significant difference ($p \leq 0.05$) in taste was observed between *masa* produced by single starter organisms of *S. cerevisiae*.

Conclusion

This study has complemented the knowledge on maize utilization for human food and has given an insight into microorganisms responsible for the traditional fermentation of maize for *masa* production. The use of lactic acid bacteria isolates from the uncontrolled fermentation process as starter organisms to ferment the laboratory

Table 1. Proximate composition of fermented maize meal for *masa* production through traditional fermentation

Parameters (%)	BEMS	MUMS	OEMS	OAMS	SAMS	<i>L.</i> <i>plantarum</i>	<i>P.</i> <i>acidilactici</i>	<i>L.</i> <i>fermentum</i>	<i>S.</i> <i>cerevisiae</i>
Moisture content	59.20	57.00	57.80	58.85	59.25	55.5	54.0	54.9	52.5
Ash	0.51	0.41	0.43	0.49	0.50	0.58	0.51	0.50	0.49
Crude fibre	0.34	0.28	0.27	0.29	0.32	0.54	0.44	0.52	0.40
Ether extract	0.98	0.88	0.93	0.97	1.00	1.35	1.31	1.25	1.30
Crude protein	4.78	4.01	3.94	4.28	4.70	4.29	4.20	4.16	4.21
Carbohydrate	34.19	37.42	37.15	35.12	34.23	37.7	39.5	38.7	41.1

Crude protein= % Nitrogen X 6.25.

BEMS: Bere masa Sample

MUMS: Muslim masa Sample

OEMS: Oje masa Sample

OAMS: Oja Oba masa Sample

SAMS: Sasa masa Sample.

Table 2. Sensory evaluation of *masa* inoculated with starter cultures of *Lactobacillus sp.*, *Pediococcus* *Saccharomyces* and *masa* produced by traditional fermentation.

Code	Organoleptic attributes scored					
	Colour	Texture	Taste	Sourness	Appearance	General acceptability
101	3.89 ^{abc}	4.11 ^a	3.44 ^{bc}	3.56 ^a	3.89 ^{ab}	4.00 ^{ab}
102	3.56 ^{abc}	3.67 ^{ab}	3.44 ^{bc}	3.56 ^a	3.89 ^{ab}	3.56 ^{ab}
103	3.22 ^{bc}	3.44 ^{abc}	3.33 ^d	3.58 ^a	3.89 ^{ab}	3.78 ^{ab}
104	4.00 ^{ab}	3.33 ^{abc}	4.33 ^a	3.56 ^a	3.56 ^{ab}	4.33 ^a
105	3.22 ^{bc}	3.44 ^{abc}	3.78 ^{ab}	3.56 ^a	3.89 ^{ab}	4.11 ^a
106	3.22 ^{bc}	3.33 ^{abc}	4.11 ^{ab}	3.50 ^a	3.89 ^{ab}	2.89 ^c
107	3.67 ^{abc}	3.67 ^{ab}	3.89 ^{ab}	3.44 ^a	4.00 ^{ab}	4.22 ^a
108	4.22 ^a	3.78 ^{ab}	4.00 ^{ab}	2.58 ^c	3.22 ^a	2.22 ^c
109	3.44 ^{abc}	3.25 ^{bc}	4.56 ^d	3.53 ^a	4.00 ^{ab}	4.22 ^a
110	3.00 ^c	2.78 ^c	4.10 ^{au}	3.40 ^a	3.80 ^{au}	3.89 ^{au}

Values represent the mean scores

Mean having the same superscripts within each column do not differ significantly ($P \leq 0.05$).

101: *Masa* produced with *L. plantarum*

102: *Masa* produced with *P. acidilactici*

103: *Masa* produced with *L. fermentum*

104: *Masa* produced with *L. plantarum* + *P. acidilactici*

105: *Masa* produced with *L. plantarum* + *L. fermentum*

106: *Masa* produced with *P. acidilactici* + *L. fermentum*

107: *Masa* produced with *L. plantarum* + *P. acidilactici* + *L. fermentum*

108: *Masa* produced with *S. cerevisiae*

109: *Masa* produced with *L. plantarum* + *P. acidilactici* + *L. fermentum* + *S. cerevisiae*

110: *Masa* produced by uncontrolled natural fermentation

prepared *masa* suggested the most important lactic acid bacteria species and yeast that are involved in the fermentation process.

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