

Nutraceutical Potential and Sensory Acceptability of Unripe Plantain-Millet Composite Flour Blends

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Abstract

There is an increasing need globally to eat foods that contain arrays of health promoting phytochemicals as alternatives to synthetic drugs. Therefore, the present study sought to assess the nutritional and antioxidant potentials of unripe plantain (UP) and millet (M) composite blends (UP: M); 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100. Some of the evaluated proximate indices (fat, protein, and fiber) gave high values as the composition of millet increases in the blends. On the contrary, the result showed an increasing value in the level of some minerals (sodium, calcium, potassium and magnesium) as the inclusion of unripe plantain increases in the blend, with the exception of phosphorous. Physico-chemical properties of the blends did not show any particular trend with the proportion of either plantain or millet in the blend. The result of the sensory properties of the porridge prepared from the blend showed a varied rating, with better overall acceptability for two blends (40UP:60M, 20UP:80M). HPLC/DAD analyses of millet and unripe plantain revealed the presence of some flavonoids and phenolic acids. Antioxidant indices were also high as the percentage inclusion of millet increases in the blend. Results from this investigation revealed that millet contributes higher antioxidant potential and is a better source of protein and fat, while unripe plantain have higher amount of minerals. This by implication is that the combination of these plant food most especially two of the blends (40UP:60M and 20UP:80M) would serve well as a functional food which could be harnessed as in the management of free radical mediated diseases.

Keywords: Antioxidant potential; Nutritional value; Phenolic composition; Sensory properties; Millet; *Musa paradisiaca*

1.0. Introduction

The medicinal value of plant food have assumed a more important dimension in the past decades owing largely to the discovery that their extracts contain not only minerals but also a diverse array of secondary metabolites with antioxidant potentials (Ahenkora *et al.*, 1996; Akinmoladun *et al.*, 2007). These antioxidants have been implicated in the therapeutic effects of several plants and vegetables that are used in traditional medicine (Ames *et al.*, 1993; Kumar *et al.*, 2005; Marthur and Marthur, 2001). Plant based antioxidants are now preferred to synthetic ones because of safety concerns. These factors have thus inspired the widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals or polyphenols present in them and the utilization of these antioxidants. More so, knowledge of the chemical composition of a plant together with its antioxidant activity will give a fair estimate of its therapeutic potential (Akinmoladun *et al.*, 2007).

Plantain belongs to the *Musaceae* family, a rhizomatous perennial crop and is cultivated in many tropics and subtropical countries of the world. It ranks third after yams and cassava for sustainability in Nigeria (Akomolafe and Aborisade, 2007). The nutritional potential and functional properties of starch of ripe and unripe plantains have been evaluated (Izunfuo and Omuaru, 2006). It is also high in total dietary fibre content especially in hemicelluloses (Kayisu *et al.*, 1981), which is higher than most fruits and vegetables. Unripe plantain meal is usually consumed by Nigerian diabetics to reduce postprandial glucose level (Obob, 2010; Oyewole and Adewunmi, 2003; Pari and Maheswari, 1999).

Millet is one of the four most important cereals (rice, maize, sorghum and millets) grown in the tropics. It is richer in fat content as compared to most grains, 75% of the fatty acids are unsaturated. It is also rich in B-

vitamins, potassium, phosphorous, magnesium, iron, zinc, copper and manganese with high quality protein (Klopfenstein and Hosney, 1995), and contains high amount of antioxidants. Millet is a rich source of energy which is comparable with commonly consumed cereals such as rice, wheat, maize and sorghums. It has high fiber content, and most of the dietary fiber is insoluble; α -amylase activity is 8 to 15 times greater in millet than in wheat (Sheorain and Wagle, 1973). Due to its chemical composition, millet has been attributed to having several health promoting abilities which includes diabetes, anemia constipation, cancer, non-communicable diseases and allergies (Nambiar *et al.*, 2011). Millet is useful for people who are suffering from atherosclerosis, diabetic and heart disease (Gélinas *et al.*, 2008).

In the 1960s and 1970s, composite blends very often found themselves at the focus of attention in European and International research. However, in the developing countries the use of composite blends offers a better use of domestic agricultural products (Bugusu *et al.*, 2001), with the overall effect of enhancing the use of the food product as a functional food. Recently, consumers' awareness of the need to eat high quality and healthy foods is increasing (Ndife and Abbo, 2009).

Therefore, the present investigation seeks to assess the nutraceutical potential and sensory acceptability of unripe plantain and millet composite blends with a view to developing a functional food that could be explored in the management of free radical mediated diseases.

2. Materials and Methods

2.1. Collection and Identification of Samples

Millet and Plantain were sourced from Akure main market (Oja Oba), Akure, Ondo State Western Nigeria respectively and were identified and authenticated in the Department of Crop, Soil and Pest Management, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria. The samples were weighed, washed, drained and dried. Millet was sorted to remove dirty particles, dried and ground to fine powder while the plantain was cut into small slices and steamed at 100°C for 10 minutes to prevent browning of the flesh. The plantain sample was then cooled, air dried and ground to fine powder using milling machine. The Unripe Plantain (UP) - Millet (M) composite flour of different ratio (100UP:0M; 80UP:20M; 60UP:40M; 40UP:60M; 20UP:80M; 0UP:100M) were prepared and stored in sealable bag before use.

2.1.2 Extraction of Sample for Antioxidant Assay

Phenolic compound extraction as modified by Hsu *et al.* (2003); 10 grams of millet-plantain flour blends was mixed with 80ml methanol and kept overnight. The suspension was filtered and the filtrate was diluted to 100ml with methanol and the extracts were then stored in amber bottles and refrigerated for prior analyses.

2.2 Nutritional Studies

2.2.1 Proximate Analysis

Proximate composition (moisture, proteins, fat, carbohydrates and ash) of the various proportions of unripe plantain-millet blends were determined by the standard methods (AOAC, 1990). The crude protein content ($N \times 6.25$) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Total carbohydrates were calculated by difference.

2.2.2 Mineral Analysis

Five grams (5g) of the sample was ashed in an electric furnace at 550°C for 24hours. The resulting ash was cooled in desiccators and weighed. The ash was dissolved with 2ml of concentrated HCl and few drops of concentrated HNO₃ were added. The solution was placed in boiling water bath and evaporated almost to dryness. The content was then transferred to 100ml volumetric flask and diluted to volume with deionized water. Appropriate dilution was made for each element before analysis. The mineral analysis carried out on the sample were calcium, magnesium, potassium, sodium, phosphorus, zinc, copper and iron contents were quantified using Buck Atomic absorption spectrophotometer model 210A, as described by methods (AOAC, 1990) [19].

2.2.3 Starch and Free Sugar determination

The method described by Dubois *et al.* (1956) was used. This involves weighing 0.020g finely ground sample into centrifuge tubes and wetted with 1ml of ethanol. 2ml of distilled water was added, followed by 10ml

hot ethanol. The mixture was vortexed and centrifuged at 2000rpm for ten minutes. The supernatant was collected and used for free sugar analysis, while the residue was used for starch analysis. To the residue was added 7.5ml of perchloric acid and allowed to hydrolyze for 1 hour. It was diluted to 25ml with distilled water and filtered through Whatman No 2 filter paper. From the filtrate 0.05ml was taken, made up to 1ml with distilled water, vortexed and ready for color development as was described for standard glucose curve preparation. The supernatant was made up to 20ml with distilled water, an aliquot of 0.2ml was taken and 0.5ml (5% phenol) and 2.5ml concentrated sulphuric acid was subsequently added. The sample was allowed to cool and the absorbance read on a UV/Visible at 490nm wavelength.

2.2.4 Amylose Determination

The method described by Juliano (1971) was used. 0.1g of flour sample or standard was weighed into a centrifuge tube. To this, 1ml of 95% ethanol and 9ml 1N NaOH were carefully added, the test was covered and the content was mixed very well on a vortex mixer. Thereafter, the samples were heated for 10 minutes in a boiling water bath to gelatinize the starch, and then allowed to cool to room temperature. 10 times dilution of the extract was made by taking 1ml of the extract and make up to 10ml with 9ml of distilled water. An aliquot of 0.5ml was taken from the diluents for analysis. 0.1ml of Acetic acid solution and 0.2ml of iodine solution were added to the diluents taken. The volume was made up to 10ml with 9.2ml of distilled water. The test mixture was left for 20mins for color development after which it was vortexed and the absorbance was read at 620nm.

2.2.5 Sensory Evaluation

The six samples obtained from the different percentage composition of unripe plantain -millet composite flour were made into thick gruel (paste) using about 50g of flour and 15ml of boiling water. The method of Larmond (1977) was used for the sensory evaluation; The samples were rated on the following quality attributes; taste, after taste, appearance, aroma, mouth feel, firmness, swallow ability and overall acceptability using 9 point hedonic scale. The score obtained were subjected to analysis of variance of 5% level of significance and mean separated using Duncan Multiple Range Test.

2.2.6 Physico-chemical properties

The bulk density of the samples was determined by the method of Okaka *et al.* (1991). The swelling power and total soluble solid was determined by the method described by Tester and Morrison (Tester and Morrison, 1990). The water absorption capacity of the samples was determined by standard method (Lawal and Adebowale, 2005). The pH of all the samples was determined using the method described by AOAC (1990).

2.3 Antioxidant Indices

2.3.1 Total Phenolic Estimation

The total phenolic content of the extracts was determined by the Folin-Ciocalteu assay as described by Waterman and Mole (1994). The hydro-alcoholic extract (0.25 ml), was placed in a 25 ml volumetric flask and 5 ml distilled water was added. Folin-Ciocalteu's phenol reagent (1.25 ml) was added and mixed. After 2 min, 3.75 ml 20% (w/v) sodium carbonate solution was added. The contents were mixed and distilled water was added to volume and mixed. The mixture was left to stand for 2 h after addition of the sodium carbonate for which the absorbance of the mixture was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). The standard used was tannic acid and the results which were determined in triplicates were expressed as mg tannic acid equivalents per gram of the sample.

2.3.2 Total Flavonoid Content

The total flavonoid content of the extract was determined using a slightly modified method reported by Meda *et al.* (2005). Briefly, 0.5mL of appropriately diluted sample was mixed with 0.5mL methanol, 50 μ L of 10% AlCl₃, 50 μ L of 1mol L⁻¹ potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid content was calculated using quercetin as standard by making use of a seven point standard curve (0-40 μ g/ml or 0-100 μ g/ml), the total flavonoids content of samples was determined in triplicates and the results were expressed as mg quercetin equivalent per gram of the sample.

2.3.3 Reducing Power

The reducing power of the extracts was determined by assessing the ability of each extract to reduce FeCl₃ solution as described by Oyaizu (1986). Briefly, appropriate dilution of each extract (2.5 ml) was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. Each mixture was

incubated at 50°C for 20 min and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 353 x g for 10 min. 5ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant power was determined in triplicate and expressed as mg ascorbic acid equivalent/g of the sample.

2.3.4 ABTS antiradical assay

Antioxidant activity of the extracts was determined using the 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ABTS antiradical assay (Awika *et al.*, 2003). The ABTS^{•+} (mother solution) was prepared by mixing equal volumes of 8mM ABTS and 3mM potassium persulphate (K₂S₂O₈) (both prepared using distilled water) in a volumetric flask, which was wrapped with foil and allowed to react for a minimum of 12 h in a dark place. The working solution was prepared by mixing 5 ml of the mother solution with 145 ml phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-carboxylic acid) standard solutions (100–1000 µM) were prepared in acidified methanol. The working solution (2.9 ml) was added to the methanolic extracts (0.1 ml) or Trolox standard (0.1 ml) in a test tube and mixed with a vortex. The test tubes were allowed to stand for exactly 30 min. The absorbance of the standards and samples were measured at 734 nm with a Lambda EZ150 spectrophotometer. The results which were determined in triplicates were expressed as µM Trolox equivalents/g sample, on dry weight basis.

2.3.5 DPPH antiradical assay

The DPPH assay was done according to the method of Brand-Williams *et al.* (1995), with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20°C prior analysis. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.1 units at 515 nm using the spectrophotometer. Phenol extracts (300µl) were allowed to react with 2700µl of the DPPH solution for 6 h in the dark. Then the absorbance was taken at 515 nm. Results which were determined in triplicates were expressed in µM Trolox Equivalent/g sample. Additional dilution would be needed if the DPPH value measured was over the linear range of the standard curve.”

2.3.6 OH Radical scavenging ability

The ability of the extract to prevent Fe²⁺/H₂O₂ induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1985). Briefly, freshly prepared extract (0-100µl) was added to a reaction mixture containing 120µl, 20mM deoxyribose, 400µl, 0.1M phosphate buffer pH 7.4, 40µl, 20mM hydrogen peroxide and 40µl, 500mM FeSO₄, and the volume was made to 800µl with distilled water. The reaction mixture was incubated at 37°C for 30min and stopped by the addition of 0.5ml of 2.8% TCA. This was followed by the addition of 0.4ml of 0.6% TBA solution. The reaction tubes were subsequently incubated in boiling water for 20min. The absorbance was measured at 532nm in spectrophotometer and the percentage radical inhibition which was determined in triplicates was subsequently calculated.

2.4 Quantification of compounds by HPLC-DAD in Unripe Plantain and Millet

Reverse phase chromatographic analyses were carried out under gradient conditions using C₁₈ column (4.6 mm x 150 mm) packed with 5µm diameter particles; the mobile phase was water containing 1% acetic acid (A) and acetonitrile (B), and the gradient program was started with 13% of B until 10 min and changed to obtain 20%, 30%, 50%, 70% and 100% B at 20, 30, 40, 50 and 60 min, respectively, following the method described by Boligon *et al.* [32], with slight modifications. The samples extract were analyzed at a concentration of 20 mg/mL. The presence of six compounds was investigated, namely, gallic acid, caffeic acid, ellagic acid, quercetin, isoquercitrin and rutin. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.6 ml/min; injection volume 40 µl and the wavelength were 254 nm for gallic acid, 327 nm for caffeic and ellagic acids, and 365 nm for quercetin, isoquercitrin, and rutin. The samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.030 – 0.250 mg/ml for quercetin, isoquercitrin and rutin; and 0.050 – 0.350 mg/ml for caffeic, ellagic and gallic acids. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Boligon *et al.* (2012). LOD and

LOQ were calculated as 3.3 and 10 σ/S , respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.5 Statistical Analysis

All experiments were performed in triplicates. Analysis at every time point from each experiment was carried out in triplicate. Means, standard errors and standard deviations were calculated from replicates within the experiments and analyzed using Microsoft Excel XP.

3. Results and Discussion

Foods and nutrients play a vital role in maintaining the health of the individual and in reducing the risk of various diseases. There is a global acceptance of this fact and this formed a relationship between "nutrition" and "health" which is known as "nutraceuticals". Nutraceuticals are medicinal foods that play a role in maintaining well being, enhancing health, modulating immunity and thereby preventing as well as treating specific diseases. Thus, the field of nutraceuticals can be envisioned as one of the missing blocks in the health benefit of an individual.

The proximate composition of the various blends is presented on Table 1. The 100M-0 UP blend had the least percentage moisture content (5.38%), carbohydrate (74.53%), ash(1.15%) and a highest protein (10.48%), fiber (0.69%) and fat (7.05%) content compared to whole unripe plantain and the other blends. There is a progressive increase in the protein content of the composite flour blend with increase in millet flour composition, and this could be attributed to high protein content of millet compared with unripe plantain. This is agreement with previous report that revealed a higher protein level in millet compared with unripe plantain (Léder,2004) . The result obtained from the composite blend revealed a high amount of fat as the composition of millet increases in the blend. Burton *et al.* 1972 and Rooney, 1978, reported that the consumption of millet should be encouraged since it contain the type of fat that is easily used by the body system and the presence of phospholipids in millet is useful in brain function, behavioral disorder and stress . This observation is in agreement with the reports of Singh *et al.*, 2005 and Premavalli *et al.*, 2005, that reported an increasing level of fat with increasing amount of millet flour in wheat-millet blends. The carbohydrate, moisture and ash content of whole millet flour compared favorably with previous reports (Arisa *et al.*, 2013; Adeolu and Enesi, 2013), though with a relatively higher level of protein.

The result of the mineral content (mg/g) of the various blends is as presented in Table 2. The result showed that a high unripe plantain in the blend bring about an increasing value of some evaluated minerals (Ca, Mg, P and Fe). The 100UP blend recorded the highest level of Ca (7566), Mg (2496), K (48600) and Na(14900) respectively, while the least amount of Ca (4655), Mg (1838), K (29600) and Na(8943) was recorded for 100M. The 100M flour blends have the highest content of P(1052) and value is in agreement with previous reports (Stover *et al.*, 1987) .

The starch: sugar and amylose: amylopectin content is presented in Table 3, Starch is composed of two major polysaccharides, amylose and amylopectin (Birkett and Brown, 2007). The result of the amylose: amylopectin indicate that 100M flour (4.87 \pm 0.02) had the highest amylose: amylopectin while 100UP had the lowest amylose: amylopectin ratio (2.45 \pm 0.01). Behall and Howee (1995), reported that the consumption of high amylose: amylopectin foods normalized insulin response and even lowered glucose response than low amylose: amylopectin foods and they also empty more slowly from the stomach therefore in this result it was found that 100M had the highest ratio. Behall *et al.*, 1989 also reported that high amylose:amylopectin normalized and control insulin in normal subjects Therefore, it could be hypothesized that the more the millet in the blend the better it will empty more slowly in the stomach, normalized insulin response and even lowered glucose response than 100UP. 100UP flour have the highest starch:sugar (54.61 \pm 1.05) while 100M flour recorded the least ratio (16.01 \pm 0.17). The highest sugar: starch (54.61 \pm 1.05) was recorded for 100UP, while the least was recorded for 100M (16.01 \pm 0.17). Sugars are part of carbohydrates and are an important part of our diet, it has been reported that high intake of sugars are known to have negative influence on the incidence of type 2 diabetes and also possible mechanisms through which sugars can cause hypertension (Johnson *et al.*, 2007).

The physico-chemical properties are those characteristics that govern the behaviour of nutrients in food during processing, storage and preparation as they affect food quality and acceptability (Onwuka, 2005), and they also determine the application and use of food material for various food products. The physicochemical property of the whole flour and the composite blends is as shown in Table 4. The bulk density values (g/ml) were found to be between 0.39 - 0.76. 100UP flour was denser than all other blends (0.76g/ml) and 40UP-60M flour

blend had the least value (0.39g/ml). Bulk density is generally affected by the particular size and the density of the flour and it is very important in determining the packaging requirements and the higher the bulk density the denser the flour (Adebowale *et al.*, 2008). Eleazu and Okafor, 2012 reported that the relatively high bulk density of unripe plantain flour suggests its suitability as a drug binder in pharmaceuticals industry. Total soluble solid of 40UP-60M ranked as the highest (88.82%) and the least was recorded for 100M (87.13%). In the swelling capacity 20UP-80M flour blend recorded the highest capacity (3.25ml) and 60UP-40M flour blend the least swelling capacity 1.00ml. Swelling power indicates how much water a product can absorb to swell in the presence of heat. Also 40UP-60M flour blend recorded the highest water absorption capacity (3.58ml) and 100M flour with least (2.23ml). Water absorption capacity describes flour-water association ability under limited water supply. Therefore water absorption capacity is important in bulking and consistency of products as well as in baking application. Niba *et al.*, 2001 further describe water absorption capacity as an important processing parameter that has implication for viscosity. The result equally revealed that 80UP -20M had the highest viscosity (1.85p.c) and 20UP-80M showed the least viscosity (0.51p.c).

Based on the fifteen points hedonic scale used to carry out the sensory evaluation is showed in Table 5. There was a significant decrease ($P < 0.05$) in the sensory rating of the porridge with increase in millet level. 100% unripe plantain flour had the highest rating of taste, after taste, appearance, aroma, mouth feel, firmness, swallow ability and overall acceptability and 100% millet flour had the least rating. On the overall, the higher the amount of unripe-plantain in the composite blend the better the overall acceptability.

The result of the antioxidant indices (Total Phenol, DPPH* scavenging power, ABTS* scavenging power, OH* scavenging power and Total Flavonoid) of the composite blends is as shown in Table 6. The antioxidant indices of the composite blends showed an increasing value as the composition of millet flour increases in the blends, with the exception of OH* scavenging power. The observed similarity of the evaluated antioxidant activities with the total phenolic content is in agreement with previous reports which established a positive correlation between total phenolic content and antioxidant indices of plant foods (Dlamini *et al.*, 2007; Siatka and Kašparová, 2010). On the overall, the highest antioxidant power was recorded for 100UM while the least value was recorded for 100UP total phenolic content value. The increasing value with increasing millet composition is probably due to the additive interaction of the constituent Polyphenol (Eleazu and Okafor, 2012), since the total phenolic content of millet is higher than that of unripe plantain.

The result of the phenolic analyses of unripe plantain and millet with the aid of HPLC- DAD is shown in Table 7 and Figure 1. The result revealed the presence of some flavonoids (rutin, isoquercitrin, and quercetin) and phenolic acids (gallic acid, caffeic acid, ellagic acid) in both unripe plantain and millet. Quantitative estimation of phenolic compounds in unripe plantain showed quercetin (51.83 mg/g) to be the most abundant phenolic compound while the least phenolic compound was shown to be gallic acid (3.75 mg/g). Isoquercetin (45.73mg/g) was observed to be the most abundant phenolic compound in millet and the least phenolic acid was also shown to be gallic acid (4.23mg/g). Millet has been reported to contain both flavonoids and phenolic acid which are located in the pericarp, testa, aleurone layer and endosperm (Hahn *et al.*, 1984; Hilu *et al.*, 1978; McDonough *et al.*, 1986). The phenolic acid and flavonoids which are present in unripe plantain and millet flour are reported by Duenas *et al.*, (2005) to have a positive role in strengthening the capillary walls, thinning blood by reducing the agglutination of RBCs, even preventing cancer and also have anti-inflammatory properties. Therefore both flavonoids and phenolic acids are known to be highly active antioxidant.

4. Conclusion

The studies showed that blends with high millet flour had the highest protein, fat, dietary fibre, amylose: amylopectin, low sugar: starch and high antioxidant properties than the blends with high amount of unripe plantain flour, while blends with high amount of unripe plantain is rich minerals and contribute better to the overall sensory acceptability and these can be beneficial in promoting good health. The presence of phenolic compounds and phytochemicals of millet flour greatly contribute to the antioxidant potential which helps to neutralize and counteract the effects of free radicals. On a holistic assessment, 40UP-60M and 20UP-80M blend containing both unripe plantain and millet showed relatively fair and consistent amylose: amylopectin with low sugar: starch, a reasonably high level of protein, fibre and fat, and a high antioxidant activities, with good sensory acceptability. This by implication is that the two blends could be a valuable functional food which could be explored in the management of free radical related diseases.

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Table 1: Proximate Composition (%) of plantain and millet flour blend

Sample	Moisture	Ash	Fat	Crude Fiber	Crude Protein	CHO (By Diff)
A	9.14±0.01 ^f	1.73±0.03 ^f	0.91±0.01 ^a	0.19±0.01 ^c	3.84±0.10 ^a	84.19±0.07 ^a
B	8.81±0.01 ^e	1.48±0.01 ^d	0.46±0.01 ^b	0.13±0.01 ^a	5.38±0.10 ^b	83.75±0.11 ^e
C	8.34±0.01 ^d	1.53±0.01 ^e	1.67±0.02 ^c	0.16±0.02 ^b	5.84±0.11 ^c	82.46±0.12 ^d
D	8.08±0.01 ^c	1.26±0.01 ^b	2.97±0.01 ^d	0.49±0.01 ^e	6.87±0.09 ^d	80.32±0.11 ^c
E	5.38±0.02 ^a	1.33±0.01 ^c	6.76±0.01 ^e	0.31±0.01 ^d	8.16±0.09 ^e	79.05±1.59 ^b
F	6.10±0.02 ^b	1.15±0.02 ^a	7.05±0.02 ^f	0.69±0.02 ^f	10.48±0.09 ^f	74.53±0.12 ^a

Means ± SE followed by different letter in a column are significantly different ($p < 0.05$) by Duncan Test A: 100 Unripe plantain-0millet;

B:80 Unripeplantain-20millet;C:60 Unripeplantain-40millet;D:40Unripeplantain-60millet;E:20Unripe plantain-80millet;F:0Unripe plantain-100millet

Table 2: Mineral composition (mg/kg) of Plantain and Millet composite flour blend

Sample	Na ⁺	K ⁻	Ca ²⁺	Mg ²⁺	P
A	14900±6.00 ^f	48600±6.81 ^f	7566.33±30.93 ^f	2496.67±5.51 ^f	496±1.73 ^a
B	14500±3.46 ^e	44100±6.56 ^e	6877.33±6.66 ^e	2290.67±4.04 ^e	592.33±4.51 ^c
C	14100±6.50 ^d	41200±11.79 ^d	5131.33±2.89 ^d	2040.33±4.04 ^d	507.67±4.16 ^b
D	13300±12.50 ^c	36500±10.02 ^b	4979±3.00 ^b	1891±3.00 ^c	884±4.58 ^d
E	9993.67±10.26 ^b	36900±4.16 ^c	5312.67±8.76 ^d	1868±6.00 ^d	975.33±3.79 ^a
F	8943.67±9.45 ^a	29600±10.44 ^a	4655±5.57 ^a	1838±1.53 ^a	1052±4.24 ^f

Means ± SE followed by different letter in a column are significantly different ($p < 0.05$) by Duncan Test

A:100 Unripe plantain-0millet;B:80 Unripe plantain-20millet;C:60 Unripe plantain-40millet;D:40 Unripe plantain-60millet;E:20 Unripe plantain-80millet;F:0 Unripe plantain-100 millet

Table 3: Starch: Sugar and Amylose: Amylopectin of Unripe Plantain and Millet Composite Blends

Sample	% Starch	% Sugar	Starch: Sugar	% Amylopectin	%Amylose	Amylopectin: Amylose
A	60.40±0.11 ^f	1.11±0.03 ^a	54.61±1.05 ^f	71.04±0.11 ^a	28.97±0.11 ^f	2.45±0.01 ^a
B	57.03±0.11 ^e	1.87±0.03 ^b	30.45±0.44 ^e	76.42±0.36 ^b	23.59±0.36 ^e	3.24±0.06 ^b
C	55.46±0.10 ^d	1.96±0.02 ^c	28.32±0.30 ^d	80.09±0.10 ^c	19.91±0.10 ^d	4.03±0.03 ^c
D	53.42±0.21 ^c	2.17±0.02 ^d	24.61±0.17 ^e	81.29±0.10 ^d	18.71±0.10 ^e	4.35±0.03 ^d
E	52.18±0.31 ^b	2.51±0.03 ^e	20.75±0.24 ^b	82.10±0.10 ^e	17.90±0.10 ^b	4.59±0.05 ^e
F	51.46±0.21 ^a	3.22±0.05 ^f	16.01±0.17 ^a	82.95±0.05 ^f	17.05±0.05 ^a	4.87±0.02 ^f

Means ± SE followed by different letter in a column are significantly different ($p < 0.05$) by Duncan Test.

A:100 Unripe plantain-0 millet; B:80 Unripe plantain-20millet; C:60 Unripe plantain-40millet;

D:40 Unripe plantain-60millet; E:20 Unripe plantain-80millet;F:0 Unripe plantain-100 millet

Table 4: Physico-Chemical Properties of Plantain-Millet Composite Flour Blend

SAMPLE	pH	TSS (%)	SC (ml)	B.D (g/ml)	VISCO (p.c)	WAC (ml)
A	6.41±0.005 ^a	87.39±0.295 ^a	3.00±0.00 ^a	0.76±0.001 ^f	0.70±0.000 ^b	3.35±0.050 ^e
B	6.34±0.005 ^b	87.26±0.570 ^a	1.25±0.25 ^a	0.67±0.000 ^e	1.85±0.050 ^e	3.40±0.000 ^e
C	6.36±0.000 ^e	88.05±0.220 ^b	1.00±0.00 ^a	0.69±0.000 ^d	1.51±0.005 ^d	3.55±0.050 ^d
D	6.41±0.01 ^e	88.82±0.025 ^c	2.75±0.25 ^c	0.39±0.005 ^a	1.02±0.000 ^e	3.58±0.025 ^d
E	6.38±0.005 ^d	88.61±0.005 ^e	3.25±0.25 ^d	0.70±0.0005 ^e	0.51±0.010 ^a	3.10±0.100 ^b
F	6.32±0.005 ^a	87.13±0.055 ^a	2.00±0.00 ^b	0.61±0.005 ^b	0.71±0.005 ^b	2.23±0.025 ^a

Sample with the same number of superscript are not scientifically different at 5% probability level. Sample: A:100Unripe plantain flour;

B:80Unripe plantain flour-20 millet flour;C:60 Unripe plantain flour-40 millet flour; D:40% Unripe plantain flour:60 millet flour;

E:20Unripe plantain flour:80 millet flour;F:100millet flour TSS: Total Soluble Solid; SC: Swelling Capacity B.D: Bulk Density;

VISCO: Viscosity; WAC: Water Absorption Capacity

Table 5: Sensor Evaluation of Unripe Plantain-Millet Composite Flour Blend

Quality attributes	A	B	C	D	E	F
Taste	8.14±0.77 ^d	7.43±0.76 ^d	6.57±0.76 ^c	5.50±1.16 ^b	4.43±1.55 ^a	3.64±1.50 ^a
After taste	8.07±1.07 ^d	7.43±1.28 ^d	6.43±0.94 ^c	5.50±1.22 ^{b,c}	4.71±1.33 ^b	3.57±1.70 ^a
Appearance	7.86±0.77 ^d	7.07±0.99 ^{cd}	6.29±0.61 ^{b,c}	5.50±1.16 ^b	4.42±1.28 ^a	3.64±1.82 ^a
Aroma	8.00±0.78 ^e	7.14±0.86 ^d	6.21±0.69 ^c	5.86±0.77 ^c	4.57±1.09 ^b	3.64±1.78 ^a
Mouth feel	8.00±0.39 ^d	6.92±0.92 ^c	6.14±0.53 ^c	5.26±1.20 ^b	4.50±1.56 ^{ab}	3.86±1.56 ^a
Firmness	8.21±0.97 ^d	7.21±0.97 ^c	6.79±0.80 ^c	5.50±0.94 ^b	4.92±1.21 ^b	3.79±1.67 ^a
Swallow ability	7.86±0.36 ^e	7.14±0.66 ^{de}	6.57±0.94 ^{cd}	5.79±1.25 ^{b,c}	5.14±1.51 ^b	4.14±1.99 ^a
Overall acceptability	8.42±0.51 ^f	7.29±0.99 ^e	6.50±0.94 ^d	5.71±1.27 ^c	4.79±1.42 ^b	4.00±1.71 ^a

Sample with the same number of superscript are not scientifically different at 5% probability level. Sample A:

100%Unripe plantain flour;B:80%Unripe plantain flour-20 millet flour;C:60 %Unripe plantain flour-40 millet flour;

D:40% Unripe plantain flour;60 millet flour;E:20%Unripe plantain flour;80 millet flour;F:100millet flour

Table 6: Antioxidant Indices of Unripe Plantain-Millet Composite Blends

Sample	Total Phenol (mg TAE/g)	DPPH (µmol Trolox Eq/g)	ABTS (µmol Trolox Eq/g)	Total Flavonoid (mg Ascorbic acid Eq/g)	OH [•] Assay (%)
A	0.0029±0.002 ^a	0.00024±0.000015 ^a	0.0885±0.00013 ^a	0.54±0.004 ^a	66.75±2.02 ^a
B	0.0243±0.002 ^b	0.00027±0.000003 ^a	0.0888±0.00009 ^b	0.87±0.005 ^b	59.56±1.30 ^d
C	0.0442±0.003 ^c	0.00034±0.000026 ^b	0.0893±0.00017 ^c	1.14±0.017 ^b	57.11±1.15 ^{cd}
D	0.0558±0.003 ^d	0.00047±0.000029 ^c	0.0897±0.00011 ^d	1.34±0.024 ^c	55.23±3.00 ^{bc}
E	0.0683±0.005 ^e	0.00059±0.000019 ^d	0.0899±0.00005 ^e	1.55±0.055 ^d	52.10±2.03 ^b
F	0.0916±0.004 ^f	0.00068±0.00002 ^e	0.0902±0.00011 ^f	1.63±0.047 ^e	45.85±2.44 ^a

Value are given as mean ± SD of independent experiments performed in triplicate. Sample with the same number of superscript are not scientifically different at 5% probability level. Sample:A:100%Unripe plantain flour;B:80%Unripe plantain flour-20 millet flour;C:60 %Unripe plantain flour-40 millet flour;D:40% Unripe plantain flour;60 millet flour;E:20%Unripe plantain flour;80 millet flour;F:100millet flour

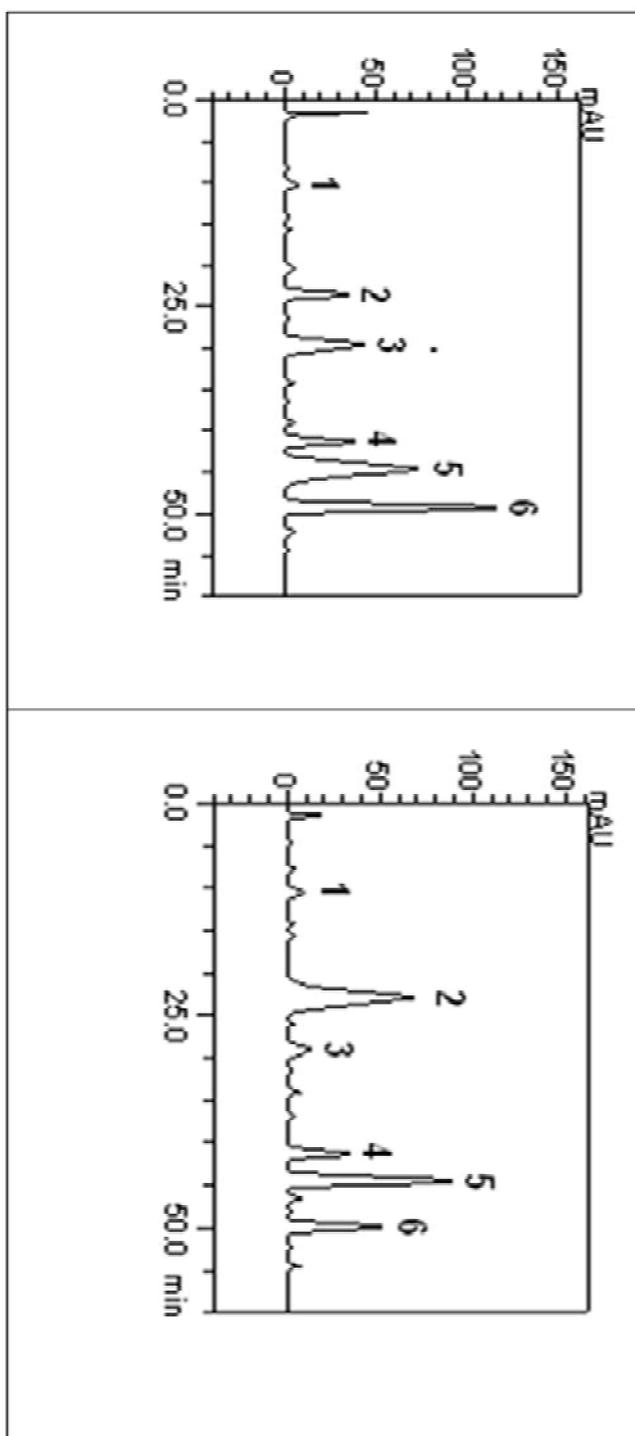


Figure 1: HPLC-DAD Phenolic Chromatogram of (a) Urripe Plantain- 1: Gallic acid; 2: Caffeic acid; 3: Ellagic acid; 4: Rutin; 5: Isoquercitrin; 6: Quercetin (b) Millet: 1: Gallic acid; 2: Caffeic acid; 3: Ellagic acid; 4: Rutin; 5: Isoquercitrin; 6: Quercetin.

Table 7: Phenolic Composition of Unripe Plantain and Millet Extract

Sample/ Compound	mg/g	%	LOD (µg/ml)	LOQ (µg/ml)
<u>Unripe Plantain</u>				
Gallic acid	3.75±0.01 ^a	0.37	0.015	0.049
Caffeic acid	18.90±0.02 ^b	1.89	0.018	0.059
Ellagic acid	20.65±0.02 ^b	2.06	0.036	0.119
Rutin	19.36±0.01 ^b	1.93	0.042	0.138
Isoquercitrin	40.71±0.03 ^c	4.07	0.021	0.071
Quercetin	51.83±0.01 ^d	5.18	0.007	0.024
<u>Millet</u>				
Gallic acid	4.23±0.03 ^a	0.42	0.015	0.049
Caffeic acid	39.15±0.01 ^b	3.91	0.018	0.059
Ellagic acid	8.67±0.01 ^c	0.86	0.036	0.119
Rutin	18.95±0.01 ^d	1.89	0.042	0.138
Isoquercitrin	45.73±0.02 ^e	4.57	0.021	0.071
Quercetin	26.81±0.03 ^f	2.68	0.007	0.024

Results are expressed as mean ± standard deviations (SD) of three determinations.

Averages followed by different letters differ by Tukey test at $p < 0.05$. LOD: Limit of detection;

LOQ: Limit of quantification.

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