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Prevention of Product Blackening by Inhibition of Polyphenol Oxidases in Plantain Fruits(*Musa paradisiaca*)

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ABSTRACT
Plantain fruits suffered post-harvest losses at glut season sandwhen overripenedare rejected while the prices get reduced ridiculously. Drying of Plantain as an ultimate method of preservation suffered setback because of products blackening often caused by enzymatic reaction of polyphenol oxidases (PPO) and the phenolic contents. This research is aimed at improving the quality of the product by limiting extent of blackening. Matured fresh unripe plantain fruit 2 to 3mm disk for werepre-treatment with blanching at 50^0Cto65^0C temperaturesand administration of Ascorbic acid in the range of 1.5%, to 2.5% v/v liquorconcentrations. The samples were dried to constant weight with less < 15% w/w of water content. The pastry from powdered dried plantain samples were analysed for browning index, nutritional and sensory (colour and texture) qualities. A combination of blanching at 65°C with ascorbic acid (2.5%) for 30 min was found to be the most efficient in inhibiting the PPOs when compared to other pre-treatments. The proximate analysis of Fresh (FP) and Blanched (BP) plantain products revealed increased Carbohydrate, Ash, Protein and Fibre content over samples not pre-treated. Pre-treatment of plantain with blanching and ascorbic acid improved the product qualities including colour retention hence more acceptable.A reduction in the processing time from the slicing to powder production, improved the appearances the most and this will increase its acceptance generally.
Keywords: plantain; blanching; ascorbic acid; polyphenol oxidase; inhibition

I. INTRODUCTION

Plantain (Musa species) are arable crops and the fruits are rich in different degrees of carbohydrate as it contained high starch content when unripe but when ripe contained higher percentages of fructose, minerals and vitamins.In west and central Africa, about 70 million people are estimated to derive more than a quarter of their food energy requirement from plantain and Nigeria is the leading producer of plantain in West Africa region with an estimated annual production of over three million metric tonnes (FDA, 2005). It is grown extensively in the southern parts of Nigeria but about 35 to 72% of this production is wasted to post-harvest losses (Kaanane, and Labuza, 1989; AFPGEAN).

In thecoastal region of West Africa are good sources of nutrients, the utilisation of Musa speciesare enormous and they are used for different dishes, cooked or eaten in roasted forms, pastry or dried into chips or powdered for storages. However, the products become unpopular because of the blackening, resulting from oxidation of the polyphenyl group asthey loose their colour status in the course of processing. They are also perishable commodities as a result of high-water contents when ripe and this enables microorganism to grow easily thus causing disintegration and rot within a short period of time after harvest.

Plantaindepreciates due to rapid respiration after harvest and senescence. Consequently, they are processed into flour in order to extend their shelf life. Over the years, this has been done by sun drying for storage or pulverized into flour for pastry making popularly known as 'Amala' in the South west region of Nigeria before consumption. There are some constraints associated with the usualsun drying process such as slow dryingrate, uncertainty of the weather and uneven drying (Arinolaet al., 2016).Discolouration of the final product when compared to that of fresh plantain made it unpopular andundesirable.Theassociated discolouration (blackening or deep browning) of final product was reported to be a result of Enzymatic browning reaction wasalso reported to affect theflavour, and nutritional value of the products with consequential economic losses when not sold to consumers in time (Corzo-Martínez, 2012; Holderbaum, 2010 andEscalante et al., 2018).

This browning reaction usually occurs during processing as a result of the enzyme activity of polyphenol oxidase which is also known as tyrosinase. It is a copper-containing protein that belongs to the group of

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oxidoreductaseswhichcatalyze the hydroxylation of monophenols to o-diphenols and oxidation of p- and o-diphenols to p- and o-quinones(Golan and Whitaker, 1984).Enzymatic browning makes the product dark, limits the storage and contributes to reduction of its organoleptic quality.Major factors that determine the rate of enzymatic browning of fruits and vegetables are the concentrations of both polyphenol oxidases (PPO) and phenolic compounds present, as well as the prevailing media acidity (pH), temperature and ambient oxygen content.

The stability of polyphenol oxidases (PPO) was found to differ with species and the prevailing temperature.Liu et al., (2007) showed that PPO activity decreased continuously with increased temperatures. A complete inactivation was reported at higher temperature treatment between 60ºC and 75ºC for 10 to 30 min. (Gisele et al, 2010 and Jiang 2001). However, blanching pre-treatment for enzyme inactivation in vegetables and fruitsabove 50°C may result in undesirable colourand changes in the texture.

Blanching is a short heat pre-treatment to which food materials may be subjected for maintenance, or to inactivate quality-changing enzymes to reduce deterioration reactions of quality attributes such as colour and texture, with positive effects of annihilating the surface microbes(Jaiswal et al, 2012;Caciano and Renata, 2012)). Many compounds may be used as well to reduce polyphenol oxidase (PPO) browning in foods, one of such is application of sulphating agents which are found to be very effective and inexpensive, but they are toxic to human health(McGhie et al., 2005). Due to its adverse health effects, there has been growing interest in the use of non-sulfite anti-browning agents to replace sulfitespreservatives (Ozoglu and Bayindirli, 2002).

Recently, one of the most commonly used compounds is ascorbic acid and its derivatives such as cysteine and glutathione.Ascorbic Acid (Vitamin C): is effective in preventing discolouration in most fruits. Not only does it preserve natural colour and flavour of fruits, but it adds nutritive value as well. Ascorbic acid in powdered form is available at some drugstores or where freezing supplies are sold. Ascorbic acid tablets may be more readily available and less expensive, but are more difficult to dissolve. They do need to be finely crushed before use. These are anti-browning agents that were discovered to possess the ability to effectively combat and control enzymatic browning. McEvily et al, (1992) also recorded that ascorbic acid reduces o-quinones to diphenols and prevents the formation of browning pigments and that PPO was completely inhibited at a pH below 3. These compounds takes effect in anti-browning processes either by reducing the formed o-quinones instantly to the original substrate (colourless diphenols) at high concentration (0.5 to 4%) or by reacting irreversibly with o-quinones to form stable colourless products(Holzwarth et al., 2012). However, the effect of ascorbic acid is found to be temporary, since once added, it is completely oxidized and O-quinones could accumulate, leading to browning pigment formation (Ozoglu and Bayindirli, 2002; Jang et al., 2011).Nature Seal, which contains mainly calcium ascorbate, had also been widely used for browning control of cut apples. (Abbott et al, 2004).

Other techniques that had been explored includes; using acids eg Lemon juice and other acids lower the pH and remove the copper cofactor necessary for the responsible enzymes to function;Jiang (2001) found that the PPO activity in litchi was maximum at pH 6.8 with 4-methylcatecol, while below pH 4.0 no enzyme activity was detected. Sellés-Marchart et al. (2006) reported that browning of litchi fruit can be controlled by applying acid or alkaline solution treatments.Several methods have been employed to prevent or inhibit enzymaticbrowning of plantain (and other foods), but each of this methods are based on two foundations, either by inactivating the necessary ingredients “enzyme” or by removing essential components (most often oxygen) from the product using antioxidants.

Information about the effects of enzymatic browning on the proximate composition, functional, colour and pasting properties of plantain flours with respect to the most effective drying methods is very uncommon while the control is very important. It is imperative to preserve the colour of our processed final product because it is a significant attribute that influences consumer’s decision on the acceptance. Hence, main objective of this study was to analyse the influence of temperature and ascorbic acid concentration on the sensory qualities (colour) and functional composition of unripe plantain.

II. MATERIALS AND METHODS

Matured fresh unripe plantain fruit was procured from the local market in Epe. The recipe was prepared by peeling and cutting it into disks of about 2-3mm thickness using a sharp knife and kept in a desiccant. Ethanol and ascorbic acid used in the investigation were of analytical grade which were procured from standard local suppliers.

Experimental Procedure

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The treatment solution concentration was prepared by weighing known mass varying mass of ascorbic acid (1.0g, 1.5g, 2.0g and 2.5g) and dissolving in 100ml of water. A 100g each of plantain rings were subjected to three different treatment plans; The control experiment had the samples plantain disks processedinto flour without any pre-treatment; thesecond batch were blanched in hot water of about 50 ^{o}C and 60 ^{o}C with the varying concentrations of the ascorbic acid treatmentfor 30 minutes in each of the cases.

Treated samples of plantain chips were immediately drained, and moped with paper towel to remove any foreign material and subjected to two forms of drying. The first batch was sun dried, while the other was dried in an oven at 50^{0}Ctill constant mass is recorded. This was achievedabout 15 hours of continuous drying in the oven. The samples were immediately crushed andmilled into flour in a hammer-millinto fine grained powder and packaged in air tight containers forpastes preparation and further analysis.

About 100g each of the powdered samples were prepared into thick paste of homogenous texture in a 250ml Beaker of heated water at 100 deg C. Products from the sample cases were compared with the controlled and were evaluated using a 7-point hedonic scale ranked between extremely like and extremely dislike for 1 and 7 points scale respectively. Each sample was assessed for taste, texture, appearance and general acceptability on clean foil paper by individuals familiar with the product under fluorescent light at room temperature (25°C)..

2.3 Browning Evaluation

The effectiveness of treatment on inhibiting browning of plantain fruit was carried out using the assessment ofSupapvanich et al., (2011). 1 g of dried treated plantain powder was extracted using 20 mL of 65% (v/v) ethanol. The mixture was left in a room of 27^{o}C for 30 min. The extract was filtered with cotton into a test tube and the absorbance of extract was measured at 420 nm using a spectrophotometer (Biowave 2, Denville, UK).

2.4 Analysis of Proximate Content

The proximate parameters such as ash, crude fat, crude fibre, crude protein and carbohydrate contents were determined using the procedure described by AOAC, 1990. The moisture content in each of the samples was determined by drying known weight of sampleto constant weight in the oven at about 105^{o}C. The differences weredetermined and the percentage moisture content calculated were recorded.

The ash content was determined for each sample by the incinerating known weight of samples in a muffle furnace maintained at 550^{o}C for 5 –8 hours while the crude fibre content was obtained by digesting 2g of the samples with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550^{o}C for 5 –8 hours. The crude protein (% total nitrogen x 6.25) was determined usingKjeldahl method, with2g of each of the samples. The crude lipid content was also determined by exhaustively extracting 10g of each sample in a Soxhlet apparatus using n-hexane as the extracting solvent and the carbohydrate content was determined by deducting the total percentage of moisture, ash, fibre, fat and protein from 100g.

III. RESULTS AND DISCUSSION

3.1Effect of Ascorbic Acid and Heat Treatment on Browning of Plantain Flour

Fresh plantain fruits is highly susceptible to enzymatic browning when it comes in contact with air. Plant responds spontaneously to Browning reactiondue to enzymatic oxidation of Dopamine by polyphenol oxidase leading to the production of brown pigments (Ranveer et al., 2010). Based on physicochemical characteristics and the thermal stability of PPOs from plantain, the two methods developed to counter the browning of the puree involved the use of blanching atalone was considered and secondly blanching combined with ascorbic acid Powdered samples of the plantain were prepared into pasteryandmeasured for the extent browning.

The sample were labelled as follows: **B$_1$**untreated plantain, **Ba$_1$**blanching with ascorbic acid at 50^{0}C, **Ba$_2$**blanching with ascorbic acid at 50^{0}C, **Bb$_1$**blanching with ascorbic acid at 65^{0}c, **Bb$_2$**blanching with ascorbic acid at 65^{0}c, **Bc$_1$**blanching at 65^{0}c and **Bc$_2$**blanching at 65^{0}c. Samples with subscript 1 were sun dried while the ones with 2 were oven dried.

3.2: Effect of blanching and Ascorbic Acid concentration

Table 1 showed the gravimetric analysis of theweight of samples during drying and it can be observed from the table that there is little or no difference in their overall weight loss percent. The slight variation perhaps due to the effects of the ascorbic acids introduced into the medium as witnessed as the weightsof the samples and the respective percentage water loss which were minimal. However, at higher temperature of 65 ^{o}C,

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the percentage water loss were higher average was recorded as shown in Table 1:

Table 1: Effects of Ascorbic Acid concentration and Temprature on 100g

S/N	Asc Acid (mg)	50 C	% water loss	60 C	% water loss
1	0	47.9	52.1	47.9	52.1
2	1.5	46.6	53.4	47.85	52.15
3	2	46.9	53.1	47.6	52.4
4	2.5	46.1	53.9	47.8	52.2

In the present investigation, the data in Table 2 reveal the effect of different anti-browning pre-treatments on browning of unripe plantain flour. It was observed from the data that the control recorded a browning of 0.476 Refractive Index, and it was the lightest of the samples. These values were compared against the treated plantain sample to ascertain the extent of inhibition of browning. In the case of $Ba_1$and Ba_2as against the control (B_1)The activity of PPOwhich were supposed to reduce the. It can be observed that there is an increase in PPO activity as time progresses despite these treatments which is in contradiction to the report of Arlette et al, (2018) who observed thatthere was a 90% reduction in the activity of PPOs from plantain at times ranging from 31 to 47.27 min at 60°C when heat treatment was combined with the addition of ascorbic and citric acid.However, reversal of the effects of the Ascorbic acid may be reversed by oxidation

Similarly, a mild heat treatment of 40°C and 5 min was reported as promising approach to inhibit browning in sliced button mushrooms (Zhang et al, 2017). This could be due to the fact that the heat treatment didn't stop the activity of PPO but only lowered its power and the other fact was that the ascorbic acid was only able to hinder the process for a while by reducing the o-quinones that polymerizes to produce brown pigments without affecting the enzymatic activity. This was also supported by Tortoe et al., (2007) who observed that the effect of ascorbic acid can be considered temporary because it is oxidized irreversibly by the reaction with pigment intermediates, endogenous enzymes, and metals such as copper. As a result, the browning reaction continued throughout the drying, milling and storage period before being analysed. It is observed that the samples labelled 2 (dried with oven) had lower absorbance values when compared to sample 1 which took lesser time to dry completely. The same can be said for the other samples, but combination of blanching at 65°C with ascorbic acid (2.5%) for 30 min was found to be the most potent inhibitor of PPOs when compared to other treatments.

3.2 Appraisal of Extent of Blackening

The sample products of the dried and powdered plantain samples were prepared under the same conditions the observe the effects of the different concentrations of ascorbic acid and the blanching. Some differences observed in colour of the samples were evaluated by subjecting the pastry obtained to Absorption spectra, the pictorial observations were also as presented in Table 2.

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Table 2: Effects of the Ascorbic and blanching on the respective samples

SN	Samples	Sample Description	Mean Absorbance	Pictorial views of samples	Remarkes
1	B_1	Control sample	0.476		
2	Ba_1	1.5g Ascorbi conc 50 C	1.252		
3	Ba_2	1.5g Ascorbi conc 50 C	1.051		
4	Bb_1	1.5g Ascorbi conc 50 C	1.217		
5	Bb_2	1.5g Ascorbi conc 50 C	0.993		
6	Bc_1	1.5g Ascorbi conc 50 C	1.028		
7	Bc_2	1.5g Ascorbi conc 60 C	1.082		
8		No pretreatment	0.560		
9		Freshly Prepare without pre-treatment or Powdered	0.414		

3.3 Proximate Analysis of Samples

The proximate composition of the samples is shown in Table 3. The proximate composition of the unripe plantain revealed that the carbohydrate had the highest value followed by the moisture content, protein, fat, ash and fibre in the fresh plantain sample. When compared to the values from the blanched plantain samples, the result revealed that the fresh unripe plantain (FP) and blanched unripe plantain (BP) contain varying percentages of carbohydrate, protein, fat, moisture, ash and crude fibre. The moisture content of the **FP** (15.30%) was greater than **BP** (11.50%), protein of the **FP** (3.22%) was lesser than **BP** (3.62%)**,** fat of the **FP** (2.90%) was greater than **BP** (2.56%)**,** ash of the **FP** (2.53%) was lesser than **BP** (3.05 %) and fibre content of the **FP** (0.32%) was lesser than **BP** (2.63%). Fibre had the lowest proportion in **FP** (0.32%) while fat was the lowest in **BP** (2.63%)**.**The moisture level of the samples was very high compared to the value of 9.03% reported by Falola et al, (2011). The moisture content of foods or its processed products could be used to predict its freshness and shelf life.High moisture content subjects' food items to increased microbial spoilage and short shelf life, which can lead to its deterioration Adepoju and Onasanya, (2008). According to Luzia and Jorge (2014), the proximate composition of fruits can be influenced by several factors, including variety, cultivar, maturity, climate and geographical condition of production, handling during and post-harvest, processing and storage. Furthermore, the species type, growing conditions, and the interaction between the soil and environmental characteristics especially the weather condition may also influence directly the composition of the fruits.

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Table 1: Proximate composition of unripe plantain per 100 g samples

Sample fresh Plantain sample (FP)	Moisture	Ash	Fibre	Fat	Protein	Carbohydrate
%	15.30	2.53	0.32	2.90	3.22	75.74
Blanched Plantain sample (BP)	11.50	3.05	2.63	2.56	3.62	76.18

*****% dry weight basis

IV CONCLUSION

A combination of blanching at 65°C with ascorbic acid (2.5%) for 30 min was found to be the most potent inhibitor of PPOs when compared to other treatments. However, the physicochemical properties and thermal stability of these PPOs activities, treatments carried out on the plantain samples led to a reduce and temporary halt in browning activity of the plantain samples. The thermal treatment of the samples combined with the addition of ascorbic acid would have had good effects on the organoleptic quality (colour) of the generated "Amala" plantain semolina if the drying was done in a short period of time after the treatment. This would have eliminated the medium in which reaction reversal would take place. It is recommended that more research be done into a better way by which this reaction can be halted and drying completed as soon as possible. It isbelieve that this approachimproved the colour of plantain for better acceptance and thus contribute to food security on a global scale.

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