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Effect of Blanching on the Microbiological and Nutritional Qualities of Fermented Breadfruit Cowpea blends: A formulated Weaning Diet.

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ABSTRACT

This study investigates the effect of blanching on the microbiological and nutritional qualities of fermented breadfruit cowpea blends. Samples of breadfruit were processed into blanched fermented breadfruit cowpea (Sample BA) and unblanched fermented bread fruit cowpea blends (Sample UB) using spontaneous fermentation process. The microbial content, pH, and the total titratable acidity (TTA) of the fermented blends were monitored for 48 hours using standard procedures. The proximate composition and anti-nutrient content were also determined by standard methods. The pH of the fermented breadfruit cowpea blends decreased as fermentation time increased while the % TTA increased as fermentation time increases with Sample UB having the highest TTA of 1.22 % and least pH (3.20) after 48 hours. The microbial count increased in all fermented breadfruit cowpea samples but higher in unblanched fermented blends while enteric bacteria were completely suppressed after 48 hours of fermentation. Some microorganisms such as Lactobacillus *plantarum, L. brevis, L. delbrueckii, Bacillus spp., Staphylococcus aureus, Candida spp., B. cereus, B. subtilis* and *Saccharomyces cerevisae* were isolated and identified from the fermented blends. The highest crude protein content (6.01%) was observed in Sample BA at 48 hours but not significantly different (*p≤0.05*) from sample UB (5.99%). The anti-nutrient content decreased as fermentation time increased in all fermented samples with more reductions in Sample BA. Blanching breadfruit samples prior to fermentation resulted in blends with high protein content, reduced enteric count, low carbohydrate content with highly reduced anti-nutrient content.

INTRODUCTION

Weaning is a gradual process of introducing solid food to an infant's diet alongside breast milk from the age of three to four months, since breastfeeding alone cannot meet the infant nutritional requirements (Oduro *et al*., 2007). In Nigeria, traditional weaning food consist of cereal grains prepared form either millet, sorghum, maize, referred to as "Ogi" or "Akamu" which is of poor nutritional value (Msheliza *et al*., 2018). The major problem associated with infant during transitional phase of weaning is protein energy malnutrition (PEM) which results into condition such as marasmus or kwashiorkor (Salmon *et al*., 2008). Poor feeding practices as well as lack of suitable complementary foods are responsible for under nutrition with poverty exacerbating the whole issue (Adepeju *et al*., 2014). Apart from cereals and roots, breadfruit is one of the commonest staple foods in the tropics especially in the South-West, Nigeria (Ijarotimi and Aroge, 2005).

Breadfruit (*Artocarpus atilis*) has a considerable untapped potential as a nutritious food particularly among the low-income groups of the society in developing countries, and has an advantage over cereals and roots as it yields two or three times as much minerals and vitamins as cereals and roots (Amusa *et al.*, 2002). Moreover, it provides an excellent source of calories for the diet; it is also a rich source of fiber, vitamins such as vitamin C, minerals such as potassium, and phytochemicals such as flavonoids (Innocent *et al.,* 2016). It is one of the principal sources of energy protein, vitamins and minerals.

Also, its carbohydrate, protein, fat and mineral contents is comparable and in some cases superior to some cereals or food grains (Adebowale *et al.,* 2008). Breadfruit powder or flour is reported to contain about 76.7% carbohydrate (Morton, 1987), 17.1% protein, 11% fat, 3.0% ash and 0.1% crude fiber (Akubor *et al*., 2000). Composite meal has been reported to be formed from breadfruit and some plant materials. Supplementation of breadfruit with cowpea which has a potential of reducing protein-energy malnutrition during the weaning age has also been reported (Ariahu *et al.,* 1999).

There is need to investigate the effect of blanching on the microbiological and nutritional qualities of breadfruit cowpea based weaning diet.

MATERIALS AND METHODS

Sample collection

Twenty (20) freshly harvested, matured and unripe breadfruit (*Artocarbus atilis*) weighing 15kg were purchased from a local market in Ile-Ife, Osun State South West Nigeria and transported to the Postgraduate Laboratory of Department of Microbiology, University of Ibadan. The breadfruit samples were stored in clean polyethylene bag at 4^0C for further analysis.

Sample treatment

Preparation of breadfruit flour

Fresh unripe Breadfruit were thoroughly washed with sterile distilled water, manually peeled, and diced into sizes. The diced fruits were divided into two parts, First half was blanched in boiling water (100°C) for 10 minutes, drained, oven dry at 40°C for 24 hours, and milled using sterile blender ,and then sieved to obtain blanched breadfruit flour (Adepeju *et al.,* 2014). The second half was used soaked in sterile distilled water for 10 minutes, decanted, drained and dried at 40°C for 24 hours, milled and sieved to obtained unblanched breadfruit flour (Wakil *et al.,* 2008).

Product Development

Development of blanched fermented breadfruit cowpea blends

500g of Blanched Breadfruit flour was weighed into a clean beaker. The Breadfruit flour was reconstituted in sterile distilled water at 30% (w/v) (Livingstone *et al.,* 1993) containing 10 g of cowpea flour, and allowed to ferment spontaneously at 30±2°C for 48 hours on the laboratory bench. Samples were taken at regular intervals during fermentation (0, 24 and 48 hours) to assess the microbial load/quality, nutritional quality and the physicochemical parameters (Total Titratable Acidity and pH) of the fermenting slurry. The sample was allowed to dry to obtain blanched fermented breadfruit cowpea diet (Sample BA)

Development of unblanched fermented breadfruit cowpea blends

The fresh unblanched Breadfruit flour was weighed into a clean container steeped in sterile distilled water at 30% (w/v) (Livingstone *et al.,* 1993) containing 10g of cowpea flour, and allowed to ferment spontaneously at 30±2°C for 48 hours on the laboratory bench. Samples were taken at regular intervals during fermentation (0, 24 and 48 hours) to assess the microbial load/quality, nutritional quality and the physicochemical parameters (Total Titratable Acidity and pH) of the fermenting blends. The fermented sample was then drained, dried and sieved to obtain fine unblanched fermented breadfruit cowpea diet (Sample UB).

Microbiological analysis of the fermented blends

The analysis of microbial load was carried out on the fermenting slurry using the pour plate method as described by Prescott *et al.,* (2014). The media used are De Man Rogosa and Sharpe (MRS) agar for Lactic Acid Bacteria (LAB), Nutrient agar for aerobic bacteria, MacConkey agar for enterobacteria, Mannitol salt agar for Staphylococcus aureus, and Yeast extract agar (containing little concentration of streptomycin) for fungi counts. This was carried out using pour plate method. Identification of isolates was based on the established methods using standard procedures as important biochemical tests and morphological observations.

Determination of pH and Total Titratable Acidity (TTA)

The pH of the fermented samples was determined using the calibrated pH meter (Jenway 3520) while the TTA was determined by titration according to the method of A.O.A.C (1990).

Proximate analysis

Proximate composition of all fermenting samples, were analyzed according to the official methods of analysis described by Association of Official Analytical Chemist (A.O.A.C, 2005). All analysis was carried out in triplicate.

Statistical Analysis

All the data obtained were subjected to statistical analysis using analysis of variance (ANOVA) and Duncan's multiple range test using the Gen Stat Software. The mean and test of significance were determined at $p \leq 0.05$.

RESULTS

Microbiological Analysis

Table 1 shows that the lactic acid bacteria count increased as the fermentation time increased in all samples with highest count (5.12×10^{13} CFU/mL) observed in sample BA after 48 hours of fermentation and the least count (1.14×10^{10}) observed in sample BA after 24 hours. Colony count on nutrient agar for aerobic bacteria showed that there was an increase in aerobic bacteria count as fermentation time increases with the highest load (7.89×10^{15} CFU/mL) observed in unblanched fermented breadfruit cowpea blends (Sample UB) after 48 hours, while the lowest aerobic bacteria count was observed in sample BA (1.96×10^{6} CFU/mL) at 0 hour. The enteric count on MacConkey agar decreased as fermentation time increases and total elimination recorded by 48 hours for both samples BA and UB. The unblanched breadfruit cowpea blends (UB) had the highest yeast count (6.1×10^{14} CFU/mL) while sample BA had the lowest yeast count (6.0×10^{5} CFU/mL) after 48 hours of fermentation.

The Lactic acid bacteria isolated from the fermenting samples were identified phenotypically as *L. plantarum, L. mensentorides, L. delbrueckii, and L. brevis*. The aerobic organism isolates as *Bacillus* spp*., Pseudomonas* spp*., Staphylococcus aureus, Bacillus cereus, Bacillus subtilis,* and *Bacillus* spp*.* The fungi isolates are *Saccharomyces cerevisiae* and *Candida* spp*.*

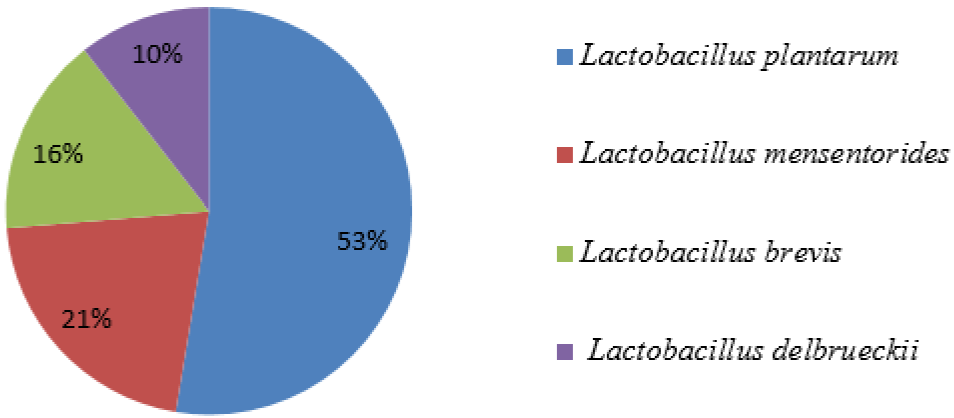
TABLE 1: Microbial Load (CFU/mL) of the Formulated Weaning Blends

		Fermentation time (hrs)/Microbial count (CFU/mL)		
Sample code	Microbial type	0	24	48
BA	LAB	NG	1.14×10^{10}	5.12×10^{13}
	Aerobic Bacteria	1.96×10^{6}	2.14×10^{7}	2.93×10^{7}
	Yeast	NG	1.0×10^{3}	6.0×10^{5}
	Enterics	1.00×10^{5}	1.4×10^{3}	NG
UB	LAB	NG	1.38×10^{10}	2.65×10^{15}
	Aerobic Bacteria	5.3×10^{7}	1.12×10^{11}	7.89×10^{15}
	Yeast	1.0×10^{5}	3.0×10^{8}	6.1×10^{14}
	Enterics	1.66×10^{5}	1.25×10^{5}	NG

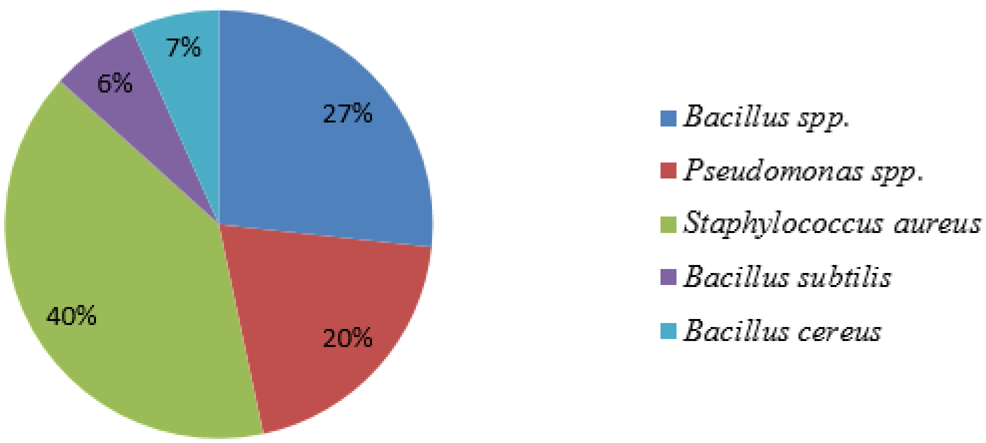
KEY: NG= No Growth
Sample BA: Blanched Fermented Breadfruit cowpea blends
Sample UB: UnBlanched Fermented Breadfruit cowpea blends

Distribution of Probable Organisms during Fermentation:

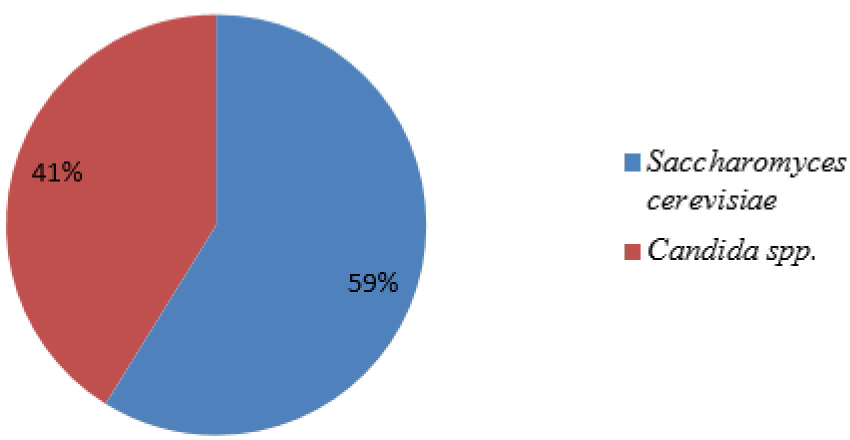
The organisms isolated within the period of fermentation were probably identified based on biochemical characteristics and sugar fermentation ability of all the isolates. Fig 2a, Fig 2b and 2c shows the distribution of Lactic Acid Bacteria (LAB), aerobic bacteria and yeast respectively during fermentation of the samples. *L. plantarum* had the highest percentage (53%) of occurrence and *L. delbrueckii* had the least percentage (10%) of occurrence of LAB isolates as shown in Fig 2a. From the aerobic bacteria *Staphylococcus aureus* had the highest percentage (40%) occurrence, followed by *Bacillus* spp*.* with (27%) and the least percentage occurrence was recorded in *Bacillus subtilis* with (6%) as shown in fig 2b. *Saccharomyces cerevisiae* had the highest percentage (67%) of occurrence while *Candida* spp. had the least percentage (33%) occurrence of the yeast isolates as shown in fig 2c.



(a) Lactic acid bacteria



(b) Aerobic Bacteria



(c) Yeasts

Fig: 2a-c: Occurrence (%) of Lactic acid bacteria, Aerobic bacteria and yeast during fermentation of breadfruit cowpea blends.

Chemical Analysis

The result of the chemical analysis in Table 2 shows that the pH of all the fermented samples decreased as the fermentation time increased. The least pH (3.20) was observed at 48 hours in sample BA while the highest pH (6.58) was observed at 0 hour in sample UB. The titratable acidity increased as the fermentation time increased. Sample BA has the highest lactic acid content of 1.22% after 48 hours while Sample BA had the least lactic acid content of 0.18% after 0 hours.

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TABLE 2: Effect of Fermentation Time on the pH and Titratable Acidity of the Fermented Blends

Sample code	Parameter	Fermentation Time (hrs) 0	24	48
BA	pH	5.72	4.71	3.20
UB		6.58	4.25	4.01
BA	%TTA	0.18	0.68	1.22
UB		0.20	0.71	0.84

Key:
Sample BA: Blanched Fermented Breadfruit cowpea blends
Sample UB: UnBlanched Fermented Breadfruit cowpea blends

Effect of Blanching on the Proximate Analysis of the Fermented Blends

The result in table 3 revealed that blanched fermented breadfruit cowpea blend (sample BA) had the highest protein (6.01%) at 48 hour fermentation, and the least protein content (3.65%) was observed in Sample BA at 0 hour of fermentation. The highest crude fat (1.40%) was recorded in fermented Sample UB at 48 hours while the least crude fat content (0.55%) was observed in Sample BA at 0 hour of fermentation. The highest carbohydrate content (80.18%) was recorded in Sample UB at 0 hour of fermentation and the least carbohydrate content (35.49%) was observed in fermented blanched breadfruit sample BA at 48 hours of fermentation. The least crude fibre content (2.65%) was recorded in fermented Sample UB at 48 hours which was not significantly different from fermented Sample BA (2.70%). Statistical analysis showed that the crude fibre of fermented Samples BA and UB were not significantly different from each other (at $p \leq 0.05$) at 48 hours while blanching significantly ($p \leq 0.05$) affect crude protein and carbohydrate content of all fermented Breadfruit cowpea blends. Blanched Sample BA recorded higher protein content as the fermentation time increases. Generally, blanching and fermentation increases the proximate parameters of the blends except for the Moisture, ash and carbohydrate parameters that show a decrease as fermentation time increased.

TABLE 3: Effect of blanching on the proximate composition of fermented breadfruit cowpea blends

		Proximate Content (%)					
Processing method	Sample code	Crude protein	Crude fat	Crude Fibre	Ash	Moisture	Carbohydrate
Blanched	BA(0hr)	3.65 ±0.04^{c}	0.55 ±0.01^{d}	4.41 ±0.01^{b}	2.23 ±0.02^{b}	8.70 ±0.01^{d}	63.98 ±0.06^{c}
	BA(24hrs)	4.05 ±0.01^{b}	0.95 ±0.00^{b}	3.61 ±0.00^{c}	2.10 ±0.00^{c}	7.83 ±0.02^{e}	40.47 ±0.01^{d}
	BA(48hrs)	6.01 ±0.01^{a}	1.24 ±0.00^{b}	2.70 ±0.00^{e}	0.83 ±0.00^{e}	6.83 ±0.01^{f}	35.49 ±0.02^{e}
Unblanched	UB(0hr)	4.00 ±0.01^{b}	0.71 ±0.12^{c}	4.80 ±0.12^{a}	2.40 ±0.01^{a}	21.00 ±1.15^{a}	80.18 ±1.15^{a}
	UB(24hrs)	4.55 ±0.01^{b}	1.20 ±0.01^{b}	3.78 ±0.01^{d}	2.21 ±0.01^{b}	18.30 ±0.00^{b}	72.59 ±0.02^{b}
	UB(48hrs)	5.99 ±0.05^{a}	1.40 ±0.01^{a}	2.65 ±0.00^{e}	0.99 ±0.01^{d}	15.88 ±0.01^{c}	64.10 ±0.02^{c}

Means ± Standard deviation. Means within a column with same letter(s) are not significantly different at $p \leq 0.05$ level using Duncan's Multiple Range Test (DMRT)
KEY: Sample BA: Blanched Fermented Breadfruit cowpea blends
Sample UB: Unblanched Fermented Breadfruit cowpea blends

Effect of Blanching and Fermentation on the Anti-nutrient Content of Breadfruit cowpea blends.

Table 4 shows the result of blanching and fermentation on the anti-nutrient content of the formulated blends. The fermented Sample BA had the highest polyphenol (0.75 mg/g) at 0 hr and fermented Sample UB had the least polyphenol content (0.34 mg/g) after 48 hrs of fermentation. The highest oxalate content (6.57 mg/g) was recorded in the fermented Sample BA and the least oxalate content (0.60 mg/g) was observed in fermented sample UB at 48 hrs. Sample BA had the highest phytate content (14.12 mg/g) and the least phytate content (0.51 mg/g) was recorded in fermented Sample UB at 48 hrs. Highest tannin content (29.32 mg/g) was recorded in Sample BA and the least tannin content (0.28 mg/g) was recorded in Sample UB at 48 hrs.

Statistical analysis showed that the polyphenol content of Samples BA and UB were not significantly different from each other ($p \leq 0.05$) at 0 hr of fermentation while the processing method and supplementation significantly affect ($p \leq 0.05$) phytate, oxalate and tannin contents of the formulated breadfruit cowpea blends. Blanched blends had higher anti-nutrient content while fermentation resulted in decrease in anti-nutrient content of both blanched and unblanched blends.

Table 4: Anti-nutrient Content (mg/g) of Spontaneously Fermented Breadfruit cowpea blends

		Antinutrient content (mg/g)			
Processing	Sample code	Polyphenol	Phytate	Oxalate	Tannin
Blanched	BA(0hr)	0.75 ± 0.01^a	14.12 ± 0.01^a	6.57 ± 0.01^a	29.32 ± 0.01^a
	BA(24hrs)	0.54 ± 0.01^c	10.11 ± 0.01^b	5.47 ± 0.12^b	16.71 ± 0.01^b
	BA(48hrs)	0.49 ± 0.01^d	6.59 ± 0.01^c	3.10 ± 0.01^c	15.99 ± 0.01^c
Unblanched					
	UB(0hr)	0.73 ± 0.01^a	0.63 ± 0.01^d	0.76 ± 0.01^d	0.98 ± 0.01^d
	UB(24hrs)	0.64 ± 0.01^b	0.54 ± 0.01^e	0.63 ± 0.01^e	0.71 ± 0.01^e
	UB(48hrs)	0.34 ± 0.01^e	0.51 ± 0.01^e	0.60 ± 0.01^e	0.28 ± 0.01^f

Means ± Standard deviation. Means within a column with same letter(s) are not significantly different at $p \leq 0.05$ level using Duncan's Multiple Range Test (DMRT)

KEY: Sample BA: Blanched Fermented Breadfruit cowpea blends
Sample UB: Unblanched Fermented Breadfruit cowpea blends

DISCUSSION:

Spontaneous fermentation of breadfruit (*Artocarbus atilis*) was brought about by both bacteria and fungi; this is in agreement with the report by Adegbehingbe *et al.* (2017). The increase in LAB and aerobic bacteria counts on MRS agar and Nutrient agar respectively as the fermentation time increases can be attributed to suitable environmental condition, probable reduction of growth inhibitors and nutritional composition of breadfruit; this is similar to the observation of Ojokoh *et al.* (2013) who reported a similar increase in the growth of lactic acid bacteria and aerobic bacteria as the fermentation time increase while fermenting African breadfruit seeds. There was a reduction and subsequent elimination of enteric bacteria as the fermentation time increases. This could be as result of reduction in pH of the fermenting medium as the fermentation time increases (Dong-Ho *et al.,* 2004). Also, the activities of lactic acid bacteria in the fermenting medium could bring about antimicrobial effect on pathogenic microorganism (Adegbehingbe, 2014). *L. plantarum* was the most predominant lactic acid bacteria in this study, and this is in agreement with the findings of Oyedeji *et al.,* (2013) who reported *L. plantarum* as the most predominant organism in fermentation responsible for lactic acid production. The presence of *Staphylococcus aureus* could be indicative of inadequate precautionary measure during processing of breadfruit or cowpea flour, this is in agreement with the report by Ojokoh *et al.* (2013).

The observed decrease in pH of the fermenting samples and simultaneous increase in the total titratable acidity of the blends is a desirable development because growth of many Gram negative bacteria and acid sensitive food borne pathogens will be inhibited at low pH. This could be due to the dominance of Lactic Acid Bacteria (LAB) in the fermenting medium (Wakil and Kazeem, 2012). A similar increase in total titratable acidity and decrease in pH had been reported by Ariahu *et al.* (1999) who reported that degradation of carbohydrate by the dominating LAB during fermentation results in acidification of the product.

The analysis of the proximate content of the blends showed that there was an increase in the protein content of the blanched samples during fermentation; although the protein content of the unblanched fermented breadfruit cowpea blend was also high. This may be due to the presence of cowpea flour in the blends. This is in contrast with the report of Ndidi *et al.,* (2014), who reported a decrease in protein content of African yam bean (*Sphenostylis stenocarpa*) due to heat treatment. The analysis of the proximate composition of the fermented samples indicates that the crude fiber, ash and carbohydrate content decrease after fermentation, this is in agreement with the report of Igbabul *et al.,* (2014) and Ojokoh (2014).

The decrease in carbohydrate content of fermented blanched samples BA and UB may be due to the utilization of some of the sugar by the fermenting lactic acid bacteria (Wakil and Kazeem, 2012).

The observed decrease in the ash content of all the fermented breadfruit cowpea blends may be due to the activities of microorganisms involved in the breaking down of sample components into absorbable forms during fermentation. This observation is in contrast with the work of Okafor *et al.* (2018) who reported an increase in ash content of fermented blends. The observed reduction in crude fibre in samples BA and UB may be due to enzymatic breakdown of the fibre during fermentation as reported by Olatubi and Ojokoh (2015). This study shows that the anti-nutrient content of the breadfruit cowpea blends reduced after fermentation, a similar decrease in anti-nutritional content has been reported by Adeyeye *et al*. (2020). The reduction observed in the anti-nutrient content may be due to microbial activities during fermentation (Ojokoh, 2014). The presence of *Saccharomyces cerevisiae and Lactobacillus sp* have been reported to enzymatically hydrolyze some anti-nutrient content during fermentation (Sanusi *et al.,* 2013).

CONCLUSION

The result of this research shows that blanching breadfruit prior to fermentation resulted in blends with high protein content, reduced enteric count, reduced carbohydrate content with highly reduced anti-nutrient content. This process could be used in the food industry to reduce malnutrition.

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