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Full Length Research Paper

Chemical Composition, antioxidant activity, functional properties and inhibitory action of unripe plantain (M. *Paradisiacae*) flour

Eleazu, Chinedum[1]. O., Okafor, Polycarp, N[2]., Amajor, John[2], Awa, Ezinne[1]., Ikpeama, Ahamefula. I[1]. and Eleazu, Kate. C.[1]

[1]Biochemistry Department, National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria.
[2]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

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In this study, an evaluation of the chemical composition, antioxidant activity, functional properties and inhibitory action of the extract of unripe plantain flour on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was carried out. Chemical analysis of the flour showed that it contained significant quantities of dry matter (48.00 ± 3.96%) and starch (31.10 ± 0.44%) but was low in phenol (1.42 ± 0.03%), protein (3.15 ± 0.042%), ash (5.50 ± 0.42%) and total soluble sugar (0.64 ± 0.001%) ($p < 0.05$). The antioxidant activity of the extract as determined by the quantities of peroxidase (52 ± 0.00%) and reducing power tests indicated that the flour had a strong antioxidant activity while the inhibitory capacities of the extract and quercetin on DPPH radical were 78.57% and 5.32ug/ml respectively. Analysis of the phytochemical compositions of the using the Association of Official Analytical Chemists (AOAC) and the gravimetric method of Harbone showed that it contained 1.58 ± 0.04% tannin, 1.82 ± 0.05% saponin, 1.37 ± 0.05% alkaloid and 0.98 ± 0.00% flavonoid. The unripe plantain flour was found to have good functional properties in addition as indicated by the values obtained for water solubility index (3.38 ± 0.007%), water absorption capacity (1.25 ± 0.35g/g) and bulk density (0.94 ± 0.014gml^{-1}).These findings suggest that unripe plantain could serve as a good source of natural antioxidants with free radical scavenging activity. In addition, it could have a wider utility in alcohol production, food and sugar industries and as a drug binder and disintegrant in pharmaceuticals.

Key words: Antioxidant Activity, Chemical Composition, Functional Properties, Plantain flour, Reducing power, Percentage inhibition.

INTRODUCTION

Plantain (*M. paradisiacae*) is a staple food crop in West Africa where its starchy fruits are generally cooked or fried before consumption. During unripe plantain ripening, the starch is converted to reducing sugars and sucrose (Lii et al., 1982).

The medicinal value of plants 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 (Akinmoladun et al., 2007., Ahenkora et al., 1998). 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) As these compounds are predominantly found in fruit tissues, it would be worthwhile investigating the nature of polyphenols that are present in them. In addition, 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

*Corresponding author. E-mail: eleazon@yahoo.com.

Table 1. Physicochemical properties of unripe plantain flour.

Parameter	Percentage composition
Dry matter	48.00 ± 3.960
Moisture content	58.98 ± 0.22
Starch	31.10 ± 0.44
Phenol	1.42 ± 0.030
Protein	3.15 ± 0.042
Ash	5.50 ± 0.42
Total soluble sugar	0.64 ± 0.001

Values in the table are the means ± standard deviations of triplicate experiments.

estimate of its therapeutic potential (Akinmoladun et al., 2007).

Since unripe plantain flour is used by the traditional medical practioners in Nigeria in the dietary management of diabetes mellitus and other disease conditions (Eleazu et al., 2010) and being that free radicals have been associated with some of these disorders, we therefore decided to investigate the chemical composition, antioxidant activity and functional properties of unripe plantain flour.

MATERIALS AND METHODS

Sodium dihydrogen orthophosphate (NaH_2PO_4), Disodium hydrogen orthophosphate (Na_2HPO_4), Phenol, Quercetin and DPPH used were products of Sigma Chemical Company (UK) used were products of Sigma Chemical Company (Uk). Peroxidase used was purchased from Horseradish. All other chemicals used were purchased from Associated Laboratories, Aba, Abia State, Nigeria. The unripe plantain used was bought locally from the market in Umuahia, Abia State, Nigeria. It was thoroughly washed, peeled and freeze dried in a freezer for 48 h.

Preparation of plant materials for analysis

The peeled portion of the unripe plantain was ground into flour using a food processor and the flour was then used for the analysis.

Physicochemical composition of unripe plantain flours

The AOCA methods (1990) were used in the determination of the dry matter, moisture, starch, phenol, crude protein and ash contents of the unripe plantain flour.

Determination of total soluble sugars

The phenol-sulfuric acid method (Dubois *et al*., 1979) as modified by Eric (2001) was used in the determination of the total soluble sugar content of the flour. Briefly, 5ul of aqueous extract of the flour (which was obtained by dissolving 0.1g of flour in 100mls water and centrifuging at 3000 x g for 10mins since cellulose reacts with the assay) were added to 500ul (4%) phenol + 2.5ml (96%) sulfuric acid . The resulting solution was further re-diluted in 1ml of water and the OD read spectrophotometrically at 490nm against a reagent blank which contained 500ul (4%) of phenol and 2.5ml (96%) of sulfuric acid. The concentration of the sample was extrapolated from a standard curve obtained by serially diluting 1mg/ml glucose standard to varying concentrations (0.05 - 0.5ug/ml) in 0.05 increments which was further re-diluted with 1ml of water each and results results were expressed in percentage.

Phytochemical composition of unripe plantain flour

The gravimetric method of Harbone (1967) was used in the determination of the total alkaloid content, while the AOAC method (1984) was used in the determination of other phytochemical constituents of the sample.

Assay of DPPH radical scavenging activity

The free radical scavenging activity of the plantain extract was determined using the modified method of Blois (1985). 1 µl of different concentrations (500, 250,125, 62.5 and 31.25 µg/ml) of extracts and standard quercetin was added to 1 ml of 0.3 mm DPPH in methanol to bring the final concentration to 250, 125, 31.25 and 15.62 µg/ml. The mixture was vortexed and incubated in a dark chamber for 30 min and the absorbance was read at 517 nm against a DPPH control which contained 1 ml of methanol. The percentage inhibition was calculated as:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control - absorbance of sample}}{\text{Absorbance of control}} \times \frac{100}{1}$$

Assay of total antioxidant activity

The total antioxidant activity was measured according to the method described by Hsu et al. (2003). 0.2 ml of peroxidase + 0.2 ml of H_2O_2 (50 µm) + 0.2 ml ABTS (100 µm) + 1 ml distilled water were mixed together and left in the dark to form a bluish green complex.

After adding 1 ml of the methanolic plantain flour extract, the absorbance was measured at 734 nm to represent the total antioxidant activity.

Determination of reducing power

The reducing property of the unripe plantain flour was determined by assessing the ability of the sample extract to reduce $FeCl_3$ solution as described by Ademiluyi and Oboh (2008) with modifications. Briefly, 2g of the unripe plantain flour was dissolved in 40mls of methanol and filtered with Whatmann No1 filter paper. The resulting solution was made up to 50mls with methanol. Appropriate dilutions 1.25- 6.25ml (in 1.25ml increments in duplicates) of unripe plantain extract (corresponding to concentrations 2, 4, 6, 8, 10mg/ml) were mixed with 0.5ml (instead of 2.5ml) phosphate buffer(0.2M, pH 6.6) and 2.5ml potassium ferricyanide (1% v/w) in a test tube and reacted for 20mins at 50^oc. The tubes were cooled immediately using crushed ice and 0.5ml (10%) Trichloroacetic acid (instead of 2.5ml) was added and subsequently centrifuged at 3000g for 10mins. After centrifugation, 1ml of the supernatant was mixed with 1ml of distilled water and 0.1ml of (0.1%) ferric chloride and reacted for 10mins. The absorbance was taken at 700nm against a reagent blank using a spectrophotometer. Increased absorbance reading indicated increased reducing power.

Functional properties

The method of Wang and Kinsella (1976) was used in bulk density determinations while the method of Anderson and Sefa-Dedeh (2001) was used in the determination of water absorption capacity and water solubility index.

Statistical analysis

Statistical analysis was conducted using the means ± STD of three experiments. Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

In the study carried out, the physicho-chemical composition of the locally unripe plantain flour showed that it contained significant quantities of dry matter (48.00 ± 3.96) (Table 1). The dry matter content of plantain fruit relates to good cooking quality (Vuylsteke *et al*.,1997). Higher dry matter contents suggests better cooking qualities and extended storage lives.

Because of the limited supply of fossil fuels and other possible difficulties like political, technical and security problems, all countries in the world have been trying to explore possible energy resources. Alcohol, one of the many found substitute energy resources is especially attractive because starch or sugary biomass can produce it. Starch on its own, also has a wide application in both food and sugar industries (Amajor *et al*., 2011). The high content of starch obtained from the processed flours of the unripe plantain as represented in Table 1 is a significant finding in this study as it shows the wider utility of this unripe plantain flour in alcohol production on one hand and its application in both food and sugar industries on the other hand.

In recent years, phenolics have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals, the formation of which is associated with the normal metabolism of aerobic cells (Oboh and Rocha, 2007). That the phenolic compounds present in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals has been reported (Kirkosyan *et al*., 2003). The low phenolic content of the unripe plantain flour as shown in Table1 is another significant finding in this present study as it suggests that the antioxidant activity of the unripe plantain flour could have been as a result of other Phytochemical constituents of the flour.

The low crude protein content obtained in the plantain flour as represented in Table 1 are in accordance with previous studies (Brakohiapa *et al*., 2001., Agunbiade et al., 2006).

Since a healthy adult needs about 0.75g of protein per kg per day, unripe plantain diet alone cannot meet adult protein diet need.

The unripe plantain flour was found to contain low quantities of ash which reflected its mineral contents (Table1). Plantains have been reported to contain low quantities of minerals (Ketiku, 1973., Ahenkora, 1998).

Oligosaccharides are hydrolyzed by concentrated sulfuric acid during the phenol–sulfuric assay and form monomers, namely glucose, fructose and galactose which are reducing sugars. Reducing sugar refers to any sugar that has an aldehyde group or is capable of forming one in solution through isomerisation (Anderson and Sefa-Dedeh, 2001). Glucose, mannose and lactose are reducing sugars. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus. Although the mechanisms underlying the alterations associated with diabetes mellitus are presently not well understood, hyperglycemic levels lead patients to an increased oxidative stress (Kaneto *et al*., 1996) because the production of several reducing sugars (through glycolysis and polyol pathways) is enhanced. These reducing sugars can easily react with lipids and proteins (non-enzymatic glycation reaction), increasing the production of reactive oxygen species (ROS). The low sugar content of the unripe plantain flour used suggests that it could serve the purposes for which its been used in diabetics.

Table 2. Phytochemical composition of unripe plantain flour.

Phytochemical	Percentage composition
Tannin	1.577±0.004
Alkaloid	1.37±0.048
Saponin	1.827±0.0042
Flavonoid	0.981± 0.0014

The values in the table were obtained by calculating the average of 3 experiments ± standard deviation.

Table 3. Total antioxidant activity of unripe plantain flour/percentage inhibition on DPPH radical.

Antioxidant/DPPH	Activity/inhibition (%)
Peroxidase	52
DPPH Radical	78

Each value in the table was derived by calculating the average of three experiments ± standard deviation.

Table 4. Functional properties of unripe plantain flour.

Parameter	Composition
Water solubility index (%)	3.38 ± 0.007
Water absorption capacity (g/g)	1.25 ± 0.35
Bulk density (gml^{-1})	0.94 ± 0.014

Values in the table are the average of three experiments ± standard deviation.

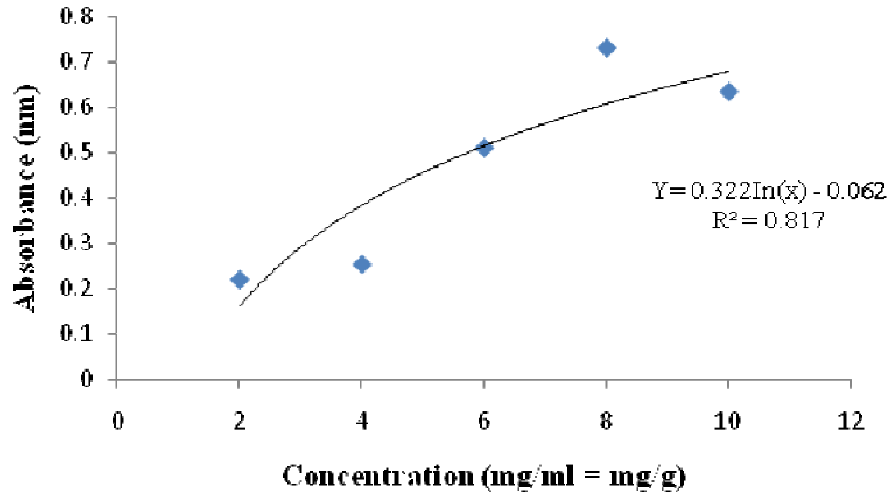

Figure 1: Reducing power of unripe plantain flour. Increased absorbance reading indicates increased reducing power.

Phytochemical composition of unripe plantain flour

The phytochemical composition of the unripe plantain flour showed that it contained significant quantities of saponins, flavonoids, alkaloids and tannins (Table 2).

Saponins are known to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic permeabilization of the intestine and paralysis of the sensory system) properties (Price *et al*., 1987). However, the levels of saponin in the flour are quite too low to cause any deleterious effects.

Flavonoids, alkaloids and tannins are polyphenolic compounds with antioxidant properties. In addition, phenolic compounds existing in plants are also responsible for their contribution to color, sensory and antioxidant properties of food (Robbins, 2003).

The present study shows that unripe plantain flour contains low quantities of phenolics.

Inhibitory activity of unripe plantain flour

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Liu *et al*., 2008).Substances with IC_{50} values lower than 20ug/ml are considered to possess high antioxidant activities. Higher IC_{50} values denote lower antioxidant activities .The high scavenging activity of the extract of unripe plantain flour as determined is another significant finding in this study (Table 3). This could be attributable to the phytochemical and phenolic constituents of the flour though present in low levels.

Total antioxidant activity of unripe plantain flour

The extract of unripe plantain flour was found to have a high antioxidant activity as seen from the value obtained for peroxidase (Table 3). This could be attributable to the presence of phenols and phytochemicals (which are high potency antioxidants with free radical scavenging activities) in the unripe plantain flour though their levels were low. These results obtained show that unripe plantain flour is a potential source of natural antioxidants and could be of medicinal purposes in treatment of ailments implicating reactive oxygen species and oxidative stress.

Reducing power tests

Reducing power is a measure of the ability of the extracts to reduce Fe^{3+} to $Fe^{2+.}$ Substances which have reduction potential react with potassium ferri-cyanide ($Fe^{3+)}$ to form potassium ferro-cyanide (Fe^{2+}) which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm. Reducing power has become one of the antioxidant capability indicators of medicinal plants (Duh and Ten, 1997) as it may accord with overall antioxidant activity. This is because antioxidants are strong reducing agents and this is principally because of the redox properties of their hydroxyl groups and the structural relationships of any parts of their chemical structure (Oboh and Rocha, 2007; Eleazu et al., 2011). Benzie and Strain (1999) also. considered antioxidants as any specie that reduces the oxidizing species that would otherwise damage the substrates. Results obtained in Figure 1 indicate that the flour has a strong antioxidant activityFunctional properties are those characteristics that govern the behavior of nutrients in food during processing, storage and preparation as they affect food quality and acceptability (Onwuka, 2005). Some important functional properties that influence the utility of certain foods are water solubility index, water absorption capacity , bulk density, etc.

Water solubility index measures the amount of free molecules leached out from the starch granule in addition to excess water and thus reflects the extent of starch degradation (Onwuka, 2005). Results obtained in Table 4 indicate high starch degradation in the unripe plantain flour.

Water Absorption Capacity measures the volume occupied by the starch granule after swelling in excess of water and results obtained indicate good water absorption capacity.

Bulk density is a function of particle size while particle size is inversely proportional to bulk density. The relatively high bulk density of the unripe plantain flour as observed in table 3 is another significant finding in this study as it suggests its suitability as a drug binder and disintegrant in pharmaceuticals.

Conclusion

Unripe plantain contains significant quantities of dry matter but low in ash, phenol, reducing sugar and protein which has been demonstrated in this study. It was found to be a good source of natural antioxidants with free radical scavenging activity and this could be used for treating diseases implicating free radicals. Its good functional properties and starch contents indicate the wider utility of the flour in alcohol production, food and sugar industries, and as a drug binder and disintegrant in pharmaceuticals.

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