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Phytochemical studies of wheat-plantain composite flours enriched with velvet beans flours

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ABSTRACT: Wheat, which is an expensive grain, is not adequate to meet the nutritional requirements of the end users. Hence, enrichment of wheat with easily affordable underutilized legumes and fruits that have superior nutritional value is a good approach to producing functional foods and this is also a good way to reduce the cost of wheat importation. Therefore, this study aimed to evaluate the phytochemical composition of the flour blends processed from wheat-plantain flour blends enriched with velvet bean. Wheat, plantain and Velvet flours composite were prepared in the ratio 240:37.5:22.5, 210:60:30 and 150:105:45 respectively and 100% wheat flour was used as the control. Phytochemicals revealed that total phenolic, total flavonoids, total carotenoids, L-Dopa, DPPH and FRAP ranged from 41.22 – 383.09 mg/GAE/100 g, 42.94 – 83.11 mg/CE/100 g, 0.41– 1.66 mg/100 g, 0.91 – 1.21%, 33.12 – 46.85 mg/AAE/100 g and 4.05 – 6.29 mg/FeSO4/100 g, respectively. Addition of plantain and velvet beans flour blends to the wheat flour significantly ($p < 0.05$) enhanced the phytochemical properties of the flour blends. The blends could therefore be used to produce any functional food. Hence, its food applications are highly recommended.

Keywords: DPPH, FRAP, L-dopa, nutritional value, total carotenoids, total flavonoids, total phenol.

INTRODUCTION

Wheat (*Triticum aestivum* L.) belonging to the Poaceae family, is the second most frequently grown cereal grain behind rice for human consumption (Chung *et al*., 2022). This crop may be grown in Mediterranean, temperate, and subtropical climates due to its extensive genetic diversity. Global worldwide production of wheat has been reported as 778.6 million metric tons in 2021/2022 (Ammar *et al*., 2023). Wheat flour is widely used due to its gluten content which is a protein that facilitates excellent dough formation and elasticity; a characteristic feature absent in other flours (Wujun, 2019).

The plantain plant (*Musa paradisiaca*) is a gigantic herb that springs from an underground stem, or rhizome. Most varieties are 3–10 metres (10–33 feet) tall and have a conical false “trunk” formed by the leaf sheaths of long spirally arranged leaves. The fruit which is green to brown-yellow, is typically larger than the common banana and is borne in bunches. (Britannica, 2024). It is an important staple food in Central and West Africa (Makanjuola *et al*., 2013). Plantains are abundant in Nigeria and other developing countries. Plantain fruit is composed of 75% different elements and 32% of carbohydrates and it also contains several vitamins including A, B, C and is very low in protein and fat but rich in minerals particularly iron. Also, it is free from cholesterol, high in fibre and low in sodium (Adewole and Duruji, 2010). Because plantains have poor amino acid profile, it should be supplemented with protein rich food crops like legumes. The resulting products would be rich in both protein and carbohydrates.

Velvet bean (*Mucuna pruriens*), belongs to the Fabaceae family, it is part of various legumes which is not commonly used by people as a result of antinutrients. This level of anti-nutrients could be reduced by boiling and other prescribed methods such as drying, soaking etc (Samtiya, 2020). Velvet bean is commonly grown in the tropical and subtropical part of the world. It has been reported to be a great source of dietary protein because it has a higher per-centage of protein (about 26%), and it is easily digestible

compared to other annual leguminous crops (Janardhanan *et al*., 2003). Velvet bean can also help in stress management and improvement of semen quality (Shukla, 2007). One of the possible ways to promote the use of velvet bean could be its utilization in formulations for mass consumer goods, such as bread, etc. These beans were grossly under-utilized in most countries especially in Nigeria due to low level of awareness of the numerous benefits derivable from them. Therefore, the purpose of this study was to evaluate the phytochemical composition of the flour processed from wheat-plantain flour blends enriched with velvet beans.

MATERIALS AND METHODS

Plantain, velvet bean and wheat flour were purchased from Owode market, Offa, Kwara State, Nigeria. The equipment used was made available from the Department of Food Technology, Federal Polytechnic Offa, Nigeria. All chemicals that were used are of food standard and analytical grade.

Sample preparation

Preparation of plantain flour

Plantain (*Musa paradisiaca*) flour was prepared following the processing steps described by Kure *et al.* (2012). Plantain fingers were separated from the bunches, washed, peeled manually and sliced to (2 mm thickness) using a stainless-steel kitchen slicer. The sliced chips were blanched at 70°C for 5 min, and dried in a cabinet drier at 50°C for 48 hours. The dried slices were milled, sieved and packaged in a low density polyethylene bag; and stored at ambient conditions for subsequent use (Figure 1).

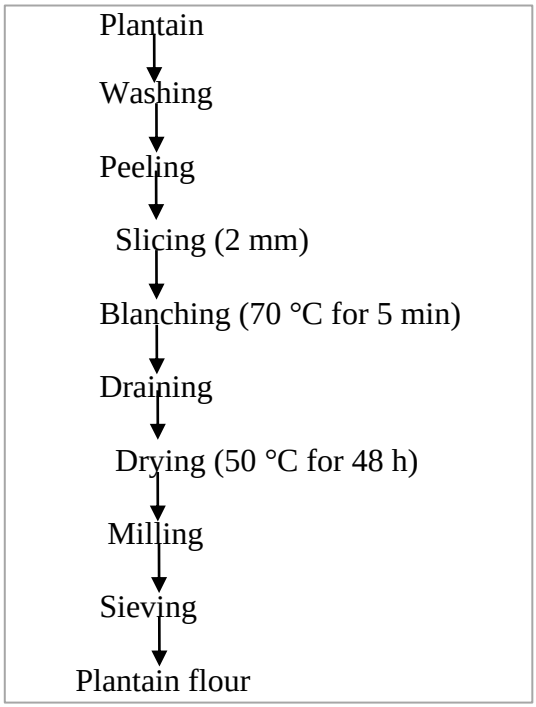

Figure 1. Flow chart for the production of plantain flour (Source: Kule *et al*., 2021).

Preparation of boiled-velvet beans into flour

Velvet beans (*Mucuna pruriens)* were processed into flour as described by Balogun and Olatidoye (2010). About one thousand grams (1000 g) of matured velvet beans seed were sorted cleaned to remove extraneous materials like stones and defective seeds. The seeds were introduced into already boiling distilled water (1000:4000 g/ml) and boiled for 30 minutes. The seeds were dehulled manually and washed thoroughly under running water and drained. The seed was oven-dried at 50°C for 24 hours and milled into flour (300 µm) (Figure 2).

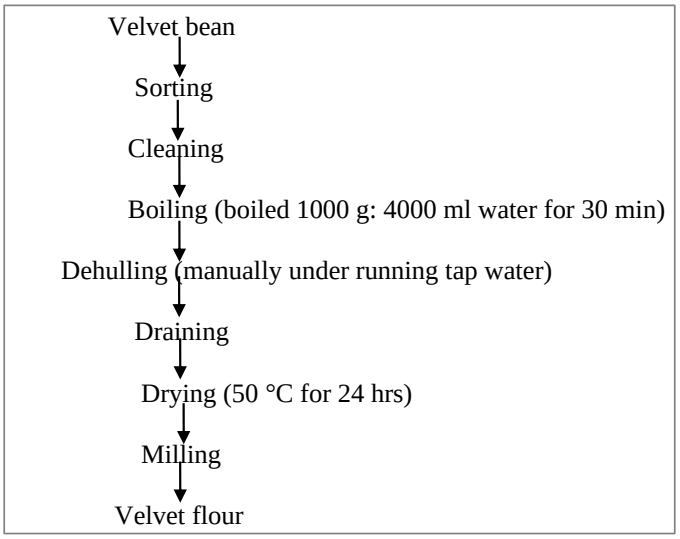

Figure 2. Flow chart for the production of Soaked-velvet bean flour (Source: Balogun and Olatidoye, 2010).

Composite flour preparation

Boiled-velvet bean flour and plantain flour composite flours were prepared by blending them with wheat flour. The composite flour of wheat-plantain-velvet beans 240:37.5: 22.5, 210:60:30 and 150:105:45 respectively and 100% wheat flour was used as the control.

Determination of phytochemicals

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and

plant virus infections and also consumption by insects and other animals. Some phytochemicals have been used as poisons and others as traditional medicine. These include;

Total flavonoids

Total flavonoid was studied by the method described by Peixoto Sobrinho *et al.* (2008) with slight modification. One gram (1.0 g) of sample was weighed into a conical flask. Add 50 ml of 80% methanol was added. It was extracted by placing on a hot plate at low temperature for 30 minutes while stirring. It was allowed to cool and filtered into a 100 ml volumetric flask**.** It was made up to mark of 100 ml with 80% methanol. 3 ml of extract was pipetted into a test tube and 0.1 ml of 10% $AlCl_3$ was added. Then, 0.1 ml Na – K tartar ate was added to it before 3ml of distilled water was added**.** Then it was shaken properly to mix. The solution was read absorbance at 415 nm. Procedure No 3 – No 9 was repeated for routing standards of concentrations 5, 10, 15, 20 mg/l. A standard curve was plotted for the routing standard. The concentration of the samples was determined by extrapolating the absorbances down the concentration axis. Total flavonoid is calculated as follows.

$$\text{Total Flavonoid (mgRE/Kg)} = \frac{\text{conc. obtained (mg/l) x TVE x DF}}{\text{Sample weight}}$$

Where: TVE = total volume of extract, and DF = Dilution factor. If not diluted, then DF = 1

Determination of total carotenoid

Determination of total carotenoids of the flour samples were carried out by following the method adopted by Hussain *et al.* (2022). Control biscuits and biscuits developed with different ratios of pumpkin parts 25 g each, was taken in vessel covered with aluminum foil. Exact 80 ml of extraction solvent, acetone / n-hexane (1:1, v/v) was added to the vessel and shaken well. Through separation technique, release of organic phase was carried out. Again, 15 mL of acetone / n-hexane (1:1, v/v) was added to repeatedly carryout the aqueous phase extraction until the colourless appearance was achieved. Anhydrous sodium sulphate was used to dehydrate the organic phase followed by the spectrophotometric analysis of extracts at 450 nm. Total carotenoids were expressed as mg/100 g of flour samples.

Determination of total phenolic

Method of Hussain *et al.* (2024) was used to determine the total phenol of the composite flour sample. Sample was diluted with 0.5 mL 0.5N Folin-Ciocalteu reagent at ambient temperature, and then $NaCO_3$ (75 g/L) was added to make the medium alkaline due to the oxidation–reduction reaction between the phenolic compounds and the Folin reagent. These mixtures were then kept at 23°C for a time period of 2 hour before measuring absorbance. The absorbance at 760 nm was measured with a spectrophotometer (U 2900 Hitachi, Japan). Total phenolic contents were calculated as mg gallic acid equivalents (GAE) per 100 mL juice using gallic acid as a reference. Three replicates of absorption values were recorded, and mean was calculated.

Determination of scavenging power using DPPH

DPPH free radical scavenging activity was determined using the standards illustrated by Hussain *et al.* (2023). DPPH of a small quantity of 0.01 g of the sample was poured into a 25 mL flask having methanol and water solution (80:20 vol/vol). An ascorbic acid calibration curve was also made. In microplates, 100 µl of each sample was taken and then 2 mL solvent and 250 µl DPPH were added. The whole mixture was shaken and mixed thoroughly and placed in the dark at ambient temperature for approximately 30 minutes. The results were expressed as mg of ascorbic acid equivalent/100 g powder for 30 minutes in the reaction after the mean value was computed for each sample

Determination of scavenging activity using FRAP

The ferric reducing power of the samples was determined according to the method of Olaoluwa *et al.* (2024). Experimental sample was dissolved in 0.2 M phosphate buffer, pH 6.6; an aliquot (250 µL) was mixed with 250 µL of 1% potassium ferricyanide solution. The mixture was thoroughly mixed using a vortex machine and heated at 50°C for 20 minutes. After incubation, 250 µL of 10% trichloroacetic acid (TCA) was added followed by 50 µL o f 0.1% ferric chloride dissolved in double distilled water and then 200 µL of distilled water was added. The solution was allowed to stand for 10 minutes at room temperature, after which it was centrifuged at 1000 * g for 10 minutes. An aliquot (200 µL) of the 96-well plate and the absorbance was measured at 700 nm.

Determination L- Dopamine

About 0.5 g of samples was weighed into 100 ml beaker; 9.5 ml of 0.1N HCl was added using 10 ml measuring cylinder. The mixtures were transferred to a blender and blended for 1 minute. 1 ml aliquots of these resulting suspensions were transferred into 30 ml test tubes and 9 ml of carbon suspension were added (The carbon suspension was made by dissolving 1.5 g of activated carbon (Norit A) in 1 litre of 0.1N HCl using a magnetic stirrer). The mixtures were agitated for 15 minutes on a boiling water bath and cooled. The cooled solutions were then filtered into 100 ml conical flasks, 3 ml of EDTA

diluent were added, and were followed by addition of 5 ml of iron II reagents (This was made by dissolving 2 g $FeCl_3.6H_2O$ in 500 ml 0.05N – HCl). The mixtures were allowed to stand for 5 mins to develop colour before reading on the spectrophotometer. Standard Levo Dopa (3,4-dihydroxyphenylalanine) solution of range 5 to 20 ppm were prepared from 100 ppm stock levo Dopa (3,4-dihydroxyphenylalanine) solution (Cabiochem Grade A) in 0.1NHCl to obtain a gradient factor. The absorbance of extracted samples as well as standard levo Dopa solution of range 5 ppm to 20 ppm were read at a wavelength 530 nm on a Spectronic 21D Spectrophotometer. The % L-Dopa was calculated using the formula:

$$\% \text{ L} - \text{Dopa} = \frac{\text{Absorbance of sample x Gradient factor x } 0.72}{\text{Weight of sample x } 1000}$$

Statistical analysis

Data generated from this study were analyzed using Analysis of Variance (ANOVA). Values were expressed as mean ± standard error of mean (SEM) from three determinations. Differences in mean were compared using Duncan multiple test range. P<0.05 was considered significant (Iwe, 2002).

RESULTS AND DISCUSSION

Table 1 shows the result of phytochemical properties of flour blends. Total phenolic samples varied from 41.22 to 383.09 mg GAE/100 g with sample CSA (control) having the lowest value (41.22 mg GAE/100 g) and CSD having the highest value (383.09 mg GAE/100 g). The result showed significant ($p < 0.05$) increase in the value of total phenolic as the addition of velvet beans increases. The differences in the present study might be due to the different formulation used, age of raw materials, environment, soil type, and climate (Ram *et al*., 2013; Guevara-Figueroa *et al*., 2010) and results were in agreement with those of Guevara-Figueroa *et al*. (2010) for commercial varieties of nopal (Blanco and Manso) with 5.25 and 11.7 mg GAE/g.

Total flavonoids are group of natural substances with variable phenolic structures which contain anti-carcinogenic properties; they also displayed antioxidant activities, free radical scavenging properties, heart disease prevention and exhibit potentials for anti-immunodeficiency virus (Vu *et al*., 2017). Flavonoids content values ranged from 42.94 - 83.11 (mg CE/100 g). Sample CSD had the lowest value (42.94 mg/100 g) while the highest value (83.11 (mg CE/100 g) was observed in sample CSC. There were significant differences between the flavonoids contents of the composite flours at 95% confidence level with values ranging from 42.94 - 83.11 mg/100 g. As the percentage of plantain and velvet beans flours increased, so did the flavonoids increase. Findings here are not in consonance with the values (0.23 – 0.89 mg CE/100 g) for wheat-pearl millet-*Andrographis paniculata* leaf flour blends by Ishola *et al.* (2022). The high flavonoids contents of the composite flours in this work could offer anti-inflammatory, anti-cancer and anti-hypertensive potentials (Arukwe *et al.*, 2012).

The mean results for the total carotenoid varied from 0.41 to 1.66 mg/100 g with sample CSA (control) had the lowest value (0.41 mg/100 g) while sample CSC (210 g wheat flour + 60 g plantain flour + 30 g velvet bean) had the highest value (1.66 mg/100 g). The result showed significant differences between the samples ($p<0.05$). There were increments in total carotenoids of the composites with increased level of plantain-velvet bean flours substitution. The reports in this study are slightly in agreement with the values (0.80 – 1.27 mg/100 g) reported by Sodipo *et al.* (2020) for provitamin-A-biofortified maize-germinated lentil seeds complementary diets but lower than the findings of Barakat and Ghazal (2016) for *Moringa oleifera* seeds (13.53 – 19.56 mg/100 g).

The Levodopa or L-3,4 dihydroxyphenylalanine (L-Dopa) content of the composite flour samples ranged between (0.91 – 1.21%) with CSD (150 g wheat flour, 105 g plantain flour and 45 g velvet bean flour) having the highest value (1.21%) while the least value (0.91%) was observed in CSA (300 g wheat flour). There were significant differences ($p < 0.05$) between the L-Dopa of the composite flours. The result showed significant ($p < .05$) increase in L-Dopa of the composite flours with increase in level of velvet bean flour substitution. This is due to the commendable amount of L-Dopa (72.5 kg/g DM) found in velvet bean. L-DOPA is the precursor of dopamine, noradrenaline and adrenaline, neurotrans-mitters; hence, its presence in the composite flours of this study would help in treatment of diseases with deficit in neuronal cholinergic transmission, such as Parkinson's disease. The viability of L-Dopa in *Mucuna pruriens* for treatment of parkinson's disease has further contributed to its commercialization especially as powder or capsule (Altemimi *et al.*, 2017). The findings of this study are lower than the values (3.75 – 4.36%) reported for raw local velvet bean varieties by Nyirenda *et al.* (2003). Variation in values could be attributed to the inclusion of wheat and plantain flour which might have reduced the L-Dopa content of the composite flours.

DPPH (2, 2-diphenyl2-picrylhydrazyl hydrate) is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Tiruneh *et al.*, 2018). The DPPH (2, 2-diphenyl2-picrylhydrazyl hydrate) scavenging activity of the composite flours ranged between 33.12 – 46.85 (mg/AAE/100 g). There were significant differences ($p <$ j0.05) between the DPPH of the composites. CSD (150 g wheat flour, 105 g plantain flour and 45 g velvet bean flour) had the highest DPPH (46.85 mg/AAE/100 g) while the lowest DPPH was observed in CSA (300 g wheat flour). Values of DPPH in the composite flours gradually

Table 1. Result of phytochemicals in the composite flour blends.

Sample	CSA	CSB	CSC	CSD
Total phenolic (mg/GAE/100 g)	41.22±0.36[a]	190.93±0.11[b]	329.15±0.47[c]	383.09±0.28[d]
Total flavonoid (mg/CE/100 g)	60.07±0.18[b]	76.70±0.58[c]	83.11±0.02[d]	42.94±0.08[a]
Total carotenoid (mg/100 g)	0.41±0.01[a]	0.49±0.00[b]	1.66±0.03[d]	1.13±0.00[c]
L-Dopa (%)	0.91±0.03[a]	1.00±0.03[b]	1.07±0.02[c]	1.21±0.02[d]
DPPH (mg/AAE/100 g)	33.12±0.31	38.91±0.13	42.71±0.05	46.85±0.15
FRAP (mg $FeSO_4$/100 g)	4.05±0.02	5.34±02	5.93±0.05	6.29±0.03

Values are means ± standard deviation of measurements. Different letter in the same column indicates significant different ($p<0.05$). **Key:** Sample CSA = 300 g wheat flour; Sample CSB = 240 g wheat flour + 37.5 g Plantain flour + 22.5 g velvet bean; Sample CSC = 210 g wheat flour + 60 g Plantain flour + 30 g velvet bean; Sample CSD = 150 g wheat flour + 105 g Plantain flour + 45 g velvet bean.

increased with increased level of plantain-velvet bean flour supplementation. The reports of this study are higher than the findings of Antonic *et al.* (2021) for waffle with added grape flour (2.91 – 26.65 mg AAE/100 g) but lower than those obtained for DPPH of wheat-tacca composite flour biscuits (59.90 – 78.40 mg AAE/100 g) by Ojewumi *et al.* (2021).

The FRAP (ferric reducing antioxidant power), the ability to reduce Fe^{3+} exhibited by the composite flours, ranged from 4.05 to 6.29 mg/$FeSO_4$/100 g. Increase in the level of supplementation significantly ($p < 0.05$) improved the FRAP ability of the composite flours. CSD (150 g wheat flour, 105 g plantain flour and 45 g velvet bean flour) exhibited the highest level of the highest Fe^{3+} reduction power while the least Fe^{3+} was observed in CSA (300g wheat flour). The result showed that Fe^{3+} reducing abilities were in dose dependent manners which increased with increase in the substitution of velvet bean-plantain flour with wheat flour. The findings in this study corroborate the report of Ojewumi *et al.* (2021) for wheat-enzymatically modified tacca flour biscuit (3.03 – 7.46 mg/$FeSO_4$/100 g) but lower than the reports (6.27 – 30.35 mg/$FeSO_4$/100 g) of Ishola *et al.* (2022) for FRAP of wheat-pearl millet-*Andrographis paniculata* leaf flour. The higher FRAP abilities of composites in this study indicate their better capabilities in donating electron and free radicals to form stable substances, thereby interrupting the free radical chain reactions. The ferric reducing power is considered a defense mechanism which is related to the ability of the antioxidant agents to transfer electron or hydrogen atom to oxidants or free radicals (Ogunmoyole *et al*., 2009).

Conclusion

The results of this study show that addition of the blends of plantain-velvet beans flours significantly improved the phytochemical composition of the final flour blends. The study herein as demonstrated a great potential for its use for the production of functional foods. The flour blends are excellent source of phytochemicals, a future prospect for pharma-food industries. Food applications of these flour blends and storability of its products are recommended.

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