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Possible minimal post-processing losses of pro-vitamin A and ascorbic acid in short-term stored wholesome cassava flour

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Abstract

Wholesome cassava flour samples produced from freshly harvested tuberous roots of two white fleshed cassava varieties (TME 419 and TMS 30572) and a yellow fleshed variety (UMUCASS 38) and stored in sealed black polyethylene bags (each containing $\cong 200$ g sample) at ambient room temperature (26-32°C) were used to determine possible post-processing losses on their ascorbic acid (vitamin C), carotene (pro-vitamin A) and cyanide contents. Results showed that the experimental fresh flour samples from the white fleshed varieties had 8.00-8.13mg/100g ascorbic acid, 0.53-1.13 μ g/g carotene and 4.65-4.74mg/kg cyanide contents while the yellow fleshed cassava flour had 9.73mg/100g ascorbic acid, 14.35 μ g/g carotene and 5.75mg/kg cyanide contents. Four weeks storage of the flour samples under dark conditions gave no significant decrease ($P=0.05$) in the carotene content of all the experimental samples. Minimal significant ($P=0.05$) reductions were observed only in the ascorbic acid content of the yellow cassava (UMUCASS 38) sample (9.73mg/100g to 6.67 mg/100g). The variation in the cyanide content of all the varietal samples (4.65-5.75 mg/kg to 3.03-4.92 mg/kg) were also minimally significant ($P=0.05$) after four weeks of storage. These experimental wholesome dry flour samples maintained near stable packed bulk density (0.63-0.67g/cm³) and swelling index (1.25-1.41) throughout the storage period of four weeks.

1. Introduction

Cassava or manioc (*Manihotesculenta*) is extensively cultivated as an annual crop in tropical and subtropical regions of the world for its edible starchy tuberous roots (Ugwu and Ukpabi, 2002; Burrell, 2003; Ukpabi, 2008). Cassava ranks very high among crops that convert the greatest amount of solar energy into carbohydrate per unit geographic area (about 40% higher than rice and 25% more than maize) with the result that cassava is the cheapest source of calories for both human nutrition and animal feeding (Burrell, 2003). The edible cassava root, as a root crop, also contains

the anti-scurvy vitamin C or ascorbic acid, unlike cereal crops such as wheat, rice and maize (Davidson *et al.*, 1975; Okaka and Okaka, 2001).

Nigeria is not only the world's largest producer of cassava (Ukpabi, 2009) but also has the crop's processed products (e.g. gari, lafun and cassava flour) largely found in the daily meals of many Nigerians. Adequate cassava processing is known to detoxify the toxic cyanogenic glycosides or glucosides (linamarin and lotastralin) present in the crop (Bokanga, 1995; Cardoso *et al.*, 2005) to safe levels of less than 10 parts per million (ppm) (FAO/WHO, 1991). Cassava is presently undergoing a transition from a mere subsistence crop found in the fields of peasant farmers to a commercial crop that is grown in large quantities in commercial farms in Nigeria, as it is a source of raw materials for a number of industrial products such as cassava starch, bio-ethanol and cassava flour (Ukpabi, 2008; Ukpabi, 2009). Cassava flour as a raw material for the bakery industry is fast gaining recognition as a viable partial substitute for wheat in bread baking in Nigeria (Taiwo, 2006; Oti and Ukpabi, 2007).

Recent research efforts at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and National Root Crops Research Institute (NRCRI), Umudike, Nigeria have led to the development of yellow fleshed cassava roots that have appreciable quantity of pro-vitamin A or carotenes (through bio-fortification). The pro-vitamin A rich yellow cassava varieties are proposed to be used as a tool in combating vitamin A deficiency (VAD) syndrome especially amongst malnourished women and children in resource poor homesteads. Unfortunately, Ukpabi *et al.* (2012) confirmed earlier local observation on pro-vitamin A storage losses in a stored wholesome cassava floury product (gari) that was enriched with the pro-vitamin A rich palm oil. This study was therefore carried out to investigate the feasibility of producing wholesome cassava flour that will have near stable major micronutrients or vitamins contents of the processed crop in short term storage period that is required for the local end users to possibly finish using the product. This investigation is also aimed at getting data that will help in properly advising local cassava flour producers on relevant post-processing quality attributes of their floury product.

2. Materials and Methods

2.1. Sources of Materials

The two experimental white fleshed cassava varieties (TME 419 and TMS 30572) and a yellow fleshed variety (UMUCASS 38) were randomly harvested at 12 months after planting from the experimental plots of Cassava Programme, National Root Crops Research Institute, Umudike, Nigeria. The analytical chemicals and reagents used in this study were manufactured by BDH (British Drug Houses), Poole, England.

2.2. Cassava Flour Processing

The cassava flour samples from the tuberous roots of the respective experimental varieties were prepared in a near dark food laboratory (with windows covered with black curtains) within 15 hours from the time of field harvest of the roots. Figure 1 shows the unit and subunit operations used in the production of the cassava flour samples based on the modified method of Ukpabi (2008). The peeling of the fresh cassava roots was done manually with sharp kitchen knife while washing was done also manually with clean water. The grating was done mechanically with a grater (a Field Marshal model with 7.5 Horse power diesel engine). Dewatering of the grated cassava pulp (bagged in sacks) was done with a screw press. The pulverized dewatered cassava mash was effectively dried at 65°C to brittleness in an electric hot air, thermo-regulated oven (Gallenkamp, BS model Ov-160). Milling of the dry cassava mash was done with a single disc attrition mill (A446A model) while 250µm mesh sieve was used to get the fine flour for each experimental cassava variety. The various flour samples obtained (with ≤8% moisture content) were packaged in black polyethylene bags (with the open ends sealed up) and labelled accordingly.

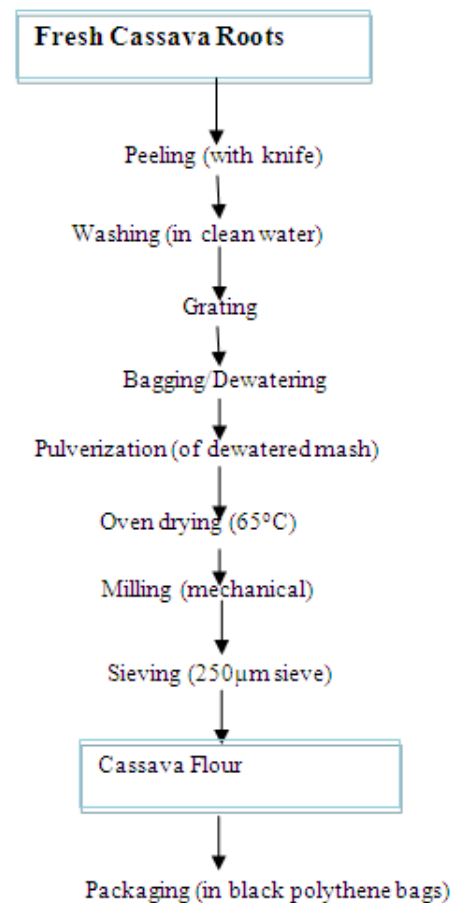


Figure 1. Flow chart for the production of the experimental cassava flour.

2.3. Post Processing Flour Storage

The experimental cassava flour samples were packaged in black low density polyethylene (LDPE) bags and stored in a metallic cabinet kept in a dark room at 26-32°C. Each of the LDPE bags contained about 200g of the flour samples, and the short term storage periods for laboratory analysis were zero, 14 and 28 days.

2.4. Laboratory Analyses

The total carotene, ascorbic acid and cyanide contents of the randomly collected experimental roots (after peeling) and flour samples were analyzed in triplicates in the laboratory. The carotene content was determined spectrophotometrically with the HarvestPlus method (Rodriguez-Amaya and Kimura, 2004). Acetone and petroleum ether were sequentially used as the extraction solvents (with light exclusion) while the readings with the spectrophotometer (Jenway 6406, England) were done at $\lambda 450$ nm (with 1 cm glass cuvette).

The carotene content was calculated as follows:

$$\text{Carotene content } (\mu\text{g/g}) = \frac{A \times V \times DF \times 10^4}{A^{1\%} \times \text{Sample weight(g)}}$$

Where A= absorbance

V= Volume of extract

DF = Dilution factor

10^4 = constant

$A^{1\%}$ =Absorption coefficient of β -carotene in petroleum ether= 2592

The titration method, as described by James (1998), was used to determine the ascorbic acid content of the fresh peeled tuberous roots and flour samples using 2,6-dichlorophenolindophenol (DCP) blue dye as an indicator to get the titer values (at 15 seconds persistent pinkish end points) with liquid extracts of the samples. Freshly prepared standard ascorbic acid solution was used to calculate equivalent to 1ml of the DCP dye solution.

The total cyanide content of fresh peeled cassava roots and flour samples was determined by the colorimetric Alkaline Picrate method of Ikediobi *et al.* (1980) as modified by Onwuka (2005). The yellowish alkaline picrate solution was obtained by dissolving 1g picric acid and 5g Na_2CO_3 in distilled water. The liquid extract filtrate (1.0 ml) from the cyanide extraction process was added to 4.0ml alkaline picrate solution in a test tube and corked. The mixture was incubated at 50°C for 5 minutes to allow for colour development. After colour development (from yellowish colour to reddish colour) and cooling, the absorbance was read at 490nm wavelength with UV/visible spectrophotometer (Jenway 6405, England). Diluted potassium cyanide (KCN) was used to prepare the standard curve that was employed to calculate the cyanide content of the experimental samples.

Swelling index determination of the experimental flour samples was done in triplicates with the method of

Ukpabi and Umeh, (2001). In this determination, 20g flour sample was gradually put into a 500ml measuring cylinder containing 100ml distilled water. The initial volume (V_1) of the flour sample in the measuring cylinder and its final volume (V_2) after the sample had been allowed to swell in the water medium for 24hrs were used to calculate the swelling index as follows:

$$\text{Swelling index} = \frac{\text{final volume}(V_2)}{\text{Initial volume}(V_1)}$$

The method of Okezie and Bello, 1988 as modified by Ukpabi and Umeh (2001), was used to determine the packed bulk density of the flour samples. Previously tared 100ml measuring cylinder was gently filled with 50g of the sample and then gently tapped on a laboratory bench several times until a constant volume was obtained. This final volume (V_2) was then used for the calculation of the packed bulk density as follows:

$$\text{Packed bulk density (g/ml)} = \frac{\text{weight of sample(g)}}{\text{volume } (V_2) \text{ of the sample(ml)}}$$

2.5. Statistical Analysis

Statistical Analysis System (SAS) PC software (License site 0822206002) belonging to International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria was used for the mean separations and other statistical analysis.

3. Results and Discussion

Table 1 shows the dry matter and carotene contents of the fresh roots of the experimental yellow fleshed cultivar (UMUCASS 38) and white fleshed cultivars (TME 419 and TMS 30572). It was observed that the yellow fleshed root had carotene content that was significantly ($P=0.05$) higher than the control cultivars or varieties which had whitish flesh colour. Carotenes are known to impart yellow to orange colour (based on concentration and type) on fresh plant materials (Rodriguez-Amaya, 1999; Rodriguez-Amaya and Kimura, 2004). On dry matter basis, the disparity between the carotene content of the yellow fleshed cassava genotype over the control white fleshed genotypes seemed relatively large. It was also observed that the mean moisture content of the yellow fleshed variety was up to 10% higher than the moisture values got for the experimental white fleshed cassava varieties (Table 1). The anti-scurvy ascorbic acid content of the fresh yellow fleshed cassava was also found to be significantly ($P=0.05$) higher than those of the white fleshed roots with the obtained cyanide potentials of all the experimental roots (Table 1) indicating that they all have appreciable amount of cyanogenic glycosides that need adequate detoxifying treatments to make them wholesome for human consumption (Cardoso *et al.*, 2005).

Table 1. Mean moisture and carotene contents of the fresh experimental cassava roots*.

Variety	Moisture Content (%)	Carotene**(µg/g)	Ascorbic acid**(mg/100g)	Cyanide (mg/kg)
TME419	62.60a	1.37c(3.66c)	19.54b(52.25c)	51.58b(137.91c)
TMS 30572	64.90a	1.93b(5.49b)	20.47b(58.32b)	54.87 b(156.32b)
UMUCASS 38	76.74b	5.78a(24.85a)	23.33a(100.30a)	74.99a (322.39a)

*Values in the same column with different letters are significantly different (P=0.05); **Values in brackets are on dry matter basis.

However, proper processing of high cyanide cassava roots into flour is known to lead to natural detoxification that drastically reduce the quantity of the poisonous and anti-nutritional cyanogenic glycosides to innocuous level for human consumption (Bokanga, 1995; Ukpabi, 2008). For example, the abrasive grating activity during the processing of the cassava roots cause the cassava cellular membranous linamarase enzyme molecules (Mponget *al.*, 1990) to come into contact with the cytoplasmic cyanogenic glycosides leading to their detoxifying hydrolysis (Bokanga, 1995). Food processing can also reduce the nutritionally beneficial carotene and ascorbic acid contents of plant food materials based largely on the processing methods and the nature of the raw material (Lee and Kader, 2000; Rodriguez-Amaya and Kimura, 2004; Omodamiro *et al.*, 2012)). Though the carotene content of the yellow cassava flour was found to be significantly (P=0.05) higher than those of the white cassava flour samples, the ascorbic acid contents of the three experimental cassava flour samples did not differ significantly (P=0.05) (Table 2). The processing loss of the water soluble ascorbic acid in this investigation seem to be more than that of the fat soluble carotene (Tables 1 and 2) probably largely due to the dewatering operation of the experimental processing system (Fig. 1). The obtained cyanide content of all the freshly prepared experimental flour samples (4.65-5.75mg/kg) showed in Table 2 is below the recommended safe level of 10mg/kg cyanide content for edible cassava flour (FAO/WHO/ 1991). Furthermore, the processing method used in the experimental flour production (Ukpabi, 2008) that involved both the dewatering and oven drying operations might have helped to drastically reduce the cyanide content of the product as the released HCN (during the hydrolytic detoxification of the endogenous cyanogenic glucosides) is both volatile (in tropical temperatures of $\geq 26^{\circ}\text{C}$) and highly soluble in water.

Table 2. Mean carotene, ascorbic acid and cyanide contents of the freshly prepared cassava flour samples.

Variety	Carotene(µg/g)	Ascorbic acid(mg/100g)	Cyanide(mg/kg)
TME419	0.53c	8.00a	4.74 b
TMS 30572	1.13b	8.13a	4.65b
UMUCASS 38	14.35a	9.73a	5.75a

Values in the same column with different letters are significantly different (P=0.05).

Light and oxygen have been observed to reduce the vitamin A value of processed carotene-rich food products

during storage (Rodriguez-Amaya, 1999; Vásquez-Caicedo *et al.*, 2007). In this study, it was found that light exclusion and packaging of 200g flour samples with sealed polyethylene sheets could give minimal non-significant (P=0.05) carotene reduction during four weeks of storage (Table 3). Due to the fact that head space oxygen in packaged food materials assist in carotene degradation during storage (Vásquez-Caicedo *et al.*, 2007), the packaging of yellow cassava flour in bulkier sizes of above 200g in the absence of light and possible air or oxygen exclusion could lead to a better retention of carotene in the product. On the other hand, the mean ascorbic acid content of the flour samples remained significantly indifferent (P=0.05) for two weeks in yellowish UMUCASS 38 flour sample and four weeks in the whitish TMS30572 and TME419 samples. Though oxidation is generally known to be involved in the primary degradation of ascorbic acid (with accompanying dehydration and polymerization to form nutritionally inactive products), there is a need to further investigate for any possible interrelationship between carotene and ascorbic acid degradations during the storage of food materials rich in these important micronutrients. This is based on the fact that both carotene and ascorbic acid molecules are antioxidants or oxygen scavengers (Damodaran *et al.*, 2008).

Table 3. Effect of storage on mean carotene and ascorbic acid contents of the cassava flour samples.

Storage Period(Weeks)	Carotene(µg/g)			Ascorbic Acid(mg/100g)		
	TME 419	TMS 30572	UMUC ASS 38	TME 419	TMS 30572	UMUC ASS 38
0	0.53a	1.13a	14.35a	8.00a	8.13a	9.73a
2	0.49a	1.04a	14.08a	7.47a	7.20a	8.40a,b
4	0.45a	0.99a	12.78a	6.13a	5.73a	6.67b

Values in the same column with different letters are significantly different (P=0.05).

Based on the fact that FAO/WHO (1991) gave the safe level of cyanide content of processed foods as 10ppm or mg/kg HCN the cyanide values in Table 4 indicate that all the experimental flour samples remained wholesome (in terms of cyanide toxicity) throughout the storage period with all the samples even having significantly (P=0.05) reduced cyanide contents at the end of the four weeks storage period. Owuamanam *et al.* (2010) had earlier indicated that longer fermentation period reduces cyanide concentration during the production of a dry fermented cassava granular product known as gari. Therefore, it is probable that minute fermentation might have taken place during the storage of the dry experimental cassava flour

samples. Ukpabi *et al.* (2012) also found that gari packaged with sealed LDPE film with water vapour laden air space had minute increase in its moisture content during two months storage period. We suggest a future detailed investigation on the possible effect of this enhanced moisture content of stored cassava floury materials on their obtained cyanide contents and even on those of carotene and ascorbic acid contents.

Table 4. Effect of storage on the mean cyanide (mg/kg) content of the cassava flour samples

Storage period(weeks)	TME 419 flour	TMS 30572 flour	UMUCASS 38 flour
0	4.74a	4.65a	5.75a
2	4.67a	4.21b	5.43b
4	3.03b	3.28c	4.92c

Values in the same column with different letters are significantly different (P=0.05).

Table 5. Effect of storage on the mean swelling index and packed bulk density of the cassava flour samples.

Storage period(weeks)	TME419 flour		TMS 30572 flour		UMUCASS 38 flour	
	Swelling index	Bulk density	Swelling index	Bulk density	Swelling index	Bulk density
0	1.40a	0.65a	1.37a	0.64a	1.41a	0.67a
2	1.34b	0.63a	1.34a	0.63a,b	1.38a,b	0.64a,b
4	1.25c	0.63a	1.26b	0.62b	1.31b	0.62b

Values in the same column with different letters are significantly different (P=0.05).

4. Conclusion

Production of cassava flour (especially with yellow fleshed cassava variety) under light exclusion (using unit operations that include: grating, dewatering and oven drying) gives appreciable retention of carotene molecules (pro-vitamin A) in the wholesome food product. The retention of carotene (pro-vitamin A) and ascorbic acid (vitamin C) composition of cassava flour samples during storage can also be enhanced through packaging with materials that have potentials of excluding light. It is therefore suggested that yellow cassava flour should be particularly stored under light exclusion in appropriate economically feasible packaging materials and volumes that will minimize post-processing carotene and ascorbic acid degradations.

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Edible wholesome floury materials are usually mixed with water before being used in numerous food preparations. The swelling index of a floury material, which indicates its ability to swell in water, can be slightly affected by storage (Ukpabi and Ndimele, 1990). Table 5 shows that the reduction effect of storage on the swelling index of the experimental flour samples did not reach four percent in all the experimental flour samples throughout the time of storage. Though Ukpabi and Ndimele (1990) also observed slight depreciation in the bulk density of floury materials during storage, the obtained results in Table 5 showed that the decrease in the packed bulk density of the wholesome experimental flour samples only ranged from 3.08% to 7.46%. Generally, the knowledge of the bulk density of processed floury foods in the food industry helps food technologists to know the quantity (net weight) of food materials to be put in packaging materials with known volume capacity.

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