Phenolic Content, Polyphenol Oxidase Activity And Antioxidant Scavenging Activity In Three Species Of Plantain In Ghana

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Abstract- Plantains and Bananas (*Musa* spp.) are grown in more than 120 countries in the tropical and subtropical regions of the world. In Ghana, plantain is used like a starchy food similar to yams. Plantain (Musa *paradisica)* is found to contain some phenolic compounds. Polyphenols are a large group of non-nutrient compounds that are found in plants and they have antioxidant properties. Consumers have developed interest in organic foods that have this property due to their perceived health benefits. Three cultivars, Apantu, Oniaba and Apem at three stages of ripeness; unripe, firm ripe and fully ripe were used. Phenolic compounds were extracted with 70% Ethanol. Standard methods were employed in determining the PPO (Polyphenol Oxidase) activity. The results showed that the total phenolic content was highest in unripe Apem (448 ± 18.45mg GAE/g DW) and Apantu had the least value of 117.70 ± 4.35mg GAE/g DW. Oniaba had 169.1 ± 11.25mg GAE/g DW. Apart from Apantu that recorded a decrease in the total phenolic content at the fully ripe stage, Oniaba and Apem recorded increases in their total phenolic content. Processing (boiling, drying and milling) had an effect on the total phenolic content of the studied samples. Apantu and Oniaba had increases in their total phenolic content but Apem had a decrease in the total phenolic content. The polyphenol oxidase activity was dependent on the stage of ripeness and the cultivar of plantain studied. The highest PPO activity was observed in the fully ripe Apem and the least was in unripe Apem. The fully ripened samples recorded the highest PPO activity followed by the firm ripe sample with unripe sample having the least. Phenolic compounds determined in the pulp and peels were Gallic acid, Catechol, Dopamine hydrochloride and Catechin. The concentrations of these phenolic compounds were found in varying concentrations in the pulp and the peels and also in the cultivars. The antioxidant scavenging activity affected by senescence of the pulp with fully ripe 'Oniaba' having the highest (51.40 ±0.68) percent antioxidant scavenging activity.

Index Terms- Polyphenol, Polyphenol oxidase, Polyphenol oxidase activity, Antioxidant scavenging activity

I. INTRODUCTION

Plantain (*Musa paradisiaca*), a plant of the banana family (Musaceae) is closely related to the common banana (*M. sapientum*). The botanical classification of plantains and bananas is so complicated that plantain is variously viewed as a subspecies of banana, and the banana as a subspecies of plantain (Oke *et al*., 1998). Plantain has a maximum of starch before it ripens, it is usually cooked green, either boiled, roasted or fried. It may also be dried for later use in cooking or ground for use as a meal (FAO document repository). The plantain meal can be further refined into flour. In some parts of East Africa plantain is a staple food and beer-making crop, notably in Central and Eastern Uganda and Tanzania (formerly Tanganyika). The plant is believed to have originated from South-East Asia. (Encyclopædia Britannica, Inc. 2011).

Phytochemicals are a large group of non-nutrient substances present in plants. They are biologically active and they play an important role in the interaction of plants with their environment as well as having some health promoting impacts (Schreiner *et al*., 2006).

Natural resources such as fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest to consumers and in the food industry because of their perceived benefits beyond basic nutrition. Such food sources have been shown to retard oxidative degradation of lipids and thereby improving and enhancing the quality of food beyond its nutritional value (Javanraedi *et al.*, 2003). Therefore, the study of non-nutrient compounds such as phenolic acids, flavonoids and high molecular tannins as natural antioxidants have greatly increased (Siddhuraju and Becker, 2007).

In recent times, attempts are being made to produce convenience foods from starchy foods such as corn flour, cassava flour, yam flour and plantain flour. However, processed plantain flour has been observed to darken upon storage, probably due to the interactions of polyphenols and polyphenol oxidases or non-enzymatic factors. To be able to solve the browning problem of plantain flour during storage, it is important to characterize the polyphenols present in plantains, their stability during processing into precooked flours, as well as the polyphenol oxidase activity of plantains.

The aim of the study is to determine the phenolic contents of the three species of plantain in Ghana.

II. Materials and Methods

Collection of Plant Materials

Three cultivars of Plantain, namely false horn plantain, intermediate plantain and French plantain with the local names *Apantu*, *Oniaba* and *Apem* respectively were obtained from the Agricultural Research Center of the University of Ghana, Legon at Kade in the Eastern Region of Ghana.

Sample Preparation

The matured samples were harvested from the same field and transported immediately to the laboratory. The samples were cleaned and washed with distilled water.

Laboratory Analysis

Extraction of Phenolic Compounds from the Freshly Harvested Plantain Pulp.

The extraction procedure used was that by Alothman *et al.*, (2008) with slight modification. The samples were washed in distilled water and peeled using a stainless-steel kitchen knife. 100 g of plantain pulps were diced into small sizes and blended for 3 minutes and then extracted with 200 ml of 70% Ethanol while shaking (GALLENKAMP orbital shaker, United Kingdom) at 100 rpm for 20 minutes at room temperature (25 ± 1ºC). The extract was then filtered using a clean muslin cloth and centrifuged (DENLEY BS 400 Centrifuge, England) at 4750 g for 15 minutes. The supernatant was concentrated at 60ºC using a rotary evaporator (BÜCHI ROTAVAPOR R-114). The crude extracts were collected after 3 hours and stored at 4ºC in the dark. Light exposure was avoided throughout the extraction process. The extracts were stored in a dark container at refrigeration temperature (6°C) until further analysis. The extraction process was carried out in triplicate

Extraction of Phenolic compounds from the firm ripe plantain

The pulp of the firm ripe plantain varieties were cleaned and washed with distilled water. 100g of the pulp was weighed and taken through the extraction procedure.

Effect of Processing on the Phenolic Compounds

Extraction of Phenolic compounds from unripe Plantain flour

The pulp of the freshly harvested plantain varieties were oven dried (Wagtech, Model; GP/150/SS/250/HYD, DB THERMAL systems Ltd) at 60ºC for 48 hours. The dried pulp was milled into flour using a hammer mill (Christy and Norris Ltd, Chelmsford England, equipped with sieve size 5µm). 50g of the flour was weighed and 150ml of 70% ethanol used for the extraction.

Extraction of Phenolic Compounds from Unripe Pre- Cooked Plantain Pulp Flour

The fresh unpeeled plantain varieties were boiled for 7 minutes. The pre-cooked plantain was allowed to cool after which the peels were removed and the pulp oven dried (Wagtech, Model; GP/150/SS/250/HYD, DB THERMAL systems Ltd) at 60ºC for 48 hours. The dried pulp was milled into flour using a hammer mill (Christy and Norris Ltd, Chelmsford England, equipped with sieve size 5µm). 50g of the flour was weighed and taken through the procedure of extraction.

Extraction of Phenolic Compounds from Fully Ripe Plantain Pulp

The plantain was stored in an airy room for 14 days and allowed to ripen. The ripe plantain was peeled and 100g of the pulp weighed. It was then taken through the extraction procedure.

Determination of Total Phenolic Contents in the Plantain Samples

The total phenolic compound contents were determined by using Folin–Ciocalteu reagent assay (Singleton and Rossi, 1965). 0.1 ml extract (contains 0.1 mg extract) was mixed with water (46 ml). 1 ml of Folin–Ciocalteu reagent was added and mixed thoroughly. 3 ml of Na_2CO_3 (7%) was added to the mixture. The absorbance was measured at 760 nm using Cecil 3042 spectrophotometer. A standard curve was prepared using gallic acid in ethanol to cover a range of 0–100 µg/ml. The concentration of total phenolic compounds in the extracts was determined as mg gallic acid equivalent. The data were presented as the average of triplicate analyses.

Total antioxidant activity of the extracts (Radical scavenging activity)

DPPH (2, 2 –diphenyl-1- picrylhydrazyl) assay

The effect of the extracts and standard antioxidants on DPPH radical was estimated according to the method of Blois (1958) with slight modification wherein the bleaching rate of a stable free radical DPPH is monitored at a characteristic wavelength in the presence of samples.

An amount of 0.5ml of 0.1mM ethanolic solution of DPPH was added to 3.0ml of all the extract samples or standard antioxidants solution (50-500µg/ml) in water. The mixture was shaken vigorously and kept standing at room temperature for 30minutes in the dark, the absorbance of the mixture was measured at 517nm using Cecil 3042 spectrophotometer. The decrease in absorbance indicates an increase in DPPH radical scavenging activity. This activity was calculated by using the equation below;

$$\text{DPPH scavenging Effect (\%)} = [(A_0-A_1)/A_0\ ]*100$$

Where A_0 is the absorbance of control and A_1 is the absorbance of the extract or standard.

Standard Mixture Preparation

A standard mixture of Catechin, Epicatechin, Gallic acid, Chlorogenic acid, Catechol, Caffeic acid, Dopamine HCl, P-Cresol, was prepared into a stock solution of 200µg/ml using the mobile phase. The stock solution was diluted to 100µg/ml and 50µg/ml for the calibration graph.

Sample Preparation

One ml of the Rotary evaporated extracts was filtered through a sterile 0.45µm cellulose membrane filter (Nalgene, Nalg Nunc International Corporation U.S.A).

Polyphenol Oxidase (PPO) Activity

The method adopted by Montgomery and Sgarbieri (1975) was employed. Five grams of plantain pulp were homogenized with 0.6 g of Polyvinylpyrrolidone (PVPP) and 20 ml of 50mM

(pH 7) phosphate buffer. This was then filtered and centrifuged at 10,000×*g* at 4 °C for 15 min. The activity was measured with 2.85 ml of 0.2mM (pH 7) phosphate buffer, 50 µl of Catechol (60 mM) as a substrate and 100µl of enzymatic extract. The mixture was maintained at 25 °C and the change in absorbance was read over 3 min at 420 nm. Activity was expressed as units of activity (UA) in which one unit of PPO was defined as the change in one unit of absorbance per second.

III. Results and Discussion

Total Phenolic Content in Plantain Pulp

The total phenolic content in the plantain cultivars are shown in Figure 1. Results for the unripe plantain samples showed that Apem had the highest total phenolic content (440 mg GAE/g DW) and Apantu had the least value of 120 mg GAE/g DW.

In the firm ripe plantain samples, Apem recorded the highest total phenolic content (280 mg GAE/g DW) with Oniaba having the least value of 250 mg GAE/g DW in Figure 4.1. When the unripe samples were compared with the firm ripe samples, Apantu had an increase of 150 mg GAE/g DW in the total phenolic content and Oniaba (85 mg GAE/g DW) but Apem recorded a decrease of 150 mg GAE/g DW in the total phenolic content. Romani *et al.* (2002) have indicated that a quantitative variation in phenolic compound content could be due to variety, different agronomic conditions, and tissue type.

Analysis of variance for the total phenolic content of the various cultivars indicated that the total phenolic content of the samples were significantly ($p<0.05$) affected by the interaction of the cultivar type and the stage of ripeness of the plantain varieties. The difference might be due to their genomic variation because all the plants were exposed to the same agronomic conditions.

The total phenolic content of the unripe samples were compared with their peels to find out whether the peels will also show a similar result but from figure 4.2, the results were different. Apantu recorded the highest phenolic content of about 3000 mg GAE/g DW and this was followed by Oniaba (2000 mg GAE/g DW) and finally Apem (1000 mg GAE/g DW).

The Effect of Maturity and Ripening on The Phenolic Content of The Plantain Pulp

The results in Figure 1 showed that Apem had the highest (220 mg GAE/g DW) total phenolic content among the fully ripened samples and the least was Oniaba (160 mg GAE/g DW). There was a general decrease in total phenolic content from the unripe and/or firm ripe stage to the fully ripe stage. Ngoh-Newilah *et al*., (2010) reported a decrease in total phenolic content in some ripe Musa hybrids ('CRBP755' and 'DS11') in Cameroun and an increase in other cultivars ('Bita3', 'CRBP39' and 'FHIA21') from the unripe stage through the ripe stage to the fully ripe stage. It was explained that the postharvest metabolisms either enhance some metabolites synthesis for a determined period according to the environment and the genotype, or stimulate degradation of existing/synthesized substances leading to many secondary compounds found in fruits such as bananas and plantains (Robards *et al.,* 1999). The decrease in total phenolic content with fruit ripening was also reported in banana (Ibrahim *et al*., 1994), mango (Abu-Goukh and Abu-Sarra, 1993) and date (Al-Ogaidi and Mutlak, 1986).

According to Taylor (1993), the decrease in polyphenol content of guava fruit causes a loss in astringency during ripening of the fruit. The increase in antioxidant activity as the fruit matured was due to the breakdown of starch to glucose which was used in the biosynthesis of ascorbic acid. The difference between the maximum and the minimum total phenolic content was about 50 mg GAE/g DW. There is a possibility that as the pulp ripens, polyphenol oxidase gets gradual access to the phenolic compounds and this causes a decrease in the total phenolic content.

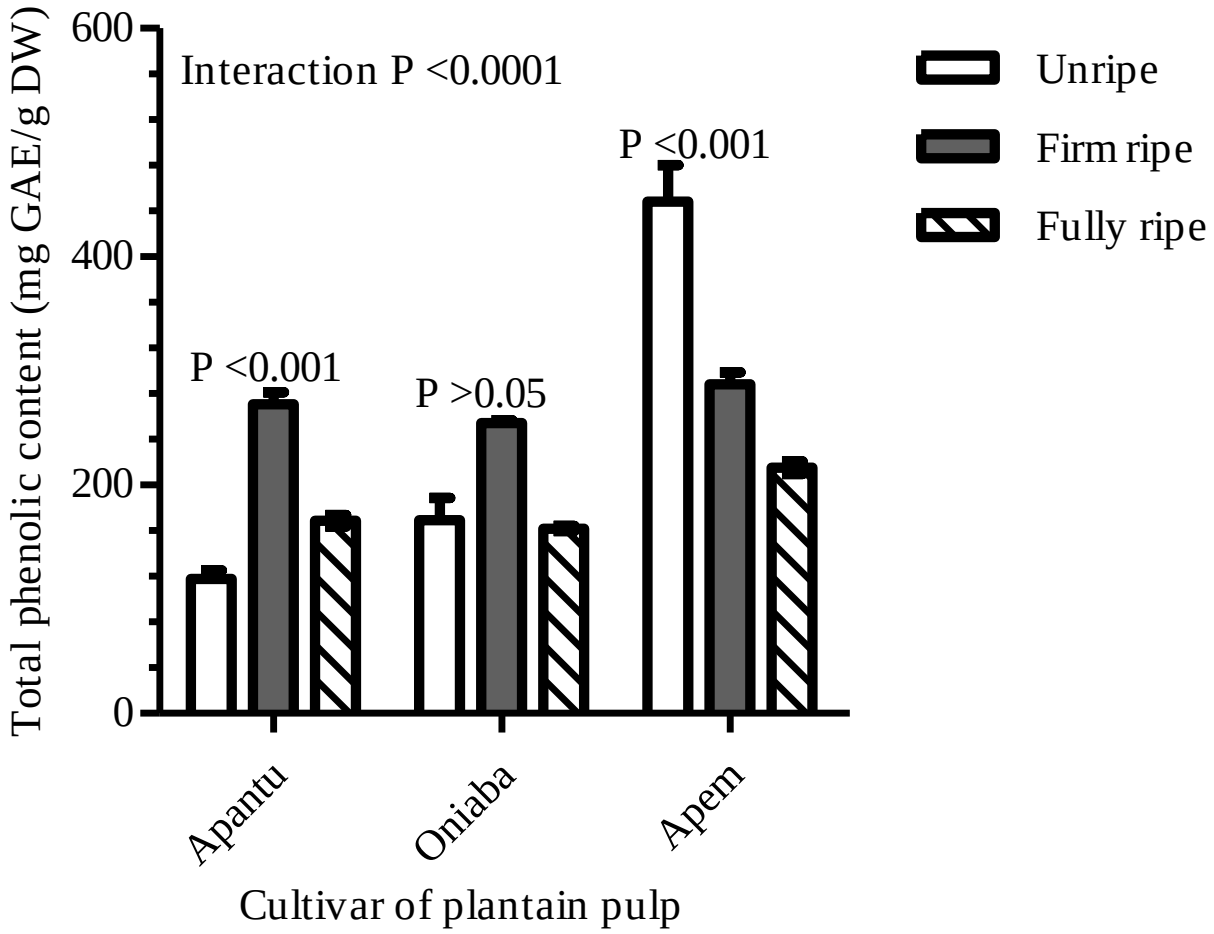

Figure 1:

Total Phenolic Content in Different Cultivars Of Plantain

The Effect of Processing (Boiling and Drying) on the Phenolic Content of Plantain Flour.

The data in Table 1 showed that processing had an effect on the phenolic content of the plantain cultivars. In the raw, dried and milled plantain into flour termed as (FDM) process, there were increases in the total phenolic content of Apantu (80 mg GAE/g DW) and Oniaba (47 mg GAE/g DW) with the exception of Apem which recorded a decrease of 107 mg GAE/g DW in the total phenolic content. Within the FDM there were significant differences ($P < 0.05$) between Apantu and Apem and also Oniaba and Apem. Apantu had the least (199.00 ± 2.11mg GAE/g DW) total phenolic content with Apem having the highest (341.00 ± 34.6mg GAE/g DW) total phenolic content although not as high as that of the unripe Apem (440 mg GAE/g DW).

The boiled, dried and milled plantain termed as (FBDM) process, recorded higher values when compared with that of the unripe plantain cultivars. Oniaba had the highest phenolic content of 187.2 ± 3.66mg GAE/g DW and Apantu with the least 166.9 ± 0.96mg GAE/g DW.

Table 1: Total Phenolic Content of the Extract of Plantain Flour from Pulp

Cultivar	Total phenolic content (mg GAE/g DW)	
	FDM + Mean ±SE	FBDM # Mean ±SE
Apantu	199.00±2.11[a]	166.90 ±0.96[a]
Oniaba	216.00 ±3.10[a]	187.20 ±3.66[b]
Apem	341.00 ±34.6[b]	

GAE- Gallic Acid Equivalent, DW- Dry Weight. FDM-fresh, dried and milled plantain pulp, FBDM-fresh, boiled, dried and milled. Values in the same column with different letters are significantly different at $P < 0.05$. +- analysis was done using one-way ANOVA, #- analysis was done using unpaired t-test. (n=3)

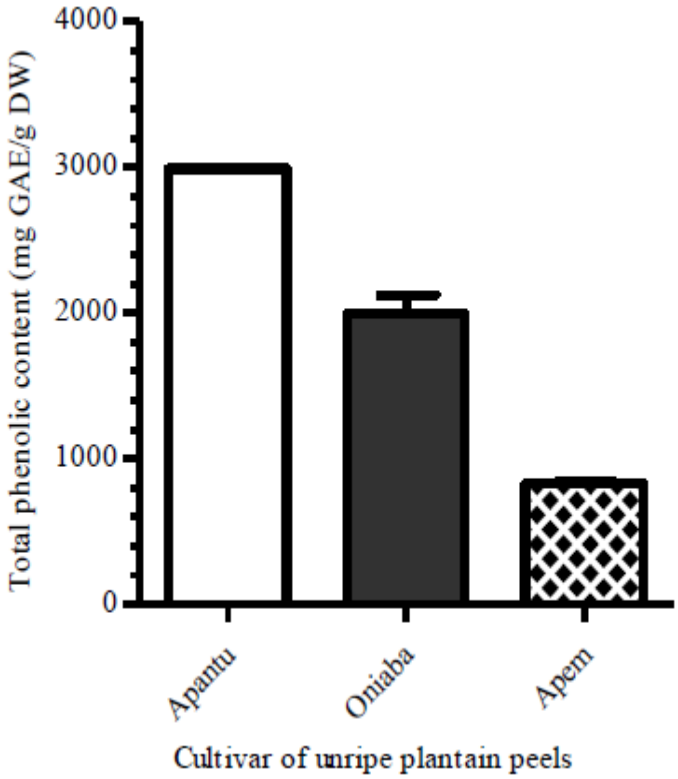

Figure 3: Total phenolic content in unripe plantain peels.

Polyphenol Oxidase Activity

The results shown in figure 4 show the Polyphenol Oxidase activity of the three plantain cultivars in accordance with the methods used. The Polyphenol Oxidase (PPO) activity of the Plantain was determined during various stages of ripening. Activity was expressed as units of activity (UA) in which one unit of PPO was defined as the change in one unit of absorbance per second.

In general, there was an increase in the PPO activity within the same cultivar from the unripe state to the fully ripe state. Similar findings have been reported for a local variety of plantain, 'Ogbutu', by Omuaru *et al.* (1990) and some banana varieties by Jayaraman and Ramanujao (1987). Within the fully ripe stage, Apem recorded the highest PPO activity (0.0402 UA) with Oniaba being the least (0.00244 UA).

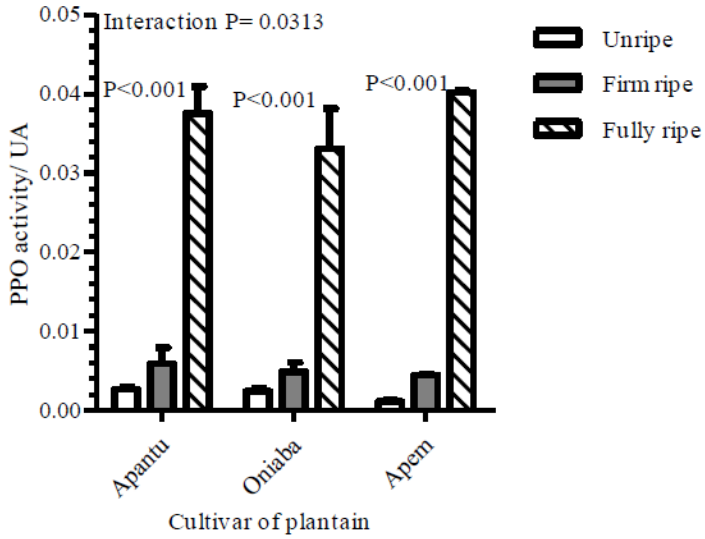

Figure 4: Polyphenol Oxidase Activity of Plantain Extract.

From Apantu to Apem, there was a decrease in the PPO activity within the unripe stage and within the Firm ripe stage in figure 4. This observation was different in the fully ripe stage. The Polyphenol Oxidase activity in the firm ripe samples was intermediate between the PPO of the unripe and the fully ripe samples. The PPO activity was highest in the fully ripe stage because during ripening and senescence, there is a break down in the cell compartmentation and therefore the PPO get in contact with the substrate (polyphenols) and thus act on the polyphenols but in the unripe samples, the cell compartmentation is intact and this prevent the direct contact of the enzymes with the polyphenols (substrate). The enzyme activity is reduced and this was observed in figure 4.

Free radical-scavenging activity of Plantain extracts using DPPH (Antioxidant activity assay)

In table 2, the percentage scavenging activity of the fresh samples ranged from about 16% to about 51% for the unripe samples. Antioxidants are known to interrupt the free radical chain of oxidation by donating hydrogen from phenolic hydroxyl groups and to form stable products, which do not initiate or propagate further oxidation of lipids. DPPH radical is often used as an indicator in testing hydrogen-donation capacity and thus antioxidant activity (Dorman *et al*., 2003).

Table 2: Antioxidant activity of pulp extract of Plantain samples

Cultivar	% Scavenging Activity		
	Unripe	Firm ripe	Fully ripe
	Mean ± SE	Mean ± SE	Mean ± SE
Apantu	46.60 ± 4.11^{a}	20.50± 4.11^{a}	39.70± 4.11^{a}
Oniaba	34.20 ±0.00^{b}	28.80 ±1.37^{b}	51.40 ±0.68^{b}
Apem	15.80 ± 2.05^{c}	24.00 ± 2.05^{a}	43.80 ± 1.37^{a}

Post hoc test was by Tukey's. Values in the same column with different letters are significantly different at $P < 0.05$. Values are reported as Mean ± SE (n=3).

Apantu had the highest (46.60 ± 4.11) percentage scavenging activity followed by Oniaba (34.20 ±0.00) with Apem recording the least value (15.80 ± 2.05). The difference in the percentage scavenging activity was due to the different cultivars used and this is in line with Prior *et al*., 1998, since they all had different polyphenol contents.

There was a decrease in the percentage scavenging activity of the Firm ripe plantain samples of Apantu (26.1) and Oniaba (5.4) in comparison with the fresh and raw samples but that of Apem recorded a percentage increase of 8.2.

There was a general increase in the percentage scavenging activity of the fully ripe plantain samples of Apantu, Oniaba and Apem and the percentage rise were 19.20, 22.60 and 19.8 respectively. Although there were increases in percentage scavenging activity from the Firm ripe samples to the fully ripe samples, there were no significant differences ($P < 0.05$) among the means of the fully ripe plantain cultivars. Free radicals are thought to play an essential role in fruit ripening and senescence, especially those derived from oxygen, and a higher relative concentration of phenolics in the older leaves in relation with the young ones has been revealed (Torras-Claveria L. *et al*., 2011; Ivanova D.G., Singh B.R., 2003), indicating that phenolic derivatives play a prominent role in tissue senescence (Tamagnone L., *et al*., 1998).

When the fresh unripe samples were compared with the fully ripe samples, it was noticed that apart from Apantu (6.9) which had a reduction in the percentage scavenging activity Oniaba (17.2) and Apem (28) recorded an increase in their percentage scavenging activity. The increase in percentage scavenging activity after storage was due to several reasons. After harvest, ascorbic acid is still being synthesized in the living fruit tissues and it also acts as antioxidant. It is known that fruit ripening continues after harvest and this process leads to significant changes in the contents of the antioxidants.

Yan *et al*., (2006) reported a lower antioxidant scavenging activity for banana when compared with other fruits like guava, dragon fruit, star fruit, orange and sugar apple but among the fruits studied banana had the highest chelating power.

In figure 1, unripe Apem recorded the highest total phenolic content but this did not commensurate with the percentage scavenging activity. The percentage scavenging activity of the unripe plantain showed a reverse of what was observed in the total phenolic content. A similar observation was made by Barros, *et al.*, 2009. It was suggested that other compounds different than phenolic acids are present and react with the Folin–Ciocalteu reagent and also contribute to their antioxidant properties.

IV. Conclusion

The main conclusions from this study include that, the total phenolic contents were highest in the pulp of the unripe samples with Apem recording the highest followed by Oniaba and Apantu. In the processed pulp flour, Apantu FDM (341.00 ±34.6 mg GAE/g DW) was highest and the least was Apantu FBDM (166.9±0.96 mg GAE/g DW). The peels of the samples recorded the highest total content and this was determined in Apantu, Oniaba and Apem (figure 2). PPO activity was also highest in the fully ripe samples. The following phenolic compounds were found in the samples studied; Gallic acid, Catechin, Catechol, Dopamine in varying concentrations. The antioxidant scavenging activity affected by senescence of the pulp with fully ripe 'Oniaba' having the highest (51.40 ±0.68) percent antioxidant scavenging activity.

V. Recommendation for Further Work

Further study should be done to fully characterize the polyphenols and identify those with high percentage scavenging activity.

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