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COMPARATIVE EVALUATION OF THE MICROFLORA AND BIOCHEMICAL CONSTITUENTS OF SORGHUM-AFRICAN BREADFRUIT BLENDS FOR COMPLEMENTARY FOODS

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Complementary foods formulated from locally obtainable, underutilized, low-priced sorghum and African breadfruit seeds were assessed for their microbial quality and nutritional values. The fermented gruel produced from sorghum (*Ogi-baba*) and African breadfruit flour blends were mixed in varying ratios of 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (w/w.) respectively. The microbiological quality, changes in pH and titratable acidity and proximate compositions of the blends were determined using standard analytical methods. The energy value was evaluated using the Atwater factor. Bacteria isolated from the samples were *Bacillus* species, *Escherichia coli, Pseudomonas* species, *Staphylococcus aureus, Klebsiella* species and *Lactobacillus* species while fungal isolates include *Saccharomyces* species, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* species. The colony-forming units of the bacteria and fungi investigated in the supplemented samples ranged from 1.1×10^5 $\pm0.00^f$ to $10.9\times10^7\pm10.04^c$ cfu/g over the 96 hours of fermentation period. The most predominant bacteria and yeast genera were *Lactobacillus* and *Saccharomyces*, respectively in all the blends persisting throughout the fermentation period. The pH of the fermenting samples decreased with a concomitant increase in the titratable acidity with an increase in percentage supplementation and fermentation time. The supplemented product shows significant ($p<0.05$) increases in the crude protein (18.92 ± 0.02^c for 50%), fat (10.36 ± 0.02^c for 50%), ash (6.55 ± 0.03^b for 50%), and fiber (1.92 ± 0.00^d for 50%) contents with a corresponding decrease in the carbohydrate and moisture content as the levels of substitution with African breadfruit increases from 10% to 50%. The energy value ranged from 340.99 ± 0.11^d to 381.76 ± 0.15^b kcal/100 g. The use of African breadfruit to supplement sorghum has been shown to have a considerable nutritive effect. Therefore, sorghum- African breadfruit flour blends can serve as a constituent of traditional weaning and adult meals which are low-priced compared to formulated foods.

Keywords: Complementary food, Sorghum, African breadfruit, Proximate, Microflora.

INTRODUCTION

Complementary feeding refers to the introduction of nutritionally adequate and hygienically prepared foods to infants from six months of age along with continued breastfeeding (1). It is proven that exclusive breast-feeding is the ideal feeding for infants from birth till six months of age while complementary foods are integrated into the feeding from six months till two years of age (2). As infants grow, it is crucial to introduce nutrient-rich complementary or weaning foods that would enable them to meet the nutritional needs of growing infants in order to avoid retarded or stunted growth.

Majority of the complementary foods (commercial and homemade) consumed in most developing countries are usually made from cereals. Commercially fortified foods are often beyond the reach of the poor resulting in the use of homemade complementary foods which have been reported to be poor in protein content and deficit in essential nutrients that are required for the proper and rapid growth of the child (3, 4). The intake of foods deficit in the required amount and quality of proteins can lead to various forms of malnutrition in children (5, 6). Proper combination and formulation are imperative for increased quality of homemade complementary foods. Consequently, in order to improve the nutritional quality and increase the accessibility of affordable quality complementary foods, the inclusion of high protein crops and inexpensive nutrient-rich food materials in its production is imperative (4, 7).

Sorghum [*Sorghum bicolor* (L) Moench] is a major crop of the semi-arid tropics of Africa and Asia, grown almost everywhere in the world. Sorghum belongs to the family Poaceae and is ranked the fifth most important grain crop after wheat, maize, rice and barley (8). The crop provides a good source of energy, antioxidants and is gluten-free (9, 10, 11). Sorghum is used in making a variety of food products such as: bread, porridge, pancakes, muffins and fermented foods like 'buchera', 'kunu-zaki', 'koko' and 'ogi-baba' (12). Sorghum *ogi* (*ogi-baba*) produced from the natural fermentation of cereals using simple processing methods is an important food majorly used for weaning infants in West Africa (13, 14). It is generally high in carbohydrates and low in protein content as a result of the loss of important nutrients during its processing (15). This results in nutritional contents that are inadequate and unable to meet the dietary needs of infants (16). This has led to a variety of studies on the enhancement of the nutritional value of *ogi-baba*.

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African breadfruit (*Treculia africana*), is a tropical African leguminous crop of the family *Moraceace* and is widely distributed in Senegal, Sudan and Angola. In Nigeria, it is an important food crop that is widely cultivated in the humid Southeastern ecological zone (17, 18). The crop serves as an important reservoir of essential food nutrients when common sources of other food nutrients are unavailable or out of season. The seed which is the edible part of this fruit possesses a rich protein source (25-35%) (19); the raw seed contains a good number of vitamins and minerals, 10% oil, and 40-45% carbohydrates (17, 20, 21). Before consumption, this legume (African breadfruit) is subjected to heat treatment which is one of the unique characteristics that differentiate it from other fruits. The seeds are prepared in various forms such as: boiling, roasting, frying, boiling and pounding, processed into non-alcoholic beverage, starch or flour. They are consumed as snack, side dish (decanted juice) or porridge (22, 23). Microorganisms such as: *Bacillus subtilis, Erwinia* sp., *Micrococcus* sp., *Streptococcus* sp., *Staphylococcus aureus; Lactobacillus plantarum; Laeuconostoc esenteroides; Aspergillus niger* and *Rhizopus stolonifer* have been reported to be associated with the deterioration and fermentation of African breadfruit (22, 24, 25, 18).

A number of studies have shown that supplementing cereals with legumes or tubers with vegetables and food sources from animals tends to be nutritionally superior and better supported growth and development rather than the single diets of cereal meals (26).

For instance, the nutritional content of fermented cereal foods has been improved by supplementing with legumes such as 'ugba', bambara groundnut, soy-beans, lima-beans, chickpea and beniseeds (27, 28, 29). Hence, this study is aimed at determining the microbial quality and nutritional constituents of complementary food formulated from sorghum-African breadfruit.

MATERIALS AND METHODS

Sample Collection. Sorghum (*Sorghum bicolor*) grains and African breadfruit (*Treculia africana*) used in this study were obtained from a local market in Umuahia, Abia State, Nigeria. The samples were collected in clean polythene bags and transported to the laboratory for analysis. They were monitored at the points of preparation from zero (0) to 96 hours of fermentation.

Preparation and Fermentation of Fermented Sorghum Flour (*Ogi*). One Kilogram (1 kg) of cleaned and sorted sorghum grains were soaked in water for about 96 hours at 28°C. The soaked grains were washed and wet-milled into a smooth paste. The milled grain was sieved through a 200 µm pore sieve size to obtain the slurry. In order to develop its characteristic sour taste, the filtrates were allowed to ferment (secondary fermentation) for about 24-72 hours (30).

Preparation and Fermentation of African Breadfruit Seed Flour. Freshly harvested African breadfruit seeds were sorted, cleaned and measured (2 kg). The sorted seeds were parboiled for 20 minutes, drained and dehulled manually and then wet milled using a commercial milling machine. The sample was sieved through a mesh of 425 µm pore sieve size to obtain the residue flour (31) to be used for product formulation and analysis.

Formulation of Fermented Sorghum Flour (Ogi)-African Breadfruit Complementary Foods. Five different blends of sorghum flour (*Ogi*)-African breadfruit seeds complementary foods in the ratios 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (w/w) respectively were produced. The 100% sorghum *Ogi* served as the control sample. Each sample was thoroughly mixed into homogenous flour, packaged in an airtight container, labeled and stored at ambient temperature (27±2ºC) prior to analysis.

Microbiological Analysis of Samples.

Enumeration of Isolates. At different stages of the fermentation process, samples of sorghum (*Ogi*)-African breadfruit seed flours were collected in duplicate. The number and type of microorganisms (bacteria and fungi) per ml of the fermenting samples were estimated daily for 96 hours by pour plate method using the serial dilution technique. The dilutions were prepared using sterile peptone water and after appropriate dilutions were made, 1 ml of the selected diluents were pour plated in duplicate plates on Nutrient agar (NA), MacConkey agar (MCA), Mannitol salt agar (MSA) and De Man Rogosa Sharpe agar (MRS) for aerobic bacteria count, total coliform count, total Staphylococci spp. count and total lactic acid bacteria count, respectively. Sabouraud Dextrose agar (SDA) with Chloramphenicol (250 mg/100 ml) was used for total mold count while the medium was adjusted to pH 3.5 with tartaric acid for total yeast count. Incubation of NA, MCA and MSA plates were at 37℃ for 24 hours; MRS plates were incubated under anaerobic conditions in an anaerobic jar at 30℃ for 48-72 hours and SDA plates were incubated at 28℃ for 72-96 hours. Afterwards, the colony-forming units (cfu/g) of the isolates were calculated (32).

Identification of Isolates. Pure cultures of each isolate were obtained by sub-culturing randomly selected distinct colonies onto suitable media and incubated appropriately. The selected bacterial isolates were identified based on morphological, microscopic and biochemical characteristics (32, 33) while the fungi isolates were identified according to the laboratory manual of (34) and the yeast using (35) method.

Physico-Chemical (pH and Total Titratable Acidity) Analysis of Samples. The fermenting samples were taken every 24 hours during the fermentation period and analyzed in duplicates according to the methods of (36) and (37). The pH of the samples was measured on a Jenway pH meter calibrated with KOH buffer solutions of pH 7.0 and 4.0. The determination of total titratable acidity (TTA) was done by titrating (50 ml) the homogenized sample against 0.1 N NaOH using 1 drop of phenolphthalein as an indicator and the values were expressed as percent lactic acid.

Proximate Composition and Energy Content of Samples. The different formulations of African breadfruit- supplemented *Ogi* samples were analyzed after the 96 hours of fermentation period for moisture, total ash, crude fibre, crude protein, crude fat and carbohydrate content using standard methods described by (36). Difference method was used in determining the total Carbohydrate content while Atwater factor was used in calculating the energy content (kcal/100 g).

Statistical Analysis. Data obtained in replicates of two were subjected to one-way ANOVA, as well as Mean, and Standard deviation from the means. The means were separated at $p<0.05$ using Duncan Multiple Range Test employing the SAS program.

RESULTS

The microbial counts during the fermentation of sorghum-African breadfruit blends are shown in Table 1. The colony-forming units of the organisms were investigated for a period of 96 hours and the value of the colony-forming units per gram/milliliter ranged from $1.1 \times 10^5 \pm 0.13^b$ to $10.9 \times 10^7 \pm 10.04^c$ cfu/g. The total bacteria count ranged from $3.3 \times 10^7 \pm 0.04^e$ to $10.9 \times 10^7 \pm 10.04^c$ cfu/g, total Staphylococci count from $1.3 \times 10^5 \pm 0.02^f$ to $5.8 \times 10^6 \pm 0.70^a$ cfu/g, total lactic acid bacteria count from $3.1 \times 10^6 \pm 0.14^c$ to $10.7 \times 10^6 \pm 0.01^d$ cfu/g, total coliform count from $1.6 \times 10^7 \pm 0.04^d$ to $8.8 \times 10^7 \pm 0.71^a$ cfu/ml, while the total yeast count ranged from $1.1 \times 10^5 \pm 0.00^f$ to $5.7 \times 10^6 \pm 0.14^b$ cfu/g and total mold count from $1.1 \times 10^5 \pm 0.13^b$ to $5.7 \times 10^6 \pm 0.01^a$ cfu/g.

Table 2 showed the occurrence of microorganisms isolated from sorghum-African breadfruit blends throughout the fermentation period of 96 hours. The most occurring bacteria and yeast isolates were *Lactobacillus* species and *Saccharomyces* species respectively while the least occurring bacteria and mold were *Pseudomonas* species and *Rhizopus* species respectively. Six bacteria (*Bacillus* species, *Escherichia coli*, *Pseudomonas* species, *Lactobacillus* species, *Staphylococcus aureus* and *Klebsiella* species) and four fungi (*Saccharomyces* species, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* species) were isolated from the blends of sorghum-African breadfruit seed.

The results of the pH and TTA of the fermenting blends of sorghum supplemented with African breadfruit seed are presented in Figures 1 and 2. The pH decreased with an increase in percentage supplementation and fermentation time. The total titratable acidity (TTA)

increased from 0 hour to 72 hours and then slightly decreased at 96 hours.

Table 3 depicts the result of proximate composition and energy value of complementary food from different blends of sorghum-African breadfruit seeds. Significant ($p<0.05$) increases were observed in the crude protein, fat, ash and fiber contents with a corresponding decrease in the carbohydrate and moisture content as the levels of substitution with African breadfruit seed increases.

Table 1: Microbial Counts of the Fermenting Sorghum-African Breadfruit Blends.

Microbial counts (cfu/gm)	Ratios	Fermentation Time (Hours) 0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Aerobic bacteria count	A (100:00)	$5.6 \times 10^7 \pm 0.01^d$	$8.3 \times 10^7 \pm 0.70^a$	$9.9 \times 10^7 \pm 0.05^c$	$10.9 \times 10^7 \pm 0.04^c$	$8.3 \times 10^7 \pm 0.14^d$
	B (90:10)	$4.1 \times 10^7 \pm 0.11^c$	$6.8 \times 10^7 \pm 0.04^d$	$8.2 \times 10^7 \pm 0.70^a$	$9.0 \times 10^7 \pm 0.01^d$	$7.9 \times 10^7 \pm 0.70^b$
	C (80:20)	$3.9 \times 10^7 \pm 0.00^e$	$4.5 \times 10^7 \pm 0.12^c$	$6.1 \times 10^7 \pm 0.12^b$	$7.6 \times 10^7 \pm 0.01^e$	$9.4 \times 10^7 \pm 0.70^a$
	D (70-30)	$6.4 \times 10^7 \pm 0.70^a$	$5.7 \times 10^7 \pm 0.01^f$	$7.0 \times 10^7 \pm 0.03^e$	$8.2 \times 10^7 \pm 0.70^a$	$6.5 \times 10^7 \pm 0.70^c$
	E (60-40)	$4.7 \times 10^7 \pm 0.12^b$	$5.2 \times 10^7 \pm 0.13^b$	$7.8 \times 10^7 \pm 0.01^f$	$9.6 \times 10^7 \pm 0.01^d$	$7.3 \times 10^7 \pm 0.14^e$
	F (50-50)	$4.0 \times 10^7 \pm 0.01^d$	$3.3 \times 10^7 \pm 0.04^e$	$4.5 \times 10^7 \pm 0.04^d$	$6.9 \times 10^7 \pm 0.12^b$	$7.0 \times 10^7 \pm 0.01^f$
Staphylococci count	A (100:00)	$1.7 \times 10^5 \pm 0.12^c$	$3.1 \times 10^6 \pm 0.04^d$	$4.7 \times 10^6 \pm 0.01^d$	$2.9 \times 10^6 \pm 0.14^a$	NG
	B (90:10)	$2.2 \times 10^6 \pm 0.60^b$	$3.0 \times 10^6 \pm 0.12^b$	$4.3 \times 10^6 \pm 0.01^d$	$3.6 \times 10^6 \pm 0.01^e$	NG
	C (80:20)	$3.1 \times 10^6 \pm 0.02^e$	$4.4 \times 10^6 \pm 0.04^c$	$5.8 \times 10^6 \pm 0.70^a$	$2.2 \times 10^6 \pm 0.04^c$	NG
	C (70-30)	$1.3 \times 10^5 \pm 0.02^f$	$3.9 \times 10^6 \pm 0.01^e$	$5.1 \times 10^6 \pm 0.70^{ab}$	$3.5 \times 10^6 \pm 0.01^e$	NG
	D (60-40)	$3.6 \times 10^6 \pm 0.70^a$	$4.2 \times 10^6 \pm 0.00^f$	$5.0 \times 10^6 \pm 0.04^b$	$3.1 \times 10^6 \pm 0.02^d$	NG
	E (50-50)	$2.5 \times 10^6 \pm 0.04^d$	$4.9 \times 10^6 \pm 0.12^a$	$5.2 \times 10^6 \pm 0.01^c$	$3.4 \times 10^6 \pm 0.12^b$	NG
Lactic Acid Bacteria (LAB) count	A (100:00)	$4.1 \times 10^6 \pm 0.14^b$	$5.5 \times 10^6 \pm 0.02^d$	$9.3 \times 10^6 \pm 0.12^a$	$8.0 \times 10^6 \pm 0.70^a$	$6.8 \times 10^6 \pm 0.01^e$
	B (90:10)	$6.7 \times 10^6 \pm 0.01^d$	$8.2 \times 10^6 \pm 0.14^b$	$10.7 \times 10^6 \pm 0.01^d$	$8.9 \times 10^6 \pm 0.14^c$	$7.6 \times 10^6 \pm 0.02^c$
	C (80:20)	$5.0 \times 10^6 \pm 0.01^e$	$7.7 \times 10^6 \pm 0.01^e$	$6.9 \times 10^6 \pm 0.01^e$	$6.2 \times 10^6 \pm 0.10^e$	$4.7 \times 10^6 \pm 0.12^b$
	D (70-30)	$5.6 \times 10^6 \pm 0.70^a$	$6.0 \times 10^6 \pm 0.01^e$	$8.4 \times 10^6 \pm 0.12^b$	$7.5 \times 10^6 \pm 0.14^d$	$5.2 \times 10^6 \pm 0.02^d$
	E (60-40)	$4.9 \times 10^6 \pm 0.14^b$	$5.8 \times 10^6 \pm 0.14^c$	$7.9 \times 10^6 \pm 0.00^f$	$5.3 \times 10^6 \pm 0.70^b$	$4.3 \times 10^6 \pm 0.01^f$
	F (50-50)	$3.1 \times 10^6 \pm 0.14^c$	$4.2 \times 10^6 \pm 0.70^a$	$6.6 \times 10^6 \pm 0.02^c$	$4.9 \times 10^6 \pm 0.70^b$	$3.6 \times 10^6 \pm 0.70^a$
Coliform count	A (100:00)	$4.9 \times 10^7 \pm 0.13^b$	$5.1 \times 10^7 \pm 0.70^b$	$3.2 \times 10^7 \pm 0.01^f$	$2.0 \times 10^7 \pm 0.70^a$	$1.6 \times 10^7 \pm 0.04^d$
	B (90:10)	$9.0 \times 10^7 \pm 0.11^c$	$7.9 \times 10^7 \pm 0.00^f$	$8.8 \times 10^7 \pm 0.71^a$	$5.3 \times 10^7 \pm 0.14^b$	$4.0 \times 10^7 \pm 0.12^c$
	C (80:20)	$6.3 \times 10^7 \pm 0.01^d$	$4.6 \times 10^7 \pm 0.14^d$	$6.1 \times 10^7 \pm 0.14^c$	$4.5 \times 10^7 \pm 0.14^b$	$2.9 \times 10^7 \pm 0.14^b$
	D (70-30)	$5.5 \times 10^7 \pm 0.70^a$	$8.2 \times 10^7 \pm 0.70^a$	$7.0 \times 10^7 \pm 0.02^e$	$6.3 \times 10^7 \pm 0.02^d$	$5.8 \times 10^7 \pm 0.70^a$
	E (60-40)	$3.7 \times 10^7 \pm 0.01^e$	$4.1 \times 10^7 \pm 0.01^e$	$4.9 \times 10^7 \pm 0.12^d$	$3.4 \times 10^7 \pm 0.12^c$	$1.9 \times 10^7 \pm 0.02^e$
	F (50-50)	$2.0 \times 10^7 \pm 0.00^f$	$3.8 \times 10^7 \pm 0.70^c$	$5.2 \times 10^7 \pm 0.70^b$	$4.1 \times 10^7 \pm 0.01^e$	$3.3 \times 10^7 \pm 0.01^f$
Yeast count	A (100:00)	NG	$1.3 \times 10^5 \pm 0.12^c$	$2.5 \times 10^6 \pm 0.01^c$	$4.5 \times 10^6 \pm 0.14^{bc}$	$5.2 \times 10^6 \pm 0.12^c$
	B (90:10)	NG	$1.7 \times 10^5 \pm 0.01^e$	$2.0 \times 10^6 \pm 0.70^a$	$4.3 \times 10^6 \pm 0.70^a$	$3.6 \times 10^6 \pm 0.01^e$
	C (80:20)	NG	$2.0 \times 10^6 \pm 0.70^a$	$3.9 \times 10^6 \pm 0.00^d$	$2.7 \times 10^6 \pm 0.04^c$	$1.1 \times 10^5 \pm 0.00^f$
	D (70-30)	NG	$1.6 \times 10^5 \pm 0.04^d$	$3.1 \times 10^6 \pm 0.00^e$	$3.9 \times 10^6 \pm 0.02^d$	$2.0 \times 10^6 \pm 0.02^d$
	E (60-40)	NG	$1.9 \times 10^5 \pm 0.13^b$	$3.2 \times 10^6 \pm 0.04^b$	$4.6 \times 10^6 \pm 0.01^e$	$3.1 \times 10^6 \pm 0.14^b$
	F (50-50)	NG	$1.3 \times 10^5 \pm 0.70^{ab}$	$2.9 \times 10^6 \pm 0.00^e$	$5.7 \times 10^6 \pm 0.14^b$	$4.0 \times 10^6 \pm 0.70^a$
Mold count	A (100:00)	$1.1 \times 10^5 \pm 0.13^b$	$1.5 \times 10^5 \pm 0.70^a$	$3.0 \times 10^6 \pm 0.01^d$	$2.2 \times 10^6 \pm 0.00^d$	NG
	B (90:10)	$1.8 \times 10^5 \pm 0.02^d$	$2.3 \times 10^6 \pm 0.70^a$	$3.7 \times 10^6 \pm 0.01^c$	NG	NG
	C (80:20)	$2.0 \times 10^6 \pm 0.02^d$	$3.3 \times 10^6 \pm 0.12^c$	$2.5 \times 10^6 \pm 0.04^b$	NG	NG
	D (70-30)	$3.5 \times 10^6 \pm 0.01^e$	$5.0 \times 10^6 \pm 0.04^d$	$3.9 \times 10^6 \pm 0.70^a$	$4.0 \times 10^6 \pm 0.02^a$	$5.7 \times 10^6 \pm 0.01^a$
	E (60-40)	$2.9 \times 10^6 \pm 0.70^a$	$4.0 \times 10^6 \pm 0.02^e$	$3.4 \times 10^6 \pm 0.70^a$	$1.9 \times 10^6 \pm 0.02^b$	NG
	F (50-50)	$4.9 \times 10^6 \pm 0.02^c$	$5.5 \times 10^6 \pm 0.12^b$	$3.7 \times 10^6 \pm 0.01^c$	$4.4 \times 10^6 \pm 0.00^c$	$5.6 \times 10^6 \pm 0.12^a$

Note: Values are the mean ± standard deviation of two replication of each parameter. Values with different superscripts down a column are significantly different from each other. NG= no growth.

Table 2: Occurrence of Microbes Isolated from the Sorghum-African Breadfruit Blends.

Isolates	Fermentation Time (Hours) 0 Hour						24 Hours						48 Hours						72 Hours						96 Hours					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Bacillus species	+	+	-	-	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-
Escherichia coli	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Pseudomonas species	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactobacillus species	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-
Klebsiella species	-	-	-	+	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-
Saccharomyces species	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	+	+	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	-	+
Rhizopus species	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

Note: A: 100:0 = 100 % Sorghum; B: 90 % Sorghum: 10% African Breadfruit; C: 80% Sorghum: 20% African Breadfruit; D: 70% Sorghum: 30% African Breadfruit; E: 60% Sorghum: 40% African Breadfruit; F=50% Sorghum: 50% African Breadfruit. + = Present, - =Absent.

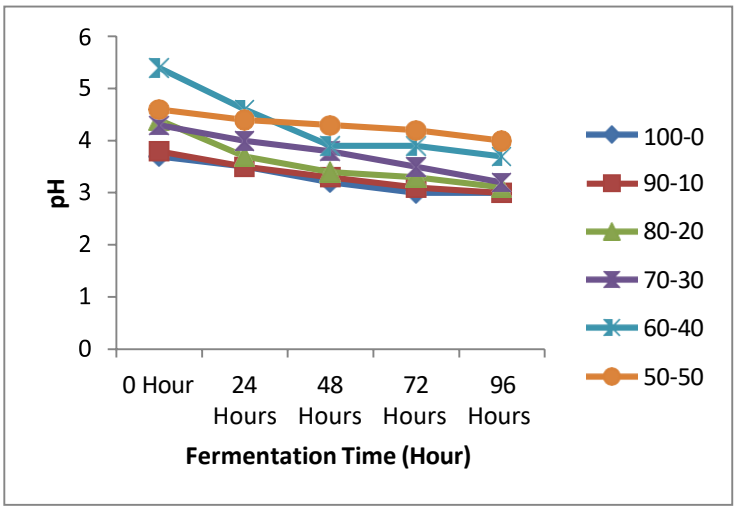

Figure 1: Changes in pH of the fermenting Sorghum-African Breadfruit Blends.

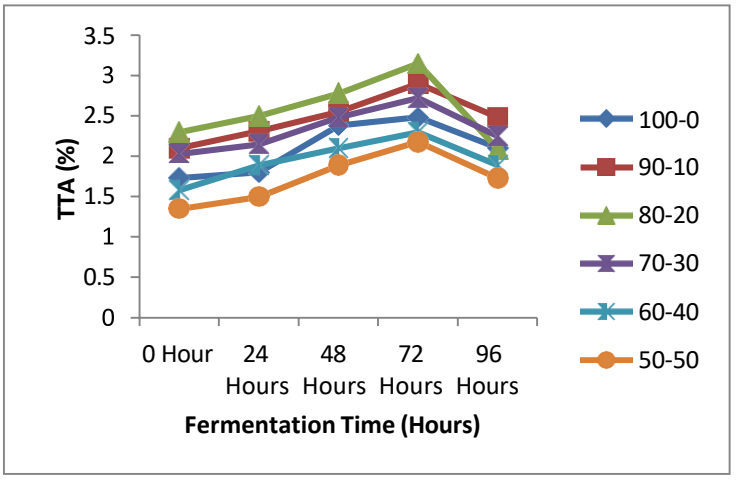

Figure 2: Changes in Total Titratable Acidity (TTA) of the fermenting Sorghum-African Breadfruit Blends.

Table 3: Proximate Composition (%) and energy value (kcal) of Complementary Food from Sorghum (*Ogi*) -African Breadfruit Blends.

Fortified Ratio (%)	Carbohydrate (%)	Crude Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Moisture Content (%)	Energy(kcal)
100-0	63.85±0.17^{a}	12.33±0.00^{f}	4.03±0.01^{d}	5.03±0.07^{a}	0.51±0.01^{c}	14.12±0.07^{a}	340.99±0.11^{d}
90-10	62.50±0.07^{b}	13.08±0.01^{d}	4.83±0.00^{e}	5.05±0.00^{e}	0.53±0.01^{c}	14.01±0.03^{b}	342.85±0.07^{f}
80-20	62.35±0.00^{f}	13.42±0.03^{b}	5.13±0.00^{e}	5.07±0.00^{e}	0.61±0.00 ^{e}	13.42±0.01^{c}	349.25±0.13^{c}
70-30	59.51±0.03^{d}	15.08±0.14^{a}	7.03±0.07^{b}	5.40±0.01^{d}	0.93±0.03^{b}	12.05±0.01^{c}	361.63±0.09^{e}
60-40	56.25±0.04^{c}	17.05±0.00^{e}	9.45±0.14^{a}	5.95±0.01^{c}	1.28±0.03^{a}	10.03±0.01^{d}	378.25±0.18^{a}
50-50	53.23±0.01^{e}	18.92±0.02^{c}	10.36±0.02^{c}	6.55±0.03^{b}	1.92±0.00 ^{d}	9.04±0.00^{e}	381.76±0.15^{b}

Note: Values are the mean ± standard deviation of two replication of each parameter. Values with different superscripts down a column are significantly different from each other.

DISCUSSION

In this study, the different microorganisms that were encountered and isolated during the fermentation of sorghum *Ogi*–African breadfruit blends may be a result of the nature of fermentation which is uncontrolled and spontaneous. Similar microorganisms have been reported to be associated with fermented products and also served as inoculum for their natural fermentation process (38, 39, 40).

The dominance of lactic acid bacteria throughout this study period could be due to increased acidic and reduced pH levels of the microbial environment thereby having an inhibitory effect on the growth of spoilage and pathogenic microflora. This explains the decrease in the microbial loads of some bacteria isolated as different microbes tolerate acid medium differently; it encourages their growth while in others it antagonizes and kills them. The presence and increase in the yeasts population could be attributed to their ability to adapt to the condition for its growth created by the decrease in the pH. This has been reported by several authors (27, 41, 42). Their presence in fermenting foods has been found to aid aroma improvement and flavor development of fermented foods (43). The presence of *Staphylococcus* which is a normal flora of the skin and nasal cavity of man could probably be introduced from air, during the washing of grains, human handlers and other materials used for the processing of the product.

The molds isolated in this study are commonly present as contaminants in cereals and could be introduced from sources such as utensils, the environment or the processing water (44). The disappearance of molds during the fermentation period could be attributed to the low oxygen tension in the fermenting environment. The genera of *Penicillium*, *Aspergillus*, *Mucor* and *Rhizopus* have been isolated by previous authors during the fermentation of maize for *Ogi* production (45); (46) and (41).

To a large extent, the population and diversity of microorganisms during fermentation play both essential and deleterious roles in the fermentation of its products. The differences in the occurrence or population of the various microorganisms (Table 2) could be linked to the acidic nature of the medium. This is, however, a result of a persistent increase in the population of lactic acid bacteria and yeast (*Saccharomyces* species) throughout the fermentation period while bacteria such as *Pseudomonas* species and mold were eliminated after 24 – 48 hours of the fermentation period.

A decrease in the pH values and a rise in the total titratable acidity during the fermentation of the sample blends were observed. These findings agree with (27) and (47) who observed similar trends in fermented maize blended with *ugba* and 30% Bambara groundnut respectively. A similar increase in acid production had been observed (48) during the production of weaning food from maize-cowpea blends. This could be due to enhanced microbial proliferation and production of organic acids from available nutrients by the fermenting lactic acid bacteria, creating a favorable condition for lactic acid bacteria and yeast and in turn inhibiting the growth of spoilage organisms. The increase in titratable acidity is essential in preventing the growth of undesirable microorganisms that can cause poor fermentation (47).

On the nutritional composition, there was an increase in the contents of the crude protein, fat, ash, and fiber of the sorghum *ogi* when supplemented with various blends of African breadfruit seed flour. The result is consistent with other reports by (27) and (4) on the quality improvement of cereals. The protein content obtained was higher than those obtained by (17), who reported a value of 12.55 at a 65:20:15 ratio of cooking banana flour, African breadfruit seed flour and broken rice flour. The increase in protein contents of the fermented blends with increased addition of African breadfruit seed flour conforms to earlier reports of (27) and (49) supplementing *Ogi* with *ugba* and *Ogi-baba* with lima-bean respectively. The relatively higher protein content of this complementary food from sorghum *Ogi*-African breadfruit seed would improve the protein intake of infants. The increase in protein may be attributed to the increased activities and growth of microorganisms in the form of single-cell protein (50, 51). The protein content is the major component of body tissues and a crucial nutrient required for growth.

The increase in the fat contents with increased ratios of African breadfruit seed addition could be due to the high level of fat in African breadfruit seeds and the activities of the fermenting organisms. The result of this study contrasts with the earlier report of (4) who observed a significant increase in the proportion of fermented breadfruit-soybean flour blends with lower values of 3.05 to 4.72% at a ratio of 10 to 40. The presence of fat in complementary food increases the energy density of food, serves as an important component of cell membranes and transport vehicle for fat soluble vitamins which are required for adequate development of infants and their normal growth (52). The ash content increased significantly ($p<0.05$) as the amount of African breadfruit seed addition increased. The ash contents of the samples were higher than the values (2.50 – 3.53 g/100 g) reported by (17) for complementary food made from a combination of cooking banana, African breadfruit seed and broken rice flours. The increase in ash contents of these complementary food blends is an indication that African breadfruit seed would be a good source of mineral content. This therefore, suggests that the ash content of sorghum *Ogi* can be improved by the incorporation of African breadfruit seeds. Ash content is an indication of the level of minerals in food and an index of the total mineral elements in each food sample. Minerals are essential nutrients which serve various important metabolic functions (53). The crude fiber content was significantly ($p<0.05$) higher as the proportion of African breadfruit seed addition increased but were in correlation with the (54) standard of not more than 5%. The values obtained in this study were lower than the values (4.92 – 5.86%) reported by (4) for breadfruit and soybean flour but were comparable

with those obtained by (27) for *Ogi* and *ugba.*

The carbohydrate content was reduced with an increase in the level of African breadfruit seed inclusion. This agrees with the earlier observation of (14) and (49) that addition of legume decreases the carbohydrate content of sorghum based traditional foods. The energy value of the samples, which ranged from 342.85 to 381.76 kcal/100 g, increased when supplemented with various amounts of African breadfruit seed flours. Although, there was a reduction in the carbohydrate content of the complementary foods, the increase in energy value of the samples could be due to the significant contribution of African breadfruit seed to the protein and fat contents of the foods. Carbohydrates are good energy sources which contribute to the energy value of food samples.

The moisture contents of all the complementary blends decreased with an increased level of African breadfruit seed addition. Also, (51) observed lower moisture content during the fortification of cowpea with bread fruit blends. The low moisture content of the food sample indicates that it would have a good keeping quality and enhanced storage stability by preventing the growth of food spoilage microorganisms (55).

CONCLUSION

The present study has provided information on the nutritional quality of locally produced complementary blends of *Ogi-baba*-Africa breadfruit, revealing the presence of lactic acid bacteria, yeasts and some pathogens which were not directly associated with the fermentation process. The study established that formulation of complementary foods from sorghum and African breadfruit seeds flour blends brought about significant increases in the nutritional composition with higher crude protein, fat, ash and fiber contents and a notable reduction in the carbohydrate and moisture contents when compared to sorghum alone (control). This further revealed the possible utilization of Africa breadfruit seeds as a constituent of traditional weaning meals and adult food which will alleviate the symptoms of diabetes and protein deficiency mostly common in developing countries.

AUTHOR CONTRIBUTIONS

IVO conceived and designed the experiments; OCA performed the experiments; IVO analyzed the data and wrote the paper. Both authors read and approved the final manuscript.

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