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Effect of Storage Relative Humidity on Some Chemical Composition and Browning Development of Treated Cocoyam (*Colocasia esculenta***) Corm Flour**

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Authors' contributions

Author JEO designed the work, carried out statistical analysis and prepared the manuscript in its present form; author AUGO carried out the laboratory analysis while author ECI interpreted and discussed the research findings. The financial burden of the research was borne by all authors. *All authors read and approved the final manuscript.*

Original Research Article

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ABSTRACT

Aim: Some unstable compounds namely ascorbic acid, anthocyanin and reducing sugar contents were used to monitor the stability of differently processed cocoyam flours stored under different relative humidities.

Place and Duration of Study: Federal Polytechnic Idah, Nigeria; January to August 2011.

Methods: Peeled cocoyam corm slices (3–5cm thick) were washed and divided into three parts that were respectively sundried (NT), blanched and sundried (BT); and sulphited, blanched and sundried (SBT). The milled samples (<200um) were stored under different relative humidities (RH) and analysed at time intervals for ascorbic acid (AA), anthocyanin (ACY) and reducing sugar contents; and for alcohol soluble colour (non-enzymatic browning) developments.

Results: Ascorbic acid content of cocoyam flour ranged from 8.65mg/100g in NT to 14.13mg/100 in SBT while anthocyanin and reducing sugar contents ranged from 8.62

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and 317.5mg/100g, respectively, in NT to 13.37 and 302.0mg/100g in BT flours. These values decreased with increasing storage RH and time. At seventh month, AA was retained in NT flour stored only at 11% RH and in BT and SBT flours stored at ambient, 11% and 33% RH. Similarly, only storage at 11% RH retained ACY for 5 months in NT and for 7 months in BT and SBT cocoyam flours. Reducing sugar decreased with increasing storage RH and time. Initial absorbance of flour alcohol extract was highest in NT and least in SBT, indicating greater browning in the former. Regression analysis revealed that the rate of non-enzymatic browning development was lower in the SBT cocoyam flour; and seemed to be higher in BT flour than in NT at RH of 53% and below, and vice versa at RH above 53%. Correlation coefficients of reducing sugar contents and non-enzymatic browning development were significant (*P=0.05*) in RH conditions studied. **Conclusion:** Cocoyam flour stability was improved by sulphiting and blanching treatments and low RH storage.

Keywords: Cocoyam; blanching; sulphiting; ascorbic acid; anthocyanin; reducing sugar; non-enzymatic browning; relative humidity.

1. INTRODUCTION

Taro, *Colocasia esculenta*, regarded as third most important root crop after yam and cassava in West Africa [1] has high content of protein and amino acids than any tropical root/tuber crops [2]. It's small sized starch granules, which results in high digestibility, makes it useful for preparation of infant foods in Hawaii and other Pacific Islands [3,4]. According to Huang et al. [5] and Amon et al. [6], the combination of small size granules and high soluble dietary fibre content makes taro corm a good source of carbohydrate for extruded special products as infant weaning diets and low glycemic index food.

Although widely available and seemingly nutritionally superior to other roots and tubers, cocoyam is greatly underutilized probably due to its acridity factor, high rate of post harvest losses and lack of proper scientific attention to this problem. This has contributed to more than 70% drop in annual production rate in Cameroon [7]. Considering the nutritional and chemical composition report, Owuamanam et al. [8] is of the opinion that cocoyam, if fully exploited, would enhance food security of people living in the tropics. To overcome the post harvest losses, the corms and cormels may be processed into flour, which stores much longer [9,10]. Besides, the aroid flour could be advantageous in the preparation of myriad products by food development industry since it could be used in dehydrated soup formulation, baked goods, formulation of baby foods, snacks and breakfast products; and serves as useful source of starch not only for food items but also for other industries such as drug, textile, paper, oil production and bread making. Conversion of cocoyam corms and cormels to flour could also improve its competitiveness alongside other root and tuber crops and enhance the application in other food systems and improve marketing potential.

Conversion of cocoyam corms/cormels into flour though can help diversify the use especially in food systems, is not an end to the problem of its storage. The storage relative humidity is a very important factor. According to Kumar and Balasubrahmanyam [11], the moisture content considered to be the safest with reference to good storage stability of food is that between the monolayer value and that corresponding to a water activity of 0.6 to 0.64 (relative humidity of 60 – 64%). Monolayer moisture content is the minimal amount of water bound to active sites and guarantees the stability of flour during storage [12]. Kumar and Balasubrahmanyam [11] stated that it is preferable to have the initial moisture content at or

slightly above the monolayer value for extending the shelf life with minimal deterioration as drying to lower moisture below this value requires large amount of extra heat in addition to the heat of evaporation. Onwuka [13] listed the monolayer range of 0.0320 to 0.160 g H₂O/ g solid for some dry starchy flour. Owuamanam et al. [8] reported a monolayer range of 0.0353 to 0.0471 g H2O/ g solid for different cultivars of *Colocasia esculenta* corm flour whereas Nwanekezi et al. [14] reported a range of 0.0367 to 0.0787g H_2O/g solid for various cocoyam cormel flours. At 25ºC and 35ºC, the monolayer values of taro flour estimated by Nurtama and Lin [15] were 3.5794% and 3.6620%, respectively. A preliminary analysis in this work revealed a monolayer value of 3.57%, which corresponds to water activity slightly below 0.11 (11% relative humidity).

The effective storage life of food can be established from an enormous body of information concerning changes in colour, odour, flavour, texture, nutrients, moisture content, staling, rancidity and overall changes in product acceptability [16]. Cocoyam corms contain some unstable compounds that can serve as indices of its storage stability. Such compounds, among others, include ascorbic acid that is regarded as most unstable nutrient present in foods [17]; anthocyanin, which is a colour pigment of cocoyam and potential antioxidant [18,19] and reducing sugars. These compounds contribute to non-enzymatic browning development in foods [20,21]. This research was aimed at determining the effect of storage relative humidity on the aforementioned compounds and the development of alcohol soluble colour (non-enzymatic browning) of different processed cocoyam flours.

2. MATERIALS AND METHODS

2.1 Raw Material Procurement

Freshly harvested corms and cormels of taro (cocoyam) cultivar identified at the Department of Botany of University of Nigeria, Nsukka as *Colocasia esculenta* var. *esculenta* was purchased from Ekuluoko market at Afulugo in Igalamela/Odolu Local Government Area of Kogi State, Nigeria. The cocoyam was stored in a shade under a cashew tree and processed into flour within one week of procurement.

2.2 Processing of Cocoyam into Flour

Cocoyam flour was produced following a traditional method with slight modification. Taro corms were washed, peeled into bowl containing tap water to prevent or limit discolouration of the corms, cut into 3-5 mm thick slices and washed again to remove mucilage from the cut surfaces. The taro slices were divided into three equal parts. The first part was sun-dried without any treatment (NT). The second was blanched for 5 minutes in equal quantity of boiling tap water (w/v) before sun-drying (BT). The third was soaked for 4 hours in 0.025% (250 ppm) sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) solution) and blanched in the same solution for 5 minutes (SBT). The treated (BT and SBT) and untreated (NT) cocoyam slices were sundried for 3 days (34 \pm 2°C) on stainless steel trays placed on corrugated iron sheet 2 m above the ground. The dried cocoyam slices were milled using № 1A premier grinding mill driven by a lister engine (Model RLA 201-80014, UK). The resulting cocoyam granules were sieved with Tyler Standard Screen mesh number 65 to give granule/ flour particle size below 0.208 mm.

2.3 Storage of Cocoyam Granules/Flours

The storage relative humidities (RH) for the study were chosen to spread between 11 and 75%. Hence, storage was carried out under RH of 11, 33, 53, 67 and 75%. The RHs were achieved with saturated solutions of lithium chloride (11%), magnesium chloride (33%), magnesium nitrate (53%) and sodium chloride (75%) [13,22]. Combined saturated solutions of potassium nitrate and ammonium chloride, which gives 67.2–70% RH at temperature range of 25ºC to 35ºC was also chosen because at 30ºC, the RH will be slightly below 70%, which is critical to many microorganisms. Control samples were stored at ambient temperature (32 \pm 2°C) in loosely closed sorption jar void of saturated salt solution. Dry and wet bulb thermometers were installed on the laboratory bench for estimation of the storage relative humidity of the control samples.

2.4 Methods of Analysis

2.4.1 Ascorbic acid content determination

Ascorbic acid content was determined using the indophenols titration method [23]. Five grammes of cocoyam sample was macerated with 25ml of 20% metaphosphoric acid in a blender (Philip, type HR 1731, Brazil). The macerate was transferred into a beaker. The blender cup was rinsed with 25ml metaphosphoric acid and added to the beaker. The macerated sample was allowed to settle for 10 minutes before decanting. To 20 ml of decanted extract, 5ml of acetone was added and the mixture titrated with standard indophenols solution. The ascorbic acid content was then calculated as mg per 100g of sample as stated by AOAC [24].

% Ascorbic acid = Z x (F/E) x (V/Y) x 100

Where $Z =$ volume (ml) of sample titration (titre)

- $F =$ weight (mg) ascorbic acid equivalent to 1.0ml indophenols standard solution
- E = weight (g) of sample assayed
- $V =$ initial assay solution volume (ml)
- Y = volume (ml) of sample aliquot titrated

2.4.2 Determination of total anthocyanin

Total anthocyanin (Acy) was determined using the method of Fuleki and Francis [25] with slight modification. Five grammes of cocoyam flour was macerated with 50ml of 1.5 N HCl in 95% ethanol (15:85), which pH was adjusted to 1.0, in a Philip blender (type HR 1731, Brazil) at full speed (2000 rpm) for 5 min. The sample was transferred quantitatively to a 400 ml beaker using approximately 50ml of extracting solvent for washing the blender jar. The beaker was covered with aluminum foil and stored overnight at 4° C. The sample was filtered by suction on Whatman № 1paper through a Buchner funnel. The beaker and the residue on the filter paper were washed repeatedly with the extracting solvent until approximately 150 ml of extract was collected. The extract was transferred into 200ml volumetric flask and made up to volume. This extract was kept in the dark to equilibrate for 2h before the optical density (O.D.) was measured in 1cm cuvette at 535nm (Spectronic 21D, Milton Roy, Belgium). The total anthocyanin (T ACY) content was calculated in mg using the average extinction coefficient for cranberry anthocyanin [26].

T ACY in mg per 5g = $0.D. x \frac{TeV}{W} x 1$ W 98.2 Where O.D. = optical density (absorbance) TEV = total extract volume W = weight of sample 98.2 = average extinction coefficient for cranberries divided by ten

The value obtained was multiplied by 20 to express as per 100g.

2.4.3 Reducing sugar determination

Reducing sugar was determined by the method described by AOAC [24]. Two grammes of cocoyam sample was equilibrated with 10ml of 80% ethanol for 10 minutes, stirred and filtered through № 1 Whatman filter paper. The container and the residue were washed with 10ml of the extracting solvent. Then, 5ml of the extract was pipette into 75ml test tube (Pyrex 1 x 8'). Exactly 10ml of 0.1 N potassium ferricyanide $[K_2(Fe(CN)_3)_6]$ was added to the test tube, mixed and immersed in vigorously boiling water bath so that the liquid in the tube is 3- 4cm below the surface of boiling water. After exactly 20 min in boiling water, the tube and its contents were cooled under running tap water and transferred into a 200ml conical flask. The test tube was rinsed with 25ml of acetic acid salt (OHAC-NaCl) solution, added to the flask and mixed thoroughly. Then, 2ml of starch solution and 1ml of potassium iodide (KI) solution were added and the mixture titrated with 0.1 N solution of sodium thiosuphate (Na₂S₂O₃.5H₂O) until blue colour completely disappeared. The titre volume (ml) of Na₂S₂O₃ was subtracted from 10ml of $K_2(Fe(CN_3)_6$ solution used. The maltose equivalent (in mg) of the difference was deduced from Table 939.03 of AOAC [24]. The result was multiplied by the dilution factor and expressed as the mg of maltose per 100g of sample.

2.4.4 Determination of alcohol soluble colour

Alcohol soluble colour was determined by measurement of absorbance of clarified extract of cocoyam flour [27] with slight modification. Ten milliliters of 50% ethanol was vigorously mixed with 0.5 g sample of cocoyam flour in a 15ml centrifuge tube for 1 minute. The suspension was then centrifuged for 5 minute at top speed (2000rpm) using a centrifuge (Shermond, England). The absorbance of the supernatant was measured at 390 nm with Spectron 21D spectrophotometer. The absorbance at 390nm was used as an indication of degree of non-enzymatic browning.

2.4.5 Statistical analysis

Statistical tools namely analysis of variance (ANOVA), regression and correlation was used to analyze the data. Significant means were separated using Tukey's Least Significant Difference (LSD) test [28]. All statistical analyses were carried out at 5% confidence level.

3. RESULTS AND DISCUSSION

3.1 Ascorbic Acid and Anthocyanin

The ascorbic acid retention in cocoyam flour stored under various relative humidity (RH) conditions is shown in Table 1. Ascorbic acid (AA) content of cocoyam flours decreased with increasing storage RH and progressing storage time. Storage at 67% and 75% could not ensure AA retention in the flours after one month irrespective of processing treatments (NT, BT and SBT). AA was not retained in the NT flour after 3 months and in the BT and SBT cocoyam flours after 5 months storage under 53% RH. At the $7th$ month, AA could be retained in the NT flour stored only at 11% RH and in the BT and SBT flours stored at ambient, 11% and 33% RH. However, based on the results presented in Table 1, it may not be pertinent to conclude that losses were higher in the NT flour than the BT and SBT flours considering the initial 8.65mg/100g of the NT flour, 13.24mg/100g of BT and 14.13 mg/100g of SBT flours. Hence, the rate of ascorbic acid degradation was calculated using regression analysis.

The result presented in Table 3 showed that the rate of destruction of ascorbic acid was higher in NT flour than in its blanched (BT and SBT) counterparts at every storage RH. This could be attributed to the continued activity of ascorbic acid oxidase in the flour. Although greater ascorbic acid was retained in the sulphited cocoyam (SBT) flour, its rate of destruction seemed to be slightly higher in the flour during storage than in BT flour (Table 3). Also, the destruction rate increased with increasing storage RH. The later observation agreed with the observations [29,18,30] that stability of ascorbic acid in various dry foods is a function of water content and water activity. Karel and Nickerson [31] had reported that all adsorbed water including the water present in monomolecular layer appears to be available for the reaction resulting in the destruction of ascorbic acid. According to Kirk et al. [18], dissolved oxygen in food system is a function of water activity because, as the moisture content was increased, the viscosity was reduced resulting in more mobility of the reactants, hydration of catalysts and swelling of solid matrices exposing new catalytic sites. Sharma et al. [32] reported that ascorbic acid content of lemon juice concentrates experienced some loss during storage due to their light and heat sensitivity and involvement in browning reaction. The retention of ascorbic acid has been taken as an index of retention of the original nutritive and quality values of processed food [29].

3.2 Anthocyanin

Like ascorbic acid, there were losses of anthocyanin (ACY) of the cocoyam flours during storage at every storage RH conditions (Table 2). Greater losses were observed in the flours stored under high RH conditions. Storage at 75% RH could not guarantee the retention of ACY in NT cocoyam flour within the first month of storage. In the same way, storage at 67% and 53% RH could not retain any ACY after 3 months of storage. Only storage at 11% RH could retain the ACY for 5 months in NT cocoyam flour and for 7 months in the blanched (BT and SBT) cocoyam flours. Tonon et al. [33] reported that anthocyanins are very unstable to processing and storage since they are sensitive to factors like temperature, light, pH, oxygen and others. The presence of oxygen and interactions with other components like sugars and ascorbic acid also affect anthocyanin stability [34]. Kirk et al*.* [18] have reported that dissolved oxygen in food system is a function of water activity because, as the moisture content was increased, the viscosity was reduced resulting in more mobility of the reactants, hydration of catalysts and swelling of solid matrices exposing new catalytic sites. Agudelo- Laverde et al. [20] studied the effect of water activity on the chromatic attributes of dehydrated straw berries and observed that anthocyanin degradation increased as increasing water content and that browning reaction and pigment degradation were accelerated at 75% relative humidity. According to Tonon et al. [33], both temperature and water activity negatively affected anthocyanin stability of spray-dried acai (*Euterper oleracea* Mart.); the antioxidant activity also decreased with increasing water activity. However, Somboonkaew and Terry [35] reported that anthocyanin level of 80% RH-treated litchi 'kom' fruit pericarp tissue were significantly lower than litchi held under higher RH conditions. Due

to the observed proportionality between the speed of red colour disappearance from anthocyanins and the velocity of free sugar formation in *Musa acuminata*, Roobha et al. [34] attributed the main cause of pigment colour loss to anthocyanin hydrolysis.

During storage of the flour, the rate of ACY degradation was higher than that of ascorbic acid (Table 3). Among the cocoyam flour treatments, it was highest in NT flour. The higher degradation rate of ACY in NT flour could be associated with the activity of hydrolytic enzymes, which may have catalyzed the hydrolysis of the ACY to anthocyanidin and simple sugars.

3.3 Reducing Sugars

The effect of storage RH on the reducing sugar content of the various processed (NT, BT and SBT) cocoyam flour is shown in Table 4. The NT flour had the highest reducing sugars. Leaching of the sugars into the blanching water may have been the cause of the lower reducing sugar content of the blanched cocoyam flours (BT and SBT). The reducing sugar content of SBT cocoyam flour was slightly higher than that of BT flour even in the course of storage at every RH studied. The loss of these sugars increased with increasing storage RH and time. This could be attributed to the increasing involvement of reducing sugar in Maillard reaction browning since its mobility, as reactant, increased with increasing moisture content [18,19]. Also, both ascorbic acid and anthocyanin are antioxidants [33] and may have served as shields to oxidation of reducing sugar. Their decrease with increasing RH should certainly increase the loss of reducing sugar. The difference in the reducing sugar content of samples of BT and SBT cocoyam flours stored under ambient (Table 6), 11% and 33% RH remained insignificant $(P=0.05)$ even at the $3rd$ month of storage. The correlation coefficients of the reducing sugar content and non-enzymatic browning reaction are significant at 5% confident level in all the storage RH conditions studied (Table 5). Sulphiting neither prevented the loss of reducing sugars (Table 4) nor the development of non-enzymatic browning (Figure 3) but rather reduced their rate of loss and development, respectively.

Table 1. Ascorbic acid retention (mg/ 100g cocoyam) in cocoyam flours stored at various relative humidities

Values (± SD) are means of triplicate determinations; -, not detected; Given in Table 6*

Table 2. Anthocyanin retention (mg/100g cocoyam) in cocoyam flours stored under various relative humidities

*Values (± SD) are means of triplicate determinations; -, not detected; * Given in Table 6*

Table 3. Rate of ascorbic acid and anthocyanin degradation, and non-enzymatic browning development in cocoyam flours stored under different relative humidities

Given in Table 6. ND, not determined. † Values were not estimated from regression curve but from observed points since n = 2.

Table 4. Reducing sugar content (mg/100g, dry weight basis) of treated and non-treated cocoyam flours stored under different relative humidities

*different (P = 0.05). * Shown in Table 6.*

3.4 Alcohol Soluble Colour

The measurement of alcohol soluble colour at 390nm indicates the degree of non-enzymatic browning. The effect of storage RH and storage time in differently processed (NT, BT and SBT) cocoyam flours are shown in Figures 1-3. The absorbance of the NT flour extract was highest while that of SBT was lowest. This indicates that the non-enzymatic browning was highest in NT flour and lowest in SBT flour. Some factors known to influence the browning process include temperature, time, pH, oxygen content, amino acids, sugars and trace metals [36]. According to Kopelman et al. [37], this non-enzymatic browning is associated with the Maillard reaction between reducing sugars and amino compounds, acid caramelization of sugars and the degradation of ascorbic acid. Lloyd [36] reported that L ascorbic acid (vitamin C) has a role in the non-enzymatic browning of foods although the mechanism remains obscure; browning might occur from direct participation of ascorbic acid or reactions between degradation products of the vitamin and free amino acids or other compounds. The absorbance of the cocoyam flour extract at 390 nm could, therefore, be a cumulative contribution of Maillard reaction, acid caramelization of sugars and the degradation of ascorbic acid in varying degrees. Ascorbic acid may have led to the higher absorbance of the NT flour considering the greater rate of loss (Tables 1 and 3) in the NT flour. Moreover, the brown pigment of degraded anthocyanin, the major pigment of cocoyam, has its absorbance at maximum wavelength of 422 nm [38].

It is likely that the spectra of the absorbance may have extended to 390 nm thereby increasing the absorbance of NT flour extract at this wavelength. In the same way, the lower non-enzymatic browning of the BT than NT flour could be associated with lesser acid caramelization of sugar and lesser ascorbic acid and probably anthocyanin degradation, considering the greater ascorbic acid and anthocyanin retention (Tables 1 & 2) in the BT flour.

Relative Humidity (%)	Treatments		
	None	Blanched	Sulphited & blanched
Ambient [*]	-0.991	-0.974	-1.000
11	-0.934	-0.980	-0.922
33	-0970	-0.992	-0.976
53	-0.996	-0.998	-0.991
67	-0.936	-0.998	-0.998
75	-0975	-0.991	-0.998

Table 5. Correlation coefficient of the absorbance (390 nm) of alcohol soluble colour (non-enzymatic browning) and the reducing sugar content of cocoyam flour stored under various relative humidity conditions.

*t-value significant (α level 0.05, 3df) *shown in Table 6*

Table 6. Summarized ambient† temperature and relative humidity conditions of control samples

† The abient conditions are of a laboratory in the Federal Polytechnic, Idah, Nigeria, where the research was carried out.

Figs. 1-3 revealed that browning increased with storage RH and storage time. The occurrence of browning at the RH of 11% gave credence that water present in a monomolecular layer is available for browning reaction [31], considering the monomolecular layer of 3.57%, which corresponds to water activity slightly below 0.11 (11% relative humidity) observed in the preliminary analysis of this work. Contrary to expectation, sulphiting neither prevented nor delayed browning during storage except for storage at ambient (Table 6) and 11% RH where a lag phase of one month was observed before the development of browning. The rate of browning was calculated using regression analysis to determine the slope of the straight lines relating browning to storage time. The result presented in Table 3 revealed that the rate of development of non-enzymatic browning was generally lower in the sulphited cocoyam (SBT) flour. The rate of the browning development seemed to be higher in BT flour than in NT at RH of 53% and below, and vice versa at RH above 53%. Non-enzymatic browning is one of the most troublesome irreversible changes that accompany the dehydration of food products, leading to loss of acceptable colour, development of off flavour and loss of nutritive value [39]. Kopelman et al. [37] reported that the shelf life of citrus powder is reduced by non-enzymatic browning associated with Maillard reaction between reducing sugars and amino compounds, acid caramelization of sugars, and degradation of ascorbic acid.

Fig. 1. Absorbance of alcohol soluble colour of non-treated cocoyam flour

Fig. 2. Absorbance of alcohol soluble colour of blanched cocoyam flours

Fig. 3. Absorbance of alcohol soluble colour of sulphited and blanched cocoyam flour

4. CONCLUSION

Blanching and its combination with sulphiting favoured the preservation of ascorbic acid and anthocyanin during processing and storage. Ascorbic acid and anthocyanin degradations were lower in the blanched (BT) and sulphited-blanched (SBT) flour than in the non-treated (NT) flour at every storage RH. The degradations were higher at higher storage RH. Storage at 11% RH guaranteed the retention of anthocyanin at fifth month in the NT and at the seventh month in the BT and SBT. Ascorbic acid was also retained at the seventh month in the NT flour stored at 11% RH, and BT and SBT flours stored at ambient, 11% and 33% RH. Reducing sugar content was highest in NT flour and least in BT flour; and also decreased during storage. The decrease was greater at higher storage RH. Non-enzymatic browning development was higher in the NT flour and increased with increasing storage RH. At every storage RH, the correlation of reducing sugar and browning development was high.

COMPETING INTEREST

The authors hereby declare that there is no competing interest in this researched work.

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