Evaluation of fermented African yam bean flour composition and influence of substitution levels on properties of wheat bread

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Abstract: The composition (proximate, amino acids, *in vitro* protein digestibility [IVPD]), antinutritional factors (ANFs), functional properties, and antioxidant activity of fermented African yam bean flour (FAYBF) were determined in this study, and the effect of substituting FAYBF on the properties (nutritional, physical, and functional) of bread was investigated. Fermentation significantly ($P \leq 0.05$) increased the levels of nutrients, IVPD, total phenolic content (TPC), and antioxidant activity in the flour, with significant ($P \leq 0.05$) reduction in ANFs. The water absorption capacity (WAC) and oil absorption capacity (OAC), and swelling capacity of the flour increased after fermentation, while bulk density decreased. Substitution of wheat flour with FAYBF increased WAC and OAC, while peak viscosity decreased. Composite breads had higher nutritional, IVPD, TPC, and antioxidant activity than 100% wheat bread. The study demonstrates that FAYBF could be explored for the preparation of wheat-based bread, with reduced gluten levels.

Keywords: African yam bean flour, bread, fermentation, legume, nutritional composition

Practical Application: Bread is a staple food and this study can assist in increasing the utilization of neglected leguminous crops as well as addressing the challenge of malnutrition, prevalent in developing countries.

1. INTRODUCTION

According to Azeke, Fretzdorff, Buening-Pfaue, Holzapfel, and Betsche (2005), the African yam bean (AYB, *Sphenostylis stenocarpa*) is considered an underutilized leguminous plant in the humid tropics, mostly cultivated in Central, East, and West Africa for its seeds. The seeds are known to contain up to 29% protein, 4.7 to 5.3% crude fiber, and 50% carbohydrate, and are a rich source of most essential micronutrients (Eromosele, Arogundade, Eromosele, & Ademuyiwa, 2008). AYB seed protein contains an appreciable amount of most essential amino acids (Oshodi, Ipinmoroti, Adeyeye, & Hall, 1995), with relatively higher levels of some essential amino acids when compared to some other legumes (Ene-Obong & Carnovale, 1992).

AYB seeds, like most legumes, have been associated with several factors that limit their utilization in food products. These include their hard-to-mill and hard-to-cook attributes as well as long cooking time, which negatively affect their utilization and limit their consumption (Gwala et al., 2019). Aside these aforementioned factors, the presence of α-galactosides, soluble fiber (Oboh et al., 2000), and antinutritional factors (ANFs) decreases protein digestibility and nutrient bioavailability (Ene-Obong, 1995).

To address the challenges limiting the use of AYB seeds, some processing methods have been adopted and these include soaking, dehulling, germination, heat treatment, and natural fermentation (Azeke et al., 2005; Ene-Obong & Obizoba, 1996; Oboh et al., 2000). Fermentation is an age-long food processing technique that reduces ANFs and flatulence and also improves the nutritional quality, health promoting properties, and antioxidant activity of legumes (Adebiyi, Njobeh, & Kayitesi, 2019; Chandra-Hioe, Wong, & Arcot, 2016; Olukomaiya et al., 2020). Particularly, solid-state fermentation (SSF) with yeast (*Saccharomyces cerevisiae*) has been identified as a viable bioprocessing method reported to improve the functionality of foods (Adebo, 2020; Ilowefah, Bakar, Ghazali, & Muhammad, 2017; Moreno, Cuevas-Rodriguez, Milan-Carrillo, Cardenas-Valenzuela, & Barron, 2004). Although SSF of AYB seed has been studied, particularly regarding the flatulence potential of the flour (Azeke, Fretzdorff, Buening-Pfaue, & Betsche, 2007), the effect of SSF on the nutritional composition, ANFs, antioxidant activity, and functional properties of AYB seed has not been studied.

In recent times, there have been numerous research efforts to provide cheap and value-added quality food ingredients with potential health benefits. In developing countries, there is growing research interest in fortifying wheat bread with processed legumes, especially fermented legume flour, to increase nutrient the intake of high-quality protein and bioactive ingredients, and to take advantage of bread as a carrier of health-promoting compounds (Shrivastava & Chakraborty, 2018). Though there are few reports

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doi: 10.1111/1750-3841.15527

Journal of Food Science A Publication of the Institute of Food Technologists

on the inclusion of fermented legume flour in wheat-based bread (Bourré et al., 2019; Hallén, Ibanoğlu, & Ainsworth, 2004; Kefalas et al., 2009; Rizzello, Calasso, Campanella, De Angelis, & Gobbetti, 2014; Shrivastava & Chakraborty, 2018), to the best of our knowledge, fermented African yam bean flour (FAYBF) as an ingredient in bread making has not been explored. Therefore, the objective of the study was to determine the effect of SSF on the functional properties, nutritional composition, and ANFs of AYB flour and to investigate the nutritional composition and the functional properties of the breads prepared from wheat-FAYBF blends.

2. MATERIALS AND METHODS

2.1 Materials

AYB seeds (brown variety) and food grade yeast (*S. cerevisiae*) (Angel Yeast Co., Yichang Hubei, China) were purchased from local markets in Nigeria. All chemicals used were of analytical grade.

2.2 Preparation of RAYBF

The AYB seeds were cleaned to remove dirt and debris, and the cleaned seeds were subsequently steeped (in order to increase the moisture content of the seeds and facilitate dehulling) in water (1:4, w/v) for 12 hr and drained. The soaked beans were manually dehulled, washed with water, and dried (Gallenkamp 300 series, Widnes, Cheshire, UK) at 45 °C for 24 hr. The dried seeds were milled and sieved (mesh size 100 μm) to obtain raw African yam bean flour (RAYBF).

2.3 Solid state fermentation of RAYBF

Fermented AYBF was prepared as described by Ilowefah et al. (2017). To 1 g of dry yeast (*S. cerevisiae*), 65 mL of water was added, and the suspension was poured into 100 g of the RAYBF and mixed for 2 min. The resulting mixture was covered with aluminum foil (Magic Wrap, Yuyao, Zhejiang, China) and fermented in a fermentation cabinet (National MEG CO, Lincoln, NE, USA) for 16 hr at 27 °C and 85% relative humidity. The fermented AYB batter was dried (Gallenkamp 300 series, Widnes, Cheshire, UK) at 45 °C for 24 hr, and the dried flour was blended and sieved (mesh size 100 μm) to obtain FAYBF.

2.4 pH determination

Deionized water (8 mL) was added to 2 g of the flour. The pH of the mixture was measured by using a calibrated pH meter (PHS-25; TECHMEL, Texas, USA).

2.5 Functional properties analysis

2.5.1 Bulk density. The flour samples were weighed into a 10-mL graduated cylinder and continuously but gently tapped until there was no further diminution in the sample level. The bulk density (g/cm^3) was expressed as the weight of sample per volume of sample (Escamilla-Silva, Guzman-Maldonado, Cano-Medinal, & Gonzalez-Alatorre, 2003).

2.5.2 Swelling capacity. The samples were filled up to the 10 mL mark in a 100-mL graduated cylinder, while distilled water was added to bring the total volume to 50 mL. The top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. The suspension was later inverted after 2 min and allowed to stand for a further 30 min. The volume occupied by the sample was taken after 30 min as the swelling capacity (SC) (Okaka & Potter, 1977).

2.5.3 Water and oil absorption capacity. For water absorption capacity (WAC), 10 mL of distilled water was added to 1 g of flour in a weighed centrifuge tube, mixed, and kept at ambient temperature for 30 min. The resulting mixture was centrifuged at 2,000 × *g* for 10 min and the supernatant decanted. For oil absorption capacity (OAC), refined sunflower oil replaced the water. Both WAC and OAC were calculated as the difference between the initial and final weights after the water/oil added has been decanted (Sosulki, Garratt, & Slinkard, 1976).

2.5.4 Pasting parameters. Pasting parameters were determined using a rapid visco analyzer (RVA) (Newport Scientific Pty Ltd, New South Wales, Australia) according to Chinma et al. (2016). Briefly, 2.5 g of flour was weighed into a canister and 25 mL of distilled water was added. The suspension was mixed, placed in RVA, initially kept at 50 °C for 1 min, then heated to 95 °C at 12.2 °C/min and held at 95 °C for 2.5 min. It was later cooled to 50 °C at the rate of 11.8 °C/min and held for 2 min. The RVA parameters determined were paste viscosities (peak, trough, breakdown, final, and setback viscosity), peak time, and pasting temperature.

2.6 Bread preparation

Bread was prepared following the straight-dough method of Chinma et al. (2016) with slight modification in terms of the quantity of flour and ingredients used. The recipe was as follows: 200 g wheat flour (WF) (Golden Penny Plc., Lagos, Nigeria), 10 g shortening (margarine) (Blue Band, Unilever Plc., Lagos, Nigeria), 5 g sugar (Dangote Refinery Plc, Lagos, Nigeria), 2 g salt (Dangote Refinery Plc, Lagos, Nigeria), 5 g dry yeast (*S. cerevisiae*) (Angel Yeast Co., Yichang Hubei, China), and 125 mL water. Appropriately weighed composite flours (100% WF, 95% WF:5% FAYBF, 90% WF:10% FAYBF, 85% WF:15% FAYBF and 80% WF: 20% FAYBF), sugar and salt were poured into a bread mixer and thoroughly mixed for 10 min. Yeast (*S. cerevisiae*) was suspended in 5 mL of water at room temperature for 3 min before added into the mixer. Margarine was added and mixed for 3 min. The dough formed after proper mixing was weighed, cut into uniform pieces (100 g), manually kneaded, molded, placed in greased baking pans and proofed (Gallenkamp 300 plus series; Widnes, Cheshire, UK) at 35 °C for 36 min. After fermentation, baking was done in a thermostatically controlled baking oven (Gallenkamp, UK) at 180 °C for 35 min. Breads were cooled at ambient temperature for 2 hr prior to further analysis.

2.7 Nutritional analysis

Moisture, protein, fat, ash, and fiber content were determined according to AOAC (2005) methods, while total carbohydrate content was calculated by difference. Moisture content was determined by drying at 105 °C in an oven (Gallenkamp 300 series; Widnes, Cheshire, UK) until constant weight (method No. 925.09B). Protein was determined using the micro-Kjeldahl method, after the three steps of digestion, distillation, and titration. The nitrogen value was corrected and multiplied by a factor of 6.25 to obtain the protein value (method No. 992.23). Ash was determined by incinerating the samples in a muffle furnace at 550 °C for 24 hr (method No. 923.03). Crude fat was estimated by extraction of the sample with petroleum ether in a Soxhlet extraction apparatus (method No. 920.39C), while crude fiber was determined by digesting the sample with diluted acid and alkali (method No. 962.09E). Carbohydrate content was determined by the difference. Total energy value was calculated using the Atwater factors [energy value (kcal) = (% protein × 4 + %

carbohydrate × 4 + % fat × 9)] as described by FAO (2003). Mineral (calcium (Ca), iron (Fe) magnesium (Mg), and zinc (Zn)) compositions were determined using an atomic absorption spectrophotometer (Perkin-Elmer Model 2380, USA), while phosphorus (P) and potassium (K) were determined using the flame photometric method (AOAC 2005).

Amino acid composition of the flour and bread samples was determined using the Pico-Tag method described by Chinma, Ilowefah, Shammugasamy, Ramakrishnan, and Muhammad (2014). The flour and bread samples were hydrolyzed at 116 °C with 6 M HCl for 24 hr. The *in vitro* protein digestibility (IVPD) of the samples was determined using a modified method described by Tanaka, Adoracion, Juliano, and Bechtel (1978). Briefly, flour (200 mg) was added to a 100-mL Erlenmeyer flask containing 35 mL 0.1 M sodium citrate tribasic dihydrate (pH 2.0) with pepsin (1.5 g pepsin/L). The mixture was incubated in a water bath at 37 °C for 2 hr, centrifuged at 10,000 × *g* for 15 min, and the supernatant decanted. The residue was washed, dried, and analyzed for nitrogen content. The IVPD was calculated as the percentage of protein in the supernatant divided by the total protein content of the sample.

2.8 Determination of antinutritional factors

Phytic acid was determined as described by AOAC (2005). The quantity of phytate was calculated from the standard curve (using phytic acid), with results expressed as milligram phytic acid per gram. Tannin content was determined using the Folin–Denis method (AOAC, 2005). Tannic acid was used as standard. Results obtained were expressed as gram per 100 g dry sample. Trypsin inhibitory activity (TIA) was determined as described by Liu and Markakis (1989) using Nα–benzoyl–L–arginine 4–nitroanilide hydrochloride (BAPA) at 0.04% (w/v) as the trypsin substrate. Trypsin inhibitor activity (TIA) was expressed as trypsin inhibitor unit/mg sample. The absorbance was read at 410 nm, using a spectrophotometer, and results were expressed as the amount of inhibitor that reduced the absorbance per minute of the standard reaction by 0.01 (Liu & Markakis, 1989).

2.9 Total phenolic content and antioxidant activity

Methanolic extract (ME) of RAYBF, FAYBF and bread samples was prepared according to the method of Chinma et al. (2014). Briefly, 0.2 g of each sample was extracted twice with 4 mL of 80% methanol, placed in a shaking water bath at 40 °C for 2 hr and centrifuged at 2 000 × *g* for 10 min. The total phenolic content (TPC) was determined according to the method described by Singleton and Rossi (1965) and results were expressed in milligrams of gallic acid equivalent per gram of dry weight. The DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging activity was determined using the method of Brand-Williams, Cuvelier, and Berset (1995). A 2.8 mL of DPPH solution was added to 0.2 mL of a standard/sample extract, the mixture was kept in the dark for 30 min, and the absorbance was measured at 516 nm. Trolox was used as standard and the DPPH radical scavenging activity was expressed as Trolox equivalents (μmol TE/g of dry sample).

2.10 Determination of physical properties of bread

The weight and the loaf volume of respective bread loaves were determined by adopting the rapeseed displacement method (AACC, 2000). Specific volume (SV) was calculated by dividing loaf volume by loaf weight (AACC, 2000).

Table 1– pH, proximate composition (dry basis), mineral composition, amino acids, in vitro protein digestibility, antinutritional factors, total phenolic content, and antioxidant activity of raw and fermented African yam bean flour.

Parameters	Raw flour	Fermented flour
pH	6.45 ± 0.01[a]	5.89 ± 0.01[b]
Proximate composition		
Moisture (%)	9.21 ± 0.30[a]	9.32 ± 0.22[a]
Protein (%)	21.33 ± 0.56[b]	25.02 ± 0.28[a]
Fat (%)	5.80 ± 0.07[a]	4.34 ± 0.04[b]
Ash (%)	3.14 ± 0.10[b]	3.57 ± 0.01[a]
Fiber (%)	5.20 ± 0.05[a]	4.43 ± 0.02[b]
Carbohydrate (%)	55.32 ± 0.11[a]	53.28 ± 0.06[b]
Minerals		
Calcium (mg/100 g)	86.34 ± 0.51[b]	98.22 ± 0.30[a]
Iron (mg/100 g)	4.55 ± 0.20[b]	6.90 ± 0.25[a]
Magnesium (mg/100 g)	161.20 ± 0.75[b]	175.35 ± 0.49[a]
Phosphorus (mg/100 g)	280.46 ± 0.50[b]	290.56 ± 0.80[a]
Potassium (mg/100 g)	1462.11 ± 1.01[b]	1483.74 ± 0.93[a]
Zinc (mg/100 g)	2.68 ± 0.20[b]	3.58 ± 0.11[a]
Essential amino acids		
Histidine	4.13 ± 0.03[b]	4.26 ± 0.02[a]
Isoleucine	4.48 ± 0.01[b]	4.79 ± 0.04[a]
Leucine	7.66 ± 0.00[b]	7.82 ± 0.01[a]
Lysine	7.48 ± 0.01[b]	7.63 ± 0.01[a]
Methionine	1.19 ± 0.00[b]	1.29 ± 0.02[a]
Phenylalanine	5.56 ± 0.03[b]	5.71 ± 0.01[a]
Threonine	5.94 ± 0.01[a]	5.75 ± 0.03[b]
Valine	5.38 ± 0.04[a]	4.51 ± 0.01[b]
Nonessential amino acids		
Alanine	4.42 ± 0.01[a]	4.40 ± 0.01[a]
Arginine	5.27 ± 0.02[a]	5.12 ± 0.02[b]
Aspartic acid	11.33 ± 0.07[a]	11.30 ± 0.04[a]
Cysteine	1.85 ± 0.01[a]	1.87 ± 0.01[a]
Glutamic acid	15.61 ± 0.12[a]	15.40 ± 0.07[b]
Glycine	4.77 ± 0.01[a]	4.78 ± 0.00[a]
Proline	4.82 ± 0.03[b]	4.93 ± 0.02[a]
Serine	5.03 ± 0.02[b]	5.70 ± 0.03[a]
Tryosine	4.11 ± 0.01[b]	4.27 ± 0.01[a]
Total amino acid	99.02	99.53
IVPD (%)	71.02 ± 0.65[b]	78.14 ± 0.57[a]
Antinutritional factors		
Phytic acid (mg/g)	1.33 ± 0.18[a]	0.80 ± 0.11[b]
Tannin (mg/100 g)	1.26 ± 0.10[a]	0.99 ± 0.50[b]
TIA (TIU/mg)	3.24 ± 0.11[a]	1.36 ± 0.15[b]
Total phenolic content and antioxidant activity		
TPC (mg GAE/g sample)	2.75 ± 0.11[b]	13.96 ± 0.23[a]
DPPH (μmol TE/g)	1.48 ± 0.13[b]	12.35 ± 0.29[a]

Notes. Values represent mean and standard deviation of triplicate determinations. Values in the same row with different superscript are significantly ($P < 0.05$) different. TIA, trypsin inhibitory activity; TPC, total phenolic content; DPPH–1, 1–diphenyl–2–picryl–hydrazil radical scavenging activity; IVPD, *in vitro* protein digestibility.

2.11 Color profile of bread crumb

Bread crumb color attributes were measured using a chroma meter (CR-410; Konica-Minolta, Tokyo, Japan) and color parameters determined were lightness (*L*), redness (+*a*), greenness (−*a*), yellowness (+*b*), and blueness (−*b*). A white tile with *L*, *a*, and *b* values of 97.30, 0.10, and 0.13, respectively, was used as standard.

2.12 Statistical analysis

All analyses were conducted in triplicates and data obtained were subjected to analysis of variance (ANOVA) using SPSS version 20 (IBM, Armonk, NY, USA). Differences among the means of the investigated parameters were separated using Tukey's test at 5% probability.

Table 2–Functional properties of raw African yam bean flour, fermented African yam bean flour, wheat, and fermented African yam bean flour blends.

Parameter	RAYBF	FAYBF	100WF	95WF: 5FAYBF	90WF: 15FAYBF	85WF: 10FAYBF	80WF: 20FAYBF
Bulk density (g/cm^3)	0.70 ± 0.02^a	0.59 ± 0.01^b	NA	NA	NA	NA	NA
Water absorption capacity (g/g)	1.02 ± 0.01^g	1.86 ± 0.03^f	2.08 ± 0.07^e	2.30 ± 0.01^d	2.35 ± 0.01^c	2.38 ± 0.01^b	2.43 ± 0.05^a
Oil absorption capacity (g/g)	1.24 ± 0.01^b	1.20 ± 0.01^c	1.01 ± 0.01^e	1.14 ± 0.01^d	1.16 ± 0.02^d	1.14 ± 0.01^d	1.80 ± 0.02^a
Swelling capacity (mL/g)	0.48 ± 0.03^d	0.64 ± 0.01^c	0.83 ± 0.01^a	0.80 ± 0.02^a	0.75 ± 0.03^b	0.73 ± 0.01^b	0.72 ± 0.01^b

Notes.Values represent mean and standard deviation of triplicate determinations. Values with different superscript in a row are significantly ($P \leq 0.05$) different from each other. RAYBF, raw African yam bean flour; FAYBF, fermented African yam bean flour; 100WF, 100% wheat flour; 95WF:5FAYBF, 95% wheat flour:5% fermented African yam bean flour; 90WF:10FAYBF, 90% wheat flour: 10% fermented African yam bean flour; 85WF:15FAYBF, 85% wheat:15% fermented African yam bean flour; 80WF:20FAYBF, 80% wheat flour:20% fermented African yam bean flour; NA, not applicable.

Table 3–Proximate composition (on dry basis), mineral composition, amino acids, in vitro protein digestibility, total phenolic and antioxidant activity of bread supplemented with fermented African yam bean flour.

Parameter Proximate composition	100WF	95WF: 5FAYBF	90WF: 10FAYBF	85WF: 15FAYBF	80WF: 20FAYBF
Moisture (%)	30.47 ± 1.06^b	29.70 ± 0.97^c	26.39 ± 1.72^d	31.32 ± 0.67^a	31.40 ± 0.90^a
Protein (%)	11.83 ± 0.19^e	13.05 ± 0.14^d	14.29 ± 0.24^c	15.60 ± 0.17^b	17.15 ± 0.11^a
Ash (%)	1.25 ± 0.01^e	1.68 ± 0.05^d	1.71 ± 0.10^c	1.87 ± 0.04^b	1.99 ± 0.05^a
Fiber (%)	2.04 ± 0.08^e	2.30 ± 0.05^d	2.48 ± 0.03^c	2.60 ± 0.02^b	2.98 ± 0.02^a
Fat (%)	3.70 ± 0.03^e	4.14 ± 0.06^d	4.35 ± 0.11^c	4.73 ± 0.08^b	4.94 ± 0.05^a
Carbohydrate (%)	50.71 ± 0.20^a	49.13 ± 0.06^b	50.78 ± 0.13^a	43.88 ± 0.06^c	41.54 ± 0.11^d
Energy value (kcal)	281.30 ± 1.13^b	285.98 ± 1.77^a	277.67 ± 1.90^e	280.49 ± 1.24^c	279.22 ± 1.47^d
Minerals					
Calcium (mg/100 g)	43.94 ± 0.42^e	44.50 ± 0.20^d	46.28 ± 0.41^c	47.66 ± 0.29^b	48.10 ± 0.85^a
Iron (mg/100 g)	2.06 ± 0.11^e	2.94 ± 0.13^d	3.23 ± 0.10^c	4.11 ± 0.14^b	4.62 ± 0.10^a
Phosphorus (mg/100 g)	241.80 ± 2.11^e	256.53 ± 2.08^d	269.66 ± 1.17^c	272.80 ± 0.89^b	280.94 ± 1.66^a
Potassium (mg/100 g)	266.25 ± 1.23^e	280.10 ± 0.95^d	311.42 ± 1.17^c	328.36 ± 1.20^b	342.70 ± 1.03^a
Magnesium (mg/100 g)	78.33 ± 0.60^e	81.27 ± 0.74^d	98.50 ± 0.82^c	103.42 ± 0.50^b	112.70 ± 0.49^a
Zinc (mg/100 g)	0.79 ± 0.02^e	1.02 ± 0.01^d	1.18 ± 0.01^c	1.27 ± 0.02^b	1.44 ± 0.01^a
Essential amino acids					
Histidine	1.17 ± 0.01^e	1.32 ± 0.02^d	1.51v0.01^c	1.80 ± 0.04^b	2.11 ± 0.02^a
Isoleucine	0.63 ± 0.05^e	1.02 ± 0.00^d	1.29 ± 0.03^c	1.45 ± 0.02^b	1.79 ± 0.01^a
Leucine	3.10 ± 0.07^e	3.31 ± 0.04^d	3.54 ± 0.06^c	3.66 ± 0.08^b	3.81 ± 0.07^a
Lysine	1.35 ± 0.05^e	1.74 ± 0.03^d	2.23 ± 0.08^c	2.45 ± 0.01^b	2.76 ± 0.01^a
Methionine	0.84 ± 0.02^d	1.19 ± 0.01^c	1.40 ± 0.03^b	1.66 ± 0.05^a	1.72 ± 0.06^a
Phenylalanine	2.40 ± 0.01^e	2.63 ± 0.03^d	2.89 ± 0.01^c	3.13 ± 0.02^b	3.54 ± 0.03^a
Threonine	1.29v0.00^e	1.41 ± 0.05^d	1.60 ± 0.01^c	1.79 ± 0.03^b	1.97 ± 0.02^a
Valine	0.67 ± 0.05^e	1.02 ± 0.01^d	1.29 ± 0.03^c	1.42 ± 0.02^b	1.66 ± 0.04^a
Nonessential amino acids					
Alanine	1.08 ± 0.00^e	1.24 ± 0.01^d	1.40 ± 0.01^c	1.65 ± 0.04^b	1.81 ± 0.01^a
Arginine	1.54 ± 0.09^e	1.70 ± 0.05^d	1.98 ± 0.07^c	2.13 ± 0.10^b	2.50 ± 0.06^a
Aspartic acid	1.99 ± 0.00^e	2.21 ± 0.01^d	2.53v0.02^c	2.90 ± 0.01^b	3.22 ± 0.03^a
Cysteine	0.25 ± 0.01^e	0.43 ± 0.03^d	0.81 ± 0.06^c	1.10v0.05^b	1.46 ± 0.02^a
Glutamic acid	4.81 ± 0.04^e	5.15 ± 0.05^d	5.44v0.10^c	5.96 ± 0.08^b	6.23 ± 0.06^a
Glycine	2.20 ± 0.01^e	2.46 ± 0.03^d	2.67 ± 0.05^c	2.80 ± 0.03^b	2.94 ± 0.03^a
Proline	5.95 ± 0.14^e	6.20 ± 0.10^d	6.48 ± 0.13^c	6.53 ± 0.10^b	6.70 ± 0.12^a
Serine	1.18 ± 0.01^c	1.32 ± 0.00^b	1.55 ± 0.01^{ab}	1.69 ± 0.01^a	1.87 ± 1.01^a
Tryosine	1.36 ± 0.00^e	1.60 ± 0.01^d	1.84 ± 0.01^c	1.97 ± 0.00^b	2.11 ± 0.02^a
IVPD (%)	77.05 ± 0.88^e	80.19 ± 0.73^d	81.70 ± 0.65^c	82.16 ± 0.53^b	82.60 ± 0.92^a
Total phenolic content and antioxidant activity					
TPC (mg GAE/g)	0.44 ± 0.05^b	0.77 ± 0.02^a	0.85 ± 0.08^a	0.93 ± 0.06^a	0.98 ± 0.04^a
DPPH(μmol TE/ 100 g)	101.60 ± 0.83^e	133.42v0.77^d	140.16 ± 0.94^c	152.10 ± 0.58^b	170.47 ± 0.63^a

Notes.Values represent mean and standard deviation of triplicate determinations. Mean values with different superscript in a row are significantly ($P \leq 0.05$) different from each other. 100WF, 100% wheat flour (control sample); 95WF:5FAYBF, 95% wheat flour:5% fermented African yam bean flour; 90WF:10FAYBF, 90% wheat flour:10% fermented African yam bean flour; 85WF:15FAYBF, 85% wheat: 15% fermented African yam bean flour; 80WF:20FAYBF, 80% wheat flour:20% fermented African yam bean flour; TPC, total phenolic content; DPPH–1, 1-diphenyl-2-picryl-hydrazil radical scavenging activity.

3. RESULTS AND DISCUSSION

3.1 Proximate composition, antinutritional factors, total phenolic content, and antioxidant activity of raw and fermented flour

The proximate composition, amino acids, *in vitro* protein digestibility (IVPD), ANFs, TPC, and antioxidant activity of RAYBF and FAYBF are presented in Table 1. There was a reduction in pH of RAYBF (6.45) after fermentation (5.89), which is in line with the reports of Chandra-Hioe et al. (2016) for fermented chickpea and faba bean flour. The reduction in pH value after fermentation may be due to the degradation of carbohydrate and nutrients by microorganisms, resulting in the accumulation of organic acids, which increased the acidity. RAYBF had an initial protein content of 21.33%, which increased by 17.3% in FAYBF. This result contradicts the findings of Ene-Obong and Obizoba (1996),

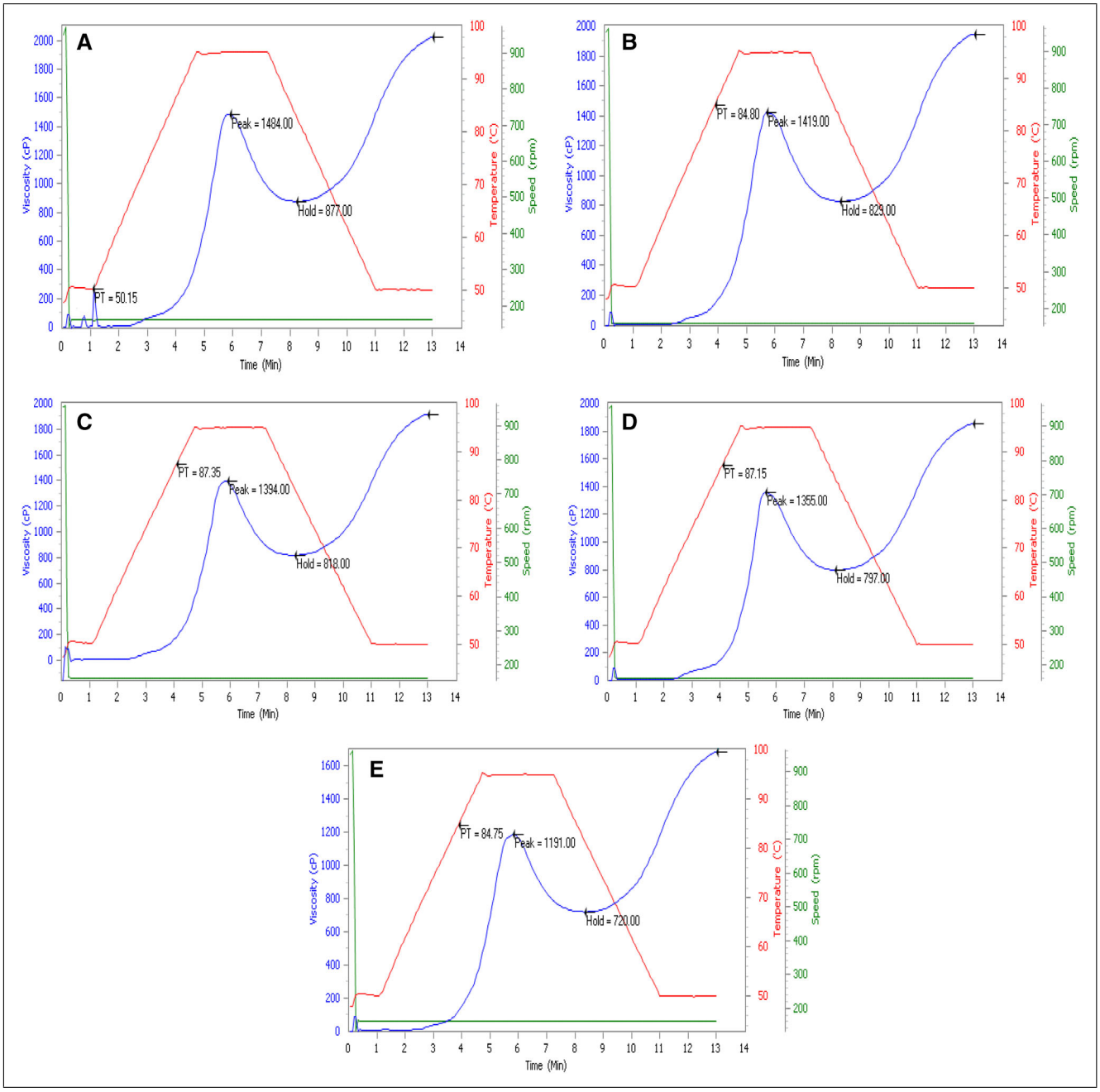

Figure 1–Pasting profiles of (A) 100% wheat flour, (B) 95% wheat flour:5% fermented African yam bean flour, (C) 90% wheat flour:10% fermented African yam bean flour, (D) 85% wheat flour:15% fermented African yam bean flour, and (E) 80% wheat flour:20% fermented African yam bean flour.

who reported that fermentation had no effect on the crude protein content of the AYB, though an increase in protein content during lupin fermentation with dry yeast has been reported (Kasprowicz-Potocka et al., 2016). The increase in the protein content of FAYBF could be attributed to a decrease in the carbon ratio in the total mass (Onyango, Noetzold, Bley, & Henle, 2004). Fermenting microorganisms are known to utilize carbohydrates as an energy source and produce carbon dioxide as a by-product and, as such, cause the nitrogen in a fermented product to be concentrated, thereby increasing the proportion of protein in the total mass (Cui, Li, & Liu, 2012). In addition, the increase in protein content may be due to yeast proliferation or accumulation of its cells (Ilowefah et al., 2017).

Fat content of RAYBF was observed to reduce by 25.17% after fermentation, which may be attributed to the breakdown of lipids by lipase enzymes during fermentation (Adebiyi et al., 2019) and use of lipids by fermenting organisms as food source. Ash content in FAYBF was increased by 13.69% compared to RAYBF, which may be attributed to an increase in dry matter loss caused by enzyme activities and yeast proliferation. Fiber content in FAYBF decreased by 15.78% compared to RAYBF and may be ascribed to enzymatic degradation of fiber during fermentation. Carbohydrate content in RAYBF (55.32%) decreased after fermentation compared to FAYBF (53.28%). The decrease in carbohydrate content of FAYBF may be ascribed to the use of carbohydrate-related compounds as energy source by microorganisms due to increased

Table 4–Bread volume and color attributes of bread substituted with fermented African yam bean flour.

Parameter	100WF	95WF: 5FAYBF	90WF: 10FAYBF	85WF: 15FAYBF	80WF: 20FAYBF
Specific volume (cm^3/g)	2.13 ± 0.01^a	2.06 ± 0.01^b	1.56 ± 0.01^c	1.39 ± 0.01^d	1.38 ± 0.01^d
Bread crumb color					
L	82.64v0.17[a]	79.90 ± 0.23^b	75.22 ± 0.14^c	68.53 ± 0.21^d	60.15 ± 0.17^e
a	3.15 ± 0.03^e	4.48 ± 0.02^d	5.40 ± 0.01^c	6.11 ± 0.04^b	6.70 ± 0.01^a
b	11.02 ± 0.13^e	13.14 ± 0.09^d	15.90 ± 0.11^c	16.38 ± 0.09^b	17.20 ± 0.12^a

Notes. Values represent mean and standard deviation of three replicates. Mean values with different superscript in a row are significantly ($P \leq 0.05$) different from each other. 100WF, 100% wheat flour (control sample); 95WF:5FAYBF, 95% wheat flour:5% fermented African yam bean flour; 90WF:10YFAYBF, 90% wheat flour:10% fermented African yam bean flour; 85WF:5FAYBF, 85% wheat:15% fermented African yam bean flour; 80WF:20YFAYBF, 80% wheat flour:20% fermented African yam bean flour. *L*, lightness; *a*, redness; *b*, yellowness.

activity of α-amylase, causing the hydrolysis of polysaccharides into glucose (Olukomaiya et al., 2020).

Mineral content results clearly revealed that fermentation significantly ($P < 0.05$) increased the mineral content of fermented flour (Table 1). Our result contradicts the findings of Ene-Obong and Obizoba (1996) who observed little or no changes in mineral content of AYB after fermentation. Chawla, Bhandari, Sadh, and Kaushik (2017) reported that SSF increased mineral (Fe and Zn) of black-eyed peas. The increase in mineral content of fermented AYB flour may be attributed partly to the reduction in ANFs, especially phytic acid (Table 1). According to Adebiyi et al. (2019), the mechanism by which fermentation increases the mineral content and bioavailability is related to the reduction of phytic acid and other ANFs. This is in tandem with observed reductions in the contents of phytic acid, tannin, and trypsin inhibitor by 39.85, 21.43, and 58.02%, respectively (Table 1), which could be attributed to enzyme production by microorganisms during SSF. Phytic acid and tannin contents recorded in FAYBF were lower than the values (6.87 mg/g phytic acid and 3.44 mg/g tannin) in African yam seeds fermented with *L. plantarum Lactobacillus* (Azeke et al., 2005). The *S. cerevisiae* used in this study might also have exhibited extracellular phytase activity, degrading the phytic acid. Such significant phytase activity of yeasts, including *S. cerevisiae*, has been reported in the literature, with some studies reporting phytase encoding genes in yeast strains (Greppi et al., 2015; Hellström, Almgren, Carlsson, Svanberg, & Andlid, 2012; Nuobariene, Hansen, Jespersen, & Arneborg, 2011).

Total phenolic content of FAYBF increased by 80.3% compared to RAYBF, which is consistent with a previous report of increased TPC after SSF of soybean with *Bacillus subtilis* (Dai et al., 2017). The increase in TPC may be attributed to the activity of microorganisms and/or inherent enzymes during fermentation leading to the release of bound phenolics to free forms as well as synthesis/liberation/generation of other phenolic compounds (Adebo & Medina-Meza, 2020). The breakdown of lignin present in the cell wall of food crops has also been reported to contribute to increase in TPC (Kupski et al., 2012). The DPPH radical scavenging ability of FAYBF is 6.2-fold higher than RAYBF. This implies that the scavenging DPPH ability of FAYBF was stronger than that of RAYBF. This observation is generally in agreement with reports on increased DPPH value after fermentation of leguminous products. An increase in TPC with an increase in antioxidant activity agrees with similar studies on fermented products (Sanjukta & Rai, 2016; Verni, Verardo, & Rizzello, 2019; Watanabe, Fujimoto, & Aoki, 2007). While phenolic compounds are known antioxidant-related compounds, other bioactive compounds generated during the fermentation process could also have contributed to this observation (Adebo, Njobeh, & Kayitesi, 2018; Kupski et al., 2012; Sanjukta & Rai, 2016).

3.2 Amino acid composition and IVPD of raw and fermented flour

It was observed that fermentation increased some of the essential and nonessential amino acids as compared to the raw flour (Table 1). Similar findings have been reported in similar fermented leguminous products (Adebiyi et al., 2019; Dai et al., 2017). According to Urga, Fite, and Biratu (1997), the formation of soluble products and monomers after fermentation can enhance amino acid levels in food. During yeast metabolism, the assimilation of some amino acids occurs as well as conversion into other amino acids (Pinu, Edwards, Gardner, & Villa-Boas, 2014), leading to an increase in the contents of other amino acids. Leucine was the most abundant essential amino acid in raw and fermented flour, while glutamic acid was the most dominant nonessential amino acid (Table 1).

The IVPD of RAYBF and FAYBF was 71.02 and 78.18%, respectively. Chandra-Hioe et al. (2016) reported increased IVPD of chickpea and faba bean flour after fermentation. The increase of IVPD may be attributed to proteolysis, which occurs during legume fermentation (Rizzello et al., 2014). In addition, reduced contents of ANFs after fermentation, as recorded in Table 1, might have caused increased IVPD. Further to this could be the increased availability of amino acids in the fermented product, as also reported by Angulo-Bejarano et al. (2008). This corroborates the report that during fermentation, the interactions of ANFs and proteins are altered, making the protein functional groups more susceptible to proteolytic attack, leading to increased IVPD (Chitra, Singh, & Rao, 1996).

3.3 Functional properties

Table 2 shows the functional properties of RAYBF, FAYBF, and wheat FAYBF blends. Bulk density of FAYBF was decreased by 15.71% compared to RAYBF. This is in accordance with decreased bulk density in pigeon flour after fermentation (Adebowale & Maliki, 2011). Reduction in bulk density after fermentation has been attributed to a breakdown of complex structures, such as carbohydrates and proteins, into smaller units, which yields less bulky flour (Adebiyi, Obadina, Mulaba-Bafubiandi, Adebo, & Kayitesi, 2016). This probably accounted for the reduction in the bulk density recorded in FAYBF. The WAC increased by 82.35% after fermentation, compared to raw flour. The result is consistent with a previous report on increased WAC after SSF of black-eyed pea flour (Chawla et al., 2017). The increase in WAC in fermented flour may be attributed to the unfolding and modification of macromolecules of the flour during fermentation. The modification exposes the hydrophilic domains and amino acid residues of proteins and other macromolecules that have high affinity of interactions with aqueous medium (Chawla et al., 2017). On the other hand, WF had higher WAC (2.08 g/g) than FAYBF

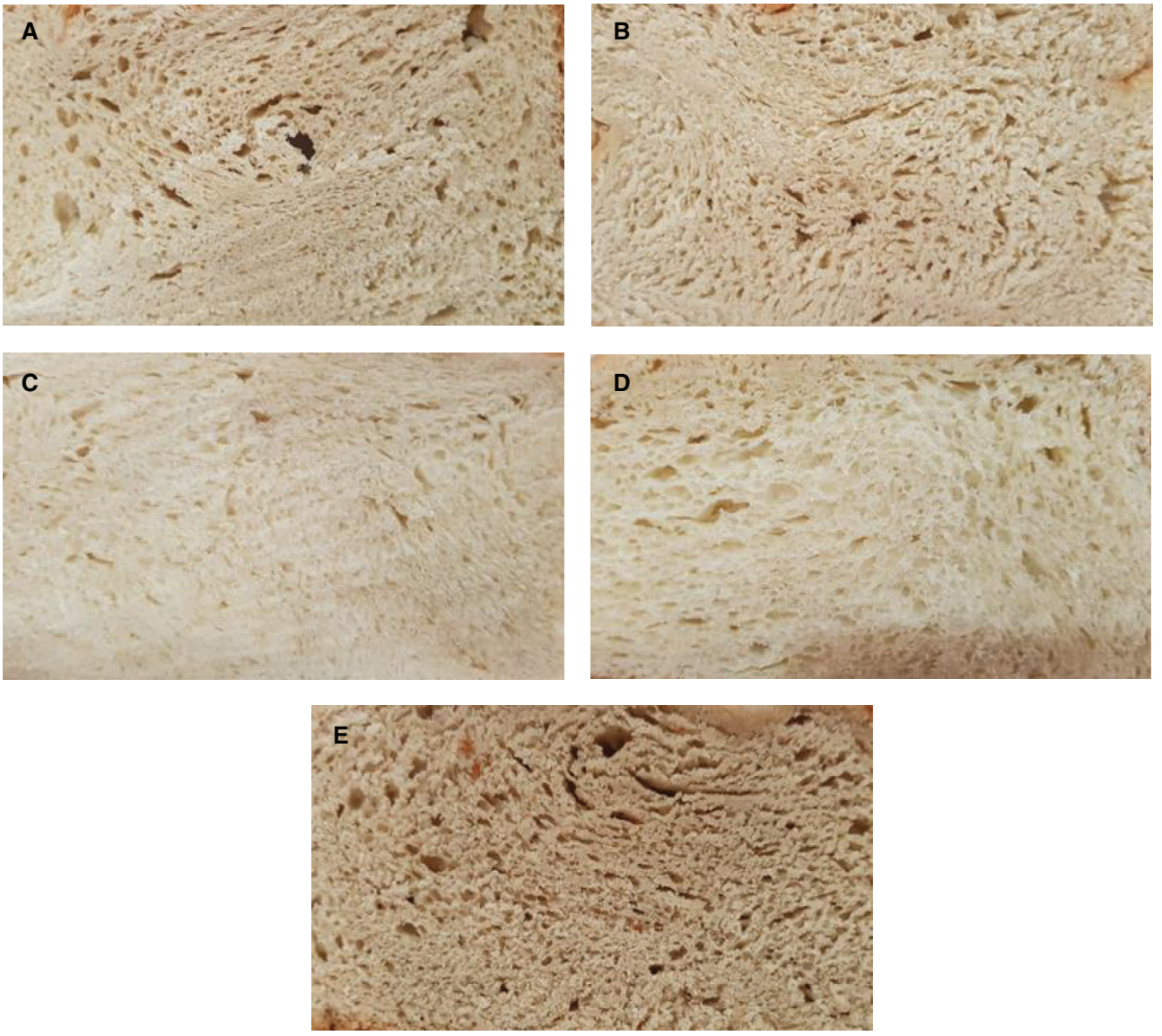

Figure 2–Cross-section of bread samples (A) 100% wheat bread (control), (B) 95% wheat flour: 5% fermented African yam bean flour, (C) 90% wheat flour:10% fermented African yam bean flour, (D) 85% wheat flour:15% fermented African yam bean flour, and (E) 80% wheat flour:20% fermented African yam bean flour.

followed by RAYBF, which could be attributed to the high starch content of WF. WF had lower WAC than the blends, which increased with increasing substitution of FAYBF. Higher WAC could be attributed to the loose structure of starch polymers, while lower values suggest compactness of the starch fractions (Adebowale, Sanni, & Awonorin, 2005).

The OAC in RAYBF increased by 39.13% after fermentation (Table 2). This observation agrees with increased OAC in fermented chickpea (Chandra-Hioe et al., 2016). The wheat FAYBF blends had higher OAC compared to 100% WF. Good OAC of flour is an important attribute for the improvement of flavor and mouthfeel in baked products (Ahmed et al., 2019). Fermentation significantly ($P < 0.05$) increased the SC of AYB compared to raw flour. This result is in line with increased SC recorded in fermented lupin flour (Olukomaiya et al., 2020). On the other hand, SC of wheat FAYBF blends ranged from 0.72 to 0.83 mL/g. The 100% WF had the highest SC value, while 80WF and 20FAYBF had the lowest value. The decrease in SC may be partly attributed to the interaction between protein and starch-related structures of the blends, which probably influenced the SC.

3.4 Pasting properties of wheat and FAYBF blends

The pasting profiles of wheat and FAYBF blends are presented in Figure 1. Substitution of WF with FAYBF reduced the pasting viscosities of WF. The reduction in pasting viscosities of WF with an increasing level of FAYBF may be attributed partly to increased protein content in the blends, which caused restricted swelling of starch granules that reduced their pasting viscosity. This observation corroborates the reduction in SC of wheat FAYBF blends (Table 2). Furthermore, the reduction in pasting viscosities of the composite blend compared to 100% wheat could be attributed to the high fat content recorded in FAYBF, which probably led to a reduction in viscosity through the formation of lipid–amylose complexes. From Figure 1, it was observed that the pasting

temperature of the composite flours was higher than for WF. This may be attributed partly to higher resistance to swelling and high protein content. There was no significant difference ($P \geq 0.05$) in peak time of 100% WF compared to the blends.

3.5 Proximate composition, TPC, and antioxidant activity of breads

Table 3 shows the proximate composition, TPC, and antioxidant activity of breads. A significant ($P < 0.05$) decrease in moisture content was recorded in bread supplemented with 5 and 10% FAYBF, and thereafter it increased with an increasing FAYBF level. A significant ($P < 0.05$) increase in the protein, ash, fiber, and fat content of bread was recorded with an increasing FAYBF level. This may be due to the higher value of these constituents in FAYBF compared to WF (Table 1). Substitution of 5, 15, and 20% FAYBF caused a significant reduction in the carbohydrate content compared to the control. The reduction in carbohydrate content may be attributed to a reduction in the starch content caused by the addition of a higher protein flour (FAYBF). Substitution of 10% to 20% FAYBF in bread caused a reduction in the energy value compared to the control (Table 3). The low carbohydrate and energy values recorded in composite breads could be advantageous for individuals on special diets for weight control.

Ca, Mg, Fe, P, K, and Zn content of breads ranged from 43.94 to 48.10, 78.33 to 112.70, 2.06 to 4.62, 241.80 to 280.94, 266.25 to 342.70, and 0.79 to 1.44 mg/100 g, respectively. Mineral content of the breads increased with the increasing level of FAYBF with the highest value at 20% FAYBF substitution. The increased mineral content of composite bread samples could be attributed to the higher mineral content in FAYBF, compared to WF.

The TPC of bread ranged from 0.44 (control) to 0.98 mg GAE/g (bread containing 20% FAYBF) (Table 3). The TPC of bread containing 5, 10, 15, and 20% FAYBF increased by 75, 93.18, 111.36, and 122.73%, respectively, compared to the control (Table 3). Similarly, the DPPH radical scavenging ability of bread substituted with 5, 10, 15, and 20% FAYBF increased by 31.32, 37.95, 49.70, and 67.79%, respectively, compared to the control. Higher TPC and DPPH radical scavenging activity of composite breads could be due to the higher content of phenolics in FAYBF compared to WF (68.27 mg GAE/ 100 g).

3.6 Amino acid composition and IVPD of bread

The amino acid composition together with IVPD gives an indication of the nutritive value of a food product (Sá, Moreno, & Carciofi, 2019). The amino acid composition and IVPD of the obtained breads are presented in Table 3. The substitution of FAYBF in bread increased most of the amino acids. The lysine content of bread substituted with 5, 10, 15, and 20% FAYBF increased by 28.89, 65.19, 84.48, and 104.44%, respectively, compared to 100% wheat bread. Lysine plays a key role in protein synthesis, which is important for growth and maintenance of the body (Adebiyi et al., 2019). The major essential amino acids in breads were leucine and phenylalanine, while glutamic acid was the predominant nonessential amino acid. The IVPD of 100% bread was 77.05%, while composite breads ranged from 80.19 to 82.6%. The substitution of WF with FAYBF increased the IVPD. The increased IVPD could be attributed partly to the inclusion of fermented flour in bread with a high protein quality. Rizzello et al. (2014) reported an improvement (68.98 to 77.85%) in the IVPD of bread supplemented with fermented chickpea, lentil, and bean flours.

3.7 Bread characteristics

SV and crumb color profile of bread are presented in Table 4. SV indicates the porosity of the bread (Haber, Mishyna, Martinez, & Benjamin, 2019), and high SV means that the bread contains more gas cells, which make the texture softer. The 100% wheat bread had the highest SV (2.13 cm^3/g), while bread containing 20% FAYBF had the lowest value (1.38 cm^3/g). The SV result is in agreement with a previous report on decreased SV of breads enriched with defatted grasshopper powder (Haber et al., 2019). The reduction in SV of composite breads could be attributed partly to the gluten dilution effect caused by the addition of fermented AYB flour. The reduction of gluten (caused by the substitution of FAYBF) or the interaction between gluten and fiber could result in weakening the wheat dough structure and a reduction in the dough's ability to retain carbon dioxide (Fendri et al., 2016).

Color is a vital sensory attribute that determines consumers' acceptability of baked products. From Table 4, it was observed that *L* value of breads decreased from 82.65 to 60.15 with the addition of FAYBF, while *a* and *b* values increased from 3.15 to 6.70, and 11.02 to 17.20, respectively. This means that the addition of FAYBF caused a darker or brownish color of the bread crumb as shown in Figure 2. The lower *L* and higher *a* and *b* in composite bread could be attributed to caramelization and Maillard reactions during baking due to the high content of protein and lysine in composite bread (Chinma, Ilowefah, Shammugasamy, Mohammed, & Muhammad, 2015).

4. CONCLUSION

This study suggests that SSF improved the nutritional composition, antioxidant activity, and reduced antinutritional factors of RAYBF. SSF increased SC, WAC and OAC, with a decrease in the bulk density of the flour. Pasting properties of WF were influenced by substitution of 5 to 20% FAYBF. The substitution of WF with FAYBF influenced the pasting properties of WF. However, there was an improvement in the nutritional composition, the total phenolic content and the antioxidant activity of composite breads compared to the control. SV and crumb color attributes of breads were influenced by the addition of FAYBF. Future studies are still recommended to determine the textural and rheological properties of the bread, further health promoting properties of the product as well as comprehensive consumer acceptability and descriptive sensory analyses.

AUTHOR CONTRIBUTIONS

Conceptualization, supervision, and project administration: C.E. Chinma, H.A. Oboh, O.A. Adebo, and N. Danbaba. Funding acquisition and resources: C.E. Chinma. Formal analysis, investigation, and methodology: S.O. Azeez, H.T. Sulayman, K. Alhassan, S.N. Alozie, H.D. Gbadamosi, and J.C. Anuonye. Writing and original draft: H.A. Oboh, N. Danbaba, S.O. Azeez, H.T. Sulayman, K. Alhassan, S.N. Alozie, H.D. Gbadamosi, and J.C. Anuonye. Writing, and review and editing: C.E. Chinma and O.A. Adebo.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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