EFFECTS OF FERMENTATION AND EXTRUSION ON THE PROXIMATE COMPOSITIONS AND ORGANOLEPTIC PROPERTIES OF SWEET POTATO (*IPOMOEA BATATAS*) AND BENISEED (*SESAMUM INDICUM*) BLENDS

ABSTRACT

Fermentation and extrusion have been proven to increase the nutritional value of foods by reducing the water-binding capacity of cereal flour Thus, the effect of fermentation and extrusion on the microbiological qualities, proximate compositions and organoleptic properties of orange flesh potato and beniseed blends were investigated using standard methods .The blended samples were prepared in four combinations (A=100g sweet potato; B = 70g sweet potato + 30g beniseed; C= 60g sweet potato + 40g beniseed, D = 50g sweet potato + 50g Beniseed) and separated into four batches (i.e. first batch = preconditioned and fermented; second batch = extruded; third batch = fermented and extruded; and fourth batch = unfermented/unextruded). The blended samples were fermented for 72 hours using solid state fermentation. The bacteria isolated include *Bacillus subtilis, Lactobacillus Plantarum, Lactococcus lactis* and *Staphylococcus aureus* while fungi include *Mucor mucedo, Aspergillus niger, Penicillium chrysogenium, Aspergillus flavus, Geotrichum* spp*, Mucor mucedo* and *Alternaria alternate.* The results of the proximate composition of the fermented and extruded blends showed a significant difference as compared with the unfermented and unextruded blends. The moisture content was highest in fermented extruded 50% sweet potato + 50% beniseed (18.61%) and least in the unfermented unextruded 50% sweet potato + 50% beniseed (4.0%). Fermentation also helps to increase the protein content and the highest was observed in composite bleed containing 50% sweet potato + 50% beniseed which increased from 2.88% to 8.75%. Extrusion also increased the protein content. The highest protein content was observed in the composite blend that was extruded and fermented (18.61). The carbohydrate content was highest in the unfermented unextruded 50% sweet potato + 50 beniseed (84.04%). The crude fat content was highest in the fermented unextruded 100% sweet potato blends (21.50%) and least in fermented extruded 50% sweet potato + 50 beniseed (2.0%). The sensory evaluation of the samples showed a good preference for the fermented-extruded samples. Findings from this research have established that orange flesh potato and beniseed blends can be fermented and extruded to produce food of enhanced nutritional value.

Keywords: Sensory, Extrusion, Fermentation, Composition

INTRODUCTION

Sweet potato *(Ipomoea batatas. L.)* is an important tuber crop grown in the tropics, subtropics and warm temperate region of the world for it edible storage root (Camire *et al.,* 1993). The crop contains some nutrients which are important and essential for the body, outranking most carbohydrate foods in vitamins, minerals, dietary fiber and protein content (Liu *et al*., 2011). Sweet potato (*Ipomoea batata* (L) Lam) is an important tropical root crop. It belongs to the Morning-glory family known as convolulaceae and is originated from Latin America (Vanhijum *et al*., 2013). It is the sixth most important world food crop after rice, wheat, potatoes, maize and cassava and the fifth most important food crop in developing countries after rice, wheat, maize and

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cassava (FAO, 2005). The crop can be considered promoting nutritional security particularly in agriculturally backward areas. Besides carbohydrates, it is a rich source of lipid, protein, carotene and calcium (Liu *et al*., 2011).

Fermentation and extrusion improve the nutritional value of foods by reducing the water-binding capacity of cereal flour. This allows the fortified to have a free-flowing consistency even with high proportion of flour. Extrusion has been reported as an effective processing treatment to improve the nutritional quality of cereals (Amadou *et al*., 2011). In the developing world, fermentation is one of the oldest technologies used for food processing and preservation. It can be described as a desirable process of biochemical modification of primary food products brought about by microorganisms and their enzymes (Muchoki *et al.,* 2010.) Extrusion cooking technology has been described as a process in which raw materials are heated and worked upon mechanically while passing through compression screws (Iwe, 2003).

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 (Anuonye, 2012). The rural consumption of beniseed in Nigeria is partly due to high cost of animal protein; however, its usefulness as ingredient in different food formulations is limited probably due to the non-availability of nutritional information. Hence, the objective of this research is to determine the effect of fermentation and extrusion on the proximate compositions and organoleptic properties of orange flesh potato and beniseed blends.

METHODS

Collection of Samples

Fresh Orange fleshed tubers of sweet potato were obtained at Agricultural and Rural Management Training institute Kwara State, Nigeria. Dried grains of Beniseeds were purchased at a local market in Kogi State, Nigeria.

Processing of Sweet Potato Flour

Orange flesh sweet potatoes were sorted, washed with cleaned water to remove soil and dirt; these were then peeled by using clean kitchen knife. The sweet potatoes were chipped into slices of about 2mm using kitchen knife and it was soaked in cleaned water for 15 hours to remove the sweetness and disallow enzymatic browning. After 15 hours, the chips were then sundried for 5days. The dried chips were then milled and sieved to obtained flour.

Processing of Beniseed Flour

Dried grains of beniseed were sorted, winnowed, washed with cleaned water,

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dehulled by soaking in clean water for 24 hours .The dehulling was done manually by the use of mortar and pestle. These were then washed again to remove chaffed and sundried for 5days. The dried grains were milled which formed paste and were defatted with n-Hexane by the use of soxhlet extraction to obtained oil and powdered samples. These were then oven dried at 45^0C for 7hours. The dried samples were then re-milled and sieved to flour

Formation of Sweet Potato Beniseed Blends

The experimental composite flour was formulated using a substitution method (Table 1). Orange flesh sweet potato (OFSP) and Beniseed (BS) composite flours for experiment, the sweet potato (peeled) and beniseed (dehulled, defatted) were washed, dried, milled and mixed at different proportions of 100:0, 70:30, 60:40 and 50:50 respectively as shown in Table 1. The blends were separated into four batches, the first batch were fermented, the second batch were extruded, and the third batch were fermented and extruded, while the fourth batch were unfermented and unextruded which serve as control.

Table 1: Formation of composite flour

Formation	% of Ingredient Formation	
	OFSP	BS
OFSP-1 (0%)	100	0
OFSP-2 (30%)	70	30
OFSP-3 (40%)	60	40
OFSP-4 (50%)	50	50

Fermentation and Extrusion of Flour Blends

Samples were soaked at different concentrations in a transparent sterile container of distilled water and allowed to incubate for 0, 24, 48, 72 and 96 hours respectively at 25^oC. The pHs, total titratable acidity (TTA) of the samples were monitored before and during the process of fermentation for 0-72 hours using AOAC (2005) method. The fermentation processes were terminated by oven drying at 60^0 C for 24 hours before the samples were subjected to subsequent analyses. The extrusion process was carried out in a Brabender 20DN single screw laboratory extruder (Brabender OHG, Duisburg, Germany) having a uniform tapered screw with a nominal compression ratio of 2:1, diameter 19 mm, length to diameter 20:1, die diameter 3 mm and screw speed at feed inlet which was kept constant at 30 rpm. Electrical heating was applied to the three barrel zones along the screw. The screw speed was maintained at 200 rpm. Two batches of samples were subjected to extrusion cooking. The first batch consists of the unfermented blends, while the second batch was the fermented blends. The blends were hydrated and preconditioned

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by adding 10 ml of water to 100g of the sample and manually mixed in a sterile bowl to ensure even distribution of water and form dough. The dough was extruded using a Brabender 20DN single screw laboratory extruder (Brabender OHG, Duisburg, Germany). All the extrudates were air dried for 12 hours after which they were stored at 38±2°C in sterile polyethylene bags and kept in properly labeled air tight containers

Microbiological Analysis of the Samples

Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while De Man Rogosa sharpe 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°C for 18 to 24 hours, fungal plates were inverted and incubated at 24°C for 48 to 72 hours. De Man Rogosa sharpe agar plates were incubated at 32°C for 18 to 24 hours anaerobically. The organisms were characterized based on biochemical and morphological observations according to the methods of Fawole and Oso (2007)

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 value 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, mixed and filtered. 10 ml of the filtrate was measured into a beaker and 2 drops of phenolphthalein indicator was added. 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: TTA= Titre value × 9 mg/100. The pH and TTA of the samples were carried out according to the method described by AOAC (2012).

Proximate Composition

All samples were analyzed for Moisture, Ash, Fat, Protein, Crude fiber and Carbohydrate determined by difference according to the method described by AOAC (2012).

Sensory Evaluation

The sensory evaluation was done by the method of panel of 15 judges (Larmond, 1977), samples of the raw flour blend, extruded unfermented (EUF), fermented extruded (FE) flour blend and fermented unextruded flour blend (FUE) made into pasta-like, then boiled and were served to

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the panel. The panels rated the samples based on the colour, aroma, texture, taste and overall acceptability by grading them on a seven-point hedonic scale (1= strongly disliked, 2= moderately disliked, 3= slightly disliked, 4= indifferent, 5= slightly liked, 6= moderately liked, 7= strongly liked (Granato *et al.,* 2010).

Statistical Analysis

Statistical analyses of the Data were obtained using SPSS statistical software (SPSS for window version 20). Data obtained as mean standard deviations were analysed by Analysis of Variance (ANOVA), followed by Duncan's New Multiple Range Test(P<0.05) to determine the significant differences between the mean values.

RESULTS

Morphological and biochemical characterization of microorganisms isolated

The biochemical tests carried out on the bacterial isolates (Table 2) are; Gram stain, Cell shape, Urease, Methyl Red, Oxidase, Citrate, Motility, Indole, Catalase, Sugar Fermentation, Coagulase, Spore Staining, Nitrate reduction and Starch hydrolysis. All the isolates showed different biochemical properties and morphologically features. The morphological characteristics features of fungal isolates during natural fermentation are also shown on Table 3

Type of bacterial and fungal isolates in the fermented samples

Details of the bacteria isolated during the fermentation of sweet potato and beniseed blend are shown in Table 4. The bacteria isolated during fermentation include *Bacillus subtilis, Bacillus* spp*, Lactococcus* spp*, Lactobacillus* spp*, Micrococcus* spp and *Lactobacillus* spp while the fungi isolated as shown on Table 3 include *Mucor mucedo. Aspergillus* niger, *Penicillium chrysogenium, Aspergillus flavus, Geotrichum* spp, *Aspergiluss fumigatus, Rhizopus oryzae* and *Alternaria alternate*.

Bacterial occurrence during fermentation of the samples

At 0 and 24 hours of fermentation, for all the samples, *Bacillus* species were the most isolated organism. The succession of bacteria during the fermentation of sweet potato and beniseed flour blends is shown in Table 4. Toward the later stage of the fermentation process, Lactic acid bacteria predominate and *Lactobacillus* spp and *Lactococcus* spp were the most isolated organisms at 72 and 96 hours.

Microbial load of microorganisms isolated during fermentation

The microbial load of the fermented samples are shown in Table 5. The total bacteria and and lactic acid count was highest in Fermented extruded sample (60% sweet potato: 40% beniseed) with 67 X 10^5 cfu/ml and 63 X 10^5 cfu/ml respectively while the fungi count was

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highest in Fermented sample (50% sweet potato : 50% beniseed) with 6 X10^{1} sfu/ml.

Changes in pH and Titratable acidity during the fermentation of the samples

The pH of the samples subjected to fermentation decreased with increase in the fermentation duration (Table 6). In sample F1, F2, F3 and F4, the pH values decreased from 5.8, 5, 5, and 5.3 at day 1 to 4.6, 4.6, 4.5 and 4.23 respectively on the last day of fermentation. The titratable acidity of the samples subjected to fermentation increased with increase in the fermentation duration. In sample F1, F2, F3 and F4, the titratable acidity values increased from 0.0201, 0.0201, 0.0134 and 0.0134 at day 1 to 0.1005, 0.0737, 0.0670 and 0.0603 respectively on the last day of fermentation. Variations in titratable acidity (TTA) during fermentation are represented in Table 7.

Proximate Compositions of Sweet potato and beniseed Flour Blends

The moisture content of Sweet potato and beniseed flour blends are represented in Table 8. Raw flour blend had the lowest moisture content with values ranging from 4.0 % to 5.80 %. Fermented samples had the highest moisture content ranging from 5.0 % in fermented extruded sample (70% sweet potato: 30% beniseed), to 13.0 % in fermented sample (100% sweet potato).

The variations in protein content of sweet potato and beniseed flour blends are shown in Table 8. There was significant ($P \leq 0.05$) difference in the raw flour blends with values ranging from 2.23 % to 4.90%. Fermented samples recorded significant difference ($P \leq 0.05$) for all the blends with values ranging from 5.91% in fermented sample (100% sweet potato) to 18.61% in fermented extruded sample (50% sweet potato : 50% beniseed). The extruded unfermented exhibited protein content ranging from 3.33 % to 4.90%. Extruded fermented samples exhibited significant difference ($P \leq 0.05$) among all the blends with values ranging from 7.34 % to 18.61%.

The crude fibre content of the sweet potato and beniseed flour blends are shown in Table 8. There was significant difference ($P \leq 0.05$) in the crude fibre content of the blends. The crude fibre of the raw blends range from 1.1% to 2.0%. Fermented blends had the highest crude fibre content ranging from ranging from 2.4% in fermented extruded sample (70% sweet potato: 30% beniseed), to 4.0% in fermented sample (70% sweet potato: 30% beniseed). Extruded unfermented blends had crude fibre content ranging from 1.2% to 2.0%. Extruded fermented blends ranged from 2.4% to 3.85%.

The fat content of sweet potato and beniseed flour blends are shown in Table

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8. There was significant ($P \leq 0.05$) difference in the fat content of the raw flour blends with values ranging from 9.50% to 12.00% . The fermented samples had the highest range in fat content with values ranging from 2.00% to 22.00%. There were significant ($P \leq 0.05$) differences in the extruded unfermented blends with values ranging from 6.00% to 9.00%. Fat content of extruded fermented samples had the least ranged in value from 2.00% to 5.00% .

The carbohydrate content of sweet potato and beniseed flour blends are shown in Table 8. Carbohydrate content of raw flour blends ranged from 70% to 78%. The fermented blends had carbohydrate content ranging from 53.97% to 67.55%. Extruded unfermented blends had carbohydrate content ranging from 74.72 % to 79.50 %. There is no significant difference in the carbohydrate content of the extruded fermented blends with values ranging from 65.11% to 67.55 %.

Sensory Evaluation of Sweet potato and Beniseed Blends

The result obtained in the evaluation demonstrated that there was no significant difference in the blends for colour, texture, aroma, taste and overall acceptability. Fermented blends (50% sweet potato : 50% beniseed) and extruded-fermented blend (50% sweet potato : 50% beniseed) recorded the highest values for colour, texture, aroma, taste and overall acceptability while the Fermented unextruded blends (100% sweet potato) and extruded-fermented blend (100% sweet potato) recorded the lowest values for colour, texture, aroma, taste and overall acceptability. This result is represented in Table 9.

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Table 2: Colonial, morphological and biochemical characterization of bacterial isolates from fermented broth cultures

Isolate No	Colony Morphology	Gram's Reaction	Catalase	Coagulase	Motility	Mannitol	Glucose	Fructose	Maltose	Lactose	Sucrose	Citrate	Indole	Spore Forming	Methyl Red Test	Starch hydrolysis	Urease test	Probable Identity
1	Cream, circular, opaque, flat, rough	+	+	NA	+	+	AG	AG	AG	AG	AG	+	-	+	-	+	-	*Bacillus subtilis*
2	Cream, circular, opaque, flat, rough	+	+	NA	+	+	AG	AG	AG	A	A	+	-	+	-	+	-	*Bacillus* spp
3	Circular, opaque, convex, cream, smooth colonies	+	-	-	-		A	AG	AG	A	AG	-	-		+	-	+	*Lactobacillus* spp
4	Cream, circular, smooth, entire	+	-	NA	-	-	AG	A	A	A	AG	-	-			-		*Lactococcus* spp
5	Cream, circular, raised and smooth	-	+	-	+	-	A	A	-	-	A		-	NA		+		*Micococcus* spp
6	Circular, translucent, convex, creamy, smooth colonies	+	-	-	-		AG	A	AG	AG	AG	-	-			-		*Lactobacillus* spp

Keys:

(+) = **positive** (AG) = **Acid and Gas**

(-) = **negative** (A) = **Acid** (NA) = **not applicable**

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Table 3: Morphological characteristics of fungal isolates during fermentation

Isolate code	Morphological Characteristics	Microscopic Characteristics	Suspected fungi
F11	of white spread on the whole plate, wooly like structure	Aseptate with spore not enclosed	***Mucor mucedo***
F12	White at basement with black grey wooly like white at top	Aseptate, spore surrounding the sporangiospore	***Aspergillus* niger**
F13	Greyish and whitish cotton like	Sporangiospore sounded with spores	***Penicillium chrysogenium***
F21	Well-developed strand and greenish in nature	Spores enclosed and aseptate also with naked spores	***Aspergillus flavus***
F22	Creamy round with rough surfaces	Naked spores	***Geotrichum* spp**
F23	Of white cotton like strand spread throughout the plate	Septate with spore in the middle	***Mucor mucedo***
F31	Well-developed strand and greenish with white basement	Spores enclosed and aseptate also with naked spores	***Aspergiluss fumigatus***
F32	Creamy round with rough surfaces	Naked spores	***Geotrichum* spp**
F33	Of white cotton like strand spread throughout the plate	Septate with sore in the middle	***Rhizopus oryzae***
F41	Wooly like strand whitish spread throughout the plate	Aseptate interwoven with each other and spore with rhyzoid	***Rhizopus oryzae***
F42	Milky colour like oval shape,	Spore not enclosed and scattered	***Geotrichum* spp**
F43	Pink on plate	Pink colour with spores	***Alternaria alternate***

Key:
F_1 = Fermented sample (100%), F_2 = Fermented sample (70% - 30%), F_3 = Fermented sample (60% - 40%), F_4 = Fermented sample (50% - 50%)

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Table 4: Bacterial succession during the fermentation of Sweet potato and beniseed blends

Sample codes	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours
F1	*Bacillus* spp, *Bacillus subtilis*	*Micrococus* spp *Bacillus* spp	*Micrococus* spp, *Lactobacillus* spp *Lactococcus* spp,	*Lactobacillus* spp *Lactococcus* spp,	*Lactobacillus* spp *Lactococcus* spp
F2	*Bacillus* spp.	*Micrococcus* spp, *Bacillus* spp	*Lactobacillus* spp *Lactococcus* spp,	*Lactobacillus* spp *Lactococcus* spp,	*Lactobacillus* spp *Lactococcus* spp
F3	*Bacillus subtilis, Bacillus* spp	*Bacillus* spp	*Micrococus* spp, *Lactobacillus* spp *Lactococcus* spp *Lactobacillus* spp	*Lactobacillus* spp *Lactococcus* spp *Lactobacillus* spp	*Lactobacillus* spp *Lactococcus* spp
F4	*Bacillus subtilis, Bacillus* spp	*Bacillus* spp	*Micrococus* spp, *Lactobacillus* spp *Lactococcus* spp *Lactobacillus* spp	*Lactobacillus* spp *Lactococcus* spp *Lactobacillus* spp.	*Lactobacillus* spp *Lactococcus* spp

Keys:
F_1 = Fermented sample (100%), F_2 = Fermented sample (70% - 30%), F_3 = Fermented sample (60% - 40%), F_4 = Fermented sample (50% - 50%),

Table 5: Microbial load of Fermented sample

Sample codes	Total LAB count (cfu/ml)	Total bacterial count (cfu/ml)	Total fungal count(sfu/ml)
F1	$16 X10^{5}$	$28 X10^{5}$	$3 X10^{1}$
F2	$40X10^{5}$	$280 X10^{5}$	$2 X10^{1}$
F3	$147 X10^{5}$	$158 X 10^{5}$	$1 X 10^{1}$
F4	$30 X10^{5}$	$170 X 10^{5}$	$3 X 10^{1}$
FE1	$28 X10^{5}$	$40 X 10^{5}$	$5 X10^{1}$
FE2	$32 X10^{5}$	$41 X 10^{5}$	$6 X10^{1}$
FE3	$63 X10^{5}$	$67 X 10^{5}$	$5 X10^{1}$
FE4	$57 X10^{5}$	$33 X 10^{5}$	$6 X10^{1}$

Keys:
F_1 = Fermented sample (100%), F_2 = Fermented sample (70% - 30%), F_3 = Fermented sample (60% - 40%), F_4 = Fermented sample (50% - 50%),.

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Table 6: Effects of fermentation on the pH of fermented sample

Samples	Durations (Days)			
	1	2	3	4
F1	5.8	5.5	4.7	4.6
F2	5	4.9	4.6	4.6
F3	5	4.9	4.5	4.5
F4	5.3	4.8	4.48	4.23

Keys:
F_1 = Fermented sample (100%), F_2 = Fermented sample (70% - 30%), F_3 = Fermented sample (60% - 40%), F_4 = Fermented sample (50% - 50%),

Table 7: Effects of Fermentation on the Titratable Acidity of Fermented Sample

Samples/	**Durations (Days)**			
	Day 1	**Day 2**	**Day3**	**Day4**
F1	0.0201	0.0335	0.0737	0.1005
F2	0.0201	0.0335	0.0677	0.0737
F3	0.0134	0.0402	0.0603	0.0670
F4	0.0134	0.0402	0.0469	0.0603

Keys:
F_1 = Fermented sample (100%), F_2 = Fermented sample (70% - 30%), F_3 = Fermented sample (60% - 40%), F_4 = Fermented sample (50% - 50%),

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Table 8: Effects of fermentation and extrusion on the proximate composition of samples

Sample	Crude Fibre %	Ash %	FAT %	Moisture %	Protein %	Cho %
F1	3.92±0.04	5.50±0.16	21.50±0.21	9.20±0.20	5.91±0.16	53.97±0.07
F2	4.00±0.19	4.00±0.09	22.00±0.21	9.00±0.03	7.69±0.04	57.31±0.03
F3	2.5 ±0.01	4.41±6.02	14.50±0.16	5.00±0.17	8.40±0.12	65.19±0.09
F4	2.7 ±0.02	4.57±0.05	15.50±0.21	5.50±0.21	8.75±0.03	62.98±0.04
FE1	3.85±0.02	5.00±0.11	5.00±0.09	13.00±0.02	8.04±0.09	65.11±0.03
FE2	2.4 ±0.04	4.31±0.02	3.50±0.21	8.90±0.06	10.34±0.05	65.55±0.18
FE3	2.7 ±0.06	4.93±0.02	3.50±0.14	6.20±0.02	16.35±0.03	66.32±0.02
FE4	3.0± 0.06	4.85±0.02	2.00±0.12	6.00±0.09	18.61±0.02	65.54±0.04
UFE1	1.6 ±0.03	4.00±0.03	7.00±0.19	5.50±0.2	3.96±0.01	77.94±0.02
UFE2	1.8 ±0.03	4.50±0.02	8.00±0.16	4.40±0.16	3.33±0.06	77.97±0.06
UFE3	1.2 ±0.02	5.50±0.02	9.00±0.01	5.90±0.21	3.68±0.05	74.72±0.02
UFE4	2.0 ±0.05	6.0±0.09	6.00±0.03	5.60±0.16	4.90±0.05	79.50±0.02
UFUE1	2.0 ±0.02	3.79±0.02	9.50±0.12	4.00±0.14	2.66±0.05	78.05±0.03
UFUE2	2.05±0.03	6.50±0.02	12.00±0.16	5.80±0.11	2.23±0.01	70.42±0.06
UFUE3	1.1 ±0.02	5.61±0.08	9.50±0.20	5.50±0.21	3.21±0.02	75.08±0.05
UFUE4	1.5±0.03	4.08±0.03	10.50±0.02	4.0±0.16	2.88±0.02	77.04±0.02

Keys: F_1 = Fermented sample (100%), F_2 = Fermented sample (70% - 30%) F_3 = Fermented sample (60% - 40%), F_4 = Fermented sample (50% - 50%), FE_1 = Fermented Extruded (100%), FE_2 = Fermented Extruded (70% - 30%), FE_3 = Fermented Extruded (60% - 40%), FE_4 = Fermented Extruded (50% - 50%), UFE_1 = Unfermented Extruded (100%) , UFE_2 = Unfermented Extruded (70% - 30%), UFE_3 = Unfermented Extruded (60% - 40%), UFE_4 = Unfermented Extruded (50% - 50%), $UFUE_1$ = Unfermented Unextruded (100%), $UFUE_2$ = Unfermented Unextruded (70% - 30%), $UFUE_3$ = Unfermented Unextruded (60% - 40%) , , $UFUE_4$ = Unfermented Unextruded (50% - 50%)

Legend: Values are means ± SEM (Standard error of mean) of triplicates, values in the same column carry same superscript are not significantly different according to new Duncan's multiple range test at $p \leq 0.05$.

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Table 9: Sensory Acceptability of Fermented Samples

Samples	Colour	Texture	Taste	Overall
F1	7.0[a]	6.6[a]	6.8[a]	6.8[a]
F2	7.4[a]	7.2[a]	7.0[a]	7.4[a]
F3	7.2[a]	6.8[a]	7.0[a]	7.0[a]
F4	7.4[a]	7.2[a]	7.2[a]	7.4[a]
FE1	6.8[a]	7.0[a]	6.8[a]	6.8[a]
FE2	7.2[a]	7.2[a]	7.0[a]	7.0[a]
FE3	7.1[a]	7.0[a]	7.0[a]	7.0[a]
FE4	7.5[a]	7.4[a]	7.8[a]	7.4[a]

Keys: F_1 = Fermented sample (100%), F2 = Fermented sample (70% - 30%) F_3 = Fermented sample (60% - 40%), F_4 = Fermented sample (50% - 50%), FE_1 = Fermented Extruded (100%), FE_2 = Fermented Extruded (70% - 30%), FE_3 = Fermented Extruded (60% - 40%), FE_4 = Fermented Extruded (50% - 50%), UFE_1 = Unfermented Extruded (100%) , UFE_2 = Unfermented Extruded (70% - 30%), UFE_3 = Unfermented Extruded (60% - 40%), UFE_4 = Unfermented Extruded (50% - 50%), $UFUE_1$ = Unfermented Unextruded (100%), $UFUE_2$ = Unfermented Unextruded (70% - 30%), $UFUE_3$ = Unfermented Unextruded (60% - 40%) , , $UFUE_4$ = Unfermented Unextruded (50% - 50%)

DISCUSSION

This study evaluates the effect of fermentation and extrusion on the nutritional composition of sweet potato (*Ipomoea batatas*) and beniseed (*Sesamum indicum*) blends. Also, the effects of fermentation and extrusion on the microbial quality of the blends were also determined. There were varied microbial populations during the fermentation of sweet potato and beniseed flour blends. The microorganisms that were present in the fermenting media all had the ability to utilized carbohydrate, protein and fat which were the major component of sweet potato and beniseed. These isolated organisms were also similar to those isolated by Ojokoh and Udeh (2014) from legume supplemented products. *B. subtillis* was the most frequently occurring organism during the first two day of the fermentation of all the blends and could be as a result of the fact that *B. subtillis* have proteolytic ability to break down the high oil and protein content of beniseed (Enujiugha, 2009). *Bacillus subtillis* has been associated with fermenting locust bean for iru production (Antai and Ibrahim, 1986) and for fermenting soy bean for natto production (Antai and Ibrahim, 1986) and this also agrees with the observation of Isu and Njoku, (1997) that *Bacillus* species

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constitute over 95% of the total microbial population density in ugba fermentation. However, *B. subtillis* and other organism isolated during the early stage of the fermentation disappeared toward the later stage of the fermentation. This could be as a result of lactic acid bacteria that were predominant towards the latter stage of the fermentation of all the blends probably because lactic acid bacteria grow best at a reduced pH that could prevent or inhibit the growth of other organism in the fermenting medium. This agrees with the observation of Enujiugha (2009), who reported that lactic acid bacteria produces acid medium during fermentation to inhibit the growth of other microbes that cannot grow in acidic medium.

As fermentation of sweet potato and beniseed flour blends progressed, the pH of the samples decreased. Odion-Owase *et al.* (2018) also recorded decrease in pH during the fermentation of pigeon pea. The lowering of pH could be due to the high carbohydrate composition in sweet potato and beniseed blends which might have been degraded to organic acids. This finding is similar to that of Hassan *et al.* (2015) who stated that the decrease in pH may be as a result of the activities of microorganisms on the fermentable substrate which led to the hydrolysis of complex organic compounds of the substrate thereby producing acid and ethanol. The acids produced led to a decrease in pH and increase in total titratable acidity (Hassan *et al.,* 2015). The fermentation results of this research suggest that it is acidic fermentation where pH of fermenting media decreases with increase in total titratable acidity (TTA).

The protein increased with increasing level of beniseed flour substitution indicating nutrient enhancement. This could obviously be due to the significant quantity of protein in beniseed. The increase in protein content is similar to some other research in which leguminous food flour was used in supplementation, such as in Ogi supplemented with cowpea (Ashaye *et al.* 2001) and acha and cowpea blends (Abiodun and Ogugua, 2012). Increase in the protein content of fermented unextruded blends could be as a result of fact that fermentation result in the liberation of nutrients locked in plant structures and cells by indigestible materials, and the fact that microorganisms are not only catabolic, breaking down more complex compounds, but they also are anabolic synthesizing several complex growth factors during fermentation. Increase in the protein content of fermented unextruded blends was also reported by Osundahunsi (2009). Jeff-Agboola and

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Oguntuase (2006) reported that microorganisms are found to increase the protein content of the samples on which they grow. More so, many microorganisms make use of carbohydrates as energy concentration, thereby increasing the fermenting mass (Onyango *et al.,* 2004). There was a moderate increase in the protein content of unfermented extruded blends. Increase in protein content of unfermented extruded blends corresponds with the findings of Abiodun and Ogugua (2012) in the evaluation of extruded snacks from blends of acha and cowpea. Moisture content of any product is measured for various reasons including legal and label requirements, economic importance, food quality, better processing operations and storability. The stable moisture content of the raw sweet potato blends prior to fermentation and extrusion indicates the storability and shelf life of the samples if properly packaged (Odom *et al.,* 2013). Increase in moisture content of fermented and extruded blends may be due to hydration.

Moderate increase in the moisture content of unfermented extruded, fermented unextruded and fermented extruded blends may cause reduction in cooking time and fuel consumption. Similar result was also reported by Oladunmoye (2007) during fermentation of locust beans. The carbohydrate content of the raw blends decreased with increase in beniseed which could be as a result of the low carbohydrate content of beniseed when compared to sweet potato. Abiodun and Ogugua (2012) also reported similar result when a high carbohydrate product is fortified with a low carbohydrate food product. Reduction in the carbohydrate content of fermented unextruded blends could be as a result of utilization of carbohydrate by microorganisms during fermentation for energy production (Anuonye *et al*., 2009).

Crude fibre gives bulk to food and aids in regulating physiological functions in the body. Result from this research showed that extrusion reduce the crude fibre content of the blend as unfermented extruded and fermented extruded blends had low crude fibre content. Fermented unextruded blends had the highest crude fibre content. The result of the fermented unextruded blends compares favourably with the work of Eze and Ibe (2005) on the effect of fermentation on the nutritive value of *B. eurycoma* “Achi”. The fermentation process involves the conversion of materials to the peculiar 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

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microorganisms were not separated from the biomass, the increase in fibre could be due to such conversion of materials to peptidoglycan by the microorganisms (Eze and Ibe, 2005). Fat content was highest in fermented unextruded blends. This could be as a result of the metabolic activities of the fermenting microorganisms. Emmanuel *et al.* (2017) also reported increase in fat content of fermented locust bean seed flour and that it was attributed to the increased activities of lipolytic enzymes which hydrolyze fat to glycerol and fatty acid. 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 linoleic and linolenic acid, which are essential fatty acids. Kpodo *et al.* (2016) reported that oil seeds are susceptible to lipid oxidation leading to the formation of numerous aldehydes, acids, ketones and alcohols responsible for rancid and off-flavours in peanut products. These long chained fatty acids are highly susceptible to oxidation which results from application of temperature during extrusion (Ranjit and Subha, 2014).

The result obtained in the sensory evaluation demonstrated that there was no significant difference in the blends for colour, texture, aroma, taste and overall acceptability. Fermented blends (50% sweet potato : 50% beniseed) and extruded-fermented blend (50% sweet potato : 50% beniseed) recorded the highest values for colour, texture, aroma, taste and overall acceptability while the Fermented unextruded blends (100% sweet potato) and extruded-fermented blend (100% sweet potato) recorded the lowest values for colour, texture, aroma, taste and overall acceptability. A higher sensory score observed in fermented blends and fermented-extruded could be that fermentation contributes to the aroma, taste, texture and colour of foods positively.

CONCLUSION

From the results of this research, Variation in the ratio of beniseed used in supplementing sweet potato had a notable effects in increasing its nutritional composition such as protein, vitamin and minerals of sweet potato. This is a welcome development in area where the cost of purchasing high proteinous food is high. Furthermore, fermentation and extrusion helps to increase the nutritional and sensory attributes of sweet potato and beniseed blends and hence, a means of resolving issues related to

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malnutrition associated with consumption of sweet potato flour due to its low protein content, supplementing it with foods rich in proteins such as beniseed will solve this problem.

REFERENCES

Abiodun, A. O. and Ogugua, C. A. (2012). Evaluation of extruded snacks from blends of acha (*Digitaria exilis*) and cowpea (*Vigna unguiculata*) flours. *Agricultural and Engineering International CIGR Journal*. 14 (3): 210-217.

Amadou, I., Gbadamosi, O. S., and Le, G. W. (2011). Millet based traditional processed foods and beverages: A review. *Cereal Foods World*. 56: 115-121.

Antai, S.P. and Ibrahim, S.H. (1986). Microorganisms associated with African locust bean (*Parkia filicoidea* Welw) fermentation for 'dadawa' production. *Journal of Applied Bacteriology*, 61: 145-148.

Anuonye, J. C. (2012). Some functional properties of extruded acha/soybean blends using response analysis. *African Journal of Food Science*. 6(10): 269-279

Anuonye, J. C., Onu, J. O., Egwin, E. and Adeyemo, S. O. (2009). Nutrient and anti-nutrient composition of extruded acha/soybean Blend. *Journal of Processing*. 34: 680-691.

AOAC, (2012). Official methods of analysis of the Association of Official Analytical Chemists international. 19th edition. Gathersburg, Maryland, U.S.A.

Ashaye, O. A., Fasoriyo, S. B., and Lawal, R. O. (2001). The effect of fortification on the composition and sensory attributes of cowpea-amala. *Journal of Nutritional Food Science.* 31: 88-91.

Association of Official Analytical Chemists (AOAC) (2005). Official Methods of Analysis. 1p 9th Edition, Association of Official Analytical Chemists, Washington DC.

Camire, M. E., Zhao, J. and Violette, D. A. (1993). *In vitro* binding of bile acids by extruded potato peels. *Journal of Food Chemistry*. 41(12): 2391-2394

Emmanuel, I., Aladesanmi, O., Adekunle, O., and Oluwafemi, O. (2017). Effect of fermentation on the physicochemical properties and nutritionally valuable minerals of locust bean (*Parkia biglobosa*). *American Journal of Food Technology.* 12: 379-384.

Enujiugha, V. N. (2009). Major fermentative organisms in some Nigerian soup condiments. *Pakistan Journal of Nutrition*. 8(3): 279-283.

Eze, S. O. and Ibe, O. J. (2005). Effect of fermentation on the nutritive value of *B. Eurycoma* "Achi". Chemical Society of Nigeria. 30: 1-5.

FAO, (2005). Production Yearbook for 2005. FAOSTAT Data, Food and Agriculture Organisation of the United Nations, Rome.

Fawole, M. O. and Oso, B. A. (2007). Laboratory manual of microbiology. 5thedition. Spectrum Books limited, Ibadan, Nigeria. 22-23.

GRANATO, D. *et al.* (2010). Physical stability assessment and sensory optimization of a dairy-free emulsion using response surfacemethodology. Journal of Food Science, v. 73, p. 149-155.

UNDER PEER REVIEW

Hassan, G. F., Yusuf, L., Adebolu, T. T., and Onifade, A. K. (2015). Effect of fermentation on mineral and anti-nutritional composition of cocoyam. *Sky Journals of Food Science.* 4(4): 42-49.

Isu, N.R. and Njoku, H.O. (1997). An evaluation of the micro flora associated with fermented oil bean (*Pentaclethra macrophylla* Benth) seeds during Ugba production. *Plant Foods for Human Nutrition* 51: 145-157.

Iwe, M. O. (2003). The science and technology of soybean: Chemistry nutrition processing and utilization. 1st Edition. Rejoint Communication Services Limited. Enugu State. Nigeria. Page 680.

Jeff-Agboola, Y. A., and Oguntuase, O. S. (2006). Effect of *Bacillus sphaericus* on proximate composition of soybean (*Glycine max*) for the production of soyiru. *Pakistan Journal of Nutrition.* 5: 606-607.

Kpodo, F., Afoakwa, E., Saalia, K., and Amoa, B. (2016). Changes in physico-chemical characteristics and volatile flavor components of different yoghurt products made from soy, peanuts and cow milk. *African Journal of Food Agriculture, Nutrition and Development.* 16(4): 11278-11294.

Larmond, E. (1977). Laboratory methods of sensory evaluation of foods. Publication 1673. Canada. Department of Agriculture. Ottuwa. 73.

Liu, S., Han, Y., and Zhou, Z. J. (2011). Lactic acid bacteria in traditional fermented Chinese foods. *International Journal of Food Research.* 44: 643–651.

Muchoki, C. N., Imungi, J. K., and Lamuka, P. O. (2010). Changes in beta-carotene, ascorbic acid and sensory properties in fermented, solardried and stored cow-pea leaf vegetables. *African. Journal of Food Agricultural Nutrition and Development*. 7:16–26.

Odion-Owase, E., Ojokoh, A. O., and Oyetayo, V. O. (2018). Effect of different fermentation methods on the microbial and proximate composition of pigeon pea (*Cajanus cajan*). *Microbial Research Journal International.* 23(1): 1-6.

Odom, T. C., Udensi, E. A. and Iwe, M. O. (2013). Nutritional evaluation of unripe *Carica papaya*, unripe *Musa paradisiaca* and *Mucuna cochichenesis* weaning food formulation. *European Journal of Biological and Medical Science.* 1(1): 6-15.

Ojokoh, A. O., and Udeh, E. N. (2014). Effects of fermentation and extrusion on the proximate composition and organoleptic properties of sorghum- soya blends. *African Journal Biotechnology*. 13(40): 4008-4018

Oladunmoye, M. K. (2007). Effects of fermentation on nutrient enrichment of locust beans (*Parkia biglobosa*, Robert bam). *Research Journal of Microbiology.* 2(2): 185-189.

Onyango, C., Neotzold, H. Bley, T. and Henle, T. (2004). “Proximate Composition and Digestibility of Fermented and Extruded Uji from Maize-Finger Millet Blend”. 37(8): 827 -832.

Osundahunsi, O. T. (2009). Scanning electron microscope study and pasting properties of ripe and unripe plantain. *Journal of Food and Agricultural Environ*ment. 7(4): 182-186.

UNDER PEER REVIEW

Ranjit, B. and Subha, G. (2014). Extrusion technique in food processing and a review on its various technological parameters. *Industrial Journal of Science Research Technology*, 2(1):1-3.

Vanhijum, S.A., Vaughan, E.E. and Vogel, R.F. (2013). Application of state-of-art sequencing technologies to indigenous food fermentations. *Current Opinion in Biotechnology,* 24: 178–186

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