MICROBIOLOGICAL CHANGES DURING THE PRODUCTION OF MAIZE-*ACHA MASA* FORTIFIED WITH SOYBEAN

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Abstract
The study investigated the effect of maize substitution with acha and the fortification with soybean on the microbiological characteristics of the fermenting masa. Total viable counts, lactic acid bacteria counts, yeast counts, pH and titratable acidity were determined and the associated microorganisms were isolated and identified. The results showed that total viable counts, lactic acid bacteria counts and yeast counts were 6.11 - 14.28 log cfu.g^{-1}, 7.00 - 14.98 log cfu.g^{-1} and 2.60 - 5.54 log cfu.g^{-1} respectively during fermentation. Lactic acid bacteria isolates were identified as Lactobacillus acidophilus, Leuconostoc mesenteroides, Lactobacillus plantarum, Pediococcus dextrinicus and Lactobacillus delbrueckii. Bacillus substilis, Bacillus megaterium, Bacillus pumilus and Micrococcus luteus were also isolated. Yeast isolates were identified as Saccharomyces cerevisiae, Brettanomyces claussenii and Torulopsis versatilis. Aspergillus niger was isolated at the beginning of fermentation. The pH was within the range of 2.47 - 6.43 and the titratable acidity was 0.68 % -1.34 %. The pH decreased while the titratable acidity increased during the period of fermentation. Masa produced from 100 % acha generally had higher lactic acid bacteria count than 100 % maize masa. Fortification of masa with soybean generally increased the lactic acid bacteria count. Masa samples fortified with 20% soybean had higher lactic acid bacteria and yeast counts during the period of fermentation.

Keywords: Lactic acid bacteria, fermentation, fungi, *acha*, titratable acidity

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1. INTRODUCTION

Masa is a cereal-based spontaneously fermented cake popularly consumed as an adjunct to breakfast porridges (Owuzu-Kwarteng and Akanbada, 2014). It is produced from maize (*Zea mays*), millet (*Pennisetum glaucum*) or rice (*Oryza sativa*) depending on the producer's preference. It is common in the Northern and South-western part of Nigeria. The brown crisp edges and the mild sour taste are considered by many consumers as the quality attribute required of *masa* (Ayo *et al.*, 2014). Different types of cereal grain used for *masa* production have been reported to have different effects on physical qualities of *masa* such as thickness, weight, volume, volume index and sensory characteristics such as taste, aroma, texture and degree of sourness (Igwe *et al*., 2013).

A food is considered fermented if one or more of its constituents has been acted upon by microorganisms so that the chemical composition of the final product is considerably altered (Abiose *et al.*, 1982). Fermentation is also known to soften food texture and alter its composition in such a way that it will require minimal energy both in the cooking and preservation process (Chelule *et al*., 2010). It is one of the important techniques employed to extend the shelf life of raw food materials, to develop and add aroma to a variety of diets (Achi, 2005; Isabel *et al.*, 2005). It is an environment friendly process, produces less waste and is easy to manage under household conditions as well as on industrial scale (Ramachandran *et al.*, 2003). Fermentation of cereals by lactic acid bacteria has been reported to increase acidity, total free amino acids and their derivatives by proteolysis and/or by metabolic synthesis. pH is also reduced thereby inhibiting pathogenic organisms (Mugula *et al.*, 2003; Kohajdová and Karovičová, 2007).

Maize (*Zea mays*) is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production. It ranks third in the world after rice and wheat and

ranks fourth after millet and rice in Nigeria. Its production is quite common in all parts of Nigeria, from the north to the south, with an annual production of about 5.6 million tones (Abdulrahaman and Kolawole, 2006). Preparation and uses of the maize grains vary from group to group. It can be cooked, roasted, fried, ground, fermented, pounded or crushed to prepare food such as pap, *tuwo*, *dokunnu*, *masa*, *abari*, *aadun ogi* and a host of others. (Adegunwa *et al.*, 2011; Oladejo and Adetunji, 2012).

Acha (*Digitaria exilis*) is a highly nutritious cereal crop of West African origin belonging to the family *Graminaea* (Harlan, 1993; Jideani, 1999; Chukwu and Abdul-Kadri, 2008; Satimehin and Philip, 2012). It is also known as *Fonio*, *Findi*, *Funde*, *Pom*, *Kabug* and hungry rice in different West African countries (Oyetayo and Agbaje, 2012). In Nigeria, various processing methods such as cooking, boiling, roasting and fermenting are involved (Oyetayo and Agbaje, 2012). It does not contain any glutenin or gliadine proteins which are the constituents of gluten, making this cereal suitable for people with gluten intolerance (Harlan, 1993; Jideani, 1999; Ayo *et al.*, 2014). The aim of this study is to determine the effects of substitution of *acha* and the soybean fortification on the microorganisms during the production of *masa*.

2. MATERIALS AND METHODS

Raw materials

Acha was obtained from Zaria, Nigeria while quality protein maize (Ile-1-OB) and soybeans (TGX 1740 2E) were obtained from the Institute of Agricultural Research and Training, Ibadan, Nigeria.

Preparation of *masa*

Masa was produced using the modified method of Owuzu-Kwarteng and Akabanda (2014). Maize and *acha* were cleaned, weighed, washed, steeped in water for 12 h at room temperature, washed and drained. Soybean was cleaned and steeped in water for 2 h at room temperature, blanched for 20 min in boiling water and dehulled by hand. The hulls were separated from the cotyledon and drained. Maize, *acha*, and soybean were mixed at ratios: 100:0:0, 0:100:0, 70:20:10, 60:30:10, 60:20:20, 50:40:10, 50:30:20, 40:40:20 and then milled. The batter obtained was divided into three portions. One third of each ground sample was mixed with equal volume of water and then pregelatinized. The pregelatinized portions were mixed with the uncooked two third portions and the resulting batter were spontaneously fermented for 24 h at room temperature and fried in hot vegetable oil.

Microbiological analysis

Fermenting *masa* samples were subjected to microbiological analysis at 6 h interval for 24 h using the pour plate method. Samples (5 g) were weighed into stomacher bag and homogenized with 45 ml of sterile peptone water. The resulting mixture was serially diluted and 1.0 ml of appropriate dilution was dispensed into Petri dish. Nutrient Agar (NA) was used for enumeration of total viable count, de Man Rogosa and Sharpe (MRS) Agar for Lactic acid bacteria count and Potato Dextrose Agar (PDA) for fungi count (Harrigan and McCance, 1976; Harrigan, 1998). Plates were incubated for total viable count at 35^{o}C for 24 h, lactic acid bacteria counts at 35^{o}C for 72 h, yeast and mould count at 25^{o}C for 3 to 5 days (Harrigan, 1998). Following incubation, the colonies on each plate were counted using a Gallenkamp colony counter and pure isolates of the representative colonies were obtained by streaking on media of their primary isolation, incubated appropriately and kept in agar slant under refrigeration condition.

Bacteria isolates were identified using cultural characteristics such as luminescence, shape, elevation, colonial edge, consistency, surface and pigmentation. Biochemical tests such as catalase test, oxidase test, sugar fermentation test (glucose, fructose, lactose, sucrose, maltose, salicin, arabinose, trehalose and galactose), production of carbon dioxide from glucose, nitrate reduction, indole production, methyl red test, production of ammonia from peptone, production of ammonia from arginine,

Voges-Proskauer test and production of hydrogen sulphide were carried out following the scheme of Harrigan and McCance (1976) and Wood and Holzapfel (1995). Yeast isolates were identified using colony characteristics (size, color, elevation, shape, texture, margin, and surface type). Cell shape, size, type of budding and cell aggregation were determined by microscopy (Leica DM500 Model 13613210), and the ability of isolates to assimilate various carbon sources and nitrate were assessed (Beech *et al.*, 1968; Barnett *et al.*, 2000). Mould isolates were identified based on morphological characteristics such as the color of growth on agar, color of mycelium and texture. As well as microscopic (Leica DM500 13613210) features such as hyphae (septate or non septate), characteristics of spore head (size, shape and arrangement), mode of reproduction and presence of special structure such as foot cell or rhizoids were also employed (Harrigan and McCance, 1976, Harrigan, 1998).

Statistical analysis

Data obtained were subjected to descriptive and inferential statistics (Analysis of Variance) using SPSS (version 17 incorporation, Chicago, Illinois, USA). Means of samples was separated using Duncan Multiple range Test.

3. RESULTS AND DISCUSSION

Changes in Total Viable Count during Fermentation of *Masa*

The total viable counts were within the range of 6.11 and 14.28 log cfu.g $^{-1}$ during the period of fermentation (Table 1). There was a general increase from the beginning of fermentation to 12 h followed by a decrease until 24 h. Total viable counts increased in 100 % maize from 6.11 cfu.g $^{-1}$ at 0 h to 10.17 cfu/g at 6 h and 12.29 cfu.g $^{-1}$ at 12 h and then decreased to 11.67 cfu.g $^{-1}$ at 18 h and 10.04 cfu/g at the end of fermentation. Counts also increased in 100 % *acha* from 8.19 cfu/g at 0 h to 9.76 cfu.g $^{-1}$ at 6 h and 14. 00 cfu.g $^{-1}$ at 12 h of fermentation and thereafter decreased to 10.64 cfu.g $^{-1}$ at 18 h and 9.97 cfu.g $^{-1}$ at 24 h. Total viable counts was significantly lower ($p < 0.05$) in 100 % maize than in 100 % *acha* at the beginning of fermentation but was higher in 100 % maize than in 100 % *acha* at the end of fermentation. *Masa* samples fortified with soybean had higher counts at the beginning of fermentation and the counts were also higher in samples fortified with 20 % soybean than in 10 % soybean after 12 h (13.17 to 14.00 log cfu.g $^{-1}$) to 18 h (13.13 to 14.28 log cfu.g^{-1}).

Table 1 Total viable counts (log cfu. g^{-1})

	Hour of fermentation				
Sample	0	6	12	18	24
100 % M	6.11±0.06^{h}	10.17±0.03^{e}	12.29±0.05^{f}	11.67±0.04^{g}	10.04±0.06^{a}
100 % A	8.19±0.09^{g}	9.76±0.04^{g}	14.00±0.03^{c}	10.64±0.03^{h}	9.97±0.04^{c}
70 % M: 20 % A: 10 % S	8.53±0.05^{e}	10.17±0.05^{e}	13.17±0.04^{e}	11.99±0.07^{f}	9.39±0.02^{h}
60 % M: 30 % A: 10 % S	8.91±0.03^{a}	11.18±0.03^{a}	13.17±0.06^{e}	12.29±0.03^{e}	9.43±0.01^{g}
60 % M: 20 % A: 20 % S	8.74±0.06^{c}	10.76±0.01^{d}	14.28±0.01^{a}	13.02±0.08^{c}	9.89±0.02^{e}
50 % M: 40 % A: 10 % S	8.83±0.01^{b}	10.83±0.07^{c}	14.00±0.04^{c}	12.59±0.02^{d}	9.84±0.04^{f}
50 % M: 30 % A: 20 % S	8.34±0.08^{f}	10.15±0.04^{f}	13.31±0.06^{d}	13.35±0.08^{a}	9.93±0.03^{d}
40 % M: 40 % A: 20 % S	8.78±0.11^{d}	10.91±0.02^{b}	14.16±0.04^{b}	13.27±0.04^{b}	10.00±0.01^{b}

M: Maize; A: *Acha*; S: Soybean

Values are means of three replicates ± standard error. Means followed by different superscript in the same column are significantly different at $p < 0.05$

The initial increase may be attributed to multiplication of microorganisms due to the presence of metabolizable nutrients while the decrease may be due to production of metabolites and depletion of some limiting nutrients and that made the environment unfavorable for their growth and activities.

This trend was also reported by Ezeama and Ihezie (2006) during fermentation of rice *masa* fortified with soybean.

Changes in Lactic Acid Bacteria Count during Fermentation of *Masa*

Lactic acid bacteria counts ranged between 7.00 and 14.98 log cfu.g $^{-1}$ during the period of fermentation (Table 2). There were a generally increase from 0 h to 12 h followed by a decrease from 12 h to 24 h. Counts were lowest in *masa* produced from 100% maize (7.00 to 12.82 log cfu.g $^{-1}$) from the beginning to the end of fermentation. Counts were significantly higher ($p < 0.05$) in *masa* samples fortified with soybean at 18 h (12.25 to 14.38 log cfu.g $^{-1}$) and 24 h (10.99 to 111.41 log cfu.g^{-1}) than 100 % *acha* (13.34 at 18 h and 10.1 cfu.g $^{-1}$ at 24 h). This increase may be attributed to the high amino acid content of soybean which could be easily utilized by lactic acid bacteria. According to Owuzu-Kwarteng and Akabanda (2014), the increase in protein content of fermented batter may have contributed with additional amino acids and consequently accelerating the growth of lactic acid bacteria. Lactic acid bacteria was likely responsible for rapid acidification of the raw material through the production of organic acids which exerts a positive effect on food through their activities during fermentation by imparting desirable flavors and inhibiting a variety of food spoilage and pathogenic organism (Fowoyo and Ogunbanwo, 2010; Okpara *et al.*, 2014).

Changes in Yeast Count during Fermentation of *Masa*

Yeast counts of fermenting *masa* ranged between 2.60 and 5.54 log cfu.g $^{-1}$ (Table 3). There was generally no growth at the beginning but visible colonies started appearing at 6 h of fermentation. The counts were higher in 100 % *acha* (3.17 to 3.88 cfu/g) than in 100 % maize (2.60 to 3.11 cfu.g $^{-1}$) from 6 h to 12 h while higher counts were recorded in 100 % maize (3.32 to 4.28 log cfu.g $^{-1}$) than in 100 % *acha* (3.17 to 4.22 log cfu.g $^{-1}$) at 18 h and 24 h. Counts were significantly higher ($p < 0.05$) in samples containing 20 % soybean (5.38 to 5.54 cfu.g $^{-1}$) at the end of fermentation. This increase may be due to the reduction in pH which made the environment conducive for yeast multiplication. Symbiotic relationships exist between yeasts and lactic acid bacteria during fermentation, the bacteria provide the rapid acidic environment and the yeast provide essential metabolites such as pyruvates, vitamins and amino acids for the bacteria (Gadaga *et al.*, 2001; Owuzu-Kwarteng and Akabanda, 2014). According to Edema *et al.* (2005) *Saccharomyces cerevisiae* is important for good batter leavening and bread viscosity.

Table 2 Lactic acid bacteria counts (log cfu. g^{-1})

	Hour of fermentation				
Sample	0	6	12	18	24
100 % M	7.00±0.03^{h}	9.56±0.04^{g}	12.65±0.03^{h}	12.77±0.03^{h}	10.07±0.02^{f}
100 % A	8.26±0.01^{d}	10.31±0.06^{d}	13.34±0.02^{f}	12.82±0.07^{g}	10.01±0.05^{g}
70 % M: 20 % A: 10 % S	8.10±0.05^{f}	10.16±0.04^{f}	13.08±0.03^{g}	12.97±0.04^{f}	11.41±0.04^{b}
60 % M: 30 % A: 10 % S	8.43±0.02^{b}	10.38±0.05^{c}	13.69±0.05^{e}	13.44±0.01^{d}	11.32±0.06^{d}
60 % M: 20 % A: 20 % S	7.38±0.03^{g}	10.89±0.03^{a}	14.14±0.04^{d}	13.96±0.04^{c}	11.38±0.04^{c}
50 % M: 40 % A: 10 % S	8.41±0.01^{c}	10.25±0.02^{e}	14.20±0.07^{c}	13.25±0.05^{e}	10.99±0.03^{e}
50 % M: 30 % A: 20 % S	8.25±0.04^{e}	10.89±0.03^{a}	14.98±0.02^{a}	14.08±0.03^{b}	11.47±0.04^{a}
40 % M: 40 % A: 20 % S	8.68±0.04^{a}	10.88±0.02^{b}	14.76±0.05^{b}	14.38±0.05^{a}	11.38±0.04^{c}

M: Maize; A: *Acha*; S: Soybean

Values are means of three replicates ± standard error. Means followed by different superscript in the same column are significantly different at $p < 0.05$

Table 3 Yeast counts (Log cfu. g^{-1})

	Hour of fermentation				
Masa samples	0	6	12	18	24
100 % M	Nil	2.60±004^{a}	3.11±0.04^{g}	3.32±0.03^{f}	4.28±0.03^{e}
100 % A	Nil	3.88±0.01^{a}	3.19±0.08^{f}	3.17±0.06^{h}	4.22±0.04^{f}

70 % M: 20 % A: 10 % S	Nil	3.49±0.03[a]	3.46±0.05[c]	4.18±0.09[d]	4.16±0.06[g]
60 % M: 30 % A: 10 % S	Nil	3.20±0.05[a]	2.82±0.10[d]	3.48±0.02[e]	4.76±0.03[d]
60 % M: 20 % A: 20 % S	Nil	3.08±0.10[a]	3.76±0.04[b]	4.73±0.10[b]	5.38±0.06[b]
50 % M: 40 % A: 10 % S	Nil	3.61±0.04[a]	3.40±0.01[e]	3.25±0.03[g]	3.84±0.04[h]
50 % M: 30 % A: 20 % S	Nil	3.89±0.05[a]	4.10±0.06[a]	4.99±0.10[a]	5.54±0.07[a]
40 % M: 40 % A: 20 % S	Nil	3.30±0.03[a]	2.72±0.08[h]	4.49±0.07[c]	5.17±0.04[c]

M: Maize; A: *Acha*; S: Soybean
Values are means of three replicates ± standard error. Means followed by different superscript in the same column are significantly different at $p < 0.05$

Table 4 Identified Microbial Isolates during Fermentation of *Masa*

	Time of Fermentation																															
	0 h								6 h								12 h								18 h								24							
Isolated bacteria	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Leuconostoc mesenteroides	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pediococcus dextrinicus	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactobacillus plantarum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus acidophilus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus delbrueckii	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bacillus subtilis	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus pumilus	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-
Bacillus megaterium	-	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus luteus	+	-	+	+	+	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Torulopsis versatilis	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
Brettanomyces claussenii	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A: 100% maize; B: 100 % *acha;* C: 70 % Maize: 20 % *Acha*: 10 % Soybean; D: 60 % Maize: 30 % *Acha*: 10 % Soybean; E: 60 % Maize: 20 % *Acha*: 20 % Soybean; F: 50 % Maize: 40 % *Acha*: 10 % Soybean; G: 50 % Maize: 30 % *Acha*: 20 % Soybean; H: 40 % Maize: 40 % *Acha*: 20 % Soybean

Identification of microbial isolates

Microbial isolates identified during the fermentation of *masa* are shown in Table 4. Lactic acid bacteria and *Saccharomyces cerevisiae* were dominant during the period of fermentation. *Lactobacillus plantarum, Lactobacillus acidophilus* and *Lactobacillus delbrueckii* were identified throughout the period of fermentation while *Leuconostoc mesenteroides* and *Pediococcus dextrinicus* were only identified at the initial stage of fermentation. *Lactobacillus plantarum* has been identified as the dominant organism at the end of several natural lactic acid fermentations and had superior ability to utilize substrate including dextrins (Adepoju *et al.*, 2012). The absence of *Leuconostoc mesenteroides* after 12 h may be due to the reduction in pH which

made the environment unsuitable for its activities. Sanni and Adesulu (2013), Fowoyo and Ogunbanwo (2010) reported the presence of *Lactobacillus plantarum, Lactobacillus* sp., *Pediococcus* sp. and *Leuconostoc mesenteroides* during production of *masa* from spontaneously fermented maize. Lactic acid bacteria are prominent in fermentation of food. They utilize the available sugar for the production of organic acids such as lactic acid, butyric acid, acetic acid and other metabolites which improve the aroma, taste, texture and shelf life of the fermented food. They also inhibit the growth and activities of pathogenic microorganisms by producing antimicrobial substances known as bacteriocin. Their common occurrence in food contributes to their acceptance (Aguirre and Collins, 1993). *Bacillus pumilus, Bacillus subtilis, Bacillus megaterium* and *Micrococcus luteus* were also identified during the period of fermentation though not consistent. Inconsistency in their presence during fermentation could be because they are not very important in the fermentation process. *Bacillus, Lactobacillus* and *Micrococcus* species have been identified during production of rice-soybean *masa* and fermented legumes (Ezeama and Ihezie, 2006; Farinde *et al.*, 2014).

Saccharomyces cerevisiae was isolated from all samples from 6 h to 24 h of fermentation. *Torulopsis versatilis* and *Brettanomyces claussenii* were present in all samples at 24 h. *Saccharomyces cerevisiae* have been isolated from acidic fermentation of plant substrates and has been reported as the prominent yeast in sourdough. Yeast has been isolated from many spontaneously fermented plant products and has been reported to make useful contribution to flavor and aroma of the fermented product (Owusu-Kwarteng *et al.*, 2010). According to De Vuyst *et al.* (2014), the stability of some sourdoughs depends on the specific synergy betweencertain species of yeasts and lactic acid bacteria. Ezeama and Ihezie (2006) also reported the predominance of lactic acid bacteria, *Micrococcus* and *Saccharomyces cerevisiae* after 18 h of fermentation of rice-soybean *masa*. *Aspergillus niger* was present in all samples at the beginning of the fermentation and disappeared after 6 h. This may be due to the depletion of oxygen since *Aspergillus niger* is aerobic in nature (Dragisa *et al.*, 2007).

Changes in pH during Fermentation *Masa*

The pH values of fermenting *masa* samples are shown in Figure 4. The pH ranged between 2.47 and 6.43 during the period of fermentation. There was general decrease from 0 h to 18 h and consequently pH values increased at 24 h. It decreased in *masa* produced from 100 % maize from 6.43 at 0 h to 4.49 at 6 h, 4.05 at 12 h, 3.45 at 18 h and increased to 3.86 at 24 h. It decreased in *masa* produced from 100 % *acha* from 5.59 at 0 h to 3.35 at 6 h, 3.19 at 12 h, and 2.47 at 18 h and increased to 3.29 at 24 h. It was higher in *masa* produced from 100 % maize than in 100 % *acha* throughout the period of fermentation. The pH decreased with the increase in addition of *acha* and increased with the increase in fortification with soybean. It was lowest in *masa* produced from 100 % *acha* and highest in 60 % maize: 20 % *acha*: 20 % soybean throughout the period of fermentation. The decrease in pH may be due to the growth and activities of fermenting microorganisms which resulted in the production of organic acids such as lactic acid and acetic acid. Many studies have reported a decrease in pH during fermentation of *masa* (Owuzu-Kwarteng and Akabanda, 2013; Sanni and Adesulu, 2013).

Changes in Titratable Acidity during Fermentation of *Masa*

Titratable acidity of *masa* during fermentation is shown in Fig. 2. It ranged between 0.68 to 1.34 %. There was a general increase from 0 h to 18 h but the titratable acidity decreased at 24 h. It increased in *masa* produced from 100 % maize from 0.65 at 0 h to 0.84 at 6 h, 0.90 at 12 h, 1.28 % at 18 h and then decreased to 0.99 % at 24 h. It decreased in 100 % *acha* from 0.95 at 0 h to 1.10 mg/g at 6 h, 1.15 % at 12 h and 1.34 % at 18 h and decreased to 1.12 mg/g at 24 h. It was significantly higher ($p < 0.05$) in *masa* produced from 100 % *acha* than 100 % maize throughout the period of fermentation. *Masa* samples fortified with 20 % soybean had

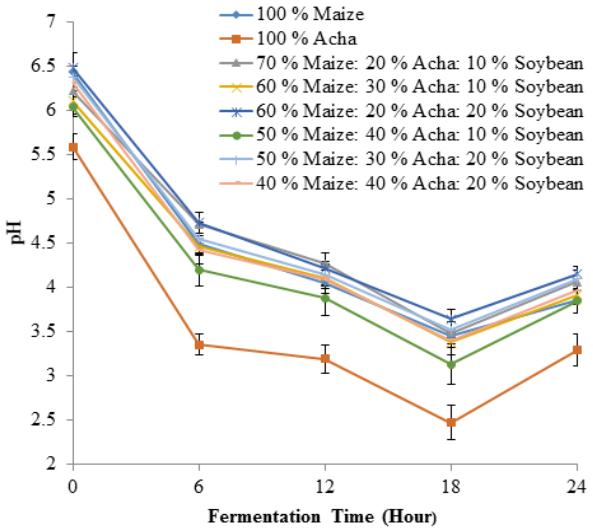

Fig. 1 Changes in pH of *masa* during fermentation

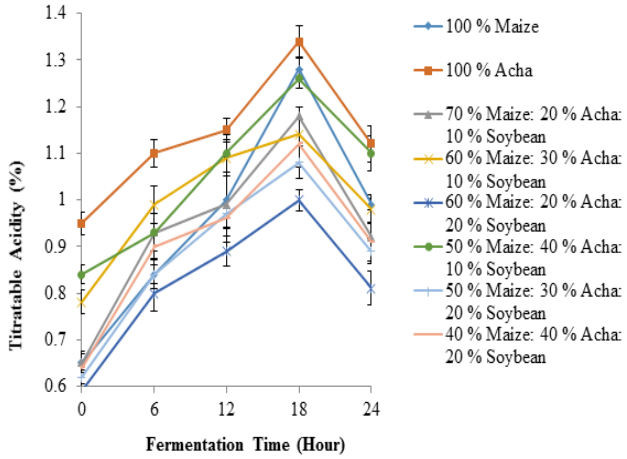

Fig. 5 Changes in titratable acidity during fermentation of *masa*

lower titratable acidity than samples fortified with 10 % soybean. The titratable acidity increases as the pH decreases during the period of fermentation. The increase in the production of organic acid by the fermenting organisms could be responsible for the increase in titratable acidity. Many researchers attributed the increase in titratable acidity to the production of acid from sugar by various metabolic microorganisms (Owuzu-Kwarteng and Akabanda, 2013; Sanni and Adesulu, 2013).

4. CONCLUSION

Lactic acid bacteria and *Saccharomyces cerevisiae* were dominant during the period of fermentation. Both identified lactic acid bacteria and yeasts are important in improving the rheological properties, digestibility, aroma, shelflife and acceptability of fermented food. Association of yeast and lactic acid bacteria is very important in traditionally fermented products because lactic acid bacteria produce acids which make the environment suitable for yeast growth while the yeast produces amino acids for lactic acid bacteria. The decrease in pH in *masa* samples during fermentation inhibited the growth and activities of pathogenic organisms thereby making *masa* fit for consumption. *Acha* can be used as a substitute for maize in the production of *masa* as a way of promoting its utilization and fortification of *masa* with soybeans will improve its nutritional composition since soybean is high in protein.

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