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Processing Effects on the Total Carotenoid Content and Acceptability of Food Products from Cultivars of Biofortified Cassava (*Manihot esculenta* crantz)

Oluranti, O.M., Badejo, A.A and Fagbemi, T.N.*

Department of Food Science and Technology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria. *Corresponding Author: tnfagbemi@futa.edu.ng

ABSTRACT

Cassava (Manihot esculenta) is generally low in vitamins and micronutrients. The biofortified varieties are high in Vitamin A precursors. This study was conducted to determine the effects of traditional processing methods on the proximate composition, total carotenoid content and consumer acceptability of four different cassava products (fufu, gari, lafun and pupuru) commonly consumed in West Africa from three cultivars of low-cyanide biofortified cassava roots- TMS 01/1368, TMS 01/1371 and TMS 01/1412. The carotenoid contents were determined before and after traditional processing in order to establish the effect of processing on carotenoid retention. The acceptability of the processed biofortified cassava products was determined by sensory evaluation. All the products have comparable ash, crude protein and crude fibre contents. The carotenoid contents of the raw dried cassava cultivars ranged from 17.9µg/g to 27.11µg/g for the three cultivars. The carotenoid contents of gari ranged between 4.78 and 14.82 µg/g, for lafun, 9.33 and 11.53 µg/g; pupuru, 5.80 and 12.81 µg/g and fufu, 10.6 and 17.5 µg/g. Fufu from TMS 01/1412 had the highest carotenoid content with 98% retention compared to less than 60% retention in the other two varieties. The sensory evaluation showed that all the products were generally acceptable with no significant difference (p<0.05) across the varieties of the cassava.

Keywords: *Carotenoid, Lafun, Gari, Fufu, Pupuru, Biofortified cassava*

INTRODUCTION

The teaming population of Sub-Saharan Africa subsists on mainly starchy foods such as cassava, maize and yam (Gegios *et al.*, 2010). Cassava (*Manihot esculenta* Crantz), grown mainly by small scale farmers, thrives in marginally fertile soils and can tolerate wide range of rainfall. The edible portions of cassava are the roots and leaves constituting 50% and 6% of the mature cassava plant respectively. The roots are highly priced than the leaves in many developing countries. Nutritionally, the root is starchy, containing about 60-70% moisture and 32-35% total carbohydrate of which 80% is starch with greater proportion as amylopectin (Rawel and Kroll, 2003). It has varying amounts of crude fat (0.1-0.3 %), protein (1-3%) and fibre (1.5%) depending on the age and variety (Charles *et al.*, 2005).

Conventional varieties of cassava are deficient in micronutrients such as vitamin A, iron, and zinc thus limiting their use as a single food considering the importance of these micronutrients to humans. This is the major cause of hidden hunger in most parts of Sub-Saharan Africa. At present, more than 600 million people rely on cassava as a vital food source in the tropical countries (Fagbemi, 2012) with over 250 million children under the age of five suffering from vitamin A deficiency (Tanumihardjo, 2008; Gegios *et al.,* 2010). Vitamin A is an essential micronutrient for the normal functioning of visual system, growth and maintenance of epithelial cellular integrity. Cassava bio-fortified with micronutrients could be a sustainable approach to control micronutrient malnutrition and this could reach the public especially in the tropics through some of the key cassava-based food products such as *fufu*, *gari*, *pupuru*, *abacha* and *lafun*. Cyanide is a toxic factor in cassava that is limiting its use. The cyanide content of the root and leaves range from 10 to 1,300 mg cyanide equivalents per kilogram (Montagnac *et al*., 2009). High cyanide intake from the consumption of insufficiently processed cassava has also been reported as a possible aetiologic factor in some diseases such as Konzo (spastic paraparesis) and glucose intolerance (Nzwalo and Cliff, 2011).

Cassava farming populations have empirically developed several processing methods for stabilizing cassava and reducing its toxicity. Fermentation, which is part of almost all these processes, is widely used to transform and preserve cassava because of its low technology and energy requirements and the unique organoleptic qualities of the final products (Nweke, 2002). This study was carried out to determine the effect of traditional processing techniques on the proximate composition and carotenoid contents of four different cassava products developed from biofortified cassava cultivars.

MATERIALS AND METHODS

Source of Materials and Treatment

Three low cyanide bio-fortified yellow cassava varieties (Tropical Mosaic Series; (TMS 01/1368, TMS 01/1371 and TMS 01/1412) were obtained from the research farm of the International Institute of Tropical Agriculture, Ibadan, Nigeria. The tubers were peeled and sliced to 5 mm thickness before drying for 24 hours at 60°C to obtain cassava chips.

Production of Cassava-Based Traditional Foods

The roots of the cassava cultivars were subjected to the two different traditional processing techniques commonly used in the production of the various products. The roots were washed and soaked either with or without the peels in the production of *fufu*, *lafun* and *pupuru*.

***Production process of fufu*:** Cassava tubers were peeled, cut into small sizes, steeped in water to ferment for three days. At the end of the fermentation, the peels were manually removed (for the batch soaked with peels). The fermented mass in both treatments were mashed manually and sieved by adding water to the retted mass to remove the fibres using cheese cloth. After 24 hr the water on top of the sediment was decanted and the remaining mass (mainly starch) was dewatered using hydraulic press and the cake was sun dried for 2 days.

***Production process of lafun*:** Fresh cassava roots were manually peeled and cut into chunks before steeping in water for 4 days to ferment. The fermented roots were then broken up into small pieces (the peels were manually removed as appropriate), and sun dried for 3 days. The dried pieces were milled into flour. Unlike *fufu*, the fibers in the retted roots for *lafun* were dried along with the mash and later sieved out. Thus, *lafun* has more coarse particles than *fufu*.

***Production process of pupuru*:** The cassava roots were peeled, washed, cut into chunks, and steeped in water to ferment for 4 days. The fermented cassava mash (with the peels manually removed as appropriate) was removed from the water into a jute bag to dewater by pressing. The dewatered mass was sifted and later roasted on a hot ceramic clay pot before being milled into *pupuru* flour.

***Production process of gari*:** Freshly harvested cassava roots were washed, peeled and grated into mash. The mash was packed into sacks and left for 2 days in a fermentation trough to ferment. The fermented mash was dewatered by placing under hydraulic press. The dewatered cakes were pulverized and sieved to separate the fibrous materials. The sieved flour was then roasted in a large, shallow stainless steel pan with constant stirring until creamy, free-flowing granules were obtained. In the second processing technique, the roasting was partially done after sun drying. The final *gari* was spread on a stainless steel tray to cool and then sieved to obtain granules of uniform particle size.

Chemical Analyses

Proximate analysis

Samples of dried cassava chips, *fufu, lafun, pupuru* and *gari* were analyzed for moisture, crude protein, total ash, crude fibre and carbohydrate contents by AOAC (2000). Protein content was determined by the Kjeldahl-Nitrogen analysis procedure, using 6.25 as a conversion factor while the crude fat was determined using the Soxhlet extractor. The carbohydrate content was obtained by difference.

Determination of Total Carotenoid Content

The total carotenoid content of the samples was determined using spectrophotometric method as described in AOAC (2000). Each sample (2.5g) was weighed into conical flask followed by the addition of 30ml hexane, 20ml ethanol and 2ml of 2% NaCl. Extraction was performed by thoroughly mixing the content of the conical flask for 30 min. The mixture was then transferred into a separating funnel and allowed to stand for 10 min for complete extraction of carotenoid. The absorbance of the filtrate at 436nm was then determined using spectrophotometer.

Sensory Evaluation

The *fufu*, *lafun* and *pupuru* powders were prepared traditionally by stirring in hot water to make the gelatinized balls that could be eaten or swallowed. Only *gari* was presented as it was after final processing for sensory analysis. The samples were coded and served to fifteen semi-trained panelists. Each panelist was given coded samples to evaluate for taste, aroma, appearance and overall acceptability on a 9-point Hedonic scale where '1' was used to denote 'Extremely Dislike' and '9' 'Like extremely'.

Statistical Analysis

All determinations were carried out in triplicate and error reported as standard deviation from the mean. All data were subjected to analysis of variance and significance accepted at $p \leq 0.05$. The means were separated using Duncan Multiple Range Test with SPSS package (version 17.0).

RESULTS AND DISCUSSION

The moisture content of the chips made from bio-fortified cassava varieties ranged between 2.85 and 3.12g/100g but there was no significant difference ($p \leq 0.05$) between the three cultivars (Table 1). TMS01/1412 was significantly higher in fat and protein contents than the other varieties (Table 1). The dried chips had protein contents ranging from 3.2 to 5.6%. While TMS 01/1412 had the highest value, there was no significant difference between the other two varieties. However, these values were more than 100 folds higher than the values reported for chips produced from three other different varieties of white cassava (Abera and Rakshit, 2003). Cassava has always been processed or modified to improve its utilization (Abera and Rakshit, 2003; Fagbemi *et al*., 2015).

Table 1: Proximate composition (g/100g) of dried chips produced from biofortified cassava varieties

Sample	Moisture	Total Ash	Crude Fibre	Crude Fat	Crude Protein	Carbohydrate
TMS 01/1368	2.85+0.06[a]	3.30+0.30[a]	2.77+0.15[b]	1.30+0.20[b]	3.30+0.30[b]	86.48+0.42[a]
TMS 01/1371	2.93+0.19[a]	3.25+1.05[a]	3.53+0.32[a]	3.20+0.70[a]	3.20+0.10[b]	83.89+0.21[b]
TMS 01/1412	3.12+0.22[a]	1.91+0.85[b]	3.87+0.06[a]	1.48+0.43[b]	5.60+0.80[a]	84.02+0.64[b]

Values are Mean± SEM; Values with different alphabet within the column are significantly different P<0.05

The processing techniques in this study had no significant effect on the proximate composition of *fufu* made with cassava tubers that were soaked with or without the peels (Table 2). However, TMS 01/1412 had the highest crude fibre content and there was no significant difference between the two *fufu* products made from this variety. The protein content (3.21±0.55 g/100g) was higher in the *fufu* when the cassava was soaked with the peels for TMS01/1412. The protein contents recorded for all the products, irrespective of the variety of cassava, were higher than the values of 1.2% - 1.8% recorded for the different genotypes earlier studied (Abera and Rackshit, 2003; Charles *et al*., 2005).

Table 2: Proximate composition (g/100g) of fufu produced from biofortified cassava varieties

Sample	Moisture	Ash	Fibre	Fat	Protein	Carbohydrate
TMS 01/1368	8.16+0.22[b]	0.28+0.08[d]	2.30+0.13[c]	1.16+0.08[c]	2.57+0.15[b]	85.53+0.89[a]
TMS 01/1371	7.99+0.07[c]	0.48+0.03[c]	1.57+0.11[e]	1.80+0.09[a]	2.17+0.11[c]	85.99+0.08[a]
TMS 01/1412	8.18+0.25[b]	0.81+0.06[a]	3.54+0.27[a]	1.20+0.10[c]	1.64+0.07[d]	84.63+0.64[a]
TMS 01/1368 (P)	8.16+0.19[b]	0.82+0.09[a]	1.44+0.14[e]	1.39+0.19[b]	3.39+0.13[a]	84.80+0.99[a]
TMS 01/1371 (P)	8.33+0.09[b]	0.59+0.07[b]	1.89+0.16[d]	1.43+0.11[b]	3.23+0.09[a]	84.53+0.45[a]
TMS 01/1412 (P)	9.22+0.14[a]	0.55+0.03[b]	2.89+0.09[b]	1.46+0.40[b]	3.21+0.15[a]	83.66+0.49[a]

(P) cassava varieties fermented with the peels. Values are Mean±SEM. Values with different alphabet within the column are significantly different P<0.05

Gari produced from TMS01/1412 that was partially toasted after sun drying had the highest crude fibre content of 4.82 g/100g (Table 3).

Table 3: Proximate composition (g/100g) of gari produced from yellow cassava varieties

Sample	Moisture	Ash	Fibre	Fat	Protein	Carbohydrate
TMS 01/1368	8.55±0.24[b]	0.51±0.11[d]	3.39±0.19[c]	0.75±0.20[c]	3.70±0.15[a]	83.10±1.31[a]
TMS 01/1371	7.45±0.39[c]	0.95±0.04[c]	3.46±0.13[c]	1.25±0.25[b]	3.17±0.25[b]	83.72±0.74[a]
TMS 01/1412	8.58±0.23[b]	1.13±0.11[bc]	3.43±0.20[c]	2.31±0.75[a]	3.88±0.25[a]	80.67±1.47[ab]
TMS 01/1368 (P)	8.54±0.36[b]	1.40±0.12[b]	3.00±0.02[d]	2.17±0.20[a]	3.81±0.82[a]	81.08±1.05[ab]
TMS 01/1371 (P)	8.08±0.26[c]	0.97±0.12[c]	3.70±0.01[b]	1.24±0.05[b]	3.20±0.10[b]	82.82±0.33[a]
TMS 01/1412 (P)	9.06±0.37[a]	1.86±0.21[a]	4.82±0.26[a]	1.22±0.17[b]	3.11±0.02[b]	80.03±0.66[b]

(P) cassava varieties processed by partial sun drying. Values are mean±SEM. Values with different alphabet within the column are significantly different P<0.05

The crude fibre contents obtained for other varieties in this study compared favourably with 1.5 % to 3.5 % earlier reported for some other varieties of cassava (Charles *et al*., 2005), and they were higher than values reported for some white cassava (Abera and Rakshit, 2003). TMS01/1371 soaked with peels and used for *lafun* had the highest ash content of 3.74 g/100g (Table 4). This value was higher than values reported earlier for other varieties of cassava (Abera and Rackshit, 2003; Charles *et al*., 2005). All the products in this study have ash content ranging from 0.28 % to 3.74 %, although higher than values reported by Abera and Rackshit (2003), they compare favourably with values reported by Charles *et al*. (2005). There were no significant differences in the ash, protein and fibre contents of *pupuru* irrespective of the processing method and variety of cassava used (Table 5).

Table 4: Proximate composition (g/100g) of lafun produced from yellow cassava varieties

Samples	Moisture	Ash	Fibre	Fat	Protein	Carbohydrate
TMS 01/1368	6.89±0.36[d]	1.29±0.33[b]	2.86±0.09[a]	1.33±0.18[a]	3.30±0.25[a]	84.33±0.07[a]
TMS 01/1371	7.71±0.21[cd]	1.98±0.18[b]	2.76±0.43[a]	1.28±0.15[a]	1.47±0.40[c]	84.79±1.09[a]
TMS 01/1412	9.38±0.70[b]	1.66±1.56[b]	2.82±0.28[a]	1.45±0.33[a]	2.96±0.07[b]	81.73±1.87[b]
TMS 01/1368 (P)	8.41±0.78[a]	2.31±0.25[b]	3.14±0.17[a]	1.76±0.91[a]	3.80±0.53[a]	80.58±2.04[b]
TMS 01/1371 (P)	10.38±0.53[b]	3.74±0.13[a]	3.06±0.23[a]	1.27±0.14[a]	3.43±0.29[ab]	78.12±0.46[c]
TMS 01/1412 (P)	9.70±0.11[a]	2.30±0.07[b]	3.05±0.25[a]	1.48±0.28[a]	3.18±0.28[ab]	80.29±0.55[bc]

(P) cassava varieties fermented with the peels. Values are mean±SEM. Values with different alphabet within the column are significantly different P<0.05

Table 5: Proximate composition (g/100g) of pupuru produced from yellow cassava varieties

Samples	Moisture	Ash	Fibre	Fat	Protein	Carbohydrate
TMS 01/1368	10.33±0.09[a]	0.58±0.04[a]	3.17±0.03[ab]	1.39±0.06[ab]	3.01±0.01[a]	81.53±0.70[abc]
TMS 01/1371	8.68±1.14[b]	0.83±0.20[a]	3.27±0.98[ab]	1.41±0.47[ab]	2.89±1.20[a]	82.92±0.88[a]
TMS 01/1412	11.02±0.68[a]	1.01±0.14[a]	3.25±0.32[ab]	1.16±0.13[b]	3.67±0.76[a]	79.90±1.46[c]
TMS 01/1368 (P)	10.16±0.58[a]	0.65±0.22[a]	2.70±0.04[b]	1.23±0.16[b]	4.27±0.58[a]	80.99±0.81[abc]
TMS 01/1371 (P)	8.77±0.49[b]	0.64±0.37[a]	2.40±0.04[b]	2.59±1.29[a]	3.50±0.87[a]	82.10±1.62[ab]
TMS 01/1412 (P)	9.88±0.11[a]	1.04±0.14[a]	3.63±0.52[a]	1.95±0.93[ab]	3.41±0.10[a]	80.09±0.80[bc]

(P) cassava varieties fermented with the peels. Values are mean±SEM. Values with different alphabet within the column are significantly different P<0.05

Cassava chips from TMS 01/1371 had carotenoid content of 27.11 μg/g and were significantly higher than all the other varieties. TMS 01/1412 had the lowest carotenoid content of 17.9μg/g in the dried chips (Table 6). When the different varieties were processed into *gari*, *fufu*, *lafun* and *pupuru*, the carotenoid contents of the products varied depending on the product. Toasted *gari* from TMS 01/1412 had significantly higher carotenoid content of 14.82 μg/g with 83% retention. The partially sundried *gari* had drastically reduced carotenoid contents in all the TMS varieties. This finding is similar to that reported for tomato treated with sunlight for 21 days that showed drastic reduction in the carotenoid content (Liu *et al*., 2009), because the sunlight might not be a specific regulator of carotenoid synthesis. *Lafun* made from TMS 01/1368 gave the highest carotenoid content irrespective of the processing technique with almost 60% retention (Table 6). *Pupuru* had carotenoid contents ranging from 6.81 to 12.81 μg/g depending on the processing method and the cassava variety. Although the retention was higher in *pupuru* made from TMS 01/1412, *pupuru* made from TMS 01/1371 had the highest carotenoid contents of 12.81 μg/g. Among all the products, *fufu* made from TMS 01/1412 had the highest carotenoid contents and retention level of up to 98% when processed without the peels (Table 6). Conventional cassava is practically devoid of carotenoid (Gegios *et al*., 2010). The yellow bitter cassava studied by Oliveira *et al*. (2010) showed carotenoid content of 3.65-18.92 μg/g and processing resulted in up to 50% degradation in the carotenoid contents. The sweet yellow cassava cultivars had carotenoid content ranging from 2.64 - 14.15 μg/g (Carvalho *et al*., 2012). When subjected to different cooking methods, the carotenoid retention varied and up to 60% was recorded but no cooking method could be said to have the best retention (Carvalho *et al*., 2012). When yellow sweet potato was processed by cooking for 20-30 min, carotenoid retention was found to range between 83 and 92% (Van Jaarsveld *et al*., 2006). Aman *et al* (2005) reported that food processing can cause losses of carotenoids due to degradation, isomerization, and oxidation. Also, processing particularly disrupts the plant matrix, including the cellular compartments and binding proteins that serve to protect and stabilize the carotenoid pigments. The relatively good retention of total carotenoids upon the processing of the various cultivars used in this study provides experimental support for the feasibility of cassava biofortification as a means to alleviate vitamin A deficiency.

Table 6: Total carotenoid contents (μg/g) and retention (%) after processing cassava to different products

Cassava products	TMS 01/1368		TMS 01/1371		TMS 01/1412	
	Carotenoid content (μg/g)	Retention (%)	Carotenoid content (μg/g)	Retention (%)	Carotenoid content (μg/g)	Retention (%)
Dried Cassava chips	19.62±1.51^{b}	100	27.11±1.41^{a}	100	17.9±1.84^{b}	100
Gari (toasted dried)	11.33±0.35^{b}	57.75	11.28±0.43^{b}	41.6	14.82±0.10^{a}	82.79
Gari (partially sundried)	4 .78±0.10^{c}	24.36	7.74±0.08^{a}	28.55	6.30±0.06^{b}	35.19
Lafun (soaked without peel)	10.44±1.65^{a}	53.21	10.70±1.47^{a}	39.47	9.9±1.96^{a}	55.31
Lafun (soaked with peel)	11.53±1.13^{a}	58.77	9.33±0.21^{b}	34.42	9.84±2.04^{b}	54.97
Pupuru (soaked without peel)	9.80±0.92^{c}	49.94	12.81±0.26^{a}	47.25	10.87±1.22^{b}	60.73
Pupuru (soaked with peel)	6.81±0.45^{b}	34.71	9.51±0.82^{a}	35.08	7.15±0.08^{b}	39.94
Fufu (soaked without peel)	12.12±1.23^{c}	61.77	15.67±1.15^{b}	57.81	17.50±1.01^{a}	97.76
Fufu (soaked with peel)	11.77±0.59^{a}	59.99	10.16±0.72^{b}	37.48	11.67±0.93^{a}	65.19

Values of carotenoid are mean±SEM. Values with different alphabets across the row on carotenoid contents are significantly different P<0.05

It was reported earlier that *fufu* processed by different methods was preferred differently by the consumers (Tomlins *et al*., 2007). In the present study although two processing techniques were used for each of the products, they were scored the same during sensory analyses thus only the result of one technique is shown in Table 7. Apart from *lafun* that had significantly higher values for taste, aroma, texture and overall appearance in TMS 01/1368, all the other products had significantly higher values in all the parameters measured from TMS 01/1412 (Table 7). Peeling enhances carotenoid retention in all the products made from the biofortified cassava.

Table 7: Sensory evaluation of *fufu*, *gari*, *lafun* and *pupuru* made from biofortified cassava

Products/Parameters	TMS 01/1368	TMS 01/1371	TMS 01/1412
Fufu (Taste)	6.53±0.84^{a}	5.93±0.90^{a}	6.67±1.04^{a}
Fufu (Aroma)	6.06±2.01^{a}	6.20±1.32^{a}	6.93±1.70^{a}
Fufu (Appearance)	7.00±1.55^{a}	6.13±1.59^{a}	6.40±1.63^{a}
Fufu (OA*)	6.40±1.72^{a}	6.06±1.50^{a}	6.93±1.03^{a}
Gari (Taste)	6.06±1.37^{a}	6.20±1.42^{a}	7.13±1.24^{a}
Gari(Aroma)	6.40±1.80^{a}	5.80±1.65^{a}	6.66±1.34^{a}
Gari (Appearance)	7.80±0.80^{a}	6.40±0.63^{b}	7.60±0.29^{a}
Gari (OA*)	7.40±0.45^{a}	6.60±0.41^{b}	7.73±0.91^{a}
Lafun (Taste)	6.60±1.50^{a}	6.53±1.50^{a}	6.26±1.38^{a}
Lafun (Aroma)	6.40±1.45^{a}	5.86±1.53^{a}	5.73±1.62^{a}
Lafun (Appearance)	6.73±1.27^{a}	6.53±1.45^{a}	5.73±1.08^{b}
Lafun (OA*)	6.93±0.43^{a}	6.33±0.49^{a}	5.93±0.48^{a}
Pupuru (Taste)	6.67±1.71^{a}	6.13±1.68^{a}	6.73±1.38^{a}
Pupuru (Aroma)	7.20±1.52^{a}	5.93±1.83^{b}	6.86±1.84^{a}
Pupuru (Appearance)	6.53±1.92^{ab}	6.13±0.13^{b}	7.33±1.63^{a}
Pupuru (OA*)	6.80±1.85^{a}	6.06±1.94^{a}	7.13±1.80^{a}

Values are Mean±SEM; Values with different alphabet within the row are significantly different P<0.05. *Overall Acceptability

CONCLUSION

It can be concluded from the study that the various traditional processing techniques applied to the TMS varieties of yellow cassava had varying effects on the food products. The crude fibre contents were significantly influenced particularly in *fufu* and *gari*, but not in *pupuru* and *lafun*. *Fufu* that was processed by peeling had the best carotenoid retention in all the three different cultivars. With the exception of *gari*, there were no significant differences (p<0.05) in the overall acceptability of all the food products from the three different yellow cassava cultivars.

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