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Effects of Fermentation and Extrusion on the Proximate and Organoleptic Properties of Cowpea-plantain Flour Blends

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Authors' contributions

This work was carried out in collaboration between both authors. Author FAO designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Author OAO managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to investigate effects of fermentation and extrusion on the chemical and sensory properties on blends of unripe plantain flour and cowpea flours. Three ratios (100:0, 80:20 and 60:40) of unripe plantain and cowpea flour were fermented and extruded. A total of 18 organisms were isolated during fermentation of plantain: cowpea blends. They include eleven (11) bacteria, four (4) moulds and three (3) yeasts. These are *Bacillus subtilis, Bacillus licheniformis, Micrococcus luteus, Enterobacter cloacae, Proteus mirabilis, Staphylococcus aureus, Leuconostoc mesenteroides, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus lactis, Aspergillus niger, Aspergillus flavus, Rizopus stolonifer, Trichoderma viride, Candida utilis, Geotrichum candidum* and *Saccharomyces cerevisiae.* The pH and total titratable acidity (TTA) varied significantly during fermentation. The proximate composition of the raw blends, fermented, extruded and fermented- extruded samples were assessed. The crude protein and moisture content increased in the fermented, extruded and fermented- extruded samples; whereas fermentation and extrusion reduced the carbohydrate content. Fat, crude fibre and ash contents

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varied among all the samples. The sensory evaluation of the samples showed a good preference for fermented- extruded samples in terms of texture and aroma.

Keywords: Fermentation; extrusion; cowpea; plantain.

1. INTRODUCTION

The problem of malnutrition is predominant in Nigeria due to deficiency of protein and calories and protein-calories sources of vegetable origin have been proposed as a solution to this problem [1].

Plantains (*Musa paradisiaca*) are used as an inexpensive source of calories in Nigeria and many African countries [2]. It is an important starchy staple and commercial crop in the West and Central Africa. According to FAO [3], over 2.3 billion kilograms of plantains are produced in Nigeria annually. However, about 35 to 60% post-harvest losses had been reported and attributed to lack of storage facilities and inappropriate technologies for food processing [3]. Unripe plantain is traditionally processed into flour in Nigeria and in other west and central African countries. The flour produced is mixed with boiling water to prepare an elastic pastry (“amala*”* in Nigeria). Unripe plantain is often recommended to diabetic patients because it contains low glycemic index carbohydrate [4]. Despite the nutritional importance of this crop, it has been discovered to contain low amount of protein.

Cowpea (*Vigna unguiculata*) is a drought tolerant food crop, well adapted in varieties of climates and soils. This crop is widely cultivated throughout the tropics and subtropics, particularly in west and central Africa, with an annual production of 3 billion kilograms [5]. Its grains are used to prepare many traditional foods such as moin-moin, akara, and gbegiri (soup). Cowpea grains have been used to fortify cereal-based weaning foods, in which they formed complementary amino acid profiles and improved protein quality [6]. The potential for incorporation and utilization of cowpea as a source of protein in diets serves as the basis for the need of adequate processing.

While many foods can be eaten raw, many also undergo some form of preparation for reasons of safety, palatability, texture, or flavor and digestibility. At the simplest level this may involve washing, cutting, trimming, or adding other foods or ingredients, such as spices. It may also involve mixing, heating or cooling, pressure cooking, fermentation, extrusion or combination with other food. Fermentation is one of the oldest methods of food preservation, and embedded in traditional cultures and village life. Fermentation enhances the nutrient of food through biosynthesis and bioavailability of vitamins [7,8]. Fermented foods are described as palatable and wholesome and are generally appreciated for attributes, their pleasant flavours, aromas, textures, and improved cooking and processing properties [9]. Extrusion cooking technology has been described as a process in which raw materials are heated and worked upon mechanically while passing through compression screws and is forced through a die [1]. Extrusion cooking has been used as an important technique for modification and manufacture of a wide variety of traditional and novel foods and food blends [10-13]. Blended foods are usually precooked by extrusion so that less cooking time is required and to increase shelf life. Although the effect of extrusion on the nutritional composition of blends is not clear, Abiodun and Ogugua [14] repoted an increase in protein content of extruded blends in the evaluation of extruded snacks from blends of acha and cowpea.

Nutritional benefits of cowpea and plantain flour are limited without fermentation. In many instances, the use of only one method of processing may not impact desired level of improvement of staple foods. Therefore, food processing technologies such as extrusion cooking coupled with fermentation can provide alternatives for improving the nutritional quality of food. To maximize the nutritional benefits of cowpea and plantain flour, there is need to determine the effects of fermentation and extrusion on these staple foods. The objective of this research is to determine the effect fermentation and extrusion on the proximate and organoleptic properties of cowpea and plantain flour blends.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Green matured plantain and cowpea seeds used for this study were obtained from a local market in the Southwestern part of Nigeria.

2.2 Processing of Unripe Plantain Flour

The unripe plantain was sorted for maturity and cleaned by washing with water. The clean unripe plantains were peeled and sliced thinly into 2 mm diameter and sun dried for 72 hours. The dried unripe plantain was then fed into a Bentall attrition mill (Model 200L090). The milled flour was sieved with 0.25 mm mesh sieve into fine flour and kept in an air tight container.

2.3 Processing of Cowpea Flour

Cowpea seeds were cleaned by sorting out dirt and stones. The cleaned cowpea seeds were coarsely milled to separate the coat from the cotyledon. The husk was separated from the seed by blowing air into it. The dehalled cowpea seeds were milled into fine flour using an attrition mill after which it was sieved through 0.25 mm mesh. The cowpea flour was kept in an airtight container.

2.4 Formation of Cowpea-plantain Blends

The unripe plantain and cowpea flours were formulated in the ratio of (unripe plantain: cowpea) 100:0; 80:20; and 60:40.
Sample A (100:0) = 100% unripe plantain flour
Sample B (80:20) = 80% unripe plantain flour and 20% cowpea flour
Sample C (60:40) = 60% unripe plantain flour and 40% cowpea flour

2.5 Fermentation and Extrusion of Flour Blends

A batch of the flour blend was fermented using semi- solid state fermentation for 72 hours. 70 ml of sterilized water was added to 100 g of each sample in cleaned containers and properly sealed. The fermentation process was terminated by oven drying at 60℃ for 24 hours. Two batches of samples were subjected to extrusion cooking. The first batch consists of the unfermented blends. The blends were hydrated and preconditioned by adding 50 ml of water to 1000 g of the sample and manually mixed in a sterile bowl to ensure even distribution of water. The samples were extruded using a Brabender 20DN single screw laboratory extruder (Brabender OHG, Duisburg, Germany). The second batch of the samples consists of the fermented samples. The fermented samples were also extruded using a Brabender 20DN single screw laboratory extruder (Brabender OHG, Duisburg, Germany). The samples were extruded at 100℃, 20 revolution per minute and feeding rate of 30 kg/h. All the extrudates were air dried for 12 hours after which they were stored at 32℃ in sterile polyethylene bags and kept in properly labeled air tight containers. The control which consists of the raw blends which were neither fermented nor extruded was kept in air tight containers.

2.6 Microbiological Analysis of the Samples

Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while De Man Rogosasharpe agar was used to isolate lactic acid bacteria. Techniques were enumerated by using appropriate serial dilution and pour plate techniques. The bacterial culture was incubated at 37℃ for 18 to 24 hours, fungal plates were inverted and incubated at 24℃ for 48 to 72 hours. De Man Rogosasharpe agar plates were incubated at 32℃ for 18- 24 hours anaerobically. The organisms were characterized based on biochemical and morphological observations according to the methods of Sneath et al. [15] and Cowan and Steel [16].

2.7 Determination of pH and TTA

The pH of all fermenting samples was determined at 24 hours interval using a pocket size pH meter. A 1 g of the sample was dissolved in 10 ml of distilled water and filtered. The pH meter was calibrated with buffer solutions of pH 4, 7 and 9, this was followed by dipping the electrode of the pH meter into the sample solution and the observed pH was read and recorded in triplicates. The total titratable acidity of the fermenting samples was determined at 24 hours interval. A 2 g of macerated sample was weighed into a beaker. 20 ml of distilled water was added to it, it was mixed and filtered. 10 ml of the filtrate was measured into a beaker and 2 drops of phenolphthalein indicator was added into it. This was titrated with 0.1 M sodium hydroxide (NaOH) solution and the titre value was read. Total titratable acidity was expressed as percent (%) lactic acid. The acidity was calculated as stated below:

$$\text{TTA} = \text{Titre value} \times 9\ \text{mg}/100$$

2.8 Proximate Composition

All the samples were analyzed for moisture, ash, crude fibre, protein (N*6.25), crude fat and the carbohydrate determined by difference according to the method described by [17].

2.9 Sensory Evaluation

The sensory evaluation was done by the method. A panel of 10 judges (untrained but familiar with extruded products such as pasta, noodles, breakfast cereal quality char acteristic) was set up. Coded samples of the raw flour, extruded unfermented flour, fermented unextruded flour and fermented extruded flour were served to the panelists. The panelists were asked to rate the samples based on the colour, aroma, texture and overall acceptability by scroring them on a seven-point hedonic scale.

2.10 Statistical Analysis

All analyses were performed in triplicates. The data obtained were subjected to one way analysis of variance (ANOVA) while differences in mean were determined using Duncan's New Multiple Range Test (DMRT). All data analyses were done with SPSS 16.0 version.

3. RESULTS

3.1 Microorganisms Isolated During Fermentation of Cowpea-plantain Flour Blends

A total of eighteen (18) microorganisms were isolated during the fermentation of cowpea and unripe plantain flour blends. These comprise of eleven (11) bacteria, four (4) moulds and three (3) yeasts. These are *Bacillus subtilis* (sample A and C)*, Bacillus licheniformis* (sample B and C)*, Micrococcus luteus* (sample B)*, Enterobacter cloacae* (sample B)*, Proteus mirabilis* (sample B and C)*, Staphylococcus aureus* (sample A and C)*, Leuconostoc mesenteroides* (sample A and C)*, Lactobacillus bulgaricus* (sample A and B)*, Lactobacillus casei* (sample B)*, Lactobacillus fermentum* (sample C),*Lactobacillus lactis* (sample B)*, Aspergillus niger* (sample A)*, Aspergillus flavus* (sample A)*, Rizopus stolonifer* (sample A, B and C)*, Trichoderma viride* (sample B)*, Candida utilis* (sample A)*, Geotrichum candidum* (sample B) and *Saccharomyces cerevisiae* (sample B and C)

3.2 Changes in pH During Fermentation of Cowpea: Plantain Flour Blends

The pH variations during the fermentation of cowpea- plantain flour blends are shown in Fig. 3. The pH of sample A slightly increased from 6.84±0.02 to 6.89±0.01 at 24 hours but gradually decreased to 6.3±0.00 and 5.18±0.00 at 48 hours and 72 hours respectively. Sample B gradually decreased from pH 6.88±0.01 at 0 hour to 6.23±0.01, 6.01±0.01 and 5.31±0.01 at 24 hours, 48 hours and 72 hours respectively. Sample C also decreased from pH 7.41±0.01 at 0 hour to 6.80±0.01, 5.86±0.01 and 5.36±0.02 at 24 hours, 48 hours and 72 hours respectively.

3.3 Changes in Total Titratable Acidity (TTA) During Fermentation of Cowpea: Plantain Flour Blends

Variations in the total titratable acidity (TTA) during fermentation of cowpea- plantain flour blends are shown in Fig. 4. Sample A had initial TTA of 0.011 at 0 hour which increased to 0.022±0.00 at 24 hours to 0.025±0.00 at 48 hours and finally to 0.043±0.00 at 72 hours. Sample B increased from 0.018±0.00 at 0 hour to 0.032 at 24 hours and decreased slightly to 0.018 at 48 hours which later increased to 0.079 at 72 hours. Sample C had initial TTA of 0.014 which increased to 0.065 at 24 hours and decreased to 0.054 at 48, it finally increased to 0.086 at 72 hours.

3.4 Changes in Proximate Composition of Cowpea-Plantain Samples

<u>3.4.1 The moisture content of cowpea-plantain flour blends</u>

The moisture content of cowpea- plantain flour blends are shown in Fig. 3. Raw flour (RF) blends had the lowest moisture content ranging from 4.00±0.03 to 6.40±0.02 in sample A to C. There was no significant difference (P≤0.05) in the moisture content of sample A and C of the raw flour blends but sample B was significantly different (P≤0.05). The moisture content of unfermented extruded blends ranged from 8.20±0.06 to 12.24±0.10 in samples A to C. The moisture content of fermented unextruded blends ranged from 12.24±0.10 to 13.00±0.03. Fermented extruded blends exhibited moisture content ranging from 6.10±0.11 for sample B to 8.10±0.03 for sample A and C.

<u>3.4.2 The protein content of cowpea- plantain flour blends</u>

The changes in protein content of the cowpea-plantain blends are shown in Fig. 4. There was significant difference (P≤0.05) in the protein content of all the blends except 100% raw plantain flour (RFA) and unfermented extruded 100% plantain (UEA).

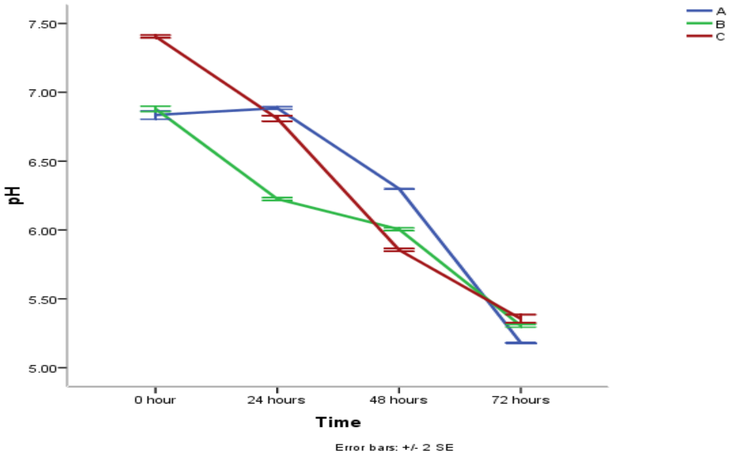

Fig. 1. pH variations during fermentation of cowpea-plantain flour blends

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour

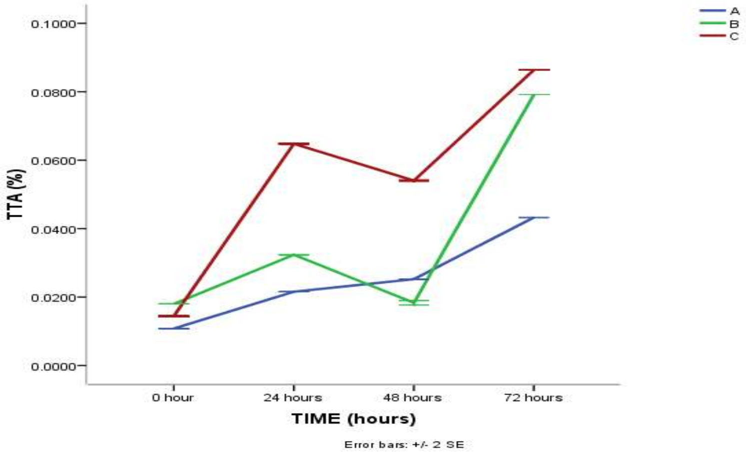

Fig. 2. Total titratable acidity (TTA) in percent lactic acid

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour

Fermented extruded blends recorded the highest protein content with values ranging from 3.06±0.03 to 12.10±0.08. Protein content of fermented unextruded blends ranged from 2.98±0.06 to 11.44±0.08. Unfermented extruded blends exhibited protein contents ranging from 2.62±0.09 to 9.11±0.03. Raw blends had the least protein content with values ranging from 2.62±0.11 to 8.75±0.12.

3.4.3 The carbohydrate content of cowpea-plantain flour blends

The carbohydrate content of the blends is represented in Fig. 5. Carbohydrate content of raw flour blends range from 82.80±0.88 to 88.88±0.09 in sample C to A. Unfermented extruded blends had carbohydrate content ranging from 72.66±0.23 to 82.70±0.18. Fermented unextruded samples had the least moisture content with values ranging from 60.27±0.21 to 72.47±0.07. Fermented extruded blends had carbohydrate content ranging from 75.43±0.05 to 84.25±0.13.

3.4.4 The fat content of cowpea- plantain flour blends

The variations in the ash content of cowpea-plantain blends are shown in Fig. 6. There was a significant difference (P≤0.05) in the fat content of the blends. Fermented unextruded blends had the highest fat content with values ranging between 4.89±0.10 and 5.80±0.02. Raw blends had the least fat content with values ranging from 1.19±0.03 to 1.99±0.04. Unfermented extruded blends had fat content ranging from 2.19±0.09 to 5.50±0.13. Fat content of fermented extruded blends range from 1.90±0.02 to 3.18±0.05.

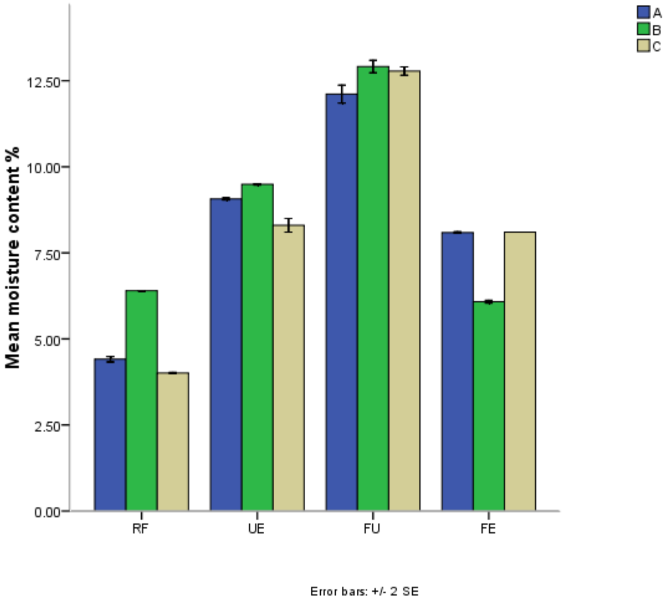

Fig. 3. Moisture content of cowpea-plantain blends

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour; RF= Raw Flour Samples; UE= Unfermented Extruded blend Samples; FU= Fermented Unextruded Samples; FE= Fermented Extruded Samples

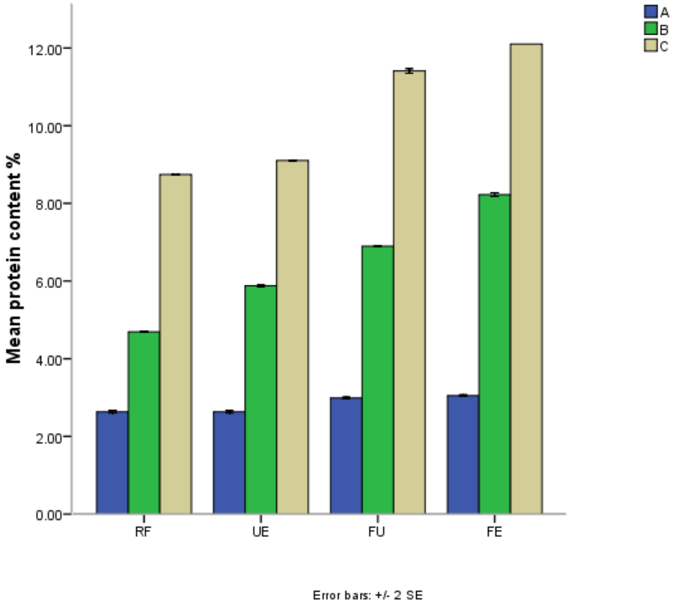

Fig. 4. Protein content of cowpea-plantain blends.

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour; RF= Raw Flour Samples; UE= Unfermented Extruded blend Samples; FU= Fermented Unextruded Samples; FE= Fermented Extruded Samples

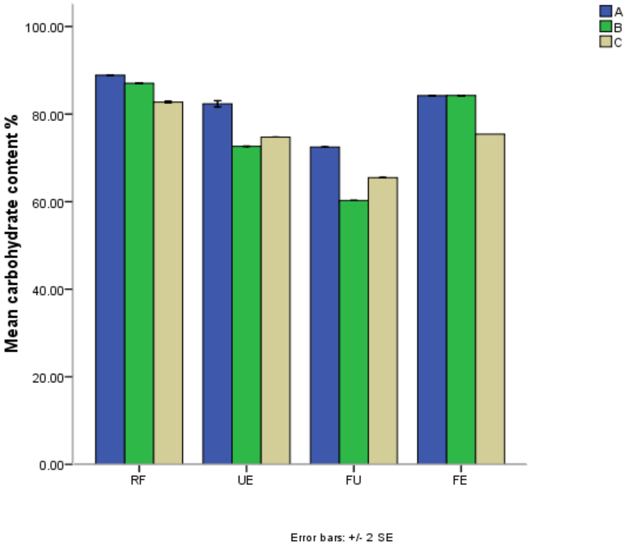

Fig. 5. Carbohydrate content of cowpea-plantain blends

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour; RF= Raw Flour Samples; UE= Unfermented Extruded blend Samples; FU= Fermented Unextruded Samples; FE= Fermented Extruded Samples

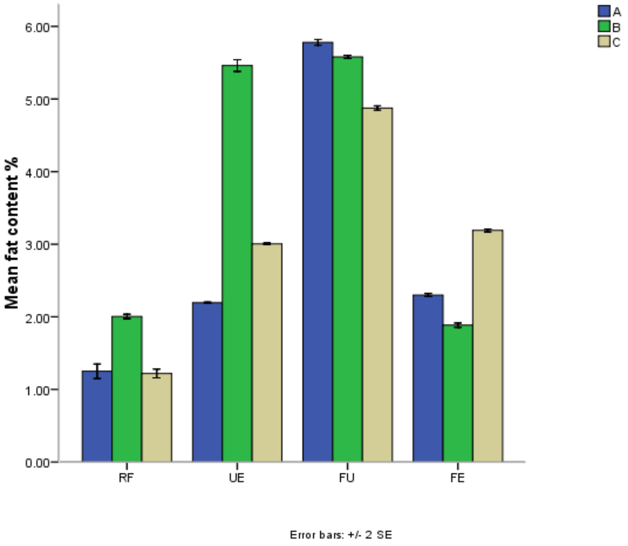

Fig. 6. Fat content of cowpea-plantain blends

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour; RF= Raw Flour Samples; UE= Unfermented Extruded blend Samples; FU= Fermented Unextruded Samples; FE= Fermented Extruded Samples

3.4.5 The crude fibre content of cowpea-plantain flour blends

The crude fibre content of cowpea- plantain blends are shown in Fig. 7. There was a significant difference (P≤0.05) in the crude fibre content of the blends. The crude fibre contents of the raw blends range from 0.10±0.04 to 0.41±0.01. Unfermented extruded blends had crude fibre content ranging from 0.64±0.02 to 0.82±0.02. Fermented unextruded blends had values ranging from 1.00±0.03 to 1.12±0.02. Fermented extruded blends had crude fibre ranging from 0.11±0.02 to 0.43±0.01.

3.4.6 The ash content of cowpea- plantain flour blends

The ash content of the cowpea- plantain blends are shown in Fig. 8. There is a significant difference in the ash contents of the samples. The ash content of the raw blends range from 1.73±0.03 to 2.85±0.13. Unfermented extruded blends had values ranging from 2.29±0.01 to 3.69±0.05. The ash content of fermented unextruded blends ranged from 2.03±0.02 to 2.69±0.09. Fermented extruded blends had ash content ranging from 1.90±0.08 to 2.60±0.07.

3.4.7 Sensory evaluation of the blends

The result obtained in the sensory evaluation indicated that there was no significant difference in the colour of the raw blends and unfermented extruded blends. Fermented unextruded blends and fermented extruded blends recorded significantly low colour. There was no significant difference in the texture of raw blends and unfermented extruded blends. Fermented blends recorded significantly high values in terms of aroma. Raw blends and fermented extruded blends recorded high values for overall acceptability. This result is represented in Table 2.

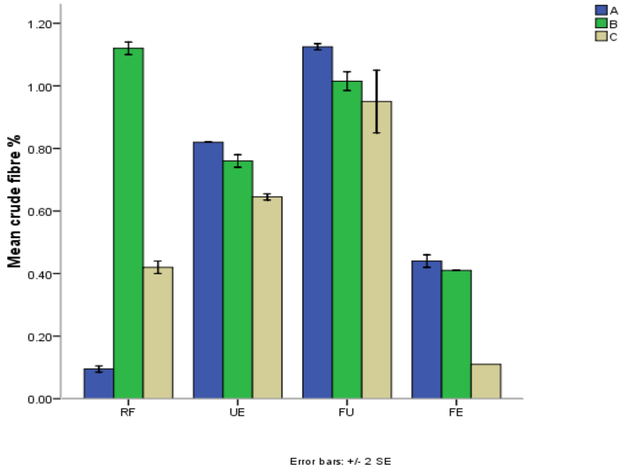

Fig. 7. Crude fibre content of cowpea-plantain blends

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour; RF= Raw Flour Samples; UE= Unfermented Extruded blend Samples; FU= Fermented Unextruded Samples; FE= Fermented Extruded Samples

Table 1. Chemical composition of cowpea-plantain blends

Sample	Moisture content	Protein content	Carbohydrate content	Fat content	Fibre content	Ash content
RFA	4.45±0.05	2.62±0.11	88.88±0.09	1.20±0.08	0.10±0.04	2.75±0.07
RFB	6.40±0.02	4.70±0.06	87.05±0.10	1.99±0.04	0.11±0.02	1.73±0.03
RFC	4.00±0.03	8.75±0.12	82.80±0.88	1.19±0.03	0.41±0.01	2.85±0.13
UEA	9.05±0.20	2.62±0.09	82.70±0.18	2.19±0.09	0.82±0.02	2.55±0.04
UEB	9.50±0.20	5.88±0.05	72.66±0.23	5.50±0.13	0.77±0.01	3.69±0.05
UEC	8.20±0.06	9.11±0.03	74.76±0.19	3.00±0.10	0.64±0.02	2.29±0.01
FUA	12.24±0.10	2.98±0.06	72.47±0.07	5.80±0.02	1.12±0.02	2.03±0.02
FUB	13.00±0.03	6.82±0.05	60.27±0.21	5.58±0.12	1.03±0.01	2.30±0.05
FUC	12.82±0.12	11.44±0.08	65.45±0.11	4.89±0.10	1.00±0.03	2.69±0.09
FEA	8.10±0.03	3.06±0.03	84.22±0.10	2.29±0.05	0.43±0.01	1.90±0.08
FEB	6.10±0.11	8.25±0.01	84.25±0.13	1.90±0.02	0.41±0.03	2.09±0.10
FEC	8.10±0.00	12.10±0.08	75.43±0.05	3.18±0.05	0.11±0.02	2.60±0.07

**Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different (P≤0.05)*

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour; RF= Raw Flour Samples; UE= Unfermented Extruded blend Samples; FU= Fermented Unextruded Samples; FE= Fermented Extruded Samples

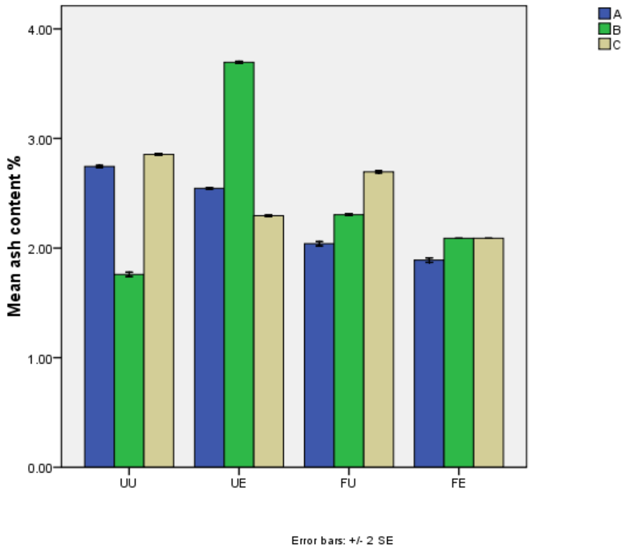

Fig. 8. Ash content of cowpea-plantain blends

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour; RF= Raw Flour Samples; UE= Unfermented Extruded blend Samples; FU= Fermented Unextruded Samples; FE= Fermented Extruded Samples

Table 2. Sensory evaluation of cowpea-plantain blends

Sample	Colour	Texture	Aroma	Overall acceptability	Total
RFA	6.40±0.70^{e}	6.30±0.48^{d}	5.40±1.26^{cd}	6.00±0.67^{e}	24.10^{e}
RFB	6.20±0.79^{e}	6.20±0.63^{cd}	4.70±0.95^{bc}	6.00±0.82^{e}	23.10^{d}
RFC	5.90±0.88^{e}	5.90±0.74^{bcd}	3.80±0.92^{a}	3.50±0.85^{a}	19.10^{b}
UEA	6.10±0.74^{e}	5.40±0.70^{abcd}	5.00±0.82^{bc}	5.20±0,92^{cde}	21.70^{c}
UEB	5.60±0.70^{e}	5.40±1.07^{abcd}	4.20±1.14^{ab}	5.10±0.74^{cd}	20.30^{bc}
UEC	5.60±0.84^{e}	5.00±0.67^{ab}	4.30±0.95^{ab}	4.10±0.88^{ab}	19.00^{a}
FUA	4.50±1.18^{d}	5.30±0.82^{abc}	6.20±0.63^{d}	4.50±0.53^{bc}	20.50^{bc}
FUB	3.40±1.51^{ab}	4.80±1.03^{a}	5.30±0.82^{cd}	4.80±0.63^{bcd}	18.30^{a}
FUC	2.70±0.95^{a}	5.20±0.79^{ab}	5.90±0.88^{d}	5.20±0.79^{cde}	19.00^{b}
FEA	4.70±0.67^{d}	4.40±1.78^{a}	6.20±0.63^{d}	5.40±0.97^{de}	20.70^{bc}
FEB	4.30±0.48^{cd}	4.90±1.10^{ab}	6.10±0.74^{d}	5.20±1.03^{cde}	20.50^{bc}
FEC	3.60±0.97^{bc}	5.40±1.17^{abcd}	6.20±0.79^{d}	4.70±1.25^{bcd}	19.90^{b}

**Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different (P≤0.05)*

A= 100% Plantain flour; B= 80% plantain flour and 20% cowpea flour; C= 60% plantain flour and 40% cowpea flour; RF= Raw Flour Samples; UE= Unfermented Extruded blend Samples; FU= Fermented Unextruded Samples; FE= Fermented Extruded Samples

4. DISCUSSION

The microbial flora of the fermenting media was heterogeneous comprising of eleven (11) bacteria, four (4) and three (3) yeasts. This is similar to the findings of Ojokoh and Udeh [18] that legume supplemented products had a greater microbial diversity and higher microbial populations. The decrease in pH may be as a result of the activities of microorganisms on the fermentable substrate which lead to the hydrolysis of complex organic compounds of the substrate thereby producing acid and ethanol. The acids produced leads to decrease in pH and increase in total titratable acidity which consequently resulted in decreasing microbial load [19]. Similar results were reported by Hassan et al. [19] and Ojokoh and Udeh [20]. However, the result of this fermentation research suggests that it is a lactic type where pH of fermenting media decreases with increase in total titratable acidity (TTA).

Moisture content is one of the most important and commonly measured properties of different food products. It is measured for various reasons including legal and label requirements, economic importance, food quality, better processing operations and storagability. The stable moisture content of the raw blends prior fermentation and extrusion indicates the storagability and shelf life of the samples if properly packaged [21-23].

The protein increased with increasing level of cowpea flour substitution indicating nutrients enhancement with cowpea flour substitution. This could obviously be due to the significant quantity of protein in cowpea seeds. The high protein content in plantain- cowpea blends will be of nutritional importance in most developing countries, Nigeria inclusive where many people can hardly afford high proteinous foods because of the costs. The increase in protein content is similar to some other research study in which cowpea flour was used in supplementation, such as in ogi supplemented with cowpea [24] and acha and cowpea blends [14]. There was a moderate increase in the protein content of unfermented extruded blends. This is contrary to the findings of Oguntunde and Shoola [25] that extrusion cooking caused reduction in protein and carbohydrate. Increase in protein content of unfermented extruded blends corresponds with the findings of Abiodun and Ogugua [14] in the evaluation of extruded snacks from blends of acha and cowpea. Protein content of fermented unextruded blends ranged from 2.98±0.06% to 11.44±0.08%. Increase in the protein content of fermented unextruded blends could be as a result of protein synthesis by microorganisms during fermentation which contribute to high value in fermented samples. This increase could be attributed to the increase in microbial mass during fermentation, causing extensive hydrolysis of the protein molecule to amino acid and other simple peptides [26]. Another reason for the increase in protein content may be due to the structural proteins that are integral part of the microbial cells [27]. The apparent increase in growth and microbial proliferation of microorganisms in the form of single cell protein of the normal flora may account for the observed trend in crude protein [28]. This trend was also reported by Michodjehoun et al. [29] and Igbabul et al., [26]. Increase in the protein content of fermented extruded blends was also reported by Osundahunsi [30]. Jeff- Agboola and Oguntuase [31] reported that microorganisms are found to increase the protein content of the samples on which they grow. Fermented extruded blends recorded the highest protein content with values ranging from 3.06±0.03 to 12.10±0.08. This is in agreement with the findings of Ojokoh and Udeh [20] in which extruded fermented blends had the highest protein contents.

The carbohydrate content of the raw blends decreased with increase in cowpea. Abiodun and Ogugua [14] also reported decrease in the carbohydrate content of raw blends of acha and cowpea flour. Reduction in the carbohydrate content of fermented unextruded blends could be as a result of utilization of carbohydrate by microorganisms during fermentation. Decrease in carbohydrate content of fermented samples may be because it was used up as the main source of energy during fermentation.

Fat is one of the major components of food that provides essential lipids and energy. Lipid constituents are the major determinants of overall physical characteristics of food such as aroma and texture. Fat content was highest in fermented unextruded blends. This could be as a result of the metabolic activities of the fermenting microorganisms. Reduction in the fat content of unfermented extruded and fermented extruded blends could be due to lipid oxidation. Lipid oxidation can reduce the nutritive quality of food by decreasing the content of essential fatty acids, such as linolenic acid and linoleic acid, which are essential fatty acids. These long- chained fatty acids are highly susceptible to oxidation which results from application of high temperature during extrusion [32].

Fibre is an indigestible component of food material that helps in improving roughage and bulk as well as contributes to a healthy condition of the intestine [33,23].Crude fibre gives bulk to food and aids in regulating physiological functions in the body. Fermented unextruded blends had the highest crude fibre content but unfermented extruded and fermented extruded blends had low crude fibre content. This implies that extrusion had negative impact on the crude fibre content of the blends. The result of the fermented unextruded blends compares favourably with the work of Eze and Ibe [34] on the effect of fermentation on the nutritive value of *B. Eurycoma* “Achi” where an increase in fibre content for the fermented sample was reported. The reason for unexpected increase in fibre content for the fermented samples may be due to the activities of microorganisms. The fermentation process involves the conversion of materials to the needs of the microorganisms, which include the bacterial cell wall. The bacterial cell wall is made of peptidoglycan or murein, which is a polysaccharide like cellulose. As the microorganisms were not separated from the biomass, the increase in fibre could be due to such conversion of materials to peptidoglycan by the microorganisms [34].

Ash is an inorganic residue remaining after the removal of water and organic matter which provides a measure of total amount of minerals in the food component. Fermentation caused a significant (P≤0.05) reduction in the ash content of the samples. Michodjehoun et al. [29] also reported a decrease in ash content during fermentation of ‘‘Gowe’’, a traditional food made from sorghum, millet or maize. This also corresponds with the report of Omafuvbe et al. [35].

The result obtained in the sensory evaluation indicated that there was no significant difference in the colour of the raw flour blends (RF) and unfermented extruded blends (UE). Fermented unextruded blends and fermented extruded blends recorded significantly low values for colour. There was no significant difference in the texture of raw blends and unfermented extruded blends. Fermented blends recorded significantly high values in terms of aroma. Raw blends and fermented extruded blends recorded high values for overall acceptability. The low values obtained for colour in fermented blends may be as a result of browning which occurred during fermentation. It was observed that fermentation enhanced the aroma of the blends. There is no significant difference (P≤0.05) in the texture of the flour blends. The higher organoleptic attributes of the raw blends (19.10 to 24.10) when all the attributes were summed up and compared is simple to explain. The judges were familiar with raw plantain and cowpea flour. On the other hand, the similarity in organoleptic attributes between the raw flour blends and unfermented extruded blends indicates that the samples were equally liked. The judges had preference to colour for the raw flour blends over the other test samples while fermented unextruded and fermented extruded blends had highest preference for aroma. The fermented blends had better flavour than other test blends while raw blends had the highest colour. Based on these, they were much more acceptable. This is not surprising because it is known that appearance of food evokes the initial response and flavour determines the final acceptance or rejection of the product by the consumer [36,37]. The improved flavor of fermented blends may be as a result of fermentation and mutual supplementation effect of food nutrients.

5. CONCLUSION

The investigation so far revealed that the blending of unripe plantain and cowpea has the potential of producing enriched complementary food for teeming undernourished children of developing countries especially Nigeria. Also, fermentation and extrusion of cowpea-plantain blends improved the nutritional quality of the samples compared with the raw blends.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anuonye JC. Some functional properties of extruded acha/soybean blends using response surface analysis. Afr. J. Food Sci. 2012;6(10):269-279.
2. Akubor PI, Adamolekun, FO, Oba CO, Obari H, Abudu IO. Chemical composition and functional properties of cowpea and plantain flour blends for cookie production. Plant Foods Hum Nutr. 2003;58(3):1-9.
3. FAO. Food and Agriculture Organisation of the United Nations. Joint meeting of the fourth session of the sub-group on bananas and the fifth session of the sub-group on tropical fruits held in Rome, 9 – 11th December; 2009.
4. Agama-Acevedo E, Islas-Hernandez JJ, Pacheco-Varga G, Osorio-Diaz P, Bello-Perez LA. Starch digestibility and glycemic index of cookies partially substituted with unripe banana flour. Food Sci. Tech. 2012;46(1):177-182.
5. Onyenekwe PC, Njoku GC, Ameh DA. Effect of cowpea processing methods on flatus causing oligosaccharides. Nutr. Res. 2000;20:349-358.
6. Bressani R. Nutritive value of cowpea. In: Cowpea Research, Production and Utilization. Chichester: John Wiley and Sons. 1985;353–359.
7. Gabrie RAO, Akharaiyi FC. Effect of spontaneous fermentation on the chemical composition of thermally treated jack bean (*Canavalia ensiformi* L.). Int. J. Biol Chem. 2007;1(2):91-97.

8. Ijarotimi OS. Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (*Triticumaetivum*). J. Cereals Oilseeds. 2012;3(3):35-47.
9. Holzapfel WP. Appropriate starter cultures technologies for small- scale fermentation in developing countries. Int. J. Food Microbiol. 2002;75:197-212.
10. Jisha S, Sheriff JT, Padmaja G. Nutritional, functional and physical properties of extrudates from blends of cassava flour with cereal and legume flours. Int. J. Food Prop. 2010;13(5):1002-1011.
11. Nwabueze TU, Iwe MO, Akobundu ENT. Unit operations and analyses for African breadfruit based spaghetti type products at extreme process combinations. J. Food. Tech. 2007;5(1):87-94.
12. Oluwole OB, Olapade AA. Effect of extrusion cooking of white yam and bambara-nut blend on some selected extrudate parameters. Food Nutr. Sci. 2011;2(6):599-605.
13. Sobota A, Rzedzicki Z. Effect of the extrusion process of corn semolina and pea hulls blends on chemical composition and selected physical properties of the extrudate. Int. Agrophys. 2009;23:67-79.
14. Abiodun AO, Ogugua CA. Evaluation of extruded snacks from blends of acha (*Digitaria exilis*) and cowpea (*Vigna unguiculata*) flours. Agric. Eng. Int: CIGR Journal. 2012;14(3):210-217.
15. Sneath PHA, Mair NS, Sharpe ME, Holt JG. Bergey's manual of systemic bacteriology. Williams Wilkins Co., Baltomore. 1986;1123.
16. Cowan ST, Steel KJ. Bergey's manual of determinative microorganisms. 4th edition. Cambridge University Press. 1990;58.
17. AOAC. Official methods of analysis of the Association of Official Analytical Chemists international. 19th edition. Gathersburg, Maryland, U.S.A; 2012.
18. Ojokoh AO, Udeh EN. Microorganisms associated with the natural fermentation of extruded sorghum- soya blends. J. Pure Appl. Microbio. 2012;6(2):589-596.
19. Hassan GF, Adebolu TT, Onifade AK. Effect of fermentation on mineral and anti-nutritional composition of cocoyam (*Colocasia esculenta* Linn). Sky J. Food Sci. 2015;4(4):42-49.
20. Ojokoh AO, Udeh EN. Effects of fermentation and extrusion on the proximate composition and organoleptic properties of sorghum- soya blends. Afr. J. Biotechnol. 2014;13(40):4008-4018.
21. Aremu MO, Olaofe O, Akintayo ET. A comparative study on the chemical and amino acid composition of some Nigerian under-utilized legume flours. Pak. J. Nutr. 2006;5(1):34–38.
22. Alozie YE, Umoh IB, Eyong EU. Comparative biological evaluation of *Dioscorea dumentorum* varieties and cereals (maize and rice) flours in weaning wistar albino rats. Niger. J. Nutr. Sci. 2008;29:111-120.
23. Odom TC, Udensi EA, Iwe MO. Nutritional evaluation of unripe *Carica papaya*, unripe *Musa paradisiaca* and *Mucuna cochichenesis* weaning food formulation. Euro. J. Biol. Medical Sci. 2013;1(1):6-15.
24. Ashaye OA, Fasoyiro SB, Kehinde RO. Effect of processing on the chemical and sensory quality of ogi fortified with full fat cowpea flour. Moor J. Agric. Res. 2000;1: 115-123.
25. Oguntunde AO, Shoola FK. Effects of extrusion cooking on selected properties of soybean. Niger. Food J. 1999;11:9-15.
26. Igbabul BD, Bello FA, Ani EC. Effect of fermentation on the proximate composition and functional properties of defatted coconut (*Cocos nucifera* L.) flour. Sky J. Food Sci. 2014;3(5):34–40.
27. Tortora GJ, Funke BR, Case CL. Microbiology: An introduction. 7th Edn., Benjamin/Cummings Publishing Company, Inc., USA. 2002;887.
28. Oboh G. Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisae* and *Lactobacillus* sp. solid media fermentation techniques. Electron. J. Biotechnol. 2006;9:46-49.
29. Michodjehoun-Mestres L, Hounhouigan JD, Dossou J, Mestres C. Physical, chemical and microbiological changes during natural fermentation of ''gowe'' a sprouted or non-sprouted Sorghum beverage from West Africa. Afr. J. Biotechnol. 2005;4(6):487-496.
30. Osundahunsi OF. Functional properties of extruded soybean with plantain flour blends. J. Food Agric. Environ. 2006;4(1): 75-60.
31. Jeff- Agboola YA, Oguntuase OS. Effects of *Bacillus sphaericus* on the proximate composition of soybean (*Glycine max*) for the production of iru. Pak. J. Nutr. 2006;5(6):606-607.

32. Ranjit B, Subha G. Extrusion technique in food processing and a review on its various technological parameters. Ind. J. Sci. Res. Tech. 2014;2(1):1-3.
33. Potter NN, Hotchkiss JT. 'Food Sciences' 5th edition, CBS Publishers and Distributors, New Delhi. 2004;55-56,63, 347,583.
34. Eze SO, Ibe OJ. Effect of fermentation on the nutritive value of *B. Eurycoma* "Achi". Chemical Society of Nigeria. 30:1-5.
35. Omafuvbe BO, Falade OS, Osuntogun BA, Adewusi SRA. Chemical and biochemical compositon changes in African locust beans Parkiabiglobosa and melon (*Citrullus vulgaris*) seeds during fermentation to condiments. Pak. J. Nutr. 2004;3:140-145.
36. Nnam NM. Evaluation of complementary foods based on maize, ground nut, pawpaw and mango flour blend. Niger. J. Nutr. Sci. 2002;23:1-4.
37. Teratanavat R, Neal HH. Consumer valuations and preference heterogeneity for a novel functional food. J. Food. Sci. 2006;71:533-534.

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