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Retention of pro-vitamin A carotenoid in composite bread baked with high quality cassava flour from yellow-fleshed cassava root

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ABSTRACT

Background: As one of the most widely consumed foods, bread is one of the most important agricultural products. Bread made from high-quality cassava flour is consumed in some parts of Sub-Sahara Africa (SSA). The bread has no pro-vitamin S carotenoids (pVAC) due to the use of artificial colorants. Consequently, there is a need for the use of pVAC rich foods for bread production. Foods that are rich with pro-vitamin A carotenoids can be converted into retinol in the human body and whose bioconversion contributes to the reduction of vitamin A deficiency diseases (VAD). VAD has caused annual loss of life in SSA, especially in Nigeria. The yellow-fleshed cassava root might contribute to the reduction of this disease. The high quality yellow cassava flour (YHQCF) produced from yellow-fleshed cassava root may contribute to the pVAC composition of bread. As a result, there is a need for the evaluation of the retention of pVAC in composite bread baked with high quality cassava flour from yellow-fleshed cassava roots.

Methods: The YHQCF was produced from TMS01/1368 cassava variety. The bread loaves consisted of 20% and 100% YHQCF and were produced by mixing the sugar, margarine, yeast, improver, and salt with the composite flour and YHQCF respectively, after which water was added and mixed to get the homogenous dough. The dough was proofed for 2.5 hours, kneaded, cut into shape, placed in a lubricated baking pan, and baked at 200^{o}C for 30 min. Analyses of the pro-

vitamin A (cis and trans-β carotene) and dry matter content were carried out on all the samples, including samples from the YHQCF production steps using standard methods. The samples from the YHQCF production steps were chosen and analyzed for pVAC in order to check the levels of degradation of the pVAC from the raw cassava root to using the root for flour production and the quantity of pVAC retained when 100% of the YHQCF is used for bread production compared to 20% composite. The β-carotene nutrient retention of the bread was also calculated.

Results: The results demonstrated how the total pVAC content of the raw yellow-fleshed cassava root was 16.83 µg/g dry basis with 29% dry matter (DM) content. Subsequent processing by peeling, washing, grating, and dewatering into granules (56% DM) caused 48% reduction in the pVAC content which was reduced to 40% after drying and milling the dried grits into YHQCF (97% DM). Preparation of recipe for bread demonstrated how the 20% composite flour dough (61% DM) contained 0.29 µg/g db pVAC representing 1.72% retention, which was later reduced to 0.25 µg/g db pVAC or 1.49% retention after baking (62%DM). On the other hand, bread loaves baked from 100% YHQCF (67% DM) retained 0.74 µg/g db pVAC representing 4.40% of the 16.83 µg/g db pVAC in the starting raw material.

Conclusions: The bread produced from 100% YHQCF may contribute to the pro-vitamin A status of bread consumers in SSA more than the 20% YHQCF composite. However, both bread samples are low in pVAC. In order to attain the required retinol equivalent level after bioconversion in the human body, consumption of other foods rich in vitamin A would be required
to attain the required retinol equivalent level after bioconversion in the human body but can be enhanced if consumed with other foods rich in vitamin A.

Keywords: High quality cassava flour; composite flour; Bread; Pro-vitamin A carotenoid; Nutrition

BACKGROUND

Bread is one of the most important fast foods consumed daily in Sub-Sahara Africa (SSA), especially in Nigeria. The wheat used to produce bread and other confectionaries is imported, making Nigeria one of the highest importers of wheat in the world [1-3]. The foreign exchange expenditure on wheat importation is adversely affecting public investment in development and human welfare. As a result, most SSA countries are looking for ways to process locally sourced flours that can be used to produce bread that meets the sensory quality characteristics desired by the population and contributes to nutrition. Researchers have demonstrated the suitability of high quality cassava flour (HQCF) produced from white-fleshed cassava roots as an alternative to substitute wheat flour in composite flours [4-9] to reduce wheat importation. However, there is more research needed on the use of cassava flour for bread production.

Cassava roots are known to be low in micronutrients such as vitamin A, iron, and zinc. Micronutrient deficiencies threaten the lives of millions of poor households and those located in remote rural areas of SSA often not targeted by fortification programs. An evaluation of nutritional and health benefits of increased vitamin A status of yellow-fleshed cassava roots (YfCR) was studied by Manyong et al. [10] for at-risk-target-groups using Disability-Adjusted Life Years (DALYs) approach. The researchers observed that vitamin A deficiency (VAD) causes an annual loss of about 553,000 years of 'healthy' life in Nigeria with children constituting more than 40%. Researchers also discussed how YfCRs would reduce VAD optimistically by 29%, 76%, and 20% for children, pregnant women, and lactating women respectively. Therefore, research and development efforts aimed at bio fortifying cassava roots is a powerful strategy in the fight against micronutrient malnutrition or hidden hunger from micronutrient deficiencies. Moreover, the African government and international investors at the local and national levels should support efforts to improve standards of living in SSA.

Although work has been done on the consumer acceptability of some cassava products produced from laboratory-made yellow-fleshed cassava flour [11-13], there is still work required for commercially produced yellow-fleshed high quality cassava flour (YHQCF). Specifically, there is a need for product development and retention of pro-vitamin A carotenoids (pVAC). Substances that can be bioconverted into vitamin A in the human body and use for the reduction of VAD are called pVAC. Additionally, pVAC has an important nutritional role as the principal precursor of vitamin A, which is involved in vision, cell differentiation, synthesis of glycoprotein, mucus secretions from epithelial tissues, reproduction, and the overall growth and development of bones [14]. The retention of pVAC during industrial processing of fresh cassava to flour and eventual baking of the flour into bread remain a challenge. Consequently, evaluating the retention of pVAC during commercial processing of YfCRs to YHQCF and the retention in bread produced from the flour will contribute to our understanding of the potential usability of provitamin A cassava varieties to produce manufactured nutrient-enhanced food items, which may contribute to the reduction of VAD.

Therefore, we evaluated the retention of pro-vitamin A carotenoid in composite bread baked with high quality cassava flour from yellow-fleshed cassava roots.

Materials and methods

Materials

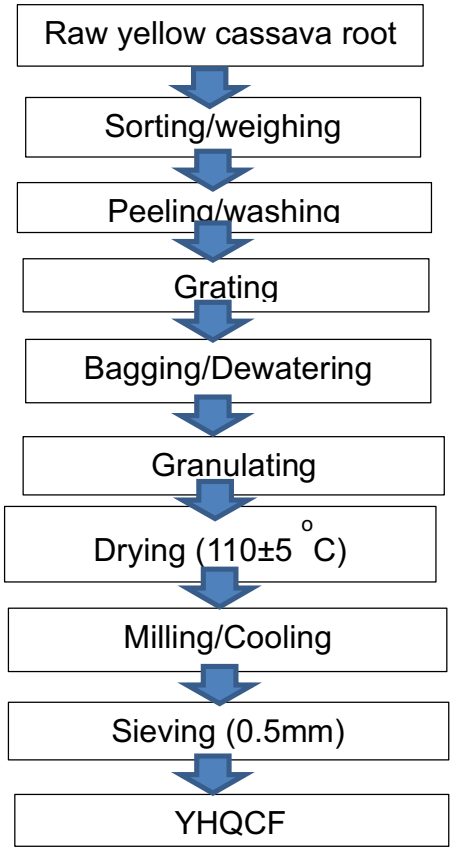
The cassava variety (TMS01/1368) used to produce the YHQCF was obtained from the research farm of the International Institute of Tropical Agriculture, Ibadan, Nigeria. Bread ingredients - sugar, margarine, yeast, improver (ethylene diamine tetra-acetic acid), salt, and wheat flour - were purchased from a local market.

Methods

Production of high quality cassava flour from yellow-fleshed cassava roots

The YHQCF was produced using commercial machinery at Niji Lucas cassava processing factory Ilero, Oyo State, Nigeria, according to the method described by Abass et al [15] (Figure 1).

Figure 1. Production of yellow high quality cassava flour (YHQCF) [15].



Production of bread

The bread loaves, which consist of 20% and 100% YHQCF were produced by mixing the sugar (100 g), margarine (50 g), yeast (7 g), improver (3 g), and salt (16 g) with the composite flour (200 g YHQCF and 800 g wheat flour) and the YHQCF respectively, after which water (555 ml) was added and mixed to get the homogenous dough. The dough was allowed to proof for 2.5 h. Then the dough was kneaded, cut into shape, placed in a lubricated baking pan, and baked at 200 ^{o}C for 30 min. The dough was allowed to cool and packaged in low-density polyethylene bag prior to further study.

Carotenoid extraction, identification, and quantification

To determine the β-carotene and its trans and cis-isomers, approximately 15 g of each sample and 3 g of Celite 454 (Tedia, Ohio, USA) were weighed. Successive additions of 25 ml of acetone were performed to obtain a paste, which was transferred to a sintered funnel (5 μm), coupled to a 250 ml Buchner flask, and filtered under vacuum. This procedure was repeated three times until the sample became colorless. Then the extract was transferred to a 500 ml separation funnel containing 40 ml of petroleum ether. The acetone was removed through the slow addition of

ultrapure water (Millipore) to prevent emulsion formation. The aqueous phase was discarded. The procedure was repeated four times until no residual solvent remained. The extract was then transferred through a funnel containing 15 g of anhydrous sodium sulphate and made up of 50 ml with petroleum ether [16]. To identify and quantify β-carotene and its trans and cis-isomers, 2 mL was removed from the extract and dried in an amber flask under nitrogen flow [16]. The sample was diluted in 100 µl of acetone under shaking in a vortex mixer (Genie 2-Scientific Industries) and transferred to a 2-ml amber flask for High-Performance Liquid Chromatography (HPLC) analysis. The concentration of β-carotene and its trans- and cis-isomers was determined using:

$$\mathbf{C\ (\mu g/g)} = \frac{\mathbf{A_x} * \mathbf{C_s\ (\mu g/ml)} * \mathbf{V\ (ml)}}{\mathbf{A_s} * \mathbf{P(g)}}$$

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

β-carotene nutrient retention

The β-carotene nutrient retention of the samples was calculated as reported by Li et al. [17]. The Apparent Nutrient Retention (ANR) is defined as the ratio of the nutrient content retained in the processed portion to the content of nutrient in the raw portion and is related to the loss of nutrients.

The ANR was calculated using the following formula:

$$\text{ANR} = \frac{\text{Nutrient content per g of processed food}}{\text{Nutrient content per g of raw food}} \text{ x } 100$$

Dry matter content

The dry matter content of the samples was determined by drying triplicate 5 g samples at 105 ºC until constant weight in an air-ventilated oven (draft air Fisher Scientific Isotemp^R Oven model 655F) for a minimum of 24 h [18].

Statistical Analysis

Analysis of variance (ANOVA) and separation of the mean values (using Duncan's Multiple Range Test at $P < 0.05$) were calculated using Statistical Package for Social Scientists (SPSS) software (version 21.0).

Results and Discussions

The results in Table 1 demonstrated how the total pVAC content of the raw biofortified cassava roots was 16.83 µg/g dry basis with 29% dry matter (DM) content. Subsequent processing by peeling, washing, grating, and dewatering into granules (56% DM) caused 48% reduction in the pVAC content, which was reduced again to 40% after drying and milling the dried grits into yellow HQCF (97% DM) (Table 1). Preparation of recipe for bread using YHQCF (200g), wheat flour (800g), salt (16 g), sugar (100g), butter (50 g), and yeast (7 g) mixed with 555 mL water might have diluted the pVAC content per unit dry matter. The the 20% composite flour dough (61% DM)

contained 0.29 µg/g db pVAC representing 1.72% retention, which was later reduced to 0.25 µg/g db pVAC or 1.49% retention after baking (62%DM). On the other hand, bread loaves baked from 100% yellow HQCF (67% DM) retained 0.74 µg/g db pVAC representing 4.40% of the 16.83 µg/g db pVAC in the starting raw material.

Table 1: Retention of provitamin A carotenoid at each processing step of yellow-fleshed high quality cassava and its use in composite bread

Samples	Cis-β carotene (µg/g db)	trans-β carotene (µg/g db)	pVAC (µg/g db)	Dry Matter (%)
Unpeeled cassava	8.44± 0.04a	8.40 ±0.18a	16.83 ±0.14a	29.26±1.06h
Peeled cassava	7.72 ±0.30b	7.69 ±0.15b	15.40 ±0.45b	30.54±0.30g
Cassava mesh	4.96 ±0.21c	5.50 ±0.26c	10.45 ±0.46c	25.94±0.63i
Pressed Cake	5.03 ±0.22c	4.96 ±0.22d	9.98 ±0.44c	52.08±0.37f
Pulverised grit	3.90 ±0.00d	4.10 ±0.00e	8.00 ±0.00d	56.46±0.51d
Dried grit	3.83 ±0.69d	3.78 ±0.25ef	7.61 ±0.94de	96.80±0.02a
HQCF	3.46±0.12d	3.31±0.32f	6.77 ±0.44e	96.84±0.15a
Dough 20% HQCF:80% wheat flour	0.15 ±0.00e	0.14 ±0.00g	0.29 ±0.01f	61.02±0.08c
Dough 100% HQCF	0.53 ±0.14e	0.51 ±0.13g	1.04 ±0.27f	54.00±0.06e
Bread 20% HQCF:80% wheat flour	0.13 ±0.01e	0.12 ±0.01g	0.25 ±0.02f	61.77±0.04c
Bread 100% HQCF	0.39 ±0.00e	0.35 ±0.00g	0.74 ±0.00f	67.47±0.16b
P-level	***	***	***	***

***P≤0.001, pVAC-Pro-vitamin A carotenoid
Means with the same letters on the same column are not significantly different at p≤0.05

The reduction in the pVAC contents in each of the unit operations involved in the production of the YHQCF from yellow-fleshed cassava root of the present study supported the results of Rodriguez-Amaya [18, 19]. This researcher reported how alteration or loss of carotenoids occurs during processing and storage of foods through physical removal (e.g. peeling), geometric isomerization, and enzymatic or non-enzymatic oxidation. Although pVAC contents reduced gradually at each processing step during processing of fresh cassava roots to bread, some processing steps such as elevated temperatures like drying, milling, and baking do not appear to have a significant reducing effect on pVAC contents. In fact, the peeling of roots and two other processing steps that involved size reduction—grating of roots and pulverization of pressed cake—which results in an increase in surface area and porosity of in-line products had a significant reducing effect on pVAC contents. These results support the observations of Dutta et al. [20].

The daily pro-vitamin A intake recommended by the FAO is 250 to 400 retinol activity equivalents (RAE) for children, 575 to 725 RAE for adolescents, and 750 RAE for adults [20]. IITA [21] discussed how the RAE conversion ratio is 3.7 µg of β-carotene to 1 µg of retinol. This

refutes the previous estimate of 12 μg of β-carotene in cassava being equivalent to 1 μg of retinol reported by the United State Institute of Medicine [22]. Using the IITA [21] standard, the RAE/ 100 g (calculated as the percentage of the pVAC content divided by 3.7) of the 20% composite bread (7 RAE/ 100 g) and 100% YHQCF bread (20 RAE/ 100 g) was very low. This implied that the bread could be consumed with foods high in pVAC in order to achieve the FAO recommended daily intake of pro-vitamin A.

The study also revealed that 93% of total pVAC in raw cassava was lost during processing into flour, suggesting that much higher concentrations of pVAC need to be targeted in the ongoing biofortification programs to increase the pVAC in bread produced from YHQCF for the benefit of the consumers.

CONCLUSIONS

The bread produced from 20% composite and 100% yellow high quality cassava flour may contribute to the pro-vitamin A status of bread consumers if consumed with other foods rich in pro-vitamin A in order to achieve the FAO recommended daily intake for the reduction of vitamin A deficiency diseases.

Competing Interests: The authors declare no conflict of interest.

List of Abbreviations: SSA, Sub-Sahara Africa; pVAC, Pro-vitamin A carotenoids; VAD, Vitamin A deficiency diseases; YHQCF, Yellow high-quality yellow cassava flour; DM, Dry matter; HQCf, High quality cassava flour; YfCR, Yellow-fleshed cassava roots; VAD, vitamin A deficiency; DALYs, Disability-Adjusted Life Years; ANOVA, Analysis of variance

Authors' Contributions: WA and ABA designed the research. WA, ABA, and BMD performed the experiment. WA, ABA, and BMD prepared the manuscript.

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