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Retention of Provitamin A Carotenoids in High β-Carotene Maize (Zea mays) During Traditional African Household Processing

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High β-carotene maize, biofortified with β-carotene through plant breeding, is being developed as a cost-effective, sustainable agronomic approach to alleviating the problem of vitamin A deficiency in Africa. We used high β-carotene maize (10.49 ± 0.16 μg β-carotene/g) to prepare traditional maize porridges and compared the carotenoid contents in the following: (1) whole kernels; (2) wet milled flour; (3) wet milled flour, fermented; (4) wet milled flour, cooked; (5) wet milled flour, fermented and cooked. The cumulative losses of β-carotene in the final, cooked products were 24.5% (95% CI 22.8–26.2%) and 24.8% (95% CI 23.1–26.5%), for the fermented and unfermented porridges, respectively. Thus, fermentation, a traditional technology with documented nutritional and other health benefits, does not adversely affect the retention of β-carotene in porridges prepared with high β-carotene maize. The relatively good retention of β-carotene during traditional maize processing provides additional experimental support for the feasibility of maize biofortification as a means to alleviate vitamin A deficiency.

KEYWORDS: Africa; β-carotene; biofortification; carotenoid; fermentation; maize; ogi; porridge; processing; vitamin A; *Zea mays*

INTRODUCTION

Africa has the second highest prevalence of vitamin A deficiency (28−35% of children, based on the World Health Organization-defined subregion) following South-East Asia (*1*). Vitamin A deficiency causes impaired vision in many areas of the developing world and is the leading cause of acquired blindness in children (*1*). More than 33% of early childhood cases of xerophthalmia (1.6 million) and 17% of cases of maternal night blindness (1.1 million) are found in Africa (*2*). Vitamin A deficiency is also a major global public health problem that affects approximately 127 million preschool children (serum retinol <0.70 μmol/L or displaying abnormal impression cytology) and more than 7.2 million pregnant women (serum or breast milk vitamin A concentrations <0.70 μmol/L) worldwide (*2*). Apart from its ocular manifestations, the nonocular systemic consequences of vitamin A deficiency include increased infectious morbidity and mortality, growth retardation, and anemia. The devastating effects of vitamin A deficiency are attributed to over 4–6% of the entire disease burden in Africa (*1*).

More than any other continent, Africa depends on maize as a food source (*3*). In southern Africa, maize has become the most important staple food and supplies more than 50% of the energy in local diets (*3*). Global statistics for cereal consumption calculated by the World Health Organization indicate average total cereal consumption in the African diet is 291.7 g/person/day, including an average maize consumption of 106.2 g/person/day (*4*). This average per capita maize consumption is more than twice that calculated for any of four other regional diets: European, Far Eastern, Latin American, or Middle Eastern (*4*). The majority of maize produced and consumed in Africa is white maize, which is essentially devoid of yellow carotenoid pigments, including those that serve as a source of provitamin A. For those African countries for which data is available, the estimated average share of white maize in total maize production ranges from 90 to 100%; the exception being Côte d'Ivoire, in which the average share of white maize is 70% (*5*).

Maize is one of six key staple food crops that are the focus of an international agronomic effort to combat micronutrient malnutrition in developing countries through biofortification (*6*).

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High-provitamin A, yellow maize genotypes are being developed through plant breeding to produce kernels that have enhanced contents of β-carotene and other provitamin A carotenoids, as well as other micronutrients. Ultimately, the seeds will be distributed to poor farmers who will save and share the seeds over generations as a cost-effective, renewable resource to alleviate micronutrient deficiencies. An increment in the provitamin A contents in the maize kernel is expected to have a substantial health impact in Africa given the large daily maize consumption by entire families, including children and women who are most vulnerable to vitamin A deficiency (*6*). For example, the National Food Consumption Survey (NFCS) in South Africa indicated more than 90% of children 1–9 years of age consume maize porridge (*7*).

Fermented maize flour is used to prepare porridges as well as a variety of other staple foods in Benin, Ghana, Nigeria, and Togo (*8*). Maize-meal (corn flour) porridge is considered the most important weaning food for infants in West Africa, although it is also consumed by older children and adults (*9*). Our objective was to determine the retention of the major provitamin A carotenoids, α-carotene, β-carotene, and β-cryptoxanthin, in high β-carotene maize during traditional processing. We used a fermented maize porridge, *ogi*, to model steps used in indigenous maize processing in West Africa. The traditional production of ogi involves soaking of dried whole maize kernels, milling, addition of water to form a wet flour or dough, and spontaneous (noninoculated) fermentation (24–72 h) (*8*). The ogi is then cooked with added water to prepare hot porridges.

MATERIALS AND METHODS

Maize. To generate the high β-carotene maize, a DeExp × CI.7 single-cross hybrid was cross-pollinated with BC Orange, an experimental synthetic maize population derived from three different inbred lines and developed at the University of Illinois. The kernels of the resulting (DeExp × CI.7) × BC Orange hybrid were used in this study. The high β-carotene maize hybrid was developed by Dr. Torbert Rocheford, Department of Crop Sciences, University of Illinois, Urbana, IL, and was grown in the facilities of the University of Illinois Research and Education Center in Urbana. The maize was harvested at maturity and dried at low temperature (40 °C) for 66 h. In a preliminary study of intercob variability of carotenoid concentrations among kernels isolated from 24 individual cobs, we found the carotenoid concentrations to be (mean ± SD, μg/g dry weight): α-carotene, 0.95 ± 0.24; β-carotene, 12.74 ± 1.39; β-cryptoxanthin, 1.62 ± 0.21; lutein, 11.46 ± 1.51; and zeaxanthin, 8.38 ± 1.10. The coefficient of variation (CV) for the concentration of total β-carotene (cis- and trans- isomers), the predominant provitamin A carotenoid, was 10.9%.

For the current study, five replicate subsamples of 505 g were taken from a well-mixed bulked sample of cleaned whole maize kernels. From each 505 g replicate, a 5 g aliquot of kernels was removed for baseline analysis of carotenoid concentrations and moisture content.

Soaking and Milling. The replicate subsamples ($n = 5$) each containing the remaining 500 g of maize kernels were soaked in tap water (1:1 by weight) for 24 h at room temperature (30 °C) while protected from light. The hulls floated to the surface and were removed with excess water by decanting. The soaked kernels were then drained and milled at 10,000 rpm × 30 s (Grindomix GM 200, Retsch GmbH, Haan, Germany) into a fine wet flour to pass through a USA #40 standard sieve with a pore size of 416 μm. An aliquot of the wet flour was immediately analyzed for carotenoid concentrations and moisture content.

Fermentation. To prepare the fermented porridges ($n = 5$), a 300 g portion of the wet flour was removed from each replicate batch, and 100 g of water was added. The wet flour was allowed to spontaneously ferment at room temperature (30 °C) in the dark for 48 h (solid state fermentation) (*9*). An aliquot of the fermented wet flour was immediately analyzed for carotenoid concentrations and moisture content.

Cooking. *Cold-water Procedure.* To prepare the unfermented and fermented porridges, a portion of the wet unfermented and fermented flours, respectively, from each replicate batch ($n = 5$) was mixed with cold water to form a slurry. The volume of added water was adjusted to a predetermined amount to produce equivalent final moisture contents in the unfermented and fermented porridges after cooking. For the unfermented porridge, 300 g of water was added to a 250 g portion of the unfermented wet flour. For the fermented porridge, 160 g of water was added to a 250 g portion of the fermented, wet flour. The resulting slurries were then heated from a temperature of 30 to 93 °C over 3 min, held at 93 °C with continuous stirring for 9 min, then cooled to room temperature (approximately 2–3 h) while protected from light. To determine the effect of moisture content on carotenoid retention, a thin porridge was also prepared from each replicate batch ($n = 5$) of unfermented wet flour. To prepare the thin porridge, 250 g of water was added to a 76.8 g portion of the unfermented wet flour; the remaining cooking protocol was identical to that described above. An aliquot of each cooled porridge was immediately analyzed for carotenoid concentrations and moisture content.

Cold-boiling-water Procedure. In a separate experiment, we compared the cold-water cooking procedure and a cold-boiling-water cooking procedure that is also commonly practiced in African households (*10*). In the cold-boiling-water cooking procedure, the wet milled flour is mixed with water to form a slurry. The slurry is then poured into boiling water and cooked for a specified time. Three replicate subsamples of 505 g were again taken from the same well-mixed bulked sample of clean whole maize kernels. We followed the same procedures described above to produce three replicate batches of fermented wet flour. From each replicate, porridge was prepared using both the cold-water cooking procedure and the cold-boiling-water cooking procedure. In the cold-water cooking procedure, 100 g of the fermented wet flour was mixed with 300 g of cold water. The resulting slurry was then heated from a temperature of 30 to 93 °C over 3 min, and held at 93 °C with continuous stirring for 9 min, as described above. In the cold-boiling-water cooking procedure, 100 g of the fermented wet flour was mixed with 100 g of cold water to form a paste. The paste was then added to 200 g of boiling water and cooked with continuous stirring for 9 min. Each porridge was cooled to room temperature (approximately 2–3 h) while protected from light. An aliquot of each cooled porridge was immediately analyzed for carotenoid concentrations and moisture content.

Carotenoid Analysis. Representative samples from each processing step were analyzed for carotenoid concentrations, including dried whole kernels, milled wet flour, fermented milled wet flour, unfermented porridge, and fermented porridge. Samples were extracted and analyzed in duplicate for five major carotenoids: α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin. The major isomers of β-carotene — 9-*cis*-, 13-*cis*-, 15-*cis*-, and all-*trans*-β-carotene — were also quantified to evaluate isomerization during processing.

Carotenoids were extracted using a modification of the method by Granado et al. (*11*). Prior to analysis, dried maize kernels were ground to a fine flour in an analytical mill (A11 Basic, IKA, Wilmington, NC). A 1.0 g sample of finely ground or processed maize was transferred to a 40 mL screw-capped culture tube, and 6.0 mL of methanol containing 0.1 g of butylated hydroxytoluene/L was added. The tube was loosely capped and heated in a water-filled beaker in the dark with magnetic stirring at 50 °C for 15 min. The mixture was allowed to cool before 6.0 mL of tetrahydrofuran containing 0.1 g of butylated hydroxytoluene/L was added. The tube was vortexed for 90 s, and the contents were allowed to stand for 5 min to allow fine particles to settle. A 0.5 mL aliquot of the upper clear extract were transferred to a 25 mL screw-capped test tube, and 1.0 mL of 40% methanolic potassium hydroxide containing 0.1 M pyrogallol was added. Saponification was carried out at room temperature under argon with continuous vortexing for 5 min. Then, 2 mL of HPLC-grade water were added to wash the potassium hydroxide out of the maize extract. An internal standard, β-apo-8′-carotenal (Fluka, Milwaukee, WI) in methanol, was added, followed

by 4 mL of hexane/methylene chloride (5:1 v/v) containing 0.1 g of butylated hydroxytoluene/L. After the tube and conents were vortexed for 90 s, the tube was centrifuged for 5 min at 700*g*. The upper organic phase was collected into a 10 mL disposable test tube, and the contents were evaporated to dryness in a speed vacuum evaporator (Model SPD 131 DDA, Thermo Electron, Milford, MA) with a universal vacuum system (UVS 800 DDA, Thermo Electron). The dried extract was reconstituted in 100 μL of methyl-tert-butyl ether followed by 300 μL of methanol. A 100 μL aliquot was injected into the HPLC system.

The HPLC system included a 717Plus autosampler with the temperature control set at 5 °C, two 515 solvent delivery systems, and a 2996 photodiode array detector (Waters Corporation, Milford, MA). The system was controlled by Empower chromatography manager software (version 1, Waters Corporation). The carotenoids were separated on a 5 μm C_{30} Carotenoid Column (4.6 × 250 mm; Waters Corporation) and eluted using 100% methanol (containing 1 g ammonium acetate/L) (Solvent A) and 100% methyl-*tert*-butyl ether (MTBE) (Solvent B). The following gradient was used: 0−15 min, 100% Solvent A; 15−25 min, linear gradient to 10% Solvent B; 25−35 min, linear gradient to 30% Solvent B; 35−55 min, linear gradient to 50% Solvent B. The flow rate was 1.0 mL/min. Peak area integration was at 453 nm. All solvents were HPLC grade and were purchased from Fisher Scientific, Fairlawn, NJ. Calibration standards for α-carotene, β-cryptoxanthin, lutein, and zeaxanthin were purchased from CaroteNature (Lupsingen, Switzerland); all-*trans*-β-carotene was purchased from Fluka (Milwaukee, WI). These standards were used to generate internal standard calibration curves. *Cis*- and *trans*-β-carotene isomers were quantified using the all-*trans*-β-carotene calibration curve.

Moisture Analysis. The moisture contents of the samples were determined by oven drying according to the Association of Official Analytical Chemists (AOAC) method for plant materials (Method 934.01; *12*). The 2 g test portions were dried to constant weight at 100 °C.

Apparent and True Retentions. Nutrient retention may be calculated as either apparent or true retention. Apparent retention is defined by calculations based on the nutrient contents of the moisture-free raw and cooked foods (*13*). Because apparent retention is calculated on a dry weight basis, it provided a straightforward approach to correct for changes in the carotenoid concentrations in the maize resulting from gains in moisture content during soaking, fermenting, and cooking. The apparent retention calculations assume that solids are not lost to a significant extent, for example, by leaching into the steep water, which is later discarded. Loss of dry matter during preparation of ogi porridge is reported to be about 13–18% (*14, 15*). If there is a disproportionate loss of solids relative to carotenoids during maize processing, apparent retention could overestimate carotenoid retention. To identify and avoid such potential bias, we used both apparent and true retention to calculate carotenoid retention during cooking. The percent true retention was calculated according to Murphy et al. (*13*).

Statistical Analyses. *Apparent versus True Retention*. The absolute differences (true retention minus apparent retention) at individual processing steps were analyzed as a randomized complete block design with the sample replicate as the fixed block and with the processing step as the treatment variable. A difference in apparent versus true retention was detected when the *p*-value obtained from the overall F-test was significant. *Apparent Retention*: For subsequent statistical analyses, carotenoid retentions were expressed on a dry weight basis. The percent loss of a carotenoid between two sequential processing steps was calculated as the change in the concentration of the carotenoid as a percentage of the initial concentration of the carotenoid in the whole, dried maize kernels. Outcome variables (carotenoid concentration, percent loss) were analyzed as a randomized complete block design with the sample replicate as the fixed block and with the processing step as the treatment variable. When the *p* value obtained from the overall F-test was significant, differences among processing steps were determined by *t*-tests. The post hoc comparisons used the Bonferroni adjustment for multiple comparisons. *Cis- versus Trans-*β*-carotene*: To evaluate the effect of each processing step on the isomeric composition of the β-carotene in the maize, we calculated the ratio of the total *cis*-β-carotene concentration (9-*cis* plus 13-*cis* isomers) and the total β-carotene concentration (cis plus trans isomers). This outcome variable was then analyzed as a randomized complete block design, as described above. *Cold-water- versus Cold-boiling-Water-Processing:* The carotenoid retentions after cold-water and cold-boiling-water processing were compared as a trivial randomized complete block design with sample replicate as the fixed block, processing method as the treatment variable, and two treatments per block. Statistical analyses were performed using SAS (version 9.1; SAS Institute, Inc., Cary, NC). Data are presented as the mean ± SD. Differences with $p \leq 0.05$ were considered significant.

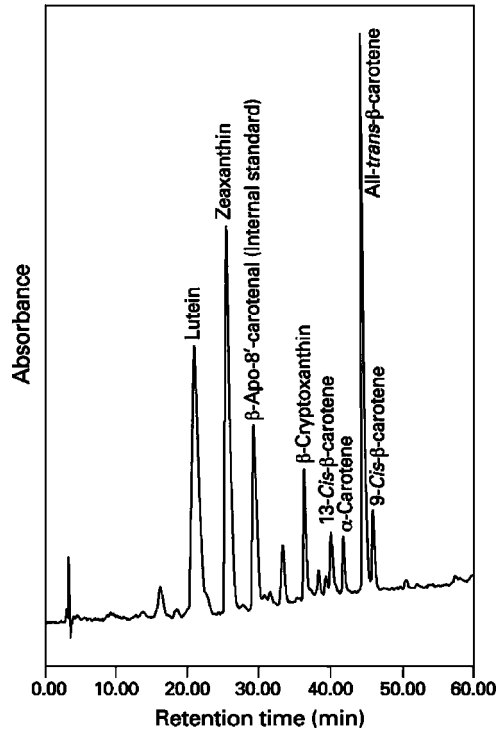

Figure 1. The HPLC chromatographic profile of the carotenoids in an extract of high β-carotene maize.

RESULTS

Carotenoid Profile. A representative chromatogram of the carotenoid profile in the high β-carotene maize kernels is shown in **Figure 1**. As expected, the major provitamin A carotenoid in the maize was all-*trans*-β-carotene. The total β-carotene concentration, 10.49 ± 0.16 μg/g dry weight, was more than 15 times higher than the mean concentration reported in a survey of 44 sweet and dent corn lines, 0.68 μg/g dry weight (*16*). The raw kernels contained two prominent cis isomers of β-carotene, 9-*cis* (1.54 ± 0.10 μg/g dry weight) and 13-*cis* (1.20 ± 0.05 μg/g dry weight). The 9-*cis* isomer of β-carotene is also detected in native plastids of other plants; 9-*cis*-β-carotene was reported to be the major cis-isomer of β-carotene in native chloroplasts of spinach (*17*). The concentrations of α-carotene (0.79 ± 0.01 μg/g dry weight) and β-cryptoxanthin (1.73 ± 0.19 μg/g dry weight), the remaining two provitamin A carotenoids, in high β-carotene maize were considerably lower than that of β-carotene. However, they substantially exceeded the mean α-carotene and β-cryptoxanthin concentrations reported in the published survey of maize lines, 0.16 μg/g dry weight and 0.55 μg/g dry weight, respectively (*16*).

Moisture Content. As expected, the moisture content of the maize increased during each processing step: soaking, fermentation, and cooking ($p < 0.0001$). The moisture contents were 14.5 ± 0.5% for the raw whole maize kernels, 35.2 ± 2.3% for the wet milled flour, and 49.0 ± 1.2% for the fermented wet milled flour. The moisture contents of the unfermented and

Table 1. Carotenoid Concentrations after Steps in the Processing of β-Carotene-rich Maize[a,b]

	μg/g dry weight				
processing step	α-carotene	β-carotene[c]	β-cryptoxanthin	lutein	zeaxanthin
whole kernels	0.79 ± 0.01 a	10.49 ± 0.16 a	1.73 ± 0.19 a	11.71 ± 0.16 a	9.20 ± 1.08 a
wet milled flour	0.73 ± 0.03 b	9.72 ± 0.09 b	1.56 ± 0.08 b	11.03 ± 0.45 a	8.67 ± 0.39 a
		Fermented Porridge			
wet milled flour, fermented	0.62 ± 0.03 c	8.65 ± 0.21 c	1.32 ± 0.08 cd	8.85 ± 0.24 b	6.73 ± 0.29 b
wet milled flour, fermented and cooked	0.56 ± 0.02 d	7.92 ± 0.17 d	1.22 ± 0.11 c	7.90 ± 0.29 c	5.88 ± 0.21 bc
		Unfermented Porridge			
wet milled flour, cooked	0.59 ± 0.02 cd	7.89 ± 0.18 d	1.28 ± 0.07 cd	8.29 ± 0.66 bc	6.08 ± 0.54 bc
		Unfermented Porridge — Thin Gruel			
wet milled flour, cooked	0.61 ± 0.04 c	8.16 ± 0.20 d	1.39 ± 0.10 d	8.50 ± 0.55 bc	5.78 ± 0.30 c

[a] Values are means of five replicates ± SD. [b] Mean values having the same letters within a column are not significantly different, $P < 0.05$ (Bonferroni post hoc test). [c] Total β-carotene (sum of cis and all-trans isomers).

Table 2. Carotenoid Losses after Steps in the Processing of β-Carotene-rich Maize[a]

	losses (%)				
	α-carotene	β-carotene[b]	β-cryptoxanthin	lutein	zeaxanthin
		Fermented Porridge			
total losses	28.9 (26.0, 31.8)	24.5 (22.8, 26.2)	29.1 (25.1, 33.1)	32.5 (28.0, 37.0)	35.5 (28.6, 42.4)
after soaking and milling	7.1 (4.2, 10.0)	7.3 (5.6, 9.0)	9.5 (5.5, 13.5)	5.7 (1.3, 10.2)	4.8 (−2.1, 11.7)
after fermentation	14.4 (11.5, 17.3)	10.2 (8.5, 11.9)	13.9 (9.8, 17.9)	18.7 (14.2, 23.2)	21.5 (14.6, 28.4)
after cooking, fermented wet flour	7.4 (4.5, 10.4)	6.9 (5.2, 8.6)	5.8 (1.8, 9.8)	8.1 (3.6, 12.6)	9.3 (2.4, 16.2)
		Unfermented Porridge			
total losses	25.3 (22.4, 28.2)	24.8 (23.1, 26.5)	25.3 (21.3, 29.3)	29.2 (24.7, 33.7)	33.7 (26.8, 40.6)
after soaking and milling	7.1 (4.2, 10.0)	7.3 (5.6, 9.0)	9.5 (5.5, 13.5)	5.7 (1.3, 10.2)	4.8 (−2.1, 11.7)
after cooking, unfermented wet flour	18.2 (15.3, 21.1)	17.5 (15.8, 19.2)	15.8 (11.8, 19.8)	23.4 (19.0, 27.9)	28.9 (22.0, 35.8)

[a] Values are means of 5 replicates; 95% CI in parentheses. Losses are expressed as a percentage of the initial carotenoid contents in the raw whole kernels. [b] Total β-carotene (sum of cis- and all-trans isomers).

fermented porridges were nearly identical, 60.2 ± 0.9% and 61.5 ± 0.4%, respectively. Thus, we avoided potential confounding effects of moisture content when comparing the carotenoid retention in the unfermented and fermented porridges. The moisture content of the thin, unfermented porridge (gruel) used to evaluate the effect of moisture content on carotenoid retention was 74.3 ± 0.8%.

In experiment 2, which compared the cold-water cooking procedure and a cold-boiling-water cooking procedure, the moisture contents of the fermented porridges were equivalent, 76.0 ± 0.3% for the cold-water cooking procedure and 75.6 ± 0.2% for the cold-boiling-water cooking procedure.

Apparent versus True Retention. There were no statistically significant differences in percent carotenoid retention when calculated as apparent or true retention. No significant differences were detected for any of the carotenoids during any of the processing steps (data not shown). Because data analysis of carotenoid concentrations expressed on a dry weight basis is more straightforward, subsequent data analyses were based on apparent retention.

Carotenoid Retention. Table 1 shows carotenoid concentrations (μg/g dry weight) in high β-carotene maize during the following stages in home processing to prepare porridge: (1) whole kernels; (2) wet milled flour; (3) wet milled flour, fermented; (4) wet milled flour, fermented and cooked (fermented porridge; 60% moisture); (5) wet milled flour, cooked (unfermented porridge; 60% moisture); (6) wet milled flour, cooked (unfermented porridge — thin gruel; 74% moisture). A comparison of the carotenoid concentrations in the latter two porridges with different moisture contents enabled us to investigate potential effects of moisture content on carotenoid retention. Because the carotenoid concentrations in these two porridges expressed on a dry weight basis were not significantly different, there was no effect of moisture content. Thus, data for the thin porridge (74% moisture) were not included in **Table 2**.

Table 2 shows the carotenoid losses expressed as a percentage of the initial carotenoid contents in the raw whole maize kernels. There were small but significant losses of each of the three provitamin A carotenoids, α-carotene, β-carotene, and β-cryptoxanthin, during the initial processing step, which was soaking and milling to produce wet milled flour. This processing step did not result in significant losses of lutein and zeaxanthin. During the preparation of the fermented porridge, there were significant losses of each of the five carotenoids during the fermentation step. Mean carotenoid losses during the fermentation step ranged from 10.2% for β-carotene to 21.5% for zeaxanthin. Despite these losses during fermentation, the cumulative losses of each carotenoid in the final products, fermented and unfermented porridge, were not different. For example, the mean cumulative β-carotene losses in the fermented and unfermented porridges were 24.5% and 24.8%, respectively. When the unfermented porridge was prepared by omitting the fermentation step, the loss of β-carotene during the cooking step increased from 6.9% for the fermented porridge to 17.5%. Thus, carotenoid losses during the fermentation step were compensated by lower losses during the cooking step for the fermented as compared with the unfermented porridge. The same trend of higher losses occurring during cooking of the unfermented porridge was observed for all carotenoids.

Distribution of β-Carotene Isomers. The effects of processing on the quantitative distribution of cis and all-trans isomers of β-carotene in high β-carotene maize are shown in **Table 3**. In the cooked porridges, fermented and unfermented, there were small increases in the percent cis isomers (9-*cis* plus 13-*cis*) relative to the total β-carotene content. Because of the high precision among replicates, these minor changes in cis isomer

Table 3. Quantitative Distribution of β-Carotene Isomers in Dried and Processed Kernels of β-Carotene-rich Maize[a]

processing step	μg/g dry weight					
	all-*trans*	13-*cis*	9-*cis*	total *cis*	total β-carotene	% *cis*[b]
whole kernels	7.75 ± 0.03	1.20 ± 0.05	1.54 ± 0.10	2.74 ± 0.14	10.49 ± 0.16	26.1 ± 0.01 a
wet milled flour	7.27 ± 0.03	1.11 ± 0.05	1.35 ± 0.04	2.46 ± 0.08	9.72 ± 0.09	25.3 ± 0.01 a
wet milled flour, fermented	6.43 ± 0.11	1.01 ± 0.07	1.21 ± 0.06	2.22 ± 0.13	8.65 ± 0.21	25.6 ± 0.01 a
wet milled flour, cooked	5.47 ± 0.11	1.17 ± 0.05	1.25 ± 0.04	2.42 ± 0.09	7.89 ± 0.18	30.7 ± 0.01 b
wet milled flour, fermented and cooked	5.66 ± 0.08	1.05 ± 0.07	1.21 ± 0.06	2.26 ± 0.12	7.92 ± 0.17	28.5 ± 0.01 c

[a] Values are means of five replicates ± SD. [b] Mean values having the same letters within a column are not significantly different, $p < 0.05$.

distribution were statistically significant. Cis–trans conversions have nutritional consequences in terms of the bioavailability (*18*) and bioconversion of the β-carotene to vitamin A. As compared with *trans*-β-carotene, the cis isomers have lower provitamin A activity (*19*). However, the increases in the percent cis isomers that occurred during the cooking step were too small to be of practical significance in terms of the provitamin A value of the β-carotene in the maize.

Cold-water Cooking versus Cold-boiling-water Cooking Procedures. In experiment 2 we did not find significant differences for any of the five carotenoids when the fermented porridges were prepared using the cold-water cooking procedure as compared with the cold-boiling-water cooking procedure. The respective carotenoid concentrations (mean ± SD; μg/g dry weight) in the fermented porridges prepared by the cold-water cooking procedure and the cold-boiling-water cooking procedure were 0.57 ± 0.01 and 0.59 ± 0.01 μg/g α-carotene, 7.84 ± 0.07 and 7.91 ± 0.05 μg/g total β-carotene, 1.42 ± 0.05 and 1.41 ± 0.04 μg/g β-cryptoxanthin, 8.55 ± 0.13 and 8.60 ± 0.23 μg/g lutein, and 5.24 ± 0.08 and 5.45 ± 0.19 μg/g zeaxanthin. The respective concentrations of the cis-isomers of β-carotene were 1.45 ± 0.02 and 1.46 ± 0.02 μg/g 13-*cis*-β-carotene and 1.53 ± 0.09 and 1.47 ± 0.04 μg/g 9-*cis*-β-carotene. Thus, these two commonly used cooking procedures would be expected to result in equivalent losses of provitamin A carotenoids. The pH of the fermented wet flour used to prepare the porridges was 4.0, when measured directly by a glass electrode pH meter (Model 420, Thermo Orion, Inc., Beverly, MA).

DISCUSSION

Semiliquid maize-based gruels (gruels of fermented maize) are traditionally used to complement breast milk for infants in Sub-Saharan Africa. However, these infant gruels have poor protein quality, low energy density (25–50 kcal/100 g or 105–209 kJ/100 g) (*10*), and low micronutrient density. Biofortification of maize with provitamin A carotenoids and other micronutrients is an important step toward improving the nutritional quality of these weaning foods.

Detailed knowledge of the retention of provitamin A carotenoids during traditional processing steps is a prerequisite for predicting the efficacy of biofortification in combating vitamin A deficiency. Such knowledge also provides a basis for strategies to enhance carotenoid retention, thereby enhancing the provitamin A value of the processed maize. We investigated carotenoid retention during steps in the traditional processing of high β-carotene maize to prepare West African maize porridges. The processing steps included soaking and wet milling of the kernels, fermentation of the wet flour, and cooking.

Soaking the dried kernels in water followed by wet milling to produce a fine flour resulted in losses of provitamin A carotenoids that, although statistically significant, were not substantial (<10%). Lipoxygenases in grains and vegetables catalyze lipid oxidation and the cooxidation and bleaching of pigments such as carotenoids and chlorophylls (*20*). During soaking of the dry maize kernels for 24 h in the dark and subsequent wet milling, we did not detect substantial lipoxygenase activity, as reflected in losses of carotenoids. L1 and L2 lipoxygenase isoforms accumulate in the dry embryos of maize seeds (*21*). The activity of both lipoxygenase and linoleate hydroperoxide isomerase is reported to be low in endosperm extracts of crib-dried maize (*22*). In maize, the carotenoids are located primarily in the endosperm. During the soaking step, the compartmentalization of lipoxygenases, as well as most of the lipid, in the embryo of the intact kernels may protect the carotenoids from bleaching. After wet milling, the compromised cellular integrity and favorable moisture environment would be expected to promote the activity of endogenous lipases and lipoxygenases, leading to lipolysis, fatty acid oxidation, and bleaching of carotenoids (*23*). Because our samples were analyzed immediately after milling, losses due to these endogenous fat-metabolizing enzymes would not have been apparent until the subsequent processing step. After harvest, the maize was dried to acceptable moisture levels at low temperature (40 °C) with heated air. The activities of lipoxygenase decrease upon high-temperature (116 °C) air drying (*24*). However, there was no significant difference in the lipoxygenase activity in the germ of maize kernels that were dried at room temperature (ca. 25 °C) as compared with those dried at 88 °C. Thus, the mild temperature used to dry the maize kernels in this study is unlikely to have inactivated lipoxygenase-mediated carotenoid degradation.

Throughout Africa, fermentation is a traditional component of cereal processing (*8*). Fermentation of cereal staples is a recommended processing strategy to enhance micronutrient bioavailability for infants and children in developing countries (*25*). Fermentation provides an optimal pH for enzymatic degradation of phytate, which may increase the amount of soluble calcium, iron, and zinc (*9*). Microbial synthesis during the fermentation of maize meal improves the concentrations of the limiting indispensable amino acids, lysine and methionine (*26*). Fermentation also contributes to the safety, shelf life, and acceptability of maize-based foods (*9*). The antimicrobial activity caused by the growth of lactic acid bacteria is primarily due to a decrease in pH (*27*).

During preparation of the fermented porridge, significant losses of each carotenoid occurred during the fermentation step. Losses during fermentation ranged from 10.2% (95% CI 8.5–11.9%) for β-carotene to 21.5% (95% CI 14.6–28.4%) for zeaxanthin. During fermentation, the pH of wet maize flour (dough) drops from 6.1 to about 4.1 within the first 24 h (*28*). The measured pH of our fermented maize flour was 4.0. Carotenoids are stable to changes in pH in foods over the range pH 2–7 (*29*). Thus, it is unlikely that the decrease in pH during fermentation accounts for the observed carotenoid losses. During the tissue disruption that occurred with milling, lipoxygenases were released from the germ and brought into contact with

carotenoids in the endosperm. Initial pH conditions would have been favorable, because lipoxygenase in maize germ has a pH optimum of 6.0–7.0. Little lipoxygenase activity is observed at pH 4.0 using lipoxygenase partially purified from maize germ (*30*). In West Africa, the typical fermentation period is 48–72 h (*8*). If lipoxygenase contributed to carotenoid losses, the losses would be expected to subside within the first 24 h of fermentation, because the pH would no longer be favorable for lipoxygenase activity. By producing a low pH not conducive to lipoxygenase activity and cooxidation of carotenoids, fermentation may promote carotenoid retention during storage of the wet maize flour.

As expected, given the susceptibility of carotenoids to heat, losses occurred during the cooking step. Incorporation of oxygen during mixing would also have contributed to the direct oxidation of carotenoids as well as oxidation of unsaturated fatty acids to peroxides (*20*). The carotenoid losses during cooking were 2.5- to 3-fold lower in the fermented porridge than in the unfermented porridge (**Table 2**). However, the lower losses of the β-carotene and the other carotenoids during the cooking step in the fermented porridge were compensated by the previous losses of these carotenoids during the fermentation step. In the unfermented porridge, the fermentation step, and its accompanying losses of carotenoids, was omitted. However, losses of carotenoids during cooking were higher in the unfermented porridge. As a result, the cumulative losses of β-carotene and other carotenoids in the final products, fermented and unfermented porridges, were not significantly different (**Table 1**). Our findings indicate that fermentation, a traditional technology with documented nutritional and other health benefits, does not adversely affect the retention of provitamin A carotenoids in porridges prepared with high β-carotene maize.

The fact that near-identical carotenoid losses occurred regardless of processing method suggests the existence of a labile "pool" of carotenoids in high β-carotene maize. If not partially lost due to inclusion of a fermentation step, the entire labile pool was lost during the cooking step in the unfermented porridge. Although the carotenoid profile of the maize is most noteworthy for its high *trans*-β-carotene content, biosynthesis of each of the 5 major carotenoids is likely to be upregulated in this genotype. β-Carotene is the biosynthetic precursor of both β-cryptoxanthin and zeaxanthin. As noted above, the α-carotene content of the maize is higher than commonly found in conventional maize. Because α-carotene is the precursor for lutein biosynthesis, it is probable that lutein biosynthesis is also at least modestly enhanced in this maize. Carotenoids are present in substantial concentrations in the amyloplasts of yellow maize (*31*). Within plastids, there is a close link between the machineries for carotenoid biosynthesis and sequestration (*32*). Carotenoids are stabilized by the protein and lipid molecules in their proximity. In addition, the ultrastructural organization of plastids responds to the extent of carotenoid accumulation (*32*). In high β-carotene maize, the overaccumulation of carotenoids would be expected to alter their own sequestration, as well as possibly plastid structure, potentially leading to greater lability of the overaccumulated carotenoids.

Food processing can cause losses of carotenoids due to degradation, isomerization, and oxidation. Processing also disrupts the plant matrix, including the cellular compartments and binding proteins that serve to protect and stabilize the carotenoid pigments (*17*). However, when carotenoids are retained after processing, disruption of the plant matrix also markedly enhances their potential for intestinal absorption (bioaccessibility) (*33*). The soaking and cooking steps in traditional maize processing soften the plant matrix and would be expected to enhance the release and bioaccessibility of provitamin A carotenoids for absorption and bioconversion to vitamin A. The milling step converts the softened matrix to fine particles and disrupts cellular structures, which is essential for the bioaccessibility of the embedded carotenoids.

We conducted a systematic study under controlled laboratory conditions of the retention of provitamin A carotenoids in high β-carotene maize as traditionally processed to prepare ogi maize porridge. In households, maize kernels are typically soaked and fermented in earthenware, plastic, or enamel pots (*34*), so there is minimal direct light exposure. When ogi is subsequently cooked with added water to prepare porridges for immediate consumption, the duration and intensity of heat exposure would be expected to resemble conditions in our study. However, our study did not evaluate the retention of provitamin A carotenoids in ogi during storage. After the typical fermentation phase, ogi is often stored at room temperature (26–27 °C) and used progressively over many days while fermentation continues (*35*). Community-based studies are ongoing in rural Sub-Saharan Africa to investigate pre- and postharvest losses of provitamin A carotenoids in high β-carotene maize, including losses during processing and storage under typical household conditions.

We found that only modest losses of provitamin A carotenoids in high β-carotene maize could be directly attributed to household processing steps in the preparation of fermented maize porridges, which are traditional weaning foods in Sub-Saharan Africa. A recent study in an animal model, the Mongolian gerbil, found that the β-carotene in high β-carotene maize is highly bioavailable and efficacious in maintaining liver vitamin A stores (*36*). Together, these initial studies support the feasibility of maize biofortification as a means to alleviate vitamin A deficiency. A remaining feasibility question relates to the acceptability of the yellow/orange high β-carotene maize by African consumers of white maize.

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