Evaluation of Protein Quality in Blends Prepared from Commercial Wheat (*Triticum aestivum*) and Yellow Maize Flours (*Zea mays*) and African Walnut (*Tetracarpidium conophorum*) Protein Isolate

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Abstract. African walnut seed is an underutilized crop gaining attention by the researchers in developing countries like Nigeria due to poor protein consumption. Based on preliminary study, the blends of Commercial Wheat Flour (CWF) (30-50%), Yellow Maize Flour (YMF) (20-30%) and African Walnut Protein Isolate (AWPI) (10-20%) were prepared using optimal mixture design of response surface methodology to generate twenty composite flours. The 100% commercial wheat flour serves as control. The composite flours were subjected to proximate composition using standard method. Among the twenty composite flours, three flour blend samples 4, 8 and 11 with superior qualities as assessed by highest protein and crude fibre, and lowest fat content were selected and further evaluated for amino acid profiling and in-vitro protein digestibility using standard procedure. Data obtained were subjected to ANOVA at $\alpha_{0.05}$. The result of the flours showed that AWPI had the highest protein (90.80%) content. The flour blends were significantly different in their moisture (8.33-9.17%), protein (17.27-31.27%), fat (0.86-1.20%), ash (1.60-2.22%), crude fibre (0.70-1.16%) and carbohydrate (49.16-70.36%) contents. Among the samples with superior qualities assessed by highest protein (31.27%) and crude fibre (1.16%), and lowest fat (0.86%), sample 11 scored highest values in lysine (4.6150%) and tryptophan (1.1480%) as well as in-vitro protein digestibility (78.31%). However, the best proportion of flours that can improve protein quality in our Nigerian food industry, homes and ready-to-eat snacks was established.

Key words: Composite blends, African walnut seed, Protein quality, Yellow maize flour, Commercial wheat flour

Introduction

Cereals are generally limiting in sulphur-containing amino acids such as lysine and tryptophan. Based on this fact, the nutritional composition of composite blend has to be improved (Akubor, 2003; Hooda & Jood, 2005). Several researches have been conducted on production of various blends such as wheat and fonio grains (McWatters *et al.*, 2003), millet and pigeon pea (Eneche, 2003), wheat and plantain (Mepba *et al.*, 2007), wheat and African walnut flour (Awofadeju *et al.*, 2015) and maize and pigeon pea (Echendu *et al.*, 2004).

Wheat is relatively low in total protein, lysine, tryptophan and beta-carotene while maize is rich in beta-carotene. Maize plays a major role in nutrition of many countries. It is widely used for human nutrition as a source of flour, starch and oil. It is used in production of several food products like bread, tortillas, snacks, beverages, pancakes and porridges (Enyisi *et al.*, 2014). White, red and yellow maize are the major types of maize but the yellow one is mostly consumed by human and animal. The yellow maize, in contrast to others, supplies beta-carotene (provitamin A) which improves vision in kids and grown-up as well as xanthophylls (Enyisi *et al.*, 2014). Maize is a good source of carbohydrate (65-70%), protein (8.8%); low in fibre and minerals, and deficient in lysine and tryptophan (Shaita *et al.*, 2017) but rich in African walnut seed.

African walnut seed is currently not a staple crop as it still grows in the wild. Consequently, it can be classified as one of the neglected and underutilised crops that are beginning to gain attention among scientists in Africa. This growing interest in African walnut seed is necessitated by the persistent menace of malnutrition which has been earmarked as one of the major challenges facing developing nations (IFPRI, 2002: Muller & Krawinkel, 2005: Bhat & Karim, 2009). The supplementation of these crops with African walnut protein isolate would have considerable improvement in protein content, nutritional value and even serve as better food than the cereal alone. The blends would bring about balancing of amino acids, a process called "protein complementation" (Potter and Hotchkiss, 2006). However, the objective of this study was to evaluate the protein quality of the cereal blends fortified with African walnut protein isolate.

Materials and Methods

Materials

Yellow maize and African walnut kernels were bought at Oje market in Ibadan. Commercial wheat flour (Golden Penny, Flourmills of Nigeria Limited), and packaging material were purchased at a supermarket in Ibadan, Oyo state, Nigeria.

Methods

Processing of maize into flour. The maize grains were separated from plant debris, stones and other foreign materials then washed in water to remove dust and tiny particles present and later air-dried for 5 days. The dried grains were milled to flour with the use of commercial grinder (Heavy duty electric Grinder, Model MG182/00) and sieved through a 40 mesh screen. The flour was packaged in high density polyethylene bags (0.77 mm thickness) and heat sealed. Figure 1 described the processing of yellow maize.

Whole maize grain
↓
Sorting
↓
Washing
↓
Drying
↓
Milling
↓
Sieving
↓
Maize flour

Figure 1. Processing of maize into flour (Olanipekun *et al., 2015)*

Preparation of African walnut protein isolates (AWPI). Walnut kernel was washed thoroughly to remove any adhering contaminants, cooked for 15 min for easy removal of shell and to reduce toxic constituent, cooled, de-shelled, and cut into smaller sizes (3 mm) to increase the surface area and oven dried at 55 ^{o}C for 24 hr. Dried nut was grinded into flour using a Qlink grinder (Model No. QBL – 1861A; Turinar Corp. No 1682, Fu-Yong Ave, Nan-Tun County, Shang-Hai, China). The flour (100 g) was defatted with 400 ml hexane and dried in a fume hood at room temperature for 24 hr. The defatted walnut was grinded to pass through 150 meshes, packaged and stored till further use. The protein isolate derived from walnut seed was prepared by the use of an alkaline extraction followed by an acid precipitation according to the method adopted by Wagner *et al*. (2000) with a slight modification.

Defatted walnut flour (DFWF) was suspended with up to 10 fold (w/v) de- ionized water coupled with 1mol/L^{-1} NaOH which was added to reach pH 8.5. Protein was extracted by incubating the suspension for 2 hr at room temperature. The dispersion obtained was centrifuged at 4800 g for 20 min. The supernatant was harvested and protein was precipitated by adding 2mol/L^{-1} HCL until a pH value of 4.8 was reached. After centrifuging at 4800 g for 20 min at 4 ^{o}C, the precipitate was washed with de-ionized water and dialyzed 3 times at 4 ^{o}C against 0.2mol/L-1 phosphate buffer, pH 8. The protein content of walnut protein isolates was determined by micro-kjeldhal method (N x 6.25).

Experimental Design

An Optimal mixture design of Response Surface Methodology (RSM) (Design Expert 8.0.3.1., Stat-Ease Inc., Minneapolis, USA trial version) was adopted of which the variables were wheat flour (30-50%), yellow maize flour (20-30%) and African walnut protein isolates (10-20%); it generated 20 experimental runs (Table 1).

Table 1. Composite flours obtained from the optimal mixture model of RSM

Run	Wheat flour (%)	Yellow maize flour (%)	African Walnut protein isolate (%)
1	50.00	30.00	20.00
2	55.87	23.17	20.95
3	50.00	31.25	18.75
4	42.86	42.86	14.29
5	50.00	31.25	18.75
6	58.69	25.82	15.49
7	50.00	31.25	18.75
8	55.56	22.22	22.22
9	37.50	37.50	25.00
10	55.56	33.33	11.11
11	45.24	28.27	26.48
12	50.00	33.33	16.67
13	50.00	31.25	18.75
14	42.86	28.57	28.57
15	62.50	25.00	12.50
16	36.69	39.57	23.74
17	45.24	37.79	16.97
18	55.87	34.92	9.21
19	50.00	31.25	18.75
20	50.00	31.25	18.75

Proximate composition of flours and blends. The crude protein, fat, moisture, fibre, ash and carbohydrate were determined according to AOAC (2005) methods on triplicate samples. Carbohydrate was determined by differences.

Spectrophotometric determination of amino acids on selected blends. Principle: Ninhydrin combines with amino acids to form colored complexes, the intensity of whose colours depend on the amount of amino acid present.

Reagents: 0.1mol standard solutions of different amino acids (i.e. Alanine, Aspartic acid, Leucine, Isoleucine, Lysine, Methionine, Glycine, Threonine, Valine, Glutamic acid, Cysteine, Tryptophan, Phenylalanine, Ornithine, Tyrosine, Histidine, Serine, Proline, Asparagine, Pyrolysine); Acetate buffer at pH 5.5, Methyl cello solve (ethylene glycol mono methyl), 50% ethanol (V/V), Hydrindantin, Ninhydrin reagent (which was prepared by dissolving 0.8 g of Ninhydrin, 0.12g of Hydrindantin in 30ml of methyl cello solve and 10ml of acetate buffer prepare fresh and store in a brown bottle).

Preparation of sample by hydrolysis. A grounded sample of 1g was weighed into a stopper 250 ml conical flask, 100 ml of 6M HCl was added to the sample and heated in an oven or incubated for 16hrs to hydrolyse the sample. The mixture obtained was filtered through a double layered Whatman No. 42 Filter paper into another 250 ml conical flask and stopper. The hydrolysate obtained was stored at -4 ^{0}C prior to analysis if analysis is not done immediately.

Determination: 2ml of the above hydrolysate was pipetted into a 30ml test tube.10ml of buffered ninhydrin reagent was added, heated in a boiling water bath for 15min, cooled to room temperature and 3ml of 50% Ethanol was added immediately. 0-5µg/ml working standard amino acids was prepared from each standard solution of amino acids to get the gradient factor from the calibration curve for each amino acid. The working standards were heated with the buffered ninhydrin reagent as done with the sample hydrolysate above. The absorbance or transmittance of sample buffered heated hydrolysate and working standards were measured at the wavelength of colour developed by each amino acids.

$$Amino\ acid\ \% = \frac{Absorbance\ of\ sample\ x Gradient\ factor\ x\ Dilution\ factor}{1000}$$

Determination of In-vitro protein digestibility (IVPD) on selected blends. Using the procedure adopted by Mertz *et al.* (1994) and Aboubacar *et al*. (2001), the protein digestibility in the selected blend samples was determined. The flour sample (200 g) was measured into an Erlenmeyer flask and mixed with 35 ml of porcine pepsin (1.5 g of pepsin in 0.1 M KH_2PH_4, pH 2.0). Samples were digested for 2h at 37 ^oC in a shaking water bath. Digestion was stopped by addition of 2 mls of 2M NaOH. Samples were centrifuged (1000rpm, 40 ^oC) for 20 min and the supernatant was discarded. The residues was washed and centrifuged twice with 20 ml of buffer (0.1M KH_2PO_4, pH 7.0). Undigested nitrogen will then be determined using Kjeldhal method. Digestible was calculated as % digestibility = (N in sample- Undigested N) /N in sample x 100.

Statistical Data Analysis

The data was analyzed with the use of one-way analysis of variance (ANOVA). Also, the mean separation was done by the Duncan's multiple range tests using the Statistical Package for the Social Sciences (SPSS) 16.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Proximate Composition of Flours

Proximate composition of the flours is presented in Table 2. The fat content ranged from 0.70 to 20.20%. The fat content for the four flour samples were significantly different ($p<0.05$) from each other. Defatted African walnut flour (DAWF) had the highest fat content while African walnut protein isolates (AWPI) scored the least content. The results recorded in this study are at variance from those of Mao and Hua (2012); Iyenagbe *et al.* (2017) reported 1.80 and 0.23%; 3.87% and 3.38% for DAWF and AWPI, respectively. The differences in the results may be attributed to factors in varieties of walnut. The highest fat content in DAWF could be attributed to the high percentage of oil in African walnut seed compared to other flours which was justified by Enujiugha and Ayodele-Oni (2003). Also, Awolu *et al.* (2015) described high fat content as a factor influencing the life span of flour due to oxidative activities. Based on this, DAWF was used as the starting point for the preparation of AWPI to achieve low fat content as recorded in this study.

Table 2. Proximate composition of flours

Parameters (%)	DAWF	AWPI	YMF	CWF
Moisture content	$8.90^{b} \pm 0.20$	$8.43^{c} \pm 0.06$	$9.07^{b} \pm 0.15$	$10.67^{a} \pm 0.02$
Protein	$26.80^{b} \pm 0.15$	$90.80^{a} \pm 0.25$	$9.63^{d} \pm 0.15$	$10.49^{c} \pm 0.04$
Fat	$20.20^{a} \pm 0.15$	$0.70^{d} \pm 0.15$	$2.03^{b} \pm 0.58$	$1.22^{c} \pm 0.02$
Ash	$3.60^{a} \pm 0.15$	$0.13^{d} \pm 0.06$	$1.53^{b} \pm 0.15$	$0.63^{c} \pm 0.02$
Crude fiber	$1.53^{b} \pm 0.06$	ND	$2.20^{a} \pm 0.15$	$0.30^{c} \pm 0.20$
Carbohydrate	$39.10^{c} \pm 0.31$	ND	$75.60^{a} \pm 0.31$	$74.40^{b} \pm 0.20$

Results represent the average of three replicates ± SD, the means with the same superscripts in a column are not significantly different ($p>0.05$); ND- not detected

DAWF = Defatted African Walnut Flour

AWPI = African Walnut Protein Isolate

YMF = Yellow Maize Flour

CWF = Commercial Wheat Flour

The values of YMF achieved were in agreement with Ijabadeniyi and Adebolu (2005) and Enyisi *et al.*, (2014 a and b) for varieties of maize. Moisture content of DAWF, AWPI, YMF and CWF were 8.90, 8.43, 9.07 and 10.67% respectively. They were significantly different ($p<0.05$) from each other except DAWF and YMF that presented opposite situation. It was observed that de-fatting decreased the moisture content of AWPI because it was subjected to drying under mild temperature (45 ^{O}C for 3 days) and food materials with low moisture content have the benefit of been preserved for an appreciable period of time. The value achieved for DAWF in this work was low (8.90%) and AWPI had high value (8.43%) compared to 9.20 and 4.50% (Mao and Hua, 2012), respectively.

The moisture content in YMF and CWF may be attributed to different factors such as agronomic, environmental factors and storability. However, the moisture content of the flour was consistent with values 9 – 19 % reported in the literature (Enyisi *et al.*, 2014 a and b). It was discovered that flour containing above 12 % moisture cannot be stored for a longer period of time compared to food materials with low moisture content (Edema *et al.*, 2005). The total sample was carried in a regular moisture content of dried food of 11 %. Hence, the water content of about 12 % is specified in flour and its related products.

The protein contents were 26.80, 90.80, 9.63 and 10.49 % for DAWF, AWPI, YMF and CWF, respectively. They were significantly different ($p<0.05$) from each other. It was observed that value of AWPI recorded in this work was higher and DAWF lower than 90.50 and 52.51% reported by Mao and Hua (2012), respectively. More so, Iyenagbe *et al.* (2017) obtained higher value (58.86 %) for DAWF when compared to the value achieved in the present work, while AWPI obtained in this study was higher than the 86.92 % reported by earlier scientist. This could be attributed to the various issue namely varieties as well as origin which can affect protein constituent. The value recorded for DAWF in this study falls within the range of 25.30 - 27.31% reported by (Aremu *et al.*, 2006: Omosuli *et al.*, 2009). Accordingly, Oyenuga (1997) recorded the values of maize flour between 9.60 and 10.70 % which was in agreement with the values of YMF in this study. Furthermore, values reported and published on various maize varieties (Ijabadeniyi & Adebolu, 2005; Enyisi *et al.*, 2014 a&b) also concurred with this work.

Ash contents for DAWF, AWPI, YMF and CWF were significantly different ($p<0.05$) from each other. DAWF had the highest value followed by YMF. The ash content of AWPI was the lowest (0.13) while DAWF had the highest (3.60). The ash values recorded for DAWF and AWPI in this work contradict the values reported by Mao and Hua (2012). They reported DAWF and AWPI contents to be 1.94 and 2.27 %. It was found that the higher the proportion of ash contents the higher the mineral content present in flours.

The values for crude fibre contents of DAWF, AWPI, YMF and CWF were significantly different ($p<0.05$) from each other. There was no value recorded for AWPI and this tally with the work of Iyenagbe *et al.* (2017). The value of crude fiber recorded was justified by the work of Monilola *et al.* (2017); it is also similar to those of other oil seeds (Enujiugha and Ayodele-Oni, 2003). The percentage of fiber in YMF was 2.20%. The result obtained was in accordance with the observations of Ijabadeniyi and Adebolu (2005), who deduced a fibre content of maize to fall between the ranges of 2.07 – 2.97 % for maize variety in Nigeria.

Carbohydrate contents were 39.10, 0.00, 75.60 and 74.40 % for DAWF, AWPI, YMF and CWF, respectively. They were significantly different ($p<0.05$) from each other. There was no value recorded for AWPI while DAWF was lower compared with YMF (75.60 %) and CWF (74.40 %). The results were not unexpected since YMF and CWF are mainly composed of carbohydrate. The results revealed that African walnut protein isolate is rich in protein having moderate amount of ash which was reflected in DAWF. The chemical composition used in the extraction of protein isolate is an easy and suitable way of explaining the purity of other components such as fat, ash, carbohydrate and fiber which are highly needed. Furthermore, results show that African walnut protein isolate had no value for crude fiber and carbohydrate.

More so, all the values obtained were suitable as it signify the concentration of the protein isolates recovered. The highest value (90.80%) in protein content recorded was close to 91.83% for pigeon pea protein isolate discovered by Monilola *et al.* (2017) which was isolated through methanol precipitation compared to 90.65% value achieved from pigeon pea protein isolate isolated through alkaline isoelectric precipitation technique (Olawunmi *et al.,* 2012). However, carbohydrate and crude fibre present in other flours will complement AWPI when blended together. Also, proportion of protein isolate achieved show that it could be added to food like ice cream, confectionery products, and baby food to enhance the protein pool and complement each other.

Proximate Composition of the Blends

The proximate composition of the blends is shown in Table 3. Moisture content of the blends ranged from 8.33 to 9.17%. Run 14 had the lowest content, while runs 3, 5, 7, 13, 19 and 20 had the highest content (9.17%). The values obtained for moisture contents in this literature could be justified with the values 1.47 – 9.51% reported by Venkatachalam and Sathe (2006). Moisture content is a crucial factor in flour which appreciably alters the life span of food product. The percentage moisture content recorded in this work was lower than the minimum limit (10%) reported by Shahzadi *et al.* (2005) which indicate that the composite flour can be used to make a shelf stable product (Akhtar *et al.,* 2008; Elleuch *et al.,* 2011). The moisture content of flour should be less than 10% as obtained in the work.

The response surface curve (3D plot), of the effects of moisture content is presented in Figure 2. The result in Figure 2a showed that there is no moisture retention, and this will prolong the shelf life of the blends. These could be an added advantage using the blends in composite flour development. The Lack of Fit was not significant, showing that the quadratic regression equation could forecast the response model to be true. The significant model terms (B, C, A^2, $B^{2,}$ C^2 and AC) are interpreted to mean that the model could have a power on the response value. The model "Prob>F" value were less than 0.05 showing that the model is noticeable. Model calibration coefficient Adj – R squared is 0.8577 which means that the model can be used to explain the change in 85.77% response value and only about 9.91% of the adequate precision could explain the model, proposing that the model can be used to navigate design as the ratio was greater than 4.

The R-squared (0.9251) is equal to the percentage suggested by Koocheki *et al.* (2009); that values of R^2 should not be less than 85%, or less than 75% by Chauhan and Gupta (2004) which is acceptable for fitting a model. In this study, the models developed indicated that R^2 values obtained showed appropriateness of the developed model equation in predicting the moisture content in the composite blends where the independent variables are mathematically combined. The equation below shows the actual value of moisture content.

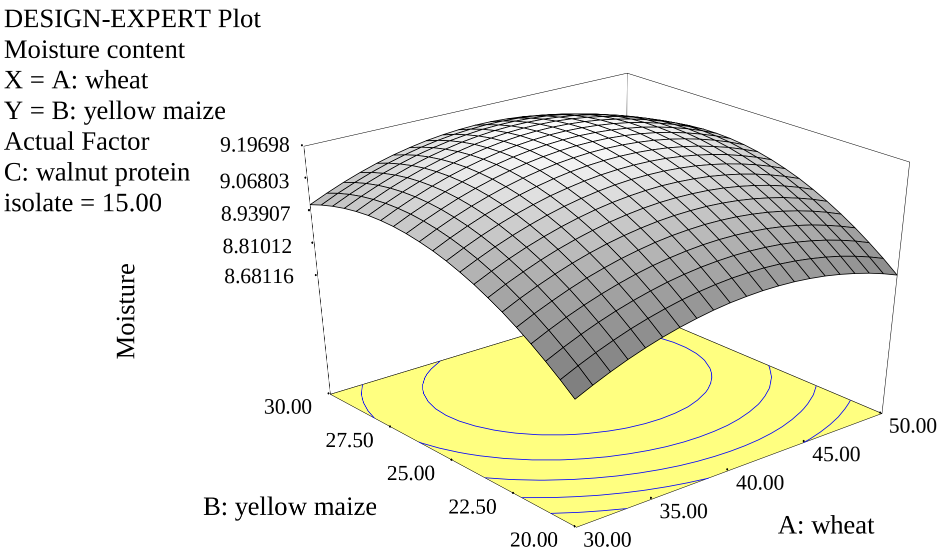
Moisture content = $9.17 + 0.024A + 0.13B + 0.084C – 0.15A^2 – 0.18B^2 – 0.15C^2 – 0.014AB + 0.14AC + 0.039BC$

The protein content of the composite blends ranged from 17.27 to 31.27%. The highest occurred in run 11 while run 18 scored lowest value. Other runs had their values being around 18 to 29 %. More so, the value of run 17 is very close to run 11. However, the formulation of three different types of flour into composite blends yields best proportion in run 11 in terms of highest protein, crude fibre and lowest fat content. The expected increase in the protein content of the blends was the reason for the formulation, such that the final products will not only have higher protein content but higher protein quality. Studies have also shown similar increase in the protein content of walnut meal and isolate (Mao and Hua, 2012); and values (19.30 - 39.29%) of walnut meal (Mostafa and Awad-Allah, 2013). African walnut protein isolate is found to be an excellent source of protein enhancing the protein content of the blend.

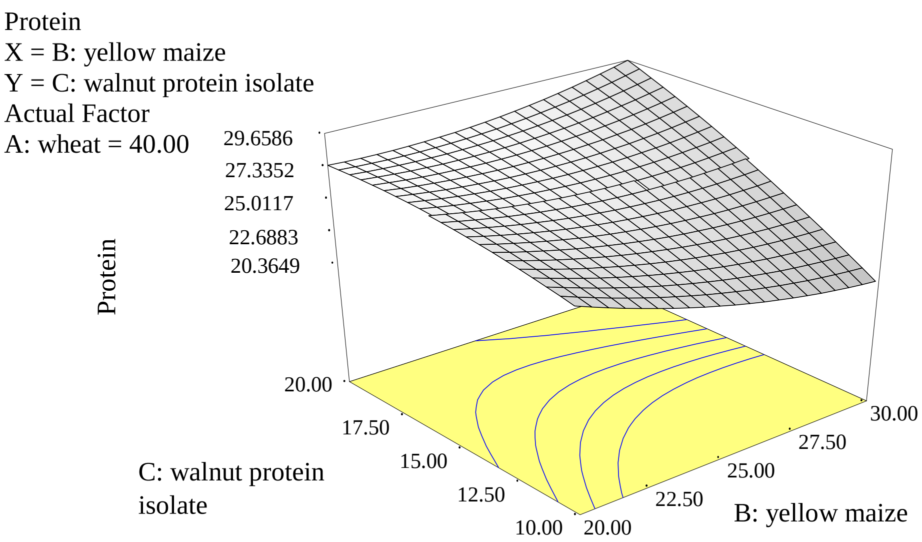
Table 3. Proximate composition of blends

Run	A (g)	B (g)	C (g)	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)	Fibre (%)	CHO (%)
1	50.00	30.00	20.00	9.03±0.15	27.47±0.15	1.06±0.05	2.03±0.15	1.03±0.05	59.36±0.21
2	55.87	23.17	20.95	8.43±0.15	26.63±0.15	1.06±0.15	1.93±0.15	0.73±0.05	61.20±0.61
3	50.00	31.25	18.75	9.17±0.15	25.53±0.15	1.06±0.05	1.90±0.10	0.80±0.10	61.53±0.23
4	42.86	42.86	14.29	8.76±0.15	21.03±0.15	1.03±0.05	2.16±0.06	1.16±0.15	65.83±0.35
5	50.00	31.25	18.75	9.17±0.15	25.53±0.15	1.06±0.05	1.90±0.10	0.80±0.10	61.53±0.23
6	58.69	25.82	15.49	8.73±0.15	22.63±0.27	0.96±0.10	2.00±0.10	0.76±0.15	65.23±0.49
7	50.00	31.25	18.75	9.17±0.15	25.53±0.15	1.06±0.05	1.90±0.10	0.80±0.10	61.53±0.23
8	55.56	22.22	22.22	8.80±0.10	27.40±0.20	0.86±0.15	1.86±0.15	0.70±0.10	60.36±0.47
9	37.50	37.50	25.00	8.67±0.15	26.76±0.15	1.10±0.10	2.53±0.15	0.87±0.15	60.07±0.21
10	55.56	33.33	11.11	8.63±0.15	18.63±0.21	1.00±0.10	1.60±0.10	0.96±0.15	49.16±0.21
11	45.24	28.27	26.48	9.03±0.15	31.27±0.15	0.90±0.05	2.66±0.15	0.76±0.05	55.30±0.10
12	50.00	33.33	16.67	8.63±0.15	22.97±0.15	1.16±0.05	2.13±0.15	0.93±0.15	64.16±0.21
13	50.00	31.25	18.75	9.17±0.15	25.53±0.15	1.06±0.05	1.90±0.10	0.80±0.10	61.53±0.23
14	42.86	28.57	28.57	8.33±0.15	27.57±0.21	1.03±0.05	2.63±0.15	0.83±0.05	59.60±0.26
15	62.50	25.00	12.50	8.50±0.10	19.24±0.10	1.17±0.05	2.20±0.15	0.73±0.05	54.70±0.26
16	36.69	39.57	23.74	8.87±0.05	29.67±0.20	1.20±0.10	2.43±0.05	0.83±0.05	56.97±0.29
17	45.24	37.79	16.97	9.00±0.10	30.07±0.15	1.13±0.10	2.30±0.15	1.00±0.10	56.50±0.45
18	55.87	34.92	9.21	8.53±0.15	17.27±0.20	1.13±0.15	1.76±0.25	0.93±0.05	70.36±0.38
19	50.00	31.25	18.75	9.17±0.15	25.53±0.15	1.06±0.05	1.90±0.10	0.80±0.10	61.53±0.23
20	50.00	31.25	18.75	9.17±0.15	25.53±0.15	1.06±0.05	1.90±0.10	0.80±0.10	61.53±0.23

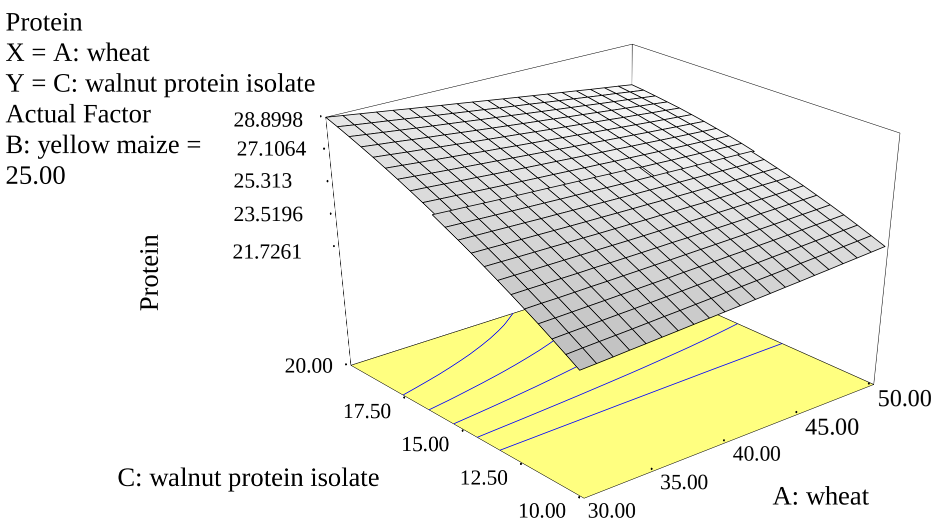
Note: A-wheat; B-yellow maize flour; C-walnut protein isolate; CHO-carbohydrate



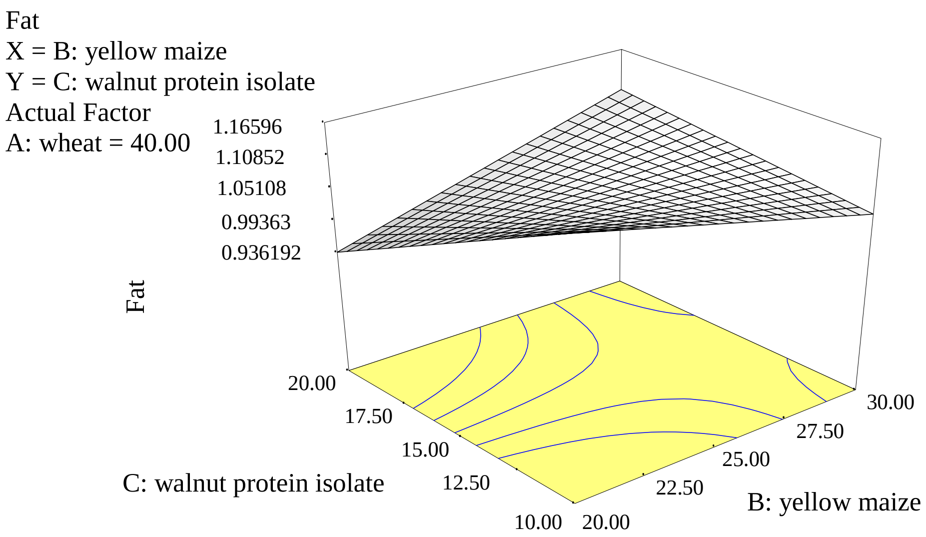
a. Moisture Content



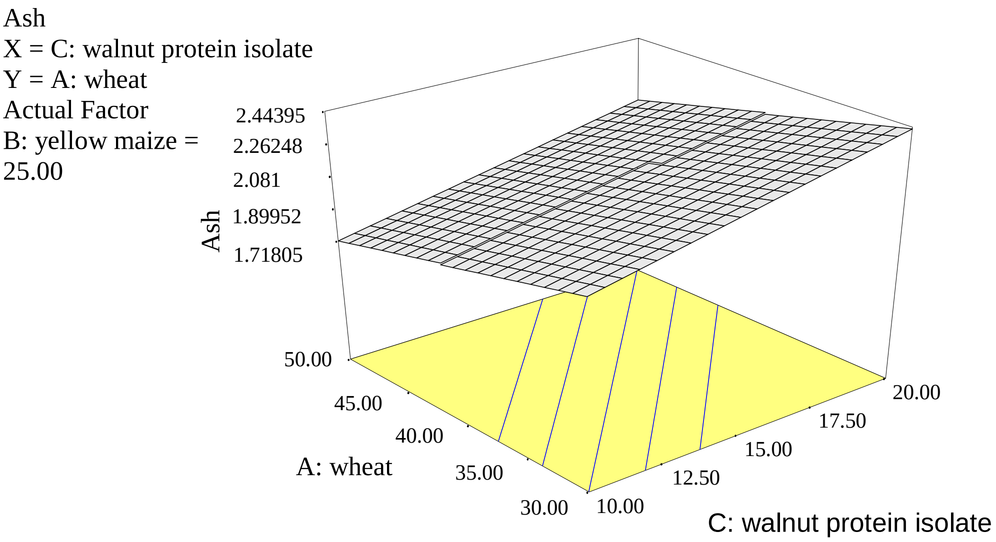
b (i). Protein Content (Yellow maize versus African Walnut Protein Isolate)



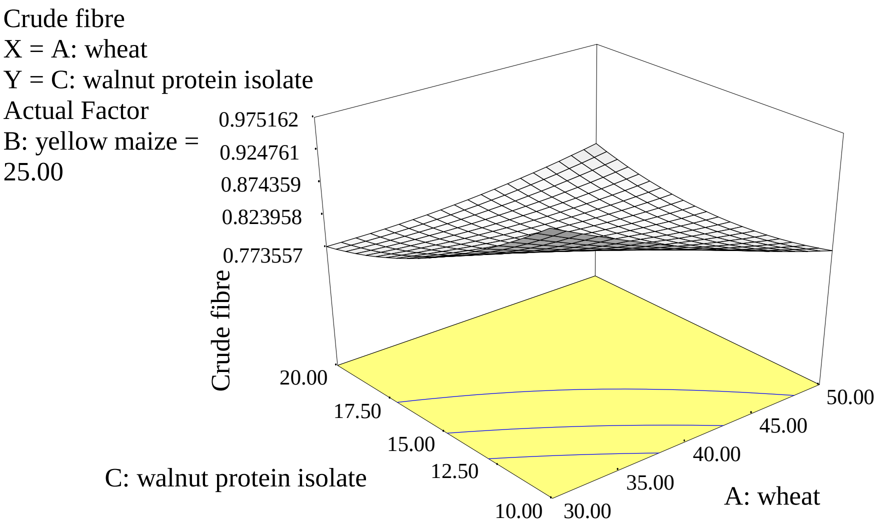
b (ii). Protein Content (Commercial Wheat flour versus African Walnut Protein Isolate)



c. Fat Content (Yellow maize flour versus African Walnut Protein Isolate)



d. Ash Content (African Walnut Protein Isolate versus Commercial Wheat Flour)



e. Crude Fibre Content (Commercial Wheat Flour versus African Walnut Protein Isolate)

Figure 2 (a–e). 3D surface plots showing the effect of proximate composition on blends (commercial wheat flour, yellow maize flour and African walnut protein isolate) and dependent [(a) moisture (b) protein (c) fat (d) ash (e) crude fibre)] variables displaying significant differences

The model term (C) was significant as this implies that the independent variable is African walnut protein isolate. The significant model term of "C" ($P<0.05$) implies that it could have the power to affect the response value well and accurate enough to predict the responses. A negative value of predicted R^2 for protein contents implies that it could only be described by means and a better predictor for the responses. The adequate precision value is 5.613 which indicate an adequate signal; and the values suggested that the model can be used to navigate design space. The value for R-squared and Adjusted R-squared were 0.6275 and 0.2922 respectively.

The 3D plot graph in Figure 2b (i) and (ii) showed an increase in the protein content of the blends as substitution of protein isolate from African walnut protein isolate and yellow maize flour increases. Again, increase in the substitution of AWPI and CWF decreased the protein content.

The final equation is showed in equation below:

Protein content = 25.55 – 0.33A – 0.80B + 2.74C + 0.026A^2 + 0.85B^2 – 0.59C^2 – 1.40AB – 0.85AC + 1.91BC

The fat content ranged from 0.86 to 1.20% (samples 8 and 16), respectively. High fat content would adversely influence the shelf life of the blends due to oxidative activities (Awolu *et al.,* 2015). The relatively low fat content of the composite blends makes them suitable raw materials in the formulation of food products. The values of fat content in this study agreed with the value recorded for soy-protein isolate and composite blends 0.59 – 1.63 % (Adeyeye *et al.,* 2017). The significant "Lack of Fit" value of 0.022 is obtained. The significant model terms (A, C and BC) revealed that they could have high impact on the response value. The model "Prob>F" value was less than 0.05 which implied that the model test is good. Model calibration coefficient Adj – R squared is 0.7626 which means that the model can explain the change in 76.26% response value.

The model was significant ($p<0.05$) with p-value 0.0002, 0.0003 and 0.0003 which could be accounted for only 83.75% ($R^2 = 0.8375$). The results suggested that the values were acceptable for fitting model. Also, the value of R^2 and Adj – R^2 recorded in this work shows the absolute development of a model equating the forecast of fat contents in the composite blends. The response surface plot is shown in Figure 2c, revealing that the increase in yellow maize substitution induced the increase of fat content in the blends more than African walnut protein isolate. At 20% of African walnut protein isolate substitution, the value for fat content is 0.94 while at 30% incoporation of yellow maize flour is 0.99. The R-squared and Adjusted R-squared are 0.8607 and 0.7354 respectively.

The final equation is stated below:

Fat content = 1.06 – 0.054A + 6.424x10^3B – 0.044C – 6.128x$10^3$$A^2$ + 9.782x$10^3$$B^2$ – 7.896x$10^3$$C^2$ + 0.011AB – 0.024AC + 0.071BC

The ash content ranged from 1.60 to 2.66%. Run 10 had the lowest content while the highest value was observed in run 11. The remaining composite blends ranged between 1.76 to 2.63%. Ash contents recorded in this work are in agreement with (Ohizua *et al.*, 2017). Ash is an indication of mineral contents of foods. The response surface graph is described in Figure 2d which shows a significant linear effect. Ash content in the blends increased as there is an increase in African walnut protein isolate and wheat flour. The final equation is presented below:

Ash content = 1.90 – 0.18A + 8.953x10^3B + 0.18C + 0.100A^2 + 0.065B^2 + 0.098C^2 – 0.045AB – 0.097AC + 0.080BC

The crude fibre content ranged from 0.7 to 1.16%. Run 8 scored lowest while run 4 had highest value. The remaining runs varied between 0.73 – 1.03%, which aligned with Adeyeye *et al.* (2017) observation. The crude fibre increased as the yellow maize flour in the composite blends increases. This indicates that the composite blends are rich in fibre which could be used in processing of functional food products. Intake of fiber-rich food (soluble fibre) was said to reduce blood cholesterol, diabetes, high blood pressure and excess fat in the body (Chukwu *et. al.*, 2013; Jaja and Yahere, 2015) while insoluble fibre fight against constipation and reduce the risk of colon cancer (Islam *et al.*, 2007). However, these values are still low for rich-fibre foods when compared with the suggested ranges of 20 – 35 g/day for adults and elders and 5g/day for children (Marlett *et al.*, 2002; Dhingra *et al.*, 2012; Neha and Chandra, 2012). In spite of that, the composite blends have non negligible contents which are able to contribute to the fibre recommended daily allowance (RDA).

The significant model terms A, B, C, B^2 and AC makes the independent variables have a significant effect to the response surface value. The model "Prob>F" value was significant with the p-values of 0.0312, <0.0001, 0.0085, 0.0251, 0.0599 and 0.0162 respectively. The values could be accounted for only 89.30% (R^2 = 0.8930). The values less than 0.05 were obtained which means that the model test is good. Model calibration coefficient Adj- R squared is 0.7967 which implies that the model can explain the change in 79.67% response value. The values obtained in this study claimed that the values are acceptable for fitting a model. Adequate precision value is greater than 4 which indicate that the design is adequate. The 3D response surface plot is showing the effect of variables on crude fiber as shown in Figure 2e; all variables had positive effect on the fiber content. The crude fibre increased with decrease in African walnut protein isolate and wheat flour substitution. At 10% substitution of African walnut protein isolate, crude fiber increased to 0.80 while at 20%, the crude fiber decreased to 0.78. However, the crude fiber increases with decreased in African walnut protein isolate. The final equation is described as thus:

$$\text{Crude fibre} = 0.80 - 0.036A + 0.094B - 0.047C + 0.012A^2 + 0.037B^2 + 0.029C^2 + 0.036AB + 0.054AC - 0.011BC$$

Carbohydrate content ranged from 49.16 to 70.36% for run 10 scoring the lowest while run 18 scored highest among the blends. The values observed in this work are in agreement with values recorded for unripe cooking banana, pigeon pea, and sweet potato flour blends (Ohizua *et al.*, 2017). It was observed that decrease in the addition of AWPI and ratio of CWF and YMF increased the carbohydrate content of the blends. This result from the fact that carbohydrate content was not detected in AWPI flour. This explains that the 2FI model could predict the response value well. A negative predicted R- squared obtained implies that they could only be described by their means. The final equation is stated below:

$$\text{Carbohydrate} = 61.59 - 0.89A - 0.90B - 1.45C - 0.52A^2 - 1.31B^2 + 0.093C^2 - 1.08AB + 3.27AC + 0.42BC$$

Considering the best three blend samples as assessed by high protein and fibre, and low fat, samples 4, 8 and 11 were selected and further used for the evaluation of amino acid profiling and in-vitro protein digestibility.

Amino Acid Profile of Selected Composite Blends

The amino acid profile of three best selected composite blend samples is shown in Table 4. Essential and non-essential amino acid contents of the blend samples were determined. The concentration of histidine, lysine and proline were particularly the most abundant amino acids which occur in sample 11 while control sample appeared lowest. The values in sample X appeared lower when compare with composite blends. The differences in value justify the inclusion of under-utilized locally grown crops (AWPI) added to wheat flour. The content of histidine and lysine obtained in this study was higher compared to Iyenagbe *et al.* (2017) with the value that ranges between 3.08 and 3.75 for boiled conophor nuts isolates while proline concentration of the conophor nut isolate was higher (7.52) than the value recorded in this work. The variation in value could be attributed to cultivars, origin and environment. Among the three desired blend samples, sample 11 scored highest in all the amino acids profiling except alanine and tyrosine. In sample 8, alanine and tyrosine had highest composition of 2.3187 and 0.3010, respectively while control sample had the least value. The highest value of essential and non-essential amino acids recorded in sample 11 could be due to the quantity of AWPI substituted in the blends which could influence protein composition in the products. In sample 4, proline was having the highest value of 2.5780 followed by histidine 1.9757 while ornithine had least value, respectively. Control sample was having the lowest scores in the amino acid profiling. Tryptophan was not detected in control sample but highest in sample 11.

Table 4. Amino acid profile of selected blends (%)

Amino acid profile	Sample X	Sample 4	Sample Run 8	Sample 11
Alanine	0.2330 ± 0.002	0.2340 ± 0.002	2.3187 ± 0.002	0.9587 ± 0.002
Cysteine	0.1201 ± 0.002	0.1027 ± 0.002	0.0817 ± 0.002	0.2520 ± 0.002
Histidine	0.2713 ± 0.002	1.9757 ± 0.005	0.6197 ± 0.002	4.0177 ± 0.003
Isoleucine	0.3430 ± 0.002	0.1357 ± 0.002	0.0657 ± 0.003	1.2277 ± 0.002
Leucine	0.0870 ± 0.001	0.0757 ± 0.003	0.5211 ± 0.002	1.2107 ± 0.002
Lysine	0.1776 ± 0.003	0.8880 ± 0.002	2.5430 ± 0.003	4.6150 ± 0.002
Methionine	0.0911 ± 0.001	0.2920 ± 0.002	0.4600 ± 0.020	1.6407 ± 0.003
Phenylalanine	0.3957 ± 0.003	0.2967 ± 0.002	0.4600 ± 0.020	1.4397 ± 0.002
Threonine	0.0280 ± 0.002	0.0920 ± 0.129	0.2600 ± 0.002	0.4487 ± 0.002
Tyrosine	0.0177 ± 0.002	0.1240 ± 0.161	0.3010 ± 0.002	0.0701 ± 0.093
Trytophan	ND	0.2277 ± 0.003	0.1870 ± 0.003	1.1480 ± 0.002
Valine	0.5311 ± 0.002	0.3400 ± 0.002	0.7260 ± 0.002	1.4527 ± 0.003
Total essential amino acids	2.2956	4.7842	8.5439	18.4817
Arginine	0.2530 ± 0.002	0.7110 ± 0.003	0.7030 ± 0.002	2.3480 ± 0.002
Aspartic acid	0.4143 ± 0.002	0.9530 ± 0.003	0.1757 ± 0.002	1.9110 ± 0.001
Glutamic Acid	0.0123 ± 0.002	0.1547 ± 0.003	0.077 ± 0.002	0.3917 ± 0.002
Glycine	0.2211 ± 0.003	0.5237 ± 0.003	0.3307 ± 0.002	1.8930 ± 0.003
Ornithine	0.0100 ± 0.000	0.0360 ± 0.000	0.0590 ± 0.001	0.7180 ± 0.002
Pyrolysine	0.0312 ± 0.000	0.7277 ± 0.003	0.3207 ± 0.002	1.2327 ± 0.002
Proline	0.6544 ± 0.002	2.5780 ± 0.003	0.9697 ± 0.003	4.4860 ± 0.002
Serine	0.0119 ± 0.000	1.0527 ± 0.003	0.4620 ± 0.002	1.7490 ± 0.002
Total non essential amino acids	1.6082	6.7368	3.0978	14.7294

Sample 4 – 42.86 g CWF, 42.86 g YMF, 14.29 g AWPI; Sample 8 - 55.56 g CWF, 33.33 g YMF, 11.11 g AWPI; Sample 11 – 36.69 g CWF, 39.57 g YMF, 23.74 g AWPI: Sample X – 100 % commercial wheat flour

The main function of lysine is to contribute to protein production which is crucial for growth and maintenance of the body. All essential amino acids were significantly present in the three samples. Essential amino acid (lysine) is needed in the body for healing and growing of hair and nails; any deficiency in these essential amino acids will hinder recovery process (Zuraini *et al.*, 2006). Earlier studies have shown the effect of heat on the processing of amino acids in conophor nuts isolate (Iyenagbe *et al.*, 2017). More so, high temperature treatments was said to reduce lysine bioavailability due to formation of Maillard reaction in hazelnut (Ozdemir *et al.*, 2001). Therefore, the heat processing of African walnut seed into various recipes either flour, protein isolate or snacks must be considered as to retain the amino acid profile. The total essential and non-essential amino acid observed was 18.4817 and 14.7294 (sample 11); followed by sample 4 (6.9932 and 6.7368), sample 8 (5.3212 and 3.0978) and least in sample X (3.3093 and 1.6082).

These values were lower to 36% considered for an ideal protein (FAO/WHO 2010). Nevertheless, the concentration achieved in this study cannot be ignored. Hence, the flour blends substituted with African walnut protein isolate can be regarded as good quality protein. The concentration of arginine/lysine obtained in this work are higher to the values 2.31, 1.40, <1, 0.44 and <3 reported for hemp, soybean, kidney bean, casein and rice protein isolates, respectively (Tang *et al.*, 2006; Yang *et al.*, 2012; Mundi and Aluko, 2012, 2013). High ratio of arginine/lysine in the diet produces beneficial hypo-cholesterolemic effects in improving the cardiovascular health (Malomo *et al.*, 2014) and also helps in hypertension regulation (Vallabha *et al.*, 2016). Arginine is a nutrient that speeds protein synthesis, helps build muscle and nitric oxide in the body. According to the National Institutes of Health, arginine may be effective for treating erectile dysfunction, lowering high blood pressure and improving wound healing (Malomo *et al.*, 2014). Thus the present result showed that the composite blends might have positive effects on the cardiovascular system as well as enhance nutrient pool of the ready-to-eat snacks.

In-vitro Protein Digestibility (IVPD)

The in-vitro protein digestibility of composite blends is shown in Table 5. The in-vitro protein digestibility of the flour blends ranged from 66.36 to 78.31% for samples 4 and 11 while control sample was 21.11%. The IVPD of 100% commercial wheat flour was significantly lower to composite blends. These values were within the range reported by Giami *et al.* (1999). The IVPD of flour blends produced from substitution of AWPI, CWF and YMF compared favorably with the digestibility of cashew nut flour (74.3 - 82.8%) reported by Fagbemi *et al.* (2006) and some oil seeds like cotton meal (Hsu *et al.*, 2003).

Table 5. In-vitro Protein Determination of composite blends

Sample	In vitro Protein Digestibility (%)
X	$21.11^{d} \pm 0.01$
4	$66.36^{c} \pm 0.015$
8	$72.80^{ab} \pm 0.020$
11	$78.31^{a} \pm 0.020$

Sample 4 – 42.86 g CWF, 42.86 g YMF, 14.29 g AWPI; Sample 8 - 55.56 g CWF, 33.33 g YMF, 11.11 g AWPI; Sample 11 – 36.69 g CWF, 39.57 g YMF, 23.74 g AWPI: sample X – 100% commercial wheat flour

Conclusion

African walnut protein isolates, commercial wheat and yellow maize flours were blended together using response surface methodology. The proximate composition of flours, composite blends and protein quality of the composite blends were determined. Among the composite blends subjected to analysis, three desirable flour blends, samples 4, 8 and 11were selected based on highest protein and fibre, and lowest fat content. The study showed that sample with proportion of flours in sample 11 (36.69 g CWF, 39.57 g YMF, 23.74 g AWPI) scored highest in proximate and protein quality (amino acid profiling and in-vitro protein digestibility), followed by samples 8 and 4. In conclusion, the aforementioned proportion of flours (sample 11) had overall best result which could be useful in food industry and fight against protein deficiency.

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References

Aboubacar, A, Axiell, J. D, Huang, C. & Hamaker, D. (2001). A rapid protein digestibility assay for identifying highly digestible sorghum lines. *Cereal Chemistry, 78*(2): 160-165.

Adeyeye, S. A. O., Adebayo-Oyetoro, A. O. & Omoniyi, S. A. (2017). Quality and sensory properties of maize flour cookies enriched with soy-protein isolate. *Cogent Food and Agriculture, 3*: 1278827.

Akhtar, S., Anjum, F., Rehman, S., Sheikh, M. & Farzena, K. (2008). Effect of fortification on the physico-chemical and microbiological stability of whole wheat flour. *Journal of Food Chemistry, 112*: 156-163.

Akubor, P. (2003). Functional properties and performance of cowpea, plantain and wheat flour blends in biscuits. *Journal of Plant, Food and Human Nutrition, 58*: 1-8.

AOAC (2005). Official Methods of Analysis. 15th Ed. *Association of Official Analytical Chemists,* Washington DC.

Aremu, M. O., Olonisakin, A., Bako, D. A. & Madu, P. C. (2006). Compositional studies and physicochemical characteristics of cashew nut (Anarcadium occidentale) flour. *Pakistan Journal of Nutrition, 5*(4): 328-333.

Awofadeju, O. F., Awe, A. B., Adewumi, O. J., Amadi, B.O. & Oluwatoke F. O. (2015). Nutritional and organoleptic evaluation of cookie produced from wheat flour and African walnut (*Tetracarpidium conophora*) flour blends. *Elixir Food Science, 86*: 34898-32900.

Awolu, O. O., Oluwaferanmi, P. M. Fafowora, O. I. & Oseyemi. G. F. (2015a). Optimization of the extrusion process for the production of ready-to-eat snack from rice, cassava and kersting's groundnut composite flours. *LWT Food Science Technology, 64*: 18–24.

Bhat, R. & Karim, A. A. (2009). Exploring the nutritional potential of wild and underutilized legumes. *Comprehensive reviews in Food science and Food safety, 8*(4), 305-331.

Chauhan, B. & Gupta, R. (2004). Application of statistical experimental design for optimization of alkaline protease production from Bacillus sp RGR-14. *Process Biochem*istry, *39*: 2115-2122

Chukwu, B. N., Ezebuiro, V. O., Samuel, E. S., & Nwachukwu, K. C. (2013). Gender differential in the incidence of diabetes mellitus among the patients in Udi local government area of Enugu state, Nigeria. *Mediterranean Journal Social Science, 4*: 131–138.

Dhingra D., Michael M., Rajput H & Patil R. T. (2012). Dietary fibre in foods: A review, *Journal of Food Science and Technology, 49*(3): 255-266.

Echendu C. A., Onimawo I. A. & Somtochi, A. (2004). Production and Evaluation of Doughnuts and Biscuits from Maize- Pigeon pea Flour Blends. *Nigerian Food Journal, 22*: 147-153.

Edema M. O., Sanni, L. O. & Abiodun, I. (2005). Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria. *African Journal Biotechnology, 4*(9): 911-918.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia. H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, Technological, Functionality and Commercial Applications. *Revised Food Chemistry, 124*: 411–421.

Eneche, E. H. (2003). Biscuit making potentials of millet/pigeon pea flour blends. *Plant Foods Human Nutrition, 54*: 21-27.

Enujiugha, V. N. & Ayodele-Oni, O. (2003). Evaluation of oil nutrient and anti-nutrients in lesser known underutilized oil seeds. *International Journal of Food Science and Technology, 38*: 525-528.

Enyisi, I. S., Umoh, V., Whong, C., Abdullahi, I. & Alabi, O. (2014a). Chemical and nutritional value of maize and maize products obtained from selected markets in Kaduna State, Nigeria. *African Journal Food Science and Technology, 5*(4): 100-104.

Fagbemi, T. N., Oshoudi, A. A. & Ipinmoroti, K. O. (2006). Effects of processing in the functional properties of full fat and defatted fluted pumpkin seed flours. *Journal Food Technology, 4*: 70-79.

FAO/WHO (2010) Fats and fatty acids in human nutrition: report of an expert consultation. FAO Food and Nutrition Paper, 91, 10-14 Nov. 2008. Geneva.

Giami, S. Y., Adindu, M. N. & Akusu, M. O. (1999). Effect of processing methods on fluted pumpkin (*Telfairia occidentalis)* and other chemical constituents. *Proceedings 23rd Annual NIFST Conference,* Abuja (pp. 127-129).

Hooda, S. & Jood, S. (2005). Organoleptic and nutritional evaluation of wheat biscuits supplemented with untreated and treated fenugreek flour. *Food Chemistry, 90*: 427-35.

Hsu, C. L., Chen, W., Weng, Y. M. & Tseng, C. Y. (2003). Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry*, *83*.1: 85-92.

IFPRI-International Food Policy Research Institute (2002). Sustainable Food Security for all by 2020. In: *Proceedings of an International Conference*, September 4-6, 2001, Bonn Germany (pp. 16-34).

Ijabadeniyi, A. & Adebolu. T. (2005). The effect of processing methods on the nutritional properties of ogi produced from three maize varieties. *Journal Food Agriculture Environment, 3*: 108-109.

Iyenagbe, O. David, Sunday, A. Malomo, Atinuke, O. Idowu, Adebanjo A. Badejo, & Tayo, N. Fagbemi (2017). Effects of thermal processing on the nutritional and functional properties of defatted conophor nut (*Tetracarpidium conophorum*) flour and protein isolates. *Food Science and Nutrition, 1*(9).

Jaja, T. & Yarhere, I. E. (2015). Risk factors for Type 2 diabetes mellitus in adolescents secondary school students in Port Harcourt, Nigeria. *Nigerian Pediatrician Journal*, *42:* 131–137.

Koocheki, A., Taherian, A.R., Razavi, S. M .A. & Bostan A. (2009). Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from Lepidium perfoliatum seeds. *Food Hydrocolloids, 23*: 2369–2379. doi:10.1016/j.foodhyd.

Malomo, S. A., He, R. & Aluko, R. E. (2014). Structural and functional properties of hemp seed protein products. *Journal of Food Science*, *79*(8), 1512–1521.

Mao, X. & Hua, Y. (2012). Composition, structure and functional properties of protein concentrates and isolates produced from walnut. *International Journal of Food Science, 13*: 1561-1568.

Marlett, J. A., McBurney, M. I. & Slavin, J. L. (2002). Position of the American dietetic association: Health implication of dietary fiber. *Journal of the Academy of nutrition and Dietetics*, *102*(7): 993-1000.

McWatters, K. H., Ouedraogo, J. B., Resurreccion, A.V. A., Hung, Y. & Phillips, R. D. (2003). Physical and sensory characteristics of sugar cookies containing mixtures of wheat, fonio (*Digitaria exilis)* and cowpea (*Vigna unguiculata)* flours. *International Journal of Food Science and Tech*nology, *38*: 403-410.

Mepba, H. D, Eboh, L. & Nwaojigwa, S. U. (2007). Chemical composition, Functional and Baking Properties of Wheat-Plantain Composite Flours. *African Journal of Food Agriculture, Nutrition and Development*, *7*(1): 1-22.

Mertz, E., Hassan, M. M., Cairuns-Whittern, C., Wrleis, A.W., Tu, L. & Axtell, J. D. (1994). Pepsin digestibility of proteins in sorghum and other cereals. *Proceedings: National Academy Science, 22*: 85-93.

Monilola K. Adenekan, Gbemisola J. Fadimu, Lukumon A. Odunmbaku & Emmanuel K. Oke (2017). Effect of Isolation Techniques on the Characteristics of Pigeon pea (*Cajanus cajan*) Protein Isolates. *Journal of Food Science and Nutrition, 6*: 146–152

Mostafa A.A. & Awad-Allah (2013. Evaluation of Selected Nuts and Their Proteins Functional Properties. *Journal of Applied Sciences Research*, *9*(1): 885-896.

Muller, O. & Krawinkel, M. (2005). Malnutrition and health in developing countries. *Canadian Association Journal, 173*(3), 279-286.

Mundi, S. & Aluko, R. E. (2012). Physicochemical and functional properties of kidney bean albumin and globulin protein fractions. *Food Research International*, *48*(1), 299–306.

Neha, M. & Chandra R. (2012). Development of functional biscuit from soy flour and rice bran. *International Journal Agriculture Food Science*, *2*: 14–20.

Ohizua, E. R., Adeola, A. A., Idowu, M. A., Sobukola, O. P., Afolabi, A. T., Ishola, R. O., Ayansina, S. O., Oyekale, T. O. & Falomo, A. (2017). Nutrient composition, functional, and pasting properties of unripe cooking banana, pigeon pea, and sweet potato flour blends. *Journal of Food Science and Nutrition*, *5:* 750-762. Doi: 10.1002/fsn3.455

Olanipekun, O. T., Olapade, O. A. Suleiman, P. & Ojo, S. O. (2015). Nutrient and analysis of abari made from composite mixture of kidney bean flour and maize flour. *Sky Journal of Food Science, 4*(2): 19-23.

Olawunmi, A., Ojukwu, M. & Eboh, B. (2012). Comparative study on the physico-chemical properties of pigeon pea *(Cajanus cajan)* flour and protein isolate. *International Journal of Agricultural and Food Science, 2:* 121–126.

Omosuli, S. V., Ibrahim, T. A. & Oloye, D. (2009). Proximate and mineral composition of roasted and defatted cashew nut flour. *Pakistan Journal of Nutrition, 8*(10): 1649–1651.

Oyenuga, V. A. (1997). *Nigeria Food and Feeding Stuffs Ibadan*. Ibadan: University Press.

Ozdemir, M., Açkurt, F., Yildiz, M., Biringen, G., Gürcan, T. & Löker, M. (2001). Effect of roasting on some nutrients of hazelnuts (*Corylus avellena* L.). *Food Chemistry*, *73*(2), 185–190.

Potter, N. & Hotchkiss, J. (2006). *Food Science*. 5th Ed. New Delhi: CBS Publishers and Distributors 376 Danyangaji.

Shahzadi, N., Butt, M. S., Rehman, S. U., & Sharif, K. (2005). Chemical characteristics of various composite flours. *International Journal of Agriculture and Biology*, *7*, 105–108

Shaista, Q., Muhammad, A., Fahrulzaman, H. & Muhammad, A. J. (2017). Comparative study for the determination of nutritional composition in commercial and non-commercial yellow maize flours. *Pakistan Journal of Botany, 49*(2): 519-523.

Tang, C.H., Ten, Z., Wang, X.S., & Yang, X.Q. (2006). Physicochemical and functional properties of hemp (*Cannabis sativa* L.) protein isolate. *Journal of Agriculture and Food Chemistry*, *54*(23), 8945–8950.

Vallabha, V. S., Tapal, A., Sukhdeo, S. V., Govindaraju, K. & Tiku, P. K. (2016). Effect of arginine/Lysine ratio in free amino acid and protein on L-NAME induced hypertension in hypercholesterolemic Wistar rats. *RSC Advances*, *6*: 73388–73398.

Venkatachalam, M. & Sathe, S.K. (2006). Chemical composition of selected edible nut seeds. *Journal Agriculture Food Chemistry, 54*(13): 4705-4714.

Wagner, J. R., Sorgentini, D. A. & Anon, M. C. (2000). Relationship between solubility and surface hydrophobicity as an indicator of modification during preparation processes of commercial and laboratory prepared soy-protein isolates. *Journal of Agricultural and Food Chemistry, 48*: 3159-3165.

Yang, L., Chen, J. H., Zhang, H., Qiu, W., Liu, Q. H., Peng, X. & Yang, H. K. (2012). Alkali treatment affects in vitro digestibility and bile acid binding activity of rice protein due to varying its ratio of arginine to lysine. *Food Chemistry*, *132*(2), 925–930.

Zuraini, A., Somchit, M. N., Solihah, M. H., Goh, Y. M., Arifah, A.K. & Zakaria, M.S. (2006). Fatty acid and amino acid compositions of three local Malaysian Channa fish spicies. *Food Chemistry, 4*: 674–678.