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EVALUATION OF ASCORBIC ACID AND SODIUM METABISULPHITE AS INHIBITORS OF BROWNING IN YAM (D. rotundata) FLOUR PROCESSING

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Abstract
The inhibition of polyphenol oxidase (PPO) and browning by ascorbic acid and sodium metabisulphite in the processing of yam flour (elubo) was investigated. The physicochemical properties and organoleptic score of flour meal (amala) samples were also evaluated using standard methods of analysis. The yam samples with or without inhibitors were steeped in water for 12hrs at 30^0C, 40^0C and 50^0C. Polyphenol oxidase activity and browning index were monitored at 2hrs interval. The study revealed that browning is temperature-dependent in all the yam samples. Sodium metabisulphite completely inhibited browning in the processed yam flour. Polyphenol oxidase activity and browning index were positively (53.2%) correlated. Browning and PPO activity, temperature and steeping time were also negatively correlated. However, a significant increase ($p<0.05$) in the calorific energy of yam sample with sodium metabisulphite was observed. The organoleptic score of amala produced from test samples revealed that there was no significant difference ($p>0.05$) in the flavour, taste and texture of amala made from yam samples processed with or without inhibitors. However, significant difference ($p<0.05$) in colour exist among the amala samples. Amala made from yam samples processed with sodium metabisulphite was white at all processing temperatures and time compared with the control samples. Those made from yam samples processed with ascorbic acid was darker compared to the control. This study thus, revealed that PPO and browning were effectively inhibited in processed yam flour by sodium metabisulphite as shown by white amala obtained after treatment with the inhibitor. Ascorbic acid did not inhibit PPO in yam flour. The work concludes that sodium metabisulphite completely inhibits PPO and produce amala that is white, and its acceptability was comparable to the normal dark amala produced by conventional process.

Keywords: browning, physicochemical, polyphenol oxidase, yam, solubility, inhibition

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1. INTRODUCTION

Yam (*Discorea spp.*), is a multi-species crop that originated principally from Africa and Asia before spreading to other parts of the world (Hahn, et al 1987). It belongs to the family Discoreacea (Coursey, 1983 and Ayensu, 1972) and serves as a staple crop in West Africa (Asiedu, et al 1992). The yam tuber, which is the most important part of the plant, can be stored longer than other root tuber crops, hence ensuring food security even at times of general scarcity. It is the third most important root and tuber crop after cassava and sweet potatoe (Fu et al, 2005). The crop is of major importance in the diet and economic life of people in West Africa, the Caribbean Islands, parts of Asia, and Oceania (Revindran and Wanasundera, 1992, Girardin et al, 1998). Yam is an elite crop, preferred over other root and tuber crops in West Africa and a choice during ceremonies and festivities (Hanhet al, 1987).Yam is consumed in different forms, particularly boiled, fried, roasted, as pounded yam and yamporridge. The study by Asumugha et al(2007) found that for all income levels, yam consumption represented more than 50% of totalexpenditure on roots and tubers.

In addition to traditional dried yam products, 'new' processed yam products (e.g. poundo yam) haveentered the market in recent years and are consumed due to convenience factors as affluence rises among certain sectors of the population.

West Africa is the leading producer of yam, grows over 90% of the worldwide production (40 million tones fresh tubers per year), followed by the West Indies where Jamaica is the leading producers (Dipeolu et al, 2002; FAOSTAT, 2004).

Table 1: Global Yam Production 2008

Location	cultivated area ('000 ha)	yield (t/ha)	production ('000t)	percentage of Total
World	4,928	10.5	51,778	100
Africa	4,718	10.6	49,833	96.3
West Africa	4,443	10.8	48,101	93.0
Nigeria	3,045	11.5	35,017	67.7
Cote IIvoire	820	8.5	6,933	13.4
Ghana	299	11.9	3,550	6.9
Benin	205	8.8	1,803	3.5
Togo	63	0.2	638	1.2

Source: FAO, 2010 (in Fu et al., 2011)

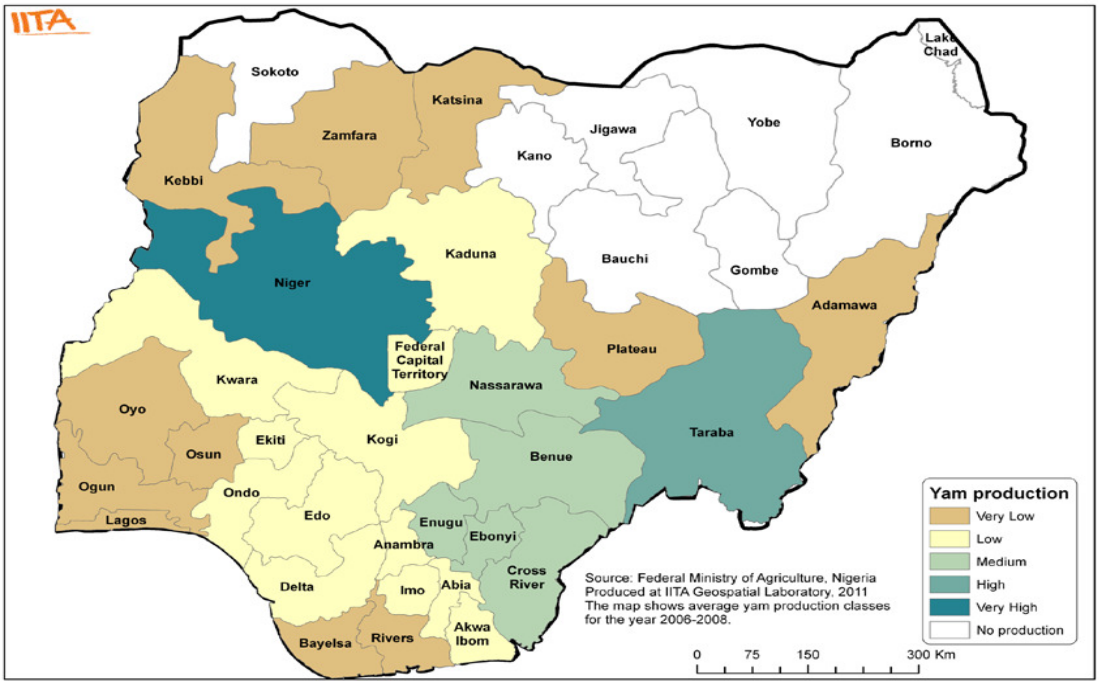

Figure 1.0: Nigeria Yam Production 2008 (Source: IITA)

The third most important region of yam production is East Africa where Tanzania and Sudan are the major producers. Yam is also produce in Japan, Papua New Guinea, the Philippines and Panama. Nigeria is the World's largest producer of yams followed by Ghana, Cote□Ivoire and Togo (FAO, 2003). According to Kleih, et al (2012), Yam is an important staple food crop in Nigeria, produced both for household consumption and as a cash crop. Table 1 highlights the significant percentage of total global yam production from Nigeria, equating to approximately 35 million tonnes.

Both fresh tubers and yam flour are now exported from Nigeria and Ghana to developed countries such as United State of America,United Kingdom,and France etc. These are mainly patronized by emigrants from growing regions. According to the Nigeria Export Promotion council (NEPC), Nigeria realized N56 Billion from yam export in 2008 as against N37 Billion in 2007 (Osibo, 2009). However, Ghana exports the largest quantity of yams (about 12000tonnes) annually and average yam consumption per capita per day is highest in Benin Republic (364Kcal) followed

by Cote dIvoire (342 Kcal), Ghana (296 Kcal), and Nigeria (258) (IITA, 2009).

In Nigeria, yam is produced in all central and southern states. Yam is attractive to produce and trade due to the higher market value that can be obtained compared to other crops such as cassava (Fu et al., 2011). Data regarding production volumes by states varies but Niger State is consistently regarded as the largest yam producing state in the country (Figure 1.0)

Yam is a rich source of carbohydrate and also contributes to vitamins and minerals especially where it is consumed in large quantities. It is excellent source of potassium, with twice the amount as found in a medium-sized banana(Albrecht and McCarthy, 2006). They are also a good source of vitamin C, B6, folate, iron and magnesium. (Afoakwa and Sefa-Dedeh, 2001)

Yams are high in starch and contain an enzyme, alpha amylase, which converts starches to sugars as tuber matures, is stored, or when heated. Table2.0 shows the nutritional composition of yam from different authors.

There are many varieties of yam specie widespread throughout the humid tropics, but the edible yamsare derived mainly of *Dioscorea rotundata* (Opara, (1999). The most economically important of these species are: yellow yam (D*ioscorea cayenesis*) which is also native to West Africa, water yam (*Dioscorea alata*), Bitter yam (*Dioscorea dumentorum*) also called trifoliate yam because of its leaves, white yam (Dioscorea rotundata). White yam is the most viscous among all species. The tuber is roughly cylindrical in shape, the skin is smooth and brown and flesh usually white and firm (Opara, 1999).

Processing of yam flour:

Yam flour (elubo) is another way that yam can be processed. To prepare elubo, yam tubers are peeled, sliced and parboiled in water at about 60^0C. The slices are left in the water, well covered, for about 24 hours to ferment slightly. They are drained and dried under the sun to reduce the moisture content (Meatres et al, 2002).

Table 2. Nutrient contents of yam species (*Discorea spp*) per 100g fresh edible tuber portions

Nutrients(g/100g)	*D. alata*	*D. rotundata*	*D. cayenensis*	*D. esculenta*	*D. dumetortums*
% Moisture	65-78.6	50-80	60.80	67-81	67-79
% carbohydrate	22-31	15-23	16	17-25	17.25
% starch	16.7-28	16.7-28	16	25	18.25
% free sugar	0.5-1.4	0.3-1	0.4	0.6	0.2
% protein	1.1-3.1	1.1-2.3	1.1-1.5	1.3-1.9	2.8
% crude fat	<0.16-0.6	0.05-0.1	0.06-0.2	0.04-0.3	0.3
% fiber	1.4-3.8	1.0-1.7	0.4	0.2-1.5	0.3
% ash	0.7-2.1	0.7-2.6	0.5	0.5-1.5	0.7
Phosphorous (mg)	28-52	17	17	35-53	45
Calcium	28-38	36	36	12-62	52
Vitamin C (mg/100g)	2.0-8.2	6.0-12	-	-	-
Iron (mg)	5.5-11.6	5.2	5.2	0.8	-
Food energy (Kcal)	140	142	71	12	122
β-carotene (µg)	5-10	-	-	-	-
Thiamine (mg)	0.05-0.10	-	-	0.1	-
Riboflavin (mg)	0.03-0.04	-	-	0.01	-
Niacin (mg)	0.5	-	-	0.8	-

Source: Turker et al (1993); Asiedu et al, (1997) and Opara (1999).

Elubo is usually mixed with boiling water to give a smooth thick paste called amala (Akissoe et al.,2001) which is eaten with soup. Amala is a delicacy for the Yorubas of western Nigeria (Osagie et al., 1994; Orkwor, 1998). The same product is popular in Benin Republic where it is called telibo. Figure 2.0 is the flow chart for yam flour processing.

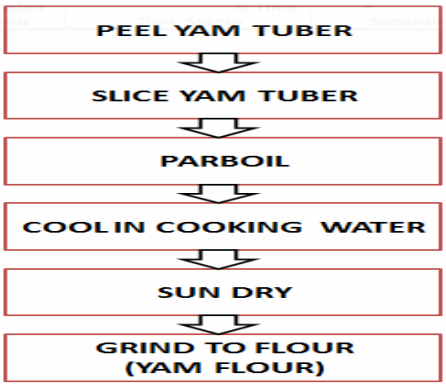

Figure 2.0 Processing Of Yam Flour

General overview of Enzymatic Browning

Appearance, flavour, texture and nutritional value are four attributes considered by consumers when making food choices. Appearance which is significantly impacted by colour is one of the first attributes by which consumers evaluate food quality.

The phenomenan of enzymatic browning has long been a source of concern to food scientists as it directly affects food quality. The colour of *amala,* a popular food all over southern Nigeria, ranges from light to dark brown and sometimes near black. However, despite its acceptability in the western part of Nigeria, its colour deters its general acceptability in other part of Nigeria particularly Northern and Eastern bloc.

This research work was therefore designed to study the effect of some phenolic oxidase inhibitors on the reduction of browning to improve on the texture and general appearance of the product thereby increasing the general acceptability of *amala*.

2. MATERIALS AND METHODS

Collection of samples

Yam tubers (*D. rotundata*) were bought from the opened Minna Central market.

Reagents

Ascorbic acid (0.02M) (anar, BHD chemicals Ltd Poole England) ,0.02M Sodium Metabisulphite (GPR, May & Baker Nigeria Ltd), 0.05M Potassium Phosphate Buffer (anar, BDH chemicals Ltd.Poole), Ethanol (absolute) (anar, sigma Aldrich laboratories Germany), Folin-Ciocalteau reagent, egg Albumen (GPR, BDH Poole England), 0.1M sodium hydroxide (GPR, May & Baker Nigeria Ltd), 2% Sodium carbonate (GPR), May & Baker Nigeria Ltd), 0.5% copper sulphate (GPR, May & Baker Nigeria Ltd), 1% sodium potassium tartate solution (GPR, May & Baker Nigeria Ltd)), 0.02M Catechol.

Yam Flour Processing

The yams were sliced to a thickness of about 10mm. The slices were steeped in water or inhibitor solutions for 12hours at 30^{o}C, 40^{o}C and 50^{o}C. The steeped slices were sun dried then ground to flour in a blender (Crown star MC-Y44B, Trident (H.K.) Limited China)

Crude Enzyme Extraction

Yam samples (250g) were steeped in water for 12hours. The samples (1g) were thereafter homogenized in 5ml of 0.05M phosphate buffer (pH 6.5) and centrifuge at 20,000rpm for 15mins on a centrifuge (Gallenkamp England), the supernatant contained crude PPO enzyme. For PPO inhibition studies, the steps were repeated with the inclusion of 0.02M of Ascorbic Acid or Sodium Metabisulphite.

Assay of PPO Activity

The assay procedure used was based on the method of Lee and Smith, (1979) but with slight Modification. PPO activity was determined by measuring the increase in absorbance at 420nm using a Spectrophotometer (Jensway 6310). The reaction mixture contained 0.2ml of the crude enzyme solution and 2.8ml of 0.02M substrate solution in 0.05M phosphate buffer (pH 6.5) at room temperature. The control sample contained only 3.0ml of substrate solution. One unit of PPO activity was defined as the amount

of enzyme that caused an increase in absorbance of 0.001 per minute.

Calculation:

$$\text{Units/mg} = \Delta 420 \times \frac{1000}{mg\ proten!}$$

Where ΔA = change in absorbance,
1000= conversion factor

Determination of Browning Index

Ethanol (5ml) was added to 0.5g, yam flour samples and then stoppered. The contents were mixed on a Vortex mixer for 2 minutes. The mixture was allowed to stand for 10 minutes and then centrifuged at 1000rpm for 10 minutes on a centrifuge (Gallenkamp England) and the supernatant was read in spectrophotometer (Jenway 6310) at 420nm using ethanol as blank according to the method of Lee et al, (1990)

Protein Estimation

The protein concentration was determined by the method of Lowry, using egg albumin as the standard (Lowry, et al, 1951). Aliquot of protein solution were pipetted out and the total volume made up to 4ml with distilled water. To each tube, 5.5ml of alkaline solution was added and allowed to stand at room temperature for 10-15mins. Folin reagent (0.5ml) was pipette into each tube, mixing rapidly after each addition. The tubes were left for 30mins and the blue colour formed was measured in spectrophotometer (Jenway 6310) at 650nm. Blank without the protein was used and protein concentration was extrapolated from a standard protein curve.

Solubility

Yam flour sample (1.0g) was measured and transferred into a clean dried test tube and weighed (W1). It was dispensed in 50ml of distilled water using blender (Crown star MC-Y44B, Trident (H.K.) Limited China). The temperature of resultant slurry was raised to 60^0C, 70^0C, 80^0C and 90^0C for 30minutes in a regulated water bath (Griffin Britain). The mixture was cooled to room temperature and centrifuged at 500rpm for 15 minutes on centrifuge (Gallenkamp England). The supernatant (5ml) was withdrawn and dried to a constant weight at 110^0C in an oven (Gallenkamp size 2, England). The residue was then represented as the amount of sample solubilized in water. (Leach et al 1995, Akinggbala and Rooney, 1987).

Calculation:

$$\text{Solubility} = \frac{Xg}{100g\ of\ Sample\ on\ dry\ basis!}$$

Where X = The Weight Yam flour Soluble Residue. (Leat et al, 1995; Akingbala and Rooney, 1987)

Hydration Capacity

The method of Kornblum and Stoopak, (1973) was used to determine the hydration capacity. Yam flour Sample (1.0g) was placed in each of four 15ml plastic centrifuge tubes to which 10ml of distilled water was added and then stoppered. The contents were mixed on a Vortex mixer for 2 minutes. The mixture was allowed to stand for 10 minutes and then centrifuged at 1000rpm for 10 minutes on a centrifuged (Gallenkamp England). The supernatant was carefully decanted, and the sediment weighed. The hydration capacity (HC) was taken as the ratio of sediment weight to the dry sample weight.

Swelling Capacity

This was measured at the same time as the hydration capacity and calculated as follows:

$$S = \frac{[V2 - V1]}{V1} \times 100$$

Where S is the % swelling capacity, V2 is the volume of the hydrated or swollen material and V1 is the tapped volume of the material prior to hydration (Kornblum and Stoopak, 1973)

Moisture Content

Yam flour sample (5g) was dried at 1100C in an oven (Gallenkamp size 2, England) to a

constant weight. The % loss in weight was calculated as the moisture content. (AOAC, 1997)

Calculation:

$$\%\ Moisture = \frac{[(Wt\ of\ Pan + Fresh\ Sample) - (Wt\ of\ Pan + Dry\ Sample)]}{Wt\ of\ Sample} \times 100$$

Determination of Energy Value

Yam flour sample (1.0g) was weighed into a ballistic bomb metal iron crucible, pressed gently with a spatula to form a smooth level layer suitable for combustion. One end of a single strand cotton thread was tied to the platinum firing wire with sample. The bomb closure ring was filled with oxygen from the steel cylinder to the required pressure (15 atm). The firing ring was then pressed and maximum deflection was read on the galvanometer scale (XRY – 1BOxygen Bomb calorimeter) (AOAC, 1997).

Calculation:

Energy value $= ((CB - QB))/((CS - QS)))$

Where CB= calorific value of standard sample (27.63kg/g)
CS= calorific value of test sample
QB= peak galvanometer deflection of 1.0g of standard sample (12.50)
QB= peak galvanometer deflection of the test sample.

$$CS = CB - QB = \frac{Kj/g}{QB}$$

Organoleptic Score of Yam Flour Meals (Amala)

Sensory evaluation was carried out on the yam flour (amala) samples as described by Larmond, (1977). A 10-member taste panel made-up of graduate students and lecturers in the Department of Biochemistry and Chemistry, Federal University of Technology Minna, were trained to conduct the sensory analysis. A preliminary test which served as training class for members of the panel was conducted a day before the main evaluation. The purpose was to familiarize members with both hedonic and descriptor scales.

To keep the interest and morale of the panelist for the main evaluation, they were served amala with abula soup after the preliminary test to show appreciation for their service as well as a warm invitation for the main evaluation. Every member was provided with questionnaire for both objective and subjective sensory evaluation. The objective questionnaire enabled each panelist to describe the products while the subjective questionnaire requested them to give information on the degree of like or dislike of the samples. Quality characteristics including appearance, aroma, taste and the overall acceptability of the samples were evaluated based on a seven point behonic scale (where: 1=like extremely; 2= like very much; 3= like slightly; 4= neither like nor dislike; 5= dislike slightly; 6= dislike very much; 7 = dislike extremely)

Statistical Analysis

All experiments were done in 3-replicate except for organoleptic score which was an average of ten tests. Means, standard deviations correlation coefficients, regression and analysis of variance (ANOVA) on data were performed using SPSS computer software version 15.0. Means were compared using Ducan method at a probability level of 0.05 (Ducan, 1955). Relationships among measurement variables were studied using standard correlation (r^2)being correlation factor.

3. RESULTS

Polyphenol Oxidase (PPO) Activity

Polyphenol oxidase activity pattern in yam flour processed with or without inhibitors ascorbic acid and Sodium metabisulphite are shown in table 3. Ascorbic acid increased PPO activity significantly ($p<0.05$) up to 2hrs of steeping and thereafter dropped while sodium metabisulphite significantly reduced ($p<0.05$) activity compared to the control sample.

Correlation of PPO activity with browning

The correlation of PPO activity with browning index were highly moderately (53.2%) correlated.

Table 3. Polyphenol oxidase (PPO) activity and Browning index of yam tissue steeped in different PPO inhibitors*

Inhibitor	PPO Activity (Unit/mg protein)	Browning index (units/g)
Control	11618.85±15576.22[a]	36.47±7.87[a]
Yam + Ascorbic Acid	10912.38±16672.51[b]	36.67±7.87[b]
Yam + Na-metabisulphite	1069.57±652.37[c]	23.07±2.66[c]

*Values within column, with different letters superscripts are statistically different at P<0.05.Each data is mean ± SD of three replicates

Table 4.0. Influence of steeping temperature on PPO activity and browning index in yam processing*

Temperature (°C)	Activity (Units/mg protein)	Browning index (Units/g)
30	13460.93±19344.01[a]	32.25 ± 10.09[a]
40	3804.45± 2330.01[b]	30.45 ± 7.460[b]
50	2205.38 ± 2325.23 [c]	29.30 ±6.99[b]

* Values within column, with different letters superscripts are statistically different at P<0.05.Each data is mean ± SD of three replicates.

Table 5.0 Influence of steeping time on PPO activity and Browning in yam flour processing*

Time (hrs.)	Activity (Units/mg protein)	Browning index (Units/g)
2.00	13478.68±2084.47[a]	34.42±10.19[a]
4.00	5011.07±10625.80[d]	30.58±5.94[b]
6.00	5653.95±8550.85[c]	29.92±5.73[b]
8.00	6235.13±8945.98[b]	31.33±10.63[b]
12.00	2072.43±141.22[e]	27.08±6.72[c]

*Values within column, with different letters superscripts are statistically different at P<0.05.Each data is mean ± SD of three replicates

Table 6.0 Correlations of PPO Activity with Browning in yam after steeping with

Inhibitors					
		Activity (Units/mg protein)	Browning Index (Units/g)	Time (hrs)	Temp. (°C)
Activity	Pearson Correlation	1	0.53**	-0.25**	-.037**
	Sig. (2-tailed)		0.000	0.001	0.000
	N	180	180	180	180
Browning Index	Pearson Correlation	-0..53 **	1	-0.24**	-0.14
	Sig. (2-tailed)	0.000		0.001	0.054
	N	180	180	180	180
Time	Pearson Correlation	-0.25**	-0.24**	1	0.000
	Sig. (2-tailed)	0.001	0.001		1
	N	180	180	180	180
Temp.	Pearson Correlation	-0.32**	-0.14	0.000	1
	Sig. (2-tailed)	0.000	0.054	1.000	
	N	180	180	180	180

**Correlation is significant at the 0.01 level (2-tailed)

Regression

Table 6.0 shows the regression of PPO activity and browning of yam flour processed with or without inhibitors. The regression equations are:

PPOActivity = 34624.327 + 562.78Temp – 878.59 Time

Browning Index = 40.35 – 0.157 Temp – 0.59 Time

Table 7. Regression coefficients of PPO Activity

Model	Unstandardized coefficients		standardized coefficients	T	Sig
	B	STD Error	Beta		
Constant	34624.327	4404.807	-	7.861	0.000
Temperature	-562.777	101.125	-374	-5.565	0.000
Time	-878.590	239.959	-246	-3.661	0.000

Dependent variable: Activity
Activity = 34624.327 – 562.78Temp. - 878.597Time

Table 8. Regression coefficients[a] of Browning index

Model	Unstandardized coefficients		Standardized coefficients	T	Sig
	B	STD Error	Beta		
Constant	40.35	3.21	-	12.56	0.000
Temperature	0.15	0.07	-0.14	-2.00	0.047
Time	-0.59	0.18	-0.24	-3.38	0.001

Dependent variable: Browning index; Browning index = 40.35 – 0.15Temp – 0.59 Time

Physiochemical properties

The physiolochemical properties of the processed yam flour samples are presented in table 9.0 and 10.0. The result indicated that there is no significant difference ($p>0.05$) in the moisture content, hydration capacity, swelling capacity and solublity of the yam tissues with or without inhibitors. However, a significant increase ($p<0.05$) in the energy of the yam tissues steeped with sodium metabisulphite was observed.

Organoleptic score of *amala*

The organoleptic score of yam flour meal (amala) produced from samples are presented in Table11.0. The result revealed that there is no significant difference ($p>0.005$) in the taste, colour, texture and flavor of amala produced from yam samples processed with or without inhibitors. However, there was significant difference ($p<0.05$) in colour among all the amala samples.

That is, amala made from yam samples processed with sodium metabisulphite was white throughout steeping temperatures and time compared to the control samples. Amala made from yam samples processed with ascorbic acid gave a golden brown colour compared to the control (even darker than the control). Generally, amala produced from yam steeped with sodium metabisulphite was the most acceptable of all the samples.

4. DISCUSSION

Polyphenol oxidase (PPO) Activity

Browning is one of the most important colour reactions that affects fruits, vegetables and sea foods. It has been found to be as a result of the presence of the enzyme, Polyphenol Oxidase. It is a surface phenomenon requiring molecular oxygen and specific phenolic substrates (Macheix et al., 1991, Nicholas et al., 1994; Sheen and Calvert 1969).

The existence of physical browning process in plant tissue is well documented (Wolfrom, et al 1974) most of which have shown to be enzymatic. Various techniques and mechanisms have been developed over the years for the control of these undesirable enzyme activities. These techniques attempt to eliminate one or more of the essential components (oxygen, enzyme, copper or substrate) from the reaction. Sulfahydryl compounds such as sodium metabisulphite have been investigated as inhibitors of enzymatic browning. The formation of Quinone-sulphite complexes prevents the Quinone polymerization (Embs and Markakis, 1985).

Table 9. Physiochemical properties of yam steeped in different PPO inhibitors*

Inhibitor	Moisture Content (%)	Hydration capacity (g/g)	Swelling capacity (g/g)	Energy Value (Kj/g)
Control	6.75±0.25 [a]	1. 79±0.26[a]	82.60±5.40[a]	27. 933±2.00[a]
Yam + ascorbic acid	6.75±1.25[a]	1.57±0.30[a]	75.35±1.85[a]	17.605±3.00[a]
Yam + Na-metabisulphite	6.25±0.25[a]	1.76±0.07[a]	77.75±4.25[a]	29.058±1.00[a]

*Values within column, with different letters superscripts are statistically different at P<0.05. Each data is mean ± SD of three replicates

Table 10. Solubility of yam steeped in different PPO inhibitors at different temperatures*

Inhibitor	Solubility	Temperature (^{O}C)	Solubility
Control	0.3883±0.50[a]	60.00	1.0400±1.5E-02[a]
Yam + ascorbic acid	0.2700±0.36[b]	70.00	0.1300±0.3232[b]
Yam + Na-metabisulphite	0.2683±0.27[c]	90.00	0.0133±0.3E-02[c]

*Values within column, with different letters superscripts are statistically different at P<0.05. Each data is mean ± SD of three replicates

Table 11. Organoleptic evaluation of yam flour meal (amala) steeped in different PPO inhibitors*

Samples	Taste	Colour	Texture	Flavour	Overall Acceptability
Control	2.8±1.03[a]	2.8±1.03[a]	2.9±0.88[a]	3.3±0.48[a]	2.3±1.16[a]
Yam + ascorbic acid	3.7±1.42[a]	3.4±2.01[a]	3.0±1.05[a]	3.6±0.70[a]	2.9±1.85[a]
Yam + Na-metabisulphite	2.9±0.88[a]	1.6±0.70[b]	2.9±0.88[a]	3.5±0.53[a]	1.5±0.71[b]

*Values within column, with different letters superscripts are statistically different at P<0.05. Each data is mean ± SD of three replicates

A further action of sulfhydryl compounds on PPO may be directly inhibiting the enzyme by combining irreversibly with copper at the active site of the enzyme (Voleroet et al, 1991) thus, inhibiting the enzyme. Result in this study showed that, there were significant differences ($p<0.05$) in PPO activities in yam samples processed with different inhibitors compared with the control irrespective of temperature and time. Statistical analysis revealed that PPO activity of the control is significantly higher than those of the yam samples processed with inhibitors. This implies that the inhibitor studied were able to inhibit PPO activities in the yam tissues. Among yam samples processed with inhibitors, those processed with Ascorbic acid had higher PPO activity than those possessed with sodium metabisulpite (ascorbic acid > sodium metabisulphite). This implies that sodium metabisulphite (0.02M) effectively inhibited PPO activity more than ascorbic acid which partially inhibited PPO activity at the concentrations used. The result showed that inhibition of PPO increased with steeping time. Highest inhibition was recorded at 12hrs. It showed that inhibition of PPO increased with rise in temperature with highest inhibition recorded at 50^{0}C and 30^{0}C. Omidiji and Okpuzor, (1996) also observed that inhibition of PPO activity in yam tissue were maximal at 12hrs of incubation. They also reported that yam processing techniques at ambient temperature in the presence of PPO inhibitors should be completed within 12hrs before the onset of non-enzyme related browning.

This value (30^0C) is similar to the optimum temperature of the PPO of *Dioscorea opposite* (Shuji, et al, 2006) and another species of yam tuber (Ikediobi and Obasuyi, 1982; Omidiji and Okpur, 1996). This implies that rise in temperature denatured PPO enzyme.

Browning index

The browning index indicates the proportion of oxidized phenols (Jeong, et al, 2008). In the present study, there was significant differences ($p<0.05$) in browning index of yam flour (elubo) made from yam samples processed with inhibitors compared with the control irrespective of temperature and time.

Statistical analysis revealed that browning index of elubo made from yam samples processed with

Ascorbic acid and those of the control were not significantly different ($p>0.05$) but were significantly higher than those of elubo made form yam samples processed with sodium metabisulphite. This implies that sodium metabisulphite appears to be a potent inhibitor for preventing browning in yam flour processing. However, physical observation showed that 0.02M Ascorbic acid did not inhibit browning in the processed yam flour rather; it increased browning compared to the control. The study therefore, showed that ascorbic acid is undesirable in reducing tissue browning in processing yam flour (elubo) even though it is a PPO inhibitor in other tissues e.g. apples, quince, loquat, etc. Ascorbic Acid, an antioxidant, is more readily oxidized than phenols and thus tends to form deep brown colour even before the phenols are used as substrates (Vamos-Vigyazo, 1981). The enhancement of browning throughout incubation in the presence of micro molar quantity of ascorbic acid therefore suggests an involvement of an in vivo oxidisable phenolic pool in the browning process (Omidiji and Okpuzor, 1996) .The studies showed that Ascorbic acid did not prevent browning in yam tissues while sodium metabisulphite completely prevented browning in yam tissues.

Among compounds that inhibit PPO activity, sodium and potassium metabisulphite and sulphur dioxide(SO_2) are amongst the most effective and have been used in food industries for many years. However, restrictions of sulphite usage in foods associated with consumer concern about its safety generate the need for substitutes (Rocha and De Moris, 2005). Therefore, alternative chemicals without toxic effects are needed, such as sulfhydryl (SH or Thiol), Ascorbic acid, and citric acid. These compounds have potential to be used commercially, as substitute to sulphite as anti-browning agent, to prevent enzymatic browning in processed fruit products.

The result in this study also showed that inhibition of browning increased with time with highest inhibition recorded at 12hrs. The inhibition of browning increased with rise in temperature with highest inhibition rendered at 40^oC and 50^oC. Omidiji and Okpuzor, (1996) also observed that browning in *D.rotundata, D. esculenta, D. alata and D. cayensis*, were maximal at 30^oC which later declined with rise in temperature.

Correlation of PPO Activity and Browning Index

Polyphenol oxidase (PPO) activity and browning index were significantly, positively and moderately (53.2%) correlated. This implies that as PPO activity increases, the browning index of the yam flour (elubo) increases significantly and vice versa. PPO activity, steeping time, and Browning index were significantly, negatively and weakly correlated. This implies that as the steeping temperature does not influence PPO activity. Jeong et al, (2008) also observed positive correlations between PPO activity and browning index for all their treatments with ascorbic acid.

Regression

From linear regression model, the equation for the relationship between activity, time and temperature and between browning index, time and temperature in this study were established as;

Activity = 34624.327 – 562.78Temp – 878.59Time.

Browning index = 40.35 – 0.15Temp – 0.59Time.
R^2 = 0.200 for activity and 0.080 for browning index.
Thus, indicating that temperature and time are important variables in predicting PPO activity and browning index in yam flour processing. This model can also be used for a scale up operation.

Physiochemical characteristics

Quantifying physiochemical properties are important for food processing and quality, because they influence functional properties of flour (Moorthy, 1994; Gerard et al., 2001; you and Izidorczyk, 2002) which in turn affect the textural quality of food products.
In the present study, test samples were not significantly different ($p>0.05$) in terms of moisture content, hydration capacity, swelling capacity and solubility when compared to that of the control sample. The moisture content of a food sample reflects the amount of solid matter in the sample. The higher the moisture content, the higher the rate of spoilage.
The data in this study indicated that moisture content of the processed yam flour (elubo) ranged between 6.25 – 6.75%. Okaka and Okechuckwu, (1993) also obtained values less than 10% and stated that ≤10% moisture content is needed for prolonged shelf life (up to 6 months) for well package dehydrated yam products. This suggests that the processed elubo samples may be stored for a long period of time without fear of spoilage.
High swelling powers were also observed for all the test samples. This is consistent with the work of Walter, (2002) who observed that *D. rotundata* had higher swelling power in comparism to other yam species. This high swelling has linked to low amylose content; due to low reinforcement of internal network by amylose molecules (Lorenze and Collins, 1990; Richardson et al, Hoover, 2001). Riley et al (2006) also observed higher swelling power in yam varieties which had lower amylose content. According to Jane and Chen, (1992), amylopectin contributes to granule swelling while amylose and lipid contents inhibit it. Highly associated starch granules with an extensive and strongly bonded micelles structure also exhibit resistance toward swelling (Leach et al., 1959) thereby exhibiting low swelling capacities.
Carbohydrate supplies energy to cells such as brain, muscle and blood. It contributes to fat metabolism and spare protiens as energy source. It also acts as mild natural laxatives for human beings and generally adds to the bulk of the diet (Gordon, 2000; Gaman and Sherrington, 1996). The data in this study also showed that there were significant differences ($p<0.05$) in calorific energy values between yam samples processed with inhibitors and the control. Statistical analysis revealed that yam samples processed with sodium metabisulphite was significantly higher in calorific energy than other inhibitors. In the same vein, the control was significantly higher in calorific energy than samples processed with ascorbic acid. Result in this study also showed that there was no significant difference (<0.05) in solubility between yam samples processed with inhibitors and control but statistical analysis revealed that irrespective of temperature, the solubility of the control was significantly higher than those of yam flour made from yam samples processed with inhibitors. Statistical analysis also revealed that solubility irrespective of inhibitor at $60^{O}C$ with decrease in temperature. (ie $60^{O}C>70^{O}C>80^{O}C$ &$90^{O}C$). However, solubility at $80^{O}C$ and $90^{O}C$ were not significantly different from each other. Thus, solubility of elubo decreases with increase in temperature. This implies that the addition of inhibitor did not change the physiochemical properties of yam flour except that calorific energy which increased upon processing with sodium metabisulphite.

Organoleptic score of amala

People's expectation and evaluation of food always starts with visual inspections (Hutchings,2003) . consumers consciously or unconsciously examine food in the first place by appearance including colour, visual structure and surface texture.
Temporal factors such as climate, place and

physical condition can affect the evaluation as well as individual background. In the next step, consummers identify safety of the food followed by assessment of flavor and texture. The final step of food evaluation before eating is to guess pleasant and satisfaction derived by eating the food. Dubose et al (1980) reported that colour is a very important sensory attribute of most foods since it influences the consumers first judgment and provides sensory information, which may interact with the gustory olfactory and textural cues to determine the overall acceptability. Francis (1980) also remarked that when the colour is unappealing, consumers are unlikely to be able to judge the flavour or texture as favourable.

In the present study, there was no significant difference ($p>0.05$) in taste, texture and flavour between amala made from samples processed with inhibitors and the control. However, significant differences ($p<0.05$) in colour existed between amala made from yam samples processed with inhibitors and the control. Statistical analysis showed that the colour scores for the control and amala made from yam samples processed with ascorbic acid had no significant difference from one another. However, the colour scores of control and amala from yam samples processed with ascorbic acid were significantly higher than those processed with sodium metabisulphite.

Also, physical observation showed that browning was completely inhibited in the presence of sodium metabisulphite hence; amala made from yam samples processed with sodium metabisulphite was completely white throughout steeping period compared to the control. However, browning was enhanced in the presence of ascorbic acid thus, yam samples processed with ascorbic acid produced amala that was darker than those of the control throughout steeping period.

The taste attributes of all the test samples were alike (slightly sweet) according to the panelist. This is not surprising because any trace of bitter principle in the tuber got in the water during steeping. The panelists also recorded similar flavour and texture for all the samples. The test samples were very elastic in textures hence there acceptability by the panelists.

The study also showed that there was significant difference ($p<0.05$) in acceptability between amalamade from yam samples processed with inhibitors and control. Statistical analysis revealed that the general acceptability of the control was not significantly different from amala made from yam samples processed with inhibitors but among the inhibitors. However, there exist difference in the general acceptability of amala made from yam samples processed with ascorbic acid and those processed with sodium metabisulphite. According to the seven point hedonic scale, the lower the value scored, the higher the acceptability. This therefore implies that amala made from yam samples processed with the inhibitors were accepted in the same manner as the control but within the process groups, amala made from yam samples processed from sodium metabisulphite was preferred to amala made from yam processed with ascorbic acid.

Generally speaking, the lower the values recorded for each sample, the higher the quality and its acceptability according to the seven hedonic scales. Therefore, amala obtained from yam samples processed with sodium metabisulphite was the best and the most acceptable of all the amala samples.

5. CONCLUSIONS

Polyphenol oxidase (PPO) was effectively inhibited in processed yam flour (elubo) by sodium metabisulphite which also resulted in inhibition of browning and the production of white amala as shown by the white amala obtained after treatment with inhibitor. Ascorbic acid however, did not inhibit PPO in elubo. Also, PPO activity and browning index were significantly, positively and moderately (58.2%) correlated.

The addition of inhibitors did not change the physiochemical properties of the processed elubo except that colorific energy was increased when processed with sodium metabisulphite. In conclusion, of all the yam flour samples, amala made from yam issues

steeped with sodium metabisulphite was the most accepted.

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