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In vitro antioxidants and anti-inflammatory potentials of high protein-fibre cookies produced from whole wheat, sweet potato, rice bran and peanut composite flour blends

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Abstract

The growing demand for low-cost and functional snacks in many developing nations called for interest in the use of locally grown crops as substitutes for costly imported wheat flour. The amino acid composition, antioxidant and anti-inflammatory properties of the cookies from whole wheat, sweet potato, rice bran and peanut (56.25:18.75:5:20; 37.50:37.50:5:20; 18.75:56.25:5:20% as WPRG 1, WPRG 2, WPRG 3) composite flour blends, respectively, were obtained in this study. The 100% whole wheat and 100% refined flours served as control 1 and 2, respectively. The level of hydrophobic and aromatic amino acids was significantly ($p < 0.05$) high in WPRG 2 (~ 30 and ~ 10%), respectively when compared to others. However, the branched chain amino acids and Fischer ratio was significantly ($p < 0.05$) high in WPRG 1 (11.40% and 1.29), respectively, which could have contributed to their improved bioactivities. Notably, the composite cookie samples WPRG 1, 2 and 3 had higher hydroxyl (73.86 – 84.16%), DPPH (76.52 – 84.60%) radical scavenging as well as ferric reducing antioxidant (0.64–0.87 mmolFe^{2+}/mg) properties than the control samples WWF and CWF, respectively. On the contrary, the metal chelating activities of the cookies WPRG 1–3 were not significantly ($p > 0.05$) different from control samples WWF and CWF. The improved amino acid profile and enhanced antioxidant properties of the composite cookies might have effectively influenced their anti-inflammatory properties (IC_{50}; < 26 µg/ml) when compared to the control samples (IC_{50}; ~ 40 µg/ml), respectively. Hence, the cookies that comprised of antioxidants and anti-inflammatory potentials needed in human health, were acceptable by the consumers.

Keywords Cookies, Anti-inflamatory, Oxidative stress, Hydroxyl radicals, Cyclooxygenase, Lipoxygenase

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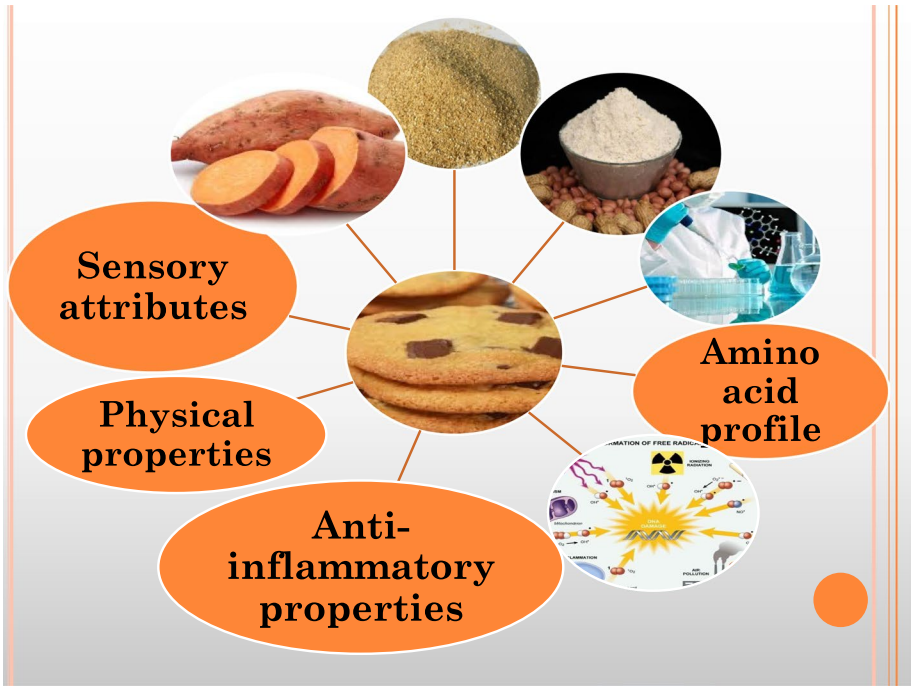
Graphical Abstract



Introduction

Oxidative stress and associated oxidative damages were mediators of vascular injury and inflammation in the initiation, progression and clinical implications of many cardiovascular diseases, including hypertension, hyperlipidemia and diabetes (Akinbode et al. 2023). Oxidative stress played a significant role in many human diseases, including inflammations (Ou et al. 2001). The antioxidants worked by chain breaking, reduction of the concentration of reactive oxygen species and by scavenging the initial radicals (Malomo et al. 2020). Interest has also been directed towards oxidative stress and reduced bioavailability of nitric oxide (NO), as a result of systemic and localized inflammatory responses, which led to vasoconstriction and impaired vascular function (Ma et al. 2013). It is generally associated with an increase of protein denaturation, vascular permeability and membrane alteration, etc. (Ferrero-Millani et al. 2007). In the case of inflammation, the nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of medications commonly administered to manage pain and inflammation (Akinbode et al. 2023). Notably, the NSAIDs acted by inhibiting the intracellular cyclo-oxygenase enzymes, which has two isoforms (COX-1 and COX-2). Most of the drugs are available over the counter in many countries while the rest needed prescription, which are included but not limited to ibuprofen, diclofenac, naproxen, celecoxib, etc. (Ferrero-Millani et al. 2007). Several side effects have been associated with frequent administration of NSAIDs, much particularly in the gastrointestinal (GI) tract, where they caused bleeding, intestinal perforation and peptic ulcer (Akinbode et al. 2023). Therefore, an effective nutritional therapy from locally produced and underutilized food crops, such as sweet potato, peanut and rice bran, that is devoid of all these occurring complications, is thereby preferred in the management of both acute and chronic inflammations in the body. Thus, the present work is aimed to exploit the potentials of some of the indigenous, locally grown and low-cost produced crops (sweet potato and peanuts) with industrial waste products (rice bran) to produce a well-nutritious, healthy and disease-management-targeted cookies for some health-challenged individuals.

The cookies consumption is rated as part of the cereals-based food besides bread, due to its wide availability, as a ready-to-eat, convenient and inexpensive food products with important digestive cum dietary components (Malomo & Udeh 2018). Cookies are nutritively obtained from dough or batter, which is products of oven heat treatment (Akinbode et al. 2023). Report showed a large reliance on wheat cereal importation despite the growing consumption rate of these flour-based baked

products (Arise et al. 2020). Moreso, the competitive market, increase in demand of food products that were healthy, natural but nutri-functional have caused several attempts in improving the bio-nutritive and functionality of cookies by modifying its nutritional compositions. Thus, this is geared towards increasing the cookies protein and fibre contents for quality and functional bioactivities. Staple crops like yam, cassava, cocoyam, sweet potatoes, oilseeds, etc. have been reported as good and potential ingredients for baked foods (Olugbuyi et al. 2023; Arise et al. 2020; Malomo & Udeh 2018). The potentials of various indigenous, locally grown, underutilized and low-cost produced crops have been exploited in the production of cookies in Nigeria, so as to propagate the utilization and value-addition to these crops. For instance, Mepba et al. (2007) had previously investigated the baking potentials of wheat-plantain composite flours for cookies production. Furthermore, another study (Inyang & Nwabueze 2020) reported the production of cookies from *Acha*-green banana composite flour blends. Besides, Malomo and Udeh (2018) reported the utilization wheat-unripe plantain-crayfish composite flours in producing cookies with high ratings of consumer acceptability.

Sweet potato (*Ipomea batatas* L.) has been sources of most macro- and micronutrients, such as starch, protein and vitamin A. Its ability to thrive on poor soil makes it suitable for tropical soil, where fertilizer is not much available and this is because the crop's roots penetrate deep into the soil to make use of residual fertilizer from previous crops cultivation (Alawode and Idowu 2017). Peanut (*Arachis hypogeal* L.) is a cheap and locally available crop reputed for its high protein content and substantial level of amino acid. It contained about 40% protein, digestible and cholesterol-free oil with very high polyunsaturated fatty acids (Sani et al. 2023). The crop is easily processed into different product to serve as an ideal source of protein supplementation to starchy food in most home diets in Nigeria (Sani et al. 2023). Such products like cookies, bread, cake, pastries, peanut butter, etc. were produced for significant dietetic, industrial, medical and agricultural purposes (Olaoye et al. 2006). Rice (*Oryza sativa L.*) bran is a by-product from the rice processing industry, and is the cuticle existing between the rice and the husk of the paddy that made up the embryo and endosperm (about 80%) of the grains (Bhosale & Vijayalakshmi 2015). Its recent use is greatly important to the food and human health industrial applications beacuse, large amounts of rice outer layers were removed with most important nutrients being concentrated in the bran (most especially the B-vitamins) during the rice processing (Quereshi et al. 2000).

To our knowledge, there existed scanty information on the use of composite flours from combination of whole wheat, sweet potato, rice bran and peanut to produce cookies despite their resultant higher protein and fibre contents. Besides, functional requirements for enzyme inhibitors from whole wheat, sweet potato, rice bran and defatted peanut composite cookies have not been reported as well. This study is thus aimed to investigate the antioxidants and anti-inflammatory potentials of cookies from whole wheat (*Triticum monococcum* L.), sweet potato (*Ipomoea batatas L.*), rice (*Oryza sativa L.*) bran and peanut (*Arachis hypogaea L.*) composite flour blends.

Materials and methods

Experimental materials

The freshly harvested matured sweet potato (*Ipomoea batatas* L.), whole wheat (*Triticum monococcum* L.), peanut grains (*Arachis hypogaea* L.) and other baking ingredients (egg, salt, veg oil, etc.) were obtained from Erekesan market, Akure, Nigeria. The rice bran was procured at a local rice mill in Ise Ekiti, Nigeria. All experimental crops were properly authenticated at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Nigeria. The analytical chemicals and reagents used were procured from Sigma-Aldrich, London, UK and Fischer Chemicals, USA.

Production of flours

The freshly harvested matured sweet potato were sorted, peeled, cut to size, blanched, oven-dried and milled into flour (Alawode and Idowu 2017). The whole wheat seeds were sorted, dried milled, sieved and packaged as whole wheat flour (Malomo & Udeh 2018). The rice bran was washed, oven-dried and also milled to flour (Olugbuyi et al. 2023). The peanuts were sorted, dried, milled, defatted, dried, then re-milled and sieved through a 200 µm mesh sieve prior further analysis (Sani et al. 2023). The composite flour blends (Table 1) of whole wheat, sweet potato, peanut and rice bran were produced using Nutri survey linear programming software (with 18–20% protein, 10% fibre and 55–65% carbohydrate recommended for the diabetic patients).

Production of cookies

The cookies were produced using the previously described method (Malomo & Udeh 2018). The ingredients used were mixed together, added to the dough and mixed (for 15 min) to form a uniform smooth paste, further rolled, cut into shapes, placed on a greased baking foil paper and kept at a normal room temperature (for 2 h) for proper dough leavening. The leavened doughs were baked in a hot air oven (184 ^{0}C; 20 min) until a very

Table 1 Different composite flour formulations obtained from the whole wheat, sweet potato, rice bran and peanut flour blends (%) using Nutri survey linear programming software

Samples/Flours	Whole Wheat	Sweet Potato	Rice Bran	Peanut	CWF	Total
WPRG 1	56.25	18.75	5	20	0	100
WPRG 2	37.50	37.50	5	20	0	100
WPRG 3	18.75	56.25	5	20	0	100
WWF (Control 1)	100	0	0	0	0	100
CWF (Control 2)	0	0	0	0	100	100

Key:

WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut

WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut

WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut

WWF Whole wheat flour, *CWF* Commercial wheat flour

light brown colour is obtained. The hot cookies were cooled and packaged in high-density polyethylene bag.

Determination of amino acid compositions

Amino acid content was determined using Pico-Tag method as previously described and modified methods as reported by Malomo et al. (2020) by following the procedures viz:

Defatting of sample: Two grams (2 g) of the dried sample was weighed into extraction thimble and the fat extracted with chloroform/methanol (1:1 v/v) using soxhlet extraction apparatus.

Hydrolysis of the Sample: 40 mg of each defatted sample was separately weighed into glass ampoules. 7 mL of 6 M HCl, was added and oxygen expelled by passing nitrogen into the glass ampoules were then sealed with flame and put into an oven pre-set at 105 °C and left for 22 h to hydrolyze. The ampoules were then allowed to cool at 40 °C under vacuum in a rotary evaporator. Residues were dissolved in 5 mL acetate buffer (pH 2.0) and stored in plastic samples bottle at 4 °C until required.

Loading of the hydrolysate into the TSM-1 analyzer: Ten microliter of each hydrolysate was dispensed into the cartridge of the analyser. The amino acid analysis was done by ion–exchanger chromatography (Moore et al. 1958) using a Technicon Sequential Multi-Sample Amino Acid Analyser (Technicon Instruments Corporation, New York, USA). The analyser then separated and analysed free acidic, neutral and basic amines, which lasted for 76 h. Norleucine was employed as the internal standard. Ten micro-liter (10 µL) of the standard solution mixture of the amino acid was also loaded into the analyser. Values of both the standard and samples were recorded and printed out as chromatogram peaks by the chart recorder.

Calculation from the peaks: The net height of each peak produced on the chromatogram (each representing amino acid) was measured. The half-height of each peak was located and the width of the peak at half-height was measured. Approximate area of each peak was then obtained by multiplying the height with the width of the half height.

The cysteine and methionine were converted into cysteic acid and methionine sulfone by oxidizing with performic acid as described by Toran et al. (2006). The oxidized samples were then subjected to acid hydrolysis (6 M HCl, 105–110 °C for 24 h). After derivatization with phenylisothiocyanate, reverse phase HPLC separation was carried out on the samples at 48 °C and with UV detection.

The tryptophan was estimated by the ninhydrin method of Pinter-Szakacs and Molnan-Perl 1990. Sample (1.0 g) was introduced into a 25 ml polypylene test tube with caps and 10 ml of 0.075 M NaOH was added and mixed until there were no lumps. The dispersion was shaken for 30 min and centrifuged at 5000 xg for 10 min and the supernatant was transferred to a clean test tube. To 0.5 ml of supernatant, 5 ml of ninhydrin reagent (1.0 g of ninhydrin in 100 ml mixture of 37% HCl and 96% HCOOH at a ratio of 2:3) was added and then solution was incubated at 35 °C for 2 h. It was then cooled to room temperature and the volume was made up to 10 ml with diethyl ether, thoroughly mixed with a Vortex mixer and thereafter filtered. The absorbance of the filtrate was read at 380 nm. A standard tryptophan curve was prepared using 0 ~ 100 µg tryptophan. From the standard graph, the concentration of tryptophan was calculated and expressed as g /100 g protein.

Determination of hydroxyl radical scavenging activity

The deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium according to previously described procedures of Malomo et al. (2020). The reaction mixture containing $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) at various concentrations of samples in 1 ml final reaction volume was made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 h at 37 °C. The mixture was heated at 95 °C in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally, the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. The absorbance of supernatant was measured at 532 nm while ascorbic acid was used as the positive control. The activity is thus, calculated as shown in Eq. 1:

$$\%\ \text{Hydroxyl Scavenging Activity} = \left[\frac{(A_c - A_s)}{A_c}\right] X100 \quad (1)$$

Where A_c is the absorbance of control and A_s the absorbance of the extract.

Determination of DPPH scavenging radical activity

The radical scavenging activities of the cookies were determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) as previously described by Malomo et al. (2020). Briefly, 1 ml of 0.3 mM DPPH in methanol was added to 1 ml of different concentrations of the sample or standard (ascorbic acid) in a test tube. The reaction of DPPH with an antioxidant compound that could donate hydrogen, led to its reduction. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. The mixture was mixed and incubated in the dark for 30 min after which the absorbance was read at 517 nm. The activity is thus, calculated as shown in Eq. 2:

$$\%\ \text{Radical Scavenging Activity} = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] X100 \quad (2)$$

Determination of ferric reducing antioxidant power

Ferric reducing or antioxidant power was determined as described by Malomo et al. (2020). Briefly, 100 µl of the extract were mixed with 2.5 ml of 200 mmol/l phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50 °C for 20 min. Then, 2.5 mlof 10% trichloroacetic acid was added, and the tubes were centrifuged at 10,000 rpm for 10 min. After this, 5 ml of the upper layer were mixed with 5.0 ml distilled water and 1 ml of 0.1% ferric chloride. The absorbance of the reaction mixtures was measured at 700 nm while ascorbic acid was used as a positive control.

Determination of iron chelating activity

Metal chelating activity was measured according to the previously described by Malomo et al. (2020). Briefly, 0.1 mM $FeSO_4$(0.2 ml) and 0.25 mM ferrozine (0.4 ml) were subsequently added into 0.2 ml of sample for dissolution and followed by incubation at room temperature for 10 min. After incubation, the absorbance of the mixture was measured at 562 nm. The chelating activity was calculated as shown in Eq. 3:

$$\text{Metal Chelating Activity} = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] X100 \quad (3)$$

Inhibition of protein denaturation

The inhibition of protein denaturation was done according to Sakat et al. (2010) methods. 500 µL of 1% bovine serum albumin was added to 100 µL of sample extract (I mg/ml). This mixture was kept at room temperature for 10 min, followed by heating at 51 °C for 20 min. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percentage inhibition for protein denaturation was calculated as shown in Eq. 4:

$$\%Inhibition = 100 - ((A1 - A2)/A0) * 100) \quad (4)$$

Where A1 = absorbance of the sample; A2 = absorbance of product; A0 = absorbance of control.

Determination of anti-proteinase

The test was performed according to the modified method of Leelaprakash et al (2012). The reaction mixture (2 ml) contained 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 – 500 µg/ml). The mixture was incubated at 37 °C for 5 min and 1 ml of 0.8% (w/v) casein was added, incubated for additional 20 min while 2 ml of 70% perchloric acid was added to halt the reaction process and the absorbance read at 210 nm against buffer as blank.

Membrane stabilization activity

A volume of 100 µL from 10% RBC was added to 100 µL of the sample extract (1 mg/ml). The resulting solution was heated at 56 °C for 30 min followed by centrifugation at 2500 rpm for 10 min at room temperature. The supernatant was collected while the absorbance read at

560 nm. Acetyl salicylic acid was used as a positive control. Percentage membrane stabilization was calculated by the method of Sakat et al. (2010) as shown in Eq. 5:

$$\%\text{Inhibition} = 100 - ((A1 - A2)/A0) * 100) \quad (5)$$

Where A1 = absorbance of sample; A2 = absorbance of product; A0 = absorbance of control.

Nitric oxide (NO) scavenging activity

The NO scavenging activity of sample was determined by adding 400 µL of 100 mM sodium nitroprusside, 100 µL of PBS (pH—7.4) and 100 µL of of sample (1 mg/ml) according to the methods previously described by Balakrishnan et al. (2009). This reaction mixture was incubated (25 °C; 150 min) and absorbance is read at 540 nm. The activity is thus, calculated as shown in Eq. 6:

$$\%Inhibition = ((A0 - A1)/A0) * 100 \quad (6)$$

Where A0 is the absorbance of the control; A1 is the absorbance of the sample.

Anti-lipoxygenase activity

The anti-lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme (Eshwarappa et al. 2016). Test samples were dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000U/ml) and incubated (25 ^{0}C; 5 min). Thereafter, 1.0 ml of linoleic acid solution (0.6 mM) was added, thoroughly mixed whle the absorbance of the final product is read at 234 nm while diclofenac was used as standard. The activity is thus, calculated as shown in Eq. 7:

$$\%inhibition = [\{Abs\ control - Abs\ sample\}/Abs\ control]x100 \quad (7)$$

Anti-cyclooxygenase activity

The anti-cyclooxygenase activity was measured using the assay mixture containing TrisHCl buffer, glutathione, hemoglobin & enzyme. The arachidonic acid was added and terminated after 20 min incubation (37 °C) by addition of 0.2 ml of 10% TCA in 1 M HCl and mixed. About 0.2 ml of TBA was added and heated in a boiling water bath (20 min), cooled and centrifuged (1000 rpm; 3 min) while the absorbance was read at 632 nm for COX activity as previously reported (Eshwarappa et al. 2016).

Physical properties analysis

The cookie samples were analyzed for their weights, width, thickness and spread factor using the procedures described by Malomo and Udeh (2018). The weights of the samples were done with laboratory weighing balance. The average diameter of the cookies was obtained using digital venire caliper with 0.01 mm accuracy and followed by their average thickness while the spread factor was calculated as: SF = W/T (8).

Sensory evaluation

The evaluation of sensory attributes was done according to Malomo and Udeh (2018). A 100-member panelist randomly selected from the staff and students of the Federal University of Technology, Akure, Nigeria was asked to evaluate the cookies for taste, appearance, texture, mouthfeel, crumblings, aroma and general acceptability. The panelists were instructed to rate the cookie samples using 9-point hedonic scale of 9 and 1 = liked extremely and disliked extremely, respectively.

Ethical approval and consent to participate.

The study protocol for the present work was approved while the ethic clearance issued to the investigators from the Ethical Committee, School of Agriculture and Agricultural Technology of the Federal University of Technology, Akure, Nigeria with protocol number FUTA/SAAT/2022/033.

Statistical analysis

All determinations were obtained in triplicates values and the experimental errors were obtained as mean standard deviation. All data were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS) of version 21 (SPSS Inc., USA). The means were separated using the procedures of New Duncan Multiple Range Test (NDMRT) at 5% significance level.

Results and discussion

Amino acid profile of the cookies

Glutamic acid (10.52–13.30 g/100 g) and aspartic acid (7.62–12.87 g/100 g) were the predominant amino acids in the produced cookies with the highest contents in cookie sample WPRG-2 and WPRG-1, respectively as presented in Table 2. Past study reported the glutamic acid as the most abundant non-essential amino acid in most plant-based foods and invariably helped in promoting normal brain function, cognitive development in children as well as prevention of memory loss (Ijarotimi et al. 2022). The total essential amino acids (TEAA), total non-essential amino acids (TNEAA) and total amino acids (TAA) of the cookies ranged from 26.14 to 31.11; 37.20 to 45.02 and 64.63 to 76.13 g/100 g, respectively. The TAA of the cookies obtained in this study (64.63–76.13 g/100 g) were lower than the77.50–87.26 g/100 g reported for cookies produced from sorghum, orange-fleshed sweet potato and mushroom protein isolate (Akinbode et al. 2023). The higher content reported by Akinbode et al. (2023) might have been due to the inclusion of single-cell

Table 2 Amino acid profiles of cookies (mg/ 100 g)

Samples/Amino acids	WPRG 1	WPRG 2	WPRG 3	WWF	CWF	Average	± Std	#LSD ($p < 0.05$)
Aspartic acid	12.87	11.21	10.22	8.24	7.62	11.21	1.82	0.37
Threonine	2.92	2.89	2.23	2.04	2.53	2.89	0.35	1.00
Serine	4.44	4.54	4.85	4.38	4.34	4.54	0.76	0.33
Glutamic acid	12.01	13.30	11.41	11.34	10.52	13.30	2.09	1.00
Proline	2.78	2.31	2.41	2.00	2.10	2.31	0.31	1.00
Glycine	3.21	3.22	2.81	3.12	3.18	3.22	0.33	0.15
Alanine	2.52	2.76	2.18	2.57	2.58	2.76	0.36	1.00
Cystine	0.39	1.04	1.49	1.22	1.22	1.04	0.30	1.00
Valine	4.76	4.56	3.64	4.48	4.28	4.56	0.84	1.00
Methionine	2.62	2.56	2.91	2.94	2.14	2.56	0.39	1.00
Isoleucine	2.70	2.69	2.69	2.27	2.67	2.69	0.02	0.09
Leucine	3.91	4.00	3.15	3.40	3.48	4.00	0.69	1.00
Tyrosine	3.49	3.61	3.31	3.32	3.03	3.61	0.65	1.00
Phenylalanine	4.42	5.57	3.41	4.19	4.32	5.57	0.93	1.00
Histidine	3.72	3.75	3.49	3.29	3.40	3.75	0.66	1.00
Lysine	3.70	3.85	3.72	3.17	3.12	3.85	0.81	1.00
Arginine	2.88	3.03	2.19	2.28	2.61	3.03	0.19	1.00
Tryptophan	0.91	1.24	0.90	1.01	1.49	1.24	0.21	1.00

Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut; WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut; WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut

WWF Whole wheat flour, *CWF* Commercial wheat flour

protein, mushroom protein that is not present in our present cookies. Although the low TAA in the current study is still considered nutritionally good to support the nutritional and bioactive properties expected of the cookies.

Some data were estimated from the amino acid profile (Table 3) as the nutritional quality indices of the cookies produced in this present study. The hydrophobic amino acids (HAA) of the cookies ranged from 24.10 in WPRG-3 to 28.91 in WPRG-2. Factually, the increase in HAA contents would help to increase lipid solubility of the resultant cookies, thereby enhancing their antioxidant activities in disease-management (Olagunju et al. 2020). The higher levels of these factors obtained from the composite cookies could be attributed to the use of multiple plant crops, which has been reported for better improvement of essential amino acids and nutritional quality of foods (Ijarotimi et al. 2018). However, the Fischer ratio (FR) of the cookies were given as 1.08–1.29and FR has been found helpful to determine the biological activity of proteins (Ramsookmohan et al. 2020).

Table 3 Summary of the amino acid profiles of cookies

Samples/Parameters	WPRG 1	WPRG 2	WPRG 3	WWF	CWF
TAA	74.25	76.13	67.01	65.26	64.63
HAA	28.45	30.34	26.09	27.40	27.31
PCAA	10.33	10.63	9.40	8.74	9.13
NCAA	24.88	24.51	21.63	19.58	18.14
SCAA	3.01	3.60	4.40	4.16	3.36
TEAA	32.54	34.14	28.33	29.07	30.04
TNEAA	41.71	41.99	38.68	36.19	34.59
BCAA	11.37	11.25	9.48	10.15	10.43
AAA	8.82	10.42	7.62	8.52	8.84
Fischer Ratio	1.29	1.08	1.24	1.19	1.18

Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut

WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut

WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut

WWF whole wheat flour, *CWF* Commercial wheat flour, *TAA* Total amino acids, *HAA* Hydrophobic amino acids, *PCAA* Positively-charged amino acids, *NCAA* Negatively-charged amino acids, *SCAA* Sulphur-containing amino acids, *TEAA* Total essential amino acids, *TNEAA* Total non-essential amino acids, *BCAA* Branched-chain amino acids, *AAA* Aromatic amino acids

Antioxidant properties of cookies

It is well established that antioxidants, inhibit oxidation process by preventing the formation of free radicals play major roles in preventing chronic diseases such as cardiovascular diseases, diabetes, obesity and cancers (Olagunju et al. 2020). The hydroxyl (OH) radical scavenging activity of the cookie samples is presented in Fig. 1A. The properties of the samples were recorded in the ranges of 60 to 78% when compared to the common

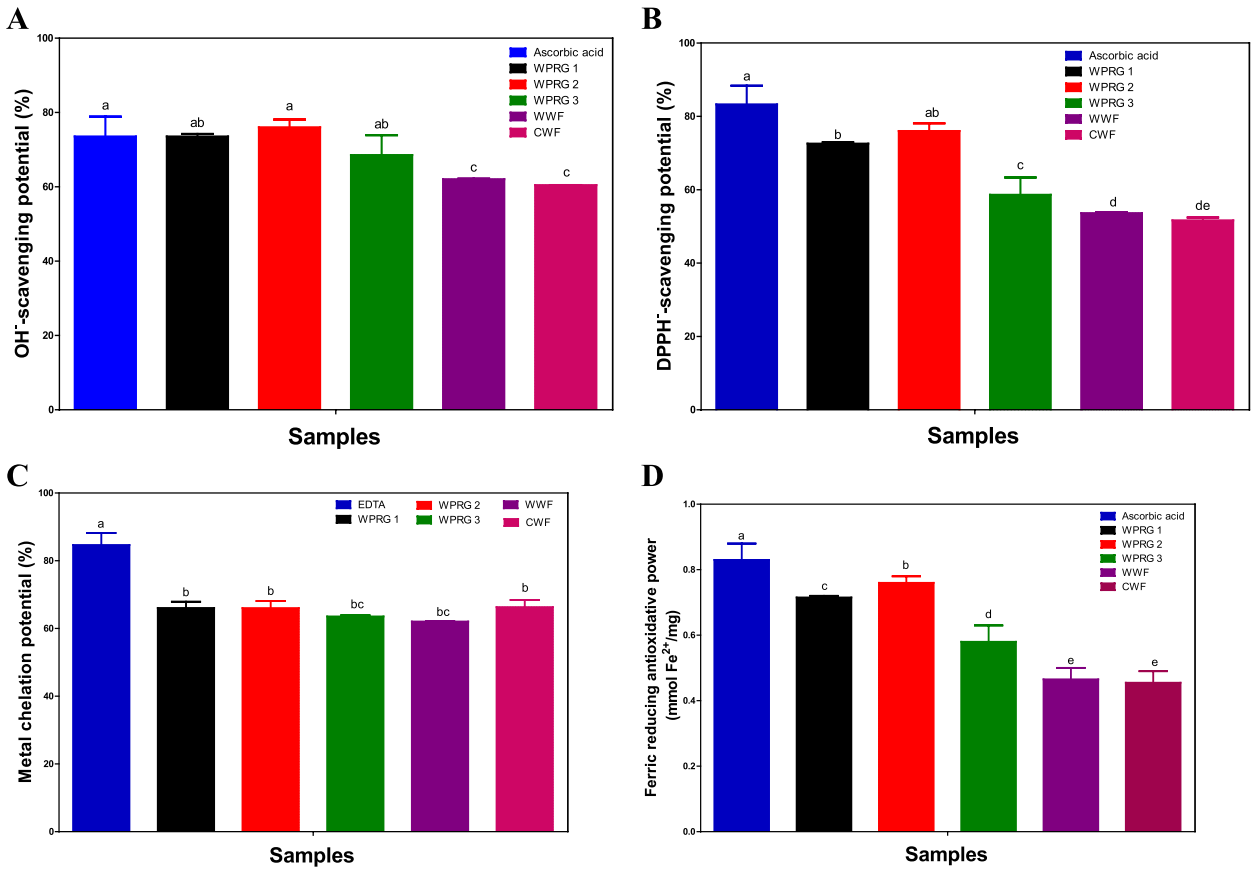


Fig. 1 **A** Hydroxyl (OH^-) radical scavenging properties of cookies. Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour. **B** $DPPH^-$ radical scavenging properties of cookies. Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour. **C** Metal (Fe^{2+}) chelation properties of cookies. Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour. EDTA = Ethylene diamine tetra acetic acid. **D** Ferric ion reducing antioxidative power (FRAP) of cookies. Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour

and well-known antioxidant, ascorbic acid (80%). It was observed that the sample WPRG 2 had the significant ($p<0.05$) highest property when compared to sample CWF (cookie from commercial wheat flour).

The DPPH radical scavenging activity of the samples is obtained in the following ranges; 50.42–80.16% as shown in Fig. 1B. It was observed that the samples shown significant ($p<0.05$) difference between each other against DPPH activities and when compared to ascorbic acid (90%). Although, it was observed that the cookie samples were significantly ($p<0.05$) less than ascorbic acid in free radical scavenging activities but they were all capable of scavenging the DPPH radicals. This observation agreed with other findings who reported that enzymatic hydrolysis of bioactive compounds enhanced its antioxidant capacities (Zhang & Lu 2019).

A similar trend of low Fe^{2+} chelation antioxidant activity was observed in WWF (60%) when compared to WPRG 2 and CWF (64.32%) as presented in Fig. 1C. It was observed that the Fe^{2+} chelation antioxidant activities of sample WPRG 1, 2 and WCF were significantly ($p<0.05$) similar to one another. This implied that the samples WPRG 1, 2 and WCF exhibited similar ability to

scavenging free radicals against free radicals thereby having the same potential to chelate Fe^{2+}.

The result presented in Fig. 1D revealed that the sample WPRG 2 also had highest ferric reducing antioxidative potentials (FRAP) when compared to others. For instance, WPRG 2, WWF and CWF had 0.80, 0.47 and 0.45 mmol Fe^{2+}/mg when compared with ascorbic acid (0.90 mmol Fe^{2+}/mg), respectively. The relative higher antioxidant potentials (~80%) of the cookie sample WPRG 2 may be attributed to its higher protein content (~18 g/100 g), hydrophobic amino acids (30.34 g/100 g) and aromatic amino acids (10.42 g/100 g) when compared to other samples. This observation agreed with other findings, that reported the efficacy of protein and amino acids profiles to enhance the antioxidant capacity, which was related to the release of bioactive compounds (Zhang et al. 2020). This finding also agreed with past study that reported on the contributions of antioxidants in diabetes and its complications (Adefegha et al. 2016). Several studies have demonstrated significant decrease of cardiovascular disease such as diabetes and hypertension with consumption of food rich in antioxidants (Adefegha et al. 2016; Odebode et al. 2017; Oluwajuyitan et al. 2020). Hence, sample WPRG 2 could help in reducing the risk of diabetic-related cardiovascular diseases.

Anti-inflammatory properties of cookies

The inhibition of protein denaturation of composite cookies at 50% inhibition (IC_{50}) is presented in Fig. 2A. It was observed that the composite cookie WPRG 2 has low IC_{50} (28 µg/ml) when compared to the control sample WWF (66 µg/ml), which is the 100% whole wheat cookies. The main cause of inflammation is denaturation of protein. Inflammation, which is generally referred to as a complex biological response of vascular tissues to harmful stimuli, has been associated with pain, and it involved in an increase of protein denaturation, an increase of vascular permeability, and membrane alteration, proteinase, among others (Ferrero-Millani et al. 2007). Therefore, this result showed that sample WPRG 2 is able to inhibit denaturation of protein more, which caused inflammation, when compared to the control.

Figure 2B showed the inhibition of trypsin activities of composite cookies at 50% inhibition. It was observed that the composite cookies especially WPRG 2 has low IC_{50} (25 µg/ml) when compared to the samples WPRG 1, 3, WWF and CWF (30, 33, 36 and 42 µg/ml), respectively. Trypsin (proteinase) had been related to anthritis, which involved inflammation or degeneration (breakdown) of body joints. Inflammation on the other hand, has also been associated with increase in trypsin activities (Ferrero-Millani et al. 2007). This result showed that sample WPRG 2 is able to inhibit trypsin activities, which mostly caused inflammation of body joints leading to arthritis.

Figure 2C showed membrane separation activities of the cookie with the potency of composite cookie WPRG 2 having lower IC_{50} (26 µg/ml) than the samples WPRG 1, 3, WWF and CWF (30, 30, 33 and 45 µg/ml), respectively. Inflammation has been strongly associated with increase in membrane alteration. Hence, sample WPRG 2 is less altered compared to the control group and this gave the sample more membrane stability that aided against inflammation than control groups (Alam et al. 2015).

Nitric oxide has been one of the indicators used when there existed inflammation in the body, for instance, an inflammation in the body could be a product of nitric oxide formation. The result presented in Fig. 2D revealed that WPRG 2 has significant ($p < 0.05$) low IC_{50} (15 µg/ml) when compared to sodium diclofenac (21 µg/ml), a well-known and common non-steroidal anti-inflammatory drug (NSAID) and the control samples WWF and CWF (42 µg/ml), respectively. The current study showcased the cookie sample WPRG 2 as a potent functional agent available to organically (without any negative health side effect) inhibit or scavenge the nitric oxide production (that has been associated with metabolic impact of serious inflammation in the body (Alam et al. 2015) more than the common NSAID (having lots of side effects, such as nausea, vomiting, itches, etc.) and samples WPRG 1 and 3 (24.41 and 34.32 µg/ml), respectively.

Cyclo-oxygenase and lipoxygenase were the dual enzymes that catalyzed the primary oxidation of unsaturated fatty acids or unsaturated fats by oxygen, leading to inflammation in the body (Ferrero-Millani et al. 2007). It was observed from the results presented in Fig. 2E and F, that the excessive activities of the dual cyclo-oxygenase and lipoxygenase were being able to be checkmated through the obtained significant ($p < 0.05$) low IC_{50} (13.85 and 17.09 µg/ml, respectively) when compared to sodium diclofenac (14.04 and 18.60 µg/ml), a well-known and common non-steroidal anti-inflammatory drug (NSAID) and the samples WPRG 1, 3, WWF and CWF (21, 20; 22.41, 23; 45, 44 and 43, 44 µg/ml), respectively.

The present study reported in Fig. 2E and F thus, showed that the cookies sample WPRG 2 could be regarded as potential agents in the inhibition of excessive activities of these two enzymes implicated in the inflammatory reactions in the body (Akinbode et al. 2023).

Physical properties of the cookies

The physical properties of the cookies were presented in Table 4. The weights and diameters of the cookies ranged from 5.18 to 6.62 g and 45.90 to 45.95 mm with samples

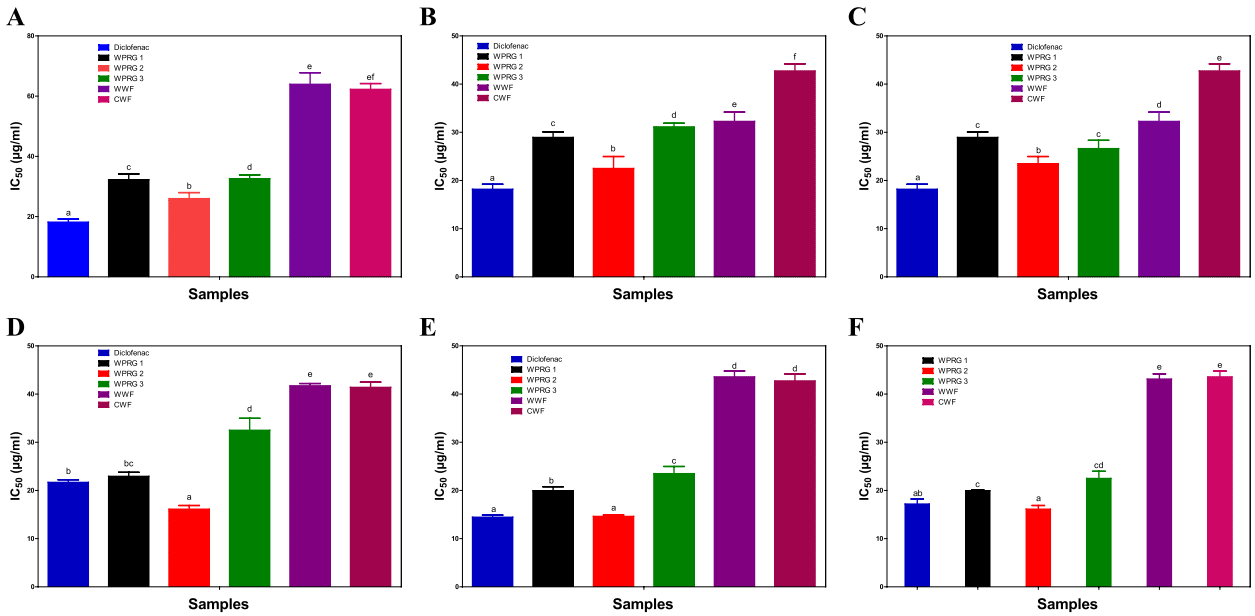


Fig. 2 **A** Inhibition of protein denaturation activity of different cookies at 50% level of inhibition concentration (IC_{50}). Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour. **B** Inhibition of proteinase (trypsin) activity of different cookies at 50% level of inhibition concentration (IC_{50}). Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour. **C** Membrane stabilization activity of different cookies at 50% level of inhibition concentration (IC_{50}). Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour. **D** Nitric oxide (NO) scavenging activity of different cookies at 50% level of inhibition concentration (IC_{50}). Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour. **E** Anti-cyclooxygenase activity of different cookies at 50% level of inhibition concentration (IC_{50}). Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour. **F** Anti-lipoxygenase activity of different cookies at 50% level of inhibition concentration(IC_{50}). Bars ($n=3$) with different letter are significantly different ($p<0.05$). Key: WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut. WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut. WWF = whole wheat flour; CWF = Commercial wheat flour

Table 4 Physical properties of cookies

Parameters/ Samples	WPRG 1	WPRG 2	WPRG 3	WWF	CWF
Weight (g)	5.18 ± 0.07^{e}	6.01 ± 0.02^{c}	6.62 ± 0.02^{a}	5.41 ± 0.01^{d}	6.51 ± 0.01^{b}
Width (mm)	45.90 ± 0.17^{a}	45.93 ± 0.06^{a}	45.95 ± 0.46^{a}	45.91 ± 0.14^{a}	45.03 ± 0.58^{a}
Thickness (mm)	5.03 ± 0.08^{a}	5.06 ± 0.28^{a}	5.03 ± 0.51^{a}	5.03 ± 0.43^{a}	5.10 ± 0.01^{a}
Spread ratio (mm)	11.96 ± 0.04^{b}	11.89 ± 0.01^{b}	12.22 ± 0.01^{a}	12.26 ± 0.02^{a}	12.24 ± 0.04^{a}

Means ($n=3$) with different letters (a, b, c and d) along the column are significantly different ($p<0.05$). Superscripts a and d are the most and least significant, respectively

Key:

WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut

WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut

WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut

WWF whole wheat flour, *CWF* Commercial wheat flour

Table 5 Sensory attributes of cookies

Samples	Appearance	Taste	Texture	Mouthfeel	Crumblings	Aroma	Overall acceptability
WPRG 1	8.20^{b}	7.70^{b}	7.45^{b}	7.90^{b}	7.75^{b}	7.50^{b}	8.20^{b}
WPRG 2	7.05^{c}	5.85^{c}	6.45^{bc}	6.55^{c}	6.40^{c}	6.65^{bc}	6.95^{c}
WPRG 3	6.95^{c}	5.25^{c}	5.75^{cd}	5.90^{c}	5.85^{cd}	6.25^{c}	6.50^{c}
WWF	6.45^{c}	3.70^{d}	4.90^{d}	4.10^{d}	5.50^{cd}	4.70^{d}	4.55^{d}
CWF	8.90^{a}	8.80^{a}	8.85^{a}	8.90^{a}	8.65^{a}	8.70^{a}	8.70^{a}

Means ($n = 100$) with different letters (a, b, c and d) along the column are significantly different ($p < 0.05$). Superscripts a and d are the most and least significant, respectively

Key:

WPRG 1 = 56.25% Whole wheat + 18.75% Sweet potato + 5% Rice bran + 20% Peanut

WPRG 2 = 37.50% Whole wheat + 37.50% Sweet potato + 5% Rice bran + 20% Peanut

WPRG 3 = 18.75% Whole wheat + 56.25% Sweet potato + 5% Rice bran + 20% Peanut

WWF Whole wheat flour, *CWF* Commercial wheat flour

WPRG 1 and WPRG 3 having the significant ($p < 0.05$) least and highest values, respectively when compared to those from common CWF. The highest diameter observed for cookie sample WPRG 3, with the lowest quantity of whole wheat flour (18.75%), might be as a result of level of gluten composed in the quantity of flour used. This undoubtedly could make the dough to flow after moulding and had resultant consequence on the diameter of the final cookies (Sharif et al. 2009). The present study also agreed with the past finding (Arogundade et al. 2023) that reported an increase in the diameter of the supplemented biscuits due to reduction in the quantity of wheat flour. However, the thickness of the cookies ranged from 5.03 to 5.10 mm with no observable difference in cookie samples WPRG 1, WPRG 3 and WWF.

The spread ratio of cookies, which is an index of particle size and wettability of flour with the category of oil used in their production (Sharif et al. 2009), ranged from 8.83 to 9.14 (Table 4). The qualities and leavening potentials of flours used in any snack formulation and production could be evaluated by the spread ratio of the snack (Adeyemo et al. 2022). Hence, the lowest spread ratio (8.83) and highest thickness (5.10 mm) obtained for sample CWF, when compared to other sample, could be due to the hydrophilic nature of the industrialized-refined flour (Chinma & Gernah 2007) being used for sample CFW.

Sensory evaluation of the cookies

Table 5 showed the sensory attributes of the cookies, which were impacted by flavour, taste, appearance, texture, colour, general acceptability and past knowledge of cookie consumption by the panelists (Malomo & Udeh 2018). The result revealed that sample WPRG 1 (cookies produced from 56.25% whole wheat + 18.75% sweet potato + 5% rice bran + 20% peanut) had the best and significant ($p \leq 0.05$) sensory attributes when compared to those from 100% whole wheat (WWF). The sample WPRG-1 also showed a closer comparative in terms of overall acceptability of the cookies and those from the commercial sources (CWF) by the panelists. The taste of the cookies was given better ratings than the other parameters, which corresponded to the previous submission of Taghdir et al. (2017) that described the taste of any food product as an essential parameter that determined its acceptability.

Conclusion

The study showed that the level of substitution of wheat flour with flour blends from indigenous but underutilized crops influenced the nutritional quality of the resultant composite cookies. The composite cookies were rich in amino acid compositions that supported the enriched antioxidant properties (> 80%) through their improved hydrophobic amino acid (~ 30%), branched chain amino acid (~ 11%), aromatic amino acid (~ 10%) and enhanced fischer ratio (> 1.0) when compared to those from the 100% wheat flour. All the experimental cookies possessed functional anti-inflammatory properties (≤ 25 µg/ml) againt the known pro-inflammatory precursor nitric oxide and dual pro-inflammatory cyclooxyemase and 5-lipooxygenase enzymes. The presence of hydrophobic amino acids, leucine and glutamic acid, in higher proportion could have enhanced the functional attributes of the composite cookies. The physical properties of the cookies revealed no significant changes in their physical features after substittution. The sample WPRG 1 (56.25% whole wheat + 18.75% sweet potato + 5% rice bran + 20% peanut) was mostly acceptable by the consumers when compared with the cookies produced from the common whole wheat and commercial flours, respectively.

Abbreviations

DPPH 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)

FRAP Ferric reducing antioxidant power

GAE Gallic acid equivalents
HCl Hydrochloric acid
HPLC High-performance liquid chromatography
TCA Trichloroacetic acid

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Authors' contributions
Udeh, C.C. performed the experiment, collected and analyzed the data as well as prepared the draft of the manuscript. Malomo, S.A. designed the experiment, supervised the study, analyzed the data, thoroughly read and edited the manuscript and served as the corresponding Author. Ijarotimi, O.S. thoroughly read and edited the manuscript and co- supervised the study.

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Availability of data and materials
Data are available upon request by contacting the authors.

Declarations

Ethics approval and consent to participate
The study protocol for the present work was approved while the ethic clearance issued to the investigators from the Ethical Committee, School of Agriculture and Agricultural Technology of the Federal University of Technology, Akure, Nigeria with protocol number FUTA/SAAT/2022/033.

Consent for publication
All authors have read and agreed to the published version of the manuscript.

Competing interests
The authors declare that they have no competing interests.

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