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Effect of Domestic Cooking on the Polyphenolic Content and Antioxidant Capacity of Plantain (*Musa paradisiaca*)

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Abstract: The effect of domestic cooking on the extractable, non-extractable, total phenolic contents as well as antioxidant capacity of ripe and unripe plantain flour was examined. Values of extractable polyphenols ranged from 2.49 to 6.11 mg/g garlic acid equivalent (GAE) for the ripe samples and between 3.46 to 7.40 mg/g GAE for the unripe samples. Total non-extractable polyphenol (TNEPP) values were between 5.5 and 15.18 mg/g sample and were significantly higher in grilled samples in both the ripe and unripe plantains. Total phenolic content varied between samples and processing methods but there was no significant difference between the grilled samples and the boiled with skin samples in the ripe plantains. Antioxidant capacities increased with increased concentration of extracts but were generally higher in the unripe samples.

Key words: Extractable polyphenols · Non-extractable polyphenols · Plantains · Domestic cooking

INTRODUCTION

Plantains are generally the larger, more angular starchy fruits hybrid triploid cultivars of the banana family intended for cooking, but also edible when fully ripe, [1]. In Nigeria and other parts of Africa and in many other places in the world, plantain (*Musa paradisiaca*) serves as a major staple food and is particularly desired for the variability in the stages of ripeness and in cooking methods. Plantains can be consumed in the unripe, fairly ripe, ripe and overripe stages. They are sometimes eaten raw by most farmers on the farm, but many households prefer to have them as boiled, grilled or fried. Sometimes steaming is done without removal of the plantain peels. Ripening stage or cooking methods used is always a function of individual preference but sometimes as a result of the fact that plantains just like bananas are very perishable and need to be consumed in good time if wastage is to be avoided.

Studies on the antioxidant capacity of plantain are very scarce in literature probably because of the assumption that it may not contribute significantly in this regard since it is a high carbohydrate food. The only work reported in relation to polyphenolic activity of plantain was done by Lewis and co-workers [2] when they identified the antiulcerogenic agent in unripe plantain banana as the natural flavonoid leucocyanidin. Unfortunately these prophylactic effects are lost when plantains are cooked [2].

It has become necessary to access the effect of cooking on total phenolic content of plantains since they are normally cooked before being consumed. It is also known that cooking induces significant changes in chemical composition, affecting the bioavailability and content of chemo-preventive compounds in vegetables [3].

The cooking methods (boiling, boiling with skin, grilling and frying) used in this work reflect the major ways in which plantains are consumed in a typical African diet. This work is being carried out because data showing the effect of domestic cooking methods on bioactive components such as polyphenols in plantain are scarce. Until recently, most data available on the polyphenol content of foods actually referred to the extractable polyphenols which meant that the total phenolic content of foods/fruits was actually underestimated. Non-extractable polyphenols are high molecular weight proanthocyanidins and phenolics associated with dietary fibre and indigestible compounds that are not taken into account in chemical and biological studies [4]. Literature are data limited for non-extractable polyphenols, [5] therefore reference to relationship to other studies are based on few data sources available.

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Simple phenols and flavonoids represent the vast majority of plant phenolics. Most of these compounds are of relatively low molecular weights and are soluble according to their polarity and chemical structure (degree of hydroxylation, glycosylation, acylation, etc.). Tannins are another group of plant phenolics and are compounds of intermediate to high molecular weight. They are highly hydroxylated molecules and can form insoluble complexes with carbohydrates and protein. Plant tannins can be subdivided into two major groups: (1) hydrolyzable and (2) condensed tannins. Hydrolyzable tannins consist of gallic acid and its dimeric condensation product, hexahydroxydiphenic acid, they are easily hydrolyzed with acid, alkali and hot water and by enzymatic action, which yield polyhydric alcohol and phenylcarboxylic acid. Condensed tannins or proanthocyanidins are high-molecular-weight polymers. Oligomeric proanthocyanidins and low-molecular weight hydrolyzable tannins are soluble in different aqueous and organic solvents, such as acetone, methanol and water. However, high-molecular-weight condensed and hydrolyzable tannins are insoluble. In addition, when tannins form complexes with protein or cell wall polysaccharides, they remain insoluble. This insolubility of tannins is responsible for significant errors in the quantification of the polyphenolic content of plants, because polyphenols usually are analyzed in extracts, often omitting the quantification of insoluble or non-extractable tannins [6].

This work therefore involved quantification of total phenolic content of plantains which includes both extractable and non-extractable contents, their changes during domestic cooking processes and the impact of cooking on antioxidant activity of extractable polyphenolic portions.

MATERIALS AND METHODS

Sample Collection and Preparation: Cultivars of ripe and unripe plantains were purchased from Leeds city market. Unripe plantains selected were full green (stage 2) while ripe plantains used were in fully ripe stage (yellow) in colour (stage 6) on the colour index scale [7]. Both ripe and unripe plantains were divided into five portions each. The peel was removed from all five portions except portion two. Portion one was and boiled, two was boiled with the skin on, three was fried while four was grilled, five was used raw. Each process was conducted three times for the four cooking methods. The samples were cut into thin slices of 2mm in diameter, freeze dried and blended into fine flour and stored way in clean plastic containers for future analysis.

Cooking Processes: *Boiling:* 150gram of sample was boiled in 300ml distilled water for 10 minutes in a clean pot with a lid.

Grilling: 150 gram of sample was grilled at high/medium heat in a grill for 30 minutes.

Frying: 150g portion of samples were cut into slices of 2mm in diameter and deep fried in vegetable cooking oil at 190°C for approximately 5 minutes.

Determination of Polyphenols

Polyphenol Extraction: Samples were extracted by vortex-mixing 1g of sample (flour) with 20ml solvent mixture of methanol: Acetone: water (5:7:8) at room temperature for 30 minutes, after which they were centrifuged at room temperature for 10minutes at 3000g. The supernatants were used for the determination of extractable polyphenols while the residues were used for the determination of non-extractable polyphenols [5].

Determination of Extractable Polyphenol Content: Extractable polyphenols were determined by the method of Folin-Ciocalteau with little modification using Gallic acid as standard, [8]. 100µl of sample was taken in a glass tube, 1.5ml of water was added and 100µl of Folin-Ciocalteau reagent was then added. The mixture was allowed to stand for 5 minutes before the addition of 300µl of 20% Sodium Carbonate solution. Tubes were incubated at 37°C for 30min and absorbance read at 765nm against a reagent blank.

Determination of Non-Extractable Polyphenol Content

Determination of Non-Extractable Polyphenols: 200mg of extraction residue was treated with 10ml HCl:Butanol 1:200 (v/v) in a boiling water bath for 180 minutes (9).Tubes were centrifuged after cooling at 2500g for 10minutes at room temperature and absorbance measured at 520nm using Cyanidine Chloride as standard (2).

Determination of Hydrolysable (Non-Extractable) Polyphenols: Hydrolysable polyphenols in the extraction residues were released by strong acid hydrolysis using 20ml methanol: sulphuric acid 9:1 (v/v) in a boiling water bath for 20 minutes [9]. Tubes were centrifuged after cooling, 2000g at room temperature and hydrolysable polyphenols were determined by Folin-Ciocalteau reagent. Results were expressed as gallic acid equivalents.

Determination of Antioxidant Property: The antioxidant activity of the plantain extracts was assessed on the basis of free radical scavenging capacity of the extracts on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) when compared with vitamin C. The method of Cavin, *et al.* [10], with some modification was used. 10, 20, 30, 50 and 100µl of sample extracts and vitamin C solution (representing 25, 50, 75, 125 and 250µg/ml, respectively) were taken in triplicate in different test tubes and 2ml 0.004% DPPH solution was added. The solutions were allowed to stand in the dark for 10 minutes after which absorbance was measured at 517nm. A control of 2ml DPPH was used while the reagent blank was 2ml of 95% methanol.

The DDPH Scavenging Capacity of Samples and Ascorbic Acid Were Calculated as Follows:

$$\% \text{ FRS of DPPH} = (A-B)/A \times 100$$

A= Absorbance of Control
B= Absorbance of Sample/ Vit C

RESULTS AND DISCUSSION

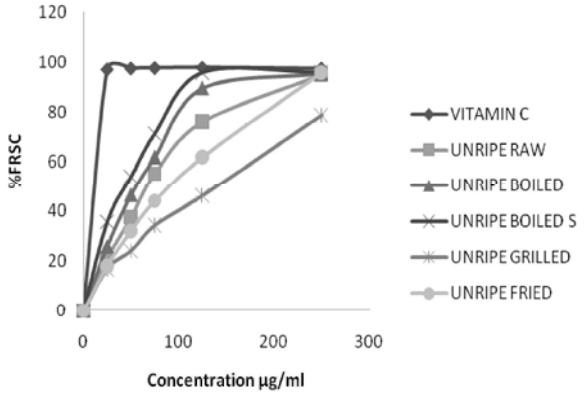
Information on table 1 shows that extractable polyphenols obtained were higher in the raw unripe samples than in the raw ripe samples, however most of the processed samples showed increases in extractable polyphenols and this is favourable disposition for food processing conditions, especially those requiring thermal treatments.

The effect of domestic cooking on the extractable and non-extractable polyphenols was widely varied in trends. The increase in soluble polyphenols in the boiled samples may be due to alterations in chemical structure/ composition, causing them to be more readily detected in the supernatants of the extract [11]. Reduction in soluble polyphenols and increase in non-extractable polyphenols in the grilled samples were observed. It is apparent therefore that each processing method results in different types of bonds being formed between phytochemicals present and cell structure which subsequently leads in higher or lower cleavage of phenolic bonds according to the type of heat applied resulting in the different responses observed in this study [12]. A similar observation to that made in this study was reported in a study involving flavonols where baking resulted in a 7-25% gain in quercetin while boiling led to an increase of 18% in quercetin concentration [13]. It is also possible that higher contents of hydolysable polyphenols in almost all the processed samples than raw samples may be due to interactions between polysaccharides and polyphenols because a similar trend was observed with potatoes and this trend is contrary to that observed in other vegetables e.g. carrot, broccoli and white cabbage which are not rich in polysaccharides [14]. One of the mechanisms proposed to explain the interactions that occur between polysaccharides and polyphenols suggests the formation of hydrogen bonds between the hydroxyl groups on the polyphenol and the oxygen atoms from the sugar of the cell wall polysaccharide: this interaction can then form dextran gels, which are able to encapsulate the polyphenols [15].

Table 1: Total polyphenol content of ripe and unripe plantains mg/g dry sample

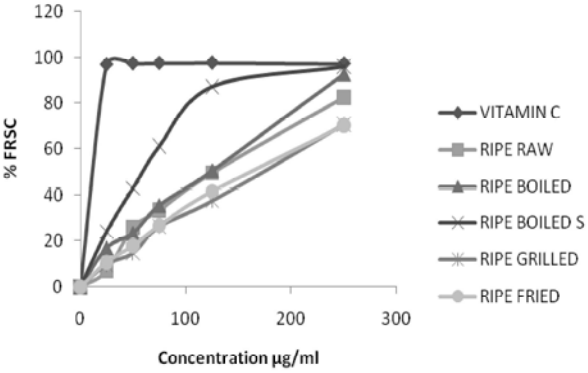
	Extractable	non Extractable			Total
Sample	EPP	HPP	NEPP	TNEPP	Polyphenolic Content
Unripe raw	$5.74^{d}\pm0.10$	$4.04^{h}\pm0.19$	$3.45^{c}\pm0.21$	$7.49^{e}\pm0.05$	$13.23^{d}\pm0.05$
Unripe boiled	$6.46^{b}\pm0.20$	$4.00^{h}\pm0.04$	$3.43^{c}\pm0.15$	$7.43^{e}\pm0.19$	$13.89^{c}\pm0.29$
Unripe boiled S	$7.40^{a}\pm0.41$	$4.31^{g}\pm0.093$	$3.76^{b}\pm0.38$	$8.06^{d}\pm0.35$	$15.47^{b}\pm0.67$
Unripe Grilled	$3.46^{g}\pm0.12$	$10.35^{a}\pm0.40$	$4.83^{a}\pm0.07$	$15.18^{a}\pm0.46$	$18.63^{a}\pm0.45$
Unripe fried	$3.77^{f}\pm0.11$	$5.71^{d}\pm0.15$	$3.39^{c}\pm0.05$	$9.10^{c}\pm0.19$	$12.87^{e}\pm0.27$
Ripe raw	$2.84^{h}\pm0.19$	$5.17^{f}\pm0.24$	$1.95^{e}\pm0.05$	$7.12^{f}\pm0.28$	$9.96^{g}\pm0.19$
Ripe boiled	$4.16^{e}\pm0.26$	$3.69^{i}\pm0.08$	$1.86^{e}\pm0.03$	$5.56^{g}\pm0.08$	$9.71^{g}\pm0.19$
Ripe boiled S	$6.11^{c}\pm0.59$	$5.37^{e}\pm0.18$	$1.95^{e}\pm0.15$	$7.32^{f}\pm0.20$	$13.43^{d}\pm0.61$
Ripe Grilled	$2.49^{i}\pm0.05$	$8.22^{b}\pm0.27$	$2.71^{d}\pm0.06$	$10.92^{b}\pm0.22$	$13.41^{d}\pm0.25$
Ripe Fried	$2.99^{h}\pm0.05$	$7.12^{c}\pm0.18$	$1.95^{e}\pm0.14$	$9.06^{c}\pm0.14$	$12.06^{f}\pm0.19$

Values are means of triplicate determinations
Boiled S-plantain boiled with skin
EPP and HPP are expressed as Gallic acid equivalent (GAE) IN SAMPLE (mg/g)
NEPP are expressed as mg/g cyanidine chloride in sample
EPP = EXTRACTABLE POLYPHENOLS, HPP = HYDROLYSABLE NONEXTRACTABLE POLYPHENOLS,
NEPP = NONEXTRACATBLE POLYPHENOLS, TOTAL NEPP (TNEPP)-SUM OF HPP AND NEPP, TOTAL PHENOLS = EPP+HPP+NEPP.
TOTAL NEPP AND TOTAL PHENOLS ARE EXPRESSED AS mg/g of sample
Values with different superscripts in the same column are significantly different while values with the same superscript in the same column are not significantly different (at 95% confidence level).



Boiled s-plantain boiled with skin.

Fig. 1: % Free Radical Scavenging Capacity of Vit C and Unripe Flowers



Boiled s-plantain boiled with skin

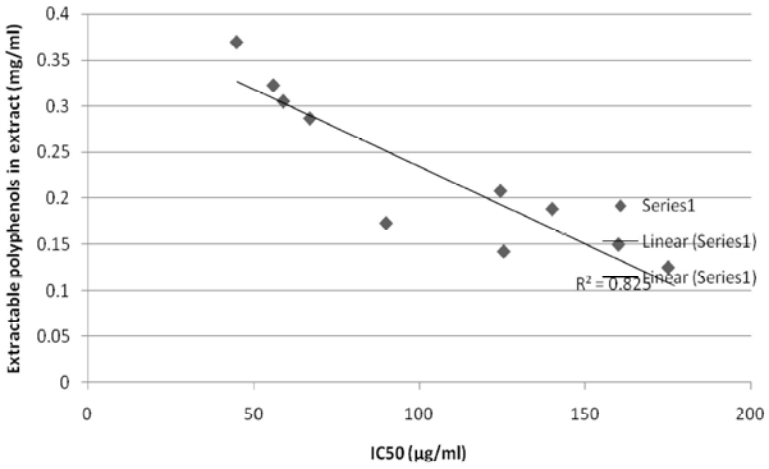
Fig. 2: % Free Radical Scavenging Capacity of Vitamin C and Ripe Flowers

Extractable Polyphenol Contents Observed Are of Reasonable Quantity: 0.287 mg GAE/ml for the raw unripe plantain is almost the same as determined for conventional carrot, 0.289 mg GAE/ml and slightly lower than for conventional Onion 0.325mg GAE/ml, [14]. Values for extractable polyphenols for raw and processed samples were lower than that obtained for potatoes (15.1mg/g GAE, dry weight), [16], which is also a high carbohydrate food. Potato however showed a decrease with boiling (13.7mg/g GAE), contrary to what was observed with both ripe and unripe plantains. This may be due to the cooking method used which involved a higher volume of water as well as the separation of the cooking water from the potatoes after boiling, [16].

Antioxidant capacity at various concentrations, (figures 1and 2) increased markedly with boiled samples, this is similar to that observed for tomatoes [17,8] and carrots [19]. It is noteworthy that plantains, carrots and tomatoes are very rich in carotenoids and the increase in antioxidant capacity observed may be linked to the release of carotenoids after cooking. On a table detailing the polyphenolic content of different foods and beverages [6] in which most of the polyphenols listed are phenolic acids and flavonoids (including anthocyanins, procyanidins, flavanones, flavanols, etc.), results of total phenolic content of plantain flours obtained in this work compares favourably with other carbohydrate rich foods on this table. Both raw and processed flours have higher phenolic content than rice, oats, corn and wheat with 8.6, 8.7, 30.9 and 22-40mg/100g respectively. They are however in a comparable range to that of Barley, millet and sorghum (1200-1500, 590-1060, 170-10,260mg/100g respectively) and could therefore compete favourably with these flours in uses that require considerations of polyphenolic content. It also shows that it will contribute a grade deal to daily intake of polyphenols and may be contribute better than most cereals if it is used in preparation of breakfast cereal or consumed wholly as a breakfast meal.

Table 2: IC_{50} Values for Vitamin C and Plantain Samples

Sample	IC_{50} (µg/ml) (mg/ml)	ExtractablePolyphenol
VITAMIN C	10	
UNRIPE RAW	67	0.286
UNRIPE BOILED	56	0.322
UNRIPE BOILED S	45	0.369
UNRIPE GRILLED	90	0.172
UNRIPE FRIED	140	0.188
RIPE RAW	125.5	0.142
RIPE BOILED	124.5	0.207
RIPE BOILED S	59	0.305
RIPE GRILLED	175	0.124
RIPE FRIED	160	0.149



Boiled s-plantain boiled with skin

Fig. 3: Correlation Graph Between Antioxidant Capacity And Phenolic Content

The free radical scavenging capacity (as indicated by the IC50 values in table 2) of the extractable polyphenols reveals that all of the samples have lower antioxidant capacity than vitamin C, with IC50 10µg/ml. Increased radical scavenging capacities were observed with the boiled samples for the unripe plantains while marked increase in free radical scavenging capacity for the ripe plantains was shown only in the boiled with skin sample. IC50 values ranging between 45-140 for most of the samples is lower than that of black pepper [20] indicating a better free radical capacity than black pepper. IC50 value for the unripe raw flour is similar to that obtained for ginger, 65.1±1.7 [20],IC50 values for the boiled samples except for the ripe boiled plantains having values ranging from 45 to 59 indicate better scavenging capacity than ginger.

Figure 3 however, indicates a that a positive correlation was observed between antioxidant capacity represented by IC5O values and extractable polyphenol content of the samples.

CONCLUSIONS

Plantains, both raw and processed have reasonably high quantities of polyphenols, hence further work on determination of constituents of the extractable and non-extractable polyphenols will be important in further accessing its potential antioxidant properties.

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