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Effect of fermentation on nutrient and anti-nutrient composition of breadfruit (*Treculia africana)* and cowpea *(Vigna unguiculata)* blend flours

A. O. Ojokoh*, M. K. Daramola and O. J. Oluoti

Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

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This research was carried out to know the effect of fermentation on the chemical composition, anti-nutrient content, pH, titratable acidity, and microbiological changes of breadfruit and cowpea blend. Breadfruit and cowpea composite flours were mixed in gram of six combinations as follows: BcA = 100:0, BcB = 90:10, BcC = 80:20, BcD = 70:30, BcE = 60:40, BcF = 50:50 and subjected to natural fermentation for 72 h. The following isolates were isolated from the fermentation; *Lacobacillus fermentum, L. acidophilus, L. bulgaricus, L. plantarum, L. dextranicum, L. rhamnosus, L. delbrueckii, L. leichemanii, L. divergens, L. reuteri, L. jenseni, L. casei, L. salivarius, L. cellubiosus, Leuconostoc messenteroide and Pediococcus* acidilactis, of which *L. plantarum* was the most dominant the throughout the period of fermentation. There was decrease in pH with increase in total titratable acidity (TTA) in all the samples. The result of the proximate analysis revealed a marginal increase in crude protein content of each sample (from 3.80 to 4.43%, from 5.83 to 6.47%, from 7.87 to 8.49%, from 9.90 to 10.53%, 11.93 to 12.56% and from 14.12 to 19.14%). There was increase in fat and crude fibre contents and decrease in carbohydrate and ash contents of the fermented samples. Results from this research also show significant reduction in anti-nutritional content which are hydrogen cyanide, oxalate and phytate, but hydrogen cyanide was not detected in the fermented sample BcF (50:50).

Key words: Breadfruit flour, cowpea flour, natural fermentation, nutrient, antinutrient.

INTRODUCTION

Numerous food products owe their production and characteristics to the fermentative activities of microorganisms. Many foods, such as cheese, sauerkraut and fermented sausages, are preserved products, in that their shelf life is considerably extended over that of the raw materials from which they are made. In addition to being made shelf-stable, fermented foods have aroma and flavour characteristics that result directly or indirectly from the fermenting organisms (Steinkraus, 2002). Fermented foods have been with us since humans arrived on earth and they will be with us far into the future As they are the source of alcoholic foods/beverages, vinegar, pickled vegetables, sausages, cheeses, yogurts, vegetable protein amino acid/peptide sauces and pastes with meat-like flavours, and leavened and sour-dough breads. Breadfruit is a species of flowering tree in the mulberry family, Moraceae, growing throughout Southeast Asia, Pacific Ocean Islands and also in the tropics such as Malaysia and Nigeria. Its name is derived from the texture of the cooked fruit, which has a potato-like flavour, similar to fresh-baked bread. African breadfruit (*Treculia africana*) is an important food crop in Nigeria. Some varieties have been studied and are appreciated for their nutritional properties because they

*Corresponding author. E-mail: tonyojokoh@yahoo.com.

are rich in carbohydrates, lipids and proteins (Rincon et al., 2004). Breadfruits just like some other legumes have been known to contain some anti-nutrients which interfere with digestive processes and prevent efficient utilization of their proteins. Some of these are protease inhibitors, heamagglutinin, lectins, saponins and flatulence factors (Osabor et al., 2009). However, they could be eliminated or reduced by some processes such as soaking, dehulling, germination and fermentation (Cheuham, 1986). Cowpea (*Vigna unguiculata*) (International Feed Number, 5-01-661) is one of several species of the widely cultivated genus *Vigna*. It is one of the most important food legume crops in the semi-arid tropics covering Asia, Africa, southern Europe and Central and South America. Cowpeas are a common food item in the southern United States, where they are often called Black-eyed pea or field peas. The largest production is in Africa, with Nigeria and Niger predominating, but Brazil, Haiti, India, Myanmar, Sri Lanka, Australia, the U.S., Bosnia and Herzegovina all have significant production Cowpeas have high protein content and constitute the natural protein supplements to staple diets. Protein quality is synergistically improved in cereal-legume blends because of the lysine contributed by the cowpea and methionine contributed by the cereals (Afoakwa et al., 2003)

The nourishment of snacks by fermentation is especially important due to the fact that many people now work outside their homes and are becoming more dependent on snacks for the supply of their daily nutritional requirements. Unfermented foods, snacks inclusive, contain complex compounds that need to be metabolized by enzymes in the stomach before absorption by the body. Fermentation which is a process involving microbial enzyme, break down these complex food compounds into simple, easily assimilated compounds. With growing concerns for diet and general health, it is not unnecessary to know the nutritional or otherwise status of this snack. The objective of this research was to investigate the effect of fermentation on the nutrient and anti-nutrient composition of breadfruit and cowpea blend.

MATERIALS AND METHODS

Source of samples

Fresh breadfruit (*Treculia africana)* used was purchased at Monday market in Ikirun, Osun State while dry cowpea seeds (*Vigna unguiculata)* were bought from a local market in Ibadan, South-West area of Nigeria.

Preparation of composite flour for fermentation

The procedure for the treatment of the legumes (Breadfruit and Cowpea) was as previously described (Wakil et al., 2008). The method involved peeling, blanching and drying and dry milling of breadfruit and removal of extraneous matter, dehulling, drying and dry milling of cowpea. The breadfruit (B)-cowpea (c) blends were formulated in ratios 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (Malleshi et al., 1989) and thus consisted of six fermented and six unfermented samples. They were labelled appropriately to avoid mixed up of samples, that is,

(BcA) B 100 g + c 0 g
(BcB) B 90 g + c 10 g
(BcC) B 80 g + c 20 g
(BcD) B 70 g + c 30 g
(BcE) B 60 g + c 40 g
(BcF) B 50 g + c 50 g

Fermentation of samples

40 ml of sterile water was added to each sample, properly mixed and allowed to ferment for 72 h at room temperature (25±2°C).

Microbiological analysis

The raw and fermenting blend samples were subjected to microbiological analysis to monitor the dynamic changes in the populations responsible for breadfruit-cowpea blends fermentation for all the samples. The standard method of Harriagan and McCance (1976) was employed. 1 g of the sample was aseptically weighed using a weighing balance and carefully introduced into 9 ml of sterile distilled water and 1 ml of the appropriate dilutions (10^3 and 10^5) was mixed with molten agar and pour–plated in duplicates on the following media: (1) Nutrient agar for estimation of bacteria (2) MRS agar for total lactic acid bacteria (LAB) incubated at 35°C for 48 h in anaerobic jars. Microbiological counts were made after incubation. Counts were expressed as log10 colony forming units (cfu) per gram of sample. Classification of isolates was based on the established methods using important biochemical and morphological observations and tests.

Physiochemical changes

The pH of the sample was measured each day with a Cambridge direct reading pH meter. Total titratable acidity (TTA) was determined on 5 ml aliquot of the sample against 0.01 N NaOH using phenol red as indicator according to AOAC (1990).

Chemical analysis

Proximate analysis of sample

Proximate analysis of the sample was performed according to 0(1995) procedures for ash, crude fibre, fat, moisture and protein using nitrogen to protein conversion factor of 6.25. Carbohydrate was determined by difference. Phytate and hydrogen cyanide were determined using AOAC (1990) methods while oxalate content was by the titrimetric method (AOAC, 1990).

RESULTS

In this study, a total of 20 bacteria were isolated from the samples before and after fermentation as shown in Table 1a and b. The predominant organism isolated was *Lactobacillus plantarum.* Figure 1 presents the result of pH. The mean pH after 24 h of fermentation was 4.02. At

Table 1a. Cultural and morphological characteristics of isolates from breadfruit – cowpea blend.

S/N	Shape	Elevation	Colour	Pigment	Surface	Identification
1	Irregular	Raised	Milky	Opaque	Wet	*Pseudomonas aeruginosa*
2	Irregular	Raised	Creamy	-	Rough	*Bacillus subtilis*
3	Irregular	Flat	Creamy	-	Rough	*Staphylococcus saprophyticus*
4	Irregular	Raised	Creamy	-	Smooth	*Flavobacterium acquatile*
5	Circular	Convex	Creamy	-	Smooth	*Staphylococcus aureus*
6	Irregular	Raised	Creamy	-	Rough	*Staphylococcus epidermidis*
7	Irregular	Raised	Creamy	-	Rough	*Flavobacterium rigense*
8	Circular	Convex	Pinkish	-	Smooth	*Bacillus cereus*
9	Irregular	Raised	Creamy	-	Rough	*Proteus vulgaris*

Table 1b. Gram's reaction, cultural and morphological characteristic of lactic acid bacteria isolated from natural fermentation of breadfruit- cowpea blend.

S/N	Gram's reaction	Cell morphology	Catalase	Motility	Identification
1	+	Rod	-	-	*Lactobacillus fermentum*
2	+	Rod	-	-	*Lactobacillus acidophilus*
3	+	Rod	-	-	*Lactobacillus dextranicum*
4	+	Rod	-	-	*Lactobacillus bulgaricus*
5	+	Rod	-	-	*Lactobacillus rhamnosus*
6	+	Rod	-	-	*Lactobacillus delbrueckii*
7	+	Rod	-	-	*Lactobacillus leichmanni*
8	+	Rod	-	-	*Lactobacillus divergens*
9	+	Rod	-	-	*Pediococcus acidilactis*
10	+	Rod	-	-	*Lactobacillus plantarum* ***
11	+	Rod	-	-	*Lactobacillus reuteri*
12	+	Rod	-	-	*Lactobacillus jenseni*
13	+	Rod	-	-	*Lactobacillus casei*
14	+	Rod	-	-	*Leuconostoc mesenteroides*
15	+	Rod	-	-	*Lactobacillus cellubiosus*

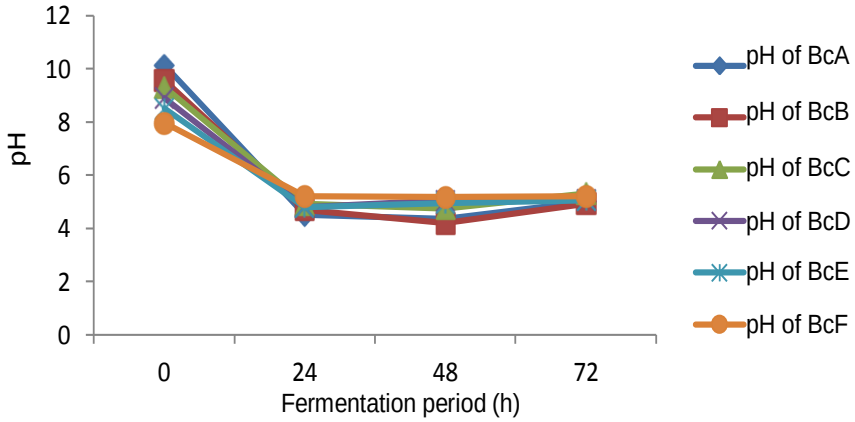

Figure 1. The graph of the pH against fermentation period. pH of BcA = pH of 100 g and 0 g breadfruit – cowpea blend; pH of BcB = pH of 90 g and 10 g breadfruit – cowpea blend; pH of BcC = pH of 80 g and 20 g breadfruit - cowpea blend; pH of BcD = pH of 70 g and 30 g breadfruit – cowpea blend; pH of BcE = pH of 60 g and 40 g breadfruit – cowpea blend; pH of BcF= pH of 50 g and 50 g breadfruit – cowpea blend.

Table 2. Total titratable acidity of each sample at different fermentation time.

Sample	Fermentation period (h)			
	0	24	48	72
BcA	ND	0.03	0.03	0.04
BcB	ND	0.02	0.06	0.05
BcC	ND	0.03	0.09	0.08
BcD	ND	0.03	0.08	0.06
BcE	ND	0.04	0.08	0.05
BcF	0.009	0.03	0.06	0.05

BcA = 100 g of Breadfruit + 0 g of cowpea, BcB = 90 g of breadfruit + 10 g of cowpea, BcC = 80 g of breadfruit + 20 g of cowpea, BcD = 70 g of breadfruit + 30 g of cowpea, BcE = 60 g of breadfruit + 40 g of cowpea, BcF = 50 g of breadfruit + 50 g of cowpea.

Table 3. Mean proximate composition (%) of all samples.

Chemical property	Unfermented sample	Fermented sample
Moisture	9.09	10.52
Protein	8.91	10.27
Fat	2.82	3.28
Ash	2.96	2.97
Fibre	2.60	2.46
Carbohydrate	73.58	70.32

Table 4a. Hydrogen cyanide content (mg/100 g) of breadfruit and cowpea blend samples.

Sample	Unfermented sample	Fermented sample
BcA	2.65	0.010
BcB	2.58	0.009
BcC	2.43	0.008
BcD	2.38	0.006
BcE	2.35	Not detected
BcF	2.28	Not detected

BcA = 100 g of Breadfruit + 0 g of cowpea, BcB = 90 g of breadfruit + 10 g of cowpea, BcC = 80 g of breadfruit + 20 g of cowpea, BcD = 70 g of breadfruit + 30 g of cowpea, BcE = 60 g of breadfruit + 40 g of cowpea, BcF = 50 g of breadfruit + 50 g of cowpea.

Table 4b. Oxalate content (mg/100 g) of bread fruit and cowpea blend samples.

Sample	Unfermented sample	Fermented sample
BcA	2.80	2.10
BcB	2.76	1.94
BcC	2.71	1.73
BcD	2.63	0.93
BcE	2.61	0.90
BcF	2.38	0.83

BcA = 100 g of Breadfruit + 0 g of cowpea, BcB = 90 g of breadfruit + 10 g of cowpea, BcC = 80 g of breadfruit + 20 g of cowpea, BcD = 70 g of breadfruit + 30 g of cowpea, BcE = 60 g of breadfruit + 40 g of cowpea, BcF = 50 g of breadfruit + 50 g of cowpea.

Table 4c. Phytate content (mg/100 g) of bread fruit and cowpea blend samples.

Sample	Unfermented sample	Fermented sample
BcA	0.91	0.84
BcB	0.86	0.81
BcC	0.84	0.73
BcD	0.74	0.70
BcE	0.70	0.64
BcF	0.61	0.41

BcA = 100 g of Breadfruit + 0 g of cowpea, BcB = 90 g of breadfruit + 10 g of cowpea, BcC = 80 g of breadfruit + 20 g of cowpea, BcD = 70 g of breadfruit + 30 g of cowpea, BcE = 60 g of breadfruit + 40 g of cowpea, BcF = 50 g of breadfruit + 50 g of cowpea.

48 h of fermentation, it was 4.75 and was 5.10 at 72 h while the mean titratable acidity at 24 h of fermentation was 0.03 and was 0.07 at 48 h and 0.06 at 72 h (Table 2). The changes in the moisture content of the samples are shown in the Table 3.There was increase in the moisture content of all the samples. The mean moisture content for unfermented samples was 9.09% and for fermented samples was (10.52%). The mean crude protein for fermented samples was 10.27% as against that of unfermented samples, which was 8.91%. The mean crude fat for fermented samples was 3.28% compared to that of unfermented samples (2.82%). The compared to that of unfermented samples (2.96%). The mean value of crude fibre for fermented samples (2.46%) was lower compared to that of unfermented samples (2.60%). There was decline in the level of carbohydrates in the samples. The mean for fermented samples was 70.32% while that of unfermented samples was 73.58%. Observed changes in the levels of anti-nutrient are recorded in Table 4a to c. There was decrease in the hydrogen cyanide of all samples. The mean hydrogen

cyanide content of fermented samples was 0.0055 mg/100 g and unfermented 2.45 mg/100 g. There was decrease in the oxalate content of all samples. The mean of the oxalate content of fermented samples was 1.41 mg/100 g, while that of unfermented was 2.65 mg/100 g. The mean value for the phyate level in the fermented samples was 0.69 m/100 g and that of unfermented samples was 0.78 mg/100 g.

DISCUSSION

The total count from all the six (6) samples on MRS medium and nutrient medium shows that Lactic Acid Bacteria colonies increase in population after 24 h of fermentation due to their acclimatization to their environment and due to their activities. There was decrease in population of other bacterial species present apart from Lactic Acid Bacteria are toxic producer; examples include *Pseudomonas* sp., *Flavobacterium* sp. and *Proteus* sp. which produce toxic metabolite that can cause infection to the consumer. The presence of *Staphylococcus* sp. could be indicative of inadequate precautionary measure during processing of raw breadfruit into flour. The presence of *Bacillus sp* in the sample is as a result of exposure of the sample during processing.

Fermentation was observed to decrease the pH and increase the titratable acidity of the fermented blends. The decrease in pH and increase in titratable acidity up to 48 h and the further increase and decrease at 72 h in pH and titratable acidity may be as a result of variations in the composition of sample supplementation. The observed increase in titratable acidity could be due to the dominance of the environment by lactic acid bacteria which degraded carbohydrates resulting in acidification. These observations are in agreement with earlier studies by Nout et al. (1989) and Ariahu et al. (1999). High titratable acidity has been reported to reduce incidence of diarrhea in infants consuming fermented legumes (Mensah et al., 1990). The breadfruit-cowpea blends will during fermentation. Some of the other bacterial species then have two important attributes, such as antimicrobial properties and high protein content. Increase in protein content was due to the activities and increase in number of micro-organisms present during fermentation while the reduction in carbohydrate content was due to the utilization of some of the sugars by fermenting lactic acid bacteria for growth and other metabolic activities. Reduction in crude fibre may be due to the enzymatic breakdown of the fibre during fermentation by lactic acid bacteria. From Table 3 fortification using cowpea and subjecting to fermentation is a means of increasing the protein and fat contents of breadfruit.

After fermentation, the anti-nutrients were greatly reduced as revealed in Tables 4a to c. The reduction observed in the hydrogen cyanide (HCN) content of the fermented samples may be as a result of microbial enzymes activities during fermentation process (Kobawila et al., 2005). The decrease in oxalate in the fermented samples may be due to the processing that the samples were subjected to coupled with activities of the microorganisms. A wide range of microflora has been known to possess phytase activity (Ojokoh, 2005) which may be partly responsible for reduction in phytic acid content in the fermenting samples.

Conclusion

Improvement in the nutritional quality and efficient reduction in the antinutriet content (HCN, oxalate and phytate) can be achieved through the fermentation of breadfruit and cowpea blend

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