

ORIGINAL CONTRIBUTION

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Plantain based dough meal: nutritional property, antioxidant activity and dyslipidemia ameliorating potential in high-fat induced rats

Timilehin David Oluwajuyitan , Oluwole Steve Ijarotimi and Tayo Nathaniel Fagbemi

Abstracts

Background: Dyslipidemia is an aberrant rise in blood lipids due to diet and lifestyle. It has implicated as the major risk factor for developing hypertension among other diseases. This study was designed to evaluate plantain based dough meal nutritional property, antioxidant activity and dyslipidemia ameliorating potential in high-fat induced rats.

Methods: The flour blends, i.e., PSC (Plantain 70%, Soycake 30%), PSR (Plantain 65%, Soycake 30%, Rice-bran 5%), PSO (Plantain 65%, Soycake 30%, Oat-bran 5%), PSRO (Plantain 60%, Soycake 30%, Rice-bran 5%, Oat-bran 5%) and controls (100% Plantain flour & Cerolina) were evaluated for chemical, antioxidants and antihyperlipidemia.

Results: Protein, fiber and energy composition varied from 2.2–4.97 g/100 g, 16.44–19.59 g/100 g and 369.7–385.5 kcal/100 g, respectively. Essential amino acid index and predicted-biological values of the foods ranged from 68.31–76.31% and 62.19–71.48%, respectively. Phenolic profiles (mg/g) were gallic acid (25.33–31.26), caffeic acid (2.75–4.61), ferulic acid (5.16–12.73), luteolin (16.31–23.60), kaempferol (21.51–30.64), quercetin (24.28–37.13), chlorogenic acid (42.25–59.78), myricetin (28.41–38.41), 3,5-dicaffeoylquinic acid (27.17–41.59) and 4, 5-dicaffeoylquinic acid (39.96–51.28). The antioxidant activity of PSRO on ABTS, DPPH, FRAP, Fe²⁺ chelation and OH free radicals was higher than other foods. Atherogenic index, coronary risk index and log (TG/HDL-conc.) of rats fed on experimental foods were lower than recommended values.

Conclusion: The study established that PSRO had higher antioxidant and anti-hyperlipidemia properties; hence, it may be suitable as a functional food.

Keywords: Functional food, Nutritional composition, Phenolic compounds, Antioxidant activity, Antihyperlipidemic

Introduction

Hypertension is a major risk factor for morbidity and premature mortality in developed and developing countries; hence, it is considered a global public health problem [1]. High concentration of Low-Density Lipoprotein cholesterol (LDL-C) in the blood has been implicated as the major risk factor for developing hypertension [2].

Hypertension is defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg [3], and it is commonly referred to as a silent killer. The prevalence of hypertension in traditional African communities, where it is once rare, is increasing [4], due to both sedentary lifestyle and regular intakes of processed diets high in salt and animal fats [5–7]. This unhealthy dietary intake has increased the prevalence of obesity, atherosclerosis-related diseases [8] and impaired fasting glucose [9], which are the major risk factors for hypertension [10]. Evidence has shown

* Correspondence: tdoluwajuyitan@futa.edu.ng
Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria

that synthetic anticholesterol and antihypertensive agents are frequently used to lower high blood cholesterol and pressure; however, these had recently been implicated with many side effects [11, 12]. In recent time, efforts have been shifted towards production of therapeutic diets high in antioxidants and fiber with non-side effects to prevent high blood cholesterol and associated diseases [12–14]. Functional foods are those modified food ingredients or conventional food, which are consumed as part of a normal diet but provides physiological benefits and/or reducing the risk of chronic disease beyond basic nutritional functions [15].

The health promoting properties of functional foods are attributed to bioactive components such as phytochemicals, peptides, fibers, etc. [16]. In recent time, there is increased in the interest of functional foods consumption patterns with the aim of reducing risk of hypertension and other chronic diseases [13]. Diets from plant origin are rich in antioxidant phytochemicals with the abilities to scavenge free radicals, and thereby inhibiting the deleterious effects of oxidative stress [17]. The drives to search for novel, cheap and effective plant-based foods with antioxidant and blood cholesterol and pressure lowering potentials become necessary. It is evidence that regular consumption of phenolic compounds present in plant-based foods is associated with health benefits in man [13]. Some phenolic compounds in foods have antioxidant activities, thereby contributing to a reduction in the risk of arteriosclerosis and cardiovascular diseases [18, 19]. Studies have shown that phenolic compounds such as gallic acid and quercetin have the potential to prevent hypertension, diabetes and other degenerative chronic diseases [18, 19].

Plantain (*Musa ABB*) is widely cultivated in Nigeria and consumed as a cheap source of energy. Nutritionally, plantain particularly unripe is a good source of carbohydrate, dietary fiber, irons, potassium and vitamins [20, 21], and regular consumption of plantain helps to prevent diabetes, hypertension and anaemia due to its high resistant starch content and low glycemic index property [22–24]. Plantain flour has been successfully added to cereals to produce bread [25], spaghetti [26] and other food products, demonstrating that its addition results in a higher resistant starch content and a lower starch digestion rate. In Southwest Nigeria, it is consumed in form of a dough meal - a prepared cooked paste from traditional diets/ plants that is food sustainable, safe, nutritious, culturally acceptable and available all year round.

Soycake (*Glycine max* L) is a by-product generated during oil extraction of soybeans oil. Soybean cake is high in protein and can be used in food formulation to prevent protein-energy malnutrition. Soybean cake is used as a protein supplement and has a good number of

advantages ranging from low cost, readily available and health benefits in man. The application of soycake in food formulation particularly with unripe plantain flour has recently been reported [27–29].

Rice bran (*Oryza sativa* L) is a by-product of rice milling; and it is rich in tocotrienol and ability to reduce total cholesterol and low-density lipoprotein (bad cholesterol). Hence, tocotrienol is used to prevent the risk of heart disease in man [30, 31]. Evidences have shown that blending rice-bran into animal formulations reduced cholesterol levels [32, 33].

Oat bran (*Avena sativa* L.) is a by-product of oatmeal and it is reported to contain β -glucan which significantly increasing blood concentrations of high-density lipoprotein cholesterol [34]. Evidence has established that regular consumption of oat-meals is beneficial to manage hypertension due to its fiber content and bioactive phytochemicals [35]. The β -glucan in oats plays an important role in preventing and managing diabetes by slowing down carbohydrate digestion and absorption of glucose in the small intestine [36].

Recently, efforts have been shifted towards production of functional foods from local food materials to manage chronic degenerative diseases [29, 37, 38]. Despite all these efforts, there is scanty information on the combination of plantain, oat bran, rice bran and soycake flour in food formulation. Hence, this study was aimed to produce and evaluates nutritional composition, bioactive phytochemicals, antioxidants and anti-cholesterol potential of diets formulated from plantain, oat-bran, rice-bran and soycake.

Materials and methods

Sources of food sample

The unripe plantains were obtained from Teaching and Research (T&R) Farm, Federal University of Technology, Akure (FUTA), Nigeria; defatted soybean cake were obtained from Rom Mill Factory, Ibadan, Nigeria; rice-bran were obtained from Igbimo Rice Processing Company, Aisegba-Ekiti, Nigeria and oat-bran were obtained from Richardson Milling Limited, Portage La Prairie, Manitoba (MB), respectively.

The food materials were obtained during the first quarter of the year, authenticated at the Department of Crop, Soil and Pest Management, FUTA, Nigeria (FUTA/CSP/AUT/2019/0087) and stored at room temperature in an air tight container after processing into flour prior to analysis.

Processing of flour and food formulations

The plantain flour was processed using method described by Mepba et al. [39] with slight modification. The plantains were manually peeled and sliced into 1 cm thick pieces, blanched (100 °C, 10 min.) in sodium

hydrogen sulphate (1.25% NaHSO₃) solution to prevent browning and drained. The food samples, i.e., soycake, rice-bran, oat-bran and blanched plantain were dried in hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 60 °C for 24 h, milled into flour (Laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and sieved through 200 mm mesh sieve (British Standard) to obtain experimental flour samples.

The flour samples, i.e., plantain, soycake, rice-bran and oat-bran flour, were blended with reference to 14 g/day protein and 5 g/day fibre (i.e., 25% of recommended daily intakes of an adult requirements) using Response Surface Methodology. The flour samples were blended as follows: PSC (Plantain 70%, Soycake 30%), PSR (Plantain 65%, Soycake 30%, Rice bran 5%), PSO (Plantain 65%, Soycake 30%, Oat bran 5%), PSRO (Plantain 60%, Soycake 30%, Rice bran 5%, Oat bran 5%). Cerolina (wheat and soybean produced by More Foods Lagos, Nigeria) and 100% Plantain flour were used as control.

Preparation of formulated food sample aqueous extracts

The aqueous extract of blended food sample was produced by dispersing each of the flour samples (500 g) into distilled water (2.5 L) and exhaustively marcerated for 12 h. After marceration, the mixture was filtered using muslin cloth and Whatman No.1 filter paper; and the filtrate was concentrated using rotary evaporator (Model 349/2, Corning Limited, England) at 40 °C for 24 h, and thereafter, the filtrate was freeze-dried to obtain food sample extract. The freeze-dried flour sample was packed in a plastic container, sealed and stored at room temperature (~ 27 °C) until required for use.

Determination of chemical composition

Proximate composition

Moisture, crude protein, fat, fiber and ash content of the food samples were determined according to AOAC methods [40]. Total carbohydrate content was calculated by subtracting the total percentage of moisture, fat, protein, fiber and ash from 100.

Mineral content

The minerals, i.e., calcium, magnesium, iron, zinc, copper and manganese, were determined using Atomic Absorption Spectrophotometer (AAS Model SP9). Sodium and potassium content were determined using flame photometer (Sherwood, U.K.) using NaCl and KCl as the standard. Phosphorus was determined by the vanado-molybdate colorimetric method of AOAC [40].

Amino acid profile

The amino acid composition of the food samples were determined using Automated Amino Acid Analyzer

(Model 6300; Beckman Coulter Inc., Fullerton, Calif., USA) as described by Mansouri et al. [41] and Hartcamp et al. [42].

Quantitative determination of phytochemical constituents of the formulated food samples

Tannins content of the food sample was determined as described by the method of Fagbemi et al. [43] with slight modification. Each of the sample extract was measured (1.0 g) and dispersed in distilled water (10 ml), vortex vigorously and centrifuged at 3000 *x* g for 10 min. The filtrate (2.5 ml) and standard tannic acid solution (2.5 ml) were separately dispersed into 50 ml flask, respectively. Into each of the flask, folin-denis reagent (1.0 ml) and saturated Na₂CO₃ solution (2.5 ml) were poured into volumetric flask, respectively. Thereafter, the mixture was diluted with distilled water to mark in the volumetric flask (50 ml) and incubated for 90 min. at room temperature. The absorbance was measured at 250 nm in an electronic spectrophotometer (Genway model 6000i). Readings were taken with the reagent blank at zero. The tannin content was calculated.

$$\text{Tannin (mg/g)} = \frac{\text{Ab} \times \text{Conc.} \times \text{Vf}}{\text{Ast} \times \text{Wt of sample} \times \text{Vol.}}$$

Where

Ab = Absorbance of test sample, Ast = Absorbance of standard solution, Conc. = Concentration of standard solution, W = Weight of sample used Vf = Total volume of extract Vol. = Volume of extract.

Flavonoid was determined by the method reported by Boham and Kocipai [44]. The flour sample (5.0 g) was mixed with 50 mL of 80% aqueous methanol in a 250 ml beaker, covered, and incubated for 24 h at room temperature. The mixture was filtered and the supernatant was discarded. The residue was re-extracted (thrice) with 50 ml of ethanol. The combined mixture was filtered with Whatman filter paper number 42 (125 mm), and the filtrate was transferred into a crucible. The filtrate was evaporated to dryness using a water bath, cooled in a desiccator and weighed until constant weight was obtained. The flavonoid was calculated.

Saponin was determined as described by the method of Obadoni and Ochuko [45]. Each of the flour samples (20 g) was poured into a 250 ml conical flask conical flask containing 100 ml of 20% aqueous ethanol. The mixture was transferred into a hot water bath maintained at 50 °C for 4 h with continuous stirring. The residue of the mixture was again extracted with another 100 ml of 20% aqueous ethanol after filtration. Thereafter, the combined mixture was filtered and concentrated in a water bath at 90 °C. To the concentrated filtrate, 20 ml diethyl ether was added and mixed

vigorously to separate the aqueous layer from the ether layer, which was discarded. This purification process was repeated twice. The separated aqueous solution was mixed with 60 ml of n-butanol. The mixture was then washed twice with 10 ml of 5% aqueous sodium chloride, and the sodium chloride layer was separated and discarded, while the left over was re-concentrated in a water bath at 90 °C for 30 min. The filtrate was then transferred into a crucible, and thereafter oven dried in hot-air oven (Gallenkamp, England) at 60 °C to a constant weight. The saponin content was calculated.

Oxalate was determined as described by the modified method of Adeniyi et al. [46]. The sample (2.5 g) was digested with 10 ml 6 M HCl at a temperature of 60 °C for 60 min. With constant stirring using a magnetic stirrer, and then filtered. To 5.0 ml of the filtrate, 1.0 ml of 5 M ammonium hydroxide solution was added (to adjust pH) until the colour of the solution changed from salmon pink colour to a faint yellow colour. Thereafter, to the solution, phenolphthalein indicator (2 drops), glacial acetic acid (3 drops) and 5% calcium chloride (5.0 ml) were added to precipitate insoluble oxalate, and the solution was allowed to incubate for 120 min. at room temperature before centrifuged at 2500 \times g 15 min. The precipitate was washed with distilled hot water, 5.0 ml of 3 M tetraoxosulphate (VI) acid was added and warmed in a water bath at 60 °C for 15 min. Freshly prepared 0.01 M potassium permanganate (KMnO₄) was titrated against 12.5 ml of the filtrate until a faint pink colour, which persisted for about 30 s. and the volume of KMnO₄ used was read from the burette reading. The oxalate content was calculated (mg/g).

$$\text{Oxalate} \left(\frac{\text{mg}}{\text{g}} \right) = V_T \times 0.9004$$

Where, VT = Titre volume (ml).

Trypsin inhibition activity (TIA) was assayed described by the modified method of Smith et al. [47]. TIA was assayed in terms of the extent to which the extract of the defatted flour samples inhibited the action of bovine trypsin (EC 3.4.21.4) on the substrate benzoyl-DL-arginine-p-nitranilide (BAPNA) hydrochloric. The food sample (1.0 g) was extracted with 50 ml, 10 mM NaOH at ambient temperature for 2 h. with continuously shaking using a mechanical shaker (GallenKamp orbital shaken Surrey, UL). To the obtained slurry, 1 M NaOH was added to adjust the pH to 9.4. The suspension was diluted to 50 ml with phosphate buffer, and into the test tube, inhibitor extract (1.0 ml), trypsin solution (1.0 ml) and 5% trichloroacetic acid (6.0 ml) were added; and the mixture was incubated at room temperature for 60 min. Thereafter, the suspension was filtered, and the absorbance of the filtrate and the blank (6 ml of 5% TCA and

4 ml buffer) were measured at 280 nm using spectrophotometer (Spectronic 601 model, Milton Roy Company, USA). Trypsin inhibitor activity was calculated in terms of mg pure trypsin (Sigma type III, lot 20H0868).

$$\text{TIA} = \frac{2.632DA \text{ mg pure trypsin inhibited g}^{-1} \text{ sample}}{\text{Weight of Sample}}$$

Where D is the dilution factor, A is the change in absorbance at 410 nm due to trypsin inhibition per ml diluted sample extract.

The phenol in the food samples was determined as described by the method of George' et al. [48] with minor modifications. The food sample (2.0 g) was defatted using 100 ml ether in a soxhlet apparatus for 90 min. The defatted food sample (1.0 g) was dispersed in 50 cm³ boiled ether for 15 min. to extract the phenolic components. Into the extracted filtrate (5.0 ml), distilled water (10.0 ml), 0.1 N ammonium hydroxide solution (2.0 ml), and concentrated amyl alcohol (5.0 ml) were added, and the mixture was left to react for 25 min. For colour development. The optical density was measured at the absorbance of 505 nm. For the preparation of phenol standard curve, 0.20 g of tannic acid was dissolving in distilled water and diluted to 200 mL mark (1 mg/ml). The standard tannin acid solution was measured into five different test tube by varying its concentration between 0.2–1.0 mg/ml. The standard tannic acid solution was then pipetted against the solution of NH₃OH (2.0 ml), amyl alcohol (5.0 ml), and distilled water (10.0 ml). The solution was thereafter made up to 100 ml volume and left to react for 25 min. For colour development. The optical density was measured with spectrophotometer (Cecil 3021 spectrophotometer, Cambridge, United Kingdom) at 505 nm. The amount of phenolic compounds was expressed as mg of gallic acid per g of extract (mgGAE/g).

The polyphenolic profiles were determined as described by Kelley et al. [49] and Provan et al. [50]. The homogenised freeze-dried formulated food samples (0.5 g) were extracted with 25 ml distilled water using homogeniser (Ultra Turrax T25 (IKA.Werke, Janke & Kunkel). The aliquot of the aqueous food sample extract (0.1 ml) was poured into GC vials and 50 μ L of an internal standard was added (3-[4-hydroxyphenyl]-1-propanol solution, 19.2 μ g/ml). Thereafter, the sample was evaporated to dryness under nitrogen and derivatised by addition of 250 μ L N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) at 60 °C for 15 min. Analysis of the samples was performed by an Agilent (Wallborn, Germany) HP series GC 6890 N coupled with a HP 5973 MS detector (EI, 70 eV), a split–splitless injector, and an HP 7683 autosampler in an HP-5 MS capillary column (5% phenyl–95% methyl siloxane, 30 m \times 0.25 mm \times

250 µm), and 3-(4-hydroxyphenyl)-1-propanol was used as the standard.

Determination of functional and pasting properties

The functional properties of flour blends, that is, bulk density, water absorption, oil absorption, foaming capacity/stability, emulsion capacity/stability and gelatinization were determined by the methods described by Iwe et al. [51]. Swelling capacity was determined according to method described by Akpata and Miachi [52]. Pasting properties of the flour blends were determined using the Rapid Visco Analyzer (RVA Model 3c, Newport Scientific PTY Ltd., Sydney) as described by AACC [53].

In-vitro antioxidant assay of the formulated food sample aqueous extracts

The in-vitro antioxidant assay of the aqueous extracts of the food sample using standard methods. The free radical scavenging activity of the food samples was determined against 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonate (ABTS) as described by Re et al. [54]. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging was determined as described by Aluko and Monu [55]. Metal chelating activity of the formulated food aqueous extract was determined using the method of Xie et al. [56]. Ferric reducing antioxidant activity (FRAP) was determined according to Zhang and Lin [57]. The hydroxyl free radical scavenging activity of the food extract was determined according to the method reported by Girgih et al. [58].

Calculation: The dietary antioxidant activity index (DAAI) were calculated for each of the formulated food samples as follows:

$$DAAI = \left(\frac{\text{Final concentration of antioxidant eg DPPH (mg/dL)}}{IC_{50}(\text{mg/dL})} \right)$$

Low antioxidant activity DAAI < 0.5, moderate antioxidant activity DAAI = 0.5–1.0, strong antioxidant activity DAAI = 1.0–2.0, very strong = DAAI > 2.0 [59].

Experimental design and determination of blood lipid profile in rats

Ethical approval

The experiments on animals were conducted in accordance with the force laws and regulations as regards animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review [60].

Experimental design

Forty male albino rats (190–200 g) were purchased from the Department of Biochemistry, Federal University of Technology, Akure, Nigeria. The rats were divided into seven groups ($n = 5$); and were housed in individual

stainless steel cages maintained at standard environmental condition of controlled temperature $24 \pm 5^\circ\text{C}$, illumination (12 h light/12 h dark cycles), relative humidity of $60 \pm 4\%$ for 7-day acclimatization.

Formulation and feeding of high fat diet to the experimental rats

After 7-day acclimatization, the experimental rats were fed on high-fat diets (HFD) for diet-induced obesity for 28 days. The high-fat diets (g/100 g) were formulated from the blending of skimmed milk (50 g), lard (30 g), cellulose (9 g), corn starch (7 g) and premix (4 g) as described by Kim et al. [61] and Vatandoust et al. [62] with slight modification. The HFD (20 g/day) and water were provided to the rats' ad libitum throughout the experimental period.

Grouping and feeding of experimental diets to the rats

- Group I – rats fed with basal diet + Satin
- Group II – rats fed with 100% Plantain flour
- Group III – rats fed with Plantain 70% + Soycake 30% (PSC)
- Group IV – rats fed with Plantain 65% + Soycake 30% + Rice bran 5% (PSR)
- Group V – rats fed with Plantain 65% + Soycake 30% + Oat bran 5% (PSO)
- Group VI – rats fed with Plantain 60% + Soycake 30% + Oat bran 5% + Rice bran 5% (PSRO)
- Group VII – rats fed with Cerolina, a commercial swallow meal

The rats were administered with the experimental diets and water ad libitum; while Satin (an anti-cholesterol agent = 10 mg/kg body weight) was administered orally to the rats for 28 days.

Determination of blood lipid profile

At the end of 28 days, the rats were sacrificed after a 12-h overnight fast, and blood sample was collected via cardiac puncture into clean test tube. Total Cholesterol (TC) was determined by the method described by Siedel et al. [63], Triglyceride concentration (TG) by the method described by Tietz [64], and HDL-cholesterol was determined using the method reported by Jacobs et al. [65]. The serum High-density lipoprotein concentration (HDL-conc.) was determined by the dextran sulphate-magnesium (II) precipitation method reported by Albers et al. [66]. Low-Density Lipoprotein (LDL-concentration), Very Low-Density Lipoprotein (VLDL-conc.), Atherogenic Index (AI) and Coronary Risk Index (CRI) were calculated:

Low-density lipoprotein (LDL)

The Low Density Lipoprotein was calculated according to the method described by Friedewald et al. [67].

$$LDL = \frac{\text{Total cholesterol} - \text{Triglycerides} - HDL}{5}$$

Very Low-Density Lipoprotein cholesterol (VLDL-C) [68].

VLDL-C (mg/dL) = Triglycerides / 2.2.

Atherogenic index (AI) [69].

$$AI = \frac{LDL\text{-cholesterol}}{HDL\text{-cholesterol}}$$

Coronary Risk Index (CRI) [70].

$$CRI = \frac{\text{Total cholesterol}}{HDL\text{-cholesterol}}$$

Where,

VLDL-C is very low-density lipoprotein cholesterol.

LDL-C is low-density lipoprotein cholesterol.

HDL-C is high-density lipoprotein cholesterol.

Data analysis

Data in triplicate readings were analysed and results presented in mean ± standard error mean (SEM). One-way Analysis of Variance (ANOVA) was used to analyze the means. Significant differences between means were determined using Duncan with level of significant at $p \leq 0.05$.

Results

Nutritional composition

The nutrient composition of the blended flour samples is presented in Table 1. The moisture content, total fiber, crude protein and energy value of the food samples ranged from 5 to 5.5 g/100 g, 2.2–4.97 g/100 g, 16.44–19.59 g/100 g and 369.7–385.5 kcal/100 g, respectively.

The mineral composition of the food products showed that phosphorous (24.37–33.36 mg/100 g) was the most abundant, while copper (0.12–0.16 mg/100 g) had the least concentration in the food products, and the values were comparatively higher than in 100% plantain flour (7.43, 0.09 mg/100 g) and cerolina (17.31, 0.09 mg/100 g). The Na/K and Ca/P molar ratios were lower than critical values of < 1, > 1, respectively. The amino acid compositions and predicted nutritional quality of the food products are presented in Tables 2 & 3, respectively. The

Table 1 Proximate composition (g/100 g DM), energy value (Kcal/100 g) and minerals (mg/100 g) of plantain, soycake, rice-bran and oat-bran flour blends and control samples

Samples	100% Plantain	PSC	PSR	PSO	PSRO	Cerolina	*Ref.
Moisture	5.10 ± 0.04 ^d	5.20 ± 0.02 ^c	5.10 ± 0.03 ^d	5.30 ± 0.02 ^b	5.50 ± 0.05 ^a	5.60 ± 0.04 ^a	< 10
Ash	2.00 ± 0.02 ^e	3.50 ± 0.01 ^c	4.40 ± 0.05 ^a	3.00 ± 0.06 ^d	4.00 ± 0.03 ^b	2.00 ± 0.04 ^e	< 3
Fat	2.00 ± 0.02 ^f	5.00 ± 0.01 ^e	6.09 ± 0.03 ^d	6.29 ± 0.05 ^c	6.87 ± 0.03 ^b	8.67 ± 0.04 ^a	10–25
Fibre	1.33 ± 0.02 ^f	2.20 ± 0.04 ^e	5.69 ± 0.03 ^a	3.19 ± 0.04 ^d	4.97 ± 0.09 ^b	3.55 ± 0.06 ^c	< 5
Protein	5.69 ± 0.09 ^f	16.44 ± 0.04 ^e	16.52 ± 0.06 ^d	19.43 ± 0.07 ^b	19.59 ± 0.04 ^a	18.01 ± 0.05 ^c	> 16
Carbohydrates	83.88 ± 0.09 ^a	67.66 ± 0.04 ^b	62.20 ± 0.05 ^c	62.79 ± 0.07 ^c	59.07 ± 0.03 ^d	62.17 ± 0.08 ^c	64
Energy	376.28 ± 0.17 ^d	381.40 ± 0.21 ^c	369.69 ± 0.15 ^e	385.49 ± 0.23 ^b	376.47 ± 0.10 ^d	398.75 ± 0.24 ^a	400–425
Minerals							
Ca	2.32 ± 0.00 ^d	2.41 ± 0.01 ^c	2.24 ± 0.01 ^e	3.27 ± 0.00 ^b	4.33 ± 0.01 ^a	2.38 ± 0.01 ^c	500
Mg	2.47 ± 0.00 ^e	2.54 ± 0.01 ^d	2.67 ± 0.01 ^c	2.85 ± 0.01 ^b	3.54 ± 0.01 ^a	2.40 ± 0.01 ^f	76
P	7.43 ± 0.01 ^f	24.37 ± 0.01 ^d	26.27 ± 0.01 ^c	33.36 ± 0.00 ^a	29.35 ± 0.01 ^b	17.31 ± 0.00 ^e	456
K	8.51 ± 0.01 ^e	9.15 ± 0.01 ^d	9.64 ± 0.01 ^c	10.12 ± 0.01 ^b	14.82 ± 0.01 ^a	7.38 ± 0.00 ^f	516
Na	4.27 ± 0.00 ^d	4.53 ± 0.01 ^c	4.59 ± 0.01 ^b	4.57 ± 0.00 ^b	4.66 ± 0.01 ^a	3.71 ± 0.00 ^e	296
Fe	2.16 ± 0.00 ^e	2.34 ± 0.01 ^d	2.83 ± 0.01 ^b	2.83 ± 0.00 ^b	2.95 ± 0.01 ^a	2.53 ± 0.01 ^c	16
Zn	2.64 ± 0.00 ^c	1.53 ± 0.01 ^f	2.38 ± 0.01 ^e	2.74 ± 0.01 ^b	2.84 ± 0.01 ^a	2.56 ± 0.01 ^d	3.20
Cu	0.09 ± 0.01 ^{bc}	0.12 ± 0.00 ^b	0.10 ± 0.01 ^b	0.15 ± 0.01 ^a	0.16 ± 0.01 ^a	0.09 ± 0.01 ^{bc}	0.89
Mn	0.19 ± 0.00 ^d	0.24 ± 0.00 ^c	0.31 ± 0.01 ^a	0.27 ± 0.01 ^b	0.29 ± 0.01 ^{ab}	0.16 ± 0.01 ^e	1.50
Na/K	0.50 ^a	0.49 ^a	0.48 ^a	0.45 ^b	0.31 ^c	0.50 ^a	< 1.00
Ca/P	0.31 ^a	0.10 ^c	0.09 ^c	0.10 ^c	0.15 ^b	0.09 ^c	> 1.00

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at $P < 0.05$

Key: 100% Plantain flour; PSC: Plantain: Soycake (70:30%); PSR: Plantain: Soycake: Rice bran (65:30:5%); PSO: Plantain: Soycake: Oat bran (65:30:5%); PSRO: Plantain: Soycake: Rice bran: Oat bran (60:30:5:5); Cerolina: a commercial flour meal. Ref: NIS [118]

Table 2 Amino acid profile (g/100 g protein) of plantain, soycake, rice bran and oat bran flour and control sample

Amino Acids	100% Plantain	PCS	PSR	PSO	PSRO	Cerolina	*Adult	*Children
Non Essential Amino Acids (NEAAs)								
Glycine	3.97 ^d	4.49 ^c	4.48 ^c	4.48 ^c	4.62 ^b	4.88 ^a	–	–
Alanine	3.73 ^f	4.46 ^d	4.48 ^c	4.44 ^e	4.50 ^b	5.14 ^a	–	–
Serine	5.24 ^f	5.51 ^b	5.33 ^e	5.48 ^c	5.45 ^d	5.66 ^a	–	–
Proline	7.45 ^a	5.05 ^d	5.07 ^c	5.09 ^b	5.10 ^b	4.04 ^e	–	–
Aspartic	9.44 ^f	12.40 ^b	12.36 ^c	11.96 ^e	12.07 ^d	14.91 ^a	–	–
Cysteine	1.59 ^a	1.07 ^f	1.16 ^e	1.32 ^c	1.21 ^d	1.35 ^b	–	–
Glutamic	24.32 ^a	17.94 ^d	17.87 ^e	18.28 ^b	18.15 ^c	15.13 ^f	–	–
Tyrosine	2.80 ^e	3.27 ^d	3.41 ^c	3.46 ^a	3.28 ^d	3.44 ^b	–	–
Arginine	5.91 ^f	7.23 ^b	7.18 ^c	7.16 ^d	7.05 ^e	8.18 ^a	–	–
ΣNEAAs	65.13 ^a	62.25 ^c	61.99 ^e	62.22 ^d	62.26 ^c	62.94 ^b	–	–
Essential Amino Acids (EAAs)								
Phenylalanine	5.12 ^c	5.10 ^d	5.17 ^b	5.18 ^b	5.22 ^a	4.62 ^e	2.50	6.90
Histidine	1.93 ^e	2.46 ^b	2.43 ^c	2.40 ^d	2.41 ^d	3.97 ^a	–	1.00
Methionine	1.52 ^b	1.26 ^f	1.41 ^d	1.47 ^c	1.37 ^e	1.63 ^a	1.50	2.70
Valine	4.55 ^e	5.00 ^b	4.97 ^d	4.97 ^d	5.06 ^a	4.99 ^c	2.60	3.80
Tryptophan	1.22 ^d	1.33 ^b	1.31 ^c	1.37 ^a	1.23 ^e	1.22 ^e	0.40	1.25
Threonine	3.48 ^f	4.10 ^b	4.07 ^c	4.01 ^d	4.13 ^a	3.66 ^e	1.50	3.70
Isoleucine	4.19 ^c	4.50 ^b	4.49 ^b	4.49 ^b	4.53 ^a	3.53 ^d	2.00	3.10
Leucine	7.34 ^d	7.73 ^a	7.72 ^a	7.64 ^c	7.70 ^b	6.61 ^e	3.90	7.30
Lysine	4.76 ^f	6.05 ^a	6.02 ^b	5.78 ^d	5.85 ^c	5.67 ^e	3.00	6.40
ΣEAAs	34.87 ^e	37.75 ^c	38.01 ^a	37.78 ^b	37.74 ^c	37.06 ^d	–	–
TAAAs	100.00	100.00	100.00	100.00	100.00	100.00	–	–

Key: 100% Plantain flour; PCS: Plantain: Soycake (70:30%); PSR: Plantain: Soycake: Rice bran (65:30:5%); PSO: Plantain: Soycake: Oat bran (65:30:5%); PSRO: Plantain: Soycake: Rice bran: Oat bran (60:30:5:5); Cerolina: a commercial flour meal; NEAAs Non-essential amino acids, EAAs Essential amino acids, TAAAs Total amino acids
*RDA of essential amino acids (mg/100 gb.w) for Adult and Children (< 5 yrs.) [119, 120]

Table 3 Predicted nutritional qualities of plantain, soycake, rice bran and oat bran flour and control sample

Parameters	100% Plantain	PCS	PSR	PSO	PSRO	Cerolina
HAAAs (g/100 g protein)	37.52 ^d	38.76 ^c	39.18 ^b	39.43 ^a	39.19 ^b	36.55 ^e
PCAAAs(g/100 g protein)	6.69 ^f	8.51 ^b	8.45 ^c	8.18 ^e	8.26 ^d	9.64 ^a
NCAAAs(g/100 g protein)	33.76 ^a	30.33 ^b	30.22 ^d	30.24 ^c	30.22 ^d	30.04 ^f
ArAAAs (g/100 g protein)	9.15 ^f	9.69 ^d	9.89 ^b	10.01 ^a	9.74 ^c	9.27 ^e
SCAA (g/100 g protein)	3.10 ^a	2.33 ^e	2.58 ^d	2.79 ^c	2.57 ^d	2.98 ^b
BCAA (g/100 g protein)	16.09 ^d	17.23 ^b	17.18 ^c	17.11 ^c	17.28 ^a	15.13 ^e
TEAA/TNEAA	0.54 ^b	0.61 ^a	0.61 ^a	0.61 ^a	0.61 ^a	0.59 ^a
PER (g/ 100 g)	2.42 ^d	2.68 ^a	2.22 ^e	2.58 ^c	2.67 ^a	2.63 ^b
EAAI (%)	62.84 ^f	67.79 ^d	68.31 ^c	68.38 ^b	76.31 ^a	67.59 ^e
P-BV (%)	54.11 ^f	62.19 ^d	62.76 ^c	62.83 ^b	71.48 ^a	61.98 ^e
NI (%)	11.32 ^d	11.14 ^f	11.29 ^e	13.40 ^c	14.83 ^a	3.85 ^b
ARG/LYS	1.24 ^b	1.20 ^c	1.19 ^c	1.24 ^b	1.20 ^c	1.44 ^a
LAAAs	Histidine	Methionine	Methionine	Methionine	Methionine	Methionine
AAAAs	Methionine	Arginine	Arginine	Arginine	Arginine	Arginine

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at $P < 0.05$

Key: 100% Plantain flour; PCS: Plantain: Soycake (70:30%); PSR: Plantain: Soycake: Rice bran (65:30:5%); PSO: Plantain: Soycake: Oat bran (65:30:5%); PSRO: Plantain: Soycake: Rice bran: Oat bran (60:30:5:5); Cerolina: a commercial flour meal. HAAAs Hydrophobic amino acids, PCAAAs Positively charged amino acids, NCAAAs Negatively charged amino acids, ArAAAs Aromatic amino acids, SAAAs Sulphur containing amino acids, BCAAAs Branch chain amino acids, EAAs Essential amino acids, NEAAs Non-essential amino acids, ARG/LYS Arginine/ lysine ratio, PER Protein efficiency ratio, P-BV Predicted biological value, NI Nitrogen index, LAAAs Limiting Amino Acids, AAAAs Abundant amino acids

essential amino acids profile, essential amino acid index (EAAI), predicted-biological values (P-BV) and nutritional index of the formulated food products ranged from 37.74–38.01 (g/100 g protein), 68.31–76.31%, 62.19–71.48% and 11.14–14.83%, respectively, and were significantly ($p < 0.05$) higher than 100% plantain flour and cerolina. The branched chain amino acids (BCAAs), aromatic amino acids (ArAAs) and Arginine/lysine molar ratios of the food products ranged from 17.11–17.28 g/100 g protein, 9.69–10.01 g/100 g protein and 1.19–1.24, respectively, and were higher than 100% plantain and cerolina, except in Arginine/Lysine ratio.

Phytochemicals and phenolic compounds

The phytochemical concentration (mg/g) in the formulated food samples showed that phenol, flavonoid and tannin varied from 11.35–18.42, 0.30–0.54 and 1.97–3.09, respectively; while phytate, oxalate and trypsin varied between 2.76–3.58, 0.54–0.72 and 5.12–10.14, respectively (Table 4). Comparatively, the concentration of these phytochemicals was lower than critical values, but were similar to that of 100% plantain flour and Cerolina.

The phenolic profile of the formulated food samples is presented in Table 5. The concentration of phenolic compounds (mg/g) in the formulated food samples were gallic acid (25.33–31.26), caffeic acid (2.75–4.61), Ferulic acid (5.16–12.73), luteolin (16.31–23.60), kaempferol (21.51–30.64), quercetin (24.28–37.13), chlorogenic acid (42.25–59.78), myricetin (28.41–38.41), 3,5-dicaffeoylquinic acid (27.17–41.59) and 4,5-dicaffeoylquinic acid (39.96–51.28). The PSRO had the highest phenolic concentration, while PSC had the lowest concentration; and the values were either higher or lower than in 100% plantain and Cerolina samples.

Functional, pasting and colour properties

The functional property of plantain, soycake, rice-bran and oat-bran composite flour and control samples is presented in Table 6. Bulk density (BD) of the blended flour samples ranged from 0.67 to 0.71 g/mL, and was significantly ($p < 0.05$) lower than 100% plantain (0.74 g/

mL) and cerolina (0.73 g/mL). Water absorption capacity of the flour samples ranged from 0.67–0.71 g/mL, and was comparable to that of 100% plantain flour and Cerolina.

The least gelation concentration (LGC), that is, the lowest protein concentration at which gel remained in the inverted tube, ranged from 6 to 8%, and the values were comparable to that of 100% plantain and Cerolina. However, lowest LGC value was observed in the products with rice-bran and oat-bran inclusion.

The peak viscosity (i.e., an index of the ability of starch-based foods to swell freely before their physical breakdown) values in this study ranged between 422 and 584 RVU, and the values were significantly lower than 100% plantain flour (2186 RVU) and cerolina (792 RVU).

The trough viscosity, i.e., the point at which the viscosity reaches its minimum during either heating or cooling processes, ranged from 362 to 500 for PSR and PSRO, respectively. These values were significantly lower than in 100% plantain flour (2065 RVU) and Cerolina (571 RVU). The breakdown viscosity of the flour samples ranged from 60 to 84 RVU, while of 100% plantain flour and Cerolina were 121 RVU and 221 RVU, respectively.

The values of final viscosity, setback and pasting temperature of the experimental flour samples ranged from 717 to 996 RVU, 355–496 RVU and 92–94 RVU, respectively, and were lower than in 100% plantain flour, but comparable to Cerolina. For the peak time, the values were 7 min. and 5.27 min. For the experimental flour samples, 100% plantain flour and cerolina, respectively.

Antioxidant activities

The antioxidant activity of the formulated food sample extracts is presented in Table 7. The antioxidant activities of the food samples against DPPH, ABTS, Fe^{2+} chelation, FRAP and OH free radical were concentration dependent, that is, as the concentration of the food sample extracts increased, the inhibitory activities of free

Table 4 Phytochemical composition (mg/g) of plantain, soycake, rice bran and oat bran flour and control samples

Parameters	100% Plantain	PSC	PSR	PSO	PSRO	Cerolina	*CV
Total Phenol	16.18 ± 0.03 ^f	19.08 ± 0.03 ^e	24.28 ± 0.04 ^c	25.97 ± 0.03 ^b	26.15 ± 0.03 ^a	21.58 ± 0.04 ^d	–
Flavonoid	0.84 ± 0.01 ^d	0.91 ± 0.01 ^c	0.92 ± 0.01 ^c	0.96 ± 0.01 ^b	1.15 ± 0.02 ^a	0.92 ± 0.01 ^c	–
Tannin	5.19 ± 0.01 ^f	5.65 ± 0.02 ^e	5.97 ± 0.01 ^c	6.04 ± 0.02 ^b	6.58 ± 0.01 ^a	5.79 ± 0.03 ^d	3.0 mg/100 g
Phytate	7.00 ± 0.02 ^a	6.18 ± 0.23 ^b	5.77 ± 0.01 ^b	4.53 ± 0.24 ^c	3.71 ± 0.03 ^d	3.71 ± 0.24 ^d	5–6 g/100 g
Oxalate	0.59 ± 0.02 ^a	0.54 ± 0.01 ^a	0.41 ± 0.02 ^b	0.32 ± 0.02 ^c	0.18 ± 0.00 ^d	0.45 ± 0.01 ^b	–
Trypsin Inhibitor	10.02 ± 0.06 ^d	8.32 ± 0.09 ^f	9.27 ± 0.02 ^e	10.61 ± 0.02 ^c	13.13 ± 0.03 ^b	17.03 ± 0.03 ^a	0.25 g/100 g

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at $P < 0.05$

Key: 100% Plantain flour; PSC: Plantain: Soycake (70:30%); PSR: Plantain: Soycake: Rice bran (65:30:5%); PSO: Plantain: Soycake: Oat bran (65:30:5%); PSRO: Plantain: Soycake: Rice bran: Oat bran (60:30:5:5); Cerolina: a commercial flour meal

*CV: phytate:calcium; phytate:zinc [121]; phytate:iron [122]; and phytate:calcium/zinc [123]

Table 5 Phenolic profile (mg/g) of plantain, soycake, rice bran and oat bran dough meal and control samples

Phenolic profile	100% Plantain	PSC	PSR	PSO	PSRO	Cerolina
Catechin	29.54 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
p-coumaric acid	nd	0.34 ^e	0.39 ^d	0.46 ^c	0.71 ^a	0.51 ^b
Epicatechin	4.30 ^a	nd	nd	nd	nd	nd
p-hydroxybenzoic acid	5.92 ^a	0.02 ^b	0.02 ^b	0.02 ^b	0.02 ^b	0.02 ^b
Gallic acid	43.79 ^a	25.33 ^e	26.51 ^d	29.80 ^c	31.26 ^b	22.90 ^f
Caffeic acid	10.74 ^a	2.75 ^d	3.30 ^c	4.61 ^b	4.61 ^b	2.55 ^e
Syringic acid	5.26 ^a	0.02 ^b	0.02 ^b	0.02 ^b	0.01 ^b	0.01 ^b
Ferulic acid	3.40 ^f	5.16 ^d	6.43 ^c	8.41 ^b	12.73 ^a	4.13 ^e
Apigenin	5.90 ^a	nd	nd	nd	nd	nd
Luteolin	18.39 ^d	16.31 ^f	18.50 ^c	21.91 ^b	23.60 ^a	17.44 ^e
Naringenin	1.32 ^a	0.01 ^b	0.01 ^b	0.01 ^b	0.01 ^b	0.01 ^b
Kaempferol	24.22 ^c	21.51 ^e	23.27 ^d	28.53 ^b	30.64 ^a	19.82 ^f
Naringenin chalcone	1.32 ^a	nd	nd	nd	nd	nd
Quercetin	23.46 ^e	24.28 ^d	29.64 ^c	32.59 ^b	37.13 ^a	24.03 ^f
Chlorogenic acid	nd	42.25 ^c	42.25 ^c	46.88 ^b	59.78 ^a	42.58 ^d
Myricetin	21.23 ^f	28.41 ^e	32.08 ^c	32.55 ^b	38.41 ^a	30.64 ^d
Isorhamnetin	2.10 ^a	0.03 ^c	0.05 ^b	0.03 ^b	0.02 ^b	0.02 ^b
3,5-dicaffeoylquinic acid	1.10 ^f	27.17 ^e	29.64 ^c	35.30 ^b	41.59 ^a	28.89 ^d
4,5-dicaffeoylquinic acid	2.65 ^e	39.96 ^c	42.25 ^c	44.20 ^b	51.28 ^a	37.32 ^d
Rutin	6.51 ^a	nd	nd	nd	nd	nd
Total	211.18 ^f	233.55 ^d	257.73 ^c	285.33 ^b	331.81 ^a	230.86 ^e

Key: 100% Plantain flour; PSC: Plantain: Soycake (70:30%); PSR: Plantain: Soycake: Rice bran (65:30:5%); PSO: Plantain: Soycake: Oat bran (65:30:5%); PSRO: Plantain: Soycake: Rice bran: Oat bran (60:30:5:5); Cerolina: a commercial flour meal; Nd: Below detectable limit

radicals' formation and oxidation of molecules against DPPH. ABTS, Fe²⁺ chelation, FRAP and OH free radical increased. However, the PSRO sample had the highest antioxidant activity when compared to other formulated food samples including 100% plantain flour, but

comparable to that of Cerolina (a control sample). The antioxidative activity (IC₅₀) of the formulated food sample extracts varied as follows: ABTS (52.67–61.23 mg/mL), DPPH (35.45–65.29 mg/mL), FRAP (57.71–60.04 mg/mL), Fe²⁺ chelation (47.29–71.05 mg/mL) and OH

Table 6 Functional and pasting properties of plantain, soycake, rice bran and oat bran composite flour and control samples

Samples	100% Plantain	PSC	PSR	PSO	PSRO	Cerolina
BD (g/mL)	0.74 ± 0.03 ^a	0.67 ± 0.00 ^b	0.71 ± 0.03 ^{ab}	0.71 ± 0.01 ^{ab}	0.67 ± 0.03 ^b	0.73 ± 0.01 ^a
WAC (g/mL)	2.50 ± 0.03 ^b	2.45 ± 0.05 ^b	2.08 ± 0.03 ^d	2.15 ± 0.03 ^c	2.70 ± 0.05 ^a	2.48 ± 0.02 ^b
SC (%)	51.43 ± 0.01 ^c	55.00 ± 0.01 ^a	48.61 ± 0.02 ^e	50.00 ± 0.01 ^d	53.75 ± 0.02 ^b	40.63 ± 0.01 ^f
LGC (%)	8.00 ± 0.00 ^a	8.00 ± 0.00 ^a	6.00 ± 0.00 ^c	6.00 ± 0.00 ^c	7.00 ± 0.00 ^b	8.00 ± 0.00 ^a
Pasting Properties						
Peak (RVU)	2186 ± 0.06 ^a	584 ± 0.04 ^c	422 ± 0.02 ^f	459 ± 0.05 ^e	500 ± 0.05 ^d	792 ± 0.06 ^b
Trough (RVU)	2065 ± 0.05 ^a	500 ± 0.03 ^c	362 ± 0.04 ^f	395 ± 0.03 ^e	432 ± 0.06 ^d	571 ± 0.05 ^b
Breakdown (RVU)	121 ± 0.03 ^b	84 ± 0.02 ^c	60 ± 0.02 ^f	64 ± 0.04 ^e	68 ± 0.07 ^d	221 ± 0.03 ^a
Final Viscosity (RVU)	3798 ± 0.16 ^a	996 ± 0.11 ^b	717 ± 0.13 ^f	848 ± 0.12 ^e	879 ± 0.15 ^d	988 ± 0.16 ^c
Set back (RVU)	1733 ± 0.03 ^a	496 ± 0.03 ^b	355 ± 0.07 ^f	453 ± 0.02 ^c	447 ± 0.08 ^d	417 ± 0.05 ^e
Peak Time (Min)	7 ± 0.02 ^a	7 ± 0.02 ^a	7 ± 0.04 ^a	7 ± 0.03 ^a	7 ± 0.02 ^a	5.27 ± 0.02 ^b
Pasting Temp. (°C)	87.3 ± 0.03 ^d	92 ± 0.06 ^c	94.45 ± 0.06 ^a	93.65 ± 0.06 ^b	94.40 ± 0.06 ^a	86.45 ± 0.02 ^e

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at $P < 0.05$

Key: PLT: 100% Plantain; PSC: Plantain: Soycake (70:30%); PSR: Plantain: Soycake: Rice bran (65:30:5%); PSO: Plantain: Soycake: Oat bran (65:30:5%); PSRO: Plantain: Soycake: Rice bran: Oat bran (60:30:5:5); CTRL: 100% Commercial dough meal. *l**: lightness/darkness; *a**: red/green; *b**: yellow/blue; *c**: brightness/dullness; *h**: hue; ΔE : total difference; WI: Degree of whiteness

Table 7 Antioxidant activities of plantain, soycake, rice-bran and oat-bran flour and control samples

Concentration						
Sample	50.00 mg/mL	75.00 mg/mL	100.00 mg/mL	IC₅₀ mg/mL	DAAI	R-square
ABTS Free Radical Scavenging Activities (mg TEAC/g)						
100% Plantain	0.0031 ± 0.0001 ^g	0.0078 ± 0.0003 ^g	0.0125 ± 0.0001 ^e	64.35 ^a	1.55	0.9507
PSC	0.0040 ± 0.0001 ^f	0.0085 ± 0.0000 ^f	0.0130 ± 0.0012 ^d	61.23 ^b	1.63	0.9429
PSR	0.0058 ± 0.0000 ^d	0.0100 ± 0.0001 ^d	0.0141 ± 0.0013 ^c	55.66 ^d	1.80	0.9349
PSO	0.0060 ± 0.0001 ^c	0.0101 ± 0.0001 ^c	0.0142 ± 0.0001 ^c	58.12 ^d	1.72	0.9313
PSRO	0.0069 ± 0.0001 ^b	0.0109 ± 0.0002 ^b	0.0148 ± 0.0003 ^b	52.67 ^e	1.90	0.9306
Cerolina	0.0049 ± 0.0000 ^e	0.0096 ± 0.0001 ^e	0.0140 ± 0.0010 ^c	58.76 ^c	1.70	0.9447
Trolox	0.0091 ± 0.0000 ^a	0.0125 ± 0.0011 ^a	0.0162 ± 0.0002 ^a	47.26 ^f	2.12	0.9104
DPPH Free Radical Scavenging Activities (%)						
100% Plantain	2.92 ± 0.05 ^g	12.08 ± 0.04 ^g	22.67 ± 0.05 ^g	72.26 ^a	1.38	0.9669
PSC	10.69 ± 0.05 ^f	28.57 ± 0.04 ^f	46.46 ± 0.05 ^f	65.29 ^b	1.53	0.9533
PSR	17.59 ± 0.04 ^d	37.61 ± 0.05 ^d	55.43 ± 0.04 ^d	60.22 ^d	1.66	0.9524
PSO	26.51 ± 0.04 ^c	41.19 ± 0.05 ^c	55.87 ± 0.04 ^c	52.26 ^e	1.91	0.9271
PSRO	53.77 ± 0.05 ^b	62.95 ± 0.04 ^b	72.24 ± 0.05 ^b	35.45 ^f	2.82	0.9094
Cerolina	13.77 ± 0.04 ^e	31.02 ± 0.05 ^e	48.27 ± 0.05 ^e	62.40 ^c	1.60	0.9457
Glutathione	77.85 ± 0.05 ^a	80.28 ± 0.05 ^a	83.13 ± 0.04 ^a	17.51 ^g	5.71	0.8798
FRAP Inhibitory Activities (mg AAE/ g)						
100% Plantain	14.24 ± 0.03 ^g	25.14 ± 0.03 ^g	42.80 ± 0.02 ^g	60.93 ^a	1.64	0.8832
PSC	15.42 ± 0.03 ^f	30.06 ± 0.02 ^f	47.21 ± 0.02 ^f	60.04 ^c	1.67	0.9241
PSR	16.74 ± 0.02 ^d	33.65 ± 0.03 ^d	50.02 ± 0.03 ^d	59.68 ^d	1.68	0.9430
PSO	17.32 ± 0.02 ^c	34.71 ± 0.02 ^c	50.35 ± 0.03 ^c	58.91 ^e	1.70	0.9490
PSRO	18.04 ± 0.02 ^b	36.70 ± 0.02 ^b	50.75 ± 0.02 ^b	57.71 ^f	1.73	0.9626
Cerolina	15.86 ± 0.02 ^e	30.48 ± 0.03 ^e	47.57 ± 0.03 ^e	60.54 ^b	1.65	0.9234
Ascorbic acid	18.88 ± 0.04 ^a	37.56 ± 0.05 ^a	51.59 ± 0.05 ^a	57.20 ^g	1.75	0.9621
Fe²⁺ Chelation Activities (mg/ mL)						
100% Plantain	4.25 ± 0.10 ^g	17.19 ± 0.10 ^f	37.90 ± 0.10 ^f	75.95 ^a	1.32	0.9691
PSC	8.44 ± 0.09 ^f	17.19 ± 0.11 ^f	37.07 ± 0.10 ^f	71.05 ^b	1.41	0.8727
PSR	15.98 ± 0.11 ^d	27.96 ± 0.10 ^d	39.82 ± 0.08 ^d	56.24 ^d	1.78	0.9343
PSO	24.49 ± 0.10 ^c	31.43 ± 0.10 ^c	43.41 ± 0.07 ^c	50.51 ^e	1.98	0.8428
PSRO	26.17 ± 0.07 ^b	39.46 ± 0.03 ^b	51.91 ± 0.10 ^b	47.29 ^f	2.11	0.9323
Cerolina	12.87 ± 0.10 ^e	26.05 ± 0.07 ^e	38.14 ± 0.10 ^e	59.33 ^c	1.69	0.9478
Ascorbic acid	39.25 ± 0.08 ^a	50.64 ± 0.09 ^a	60.27 ± 0.12 ^a	42.00 ^g	2.38	0.9354
OH Free Radical Scavenging Activities (%)						
100% Plantain	0.77 ± 0.19 ^g	16.26 ± 0.13 ^f	52.75 ± 0.13 ^g	77.24 ^a	1.29	0.9905
PSC	3.63 ± 0.06 ^f	16.26 ± 0.13 ^f	54.28 ± 0.12 ^f	76.65 ^b	1.30	0.9996
PSR	14.07 ± 0.12 ^d	36.04 ± 0.12 ^d	57.80 ± 0.12 ^d	64.53 ^d	1.55	0.9522
PSO	29.67 ± 0.13 ^c	42.42 ± 0.12 ^c	64.39 ± 0.13 ^c	55.66 ^e	1.80	0.9393
PSRO	32.75 ± 0.12 ^b	57.14 ± 0.13 ^b	80.00 ± 0.13 ^b	54.20 ^f	1.85	0.8548
Cerolina	8.35 ± 0.13 ^e	32.53 ± 0.12 ^e	54.73 ± 0.12 ^e	68.79 ^c	1.45	0.9722
Mannitol	46.59 ± 0.08 ^a	66.58 ± 0.09 ^a	88.63 ± 0.15 ^a	49.43 ^g	2.02	0.9113

Means (±SEM) with different alphabetical superscripts in the same column are significantly different at $P < 0.05$. Key: PSC: Plantain: Soycake (70:30%); PSR: Plantain: Soycake: Rice bran (65:30:5%); PSO: Plantain: Soycake: Oat bran (65:30:5%); PSRO: Plantain: Soycake: Rice bran: Oat bran (60:30:5:5); Cerolina: Commercial flour meal. DAAI: Dietary antioxidant activity index

free radicals (54.79–76.65 mg/mL) for PSRO and PSC, respectively.

Antihyperlipidemic properties

The blood lipid profile of cholesterol-induced Albino Wistar rats fed on experimental food samples and control sample is presented in Table 8. The plasma lipid profile, that is, total cholesterol TC), triglyceride (TG), high-density lipoprotein concentration (HDL-conc.), low-density lipoprotein concentration (LDL-conc.) and very low-density lipoprotein concentration (VLDL-conc.) of rats fed on the formulated food samples varied as follows: 20.11–34.61 mg/dL, 17.45–25.93 mg/dL, 12.89–23.72, mg/dL 3.73–5.87 mg/dL and 7.93–11.79 mg/dL, respectively.

Discussion

The moisture content of the flour samples was lower than recommended value (< 10) for flour samples, and this indicates that the flour samples could be stored for longer period. The amount of moisture content in the flour sample determines the growth and activities of microorganisms, hence, storage stability of the flour. Scientific findings have established that high moisture content of flour samples above 10% facilitate growth of microorganisms and food spoilage, whereas, low moisture content inhibits food spoilage and retain nutritional quality of the food products [71, 72]. The protein and fiber content of the present study food products were significantly (< 0.05) higher than 100% plantain flour, but were comparable to that of Cerolina (a commercial food

product) and other similar studies [73, 74]. The high protein and fiber content of the food products could be attributed to the inclusion of soycake, rice-bran and oat-bran, which are high in protein and fiber content than 100% plantain flour. Nutritional studies have established that a regular intake of dietary fibers is beneficial, particularly in preventing arteriosclerosis and chronic diseases like diabetes and hypertension [75, 76]. The protein content of the developed food products was higher than minimum recommended daily requirements (> 16 g/day), and this implies that the food products could be suitable for the prevention of protein-energy malnutrition particularly in children.

Mineral content of the food products in this study was comparatively lower than daily-recommended values. This implies that the food products was low in mineral composition; hence, there is a need to complement the food products intake with mineral supplements in order to prevent micronutrient deficiency. This observation was in line with previous studies, which reported that most of the African diets are low in micronutrients [77], which is responsible for high prevalence of micronutrient deficiency in many parts of developing countries including Nigeria. Deficiency of essential minerals like iron, calcium, zinc, phosphorous, sodium and potassium may lead to several health challenges such as bone deformation, anemia, electrolyte imbalance, brain and growth retardation [77].

The EAAI and P-BV of the PSRO sample were higher than other food samples including cerolina and recommended values (> 70%) for ideal food products. This

Table 8 Effect of dough meal flour from plantain, soycake, rice bran and oat bran dough meal and control sample on blood lipid profile of cholesterol-induced Albino Wistar Rats

Parameters	SAT	100% Plantain	PSC	PSR	PSO	PSRO	Cerolina	*NR
TC (mg/dL)	154.17 ± 0.38 ^a	26.85 ± 0.09 ^d	20.11 ± 0.02 ^g	22.47 ± 0.08 ^f	28.04 ± 0.02 ^c	34.78 ± 0.08 ^b	25.61 ± 0.02 ^e	< 200
TG (mg/dL)	212.10 ± 0.14 ^a	18.93 ± 0.06 ^e	17.45 ± 0.05 ^g	18.77 ± 0.02 ^f	20.53 ± 0.08 ^d	25.93 ± 0.04 ^c	28.42 ± 0.08 ^b	< 150
HDL-C (mg/dL)	42.64 ± 0.50 ^a	12.20 ± 0.06 ^g	12.89 ± 0.06 ^f	12.93 ± 0.01 ^e	17.72 ± 0.04 ^d	23.72 ± 0.04 ^b	19.48 ± 0.02 ^c	< 50
LDL-C (mg/dL)	64.39 ± 0.14 ^a	10.86 ± 0.05 ^b	3.73 ± 0.04 ^f	5.78 ± 0.06 ^e	6.21 ± 0.09 ^c	5.87 ± 0.05 ^d	0.45 ± 0.02 ^g	< 15
VLDL-conc. (mg/dL)	96.41 ± 3.10 ^a	8.60 ± 0.13 ^e	7.93 ± 0.11 ^g	8.53 ± 0.09 ^f	9.33 ± 0.21 ^d	11.79 ± 0.10 ^c	12.92 ± 0.22 ^b	
<i>Lipid Health indices of plasma</i>								
Coronary Risk Index	3.62 ± 0.0 ^a	2.20 ± 0.01 ^b	1.56 ± 0.01 ^d	1.74 ± 0.00 ^c	1.58 ± 0.02 ^d	1.47 ± 0.00 ^e	1.31 ± 0.01 ^f	3.421–8.454
Atherogenic index	1.51 ± 0.11 ^a	0.89 ± 0.00 ^b	0.29 ± 0.00 ^f	0.45 ± 0.00 ^c	0.35 ± 0.01 ^d	0.25 ± 0.00 ^e	0.02 ± 0.00 ^g	2.622–6.623
Log (TG/HDL-C)	0.69 ± 0.00 ^a	0.19 ± 0.00 ^b	0.13 ± 0.00 ^e	0.16 ± 0.00 ^d	0.06 ± 0.00 ^f	0.04 ± 0.00 ^g	0.16 ± 0.01 ^c	0.11–0.21
<i>Food intake and changes in body weight of experimental animals (g)</i>								
Food intake	1291.02 ± 5.00 ^c	472.00 ± 7.10 ^f	1142.00 ± 4.10 ^e	1403.00 ± 5.20 ^a	1289.00 ± 4.50 ^c	1239.00 ± 3.32 ^d	1344.00 ± 3.20 ^b	–
Weight gain	37.14 ± 2.31 ^b	–8.70 ± 0.03 ^f	25.70 ± 2.01 ^d	24.20 ± 3.03 ^e	37.10 ± 2.11 ^b	29.20 ± 3.01 ^c	41.50 ± 2.01 ^a	–

Key: SAT: rats fed with basal diet + Satin; PLT: rats fed with %100 Plantain; PSC rats fed with (Plantain 70% + Soycake 30%); PSR: rats fed with (Plantain 65% + Soycake 30% + Rice bran 5%); PSO: rats fed with (Plantain 65% + Soycake 30% + Oat bran 5%); PSRO: rats fed wit (Plantain 60% + Soycake 30% + Oat bran 5% + Rice bran 5%) and Cerolina. *NR: American Diabetes Association [124]

indicates that PSRO blend could be suitable as a food product to support growth in children and physiological maintenance in adults. Studies have reported that regular intakes of branched chain amino acids are health beneficial, particularly in the management of type - 2 diabetes [78]. Also, it is evident that arginine intakes play an important role in the production of nitric oxide in the body, which helps to relax arteries for the free flow of blood, hence reduces the risk of high blood pressure [79]. These findings further established nutritional quality and health benefits of the present study food products in managing diabetes and hypertension.

The phytochemical concentrations in food products in this present study were low; and this implies that the experimental food products could be suitable for human consumption without any side effects or interfering with other vital nutrients. In recent times, studies have shown that regular consumption of bioactive phytochemicals such as phenol, flavonoid, tannin, etc. in functional foods at low concentration proffers health benefits [80]. For instance, it is evident that regular consumption of plant-based foods containing flavonoid and phenolic compounds prevent degenerative chronic diseases like hypertension and diabetes; and these health benefits are attributed to antioxidant properties of these phytochemicals [80]. Antioxidants are chemical compounds capable of inhibiting the oxidation of cell molecules [81], and thereby preventing oxidative stress in animals.

Studies have shown that these phenolic compounds have antioxidative properties and ability to scavenge free radicals; hence, ability to prevent the occurrence of oxidative stress and associated chronic degenerative diseases like diabetes, etc. [82, 83]. In recent years, there is a shift of interest from using synthetic antioxidants towards natural antioxidants, due to their non-side effects [84–86]. In view of this, it may be deduced in this study that the formulated food samples are suitable as exogenous antioxidants diets, and that their regular intakes may enhance endogenous antioxidants activities to fight against excessive production of free radicals in the body [87].

The variation in BD between the blended flour samples and that of 100% plantain flour and Cerolina could be attributed to the inclusion of rice-bran and oat-bran inclusion, which might have influenced the properties of starch. Bulk density indicates the porosity of a product, and it determines the type of packaging material required [51]. It is well established that the higher the bulk density of flour sample, the denser the packaging material required [51]. The variation between WAC of these flour samples could be attributed to differences in the protein content, degree of interaction with water and conformational characteristics [88]. The low values of BD and WAC of the food products in this study have

nutritional advantage in maintaining high nutrient-energy density that is of nutritional benefits to the young children with small stomach size [89, 90]. However, flour sample with high water absorption may have more hydrophilic constituents like polysaccharides and protein, and more important in baking applications [91].

The variation in the gelling properties of these flour samples could be ascribed to different ratio of proteins, carbohydrates and lipids in their components. The lower the LGC, the better the gelling ability of the protein ingredient [92] and the swelling ability of the flour [93]. The LGC of the flour samples in this study was similar to the report of Chandra et al. [94]. The values of swelling capacity of the flour samples varied from 48.61–55%, and were higher than cerolina, but within the same range value to that of 100% plantain flour. The variation in the swelling capacity of the flours in this study could be attributed to particle sizes, processing methods or unit operations.

Pasting property (an index for predicting the ability of a food to form a paste when subjected to heat applications) is one of the most important properties that influence quality and aesthetic properties of food products [95, 96]. The low peak viscosity value that was observed in the experimental flour samples could be attributed to the inclusion of soy cake, rice-bran and oat-bran flour into plantain-based composite flour samples. High peak viscosity of processing flour reflects fragility of the swollen granules, which first swell and then breaks down under the continuous mixing of the Rapid Visco Analyzer. The low peak viscosity values noted in this study is of processing advantage, particularly for the preparation of less stiff dough products [97].

The trough viscosity values observed in this present study indicate the tendency of the plantain-based flour samples to breakdown during cooking. The breakdown viscosity is an index of the stability of the starch and a measure of the ease with which the swollen granules can be disintegrated [98]; and that the higher the breakdown viscosity, the lower the ability of the flour to withstand heating and shear stress during cooking [99].

These parameters, i.e., final viscosity, setback, peak time and pasting temperature are very important factors in determining the quality of food products. For instance, final viscosity indicates the ability of starchy foods to form viscous paste after cooking and cooling, and it is an important quality parameter used in predicting the final textural quality of starchy foods [100]. High setback values affect dough digestibility [101], while lower value indicates lower tendency of starch retrogradation [102]. The pasting temperature measures the minimum temperature needed to cook a given food sample [103], while that of peak time measures the cooking time [99] of starch.

An IC_{50} value is the measure of the effectiveness of a substance in a biochemical reaction to result in 50% antioxidant activities, the smaller the IC_{50} value the higher the antioxidant activity [104]. The low IC_{50} value observed in PSRO further established the potency of the food product as an exogenous antioxidant diet. The antioxidative ability of this food sample (PSRO) could be attributed to the synergistic effect of the flour sample combinations i.e., plantain, Soycake, oat-bran and rice-bran. Studies have earlier reported that oat-bran [105], rice-bran [106] and soycake [107], which are the most active ingredients in these formulation contain appreciable amount of bioactive compounds with antioxidant activities [108, 109].

Comparatively, the concentration of plasma lipid profile of rats fed on the formulated diets was similar to those rats fed on 100% plantain flour and Cerolina, but lower than those rats in group SAT (rats treated with Satin, an anticholesterol agent). For the health lipid indices, the values of $\log(TG/HDL\text{-conc.})$, atherogenic index (AI) and coronary risk index (CRI) ranged from 0.25–0.89 and 1.47–2.20, respectively; and were lower than normal recommended values for AI (4.4–7.1) and CRI (3.4–4.0) [110]. This finding agreed with the report of Hasanein et al. [111], who reported that the AI (2.622–6.623) and CRI (3.421–8.454) of obese rats treated with tea and cinnamon extract. The decreased in total cholesterol and low-density lipoprotein (LDL) levels couple with increased in high-density lipoprotein (HDL) levels that were observed in obese rats fed on the experimental diets in this present study could be considered health benefits for the consumers. Recently, epidemiological studies have implicated high level of total cholesterol, LDL-cholesterol, or low HDL-cholesterol as the major risk factors for the atherosclerosis-related diseases (cardiovascular diseases, diabetes, etc.) [112–114]; and are major targets for the treatment and prevention of atherosclerosis diseases [115–117].

Conclusion

This study developed and evaluated, nutritional, bioactive phytochemicals, antioxidants activities, and anti-hyperlipidemic properties of food from plantain, soycake, oat-bran and rice-bran flour. The finding established consumption of high fats diets – rich in low density lipoproteins should be avoided. As they expose the body to risk of arteriosclerosis formation. However, formulated food samples, particularly PSRO (Plantain 60%, Soycake 30%, Rice bran 5%, Oat bran 5%), had higher antioxidant and anti-hyperlipidemia activities when compared with 100% plantain flour and Cerolina (a commercial flour). Hence, this food product, that is, PSRO, may be suitable as functional food for the treatment and prevention of arteriosclerosis formation, hence, hypertension.

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Authors' contributions

IJAROTIMI Oluwole Steve & FAGBEMI Tayo Nathaniel designed the research; while implementation of the research, data analyses and manuscript preparation were done by OLUWAJUYITAN Timilehin David and IJAROTIMI Oluwole Steve. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declared that there were no conflicts of interest for the study.

Ethics approval

The Ethical Committee for Laboratory Animals of School of Agriculture and Agricultural Technology, Federal University of Technology Akure, Nigeria (Approval number: FUTA/ SAAT/2018/021) approved the study protocol.

Consent for publication

Not Applicable.

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