

## MICROBIOLOGICAL ANALYSES AND NUTRIENT COMPOSITION OF SORGHUM-BENISEED BLENDS

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### ABSTRACT

This research investigated the production of fermented gruel from sorghum (ogi-baba) fortified with beniseeds (*Sesamum indicum* L) from four different preparations designated as samples A (100% sorghum), sample B (10% beniseeds), C (15% beniseeds) and D (20% beniseeds). Bacteria that were isolated from the samples were *Lactobacillus plantarum* L. *casei*, *Bacillus subtilis*, *B. licheniformis* *Leuconostoc mesenteroides*, *Streptococcus lactis*, *Staphylococcus aureus* and while fungal isolates include *Rhizopus nigricans*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Mucor mucedo*. All the blends were dominated by *L. plantarum* and *S. cerevisiae* which persisted throughout the fermentation period while *S. aureus* and the moulds were only isolated on the first day of fermentation. Bacterial and fungal counts increased significantly in all the blends till the end of the fermentation period. The pH of the fermenting samples decreased in all the blends with lowest value in sample A (3.36) while the highest value was found in sample D (3.41). Total titratable acidity (TTA) increased with fermentation time with the highest in sample A (2.94%) while sample D had the least TTA of 2.42%. Crude protein and crude fat contents of the fermented blends increased with increase in percentage supplementation while the crude carbohydrate contents decreased with decrease in percentage supplementation. Moisture, crude ash, crude fibre contents also decreased as the fermentation progressed in all the blends. Porridges prepared from all the blends were rated above average in terms of overall acceptability but significantly rated higher in sample B. The use of beniseeds as a fortifying agent can improve nutritive value of locally fermented carbohydrate foods which are economical compared to formulated foods.

**KEYWORDS:** Fermentation, Sorghum, Beniseed, Nutrient, Lactobacillus

### INTRODUCTION

The most important staple foods for many people in the developed and developing countries are produced from cereals. In the developed countries, 70% of the cereal produced is used as animal feed while in the developing countries 68-98% of the cereal production is used for human consumption (Betschart, 1982).

Sorghum (*Sorghum bicolor*) is an ancient crop grown almost everywhere in the world. It is one of the five top cereal crops and ranks after maize (FAO, 1994). It has inferior organoleptic quality and reduced protein digestibility due to the presence of anti-nutritional factors such as tannins and phytates. The problems of anti-nutritional factors in sorghum can however be solved by adequate processing techniques such as sprouting, malting, decorticating and fermentation (Singh, 1994). Examples of fermented foods from sorghum include 'buchera', 'kunu-zaki', 'koko' and 'ogi-baba' (Adeyemi 1988; Adewusi *et al.*, 1991; Muyanja *et al.*, 2002). However, most of these fermented preparations are nutritionally poor and lack some essential amino acids.

'Ogi-baba' is a gruel produced from fermented sorghum grains. It is often used for the complementary feeding of infants and young children in Africa. The nutritional quality of 'ogi-baba' is poor in terms of crude protein content compared to the required composition of complementary food (Dewey and Brown, 2003; Lufter and Dewey, 2003; Owusu-Kwarteng *et al.*, 2010). Many attempts have been made to fortify these cereals in order to make nutritionally superior and acceptable products (Oyarekua and Adewole 2009). This has been reported to improve their protein quality and their limiting amino acid contents (Annan *et al.*, 2005).

A number of legumes that are readily available but underutilised have been reported to possess nutrient potentials that could complement fermented cereal products if properly processed and blended (Fernandez *et al.*, 2002; Compaoré *et al.*, 2011).

Beniseeds (*Sesamum indicum L.*) also known as sesame, belongs to the family *Pedaliaceae*. The seeds are mucilaginous in nature and are among the most ancient oilseeds crop known to mankind. Beniseeds are grown in some parts of the world particularly in India, China, South America and Africa (Adeniyani *et al.*, 2013). The local names of beniseeds in Nigeria include 'eluru' and 'ekuku' (Yoruba) and 'gorigo' (Ebiras). Beniseeds are used as a soup condiment in some northern Nigeria and some parts of Cross-river state (Agiang *et al.*, 2010). The seeds are also sprinkled over cakes and breads, especially in Syria and Lebanon (Momoh *et al.*, 2013).

Beniseeds are very rich in protein and minerals calcium, manganese, copper and phosphorus (Salunkhe, 1992). Beniseed oil has the highest concentration of Omega-6-fatty acids and two naturally occurring preservatives, sesamol and sesamin (Shittu *et al.*, 2007; Ayo *et al.*, 2012). Beniseed has also recognised for its medicinal properties. The seed has been reported to be good for increasing energy and prevention of aging (Hajimahmoodi *et al.*, 2008) and as a demulcent in respiratory infections and some gastrointestinal diseases. The seed powder is useful in amenorrhoea, dysmenorrhoea, ulcers and bleeding piles (Visavadiya and Narasimhacharya, 2008). Fermented liquor and methanol extract of the seeds were reported to possess antibacterial and immunostimulatory potentials (Momoh *et al.*, 2012).

Therefore, supplementation and co-fermentation of beniseeds which are rich in protein and lysine, and medicinal properties could improve the nutritional quality of the fermented cereal foods. According to Ayo *et al.*, (2010), beniseed paste, when added to a local drink, 'kunun-zaki', increased its protein, fat and energy contents by over 20%.

The aims of this research were to evaluate the preparation of ogi produced from sorghum ('ogi-baba') co-fermented with various percentages of beniseeds for their nutritive composition and sensory attributes

## MATERIALS AND METHODS

### Sample Collection

The fresh sorghum (*Sorghum bicolor*) samples were obtained from from Ibaka Market, Akungba-Akoko, while the beniseeds were obtained from Osele Market, Ikare-Akoko, Ondo State, Nigeria. These were immediately transported to the laboratory for analyses.

### Formulation of the Sorghum-Beniseed Blend

Fresh beniseeds were washed thoroughly in clean water. They were then steeped in clean water for 2 hours in plastic air-tight containers. The water was decanted after two days and was wet milled into slurry. The slurry was dried in an oven at 55°C for 48 hours.

The total weight of each blend was 500 g. The first blend (Sample A) consisted of only sorghum (100% sorghum) and served as the control. The first, second, third and the fourth blends were supplemented with 0%, 10%, 15% and 20% beniseeds respectively. Each blend was steeped in 1,500 ml of sterile distilled water for 48 hours after which the water was drained. The samples were washed and wet milled in a blender. They were then sieved using sterile muslin bags and the slurries were allowed to ferment for another 24 hours.

### Microbiological Analysis

Ten grams of each of the samples were homogenized with 90 ml sterile peptone water solution and further serially diluted. Enumeration of the total bacteria, lactic acid bacteria and fungi was carried out on daily basis using nutrient agar, deMan, Rogosa and Sharpe (MRS) agar and potato dextrose agar (PDA) respectively. Fungal plates were incubated at 25°C for 3 to 5 days while bacterial cultures were incubated at 37°C for 1 to 2 days. MRS agar plates were incubated under anaerobic conditions. Representative colonies were picked from the plates and sub-cultured by repeated streaking on their respective media until pure cultures were isolated. The isolates were characterized by cultural, morphological and biochemical tests (Olutiola *et al.*, 2000)

### Determination of Total Titratable Acidity (TTA)

The total titratable acidity of the fermenting samples was analyzed by measuring 20 ml distilled water into a beaker containing 2 g of macerated fermenting sample. Two drops of phenolphthalein (indicator) was added to the mixture. This was then titrated against 0.1ml NaOH (sodium hydroxide) and readings recorded.

### Determination of pH

An Acid-Base pH meter with glass electrodes was used to measure the pH. This was done by inserting the electrode into 10ml suspension containing 1g of the sample homogenized in 9 ml of sterile distilled water. The apparatus was standardized with a buffer solution of pH 6.9, 4.2, 9.0 before use.

### Analytical Methods

Moisture contents, crude protein contents, crude fat contents, total ash, crude fibre contents and carbohydrate contents were determined as described by AOAC (2006).

### Sensory Evaluation

Hot water was used to prepare porridges (paps) from each of the blends by stirring in the hot water until a paste was formed. The porridges were served to 20 untrained judges to evaluate the sensory qualities (aroma, colour, texture, and overall acceptability) using a seven-point hedonic scale (1 and 7 representing extremely dislike and extremely like, respectively).

### Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA), the means being tested for significance at  $p < .05$  using Duncan's multiple range (DMR) test.

## RESULTS

Seven bacterial species and four fungi were identified from the samples (Table 1) the bacteria were *Lactobacillus*

*plantarum*, *L. casei*, *Bacillus subtilis*, *Leuconostoc mesenteroides*, *Streptococcus lactis*, *Staphylococcus aureus* and *Bacillus licheniformis* while fungal isolates include *Rhizopus nigricans*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Mucor mucedo*. Lactic acid bacteria particularly *L. plantarum* and *Leuconostoc* species and the yeast *S. cerevisiae* were the most dominant microorganisms from all the blends and they persisted throughout the fermentation period (Table 1).

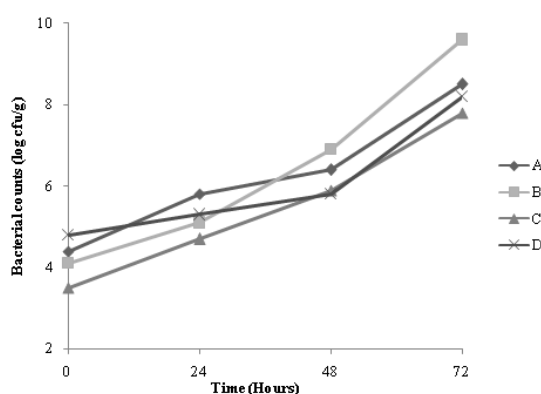
There was a significant increase in the bacterial counts increased in all the blends with the highest of 9.6 cfu/g and 7.8 cfu/g from 10% blend and 20% blend respectively (Figure 1). Fungal counts also increased in all the samples but however as the percentage of supplementation decreased, that is, the highest and the lowest counts were obtained from 0% blend (5.9%) and 20% blend (4.%) respectively (Figure 2).

The pH values decreased in all the samples (Table 2). Unblended 'ogi-baba' and 20% blend had the lowest and the highest pH of 3.36 and 3.41 respectively at the end of the fermentation period. However, the total titratable acidity (TTA) increased significantly in all the samples. The highest TTA was found in 'ogi-baba' while the least content was found in 20% blend (Table 3).

Temperature increased throughout the fermentation period in 100% 'ogi-baba' and 10% blend while decreases were observed in 15% and 20% blends after 48 hours (Table 4). The highest and the lowest temperature values in the fermented samples were found in ogi-baba (31.5°C) and 20% blend (29.0°C) respectively.

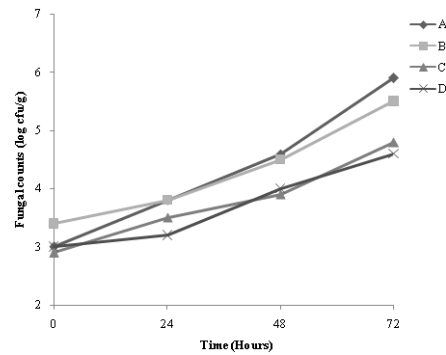
Moisture contents reduced in 'ogi-baba' and 10% blend while increased were found in 15% blend and 20% blend. Crude protein contents increased in all the samples after fermentation with the highest and the lowest content from 10% blend (14.33%) and ogi-baba (11.68%) respectively. The crude fat contents increased slightly in the entire samples. The highest and the lowest fat contents were found in 20% blend and 'ogi-baba' respectively. Total ash contents also increased while crude fibre and carbohydrate contents were found to reduce in all the samples (Table 5).

Table 6 shows the sensory evaluation of all the samples as tested by panelists. 'Ogi-baba' prepared with 10% blend was not significantly different from the 'ogi-baba' samples in all the parameters tested. All the parameters decreased as the supplementation increased in all the blends. The results of overall acceptability revealed that 10% blend could compete favourably with the unblended sample.



**Legend:** Sample A=100% sorghum, Sample B =10% beniseeds, Sample C=15% beniseeds, Sample D =20% beniseeds

**Figure 1: Bacterial Counts of the Fermenting Sorghum-Beniseed Blends**



**Legend:** Sample A=100% sorghum, Sample B =10% beniseeds, Sample C=15% beniseeds, Sample D =20% beniseeds

**Figure 2: Fungal Counts of the Fermenting Sorghum-Beniseed Blends**

**Table 1: Occurrence of Microbes Isolated from the Sorghum Beniseed Blends**

Isolates	Time (Hours)															
	0				24				48				72			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
<i>Bacillus subtilis</i>	+	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-
<i>Bacillus licheniformis</i>	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-
<i>Streptomyces lactis</i>	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Lactobacillus plantarum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Leuconostoc mesenteroides</i>	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	-
<i>Lactobacillus casei</i>	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	-
<i>Staphylococcus aureus</i>	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-
<i>Aspergillus niger</i>	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-
<i>Rhizopus nigricans</i>	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Mucor mucedo</i>	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-

**Legend:** Sample A=100% sorghum, Sample B =10% beniseeds, Sample C=15% beniseeds, Sample D =20% beniseeds

**Table 2: pH of the Fermenting Sorghum- Beniseed Blends**

Time (hours)	pH			
	A	B	C	D
0	5.92 <sup>a</sup>	5.84 <sup>a</sup>	5.76 <sup>a</sup>	5.71 <sup>a</sup>
24	4.38 <sup>c</sup>	4.57 <sup>b</sup>	4.61 <sup>a</sup>	4.62 <sup>a</sup>
48	3.72 <sup>d</sup>	3.90 <sup>c</sup>	4.12 <sup>b</sup>	4.25 <sup>a</sup>
72	3.36 <sup>c</sup>	3.39 <sup>bc</sup>	3.40 <sup>a</sup>	3.41 <sup>a</sup>

Values with the same superscript letter(s) across the row are not statistically significantly ( $P>0.05$ ) different.

**Legend:** Sample A=100% sorghum, Sample B =10% beniseeds, Sample C=15% beniseeds, Sample D =20% beniseeds

**Table 3: Total Titratable Acidity (Tta) of the Fermenting Sorghum-Beniseed Blends**

Time (hours)	(TTA)			
	A	B	C	D
0	0.92 <sup>d</sup>	0.98 <sup>c</sup>	1.02 <sup>b</sup>	1.15 <sup>a</sup>
24	1.52 <sup>d</sup>	1.59 <sup>c</sup>	1.72 <sup>b</sup>	1.79 <sup>a</sup>
48	2.41 <sup>a</sup>	2.22 <sup>b</sup>	2.17 <sup>c</sup>	2.04 <sup>d</sup>
72	2.94 <sup>a</sup>	2.85 <sup>ab</sup>	2.64 <sup>c</sup>	2.42 <sup>c</sup>

Values with the same superscript letter(s) across the row are not statistically significantly ( $P>0.05$ ) different.

**Legend:** Sample A=100% sorghum, Sample B =10% beniseeds, Sample C=15% beniseeds, Sample D =20% beniseeds

**Table 4: Temperature of the Fermenting Sorghum-Beniseed Blends**

Time (Hours)	Temperature (°C)			
	A	B	C	D
0	27.0 <sup>a</sup>	27.2 <sup>a</sup>	27.2 <sup>a</sup>	27.5 <sup>a</sup>
24	28.0 <sup>a</sup>	28.5 <sup>a</sup>	29.0 <sup>a</sup>	28.0 <sup>a</sup>
48	30.5 <sup>a</sup>	30.0 <sup>a</sup>	29.5 <sup>a</sup>	30.0 <sup>a</sup>
72	31.5 <sup>a</sup>	30.5 <sup>a</sup>	28.0 <sup>c</sup>	29.0 <sup>b</sup>

Values with the same superscript letter(s) across the row are not statistically significantly ( $P>0.05$ ) different.

**Legend:** Sample A=100% sorghum, Sample B =10% beniseeds,

Sample C=15% beniseeds, Sample D =20% beniseeds

**Table 5: Proximate Composition of the Fermented Samples**

Blends	Moisture		Protein		Fat		Ash		Fibre		Carbohydrate	
	NN	FF	NN	FF	NN	FF	NN	FF	NN	FF	NN	FF
A	10.11	9.85	7.91	11.68	4.21	4.93	1.97	1.69	2.05	1.67	73.75	70.18
B	10.92	10.13	9.22	14.33	5.16	6.91	1.99	1.96	2.26	1.16	70.54	65.51
C	11.28	12.74	10.34	13.26	6.29	7.67	1.88	0.92	2.71	1.15	67.5	64.26
D	11.96	12.85	11.17	13.21	6.71	7.88	1.76	0.86	3.32	1.29	65.08	63.91

**Legend:** Sample A=100% sorghum, Sample B =10% beniseeds, Sample C=15% beniseeds, Sample D =20% beniseeds

**Table 6: Sensory Characteristics of the Preparations**

Sample	Aroma	Colour	Texture	Taste	General Acceptability
Sample A	5.2 <sup>a</sup>	5.5 <sup>a</sup>	5.2 <sup>a</sup>	6.2 <sup>a</sup>	5.6 <sup>a</sup>
Sample B	4.9 <sup>a</sup>	5.5 <sup>a</sup>	4.9 <sup>ab</sup>	5.9 <sup>ab</sup>	5.2 <sup>ab</sup>
Sample C	4.2 <sup>b</sup>	5.3 <sup>ab</sup>	4.2 <sup>c</sup>	5.1 <sup>c</sup>	4.2 <sup>c</sup>
Sample D	3.5 <sup>c</sup>	4.8 <sup>b</sup>	3.8 <sup>d</sup>	4.4 <sup>d</sup>	4.1 <sup>c</sup>

Values with the same superscript letter(s) down a column are not statistically significantly ( $P>0.05$ ) different.

**Legend:** Sample A=100% sorghum, Sample B =10% beniseeds,

Sample C=15% beniseeds, Sample D =20% beniseeds

## DISCUSSIONS

The microorganisms that were isolated from all the 'beni-ogi' blends were peculiar to many fermented foods (Odunfa and Adeleye, 1985; Omemu, *et al.*, 2007a). Previous studies suggested that microorganisms are associated with cereal grains also served as inoculum for their natural fermentation process (Odunfa and Adeleye, 1985).

The predominance of lactic acid bacteria during the during this study has been reported by many authors on fermented cereal products (Omemu, 2011; Opere *et al.*, 2012) Lactic fermentation has been reported to reduce the levels of proteinase inhibitors in cereal porridges thereby increasing the availability of essential amino acids such as lysine, methionine and tryptophan thereby improving the protein quality of cereal grains (Holzapfel, 2002; Opere *et al.*, 2012). Previous workers have found several yeasts species in spontaneous lactic fermenting cereals including species of *Saccharomyces* and *Candida* (Jespersen, 1994). Their presence in fermenting foods has been found to improve the aroma and flavour of fermented foods (Adegbehingbe and Fakoya, 2007). The *Saccharomyces cerevisiae* which also persisted throughout the fermentation period in all the blends agreed with Vieira-Dalode *et al.* (2007) while fermenting maize for mowe production. The coexistence and symbiotic association between lactic acid bacteria and yeasts in traditional fermented products have been reported by several authors (Jespersen *et al.*, 1994; Hounhouigan *et al.*, 1993; Omemu *et al.*, 2007b). Besides they have been observed to be responsible for flavour development in fermented foods (Omemu *et al.*, 2007b; Vieira-Dalode *et al.*, 2007).

The molds isolated in the study are commonly present as contaminants in cereals. Their sources could also be from the utensils, the processing water or the environment (Oyelana and Coker, 2012). Ijabadeniyi (2007) and Omemu (2011) isolated *Penicillium* and *Aspergillus*, *Mucor* and *Rhizopus* in maize fermentation during the early stage of maize fermentation for ogi production. Jespersen (1994) attributed their early disappearance of moulds to the low oxygen tension in the fermenting porridge for 'kenkey' production. The presence of *Staphylococcus* probably originated from the air, human handlers and other materials.

The higher total titratable acidities with respect to decrease in percentage of the blends could be due to higher lactic acid bacteria in these blends. Lactic acid bacteria have been known for production of lactic acid and minor amounts of acetic and formic acids. This is one of the attributes of cereal fermented foods and beverages. A similar increase in acid production had been observed by Afoakwa *et al.* (2007) during the production of weaning food from maize-cowpea blends. Reduction in pH values of fermented foods inhibits growth of spoilage and pathogenic organisms (Opere *et al.*, 2012).

The decrease in moisture contents with increase in fermentation time was similar to Wakil and Kazeem (2012) on weaning foods produced from fermented cereal legume blends using starters. The observed increase in protein content of the blends may be due to the structural proteins that are an integral part of the microbial cell (Silhavy *et al.*, 2010).

The highest crude protein content in 10% blend might be due to the fact that the blend composition contained adequate nutrients which supported the growth of the fermenting microorganisms in forms of single cell protein. It has also been reported that *Bacillus* spp. are important producers of extracellular proteases in some fermented legumes which could hydrolyze complex plant proteins to amino acids and short chain peptides, thereby causing an increase in total nitrogen content (Fogarty and Griffin, 1973; Adegbehingbe, 2013).

The slight decrease in the ash contents of fermenting samples was in agreement with the results obtained by Esenwah and Ikenebomeh (2008) while fermenting African locust bean seeds. Loss in ash contents may be due to leaching of soluble inorganic salts into the processing water during the fermentation period (Osman, 2007; Ogonnaya *et al.*, 2010) or the fermenting microflora used it for their metabolism (Reebe *et al.*, 2000; Onweluzo and Nwabugwu, 2009).

The increase in the fat contents which might be due to the activities of the fermenting microbes (Oyewole and Odufa, 1990) was in agreement with Ayo *et al.* (2012) on his work on masa-beniseed blends.

The reduction in the total ash after fermentation agrees closely with Ayo *et al.* (2008) on effect of groundnut-maize blends on the qualities of 'masa'. They observed decrease in ash content from 2.1 to 0.8% after fermentation. The reduction in crude fibre contents of the blends after fermentation conforms with Onweluzo and Nwabugwu (2007) while fermenting pigeon pea seeds for flour production. The loss in crude fibre and ash contents could be due to their leaching into the fermenting medium or they were being metabolised by the fermenting microorganisms

The total carbohydrate content of the samples decreased steadily during the fermentation period. This is in agreement with the result of Adegbehingbe and Fakoya (2007; 2013). It may be as a result of the utilization of some of the sugars by fermenting organisms for growth and metabolic activities and also as a result of apparent increase in protein contents of the samples (Adegbehingbe, 2013).

There were no significant differences ( $P>0.05$ ) in the odour, texture and colour, and the overall acceptability of ogi-baba and 10% blend. This shows the 10% blend could compete favourably well with ogi-baba.

## CONCLUSIONS

In conclusion, the results from this work shows that the use of beniseed as a fortifying agent improved the protein contents of the blends and that 10% blend was the most acceptable among the blends. Therefore it could be used to fortify sorghum and may be recommended for solving the problem of protein-energy malnutrition (PEM) among infants in developing countries.

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