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Optimization of cereal Blend (wheat and yellow maize flours *(Zea maize)*) enriched with African walnut *(Tetracarpidium conophorum)* protein isolate for cookie making

Abiodun A. Olapade*, Oluwayemisi F. J. Awofadeju

Department of Food Technology, Faculty of Technology, University of Ibadan, Ibadan, Oyo State, Nigeria

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ABSTRACT

This study examined the effect of African walnut protein isolate, wheat and yellow maize flours on the general acceptability of cookie production. In this study, blends of African walnut protein isolate, wheat and yellow maize flours were studied in a completely randomized design assessment of the proximate composition. The variables were wheat flour (30-50%), yellow maize flour (20-30%) and African walnut protein isolate (10-20%) to generate 20 composite blends. The proximate composition and functional properties of blends and flours were determined using standard methods. Three blends of samples 50.00 g wheat flour, 30.00 g yellow maize flour, 20.00 g African walnut protein isolate; 45.24 g wheat flour, 28.27 g yellow maize flour, 26.48 g African walnut protein isolate and 45.24 g wheat flour, 37.79 g yellow maize flour and 16.97 g African walnut protein isolate as assessed by the highest protein and fiber and low fat contents, were selected from optimization results of proximate composition for production and sensory evaluation of cookies. The proximate composition of flours revealed that African walnut protein isolate was significantly higher in protein than other flours. Fat in African walnut protein isolate was the least, while crude fiber and carbohydrate contents were not detected in African walnut protein isolate. Functional analysis revealed that yellow maize flour had the highest value in water absorption capacity; yellow maize flour and African walnut protein isolate had the highest oil absorption capacity, while African walnut protein isolate scored the least in bulk density. The sample 45.24 g wheat flour, 28.27 g yellow maize flour and 26.48 g African walnut protein isolate had the highest protein and ash, with the lowest fat content among the blends. However, cookie prepared from sample 45.24 g wheat flour, 37.79 g yellow maize flour and 16.97 g African walnut protein isolate had the best overall acceptability. However, the study recommends the flour proportion of sample 45.24 g wheat flour, 37.79 g yellow maize flour and 16.97 g African walnut protein isolate in cookie production by establishing the inclusion in Nigerian food industry and as a way of reducing or alleviate malnutrition.

Introduction

Cookies are widely consumed all over the world and they represent the largest category of snack foods in most parts of the world. Generally, they are high in carbohydrates, fats and calories, but low in proteins and crude fibers. Currently, fortification of snacks has evolved in order to enhance their protein quality and the quality of other nutrients (Awolu et al., 2015a,b; Barber and Obinna-Echem, 2016) using natural anthocyanins, composite flour and emulsion filled gel based on inulin and extra virgin olive oil. Development of composite flours was meant to reduce dependency on importation of wheat and also to develop gluten-free food products suitable for gluten-intolerant people. In this regard, ready-to-eat snack

*Corresponding author E-mail: aaolapade@yahoo.com

consisting wheat, cocoyam and Bambara groundnut flour was developed (Awolu et al., 2017), while Omoba et al. (2013) optimized brewer's spent grain-plantain composite flour and cookies. The results of the researches showed that the substitution of wheat partially or fully with some other locally available crops produced composite flour with acceptable proximate and functional properties.

Wheat flour is the most valuable and majorly used in the production of cookies due to its unique properties in dough making, because of the gluten it possesses (Mepba et al., 2007). It is quite obvious that millions of people rely on wheat-based products for diverse nutritional meals on a daily basis. Thus, its importance cannot be over emphasized especially in developing countries where large number of people consumed wheat-based foods such as biscuits, bread and cookies. Wheat flour is deficient in some essential amino acids, vitamin A and minerals which necessitates its supplementation with crops that are rich in the aforementioned nutrients. Yellow maize is rich in beta-carotene, vitamins, minerals and dietary fibers, and widely used for human nutrition as a source of flour, starch and oil.

African walnut seed *(Tetracarpidium conophorum)* (Figure 1) is an underutilized crop, rich in protein, energy and essential nutrients due to high content of fats, carbohydrates, proteins, vitamins and minerals (Simsek et al., 2003; Koksal et al., 2006; Moodley et al., 2007; Alasalvar et al., 2009). The protein quality of African walnuts seed encourages the possibility of its usage as an ingredient in confectionery products (Awofadeju, 2020). The increase in its utilization is largely due to its excellent nutritional value (Ozdemir and Akinci, 2004). Previous study revealed the protein quality of African walnut protein isolate, wheat and yellow maize flours which could be included in Nigerian diet and food industry (Awofadeju, 2020); the protein quality of bread enriched with African walnut and wheat flours (Awofadeju et al., 2018), and nutritional and organoleptic evaluation of cookies produced from African walnut and wheat flours (Awofadeju et al., 2015). The non-use of this underutilized crop for human food constitutes a real economic loss since it is rich in essential nutrients which can be used as value-added ingredients. Despite its richness in lysine and tryptophan amino acids, it is also deficient in methionine which could be found in wheat and yellow maize. Nevertheless, the process of blending the three crops could bring about improved nutrient through complementation (Potter and Hotchkiss, 2006). These could enhance the wheat products with better nutrients and healthy and good functional characteristics. The aim of this study was to optimize a cereal blend (wheat, yellow maize flours and African walnut protein isolate) for cookie making and evaluate its proximate composition and general acceptability.

Fig. 1. Unshelled African walnut seed

Materials and methods

Materials

African walnut seed and yellow maize grains were purchased from local markets in Ibadan; wheat flour, granulated sugar and salt (Dangote brands, Nigeria), powdered milk (Dano), margarine (Evita, Indonesia) and baking powder (STK Industries, Nigeria) were obtained from supermarket in Ibadan, Nigeria.

Methods

Processing of yellow maize flour

The maize flour was prepared following the method of Olanipekun et al. (2015).

Processing of African walnut protein isolate (AWPI)

Walnut kernel was washed thoroughly to remove any adhering contaminants, cooked for 15 min for easy removal of shell and reducing of toxic constituents, cooled, de-shelled, sorted, cut into smaller sizes to increase the surface area and oven dried at 55 °C for 24 h to reduce the moisture content to 8%. Dried nut was ground 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 h. The defatted walnut was grounded to pass through 150 meshes (mm), packaged and stored till further use. The protein isolate derived from African walnut seed was prepared using an alkaline extraction followed by an acid precipitation according to the method adopted by Wagner et al. (2000). Defatted walnut flour (DFWF) was suspended with up to 10-fold (w/v) deionized water coupled with 1mol/LNaOH which was added to reach pH 8.5. Protein was extracted by incubating the suspension for 2 h 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 HCl until a pH value of 4.8 was reached. After centrifuging at 4800 g for 20 min at 4 °C, the precipitate was washed with deionized water and dialyzed 3 times at 4 °C against 0.2mol/L phosphate buffer, pH 8. The protein content of African walnut protein isolate was determined by micro-Kjedhal 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. The variables were wheat flour (30-50%), yellow maize flour (20-30%) and African walnut protein isolate (10-20%). The design generated 20 experimental runs. The response was proximate composition of composite blends.

Proximate analysis of blends and flours

The proximate composition (moisture, protein, fat, ash, fiber and carbohydrate) of the blends was determined using AOAC (2010) methods. Carbohydrate was determined by differences.

Functional properties of composite blends

Determination of bulk density

According to method described by Onwuka (2005), ten gram sample was measured into a 50 mL graduated measuring cylinder. The sample was packed by gently tapping the cylinder on the bench top 10 times from a height of 5 cm. The volume of the sample was recorded in equation 1.

$$Bulk\ density\ (g/mL) = Weight\ of\ sample / Volume\ of\ sample\ after\ tapping \qquad (1)$$

Determination of water absorption capacity (WAC)

The method described by Onwuka (2005) was adopted. About one gram of the flour sample was weighed into a 15 mL centrifuge tube and suspended in 10 mL of distilled water. It was shaken on a platform tube rocker for 1 minute at room temperature. The mixture was allowed to stand for 30 min and centrifuged at 1200 x g for 30 minutes. The volume of free water was read directly from the centrifuge tube as shown in equation 2.

$$WAC\ (\%) = [(amount\ of\ water - free\ water) / (Weight\ of\ sample\ x\ density\ of\ water)]\ x\ 100 \qquad (2)$$

Determination of oil absorption capacity (OAC)

The method of Onwuka (2005) was adopted. One gram of the flour was mixed with 10 mL refined corn oil in a centrifuge tube and allowed to stand at room temperature (30 ± 2 °C) for 1 h. It was centrifuged at 1600 x g for 20 minutes. The volume of free oil was recorded and decanted. Oil absorption capacity was expressed as mL of oil bound by 100 g dried flour as stated in equation 3.

$$OAC\ (\%) = \left[\frac{amount\ of\ oil\ added - free\ oil}{(Weight\ of\ sample\ x\ density\ of\ corn\ oil)}\right] x\ 100 \qquad (3)$$

Cookie preparation

From the optima mixture design, three best flour samples were selected based on protein, ash and fiber content. The flour samples 1, 11 and 17 were used for cookie preparation and sensory evaluation. Cookie was prepared following Abayomi et al. (2013). Fifteen grams of hydrogenated fat and twelve grams of granulated sugar were creamed together at medium speed until light and fluffy appearance was formed. Then 100 g of the composite flour was added with 0.5 g of salt, 2.5 g of powdered milk, 1 g of baking powder (ammonium bicarbonate) and mixed together until uniform smooth dough was obtained. The dough was allowed to rest for 20 min and rolled out to 0.2 cm thickness on a board and cut into round shape about 70 cm using a biscuit cutter. The cookie was placed in a greased tray, and baked in a preheated oven at 180 °C for 10 min.

Sensory evaluation

Sensory analysis was carried out on the cookie prepared from the blends. Twenty-five trained panelists were involved. Quality attributes evaluated were taste, appearance, crunchiness, mouth feel aroma and overall acceptability using a 9-point Hedonic scale, where 9 is like extremely and 1 is dislike extremely. The research followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the institutional human experimentation (Registration Number: UI/EC/19/0620), and that informed consent was obtained.

Statistical analysis

The data was analyzed with the use of a one-way analysis of variance (ANOVA), while, 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 was presented in Table 1. The fat content of defatted walnut flour (DWF) had the highest value, while African walnut protein isolates (AWPI) had the lowest. They were significantly different ($p<0.05$) from each other. The fat contents observed in DWF and AWPI were higher than the ones stated for DWF (1.80%) and WPI (0.23%) (Mao and Hua, 2012). Iyenagbe et al. (2017), also reported lower values of 3.87 and 3.38% for DWF and WPI, respectively. It was indicated that defatted procedure could reduce the fat content of walnut protein isolate effectively. The differences in results may be attributed to various factors and varieties of walnut. Nevertheless, DWF was used as the starting point in the processing of AWPI. The fat content in DWF revealed that walnut is a seed that has oil compared with other flour (Enujiugha and Ayodele-Oni, 2003). According to Awolu et al. (2015), he described high fat content as a factor influencing the life span of flour due to oxidative activities. Therefore, further fat extraction must be improved upon to obtain low value in defatted flour and AWPI. The values recorded were significantly different ($p<0.05$) from each other except DWF and YMF that were not significantly different ($p>0.05$). It was observed that de-fatting decreased the moisture content of AWPI, because it was subjected to drying under mild temperature (45 °C for 3 days) and food materials with low moisture have the benefit of being preserved for an appreciable period of time. The moisture content obtained for DWF was lower (8.90%), while AWPI was higher (8.43%) compared to 9.20 and 4.50% (Mao and Hua, 2012). The moisture content in YMF and WF may be attributed to different factors such as agronomic and environmental. However, the moisture content of the flour was in harmony with values 9-19% reported in the literature (Enyisi et al., 2014a; Enyisi et al., 2014b).

Table 1. Proximate composition of flours

Parameters (%)	Defatted Walnut Flour	Walnut Protein Isolate	Yellow Maize Flour	Wheat Flour
Moisture content	$8.90^{b} \pm 0.20$	$8.43^{c} \pm 0.06$	$9.07^{b} \pm 0.15$	$10.6^{a}7 \pm 0.02$
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

The AWPI had the highest protein content followed by DWF and the lowest was recorded in YMF. The flours were significantly different ($p<0.05$) from each other. It was observed that protein content of AWPI was greater and value for DWF in this course was lower than the reported values of 90.50 and 52.51% (Mao and Hua 2012). Iyenagbe et al. (2017) reported higher and lower contents of 58.86 and 86.92% for DWF and WPI, respectively. This could be attributed to various issues such as varieties as well as origin which can affect protein constituent. The value recorded for DWF in this study was within the range of 25.30 - 27.31% (Aremu et al., 2006; Omosuli et al., 2009). The earlier study in the literature on varieties of maize agreed with the values of yellow maize flour obtained (Oyenuga, 1997). However, the value reported by the scientist was between 9.60 and 10.70%. Furthermore, values reported on various maize varieties (Ijabadeniyi and Adebolu, 2005; Enyisi et al., 2014a; Enyisi et al., 2014b) concurred with this work. Defatted walnut flour had the highest ash content followed by YMF, while AWPI had the lowest. The ash values obtained for DWF was higher and AWPI content was lower than the values reported by (Mao and Hua, 2012). They reported DWF and WPI contents of 1.94 and 2.27%. The increase in ash contents was a reflection of an increase in the mineral content of the flours. Furthermore, the results revealed that African walnut protein isolate is rich in protein having moderate amount of ash which was reflected in DWF ($3.60 \pm 0.15\%$).

The value for crude fiber content in YMF was the highest among the flours. The values of the flours were significantly different ($p<0.05$) from each other. The result obtained for YMF was in accordance with the observations of Ijabadeniyi and Adebolu (2005), who deduced a fiber content of maize varieties in Nigeria to fall between the ranges of 2.07 – 2.97%. There was no value recorded for AWPI and this was in agreement with the work of Iyenagbe et al. (2017). The value of crude fiber recorded was justified by the work of Monilola et al. (2017); also similar to those of other oil seeds (Enujiugha and Ayodele-Oni, 2003). The highest carbohydrate content was found in YMF followed by WF. They were significantly different ($p<0.05$) from each other. Similarly, there was no value recorded for AWPI, while DWF was lesser compared with YMF and WF. The results were expected since YMF and WF are mainly composed of carbohydrate. Chandra and Samsher's (2013) report justified the statement.

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, the results obtained in protein, moisture, ash, fiber, fat and carbohydrate contents were suitable as it indicates the concentration of the protein isolates recovered. The highest value in protein content recorded was close to 91.83% for pigeon pea protein isolate (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).

The carbohydrate and crude fiber present in other flours will complement AWPI when blended together. Also, high content of protein in African walnut protein isolate achieved shows that it could be added to food like ice cream, confectionery products, and baby food to enhance the protein pool and complement each other.

Functional properties of flours

The functional properties of the flours are shown in Table 2. Functional properties ascertain the use of flours in several food products (Adeleke and Odedeji, 2010). The results obtained for YMF in water absorption capacity scored the highest among the flours. They were significantly different ($p<0.05$) from each other. The highest value in YMF may be as a result of low protein and high carbohydrate content present, as carbohydrate had been reported to have impact in WAC of foods (Anthony et al., 2014). It may also be attributed to the higher polar amino acid residues of protein having affinity for water molecules (Yusuf et al., 2007). The WAC properties of blends observed were lower than the values reported for flour (281.35%) and protein isolate (221.83%) extracted from Bambara groundnut (Eltayeb et al., 2011). The values were also lower than the ones stated for unripe cooking banana, pigeon pea and sweet potato flour blends (Ohizua et al., 2017), which ranged from 199.60 – 336.58%. Water absorption capacity is the ability to soak flour with water and can improve the consistency in food.

It is suitable to improve yield and consistency of food which could give body to it (Osundaunsi, 2006). The Results of WAC suggest that the flours (WF, YMF and AWPI) could be found useful in cookies production (Oladele and Aina, 2007). From the presented results, WF, YMF and AWPI flours showed favourable WAC, which could be recommended for the production of ready-to-eat food and baked products.

Table 2. Functional properties of flours

Parameters	Defatted Walnut Flour	Walnut Protein Isolate	Yellow Maize Flour	Wheat Flour
Water Absorption Capacity (%)	$128.33^{d} \pm 2.89$	$146.70^{b}\pm0.89$	$165.00^{a}\pm5.00$	$137.47^{c}\pm1.75$
Oil Absorption Capacity (%)	$113.33^{b} \pm 2.89$	$138.33^{a}\pm2.89$	$138.33^{a}\pm2.89$	$135.93^{a}\pm2.21$
Bulk density (Loosed) (g/mL)	$0.4248^{a}\pm0.00$	$0.3827^{bc}\pm0.00$	$0.3963^{b}\pm0.00$	$0.3710^{c}\pm0.02$
Bulk density (packed) (g/mL)	$0.6912^{b}\pm 0.00$	$0.6736^{c}\pm0.00$	$0.7023^{a}\pm0.00$	$0.6907^{b}\pm0.00$

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

In oil absorption capacity (OAC), DWF scored the lowest value, while AWPI and YMF had the highest, with the same values. There was no significant difference ($p>0.05$), except DWF that differed ($p<0.05$) from other flours. The higher protein contents of AWPI could have aided the protein-protein interactions that contributed to the high OAC of the isolates. The OAC properties of blends observed were lower than the values reported for flour (252.27%), but contradict protein isolate (102.29%) extracted from Bambara groundnut (Eltayeb et al., 2011). The result of AWPI and YMF in this course was higher than 102.29% reported for protein isolate extracted from Bambara groundnut. The values observed are within the range 92.93 – 154.03% stated for unripe cooking banana, pigeon pea and sweet potato flour blends (Ohizua et al., 2017). The presence of protein in the flour exposed more non-polar amino acids to the fat and increased hydrophobicity as a result of flour absorbing more oil (Oluwalana et al., 2012).

The DWF embraced the highest score in bulk density (BD) (loose), while the highest flour in bulk density (packed) was YMF. DWF and WF were the second dominant contents having the same data after DWF. The values in BD loosed were significantly different ($p<0.05$) from each other. In bulk density (packed), defatted walnut and wheat flour were not significantly different ($p<0.05$) from each other. The values of bulk density (packed) recorded for DWF and AWPI in this work are in agreement with the values (0.44 and 0.65 g/mL) reported for protein isolate obtained from raw, boiled and toasted walnut (Iyenagbe et al., 2017). Bulk density is a reflection of load at which the samples can carry if allowed to rest directly on one another. It is generally affected by the particle size and the density of the material (Okpala and Mamah, 2001).

The values of bulk density (loosed and packed) were comparable to the values 0.40 – 0.45 and 0.60 – 0.64 g/mL (Monilola et al., 2017). It has been shown that high bulk density is required for greater ease of dispersibility and reduction in thickness of paste (Amadinkwa, 2012). It also determines the mixing quality of food materials (Lewis, 1990). Low bulk density was an added advantage in preparing balancing foods as it increases nutrient and calorie density per feed of child (Akpata and Akubor, 1999; Eneche and Owheruo, 2015).

Proximate composition of composite blends

The proximate composition of composite blend is shown in Table 3. Among the composite blends, run 14 had the lowest moisture content, while run 3, 5, 7, 13, 19 and 20 had the highest. Similarly, the remaining runs had their values being around 8 and 9%. There was no significant ($p<0.05$) difference in the moisture content. The values obtained for moisture contents could be justified with the values 1.47 – 9.51% (Venkatachalam and Sathe, 2006). Moisture content is a crucial factor in flour which appreciably alters the life span of food product. The percentage of moisture content recorded in this work was lower than the minimum limit (10%) (Shahzadi et al., 2005), indicating that composite flour can be used to make a shelf stable product (Akhtar et al., 2005; Elleuch et al., 2011). The moisture content of flour should be less than 10% as obtained in the work.

The ANOVA result for moisture showed the quadratic model terms (B, C, A^2, B^2, C^2 and AC) interpreted that the model could have a power on the response value. The *lack of fit* was not significant, showing the quadratic regression equation could forecast the response model to be true. The model "Prob>F" value was less than 0.05 showing that the model is significant. Model calibration coefficient Adj – R squared is 0.8577, which means that the model can explain the change in 85.77% response value. Only about 9.91% of the adequate precision could explain the model to navigate design as the ratio was greater than 4. The R-squared (0.9251) is equal to the percentage (Koocheki et al., 2009) and values of R^2 should not be less than 85% or less than 75% (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 response surface curve (3D plot) of the effects of moisture content is presented in Figure 2. The result showed that there is no moisture retention and this will prolong the shelf life of the blends. These could be an added value using the blends in composite flour development.

Table 3. Proximate composition of blends of wheat, yellow maize flours and African walnut protein isolate

Run	A (g)	B (g)	C (g)	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
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	0.90 ± 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	0.96 ± 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	15.37	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

Results represent the average of three replicates ± SD. The mean difference is significant at 0.05 levels. Note: A-wheat; B-yellow maize flour; C-walnut protein isolate

The lowest protein content occurred in run 18, while run 11 scored the highest values. Other runs had their values being around 18 to 29%. More so, run 17 obtained a very close score to run 11. The values of the blends were significantly different ($p<0.05$). However, the formulation of three different types of flour into composite blends yield best proportion in run 11 in terms of best protein content, fat content less than 1% and the highest value in ash 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 as well. 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 an excellent source of protein enhancing the protein content of the blend.

Analysis of variance (ANOVA) for protein declared the quadratic model term of ‘C’ ($p<0.05$), where C is African walnut protein isolate, implies that it could have the power to affect the response value well and is accurate enough to predict the responses. The non-significance *lack of fit* showed that the quadratic regression equation could predict the response value well. A negative value of predicted R^2 (-1.9448) implies that it could only be described by means and a better predictor for the responses. The adequate precision value is 5.613, which indicates an adequate signal and the values suggested that the model could 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 showed an increase in the protein content of the blends when more walnut protein isolate and yellow maize flour as substitutes were used, also, the increment in the substitution of walnut protein isolate and wheat flour decreased the protein content.

The lowest fat content was observed in run 8 and the highest in run 16. The fat contents of the runs were around 1% with no significant difference ($p>0.05$) among them. High fat content would adversely influence the shelf life of the blends due to oxidative activities (Awolu et al., 2015a). The relatively low fat content of the composite blends makes them suitable flours in the formulation of confectionery 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., 1994). Also, it falls within the range of 0.51 – 2.34% for unripe cooking banana, pigeon pea and sweet potato flour blends (Ohizua et al., 2017). The ANOVA result for fat showed that (2FI) model terms (A, C and BC) explain that they could have high impact on the response value. The significant *lack of fit* value of 0.022 is obtained.

The model “Prob>F” value was less than 0.05, which implied that model test was 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, but 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. This showed that the increase in yellow maize substitution induced the increase of fat content in the blends more than walnut protein isolate. At 20% of walnut protein isolate substitution, the value for fat content is 0.94, while at 30% of yellow maize flour is 0.99.

The ash content of run 10 scored the lowest, while the highest value was observed in run 11. The remaining composite blends were within an approximate percentage of 2 to 3%. They were significantly different ($p<0.05$). Ash contents recorded in this work are in agreement with (Ohizua et al., 2017). Ash is an indication of mineral contents of foods. The ANOVA for linear regression response surface model terms for ash content (A and C) described further that the independent variables could have a significant effect to the response value. The non-significant *lack of fit* value is 0.74. The model "Prob>F" value was significant with the p-value of 0.0066 and 0.0068, but accounted for only 54.83% (R^2 = 0.5483). The adequate precision value obtained is greater than 4 and a ratio of 7.724 means that the model can be used to navigate design. The surface response graph is described in Figure 2d. Ash content in the blends increased as there is an increase in walnut protein isolate and wheat flour.

The crude fiber content of run 8 appeared the lowest, while run 4 had the highest value. The remaining runs were around 1%. There is no significant difference ($p<0.05$) within the samples which concord with (Adeyeye et al., 1994). The crude fiber increased as the yellow maize flour in the composite blends increased. This indicates that the composite blends are rich in fiber which could be used in processing of functional food products. The intake of fiber-rich food (soluble fiber) 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 fibers fight against constipation and reduce the risk of colon cancer (Islam et al., 2007). However, these values are still low for rich-fiber foods when compared with the suggested ranges of 20 – 35 g/day for adults and elders and 5 g/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 fiber recommended daily allowance (RDA).

The ANOVA result of crude fiber showed that linear and quadratic model terms A, B, C, B^2 and AC (as independent variables) have a significant effect to the response surface value. The obtained *lack of fit* value is 0.028. 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 claim that the values are acceptable for fitting a model. Adequate precision value is greater than 4 which indicates that the design is adequate. The 3D response surface plot shows the effect of variables on crude fiber as shown in Figure 2e; all variables had positive effect on the fiber content. The actual factor of maize is 25.00. The crude fiber increased with the decrease in walnut protein isolate and wheat flour substitution. At 10% substitution of walnut protein isolate, crude fiber increased to 0.80, while at 20% the crude fiber decreased to 0.78.

Carbohydrate content in run 10 scored the lowest, while run 18 had the highest value 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 increased the carbohydrate content of the blends. This is because carbohydrate content was not detected in AWPI. The ANOVA result of carbohydrate revealed the 2FI model term could predict the response value well. The *lack of fit* is not significant and a negative predicted R-squared (-1.6695) obtained implies that they could only be described by their means.

Considering the results of the high protein and fiber, and ash content, the best three blends are run 1 (50.00 g of wheat flour, 30.00 g of yellow maize flour, and 20.00 g of walnut protein isolate), run 11 (45.24 g of wheat flour, 28.27 g of yellow maize flour and 26.48 g of walnut protein isolate) and run 17 (46.04 g of wheat flour, 38.59 g of yellow maize flour, and 15.37 g of walnut protein isolate) were used for cookie making.

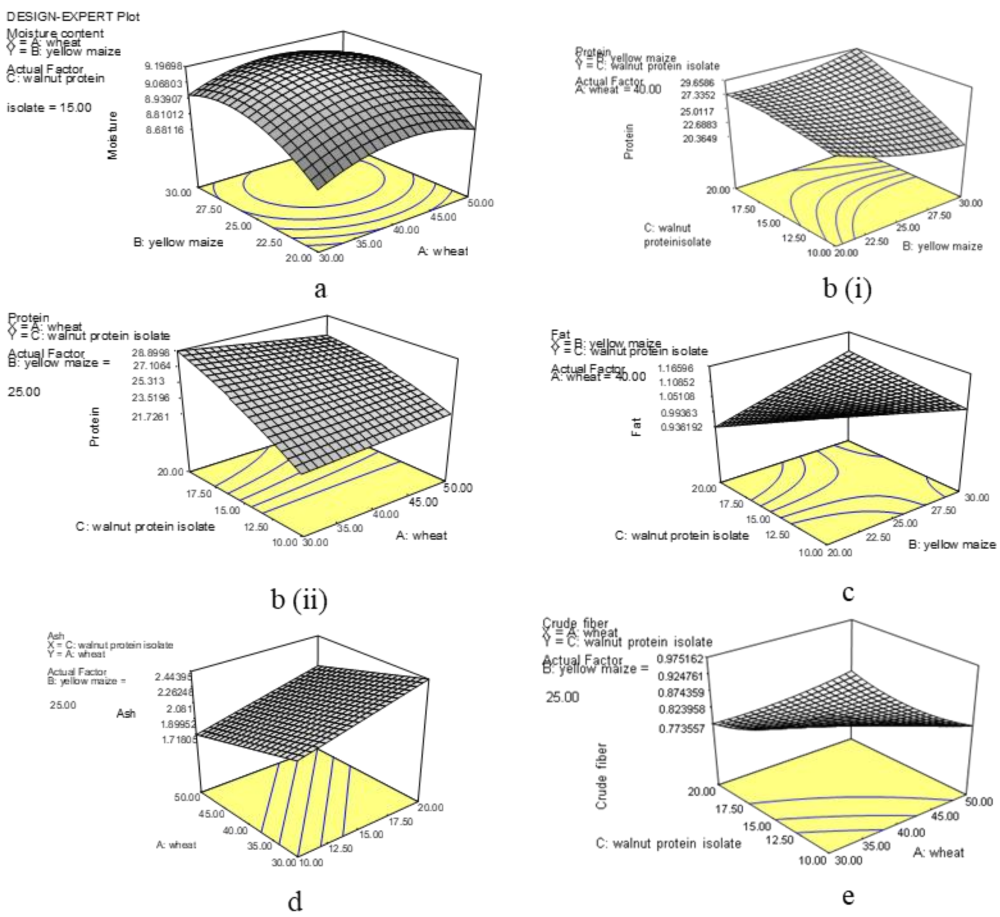

Fig. 2. a - e: 3D surface plots showing the effect of proximate composition on blends (wheat flour, yellow maize flour and walnut protein isolate) and dependent [(a) moisture (b) protein (c) fat (d) ash (e) crude fiber)] variables displaying significant differences

Sensory evaluation of wheat based cookies

Mean sensory scores of cookies prepared from wheat-yellow maize flour enriched with walnut protein isolate are presented in Table 4. It was revealed that sample 17 was generally accepted. The result also showed that moderate substitution of walnut protein isolate resulted in better overall acceptability. The incorporation of walnut protein isolate yields better taste, appearance, crunchiness, mouth feel and aroma. The statement was judged by Barber and Obinna-Echem (2016), who assessed the nutritional composition, physical and sensory properties of wheat-African walnut cookies and stated that the African walnut flour should be used successfully as a partial substitute for wheat flour at a range of 5-15%. Therefore, as the substitution of walnut protein isolate increased above 20%, the level of acceptability decreased. Sample 1 was next to sample 17 in terms of overall acceptability, while sample 11 was rated the lowest in terms of taste, crunchiness, mouth feel, aroma and overall acceptability. However, significant differences ($p<0.05$) exist between sample 11 and other samples. Consumer acceptability is a major factor for selecting a product and a texture, a taste, and an appearance of a biscuit are among the main characteristics related to quality (Omoba et al., 2013).

Table 4. Mean sensory scores of enriched cookies

Sample	Taste	Appearance	Crunchiness	Mouth feel	Aroma	Overall acceptability
1	6.27^{b}	5.53^{ab}	6.40^{b}	6.27^{b}	6.33^{b}	6.37^{b}
11	5.55^{a}	4.67^{a}	5.53^{a}	5.40^{a}	5.53^{a}	5.40^{a}
17	6.27^{b}	6.13^{b}	6.67^{b}	6.33^{b}	6.20^{b}	6.67^{b}

Mean with the same alphabet are not significantly ($p<0.05$) different

Conclusion

The findings in this study revealed that the composite blends enhanced the nutrients (protein, ash and fiber) in cookies. Therefore, it can be deduced that the blend of African walnut protein isolate, yellow maize and wheat flours could serve as a good substitute to 100% wheat flour in cookie preparation. In particular, composite flour with 45.24 g wheat flour, 37.79 g yellow maize flour and 15.37 g African walnut protein isolate showed exceptional flour and overall acceptability. This blend had an acceptable ratio of African walnut protein isolate which contributes to the overall acceptability. However, sample with 45.24 g wheat flour, 28.27 g yellow maize flour 26.48 g African walnut protein isolate was next in terms of protein and ash content. The process of formulating the flours into composite blends would bring about changes in nutrients. Also, the best proportion of composite blends for cookie making was established. Conclusively, the use of these locally grown crops will go a long way in reducing dependence on wheat flour, thereby, reducing foreign exchange used in importing wheat. It will also add more value to Nigerian bakeries and Nigeria at large.

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