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Retention during Processing and Bioaccessibility of β-Carotene in High β-Carotene Transgenic Cassava Root

Mark L. Failla,*[,†] Chureeporn Chitchumroonchokchai,[†] Dimuth Siritunga,[‡] Fabiana F. De Moura,[§] Martin Fregene,[⊥] Mark J. Manary,[¶] and Richard T. Sayre[⊥]

[†]Department of Human Nutrition, 350 Campbell Hall, The Ohio State University, 1787 Neil Ave, Columbus, Ohio 43210, United States

[‡]Department of Biology, University of Puerto Rico, Mayaguez, Puerto Rico

[§]HarvestPlus, c/o International Food Policy Research Institute, 2033 K Street N.W., Washington, D.C. 20006, United States

[¶]Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110, United States

[⊥]Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, Missouri 63132, United States

ABSTRACT: Cassava is a root crop that serves as a primary caloric source for many African communities despite its low content of β-carotene (βC). Carotenoid content of roots from wild type (WT) and three transgenic lines with high βC were compared after cooking and preparation of nonfermented and fermented flours according to traditional African methods. The various methods of processing all decreased βC content per gram dry weight regardless of genotype. The greatest loss of βC occurred during preparation of gari (dry fermentation followed by roasting) from WT and transgenic lines. The quantities of βC in cooked transgenic cassava root that partitioned into mixed micelles during in vitro digestion and transported into Caco-2 cells were significantly greater than those for identically processed WT root. These results suggest that transgenic high βC cassava will provide individuals with greater quantities of bioaccessible βC.

KEYWORDS: *β-carotene, cassava, transgenic cassava, biofortification, fufu, gari, carotenoid retention, in vitro digestion, bioacessibility, Caco-2 cells*

INTRODUCTION

Vitamin A deficiency remains a major public health problem in many countries and particularly for populations living in rural areas of central Africa, India, and Southeast Asia.[1] An inadequate supply of dietary vitamin A is a leading cause of preventable blindness, morbidity, and mortality due to infectious disease in children.[1,2] The majority of individuals in developing countries obtain vitamin A from cleavage of carotenoid precursors such as β-carotene (βC) in plant foods.[3] However, the low content of provitamin A in most varieties of high-yielding staple food crops such as maize, wheat, and cassava in developing countries generally fails to provide consumers with adequate quantities of the provitamin A. Dietary diversity, fortification, and supplementation have been successful strategies for the prevention of vitamin A deficiency for some populations, but are not feasible in remote regions lacking fiscal resources and infrastructure. Thus, low-cost, sustainable strategies are needed to increase the content and bioavailability of provitamin A carotenoids in staple food crops in those regions where vitamin A deficiency remains problematic. Biofortification has been proposed as one such strategy.[4−6] Conventional breeding and genetic engineering are approaches to generate high-yielding varieties containing increased amounts of provitamin A and other limiting nutrients in staple plant foods such as cassava.

In addition to the amount of provitamin A in the plant food, the style of processing may affect both the quantity and relative amounts of all *trans* and *cis* geometric isomers in the consumed food.[7] Retinol activity equivalence of *cis* isomers of βC is generally assumed to be half that of *all-trans*-βC and thus lowers the nutritional quality of the food product.[8] Also, the food matrix and other dietary components such as fat and fiber influence transfer of provitamin A carotenoids to mixed micelles during digestion for delivery to enterocytes and the subsequent absorption of the carotenoids and retinyl esters.[9,10]

We recently examined the relationship between βC content in conventionally bred cultivars with relatively high and low βC (ranging from <1 to 6.9 μg/g fresh weight) and the bioaccessibility of the provitamin A carotenoid as determined by the coupled in vitro digestion/Caco-2 human intestinal cell model.[11] The results showed that the quantity of βC transferred to micelles during digestion of boiled cassava and subsequently to Caco-2 cells was directly proportional to the amount of βC in the root. We also reported that the method of cooking cassava root affected retention and bioaccessibility of βC.[12] There was a marked loss of βC during roasting of fermented cassava (gari) compared to the limited loss during boiling or boiling after fermentation (fufu). The efficiency of micellarization of βC during simulated digestion of boiled cassava and gari was greater than that with fufu, suggesting that the method of cooking may also affect release of the carotenoid from the food matrix. Here, we present results from a parallel investigation of the impact of style of processing on the retention and

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bioaccessibility of βC from several lines of cassava containing transgenes that increase carotenoid biosynthesis.

MATERIALS AND METHODS

Supplies. Unless stated otherwise, all reagents and materials were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Fisher Scientific Co. (Fair Lawn, NJ, USA). Fetal bovine sera (FBS) and cell culture reagents were obtained from Invitrogen Corp. (Carlsbad, CA, USA).

Biofortification of βC in Cassava Root. βC in cassava root was enriched by expression of two transgenes, that is, *Erwinia crtB* gene, a phytoene synthase gene, and *Arabidopsis* 1-deoxy-xylulose-5-phosphate synthase (DXS) gene, under control of potato patatin root-specific promoter.[13] Tested transgenic lines and wild-type 6440 (WT) were harvested in confined fields in Puerto Rico in December 2009, washed, waxed, and forwarded to The Ohio State University.

Carotenoid and Moisture Contents of Cassava Root from Different Transgenic Plants. Upon arrival, waxed roots were stored at −80 °C. Roots were then thawed in a refrigerator (4 °C) overnight before analyses were begun. Two or three roots from each accession were peeled and quartered, and flesh from all four quarters was combined and homogenized in a food blender to minimize possible heterogeneity in the roots from a single plant.[14] Samples (5−10 g) of homogenized root also were placed in crucibles and dried at 65 °C to constant weight to determine moisture content. Five replicate samples (5−12 g) for each cassava root homogenate were transferred to 50 mL polypropylene tubes to immediately extract and analyze carotenoids as described below. Standard precautions were taken to minimize photodegradation during all procedures.

Processing Cassava Root. Upon arrival, approximately 2.5 cm sections were removed from both ends of three roots for each genotype. Each root was peeled and cut along the center of the long axis. Each of the two halves of root were then cut vertically into three sections of approximately similar size. One of the central sections from each root was stored under nitrogen at −80 °C for carotenoid analysis. Each of the other five sections from the root was pooled with the equivalent section of the other two roots from the same genotype to prepare either boiled root, fufu, gari, nonfermented flour (NFF), or fermented flour (FF) as described below. Retention of βC during processing was calculated by dividing βC content per gram dry weight (DW) of processed cassava root by βC content per gram DW of raw cassava and multiplying by 100%. This assumes that loss of βC during processing is proportional to loss of solids.

Boiling. Raw cassava root was cut into cubes (3−5 cm^3) and submerged in deionized (DI) water at room temperature in a covered bowl to remove cyanide glycosides. Water was changed every 2 h for the initial 10 h and after 24 h. Cubes were then boiled in 10 volumes of fresh DI water at 95 °C for 30 min. Boiled cubes were mashed to homogeneity.

Fufu. Raw cassava was cut to ∼15 cm lengths and transferred to a ceramic bowl. DI water (10 volumes) was added, and the bowl was covered with aluminum foil and allowed to stand at room temperature for 5 days. The softened material was manually disrupted and mashed before passing through muslin mesh cloth into a clean bowl to separate large fibers from sieved particles and water. The bowl was covered with aluminum foil and maintained at room temperature for 24 h. Water was decanted to collect the sediment. The wet fermented paste was mixed with 1.2 volumes of DI water and cooked in a stainless steel pot at 100 °C for 10 min to produce fufu.

Gari. Raw cassava was grated (stainless steel) into a ceramic bowl. The bowl containing sample was covered with black nylon cloth and allowed to ferment at room temperature for 72 h. Fermented sample was passed through cotton cloth to remove water, and the paste was sieved (pore size = 2 mm) to obtain pressed cassava cake. Pressed cake was roasted at 185 °C until granules reached 80 °C and continued at 120 °C for 20 min. After cooling, granules were pulverized and sieved (no. 14) to prepare granules <1.4 mm and >1.18 mm.

NFF. Raw cassava was cut to lengths of 5 cm. Slices were spread on a tray and dried at 37 °C for 24 h. Dried material was ground (coffee grinder) and sieved (no. 10, <2 mm) to obtain flour.

FF. Raw cassava was cut to ∼15 cm lengths and transferred to a ceramic bowl. DI water (10 volumes) was added, and bowls were covered with aluminum foil and held at room temperature for 3 days. Water was drained, and fermented cassava was cut to 5 cm lengths, spread on a tray, and dried in the oven at 37 °C for 24 h. Dried material was ground and sieved (no. 10, <2 mm) to prepare flour.

Simulated Digestion. Three-phase simulated digestion (oral, gastric, and small intestinal) was performed as described by Thakkar et al.[11] with slight modifications. A pilot experiment was performed to determine the amount of processed root to be digested to ensure adequate βC content in the micelle fraction of chyme to quantify uptake by Caco-2 cells. Wet weights of boiled cassava, fufu, and gari prepared from the transgenic roots used for digestion were 2−3, 4−5, and 1−2 g, respectively, to provide ∼5 μg of *all-trans*-βC per reaction. As the βC content of WT root was very low, 20 g of boiled cassava, fufu, and gari was digested to provide 0.4−2 μg of *trans*-βC per reaction.

Gari prepared from WT and transgenic roots was rehydrated in 20 and 10 mL, respectively, salts solution (120 mM NaCl, 5 mM KCl, 6 mM $CaCl_2$) for 30 min at room temperature. Synthetic saliva (10 mL) containing 74.6 units/mL α-amylase was added to samples of boiled cassava, fufu, and gari to initiate oral digestion in a shaking water bath at 37 °C (85 rpm, 10 min). Samples were subsequently subjected to simulated gastric and small intestinal digestion. The quantities of digestive enzymes (final concentrations = 4.8 mg/mL porcine pepsin, 1.2 mg/mL pancreatin, 0.6 mg/mL lipase) and bile extract (7.2 mg/mL) were 3 times greater than that routinely used in our laboratory[15] to ensure complete digestion of the increased mass of samples. At the conclusion of the small intestinal phase of digestion, chyme was centrifuged at 5000*g* for 35 min at 4 °C to separate the aqueous fraction from undigested material. A portion of the aqueous fraction was collected and filtered (0.22 μm pores) to prepare the micelle fraction. Aliquots of chyme and micelle fraction were stored under nitrogen gas at −20 °C until extraction and analysis of carotenoids within 2weeks. The quantity of carotenoid in chyme was divided by that in the sample of cooked root digested and normalized to 1.0 g DW of cooked cassava root. The amount of carotenoids in the micelle fraction was divided by that in the cooked root to quantify the efficiency of transfer of the carotenoids from the food matrix to mixed micelles during digestion. Aliquots of the micelle fraction were used to examine the uptake of micellar βC by Caco-2 cells.

Cell Uptake. Caco-2 human intestinal cells (0.4×10^6 cells; passages 26−29) were seeded in T75 flasks in Dulbecco's Modified Eagle Medium (DMEM) plus 15% FBS, 2 mM L-glutamine, 1% nonessential amino acids, and antibiotics (complete DMEM) until confluency. Upon achievement of confluency, the FBS content of the medium was decreased to 7.5% and fresh medium was added every second day. Cultures were used 11−14 days postconfluency.

Aliquots of the micelle fraction were diluted 1:4 with DMEM and incubated at either 37 or 0 °C for 15 min before the addition of 12 mL to flasks containing washed monolayers of Caco-2 cells. The replicate set of flasks incubated at 0 °C was included to determine nonspecific association of the carotenoids with the monolayer surface. All monolayers were washed with ice-cold phosphate buffer saline (PBS) containing 0.2% bovine albumin followed by cold PBS only. Cells were collected by scraping the monolayer into 6 mL of cold PBS followed by centrifugation (400*g*, 5 min, 4 °C). Supernatant was discarded, and the cell pellet was stored under nitrogen gas at −20 °C. Cellular uptake was calculated by subtracting carotenoid content in monolayers incubated at 0 °C from that in cells incubated at 37 °C. Results are expressed as nanograms of total βC per milligram of cell protein. These results have been normalized per gram DW of cooked root digested. Bicinchoninic acid assay (BCA; Pierce, Rockford, IL, USA) was used with bovine serum albumin as standard to determine the protein content of cell pellets (12.5 ± 0.2 mg/cell pellet).

Extraction and Analysis of Carotenoids. Carotenoids were extracted from raw and processed cassava as described by Kimura et

Table 1. Moisture and Carotenoid Contents of Roots from WT and Transgenic Cassava[a]

		total carotenoids		*all-trans*-βC		total *cis*-βC	
line	moisture (%)	μg/g FW	μg/g DW	μg/g FW	μg/g DW	μg/g FW	μg/g DW
WT	59.3 ± 0.3[b]	0.38 ± 0.01	0.93 ± 0.02	0.12 ± 0.01	0.30 ± 0.03	0.25 ± 0.02	0.64 ± 0.05
2DXS	67.4 ± 0.2	3.39 ± 0.05	10.40 ± 0.15	2.35 ± 0.18	7.21 ± 0.55	0.81 ± 0.02	2.49 ± 0.06
3DXS	76.2 ± 0.4	5.73 ± 0.07	24.08 ± 0.29	4.67 ± 0.16	19.58 ± 0.67	0.64 ± 0.09	2.69 ± 0.38
17DXS	84.2 ± 0.4	4.96 ± 0.03	31.39 ± 0.19	3.97 ± 0.09	25.13 ± 0.57	0.71 ± 0.07	4.49 ± 0.44
20DXS	83.2 ± 0.3	5.08 ± 0.02	30.24 ± 0.12	4.37 ± 0.05	26.01 ± 0.30	0.55 ± 0.03	3.27 ± 0.18
37DXS	83.3 ± 0.3	5.36 ± 0.04	32.10 ± 0.24	4.45 ± 0.15	26.65 ± 0.90	0.66 ± 0.05	3.95 ± 0.30
53DXS	81.2 ± 0.4	4.87 ± 0.04	25.90 ± 0.21	4.18 ± 0.15	22.23 ± 0.80	0.54 ± 0.04	2.87 ± 0.21
73DXS	83.2 ± 0.2	4.06 ± 0.03	24.17 ± 0.12	3.35 ± 0.09	19.94 ± 0.54	0.59 ± 0.03	3.51 ± 0.18

[a]Data are the mean ± SD for five independent extractions and analyses. [b]Low moisture content in root from WT was correlated with need for greater force to cut sections.

al.[16] Carotenoids were extracted from chyme, micelle fraction, and cell pellets according to the method of Chitchumroonchokchai et al.[15] β-Apo-8′-carotenal was used as the internal standard (IS) for both procedures. Recoveries for the two methods were 96.2 ± 2.5 and 94.5 ± 1.5, respectively. Separation and quantification of carotenoids have been described elsewhere.[15] Briefly, solvent was removed from extracts under nitrogen gas and residue was solubilized in methanol/methyl *tert*-butyl ether (1:1, v/v), filtered, and injected immediately into the HPLC system. Separation of carotenoids was achieved using a Waters YMC carotenoid C_{30} column and a Waters model 2695 HPLC with a model 2996 photodiode array detector. Carotenoids were identified by comparison of retention time and spectrum with pure (>98%) *all-trans*-βC and lutein. Six-point standard curves of the carotenoids were prepared to quantify amounts in test samples. Peaks of *cis* isomers of βC were identified by comparison of retention times and spectra with the literature, and concentrations were estimated using the extinction coefficient for *all-trans*-βC.[17]

Statistical Analysis of Data. All data were analyzed using Stata 8.0 (StataCorp., College Station, TX, USA). Descriptive statistics including mean and standard deviation (SD) were calculated for the recovery and efficiency of micellarization of carotenoids from digested foods. Means were compared using one-way ANOVA followed by Tukey's HSD posthoc test. Differences were considered to be significant at $p < 0.05$.

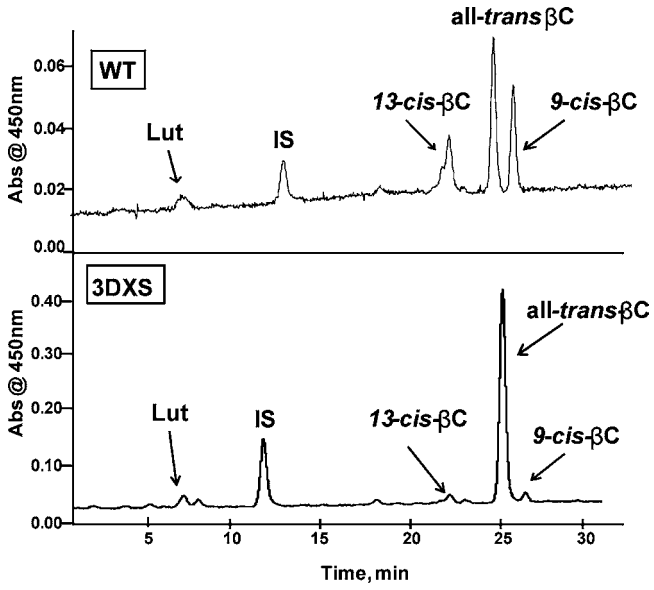

Figure 1. Representative chromatograms of carotenoid profiles in raw WT and 3DXS transgenic cassava roots. Note that the scales of the *y*-axes differ in the two panels.

RESULTS AND DISCUSSION

Profile of Carotenoids in Transgenic and WT Cassava Root. Carotenoid content of transgenic roots per gram fresh weight ranged from 3.4 to 5.7 μg, which was 9−15 times greater than that in WT (Table 1). As the moisture content of transgenic roots was significantly greater than that of WT root (Table 1), carotenoid content is also presented per gram DW. Carotenoid content per gram DW was 10−34-fold greater in transgenic roots than in WT root. βC was the most abundant carotenoid in all roots, accounting for ≥92% of total carotenoids. Thus, *all-trans*-βC content in the transgenic lines of cassava grown in Puerto Rico was similar to the high βC cultivars generated by conventional breeding and grown in Nigeria that were previously used to assess the relationship between carotenoid content in cooked cassava and βC bioaccessibility[11,12] and twice those generated in Colombia.[18] *All-trans*-βC was the predominant isomer in both WT and transgenic roots, whereas the amount of *cis* isomers of βC exceeded *all-trans*-βC in WT root (Figure 1; Table 1). 9- and 13-*cis*-βC represented 10−15% of total βC in the transgenic roots with the exception of 2DXS, which contained 26% *cis* isomers. This relative quantity is much lower than previously reported in conventionally bred high βC cultivars containing 30−45% *cis* isomers of βC.[11,12] The possibility that waxing transgenic roots before shipping attenuated postharvest isomerization of *all-trans*-βC found in the conventionally bred roots that were shipped openly from Nigeria to the United States merits consideration.

Roots from three of the seven transgenic lines (3DXS, 20DXS, and 37DXS) and WT were selected as representative transgenic lines to investigate the effects of genotype and method of processing on the retention and bioaccessibility of βC.

Effect of Method of Processing on Retention of βC. The moisture contents of boiled WT and 3DXS lines and of fufu prepared from all genotypes were greater than that of their respective raw roots, whereas the moisture content of gari was significantly less than that of raw cassava root from WT and the three transgenic lines (Table 2). βC content of cassava root per gram DW was decreased by all methods of processing. Mean loss of total βC was 24−35% per gram DW in boiled root and fufu prepared from transgenic lines (Figure 2). Dry fermentation followed by roasting (gari) of transgenic roots resulted in a loss of >60% of βC per gram DW. The ratio of *all-trans* to *cis* isomers of βC was increased in boiled cassava, fufu, and gari compared to that in raw cassava (Tables 1 and 3). 13-*cis*-βC was the most abundant isomer in cooked transgenic cassava root (Table 3). *Cis* isomers of βC accounted for 39, 41, and 30% of total βC in WT boiled root, fufu, and gari,

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Table 2. Moisture Content (Percentage of Wet Weight) of Raw and Processed Root from WT and Transgenic Cassava[a]

cooking method	WT	3DXS	20DXS	37DXS
raw	59.3 ± 0.3c	76.2 ± 0.4c	83.2 ± 0.3b	83.3 ± 0.3b
boiled	67.2 ± 0.4b	82.5 ± 0.3b	87.8 ± 0.5ab	87.9 ± 0.3ab
fufu	82.9 ± 0.5a	91.2 ± 0.2a	93.4 ± 0.2a	93.4 ± 0.4a
gari	28.4 ± 0.5d	27.6 ± 0.3d	22.9 ± 0.4c	33.6 ± 0.5c
NFF[b]	9.2 ± 0.2f	7.7 ± 0.4e	7.7 ± 0.5d	8.5 ± 0.2d
FF[b]	13.4 ± 0.4e	6.9 ± 0.4e	7.7 ± 0.2d	7.1 ± 0.3d

[a]Data are the mean ± SD for five independent extractions and analyses of raw cassava root and three extractions and analyses of indicated processed cassava root for each genotype. Different letters within a column indicate means differ significantly ($p < 0.05$). [b]NFF, nonfermented flour; FF, fermented flour.

respectively. As the retinol activity equivalence of *cis* isomers of βC is only half that of *all-trans*-βC,[12] the relative increase in *cis* isomers of βC during processing represents a decline in the nutritional quality of the cooked product. Collectively, the impact of the different methods of cooking on βC content and its isomeric profile is similar to that observed with processed cassava roots from conventionally bred high βC plants.[11,12,18]

Preparation of nonfermented flour by drying sections of transgenic roots at low temperature (37 °C) reduced βC content per gram DW by 20% (Figure 2) and generated a product with 17% *cis*-βC (Table 3). Fermentation prior to drying to produce flour yielded a product with 30% less βC per gram DW than raw transgenic cassava (Figure 2) and a mean of 26% *cis*-βC isomers (Table 3). It is expected that the amount of βC will decline further when flour is stored and used for the preparation of porridges and other cooked products.[18,19]

Bioaccessibility of βC in Processed Cassava Root. Recoveries of βC in digested samples of processed cassava root from WT and transgenic lines were 78.5 ± 2.5 and 88.6 ± 5.5%, respectively, and independent of style of processing. The quantity of βC in the micelle fractions of digested transgenic roots was much greater than that in micelle fractions of digested WT root (Figure 3). Also, the amounts of βC in micelles generated during digestion of boiled roots and fufu greatly exceeded that in gari for WT and transgenic lines. The efficiency of micellarization of βC during digestion of the various types of processed WT cassava root ranged from 27 to 31%, which was significantly ($p < 0.05$) less than that for digested processed roots (30−45%) from transgenic lines. It is possible that this apparent difference in transfer of the carotenoid to the aqueous fraction simply reflects the marked differences in the amount of βC in WT and transgenic root and the need to digest a greater mass of WT type material to provide adequate carotenoid to measure carotenoid uptake by the intestinal cells (see below). The extent of micellarization of βC during digestion of fufu slightly, but significantly, exceeded that for digested boiled cassava and gari for all genotypes. For example, 40.2% of βC in chyme generated during digestion of fufu prepared from 37DXS was present in the micelle fraction, whereas 35.5 and 36.7% of βC in digested boiled cassava and gari, respectively, prepared from this line partitioned in the micelle fraction. The enhanced micellarization of βC during digestion of fufu contrasts markedly with our previous study with conventionally generated high βC fufu in which the extent of micellarization was about half that of digested boiled cassava and gari.[12] The basis for this discrepancy is unknown, but differences in food matrices are known to affect micellarization of carotenoids.[20,21] The efficiencies of micellarization of *all-trans* and *cis* isomers of βC were similar within genotype and style of cooking (data not shown) as previously reported.[11,12]

Differentiated cultures of Caco-2 human intestinal cells were used to confirm the accessibility of βC from mixed micelles in the aqueous fraction generated during simulated digestion of

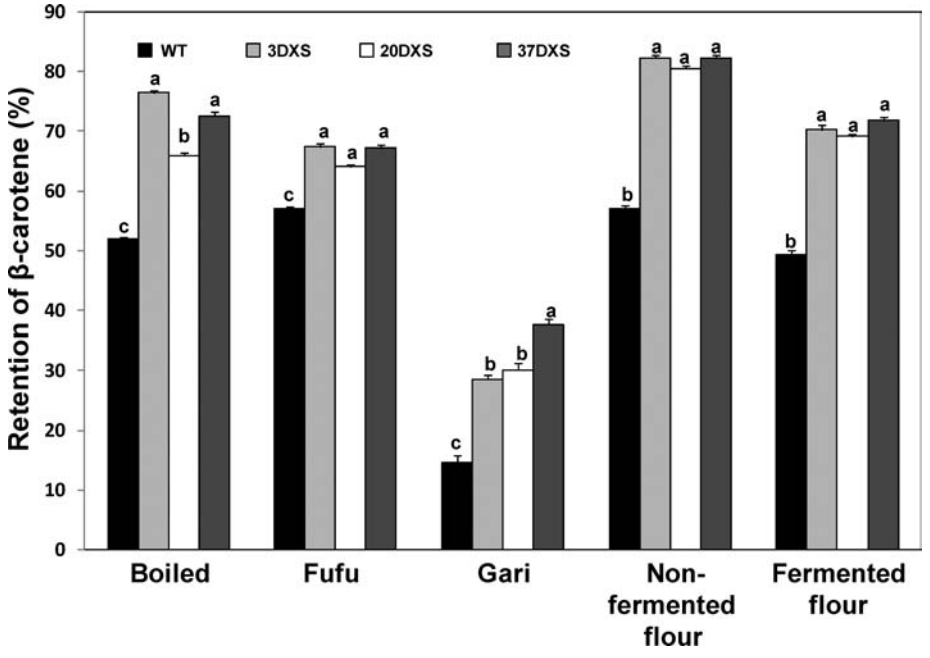

Figure 2. Processing of cassava root from WT and transgenic lines decreases βC content. Retention of βC during processing was calculated by dividing βC content per gram dry weight of processed cassava root by βC content per gram dry weight of raw cassava and multiplying by 100%. Data are the mean ± SD for three independent experiments. Different letters above error bars within columns for each method of processing indicate that means differ significantly ($p < 0.05$).

Table 3. Profile of βC Isomers in Cooked Cassava Root[a]

		μg/g DW			
genotype	cooking method	*all-trans*-βC	13-*cis*-βC	9-*cis*-βC	other *cis*-βC isomers
WT	boiled	0.22 ± 0.03	0.06 ± 0.01	0.08 ± 0.02	nd[b]
	fufu	0.23 ± 0.02	0.06 ± 0.01	0.10 ± 0.02	nd
	gari	0.07 ± 0.00	nd	0.03 ± 0.00	nd
	NFF[c]	0.21 ± 0.01	0.04 ± 0.01	0.09 ± 0.00	nd
	FF[c]	0.24 ± 0.01	0.04 ± 0.00	0.11 ± 0.00	nd
3DXS	boiled	7.48 ± 0.25	2.46 ± 0.05	1.22 ± 0.06	0.23 ± 0.05
	fufu	6.65 ± 0.42	1.73 ± 0.16	1.39 ± 0.20	0.28 ± 0.09
	gari	3.14 ± 0.08	0.54 ± 0.01	0.50 ± 0.05	0.09 ± 0.02
	NFF	6.79 ± 0.16	1.00 ± 0.03	1.27 ± 0.01	0.22 ± 0.01
	FF	7.55 ± 0.18	1.20 ± 0.04	1.45 ± 0.07	0.28 ± 0.01
20DXS	boiled	11.16 ± 0.27	3.73 ± 0.06	0.91 ± 0.06	0.20 ± 0.03
	fufu	11.27 ± 0.14	2.42 ± 0.06	1.56 ± 0.06	0.30 ± 0.06
	gari	5.73 ± 0.10	0.80 ± 0.07	0.39 ± 0.03	0.39 ± 0.00
	NFF	17.73 ± 0.22	1.50 ± 0.04	1.26 ± 0.04	0.23 ± 0.02
	FF	12.62 ± 0.28	2.51 ± 0.20	1.36 ± 0.04	0.26 ± 0.02
37DXS	boiled	13.86 ± 0.11	4.74 ± 0.59	1.03 ± 0.05	0.27 ± 0.10
	fufu	14.49 ± 0.16	2.52 ± 0.12	1.09 ± 0.08	0.34 ± 0.06
	gari	7.93 ± 0.04	1.50 ± 0.04	0.76 ± 0.02	0.15 ± 0.01
	NFF	20.78 ± 0.41	1.96 ± 0.02	1.44 ± 0.07	0.23 ± 0.04
	FF	14.91 ± 0.41	2.95 ± 0.25	1.51 ± 0.16	0.32 ± 0.07

[a]Data are the mean ± SD for three independent experiments. [b]nd, not detected (less than detection limit, <5 ng/mL). [c]NFF, nonfermented flour; FF, fermented flour.

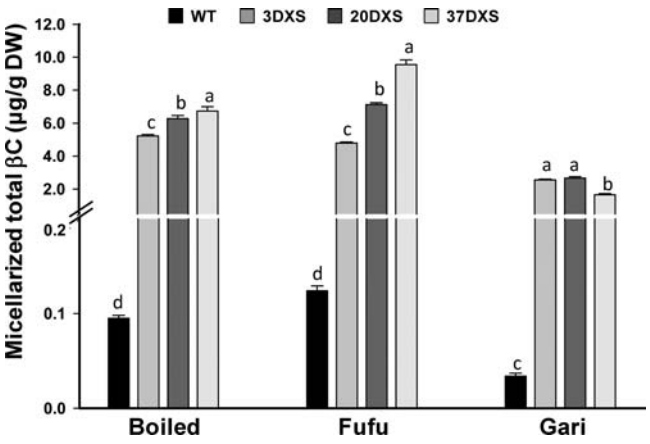

Figure 3. Quantity of βC transferred from processed cassava root to mixed micelles during simulated digestion was significantly greater for transgenic lines than for WT. Data (mean ± SD, $n = 6$ independently digested samples) have been normalized to 1.0 g DW of digested cooked root. Different letters above error bars within a specific method of processing roots indicate means for the genotypes differ significantly.

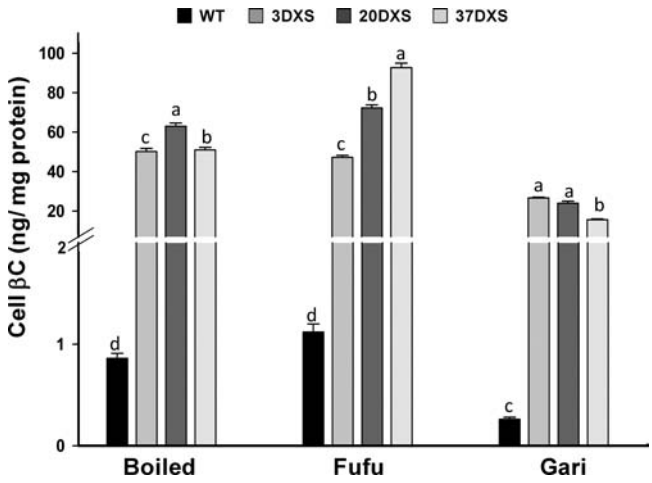

Figure 4. Cellular accumulation of βC from micelles generated during digestion of processed cassava root from transgenic lines is significantly greater than that in cells exposed to micelles from processed and digested WT root. Data are the mean ± SD for five independent cultures and have been normalized per gram DW of cooked cassava root digested. Different letters above error bars within a specific method of processing roots indicate that the means for the genotypes differ significantly ($p < 0.05$).

boiled root, fufu, and gari prepared from WT and transgenic cassava. Uptake of the carotenoid by the monolayer during the 4 h of incubation ranged from 8.3 to 12.6% of the amount present in the apical compartment. Consequently, βC concentrations in Caco-2 cells (ng/mg protein) were 57−73, 42−83, and 49−83 times greater after exposure to micelles generated during digestion of boiled cassava, fufu, and gari, respectively, that were prepared from the transgenic cassava roots as compared to identically processed WT root (Figure 4).

The above results suggest that transgenic lines of cassava with high βC content in roots, like conventionally bred[11,12] lines of cassava rich in βC, will provide consumers with proportionally greater amounts of bioaccessible provitamin A than similarly processed WT cassava. As decreases in βC content and increases in the ratio of *cis* to *all-trans* isomers of βC occur during the processing of cassava root, processing

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methods that minimize these changes should be encouraged to maximize provitamin A intake.

AUTHOR INFORMATION

Corresponding Author

*Phone: (614) 247-2412. Fax: (614) 292-1330. E-mail: failla.3@osu.edu.

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Notes

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