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Effect of Fermentation on Nutrient and Antinutrient Contents of Fermented Whole and Ground African Breadfruit (*Treculia africana*) Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. Author KTA designed the study, procured the raw materials, was involved in the microbiological and chemical analyses and wrote the first draft of the manuscript. Author SF was involved in the microbiological, chemical and statistical analyses of the study. Author BSA was in charge of literature searches and statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the microbial contents, proximate compositions and the antinutrient contents of whole and ground fermented African breadfruit seeds.
Methodology: The whole and ground samples were fermented for 72 hours. The microbial contents, pH, total titratable acidity and temperature of the samples were monitored on daily basis while their proximate composition and antinutrient contents were determined before and after fermentation.
Results: Total microbial counts increased in both samples but higher in fermented ground samples. The microorganisms isolated were *Bacillus (B.) subtilis, B. pumulis, Staphylococcus (S.) aureus, Lactobacillus (L.) plantarum, L. bulgaricus, Leuconostoc (L.) mesenteroides, Aspergillus (A.) niger, A. flavus* and *Saccharomyces (S.) cerevisiae. B. subtilis* and *L. plantarum* were isolated from both samples throughout the fermentation period. The pH of the fermented ground samples decreased

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from 6.53 to 5.25 while the fermented whole samples decreased from 6.42 to 5.73. The TTA of ground and whole samples increased from 2.34% to 3.60% and from 2.43% to 3.12% respectively. The temperature of the ground sample increased from 27.6℃ to 30.8℃ while the whole sample increased from 27.8℃ to 31.2℃. The crude protein con tents of the fermented ground and whole samples increased from 18.40% to 25.71% and 24.39% while crude fat contents reduced from 5.45% to 2.61 and 3.47% respectively. The crude ash contents had higher content in fermented ground sample (3.13%) than fermented whole sample (2.13%) while fibre contents significantly reduced from 2.66% to 1.46% and 1.87% and carbohydrate contents from 61.53% to 54.87% and 55.63% respectively. All the antinutrient contents significantly reduced in both fermented samples with more reductions in ground sample. The fermented ground sample had highest overall acceptability than the whole sample and the raw sample.

Conclusion: Improvement in the nutritional content and reduction in the antinutrient contents of the fermented samples suggest their usefulness as supplements in food and feed formulation for human and livestock.

Keywords: Breadfruit; fermentation; microorganisms; nutrient; antinutrient.

1. INTRODUCTION

The rapid increase in population in developing countries and the subsequent shortage of animal proteins call for urgent search for potential local crops which can serve as economically possible substitutes [1]. Under utilization of some wild legumes has contributed significantly to low income and dietary intake by the poor in the rural areas [2,3]. Hence, the need to exploit some of potentials in these legumes.

The use of fermentation technology for various industrial productions cannot be over-emphasized due to its contribution to the economy having long being practised since the existence of mankind [3]. Fermented foods form an intricate part of the diets of people in all parts of the world; even in Nigeria many people depend on fermented products for their daily nutritional requirements [4,5]. The traditional techniques of processing fermented foods are still being practised among the rural homes as a means of business for income generation and survival despite their shortcomings such as lack of durability and homogeneity [4]. Fermentation of different foods has different advantages such as shelf life stability, improvement in their nutrient contents and sensory properties, reduced cooking time, removal of undesirable substances and reduction in their antinutritional factors [3,6].

African breadfruit (*Treculia africana)* belongs to the family Moraceae [7,8]. It is an evergreen plant and native to many parts of West and Tropical Africa which is known as *afon, barafuta, ize, eyo, ediang* and *ukwa* in Yoruba, Hausa, Bini, Igala, Hausa and Igbo tribes of Nigeria respectively [9,10]. African breadfruit serves as nutritious feed for livestock, low cost meat substitute for poor families, fodder for animals, and wood for furniture making, pulp and paper production as well as fibre-board production [7,9]. It has also been reported to have medicinal values such as curing of malaria, cough, rheumatism, mouth-yaw, rashes, stomach disorders and regeneration of injured liver cells [9,11,12].

African breadfruit seeds can be cooked as porridge or mixed with other food stuffs such as sorghum [13]. It can also be roasted and sold with palm kernel (*Elaeis guineensis*) as a road side snack [14]. The seeds are highly nutritious and constitute a cheap source of vitamins, minerals, proteins, carbohydrates and fats, also in preparing pudding and as a thickener in weaning food for children [10,14,15]. The seeds have an excellent polyvalent dietetic value whose biological value has been reported to exceed contents of some legumes such as soybeans [16]. The extracts from the sprouted seeds have been supplemented with kunun-zaki at various concentrations and improved the protein content of the gruel [17]. The seeds have also been used as adjunct for ethanol production [18]. African breadfruit seeds contain some antinutrient factors which interfere with digestive processes and prevent efficient utilization of their proteins. They include tannins, phytates, oxalates and saponins [10,19].

However, not much work has been done on the fermentation of African breadfruit seeds. This work therefore investigates the effect of fermentation on the nutrient and antinutrient contents of whole and ground African breadfruit seeds.

2. MATERIALS AND METHODS

2.1 Source of Samples

The fresh African breadfruit pods were purchased at local market in Anambra State in South Eastern part of Nigeria and transferred to Microbiology Laboratory of the Adekunle Ajasin University in a sterile polythene bag.

2.2 Preparation of the Breadfruit Seeds

The seeds of African breadfruits were removed from the mature pods and transferred into a clean plastic bowl. The seeds were washed with several changes of clean water to reduce the sliminess of the husk. The water was drained from the seeds using a plastic basket. Unhealthy and shredded African breadfruit seeds were removed from the wholesome seeds by hand picking. The left healthy wholesome seeds were rinsed, drained and parboiled for 15 minutes for easy dehulling and prevention of the breakage of the cotyledons. The cotyledons were removed after cooling by pressing between fingers [20,21]. The dehulled cotyledons were recovered by winnowing methods using a flat tray.

2.3 Fermentation of the Samples

The dried African breadfruit seeds were processed in two ways for fermentation; the ground and the whole seed forms. The ground breadfruit sample was obtained by weighing 500 g of seeds, cooked at 70℃ for 20 minutes and allowed to cool. The seeds were milled using a sterile electrical blender with addition of 500 mL of sterile distilled water.

The whole breadfruit sample was processed by weighing 500 g of African breadfruit seeds, cooked at 90℃ for 45 minutes and allowed to cool.

Each sample was poured into a sterile plastic bucket in triplicates. Five hundred millilitre of sterile distilled water was added to the ground seed samples while 1000 mL was added to the whole seed samples. The samples were fermented using natural flora for 72 hours at ambient temperature**.**

2.4 Microbiological Analysis

Ten grams of each sample were homogenized with 90 mL sterile peptone water solution to form the stock cultures. The samples were further serially diluted to appropriate dilutions. Enumeration of the total bacteria, lactic acid bacteria and fungi was carried out on daily basis using plate count agar (Oxoid CM 325, Hampshire, UK), De Man, Rogosa and Sharpe (MRS) agar (Oxoid CM 361) and Sabouraud dextrose agar (SDA, Oxoid CM 41) respectively. Fungal plates were incubated at 25℃ for 2 to 5 days while bacterial cultures were incubated at 37℃ 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 characterized based on cultural, morphological and biochemical tests [22].

2.5 Temperature, Total Titratable Acidity and pH

The temperature of the fermenting African breadfruit seeds was monitored on daily basis for 3 days. The % total titratable acidity was determined by diluting 10 g of the sample in 90 ml of sterile distilled water. The mixture was homogenized and allowed to settle from which 20 ml would be titrated against 0.1 N NaOH using phenolphthalein as indicator. The pH was determined using a pH meter (Crison Basic model 20) calibrated with standard buffer (pH 7.0 and 4.0) [23].

2.6 Proximate Composition

The proximate composition of the fermenting African breadfruit seeds was monitored before and after the fermentation period. The parameters monitored include moisture, crude protein, crude fat, ash, crude fibre and carbohydrate contents [23].

2.7 Antinutritional Factors

The tannin content of the seeds was determined as described by Makkar et al. [24]. The phytic acid content was determined by extraction and precipitation as described by Wheeler and Ferrel [25] while method described by Day and Underwood [26] was adopted for the determination of oxalates. The methods of Obadoni and Ochukwo [27] were adopted for alkaloid and saponin determination.

2.8 Sensory Evaluation

The sensory evaluation of whole and fermented breadfruit seed was monitored according to the

method described by Iwe [28]. The fermented samples were served 20 untrained judges to evaluate the sensory qualities; texture, slimness, odour (aroma), appearance and overall acceptability. The judges evaluated the samples using an 8 – hedonic scale where 8 = like extremely 7 = like very much 6 = like moderately 5 = like slightly 4 = dislike slightly 3 = dislike moderately 2 = dislike very much and 1 = dislike extremely.

2.9 Statistical Analysis

All analyses were carried out in triplicates. Analysis of variance (ANOVA) plus Duncan's multiple range test was used for comparison of means using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago, USA). Significance was accepted at $p < 0.05$.

3. RESULTS

3.1 Microbial Counts

Table 1 shows the total microbial counts of the fermenting ground and whole breadfruit seeds. The total viable counts of the fermenting ground breadfruit seeds increased from 3.1×10^4 cfu/g to 5.1×10^7 cfu/g while the whole breadfruit seeds increased from 7.7×10^4 cfu/g to 3.6×10^6 cfu/g. The total lactic acid bacterial counts increased in the ground sample from 7.7×10^3 cfu/g to 3.1×10^6 cfu/g while an increase from 1.2×10^4 cfu/g to 1.1×10^6 cfu/g was observed in fermenting whole sample. The total fungal counts increased from 2.5×10^3 sfu/g to 4.1×10^4 sfu/g, and from 1.0×10^3 sfu/g to 2.5×10^4 sfu/g in the fermenting ground and whole breadfruit seeds respectively.

Microorganisms identified during the fermentation of the samples comprised four genera of bacteria and 2 genera of fungi. The isolates were *B. subtilis, B. pumulis, S. aureus, Lactobacillus plantarum, L. bulgaricus, L. mesenteroides, Aspergillus flavus, a. flavus* and *S. cerevisiae*. *B. subtilis* and *L. plantarum* occurred throughout the fermentation process. *S. aureus* and *Aspergillus flavus* were isolated from both samples in the first day and in whole sample only after 24 hours, while *L. bulgaricus* and *L. mesenteroides* occurred only once in the whole sample and ground sample at 0 hour and 72 hours of fermentation respectively. All the fungi were isolated from both samples in the first day of fermentation. *S. cerevisiae* and *A. niger* were isolated in ground samples and *A. flavus* in whole samples only at 24 and 48 hours of fermentation. *Aspergillus flavus* and *A. niger* were again isolated whole samples at 48 and 72 hours of fermentation respectively (Table 2).

3.2 pH, Total Titratable Acidity and Temperature of the Fermenting African Breadfruit Seeds

Fig. 1 shows the pH and total titratable acidity of the fermenting ground and whole breadfruit seeds. The pH of the ground samples decreased from 6.53 to 5.25 while the whole samples decreased from 6.42 to 5.73. The TTA of ground and whole fermenting African breadfruit samples increased from 2.34% to 3.60% and from 2.43% to 3.12% respectively.

The temperatures of both samples increased as the fermentation progressed up to the end of the fermentation period. The temperature of the ground sample increased from 27.6℃ to 30.8℃ while the whole sample increased from 27.8℃ to 31.2℃ (Fig. 2).

3.3 Proximate Composition of the Fermented African Breadfruit seeds

The results of proximate composition of the samples were shown in Fig. 3. The moisture content of the unfermented sample was 10.51% which significantly (*p>0.005*) increased to 12.21% and 12.51% in the fermented ground sample and the fermented whole sample respectively. The crude protein contents also increased significantly (*p>0.005*) from 18.48% to

Table 1. Total microbial counts of the fermenting ground and whole African breadfruit seeds

Hours	Total bacterial count (cfu/g)		Total lactic acid bacterial count (cfu/g)		Total fungal count (sfu/g)	
	GF	WF	GF	WF	GF	WF
0	3.1×10^4	7.7×10^4	7.7×10^3	1.2×10^4	2.5×10^3	1.0×10^3
24	7.6×10^4	2.4×10^5	1.6×10^4	1.5×10^4	2.8×10^3	1.6×10^3
48	5.2×10^6	2.5×10^6	2.4×10^5	2.8×10^5	2.1×10^4	4.0×10^3
72	5.1×10^7	3.6×10^6	3.1×10^6	1.1×10^6	4.1×10^4	2.5×10^4

Key: GF- Ground fermented African breadfruit seeds, WF- Whole fermented African breadfruit seeds

Table 2. Occurrence of microorganisms in the fermenting African breadfruit seeds

Isolates	Fermentation petiod (hours)							
	0		24		48		72	
	GF	WF	GF	WF	GF	WF	GF	WF
Bacillus subtilis	+	+	+	+	+	+	+	+
Bacillus pumilus	+	_	+	+	_	+	_	_
Staphylococcus aureus	+	+	_	+	_	_	_	_
Lactobacillus plantarum	+	+	+	+	+	+	+	+
Lactobacillus bulgaricus	_	+	_	_	_	_	_	_
Leuconostoc mesenteroides	_	_	_	_	_	_	+	_
Saccharomyces cerevisiae	+	+	+	_	+	_	_	_
Aspergillus niger	+	+	+	_	+	_	_	+
Aspergillus flavus	+	+	-	+	-	+	-	-

Key: +: Positive, -: Negative, GF- Ground fermented African breadfruit seeds, WF- Whole fermented African breadfruit seed

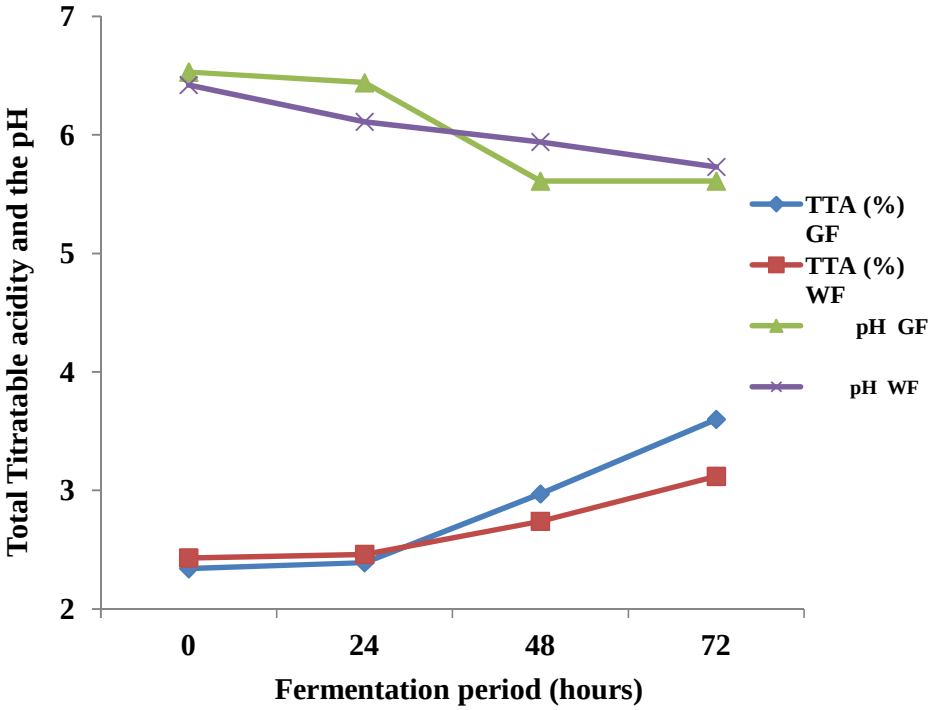

Fig. 1. The total titratable acidity and the pH of the fermenting African breadfruit seeds
Key: GF - Ground African breadfruit seeds, WF - Whole African breadfruit seeds

25.71 to 24.39% in the fermented ground sample and the fermented whole sample respectively. Similar trend was also observed in the crude ash content from 1.37% to 3.13% and 2.13% respectively but the latter was not significantly different (*p>0.005*) from the unfermented sample. However, the crude fat contents of the fermented samples decreased after the fermentation from 5.45% to 2.61% and 3.47% respectively in ground sample and whole sample. Crude fibre contents also significantly reduced (*p>0.005*) from 2.66% to 1.47% and 1.87% in ground sample and whole sample respectively after fermentation. Similar significant reductions (*p>0.005*) were also observed in the carbohydrate contents of the sample which decreased from 61.53% in the unfermented sample to 54.87% and 55.63% in ground and whole samples respectively.

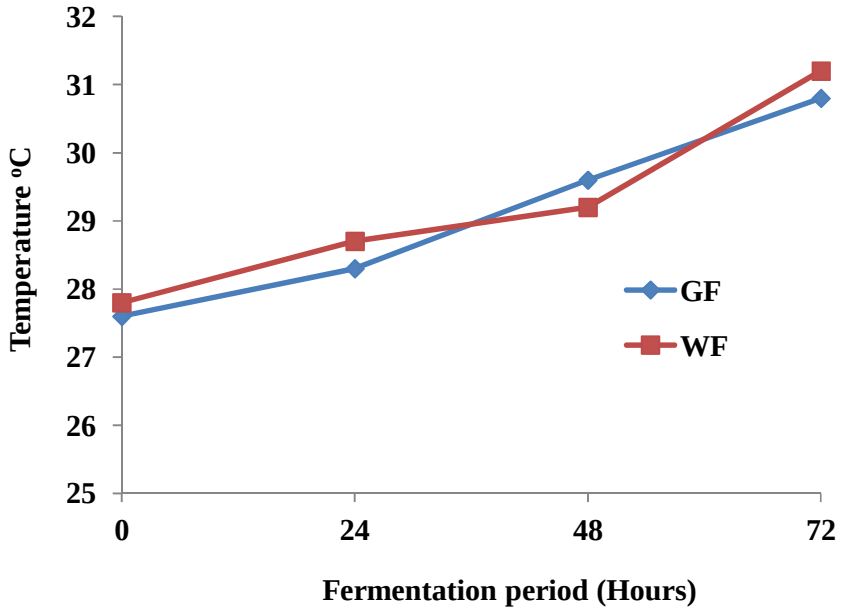

Fig. 2. Temperature of the fermenting African breadfruit seeds
Key: GF - Ground African breadfruit seeds, WF - Whole African breadfruit seeds

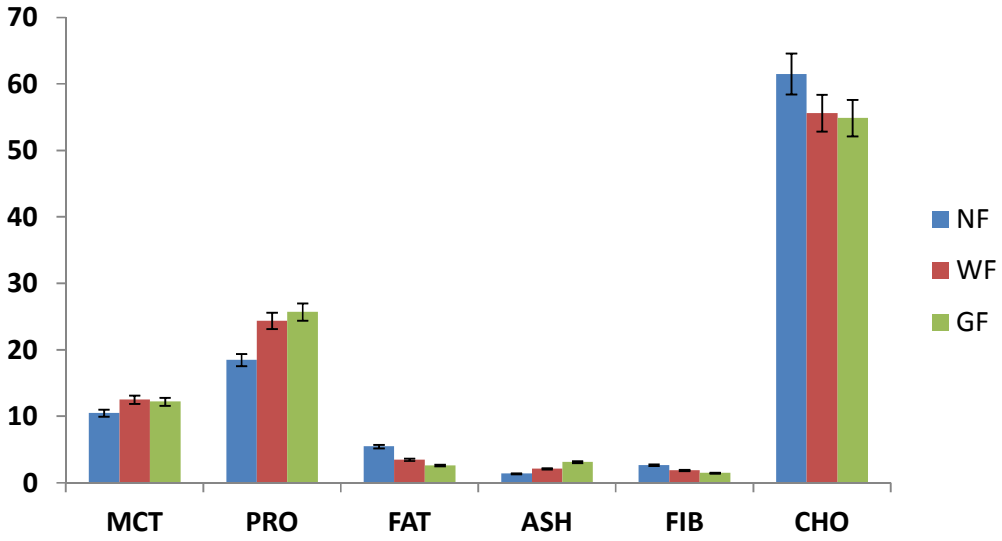

Fig. 3. Percentage proximate composition of the unfermented and fermented African breadfruit seeds
Key: NF- Non-fermented African breadfruit seeds; GF- Ground fermented African breadfruit seeds WF- Whole fermented African breadfruit seeds; MCT- Moisture content; PRO- Protein content; FAT- Fat Content; ASH- Ash content; FIB- Fibre content; CHO- Carbohydrate content

3.4 Antinutrient Contents of the Fermented African Breadfruit Seeds

The antinutrient contents decreased significantly (*p>0.005*) after fermentation in both samples with the lowest reductions in ground sample (Table 3). Oxalate contents in ground and whole fermented breadfruit decreased from 0.85 mg/g to 0.27 mg/g and to 0.31 mg/g and alkaloid contents from in ground fermented decreased

from 2.94% to 2.32% and 2.37% respectively. Saponin contents reduced after fermentation in ground and whole samples from 4.42% to 1.41% and 2.46%. The fermented ground and whole samples tannin contents decreased from 5.71 mg/100g to 2.41 mg/100g and 2.85 mg/100g while the phytate contents decreased from 6.60 mg/g to 2.39 mg/g and 2.84 mg/g respectively.

Table 3. Antinutrient contents of the unfermented and fermented African breadfruit seeds

Antinutrient	NF	GF	WF
Oxalate (mg/g)	0.85^{a}	0.27^{b}	0.31^{b}
Alkaloid (%)	2.94^{a}	2.32^{b}	2.37^{bc}
Saponin (%)	4.42^{a}	1.41^{b}	2.46^{b}
Tannin (mg/100g)	5.71^{a}	2.41^{b}	2.85^{b}
Phytate (mg/g)	6.59^{a}	2.39^{b}	2.84^{b}

Mean values with different superscripts within the same column are significantly different ($p < 0.05$)
Key: NF- Non-fermented African breadfruit seeds, GF – Ground fermented African breadfruit seeds, WF – Whole fermented African breadfruit seeds

Table 4. Sensory evaluation of the fermented African breadfruit seeds

Sensory qualities	NF	GF	WF
Texture	4.61^{b}	6.41^{a}	6.31^{a}
Sliminess	4.81^{c}	6.22^{ab}	6.72^{a}
Odour	3.21^{b}	6.61^{a}	6.52^{a}
Appearance	4.42^{b}	5.62^{a}	5.61^{a}
Overall acceptability	4.21^{c}	6.02^{a}	5.62^{a}

Mean values with different superscripts within the same column are significantly different ($p < 0.05$).
Key: NF- Unfermented African breadfruit seeds, GF – Ground fermented African breadfruit seeds, WF – Whole fermented African breadfruit seeds

3.5 Sensory Evaluation of the Fermented African Breadfruit Seeds

All the sensory parameters were significantly higher in the fermented samples than the unfermented sample. Besides, texture, odour and physical appearance of the fermented ground sample were rated higher than those of the fermented whole sample but their respective values were not significantly different ($p>0.05$). However, the sliminess was rated higher in the whole samples but they were not also significantly different ($p>0.05$). The ratings of the overall acceptability of the raw sample, fermented ground sample and the fermented whole sample were 4.21, 6.02 and 5.62 respectively.

4. DISCUSSION

The natural fermentation of African breadfruit seeds which was brought about by both bacteria and fungi has been reported in some fermented legumes. Adegbehingbe [29] isolated *Bacillus* species, *S. aureus*, *Aspergillus niger* and *S. cerevisiae* while fermenting lima bean seeds. Ajayi and Adebolu [30] isolated *Bacillus* and *Aspergillus* species from African breadfruit (*Artocarpus cummumis*) seeds during storage. Ojokoh et al. [31] also reported similar microorganisms during the fermentation of African breadfruit (*T. africana*) and cowpea blend flours. An increase in the microbial loads in both ground and the whole seed samples might be attributed to its nutritional composition of the seeds, suitable environmental condition and probably reduction of growth inhibitors in the samples as the fermentation progressed. The higher microbial counts in ground sample could be as a result grinding treatment which increased the surface area of the sample making nutrients more readily available for the fermenting microorganisms for their growth. Adegbehingbe [29] reported an increase in microbial loads while fermenting lima bean seeds. The occurrence of *B. subtilis* and *Lactobacillus* spp. might be attributed to their proteolytic action in breaking down oil in legumes and acid secretion thereby preventing the growth of unwanted microorganisms [32]. *Bacillus* spp. has been implicated as the most predominant microorganisms in many legume base fermented seeds [29,31]. The presence of *Staphylococcus* species at initial fermentation process might be due to contamination during harvesting, handling, milling and inadequate precautionary measure during processing [31].

The observed decrease in the pH of the samples and subsequent increase in the titratable acidity is a desirable development because growths of many Gram negative and acid sensitive food borne pathogens are inhibited at low pH. The increase in total titratable acidity of fermented foods during the fermentation period could be due to the ability of the fermenting organisms to secrete acids while utilizing the available nutrients for their metabolic activities [33]. This might also be due to the presence of lactic acid bacteria which degrade carbohydrate resulting in acidification [31, 34].

The temperature of the two fermented samples was found to increase slightly. This might be due to heat being generated as a result of

exothermic reactions mediated by microbial enzymes during the fermentation. Similar observation was reported by Onyimba et al. [35] on solid state fermentation of spent sorghum grains.

An increase in protein contents of the fermented sample after fermentation might be due to the proteolytic activities and increase in number of microorganisms during fermentation. The result obtained from this study was in agreement with the findings of Onyimba et al. [35] who reported protein at first week of fermentation of sweet potato leaves and spent sorghum grain.

The reduction in the fat contents of the fermented samples was in agreement with Audu and Aremu [36] while processing red kidney beans. High fat in food contents play role in the reduction of shelf life of food products. However, Okorie et al. [37] observed an increase in fat contents of composite diets prepared from sprouted and fermented millet and breadfruit seed flours which they attributed to increased activity of the lipolytic enzymes in the fermentation medium which hydrolyzed fat to glycerol and fatty acids. The reduction in crude fibre may be due to the enzymatic breakdown of the fibre during fermentation by lactic acid bacteria. This was in agreement with the findings of Ofuya and Nwajiuba [38] who reported an over 35% loss of cellulose, a major component of crude fibre, during the solid-state fermentation of cassava peel with *Rhizopus* species and *Aspergillus niger*. Amoo and Jokotagba [39] however reported an increase in crude fibre contents of *Hura crepitans* seed flour fermented with *Aspergillus niger* and *Aspergillus fumigatus.* Crude fibre is known to aid the digestive system of human. However, excess dietary fibre in diets may alter mineral metabolism, especially when phytate is present [40].

The total carbohydrate contents of the samples also decreased significantly by fermentation. This result was in agreement with results of earlier workers [41,42]. Loss in carbohydrate may be attributed to 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.

The significant reductions observed in the anti-nutrient contents of the sample might be due to leaching into the cooking water and subsequent microbial activities during fermentation [43,44]. Mixed culture of *S. Cerevisiae, Lactobacillus* sp., and some fungi have been reported to enzymatically hydrolyse some antinutrient contents in the course of fermenting foods [45, 46].

The antinutrient contents in foods have various detrimental effects when consumed by animals. Tannins which are polyphenols and polyphenolic compounds are soluble in water and also form complexes with dietary protein thereby making it indigestible. Saponin has hemolytic effect on red blood cells while oxalates and phytic acid are known to interfere with digestive processes [47,48]. Saponins have also been reported to alter cell wall permeability and therefore produce some toxic effects when ingested. Saponins have been shown to bind to the cells of the small intestine thereby affecting the absorption of nutrients across the intestinal wall [49]. The sensory properties of foods greatly contribute to their acceptability which will determine the choice of such consumables for man need. The ground and whole fermented breadfruit samples had better ratings than the unfermented sample in all the parameters considered. Besides higher ratings of fermented ground sample than the fermented whole sample might be due to the activity of microorganisms during fermentation which could be enhanced by the larger surface area of the former.

5. CONCLUSION

This study reveals the activity of microorganisms in improving the nutritional quality of the fermented breadfruit. The presence of desirable microorganisms also contributes to its sensory properties and reduction in its antinutritional content. Therefore, improvement in the nutritional quality of African breadfruit can serve as alternative cheap source of raw materials in food industries for production of children weaning food and for old age.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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