

Impact of die diameter, feed moisture, and feed composition on amino acids and mineral content of Broad bean-ighu extrudates

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Abstract The impact of die diameter, feed moisture, and feed composition on the essential amino acids and mineral content of broad bean-*Ighu* extrudates in a twin-screw extruder was investigated. Blends of broad beans flour and *Ighu* were used to produce protein-rich extruded products by using a commercial co-rotating twin screw extruder. The amino acids and mineral content of the flour blends and extrudates were determined. Twenty experimental runs were generated from three factors and three levels of three process variables: die diameter, feed moisture, and feed composition using a Box- Behnken response surface methodology. Broad bean and *Ighu* flour samples contained all the amino acids that are found in plant proteins. The blending of *Ighu* with broad bean flour increased the amino acid and mineral element content of the raw *Ighu*. Extrusion cooking reduces the essential amino acid contents of the extrudates. Extrusion cooking conditions- die diameter, feed moisture, and feed composition decreased the composition of the essential amino acids of the extrudates. Extrusion cooking of the *Ighu* /broad bean blends resulted in a decrease in the mineral content of the extrudates. No trends were observed on the effects of extrusion parameters on the extrudates' mineral contents

Keywords: Broad beans, Essential amino acid, Extrusion, *Ighu*, Minerals

Introduction

Broad bean (*Vicia faba* L.) is a legume and one of the lesser - known and under-utilized legumes consumed in the Eastern part of Nigeria. It is known by several other names such as Faba bean, Windsor bean, Horse bean, Tick bean (Deb & Paul, 2024), and *Azomiri* (Iwe & Odimegwu, 2019) in the South Eastern part of Nigeria. Broad bean ranks sixth in terms of world legume production, and they have high nutritional benefits which makes them a good source of food with protein, energy, and fiber for humans and feed for animals (Inger-Cecilia et al., 2021). The protein content of broad beans ranges from 24% to 35% (Crepon et al., 2010), making it the most protein-rich major pulse crop (Robinson et al., 2019). It is relatively high in lysine, which is an essential amino acid in humans (Khazaei et al., 2019), and contains large quantities of carbohydrates, B-group vitamins and valuable mineral micronutrients such as phosphorus, potassium, calcium, sulphur, and iron (White and Broadley, 2009;

Shunmugam et al., 2015; McClean et al., 2017). In the South Eastern part of Nigeria, broad bean is usually cooked and consumed whole or in conjunction with steeped pre-gelatinized shredded cassava slices *Ighu*, as a main meal (Iwe and Odimegwu, 2019; Odimegwu et al., 2020) or roasted and taken as a snack with coconut or palm kernel.

Cassava (*Manihot esculenta*) is among the tropical tuber crops which is one of the most vital staple food crops grown in tropical Africa, predominantly Nigeria and it is a principal source of carbohydrate for people that rely on it as their staple food. Cassava tuber contains in approximate composition about 20 – 30 % starch, 2 – 3 % protein, 75 – 80 % water, 0.1 % fat, 1.0 % fiber, and 1.5 % ash (Kim & Iida, 2023). Cassava roots has been processed in diverse ways into several edible forms such as cassava flour, *garri*, *Ighu* (pre-gelatinized shredded cassava), *fufu* etc. *Ighu* is usually eaten as a dessert in combination with groundnut or palm kernel or coconut or dry fish or meat; or made into a meal as a local salad by the addition of vegetables



(garden egg leaves), legumes, dry fish and *ugba* (Adepeju & Nwangwu, 2010). Cassava roots contains very little protein, hence its utilization with other food products such as legumes in order to complement each other's nutritional deficiencies. Cassava roots which are deficient in essential amino acids can be complemented with broad beans which are rich in essential amino acids especially lysine through extrusion of their composite blends.

Extrusion cooking has been defined as a process of mixing, homogenizing and shaping low and high moisture food materials into intermediate or finished products by forcing them through a specially designed die (Krishna et al., 2021). It is a high temperature short time process which has been used in the processing of proteinous and starchy food materials (Filli & Stading, 2020) such as cereals, legumes, roots and tubers or their composites and convert them into new snack products which can be used in feeding the ever-growing population in order to enhance food security. The effects of extrusion cooking on broad beans and its blends have been studied. Broad beans have been used in the production of wheat and faba bean extruded snack (Strauta & Muizniece-Brasava, 2016) and corn and faba bean blend extruded snack (Gimenez et al., 2013), respectively. However, these studies have concentrated on the use of single effects of extrusion processing conditions to study the nutrients, physical, functional, anti-nutrients, and structural characteristics of broad bean extrudates. Normally, during processing, both the mixture and process variables simultaneously interact to affect the physicochemical and anti-nutritional factors of food products.

The response surface methodology (RSM) is a collection of mathematical and statistical techniques which are useful for the modeling and analysis of problems, in which a response of interest is influenced by several variables (Ibham et al., 2023). One of the popular RSM designs used is the Box Behnken which explores the response surfaces covered in the experimental design, thus enhancing the efficiency and effectiveness of the optimization procedure (Wafaa et al., 2021). The response surface methodology has been adopted to study the effects of mixtures and processing conditions during food formulations and processing but its usage is still limited in evaluating the optimized properties of extrudates. RSM has been extensively used in many fields, for example in engineering, manufacturing, product development, and scientific research to improve product quality, and enhance efficiency (Wafaa et al., 2021; Ibham et al., 2023). Therefore, this study was aimed at using the Box Behnken response surface methodology design to

investigate the effect of the interaction between different extrusion cooking variables such as die diameter, feed moisture, and feed composition on the essential amino acids and mineral contents of broad bean-*Ighu* extrudates.

MATERIALS AND METHODS

Materials

Matured broad bean seeds were purchased from Afor Nmaku market in Agwu LGA in Enugu State of Nigeria. Broken seeds and extraneous materials were removed. Pre-gelatinized shredded cassava (*Ighu*) slices, processed from a cassava variety known locally as "San paper" were purchased from a local market in Isuochi, Abia State, Nigeria.

Formulation of flour blends

Broad bean (*Vicia faba*) seeds were cleaned and milled in a commercial attrition mill (IMEX 205 model, India) to produce flour. The resultant flour was sieved with a 38.12mm sieve aperture and stored in an air-tight plastic container for further analysis. *Ighu* was milled into coarse form, blended with broad bean flour by using a feed composition of *Ighu* and broad bean in the ratio of 95:5, 75:25, and 55: 45 respectively. The flour blends were moistened (with water) to 16, 20 and 24% moisture content, respectively and mixed in a laboratory-scale Hobart mixer (Hobart Corporation, Troy, Ohio, USA).

Extrusion cooking process

The homogeneous blends of *Ighu* and broad bean flour samples were extruded in a co-rotating twin screw (SLG 65 Twin screw Extruder, Jinan Saibainou Technology Development Company, China) extruder operating at a maintained screw speed of 200 rpm, length to diameter ratio of barrel of 20:1 and a screw diameter of 30mm located at Federal Polytechnic Mubi, Adamawa State, Nigeria. The homogeneous blends of *Ighu* and broad beans were introduced into the feeding zone through a conical feed hopper and pushed down to the melt and die zones with the aid of the twin screw of the extruder. The extruder was operated within the temperature range of 90 °C, 100 °C and 110 °C of the feed, melt, and die zones, respectively and extruded through a die diameter of 1.5, 3.0, and 6.0mm. The extrudates were collected, allowed to cool, and later

milled in a Corona manual blender (Landers, Y C.I.A, South Africa) into flour and packaged in polythene bags for further use.

Determination of amino acid profile of raw and extruded products

The amino acid profiles of both the raw and extruded products were determined using the method described by Benitez, (1989). The samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator, and then loaded into the Technicon Sequential Multi-sample (TSM) amino acid analyzer. Thirty to fifty (30 – 50 mg) of the sample was weighed into a glass ampoule. HCL (6N) 7mL was added, and oxygen was expelled by passing nitrogen into the ampoule (this was to avoid possible oxidation of some amino acid during hydrolysis). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 °C for 22 hours. The ampoule was allowed to cool before they were broken open at the tip, and the content was filtered through filter paper. The filtrate was then evaporated to dryness at 40 °C under vacuum in a rotary evaporator. The residue was dissolved with 5 mL of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were in the freezer. The hydrolysate 5-10 µL from each sample was loaded into the Technicon Sequential Multi-Sample (TSM) amino acid analyzer. The samples were dispensed into the cartridge of the amino acid analyzer. The period of analysis lasted for 76 minutes. Amino acids were quantitatively determined against the standard in a Technicon auto analyzer chart (No. 011-9648 - 01, Technicon Instruments Co., Tarrytown, New York), according to Benitez (1989).

Determination of mineral composition by dry ash method

The samples were dry ashed at 550 °C. The ash was boiled in 10 mL of 20% hydrochloric acid in a beaker and then filtered into a 100 mL standard flask. It was made up to the mark with deionised water. The minerals were determined from the resulting solution using Atomic Absorption Spectroscopy (AOAC, 2003). The sample (digested) was thoroughly mixed by shaking and 50 mL of it was transferred into a glass beaker of 250 mL volume. The sample was aspirated into the oxidizing air-acetylene flame or nitrous oxide acetylene flame of the atomic absorption spectrophotometer (FS

240 Varian). When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed. The absorbance of the sample was correlated to obtain the concentration of metal in the sample from the standard calibration curve plotted by the atomic absorption spectrophotometer.

Determination of phosphorous

Phosphorus was determined according to the method of Apha, (2003). In a conical flask containing 100ml of sample, 4ml of strong acid, 4ml of ammonium molybdate and 10 drops of SnCl₂ was added. The blue colour developed was measured after 10 minutes at 690nm with a spectrophotometer. Formula for determining phosphorus:

$$P = \frac{mg}{kgPO_4^{3-}} = \frac{Conc.\frac{mg}{l} \times Total\ Volume\ of\ digest}{weight\ of\ Sample} \quad (1)$$

Determination of calcium (Ca), copper (Cu), magnesium (Mg), sodium (Na) and potassium (K) by atomic absorption spectrophotometry

The digested sample was analyzed for mineral contents by atomic absorption spectrophotometer (AAS model: FS 240 Varian). Different hollow cathode lamps were used for each mineral at their respective wavelengths for each element (AOAC, 2003),

$$Conc.\ mg/kg = \frac{Conc.ppm \times Total\ Volume\ of\ digest}{weight\ of\ Sample} \quad (2)$$

Experimental design

To evaluate the effect of the extrusion variables (die diameter (X₁), feed moisture (X₂) and feed composition (X₃) on the responses (essential amino acids and minerals) of the extrudates, the Box Behnken response surface methodology was applied resulting in 13 experimental runs and seven replicates at the midpoint to reduce errors, making it a total of 20 experimental runs. Each variable was varied at three different levels: die diameter of 1.5, 3.0 and 6.0 mm, feed moisture of 16, 20, and 24% and feed composition of 5, 25 and 45%. The aim of this design was to study the linear, quadratic, interactive, and cubic effects of die diameter, feed moisture, and feed composition on the qualities (essential amino acids and minerals) of the extrudates. The design expert software version 6.0 (Stat-Ease Corporation, Minneapolis, MN, USA) was used to

analyze the data obtained from the twenty (20) experimental runs that were conducted. The polynomial regression coefficient analysis was also conducted by using the Design Expert 6.0 to relate each response (Y)

with the independent variables of linear, quadratic, interactive and cubic models of the response surface methodology. The model proposed for each response of Y was:

$$Y = b_0 + b_1 \times 1 + b_2 \times 2 + b_3 \times 3 + b_{11} \times 1^2 + b_{22} \times 2^2 + b_{33} \times 3^2 + b_{12} \times 1 \times 2 + b_{13} \times 1 \times 3 + b_{23} \times 2 \times 3.$$

Where Y = the response, X_1 = die diameter, X_2 = feed moisture, X_3 = feed composition.

b_0 = intercepts, b_1 , b_2 , b_3 are linear terms, b_{11} , b_{22} and b_{33} are quadratic terms, b_{12} , b_{13} and b_{23} are the interactive terms. The studied process variables were considered to have significant effect on the responses if the P-value < 0.05.

Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 20, IBM SPSS Statistics 20, IBM Corporation, Armonk, NY) was used to obtain mean, standard deviation and analysis of variance (ANOVA) was used to judge for significance ($p \leq 0.05$). Means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Amino acid and mineral elements composition of broad bean and Ighu flours

The results of the amino acid composition of broad bean and *Ighu* flours are presented in Figures 1 and 2 respectively. The results obtained showed that the broad bean and *Ighu* flour samples contained all the amino acids that are found in plant proteins. The values obtained for broad bean were generally higher than the values obtained for *Ighu* flour. Lysine content of broad bean was 5.78g/100g protein as against 7.50g/100g protein reported by Alghamdi, (2009). The leucine content (8.53g/100g protein) was noticeably higher than the amount reported by Alghamdi, (2009). It also exceeded the FAO/WHO, (1985) reference value (7.01g/100g protein) and FAO/WHO/UNU, (1991) reference values of 6.6mg/100 g protein of amino acids, respectively. The values of the essential amino acids (Isoleucine, methionine, phenylalanine, tryptophan, histidine, and arginine) compared favourably with the reports of other researchers such as Alghamdi, (2009) with the exception of valine (4.79g/100g protein) and threonine (3.33g/100g protein) which were low. Isoleucine (3.66g /100g protein), phenylalanine (4.43g/100g protein), methionine (1.34g/100g protein) and histidine (2.36g/100g protein) were noticeably higher than the amount reported by Alghamdi, (2009). The results of the amino acid profile of *Ighu* flour were

generally low with the exception of leucine (3.09g/100g protein), and tryptophan (0.26g/100g protein). These values were also lower than the values reported by Omeire, (2012) on the extrusion of *Ighu* with the exception of leucine which had a higher value of 3.09g/100g protein. The variation in the amino acid content of *Ighu* in this study and that of Omeire, (2012) maybe due to the variety or cultivar of cassava used and the method of processing of the cassava into *Ighu* shreds before processing into grits.

Blending of *Ighu* with broad bean flour increased the amino acid content of the raw *Ighu*. As expected, the essential amino acid contents of the raw blends increased as the quantity of broad beans increased in the blends before extrusion (Figure 1). This could be as a result of the supplementation with broad beans flour. Similar results were reported for blends of soybean and potato, African yam bean and cassava, and sorghum and cowpea respectively by Iwe et al. (2001), Omeire, (2012) and Gbenyi et al. (2016). The lysine content of raw *Ighu* grits was 1.75g/100g, and this increased to 2.17, 2.92 and 3.18g/100g respectively after blending with 5, 25 and 45% of raw broad bean flour. Such increases could be attributed to the supplementation effects of broad bean flour since broad beans has been reported to be a notable source of essential amino acids, especially lysine (Odimegwu et al., 2020). The highest values of essential amino acids was recorded for arginine (2.58, 4.30 and 5.33g/100g) while the least values (0.31, 0.68, 0.68g/100g) were recorded for tryptophan.

Also, the values obtained for mineral elements for broad bean were generally higher than the values obtained for *Ighu* flour (Figure 2). Therefore, blending of *Ighu* with broad bean flour increased the mineral elements content of the raw *Ighu*. Broad bean had been reported to contain large quantities of valuable mineral elements (Shunmugam et al., 2015; McClean et al., 2017)

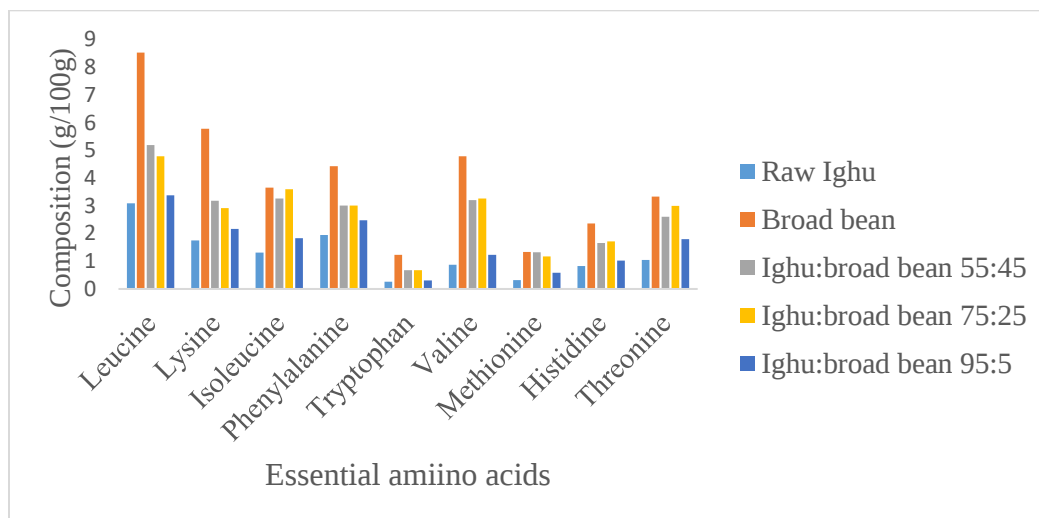


Figure 1. Essential amino acid composition of raw blends of *Ighu* and broad bean flour

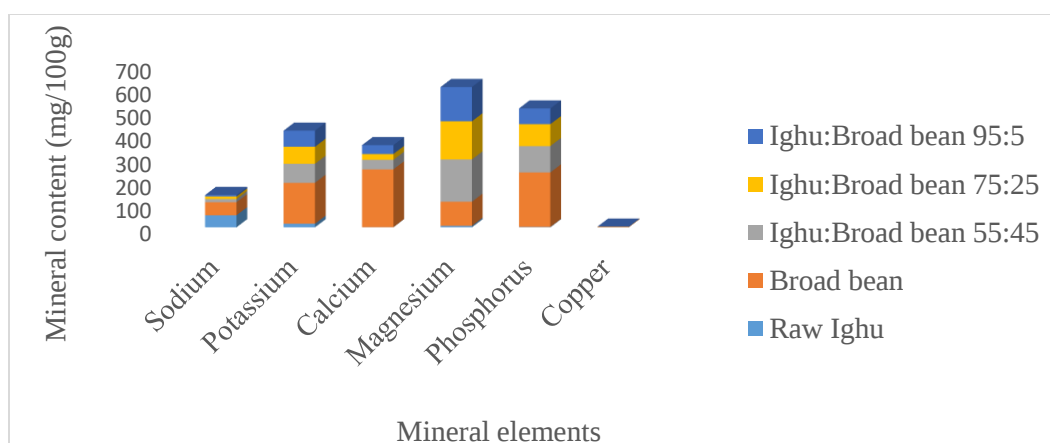


Figure 2. Mineral elements content (mg/100g) of raw blends of *Ighu* and broad bean flour

Percentage variations in essential amino acid content of raw (R) broad bean, extruded (E) broad bean, raw Ighu flour and extruded Ighu flour

The results of the percentage changes in the essential amino acid profile of the raw broad bean flour, extruded broad bean, raw and extruded *Ighu* samples are shown in Figure 3. Extrusion cooking reduced the essential amino acid contents of the extrudates of the flour samples, though all the essential amino acids in the raw blends appeared in the extrudates. This result is in line with reports by Paes and Maga, (2004) on the effect of extrusion on essential amino acids profile and colour of wheat-grain flours of quality protein maize and normal maize cultivars. Furthermore, Anuonye et al. (2010) reported the depletion of some amino acids as a result

of extrusion cooking. The essential amino acid contents obtained in this study were below the values reported in literature for a mixture of a legume with *Ighu* flour (Omeire, 2012). This variations in amino acid content of the extrudates may be due to the processing conditions of the raw materials, effects of high temperature and low moisture on the extrudates or the type of extruder used.

Reduction in essential amino acids observed in broad bean flour were 27.99% in phenylalanine, 27.51% in lysine and 20.67% in valine. The lowest reduction was observed in alanine (3.60%) while the highest reduction was encountered in phenylalanine (27.99%). These reductions were significantly ($p < 0.05$) different. Similar results have been reported in reductions in essential amino acid values of extruded

soy and sweet potato flour, extruded bread fruit, corn and soybean flours, extruded African yam bean and cassava product, and extruded sorghum malt and bambara groundnut flour respectively (Iwe et al., 2001; Nwabueze, 2007; Omeire, 2012). Such decrease in essential amino acids could be attributed to excessive maillard browning as losses up to 50% was recorded for lysine during extrusion cooking (Odimegwu et al., 2020) and therefore maillard reaction has been considered as the primary reason for the reduction of amino acid concentration in extruded grain legumes (Adeleye et al., 2020).

Extrusion cooking also reduced some of the essential amino acid content of *Ighu* flour. The highest reduction was observed in threonine (25.71%) while the lowest loss was encountered in lysine (9.14%). Extrusion cooking had no effect on the isoleucine and

phenylalanine contents of *Ighu* flour. However, a significant ($p < 0.05$) increase in the essential amino acid contents was observed in methionine (27%), leucine (33.98%), tryptophan (61.54%), and histidine (68.97%). The highest retention in essential amino acids in the extruded *Ighu* flour was observed in histidine (68.97%). From this study, it was observed that the extruded *Ighu* contained all the essential amino acids that are found in plant protein. Essential amino acid contents obtained in extruded *Ighu* in this study were in agreement with the report of Omeire, (2012), which showed losses and retention (gain) in some of the amino acids. Losses in lysine content (9.14%) observed in this study were below the value reported by Omeire, (2012). Such a decrease could be attributed to the type or die size of the extruder used in the production of the extrudates.

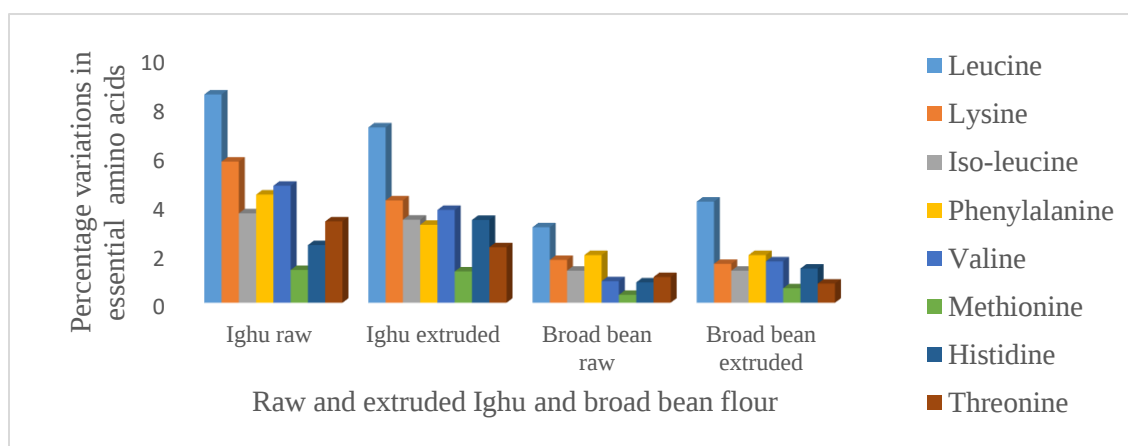


Figure 3. Percentage variations in essential amino acid content of raw broad bean, extruded broad bean, raw *Ighu* and extruded *Ighu* flours

Effect of extrusion cooking on essential amino acid content of the extrudates

The effect of extrusion cooking variables on the essential amino acids of extrudates is as shown in Table 1. It was observed that extrusion cooking decreased the composition of the essential amino acids of the extrudates when compared to the composition of the raw blends of *Ighu*/broad bean. It was also observed that Runs 6 had the highest leucine (0.52g/100g), lysine(0.37), valine (0.29g/100g) and threonine(0.22g/100g) while isoleucine(0.26), phenylalanine(0.28), methionine(0.27) and histidine(0.13) had their highest values at Runs 9, Runs 10, Runs 12, Runs 12 and Runs (2-6, 8, 10, 15) respectively.

Leucine content of extrudates

The leucine content of the extrudates ranged from 0.17 to 0.59. A published report has shown that cassava tuber is rich in arginine but low in essential amino acids (Eka, 1998). Furthermore, the essential amino acid profile of pre-gelatinized shredded cassava is generally low (Eka, 1998). The low leucine content of the extrudates in runs 3 and 7 could be attributed to low feed composition (5% broad beans and 95% *Ighu*) in the extrudates. There exist both antagonistic and synergistic effects of the process variables on the leucine content of the extrudates from the significant model terms (Equation 2).

$$\text{Leucine} = 0.48 - 0.034X_1 + 0.062X_3 - 0.027X_1^2 - 0.020X_2^2 - 0.020X_3^2 + 0.021X_2X_3 + 0.028X_1^2X_2 + 0.018X_1X_2^2 \quad (2)$$

Table 1. Essential amino acids contents of the extrudates

RUNS	DD	FM	FC	LEU	LYS	ISO	PHE	VAL	MET	HIST	THRE
1	3	16	5	0.35 ±0.00 ^{ab}	0.23 ±0.08 ^a	0.11 ±0.06 ^c	0.18 ±0.07 ^{abc}	0.06 ±0.01 ^{de}	0.16 ±0.04 ^a	0.06 ±0.00 ^b	0.05 ±0.01 ^c
2	3	16	45	0.41 ±0.10 ^{ab}	0.32 ±0.03 ^a	0.21 ±0.06 ^a	0.18 ±0.04 ^{abc}	0.06 ±0.00 ^{de}	0.21 ±0.01 ^a	0.13 ±0.04 ^a	0.11 ±0.02 ^{bc}
3	3	24	5	0.17 ±0.04 ^a	0.25 ±0.13 ^a	0.13 ±0.00 ^{bc}	0.11 ±0.06 ^c	0.05 ±0.02 ^e	0.21 ±0.04 ^a	0.13 ±0.01 ^a	0.17 ±0.03 ^{ab}
4	3	24	45	0.47 ±0.14 ^a	0.35 ±0.21 ^a	0.20 ±0.04 ^{ab}	0.12 ±0.07 ^c	0.12 ±0.01 ^{cd}	0.16 ±0.04 ^a	0.13 ±0.03 ^a	0.17 ±0.08 ^{ab}
5	1.5	20	5	0.29 ±0.00 ^{ab}	0.31 ±0.04 ^a	0.06 ±0.01 ^c	0.12 ±0.03 ^c	0.06 ±0.03 ^{de}	0.16 ±0.07 ^a	0.13 ±0.00 ^a	0.11 ±0.04 ^{bc}
6	1.5	20	45	0.52 ±0.16 ^a	0.37 ±0.11 ^a	0.20 ±0.00 ^{ab}	0.27 ±0.07 ^{ab}	0.29 ±0.00 ^a	0.21 ±0.13 ^a	0.13 ±0.01 ^a	0.22 ±0.06 ^a
7	6	20	5	0.17 ±0.00 ^a	0.28 ±0.04 ^a	0.13 ±0.01 ^{bc}	0.11 ±0.03 ^c	0.12 ±0.04 ^{cd}	0.21 ±0.03 ^a	0.06 ±0.00 ^b	0.17 ±0.07 ^{ab}
8	6	20	45	0.41 ±0.13 ^{ab}	0.29 ±0.04 ^a	0.20 ±0.06 ^{ab}	0.18 ±0.03 ^{abc}	0.17 ±0.01 ^{bc}	0.16 ±0.00 ^a	0.13 ±0.06 ^a	0.17 ±0.04 ^{ab}
9	1.5	16	25	0.23 ±0.00 ^{ab}	0.33 ±0.10 ^a	0.13 ±0.03 ^{bc}	0.12 ±0.01 ^c	0.06 ±0.02 ^{de}	0.21 ±0.04 ^a	0.06 ±0.01 ^b	0.17 ±0.03 ^{ab}
10	1.5	24	25	0.41 ±0.13 ^{ab}	0.28 ±0.07 ^a	0.26 ±0.03 ^a	0.18 ±0.01 ^{abc}	0.17 ±0.04 ^{bc}	0.21 ±0.07 ^a	0.13 ±0.00 ^a	0.22 ±0.04 ^a
11	6	16	25	0.29 ±0.08 ^{ab}	0.32 ±0.07 ^a	0.13 ±0.01 ^{bc}	0.14 ±0.03 ^c	0.17 ±0.04 ^{bc}	0.16 ±0.06 ^a	0.06 ±0.00 ^b	0.11 ±0.01 ^{bc}
12	6	24	25	0.47 ±0.11 ^a	0.32 ±0.04 ^a	0.20 ±0.01 ^{ab}	0.28 ±0.06 ^a	0.23 ±0.03 ^{ab}	0.27 ±0.04 ^a	0.06 ±0.00 ^b	0.22 ±0.01 ^a
13	3	20	25	0.52 ±0.18 ^a	0.26 ±0.03 ^a	0.13 ±0.04 ^{bc}	0.18 ±0.07 ^{abc}	0.17 ±0.01 ^{bc}	0.16 ±0.03 ^a	0.06 ±0.00 ^b	0.10 ±0.00 ^{bc}
14	3	20	25	0.41 ±0.14 ^{ab}	0.32 ±0.07 ^a	0.13 ±0.01 ^{bc}	0.14 ±0.03 ^c	0.17 ±0.06 ^{bc}	0.21 ±0.03 ^a	0.06 ±0.01 ^b	0.17 ±0.01 ^{ab}
15	3	20	25	0.52 ±0.11 ^a	0.27 ±0.03 ^a	0.13 ±0.04 ^{bc}	0.18 ±0.00 ^{abc}	0.23 ±0.03 ^{ab}	0.21 ±0.01 ^a	0.13 ±0.08 ^a	0.22 ±0.03 ^a
16	3	20	25	0.46 ±0.16 ^{ab}	0.27 ±0.04 ^a	0.13 ±0.01 ^{bc}	0.18 ±0.04 ^{abc}	0.17 ±0.03 ^{bc}	0.22 ±0.06 ^a	0.08 ±0.00 ^{ab}	0.17 ±0.04 ^{ab}
17	3	20	25	0.51 ±0.10 ^a	0.28 ±0.03 ^a	0.13 ±0.03 ^{bc}	0.17 ±0.01 ^{bc}	0.18 ±0.00 ^{bc}	0.21 ±0.06 ^a	0.08 ±0.00 ^{ab}	0.17 ±0.01 ^{ab}
18	3	20	25	0.48 ±0.20 ^a	0.27 ±0.01 ^a	0.12 ±0.03 ^c	0.14 ±0.04 ^c	0.18 ±0.03 ^{bc}	0.21 ±0.04 ^a	0.07 ±0.00 ^{ab}	0.18 ±0.03 ^{ab}
19	3	20	25	0.52 ±0.17 ^a	0.27 ±0.03 ^a	0.13 ±0.00 ^{bc}	0.14 ±0.04 ^c	0.18 ±0.03 ^{bc}	0.21 ±0.01 ^a	0.08 ±0.01 ^{ab}	0.17 ±0.04 ^{ab}
20	3	20	25	0.52 ±0.17 ^a	0.30 ±0.04 ^a	0.13 ±0.00 ^{bc}	0.14 ±0.01 ^c	0.19 ±0.03 ^b	0.21 ±0.01 ^a	0.08 ±0.01 ^{ab}	0.16 ±0.04 ^{ab}

Note: Means bearing different superscripts in the same column are significantly (p<0.05) different; DD =; Die moisture (mm); FM = Feed moisture; FC = Feed composition (%)

Lysine content of the extrudates

The lysine contents of the extrudates ranged from 0.25g/100g to 0.37g/100g. The lowest lysine value of 0.25g/100g was observed at run 3 while the highest value of 0.37g/100g was observed at run 6. The high lysine content at run 6 (45% broad bean and 55% *Ighu*) could be attributed to the high proportion of broad bean

in the sample. This finding is in agreement with the study by Gbenyi et al. (2016) and Filli et al. (2011) on the ready to eat breakfast cereals from sorghum – cowpea extrudates and fura from millet – cowpea flour respectively. It could also be attributed to the reduction in the die diameter of the extruder used in extrusion as reported by Iwe et al. (2004) on the effect of extrusion

cooking of soy-sweet potato mixtures on available lysine content and browning index of the extrudates.

Only the linear effect of feed composition and the quadratic effect of die diameter significantly ($p < 0.05$) affected the extrudate's lysine content. Both significant model terms showed synergistic effects on lysine.

The polynomial equation after removing the non-significant terms becomes:

$$\text{Lysine} = 0.28 + 0.017X_3 + 0.012X_1^2. \quad (3)$$

Isoleucine content of extrudates

The lowest isoleucine content of 0.06g/100g was observed at run 5 and the highest value of 0.26g/100g was recorded at run 10. The high value of the isoleucine could be as a result of higher feed composition. This is in agreement with the findings of Gbenyi et al. (2016). The residence time could also be another factor that affected the isoleucine content and as such Iwe et al. (2001) stated that the more viscous the mixture, the greater the difficulty to flow through the extruder die

thereby increasing the residence time. Increase in residence time will result in a greater destruction of the isoleucine content as a result of longer period of heat treatment (Paes and Maga, 2004) on the extrudates.

The linear effects of die diameter and feed composition; quadratic effects of die diameter, feed moisture and feed composition; the linear interaction of feed moisture and feed composition; the quadratic effect of die diameter and feed moisture; the interactive effect of die diameter and feed moisture and the interactive effect of die diameter and feed composition and the cubic interaction of the quadratic effect of die diameter and feed moisture; cubic interaction of the quadratic effect of die diameter and feed composition and the cubic effect of the linear effect of die diameter and quadratic effect of feed moisture are the significant model terms that significantly ($p < 0.05$) affected the extrudate's isoleucine content.

The polynomial equation after removing the non-significant terms becomes:

$$\text{Iso-leucine} = 0.13 + 0.010X_1 - 8.733\text{E-}003X_2 + 0.017X_3 + 5.927\text{E-}003X_1^2 + 0.011X_2^2 - 5.303\text{E-}003X_1X_2 - 6.187\text{E-}003X_1X_3 + 0.014X_1^2X_2 + 4.927\text{E-}003X_1^2X_3 - 6.660\text{E-}003X_1X_2^2 \quad (4)$$

Phenylalanine content of the extrudates

The phenylalanine content of 0.11g/100 g was recorded at run 7 and the highest value of 0.28g/100g was recorded at run 12. The linear effects of die diameter and feed moisture; quadratic effects of die diameter; and the cubic effect of the interaction of the quadratic effect of die diameter and feed moisture and the cubic effect of the interaction of the quadratic effect of die

diameter and feed composition and the cubic interaction of the linear effect of die diameter and quadratic effect of feed moisture are the significant model terms that significantly ($p < 0.05$) affected the extrudate's phenylalanine content. Some of the significant model terms were antagonistic (X_1 , X_2) while some were synergistic (X_1^2 , $X_1^2X_2$, $X_1^2X_3$, $X_1X_1^2$).

The polynomial equation after removing the non-significant terms becomes:

$$\text{Phenylalanine} = 0.15 - 0.015X_1 - 0.021X_2 + 8.291\text{E-}003X_1^2 + 0.018X_1^2X_2 + 0.014X_1^2X_3 + 0.012X_1X_2^2 \quad (4)$$

Tryptophan content of the extrudates

The tryptophan content of the extrudates ranged from 0.10 to 0.16g/100g. The lowest value of 0.10g/100g was recorded at run 1, and the highest value of 0.16g/100g was recorded at run 6. The high value at run 6 could be attributed to the high feed composition in the sample. The linear effect of die diameter and the interactive effect of feed moisture and feed composition were the only significant model terms that significantly ($p < 0.05$) affected the extrudate's tryptophan content ($p < 0.05$) after removing the non-significant terms,

which gave rise to equation (5) and it showed antagonistic effects.

$$\text{Tryptophan} = 0.11 - 0.012X_1 - 0.011X_2X_3 \quad (5)$$

Valine content of the extrudates

The valine content of the extrudates ranged from 0.05 to 0.29g/100g. The lowest valine value of 0.05g/100g was recorded at run 3 and the highest value of 0.29g/100 g was recorded at run 6. The high value of the extrudates at run 6 could be attributed to the high feed composition of the sample. The quadratic effects

of die diameter (X_1^2), feed moisture (X_2^2) and feed composition (X_3^2) showed antagonistic effects while the rest of the model terms showed synergistic effects.

The polynomial equation, after removing the non-significant terms, becomes:

$$\text{Valine} = 0.18 + 0.010X_1^2 - 0.017X_2^2 - 0.016X_3^2 - 0.016X_1X_3 + 8.080\text{E-}003X_1^2X_2 + 0.016X_1^2X_3 + 0.012X_1X_2^2 \quad (6)$$

Methionine content of the extrudates

Methionine content of the extrudates ranged from 0.16 to 0.27g/100 g protein. The quadratic effects of feed composition; interactive effect of die diameter and feed

moisture; interactive effect of die diameter and feed composition and the interactive linear effect of feed moisture and feed composition are the significant model terms that significantly ($p < 0.05$) affected the extrudate's methionine content.

The polynomial equation after removing the non-significant terms becomes:

$$\text{Methionine} = 0.20 - 7.955\text{E-}003X_3^2 + 9.723\text{E-}003X_1X_2 - 8.374\text{E-}003X_1X_3 - 8.839\text{E-}003X_2X_3 \quad (7)$$

Histidine content of the extrudates

The histidine content of the extrudates ranged from 0.06 to 0.13g/100g. The linear effect of die diameter, feed moisture and feed composition and quadratic

effect of feed composition are the significant model terms that significantly ($p < 0.05$) affected the extrudate's histidine content.

The polynomial model's equation, after removing the non-significant terms becomes:

$$\text{Histidine} = 0.075 - 9.927\text{E-}003 X_1 + 0.010 X_2 + 0.010 X_3 + 0.012 X_3^2 \quad (8)$$

The mineral contents of the blends of Ighu/broad bean flour and the extrudates

The results of the mineral content of the extrudates are as shown in Table 2. Extrusion cooking of the *Ighu* /broad bean blends resulted in a decrease in the mineral content of the extrudates. The result for the mineral content were in agreement with the values reported by Irving and Jideani, (1997) and Obatolu, (2002) for cereal/soybean blends. However, the extrudates were still higher in mineral composition than the raw *Ighu* grits except in its potassium and sodium content. No trend was observed on the effects of extrusion parameters on the extrudates' mineral contents although extrusion showed increases in the mineral contents of the extrudates when compared to the raw *Ighu* grits. The mineral contents of this study were comparable to some of the minerals (Ca and Na) and higher in Mg contents recorded by Anuonye et al. (2010) in acha/soyabean blend used in the production of acha/soyabean extrudates. The mineral content of blends in this study shows that the composite flour can be used in other food preparations such as in baked products. Minerals are the chemical constituents used

by the body and they play important roles in many activities in the body (Abebe et al., 2018).

Experimental variables had significant effect ($p < 0.05$) only on the sodium and potassium content of the extrudates. The quadratic effect of feed composition is the only significant ($p < 0.05$) model term that affected the extrudates' potassium content.

The polynomial model equation, after removing the non-significant terms, becomes

$$K = 11.63 - 3.12X_3^2 \quad (12)$$

Furthermore, the quadratic effect of feed moisture and feed composition was the only significant model term that significantly ($p < 0.05$) affected the extrudates' sodium content.

The polynomial equation after removing the non-significant terms becomes:

$$Na = 37.40 - 3.46X_2^2 - 7.58X_3^2 \quad (13)$$

Table 2. The mineral composition of extrudates from twin screw extrusion of blends of *Ighu* and broad bean, raw *Ighu* grits and raw broad bean flour.

Run	Die diameter (mm)	Feed Moisture	Feed Composition (%)	Calcium (mg/100g)	Magnesium (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)	Phosphorus (mg/100g)	Copper (mg/100g)
1	3	16	5	19.64±0.24	42.59±0.23	6.36±0.01	2.64±0.03	11.48±0.07	0.14±0.00
2	3	16	45	18.98±0.01	44.06±0.25	4.25±0.04	4.94±0.06	11.50±0.17	0.08±0.00
3	3	24	5	22.60±0.16	40.88±0.33	4.30±0.01	0.78±0.07	11.55±0.04	ND
4	3	24	45	13.73±0.04	42.31±0.30	2.36±0.06	3.66±0.01	11.40±0.35	0.16±0.00
5	1.5	20	5	25.61±0.40	42.31±0.44	0.48±0.00	7.61±0.01	11.70±0.02	0.7±0.04
6	1.5	20	45	14.11±0.16	37.74±0.07	2.04±0.01	24.24±0.10	11.50±0.01	0.29±0.00
7	6	20	5	22.10±0.14	39.62±0.08	2.16±0.01	25.20±0.03	11.61±0.04	0.42±0.00
8	6	20	45	19.46±0.07	39.38±0.03	0.37±0.00	19.49±0.11	11.42±0.03	0.15±0.01
9	1.5	16	25	18.89±0.13	40.35±0.21	11.00±0.03	27.91±0.01	11.55±0.04	ND
10	1.5	24	25	18.78±0.18	40.11±0.30	8.52±0.03	26.20±0.04	11.60±0.06	1.27±0.01
11	6	16	25	23.06±0.08	37.72±0.10	8.26±0.01	35.63±0.13	11.60±0.01	ND
12	6	24	25	14.60±0.14	41.36±0.23	12.51±0.01	33.40±0.06	11.65±0.08	0.32±0.01
13	3	20	25	18.26±0.18	40.33±0.47	12.07±0.10	16.63±0.04	11.41±0.00	ND
14	3	20	25	20.38±0.11	40.66±0.10	14.21±0.03	38.00±0.04	11.55±0.03	ND
15	3	20	25	18.24±0.06	35.10±0.14	14.80±0.01	44.46±0.39	5.85±0.04	ND
16	3	20	25	6.79±0.00	9.08±0.08	5.98±0.01	37.75±0.10	9.46±0.01	ND
17	3	20	25	6.62±0.03	8.10±0.04	20.50±0.20	31.28±0.18	7.39±0.03	6.03±0.00
18	3	20	25	12.00±0.16	8.98±0.01	11.32±0.03	39.23±0.30	1.03±0.00	ND
19	3	20	25	19.35±0.07	36.21±0.24	5.76±0.04	37.45±0.24	11.11±0.01	ND
20	3	20	25	15.00±0.20	38.23±0.33	14.60±0.00	41.68±0.27	5.71±0.01	1.17±0.04
<i>Ighu</i>				0.87±0.03	6.56±0.07	15.54±0.27	51.93±0.40	1.80±0.00	ND
Broad bean				248±1.75	103.20±0.28	175.5±0.85	55.98±1.10	234.00±0.48	2.21±0.30

Note: ND = Not Detected. Means bearing different superscripts in the same column are significantly (p<0.05) different

Conclusions

Incorporating broad bean flour into *Ighu* grits improved the essential amino acid content and mineral contents of the composite blends. However, the impact of the extrusion cooking variables (die diameter, feed moisture, and feed composition) affected the essential amino acids and mineral composition of the extrudates both positively and negatively. High feed moisture and feed composition were found to improve the essential amino acids of the extrudates especially that of lysine. An increase in the lysine content of the extrudates was observed as a result of a quadratic effect of die diameter. Only the quadratic effect of feed composition had no significant ($p < 0.05$) effect on the isoleucine content of the extrudates. Extrusion cooking reduced the mineral content of the extrudates, however, the mineral contents of the extrudates were higher than that of the raw *Ighu* grits. Quadratic effects of feed moisture

and feed composition had significant effects on the sodium content while the quadratic effect of feed composition had a significant effect on the potassium content of the extrudates. Thus, in this study, an improved and enriched extruded snack was developed by complementing *Ighu* grits with broad bean flour.

The production and consumption of this snack is highly recommended because of its nutrient composition which makes it suitable for use in alleviating protein energy malnutrition.

Author Contributions

Conceptualization: I.M.O. Formal analysis: O.E.N., E.F.C., O.C., A.A.N. Methodology: I.M.O. Investigation: O.E.N., E.F.C., O.C. Writing - original draft: O.E.N., E.F.C., O.C. Writing - review & editing: I.M.O., A.A.N.

Conflicts of Interest

The authors declare no conflict of interest.

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