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ASSESSMENT OF EXTRUSION TECHNIQUE ON PHYSICO-CHEMICAL PROPERTY, MICROBIAL QUALITY AND ANTI-NUTRITIONAL FACTORS OF EXTRUDED READY-TO-EAT SNACKS

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Abstract
Extruded breakfast snacks are considered microbiological and nutritionally safe to consume because the raw materials are subjected to high temperatures (higher than 150°C, which has been reported to reduce the anti-nutrients present in several legumes) and the product is dried to a minimal level of moisture content which prevents microbial growth. The formulated composite flour from African yam bean, pearl millet and tigernut produced ready-to-eat snacks (RTE) was developed using single screw extruder. The physic-chemical property, anti-nutritional factor, microbial quality and proximate composition of extruded products were evaluated. All raw materials were purchased from local open markets in Enugu and Ibadan. Results of physic-chemical property of RTE snacks ranged from 6.00- 6.15 (pH), loose and tapped bulk density (0.41- 0.59g/ml), water absorption capacity (1.90- 2.43g/ml), oil absorption capacity (1.73- 2.12g/ml) and swelling power (0.99-1.17g/ml). Anti-nutritional factor of RTE snacks showed decreasing order in oxalate and Phytate with decreasing level of African yam bean and increasing in substitution of pearl millet flours, while tannin content increased in the same order. Result of microbial quality exhibited zero coliform and acceptable bacterial counts ranging from 0.9×10^2 to 1.2×10^2cfu/g. Proximate composition of RTE snacks displayed low moisture content (10%), protein (14%), fat (6%), ash (3%), fibre (4%), carbohydrate (66 %) and total energy (391 %). The outcome of this study displayed African yam bean, pearl millet and tigernut flours complementing each other when blended in the right proportions, thereby, producing nutrient-dense breakfast snack rich in physic-chemical properties, low microbial counts and minimal anti-nutritional factors.

Keywords: Extrusion, African yam bean, Pearl millet, Tigernut, Composite flours

Introduction
Snack consumption has increased tremendously in Nigeria due to the feeding pattern of children, especially those of pre-school age. This is because of their frequent consumption of snacks in between meals, thus, their diets are often nutritionally poor. An increase in the market demand for ready-to-eat snack has attracted the attention of Research Scientists towards developing novel foods from a mixture of plant material, rich and balanced in nutrients with no adverse effect to consumers' health. Cereal based snacks are the most widely consumed food items, many of which are low in nutrient density but high in calories and/or fat content (Hess *et al.,* 2016). Nutrients and anti-nutrients of the plant material must be put into consideration before producing ready-to-eat (RTE) snacks. However, processing methods reduced the level of anti-nutritional factors and minimized micronutrients losses which are of great interest, both to manufacturers and consumers. Mechanical, thermal, or biological processes have the potential to improve the nutrient availability in foods (Hotz and Gibson, 2007). Extrusion cooking technology, a high-temperature-short-time process has been advocated for the production of half or completely-cooked, safe and acceptable foods (Guy, 2001). The process reduces the microbial count and inactivates the enzymes. It is a multi-step, multi-function thermal or mechanical process that has permitted a large number of food applications. Beneficial changes in the bioavailability and in the content of nutrients may take place during extrusion. It is being used increasingly in the food industries for the development of new products such as cereal based snacks, including dietary fiber, baby foods, breakfast cereals and modified starch from cereals (Navale *et al*., 2015). Extrusion offers many advantages over traditional food unit operations such as minimizing time, energy, and cost inputs, while adding versatility and edibility to the manufacturing process. A wide variety of different products can be produced by changing ingredients, operating conditions, and/or

minor components of the extruder (Chakraborty *et al.,* 2009). Raw materials of interest include; flour and starch granules from cereal, tubers and legumes. Extruded foods such as breakfast cereals, snacks, flakes, quick cooking pasta and texturized vegetable protein and breakfast gruel are important products from this process (Iwe, 2001; Nwabueze and Iwe 2010; Leszek, 2011). Like other processes for heat treatment of food, extrusion cooking may have both beneficial and undesirable effects on nutritional value. Some of the beneficial effects of extrusion cooking include the destruction of anti-nutritional factors and the gelatinization of starch present. However, heat-labile vitamins may be lost to varying extents. Thus, retention of vitamins and anti-nutrients post extrusion cooking is of great importance to food technologists and consumers, to assess the effects of food processing on these chemical components (Baskar and Aiswarya, 2016). More still, consumers have higher interest in snacks with good aroma, taste, appearance, texture and in addition, are nutritionally superior and healthy. These desirable product qualities are mostly influenced by conditions employed during processing such as the type of extruder, quantity of moisture in the feed material, temperature of different sections of the barrel, screw speed and feed rate into the extruder (Thymi *et al.,* 2005). The nutrient composition of African yam bean, millet and tigernut indicated that they are good sources of energy, proteins, carbohydrates and low microbial counts. Therefore, this study evaluated anti-nutrients and microbial quality of extruded RTE breakfast snacks.

Materials and Methods

African yam bean were purchased from a local market in Enugu, Enugu State. Pearl millet, tigernut, sugar, sorghum, salt and sugar were purchased from Bodija Market in Ibadan Oyo State, Nigeria. The ingredients were taken to the laboratory of Food Technology, University of Ibadan for further processing and analysis.

Processing of flours

African yam bean (AYB) flour was produced according to the method described by Enwere (1998). Sorting of beans to remove stones, dirt and other foreign material were performed manually. Sorted seeds were soaked for 24 hours at room temperature and subsequently drained, rinsed, dehulled with a mortar and pestle and oven dried at 55°C for about 15 hours. The dried beans or grains were milled into flour using disc attrition mill. Flour obtained was sieved through a 250µm British standard sieve to obtain flour of uniform size. Flour samples were packed into zip-lock bags and kept in airtight containers at room temperature (25°C) until used for extrusion processes and analyses. The millet flour was produced according to the method described by Odunfa and Adeyele (1985). Similarly, Tigernut flour was produced according to the method described by Oladele and Aina (2007). Seeds or grains were sorted manually to remove stones, dirt and other foreign material. Sorted seeds were washed and then oven dried at 55°C for 6 hours. The dried seeds or grains were milled into flour using disc attrition mill. Flour obtained was sieved through a 250µm British standard sieve to obtain flour of uniform size. Finally, flour samples were packed into zip-lock bags and kept in airtight containers at room temperature (25°C) until used for extrusion processes and analyses.

Production of sorghum malt extract

Sorghum malt extract was produced by a modified procedure of Okafor and Aniche (1980). About 5kg of white sorghum grains was steeped in tap water for 18 hours and germinated on a tray for three days at room temperature (25°C). The green malt was kilned at 55°C for 8 hours and further kilned at 65°C for 16 hours or until the shoots and roots were friable and separated from the grains. The resulting Sorghum malt was subsequently mashed by three step decoction method during which 70% of the mash was maintained at 55°C for 30 minutes, 65°C for 1 hour and lastly at 70°C for 1 hour in a hot water bath. The conditioned mash was strained through a clean muslin cloth and the filtrate (malt extract) was stored for further use.

Formulation of composite flours

The flour samples of African yam bean, pearl millet and tigernut were analyzed for proximate composition. Based on the preliminary proximate composition, four blends of flour were developed. Four batches of African yam bean, millet and tigernut flours were prepared by blending at the levels of 80% (African yam bean), 10% (millet and tigernut each) flours (80:10:10 dry basis); 70% (African yam bean), 20% (millet) and 10% (tigernut) flours (70:20:10 dry basis); 60% (African yam bean), 30% (millet) and 10% (tigernut) flours (60:30:10 dry basis); and 50% (African yam bean), 40% (millet) and 10% (tigernut) flours (50:40:10 dry basis). Names of all samples are listed in Table 1. All four different flour mixtures were blended together in their desired proportions using a laboratory scale mixer for 10 minutes at 10000rpm. After blending, the flour samples were put in zip-lock bags and stored at room temperature (25°C) until used for extrusion processes and analyses.

Preparation of ready-to-eat breakfast product

The Ready-to-eat breakfast product was produced from the composite flour blends and other ingredients (sugar, salt and water) to improve the flavor and texture as shown in Table 1.

Production of extruded ready-to-eat breakfast product

The desired proportion of composite flour in its dry states was mixed using a laboratory scale blender for 10 minutes at 10000rpm. The individual moisture contents of the composite flour was determined (on dry weight basis) using a Sartorius moisture analyzer MA-30000V3 (Göttingen, Germany). The flour (840g), malt extract (100g), sugar (50g), salt (10g) and water were mixed together using a mixer, water was gradually added until good texture was obtained. The prepared sample was subsequently extruded at a selected constant extrusion condition as follows: screw speed of 100 rpm and barrel temperature of 110°C in a fabricated laboratory single-screw extruder fitted with 2mm die nozzle. The extruder was previously set at the desired barrel temperature and allowed to run to stabilization at a screw speed of 40rpm using African yam bean flour before the experimental runs commenced. The temperature of the barrel was measured by a thermocouple inserted along the length of the extruder. The raw material was fed into the extruder barrel and the screw conveyed the flour blend along it. Further down the barrel, smaller flight-depths resisted the volume and increased the resistance to movement of the flour. As a result, it filled the barrel spaces between the screw flights becoming compressed. As it moved further along the barrel, the screw kneads the material into a semi-solid and plasticized mass during extrusion. Finally, it was forced through one restricted openings (dies) at the discharge end of the barrel as straight rope extrudates. Samples were extruded as straight rope and were collected when the extrusion process parameters reach steady state.

Table 1 Formulations of African yam bean, pearl millet and tigernut flours into blends (%)

Samples	African yam bean flour	Pearl millet flour	Tigernut flour
ORM	100	0	0
LFJ	0	100	0
CRQ	0	0	100
RPA	80	10	10
TSE	70	20	10
JLX	60	30	10
RPO	50	40	10

ORM: 100% African yam bean flour, LFJ: 100% Pearl millet flour, CRQ: 100% Tigernut flour, RPA: 80% African yam bean, 10% pearl millet and 10% tigernut flours (80:10:10 dry basis), TSE: 70% African yam bean, 20% millet and 10% tigernut flours (70:20:10 dry basis), JLX: 60% African yam bean, 30% millet and 10% tigernut flours (60:30:10 dry basis) and RPO: 50% African yam bean, 40% millet and 10% tigernut flours (50:40:10 dry basis)

Handling of extrudates

The emerging extrudates at the die nozzle was collected and cut manually to a uniform length. The extruded products was placed on a table and allowed to cool for 30 minute at room temperature. The extrudates were later dried in an air convection oven (Gallenkamp, England) at 60°C for 10 hour. The resulting dried extrudates was allowed to cool, packaged in zip lock bags and stored at room temperature till further analysis (Plate 1).

Plate 1. Extruded breakfast snack

Chemical Analysis

Proximate Composition

Moisture content, crude protein, crude fat, total ash and crude fibre for blends and extrudates were determined using the standard methods of Association of Official Analytical Chemist (AOAC, 2010) and carbohydrate was calculated by difference.

Functional Properties of extruded snacks

***pH*:** The pH of the food samples was measured according to the method described by Onwuka (2005). Ten gram (10g) of blended homogenous sample was dissolved in 100ml of de-ionized water, mixture was filtered and filtrate was measured using a pH meter.

***Bulk density*:** The Bulk density was determined following the method of Olapade *et al.,* (2011). Ten gram (10g) of flour sample was weighed into a 100ml graduated cylinder. The cylinder was tapped continuously until a constant volume was obtained and the level recorded. The bulk density was calculated as weight of flours (g) by the volume (cm^3).

***Water absorption capacity*:** The procedure of Olapade *et al.* (2011) was used. Ten milliliters (10ml) of water was added to 1.0g of each blend sample, the suspension was stirred with the aid of magnetic stirrer for 5 minutes. The suspension was transferred into centrifuge tubes and centrifuged for 30 minutes at 3,500rpm. The free water was poured in a cylinder and the volume determined. Water absorption was calculated as the difference between the initial water and the volume of the free water obtained after centrifuging.

***Oil absorption capacity*:** Oil absorption capacity (OAC) was determined following the method of Onwuka (2005). One gram of sample was mixed with 10ml of oil. The mixture was allowed to stand for 30 minutes at room temperature and centrifuge at 3500rpm for 30minutes. Oil absorption capacity was expressed as gram of oil bound per gram flour.

***Swelling power*:** Swelling power of samples was determined by the method described by Onwuka (2005). One gram of sample was measured into a 10ml measuring cylinder. Then 5ml of distilled water was added to the sample and allowed to stand for one hour. The final volume after swelling was recorded. This is calculated thus:

$$\text{Swelling index} = \frac{\text{Volume occupied by sample after swelling}}{\text{Initial volume occupied by sample before swelling}} \quad \ldots.. (1)$$

Anti-nutrient Determination

Determination of phytate

Extraction of phytate from the sample was carried out following a modified procedure of AOAC (2010). The principle of this method relies on a conversion of free phytic acid and a colorimetric measurement of the liberated organic phosphorus. Sample 2.0g was extracted with 40ml of 2.4% HCl (68.6ml of 35% hydrochloric acid) under constant shaking at room temperature (25°C) for 3 hours. All extracts were then filtered using Whatman No. 1 filter paper. The content of phytate was determined by using a spectrophotometric method, with an absorbance (A) wavelength at 640nm, outlined in AOAC (2010). The amount of phytic acid was calculated from the organic phosphorus by assuming that one molecule of phytic acid containing six molecules of phosphorus (P) was digested.

Determination of Tannin

The method of AOAC (2010) was used from different processed corms for determination of tannin. Sample 0.2g of finely ground sample was measured into a 50ml beaker, 20ml of 50% methanol was added and covered with paraffin and placed in a water bath at 80°C for 1 hour and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100ml volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. The 1mL of sample extract was pipette into 50mL volumetric flask, 20mL distilled water, 2.5 ml Folin-Denis reagent and 10ml of 17 % Na_2C0_3 were added and mixed properly. The mixture was made up to mark with distilled water, mixed thoroughly and allowed to stand for 20 minutes when bluish-green coloration developed. Standard Tannic Acid solutions of range 0-10ppm were treated similarly as 1ml of sample. The absorbance of the Tannic Acid Standard solutions and samples was read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760nm. Percentage of tannin was calculated thus:

$$\text{Tannin (\%)} = \frac{\text{Absorbance of sample x Average gradient x Dilution Factor}}{\text{Weight of sample x 10,000}} \quad \ldots \ldots (2)$$

Determination of Oxalate

Oxalate was determined using titration method (AOAC, 2010). Two grams (2g) of sample was suspended in a mixture of 190ml of distilled water in a 250ml volumetric flask, 6M HCl was added to 10ml of the suspension and heated for 1 hour at 100°C in a water bath. The mixture was cooled and made up to 250ml mark with distilled water prior to filtration. Duplicate portion of 125ml of the filtrate was measured into 250ml beakers. Each extract was made alkaline with concentrated sodium then made acid by drop wise addition (4 drops) of acetic acid until the test solution changed from salmon pink to faint yellow (pH 4-4.5) (methyl red indicator was used). Each portion was heated at 90°C to remove precipitate containing ferrous ions. The filtrate was heated again to 90°C in a hot water bath and 10ml 5% calcium chloride solution was added with continuous stirring. After heating, it was centrifuged at full speed (2,500rpm) for 5 minutes. The supernatant was decanted and dissolved in 10ml 20% (v/v) H_2SO_4 solution and the total filtrate resulting from 2g of the sample was made up to 300ml. Permaganate titration**:** Aliqout 125ml of the filtrate was heated until near boiling and then titrated against 0.05M $KMNO_4$ solution to a faint pink colour. Oxalic acid content was calculated thus:

Oxalic acid (%) = T x (Vme) (Df) x 105 ME x Mf ….. (3)

where, T = Titre of $KMNO_4$ (ml), Vme = volume - mass equivalent (1ml of 0.05M MNO4 solution is equivalent to 0.0022g anhydrous oxalic acid), Df = the dilution factor (300ml) 125ml, ME = the molar equivalent of $KMNO_4$ in oxalic acid ($KMNO_4$ redox reaction is 5), Mf = the mass of the sample used.

Microbiological analysis

Total viable bacteria and mold count was determined by pour plate method as described by Prescott *et al*. (2005). Total viable bacteria and coliform was estimated by multiplying the means of the total colonies by the dilution factor.

Statistical Analysis of Data

A one-way analysis of variance (ANOVA), least significant difference (LSD) and Duncan Multiple Range Tests were used on data obtained to determine significant differences between means and separate means respectively @ $p \leq 0.05$ level using SPSS package version 17.0. The results are expressed as mean ± standard deviation.

Results and Discussion

The Functional Properties of Extruded ready-to- eat Breakfast Snack

The pH values of the extrudates ranging from 6.00 to 6.15 showed that there were no significant differences ($p<0.05$) between the extruded ready-to-eat breakfast snack (Table 2). The value of pH recorded in this work can be grouped as low-acid foods. It was observed that there was an increase in pH of the snack as millet substitution increased from 10 to 30 with decrease in AYB substitution (80 to 60) levels. The pH is an important factor that reflects the chemical conditions of a solution and can control availability of nutrients, biological functions, microbial activity and behavior of chemicals. However, the values of pH obtained are below 7.0 (acidic) and the lower the value, the more acid the food, thereby prolonging the shelf life of extruded ready-to-eat breakfast snack. Nevertheless, the result obtained in this study was higher than 5.00-6.16 recorded by Ade-omowaye *et al.* (2008). Agunbiade and Ojezele (2010) recorded slightly lower value (4.88) for fortified breakfast cereal made from maize, sorghum, African yam bean and soybeans. The pH range observed in this study may be due to partial hydrolysis which might have occurred during soaking of the African yam bean. The highest loose bulk density was observed in sample RPA and lowest value was observed in sample MPO. The highest tapped bulk density was observed in sample STE and lowest value in sample RPA.

Table 2: Functional Properties of Extruded ready-to-eat Breakfast Snack

Parameters	RPA	STE	JLX	MPO
pH	6.00±0.20^{a}	6.13±0.15^{a}	6.15±0.06^{a}	6.06±0.58^{a}
Loosed Bulk Density (g/mL)	0.47±0.01^{a}	0.45±0.01^{b}	0.43±0.01^{c}	0.41±0.01^{d}
Tapped Bulk Density (g/mL)	0.49±0.01^{c}	0.59±0.02^{a}	0.57±0.02^{a}	0.54±0.01^{b}
Water Absorption Capacity (g/g)	1.90±0.10^{b}	2.00±0.10^{b}	2.17±0.21^{ab}	2.43±0.25^{a}
Oil Absorption Capacity (g/g)	1.73±0.15^{b}	1.90±0.10^{ab}	1.76±0.14^{b}	2.12±0.13^{a}
Swelling Capacity	0.99±0.11^{b}	1.07±0.00^{ab}	1.13±0.02^{a}	1.17±0.02^{a}

Values are means ±S.D of duplicate determinations. * SD= Standard deviation. Values in the same row with different superscripts were significantly different at 5% probability level (p< 0.05). RPA: 80% African yam bean, 10% millet and 10% tigernut flours; STE: 70% African yam bean, 20% millet and 10% tigernut flours; JLX: 60% African yam bean, 30% millet and 10% tigernut flours; MPO: 50% African yam bean, 40% millet and 10% tigernut flours

It was observed that WAC increased with the level of millet substitution as African yam bean decreased. Extruded ready-to-eat breakfast snack in sample RPA had lowest value (1.90g/g), while sample MPO was highest (2.43g/g). The oil absorption capacity (OAC) of the extrudate was highest (2.12g/g) in sample MPO. There were noticeable differences (p<0.05) among the samples. The swelling capacity of extruded breakfast snack was millet-substitution dependent as the swelling capacity increased from 0.99 to 1.17g/ml as millet ratio increased. There was no significant differences (p<0.05) among the extruded breakfast snack. The extruded breakfast snack of sample RPA was lowest, while sample MPO had optimum values.

Anti-nutritional Content of Extruded ready-to-eat Breakfast Product

The anti-nutritional content of the ready-to-eat breakfast product is presented in Table 3. Tannins are polyhydric phenols present virtually in all parts of plants and are known to inhibit trypsin, chymotrypsin, amylase, and lipase activities (Inyang and Ekop, 2015). The tannin contents of the products ranged from 1.05 to 1.21mg/100g. Lower values (0.035 to 0.130 mg/100g) were recorded for breakfast cereals made from pigeon pea and sorghum (Mbaeyi, 2005). However, values obtained were far lower than the values (14.31-15.20mg/g) reported by Ekwere *et al.* (2017) for the anti-nutrient content and in vitro protein digestibility of infant food produced from African yam bean and Bambara groundnut. The result obtained for the phytate content of the products ranged from 0.20 to 0.32mg/100g. A gradual decrease of the phytate was observed with reduction in the level of the African yam bean flour. The results obtained for the oxalate content of the products ranged from 0.11 to 0.17mg/100g. The highest value was observed in the sample RPA. The oxalate content decreased as the level of African yam bean flour decreased in the flour blends. This indicates that legume contain anti-nutritional factors which exert its effect upon ingestion. Therefore, reduction of anti-nutritional factors in legume could be achieved by soaking, heating and cooking.

Table 3: Anti-nutritional Composition of Extruded ready-to eat Breakfast Snack

Samples	Tannin	Phytate	Oxalate
RPA	1.05±0.02^{b}	0.32±0.02^{a}	0.17±0.00^{a}
STE	1.18±0.00^{a}	0.27±0.05^{b}	0.15±0.03^{b}
JLX	1.21±0.00^{a}	0.26±0.00^{b}	0.14±0.03^{c}
MPO	1.16±0.01^{a}	0.20±0.00^{c}	0.11±0.01^{d}

Values are means ±S.D of triplicate determinations. * SD= Standard deviation. Values in the same column with different superscripts were significantly different at 5% probability level (p< 0.05). RPA: 80% African yam bean, 10% millet and 10% tigernut flours; STE: 70% African yam bean, 20% millet and 10% tigernut flours; JLX: 60% African yam bean, 30% millet and 10% tigernut flours; MPO: 50% African yam bean, 40% millet and 10% tigernut flours

Microbial Quality of Extruded Ready-to eat Breakfast

The microbial properties of the breakfast products are shown in Table 4. Total bacterial count of the samples ranged from 0.9 to 1.2x10^2cfu/g, while there was no coliform growth. A bacteria count of 0.75–1.2x10^2cfu/g was reported by Nagi *et al.* (2012) for biscuit made from cereal bran compared favorably with the result observed in this study. The microbial dose of the breakfast product based on total bacterial plate counts was observed to be within acceptable limits set by International microbiological standards recommended units for foods which should be less than 10^3cfu/g. International Commission for the microbiological specification for foods according to ICMSF (1996) stated that ready-to-eat foods with plate counts between 0-10^3cfu/are acceptable, while between 10^4 -10^6cfu/g is tolerable.

Table 4: Assessment of Microbial Quality of Extruded Ready-to eat Breakfast Product (cfu/g)

Samples	Total bacterial count	Total coliform count
RPA	1.0 x 10^2	NG
STE	1.2 x 10^2	NG
JLX	0.9 x 10^2	NG
MPO	NG	NG

Values are means ±S.D of triplicate determinations. * SD= Standard deviation. Values in the same column with different superscripts were significantly different at 5 % probability level (p< 0.05). RPA: 80% African yam bean, 10% millet and 10% tigernut flours; STE: 70% African yam bean, 20% millet and 10% tigernut flours; JLX: 60% African yam bean, 30% millet and 10% tigernut flours; MPO: 50% African yam bean, 40% millet and 10% tigernut flours

Proximate Composition of African yam bean, Pearl millet and Tigernut Flours

The proximate compositions of the flours are presented in Table 5. The moisture content of the flours ranged between 10.74 to 12.63%. Sample CRQ was found to be highest in moisture content and sample LFJ appeared lowest. The protein content of the flours ranged from 6.17 to 20.82% with African yam bean having the highest protein content which is expected because it is a legume. The tigernut flour (sample CRQ) had the highest value for fat and fibre content, while there was no significant difference amongst the values for ash for all the samples. Sample LFJ had the highest carbohydrate value, while sample CRQ had the lowest value.

Table 5:Proximate Composition of African Yam Bean, Pearl Millet and Tigernut Flours

Sample	Moisture	Protein	Fat	Ash	Fibre	CHO	Total energy
ORM	12.37±0.80^a	20.82±1.92^a	2.27±0.93^b	3.32±0.33^a	3.46±0.56^b	57.76±1.46^b	-
LFJ	10.74±0.22^b	10.93±1.03^b	3.71±1.17^b	2.25±0.05^a	2.36±0.05^b	70.00±1.78^a	-
CRQ	12.63±0.33^a	6.17±0.01^c	29.31±1.54^a	3.03±0.15^a	9.87±1.26^a	46.41±1.59^c	-

Values are means ±S.D of duplicate determinations. * SD= Standard deviation. Values in the same row with different superscripts were significantly different at 5% probability level (p< 0.05). ORM- 100% African yam bean flour; LFJ- 100% pearl millet flour; CRQ- 100% tigernut flour

Proximate Composition of African yam bean, Pearl millet and Tigernut Flour Blends

The proximate composition of the blends is presented (Table 6). The moisture content of the flours ranged between 10.56 to 12.18 %. Sample JLX had highest in moisture content and sample STE lowest. There were significant (p<0.05) differences between samples RPA and STE but none between samples JLX and MPO. The moisture content obtained was however below 14% for shelf-stable storage and viability of flour blends; thus, useful for a shelf stable product (Chakraverty, 2004). Hence, there will be mold and bacterial growth if above 14%. The protein content of the flour blends decreased from 16.12 to 14.67% with increasing substitution levels of pearl millet flour. The protein content was highest in sample RPA, while lowest was observed in sample MPO. Significant (p<0.05) differences were observed among the samples.

Table 6: Proximate Composition of Varying Ratio of African Yam Bean, Pearl Millet and Tige rnut Flour Blends

Sample	Moisture	Protein	Fat	Ash	Fibre	CHO	Total energy
RPA	11.44±0.60^b	16.12±0.11^a	10.50±0.50^a	3.03±0.15^a	2.71±0.19^b	56.19±0.26^c	383.74±3.27^a
STE	10.56±0.38^c	15.57±0.08^b	9.53±0.93^{ab}	2.70±0.44^{ab}	3.19±0.21^a	57.45±0.96^{ab}	381.88±5.47^a
JLX	12.18±0.20^a	15.17±0.23^c	10.10±0.26^a	2.37±0.15^b	2.87±0.16^{ab}	58.52±0.45^{ab}	381.65±1.26^a
MPO	11.63±0.76^a	14.67±0.06^d	8.47±1.00^b	2.03±0.25^{ab}	2.43±0.25^a	59.61±1.13^a	373.35±5.04^b

Values are means ±S.D of duplicate determinations. * SD= Standard deviation. Values in the same row with different superscripts were significantly different at 5% probability level (p< 0.05). RPA: 80% African yam bean, 10% millet and 10% tigernut flours; STE: 70% African yam bean, 20% millet and 10% tigernut flours; JLX: 60% African yam bean, 30% millet and 10% tigernut flours; MPO: 50% African yam bean, 40% millet and 10% tigernut flours

The high protein content recorded in RPA was probably due to high amounts of protein associated with legumes which formed a basic amount of the flour. This result is similar to value reported by Adelekan *et al*. (2012) for use of African yam bean and shrimps in the production of maize-based cereal blends. Hence, protein is an important component that determines the rheological properties of composite flours. The fat content ranged between 8.47 to 10.50%. The highest value in a blend was recorded in sample RPA, while minimum was found in sample MPO. The high fat might be due to fat content in tigernut which reflected in the flour following Basman and Koksel (2003), who reported 24 to 30% fat wells. Result achieved in this study agreed with ranged values 1.3 to 11.0% reported by Adebayo-Oyetoro *et al.* (2017) for production and acceptability of *chinchin* snack made from wheat and tigernut flour. However, de-fatting the tigernut before utilization may yield results that are lower in fat yet higher in other nutrients. The ash content (%) of the composite flours significantly ($p<0.05$) decreased from 3.03 to 2.03% with increasing inclusion of pearl millet flour. This may be attributed to low ash content of pearl millet. Ash content is a reflection of mineral status in a sample. Nevertheless, the values were higher than 1.34-2.58% for banana-wheat composite cake (Eke *et al.,* 2007). More so, trend in results obtained in this study was in agreement with rice-based composite flour (Awolu *et al.,* 2017). The fibre content of the flour blends ranged from 2.43 to 3.19%. Significant ($p<0.05$) differences were observed among the samples. The fibre content was highest in sample STE, while lowest value was observed in sample MPO. Tigernut flour has been reported to contain substantially higher crude fibre than African yam bean and pearl millet (Sotunde *et al.,* 2021), and this may be responsible the high amount of fibre obtained in the blends. In addition, results from Ibeogu (2020) concured in production of weaning food from mung bean, pearl millet and tigernut; and Ade-Omowaye *et al.* (2008) observed increase in value as tigernut substitution increases in the formulation of wheat-tigernut based bread. Carbohydrate content of the composite flour varied significantly ($p<0.05$) and increased from 56.19 to 59.61% with increasing substitution of pearl millet. Sample MPO had highest carbohydrate, while lowest was found in sample RPA. However, all the flour blends were good sources of energy based on the proximate results. However, Ibeogu (2020), reported high values in weaning food due to increase in pearl millet inclusion. In contrary, Ade-Omowaye *et al.* (2008) reported decrease in values owing to increase in tigernut substitution in wheat-tigernut based bread. The energy value of the flour blend decreased from 383.74 to 373.35kcal/100g. The results obtained are within values reported by Kent (1983) for treated ready-to-eat breakfast cereal foods of energy value between 314.0-420.0Kcal/100g. The high energy content exhibited in sample RPA is good for product formulation like breakfast cereals.

Proximate Composition of Extruded African Yam Bean-based Ready-to eat Breakfast Snack

Table 7 shows the proximate composition of African yam bean with pearl millet and tigernut extruded breakfast snack. Results show a significant ($p<0.05$) difference in the selected extrusion variables. The moisture content varied from 8.36 to 10.06%. Sample MPO had the highest moisture content (10.06%), while sample STE had lowest (8.36 %). The value of moisture content obtained in this study is below the recommended bakery product ranging from 14 – 18% (Coutinho *et al.,* 2013) and this could prolong the shelf life of the products. However, Adebanjo *et al.* (2020) reported reduced moisture (4.5 to 3.5%) and increased fibre (2.84 to 4.53%) contents in the extrusion cooking of carrot and pearl millet. The protein content of the breakfast snack decreased significantly ($p<0.05$) from 14.88 to 12.74% with increasing substitution of millet flour. The decreasing order could be because low protein content of pearl millet flour substituted in the composite flour. There is a significant difference ($p<0.05$) among the products. The breakfast snack containing sample RPA was highest and lowest value was the snack with sample MPO. The protein contents achieved in this study were lower than the value (15.13 - 6.43%) reported by Semasaka *et al*. (2010) for extruded products from corn, millet and soybean blend. The selected extrusion variable significantly had pronounced effect on the protein content of the flour blends. During extrusion cooking, the chemical constituents of the feed material are exposed to high temperature, high shear and high pressure and these improved or damaged the nutritional quality of the protein in the extruded material by various mechanisms (Leszek, 2011). According to earlier studies, extrusion causes peptides of proteins to massively undergo unfolding and/or aggregation and this releases low molecular weight peptides thereby enhancing their digestibility. Extrusion cooking randomly disrupts the disulphide and linear linkages of peptides, hence increasing the cleavage sites of the amino acid in the molecule by proteases (Agunbiade and Ojezele, 2010). This is highly important in legume based food materials as enhancement of protein demonstrates the effect of supplementing legumes in breakfast cereals known as protein complementation.

Table 7: Proximate Composition Extruded African Yam Bean Based Ready -to eat Breakfast Snack

Parameters	RPA	STE	JLX	MPO
Moisture	10.05±0.05[a]	8.36±0.13[c]	9.78±0.18[b]	10.06±0.06[a]
Protein	14.88±0.26[a]	13.31±0.02[b]	12.96±0.03[c]	12.74±0.18[d]
Fat	5.18±0.28[b]	5.62±0.36[ab]	5.99±0.50[a]	5.52±0.29[ab]
Ash	3.03±0.15[a]	2.70±0.44[ab]	2.17±0.15[b]	2.63±0.25[ab]
Fibre	3.67±0.22[a]	3.38±0.35[a]	2.84±0.06[b]	1.94±0.05[c]
Carbohydrate	63.65±0.40[c]	64.09±0.73[b]	65.55±0.28[ab]	66.16±0.28[a]
Total energy	360.69±0.98[d]	372.15±1.42[c]	380.15±3.94[b]	391.88±0.99[a]

Values are means ±S.D of duplicate determinations. * SD= Standard deviation. Values in the same row with different superscripts were significantly different at 5% probability level (p< 0.05). RPA: 80% African yam bean, 10% millet and 10% tigernut flours; STE: 70% African yam bean, 20% millet and 10% tigernut flours; JLX: 60% African yam bean, 30% millet and 10% tigernut flours; MPO: 50% African yam bean, 40% millet and 10% tigernut flours

The selected extrusion variables significantly ($p<0.05$) affected the fat content of the flour blends. However, highest fat content was observed in sample JLX, while lowest occurred in sample RPA. This order of increase in breakfast snack could be beause of increasing substitution of pearl millet flour. According to Obilana and Manyasa (2002), pearl millet mode of preparation is not well developed because of short storage and oil content. Higher values (8.70- 14.20%) were reported for breakfast cereals made from sorghum and pigeon pea composite flour (Mbaeyi, 2005), and 8.76-9.26% for extruded adult breakfast based on millet and soybean (Coulibaly *et al.,* 2012). However, fat can be a transport vehicle for fat soluble vitamins providing essential fatty acids (n-3 and n-6polyunsaturated), which have the ability to reduce blood level of low density lipoprotein (LDL) cholesterol, thereby reducing the risk of coronary heart disease (Waniska *et al.,* 2002). The ash content showed significant ($p<0.05$) difference in variations. The highest ash content in breakfast snack occurred in sample RPA and lowest value obtained in sample JLX. Sotunde *et al.* (2021), reported that African yam bean had high ash values and this reflected in the extruded snack produced in a ratio dependent nature just as observed in this study. The ash contents of these breakfast snacks were higher than the values ranging from 1.50 - 2.50% for extruded products from corn, millet and soybean blend (Mbaeyi, 2005), but concurred with the value (3.53%) reported by Anuonye *et al.* (2010). Fibre content decreased from 3.67 to 1.94% in extruded breakfast snack as substitution of pearl millet flour increased in the blends. The carbohydrate content of the breakfast snack was significantly ($p<0.05$) different from each other with increasing substitution of pearl millet flour. The values of carbohydrate increased from 63.65 to 66.16%. The carbohydrate contents of the sample MPO was highest (66.16%), while sample RPA was significantly ($p<0.05$) lowest among the samples. According to the study of Adebanjo *et al.* (2020), in extruded flakes prepared from pearl millet and carrot, fibre content ranged from 3.71 to 4.53%. The result obtained in this study fall within the values earlier stated. Extrusion technology has been increasingly used in the production of breakfast snack which is connected to improving dietary profile of cereals (Porter, 1992; Jisha *et al*., 2010). The extruded breakfast snack has a great potential for application in diabetic foods, because fibre-rich food formulated in the diet could generate a perfect recipe for diabetic patients. The values obtained for the total energy content of the formulated samples increased from 360.69 to 391.88kcal.

Conclusion

Extrudates are good sources of physic-chemical properties, which help in enhancing good health and supply of nutrients when consumed. On the other hand, low level of anti-nutrient, especially phytic acid in the extruded ready-to-eat snack is desirable. Furthermore, extrusion process yields zero total coliform count and low bacterial count which are considered safe to eat both by children and adults.

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