Original Research Article

Nutritional and Antioxidant Properties of Resistant Starch-Based Flour Blends from Unripe Plantain, Pigeon Pea and Rice-Bran

.

ABSTRACT

Aim:
The nutrients and antioxidants of blends of native resistant starch, acetylated resistant starch, and flours of unripe plantain, pigeon pea and rice bran were investigated as possible alternatives to functional food products in managing type-2 diabetes.

Place and Duration of Study:
Food Chemistry Laboratory, Department of Food Science and Technology, and Functional Foods Laboratory, Department of Biochemistry, Federal University of Technology, Akure, Nigeria. From January, 2022 to January, 2023.

Methodology:
Native-resistant starch was isolated from plantain and was modified via acetylation. Five sample blends of native resistant starch, acetylated resistant starch, and flours of pigeon pea and rice bran were prepared, using Mixture design expert 11.0.0 and were coded as follows: PLF – (100% Plantain flour), used as a control sample; PLPRF – plantain flour: pigeon pea flour: rice bran flour (73.08:15.15:11.76); PSPRF – native plantain starch: pigeon pea flour: rice bran flour (73.08:15.15:11.76); AP1SPRF – acetylated plantain starch: pigeon pea flour: rice bran flour (73.08:15.15:11.76); AP2SPRF – acetylated plantain starch: pigeon pea flour: rice bran flour (70.67:18.15:11.18). The proximate compositions, minerals, amino acid profiles, α-amylase and α-glucosidase and in-vitro antioxidant potentials of the samples were compared with the commercial flour product, CRF (100% Cerolina flour: wheat and soybean produced by More Foods Lagos, Nigeria).

Results:
The results showed that AP2SPR had the lowest moisture content (2.40%), highest ash content (4.63%) and lowest fat content (2.42%). K, Cu, Fe. Na, Zn and P were more relatively abundant in the blends than in PLF. Generally, the chemical compositions of the flour blends improved with the addition of quality protein, which extensively enhanced the mineral contents in terms of Ca, K, Fe, Zn and P. Total essential amino acids were higher in PLF and the flour blends than CRF, ranging from 35.18 (g/100g protein) in CRF to 41.81 (g/100g protein) in PLPRF whereas their total non-essential amino acids showed an opposite trend, ranging from 58.18 (g/100g protein) in PLPRF to 64.84 (g/100g protein) in CRF. Upon acetylation, total essential amino acids improved from 39.84 (g/100g protein) in PSPRF to 41.00 (g/100g protein) in AP1SPRF and 41.32 (g/100g protein) in AP2SPRF. Both AP1SPRF and AP2SPRF were able to reduce Fe^{2+} to Fe^{3+}, scavenge DPPH, FRAP and ABTS and inhibit α-amylase and α-glucosidase activities.

Conclusion:
The flour blends of AP1SPRF and AP2SPRF studied were a good source of resistant starch and bioactive ingredients that would be useful in a variety of dietary functional food products to manage type-2 diabetes.

Keywords: Resistant starch, acetylated starch, amino acids, antioxidants

1. INTRODUCTION

The quest for quality functional foods is influenced by a lot of factors such as health issues (obesity, diabetes, cholesterol, cancer, etc.), developmental stages in life and lifestyles. The development of novel foods products, using new technologies has influenced consumers' perception on the awareness of the nutritional value and health benefits of novel foods [1].

Recently, tremendous importance has been given to functional foods, because it provides health benefits by reducing the risk of chronic diseases apart from the nutritional benefits [1].
Resistant starch (RS), basically recognized as a type of dietary fibre, is one of the most widely used ingredients in functional foods. RS is a dietary starch with the unique property of not being absorbed intact by the gut, but hydrolyzed by intestinal enzymes and fermented by bacteria in the colon to produce short-chain fatty acids like butyrate [2]. Recently, RS has drawn the interest of many researchers due to its health benefits, functional peculiarities and promising physiological benefits for non-diabetic and diabetic people, which include reduction in the amplified levels of blood glucose and insulin, restraining of colonic cancer and risks of ulcerative colitis, positive impact on bowel movements, increase in the assimilation of minerals, prebiotic actions, laxation, hypocholesterolemic and hypoglycemic effects, and enhanced fat oxidation [2,3]. Several studies have revealed that the starches modified via acetylation result in the production of food starch products with enough resistant starch content, capable of serving as raw materials for diabetes treatment [4,5]. In the acetylation process, the hydroxyl groups of the glucose monomers are converted to the groups CH_3COO^-, leading to a decrease in the gelatinization temperature, an increase or decrease in the swelling power, and solubility along with the storage stability [6].
Antioxidants are compounds that prevent the deterioration of lipids during or after food processing by scavenging the free radicals [7], thereby increasing the oxidative stability of the food, and controlling the pro-oxidants and other oxidative intermediates [8]. They are phytochemicals, vitamins and nutrients that protect our cells from damage, caused by free radicals by stabilizing or deactivating free radicals before they attack cells [8]. Consumption of foods rich in antioxidants plays a special role in the prevention and management of several human ailments, including *Diabetes mellitus* [7].
Plantain (*Musa paradisiaca*) has a thick cell wall that cannot be easily penetrated by enzymes, leading to a low glycemic index. It is a cheap source of energy, and also, medically recommended for diabetic patients, due to its high content of resistant starch [9]. Its fruit pulp has been reported to possess high antioxidant properties [10]. The extract of unripe plantain has the potential to inhibit the key enzymes linked with type-2 diabetes [11].
Pigeon pea (*Cajanus cajan*) cultivars have good quality resistant starch, minerals, medicinal and antimicrobial properties, and 36.5% protein, rich in sulphur-containing amino acids [12] in addition to excellent water retention (250.3 mL/100g), fat absorption (130 mL/100g), emulsification capacity (120%) and foaming capacity (130%) [13].
Rice bran is a by-product of the rice milling process, and it contains various antioxidants, 12-13% oil and 4.3% highly unsaponifiable components. It is also rich in dietary fibres (*α*-glucan, pectin, and gum), non-lignified cell walls, and tocotrienol, *γ*-oryzanol, and *α*-sitosterol, which lower the plasma levels of the various parameters of the lipid profile [14].
Despite the increasing research interest in the production of functional foods from local crop materials to manage chronic diseases [15], there is scanty information on the combination of plantain, pigeon pea and rice bran flours for food product formulation. Hence, this study aimed to evaluate the nutrients and antioxidants of blends of native resistant starch acetylated resistant starch, and flours of unripe plantain, pigeon pea and rice bran as possible alternatives to functional food products in managing type-2 diabetes.

2. MATERIALS AND METHODS

2.1 Sources of Materials

Matured unripe plantains (*Musa ABB*) were harvested from a local farm in Sabongida-Ora, Owan-West LGA, pigeon peas were purchased from Jattu Market, Etsako-West LGA, and rice bran was obtained from Pemos Foods, Aviele, Etsako-West LGA, Edo State. The food materials were authenticated at the Department of Crop Soil and Pest Management, the Federal University of Technology, Akure, Nigeria.

2.2 Flour Sample Preparation

The unripe plantain was processed into flour using the method of Oluwajuyitan et al. [16] with slight modifications. The plantain was manually peeled, sliced (0.5 cm thick), washed, oven dried (Plus 11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 60 ^oC for 24 h, cooled in a desiccator, milled (Rico MG 1803 Mixer Grinder, 1000 watts, India), sieved

through 200 μm mesh sieve and packaged in airtight polythene prior to analyses. The pigeon peas were handpicked, oven dried (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 60 ^{o}C for 24 h, cooled in a desiccator, milled (Rico MG 1803 Mixer Grinder, 1000watts, India), sieved through 200 μm mesh sieve and packaged in airtight polythene prior to analyses. The rice bran was washed with distilled water, drained and oven-dried (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 60 ^{o}C for 24 h, cooled in a desiccator, milled (Rico MG 1803 Mixer Grinder, 1000watts, India), sieved through 200 μm mesh sieve and packaged in airtight polythene prior to analyses.

2.3 Acetylation of Plantain Starch

Prior to acetylation of plantain starch, the native starch of the plantain was isolated according to the method described by Oladebeye et al. [17] with slight modifications. The plantains were blended, sieved through muslin cloth, supernatant decanted, the cake dried (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 60 ^{o}C for 24 h, cooled, milled (Rico MG 1803 Mixer Grinder, 1000 W, India), sieved through 200 μm mesh sieve and packaged in airtight polythene prior to modification via acetylation. For acetylation process, 100 g (dwb) of the native starch was dispersed in 200 mL of acetic anhydride in a reaction flask and stirred at 500 rpm with a mechanical stirrer for 5 min in the presence of NaOH solution (50 g NaOH/100 g water) as a catalyst at 100 °C and stirred for 1 h. To precipitate the starch, 100 mL of ethyl alcohol solution (96%) was added followed by filtration by suction with a Buchner filter funnel (Whatman filter No. 4). The residue was washed with ethyl alcohol and then with distilled water till most of the acetic anhydride was removed. The resulting paste produced by these washes was dried in an oven (Plus11 Sanyo Gallenkamp PLC, LouLoughborougheicestershire, UK) at 40 °C for 16 h up to approximately 9% moisture content [18]. Two acetylated plantain starches were prepared from the native plantain starch.

2.4 Preparation of Resistant Starch-Based Flour Blends

The flour samples of plantain, pigeon pea and rice bran were blended with reference to 14 g/day protein and 5 g/day fibre (i.e. 25% of recommended daily intakes of adult requirements), using Optimal Mixture Design Methodology. Five blended samples were prepared and coded as PLF – (100% Plantain flour); PLPRF – Plantain flour: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); PSPRF – Native Plantain starch: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); AP1SPRF – Acetylated Plantain starch: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); AP2SPRF – Acetylated Plantain starch: Pigeon pea flour: Rice bran flour (70.67:18.15:11.18). A positive control sample, CRF – 100% Cerolina flour (wheat and soybean produced by More Foods Lagos, Nigeria) was used for comparison with the formulated samples.

2.5 Determination of Proximate Compositions

The moisture, crude protein, fat, fibre and ash contents of the food samples were determined according to AOAC methods [19]. Total carbohydrate content was calculated by difference.

2.6 Determination of Mineral Compositions

Calcium, magnesium, iron, zinc, copper and manganese were determined using atomic absorption spectrophotometer (AAS Model SP9). Sodium and potassium contents were determined using a flame photometer (Sherwood, UK), using NaCl and KCl as standards. Phosphorus was determined by the vanado-molybdate colourimetric method of AOAC [19].

2.7 Amino Acid Compositions

The amino acid compositions of the food samples were determined using an Automated Amino Acid Analyzer (Model 6300; Beckman Coulter Inc., Fullerton, Calif., USA) as described by Mansouri et al. [20].

2.8 Antioxidant Activities

2.8.1 In-Vitro Antioxidant Assay

Prior to in-vitro analyses, 50 g of each of the samples was dissolved in a 250 mL flask containing distilled water. The mixture was sieved and filtered, to obtain a clear solution. The

solutions extracted were used for the in-vitro after concentrating using freeze-drying methods. The in-vitro antioxidant assays of the aqueous extracts of the food samples were carried out using standard methods. The free radical scavenging activity of the food samples was determined against 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonate (ABTS) [21]. The 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging was determined as described by Aluko and Monu [22]. The metal chelating activity of the formulated food aqueous extract was determined using the method of Xie et al. [23]. Ferric-reducing antioxidant activity (FRAP) was determined according to Zhang and Lin [24].

2.8.2 Inhibition of α-amylase Activity

The α-amylase activity was used to measure in-vitro by hydrolysis of starch in presence of α-amylase enzyme [25]. The α-amylase activity was carried out by the starch-iodine method. 10 µL of α-amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing 20 µL of 50 µg/ml concentration of extracts. After incubation at 37 °C for 10 min, 100 µL of starch solution (1%) was added and the mixture was re-incubated for 1 h. Additions of 0.1 mL of 1% iodine solution and 5 mL distilled water were done before taking the absorbance at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions.

$$\text{Inhibition of enzyme activity } (\%) = \frac{\mathrm{A} - \mathrm{C}}{\mathrm{B} - \mathrm{C}} \times 100$$

where, A = absorbance of the sample, B = absorbance of blank (without α-amylase), and C = absorbance of control (without starch).

2.8.3 Inhibition of α-glucosidase Activity

The α-glucosidase inhibition activity was performed according to the method of Tiwari et al. [26] with slight modification. The final volume of the reaction mixture was 100 µL, which contained 70 µL of phosphate buffer saline (50 mM, pH 6.8), 10 µL of 50 µg/mL concentration of extracts, and 10 µL (0.057 U) enzyme. The content was mixed, pre-incubated at 37 °C for 10 min and pre-read against the reagent blank value by spectrophotometry at 400 nm. The reaction was initiated using 10 µL of 0.5 mM substrate (i.e. p-nitrophenol glucopyranoside). Acarbose was used as a positive control. After incubation at 37 °C for 30 min, optical absorbance was measured against the reagent blank value by spectrophotometry at 400 nm. The percentage of enzyme inhibition was calculated.

$$\%\ \text{scavenging activity} = \frac{\mathrm{A_c} - \mathrm{A_s}}{\mathrm{A_c}} \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the extract.

3. STATISTICAL ANALYSIS

The statistical analysis of the data obtained was carried out with IBM SPSS 26.0 software for mean comparison, using Duncan's least significant test and one-way analysis of variance (ANOVA) at 5% significance level.

4. RESULTS AND DISCUSSION

4.1 Proximate Compositions

The proximate composition of the flour blends is presented in Table 1. Their moisture contents range between 2.40% in AP2SPRF and 10.43% in PLPRF. All the values are lower than the 10% recommended for longer storage stability of food products with no adverse effect on the quality attributes of the flour blend, except for sample PLPRF, which is higher by 0.43 [27], indicating higher shelf-life in the acetylated flour blends than the other blends [28]. The highest protein content (18.31%) is observed in CRF, being the positive control sample with wheat and soybean compositions. Among all the flour blends, the least protein content, 6.91% in AP2SPRF is higher than the 4.38% protein content in PLF. This is similar to the report that protein content can be improved with a better source of protein like bambara groundnut, soybeans and pigeon peas and this will help to boost the protein intake of consumers, who cannot afford animal-based protein [29]. The crude fibre content ranges between 2.40% in

CRF to 14.28% in PLPRF, and this high crude fibre could be due to the addition of the rice bran in the flour blends. Upon acetylation, the crude fibre is lowered from 12.91% in PSPRF to 7.32% in AP1SPRF. Fat contents of the flour blends range from 2.42% in AP1SPRF to 7.74% in CRF, which may be due to the addition of soybeans, an oil seed crop in the formulation of CRF. Also, the ash content ranges from 1.30% in PSPRF to 4.63% in AP2SPRF. High ash content in AP2SPRF (acetylated starch-based flour sample) is an indication of the relative abundance and nature of minerals [30], and the degree of digestibility of the sample [31].

Table 1. Proximate compositions of the blends from plantain flour, starch, acetylated starch with pigeon pea and rice bran and commercial product

Sample	Moisture (%)	Ash (%)	Crude Protein (%)	Fat Content (%)	Crude Fibre (%)	Carbohydrates By Difference (%)	Energy (Kcal)
PLF	8.25^{b}±0.02	1.97^{d}±0.02	4.38^{f}±0.02	2.96^{d}±0.02	3.18^{e}±0.02	78.26^{a}±1.71	377.73^{b}±0.18
CRF	5.77^{d}±0.02	1.90^{d}±0.02	18.31^{a}±0.02	7.74^{a}±0.02	2.40^{f}±0.02	63.88^{c}±0.02	415.01^{a}±0.33
PLPRF	10.43^{a}±0.03	3.48^{c}±0.02	13.85^{b}±0.02	4.98^{c}±0.02	14.28^{a}±0.02	52.98^{d}±0.02	325.51^{f}±0.35
PSPRF	6.88^{c}±0.02	1.30^{e}±0.02	10.30^{c}±0.02	5.35^{b}±0.02	12.91^{b}±0.02	63.26^{c}±0.02	357.10^{e}±0.18
AP1SPRF	2.59^{e}±0.03	4.24^{b}±0.15	8.21^{d}±0.03	2.42^{f}±0.02	7.23^{d}±0.10	75.31^{b}±0.06	372.58^{c}±0.27
AP2SPRF	2.40^{f}±0.12	4.63^{a}±0.06	6.91^{e}±0.06	2.65^{e}±0.03	9.13^{c}±0.06	74.27^{b}±0.26	365.00^{d}±1.02

PLF = 100% Plantain flour; CRF = 100% Cerolina flour; PLPRF = Plantain flour: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); PSPRF = Plantain starch: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); AP1SPRF = Acetylated Plantain starch: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); AP2SPRF = Acetylated Plantain starch: Pigeon pea flour: Rice bran flour (70.67:18.15:11.18)

Results are the means of triplicate determination ± standard deviation values in the same column with the same superscripts letters (a > b > c > d) are not significantly different (P < 0.05)

The carbohydrate content ranges from 52.98% in PLPRF to 78.26% in PLF. The carbohydrate content decreases with an increase in the percentage of protein, ash and crude fibre. The energy value ranges from 325.51 kcal/100 g in PLPRF to 415.01 kcal/100 g in CRF. High energy value has been attributed to fat content, and this is an index of the total amount of energy the human body can generate during metabolism [32]. Recently, both high-carbohydrate, low-fat (HCLF) diets and low-carbohydrate, high-fat (LCHF) diets have been identified as having no significant differences in terms of changes in lean mass, fat mass, systolic blood pressure, diastolic blood pressure, triglycerides and glucose, implying that both diets are effective for weight control and reduction of cardiovascular risk factors [33].

4.2 Mineral Compositions

The mineral compositions detected in the flour blends are sodium (Na), potassium (K), calcium (Ca), iron (Fe), copper (Cu), Phosphorus (P) and zinc (Zn) (Table 2). Dietary minerals (also known as mineral nutrients) are the chemical elements required by living organisms. Potassium is more abundant in all the minerals determined. This is the confirmation of the relative abundance of ash contents in the flour sample as indicated in (Table 1). Potassium, being the most abundant intracellular cation, maintains intracellular fluid balance and transmission of nerves in relation to sodium [34].

Table 2. Mineral compositions of the blends from plantain flour, starch, acetylated starch with pigeon pea and rice bran and commercial product

Sample	Mineral (mg/100g)						
	K	Ca	Cu	Fe	Na	Zn	P
PLF	57.53^{d}±0.04	3.23^{c}±0.02	0.04^{b}±0.02	0.33^{e}±0.03	17.25^{e}±0.02	0.69^{e}±0.02	27.63^{d}±0.03
CRF	27.75^{f}±0.02	6.19^{a}±0.02	0.11^{a}±0.02	0.55^{d}±0.02	28.53^{a}±0.04	0.99^{a}±0.02	34.04^{a}±0.02
PLPRF	67.00^{a}±0.02	2.88^{e}±0.02	0.11^{a}±0.02	1.41^{a}±0.02	18.75^{b}±0.02	0.86^{b}±0.02	32.81^{b}±0.02
PSPRF	51.00^{e}±0.02	2.93^{d}±0.03	0.08^{a}±0.02	0.84^{c}±0.02	18.25^{d}±0.02	0.70^{e}±0.03	31.60^{c}±0.02
AP1SPRF	62.75^{b}±0.02	3.38^{b}±0.02	0.10^{a}±0.02	1.14^{b}±0.02	17.00^{f}±0.02	0.74^{d}±0.02	31.60^{c}±0.54
AP2SPRF	62.23^{c}±0.25	3.38^{b}±0.03	0.09^{a}±0.01	1.17^{b}±0.06	18.57^{c}±0.06	0.81^{c}±0.01	31.55^{c}±0.09

Results are the means of triplicate determination ± standard deviation values in the same column with the same superscripts letters (a > b > c > d) are not significantly different (P < 0.05)

Dietary sodium is needed by all ages in very small quantities [35] with the view to effectively controlling the blood pressure [36]. Importantly, lowering the blood pressure implies lowering the sodium intake and simultaneously increasing the potassium intake [37]. Getting enough calcium and phosphorus from infancy to adulthood is through adequate diet, which will prevent the risk of weak teeth and bones, and fractures in later life. Additional roles played by calcium in the body are clotting of blood, neuro-sensing, contraction and relaxation of muscles, the release of hormones and maintaining normal heartbeat of appetite, fatigue, nausea, muscle cramps, sleeplessness, hyperactivity, irritability, anxiety in infants and children [38]. The Iron and the zinc are the least abundant out of all the minerals analyzed ranging from 0.33 mg/100g Fe (PLF) to 1.41 mg/100g Fe (PLPRF) and from 0.69 mg/100g Zn (PLF) to 0.99 mg/100gZn (CRF). Flour blends with high calories, nutrient density and adequate iron content as well as high nutrient bioavailability can be used to prevent iron deficiency in all ages [39].

4.3 Amino Acid Compositions

The amino acid profiles of flour blends are depicted in Tables 3-4. The total amino acids in each of the flour blends is total 100 (g/100g protein). The total non-essential amino acids of samples studied range from 58.18 (g/100g protein) in PLPRF to 64.84 (g/100g protein) in CRF while total essential amino acids are higher in PLF and the flour blends than CRF, ranging from 35.18 (g/100g protein) in CRF to 41.81 (g/100g protein) in PLPRF. Upon acetylation, total essential amino acids improve from 39.84 (g/100g protein) in PSPRF to 41.00 (g/100g protein) in AP1SPRF and 41.32 (g/100g protein) in AP2SPRF.

Table 3. Essential amino acids (g/100g protein)

Essential Amino Acid	Sample					
	CRF	PLF	PLPRF	PSPRF	AP1SPRF	AP2SPRF
Histidine	2.92^{e}	8.95^{a}	5.80^{b}	4.14^{d}	4.55^{c}	4.28^{cd}
Isoleucine	4.25^{bc}	3.91^{d}	3.89^{d}	4.12^{c}	4.43^{a}	4.27^{b}
Leucine	7.44^{a}	6.08^{c}	7.07^{b}	7.45^{a}	7.47^{a}	7.58^{a}
Lysine	4.84^{d}	5.88^{c}	6.55^{a}	5.99^{c}	6.43^{ab}	6.34^{b}
Methionine	1.60^{c}	2.11^{a}	1.86^{b}	1.71^{bc}	1.87^{b}	1.75^{bc}
Phenylalanine	5.11^{d}	4.73^{e}	7.33^{a}	7.07^{b}	6.74^{c}	7.26^{ab}
Threonine	3.37^{c}	3.95^{a}	3.71^{b}	3.67^{b}	3.89^{a}	3.72^{b}
Tryptophan	1.27^{ab}	1.37^{a}	1.05^{c}	1.10^{c}	1.15^{bc}	1.15^{bc}
Valine	4.38^{d}	4.71^{ab}	4.55^{c}	4.59^{bc}	4.79^{a}	4.65^{abc}

Means along the row with different superscripts are significantly different at the 5% level with $a > b > c$. Mean separation done by Duncan Multiple Range Test

Table 4. Non-essential amino acids (g/100g protein)

Non-Essential Amino Acid	Sample					
	CRF	PLF	PLPRF	PSPRF	AP1SPRF	AP2SPRF
Alanine	3.61^{f}	5.08^{a}	4.44^{c}	4.22^{e}	4.67^{b}	4.30^{d}
Arginine	5.77^{c}	8.25^{a}	6.76^{a}	6.32^{bc}	6.25^{bc}	6.10^{c}
Asparagine	8.95^{d}	12.53^{a}	11.26^{b}	10.34^{c}	11.29^{b}	11.06^{b}
Cysteine	2.02^{a}	1.85^{b}	1.43^{c}	1.43^{c}	1.23^{d}	1.38^{c}
Glutamine	25.68^{a}	13.79^{f}	18.20^{d}	20.70^{b}	17.42^{e}	19.44^{c}
Glycine	3.77^{c}	4.42^{a}	3.75^{c}	3.64^{c}	4.00^{b}	3.64^{c}
Proline	7.22^{a}	3.92^{f}	4.36^{e}	5.26^{b}	4.53^{d}	4.82^{c}
Serine	4.89^{d}	5.60^{b}	5.22^{c}	5.07^{c}	6.01^{a}	5.21^{c}
Tyrosine	2.93^{a}	2.86^{a}	2.76^{a}	3.19^{a}	3.30^{a}	3.07^{a}

Means along the row with different superscripts are significantly different at the 5% level with $a > b > c$. Mean separation done by Duncan Multiple Range Test

From Table 5, there is a significant increase in TEAAs, that is, lysine, leucine, isoleucine, tryptophan, arginine, threonine, valine, histidine, phenylalanine, tyrosine, methionine, obtained

in all the resistant starch-based flour samples compared to the control sample CRF (wheat and soybean-based). The increase in the amino acid profile of resistant starch-based flour blends is due to the high quality of protein present in pigeon pea flour, because legumes are protein-rich crops and have higher amino-acid composition than plantain [29]. The same trend is observed in terms of hydrophilic, hydrophobic, positively charged, branched-chain and aromatic amino acids in resistant starch-based samples, especially the native and acetylated starch-based samples compared to CRF (wheat-based). This is an added advantage as this sample can be eaten as a functional food because hydrophobic amino acids act as antioxidants by increasing the solubility of peptides in lipids, which then facilitates better interaction with free radicals [29]. The ArAA (aromatic amino acids) are made up of phenylalanine, tryptophan, and tyrosine. They are essential amino acids, which the body cannot synthesize and must therefore be consumed in foods. Except for PLF (100% plantain flour), all the plantain-based, plantain starch-based flour blends are more abundant in ArAA than CRF. However, the extent of acetylation does not have a significant difference ($P < 0.05$) in the relative abundance of ArAA in the food samples (Table 5). The respective values of EAAI, PER, BV and NI of the flour blends range from 99.92% (CRF) to 118.49% (PLF), 1.99% (PLF) to 2.65 g/100 g (AP2SPRF), 97.21 (CRF) to 117.45% (PLF) and 7.87 (AP2SPRF) to 18.30% (CRF). The values of EAAI, PER and BV show that all the plantain-based and plantain starch-based flour blends are capable of improving and supplying the adequate intake of nutrients and amino acids by infants and adults.

Table 5. Nutritional Quality

Parameter	Sample					
	CRF	PLF	PLPRF	PSPRF	AP1SPRF	AP2SPRF
TEAA	35.18^{d}	41.69^{ab}	41.81^{a}	39.84^{c}	41.29^{ab}	41.00^{b}
TNEA	64.82^{a}	58.31^{bc}	58.19^{c}	60.16^{b}	58.71^{bc}	59.00^{c}
TEAA/TNEA	0.54^{d}	0.72^{ab}	0.72^{a}	0.66^{c}	0.70^{b}	0.69^{b}
HAA-1	16.98^{b}	18.68^{a}	16.87^{b}	17.01^{b}	18.43^{a}	17.02^{b}
HAA-2	33.60^{b}	30.55^{c}	33.51^{b}	34.41^{a}	34.48^{a}	34.62^{a}
PCAA	11.36^{f}	19.92^{a}	16.79^{b}	14.35^{e}	15.64^{c}	14.93^{d}
NCAA	34.62^{a}	26.32^{e}	29.46^{c}	31.03^{b}	28.71^{d}	30.49^{b}
BCAA	16.06^{c}	14.70^{e}	15.51^{d}	16.16^{bc}	16.68^{a}	16.50^{ab}
SCAA	3.61^{b}	3.96^{a}	3.29^{c}	3.14^{c}	3.10^{c}	3.13^{c}
ArAA	9.31^{b}	8.95^{b}	11.14^{a}	11.36^{a}	11.18^{a}	11.47^{a}
EAAI (%)	99.92^{d}	118.49^{a}	115.49^{ab}	110.53^{c}	115.83^{ab}	113.84^{bc}
PER (g/100 g)	2.60^{ab}	1.99^{c}	2.45^{b}	2.58^{ab}	2.58^{ab}	2.65^{a}
BV (%)	97.21^{d}	117.45^{a}	114.19^{ab}	108.78^{c}	114.55^{ab}	112.39^{bc}
NI (%)	18.30^{a}	5.19^{f}	16.00^{b}	11.38^{c}	9.51^{d}	7.87^{e}

Means along the row with different superscripts are significantly different at the 5% level with $a > b > c$. Mean separation done by Duncan Multiple Range Test

TEAA - total essential amino acids = Leusine + Lysine + Isoleucine + Phenylalanine + Tryptophan + Valine + Methionine + Histidine + Threonine; TNEA - total non-essential amino acids = Tyrosine + Cystine + Alanine + Glutamic acid + Glycine + Aspartic acid + Serine + Proline + Arginine; HAA-1, Hydrophilic amino acids = Glycine + Tyrosine + Serine + Threonine + cysteine; HAA-2, Hydrophobic amino acids = Methionine + Alanine + Valine + Leucine + Isoluecine + Proline + Phenylalanine; PCAA – positively charged amino acids = Basic amino acids = amino acids with basic group in their side chains = Lysine + Histidine + Arginine; NCAA – negatively charged amino acids = Acidic amino acids = amino acids with carboxyl group in their side chains = Glutamic acid + Aspartic acid **OR** ASX (aspargine +aspartic acid) + GLX (glutamine+glutamic acid); BCAA- Branch chain amino acids = amino acids having aliphatic side chains with a branch = valine, isoleucine, leucine; SCAA- sulphur containing amino acids = amino acids with sulphur in their side chains = cysteine and methionine; ArAA- aromatic amino acids = amino acids with benzene ring in their side chains = phenylalanine, tryptophan and tyrosine; EAAI (%) – Essential amino acids index; PER (g/100 g) – Protein efficiency ratio; BV (%) – Biological value; NI (%) – Nutritional Index.

4.4 Antioxidant Activities

4.4.1 In-Vitro Antioxidant Assay

The scavenging activities of the acetylated starch-based samples are significantly different from other samples (Figure 1a-d). The scavenging activities of AP1SPRF and AP2SPRF are significantly higher than those of PLF, CRF and other non-acetylated samples. The results suggest that acetylation increases the DPPH radical scavenging activity of the samples.

These observations are consistent with the findings of the antioxidant activities of polysaccharides, which improve after acetylation [40–42]. The ABTS radical scavenging activities of the flour samples show that both native and modified resistant starch-based flour blends exhibit higher radical scavenging activities compared to CRF, PLF and PLPRF. The FRAP and Fe-chelating scavenging activities also follow the same trend with the acetylated samples AP1SPRF and AP2SPRF having the highest scavenging activity, indicating that the composite flours may have a better protective effect against free radicals compared to CRF (wheat-soybean based flour).

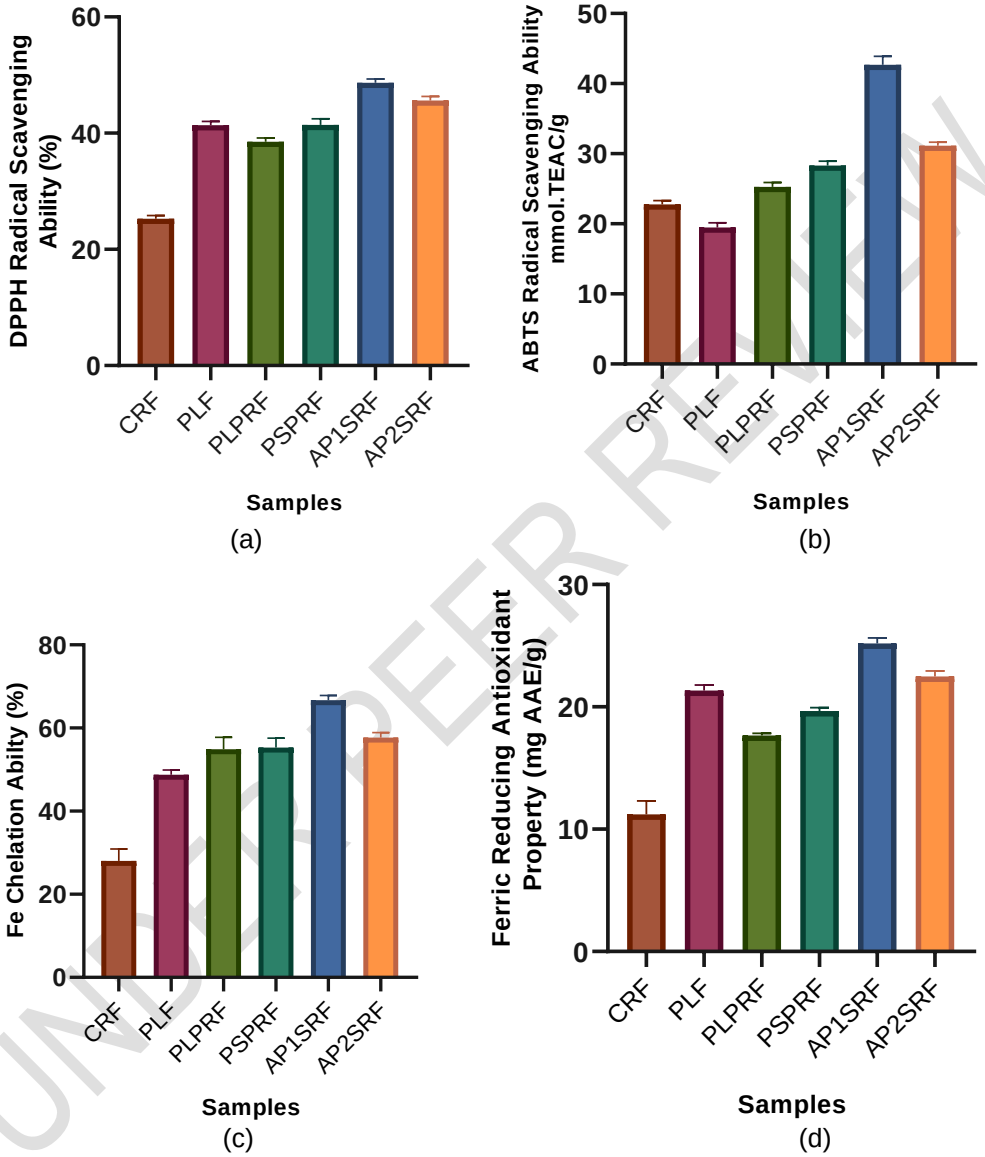


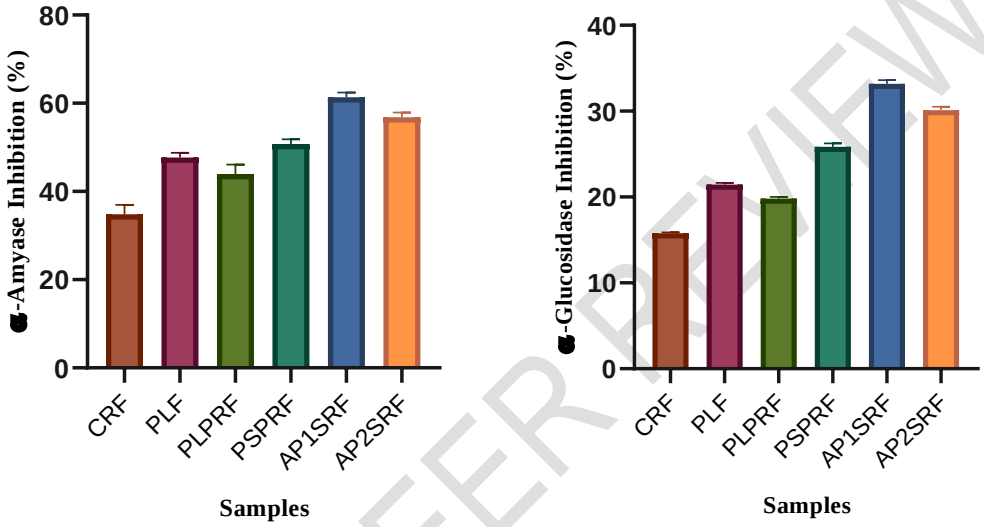
Figure 1. (a) DPPH radical scavenging ability (%), (b) ABTS radical scavenging ability (mmol.TEAC/g), (c) Fe chelation ability (%), (d) Ferric reducing antioxidant property (mg.AAE/g)

The addition of acetyl group had a significantly lifting effect on the DPPH free radical scavenging activity [43], implying a possibility of acetyl groups reducing DPPH to DPPHH, thereby preventing lipid oxidation.

4.4.2 α-amylase and α-glucosidase Inhibition Activities

The inhibitions of α-amylase and α-glucosidase of the samples studied are shown in Figure 2a, b. α-amylase and α-glucosidase are enzymes that hydrolyze carbohydrates [44]. The

addition of resistant starch in the flour blends increases the percentage inhibition of the α–amylase from 38% in CRF (commercial control sample) and 42% in PLPRF to as high as 58% in AP1SPRF and 62% in AP1SPRF. In other words, the acetylated starch-based samples, AP1SPRF and AP2SPRF, have higher α–amylase inhibition than CRF, PLF, PLPRF and PSPRF. Heating produces resistant starches (RS 2 and 3) hence, this may account for higher inhibition by the modified starches [45,46]. The same trend is observed in terms of α–glucosidase, with the highest α-glucosidase inhibition (30% and above) in acetylated starch-based flour blends (AP1SPRF and AP2SPRF) and less than 20% in the control sample (CRF). This agrees with the observations on the inhibition of α–amylase and α–glucosidase by cooked rice [45,46]. The α–glucosidase inhibitory effect exhibited by the resistant starch-based flour blends may indicate their effective potential in managing type-2 diabetes mellitus (T2DM) [46].



(a) (b)

Figure 2. (a) α-amylase inhibition (%), (b) α-Glucosidase inhibition (%)

5. CONCLUSION

This study shows that resistant starch-based flour blends (RS-type 2) have high beneficial antioxidant properties, coupled with high inhibitory effects against α-amylase and α-glucosidase activities. The increase in the amino acid and nutritional quality profiles of resistant starch-based flour blends is due to the high quality of protein present in pigeon pea flour because legumes are protein-rich crops and are richer in amino acids than plantain. The proximate and mineral compositions of the samples show appreciable indications of quality nutrition (protein, ash, fibre, energy, potassium, calcium, sodium iron and zinc). Hence, this study provides evidence for the application of resistant starch-based flour blends (AP1SPRF and AP2SPRF) as dietary functional foods.

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