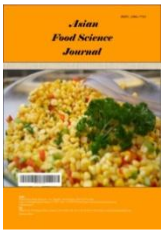

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Effect of Processing on the Antinutrient Content of Extruded Snacks from Cocoyam –Bambara Groundnut Flour Blends

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Authors' contributions

This work was carried out in collaboration among all authors. Author BDS designed the study, performed the experimental design. Author IN edited the first draft of the manuscript. Author OCE managed the literature reviews. All authors read and approved the final manuscript.

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ABSTRACT

Snacks from the blends of cocoyam and Bambara groundnut composite flour blends were developed through extrusion cooking Technology. Response Surface Methodology (RSM), formulations, and optimization of the process variables. The objective was to establish the optimum level of the effects of feed blend composition (X1), barrel temperature (X2), and feed moisture contents (X3) processing on the antinutrients composition of the composite flour blends. The responses were tannin, oxalate, phytates, and trypsin inhibitor. From the results, the tannin content ranged from 0.00 mg/100 g to 0.03 mg/100 g, oxalate 0.18 mg/100 g to 0.25 mg/100 g, phytate 0.23 mg/100 g to 0.69 mg/100 g and trypsin inhibitor 0.12mg/100g to 0.19mg/100g. These studies showed a significant difference ($P \leq 0.05$) for all the processing variables on all the responses. The studies show that high temperatures and low moisture content have the optimum effects on the antinutrient content.

Keywords: Antinutrient; extruded; snacks; cocoyam; Bambara.

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1. INTRODUCTION

Cocoyam (*Colocasia esculenta)* contributes a significant portion of the carbohydrate content of the diet in many regions of developing countries and provides edible starchy corms or cormels. Cocoyam contains 80.99% moisture, 1.0% crude ash, 5.47% crude protein, 0.02% crude fat, 1.28% fibre and 11.0% carbohydrate [1]. Nigeria is the highest producer of cocoyam, with annual production of 3,450 million metric tons annually [2]. Although they are less important than other roots crops such as yam, cassava, and sweet potatoes, they are still the main staple in some parts of the tropical and sub-tropical regions [3]. Cocoyam contains an irritating/acridity principle that causes burning discomfort. The undesirable principle must be removed through processing such as fermentation, grating, boiling, or sun-drying before being fed to animals to reduce the danger of toxicity [4]. Cocoyam reported wide variation (2.10 – 17.13 mg/100 g) in the level of these undesirable substances. Cocoyam's have nutritional advantages over root crops and other tuber crops [5]. It has a lot of crude protein than root and other tubers, the small size of its starch granules makes its starch highly digestible, its phosphorus content. Despite the nutritional importance of cocoyam as a food material, it is grossly underutilized. In some tropics and sub-tropics, cocoyam is only eaten as boiled, fried, roasted and used as flour and as a soup ingredient, and used as additives in porridge (gwate in Hausa). There is limited information on their post-harvest characteristics, contributing to the limited application of improved post-harvest technologists to maintain quality and improve marketing potential.

Bambara groundnut is a legume species of African origin [6] with subterranean fruit–set which is widespread south of the Sahara [7]. It serves as an important means of protein in the diets of a large percentage of the population in Africa, particularly for poorer people who cannot afford expensive animal protein. It serves both human and animal consumption. The plant has the potential to improve malnutrition and boost food availability. The seed makes a complete food, containing sufficient quantities of protein, carbohydrates, and fat. The Freshly harvested pods are eaten as snacks after boiling for approximately an hour [8,9]. The seeds may also be grounded into flour and baked to make small flat cakes and bread. Additionally, thin porridge and stiff porridge have been made from flour. In Eastern Africa, Bambara groundnuts are roasted and milled, and the flour is used to make soup, a relish, and a substitute. (26) HTC Bambara groundnuts require a longer boiling time (namely 3–4 hours [9] and therefore higher energy expenditure to become edible as compared to cowpea or common bean (*Phaseolus vulgaris*).

Flours obtained from other crops such as maize, millet, sorghum, cassava, potatoes, and rice had been used as a supplement to wheat flour to extend the use of local crops and reduce the cost of wheat importation [10]. Quality bread products have been made from such composite flour with other cereals and root crops [11]. [12] reported that the lack of information on the functional, chemical and nutritional properties of grain legumes grown in developing countries is responsible for the extensive use of this traditional use in food formulations. Food legumes have to be a good source of lysine, and therefore, a combination of cocoyam protein and legume protein provides an ideal source of dietary protein for humans [13]. Cocoyam is a good supplement for infants in food preparation because of the high digestibility of its starch, appreciable amounts of calcium and phosphorus (for bone building), B-complex vitamins, and provitamin A [14]. Recent research showed that cocoyam starch could be incorporated in the development of infant food which can be digested easily and accessible to low-income earners in developing countries [15]. The use of locally grown crops to produce high protein, shelf-stable, and affordable recipes in developing countries has been emphasized by international agencies as one of the suitable channels for addressing the deepening global nutrition challenges [16]. Nutritious foods to meet this requirement can be best achieved by a mixture of locally grown roots and tubers and legumes using processing techniques that are shelf-stable and acceptable consumer products using recent technologists [16,17], e.g., use of extrusion processes.

Extrusion cooking technology is a continuous high temperature short, time (HTST) food processing method in which mechanical energy is combined with thermal energy to gelatinized starch and denature proteins, plasticizing and reorganizing food material to create new shaped and textured products; and also can inactivate enzymes, destroy some active substances and reduce microbial activity [18,19] and [20] It has been used in the cereal industry to produce many foods and food ingredients such as breakfast cereals, snack foods, baby foods, pasta products, modified starches, beverages, powders, meat, and cheese analogs, textured vegetable protein and blended foods such as corn starches and grounded meats [21,22,23]. It is a technology that is robust, versatile, efficient, and low cost, has high output per unit, and short reaction time, relatively no waste generation [24]. During the extrusion process, chemical modification and structural changes occur in the raw material, such as starch gelatinization and protein denaturation [10], pigment and vitamin degradation [25], and loss of volatile compounds [26]. It is one of the latest advances in food processing technologists applied to foods [27]. It can. It can be applied to mitigate the problem associated with starch-based products in terms of improvement in functionality, physical property, and shelf stability. It provides many advantages over other process technologies regarding ready-to-eat foods of the desired shape, size, shape texture, and sensory characteristics at relatively low processing cost. The knowledge of the process in extruder operating variables, therefore, provides necessary information for the prediction of what fraction of food material will undergo a specific reaction during the extrusion process and its possible effects on the quality of finished products.

2. MATERIALS AND METHODS

The Cocoyam used for this study was obtained from Kafanchan in Jema'a Local Government Area of Kaduna State (Nigeria). The variety was identified in Root Crop Research Institute Samaru (ABU) Zaria, while the Bambara was also purchased in Kaduna Central Market.

2.1 Cocoyam Flour Preparation

Cocoyam flour was produced using the method described by [28] with slight modifications. The corms were manually cleaned, diseased corms removed and then washed, peeled, sliced, and blanched at 80°C for 4 min. The blanching process helps in removing the excess starch and the mucilage and also helps in inactivating the enzymes that are likely to cause enzymic browning. It was dried to 14% moisture content and milled using attrition mill (locally fabricated). The principles in attrition milling is that, brittle food materials such as sugars, crystal or dry grains are obtain first by compression, then by using impact forces and finally by sharing or rubbing [29]. It was then sieved with a 4.5µm laboratory sieve (Brabender OHG Duisburg type).

2.2 Bambara Ground Nut Flour Preparation

Bambara ground nut (40 kg) was cleaned and soaked in tap water at room temperature (32^0C), three times its weight by volume. The Bambara groundnut was soaked for 24hrs to facilitate dehulling, seeds were dehulled using mortar and pestle. The dehulled kernels was washed to remove the skin, oven- dried to about 14% moisture content before winnowing to have a clean dehulled seeds. The dried seeds were then milled into flour using attrition mill and sieved with 150µm laboratory sieve (Xin-Hai type). The samples were packaged in air-tight plastic container at room temperature until needed.

2.3 Blend Formulation and Moisture Adjustment

The blend formulation is derived from the experimental design (Table 1) which is formulated using Response surface methodology (RSM). The different formulations are shown in Table 2. From the formulations X_1, X_2, and X_3 represents feed blend composition, barrel temperature and feed moisture composition respectively. The coded variables for the lowest, medium and highest cocoyam feed blend composition for X_1 = 30%, 40% and 50% respectively, for the, lowest, medium and highest coded temperature variables are X_2 = 100^0C, 120^0C and 140^0C respectively. And for feed moisture content X_3 = 8%, 16%, and 24% respectively. The flour samples were blended and the calculated amount of water was added and conditioned to appropriate moisture content. It was mixed using Laboratory mixer at medium speed until it was properly mixed. The samples were put in an air tight polyethylene. The feed materials were allowed to stand for 3hrs to equilibrate at room temperature prior to extrusion exercise. The amount of water used was calculated using the equation below.

$$Y = \frac{Mf - Mi) x\, Sw}{100 - Mf} \quad (1)$$

Where; Y = Amount of water to be added (ml)
Mf = Final moisture content
Mi = Initial moisture content
Sw = Sample weight (g)

2.4 Extrusion Exercise

The blends were subjected to extrusion cooking using a single screw extruder (Brabender, Duisburg DCE-330) equipped with a DC drive of variable speed and a strain gauge torque meter. The screw geometry is constant and has a linearly tapered. Feeds were manually introduced into extruder through a screw operated conical hopper at a speed of 50rpm which ensures that the flight of the screw is filled and avoiding accumulation of feed in the hopper. This type of feeding provides a close to a maximal flow rate for the selected process parameters and the designed feed composition and moisture content. Desired barrel temperature is maintained by circulating tap water controlled by in-build thermostat and a temperature-controlled unit. Experimental samples were collected when steady state is achieved, [30,31]. The temperature was kept constant for the two extruder heating zones (1 and 2) of the barrel and the die 150, 170 and 150^{0}C respectively, the extruded samples were stored in room temperature for future analysis.

2.5 Experimental Design

A three-factor central composite design (CCD) (Box and Hunter, 1957) was adopted to study the effect of feed composition (X_1) barrel composition (X_2), and feed moisture content (X_3) on the chemical composition. (antinutrient factors) The level of each variable was established according to literature information. The independent variables and their various levels are shown in the table.

2.6 Tannin Determination

Tannin content was determined using folin-Denis colorimetric method described by [32]. 5gm sample was dispersed in 50ml of distilled water and shaken. The mixture was allowed to stand for 30min at 28^{0}C before was filtered through Whitman No 42 grade of filter paper. 2mls of the extract were dispersed into a 50 ml volumetric flask. Similarly, 2ml standard tannin solution (Tannic acid) and 2ml of distilled water were put in separate volumetric flask to serve as standard and reagent was added to each of the flask and then 2.5ml of saturated Na_2CO_3 solution was added. The content of each flask was made up to 50ml with distilled water and allowed to incubate at 260nm using the reagent blank to calibrate the instrument at zero.

Table 1. Independent variable and levels used for central composite design

Variable	**Coded variable level**				
	-α	**Low**	**Medium**	**High**	**+ α**
	-1.682	-1	0	1	1.682
Feed composition (X_1)	23.18	30	40	50	56.82
Barrel temperature (X_2)	86.36	100	120	140	153.64
Feed Moisture Content (X_3)	2.55	8	16	24	29.45

Table 2. Experimental layout in their coded and natural units

	X_1	**X_2**	**X_3**	**X_1**	**X_2**	**X_3**
1	-1	-1	-1	30	100	8.00
2	1	-1	-1	50	100	8.00
3	-1	1	-1	30	140	8.00
4’	1	1	-1	50	140	8.00
5	-1	-1	1	30	100	24.00
6	1	-1	1	50	100	24.00
7	-1	1	1	30	140	24.00
8	1	1	1	50	140	24.00
9	-1.682	0	0	23.18	120	16.00
10	1.682	0-1.682	0	56.82	120	16.00
11	0	1.682	0	40	86.36	16.00
12	0	0	0	40	153.63	16.00
13	0	0	-1.682	40	120	2.55
14	0	0	-1.682	40	120	29.45
15	0	0	0	40	120	16.00

X_1=feed composition, X_2=barrel temperature, X_3=feed moisture composition

2.7 Phytates Determination

Phytates content was determined using spectrophotometric method as describe by [33]. 0.5g of sample was weighed into 500ml flat bottom flask. The flask was placed in a shaker and the sample was extracted with 2.5% HCl for 1hr. The aliquot was filtered and 5ml of the filtrate was pipetted and diluted to 25ml with distilled water. 15 ml of NaCl was added to 10ml of the diluted sample and this was passed through an ample resin (200-400 mesh) to elude inorganic phosphorus. 15ml of 0.7M sodium chloride (NaCl) was added to the solution which was mixed on a votex mixer for 5sec. The mixture was then centrifuged for 10min. and the supernatant was read at 520 nm wave length in UV spectrophotometer. The Phytates concentration was read off from a standard curve prepared with standard inositol. Phytates was expressed in mg/100 g.

Phytates (mg) =

$$\frac{\text{conc.of phytate } (\frac{\text{mg}}{100} \text{ from standard curve}}{\text{weigt of sample}} \times \text{dilution factor} \quad (2)$$

2.8 Oxalate Determination

Oxalate was determined by the method described by [34]. This method involves three major steps; digestion, oxalate precipitation and permanganate titration.

2.9 Trypsin Inhibitors

Trypsin inhibitors of the sample was determined by spectrophotometric method of [33]. One gram of the sample was mixed with 150 ml of 0.5 M sodium chloride (NaCl) solution. The mixture was shaken for 30min at room temperature using an electric shaker. It will then be centrifuge at 3500rpm for 30min using a centrifuge (Model LC2216 Satorious, Germany) and was filtered through Whatman no. 42 grade filter paper. The resulting filtrate (extract) will then be used for the determination of trypsin inhibitors activity. Ten (10ml) each of trypsin enzyme solution was dispensed into two test tubes and labeled to represent sample and the control. 1ml of distilled water was added while 1ml of the extract was added to the sample tube. Both tubes were allowed to stand for 10min and their absorbance was measured in a spectrophotometer at a wavelength of 401nm. The number of trypsin unit inhibitor (TUI) per gram of the sample was given; TUI being equal to an increase of 0.01 absorbance unit at 410nm. It was obtained by using the formula below;

$$\text{TUI/g} = \frac{1 \times a - a}{W \times 0.01} \quad (3)$$

Where TUI/g = trypsin unit inhibitor per gram sample,
a= absorbance of control
W = weight of sample

3. RESULTS

Table 3. Result of anti-nutrient analysis of the raw materials for cocoyam and Bambara groundnut

Anti-nutrients	Cocoyam	Bambara
Tannin	0.02 ± 0.00^{a}	0.60 ± 0.00^{b}
Phytate	5.25 ± 0.01^{d}	1.76 ± 0.01^{a}
Oxalate	852.6 ± 0.01^{b}	586.2 ± 0.01^{a}
Trypsin	0.00 ± 0.00^{b}	3.00 ± 0.01^{d}

Table 4. Effects of feeds composition (x_1), barrel temperature (x_2) and feed moisture Content (x_3) on the Antinutrients of Bambara g/nut and cocoyam extrudate

Exp. Run	Ind. Variable in their natural forms					Anti-nut comp. (mg/100 mg)	
	X_1	X_2	X_3	Tannin	Oxalate	Phytate	Tryp. Inhib.
1	30	100	8	0.01	0.29	0.85	0.03
2	50	100	8	0.03	0.19	0.56	0.12
3	30	140	8	0.01	0.18	0.23	0.16
4	50	140	8	0.02	0.19	0.30	0.19
5	30	100	24	0.00	0.25	0.82	0.19

Exp. Run	Ind. Variable in their natural forms			Anti-nut comp. (mg/100 mg)			
	X_1	X_2	X_3	Tannin	Oxalate	Phytate	Tryp. Inhib.
6	50	100	24	0.01	0.20	0.62	0.12
7	30	140	24	0.01	0.22	0.69	0.17
8	50	140	24	0.01	0.25	0.69	0.17
9	23.18	120	16	0.03	0.23	0.64	0.18
10	56.82	120	16	0.02	0.19	0.52	0.19
11	40	86.36	16	0.01	0.21	0.63	0.16
12	40	153.63	16	0.01	0.22	0.63	0.17
13	40	120	2.55	0.01	0.24	0.69	0.17
14	40	120	29.45	0.01	0.20	0.55	0.13
15	40	120	16	0.00	0.22	0.65	0.16

X_1=Feed composition, X_2= Barrel temperature, X_3= Feed moisture composition

4. DISCUSSIONS

Legumes present a primary source of nutrients which is valuable but incomplete balance protein, especially in vegetarians' diets [35]. Bambara nut flour can be used in composite flour preparation and should particularly be incorporated into noodles. [36] have posited that addition of protein to foods can be achieved through Bambara flour fortification. They reported that inclusion of Bambara nut flour to noodles could take care of any nutrient deficiency that might occur in children who feeds largely on noodles without additional proteins whatsoever. The processing methods and the presence or absence of toxic factors are of great concern [35].The antinutrients content of the cocoyam/Bambara groundnut; Table 3 shows that the values of tannins ranged between 0.00mg/100g to 0.03mg/100g. The highest and the lowest values were recorded under similar processing conditions, which indicates that individual processing variables have no significant impact on tannin. Still, the extrusion process degraded the tannin content as observed from the raw product analysis (Table 2); a combination of high moisture and temperature in extrusion cooking however, has been reported to significantly reduce the number of tannins in legumes [36]. Tannins are one of the most common phenolic compounds found in beans [37]. The tannin content in all the raw materials is significantly different (Table 2), which may have informed the variance in their composition observed in the result.

The oxalate composition of cocoyam/ Bambara groundnut showed that the highest value is in run 1(0.29mg/100g) and the lowest in run 3 (0.18mg/100g) on the processing temperature of 1400c, 30%, feed blend composition and 8% moisture content. The amount recorded in raw material (Table 2) suggests that the extrusion condition has a significant impact on the oxalate content. Oxalates are a natural substance in some foods. One of the leading health challenges of oxalate is that it can bind to minerals in the gut and inhibit the body from absorbing them; they bind to calcium during digestion in the stomach and intestines [38].

The phytates content of cocoyam/Bambara groundnut extrudate ranged from 0.23mg/100 to 0.85 mg/100. The individual raw materials (cocoyam and Bambara groundnut) before extrusion showed a higher amount of Phytate; 5.25 mg/100 g and 1.76 mg/100 g, respectively (Table 2). It is possible that the reduction in Phytate is a result of the extrusion cooking conditions. Phytate (inositol hexaphosphate) is commonly found in plant-based foods. Phytate chelates with divalent metal ions and interferes with the digestion or utilization of valuable minerals, especially iron, zinc, and calcium. It is well reported that even small amounts of Phytates in food will effectively reduce iron absorption [39,40]. The composition of trypsin inhibitor of the cocoyam/Bambara groundnut extruded ranged from 0.03 mg/100 g to 0.19 mg/100 g. The highest values are obtained from the processing variables of runs 4,5 and 10 with varying extrusion conditions. The most negligible trypsin value is from a processing variable of 30% feed moisture composition, 1000c barrel temperature, and 8% moisture content. The findings did not agree with the report of [41], who recorded 6.7mg/100g of trypsin in raw Bambara groundnut. The variation may be due to variety, soil type, or the processing methods. The results obtained show that the processing heat efficiently degraded the trypsin inhibitors. [36], posits that extrusion was the best method to eliminate trypsin, chymotrypsin, a-amylase inhibitors, and haemagglutinins activity without modifying protein. [42], in their findings concluded that antinutrients that were present in legumes were reduced significantly during extrusion cooking, [43] also reported that cooking for 60mins at 1000c was sufficient to inactivate over 90% of the trypsin inhibitor activity in *Phaseolus vulgaris.* This study corroborates the findings of previous other studies on the effects of extrusion cooking on the antinutrients of foods and food products.

5. CONCLUSION

In conclusion this research work was aimed at investigating the effects of extrusion cooking process on the anti-nutritional factors of the Cocoyam-Bambara groundnut extruded snacks. The results showed that the extrusion cooking variables has a significant ($p< 0.005$) impact on the composition of the antinutrients. The various formulations has different levels of reaction (response) to the extrusion cooking variables which are X_1 = Feed composition, X_2 = barrel temperature and X_3 = feed moisture composition. Comparably, the results from the raw materials (Table 1) before the extrusion, to the result indicated a significant reduction in the anti-nutritional factors of the products after the extrusion processes thereby making the desired nutrients which may be bound as a result of some nutritional inhibitors fully available during digestion.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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