Impact of Style of Processing on Retention and Bioaccessibility of β-Carotene in Cassava (*Manihot esculanta*, Crantz)

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We previously demonstrated that the quantity of β-carotene (BC) partitioning in mixed micelles during simulated small intestinal digestion, i.e., the bioaccessibility, of boiled cassava is highly correlated with the BC content of different cultivars. However, cassava is also traditionally prepared by fermentation and roasting. These different methods of preparation have the potential to affect both the retention and bioaccessibility of BC. Here, we first compared retention of BC in boiled cassava, gari (fermentation followed by roasting), and fufu (fermentation followed by sieving and cooking into a paste) prepared from roots of three cultivars. BC content in unprocessed cultivars ranged from 6−8 μg/g wet weight, with cis isomers accounting for approximately one-third of total BC. Apparent retention of BC was approximately 90% for boiled cassava and fufu. In contrast, roasting fermented cassava at 195 °C for 20 min to prepare gari decreased BC content by 90%. Retention was increased to 63% when temperature was decreased to 165 °C and roasting was limited to 10 min. Processing was also associated with a decline in *all-trans*-BC and concomitant increase in 13-*cis*-BC. The efficiency of micellarization of all-trans and cis isomers of BC during simulated digestion was 25−30% for boiled cassava and gari and independent of cultivar. However, micellarization of BC isomers during digestion of fufu was only 12−15% ($P < 0.05$). These differences in retention and bioaccessibility of BC from cassava products prepared according to traditional processing methods suggest that gari and fufu may provide less retinol activity equivalents than isocaloric intake of boiled cassava.

KEYWORDS: Cassava; gari; fufu; processing; retention; bioaccessibility; provitamin A carotenoids; in vitro digestion; biofortification

INTRODUCTION

Eradication of vitamin A (VA) deficiency remains a global public health challenge. VA deficiency is more common in developing countries where impoverished populations lack adequate resources to diversify diets and purchase fortified foodstuffs or supplements. Such populations must rely on pro-VA carotenoids from plant foods to meet VA requirements (*1*).

Although required in small quantities, VA is essential for vision and immunocompetence as well as cellular differentiation, growth, and reproduction. Biofortification has been proposed as a sustainable strategy to combat VA and other nutritional deficiencies (*2, 3*). Biofortification of the pro-VA content of staple crops involves either the introduction of one or more limiting genes in the carotenoid biosynthetic pathway into high yielding cultivars or crossing selectively bred germplasm containing relatively high amounts of pro-VA carotenoids with agronomically important varieties.

Cassava (*Manihot esculanta*, Crantz) is one of the crops targeted for biofortification as it is consumed daily by populations in Sub-Saharan Africa. It has been estimated that 70 million people in Africa consume more than 500 kcal/day from cassava (*4*). However, currently used varieties of cassava have poor nutritional quality as the concentrations of pro-VA carotenoids, iron, zinc, and protein are very low and the roots contain toxic cyanogens (*5*).

Using an in vitro digestion model, we recently demonstrated that efficiency of incorporation of BC in cassava root into micelles during simulated small intestinal digestion, i.e., bioaccessibility, was directly proportional to the pro-VA content of different cultivars (*6*). However, cassava is also consumed

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after traditional preparation of dishes that require peeling, chopping, grating, fermentation, boiling, and roasting. For example, the preparation of gari requires fermentation of grated cassava for several days, dehydration, and roasting to produce granules that are consumed. Fufu is prepared by extended fermentation of portions of cassava roots in water followed by sieving, removing the water, and boiling the fermented paste. Such processing has the potential to decrease pro-VA content and alter its bioavailability (*7*). Our objective was to compare the retention and in vitro bioaccessibility of pro-VA carotenoids in gari roasted cassava granules), fufu (fermented, cooked cassava paste), and boiled cassava for three cultivars selected for their relatively high content of β-carotene (BC).

MATERIALS AND METHODS

Chemicals and Supplies. Unless otherwise stated, all chemicals and supplies were purchased from Sigma-Aldrich and Fisher Scientific.

Cassava Varieties. Varieties of cassava were planted at the beginning of the rainy season in 2006 and grown under rain-fed conditions in a randomized complete block design with two replications at the research farm of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Fertilizer and herbicides were not applied during growth, and hand weeding was done as needed. Roots used for this study were from three cultivars previously determined to contain relatively high amounts of total BC (*6*). Roots were harvested in summer 2007, washed, peeled, dipped in liquid nitrogen, and wrapped in aluminum foil before shipping on dry ice to The Ohio State University, Columbus, OH.

Processing of Cassava. *Boiling.* Three peeled roots of various size (small, medium, and large) were removed from the freezer and thawed overnight at 4 °C before washing with deionized (DI) water. Tips from the distal and proximal ends (1−2 cm) of the roots were removed. Cubes (3−5 cm²) were cut and submerged in 3 volumes of DI water at room temperature to remove cyanide glycosides. The water was changed every 2 h for 10 h, and then the cubes were submerged overnight in DI water. The cubes were then boiled in 10 volumes of fresh DI water for 30 min at 95 °C. After cooling to room temperature, the boiled cassava was mashed to homogeneity, and aliquots were stored in 50 mL polypropylene screw-cap tubes under nitrogen gas at −80 °C. Exposure to light was minimized throughout processing to minimize photo-oxidation.

Gari (Roasted Cassava Granules). Three roots of varying size (small, medium, and large) were selected for each of the three cultivars. After thawing, cassava roots were grated using a stainless steel grater. The mash was placed in ceramic bowls and covered with black nylon cloth to prevent exposure to light during fermentation at room temperature. After three days, the fermented mash was transferred to cotton cloth and pressed manually to remove residual water. The pressed cake was then pulverized and passed through a stainless steel mesh (U.S. Standard Sieve no. 10, pore size 2 mm in diameter) to remove fibrous materials. Next, the pulverized cake was roasted in a large, shallow stainless steel pan at either 195 °C for 20 min or at 165 °C for 5, 10, 15, or 20 min with constant stirring with a spatula to examine effect of time and temperature on retention and isomeric profile of BC (see Results). Roasted cassava granules (gari) were spread on a stainless steel tray to cool before pulverizing and sieving as described above) to obtain small sized granules for storage in 50 mL polypropylene screw-cap tubes under nitrogen gas at −80 °C.

Fufu (Fermented and Cooked Cassava Paste). One small, medium, and large root was selected for each of the three cultivars. After thawing, the peeled and washed roots were longitudinally cut to lengths of approximately 15 cm with a stainless steel knife and immersed in DI water (5 L) in a ceramic bowl for five days at room temperature. The bowls were covered with black cloth to minimize exposure to light. Cuttings were disrupted manually, and the mixture was passed through a muslin mesh cloth into a ceramic bowl to remove fiber from sieved particles and water. After 24 h, water was decanted and fermented sediment was washed once with 500 mL of DI water. Sediment was next transferred to cotton cloth and residual water removed manually.

The wet fermented paste was mixed with 1.2 volumes of DI water and cooked in a stainless steel pot with continuous stirring for 10 min at 100 °C to produce fufu. Fufu was cooled for 10 min at room temperature before storage in 50 mL polypropylene screw-cap tubes under nitrogen gas at −80 °C.

Caloric Content of Cooked Cassava. Cooked varieties of cassava (cultivar TMS 01/1371) were dried to constant mass at 105 °C. Pellets of 0.5 g dried mass were prepared for combustion in an oxygen bomb calorimeter (model 1281, Parr Instrument Company, Moline, IL). Energy content was 3.8 ± 0.1, 3.8 ± 0.1, and 4.0 ± 0.2 kcal/g dry weight for boiled cassava, gari, and fufu, respectively ($n = 3$).

In Vitro Digestion. Cassava processed according to the above methods were subjected sequentially to simulated oral, gastric, and small intestinal phases of digestion as described by Thakkar et al. (*6*). As the moisture content of cassava for each methods of preparation differed (see Results), the quantity digested was based on dry matter content. Appropriate quantities of boiled cassava, fufu, and gari were weighed (300 mg dry weight material) to initiate digestions. Gari granules were mixed with 10 parts of 95 °C DI water prior to beginning simulated digestion.

Extraction and HPLC Analysis of Carotenoids. Carotenoids were extracted from raw and processed cassava and from aliquots of digesta and micelle fraction as previously described (*6*). Extracted material was analyzed by HPLC using a Waters 2695 separation module and a Waters 2996 photodiode array detector (PDA). Separation of carotenoids was achieved using a Waters YMC Carotenoid S-5 C30 reversed-phase column (250 mm × 4.6 mm; internal diameter: 5 μm) with a Waters Nova-Pak C30 guard column. Compounds in eluate were monitored at 450 nm. Peaks were identified by comparing retention time and spectral characteristics against pure standard and available literature. Quantity was determined by comparison of peak area against a standard curve prepared with known concentrations of *all-trans*-BC. The mobile phase consisted of methanol and ammonium acetate 1 M (98:2) [solvent A] and methyl-*tert*-butyl ether (MTBE) [solvent B] with a gradient flow rate of 0.8 mL/min to 1.0 mL/min. The injection volume was 20 μL, and carotenoids were eluted using the following solvent gradient: 0−10 min, 80% A; 11−20 min, 60% A; 21−25 min, 40% A; 26−30 min, 80% A.

Statistical Analysis of Data. All statistical analyses were performed using SPSS (version 14.0, SPSS Inc., Chicago, IL). All data are expressed as means ± SEM and statistical significance was set at level α of $P < 0.05$. Means for all experiments were compared using one way analysis of variance (ANOVA) with Fisher's Protected LSD as a post hoc test.

RESULTS

Carotenoid Composition of Cultivars. Total BC content of the three unprocessed cultivars was 6.2−7.8 μg/g wet weight (25.9 ± 0.55−40.9 ± 1.10 μg/g dry weight). *All-trans*-BC was the predominant carotenoid isomer in the three cultivars. 9- and 13-*cis*-BC were present in all three cultivars and collectively accounted for 32, 45, and 30% of total BC in cultivars TMS 01/1371, TMS 01/1412, and TMS 01/1663, respectively.

Retention and Isomeric Profile of BC in Boiled Cassava, Fufu, and Gari. Dry matter represented 18−25% of the weight of peeled cassava before processing, and 19.1 ± 0.52, 45.3 ± 0.73, and 10.1 ± 0.49 of the mass of boiled cassava, gari, and fufu, respectively (**Table 1**). Thus, results are expressed per unit dry weight to compare impact of method of preparation on apparent retention of BC and the isomeric profile. Fermentation of grated cassava for three days had minimal effect on BC content with 92% retained. However, roasting the fermented paste prepared for cultivar TMS 01/1371 for 20 min at 195 °C decreased BC content by 90%. We next examined the effect of roasting the fermented cassava at reduced temperature (165 °C) for 5−20 min on BC retention. Total BC decreased 37% after roasting at the lower temperature for 5 min (**Figure 1**). Continued roasting for an additional 15 min further decreased

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Table 1. Moisture Content and Profile of BC in Uncooked Cassava[a]

cultivar	moisture (%)	all-trans-BC	13-cis-BC	9-cis-BC
			(μg/g dry weight)	
01/1371	82.0 ± 0.5 a	27.9 ± 0.87 a	5.0 ± 0.18 a	8.0 ± 0.26 a
01/1412	75.1 ± 0.3 b	17.2 ± 0.31 b	5.8 ± 0.11 a	8.5 ± 0.23 a
01/1663	78.4 ± 0.3 c	19.7 ± 0.23 b	3.5 ± 0.03 b	5.2 ± 0.07 b

[a] Significant ($P < 0.05$) differences in moisture content and concentrations of all-trans-BC and cis isomers of BC among cultivars of raw cassava are indicated by the presence of different letters within a column. Data represent mean ± SEM for three independent replicates.
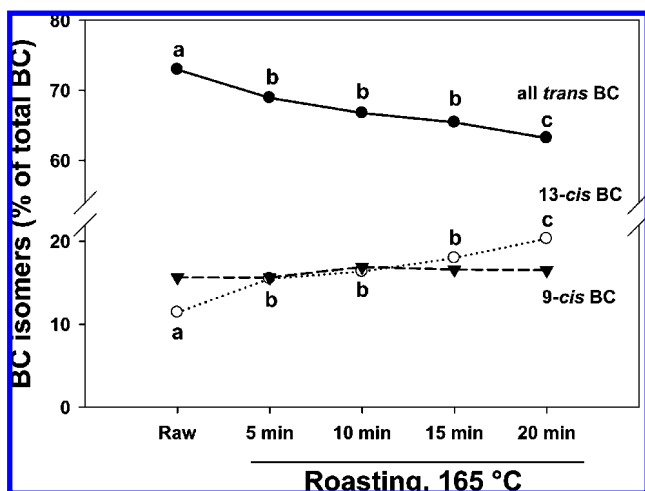

Figure 1. Relative percentage of isomers of BC in raw cassava vs fermented cassava that was roasted for indicated times to prepare gari. Data are means for three independent samples. The presence of different letters above or below points indicates significant differences ($P < 0.05$) in means for relative amount of the indicated isomer.

Figure 2. Quantity of BC isomers in raw and processed cassava (cultivar 01/1371). Data are means ± SEM for three independent preparations. Significant differences ($P < 0.05$) in the amount of all-trans and each cis isomer of BC in uncooked and differentially processed preparations are indicated by the presence of different letters above error bars.

Figure 3. Mean efficiency of micellarization of BC during simulated digestion of cassava processed in different traditional styles. Data are means ± SEM for $n = 6$. Significant ($P < 0.05$) differences in micellarization of all-trans and cis isomers of BC for the three different styles of processing are indicated by the presence of different letters above error bars. Efficiency of micellarization of all three isomers were not significantly different ($P > 0.05$) within each style of prepared cassava.

total BC content to 58% of that in the starting material. Both the total and relative amounts of *all-trans*-BC in the product further decreased with increasing time of roasting. *All-trans*-BC accounted for 69% and 63% of total BC after roasting for 5 and 20 min, respectively, at 165 °C (**Figure 1**). This loss of *all-trans*-BC was associated with a concomitant increase in 13-*cis*-BC, but no significant change in 9-*cis*-BC. For all studies presented below, fermented cassava was roasted at 165 °C for 10 min to prepare gari to compare retention and bioaccessibility with boiled cassava and fufu.

Approximately 49% of the *all-trans*-BC was lost during preparation of gari from the three cultivars. As shown in **Figure 2** for cultivar TMS 01/1371, total BC content per unit dry weight was not markedly altered by boiling raw cassava for 30 min or by fermentation followed by boiling for 10 min to prepare fufu. The results for processing the other two cultivars were very similar (data not shown). Relative amounts of cis isomers generally increased during processing. The ratio of 13-*cis*- to 9-*cis*-BC in boiled cassava (1.1−1.8) and gari (1.0−1.6) were approximately twice that in the corresponding unprocessed cultivars (0.6−0.7), whereas the ratio of these cis isomers present in raw cassava and fufu (0.8−0.9) was similar. This suggests that fermentation and the brief boiling to prepare fufu does not induce isomerization of *all-trans*- to 13-*cis*-BC.

Apparent Stability and Bioaccessibility of BC during Simulated Digestion of Processed Cassava. Recovery of BC after simulated oral, gastric, and small intestinal digestion of boiled cassava, gari, and fufu for each of the cultivars exceeded 70%. There was a trend for greater recovery of 9-*cis*-BC compared to *all-trans*- and 13-*cis*-BC in boiled
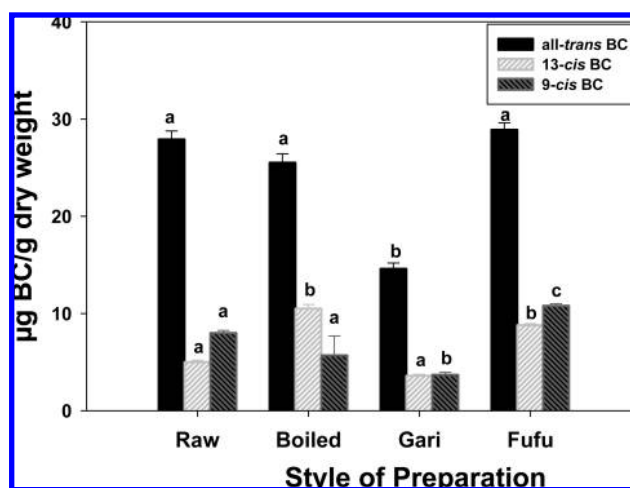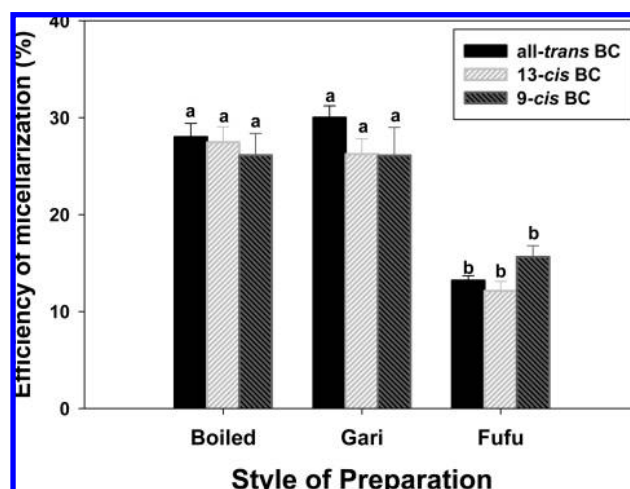
cassava and gari. It is unclear if these apparent differences reflect greater stability of 9-*cis*-BC during one of more stages of digestion, isomerization of some of the *all-trans*-BC to 9-*cis*-BC, or both.

The efficiency of micellarization of all-trans and cis isomers of BC during the small intestinal phase of digestion of boiled cassava was 25−30% (**Figure 3**) as we previously reported for these three and other cultivars of cassava harvested in summer 2006 and identically processed (*6*). Partitioning of BC isomers in micelles generated during digestion of gari was not significantly different from that for boiled cassava. However, the efficiency of micellarization of all-trans and cis isomers of BC during digestion of fufu was significantly ($P < 0.05$) less than that for boiled cassava and gari prepared from the three test cultivars (**Figure 3**).

Retention and Bioaccessibility of BC in Processed Cassava

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DISCUSSION

Carotenoids are destroyed by heat, light, and oxygen or a combination of all three (*8*). Increasing the surface area of a fruit or vegetable by chopping, grating, mashing, and oven/sun drying exposes carotenoids in the food matrix to increased light and oxygen, and baking, boiling, and roasting exposes the carotenoids to elevated temperatures. Consequently, it is not surprising that processing of plant foods is often associated with decreases in the amount of carotenoids in the consumed product. Vegetables enriched in BC, e.g., Chinese cabbage, pumpkin, and yellow sweet potato, lost an average 43% of BC after boiling at 100 °C for 15 min (*9*). Similarly, sun drying mangoes and cowpea leaves resulted in loss of 94 and 63% BC content, respectively (*10*). On the other hand, food processing can enhance the bioavailability of carotenoids (*11, 12*). For example, Livny et al. (*13*) reported that the bioavailability of BC in human subjects with ileostomy was greater during digestion of cooked pureed carrots compared to raw chopped carrots. Similarly, the efficiency of micellarization of BC during simulated digestion was increased from 3% for raw carrots to 27% for cooked carrots (*14*).

Fermentation followed by roasting is used to prepare gari from cassava root. Chávez et al. (*15*) suggested that the observed loss of 66% of BC during the preparation of gari was the outcome of extended (seven day) fermentation of the cassava mash. However, temperature and roasting time were not reported and apparently not considered. We found that fermentation of grated cassava for three days at room temperature in the preparation of gari resulted in minimal loss of total BC. However, there was a 90% loss of BC from fermented cassava during roasting at 195 °C for 20 min. This decline in BC content was limited to approximately 40% when roasting temperature was reduced to 165 °C (**Figure 1**). Similar to our results, fermentation of BC enriched maize for 48 h at 30 °C during preparation of porridge resulted in only a 10% loss of BC (*16*).

Boiling cubed cassava root or rapid boiling of fermented cassava paste to prepare fufu is also used in traditional African cooking to reduce the total cyanogen content. Apparent retention of total BC after boiling of cassava exceeded 100% on a dry weight basis for both fufu and boiled cassava in our study, suggesting that boiling has minimal impact on the retention of the pro-VA carotenoid. This is supported by the observations of Hagenimana et al. (*17*) and van Jaarsveld et al. (*18*) who reported 80% apparent retention and 88% true retention of BC, respectively, after boiling sweet potato for 30 min. K'osambo et al. (*19*) observed that the extent of loss of BC after boiling for 30 min vary by cultivars of sweet potato from 14 to 59%. There was no significant difference in retention of BC after boiling the three cultivars of cassava in the present study.

It is proposed that cooking and other processing methods increase the bioavailability of carotenoids by destroying the integrity of the cell wall and membranes of organelles in which carotenoids are located to facilitate the access of digestive enzymes for release of carotenoids from matrix into oil droplets (*11*). This is further supported by in vitro studies assessing partitioning of carotenoids into micelles. For example, Ryan et al. reported significantly increased transfer of BC from courgette, red pepper, and tomatoes upon boiling, grilling, and microwaving (*20*). When boiled samples of cassava were digested in vitro, approximately 25−30% of BC was partitioned into micelle fraction, in agreement with our previous study comparing bioaccessibility of BC from 10 cultivars of cassava (*6*). Preparation of gari also resulted in similar efficiency of transfer of BC to micelles. However, micellarization of BC was significantly lower during simulated digestion of fufu. Preparation of fufu involved a 5 day fermentation of the chopped roots in excess water, whereas grated cassava was fermented for three days in the absence of additional water to prepare gari. This somewhat surprising observation suggests that either the nature of the fermentation process or the combination of fermentation with either roasting or boiling differentially affects the release of BC from the food matrix during digestion. Further examination of the basis for the observed difference is merited.

It is noteworthy that the tested cultivars of cassava had relatively high levels of cis isomers prior to processing. Similar isomeric profile of BC from cassava was also reported by Chávez et al. (*15*). The retinol activity equivalence (RAE) of cis isomers of BC is only one-half that of *all-trans*-BC, i.e., 12 μg of *all-trans*-BC is equivalent to 1 μg of RAE (*21*). We have estimated RAE per 500 kcal of ingested boiled cassava, gari, and fufu as 96, 51, and 48 per 500 kcal, respectively, from determined energy content, retention after processing, and efficiency of micellarization. The difference is based on the greater retention of BC in boiled cassava than in gari and the more efficient micellarization of BC during digestion of boiled cassava compared to fufu. This suggests that approximately twice as many calories of gari and fufu need to be ingested to have equivalent bioefficacy as boiled cassava. Moreover, the RAE for 500 kcal of cooked cassava remains well below the DRI set by the Institute of Medicine, i.e., 400, 700, and 900 RAE for children, adult females, and adult males, respectively. The need to develop varieties of cassava containing higher amounts of BC remains evident.

Dietary fat is a known promoter of carotenoid bioavailability (*22*). Thus, addition of oil to cassava prior to ingestion or coingestion with other foods containing fat is expected to increase bioavailability of BC. Recent studies from our laboratory have shown that minimal dietary lipid (0.5−1% wet weight) is required to enhance bioaccessibility of BC (*23*), although higher amounts are required for assembly and secretion of chylomicrons, the required vehicle for transport of carotenoids to other tissues and organs (*24, 25*). Cassava has an endogenous fat content of 2−3% wet weight (data not shown). We further speculate that the addition of exogenous dietary fat will enhance BC bioavailability but not offset the limited quantity of BC in the cultivars of cassava currently available to meet the DRI of VA. Thus, strategies to further increase the pro-VA content of cassava are needed for those populations whose diet remains largely limited to this staple food.

In summary, roasting for preparation of gari is particularly troublesome as potential for major losses at higher temperature has a more adverse impact on BC content than either boiling or rapid boiling of fermented cassava (fufu). Decreasing the time and temperature of roasting during the preparation of gari is recommended, although monitoring temperature in poor socioeconomic settings likely represents a challenge and the change in texture and taste may not be readily accepted. Despite greater retention of BC during preparation of fufu compared to gari, the bioaccessibility of BC in fufu as assessed by micellarization during simulated digestion is markedly reduced and requires further investigation. Finally, greater attention is needed in the selection of cultivars containing increased amounts of BC with relatively low percentage of cis isomers in light of their lower retinol activity equivalency than *all-trans*-BC.

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preparation of gari and fufu. The assistance of William Weiss and Tonya McKelvey with analysis of caloric content of cooked cassava is appreciated. We thank Carolyn Gunther for her critical review of the manuscript.

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