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Pro-vitamin A Carotenoids Stability and Bioaccessibility From Elite Selection of Biofortified Cassava Roots (*Manihot esculanta*, Crantz) Processed to Traditional Flours and Porridges

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Abstract

Cassava biofortification programs have made significant progress in improving the pro-vitamin A carotenoid (pVAC) content; however, the nutritional impact of traditional and industrial food processing has not been fully assessed. The effects of fermentation and thermal processing on the stability and bioaccessibility of pVAC from elite selections of biofortified cassava roots were assessed to determine the potential for genotype x processing effects. Unfermented (UF) and fermented (F) flours were produced from 10 genotypes. Gari (G) flour was produced by toasting two fermented cassava genotypes. Bioaccessibility of pVAC was evaluated from finished products (porridges at 22% flour/water) via in vitro digestion. Levels of β-carotene equivalents (β-CE) were 23-43 μg β-CE/g DW among genotypes. F and Gari flours showed higher β-CE retention ($p<0.05$) during oven-drying and cooking of porridges compared with UF flours. Bioaccessibility of β-CE (ME%) was 4-15%, across the genotypes and processing. However, contrasting effects of fermentation and thermal processing effects were observed among individual cassava genotypes. These results suggest that genotypic and other factors may impact release and availability of carotenoids and that consideration of both genetics and processing may be warranted in the selection of biofortified cassava cultivars to advance into the application.

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Keywords: Cassava, biofortification, pro-vitamin A, carotenoids, bioaccessibility, micellarization efficiency

1. Introduction

Vitamin A deficiency (VAD) is a critical health issue affecting ~190 million preschool-age children and 19 million pregnant women globally.[1] VAD is the principal cause of blindness in children, as well as contributing to micronutrient related stunting, morbidity and mortality burdens in low-income countries. While dietary diversification, supplementation and fortification have been the primary strategies in addressing VAD in the developing world, there has been some success in resource-poor countries due, among different reasons, to high costs, lack of access to health care, and limited availability of centralized food-processing facilities for food fortifications.[2] Biofortification is a fourth new and complementary strategy that has emerged with the goal to develop micronutrient-dense staple crops that can be cost-effective reducing VAD in populations at risk.[3,4] Traditional staple crops including cassava, sweet potato, maize, rice, pearl millet and sorghum, have been targeted for enhancement of pro-vitamin A carotenoid (pVAC) content through conventional breeding[3,5] or genetic transformation.[6,7,8]

Cassava (*Manihot esculenta*, Crantz) is an important food crop native to Latin America, but also produced and consumed in Africa and Asia in different ways.[9] In 2008/09 production of cassava roots was the highest in Africa (51%) followed by Asia (35%) and Latin America (14%), highlighting the value of this crop particularly for African populations.[9] Traditionally cassava roots are considered an important source of carbohydrates (starch), but lack protein, fat, minerals and micronutrients including pVAC. Cassava biofortification programs have targeted the enhancement of pVAC by using a rapid-cycling recurrent selection, which has shortened the normal breeding cycle of cassava from 8 to 2-3 years.[10] The pVAC content of cassava roots has been substantially increased to levels of ~25 μg/g FW, exceeding the original breeding target of 15 μg/g FW.[10,11] Alongside this progress in breeding it is critical to consider successful implementation by including elite biofortified cassava selection in traditional and industrial food preparations that are aligned with consumers demand and trends but also allow for retention of their nutritional advantages.

Cassava roots are extensively processed within African, Asian and Latin American cultures to generate a diverse array of products including: drinks, soups, baked products, desserts, chips, thick porridges, etc.[12] Traditionally, roots are subjected to both thermal and fermentative

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processes to minimize toxic cyanogenic glucosides, extend shelf-life and facilitate production of cassava products in urban areas.[13] However, it is known that such processes likely impact the retention of original levels of pVAC in fresh roots from biofortified cultivars. For instance, previous studies have reported that pVAC content in roots from biofortified cultivars may be reduced through household style preparations by ~10% to 50% after boiling for ~30 min, and in the range of ~ 40% to 60% after frying.[14-17] Gari, a fermented-toasted product mostly consumed in Nigeria and Ghana, has been reported to have variable retention of pVAC ranging from 10% (toasting at 195ºC, 20 min) to ~ 63% (toasting at 165ºC, 5-15 min).[17] However, some variation in losses has been reported. For example, pVAC content was not significantly altered in cassava roots that were processed using in home traditional fermentation followed by boiling for 10 min to prepare Fufu (another traditional cassava product in Congo and Nigeria).[17] While style of preparation and processing appears to impact pVAC levels in cassava, it is critical to note that previous studies used widely different cultivars or genotypes making challenging to generalize findings to new and emerging elite genotypes. As such, continuous assessments are needed. Insights from a selection of the most promising biofortified genotypes selected for agronomic and processing quality is required to facilitate translation to consumers.

Beyond levels and stability of pVAC through the traditional processing/preparation chain, bioavailability of carotenoids from cassava products must be considered as the ultimate assessment of delivery. Bioavailability of carotenoids is driven by several factors including their digestive release from food matrix, efficiency of their incorporation into mixed bile salt micelles in the intestinal lumen to facilitate their transport into epithelial cells, and ultimately transport into circulation via chylomicron secretion.[18] As previously reported, food processing and preparation can impact bioavailability of pVAC. For instance, Thakkar and colleagues reported β-carotene micellarization efficiency (ME, %) for boiled cassava roots and gari to be around 25-30%, but significantly lower for fufu (15-20%).[17] However, β-carotene ME contrasted for similarly processed transgenic cassava roots into gari (35%) and fufu (40%).[19] The latter suggesting that genetic background may impact bioavailability of carotenoids.

Despite integrated efforts targeting nutritional and agronomic enhanced cassava roots to combat VAD in at-risk populations, their rapid post-harvest deterioration remains a serious concern during storage and distribution to populations living in remote places. Consequently, the success of biofortification strategies relies on the adoption of cassava roots into food processing streams that preserve not only improved pVAC level but also to extend the shelf life

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of cassava products and expand their distribution to populations in need. Although previous studies on biofortified cassava genotypes have provided insights on the stability and bioaccessibility of carotenoids during traditional and/or domestic processing, information on genotypic differences and impact of industrial processes including fermentation and roasting have not been fully assessed. With this in mind, the objective of the present study was to evaluate the stability and bioaccessibility of pVAC by following mechanized traditional processing conditions for the manufacture of fermented and unfermented flours and their incorporation into porridge preparations using elite biofortified cassava genotypes with the highest levels in pVAC and agronomic yields.

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2. Materials and methods

2.1. Chemical and standards

Enzymes and reagents were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade solvents were purchased from JT Baker (Phillipsburg, NJ) and Mallinckrodt (St. Louis, MO). Carotenoids standards (lutein, all trans-β-carotene, α & β-cryptoxanthin, α-carotene, β-apo-8-carotenal) were obtained from CaroteNature (Lupsingen, Switzerland).

2.2. Cassava germplasm

Biofortified cassava genotypes with yellow-fleshed roots were provided by the Cassava breeding program of the International Center for Tropical Agriculture (CIAT) at Palmira, Colombia. Ten of the most promising cassava genotypes (Figure S1) were selected for this study based on the highest pVAC content by using near-infrared spectroscopy predictions[20] and adequate agronomic performance. Selected genotypes showed a wide range of variation for cyanogenic potential (HCN) ranging from 100 to 940 μg/g DW. Selected cassava genotypes derived from a rapid cycling recurrent selection process for increasing pVAC content in cassava roots as described by Ceballos et al (2013).[10] Briefly, early stages of cassava breeding started by crossing yellow and white-rooted cassava accessions from the germplasm collection at CIAT, which possess cassava landraces original from different countries including Colombia (COL), Brazil (BRA), Costa Rica (CR), Malaysia (MAL), Panama (PAN), Peru (PER), and Venezuela (VEN) (Figure S2). Later, cassava genotypes with the highest carotenoids contents were selected, cloned and crossed to produce full- or half-sib families. The new resulted seeds were germinated and planted to start a new cycle. This breeding process has allowed to generate genotypes coming from low contents in TCC (2.4 μg/g FW) to higher values to date (~25 μg/g FW). Genotypes used for this study were obtained from open pollinations (SM code in the pedigree name) in which only the female progenitor is known (half-sibs), or direct controlled crosses (GM or CM code in the pedigree name), in which both the male and female progenitors are known (full-sibs). Twenty-four plants for each biofortified cultivar were planted in three different locations (8 plants per location) at CIAT Experimental Station fields. Plants were harvested 11 months after planting on late Spring (May-June) of 2015 and processed immediately as described below. Selection procedures and criteria have been published recently.[11,21]

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2.3. Processing of cassava

Processing of cassava roots to flour was conducted once by pooling together the total of roots generated from 24 plants for each genotype. This was done to ensure production with sufficient materials for all subsequent assessments and to better simulate larger scale processing. Cassava roots from the ten genotypes were peeled, washed and divided into two groups for production of fermented and unfermented flours by following African traditional processing techniques (Figure 1).[13] Fermented flours were produced by grating roots into a fine dough using a stainless-steel food processor (SKYMSEN, PA-7SE model). Grated cassava roots were packed into white woven polypropylene sack bags, dewatered by exerting constant pressure and spontaneously fermented at room temperature (~30 °C) for two days. pH of ground cassava root dropped from 6.2 ± 0.02 to 4.6 ± 0.08 (mean ± SEM) across the different genotypes after the fermentation period. Fermented roots from all genotypes were dried in a fan-forced ventilation oven at 40 °C for 2 days (Thelco Oven, model 28). *Gari* was prepared from two of the ten genotypes, SM 3762-15 and SM 3767-84, by toasting the other half of fermented cassava in a pan at ~150 °C during 15-20 min. These genotypes were selected for preparation of *gari* based on two criteria: they showed outstanding agronomic performance among the ten selected genotypes, but also had high yellow color at harvest time suggesting high carotenoids content. The second half of roots from each genotype were used to eventually produce unfermented flours which were produced by grating cassava roots into sliced pellets and dried at 40 °C for 2 days in a fan-forced ventilation oven.

Once the different processed materials were dried, they were vacuum-packed and stored at -20 °C before shipping on ice to Purdue University, West Lafayette, IN USA. All dried materials were milled to particle sizes indicated in the codex standard for edible cassava flour (The Codex Alimentarius Commission, 2016).[22] Fermented and unfermented cassava roots were milled into fine flours and sieved through a 30-mesh sieve (0.60 mm sieve). Gari is normally commercialized as a coarse flour (granular cassava food product), so gari flours were sieved through 16-mesh (1.18 mm sieve). Additionally, fine flours (30-mesh sieve) of gari were elaborated for practical comparisons. Flours were stored at -20 °C until analysis. Samples were collected after each step including grating (raw cassava), fermentation (fermented cassava), and drying the cassava for both treatments (UF and F flours) for analysis of carotenoids via HPLC. Carotenoid contents were

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reported on a dry weight basis (DW). Finally, retention (Ret) was calculated as; $Ret_{(DW)}$ = [carotenoid quantity (μg) in processed cassava/carotenoid quantity (μg) in raw cassava] *100.

2.4. Cassava porridges preparation

Model wet cooked porridges were prepared as described by Lipkie and colleagues.[23] Briefly, 10 g of cassava flour (fermented or unfermented, per triplicate each) was mixed with 25 mL distilled water and vortexed to provide a slurry. The slurry was added to 20 mL of boiling distilled water and stirred by hand with a spatula while cooking during 5 min at 100 °C. *Gari* flours were subjected to a similar preparation but following the standard culinary preparation from West and Central Africa named *Eba* (a thick paste prepared from *gari* flour). Since production of *gari* flours already included a cooking step (toasting), further cooking was avoided by preparing *Eba*. Accordingly, 10 g of *gari* were soaked in 45 mL of boiling distilled water to produce a thick paste. Porridges and Eba were cooked in triplicate using the flours previously prepared for each cassava genotype. Then, porridges (from UF, F and *Gari* flours) were cooled at room temperature, weighted in falcon tubes and stored at -80°C until analysis and *in vitro* digestions.

2.5. Carotenoids bioaccessibility

Aliquots of cassava test porridges (~8 g wet weight) were subjected to 3-stages-*in vitro* digestion as described previously by Lipkie and colleagues.[23] Canola oil was added to the cassava porridges (~5% wt/wt) to facilitate carotenoids micellarization and standardize comparative assessment of bioaccessibility. Following simulated digestion, digested samples (DG) of cassava were centrifuged at 10,000 g for 60 min at 4 °C and filtered through PTFE filters (0.22 μm pore) to isolate the micellar aqueous fraction (AQ) and stored under N_2 flush at -80 °C until analysis. Micellarization efficiency (%) was calculated by the following equation: (Carotenoids concentration in the AQ fraction / Carotenoids concentration in the DG fraction) x 100%.

2.6. Carotenoids analysis

Cassava roots and porridges were analyzed by following the procedure described by Ceballos and colleagues[10] with minor modifications. Cassava roots (~8 g of fresh material and porridges, 1 g of flours) were spiked with 50 μL of internal standard solution (β-apo-8'-carotenal in petroleum ether). 1 mL of 0.1 M NaCl solution was added only to cassava flours for hydration. Cassava materials were homogenized with 10 mL of acetone and 10 mL of petroleum ether using a

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polytron (Kinematic AG, PT-MR 2100 model) for 1 min and centrifuged at 4000 rpm for 5 min. The organic phase was collected and placed on ice. The extraction was repeated twice, and the collected organic phases were combined, washed out with 10 mL of 0.1 M NaCl three times and then dried under a N_2 flush. Dried extracts were solubilized on 1:1 methanol: ethyl acetate (0.1% BHT), filtered through PTFE filters (0.45 μm pore), and analyzed by HPLC.

Carotenoids in DG and AQ fractions obtained from in vitro digestion simulations were extracted as described by Lipkie and colleagues.[23] Briefly, 5 mL of AQ and DG solutions (2x each) were extracted with 1 mL of acetone and 3 mL of petroleum ether, followed by vortexing for 1 min and centrifugation at 3000 rpm for 5 min. The organic phase was collected, and the extraction was repeated twice in the remaining aqueous (DG and AQ) fractions. Combined extracts were dried under N_2 and resolubilized on 1:1 methanol: ethyl acetate (0.1% BHT), filtered through PTFE filters (0.45 μm pore) and analyzed by HPLC.

Carotenoids quantification was performed as described by Kean and colleagues,[24] with a HPLC (Hewlett Packard, 1090 model) equipped with a diode array detector (HP, 79880A model). Carotenoids separation was achieved by using a YMC Carotenoids (C30, 150 x 2.0 mm, 3μm) column protected by a YMC Carotenoids S-3 (20 X 2.0 mm) guard column (YMC Separation Technology). Mobile phase consisted of methanol: 1M ammonium acetate, pH 4.6, 98:2 (v:v) (Eluent A) and ethyl acetate (Eluent B). The elution gradient was as following: from 85% to 20% eluent A in 6 min, from 20% to 0% eluent A in 2 min, isocratic at 0% eluent A for 4 min, from 0% to 85% eluent A in 2 min and isocratic at 85% eluent A for 3 min (stop time). Total run time was 14 min at a flow rate of 0.37 mL/min. Carotenoids were monitored at λ=450 nm, and UV-VIS spectra was recorded between 250-600 nm. Identification of individual carotenoids was achieved by comparing retention times and UV-VIS spectra to pure carotenoids standards. Calibration curves were prepared with carotenoids standards in the 0.01-7.50 μM range (r^2=0.998-1.000). Pro-Vitamin A carotenoid concentration (reported as β-carotene equivalents, βCE) was calculated by sum of *all-E*-β-carotene + ½ [β-cryptoxanthin + α-carotene + (9, 13, 15)-Z's-β-carotene isomers].

2.7. Dry matter content

Samples of ground raw and processed cassava roots (5-10 g) were dried in a conventional oven at 105 ºC during 24 h. Analysis were run per duplicate and dry matter content was determined gravimetrically. In **Table S1** are summarized all dry matter contents (DMC) among

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genotypes through processing. In average, DMC in raw cassava genotypes was 32.11 % ± 0.85 (mean ± SEM). Those values are similar to the ones reported by Ceballos and colleagues,[10] but higher compared to transgenic cassava roots[19] and the biofortified cassava bred in Brazil.[15]

2.8. Cyanogenic potential (HCN)

Total and free cyanide content in raw and processed cassava roots were analyzed by following the enzymatic method described by Essers et al.[25]

2.9. Statistical analysis of data

All data were reported as mean ± standard error of mean (SEM) using triplicates for analysis of carotenoids in raw, processed and digested cassava, and duplicates for analysis of moisture and cyanide content. Statistical analysis on retention and micellarization efficiency of carotenoids was done using JMP®, Version 12 (SAS Institute Inc., Cary, NC). Comparison of means was performed under a full factorial model integrating effects of cassava genotypes, processing type and their corresponding interactions for each carotenoid followed by a Tukey's HSD test. Differences were considered statistically significant at $\alpha<0.05$.

3. Results and discussion

3.1. Carotenoid content and composition in roots from biofortified cassava genotypes

Around 85% of total carotenoids content (TCC) in the selected genotypes are pVAC (calculated as sum of content for all isomers (*Z* and *E*) of β-carotene over TCC), in which *All-E*-β-carotene is the predominant carotene (~70%) along (9, 13, 15)-*Z*'s-β-carotene isomers, as well as small amounts of xanthophylls lutein and zeaxanthin (xanthophylls ranged from 0.1 to 2.5 μg/g DW among cultivars). TCC, for this selected group of biofortified genotypes ranged from 31.22 ± 0.48 to 55.04 ± 0.54 μg/g DW (mean ± SEM) at harvest time (Table 1). These values are similar to those previously reported by Ceballos and colleagues,[10] but higher than those for yellow-fleshed cassava roots harvested in Nigeria reported by Thakkar and colleagues[17] and Bechoff and colleagues,[26] and for transgenic cassava roots reported by Failla and colleagues.[19] The best two genotypes, regarding TCC, were SM 3774-21 (43.27 ± 3.43 μg/g) and GM 5212-6 (55.04 ± 0.54 μg/g DW) at harvest time ($p<0.05$). These last values are similar to the ones reported by Berni and colleagues[15] for biofortified cassava conventionally bred in Brazil. pVAC reported as *All-E*-β-carotene equivalents (β-CE), ranged from 23.14 ± 0.74 to 31.12 ± 2.36 μg/g DW among nine

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cultivars at harvest time. Cultivar GM 5212-6 (42.73 ± 0.44) showed the highest β-CE content ($p<0.05$).

3.2. Cyanogenic potential (HCN) of cassava roots through processing

Cassava roots have cyanogenic glucosides such as linamarin and lotaustralin, which can release hydrogen cyanide (HCN) at levels that may be toxic and hazardous to the consumer. Consumption of high levels of cyanide may cause acute intoxication, goiters, tropical ataxic neuropathy (a nerve-damaging disorder) and irreversible paralytic disorder (konzo).[27] Proper treatment of cassava roots including soaking, fermentation and toasting, decrease content of HCN to safe levels to consumers. Since the purpose for this study was to mechanize traditional processing for pVAC-rich cassava roots and HCN is a major concern with this staple crop, we intentionally included genotypes with a wide range of HCN from a collection of around 300 genotypes harvested during the 2015 summer season. Cassava roots with >100 total HCN μg/g are considered "bitter".[28] HCN varied widely among genotypes, ranging from 101.9 ± 0.3 to 939.6 ± 2.9 μg/g (DW) for total HCN (Figure 2). As expected, total HCN decreased ~60% after spontaneous fermentation for 2 days and ~94% after oven-drying of fermented roots (for 2 days at 40ºC). Similarly, total HCN content decreased by ~95% after oven-drying (for 2 days at 40ºC) for almost all unfermented roots, except for cultivars GM 4414-5, SM 3758-43, and SM 3774-21 in which total HCN was reduced by ~40, ~35, and ~70% respectively. Toasting fermented grated cassava roots to prepare *gari* using genotypes SM 3762-15 and SM 3767-84 resulted on decreasing total HCN content in around 93% and 81%, respectively, though above the minimal safe levels for consumers.

Our results demonstrate that fermentation followed by oven-drying effectively reduced cyanide content in roots, especially for those with high cyanogenic glucosides content in raw roots (>400 μg/g DW). In average, residual HCN in cassava flours (UF flour ~ 98 and F flour ~15.83 μg/g (DW) are expected to be significantly decreased through prolonged cooking. However, fermenting cassava roots followed by oven-drying may ensure even safer levels of total HCN for human consumption. Concentrations of ≤ 10 mg/kg are considered to be safe for edible use of cassava flour.[22]

3.3. Impact of cassava roots processing on carotenoids levels

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Cassava roots are traditionally subjected to various preparation methods in countries that frequently consume this staple crop (mostly in Africa, Central- and South America). Roots are mainly consumed as fermented or unfermented flours which are purchased in local urban markets for final preparation in the household, rather than as fresh cassava due to its rapid perishability.[29,30] For the production of these commercial cassava products, the roots are subjected to several processing steps, including peeling, grating, fermentation, drying and, cooking. As each of these steps may impact carotenoid stability, retention through the processing and value chain must be established. In the present study, we report the stability of carotenoids from harvest through the production of unfermented and fermented flours as well as during their inclusion in porridge preparations made by traditional cooking methods (Table 1).

Effect of spontaneous fermentation on carotenoids retention from biofortified cassava root genotypes. Overall, fermentation significantly ($p<0.05$) decreased levels of TCC and β-CE among most of the cassava genotypes (Table 1). Retention (%) ranged from 72 to 96 for both TCC and β-CE. While genotypes SM 3762-15 and SM 3758-43 had significantly higher retention of carotenoids after fermentation (~96%), genotype GM 5212-6 maintained the highest level of TCC (43.1 ± 2.2 μg/g DW), and β-CE (34.2 ± 1.7 μg/g DW) ($p<0.05$) among fermented cassava roots. Although it was not merely testing the effect of fermentation per se on carotenoids stability, Thakkar and colleagues found that fermentation of yellow-fleshed roots did not significantly decrease β-carotene content in comparison to its original content in raw cassava roots ($p>0.05$).[17] Cassava roots were grated and fermented in a bowl covered with a black nylon cloth for three days at room temperature, followed by manually pressing to remove residual water and sieved through a stainless steel mesh.[17]

Fermentation in our study was carried out by mimicking the traditional African method for preparation of gari, which involved minimum control of light exposure, oxygen concentration, and pH. These factors are well known to induce carotenoid degradation.[31] Moreover, based on visual observations reduction of carotenoids after fermentation also may be due to physical losses during grating, pressing and fermentation of grated cassava roots. The latter in accordance with a recent work published by Bechoff and colleagues who reported significant physical losses during processing of *gari* using biofortified cassava varieties developed by IITA in Nigeria.[26] However, based on our observation that the genotype with the highest DMC (%) showed the lowest loss of TCC after fermentation (SM3762-15), suggests that

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possibly physical losses of carotenoids during grating, fermentation, and pressing may depend on the DMC (%) of cassava roots. This observation was also reported by Bechoff and colleagues[26]. Apart from that, the contribution of all these factors might not have been determinant in the carotenoid reduction observed, since all samples were subjected to the same procedure and exposure level of degradative factors. Fermentation is well known for impacting mineral availability in food matrices.[32,33] The extent to which fermentation might release enough mineral, such as iron, to induce carotenoid oxidation as has been observed even at low oxygen tensions[31], remains to be explored in more detail. Further investigation is needed to understand better whether carotenoids degradation may be promoted through such interactions.

Effect of thermal processing for elaboration of cassava flours on the retention of carotenoids in biofortified cassava roots genotypes. Unfermented and fermented roots were oven-dried for two days at 40 °C using a forced-circulation oven (Table 1). Retention of carotenoids from both unfermented and fermented oven-dried roots were compared against initial carotenoid content in raw grated cassava roots. Retention (%) of carotenoids after oven drying ranged widely for unfermented cassava roots (TCC: 21 to 80%; β-CE: 17 to 78%). In contrast, the retention (%) of carotenoids in oven dried fermented roots was more uniform (TCC: 39 to 65%; β-CE: 35 to 66%). Despite the observation that ~18% of β-CE was lost through fermentation, eight of the ten genotypes showed significantly ($p<0.05$) higher levels of β-CE in oven dried fermented cassava roots compared to the unfermented roots from the same genotype. While carotenoids levels were affected by fermentation and oven-drying, both unfermented and fermented flours of GM 5212-6 showed the highest levels of carotenoids among genotypes. Retention of carotenoids was higher ($p<0.05$) for fermented (TCC: 55.16% ± 2.97; β-CE: 54.14% ± 3.35) than unfermented roots (TCC: 44.77% ± 7.24; β-CE: 41.13% ± 7.29).

Our findings, therefore, contrast with those reported for transgenic and wild-type cassava roots,[19] where retention of carotenoids (specifically β-carotene) were slightly higher for unfermented than for fermented flours. Furthermore, Failla and colleagues[19] reported higher retention through processing. The possible explanations for these differences include differences in genotypes, and processing and preparation methods. Fermentation and oven-drying methods of cassava by Failla and colleagues[19] used a wet method where root materials were fermented under water for three days rather than fermenting dewatered grated roots, packed into white woven polypropylene sack bags under constant temperature and pressure for two days (present

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method). Fermenting under lower amount of water as well as with small particle size (as it is for grated cassava) may promote carotenoids losses since they are more susceptible to oxidative degradation either by oxygen in the environment or by components released from root matrix (e.g. different minerals), which are not rapidly leached out from the roots and subsequently may alter stability of carotenoids. Moreover, oven-drying as reported by Failla and colleagues[19] was conducted over one day at 37 ºC rather than two days at 40 ºC as in our study. It is likely that the additional exposure to oxygen and longer thermal process impacted the carotenoid profiles. These contrasting findings in our study, and even by other researchers, highlight the importance to establish the optimal conditions for processing of biofortified cassava roots (such as minimizing exposure to degradative factors like oxygen, temperature and minerals) for production of cassava flours with the ultimate goal to guarantee the maximum content of pVAC in final cassava products.

The most common traditional cassava product in some countries of Africa, predominately in Nigeria, is known as *gari*, which was prepared by toasting dewatered-grated fermented roots on a pan at ~150ºC during 15-20 using the cassava genotypes SM 3765-15 and SM 3767-84. Retention (%) of carotenoids for *gari* was slighter higher ($p<0.05$) for SM 3765-15 (TCC: 66%; β-CE: 65%) than SM 3767-84 (TCC: 54%; β-CE: 53%) (Table 2). Despite the fact fermented cassava was exposed to a higher temperature during toasting than oven-drying, retention (%) of carotenoids from *gari* was comparable to the respective fermented oven-dried counterparts within cassava genotype (SM 3765-15: ~65%; SM 3767-84: ~50), but significantly higher when compared with the unfermented equivalent (SM 3765-15: ~50%; SM 3767-84: ~20, $p<0.05$). The latter is consistent with the observations above, in which fermented roots showed higher carotenoid retentions compared to unfermented counterpart after oven-drying of grated roots. Retention (%) of carotenoids for *gari* greatly contrasts with values already reported for *gari* prepared from wild-type roots (~15%) and transgenic genotypes (~35%) toasted at 120ºC for 20 min,[19] yellow-fleshed cassava cultivars toasted at 165ºC for 10 min (~50%),[17] and cassava roots toasted in a steel pan heated by fired woods (33-65%).[26] Differences in the retention of carotenoids may be related to variations in the procedures followed to prepare *gari*, which may be a challenging aspect for the adoption of pVAC-rich cassava genotypes into traditional processing focused on keeping their improved levels in carotenoids. It is important to point out that traditional preparation of *gari* indeed varies widely even within a city or region in Africa. Populations that consume *gari* show a large diversity of local tasting preferences, these

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variations may include style on fermentation (soaked vs. dewatered/pressured), times of fermentation (1-10 days), toasting temperatures and the extent of thermal exposition, which ultimately might not be fully controlled under routine conditions. Also, variations on the retention of carotenoids may be influenced by variety as well as season and location of the cassava cultivations. Recently Bechoff and colleagues,[26] reported that retention of carotenoids for *gari* was significantly different for the same cassava cultivar planted in different locations and harvested in different seasons. These contrasting results highlight the sensitivity of carotenoids to subtle changes in post-harvest processing preparation making the development of clear recommendations important for the eventual efficacious translation of these cultivars to at-risk populations.

In consideration of household preparations, standard porridges were prepared from fermented and unfermented cassava flours to mimic further thermal exposition of cassava products under household cooking. Gari was used to make *Eba*, which is an African thick paste. Carotenoid retention (%) from unfermented and fermented cassava materials ranged from 10% to 60% (TCC) and 10% to 57% (β-CE) for porridges prepared with unfermented flours, and 6% to 51% (TCC) and 6% to 52% (β-CE) for porridges prepared with fermented flours (Table 1). *Eba* was prepared with *gari* flour yielding carotenoid recoveries of ~75 % (TCC and β-CE) for SM 3765-15 and 50% (TCC and β-CE) for SM 3767-84 (Table 2). In general, seven genotypes showed higher TCC recoveries (%) for fermented cassava porridges than the respective unfermented counterpart. Two genotypes showed better TCC retention (%) during cooking porridges with unfermented cassava flours. GM 5212-6 kept the highest content of carotenoids (~27 μg/g DW or ~48% retention for both TCC and β-CE) either for fermented and unfermented porridges among all ten genotypes ($p<0.05$). It is interesting to highlight that while GM 5212-6 showed the highest TCC values in fresh roots and retained among the highest proportion of carotenoids through processing, genotype GM 4414-5, which also showed high TCC levels in raw material and unfermented porridge, was drastically affected by fermentation with just ~6% of TCC retention for fermented porridge (2.5 μg/g DW TCC, 1.7 μg/g DW β-CE). While processing of cassava roots includes critical steps on carotenoids stability impacting the ultimate levels of pVAC delivered in different food products, the present results suggest that genetics itself also may plays an important role that merits further investigation. In other words, those genetic factors may include, but perhaps not limited to, mineral (e.g. Ca) and carbohydrates (fibers) levels that need to be schematic addressed over stability of carotenoids through processing in

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further studies in order to successfully adopt pVAC-enriched crops under typically processing procedures.

3.4. Potential pVAC provided by porridges of biofortified cassava

Cassava porridges were prepared with fermented and unfermented flours of cassava roots using selected biofortified cassava genotypes. Contents of carotenoids and potential Retinol Equivalents (REqv) provided by the cassava porridges (considering a serving size of 200 g FW of porridge) was estimated applying the recent bioconversion factor reported by La Frano et al. (2013)[34] and Zhu et al. (2015)[35] for biofortified cassava (Table 3). Most of the fermented cassava porridges (six of ten) showed significant higher contents of total carotenoids ($p<0.05$) in comparison to their unfermented pairs. The Retinol Equivalents (μg REqv/200 g FW) provided by a service size of cassava porridge range from 14.3 ± 0.6 to 163.1 ± 5.7 (fermented cassava) and 18.7 ± 0.8 to 146.8 ± 1.5 (unfermented cassava). Similarly, fermented cassava porridges (seven of ten genotypes) showed significant higher contents of REqv ($p<0.05$) in comparison to their unfermented pairs. Specifically, genotypes GM 4571-3, GM 5194-13, GM 5194-5, GM 5212-6, SM 3757-75, SM 3762-15, SM 3767-84 showed higher contents of REqv when cassava roots were fermented. The opposite was observed for genotypes GM 4414-5 and SM 3758-43, in which higher REqv contents ($p<0.05$) were noticed for the unfermented cassava porridge pairs. Genotype SM 3774-21 did not show significant differences between F and UF cassava porridges.

The potential contribution of pro-vitamin A to the Recommended Daily Allowances (RDA) for 4-to-8-year-old children (400 μg RAE/day) by the consumption of a serving size of cassava porridge was estimated as %RDA (assuming RAE = REqv), and it is presented also in Table 3. Cassava porridges may to contribute to the RDA ranging from 3.6 ± 0.2 to 40.8 ± 2.5% RDA (fermented cassava) and 4.7 ± 0.4 to 36.7 ± 0.7% RDA (unfermented cassava). Genotype GM 5212-6 showed the highest contents among genotypes either for UF and F cassava porridges (~40% of Vitamin A RDA). Incorporating biofortified cassava genotypes, such as GM 5212-6, into the diet of 4-to-8-years-old- children (especially the ones from populations in Africa where consumption of cassava reaches up to three times per day) may potentially to contribute nearly to 120% of the RDA per day in these children. While these estimations are promising for the eradication of VAD at-high risk populations, it is important to highlight that in spite of this potential, ~50% of carotenoids were reduced from harvest to the ready-to-eat porridge. This

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451 indeed emphasizes the importance of breeding genotypes with high carotenoid levels and
452 optimizing the processing of biofortified crops to delivery of pVAC in final food products.
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3.5. Bioaccessibility of carotenoids from unfermented and fermented cassava genotypes.

Porridges were prepared with fermented and unfermented flour using a traditional standardized method to ultimately test the effect of mechanized traditional processing followed by household cooking over bioaccessibility of carotenoids. Stability of carotenoids (%) through the simulated *in vitro* digestion was similar between fermented and unfermented cassava porridges across different genotypes (β-CE: F= 82.36 ± 2.21 and UF= 76.55 ± 5.06; TCC: F= 87.11 ± 2.68 and UF=81.86 ± 4.80). Recoveries of carotenoids after *in vitro* digestions exceeded 70% as reported in previous studies.[15-17,19,36]

The efficiency of micellarization (ME%) for carotenoids (β-CE, TCC, Z- βC and All-E-βC) was significantly affected by genotypes and processing methods. The genotype*processing interaction was also significant ($p<0.004$). For instance, ME% for β-CE seemed to be enhanced after fermentation ($p<0.05$) in some cases (F>UF in SM 3762-15, SM 3757-75), had negligible effects in most of genotypes ($p>0.05$) (F=UF in GM 5194-13, SM 3774-21, SM 3758-43, GM 4414-5 and GM 4571-3), and had a significant ($p<0.05$) detrimental effect in the remaining genotypes (SM 3767-84, GM 5212-6, and GM 5194-5) (Figure 3). These results tend to be consistent either for TCC, *All-E*-ßC, and *Z's*-ßC, but there were few exceptions in some genotypes. ME (%) ranged widely for unfermented roots (β-CE: 3.7 to 14.7%; TCC: 2.6 to 17.4%; *All-E*-βC: 3.3 to 13.7%; and *Z*-βC: 3.2 to 16.4%), while showing a slightly narrower window in fermented products (β-CE: 4.5 to 13.0%; TCC: 5.5 to 14.2%; *All-E*-βC: 3.8 to 12.2%; and *Z*-βC: 6.5 to 15.1%). In general, fermentation significantly decreased ME (%) among genotypes for β-CE (F=7.5 ± 0.7; UF= 9.2 ± 1.3; p=0.0078) and *All-E*-βC (F=6.8 ± 0.8; UF= 8.5 ± 1.1; p=0.01), but not for *Z*-βC (F=10.0 ± 0.8; UF= 10.4 ± 1.5; p=0.28), and TCC (F=8.8 ± 0.8; UF= 10.0 ± 1.6; p=0.11). These findings are both comparable and different to previous studies. For example, *All-E*-βC ME (%) for UF cassava porridges was lower than: *All-E*- βC ME for (UF) boiled cassava (~30%) reported by Thakkar et al.,[17] similarly processed transgenic cassava (~36%) and wild-type cassava (~30%) reported by Failla et al.,[19] some cassava genotypes (4 of 8 genotypes rounding 20 to 40%), but comparable to other cassava genotypes (4 of 8 genotypes rounding 10 to 15%) reported by Berni et al.[15] However, *All-E*-β-C ME (%)was slightly higher to values reported for (UF) boiled cassava (~6%) by Gomes et al.[16] Regarding *All-E*-βC ME (%) for fermented cassava, those results were comparable to the ones reported for *fufu* prepared using conventionally enhanced genotypes (~10%),[17] but lower than similarly processed transgenic cassava (~36%).[19]

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Our study also showed that β-CE and TCC quantity (μg/g DW) into mixed micelles similarly correlated with β-CE and TCC quantity (μg/g DW) for porridges prepared with UF cassava roots (β-CE: r=0.78, p<0.0001; TCC: r=0.79, p<0.0001) as previously reported by Thakkar et al.[36] (r=0.87, p<0.001), and Berni et al.[15] (r=0.89, p<0.01). However, lower correlations were found for roots subjected to fermentation (β-CE: r=0.52, p=0.0006; TCC: r=0.60, p=0.0006). It is important to point out that by correlating carotenoid content into micelles (from UF and F porridges) with original contents in fresh roots yields even lower correlations. For example, for unfermented roots β-CE r=0.64 (p=0.0002 and TCC r=0.61 (p=0.0004), while for fermented roots β-CE r=0.32 (p=0.09) and TCC r=0.46 (p=0.013). This suggests that higher bioaccessibility of carotenoids is not guaranteed by higher contents of carotenoids in fresh roots.

Our results strongly suggest that genotypes and processing method may interact in favour or against on the ultimate carotenoids bioavailability. Perhaps factors in the cassava matrix could be altered through the breeding process that can play an important role on stability through processing and their subsequently absorption through the intestinal cells. It is possible that variations on bioaccessibility of carotenoids may be related to fiber content. Although fiber content was not measured in cassava roots, we noticed that the texture of porridges differed among cassava genotypes (some looked as soft pastes, while others as hard thick pastes), suggesting that fiber content and/or chemical structure potentially vary along cassava genotypes. Actually, previous studies have shown that fiber contents and its nature can differ based on age and type of cassava cultivar.[37] Moreover, Cervantes-Paz et al.[38] have reviewed recently how fibers and their structural characteristics may affect carotenoids bioavailability. For instance, pectin characteristics including molecular weight, degree of methylation on pectin structure as well as hydrophobicity may influence on development of micelles through a chain of events including lipid digestion by affecting lipase activity, availability of bile salts for lipid emulsion and micelle formation, to name some. Additionally, divalent mineral cations (Ca, Mg, Zn) may be involved in the differences observed for efficiency of carotenoids micellarization among cassava genotypes. Recently, Corte-real and colleagues[39] reported that divalent mineral cations may drastically reduce bioaccessibility of carotenoids by binding bile salts and free fatty acids which are essential for micelle formation.

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Conclusions

Our findings support the hypothesis that both genotypes and processing applied to pVAC-rich cassava roots should be considered together for the ultimate goal of developing successful strategies to combat/eradicate VAD among affected populations. As our results also suggest that there may be other factors impacting the release and availability of carotenoids (e.g. fibers or minerals) that are not always considered in targeted nutrient breeding programs, it may be helpful to consider these factors as traits to monitor in the selection of the promising crops to combat VAD in at risk populations.

Conflicts of interest

There are no conflicts to declare.

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Abbreviations

β-CE: Beta Carotenoid Equivalents, **CM & GM**: code in pedigree for direct controlled crosses, **DMC**: Dry Matter Content, **DW**: Dry Weight basis, **F**: Fermented Flour, **FW**: Fresh Weight basis, **ME**: Micellarization Efficiency (%), **pVAC**: Provitamin A Carotenoids, **REqv**: Retinol Equivalents, **RDA**: Recommended Daily Allowances, **TCC**: Total Carotenoid Content, **UF**: Unfermented Flour, **SM**: code in pedigree for open pollination.

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Tables 1. Content (μg/g DW) and retention (%) of carotenoids through mechanized traditional processing of biofortified cassava genotypes[1-6]

Genotype	Treatment[2]	Carotenoid Content (μg/g DW)				(%) Ret from TCC in raw material
		all-E-βC	Z-βC[3]	βCE[4]	TCC[5]	
GM 4571-3	R	19.8 ± 0.7 ^{a}	6.7 ± 0.1 ^{a}	23.1 ± 0.7 $^{a, B}$	33.2 ± 0.9 $^{a, BC}$	
	F	16.6 ± 1.5 ^{a}	5.5 ± 0.4 ^{a}	19.4 ± 1.7 $^{ab, DE}$	27.0 ± 2.3 $^{b, B}$	81.3 ± 4.3 ^{ABC}
	OD-UF	3.9 ± 0.0 ^{d}	2.7 ± 0.0 ^{c}	5.7 ± 0.0 $^{d, E}$	8.5 ± 0.1 $^{d, F}$	25.7 ± 0.4 ^{F}
	OD-F	12.3 ± 0.1 ^{b}	6.0 ± 0.3 ^{a}	15.9 ± 0.4 $^{b, CDE}$	21.8 ± 0.8 $^{b, CD}$	65.7 ± 1.8 ^{A}
	C-UF	1.5 ± 0.1 ^{d}	1.2 ± 0.0 ^{d}	2.3 ± 0.1 $^{d, E}$	3.4 ± 0.2 $^{d, E}$	10.4 ± 0.4 ^{F}
	C-F	7.4 ± 0.5 ^{c}	4.1 ± 0.3 ^{b}	9.7 ± 0.7 $^{c, BC}$	13.9 ± 0.9 $^{c, BC}$	41.1 ± 1.7 ^{BC}
GM 5194-13	R	26.3 ± 0.3 ^{a}	2.7 ± 0.1 ^{a}	27.7 ± 0.3 $^{a, B}$	32.0 ± 0.4 $^{a, BC}$	
	F	20.5 ± 1.1 ^{b}	3.5 ± 1.7 ^{a}	22.3 ± 0.2 $^{b, BCDE}$	28.8 ± 3.9 $^{a, B}$	90.0 ± 7.1 ^{ABC}
	OD-UF	4.2 ± 0.1 ^{e}	1.6 ± 0.0 ^{a}	5.3 ± 0.1 $^{e, E}$	6.9 ± 0.2 $^{c, F}$	21.6 ± 0.3 ^{F}
	OD-F	12.1 ± 0.2 ^{c}	2.3 ± 0.0 ^{a}	13.5 ± 0.2 $^{c, F}$	15.7 ± 0.1 $^{b, EF}$	49.0 ± 0.4 ^{DE}
	C-UF	2.8 ± 0.0 ^{e}	1.6 ± 0.1 ^{a}	3.8 ± 0.1 $^{f, DE}$	5.1 ± 0.2 $^{c, DE}$	15.8 ± 0.3 ^{EF}
	C-F	7.8 ± 0.3 ^{d}	3.0 ± 0.1 ^{a}	9.5 ± 0.3 $^{d, BC}$	12.0 ± 0.5 $^{bc, BCD}$	37.6 ± 1.0 ^{C}
GM 5194-5	R	23.8 ± 0.6 ^{a}	4.4 ± 0.1 ^{a}	26.0 ± 0.5 $^{a, B}$	31.2 ± 0.5 $^{a, C}$	
	F	17.3 ± 0.9 ^{b}	3 ± 0.2 ^{b}	18.8 ± 1.0 $^{b, DE}$	23.7 ± 1.3 $^{b, B}$	76 ± 2.5 ^{ABC}
	OD-UF	6.0 ± 0.0 ^{e}	3.7 ± 0.1 ^{b}	8.3 ± 0.1 $^{d, D}$	11.7 ± 0.2 $^{d, E}$	37.5 ± 0.5 ^{E}
	OD-F	12.4 ± 0.5 ^{c}	4.6 ± 0.2 ^{a}	15.1 ± 0.6 $^{c, DEF}$	18.9 ± 0.7 $^{c, DE}$	60.4 ± 1.5 ^{ABC}
	C-UF	3.1 ± 0.2 ^{f}	2.4 ± 0.1 ^{c}	4.6 ± 0.2 $^{e, D}$	7.0 ± 0.4 $^{e, D}$	22.5 ± 0.8 ^{E}
	C-F	8.4 ± 0.0 ^{d}	3.1 ± 0.0 ^{b}	10.2 ± 0.0 $^{d, BC}$	12.8 ± 0.0 $^{d, BC}$	40.9 ± 0.3 ^{BC}
GM 5212-6	R	37.9 ± 0.4 ^{a}	9.7 ± 0.1 ^{bc}	42.7 ± 0.4 $^{a, A}$	55.0 ± 0.5 $^{a, A}$	
	F	30.3 ± 1.5 ^{b}	7.9 ± 0.4 ^{bc}	34.2 ± 1.7 $^{b, A}$	43.1 ± 2.2 $^{b, A}$	78.2 ± 2.4 ^{ABC}
	OD-UF	18.3 ± 0.8 ^{d}	13.8 ± 0.5 ^{a}	26.9 ± 1.0 $^{c, A}$	38.0 ± 0.9 $^{bc, A}$	69.0 ± 1.1 ^{B}
	OD-F	24.5 ± 0.5 ^{c}	7.2 ± 1.8 ^{bc}	28.7 ± 0.4 $^{c, A}$	35.0 ± 1.3 $^{c, A}$	63.7 ± 1.4 ^{AB}
	C-UF	11.8 ± 0.1 ^{e}	10.1 ± 0.1 ^{b}	18.0 ± 0.2 $^{d, A}$	26.8 ± 0.2 $^{d, A}$	48.7 ± 0.3 ^{B}
	C-F	17.4 ± 0.6 ^{d}	6.6 ± 0.2 ^{c}	21.1 ± 0.7 $^{d, A}$	26.5 ± 0.9 $^{d, A}$	48.1 ± 1.1 ^{A}
GM 4414-5	R	26.9 ± 2.0 ^{a}	8.4 ± 0.7 ^{ab}	31.1 ± 2.4 $^{a, B}$	40.1 ± 3.2 $^{a, BC}$	
	F	24.2 ± 0.4 ^{ab}	7.7 ± 0.0 ^{b}	28.0 ± 0.4 $^{a, B}$	35.6 ± 0.5 $^{a, AB}$	89.4 ± 4.2 ^{ABC}
	OD-UF	18.5 ± 0.2 ^{b}	11.4 ± 0.4 ^{a}	25.0 ± 0.4 $^{a, A}$	33.3 ± 0.7 $^{ab, B}$	83.5 ± 4.0 ^{A}
	OD-F	12.3 ± 0.1 ^{c}	7.1 ± 0.4 ^{b}	16.3 ± 0.5 $^{b, CD}$	21.7 ± 1.0 $^{c, CD}$	54.5 ± 2.9 ^{BCD}
	C-UF	12.2 ± 0.9 ^{c}	8.9 ± 0.6 ^{ab}	17.2 ± 1.2 $^{b, A}$	23.8 ± 1.7 $^{bc, A}$	59.7 ± 3.4 ^{A}
	C-F	1.2 ± 0.0 ^{d}	1.7 ± 0.8 ^{c}	1.7 ± 0.1 $^{c, D}$	2.5 ± 0.1 $^{d, D}$	6.2 ± 0.3 ^{E}
SM 3757-75	R	20.5 ± 0.9 ^{a}	6.2 ± 0.2 ^{a}	23.6 ± 1.0 $^{a, B}$	32.2 ± 1.3 $^{a, BC}$	
	F	15.8 ± 0.4 ^{b}	4.4 ± 0.3 ^{b}	18.0 ± 0.6 $^{b, E}$	23.1 ± 0.9 $^{b, B}$	72.0 ± 2.4 ^{C}
	OD-UF	2.8 ± 0.1 ^{e}	2.1 ± 0.1 ^{de}	4.1 ± 0.3 $^{de, E}$	6.4 ± 0.4 $^{de, F}$	19.8 ± 0.9 ^{F}
	OD-F	6.3 ± 0.1 ^{c}	3.6 ± 0.2 ^{c}	8.6 ± 0.2 $^{c, G}$	12.8 ± 0.8 $^{c, F}$	39.8 ± 1.8 ^{E}
	C-UF	1.6 ± 0.0 ^{e}	1.4 ± 0.1 ^{e}	2.5 ± 0.1 $^{e, DE}$	4.0 ± 0.1 $^{e, DE}$	12.4 ± 0.3 ^{F}
	C-F	4.6 ± 0.2 ^{d}	2.5 ± 0.1 ^{d}	6.0 ± 0.2 $^{d, CD}$	8.3 ± 0.2 $^{d, CD}$	26.0 ± 0.6 ^{D}
SM 3758-43	R	25.4 ± 0.3 ^{a}	3.5 ± 0.1 ^{a}	27.1 ± 0.3 $^{a, B}$	32.1 ± 0.5 $^{a, BC}$	
	F	24.5 ± 0.2 ^{ab}	3.5 ± 0.3 ^{a}	26.2 ± 0.3 $^{a, BC}$	30.1 ± 0.7 $^{a, AB}$	94.0 ± 1.4 ^{AB}
	OD-UF	17.1 ± 0.3 ^{bc}	5.4 ± 0.1 ^{a}	20.4 ± 0.4 $^{ab, B}$	24.8 ± 0.1 $^{ab, C}$	77.2 ± 0.7 ^{A}
	OD-F	12.8 ± 0.0 ^{cd}	5.3 ± 0.1 ^{a}	15.9 ± 0.0 $^{bc, CDE}$	21.0 ± 0.9 $^{abc, CD}$	65.4 ± 1.6 ^{A}
	C-UF	10.7 ± 0.2 ^{cd}	4.4 ± 0.1 ^{a}	13.4 ± 0.3 $^{bc, B}$	16.9 ± 0.4 $^{bc, B}$	52.7 ± 0.8 ^{B}
	C-F	9.3 ± 0.3 ^{d}	3.0 ± 1.0 ^{a}	11.6 ± 0.4 $^{c, BCD}$	14.7 ± 0.3 $^{c, BCD}$	45.8 ± 0.7 ^{AB}
SM 3762-15	R	21.4 ± 0.1 ^{a}	7.0 ± 0.0 ^{ab}	24.9 ± 0.1 $^{a, B}$	35.9 ± 0.1 $^{a, BC}$	
	F	20.4 ± 0.3 ^{a}	7.0 ± 0.1 ^{ab}	23.9 ± 0.4 $^{a, BCD}$	34.6 ± 0.5 $^{a, AB}$	96.5 ± 0.9 ^{A}
	OD-UF	9.2 ± 0.1 ^{c}	6.2 ± 0.2 ^{b}	13.1 ± 0.1 $^{c, C}$	19.4 ± 0.7 $^{c, D}$	54.0 ± 1.2 ^{C}
	OD-F	12.6 ± 0.0 ^{b}	7.9 ± 0.1 ^{a}	17.1 ± 0.0 $^{b, BC}$	24.9 ± 0.1 $^{b, BC}$	69.3 ± 0.2 ^{A}
	C-UF	6.3 ± 0.2 ^{d}	4.7 ± 0.2 ^{c}	9.1 ± 0.4 $^{d, C}$	13.5 ± 0.5 $^{c, C}$	37.6 ± 0.9 ^{C}
	C-F	8.5 ± 0.4 ^{c}	6.0 ± 0.3 ^{b}	11.9 ± 0.6 $^{c, B}$	18.2 ± 0.9 $^{d, B}$	50.5 ± 1.5 ^{A}

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664 **Table 1.** Continued

SM 3767-84	R	26.5 ± 3.2 ^{a}	4.0 ± 0.5 ^{ab}	28.5 ± 3.4 $^{a, B}$	34.6 ± 4.3 $^{a, BC}$	
	F	19.9 ± 0.3 ^{b}	3.2 ± 0.1 ^{bc}	21.5 ± 0.4 $^{b, CDE}$	25.7 ± 0.5 $^{b, B}$	75.3 ± 5.4 ^{BC}
	OD-UF	4.0 ± 0.2 ^{d}	2.6 ± 0.0 ^{cd}	5.6 ± 0.2 $^{d, E}$	7.8 ± 0.1 $^{c, F}$	23.0 ± 1.7 ^{F}
	OD-F	11.9 ± 0.1 ^{c}	4.3 ± 0.2 ^{a}	14.4 ± 0.2 $^{c, EF}$	17.9 ± 0.2 $^{b, DE}$	52.4 ± 3.7 ^{CD}
	C-UF	2.0 ± 0.1 ^{d}	1.5 ± 0.1 ^{e}	2.9 ± 0.1 $^{d, DE}$	4.5 ± 0.3 $^{c, DE}$	12.4 ± 0.7 ^{F}
	C-F	5.9 ± 0.5 ^{d}	1.8 ± 0.1 ^{de}	7.0 ± 0.5 $^{d, BCD}$	8.6 ± 0.5 $^{c, CD}$	25.2 ± 1.6 ^{D}
SM 3774-21	R	24.7 ± 2.2 ^{a}	6.8 ± 0.9 ^{a}	28.1 ± 2.6 $^{a, B}$	43.3 ± 3.4 $^{a, B}$	
	F	20.0 ± 0.8 ^{b}	3.6 ± 1.7 ^{a}	21.8 ± 1.7 $^{b, CDE}$	28.4 ± 5.9 $^{b, B}$	72.1 ± 8.6 ^{BC}
	OD-UF	9.3 ± 0.0 ^{d}	5.9 ± 0.0 ^{a}	12.9 ± 0.0 $^{cd, C}$	19.7 ± 0.2 $^{bcd, D}$	45.8 ± 2.1 ^{D}
	OD-F	14.7 ± 0.0 ^{c}	6.2 ± 0.1 ^{a}	18.3 ± 0.1 $^{bc, B}$	27.0 ± 0.6 $^{bc, B}$	62.8 ± 3.0 ^{AB}
	C-UF	5.6 ± 0.0 ^{d}	4.5 ± 0.0 ^{a}	8.3 ± 0.0 $^{d, C}$	13.5 ± 0.0 $^{d, C}$	31.5 ± 1.1 ^{D}
	C-F	7.7 ± 0.6 ^{d}	4.4 ± 0.2 ^{a}	10.1 ± 0.7 $^{d, BC}$	15.6 ± 1.0 $^{cd, BC}$	36.4 ± 1.9 ^{C}

665 [1]Data represent means ± SEM from n=3 independent observations.
666 [2]Type of processing = R: Raw grated cassava; F: Fermentation; OD-UF: Oven dried unfermented cassava; OD-F: Oven
667 dried fermented cassava; C-UF: Cooked unfermented cassava; C-F: Cooked fermented cassava.
668 [3]Z-βC = sum of 9-Z-βC, 13-Z-βC, 15-Z-βC
669 [4]βCE: β-carotene equivalent, which was calculated by sum of *all-E*-β-carotene + ½ (β-cryptoxanthin + α-carotene + *Z*-β-
670 carotene)
671 [5]TCC: total carotenoid content, sum of all quantified carotenoid species
672 [6]Presence of different letters represents significant differences in carotenoid content of retention ($p<0.05$).
673 Specifically, *lower case letters* indicate differences in specific carotenoid content, βCE and TCC through each
674 processing stage within each genotype for each column of carotenoid group (*all E*-βC, Z-βC, βCE and TCC).
675 Additionally, presence of different *upper-case letters* indicates statistically significant difference between genotypes
676 within at the same processing stage for βCE and TCC as well as for total retention.
677

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Table 2. Comparisons of Total Carotenoid Content (TCC) and β-Carotenoid equivalent (β-CE) retention (%) through traditional processing of cassava (*gari*) and mechanized traditional processing of two pVAC-rich cassava genotypes, and micellarization efficiency (ME%) from porridges made from unfermented, fermented and *gari* flours[1-3]

Carotene	Treatment	Genotypes	
		SM 3765-15	SM 3767-84
		Retention (%)	
β-CE	F	96.04 ± 0.94 ^{a*}	76.7 ± 5.42 ^{a}
	OD-UF	49.54 ± 0.28 ^{ef*}	18.73 ± 1.36 ^{d}
	OD-F	64.68 ± 0.20 ^{bcd*}	48.17 ± 3.39 ^{bc}
	T-G	64.65 ± 0.66 ^{c*}	53.11 ± 2.58 ^{b}
	C-UF	42.91 ± 6.73 ^{f*}	14.50 ± 4.01 ^{d}
	C-F	53.51 ± 5.31 ^{de*}	29.50 ± 6.11 ^{cd}
	C-G	74.53 ± 2.13 ^{b*}	46.34 ± 4.55 ^{bc}
TCC	F	96.45 ± 0.87 ^{a*}	75.34 ± 5.43 ^{a}
	OD-UF	50.98 ± 1.13 ^{d*}	21.60 ± 1.55 ^{c}
	OD-F	65.25 ± 0.18 ^{c*}	49.20 ± 3.52 ^{b}
	T-G	65.77 ± 1.09 ^{c*}	54.09 ± 2.63 ^{b}
	C-UF	37.6 ± 0.92 ^{e*}	13.06 ± 0.86 ^{c}
	C-F	50.54 ± 1.52 ^{d*}	25.19 ± 1.63 ^{c}
	C-G	77.52 ± 2.20 ^{b*}	50.39 ± 4.14 ^{b}
		Micellarization (ME%)	
β-CE	C-UF	3.73 ± 0.70 ^{b}	14.82 ± 2.41 ^{a*}
	C-F	7.49 ± 0.48 ^{b}	6.32 ± 1.10 ^{b}
	C-G	6.53 ± 2.38 ^{b}	4.50 ± 1.23 ^{b}
	C-G_c	27.25 ± 2.05 ^{a*}	15.34 ± 0.60 ^{a}
TCC	C-UF	4.74 ± 0.94 ^{b}	18.36 ± 3.78 ^{a*}
	C-F	8.93 ± 0.42 ^{b}	7.67 ± 1.24 ^{bc}
	C-G	7.68 ± 2.31 ^{b}	5.26 ± 1.15 ^{c}
	C-G_c	30.05 ± 2.09 ^{a*}	16.61 ± 0.78 ^{ab}

[1]Data represents means ± SEM from n=3 independent observations.

[2]Abbreviations: OD: Oven drying, C: Cooking porridges, UF: Unfermented cassava, F: Fermented cassava, G: *gari*, G_c: course flour, particle size 1.2 mm, all other flours were milled and sieved to a particle size of 0.6 mm (fine flour).

[3]Statistical analysis was performed by comparing retention (%) of β-CE, TCC) through processing within genotype and between genotypes within type of processing. Presence of different letters denote significant differences ($p<0.05$) in micellarization efficiency among processing within genotype and (*) denote significant differences in micellarization efficiency between pairs of cassava genotypes within same type of processing.

Table 3. Estimated impact of fermentation on the potential for biofortified cassava to provide dietary vitamin A in the form of traditional wet cooked porridges (200g serving size).[1-6]

Genotype	*Z*-βC [2]		*all-E*-βC		TCC	
	F	UF	F	UF	F	UF
GM 4414-5	32.1 ± 1.8^{f}	297.1 ± 21.2^{b*}	41.7 ± 1.4^{e}	410.8 ± 30.7^{a*}	87.8 ± 4.7^{g}	797.8 ± 57.2^{b*}
GM 4571-3	141.4 ± 8.8^{b*}	42.5 ± 1.6^{e}	253.6 ± 17.9^{bc*}	52.3 ± 1.9^{f}	477.5 ± 32.0^{bcd*}	118.0 ± 6.5^{g}
GM 5194-13	98.0 ± 2.3^{d*}	54.9 ± 2.9^{e}	258.3 ± 9.4^{bc*}	97.9 ± 1.5^{e}	398.5 ± 15.8^{de*}	174.2 ± 5.3^{f}
GM 5194-5	107 ± 1.3^{cd*}	83.5 ± 4.5^{d}	285.9 ± 0.2^{b*}	107.5 ± 6.1^{e}	434.9 ± 0.9^{cd*}	242.5 ± 13.1^{e}
GM 5212-6	213.6 ± 7.9^{a}	347.0 ± 4.6^{a*}	564.4 ± 20.7^{a*}	404.1 ± 4.8^{b}	858.6 ± 29.5^{a}	919.7 ± 7.3^{a}
SM 3757-75	85.6 ± 2.2^{de*}	45.9 ± 1.8^{e}	154.6 ± 5.6^{d*}	53.9 ± 1.6^{f}	283.2 ± 7.1^{f*}	131.1 ± 3.8^{fg}
SM 3758-43	133.4 ± 2^{bc}	152.5 ± 4.5^{c}	316.7 ± 11.3^{b}	368.8 ± 6.3^{c*}	498.0 ± 11.0^{bcd}	582.2 ± 12.6^{c*}
SM 3774-21	151 ± 6.3^{b}	158.9 ± 1.1^{c}	264.8 ± 19.9^{bc*}	197.6 ± 0.8^{d}	541.4 ± 33.5^{bc}	481.8 ± 1.4^{d}
SM 3762-15	189.8 ± 9.1^{a*}	157.0 ± 6.5^{c}	270.9 ± 14^{bc*}	210.6 ± 7.6^{d}	578.8 ± 27.5^{b*}	454.1 ± 17.5^{d}
SM 3767-84	65.0 ± 3.0^{e*}	51.3 ± 2.8^{e}	213.4 ± 17.8^{cd*}	67.9 ± 3.1^{f}	310.4 ± 16.6^{ef*}	152.2 ± 8.6^{fg}
Genotype	**βCE [3]**		**REqv [4]**		**%RDA [5]**	
	F	UF	F	UF	F	UF
GM 4414-5	60.3 ± 2.6^{e}	578.1 ± 41.4^{a*}	14.3 ± 0.6 ^{e}	137.6 ± 9.9 ^{a*}	3.6 ± 0.2 ^{e}	34.4 ± 4.3 ^{a*}
GM 4571-3	334.2 ± 22.6^{bc*}	78.4 ± 3.5^{e}	79.6 ± 5.4 ^{bc*}	18.7 ± 0.8 ^{f}	19.9 ± 2.3 ^{bc*}	4.7 ± 0.4 ^{f}
GM 5194-13	314.8 ± 11.0^{bc*}	129.8 ± 3.3^{de}	74.9 ± 2.6 ^{bc*}	30.9 ± 0.8 ^{de}	18.7 ± 1.1 ^{bc*}	7.7 ± 0.3 de
GM 5194-5	347.6 ± 0.6^{b*}	159.6 ± 8.1^{d}	82.8 ± 0.1 ^{b*}	38.0 ± 1.9 ^{d}	20.7 ± 0.1 ^{b*}	9.5 ± 0.8 ^{d}
GM 5212-6	685 ± 23.9^{a}	616.7 ± 6.5^{a}	163.1 ± 5.7 ^{a*}	146.8 ± 1.5 ^{a}	40.8 ± 2.5 ^{a*}	36.7 ± 0.7 ^{a}
SM 3757-75	204.2 ± 6.1d*	83.7 ± 2.5^{e}	48.6 ± 1.4 ^{d*}	19.9 ± 0.6 ^{f}	12.2 ± 0.6 ^{d*}	5.0 ± 0.3 ^{f}
SM 3758-43	392.3 ± 12.7^{b}	460.2 ± 9.0^{b*}	93.4 ± 3.0 ^{b}	109.6 ± 2.1 ^{b*}	23.4 ± 1.1 ^{b}	27.4 ± 0.9 ^{d*}
SM 3774-21	349.1 ± 23.2^{b}	295.4 ± 0.3^{c}	83.1 ± 5.5 ^{b}	70.3 ± 0.1 ^{c}	20.8 ± 2.4 ^{b}	17.6 ± 0.0 ^{c}
SM 3762-15	379.6 ± 19.0^{b*}	305.1 ± 12.1^{c}	90.4 ± 4.5 ^{b*}	72.7 ± 2.9 ^{c}	22.6 ± 2 ^{b*}	18.2 ± 1.2 ^{c}
SM 3767-84	251.0 ± 17.0^{cd*}	100.3 ± 4.7^{de}	59.8 ± 4 ^{cd*}	23.9 ± 1.1 ^{ef}	14.9 ± 1.8 ^{cd*}	6.0 ± 0.5 ^{ef}

[1]Carotenoids content, RAE and RDA in porridge of cassava were calculated on the basis of content of a 200 g FW serving size (means ± SEM, n=3)

[2]Z-βC = sum of 9-Z-βC, 13-Z-βC, 15-Z-βC

[3] βCE = *all*-E-βC + 1/2 Z-βC

[4]REqv (Retinol Equivalent) of pro-vitamin A carotenoids were computed considering a recent conversion factor 1 μg REqv =4.2 μg β-CE for biofortified cassava reported by La Frano et al. (2013)[34] and Zhu et al. (2015)[35]

[5] % RDA is the percentage of vitamin A contributed to the RDA (Recommended Dietary Allowances) from cassava porridges evaluated. Values were computed considering RDA of 400 μg RAE/day for 4 to 8-year-old children assuming RAE = REqv (Institute of Medicine, 2001).

[6]Presence of different letters denote significant differences ($p<0.05$) among genotypes within each measure of carotenoid type, RAE and %RDA. Presence of (*) denotes significant differences ($p<0.05$) between F and UF.

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Figure 1. Cassava processing mimicking traditional techniques as applied in current experiments. The sequence of operations for production of fermented and unfermented cassava flours. Presence of a (*) indicates the point of samples collected for analysis of carotenoids, dry matter and cyanide content.

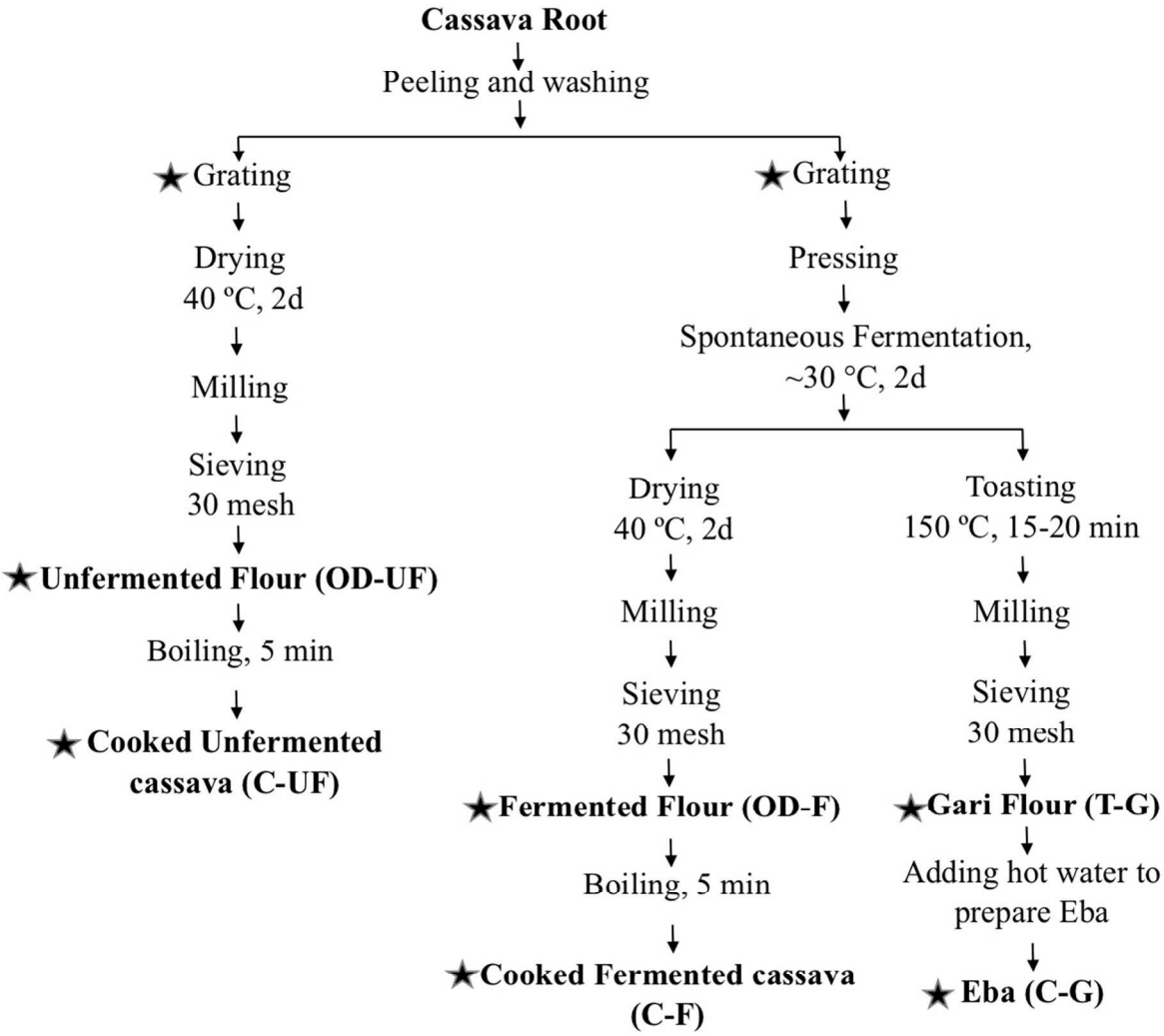

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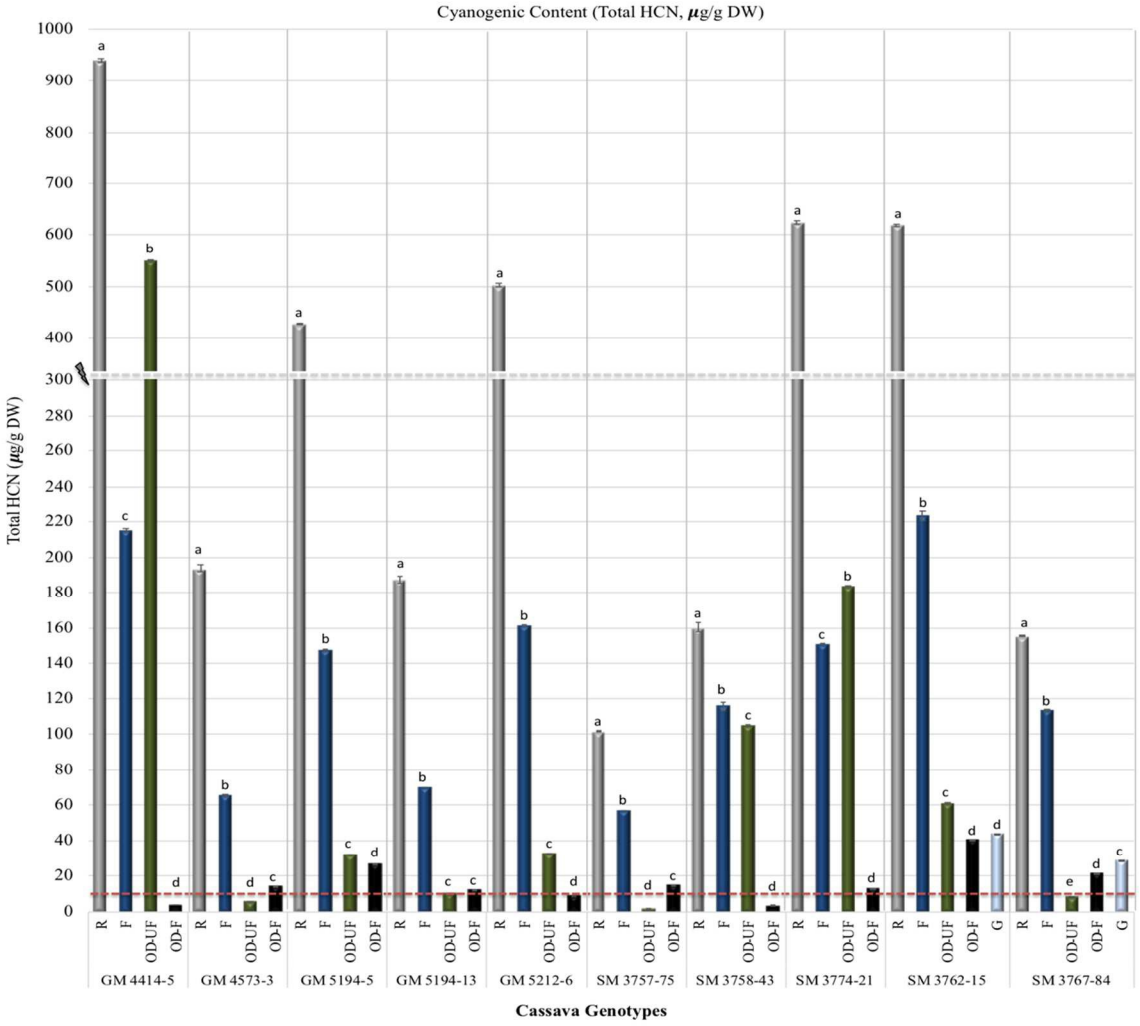
Figure 2. Total cyanogenic glycoside content (HCN, μg/g DW) among biofortified cassava genotypes through mechanized traditional processing conditions[1-3]

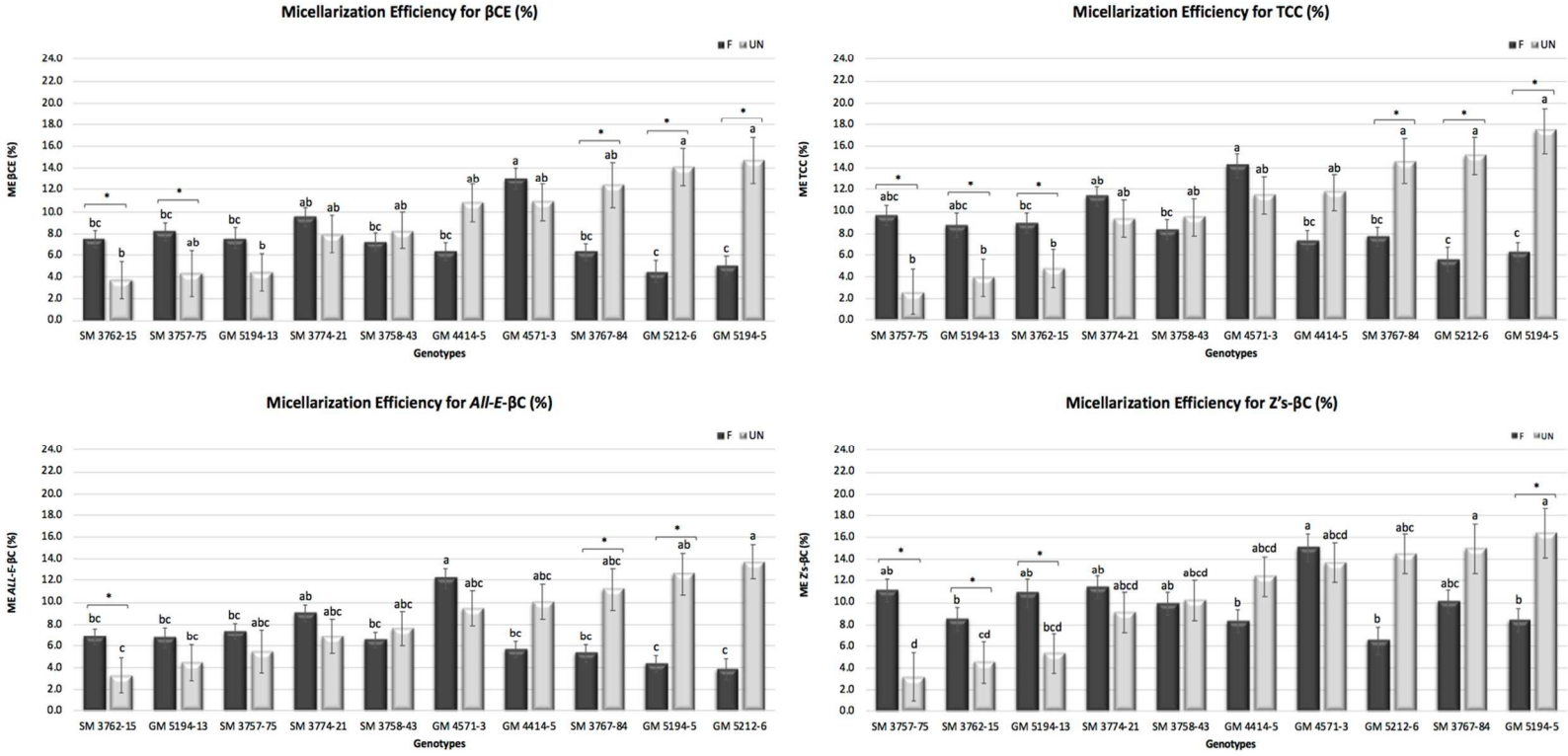


[1]Bar color: silver, R=raw cassava; dark blue, F=spontaneous-fermented cassava; green, OD-UF= unfermented oven-dried cassava; black, OD-F= fermented oven-dried cassava; light blue=toasted fermented cassava (gari).

[2]Minimal content of HCN considered safe for edible consumption of cassava flour is ≤ 10 mg/kg (The Codex Alimentarius Commission, 2016)22 as designated in the figure by the dotted red line.

[3]Presence of different letters indicates statistically significant differences of total cyanogenic content (T-HCN, μg/g DW) through processing within cassava genotypes.

Figure 3. Micellarization efficiency (ME%) of carotenoids during *in vitro* simulation of gastrointestinal human digestion of porridges made from unfermented (UF) and fermented (F) flour of biofortified cassava roots. ME%: A) β-carotene equivalent (β-CE) B) Total carotenoid content (TCC), C) *all*-E-β-carotene, D) isomers of Z-β-carotenes. [1]



[1]Data is represented as Mean ± standard error of the mean (SEM). Porridges with UF and F flours were prepared separately by triplicate (n=3) for each cassava genotype and subjected to in vitro digestion. Carotenoid analysis for DG and AQ phases for each digested porridge were repeated twice (n=2). Statistical analysis was performed under a fit model integrating cultivar and processing (UF vs F) and respective interaction. Significant differences along genotypes and processing are denoted by different letters ($p<0.05$).

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