

Production of Masa Using Maize-Sorghum Blends

Kehinde Tope Adegbehingbe

Department of Microbiology, Adekunle Ajasin University, Akungba Akoko Ondo State, Nigeria

Abstract: The study was carried out on 'masa' production from fermented maize-sorghum blends. Three 'masa' products were prepared and designated as sample A (100% maize), sample B (50% blend) and sample C (25%). They were, steeped for 6 hours, fermented for 24 hours and analysed for microbiological changes, pH, total titratable acidity (TTA), and proximate composition. Bacterial species isolated from the blends were *Lactobacillus fermentum*, *L. plantarum*, *Micrococcus luteus*, *Corynebacterium* spp, *Leuconostoc mesenteroides* and *Staphylococcus aureus*. Fungal species isolated were *Aspergillus niger*, *Fusarium* spp, *Penicillium* spp, *Saccharomyces cerevisiae*, *Candida utilis*, *Rhizopus stolonifer* and *Mucor mucedo*. *Lactobacillus plantarum* and *S. cerevisiae* were the most occurred microorganisms in all the samples. The highest fungal and bacterial counts were found in sample B while their respective lowest counts were from samples A and C. Temperature also increased with the highest and lowest increases from samples B and A respectively. Total titratable acidities increased in all the samples to 0.33%, 0.30% and 0.28% in samples B, A and C respectively. Reverse trend was observed in the pH of the samples. Crude protein and crude fat contents was highest in sample B while crude fibre and carbohydrate contents decreased in all the fermented samples. The sample B (50% blend) were observed to be a good alternative for masa production based on its protein contents and organoleptic attributes.

Keywords: cereals, sorghum, maize, fermentation, masa

1. Introduction

Fermented foods are found in diets throughout the world which were dominated by dairy beverages and cereal products. In Africa, many foods are fermented before consumption and they constitute a major component of the diets [1]. 'Masa' is a traditionally fermented food product mainly produced from maize, and sometimes from sorghum and rice. It is a popularly known food in the Northern and Southwestern parts of Nigeria. 'Masa' is still produced traditionally in the home by the local women and the fermentation is spontaneous and uncontrolled. In Nigeria, 'masa' is as popular as 'ogi' (a fermented gruel from maize) but received less attention [2] than the latter. It could be eaten with granulated oil or honey because of its sour taste. Due to the mode of production of 'masa', there are variations in the taste, flavor, sourness and the microbial load [3]. According to Odunfa [1], the fermentation process involved in the production of 'masa' is uncontrolled and occurs by chance inoculation. Microorganisms which have been isolated during 'masa' production include lactic acid bacteria such as *Bacillus* sp., *Lactobacillus plantarum*, sp., *Pediococcus* spp. and *Micrococcus* sp., and the fungi *Aspergillus* and *Penicillium*, *Rhizopus* and *Saccharomyces* sp. [4]. The production of 'masa' is, however, carried out majorly by lactic acid bacteria and the yeast *S. cerevisiae* [4; 5]. Lactic acid bacteria *Bacillus* and fungi such as *Aspergillus* and *Penicillium*, *Rhizopus* and *Saccharomyces* are the most important microorganisms involved in food fermentation [6].

There are many studies on the fermentation of cereal products [1; 7; 8] and production of 'masa' from maize [9; 3; 10]. However, there is paucity of information on the production of masa from maize and sorghum mixture. This research work sought to determine the microbial loads and proximate compositions and sensory attributes of sorghum-maize blends fermented for masa production.

2. Materials and Methods

2.1 Sample Collection

The traditional fermenting white variety of maize (*Zea mays*) and red variety of sorghum (*Sorghum bicolor*) were obtained from Ibaka market in Akungba-Akoko, Ondo State, Nigeria. They were immediately transported to the laboratory for analyses.

2.2 Fermentation Procedure

In the preparation of the samples for this research work, three different blends designated as sample A (control, 300g whole maize), sample B (150g sorghum – 150g maize, 1:1) and sample C (75g sorghum – 225g maize, 1:3). They were sorted to remove the dirt present in them. The grains were then washed with clean water thrice and rinsed with sterile distilled water. The samples were soaked in sterile distilled water in air-tight containers for 5 hours. They were then wet-milled with addition of 200 ml of sterile distilled water. They were then allowed to ferment for 24 hours.

2.3 'Masa' Preparation.

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.

2.4 Microbiological Analyses

Ten millilitres of the fermenting samples were taken at 12 hour intervals and homogenized with 90 ml sterile peptone water solution and further serially diluted appropriately using ten-fold serial dilution method. Isolation and enumeration of microorganisms present were determined using pour plate technique. Enumeration of the total bacteria, lactic acid bacteria and fungi was carried out on Nutrient agar, deMan, Rogosa and Sharpe (MRS) agar and Potato dextrose agar (PDA) respectively. Fungal plates were incubated at 25°C for 2 to 5 days while bacterial cultures were incubated at 37°C for 1 to 2 days. MRS agar plates were incubated under anaerobic conditions. The isolates were sub-cultured by repeated streaking on their respective media until pure cultures were isolated. The

isolates were identified by cultural, morphological and biochemical tests [11].

2.5 Determination of pH and Total Titratable Acidity

Changes in the pH and total titratable acidity (TTA) were assessed at 24-hour interval throughout the fermentation period. The pH was determined using a pH meter standardized with the appropriate buffer. The standard titration procedure for total titratable acidity (TTA) according to AOAC [12] was employed.

2.6 Proximate analysis

Samples of the fermented sorghum and maize were analysed by the standard procedures as adopted by AOAC [12] for total ash, crude fibre, fat, crude protein contents. Carbohydrate content was estimated by difference.

2.7 Sensory Evaluation

Masa sample prepared from each blend were served to 20 untrained judges to evaluate the sensory qualities (aroma, appearance, texture and overall acceptability) using a seven-point hedonic scale (1 and 7 representing extremely dislike and extremely like, respectively).

2.8 Statistical Analysis

Data obtained were analyzed by ANOVA and significant differences between means were compared using Duncan multiple range test with the aid of SAS/STAT program.

3. Results

Twenty four bacteria and 10 fungi were isolated during the fermentation of sorghum and maize for the production of ‘masa’. The fungi were *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor mucedo*, *Fusarium* spp., *Saccharomyces cerevisiae*, *Candida utilis* and *Penicillium citrinum* (Table 1). The bacteria isolated were *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Staphylococcus aureus*, *Corynebacterium* spp., *Leuconostoc mesenteroides* and *Micrococcus luteus*. The predominant microorganisms during the fermentation period were lactic acid bacteria mainly *L. Plantarum* and the yeast *S. cerevisiae* and isolated mostly in sample B.

Fungal counts increased in all the samples with the highest from sample B (5.7 log cfu/ml) while the lowest count was found in sample A (4.5og cfu/ml). The fungal plates were dominated by yeast colonies (Figure 1). Bacterial counts also increased in all the samples within the fermentation period (Figure 2). The highest count was also found in sample B (7.1 log cfu/ml) while the lowest count was however from sample C (6.4 log cfu/ml)

Temperature increased in all the samples. Initial temperature of all the samples was 26°C. Temperature of samples A, B, and C increased to 28.1°C, 29°C and 28.4°C respectively (Figure 3).

Total titratable acidities increased in all the samples (Figure 4). The highest content was found in sample B (0.33%) while the lowest content was found in sample C (0.28%). However the pH values of all the samples were observed to decrease as the fermentation progressed. The

lowest value of 4.12 was determined in sample B while sample C which had the highest content contained 4.45 (Figure 5).

Moisture, crude protein, crude fat and total ash contents increased in all the samples while crude fibre and carbohydrate contents were found to decrease after fermentation (Table 2). The highest crude protein and crude fat contents of 9.57% and 3.39% were observed in sample B while their respective lowest contents of 7.71% and 3.12% were determined in sample A. Sample C contained the highest ash content of 2.39% while sample A had the lowest content of 1.95%. Crude fibre content reduced most significantly in sample A (1.32%) while the lowest reduction was found in sample B (1.86%).

Table 3 shows the organoleptic properties of the blends. There were no significantly differences in all the parameters tested. Samples A and B were rated highest and lowest with respect to textural characteristics and scored 5.2 and 4.9 respectively. Sample B was the most preferred in terms of appearance (5.0) and aroma (5.5) while the preferred was sample C scored 4.6 and 5.2 respectively. Taste and overall acceptability of masa (A) with maize and 50% blend (B) were rated same and scored 5.3 and 5.5 respectively.

Table 1: Occurrence of the bacterial and fungal isolates of sorghum-maize blend during ‘masa’ production

Isolates	steeping			0 hours			12 hours			24 hours		
	A	B	C	A	B	C	A	B	C	A	B	C
	<i>Micrococcus</i> spp	-	+	+	-	+	-	-	-	-	-	-
<i>Lactobacillus fermentum</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Lactobacillus plantarum</i>	+	+	-	+	+	+	+	+	+	+	+	+
<i>Leuconostoc</i> spp	-	-	-	-	+	-	-	+	+	-	+	+
<i>Corynebacterium</i> spp	+	+	-	+	-	-	-	+	+	-	-	-
<i>Staphylococcus aureus</i>	+	+	+	-	+	-	-	-	-	-	-	-
<i>Candida</i> spp	-	-	-	-	+	-	-	+	-	-	-	-
<i>Penicillium</i> spp	+	+	+	-	+	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	+	+	+	+	+	+	-	-	-	-	-	-
<i>Fusarium</i> spp	+	+	-	-	-	-	-	-	-	-	-	-
<i>Mucor mucedo</i>	+	+	+	+	-	+	-	-	-	-	-	-
<i>Rhizopus stolonifer</i>	+	+	-	-	-	-	-	-	-	-	-	-
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+	+	+	+	+	+	+

Sample A – Maize Control (100% maize)

Sample B – Sorghum- Maize (50% blend)

Sample C – Sorghum-Maize (25% blend)

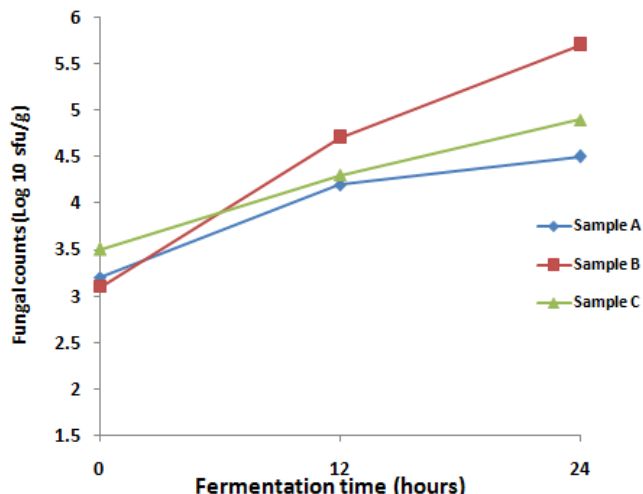


Figure 1: Fungal Growth of the fermenting Sorghum-maize blend during ‘masa’ production

Sample A – Maize Control (100% maize)

Sample B – Sorghum- Maize (50% blend)

Sample C – Sorghum-Maize (25% blend)

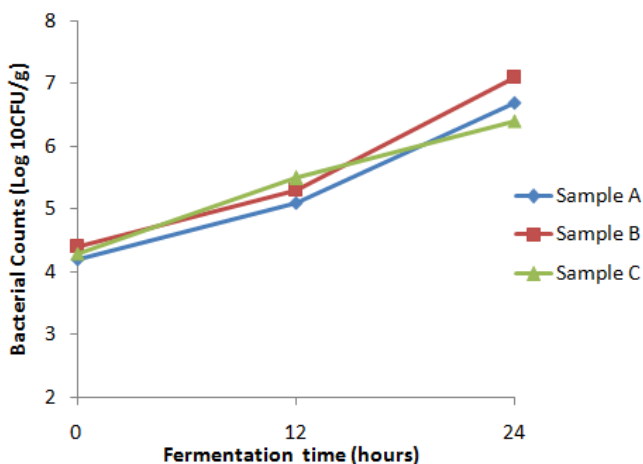


Figure 2: Bacterial Count of the fermenting Sorghum-maize blend during ‘masa’ production

Sample A – Maize Control (100% maize)

Sample B – Sorghum- Maize (50% blend)

Sample C – Sorghum-Maize (25% blend)

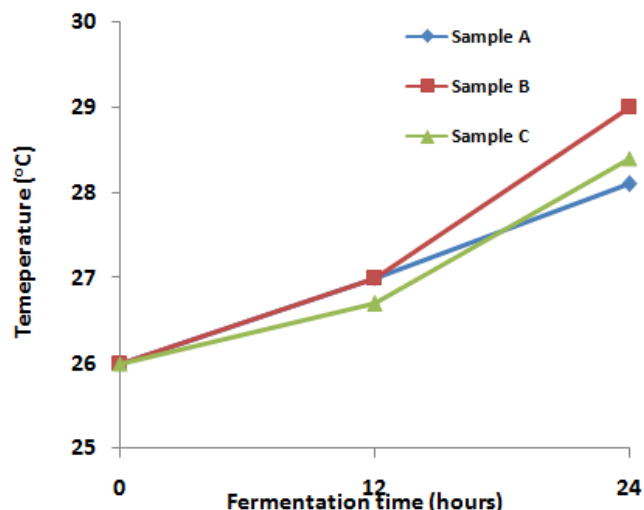


Figure 3: Changes in temperature of the fermenting sorghum-maize blend during ‘masa’ production

Sample A – Maize Control (100% maize)

Sample B – Sorghum- Maize (50% blend)

Sample C – Sorghum-Maize (25% blend)

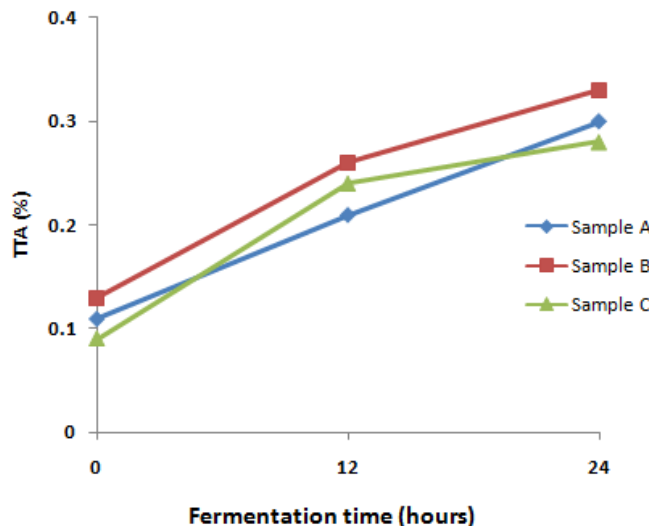


Figure 4: Total titratable acidity of sorghum-maize blend during ‘masa’ production

Sample A – Maize Control (100% maize)

Sample B – Sorghum- Maize (50% blend)

Sample C – Sorghum-Maize (25% blend)

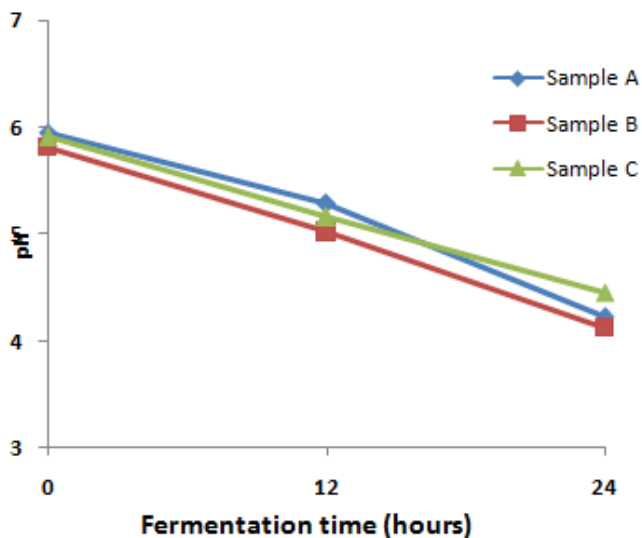


Figure 5: Changes in pH of the fermenting sorghum-maize blends during 'masa' production

Sample A – Maize Control (100% maize)
 Sample B – Sorghum- Maize (50% blend)
 Sample C – Sorghum-Maize (25% blend)

Table 2: The Proximate composition of the sorghum-maize blend during 'masa' production

Sample	Moisture		Protein		Fat		Ash		Fibre		carbohydrate	
	N	FF	N	F	N	FF	N	F	N	FF	N	FF
A	46.17 ^a	59.25 ^a	5.71 ^a	7.71 ^b	2.81 ^a	3.12 ^c	1.65 ^a	1.95 ^c	2.59 ^a	1.32 ^c	41.07 ^b	26.65 ^a
B	45.52 ^a	57.95 ^a	5.35 ^b	9.57 ^a	2.61 ^b	3.39 ^a	1.60 ^b	2.22 ^b	2.50 ^a	1.86 ^a	42.82 ^a	25.01 ^b
C	43.22 ^b	58.95 ^a	5.77 ^a	7.97 ^b	2.74 ^a	3.21 ^{bc}	1.66 ^a	2.39 ^a	2.46 ^b	1.74 ^{ab}	44.15 ^a	25.74 ^b

Samples with the same superscripts down the column are not significantly different

Keys:

Sample A – Maize Control (100% maize)
 Sample B – Sorghum- Maize (50% blend)
 Sample C – Sorghum-Maize (25% blend)

Table 3: Organoleptic properties of the 'masa' blends

Time (Hours)	Texture	Appearance	Taste	Aroma	Overall acceptability
A	5.2 ^a	4.8 ^a	5.4 ^a	5.3 ^a	5.5 ^a
B	4.9 ^{ab}	5.0 ^a	5.4 ^{ab}	5.5 ^a	5.5 ^{ab}
C	5.0 ^a	4.6 ^a	5.2 ^{ab}	5.2 ^a	5.3 ^{ab}

Samples with the same superscripts down the column are not significantly different

Keys:

Sample A – Maize Control (100% maize)

Sample B – Sorghum- Maize (50% blend)
 Sample C – Sorghum-Maize (25% blend)

4. Discussion

The differences observed in the microbial counts could be due to differences in the composition of the blends. The highest microbial counts in sample B could be due to higher sorghum in the blend. There is possibility that the sorghum might support the growth of the microorganisms than that of maize grains. High occurrences and many genera of microorganisms from the blends could be due to the sources of the substrates and the processing water. Diverse microflora has been reported to be associated with cereal grains [5]. Some fungi have been reported to be important in saccharification of carbohydrates in many substrates particularly at the steeping stage [13]. However, the subsequent disappearance of some of the microorganisms such as the moulds, *S. aureus* and *Micrococcus* has been reported by many authors who have worked on fermented cereal grain products [13; 14]. Their elimination has been attributed to antibacterial substances such as bacteriocins produced by lactic acid bacteria in the gruel.

The decreases pH and the subsequent increases in total titratable acidity of all the samples was in agreement with Vieira-Dalode *et al.* [14] while producing gowe, a fermented sorghum gruel from Republic of Benin. This could be as a result of proliferation of lactic acid bacteria and *S. cerevisiae* as the fermentation progressed. Low pH of the gruel could also be responsible for inability of pathogenic microorganisms to grow during the fermentation process. Production of lactic acid and the resulting pH decrease has been reported to be the main preserving factor in food fermentation [15; 16]. The predominance of lactic acid bacteria throughout the fermentation process was reported while producing 'kenkeh', 'agbelina' and 'kunun-zaki' all which are fermented cereal products [17; 18; 19]. Wakil and Daodu [16] reported the predominance of *L. plantarum* while fermenting maize for ogi production and attributed the souring of the gruel to the proliferation of the organism. The occurrence of *S. cerevisiae* throughout the fermentation period was in agreement with Vieira-Dalode *et al.* [14] during gowe production. *Saccharomyces cerevisiae* had been reported to contribute to flavour development in fermented foods [20; 21]. Some yeasts had been reported to show amylolytic, protease and phytase activities, apart from their role in building up of typical flavour of fermented products. The coexistence and symbiotic association between lactic acid bacteria and yeasts in African traditional fermented products have been reported by several authors [22; 23; 24].

The increase in protein content agrees with Inyang and Zakari [25] while fermenting millet. The increase in the protein content could be attributed to the structural proteins that are an integral part of the microbial cell [26; 27]. The highest protein and fat contents in sample B might be due to the fact that sorghum contains nutrient which could support the growth of the fermenting microorganisms which proliferated as single cell proteins.

The decreases in crude fibre and carbohydrate contents of the samples conform to Adegbehingbe and Fakoya [28] while fermenting cocoyam. The reduction in carbohydrate contents might be as a result of the utilization of some of the sugars by fermenting organisms, through their α - and β -amylase, for their growth [29]. It could also be due to apparent increase in protein contents of the samples [30].

The non-significant differences in the sensory attributes of the blends revealed that they could be produced with such compositions. The highest grade accorded sample B with respect to aroma could be due to the highest proliferation of lactic acid bacteria and *S. cerevisiae* in the blend. The implication of this was that production of 'masa' with 50% blend should be preferred because of its higher protein content, aroma and appearance and its non-significant differences in the remaining organoleptic properties.

5. Conclusion

Fermentation of the sorghum-maize mixture particularly the 50% blend has shown an increase in protein content and the results of their sensory attributes revealed that they could be acceptable by the consumers. Therefore, this mixture is a good substitute for the production of *masa* from maize. Further studies on *masa* production from sorghum-maize combination should include the use of starter cultures to improve the efficiency of fermentation.

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Author Profile

Dr K.T. Adegbehingbe obtained B. Tech. from The Federal University of Technology, Akure (FUTA) Nigeria before he proceeded to University of Ibadan (Nigeria) for MSc. in Microbiology. He later received P.hD in Food Microbiology from FUTA. Currently, he is a lecturer at Adekunle Ajasin University, Akungba-Akoko, Nigeria