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EFFECT OF POSTHARVEST TREATMENT OF PLANTAIN (*MUSA PARADISIACA*) WITH ALOE VERA GEL COATING ON PHENOLIC CONTENT, ANTIOXIDANT AND ZINC BIOAVAILABILITY OF PLANTAIN FLOUR.

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ABSTRACT

The aim of this study was to investigate the effect of postharvest treatment of plantain (*Musa paradisiaca*) with aloe vera gel coating on phenolic content, antioxidant and zinc bioavailability of plantain flour. The plantains were divided into three parts of ten fingers each. The first part was uncoated which served as the control, the second part was coated with 50% aloe vera gel solution while the third part was coated with 100% aloe vera gel solution of which the coated samples were allowed to air dry and all stored at room temperature ($30^oC \pm 1^oC$) for 5 days. The total phenolic content of the flour of freshly harvested plantain was found to be 4.35 µg GAE/g, it decreased significantly ($P < 0.05$) in stored untreated plantain to 2.96 µg GAE/g while the total phenolic content of the flour of stored 100% aloe vera treated plantain (4.18 µg GAE/g) was not significantly ($P < 0.05$) different from that of the freshly harvested plantain. The flour of stored 100% aloe vera treated plantain had a significantly ($P < 0.05$) higher DPPH scavenging ability, metal chelating ability and reducing power than that of the flour of stored 50% aloe vera treated and untreated plantain at the tested concentrations but not significantly ($P < 0.05$) different from that of the flour of freshly harvested plantain. The calculated [Ca][Phytate]/[Zn] molar ratios of the flour of the treated and untreated stored plantain were below the critical levels. These results indicated that treating plantain with 100% aloe vera solution prior to storage will preserve its phenolic, antioxidant qualities and zinc bioavailability.

Keywords: Plantain flour, Aloe vera, phenolic, DPPH scavenging activity, Reducing power

INTRODUCTION

Plantain (*Musa paradisiaca* Linn) is a very important fruit of the tropics and sub tropics where both ripe and unripe fruits are eaten (Adetuyi and Ajala 2010). It ranked fourth after rice, wheat and maize in order of importance; plantains are used as beverages, fermentable sugars, medicines, flavourings and cooked foods (Adepoju *et al* 2012). The world total production of plantains as at 2014 stands at 30.7 million tonnes per year and of this Nigeria ranked 5th producing 3.04 million metric tonnes (FAOSAT 2017). This might be the reason why it is widely used as staple food in Nigeria. Plantain can be made into plantain flour, cooked into a paste known as "*Amala ogede"* (Yoruba), and "*Ebue*" (Ogonis) which is eaten with any soup. Half ripe or fully ripped plantain can be processed to plantain chips; it can also be boiled and eaten with soups, sauce or vegetables (Adepoju *et al* 2012; Tchango *et al*., 1999). Varieties of foods like bread, biscuits and instant flour has been made from plantain. It was observed that wheat bread substituted with 15% plantain flour has a nutritional qualities and sensory attributes that were comparable to that of whole wheat bread (Olaoye *et al*., 2006). The major threat to the availability of plantain from old ages is post-harvest loss, since they are highly perishable agricultural products and are subjected to fast

deterioration because of their high metabolic activities after harvest (Adepoju *et al* 2012; Adetuyi and Ajala 2010). Almost 90% of the total plantain produced worldwide is consumed locally in producing countries with only 10% for export. The colour of plantain has a lot to do with the assessment of its quality by the consumer than any other single index. Hence, peel and pulp colours of plantains serve as important postharvest selection criteria. In Ghana and Nigeria consumers have developed distinct correlations between colour and the overall quality of plantain (Adetuyi and Ajala 2010).

Edible coatings provide a partial barrier to moisture and gas loss on the surface of fresh produce during postharvest storage; this ultimately slow down metabolic activities like respiration, senescence and enzymatic oxidation thereby colour, texture and volatile compounds are preserved. Chitosan, *Aloe vera*, polyvinyl acetate, mineral oils and cellulose have been observed to serve this purpose (Mahajan *et al* 2014). *Aloe vera* is a tropical and subtropical plant, it has two major liquid sources one is yellow latex (exudates) and the other a clear gel (mucilage), and these comes from the large leaf parenchymatic cells. The interest in the use of A. vera gel as a functional ingredient in the food industry has increased recently (Ni *et al*., 2004; Garcia *et al*, 2014). Aloe vera gel coatings have been found to impart a glossy appearance and better color on fruits, it also retards weight loss and prolonged storage/shelf-life of fruits (Dang *et al*., 2008). This natural product would be an innovative and interesting means for commercial application since it is a safe and environmentally friendly alternative to the use of postharvest chemical treatments such as sulfur dioxide (Serrano *et al.,* 2006, Garcia *et al*, 2014). The main objective of this study is to treat plantain (*Musa paradisiaca*) by coating it with aloe vera gel, stored it for 5 days and evaluates the effect of this postharvest treatment on the changes in the phenolic content, antioxidant properties and zinc bioavailability quality of plantain flour.

MATERIALS AND METHODS

The freshly harvested matured green plantain (*Musa paradisiaca* Linn) and Aloe vera leaves were harvested from the Ondo State University of Science and Technology Okitipupa farm.

Preparation of aloe vera coating solution

After separating aloe vera gel from the outer cortex, the colorless hydroparenchyma obtained was homogenized. The fibres in the mixture were removed by filtration. The resultant liquid is the fresh aloe vera gel. The gel was then pasteurized at 70^{o}C for 45minutes. To maintain the gel pH at 4, ascorbic acid (2.0 g\l) and citric acid (4.5 g\l) were added (He *et al*., 2005). Then 50% and 100% aloe vera gel solution were prepared. 50% aloe vera gel solution was by dissolving 50ml of aloe vera gel in 50 ml of distilled water while 100% aloe vera gel solution was 100 ml aloe vera gel.

Experimental treatments and design

The plantains were divided into three parts of ten fingers each. One part was coated with 50% aloe vera gel solution by dipping it into 50% aloe vera gel solution for 10 minutes and allowed to air dried; another one was coated with 100% aloe vera gel solution by dipping it into 100% aloe vera gel solution for 10 minutes and allowed to air dried while the last one was left uncoated. They were all stored at room temperature (30^{o}C±1^{o}C) for 5 days. The single factor experiment (post-harvest treatments) was laid out in a completely randomized design (CRD) with three replications where the plantain flour was prepared from the plantain after 5 days of storage according to the method of Mepba, *et al* (2007) and the phenolic component, antioxidant and zinc bioavailability qualities of the plantain flour was assessed.

Extract preparation

One gram (1.0 g) of the flour was soaked in 10 ml of distilled water for 3 h and the mixture was shaken intermittently. The resulting mixture was filtered using a muslin cloth. The filtrate was used for the analysis immediately.

Total phenolic determination

The total phenolic content was determined by the spectrophotometric method of Kim *et al.* (2003). 1 ml of the sample (1 mg/ml) was mixed with 1 ml of Folin–Ciocalteu phenol reagent (1:15). After 5 min, 5 ml of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 6.5ml of distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 25^0C after which the absorbance was read at 750 nm. The total phenolic content was determined from Gallic acid standard calibration curve. The estimation of the total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of dried sample.

Reducing power

The reducing power was estimated using the method of (Oyaizu 1986). 1.0 ml (0.25-1.0 mg/ml) of the extract was added to 1ml phosphate buffer (pH 6.6) and then 1ml of potassium ferricyanide (10 mg/ml) was added. The mixture was incubated at 50^0C for 20min followed by the addition of 1ml of trichloroacetic acid (100 mg/ml). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml was mixed with 1 ml of distilled water and 0.2 ml of 0.1% fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power. Ascorbic acid was used as Standard.

Metal chelating activity

The chelation of ferrous ions is estimated using the method of Dinis *et al.*, (1994). 0.1 ml (20-100 µg/ml) of the extract was added to a solution of 0.5 ml ferrous chloride (2 mM), the reaction is started by the addition of 0.2 ml of ferrozine (5mM) and incubated at room temperature for 10 min and then the absorbance is measured at 560nm. EDTA was used as a positive control.

Metal chelating ability = [(Abs control – Abs sample)/ (Abs control)] × 100

DPPH scavenging activity

DPPH radical scavenging activity was estimated according to method of Gyamfi *et al*, (1999). 1.0 (0.25-1.0 mg/ml) of sample extracts were added to 4ml of DPPH (0.025g/l prepared in methanol solution). The sample were shaken and allowed to stand in the dark for 30mins and absorbance was measured at 520nm. Ascorbic acid and Quercetin were used as the standard.

DPPH scavenging ability = [(Abs control – Abs sample)/ (Abs control)] × 100

Zinc bioavailability

The phytate content was determined by the method of Maga, (1982), this depends on the ability of standard ferric chloride to precipitate phytate in dilute hydrochloric acid extract of the plantain flour. The mineral contents were determined using an atomic absorption spectrophotometer AAS (model 372).

The method of Ferguson *et al* (1988) was used for the calculation of phytate : zinc, calcium : phytate and [Ca] [phytate]/ [Zn] molar ratios and used for the Zn bioavailability prediction.

Analysis of data:

The results of the three replicates were pooled and expressed as mean ± standard deviation. Standard deviations were calculated using spread sheet soft-ware (Microsoft Excel©, version 2013). Analysis of variance (ANOVA) was performed using Statistical Analysis System proprietary software (SAS, 2002). Duncan's multiple range test procedure as described in the SAS software was used for mean separations. Significance was accepted at $P < 0.05$. The graph was drawn with graph pad 5.0.

RESULT

Total Phenolic

The total phenolic content of the flour of stored plantain was determined and reported as µg gallic acid equivalent (GAE) per g sample as shown in Table 1. The total phenolic content of the flour of freshly harvested plantain was found to be 4.35 µg GAE/g. The flour of the stored plantain exhibited total phenolic content ranging from 2.96 µg GAE/g untreated to 4.18 µg GAE/g 100% aloe vera treated sample. The total phenolic content of the flour of plantain decreased significantly in stored untreated

plantain 2.96 µg GAE/g when compared with the total phenolic content of the flour of freshly harvested plantain 4.35 µg GAE/g. The total phenolic content of the flour of stored 100% aloe vera treated plantain (4.18 µg GAE/g) was not significantly ($P < 0.05$) different from that of the freshly harvested plantain.

Table 1: Total Phenol, Phytate and mineral content of the flour of aloe vera treated and untreated stored plantain

		storage	For	5 days
	Freshly harvested	untreated	50% Aloe vera treatment	100% Aloe vera treatment
Total phenol (µg GAE/g)	4.35±0.13a	2.96±0.12b	2.54±0.11b	4.18±0.12a
Phytate (mg/100g)	1.2±0.11a	1.1±0.1b	1.1±0.1b	1.4±0.2a
calcium (mg/100g)	5.0±0.2a	4.1±0.1b	2.3±0.1d	3.0±0.1c
zinc (mg/100g)	0.1±0.0a	0.024±0.0b	0.03±0.0b	0.1±0.0a
iron (mg/100g)	15.2±1.4a	7.8±0.8c	14.3±1.1a	10.1±0.7b

Values were the mean ±SD of triplicate. Values with the same letter in a row are not significantly different ($P < 0.05$).

Reducing Power

The reducing power of the flour extracts in Figure 1 shows to be dose dependent as the concentration of extracts increases from 250 µg/ml to 1000 µg/ml. The flour of 100% aloe vera treated stored plantain exhibited a higher reducing power than the 50% aloe vera treated and untreated sample. The flour of 100% aloe vera treated stored plantain had a similar reducing power as that of the flour of freshly harvested plantain at higher doses.

Metal Chelating Ability

The ability of the extracts of the flour of aloe vera treated and untreated stored plantain to chelate Fe^{2+} ions was evaluated and presented in Figure 2. The extracts of the flour of 100% aloe vera treated stored plantain has the highest iron chelating ability at the observed concentration of extracts of 250 – 1000 which was not significantly different from that of freshly harvested sample µg/ml.

DPPH Radical Scavenging Ability

The DPPH radical- scavenging ability of the flour of aloe vera treated and untreated stored plantain extracts at 250 – 1000 µg/ml concentrations was measured, the result is presented in Figure 3. A dose-response relationship was observed in the DPPH radical-scavenging ability of the flour of aloe vera treated and untreated stored plantain extracts; the ability increased with an increase in the concentration of the extracts. The flour of 100% aloe vera treated stored plantain recorded the highest DPPH radical-scavenging ability which was significantly ($P < 0.05$) higher than the extract of the flour of 50% aloe vera treated and untreated stored plantain.

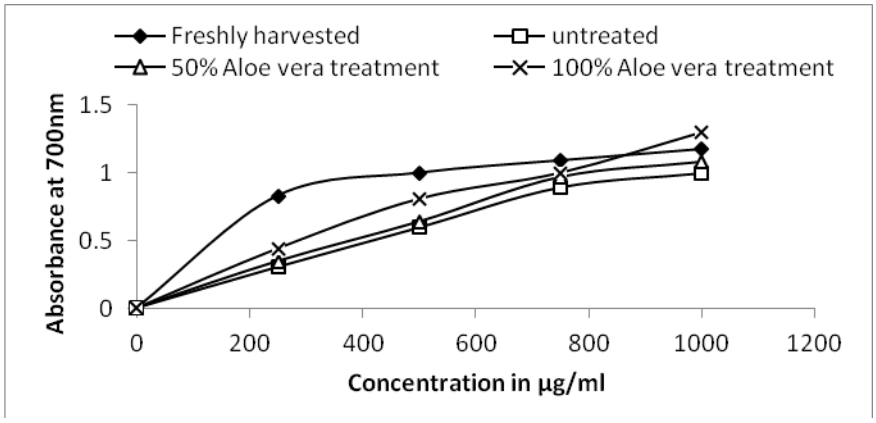

Figure 1: Reducing power of the flour of aloe vera treated and untreated stored plantain. Values represent mean ± standard deviation of triplicate determination.

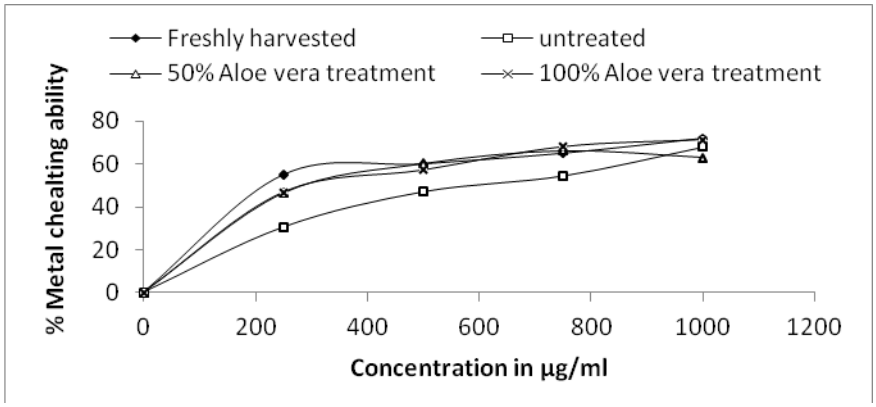

Figure 2: Metal chelating ability of the flour of aloe vera treated and Untreated stored plantain. Values represent mean ± standard deviation of triplicate determination.

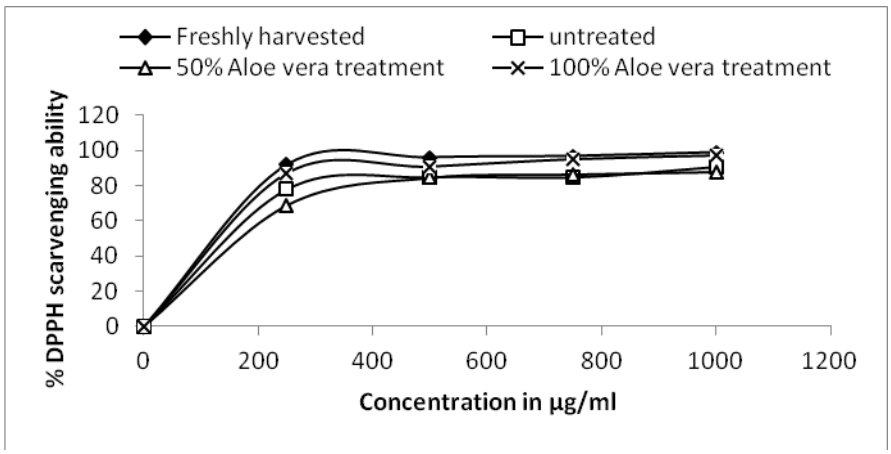

Figure 3: DPPH scavenging activity of the flour of aloe vera treated and untreated stored plantain. Values represent mean ± standard deviation of triplicate determination.

Zinc Bioavailability

The calculated zinc bioavailability of the flour of aloe vera treated and untreated stored plantain is presented in table 2. The phytate : zinc molar ratio of the flour of freshly harvested plantain was found to be 120 and that of the flour of treated and untreated stored plantain range from 140 (100% aloe vera treated plantain) to 530 (untreated plantain). The calculated calcium: phytate molar ratios of the flour of plantain ranges from 0.31 (50% aloe vera treated plantain) to 0.69 (freshly harvested plantain). The calculated [Ca][Phytate]/[Zn] molar ratio for the flour of freshly harvested plantain, 50% and 100% aloe vera treated stored plantain are 0.16 mol/kg, 015 mol/kg and 0.11 mol/kg respectively.

Table 2: Calculated phytate - zinc, calcium - phytate and [Ca][Phytate]/[Zn] molar ratios of the flour of aloe vera treated and untreated stored plantain.

	Storage for 5 days			
	Freshly harvested	untreated	50% Aloe vera treatment	100% Aloe vera treatment
Zn: Phytate	120	530	320	140
Ca: Phytate	0.69	0.62	0.31	0.35
[Ca][phytate] / [Zn]^{x}	0.15	0.53	0.16	0.11

Values represent mean ±standard deviation of triplicate determination. ^{x}mol/kg

DISCUSSION

Phenolic phytochemicals inhibit autoxidation of unsaturated lipids thus preventing the formation of oxidized low-density lipoprotein (LDL) which is considered to induce cardiovascular disease (Amic *et al.,* 2003). The total phenolic content of the flour of plantain decreased significantly in stored untreated plantain 2.96 µg GAE/g when compared with the total phenolic content of the flour of freshly harvested plantain 4.35 µg GAE/g. This reduction in the total phenolic content may be attributed to the reason that phenols are susceptible to oxidation reactions through the enzyme phenolase which turns them to quinines a product that are often extremely reactive and therefore short lived (Adetuyi *et al.,* 2008). The total phenolic content of the flour of stored 100% aloe vera treated plantain (4.18 µg GAE/g) was not significantly ($P < 0.05$) different from that of the freshly harvested plantain. This result showed that treatment of plantain with 100% aloe vera prior to storage has caused a significant reduction in the activity of the enzyme phenolase by preventing oxidation reaction.

One electron transfer (ET) and hydrogen atom transfer (HAT) made up the antioxidant capacity assays; HAT and ET assays measures the radical (or oxidant) scavenging capacity of samples and they do not look into the preventive antioxidant capacity of samples. ET assays are much easier than HAT assays because it involved colour changes as the oxidant is reduced (Phatak and Hendre, 2014; Huang *et al.*, 2005). ET assays of reducing power, metal chelating ability and 1,1-diphenyl 2 picrylhydrazyl (DPPH) radical scavenging ability were selected for the measurement of total antioxidant capacity in the flour of aloe vera treated and untreated stored plantain.

The reducing power of the flour of aloe vera treated and untreated stored plantain extracts was determined according to the ability of the extract to cause reduction in the transition metal Fe^{3+} through electron transfer to Fe^{2+} (Zarena and Sankar, 2009). The reducing power of the flour extracts showed to be dose dependent as the concentration of extracts increases from 250 µg/ml to 1000 µg/ml. The flour of 100% aloe vera treated stored plantain had a similar reducing power as that of the flour of freshly

harvested plantain at higher doses. This is expected because the phenolic content of the flour of 100% aloe vera treated stored plantain is not significantly different from that of the freshly harvested plantain. The reducing power ability is an indication that the antioxidant compounds are electron donors but this assay has a short coming of the fact that any electron-donor that has a lower redox potential when compared to the redox pair Fe^{3+}/Fe^{2+} may contribute to the reducing power value even if they do not have antioxidant properties and this will give a false high values (Tachakittirungrod, *et al.*, 2007; Nilsson *et al.*, 2005).

Bivalent transition metal ions served as catalysts of oxidative processes that can lead to hydroxyl radical formation and hydrogen peroxide decomposition reactions. This reaction can be prevented by iron chelation (Halliwell, 1997). The extracts of the flour of 100% aloe vera treated stored plantain showed to have the best iron chelating ability at the observed concentration of extracts. The iron chelating ability of the flour of 100% aloe vera treated stored plantain was not significantly ($P < 0.05$) different from the iron chelating ability of the flour of freshly harvested plantain. This may be as a result of the high phenolic content of the flour of 100% aloe vera treated stored plantain which was similar to that of the freshly harvested plantain. It has been observed by Al-Farga *et al*., (2014) and Hinneburg *et al*., (2006) that there is positive correlation between phenolic compounds and iron chelating ability.

The DPPH results are usually used to ascertain the results obtained in total phenolic (Palafox Carlos *et al.*, 2012). A dose-response relationship was observed in the DPPH radical-scavenging ability of the flour of aloe vera treated and untreated stored plantain extracts; the ability increased with an increase in the concentration of the extracts. The flour of 100% aloe vera treated stored plantain recorded the highest DPPH radical-scavenging ability which was significantly ($P < 0.05$) higher than the extract of the flour of 50% aloe vera treated and untreated stored plantain. It is to be noted that the DPPH scavenging ability of the flour of the plantain extract followed the same trend with the result obtained for reducing power and metal chelating ability where there was no significant ($P < 0.05$) difference in the scavenging ability of the flour of 100% aloe vera treated stored plantain and that of the freshly harvested plantain. The high phenolic content of the flour of 100% aloe vera treated stored plantain extract is an indication of high antioxidant capacity because phenolics always react with active oxygen radicals and lipid peroxide radicals. It has been revealed that the correlation between antioxidant capacity and phenolic content of samples are high (Aliyu, *et al.*, 2011).

The phytate : zinc molar ratio of the flour of freshly harvested plantain and that of the flour of treated and untreated stored plantain were higher than 15.0 considered as the critical value (Fergusson *et al.*, 1988; WHO 1996). This is an indication that the phytate content of the plantain flour will cause a reduction in the bioavailability of zinc. Diets having phytate : zinc molar ratio that is higher than 15 are considered to have a poor Zn bioavailability while those with phytate : zinc molar ratios that are less than 15 have a very good zinc bioavailability (WHO, 1996). The calculated calcium: phytate molar ratios of the flour of freshly harvested, treated and untreated stored plantain were lower than the critical value of 6.0 (Fergusson *et al*., 1988). The solubility of phytate and the quantity of zinc present in the complex depend on dietary calcium levels. However, phytate precipitation will not be complete until the dietary calcium : phytate molar ratios attain a value of close to 6.0. When the ratios is low, phytate precipitation will be incomplete thereby causing some dietary zinc to remain in solution (Akindahunsi and Oboh, 1999). The calculated [Ca][Phytate]/[Zn] molar ratio is a better index for the prediction of zinc bioavailability in comparison to phytate : zinc ratio because of the calcium to phytate interaction (Akindahunsi and Oboh, 1999). The calculated [Ca][Phytate]/[Zn] molar ratio for the flour of freshly harvested plantain, 50% and 100% aloe vera treated stored plantain were below the critical level of 0.5 mol/kg, which is

an indication that dietary zinc is bioavailable (Akindahunsi and Oboh, 1999). The zinc is more bio-available in the flour of 100% aloe vera treated stored plantain considering that it has the lowest index value.

CONCLUSION

It was observed in this work that treating plantain with 100% aloe vera gel solution prior to storage will preserve its phenolic, antioxidant qualities and zinc bioavailability like it is freshly harvested.

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