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Extraction and Estimation of Pectins from Unripe, Ripe and Overripe Banana (*Musa Acuminata* L.) and Plantain (*Musa Paradisiaca* L.) Peels and their Antioxidant Activities

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Pectin has several unique properties that enabled it to be use as a matrix for entrapment and/or delivery of a variety of drugs, proteins and cells. They are used in pharmaceutical as carriers in drug delivery, and in the treatment of various disease conditions.

Objectives: This study aimed at determining percentage yield of pectin, extracted from unripe, ripe, and overripe Banana (*Musa acuminata* L. Musaceae) and Plantain (*Musa paradisiaca* L. Musaceae) peels and to evaluate their antioxidant activities.

Materials and Method: Pectins were extracted from the stated samples using conventional extraction method involving alcohol precipitation, and the percentage yields were calculated. The DPPH antiradical activity and reducing capacity was assayed, using Ascorbic acid as standard.

Results: The percentage yield of pectin were obtained as 3.04±0.97%, 5.84±0.49%, 3.70±0.68% for banana and 4.65±0.70%, 6.61±0.21%, 6.91±0.86% for plantain corresponding to unripe, ripe, and overripe respectively. The extracted pectin of all the samples were observed to be statistically inferior to when compared to the standard used Ascorbic acid at all concentrations of samples prepared for both DPPH antiradical activity assay and Reducing capacity assay ($p \leq 0.005$), at 95% confidence interval.

Conclusion: The yield of banana peel pectin have an initial increase with ripening followed by a decrease in yield, while plantain peel pectin can be said to increase from its unripe stage through to its overripe stage; with plantain pectin yield greater than banana across all stages. All data showed statistical inferiority to Ascorbic acid in reducing capacity and against DPPH.

Keywords: Pectin, Banana, Plantain, DPPH, Ripening, Antioxidant

INTRODUCTION

Pectins are a group of acidic heteropolysaccharides, considered to be the most complicated plant cell wall structural polysaccharides (Ho, *et al.*, 2015). They are composed mainly of galacturonic acid units with variations in composition, structure and molecular weight. They are located in the primary cell wall and middle lamella of many plants, having highest concentration in the middle lamella, with gradual decrease from the primary cell wall towards the plasma membrane (Lara-Espinoza *et al.*, 2018).

In 1825, Pectin was first isolated and described by Henri Braconnot, a French chemist. He coined the word 'pectin', which was derived from the Greek word "pektos", meaning "rich, solidify" (Yabe, 2018).

Pectin has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabilizer (Kavitha*et*

al., 2016). Commercially, it is mostly extracted from citrus peel and apple pomace (Noreen *et al.,* 2017).
Pectins have also gained use in the pharmaceutical and biotechnology industry. The use of natural polymers, such as pectin in the pharmaceutical and biotechnology industry is increasing daily, because they are economical, readily available, non-toxic, and capable of chemical modifications, potentially biodegradable and biocompatible (Pramanik & Ganguly, 2017).

The pectin chain structure consists mainly of α-(1-4)-ɒ-galacturonic acid units forming long homogalacturonic chains interspersed by rhamnogalacturonan sections where rhamnose and galacturonic acid residues alternate. Neutral sugar units are attached to the backbone and concentrated in highly branched "hairy" regions. Part of the carboxylic groups in the galacturonic chain exist in methyl ester form (Abid*et al.,* 2017).
Pectin can therefore vary in structure from linear homogalacturonans (HG) to highly branched and complex rhamnogalacturonans (RGI and RGII), and hence can be classified according to branching degree and type of side-chains monosaccharides (Lopes *et al.,* 2016).
Pectin and pH-modified or heat modified (HM) pectin have demonstrated chemo-preventive and antitumor activities against some aggressive and recurrent cancers (Lefsih *et al.,* 2018). They are often used as biodegradable carriers for time-released drug delivery (Anderson, 2016). It is useful mainly in colon cancer targeted drug delivery formulations (Dheer *et al.,* 2017). Immunological activity analysis of Pectin isolated from *Dendrobium nobile* Lindl., suggested that the side chains, carboxyl and acetyl groups had effect on the expression of immunological activity (Wang *et al.,* 2018).Pectin (from Citrus) has been shown to reduce fasting blood glucose levels, benefit hyperlipidemia, and refine hepatic glycogen content glucose tolerance in diabetic rats. It should be expected that pectins, similar to many polysaccharides, will become a new class of hypoglycemic drugs (Minzanova *et al.,* 2018).Pectin exert effect on lowering of blood cholesterol through interference with micelle formation, lowering the rate of diffusion of bile acid and cholesterol-containing micelles through bolus, reduction in uptake of cholesterol and bile acids, increased excretion of bile acids and neutral sterols in feces (Majee *et al.,* 2018).

Pectin can be blended with various polymers either natural or synthetic or with other compounds, and this has widened its application for future prospective. Some blends include Pectin-chitosan, Pectin collagen and Pectin-ethylcellulose which have application in Drug delivery, Bone substitute and Cancer treatment respectively (Noreen *et al.,* 2017).
Another rapidly emerging use of pectin, is to detoxify the body from heavy metals, including radioactive isotopes such as Caesium (Cs) in contaminated areas such as Fukushima, in Japan, or Chernobyl, in Belarus and Ukraine (Ciriminna *et al.,* 2016).
In the United States of America, intravenous administration of aqueous modified pectin solutions (GCS-100) is now in phase III, as a new method of treating chronic inflammation (Ciriminna *et al.,* 2015).

Bananas are the fourth most important agricultural food products after rice, wheat and maize (Dahham *et al.,* 2015). Plantain is a major staple food across the world is useful in preparing food in its unripe, ripe and overripe stages (Adeyeye *et al.,* 2019).

Banana peels, as well as plantain peels represent 30-40% of the total weight of the fruit. After the processing of bananas and plantains, a large amount of peel is collected. These residues represent a serious pollution problem (Torres-Leon *et al.,* 2018), hence, extraction of Pectin biopolymer from these peels can be considered potential waste to Wealth.

Pectin has a great structural diversity according to the type of plant waste material from which it was extracted. These differences in structure can result in difference in the physiochemical properties of pectin (Dona, 2019).

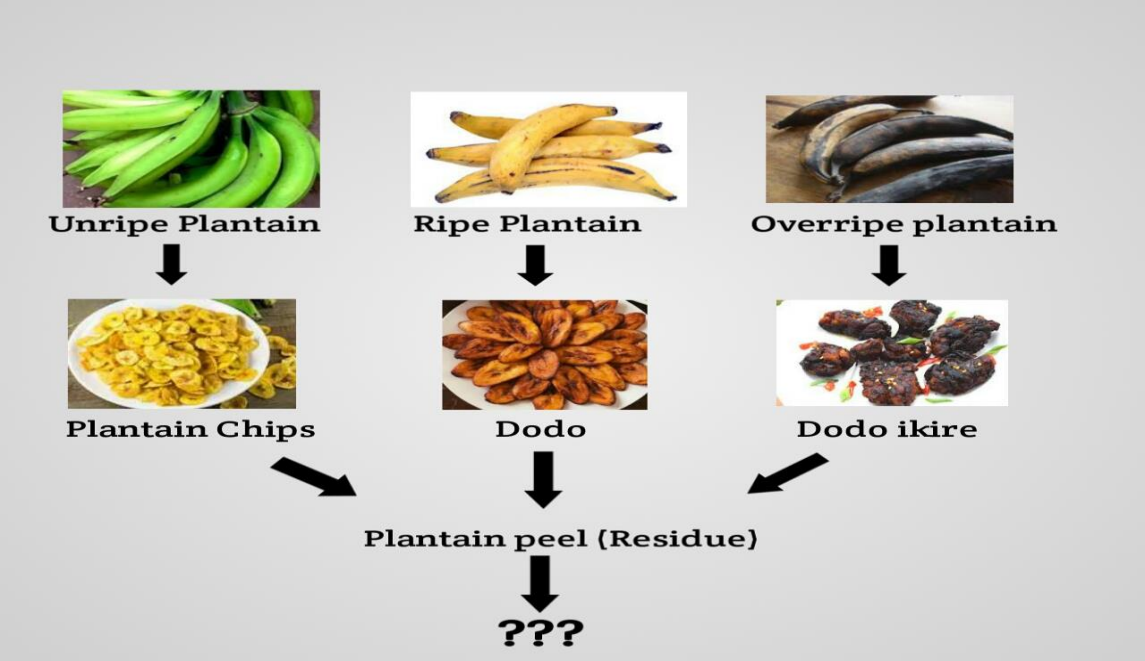

Figure 1: Plantain peels as a residue from pulp processing in Nigeria across all stages of ripening

Fruit ripening is a genetically programmed, highly coordinated process of organ transformation from unripe to ripe stage, to yield an attractive edible fruit with an optimum blend of color, taste, aroma and texture (Maduwanthi and Marapana, 2017). It is a combination of physiological and biochemical processes. Apart from changes in peel color, the rapid loss of firm texture is another noticeable change observed during ripening. It is generally accepted that the modification of the physical and chemical features of parenchyma cell walls and the loss of intracellular adhesion resulting from the dissolution of the middle lamella are the major determining factors of fruit ripening (Paniagua *et al.*, 2017).

It is therefore important to investigate the effect of ripening on the content and property of pectin. The objective of this study is to observe the yield pattern of pectin, extracted from unripe (green), ripe (yellow) and overripe (black) banana (*Musa acuminata*) and plantain (*Musa paradisiaca*) peels and evaluate the antioxidant activities of the extracted pectins.

METHODOLOGY

Reagents and Equipment

Acetone (BDH Binder, Germany), Ethanol, Sulphuric acid (H_2SO_4) (BDH Binder, Germany), Methanol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Merck KGaA, Darmstadt, Germany), Ascorbic acid, Sodium hydroxide (NaOH), Trichloroacetic acid (EMD Milliform Corporation, Germany), Fehling's solution A (Sigma Aldrich Germany), Fehling's solution B (Sigma Aldrich Germany), Naphthol, pH Meter, UV-VIS Spectrophotometer (Biomate 3, USA), Hot water bath, Oven, Analytical balance.

Plant Collection

Unripe banana *Musa acuminata* L. (Musaceae) and plantain *Musa paradisiaca* L. (Musaceae) were purchased on the 31st of July, 2019 at Idi-oro market (6.5219° N, 3.3565° E), Mushin, Lagos state, Nigeria. The material were known plants, hence authentication was not done. The fruits were divided into three, to represent the unripe, ripe and overripe fruits.

All samples were washed before the peels of the fruit were obtained. The unripe peels were peeled off the fruit pulp, cut in pieces, dried in an oven and ground. The ripening process for the other two divisions of both plant fruits was supervised. The ripe plantain peel was obtained after 5 days (6 days for banana sample) and were cut in pieces, dried in an oven and ground. The overripe plantain peels were obtained after 5 more days (6 days for banana sample) and were cut in pieces, dried in an oven and ground.

Extraction of Pectins from Plantain and Banana *(Unripe peel, ripe peel and overripe peel)*

Pectin extraction was carried out using the method of Khamsucharit, *et al.*, (2018). About 50g of plantain peel was weighed and immersed in 200ml of distilled

water. The pH of the mixture was adjusted to 4, by the addition of sulphuric acid. The mixture was heated for one hour with constant stirring, and filtered immediately while the solution was hot. The filtrate was cooled, and poured slowly into 3 volumes of acidic ethanol. The solution was stirred thoroughly for precipitation of pectin. The mixture was filtered and the extracted pectin (residue) was washed several times with small volumes of acetone to make it free from acidic ions. The product was dried, weighed and stored in a well closed container. The above procedure was performed in triplicate for each of the samples across the ripening stages. Similar procedures were carried out for the banana peels, using 30g of weighed banana peel in 120ml of distilled water. The percentage yields were calculated using the formula;

$$\%\ yield = Weight\ of \frac{extract}{weight}\ of\ peel\ X100$$ .....Equation 1

Confirmatory Test for Pectin

Molisch's Test for Carbohydrates

To 2ml of 0.5mg of sample in 2 ml of water, 3 drops of naphthol was added and shaken. Concentrated sulphuric acid, 2ml was then added slowly from the sides of the test tubes. A violet ring formed at the junction of the two confirms the test (Roopalatha and Nair, 2013). This procedure was carried out with all the pectin extracts.liquids

Fehling's Test (Test for Reducing sugar)

About 1 g of each sample was placed in individual test tubes. Distilled water, 10ml was added. The mixture was filtered and the filtrate was allowed to cool, 1ml of Fehling's solution A and B. The mixtures were then heated for 10 minutes. A brick red precipitate formed confirms the test (Roopalatha and Nair, 2013). This procedure was carried out with all the pectin extracts.

Properties in different solvents

The properties of the pectin solution weredetermined in different solvents; Ethanol, NaOH and H_2SO_4

DPPH Antioxidant Activity Assay

The antioxidant activity of one sample each of the extracts was evaluated on the basis of the radical scavenging effect of the stable 2,2 -diphenyl-1-picrylhydrazyl (DPPH)- free radical activity, in comparison with ascorbic acid standard, by a slightly modified method employed by Blois (1958).

Ascorbic acid standard (concentrations 0.2, 0.4, 0.6, 0.8, 1.0) were prepared from stock solution in triplicates using suitable dilution. 0.1mM of DPPH was prepared in methanol and 1ml of this solution was mixed with 3ml of the standard solutions in test tubes. These solutions were shaken, then they were allowed to stand for 30mins and absorbance was measured at 517nm using UV-VIS Spectrophotometer. .

Methanol (3ml) with DPPH solution (0.1mM, 1ml) was used as control. The same procedure as with the ascorbic acid standard was carried out with each of the six extract samples (Eruygur *et al.*, 2017). The experiment was performed in triplicates.

% inhibition was calculated by the formula given below:

$$Inhibition\ of\ DPPH = \frac{Ac - Aa}{Ac} X100$$ .....Equation 2

Where Ac is the absorbance of the control sample and Aa is the absorbance of the extract sample.

Reducing Capacity Assay

Using a slightly modified method as employed by Adesegun *et al.*, 2009, ferric ion reducing capacities of the pectin extracts were determined. The experiment was done in triplicate and carried out for all the six pectin extracts. Increased absorbance of the reaction mixture indicated increasing reducing power.

The pectin (0.1mg), a-Tocopherol and ascorbic acid dissolved in 1 ml of methanol was mixed with phosphate buffer (2.5 ml, 0.2M, PH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%); the mixture was incubated at 50C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 1000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

STATISTICAL ANALYSIS

Statistical analysis for the DPPH assay and the reducing capacity assay were carried out using two-way ANOVA (mixed model) using the Tukey model at an alpha value of $\alpha = 0.05$ (95%).

RESULTS AND DISCUSSION

Confirmatory Tests for Pectin

All samples were positive to Molisch and Fehling's Test (Table 1). The tests for presence of carbohydrate (Molisch's test), and reducing sugar (Fehling's test) showed a positive result for all the six samples. This result, though expected, confirms that pectins are polysaccharides. Neutral sugar units are attached to the backbone and concentrated in highly branched "hairy" regions in pectin (Abid *et al.*,2017). This statement acknowledges the presence of sugars in pectin structure.

The pectins also gave positive results to the solubility test in the various solvents confirming that the pectin from the peels were extracted as expected.

Table 1: Confirmatory test for pectin

Test	Observation	Inference
Carbohydrate (Molisch test)	A violet colour was observed at the interphase between the two layers.	Carbohydrate was present in all the six samples.
Reducing sugar (Fehling's test)	A brick red precipitate was observed.	's test Reducing sugar was present for all the samples.
Solubility in ethanol	Yellow gelatinous precipitate was obtained	Positive for the presence of pectin
Solubility in NaOH	A yellowish gel-like solution was obtained	Positive for the presence of pectin
Solubility in H_2SO_4	A whitish gelatinous solution was obtained	Positive for the presence of pectin

Yield of Pectin

The percentage yield of pectin from banana and plantain for unripe, ripe, and overripe represented as mean ± standard error of the mean. (Table 2).The extractions of pectin yield for banana peels increased from 3.04%, at its unripe stage to 5.84% at its ripe stage; then decreased to 3.70% at its overripe stage. The pectin content of plantain peels on the other hand, increased across the ripening stages–percentage yield 4.65%, 6.61% and 6.91% respectively.

In a study carried out by Castillo-Israel *et al.,* (2015), an increase in yield of pectin was observed in *saba* banana peels from unripe to ripe stage. With ripening, the connections between pectins and other cellular compounds become more fragile, making pectins more soluble, and more readily available for extraction, thus, pectin yield generally increases at first (Maduwanthi and Marapana, 2017). This mechanism could be responsible for the increase in pectin yield from unripe to ripe stage, observed in both banana and plantain peel samples.

Over-ripening of banana peel results in a decrease in yield due to the degradation of pectin under the action of enzymes: polygalacturonase, pectin methyl esterase or pectin lyase (Castillo-Israel *et al.,* 2015). In this study, banana peels pectin conformed to this decrease in yield, however, plantain peels did not. Plantain peel showed an increase instead, from ripe to overripe stage; the increase was however less rapid than that observed in the initial ripening stage. It is proposed that the enzymes responsible for degradation of pectin in plantain peels are in a probable lag phase of activity; and perhaps subsequently, enzymatic degradation actions would commence.

In all stages of maturation, pectin content in plantain peels was higher compared to banana peels. However, in a study carried out by Emaga *et al.,* (2008), a different result was obtained, where the pectin content in banana peels was higher compared to plantain peels, this can be due to the characteristics of pectin extracted from banana peels have been previously reported to depend largely on the extraction parameters as well as the varieties and maturity of the banana (Maneerat *et al.,* 2017). From this study, the yields of pectin in all samples were less than 7%. In a study carried out by Khamsucharit *et al.,* (2018), the yield of pectin from five varieties of unripe banana peel using citric acid solution ranged from 15.89 to 24.08%. The low yield obtained can be said to be due to the extraction reagents used and the conventional method employed. It is important to consider that the efficiency of advanced extraction methods such as microwave irradiation, when compared to conventional extraction methods is significantly higher (Thirugnanasambandham *et al.,* 2014).

Table 2: Percentage yield of pectin from banana and plantain peel

Sample	Mean ±SEM
Unripe Banana	3.04±0.97%
Ripe Banana	5.84±0.49%
Overripe Banana	3.70±0.68%
Unripe Plantain	4.65±0.70%
Ripe Plantain	6.61±0.21%
Overripe Plantain	6.91±0.86%

DPPH Antiradical Activity Assay

Table 3 shows the statistical data from DPPH antiradical activity assay for both banana and plantain at different stages of ripening.From the absorbance readings obtained, the percentage inhibition of each of the samples at concentrations 0.2, 0.4, 0.6, 0.8 and 1.0mg/ml were calculated. The general trend observed was an increase in percentage inhibition with increasing concentration, with the exceptions of unripe banana, unripe plantain and ripe plantain peel pectin. Unripe banana and ripe plantain peel pectin showed a reduction in percentage inhibition between concentration 0.4 and 0.6mg/ml (14.69%± 1.29 to 11.1%±2.62% and 34.19± 1.14% to 33.43± 0.55% respectively), after an initial increase in percentage inhibition observed between 0.2 and 0.4mg/ml. The increase in inhibition property of the samples however recommenced from 0.4 through to 1.0mg/ml. Unripe plantain peel pectin also, showed a sudden decrease in inhibition activity (65.22% ± 0.81 to 58.67% ± 8.05) between concentration 0.6 and 0.8mg/ml after initial increase in inhibition observed between 0.2 and 0.6mg/ml. However, the increasing percentage inhibition recommenced from 0.8 to 1.0mg/ml.

Antioxidants will react with DPPH, due to its hydrogen-donating ability at a very rapid rate. The amount of DPPH reduced could be quantified by measuring a decrease in absorbance at 517nm (Ebrahimzadeh *et al.,* 2017).Based on the statistical data obtained, the pectin's obtained are statistically different from the standard. This implies that though, the pectin have antioxidant activities, but compare to vitamin C, the standard, the samples were inferior. The mean percentage inhibition ± standard error of the mean is shown in Table 3. The mean percentage inhibition of the different pectin's obtained varies from that of the standard (ascorbic acid). An example is the value obtained for unripe plantain (73.78%) compared to the standard (95.68%) at 0.8 mg/ml concentration.

From a graph of percentage inhibition against concentration, IC_{50} of the samples were obtained. IC_{50} is the concentration of inhibitor required to produce 50% inhibition of an enzymatic reaction at a specific substrate concentration (Tom, 2018). It is used as a measure of antagonist drug potency, hence, the lower the IC_{50} value, the greater the potency – antioxidant effect in this case.The IC_{50}value of Ascorbic acid, Unripe banana, Ripe banana, Overripe Banana, Unripe Plantain, Ripe Plantain and Overripe Plantain were 0.062mg/ml, 4.824mg/ml, 1.157mg/ml, 2.552mg/ml, 0.477mg/ml, 1.320mg/ml and 0.828mg/ml respectively (See Table 4)

Unripe plantain showed the greatest antioxidant activity based on its low IC_{50} value (0.477mg/ml) compared to the other samples (IC_{50} value range 0.828mg/ml -4.824mg/ml).

Table 3: Statistical data from DPPH antiradical activity assay (Mean percentage inhibition)

Concentration (mg/ml)	**0.2**	**0.4**	**0.6**	**0.8**	**1**
Ascorbic Acid	95.87 ± 0.08	95.97 ± 0.15	96.06 ± 0.35	95.68 ± 0.17	95.68 ± 0.16
Unripe Banana	16.24 ± 0.90****	14.69 ± 1.29****	11.15 ± 2.62****	13.73 ± 0.80****	14.60 ± 0.37****
Ripe Banana	8.18 ± 2.20****	21.50 ± 2.70****	30.83 ± 1.26****	34.87 ± 2.15****	40.44 ± 1.29****
Overripe Banana	1.73 ± 1.64****	10.18 ± 2.64****	10.56 ± 1.38****	11.92 ± 1.59****	21.56 ± 4.26****
Unripe Plantain	51.79 ± 2.77****	58.31 ± 0.20****	65.22 ± 0.81****	58.67 ± 8.05****	73.78 ± 0.23***
Ripe Plantain	32.76 ± 0.22****	34.19 ± 1.14****	33.43 ± 0.55****	35.84 ± 1.63****	34.95 ± 1.55****
Overripe Plantain	24.49 ± 2.71****	33.42 ± 1.16****	41.31 ± 2.70****	48.21 ± 1.31****	54.18 ± 0.51****

*** = Significantly different from Ascorbic acid at $p \leq 0.001$)

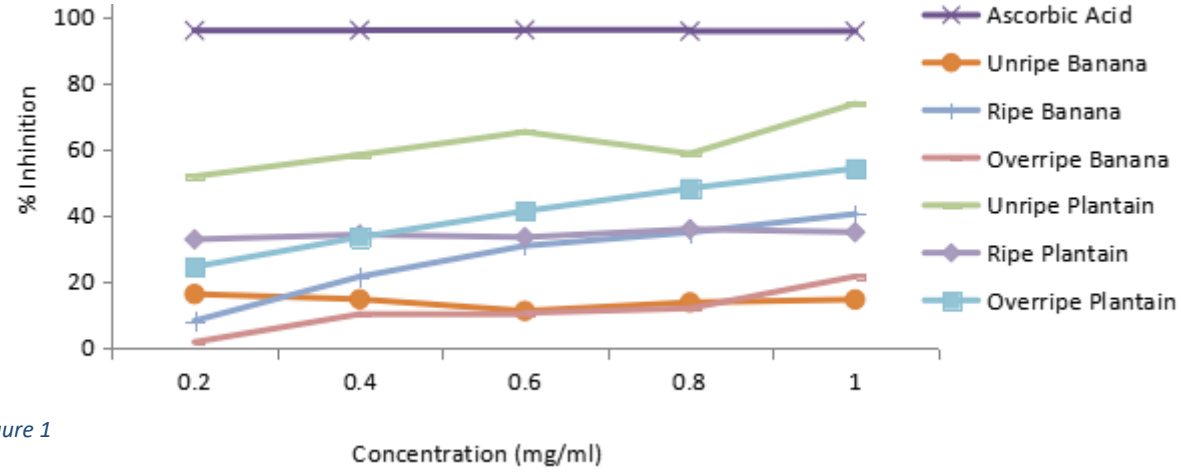

Figure 1

Figure 2: Percentage inhibition of samples against concentration

Table 4: IC_{50} value of Samples on DPPH antiradical activity

Sample	Ascorbic acid	Unripe Banana	Ripe Banana	Overripe Banana	Unripe Plantain	Ripe Plantain	Overripe Plantain
IC_{50}	0.062	4.824	1.157	2.552	0.477	1.320	0.828

Reducing Capacity Assay

Ripe banana peel pectin as well as overripe plantain showed an increase in absorbance along the concentration gradient 0.02-0.10mg/ml (0.124 - 0.261 and 0.176 to 0.349 respectively). Other samples showed irregular absorbance which did not follow the trend of increasing absorbance with increase in concentration.

Based on the statistical data obtained, all six samples were statistically inferior to Ascorbic acid in term of reducing capacity; with all samples showing statistical difference ($p \leq 0.0001$) at $p \leq 0.05$,with exceptions of ripe banana ($p \leq 0.005$), Overripe banana ($p \leq 0.001$) and Overripe plantain ($p \leq 0.005$)at concentration 0.02 with values that are about average compared to the standard. The experiment also showed that all samples of extracted banana and plantain peel pectin were statistically inferior to Ascorbic acid at concentrations 0.2 to 1.0mg/ml against DPPH. All samples at concentrations 0.02 to 0.10mg/ml were also statistically inferior to Ascorbic acid in terms of reducing capacity. Although, some of the pectin's obtained have activities that are more than moderate compared to the activity of the standard with varying level of inferiority.

Table 5: Statistical analysis for Reducing capacity assay

Concentration Samples	0.02	0.04	0.06	0.08	0.10
Ascorbic Acid	0.560 ± 0.0039	1.235 ± 0.1052	2.180 ± 0.0699	2.838 ± 0.0822	3.000 ± 0.0000
Unripe Banana	-0.067 ± 0.0207****	-0.087 ± 0.0133****	-0.008 ± 0.0460****	-0.056 ± 0.0377****	0.042 ± 0.0342****
Ripe Banana	0.124 ± 0.0272**	0.129 ± 0.0095****	0.207 ± 0.0387****	0.270 ± 0.0338****	0.261 ± 0.0493****
Overripe Banana	0.113 ± 0.0038***	0.148 ± 0.0095****	0.143 ± 0.0071****	0.171 ± 0.0028****	0.297 ± 0.0150****
Unripe Plantain	-0.055 ± 0.0354****	-0.089 ± 0.0094****	-0.075 ±0.0247****	-0.067 ± 0.0320****	-0.067 ± 0.0249****
Ripe Plantain	0.059 ± 0.0163****	0.133 ± 0.0304****	0.179 ± 0.0017****	0.175 ± 0.0435****	0.203 ± 0.0403****
Overripe Plantain	0.176 ± 0.0114**	0.208 ± 0.0135****	0.290 ± 0.0138****	0.317 ± 0.0072****	0.349 ± 0.0246****

**= Significantly different from Ascorbic acid at p=0.05

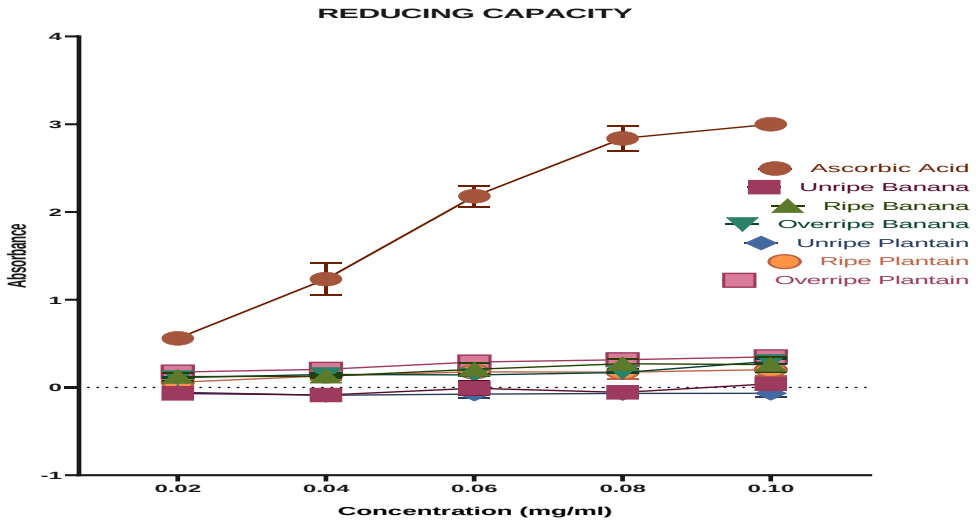

Figure 3: Absorbance against concentration for reducing capacity asssay

CONCLUSION

From the study carried out, the yield of banana peel pectin increased initially from unripe to ripe stage, then decreased from the ripe to overripe stage, while plantain peel increased across the ripening stages. In all stages of maturation, the pectin content in plantain peels was higher compared to banana peels. The study also showed that all samples of extracted banana and plantain peel pectin were statistically inferior to Ascorbic acid at concentrations 0.2 to 1.0mg/ml against DPPH. All samples at concentrations 0.02 to 0.10mg/ml were also statistically inferior to Ascorbic acid in terms of reducing capacity.

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