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TOTAL ANTIOXIDANT CAPACITY, NUTRIENT COMPOSITION, MICROBIAL LOAD AND PERCENTAGE INHIBITORY ACTIVITY OF UNRIPE PLANTAIN FLOUR

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ABSTRACT

In the present study, an evaluation of the total antioxidant capacity, nutrient composition, microbial load and percentage inhibitory activity of methanolic extract of unripe plantain flour on DPPH radical was carried out. The total antioxidant capacity of the extract as determined by the quantities of quercetin and peroxidase present was 0.532ug/ml and 52% ± 0.00 while the percentage inhibition on DPPH radical was 78.57%. Analysis of the proximate and phytochemical compositions of the flour using the AOAC methods showed that it contained 3.16 ± 0.04% protein, 0.21 ± 0.003% lipid, 5.2 ± 2.82% moisture, 5.5 ± 0.42% ash, 1.58 ± 0.04% tannin,1.82 ± 0.05% saponin, 1.37 ± 0.05% alkaloid and 0.98 ± 0.00% flavonoid. Further microbial analysis carried out revealed that the flour had good viable and fungal counts in addition as indicated by the low concentrations of *S. Aureus* and *R. Stolonifer* observed in the processed flour though the fungal counts was higher than the bacterial counts. These findings suggest that unripe plantain flour could serve as a natural source of antioxidants with free radical scavenging activity and its shelf life could be extended if packaged well and stored.

Keywords: Total antioxidant capacity, nutrient composition, microbial load, percentage inhibition, unripe plantain flour.

INTRODUCTION

Food processing is probably the most important source of income and employment in Africa, Asia and Latin America. The Food and Agriculture Organization of the United Nations has stated that value added through marketing and processing of raw materials can be much greater than the value of primary production (Anonmous, 1995). Some micro-organisms produce chemicals that color, flavor and stabilize foods, thereby increasing their storage lives (Ogunjobi *et al.*, 2005). These types of foods are important because of their increased nutritional values as well as improved flavor and aroma characteristics.

For a long period of time, plants have been a reliable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies (Kumar *et al*., 2005; Pourmorad *et al.,* 2006). The World Health Organization has recommended that this should be encouraged especially in areas where access to conventional treatment is not adequate (WHO, 1980).

Fruits and vegetables are good sources of proximates and phytochemicals such as carotenoids, flavonoids and other phenolic compounds. Studies have indicated that these phytochemicals especially polyphenols have high free radical scavenging activity, which helps to reduce the risk of chronic diseases such as cardiovascular disease, cancer, etc (Ames *et al*., 1993). In addition, phytochemicals also act as potent antioxidants in both fat soluble and water soluble body fluids and cellular components (Mathur and Mathur, 2001) and also posses biological characteristics like anti-carcinogenicity, anti-mutagenecity, anti-aging activity and anti-cholesterol activity.

Unripe plantain is a plant that is well known to the traditional medical practioners in Nigeria. It's used in the treatment of diabetic conditions and other related ailments in addition to its nutritive components.

Since free radicals have been associated with some of these disorders and being that the phytochemicals present in plants are known to possess anti-oxidative or free radical scavenging activity, the antioxidant and nutrient composition of this plant ought to be investigated.

This thus leads to the basis of this research which is designed to understudy the total antioxidant capacity, nutrient composition, microbial load and percentage inhibitory activity of unripe plantain flour.

MATERIALS AND METHODS

Chemicals

Quercetin and DPPH (2, 2 – diphenyl - 1- picrylhydrazyl) used were products of Sigma Chemical Company (UK). Peroxidase used was purchased from Horseradish. All

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other chemicals used were purchased from Associated Laboratories, Aba, Abia State, Nigeria.

Plant Materials
Unripe plantain used was bought locally from the market in Umuahia, Abia State, Nigeria. It was thoroughly washed, peeled, sliced and oven dried for 24hours at a temperature of 50^{o}C.

Preparation of Plant Materials for Analysis
The peeled portion of the unripe plantain was processed into flour using a food processor and the flour was then used for analysis.

Proximate Composition of Unripe Plantain Flour
Moisture, crude protein, crude fat and total carbohydrates were analyzed according to the AOAC (1990) methods. The values reported are means of triplicate samples with their standard deviations.

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 (1984) method 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). 1ml of different concentrations (500, 250, 125, 62.5, 31.25ug/ml) of extracts and standard quercetin was added to 1ml of 0.3mm DPPH in methanol to bring the final concentration of 250, 125, 31.25, and 15.62ug/ml. The mixture was vortexed and incubated in a dark chamber for 30minutes and the absorbance read at 517nm against a DPPH control which contained 1ml 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). 0.2ml of peroxidase + 0.2ml of H_2O_2 (50um) + 0.2ml ABTS (100um) + 1ml distilled water were mixed together and left in the dark to form a bluish green complex.

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

Microbial Analysis
The total viable counts and fungal counts of the flour were determined using the Pour plate technique (Ezeama, 2007). 5g of the flour was blended in 45ml of 0.1% peptone water (PH 7.2 ± 0.2) to obtain a dilution 1:10, from which subsequent dilutions were made and appropriate aliquot used to determine the total viable counts (TVC) on Trypton Soya Agar and Potato Dextrose Agar for total fungal count (TFC). After aerobic incubation at 35 – 37^{o}C for 18-24hours for TVC and room temperature for 2-5days for TFC, the colonies were observed and counted and results expressed as Colony forming unit per gram (Cfug $^{-1}$). Colonies were purified by sub-culturing on fresh Tryptophon Soya Agar and grams stained for morphological examination. Further biochemical tests were done for characterization and identification of the isolates. The colonies of the fungi emerging within 2 - 5days of inoculation were identified under the light microscope (x 40) and recorded. Each experiment was performed in triplicates.

Statistical Analysis
Statistical analysis was conducted using the means ± std of 3 experiments. Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

In the study carried out, the proximate composition of the locally consumed unripe plantain flour showed that it contained low quantities of ash which reflected the mineral contents of the plantain (Table1). Plantains have been reported to contain low quantities of minerals (Ketiku, 1973).

The low fat contents obtained in the unripe plantain flours were in accordance with previous reports (Agunbiade *et al.,* 2006). The low crude protein content obtained in the plantain flours were also in accordance with previous studies (Brakohiapa *et al.,* 2001). Since a healthy adult needs about 0.75g of protein per kg per day, plantains alone cannot meet adult protein diet.

The low total carbohydrate obtained in the unripe plantain flour would be expected since unripe plantain contains large amount of starch and low sugar in its green stage (Table 1). Similar results have been reported (Ahenkora *et al.,* 1998). The moisture content was also found to be in agreement with earlier reports Ketiku (1973).

Table 1. Proximate Composition of Unripe Plantain Flour.

Proximate Analyzed	Percentage Composition
Ash	5.50 ± 0.420
Carbohydrate	39.14 ± 0.212
Protein	3.15 ± 0.042
Lipid	0.21 ± 0.028
Moisture	5.2 ± 2.800

Values in the table were obtained by calculating the mean ± std of 3 experiments.

Phytochemical Composition of Unripe Plantain Flour
The phytochemical composition of unripe plantain flour showed that it contained some quantities of flavonoids, saponins and alkaloids (Table 2). Saponins are known to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic permeabilization of the intestine) 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. Phenolics have been associated with antioxidant properties of food (Robbins, 2003). It has also been reported that phenolic compounds in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals (Kirakosyan *et al.*, 2003).

The present study results show that unripe plantain flour contains considerable amount of phenolics and this implies that it may be useful in relation to diseases involving free radical reactions.

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

Each value in the table is the average of triplicate experiments ± standard deviation.

Inhibitory Activity of Unripe Plantain Flour
The high scavenging activity of the unripe plantain flour extract as observed is a major significant finding in this study (Table 3). This is attributable to the phenolic content and presence of other phytochemicals in the unripe plantain. However, we could not prove if the free radical scavenging activity came solely from the phenols present or other phytochemicals or a combination of both.

Antioxidant Activity of Unripe Plantain Flour
The high antioxidant activity of the methanolic extract of unripe plantain flour as obtained in table 3 is another significant finding in this study. This is thought to arise from the presence of phenolics and phytochemicals in the unripe plantain flour which are high potency antioxidants with free radical scavenging activities. These results obtained indicate the potentials in unripe plantain flour as a natural source of antioxidants and could be of medicinal purposes in treatment of ailments implicating free radicals.

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 3 experiments ± standard deviation.

Microbial Analysis
The Total Fungal Counts and Viable Counts of the processed unripe plantain flour as observed in table 4 were 1.6×10^2 and 3.6×10^3 colony forming units per gram ($cfug^{-1}$) respectively. The flour had higher bacterial counts than fungal counts. The processing techniques minimized contamination and oven drying helped to inhibit microbial proliferation. The micro-organisms isolated from the flour include *Staphylococc aureus* and *Rhizopus stolonifer.* Although the organisms are potential pathogenic organisms, the counts recorded are quite low to cause any health hazards. Our results obtained are consistent with FAO (2003) and this implies that the flour analyzed are quite safe for consumption and their shelf life could be extended if packaged well and stored.

Table 4. Total Viable Counts and Total Fungal Counts of Unripe Plantain Flour.

Sample	Total Viable Counts ($cfug^{-1}$)	Total Fungal Counts ($cfug^{-1}$)
Unripe Plantain Flour	3.6×10^3	1.6×10^2

Results under the table are the means of triplicate experiments and are in coliform units per gram ($cfug^{-1}$). Values are significant at $P < 0.05$.

In conclusion, that unripe plantain flour is a good source of antioxidants with free radical scavenging activity has been demonstrated in this study. Although it was found to contain *S. aureus* and *R. stolonifer* which are potential pathogenic organisms, however their concentrations are quite too small to cause any health hazards indicating good microbial load for the flour and this implies that its shelf life could be extended if packaged well and stored.

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