# EFFECT OF PRE-TREATMENT METHODS ON BETA-CAROTENE RETENTION, PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF FLOUR FROM YELLOW-FLESHED CASSAVA ROOT

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***Abstract***
*The effects of pre-treatment methods on β-carotene retention, physico-chemical properties and functional properties of high quality cassava flour (HQCF) from yellow-fleshed cassava root were investigated. The flours were prepared from peeled and sliced yellow-fleshed cassava roots. The first sample(untreated) served as the control, the second sample was blanched while the three other samples were treated using 1.0% sodium metabisulphite (NaHSO3), 1% w/v Calcium chloride ($CaCl_2$), 1%w/v citric acid solution respectively. The β-carotene retention, physico-chemical properties and functional properties of yellow cassava flour were analyzed using appropriate methods. Data obtained were subjected to Analysis of Variance and compared using Duncan Multiple Comparison Test. There existed significant ($p<0.05$) difference in the β-carotene content and $L^*a^*b^*$ colour analysis of the yellow cassava flour which ranged from 7.08-8.97 µg/g and 86.15 to 87.20, -3.08 to -2.33, 16.95 to 21.49 respectively. The citric acid pre-treated flour had the highest β-carotene content while the control had the lowest. The water absorption capacities, oil absorption capacities, pH and HCN content were significantly different while other parameters determined were not significantly different. The pH and HCN content of the flour ranged from 5.25-6.26 and 2.95-3.52 mg HCN eqv/kg, the HCN content were within the values recommended by SON (<10 mg HCN eqv/kg). However, the best quality yellow cassava flour in terms of β-carotene retention was obtained from citric acid pre-treated yellow cassava roots. Increased β-carotene (pro vitamin A) retention in turn will minimize occurrence of vitamin A deficiency (VAD) among consumers.*

**Keywords:**, *β-carotene; pre-treatment; HQCF; vitamin A deficiency, Hydrogen Cyanide*

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## 1. INTRODUCTION

Cassava (*Manihot esculanta Crantz*) is cultivated in the tropical regions such as the sub-Saharan Africa for its starchy tubers which can be processed into various food products for animals and humans. Although deficient in essential micronutrients and proteins, cassava is a quality source of carbohydrates (Aniedu & Omodamiro, 2012). Vitamin A, iron, and iodine deficiencies are the most common micronutrient deficiencies worldwide. The process of adding nutritional value to a crop is called biofortification (Tanumihardjo, 2008). Globally, vitamin A deficiency (VAD) is the leading cause of blindness among children worldwide (WHO/FAO, 2003). Cassava has however been targeted for biofortification because of its unique geographical distribution and its importance as a staple food. Recently, yellow-fleshed cassava breed which has been biofortified with beta-carotene (pro-vitamin A) were introduced to developing countries including Nigeria. Beta-carotene is a naturally occurring carotenoid, a major source of vitamin A from plant source (Mayne, 1996).

However, due to high level of cassava root's perishability, it is usually processed into a more stable product such as high quality cassava flour (HQCF). Increased effort has begun in these tropical countries to promote use of composite flours from local crops such as cassava and sweet potato in many food applications. However, HQCF has to be characterized in terms of physical, chemical and functional properties in order to ascertain its success in completely or partially replacing wheat flour (Ajibola & Olapade, 2017).

Furthermore, to ensure retention of fruits and vegetables quality, various pre-treatment methods such as physical, chemical and thermal have been investigated (Akyildiz *et al.*,

2004) (Dewanto *et al.*, 2002). Discolouration significantly affects the quality of food products and arises from two major sources. The first is the formation of brown discolouration caused by the oxidase reaction of polyphenol groups by enzymes; the second is the non-enzymatic browning (at high temperatures) that result when reducing sugars condense with amino groups (Utomo *et al.*, 2005). Several methods have been developed to eliminate enzymatic discolouration. Hoover & Miller (1973) used sodium acid pyrophosphate blanch treatment to eliminate browning. Olorunda & Kitson, (1977) eliminated discolouration in chips prepared from white flesh potatoes by dipping them in sodium sulphite. Chala G. *et al.*, (2018) used citric acid pre-treatment to evaluate and improve the nutrient content of orange fleshed sweet potato flours. Blanching, sulphites, $CaCl_2$ and Citric acid pre-treatment which have been observed to prevent nutrient loss were applied in the processing of high quality cassava flour (HQCF) from yellow-fleshed cassava roots. Hence, this study aimed to determine which pre-treatment method ensured retention of preferable quality attributes in terms of beta-carotene content, physico-chemical properties and functional properties of the HQCF.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Yellow fleshed cassava roots of variety IITA/TMS/IBA/070593 were collected from International Institute of Tropical Agriculture (IITA), Ikenne, Ogun State. All chemicals used were of analytical grade and were obtained from Sigma-Aldrich, London, United Kingdom.

#### 2.1.1. Sample Preparation

The yellow fleshed cassava roots were processed into HQCF as described by Aniedu & Omodamiro, (2012) with slight modification. Freshly harvested cassava roots were peeled, washed, sliced and divided into 5 batches. The first batch served as the control hence, untreated. The second batch was blanched in a water bath at $80^0$C for 3 mins, the third batch was dipped in 1% w\v citric acid solution for 3 mins at $20^0$C, fourth batch was also soaked in 1% w\v Calcium Chloride and finally the fifth batch was dipped in 1% sodium metabisulphite solution for 2 mins.

Each batch were afterwards grated, pressed and pulverised to increase the surface area in order to enhance drying process. The five batches were dried using a cabinet drier at $60^0$C. The dried samples were cooled, milled, sieved with a 250 µm mesh to obtain flour, packaged in low density polyethylene bags and stored appropriately.

### 2.2. Sample Analysis

#### 2.2.1. β-carotene determination

This was quantified as described by Carvalho *et al.* (2012). Fifteen milliliteres of the extract was pipetted into a concentrator tube and concentrated at $40^0$C for 25 min. in TurboVap® LV concentration workstation. The concentrate was diluted in 1 ml of dichloroethane and 1 ml of methanol. This was shaken in a vortex mixer and transferred to a 2-ml amber flask of HPLC apparatus. The apparatus was turned on and generated corresponding graph for each sample. The values obtained from the graph were input into the equation to determine total beta carotene and its isomers.

Total β-carotene (µg/g) = Ax ∗ Cs (µg/ml) ∗V (ml)/ As ∗ P(g) (6)

Where Ax = carotenoid peak area, Cs = standard concentration, As = standard area,
V = total extract volume, and P = sample weight.

#### 2.2.2. Functional Properties Determination

##### 2.2.2.1. Bulk Density determination

The bulk density of the sample was determined by the method described by Adeleke and Odedeji (2010). Fifty gram (50) g of sample was weighed into 100 ml graduated measuring cylinder. The sample was packed by gently tapping the cylinder on the bench top severally till a constant volume. The volume of the sample was recorded.

Bulk Density (g/ml) = weight of sample/ volume of sample after tapping

**2.2.2.2. Water absorption and oil absorption capacities**

Water absorption and oil absorption capacities were determined according to the method described by Olapade *et al.* (2003) with slight modification. For water absorption or oil absorption capacity, 1.5 g of the flour sample was measured into a weighed centrifuge tube, 10 ml of distilled water or refined oil was added. This was allowed to stand at room temperature for 30 mins and centrifuged at 3000 rpm for 20 mins. Water or oil absorption capacity was expressed as mass of water or oil bound by the sample.

**2.2.2.3. Dispersibility**

The dispersibility of the samples was determined as described by Chijioke *et al.* (2016). Ten grams of sample was weighed into 100 ml measuring cylinder and distilled water was added to reach 100 ml mark. The set up was stirred vigorously and allowed to settle for 3 h. The volume of settled particles was noted and subtracted from 100. The difference was recorded as % dispersibility.

**2.2.2.4. Foam capacity (FC) determination**

The method used by Sathe & Salunkhe, (1981) was used with slight modification. About 2 g of sample was mixed with 100 ml of distilled water and its volume noted. The suspension was blended with Philips blender using the lowest speed for 15 min. It was poured into a 250 ml measuring cylinder and its volume noted and recorded. Using Abbey and Ibeh (1988) formula, foam capacity expressed percentage increase in volume was calculatedas follows:

$$\text{Foam Capacity} = 100 \times \frac{\text{Volume of whipping- Volume before whipping}}{\text{Volume before whipping}}$$

**2.2.2.5. Least gelation concentration (LGC) determination**

This was evaluated using a method of Adeleke and Odedeji (2010). Dispersions of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20% (w/v) of materials in 5 ml distilled water. The dispersions were heated in a water bath at $95^0$C for 1 h, followed by rapid cooling under running tap water. The test-tubes were further cooled for 2 h at $4^0$C. The least gelation concentration (LGC) was taken as that first concentration at which the sample in the inverted test tube did not fall down or slip.

**2.2.3. Moisture content determination**

Moisture content was determined as described by AOAC (1990). Five grams of flour samples from each batch was weighed into moisture cans. The cans and its sample content were dried in an oven at $105^0$C for 3 h in the first instance. The cans was removed, cooled in a dessicator and reweighed. The weights will then be recorded. Drying, cooling and reweighing will be continued repeatedly until a constant weight is obtained by the difference. The weight of the moisture loss will be determined and expressed in percentage. It can be calculated as shown below:

$$\% \text{ moisture content} = \frac{W3-W1}{W2-W1} \times 100, \text{ where}$$

W1=weight of empty moisture can
W2=weight of can before drying
W3=weight of can + sample after drying to a constant weight

**2.2.4. Colour determination of yellow cassava flour**

The colour of yellow cassava flour samples were measured by reflectance using a colorimeter (CR200, C ILLUMINANT, $0^0$ viewing angle, ($2^0$ observing angle), 8mm aperture, Minolta, Japan). Each sample was measured in locations to determine $L^*$ (lightness) was the quantity of reflected light and chromic co-ordinate, $a^*$ (redness) and $b^*$ (yellowness). The result was expressed in $L^*$ $a^*$ $b^*$ calibrated colorimeter system according to the 1986 CIE standards (MacDougall, 2000).

**2.2.5. pH and HCN determination**

The pH of the samples was measured with a pH meter. Ten grams (10 g) of each sample

collected is homogenized in 50ml of distilled water. The resulting suspension was decanted and their pH determined using pH meter already standardized with buffer solutions of pH 4.0 and 7.0. The method of Onwuka (2005) was adopted for the determination of hydrogen cyanide (HCN).

### 2.3. Statistical analysis

All analyses were carried out in duplicate. Mean and standard deviation were obtained as data were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 20. Mean comparison and separation were done using Duncan's New Multiple Range Test (DNMRT) and LSD at ($P<0.05$).

## 3. RESULTS AND DISCUSSION

### 3.1. β-carotene contents of HQCF from various pre-treatment methods

Table 1 showed the result of the statistical analysis of the beta carotene content and colour of HQCF from pre-treated yellow fleshed cassava root of variety IITA/TMS/IBA/070593 dried at $60^0$C. Statistical Analysis showed there was significant ($p<0.05$) difference in the β-carotene content of the HQCF. The β-carotene contents ranged from 7.08-8.97 µg/g such that Citric acid pre-treated sample had the highest β-carotene content and the untreated sample had the lowest. The β-carotene content of the untreated sample is comparable to that of (Ajibola & Olapade (2017). The high β-carotene retention of citric acid pre-treated flour sample could be due to the fact that oxidation of β-carotene at $60^0$C is minimal and low activities of enzyme (peroxidases and lipoxygenases) at low pH which have a capacity to degrade β-carotene (Ahmed *et al.*, 2011). Flours pre-treated with Citric Acid from orange fleshed sweet potato (OFSP) at $55^0$C also had the highest β-carotene content (Chala *et al.*, 2018). The low β-carotene content of untreated flour is due to activities of enzymes (peroxidases and lipoxygenases) at low pH which have a capacity to degrade β-carotene (Ahmed *et al.*, 2011). Calcium chloride pre-treated sweet potato flour was observed to also retain more β-carotene than sulphite pre-treated flour (Ahmed *et al.*, 2010) which is comparable to that obtained in this yellow cassava flour.

### 3.2. L*A*B* colour of HQCF from various pre-treatment methods

Statistical Analysis from table 1 also showed significant difference ($p<0.05$) in the LAB colour analysis.

The L* (Lightness), A* (greeness) and B* (yellowness) ranged from 86.15-87.20, -2.33 - (-3.08), and 16.95-21.49 respectively. The untreated sample had the lowest lightness (L*) value and the sodium metabisulphite pre-treated sample had the highest. The untreated sample had the least b* (yellowness) value 16.95 and the Calcium chloride treated sample had the highest b* (yellowness) value of 21.49. It has been evidenced that calcium plays a major role in maintaining the quality of fruits and vegetables. Calcium chloride ($CaCl_2$) has shown its effectiveness in stabilizing surface colour of freshly cut apple slices (Ahmed *et al.*, 2010) and providing protective effect against oxidation (Lewicki, 1998) which justifies the high yellowness values. Vimala *et al.* (2011) reported that retention of carotenoids in different processing methods assists in assessing the best technique for obtaining food products of higher nutritional quality.

**Table 1: β-carotene content and LAB Colour of HQCF from various pre-treatment methods**

| Pre-treatment | β-carotene (µg/g) | L* (Lightness) | a* (greeness) | b* (yellowness) |
|---|---|---|---|---|
| No Treatment | $7.08\pm0.04^{d}$ | $87.05\pm0.09^{a}$ | $-2.33\pm0.00^{a}$ | $16.95\pm0.03^{e}$ |
| Citric Acid | $8.97\pm0.06^{a}$ | $86.46\pm0.26^{ab}$ | $-2.89\pm0.00^{c}$ | $19.77\pm0.04^{b}$ |
| Calcium Chloride | $8.88\pm0.02^{a}$ | $86.25\pm0.04^{b}$ | $-3.08\pm0.01^{d}$ | $21.49\pm0.03^{a}$ |
| Sodium metabisulphite | $8.69\pm0.06^{b}$ | $87.2\pm0.39^{a}$ | $-3.07\pm0.01^{d}$ | $19.63\pm0.02^{c}$ |
| Blanching | $8.28\pm0.02^{c}$ | $86.15\pm0.02^{b}$ | $-2.66\pm0.03^{b}$ | $18.51\pm0.04^{d}$ |

Mean duplicate determinations.
Mean values having different superscript within column are significantly different ($P<0.05$)

**Table 2: Functional properties of yellow cassava flour from various pre-treatment methods**

| Pre-treatment | BD (g/ml) | WAC (%) | OAC (%) | Dispersibility (%) | LGC (%) | FC(%) |
|---|---|---|---|---|---|---|
| No Treatment | 0.56±0.0[a] | 154.69±2.0[ab] | 147.02±1.66[a] | 67.0[a] | 6.0±0.0[a] | 2.70±0.25[a] |
| Citric Acid | 0.53±0.0[a] | 160.62±0.82[a] | 148.39±0.96[a] | 68.0[a] | 6.0±0.0[a] | 2.21±0.25[a] |
| Calcium Chloride | 0.56±0.0[a] | 159.78±0.69[a] | 150.91±0.45[a] | 69.0[a] | 6.0±0.0[a] | 2.45±0.49[a] |
| Sodium metabisulphite | 0.53±0.0[a] | 148.49±1.29[bc] | 139.49±1.43[b] | 65.0[a] | 8.0±0.0[a] | 2.45±0.00[a] |
| Blanching | 0.50±0.0[a] | 143.46±5.12[c] | 132.56±1.07[c] | 67.5[a] | 4.0±0.0[a] | 2.45±0.49[a] |

Mean duplicate determinations, BD means bulk density, WAC means water absorption Capacity, OAC means oil absorption capacity, LGC means least gelation concentration and FC means Foam capacity.
Mean values having different superscript within column are significantly different (P<0.05).

### 3.3. Functional properties of yellow cassava flour from various pre-treatment methods

Table 2 showed the result of the statistical analysis of the functional properties of the high quality HQCF. Functional properties of flours are those that directly determine their end uses. The table shows the effect of pre-treatment methods on the functional properties of the yellow cassava flour. From table 2 the following were observed:

**Water and Oil Absorption Capacities**

There were significant ($p< 0.05$) difference in the water and oil absorption capacity of the yellow cassava flour. The water absorption capacity ranged from 143.46% to 160.62% with flour from blanched sample having the lowest and flour from citric acid pre-treated sample having the highest value. The oil absorption capacity also ranged from 132.56% to 150.91% with flour from blanched sample having the lowest and flour from Calcium chloride treated sample having the highest value**.** Oil absorption is an indication of the amount of oil that can be absorbed by the physical matrix of a food. It indicates the degree of hydrophobicity (Voutsinus *et al.*, 1983) of flour. Result proved that the flour samples were high in both oil and water absorption capacities. The high water absorption capacity of some flour may be due to the higher polar amino acid residues of proteins having an affinity for water molecules (Yusuf *et al.*, 2008).

**Bulk Density**

From Table 2, flour from blanched sample had the lowest bulk density (0.50g/ml); flour from untreated sample and calcium chloride treated sample had the highest (0.56g/ml). There existed no significant difference in the bulk densities of the flours. The bulk density is an important parameter that determines the ease of packaging and transportation of particulate foods. Agunbiade & Sanni, (2003) opined that low bulk density of flour is a desirable physical attribute for saving cost in transportation and storage. The bulk density of the HQCF is comparable to values reported by Ajibola & Olapade (2017).

**Dispersibility**

Dispersibity ranged from 65 to 69% with flours from sodium metabisulphite treated sample having the lowest value and Calcium chloride treated sample having the highest value. There existed no significant difference among the samples in terms of dispersibility. The dispersibility of the HQCF is comparable to values reported by Ajibola and Olapade (2017). Dispersibility is a measure of the degree to which flour or flour blends reconstitute in water; the higher the dispersibility, the better the flour reconstitutes in water (Adebowale *et al.*, 2005). The higher dispersibility values exhibited by all the samples of HQCF were indicative of their ability to produce smooth dough in composite with wheat flour.

**Foaming Capacity**

Statistical analysis showed there were no significant differences in the foam capacity of the yellow cassava flours. The foam capacity ranged from 2.21% to 2.70% with flour from citric acid treated sample having the lowest and flour from untreated sample having the highest. The results were comparable to those reported by Adebowale *et al.* (2011). Foam is a colloid of many gas bubbles trapped in a liquid or solid. Small air bubbles are surrounded by thin liquid films. Foam can be produced by

whipping air into liquid as much and fast as possible (Sikorski, 2002). The reason why flours are capable of producing foams is that protein in flours are surface active. Soluble proteins can reduce surface tension at the interface between air bubbles and surrounded liquid. Thus, the coalescence of the bubbles is obstructed. In addition, protein molecules can unfold and interact with one another to form multilayer protein film with an increased with an increased flexibility at the air liquid interface. As a result it is more difficult for air bubbles to break, and the foams are more stabilized (Adebowale & Lawal, 2003).

**Least Gelation Concentration**

The least gelation concentration (LGC) which is defined as the lowest protein concentration at which gel remained in the inverted tube was used as index of gelation capacity (Suresh *et al.*, 2015). . There was no significant difference in the LGC of the flours. The LGC ranged from 4% to 8% with the blanched sample having the highest and sulphite pre-treated sample having the lowest.

### 3.4.pH, moisture content and Hydrogen Cyanide contents of high quality cassava flour (HQCF)

Table 3 showed the result of the statistical analysis of the pH, moisture content and Hydrogen Cyanide contents of HQCF from yellow fleshed cassava root.

The pH is an indication of the acid content of food. The lower the pH value of a food, the more acidic is the food. The pH value ranged from 5.25-6.26 which were within values of 5.72-6.01 by Ajibola & Olapade, (2017). There existed significant ($p<0.05$) difference among pH of the yellow cassava flour sample.

The HCN contents of the flour samples 2.95-3.52 mg HCNeqv/kg were within the values recommended by SON (<10 mg HCNeqv/kg) (Sanni *et al.*, 2005). The values were considerably lower than those found in *gari*, *eba*, and cooked cassava roots by Marfo *et al.* (1990). The blanched sample had the least HCN content while the untreated sample had the highest value. There existed a significant difference among the HCN content of the flour samples.

There is no consensus on the safe levels of cyanide for both human and animal consumption (Maziya-Dixon *et al.*, 2007) by scientists and international regulatory agencies. Mahungu *et al.* (1987) noted that a great danger of chronic poisoning might occur if roots with more than 150 mg HCN/kg are consumed. According to Koch *et al.* (Koch *et al.*, 1992), when the peeled portion contains <50 mg HCN/kg of freshly grated cassava, the cassava can be taken as harmless to the consumer. A concentration of between 50 mg HCN/kg and 80 mg HCN/kg may be slightly poisonous; 80–100 mg HCN/kg is toxic, while concentrations above 100 mg HCN/kg of grated cassava are fatal (Koch *et al.*, 1992) (Maziya-Dixon *et al.*, 2007). Presently in Nigeria, grating/crushing is being promoted in production of high quality cassava flour (HQCF) because it leads to the production of flour with negligible amounts of residual cyanide contents after drying. The joint FAO/ WHO Food Standards Program Codex Committee on Contaminants in Foods (JECFA) 3rd Session held in the Netherlands in 2009 concluded that a level of up to 10 mg HCN/kg in the Standard for Edible Cassava Flour (CODEX STAN 176-1989) was not associated with acute toxicity (WHO, 1993). A review of the available data by European Food Safety Authority (EFSA Journal) in 2004 arrived at a similar conclusion (JECFA, 2009).

**Table 3:pH, moisture content and Hydrogen Cyanide contents of yellow cassava flour**

| Pre-treatment | pH | HCN (mg/Kg) | M.C (%) |
|---|---|---|---|
| No Treatment | $5.85\pm0.03^{b}$ | $3.52\pm0.03^{a}$ | $10.04\pm0.01^{a}$ |
| Citric Acid | $5.76\pm0.02^{c}$ | $3.10\pm0.02^{c}$ | $10.05\pm0.00^{a}$ |
| Calcium Chloride | $5.41\pm0.02^{d}$ | $3.43\pm0.02^{b}$ | $10.06\pm0.01^{a}$ |
| Sodium bisulphate | $5.25\pm0.03^{e}$ | $3.47\pm0.01^{ab}$ | $10.02\pm0.01^{a}$ |
| Blanching | $6.26\pm0.01^{a}$ | $2.95\pm0.02^{d}$ | $10.04\pm0.01^{a}$ |

Mean duplicate determinations, HCN means hydrogen cyanide, M.C means moisture content.
Mean values having different superscript within column are significantly different ($P<0.05$)

The moisture content also ranged from 10.02 to 10.06% with the calcium chloride treated sample having the highest and the sulphite treated flour sample having the lowest. There existed no significant difference in the moisture content of the flours. FAO (FAO/WHO, 1992) had recommended a moisture safe level of 12%-13% for storage of cassava flour since high moisture content usually predispose flour to problem of formation of lumps and mould growth within a very short period of storage.

## 4. CONCLUSIONS

The effects of pre-treatment methods on β-carotene retention, physico-chemical properties and functional properties of HQCF from yellow fleshed cassava roots were investigated. Pre-treatments carried out include blanching, use of citric acid, calcium chloride, sodium metabisulphite respectively while the untreated sample served as the control. Results obtained were subjected to statistical evaluation. Use of pre-treatments showed an increase in the β-carotene and colour retention of the HQCF with the citric acid pre-treated sample retaining the most β-carotene. Increased retention of β-carotene, a precursor of vitamin A in pre-treated HQCF will help minimize the prevalence of vitamin A deficiency (VAD) in regions where these flours are utilized. All five samples including the control conformed to HQCF specifications in terms of physico-chemical properties and functional properties. Improved retention of β-carotene and other quality attributes of HQCF from yellow fleshed cassava root through the use of pre-treatment will help minimize deficiency of a significant micronutrient, Vitamin A which is a form of hidden hunger and a leading cause of blindness among children in sub-Saharan Africa.

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