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Effects of traditional African food processing on carotenoid content of [beta]-carotene-rich maize and a rapid HPLC method for the quantification of carotenoids in maize

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Effects of traditional African food processing on carotenoid content of β-carotene-rich maize and a rapid HPLC method for the quantification of carotenoids in maize

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ABSTRACT

The potential of maize (*Zea mays*) rich in provitamin A carotenoids to improve vitamin A nutrition in developing countries has been recognized and appropriate kernels have been developed. However, information on the effects of traditional food processing used by at-risk populations is scarce. In this study, the effect of traditional African food processing on the carotenoid content of β-carotene-rich maize was investigated. A rapid HPLC method for analyzing carotenoid contents of maize kernels was also developed to facilitate screening of large numbers of maize lines. In the food processing study, the concentration of all-trans-β-carotene decreased from 5.44 ± 0.97 μg/g to 3.56 ± 0.72 μg/g (dry weight basis) after soaking, fermentation and cooking ($P = 0.002$). Retention of carotenoids in the raw maize kernels after soaking for 24 h at room temperature ranged from 84.78 ± 10.68% for zeaxanthin to 75.1 ± 9.99% for all-trans-β-carotene. After the unfermented dough obtained from the soaked and ground kernels was stir-cooked for 9 min at 93 °C, the final total retention of carotenoids was 80.52 ± 13.33% for lutein, 81.44 ± 12.07% for zeaxanthin, 77.92 ± 9.97% for β-cryptoxanthin, 73.98 ± 4.55% for α-carotene and 67.86 ± 8.92% for all-trans-β-carotene. The final retention of all-trans-β-carotene was 67.58 ± 8.01% when the raw dough obtained from the soaked kernels was fermented for 48 h at room temperature. The final retention of lutein, zeaxanthin, β-cryptoxanthin, α-carotene and all-trans-β-carotene in the cooked fermented dough was 63.8 ± 8.01%, 68.0 ± 11.78%, 70.3 ± 11.57%, 71.7 ± 5.68%, and 65.6 ± 7.64%, respectively. Retention of total β-carotene (all-trans-β-carotene plus cis- and trans-isomers) averaged 66.6 ± 7.41% after soaking, fermentation and cooking. The vitamin A value of the raw maize kernels before soaking was 48.42 ± 8.3 μg Retinol Activity Equivalents (RAE)/100 g which decreased to 13.39 ± 2.6 μg RAE/100 g after soaking, fermentation and cooking (wet weight basis). It

was concluded that the vitamin A value decreased significantly during food processing, which partly reflected the dilution effect of the high moisture content of the final product.

A rapid extraction and saponification protocol was developed for accurate HPLC quantification of carotenoids in maize kernels. A published novel fast saponification protocol (Granado et al. J Food Comp Anal 2001;14:479-89) was modified for the measurement of carotenoids in maize kernels. Hot extraction, 50°C for 15 min, on a magnetic stir plate, was used to extract carotenoids from the maize kernels. Lipids were hydrolyzed in 5 minutes by exposing small volumes of extract to excess potassium hydroxide (KOH) while vortexing and then neutralized with 20% aqueous ascorbic acid. Accuracy of the developed method was evaluated by comparison with a national (SRM 2383 Baby Food Composite, National Institute of Standards and Technology, Gaithersburg, MD) standard reference material. Intra- and inter-assay precision were evaluated by using a composite of dried, ground conventional yellow maize. The measured values for 5 major carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin) were each within 1 SD of the certified values for SRM 2383. The intra-assay coefficient of variation (% CV) ranged from 2.6% for β-cryptoxanthin to 4.6% for all-trans-β-carotene. The inter-assay % CV ranged from 4.6% for lutein to 9.1% for all-trans-α-carotene. High accuracy and modest solvent needs make the rapid method a cost-effective approach to fast and precise HPLC analysis of the carotenoids in maize.

CHAPTER ONE

GENERAL INTRODUCTION

Introduction

Vitamin A deficiency disorders (VADD) are among the most widespread nutritional deficiency disorders in the developing world. VADD affect about 1.5 billion people worldwide and are significant public health problems in over 50 countries (Sommer 1995). There are another 27 countries where VADD exist in certain regions (Sommer 1995). Each year an estimated 3 to 10 million children worldwide have clinical xerophthalmia from which 300,000 go blind (Sommer 1995, Sight and Life 2002). Non-ocular systemic effects of vitamin A deficiency contribute significantly to the high prevalence of morbidity and mortality in at-risk populations (ACC/SCN 2000). Severe vitamin A deficiency is associated with a high fatality rate (60%) and marginal vitamin A deficiency is associated with a 23% increase in mortality among preschool children (McGuire 1993). Improvement of vitamin A status in deficient populations decreases all-cause mortality in children 6 months to 5 years of age by about 23% (WHO 2004), measles mortality by 50%, diarrhea mortality by 33% and the prevalence of severe xerophthalmia by 90% (AATF 2004). Improved vitamin A nutriture would be expected to prevent approximately 1.3 to 2.5 million deaths annually among children aged under 5 years (ACC/SCN 1994).

One of the approaches to alleviate micronutrient deficiencies, including vitamin A deficiency, in developing countries is biofortification (the use of biotechnology to develop staple food crops rich in micronutrients). Biofortification is a food-based approach to ensure adequate vitamin A intake through consumption of staple food crops rich in provitamin A carotenoids. The biofortification initiative is led by HarvestPlus; a global alliance of

research institutions and implementing agencies in developed and developing countries, coordinated by the International Center for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI). HarvestPlus is a global initiative supported by the Bill and Melinda Gates Foundation, USAID, World Bank, CIDA and DANIDA. Through the biofortification initiative, staple crops rich in zinc, iron, selenium, calcium and provitamin A are being developed (Graham 2003).

Maize follows wheat and rice as the most important cereal staple worldwide (FAO 1992). Enhancement of the provitamin A carotenoid content of maize will contribute substantially to provitamin A intake in at-risk populations. The yellow maize kernel contains provitamin A carotenoids (FAO/UN 1992). The carotenoids in yellow maize are present in amounts that may be genetically manipulated, whereas little or no carotenoid is found in white maize (Kimmons et al 1999). Most of the carotenoids in the conventional yellow maize kernel are found in the aleurone cell layers of the endosperm and only small amounts are in the germ (FAO/UN 1992). The potential of maize rich in provitamin A carotenoids to improve vitamin A nutrition in developing countries has been recognized and appropriate kernels have been developed through biofortification (Monsanto 2003). By means of biofortification, β-carotene content has been increased from 0.3 μg/g in conventional yellow maize to over 8 μg/g in β-carotene-rich maize (Rocheford 2003). Provitamin A carotenoids provide about 60% of the vitamin A intake in developed countries and 82 to 90% in developing countries (Granado et al 2001, McLaren and Frigg 2001), obtained mostly from fruits and vegetables (IFPRI 2000). Provitamin A carotenoid-rich foods contribute to meeting the vitamin A needs of both children and adults when eaten in sufficient quantities (Jalal 1998, Takyi 1999).

Information on the effects of maize processing on the carotenoid content of maize-based diets in at-risk populations is scarce. We investigated the effect of traditional African maize processing on the carotenoid content of β-carotene-rich maize to study its efficacy as a provitamin A source and to target provitamin A concentration in maize after processing.

The development of high carotenoid maize inbreds necessitates screening of large numbers of maize lines. Available assays for analysis of provitamin A and other carotenoids tend to be time-consuming and have lengthy saponification steps unsuitable for screening large numbers of samples. As part of this study, a rapid screening method for accurate quantification of the carotenoid content of maize was developed.

Thesis organization

This thesis presents an investigation of the effects of food processing on the carotenoid contents of β-carotene-rich maize and a rapid screening method for determining carotenoid contents of maize. This thesis is organized into four chapters. Chapter 1 presents a review of the literature. Chapter 2 is a manuscript prepared for submission for publication in the Plant Food for Human Nutrition journal. Chapter 3 is a manuscript prepared for submission for publication in the Journal of Food Composition Analysis. Chapter 4 summarizes the general conclusions for the two studies.

Literature Review

Part I. Enhancement of provitamin A carotenoid content in maize

Carotenoids derive their name because they constitute the major pigment in the edible root of the carrot plant, *Daucus carota* (Ong and Tee 1992). Approximately 600 carotenoids have been isolated from natural sources (Ong and Tee 1992). Some carotenoids (α-carotene,

β-carotene, and β-cryptoxanthin) are vitamin A precursors and thus are converted to vitamin A in the jejunal enterocytes, liver, kidney and testes (Kohlmeier 2003).

Enrichment of provitamin A carotenoid content of maize through conventional plant breeding and biotechnology is a viable strategy to alleviate the high prevalence of vitamin A deficiency in developing countries (Lindsay 2000, Parr and Bowell 2000, Schneeman 2000, Scott et al 2000, van den Berg et al 2000, Miettinen 2001). Enhancement of carotenoid content in staple crops such as maize has become feasible due to earlier work by Marrs 1981 and Armstrong et al 1990 who genetically defined the pathway for biosynthesis of carotenoids in the photosynthetic bacteria *Rhodopseudomonas capsulatand* and *Rhodobacter capsulatus,* respectively. They later determined the sequence of genes encoding the enzymes for carotenoid biosynthesis in the bacteria (Marrs 1981, Armstrong et al 1990). Another bacterium *Erwinia uredovor* containing carotenogenic genes was studied by Misawa et al 1990 who published the sequences and functions of the products of the carotenogenic genes in the bacterium. In higher plants and cyanobacteria, the four sequential desaturations of phytoene to produce lycopene are catalyzed by two enzymes, phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS) while in bacteria, such as *Erwinia uredovora*, and fungi, only one enzyme, bacterial carotene desaturase (CRTI) is required to convert phytoene to lycopene (Wurtzel 2002). The CRTI plays the role of both PDS and ZDS.

The elucidation of the genes and metabolic products of the carotenoid biosynthetic pathway in lower photosynthetic organisms provided insight into the carotenoid biosynthetic pathway in crop plants (Naik 2003). Documentation of the central isoprenoid pathway (Hirschberg 2001, Wurtzel 2002, Naik et al 2003) with emphasis on carotenoid biosynthesis

(Figure 1) has enabled genetic manipulation of maize and other crops to enhance the provitamin A carotenoid content. An over-expression of a gene in the pathway could lead to increased concentration of the desired carotenoid product. Surveys of diverse maize germplasm and F_1 hybrids revealed extensive variation in carotenoid content and composition. The variation indicates potential for enhancement of carotenoid content and composition in maize endosperm through introduction of the appropriate genes (Wurtzel 2002). Introduction of the CRTI gene into tomato resulted in 45% increase in β-carotene content as a result of concurrent upregulation of the other upstream genes (Figure 1) (Naik 2003, Romer 2000).

Through plant breeding, the β-carotene content of maize has been improved from 0.3 μg/g in conventional yellow maize to over 8 μg/g in high β-carotene maize (Rocheford 2003). The β-carotene content of maize can be improved to the 14 μg/g concentration range in the F_2 progeny and thus, breeding and selection for higher levels of β-carotene is feasible (Wurtzel 2002). Chromosome regions that influence levels of β-carotene in maize have been identified (Wurtzel 2002). Genetic engineering can be used to help develop β-carotene-rich maize for regions afflicted by VADD (Wurtzel 2002).

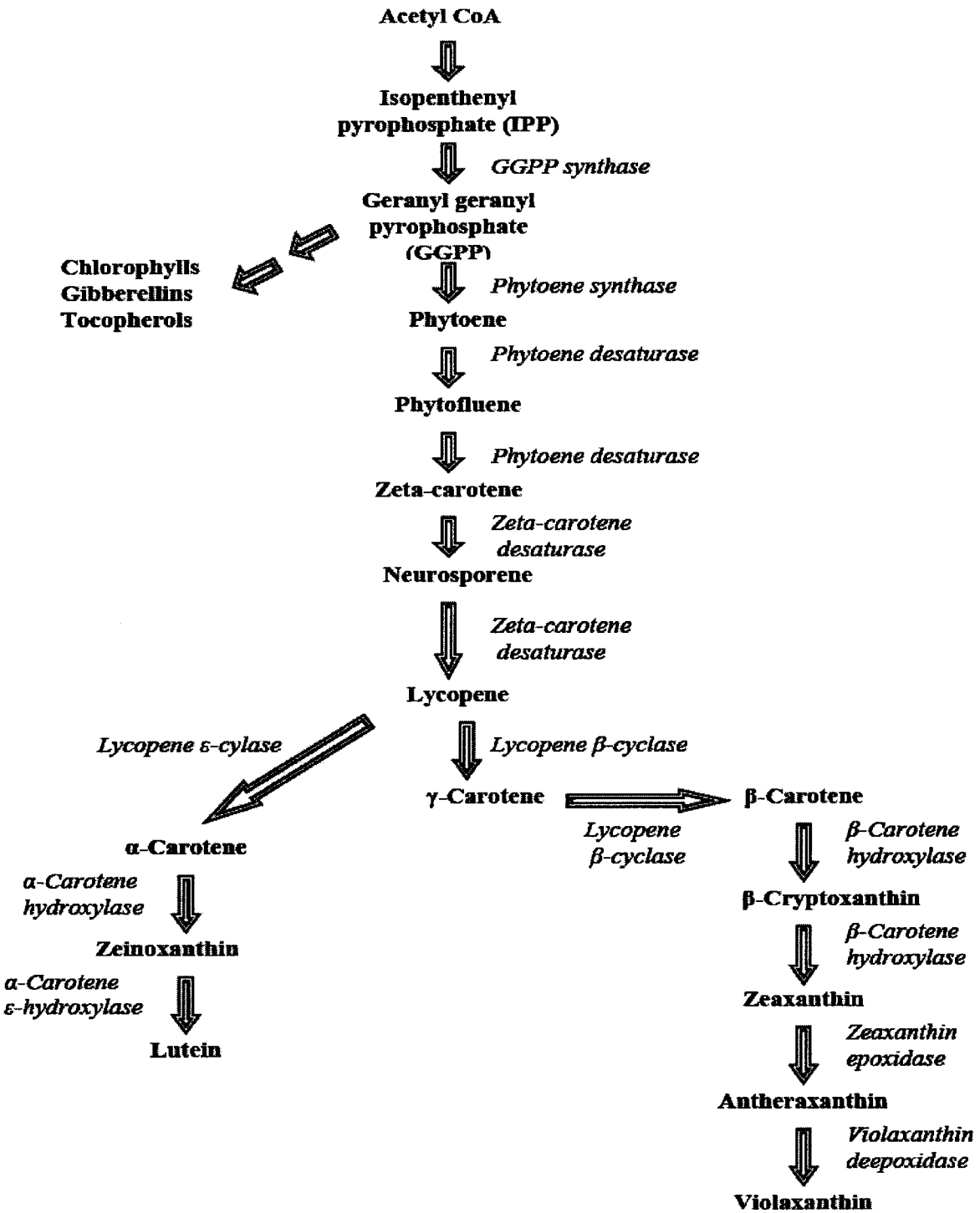

Figure 1. Major steps in the biosynthesis of carotenoids in higher plants; Biosynthesis of carotenoids from geranyl geranyl pyrophosphate.

(Sources: Hirschberg 2001, Wurtzel 2002, Naik et al 2003)

Methods for carotenoid analysis

Measurement of the provitamin A carotenoid content of biofortified staple crops requires efficient and cost-effective screening procedures. The rate of screening must keep pace with the research. One of the earliest methods for carotenoid analysis in dried maize kernels was developed by Quackenbush et al (1963). The carotenoids in maize were separated on a magnesia column and several fractions were collected. Several xanthophylls were identified, including xanthophyll esters and other pigment fractions (Quackenbush et al 1963).

The AOAC method (43.018-43.023) for carotenoid analysis involves hexane extraction and saponification in which 40% methanolic potassium hydroxide (KOH) is added and the mixture heated at 56 °C for 20 min (AOAC 1984). Livingston (1986) proposed an improvement in the AOAC method by extraction with hexane/toluene (3:1, v/v) followed by dilution to desired volume with 10% Na_2SO_4. This approach gave a higher recovery than the standard AOAC method.

Weber (1987) developed a high performance liquid chromatographic (HPLC) method for carotenoid analysis in maize in which 300 mg of ground sample was heated with ethanol for 5 min at 90 °C and then saponified with 0.08 mL 80% KOH in ethanol for 10 min at 90 °C. Carotenoids were extracted with hexane/toluene (10:8, v/v) and separated on a hand-packed 5-μm Ultrasphere silica column with a silica cartridge guard. However, the peaks for lutein and zeaxanthin were poorly resolved.

Darnoko et al (2000) using a C_{30} analytical HPLC column with a photodiode array detector obtained good resolution of the carotenoids in red palm oil by using a methyl tert-

butyl ether/methanol/water mixture as the mobile phase. The carotenoids were eluted within 45 minutes.

The procedure by Weber (1987) was modified later by Kurilich and Juvik (1999a) who simultaneously quantified carotenoids and tocopherols in extracts of maize kernels by HPLC. They subjected 600 mg of ground sample to 5 min ethanol precipitation followed by saponification with 120 μL of 80% KOH for 10 min (Kurilich and Juvik 1999a, Kurilich and Juvik 1999b). The analytes were separated by using two C_{18} reverse-phase columns in sequence: a polymeric 5-μm C_{18} reverse-phase column (Alltech OD52) followed by a monomeric 4-μm C_{18} reverse-phase column (Vydac 201TP54).

Moros et al (2002) using a C_{30} Carotenoid Column (YMC) separated, identified and quantified the major xanthophylls in maize (lutein, zeaxanthin, and *β*-cryptoxanthin) using HPLC and a photodiode array detector. Their mobile phase consisted of methyl tert-butyl ether/methanol/water (81:15:4, v/v/v). Using this solvent system, the three carotenoids, lutein, zeaxanthin, and *β*-cryptoxanthin were eluted within 25 min.

Saponification is usually employed during carotenoid analyses to eliminate interfering substances and to release esterified carotenoids. Most saponification steps take 30 min to 4 h (Granado et al 2001) or even 16 h (Ötles and Atli 2004) to complete and involve considerable volumes of solvents in the partition (Granado et al 2001). To hasten this step and conserve solvent for extraction, Granado et al (2001) developed a fast and low-cost saponification protocol for carotenoid analysis in vegetables, which they reported as the "shortcut" method. In their method, 5 mL of extract was vortexed for 3 min after addition of 0.5 mL 20% potassium hydroxide containing pyrogallol as antioxidant. This was followed by a single extraction of the carotenoids with hexane/methylene chloride (5:1, v/v).

Thus the standard protocol which requires about 4 h was decreased to about 0.5 h with an overall cost saving of about 90% (Granado et al 2001). For seven carotenoids in foods: lutein, zeaxanthin, α-carotene, β-cryptoxanthin, lycopene, α-cryptoxanthin and β-carotene, the shortcut method showed acceptable intra- and inter-assay variability similar to or better than obtained with a standard protocol. They observed a higher recovery for the carotenes and especially for lycopene when the shortcut method was compared with a standard method.

Adequate precautions must be taken during carotenoid analysis to avoid degradation and quantitative losses (Davies 1976, Schiedt and Liaaen-Jensen 1995). The precautions include completion of the analysis within the shortest possible time, use of reagent-grade chemicals, use of solvents free from harmful impurities, and use of peroxide-free ethyl ether and tetrahydrofuran and acid-free chloroform. Protection from white light, exclusion of oxygen by using a vacuum or inert gas (nitrogen or argon) and avoiding high temperature are also recommended.

Carotenoid concentrations in maize kernels

After analysis of 39 maize inbreds, Blessin et al (1963) reported ranges of 0.9 to 4.1 μg/g for carotenes and 18.6 to 48.0 μg/g for xanthophylls. Quackenbush et al (1963) evaluated 125 maize inbreds and found provitamin A contents ranging from trace to 7.3 μg/g and lutein from 2 to 33 μg/g. Among 200 maize hybrids, a range of 0.13 to 2.9 μg/g for β-carotene was observed (Egesel et al 2003).

Moros et al (2002) identified and quantified the major xanthophylls in maize, which are lutein, zeaxanthin, and *β*-cryptoxanthin. A total xanthophyll content of 21.97 μg/g was

observed for yellow dent maize. Xanthophyll concentrations were lutein, 15.7 μg/g; zeaxanthin, 5.7 μg/g; and *β*-cryptoxanthin, 0.57 μg/g. They reported that most of the xanthophylls were probably bound to the zein fraction of the proteins present in the maize kernel.

In a study on genetic variation in total carotene, iron and zinc contents of maize and cassava genotypes, Maziya-Dixon et al (2000) reported that the total carotenoid content of 16 yellow-seeded improved open-pollinated maize varieties range from 143 to 278 μg/g. A study of maize germplasm found several lines with levels of β-carotene between 13.14 μg/g and 16.18 μg/g (Rocheford, 2003).

Vitamin A activity of provitamin A carotenoids

About 50 known active provitamin A carotenoids exist (IFPRI 2000). Of these, β-carotene makes the largest contribution to vitamin A activity in plant foods (McLaren and Frigg 1997). Previously, the activity of β-carotene was considered to be one-sixth that of retinol, and for other less active provitamin A carotenoids it was 1/12 that of retinol (FAO/WHO 1988). Current studies suggest that the bioavailability of carotenoids in fruits and vegetables is much lower than previously estimated (de Pee et al 1995; de Pee and West 1996; de Pee et al 1998b; Jalal et al 1998). The bioconversion ratios of β-carotene to retinol in spinach for example were between 33:1 and 73:1, instead of 12:1 (IVACG 1999a). Studies among schoolchildren in Indonesia and breastfeeding women in Vietnam compared the increase in serum retinol resulting from feeding different types of foods (de Pee et al 1995). Based on the findings, the following apparent conversion factors were calculated:

- Retinol-rich foods (animal sources): 1 Retinol Activity Equivalent (RAE) = 1 μg retinol
- Fruits, pumpkin, orange-fleshed sweet potatoes: 1 RAE = 12 μg ß-carotene
- Dark-green leafy vegetables or carrots: 1 RAE = 26 μg ß-carotene

Based on consumption of a diet containing mixed fruit and vegetables, the retinol activity equivalence was established for conversion of provitamin A carotenoids to vitamin A (Institute of Medicine 2001). Thus:

1 RAE = 1 μg retinol = 12 μg β-carotene = 24 μg other provitamin A carotenoids.

Part II. Effects of processing on carotenoid content

Carotenoid losses due to processing

Literature on effects of food processing on the carotenoid content of maize is scarce. However, studies using other staple crops provide some insight regarding food processing effects on carotenoid retention.

Traditional methods of processing vegetables rich in provitamin A carotenoids at the household level such as thermal processing (Padmavat et al 1992), sun drying (Thane and Reddy 1997) and blanching (Negi and Roy 2000) cause significant degradation of carotenoids. As much as 90% of the provitamin A carotenoid content of foods can be destroyed through food preparation (Nestel and Nalubola 2003). Among the food preparation methods, steaming is better than boiling which in turn is better than stir-frying (Nestel and Nalubola 2003).

There is decreased retention of provitamin A carotenoids with longer processing times and higher processing temperatures. Modifications such as cooking with the lid on,

and decreasing the time between peeling or cutting and cooking/processing can improve retention significantly (Rodriguez-Amaya 1997). Better retention is also achieved when the product is protected from light and stored at a lower temperature for a shorter duration (Rodriguez-Amaya 1997).

Minimal cooking ensures better retention of provitamin A carotenoids but it also preserves the matrix in which they are sequestered and this limits their availability for absorption. Only about 10% of β-carotene may be absorbed from uncooked vegetables such as carrots (Boileau 1998).

A study of interactions between carotenoids and insoluble dietary fiber showed that the amount of α- and β-carotene bound to insoluble fiber decreased during hydrothermal processing (Borowska et al 2003). In carrots, from 52% to 64% β-carotene and from 66% to 74% of α-carotene were bound to insoluble dietary fiber. After heat treatment the quantity of α- and β-carotene bound to pectin increased by 2% and 9%, respectively.

Badifu et al (1995) studied the effects of blanching, solar-drying and storage conditions on β-carotene content in the fresh leaves of fluted pumpkin. After blanching, the concentration of β-carotene in the fresh leaves decreased from 98.9 mg/100 g to 83.8 mg/100 g. After dehydration the losses in β-carotene were in the ranges of 37.6 to 48.8% for steam-blanched, 40.5 to 51.3% for the water-blanched and 68.8 to 72.0% for the unblanched leaves (Badifu et al 1995). At ambient temperature (30°C) and at refrigeration temperature (7°C) there were significant decreases in the β-carotene contents of the leaves from 36.3 to 9.2% and 25.7 to 2.0%, respectively.

Savoy beets (*Beta vulgaris var bengalensis*) and fenugreek (*Trigonella foenum graecum*) were freeze-dried and stored for 9 months under cold storage conditions after

packaging in high density polyethylene film. Fenugreek retained 67% initial β-carotene whereas savoy beet leaves retained only 57% of the β-carotene under similar conditions (Negi and Roy, 2001). They concluded that percent retention depended on the type of vegetable involved.

Mosha et al 1997 studied the effects of traditional processing on the retention of α-carotene, β-carotene and total provitamin A carotenoids in amaranth, cowpea, peanut, pumpkin and sweet potato. The observed concentrations (mg/100 g, dry vegetables) were 26.79 to 44.74 total carotenoids, 4.16 to 19.12 β-carotene, and 0.99 to 10.26 α-carotene after processing.

Watson 1962 reported total carotenoids concentration of 4.8 μg/g in maize at harvest, which decreased to 1.08 μg/g after 36 months of storage at ambient temperature. In a study of carotenoids retention in soybeans, mean retentions of lutein and β-carotene were respectively, 92 and 73% in frozen, 62 and 62% in boiled, and 34 and 27% in freeze-dried soybeans (Simonne et al 2000).

Murphy et al 1975 developed the following equation for calculating the true retention of carotenoids after processing:

$$\%\,TR = \frac{Cc \times Gc}{Cr \times Gr} \times 100$$

Where:

TR = True retention

Cc = carotenoid concentration per g cooked food

Gc = g of cooked food

Cr = carotenoids concentration per g raw food

Gr = g of raw food

The percentage of carotenoid concentration (% Q) retained during food processing has been related as (Marcela et al 2004):

$$\%\,Q = \frac{Cc}{Cr} \times 100$$

In some cases values obtained with the %TR were also divided by the yield factors (Y) given by Y = Gc/Gr (Marcela et al 2004).

Losses of β-carotene from tubers during cooking appear to be minimal. In one study, van Jaarsveld et al (2001) found no loss of β-carotene when chopped or grated raw orange flesh sweet potato was allowed to stand 4 hours at ambient temperature. The β-carotene content of medium-sized orange flesh sweet potato ranged from 132 to 194 μg/g (van Jaarsveld et al 2001). After boiling in water with the lid on for 20 min, the true retention of β-carotene was 92%. When boiled without the lid, cooking took 30 min and the true retention was 88%. When different sizes of orange flesh sweet potato were boiled for 30 min covered with water in a closed pot, the true retention of β-carotene was 70 to 80% (van Jaarsveld et al 2001).

Increase in bioavailability due to food processing

When done appropriately, food processing can increase provitamin A carotenoid bioavailability. Cooking breaks down the structural matrix of the food and disrupts the carotenoid-protein complexes to enhance absorption of provitamin A carotenoids. For instance, β-carotene is three times more available from cooked and pureed than from raw

carrots and spinach (Rock et al 1998). Gartner et al (1997) found that lycopene bioavailability from thermally processed tomato paste was 2.5 times greater than from fresh tomatoes. In another study, lycopene was found to be more bioaccessible when thermally processed and an increase in lycopene content from 2.01 to 5.45 mg/g (from 54% to 164%) was observed after heating for 30 min at 88°C (Dewanto et al 2002). This might have occurred due to an increase in release of carotenoids from the food matrix (Dewanto et al 2002). In spite of some adverse effects of food processing on carotenoids, there is an increase in bioaccessibility of carotenoids from thermally processed vegetable products.

Effects of processing on cis-trans isomerization

The long conjugated isoprenoid carbon-carbon chain within carotenoids is susceptible to light, oxygen, heat, and acid degradation (Chen 1994). When foods are processed thermally, the *trans* double bonds become susceptible to geometric isomerization, creating a *cis* configuration. Updike and Schwartz (2003) studied the effects of thermal processing of fruits and vegetables on lutein and zeaxanthin *trans* to *cis* isomerization in broccoli, corn, peas, kale and spinach. After processing, they observed that the percent *cis* isomers of lutein and zeaxanthin increased up to 22% and 17% respectively. Processing produced an increase of 4 to 42% of the total lutein content resulting from enhanced extraction efficiency and recovery from heat treated tissues (Updike and Schwartz 2003).

Carotenoids are heat stable to at least 50 °C (Kearsley, 1981). When heated the naturally occurring *trans* double bond configuration can rearrange to the *cis* conformation. However, common unit food processing operations have only minor effects on *trans* to *cis*

isomerization of carotenoids (Kearsley, 1981). Carotenoids are stable to changes in pH in foods over the range of pH 2.0 to 7.0 (Kearsley, 1981).

Lipoxygenase co-oxidation of carotenoids

Lipoxygenase (LOX) does not act on carotenoids directly but produces hydroperoxides during its catalytic reactions with fatty acids (Kearsley 1981). The hydroperoxy fatty acids produced in turn co-oxidize and decolorize carotenoids by reacting with the highly unsaturated double bonds resulting in loss of vitamin A activity (Kearsley 1981). In a study of co-oxidation of β-carotene by soybean and pea recombinant lipoxygenase, Wu et al (1999) proposed a mechanism that involves a random attack along the alkene chain of the β-carotene. Intermediate oxidation compounds resulting from lipoxygenase action on fatty acids act as mild oxidizing agents to bleach carotenoid pigments (Hargreaves 2002). The specific substrates of lipoxygenase are polyunsaturated fatty acids containing no conjugated double bonds during stereospecific oxidation (Icard and Feillet 1997). In a study of oxido-reduction events in durum wheat pasta, Icard and Feillet (1997) reported that the bleaching of the yellow component of pasta is the result of destruction of carotenoids by a co-oxidation reaction of lipoxygenase. Borrelli et al (1999) in a study of durum wheat lipoxygenase activity and other quality parameters that affect pasta color, observed a decrease of 16.3% in semolina β-carotene content during pasta dough processing. The phase mainly responsible for pigment loss was pasta dough processing in which the isoenzyme forms LOX-2 and LOX-3, active at the dough pH of 6.6, were responsible for loss of color. Optimum pH for oxidation of linoleic acid by lipoxygenase, which yields hydroperoxides for co-oxidation of carotenoids, was 5.6-5.7,

whereas optimal pH for co-oxidation of β-carotene was 6.0 (Aziz et al 1999). Co-oxidation reactions of carotenoids are multiple isokinetic forms of a first order reaction (Jaren-Galan 1997). Enzymatic or non-enzymatic decoloring of carotenoids is a theoretical reaction that can be explained as a loss of conjugation in a sequence of conjugated double bonds (Jaren-Galan 1997).

Part III. Maize origin, processing and consumption

Maize origin, components and consumption

Maize is an important food for many people in developing countries providing significant amounts of energy and protein (FAO 1992). The maize crop is not native to Africa but was introduced by Portuguese explorers in the beginning of the 16th century (The Rockefeller Foundation 2004). After its introduction, maize has become Africa's second most important food crop second to cassava. At the beginning of the harvest season throughout Africa, considerable amount of maize is consumed fresh on-the-cob as parched, baked, roasted or boiled (IITA 2004b, Rockefeller Foundation 2004). Most African countries have populations whose per capita consumption is more than 200 g dry maize per day providing up to 45 and 59% of protein and energy intakes (Table 1) (FAO and INPhO 1998). In poor rural areas men may consume about 600 g and women about 400 g of dry maize per day (FAO and INPhO 1998). Maize is a staple food for an estimated 50% of the population in sub-Saharan Africa where it is an important source of carbohydrate, protein, iron, B vitamins, and minerals (IITA 2004b). Maize forms a starchy base in a wide variety of porridges, pastes, grits, and beer (IITA 2004b).

Among 145 countries around the world, 22 have per capita consumption of more than 100 g of maize per day (FAO 1984). Among Guatemalan children consumption varies from 64 to 120 g per day which provides about 30% of the daily protein intake and close to 40% of the daily energy intake (FAO 1992). Pre-school children were reported to consume 226 g per day from tortillas which provided 47% of energy intake (Garcia and Urrutia 1978).

The dry maize kernel consists of 83% endosperm, 11% germ, 5% pericarp and 1% as tip-cap or pedicel (IITA 2004a). The nutrient composition of the various compartments of the maize kernel differs greatly. For instance the germ of dent maize kernels contains, on a dry weight basis, 8.3%, 18.4%, 33.2%, 10.8% and 10.5% starch, protein, fat, sugar and ash, respectively while the endosperm contains 87.6%, 8.0%, 0.8%, 0.6% and 0.3% starch, protein, fat, sugar and ash, respectively (IITA 2004a, Watson 1987). Most of the protein and fat in the maize kernel is in the germ but due to the greater proportion of the endosperm, most of the protein from maize is supplied by the endosperm (FAO 1992). The whole maize kernel however, contains 73.4%, 9.1%, 4.4%, 1.9% and 1.4% starch, protein, fat, sugar and ash, respectively (IITA 2004a, Watson, 1987).

Maize fermentation and products

In the West African countries, fermented maize forms the basis of a variety of foods contributing to a large proportion of the daily carbohydrate intake (Jespersen *et al* 1994, Sanni et al 2002). During traditional African food processing, the maize grains are subjected to uncontrolled and spontaneous fermentation to prepare foods and beverages such as ogi, agidi, sekete, akadamu, kenkey and massa (Akinrele 1970, Sanni 1989, Oyeyiola

1990, Halm et al 1993). Acceptability characteristics important to consumers include appearance or color, odor, taste, sourness, texture and overall acceptability (Annan et al 2003).

A total of 106 microbial strains were isolated from different batches of fermented dough and were found to belong to the following ten genera; *Lactobacillus*, *Leuconostoc*, *Saccharomyces*, *Debaryomyces*, *Candida*, *Bacillus*, *Micrococcus*, *Klebsiella*, *Escherichia* and *Aspergillus.* Lactic acid bacteria were the predominant species (Sanni et al 2002). Hayford and Jespersen (1999) also reported that the dominant microflora in Ghanaian fermented maize dough were *L. fermentum, S. cerevisiae* and *C. krusei.* Cooking inhibits the growth of pathogenic bacteria. Growth of inoculated enteropathogenic *E. coli* and *S. typhimurium* was inhibited when fermented dough was cooked (Sanni et al 2002).

Ogi (fermented maize slurry) fermented with a mixed culture of *Lactobacillus* species and *Acetobacter* species was found to have enhanced nutrient quality in terms of increased concentrations of riboflavin and niacin above that found in unfermented grain (Akinrele 1970, Annan et al 2003). Controlled fermentation experiments using *Lactobacillus fermentum* and *Saccharomyces cerevisiae* inoculation showed that for most of the inoculated samples, the required pH of 3.7 was attained within 24 h of dough fermentation instead of 48 h as observed with spontaneous dough fermentations (Halm et al 1996, Annan et al 2003).

Organic products of fermented maize dough include lactic acid, acetic acid, esters, aldehydes and alcohols (Lindsay 1985, Stam et al 1998; Labuda et al 1997), butyric and propionic acids (Banigo and Muller 1972) and pentanoic, hexanoic, heptanoic, octanoic, benzoic dodecanoic acids (Halm et al 1993). The aroma profile of 'Uji' (fermented maize

and millet slurry) consumed in Kenya and Ethiopia, was high in hexanoic and octanoic acid as well as some alcohols including 1-propanol, 1-hexanol (Masha et al 1998).

Table 1. Consumption of maize and its contribution to daily energy and protein intakes

Country	Intake (g/person/day)	Energy (per person/day)	Protein (g/person/day)
Benin	160.5	481	12.7
Botswana	209.3	665	17.5
Cape Verde	334.1	1 052	28.0
Egypt	149.7	508	13.4
El Salvador	245.0	871	23.3
Guatemala	276.2	977	15.4
Honduras	255.9	878	22.8
Kenya	286.1	808	21.3
Lesotho	315.4	1002	26.4
Malawi	468.8	1422	37.6
Mexico	328.9	1061	27.1
Nepal	116.4	379	9.4
Nicaragua	131.0	472	11.1
Paraguay	131.2	445	11.6
Philippines	152.1	399	8.7
Romania	128.6	373	8.6
Singapore	122.2	345	8.6
South Africa	314.7	961	24.6
Swaziland	381.4	1279	33.7
Tanzania	129.1	421	10.0
Togo	136.9	411	10.8
Venezuela	118.3	339	7.4
Zambia	418.6	1226	31.3
Zimbabwe	330.9	958	25.2

(Sources: FAO and INPhO 1998)

In parts of Africa, several food preparations are made from maize (Table 2) most of which are eaten by all age groups (Mensah et al 1991). The preparation can be in the form of a maize beverage such as mahewu in South Africa or maize porridge in many parts of Africa. Dumplings such as Ugali in Kenya or kenkey in Ghana are mostly eaten by adults and form a major component of the diet. In these regions, most people consume white maize. Due to the high maize consumption, yellow maize has the potential to provide substantial amounts of β-carotene which is a source of vitamin A (FAO 1992).

Table 2. Maize-based diets in Africa and consumption by age group

	Food	Substrate	Cooked type	Consumption
Ghana	Kenkey, banku	maize	dumpling	adults
Ghana	Koko/akasa	maize	porridge	all ages
Nigeria	Ogi	maize	porridge	all ages
Ethiopia	Njera	maize	pancake	all ages
Kenya, Uganda, and Tanzania	Uji	maize, millet sorghum	porridge	all ages
Kenya	Ugali	Maize	dumpling	all ages
South Africa	Mahewu	maize	beverage	adults

(Source: Mensah et al 1991).

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CHAPTER TWO

EFFECTS OF TRADITIONAL AFRICAN FOOD PROCESSING ON CAROTENOID CONTENTS OF β-CAROTENE-RICH MAIZE

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ABSTRACT

The effect of traditional African maize processing on the carotenoid content of β-carotene-rich maize was investigated using a Ghanaian maize processing method and high performance liquid chromatograph (HPLC) analysis. The concentration of all-trans-β-carotene decreased significantly from 5.44 ± 0.97 μg/g to 3.56 ± 0.72 μg/g (dry weight basis) after soaking, fermentation and cooking (P = 0.002). Retention of carotenoids in the raw maize kernels after soaking for 24 h at room temperature ranged from 84.78 ± 10.68% for zeaxanthin to 75.1 ± 9.99% for all-trans-β-carotene. When the unfermented dough obtained from the soaked ground kernels was stir-cooked for 9 min at 93°C, the final total retention of carotenoids was 80.52 ± 13.33% for lutein, 81.44 ± 12.07% for zeaxanthin, 77.92 ± 9.97% for β-cryptoxanthin, 73.98 ± 4.55% for α-carotene and 67.86 ± 8.92% for all-trans-β-carotene. The final retention of all-trans-β-carotene was 67.58 ± 8.01% when the raw dough obtained from the soaked kernels was fermented for 48 h at room temperature. The final retention of lutein, zeaxanthin, β-cryptoxanthin, α-carotene and all-trans-β-carotene in the cooked fermented dough was 63.8 ± 8.01%, 68.0 ± 11.78%, 70.3 ± 11.57%, 71.7 ± 5.68%, and 65.6 ± 7.64%, respectively. Retention of total β-carotene (all-trans-β-carotene plus cis- and trans-isomers) averaged 66.6 ± 7.41% after soaking,

fermentation and cooking. The vitamin A value of the raw maize kernels before soaking was 48.42 ± 8.3 μg Retinol Activity Equivalents (RAE)/100 g which decreased to 13.39 ± 2.6 μg RAE/100 g after soaking, fermentation and cooking (wet weight basis). It was concluded that the vitamin A value of the finished product was decreased significantly during food processing.

KEY WORDS Carotenoid, maize, processing, retention, vitamin A

Introduction

Maize (*Zea mays*) is particularly important in the diets of consumers in Africa, especially in West African countries. It is a major staple and a regular component of diets for both adults and children [1,2]. In this region, maize is processed into many food products including ugali (dumpling) in Kenya, kenkey (dumpling) and koko (thick porridge) in Ghana, ogi (fermented porridge) in Nigeria, uji (porridge) in Uganda, Kenya and Tanzania, mahewu (beverage) in South Africa and njera (pancake) in Ethiopia. Usually the processing steps involve winnowing to clean the dried grains, soaking, milling, fermentation, addition of water to form maize dough and cooking. Some of the products are blends of toasted maize and legumes [3,4,5]. In these processes, physical, chemical and enzymatic changes occur which alter the contents of nutrients. Components altered include proteins, carbohydrates, minerals, carotenoids and vitamins [6].

The processing of maize may result in more nutritious and suitable products. Mensah et al [7] observed a decrease in phytate content from 9.9 to 4.7 μg/g after fermentation for 48 hours. In some African countries, mothers prefer to feed porridge that has been fermented for at least 24 hours to prevent childhood diarrhea [8]. Nanson and

Fields [9] noted improvements in the concentrations of available lysine, methionine, and tryptophan during the fermentation of corn meal. Fermentation improved the protein quality as well as the level of lysine in maize, millet, sorghum, and other cereals [10].

Provitamin A carotenoids are present in yellow maize kernels in amounts that can be improved genetically [11]. White maize has little or no carotenoid content [11]. Most of the carotenoids in yellow maize kernels are found in the aleurone cell layers of the endosperm and only small amounts are found in the germ [11]. The potential of maize rich in β-carotene to improve vitamin A nutrition in developing countries has been recognized and appropriate kernels have been developed through the biofortification initiative [12]. Biofortification (the use of biotechnology and conventional plant breeding to develop staple food crops rich in micronutrients) is a food-based approach to ensure adequate intake of vitamin A and other nutrients through consumption of food crops rich in provitamin A carotenoids. Given the reliance on maize as a staple, enhancement of the provitamin A carotenoid content of maize will contribute substantially to provitamin A intake in at-risk populations.

Vitamin A deficiency disorders (VADD) are a major public health problem in over 50 countries and another 27 countries where VADD exist in parts of the regions [13]. At least 5 to 10 million children develop xerophthalmia every year, and among these, a quarter go blind [13]. It is thus exigent to find workable solutions to the VADD problem.

In this study, the effects of steeping for 24 h, fermentation for 48 h and cooking on carotenoid retention in β-carotene-rich maize were studied to obtain information on the effects of traditional African maize processing on the carotenoid content of maize. The stability of the carotenoids in the maize kernels during storage was also investigated.

Materials and methods

Maize sample and carotenoid standards

The yellow maize kernels were a mixture of two lines of enhanced β-carotene contents A619 × R30 and SAPH × R30 bulked together. The maize lines were developed by Dr. Torbert Rocheford, Department of Crop Sciences, University of Illinois, Urbana, IL, and grown in the facilities of The Monsanto Company, Kihei, Hawaii.

Pure carotenoid standards (Carotenature, Basel, Switzerland) were used to prepare internal standard calibration curves. A carotenoid derivative, β-apo-8'-carotenal (Fluka Chemie, Buchs, Switzerland) was used as internal standard. All solvents were HPLC grade and were purchased from Fisher Scientific Company, Fairlawn, NJ.

Maize Processing

A traditional Ghanaian maize processing method was employed [14,16,17] (Figure 1). A total of 500 g of dry maize kernels was steeped in water for 24 h [14] at room temperature. At the end of the 24 h steeping period, the soaked maize kernels were drained using a colander and milled with a cutting mill (Grindomix GM 200, Retsch GmbH, Germany) into fine maize flour to pass through a USA #40 standard sieve pore size 416 μm [15]. After milling, the flour was made into dough by adding water to give about 50% moisture (300 g flour plus 100 g water) and covered loosely. The dough was left to auto-ferment for 48 h at room temperature [16,17] while protected from light. A 250-gram portion of the unfermented dough obtained after soaking and 250 g of the fermented dough were each stir-cooked separately into a stiff dough, locally known as "banku" in Ghana. During cooking, 100 g of water was added to 250 g of the maize dough and stir-cooked for 9 min at 93°C.

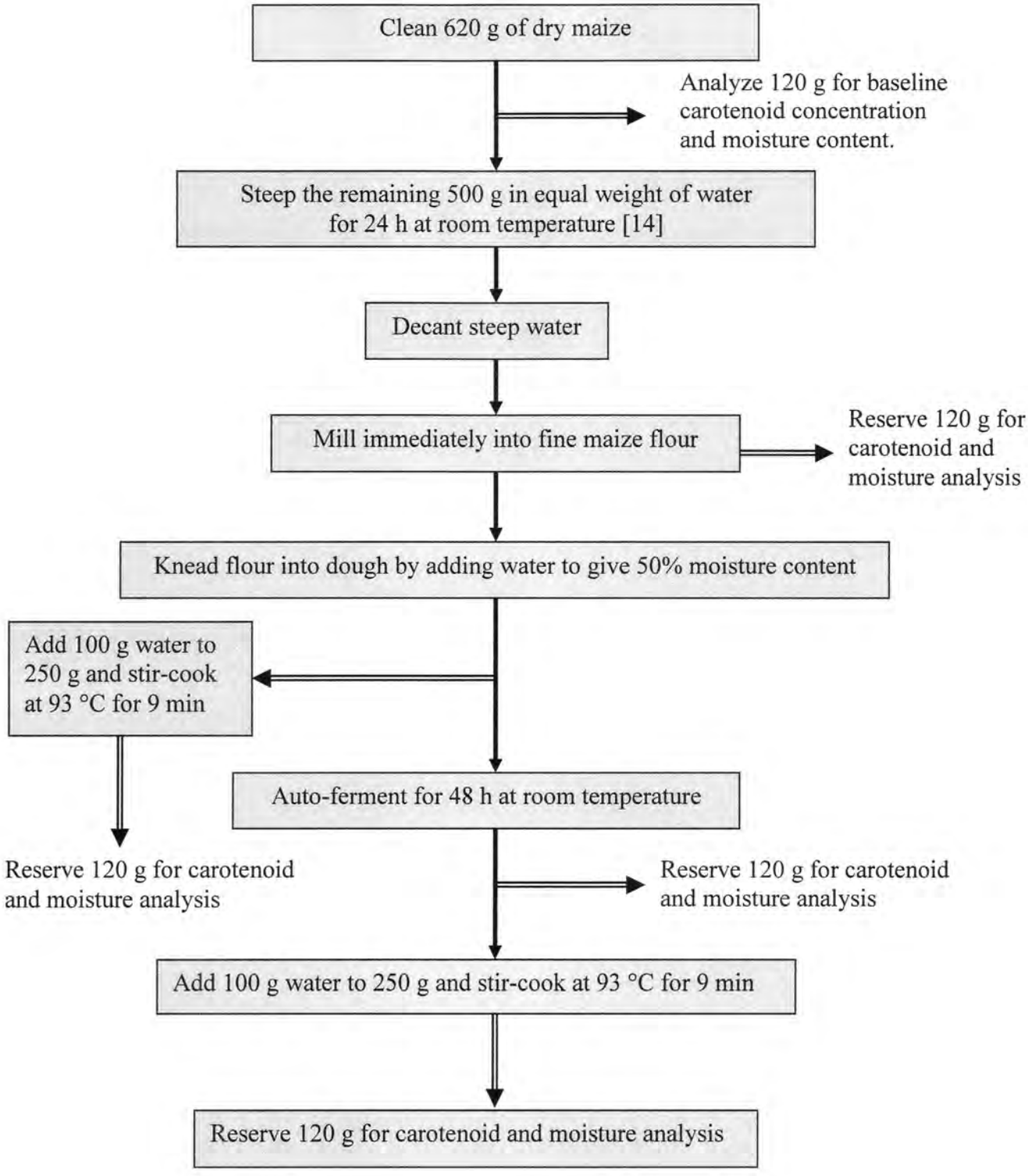

Figure 1. Flow chart for processing of β-carotene-rich maize into a fermented maize dumpling [14,16,17].

The entire processing protocol was replicated five times. As indicated in flow chart (Figure 1), 120 g samples were taken for carotenoid and moisture analysis at each critical stage of the processing. For each replicate of the processing procedure, a 120-gram aliquot of the dry maize kernels used for processing was ground into flour for baseline carotenoid analysis.

HPLC analysis of carotenoids in the maize samples

The extraction method employed was a modification of the procedure by Granado et al [18]. A 1.0-gram sample of finely-ground or processed maize was weighed into a 25.0-mL screw-capped culture tube and 6.0 mL methanol (containing 0.01% BHT) was added. The mixture was heated on a magnetic stir plate at 50 ^{o}C for 15 min while stirring continuously with magnetic stir-bar. After this step, the mixture was allowed to cool and 6.0 mL tetrahydrofuran was added and vortexed for 90 sec. The mixture was allowed to stand undisturbed for 5 min to allow fine particles to settle. Duplicate 0.5-mL aliquots of the upper clear extract were transferred into 15.0-mL screw-capped test tubes and 1.0 mL 40% methanolic potassium hydroxide (KOH) (containing 0.10 M pyrogallol) was added. The test tubes were flushed with argon for 10 sec and then vortexed for 5 min. At this point 2.0 mL 20% ascorbic acid in water (HPLC grade) was added and vortexed again for 30 sec. An internal standard (β-apo-8'-carotenal) was added and mixed well, followed by addition of 5.0 mL of a mixture of hexane/methylene chloride (5:1 v/v) (containing 0.01% BHT). The resulting solution was vortexed for 90 sec and then centrifuged for 5 min at 700 × g. The upper organic phase was transferred with a Pasteur pipette into a 10-mL test tube and completely evaporated to dryness in a vacuum evaporator (Savant SpeedVac, Forma Scientific Inc., Marietta, Ohio). The dry residue was reconstituted with 100-μL of methyl

tert-butyl ether followed by 300 μL methanol to obtain a total volume of 400 μL. A 100 μL aliquot of the reconstituted extract was injected into the high-performance liquid chromatography (HPLC) system for separation and quantification of carotenoids. Samples were analyzed in triplicate for five major carotenoids: α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin. The carotenoids were identified based on spectral data. The major cis-isomers of β-carotene; 9-cis-, 13-cis- and 15-cis-β-carotene were also determined to evaluate isomerization during processing. Five samples at different stages of processing were analyzed: raw ground dry maize, soaked ground maize, unfermented cooked maize dough, fermented maize dough and fermented cooked maize dough. In addition, the steep water after soaking for 24 h was analyzed.

HPLC system

The HPLC system (Waters Chromatography, Milford, MA) was controlled by Millennium 2010 chromatography manager software (version 2.1, Waters Corporation). The HPLC column was a C_{30} carotenoid analytical column, 4.6 × 150 mm (Waters Chromatography, Milford, MA). A 717Plus autosampler, 510 pump delivery system and 996 photodiode array detector were the other components of the HPLC system. The carotenoids were eluted using a linear mobile-phase gradient from 100% methanol (containing 1 g ammonium acetate/L) to 100% methyl-*tert*-butyl ether (MTBE) over 70 min. The mobile phase was filtered (Nylon-66 filter, 0.2 μm; Whatman International Ltd, Maidstone, England). Peak area integration was at 453 nm. The flow rate and injection volume were 1.0 mL/min and 100 μL, respectively.

Moisture Analysis

The air-oven method described by Osborne and Voogt [19] was used to determine moisture content of the raw and processed maize. Duplicate 2.0-g samples were weighed into a pre-heated and pre-weighed clean moisture dishes and placed in an oven at 100 °C overnight (at least 12 h). The moisture dishes containing the dried samples were then cooled in a desiccator and weighed. The moisture content of each sample was calculated using the following formula:

$$\text{Moisture} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where: W_1 = weight of sample before drying.

W_2 = weight of sample after drying.

Data analysis and calculations

To avoid confounding by the varying moisture contents of the samples at each stage of processing, all carotenoid concentrations were computed on a dry weight basis. Re-calculations to fresh weight basis were done when needed. The total losses of carotenoids were calculated as the difference between the carotenoid concentration of the raw and the final processed product on a dry weight basis. Losses of carotenoids at individual processing steps were calculated as the difference in concentrations between the preceding and subsequent steps. The formula by Marcela et al [20] was used for the calculation of retention of carotenoids after processing:

$$\%\,Q = \frac{Cc}{Cr} \times 100$$

Where;

% Q = percent concentration retained

Cc = carotenoid concentration per g of processed food

Cr = carotenoid concentration per g raw food

Carotenoid stability in the β-carotene-rich maize kernels was determined by comparing carotenoid concentration during storage at -20 °C at baseline and after 40 days by using two-sample *t*-test. Multiple comparison test to evaluate differences in carotenoid concentrations at indexed processing steps was done with Kenwardroger's adjustment. Differences were deemed significant at $P < 0.05$.

The U.S. Institute of Medicine retinol activity equivalence (RAE) factors [21] of 1 RAE = 1 μg retinol = 12 μg ß-carotene = 24 μg other provitamin A carotenoids (α-carotene, β-cryptoxanthin) were used to calculate the vitamin A value of the maize before and after processing.

Results

Carotenoids quantitation

Good separation of the carotenoids was achieved during HPLC analysis (Figure 2). Retention time for the carotenoids ranged from 20 min for lutein to 46 min for all-trans-β-carotene.

Moisture levels of the maize kernels and processed samples

The observed moisture content of the samples were 9.23 ± 0.72% for the raw maize kernels before soaking, 36.57 ± 2.62% for the soaked kernels, 61.69 ± 1.72% for the soaked, cooked

unfermented dough, 54.60 ± 2.53% for the soaked and fermented dough, and 62.11 ± 1.25% for the soaked, cooked and fermented dough.

Retention of carotenoids in the maize sample during processing

Focusing on all-trans-β-carotene, the major provitamin A carotenoid, there was a significant ($P < 0.0001$) decrease in concentration from 5.44 ± 0.97 μg/g to 3.56 ± 0.72 μg/g (dry weight basis) after soaking, fermentation and cooking (Figure 3). The concentrations of the other carotenoids also decreased (Table 1). The percent retention of all-trans-β-carotene after soaking for 24 h was 75.1 ± 10.0% (Table 2) and the resulting concentration was significantly lower than that in raw maize ($P < 0.0001$). No carotenoids were detected in the steep-water. Thus there was no detectable leaching of carotenoids during soaking.

After the unfermented dough obtained from the soaked kernels was stir-cooked for 9 min at 93°C, retention of carotenoids was 80.5 ± 13.3% for lutein, 81.4 ± 12.1% for zeaxanthin, 77.9 ± 10.0% for β-cryptoxanthin, 74.0 ± 4.6% for α-carotene and 67.9 ± 8.9% for all-trans-β-carotene. About 67.6 ± 8.0% of original all-trans-β-carotene content was still retained after the maize dough was fermented for 48 h at room temperature.

Retention of carotenoids in the soaked, fermented and cooked dough in relation to the raw maize kernels was: lutein, 63.8 ± 8.0%; zeaxanthin, 68.0 ± 11.8%; β-cryptoxanthin, 70.3 ± 11.6%; α-carotene, 71.7 ± 5.7%; all-trans-β-carotene, 65.6 ± 7.6% (Table 3). Soaking for 24 h resulted in the greatest decrease in the concentration of all-trans-β-carotene. This effect of soaking was similar among the remaining four carotenoids studied (Table 3) (Figure 3).

The combined losses during soaking and cooking were slightly less than the combined losses during soaking, fermentation and cooking for β-cryptoxanthin, 22.1 ± 10.0 vs 29.7 ± 11.6%; but significantly less for lutein, 19.5 ± 13.3 vs 36.2 ± 8.0%; and zeaxanthin, 18.5 ± 12.1 vs 32.0 ± 11.8%, respectively ($P < 0.05$); but not among the carotenes: α-carotene, 26.0 ± 4.5 vs 28.3 ± 5.6%; β-carotene, 32.1 ± 8.9 vs 34.4 ± 7.6%; where fermentation after soaking made little change in carotenoid retention ($P > 0.05$) (Figure 4). Soaking, fermentation and cooking all resulted in significant decreases in concentration of cis-isomers of β-carotene ($P < 0.001$) (Figure 5). Retention of total β-carotene including cis-isomers was 70.4 ± 8.4% after soaking and cooking, and 66.6 ± 7.4% after soaking, fermentation and cooking.

The traditional food processing resulted in significant loss in the vitamin A value of the β-carotene-rich maize. At baseline, the vitamin A value of the β-carotene-rich maize was 48.42 ± 8.3 μg RAE/100 g on wet weight basis with an average moisture content of 9.23 ± 0.07%. The vitamin A value decreased to 13.39 ± 2.6 μg RAE/100 g on wet weight basis with an average moisture content of 62.11 ± 1.25%.

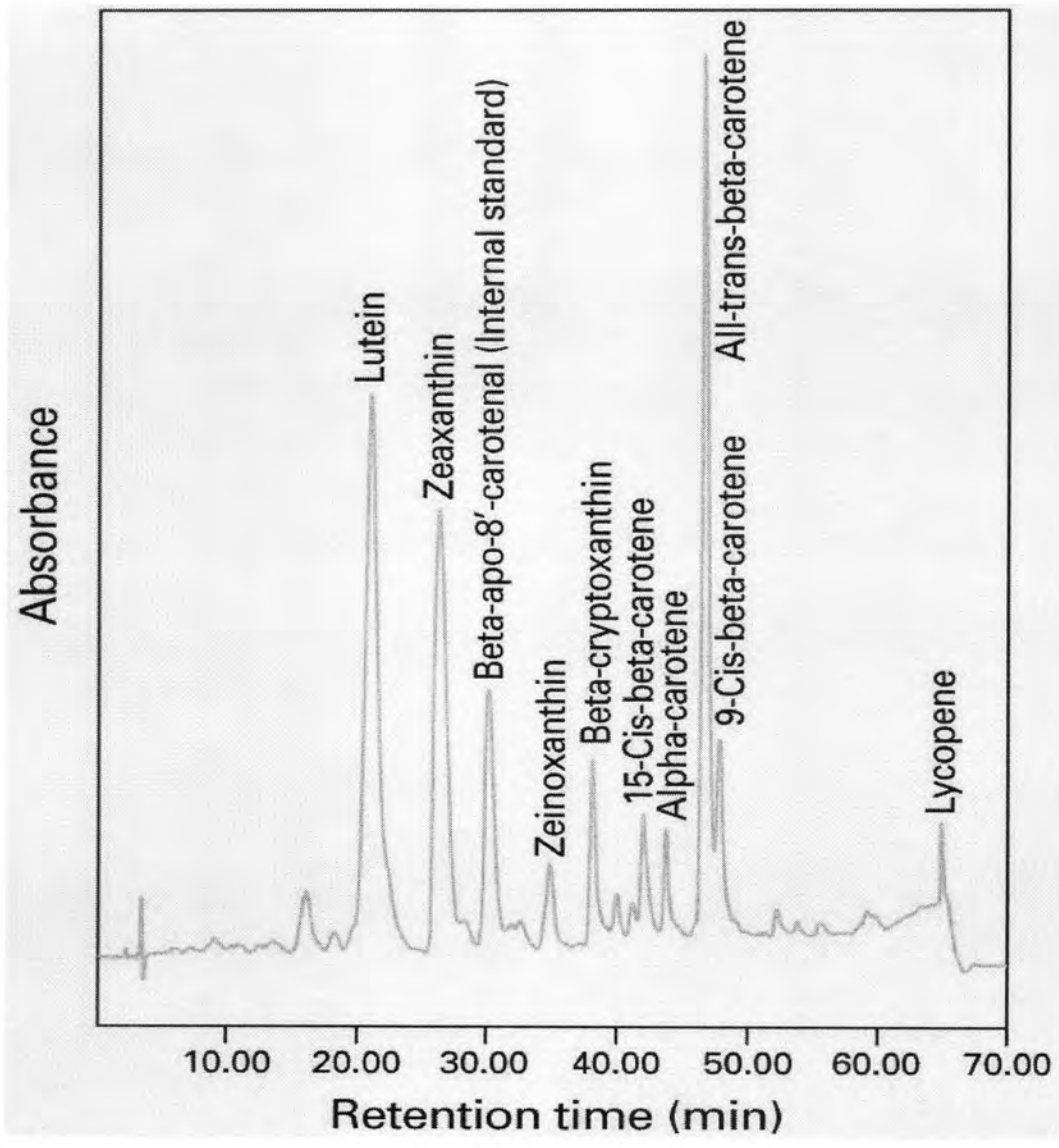

Figure 2. Elution profile of the carotenoids in β-carotene-rich maize. Separation was on a C_{30} carotenoid column (Waters Chromatography, Milford, MA). The mobile phase was a gradient from 100 % methanol containing 1 g ammonium acetate/L to 100 % methyl tert-butyl ether. The flow rate was 1.0 mL/min and detection was at 453 nm.

Carotenoid stability in the raw maize kernels during storage

There were decreases in the concentrations of carotenoids in the raw maize kernels during storage at -20°C (Table 3). The stability ranged from 95.1 ± 7.4% for zeaxanthin ($P = 0.1246$) to 80.7 ± 5.3% ($P < 0.0001$) for all-trans-β-carotene at the end of the 40 days storage period.

Table 1. Carotenoid concentrations at various stages during traditional African processing of β-carotene-rich maize*†

Carotenoids	Raw	Soaked 24 h	Soaked 24 h and cooked‡	Soaked and fermented 48 h	Soaked, fermented 48 h and cooked**
	(μg/g)	(μg/g)	(μg/g)	(μg/g)	(μg/g)
Lutein	14.76 ± 2.13[a]	12.12 ± 1.47[b]	11.54 ± 0.58[b]	10.24 ± 1.60[c]	9.40 ± 1.59[c]
Zeaxanthin	6.57 ± 1.04[a]	5.52 ± 0.70[b]	5.25 ± 0.28[b]	4.68 ± 0.73[c]	4.43 ± 0.77[c]
β-Cryptoxanthin	1.25 ± 0.17[a]	0.98 ± 0.15[b]	0.96 ± 0.09[b]	0.88 ± 0.11[b]	0.87 ± 0.14[b]
15-Cis-β-carotene	0.87 ± 0.14[a]	0.62 ± 0.14[b]	0.69 ± 0.13[b]	0.58 ± 0.08[b]	0.66 ± 0.09[b]
α-Carotene	0.67 ± 0.09[a]	0.53 ± 0.11[b]	0.49 ± 0.07[b]	0.48 ± 0.07[b]	0.48 ± 0.08[b]
All trans-β-carotene	5.44 ± 0.97[a]	4.09 ± 0.88[b]	3.57 ± 0.55[c]	3.67 ± 0.71[c]	3.56 ± 0.72[c]
9-Cis-β-carotene	1.54 ± 0.28[a]	1.00 ± 0.21[b]	1.02 ± 0.19[b]	0.94 ± 0.12[b]	0.96 ± 0.14[b]
Total β-carotene	7.91 ± 1.50[a]	5.76 ± 1.28[b]	5.42 ± 0.95[b]	5.24 ± 0.95[b]	5.25 ± 1.01[b]

*All values are on a dry weight basis.

†Means in the same row with different superscripts are significantly different, $P < 0.05$.

**Samples were cooked 9 min at 93°C.

Table 2. Retention of carotenoids during traditional African processing of β-carotene-rich Maize*

Carotenoid	Soaked 24 h	Soaked and cooked	Soaked and fermented for 48 h	Soaked, fermented and cooked
Lutein	82.46 ± 6.70	80.52 ± 13.33	69.73 ± 9.94	63.81 ± 8.01
Zeaxanthin	84.78 ± 10.68	81.44 ± 12.07	72.20 ± 13.70	67.99 ± 11.78
β-Cryptoxanthin	78.48 ± 8.89	77.92 ± 9.97	71.33 ± 11.31	70.31 ± 11.57
α-Carotene	78.12 ± 8.17	73.98 ± 4.55	72.05 ± 6.18	71.73 ± 5.68
Trans-β-carotene	75.14 ± 9.99	67.86 ± 8.92	67.58 ± 8.01	65.57 ± 7.64
Total β-carotene	72.79 ± 9.55	70.38 ± 8.36	66.57 ± 7.62	66.56 ± 7.41

*Values are percent retention ± SD.

Table 3. Concentration of carotenoids in β-carotene-rich maize at baseline and after 40 days of storage at -20°C

Carotenoids	Initial concentration	After 40 days at -20°C	Retention	P-value
	(μg/g)*	(μg/g)*	(%)	0.0004
Lutein	15.37 ± 0.44	13.29 ± 0.88	86.56± 6.29	
Zeaxanthin	6.55 ± 0.16	6.22 ± 0.44	95.14 ± 7.43	0.1246
β-Cryptoxanthin	1.27 ± 0.03	1.19 ± 0.06	93.98 ± 6.08	0.0247
α-Carotene	0.75 ± 0.05	0.61 ± 0.04	81.57 ± 8.44	0.0004
All-trans-β-carotene	5.96 ± 0.17	4.81 ± 0.34	80.66 ± 5.25	< 0.0001
Total β-carotene	8.74 ± 0.19	7.21 ± 0.48	82.62 ± 6.49	< 0.0001

*Values are mean ± SD on a dry weight basis.

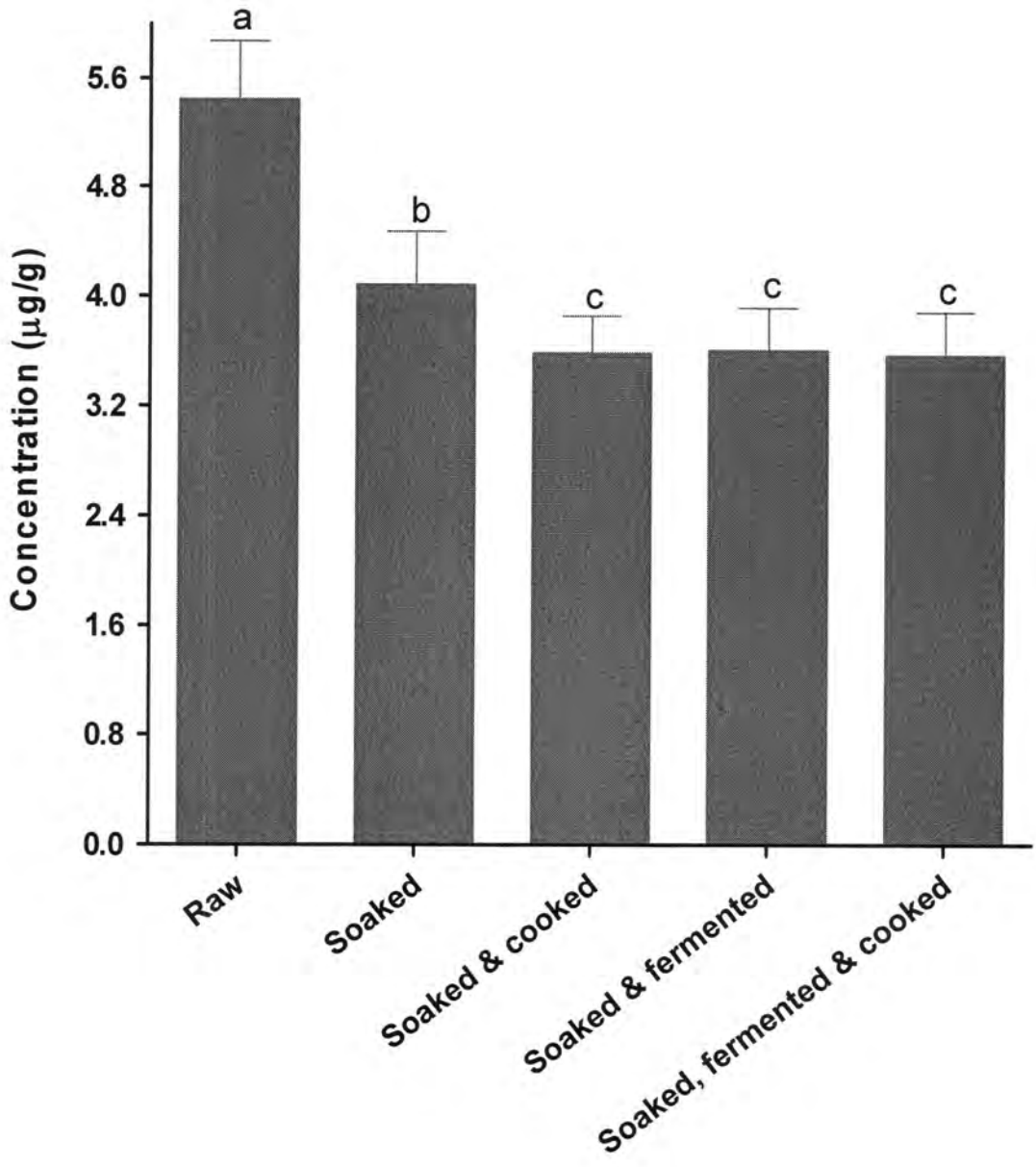

Figure 3. Retention of all-trans-β-carotene at various stages of traditional African maize processing of β-carotene-rich maize

Bars with different superscripts are significantly different; $P < 0.05$, concentrations are on a dry weight basis.

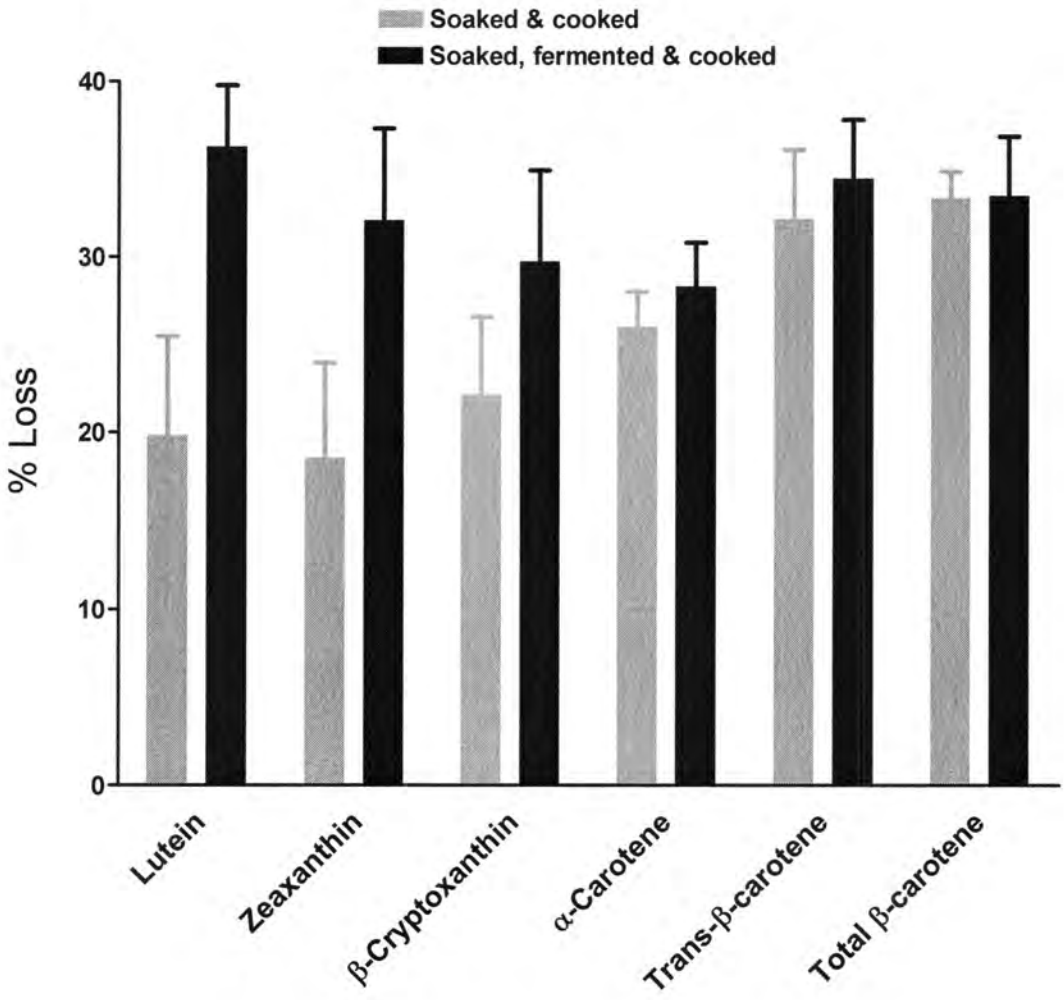

Figure 4. Combined effects of soaking, cooking and fermentation on carotenoid losses from β-carotene-rich maize during traditional African food processing

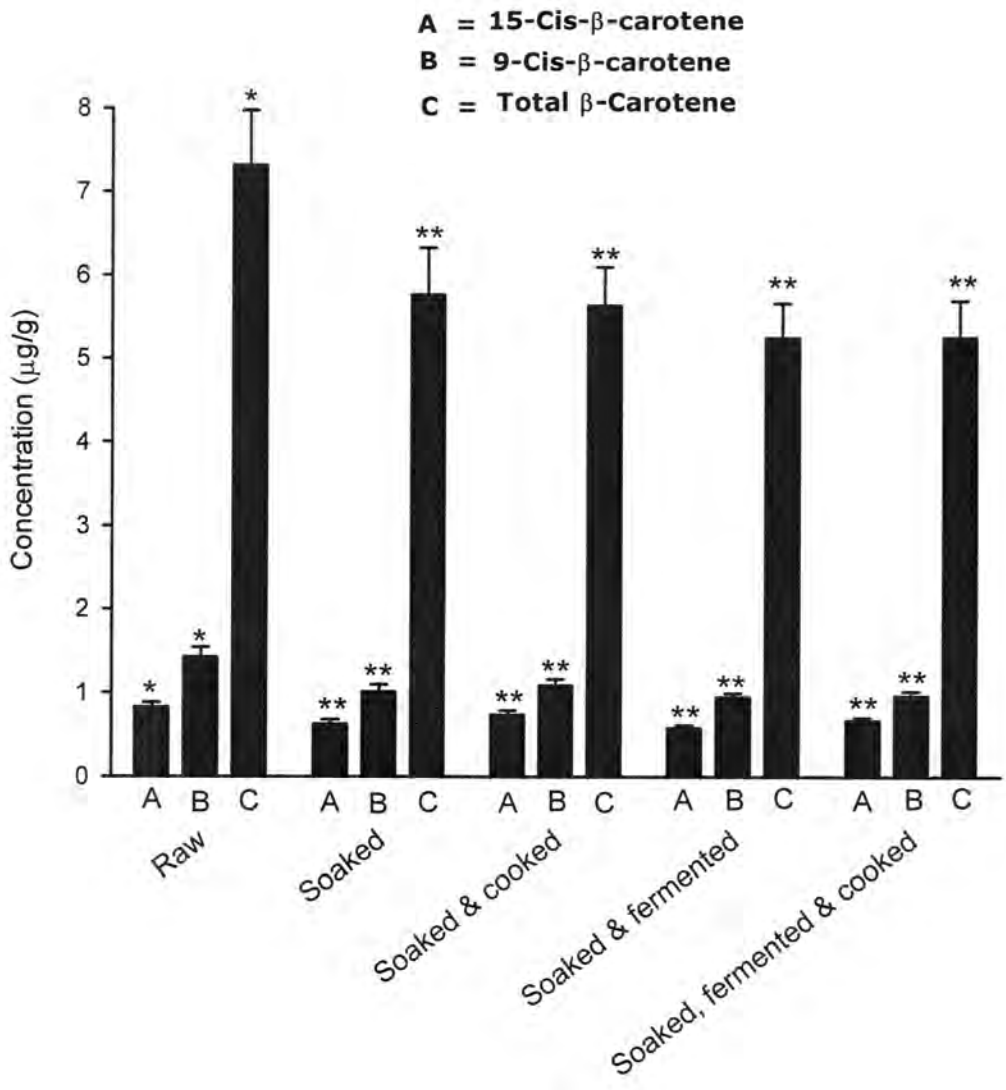

Figure 5. Concentrations of cis-isomers and total β-carotene in β-carotene-rich maize during traditional African maize processing

*Bars with different labels are significantly different; $P < 0.05$, concentrations are on a dry weight basis.

Discussion

The observed retention times for the carotenoids eluted from the β-carotene-rich maize conform to those observed for commercial carotenoid standards obtained for the study. The largest peak was for all-trans-β-carotene which eluted at 46 min. The isomers of β-carotene: 9-cis-β-carotene and 15-cis-β-carotene, were detected and quantified. The common carotenoids of conventional maize: lutein and zeaxanthin, were also quantified.

The moisture content of the raw maize kernels before soaking was within the range of published values [11]. The moisture content of the fermented maize dough observed in this study were similar to those previously reported [16,17]. The moisture content of the maize increased during soaking and subsequent processing due to imbibition of added water.

Significant losses of carotenoids were observed during processing of the β-carotene-rich maize using a traditional African method. Steeping the raw kernels in water resulted in the greatest decrease in carotenoid content. Enzymes associated with carotenoid degradation such as lipoxygenase [22] are activated when maize kernels are steeped in water. There is no direct action of lipoxygenase enzymes on carotenoids but they produce hydroperoxy fatty acids during their catalytic reactions which in turn co-oxidize and degrade carotenoids [22]. The greater decrease in carotenoid content during soaking than the other steps of processing might have been largely due to lipoxygenase action. There was little change in carotenoid contents after the soaking step during the processing. Fermentation and cooking after the fermentation step all resulted in minimal decreases in carotenoid contents, except for lutein and zeaxanthin which showed significant decreases in concentration. During fermentation, the pH of the dough drops to about 3.7 [16,23], which is below the optimum pH 6.0 for lipoxygenase co-oxidation of carotenoids [24]. However,

carotenoids are stable to changes in pH in foods over the range of pH 2.0 to 7.0 [25]. Lipoxygenase activity in the fermented dough is low due to the low pH resulting from fermentation. The short cooking time, 9 min at 93 °C, did not contribute significantly to carotenoid degradation. Lipoxygenase action, stir-cooking of the maize dough, exposure to oxygen and light all likely contributed to the significant losses of carotenoids observed in this study.

During processing of fluted pumpkin [22], it was observed that blanching resulted in a decrease in β-carotene concentration from 98.9 μg/g in the fresh leaves to 83.8 μg/g after steam- blanching. After dehydration, the losses in β-carotene were in the range of 37.6 to 48.8% for steam-blanched, 40.5 to 51.3% for the water-blanched and 68.8 to 72.0% for the unblanched leaves [22]. In a study of carotenoids retention in soybean, mean retentions of lutein and β-carotene were 92% and 73% in frozen, 62% and 62% in boiled, and 34% and 27% in freeze-dried soybeans, respectively [26]. Even though there were greater losses of carotenoids in these previous studies, the observed trend is similar to what we have observed for the β-carotene-rich maize during processing, but we did not observe significant losses during cooking.

Contrary to the degradative effect of food processing on carotenoids, under appropriate conditions, processing can increase provitamin A carotenoid bioavailability. Cooking breaks down the structural matrix of the food and disrupts the carotenoid-protein complexes to enhance absorption of provitamin A carotenoids [26]. For instance, β-carotene is three times more bioavailable from cooked and pureed than from raw carrots and spinach [27]. Gartner et al [28] found that lycopene bioavailability from thermally processed tomato paste was 2.5-times greater than from fresh tomatoes.

The calculated vitamin A value of the final product was 13.39 μg RAE. The RAE value may be interpreted that an intake of 300 g by a school-age child will supply about 40.17 μg RAE of vitamin A which is about 7% of the RDA of 600 μg RAE [21]. This estimate is based on the assumption that 12 μg β-carotene provides 1.0 μg RAE and 24 μg α-carotene or β-cryptoxanthin provides 1.0 μg RAE holds true for maize.

Significant losses of carotenoids occurred in storage at -20°C. This necessitated re-analysis of the raw maize kernels along the other samples during each replicate of the processing. Degradation of the carotenoids during storage at -20°C suggests that greater degradation could occur under ambient tropical conditions. Exposure to high humidity and intense sun light can lead to greater losses.

Deterioration of β-carotene during cold storage after freeze-drying has been reported to depend on the type of vegetable involved [29]. Retention of 67% of β-carotene was observed in fenugreek (*Trigonella foenum graecum*) and 57% in savoy beets (*Beta vulgaris var. bengalensis*) during cold storage for nine months [25].

Conclusions

Processing of the maize into either cooked dough or fermented cooked dough resulted in significant losses of carotenoids. Retention of provitamin A carotenoids in the end product (after soaking, fermentation and cooking) was: 71.7 ± 5.7% for α-carotene, 65.6 ± 7.6% for all-trans-β-carotene and 70.3 ± 11.6% for β-cryptoxanthin. Significant losses of carotenoids occurred during storage at -20 °C.

In this study, we have been able to establish the percent retention of provitamin A carotenoids and other carotenoids in β-carotene-rich maize during traditional

African food processing of maize. This information will be useful to the maize biofortification program and to vitamin A intervention programs.

Recommendations

Maize lines containing higher levels of provitamin A carotenoids should be developed so that the end product after processing will still contain high amounts of provitamin A carotenoids. An acceptability study should be done using carotenoid-rich maize to test differences in taste, smell, texture, color and overall acceptability compared with white maize traditionally favored by African consumers. The shelf life and retention of carotenoids during storage of the β-carotene-rich maize should also be tested under tropical environmental conditions. The actual vitamin A value of the β-carotene-rich maize should be determined using a human metabolic or bioefficacy study.

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CHAPTER THREE

A RAPID HPLC METHOD FOR QUANTIFICATION OF CAROTENOIDS IN MAIZE

A paper to be submitted to Journal of Food Composition Analysis

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Abstract

A rapid extraction and saponification protocol was developed for accurate HPLC quantification of carotenoids in maize kernels. A published novel fast saponification protocol (Granado et al. J Food Comp Anal 2001;14:479-89) was modified for the measurement of carotenoids in maize kernels. Hot extraction, 50 °C for 15 min, on a magnetic stir plate, was used to extract carotenoids from the maize kernels. Lipids were hydrolyzed in 5 minutes by exposing small volumes of extract to excess potassium hydroxide (KOH) while vortexing. Accuracy of the developed method was evaluated by comparison with a national (SRM 2383 Baby Food Composite, National Institute of Standards and Technology, Gaithersburg, MD) standard reference material. Intra- and inter-assay precision were evaluated by using a composite of dried, ground conventional yellow maize. The measured values for 5 major carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin) were each within 1 SD of the certified values for SRM 2383. The intra-assay coefficient of variation (% CV) ranged from 2.6% for β-cryptoxanthin to 4.6% for all-trans-β-carotene. The inter-assay % CV ranged from 4.6% for lutein to 9.1% for all-trans-α-carotene. High accuracy and modest solvent needs make the rapid method a cost-effective approach to fast and precise HPLC analysis of the carotenoids in maize and similar plant matrices.

KEY WORDS: Carotenoids, composite, extraction, maize kernels, saponification

Introduction

Enhancement of the carotenoid content of maize through biofortification has been recognized as a feasible food-based approach to improve vitamin A nutrition, especially in the developing world (IITA and Agriculture Research Service, 2004). Maize is third after wheat and rice as the most important cereal grain worldwide (FAO, 1992).

Available assays for provitamin A and other carotenoids in dried maize kernels are time-consuming and have lengthy saponification steps unsuitable for screening large numbers of samples. Saponification protocols used include heating, stirring and addition of variable quantities of KOH for stipulated times: 10% KOH for 30 min (Granado et al, 2001), 90% KOH for 10 min (Weber, 1987), 80% KOH for 15 min (Moros, 2002; Wong et al, 1998), 40% KOH for 30 min (Livingston, 1986) or for 16 h (Ötles and Atli, 2004). The saponification and extraction steps involve considerable volumes of solvents in the partition (Granado et al, 2001). The heating for prolonged periods renders most protocols unsuitable for rapid screening of large numbers of samples.

The objective of the current study was to shorten the saponification step and conserve solvents used for extraction. We adapted a fast and low-cost saponification protocol for carotenoid analysis in vegetables for use in dried maize kernels (Granado et al, 2001). A major modification of the protocol by Granado et al (2001) was the extraction of carotenoids at 50 °C for 15 min on a magnetic stir plate rather than at room temperature. The saponification involved only a 3-minute exposure of a small aliquot of extract to 40% methanolic KOH without heating. In this study, the standard protocol which takes about 4 h was reduced to about 0.5 h with overall cost savings of about 90% (Granado et al 2001).

Materials and Methods

Materials

Pure carotenoid standards (Carotenature, Basel, Switzerland) were used to prepare internal standard calibration curves. A carotenoid derivative, β-apo-8'-carotenal (Fluka Chemie, Buchs, Switzerland) was used as internal standard. All solvents were HPLC grade and were purchased from Fisher Scientific Company, Fairlawn, New Jersey. A standard reference material (SRM 2383 Baby Food Composite, National Institute of Standards and Technology, Gaithersburg, MD), β-carotene-rich maize kernels (The Monsanto Company, Kihei, Hawaii and Dr. Torbert Rocheford of the Crop Science Department, University of Illinois, Urbana, IL) and conventional yellow maize kernels (Grain Quality Laboratory, Iowa State University, Ames, IA) were obtained for method development and validation.

Rapid protocol for carotenoid analysis in dried maize kernels

A 1.0-gram aliquot of finely ground maize was weighed into a 25-mL screw-capped culture tube and 6.0 mL methanol (containing 0.01% BHT) was added. A magnetic stir-bar was added and the mixture was heated on a magnetic stir-plate at 50°C for 15 min while stirring. Then 6.0 mL tetrahydrofuran was added and vortexed 90 sec. The mixture was allowed to stand undisturbed for 5 min for the fine suspension to settle. A 0.5-mL aliquot of the upper clear extract was transferred into a 15-mL screw-capped test tube and 1.0 mL 40% methanolic KOH (containing 0.1 M pyrogallol) was added. The contents of the test tube were flushed with argon for 10 sec and vortexed for 5 min. An internal standard (β-apo-8'-carotenal) was added and mixed well, followed by addition of a 5-mL mixture of hexane/methylene chloride (5:1) (containing 0.01% BHT). The resulting solution was

vortexed 90 sec and then centrifuged 5 min at 700 × g. The upper organic phase was collected with a Pasteur pipette and evaporated to dryness in a vacuum evaporator (Savant SpeedVac, Forma Scientific Inc., Marietta, Ohio). The dry residue was reconstituted with 100 μL methyl tert-butyl ether followed by 300 μL methanol. A 100-μL aliquot was injected into the HPLC system for separation and quantification. Figure 1 shows the major differences in the rapid method compared with a standard method (Granado et al, 2001).

HPLC analysis

Analyses of extracts of the maize and SRM were performed using a Waters HPLC system (Waters Chromatography, Milford, MA) controlled by Waters Millennium 2010 Chromatography Manager software. The analytical column was a 5μ-C_{30} YMC analytical column 4.6 × 250 mm with guard cartridges (YMC Inc, Wilmington, NC). The other components of the HPLC system included a 717Plus autosampler (Millipore, Milford, MA), two 510 solvent-delivery systems and a UV-VIS 996 photodiode array detector, all from Waters. The carotenoids were eluted using a linear mobile phase gradient from 100% methanol (containing 1 g ammonium acetate/L) to 100% methyl-tert-butyl ether over 70 min. Each mobile phase was filtered (Nylon-66 filter, 0.2 μm; Whatman International Ltd, Maidstone, England). The flow rate and injection volume were 1.0 mL/min and 100 μL, respectively. Peak area integration was at 453 nm. Carotenoids were quantified using an internal standard curve. All analyses were performed under yellow light and at room temperature.

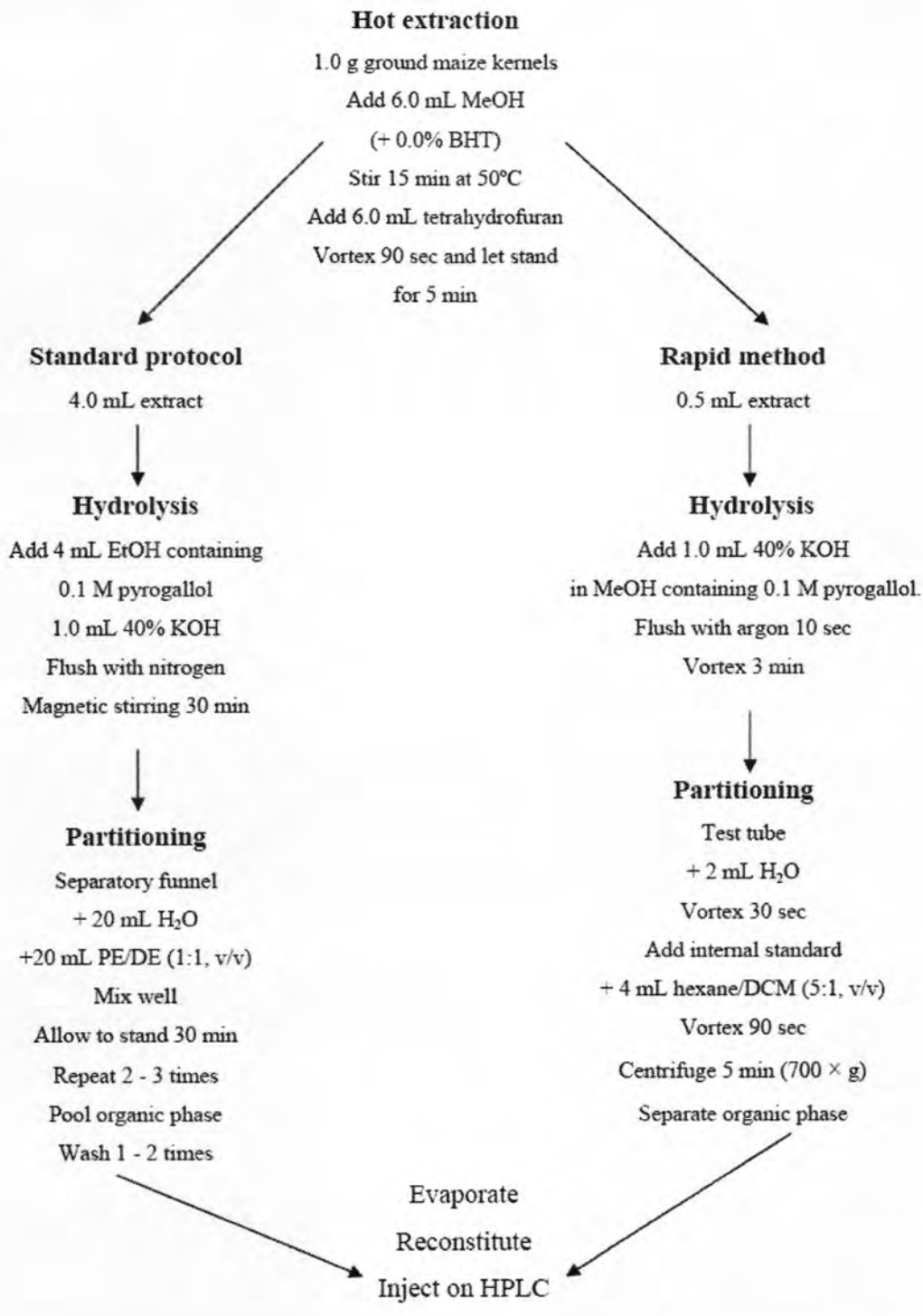

Figure 1. Schematic of the rapid and standard protocols for comparison (Source of standard method: Granado et al, 2001)

TABLE 1

Detailed protocol of the rapid method for carotenoid analysis in dried maize kernels

1	Weigh 1.0 g of ground maize into a 25-mL screw-capped culture tube.
2	Add 6.0 mL methanol (containing 0.01% BHT).
3	Add a magnetic stir-bar.
4	Extract on a magnetic hotplate at 50°C for 15 min while stirring.
5	Add 6.0 mL tetrahydrofuran.
6	Vortex 90 sec.
7	Let stand 3-5 min for fine suspension to settle.
8	Transfer an 0.5-mL aliquot of the extract into a 15-mL screw-capped test tube.
9	Add 1.0 mL 40% KOH in MeOH.
10	Flush with argon for 10 sec and cap the tubes.
11	Vortex 3 min (protect from light).
12	Vortex 30 sec.
13	Add internal standard (β-apo-8'-carotenal).
14	Add 5 mL hexane/methylene chloride (5:1 v/v) containing 0.01% BHT.
15	Vortex 90 sec.
16	Centrifuge 5 min at 700-1000 × g.
17	Remove upper organic phase and dry under vacuum.
18	Reconstitute the dried extract with 100 µL methyl tert-butyl ether followed by 300 µL methanol.
19	Inject 100 µL or a suitable volume into HPLC system.

Method development and validation

The rapid method (Table 1) was used to analyze the standard reference material, SRM 2383 Baby Food Composite, and a composite of ground conventional maize kernels to evaluate accuracy and precision, respectively. Intra- and inter-assay precision were determined. Analysis included five major carotenoids present in the maize kernels: α-carotene, all-trans-β-carotene, β-cryptoxanthin, lutein and zeaxanthin. Both the rapid method and a published method (Moros et al, 2002) were used simultaneously to analyze the β-carotene-rich maize kernels for purposes of comparison. Aliquots of conventional yellow maize kernels were analyzed to compare the effects of hot and cold extraction on carotenoid concentrations. The raw maize kernels were stored at -20°C while the ground conventional maize composite was stored at -70°C prior to analysis.

Hot and cold extraction

The importance of hot extraction during carotenoid analysis in dried maize kernels was investigated by comparing hot and cold extraction. The protocol in Table 1 was followed during the hot extraction. In the cold extraction, 1.0 g of finely ground maize was put into a screw-capped culture tube followed by 6.0 mL methanol (containing 0.01% BHT). The mixture was vortexed for 30 sec before 6.0 mL tetrahydrofuran was added and vortexed again for 5 min. Analysis was continued by following the protocol from step 7 in Table 1.

Kernel variability

Effect of sampling size on intra-assay precision was investigated by analyzing aliquots of finely ground maize prepared from 20, 30, 40, and 80 kernels. Concentrations of five major carotenoids were analyzed. Each sample was analyzed in triplicate.

Results and discussion

Biofortification approaches to alleviate malnutrition necessitate rapid screening of micronutrient-enriched staple crops. As shown in Figure 1, the duration of the entire extraction and saponification protocol is greatly shortened from about 4 h to 30 min. In the previously published method, a total reduction of 80-90% in costs was reported (Granado et al, 2001). The baseline separation of the carotenoids (Figure 2, Figure 3) indicated that complete hydrolysis of interfering esters and lipids was achieved.

The observed concentrations of carotenoids were significantly greater when hot extraction was used than when cold extraction was used ($P < 0.0001$) (Table 5). It is thus apparent that hot extraction is important for accurate analysis of carotenoids in dried maize samples. Hot extraction is a common step in published protocols for carotenoid analysis in dried maize kernels (AOAC 1984, Weber 1987, Kurilich and Juvik 1999). Heating disrupts the cellular matrix making carotenoids more accessible for extraction. However heating can also promote isomerization of β-carotene. The mild heating in our rapid method minimizes isomerization. The use of the antioxidants, ascorbic acid, BHT and pyrogallol, improves recovery of carotenoids (Kimura, 1990; Craft and Granado, 1993). Carotenoid losses due to the use of large volumes of solvents for extraction are avoided in this rapid method. The brief exposure to KOH, rapid neutralization of the KOH with ascorbic acid, and a single

extraction ensured higher recovery of carotenoids by reducing processing time. On the other hand, the use of small volumes of the extracting solvent can introduce recovery errors but such errors can be corrected with the use of an internal standard (Granado et al, 2001).

The concentrations of the five major carotenoids measured in the SRM 2383 Baby Food Composite were each within 1 standard deviation of the certified values (Table 2). The reproducibility as indicated by the coefficient of variation (% CV) was below 7% for each of the five carotenoids quantified. The SRM 2383 Baby Food Composite is prepared from cooked pureed vegetables and thus its suitability as a quality control material for the analysis of raw ground maize is uncertain. In an effort to compensate for this uncertainty, a composite of ground β-carotene-rich maize was analyzed both with the rapid method and a recently published method (Moros et al, 2002) (Table 3). In this analysis, higher concentrations were obtained with the rapid method. The higher carotenoid concentrations obtained with the rapid method may partly be due to the lower temperature used for heating, 50°C vs 85°C, and extraction of the carotenoids with hexane/methylene chloride mixture (5:1) rather than with pure hexane. The use of hexane/methylene chloride as extracting solvent in the rapid method resulted in better extraction of the xanthophylls, lutein and zeaxanthin, than the published method ($P < 0.0001$) (Table 3) (Figure 3). We did not directly compare our rapid method with the shortcut method (Granado et al 2001). However, Granado et al (2001) reported low recovery of all-trans-β-carotene in the SRM 2383 Baby Food Composite. We did not encounter this problem with our rapid method.

The intra-assay CV for the 5 carotenoids ranged from 2.6% for β-cryptoxanthin to 4.6% for all-trans-β-carotene. This shows the excellent reproducibility of the rapid method.

The inter-assay CV ranged from 4.6% for lutein to 9.1% for all-trans-α-carotene (Table 4), which also indicates good reproducibility.

Due to the high variability in the carotenoid contents of the maize lines, even from the same maize plant or cob, it was necessary to determine the sample size which resulted in the highest precision (Table 6). A sample size consisting of more than 40 maize kernels was needed for an acceptable precision (CV < 10%). The best precision was obtained with a sample size consisting of 80 kernels. Good intra-assay reproducibility is a pre-requisite for a successful analysis and hence for successful screening of maize varieties for provitamin A carotenoids.

In conclusion, the high accuracy and precision and modest solvent and sample needs make the rapid method a cost-effective approach to fast and precise HPLC analysis of the carotenoids in dried maize kernels.

TABLE 2

Concentration of carotenoids in Standard Reference Material (SRM) 2383 Baby Food Composite to evaluate accuracy of the rapid method

Carotenoid	Certified value for SRM[1]	Value obtained by rapid method	Coefficient of variation*
	(μg/g)**	(μg/g)**	(%)
All-trans α-carotene	0.85 ± 0.24	0.73 ± 0.02	3.31
All-trans β-carotene	2.40 ± 0.80	2.02 ± 0.07	3.53
Total β-carotene	3.12 ± 0.63	3.02 ± 0.08	2.81
β-Cryptoxanthin	1.38 ± 0.31	1.09 ± 0.07	6.45
Lutein	1.16 ± 0.33	1.25 ± 0.07	5.37
Zeaxanthin	0.86 ± 0.14	0.64 ± 0.05	6.00

[1]SRM 2383 Baby Food Composite, National Institute of Standards and Technology, Gaithersburg, MD.

*Number of replicates, n = 6.

**Values are means ± SD.

TABLE 3

Concentrations of carotenoids in β-carotene-rich maize determined using the rapid method and a published method

Carotenoid	Rapid method[1]	Published method[1,2]
	(μg/g)*	(μg/g)*
α-Carotene	0.544 ± 0.084	0.463 ± 0.036
All-trans-β-carotene	4.969 ± 0.110	4.239 ± 0.178
β-Cryptoxanthin	1.293 ± 0.030	1.171 ± 0.060
Lutein	15.197 ± 0.34[a]	9.447 ± 0.407 [b]
Zeaxanthin	8.311 ± 0.151 [a]	5.063 ± 0.199 [b]

[1]Number of replicates, n = 4.

*Values are means ± SD.

[2]Moros et al, 2002.

[b]Means in the same row with different superscripts are significantly different, $P < 0.05$.

TABLE 4

Inter- and intra-assay precision for carotenoids analyszed in a conventional yellow maize composite using the rapid method

Carotenoid	Intra-assay precision[1]		Inter-assay precision[2]	
	µg/g*	CV (%)	µg/g*	CV (%)
All-trans-α-carotene	0.19 ± 0.01	3.66	0.18 ± 0.02	9.07
All-trans-β-carotene	0.49 ± 0.02	4.62	0.49 ± 0.03	5.22
β-Cryptoxanthin	0.45 ± 0.01	2.55	0.41 ± 0.03	7.81
Lutein	13.72 ± 0.36	2.61	13.70 ± 0.83	4.59
Zeaxanthin	4.69 ± 0.12	2.66	4.59 ± 0.23	5.03

[1]Number of replicates, n = 4.

[2]Number of replicates, n = 27.

*Values are means ± SD.

TABLE 5

Concentrations of carotenoids in conventional yellow maize kernels using hot and cold extraction

Carotenoid[1]	Hot Extraction[2]	Cold extraction[2]
	(μg/g)*	(μg/g)*
α-Carotene	0.25 ± 0.03	0.14 ± 0.01
Trans-β-carotene	0.59 ± 0.04	0.31 ± 0.01
β-Cryptoxanthin	0.99 ± 0.06	0.60 ± 0.03
Lutein	8.18 ± 0.07	4.02 ± 0.07
Zeaxanthin	6.97 ± 0.42	3.29 ± 0.13

[1]All means for the cold extraction were significantly lower than for the hot extraction, $P < 0.05$.

[2]Number of replicates, $n = 3$.

*Values are means ± SD.

TABLE 6
Effect of sample size on intra-assay precision

	Sample size[1]							
Carotenoid	20 Kernels µg/g	%CV	30 Kernels µg/g	%CV	40 Kernels µg/g	%CV	80 Kernels µg/g	%CV
α-Carotene	0.18 ± 0.03	16.76	0.18 ± 0.02	13.91	0.18 ± 0.03	14.6	0.18 ± 0.01	3.95
All-trans-β-carotene	0.45 ± 0.07	14.46	0.37 ± 0.04	10.38	0.44 ± 0.02	5.45	0.41 ± 0.03	6.61
β-Cryptoxanthin	0.38 ± 0.06	12.66	0.34 ± 0.02	6.67	0.33 ± 0.02	5.19	0.33 ± 0.01	2.14
Lutein	13.02 ± 1.51	11.59	11.86 ± 0.53	4.49	12.12 ± 0.51	4.25	12.08 ± 0.18	1.47
Zeaxanthin	5.54 ± 0.14	2.55	5.03 ± 0.34	6.71	5.39 ± 0.50	9.36	5.15 ± 0.17	3.38

[1]Values are means ± SD for a triplicate analysis.

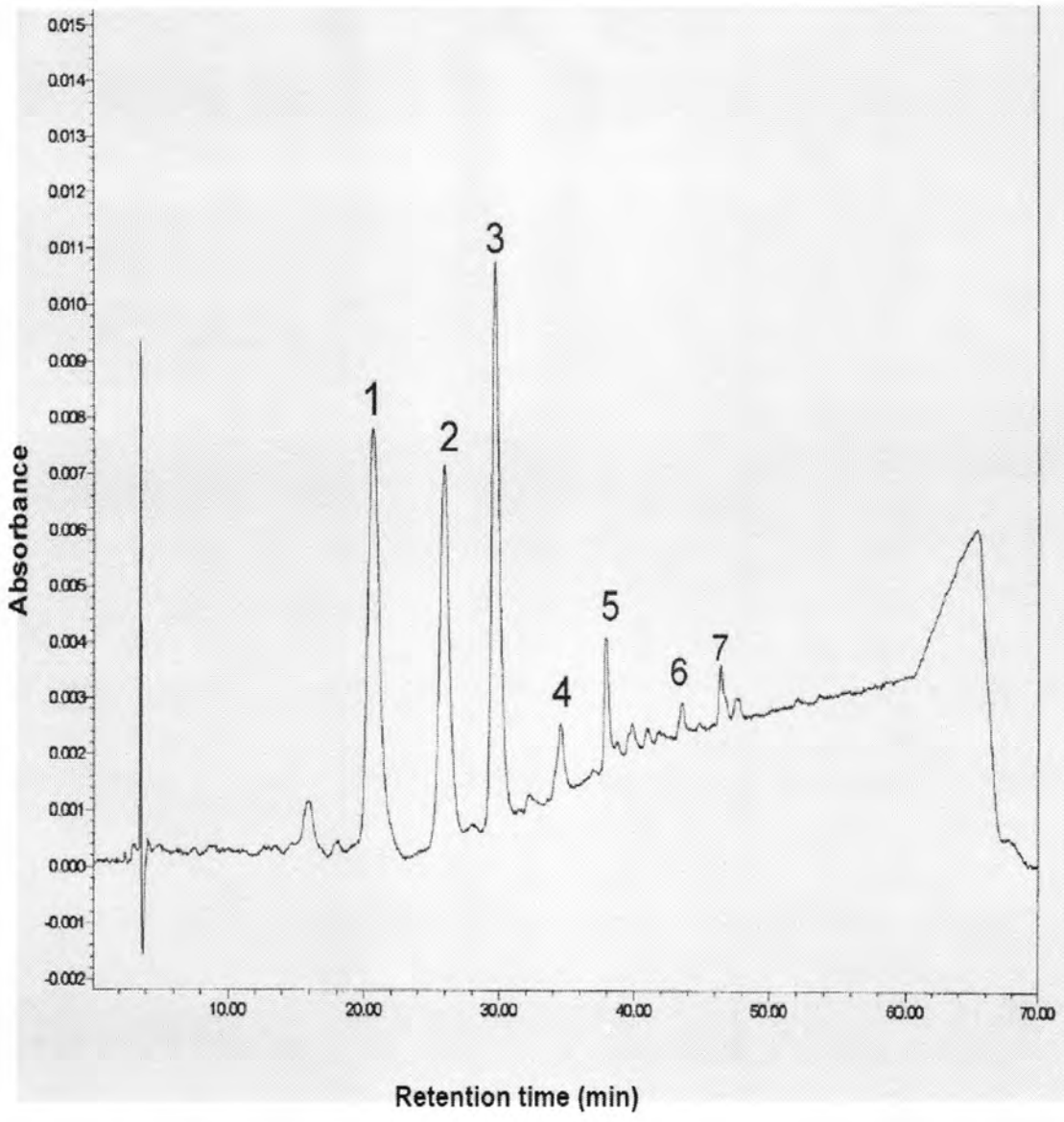

Figure 2. Elution profile of carotenoids in conventional yellow maize composite. Separation was on a C_{30} Carotenoids Column (Waters Chromatography, Milford, MA). The mobile phase was a gradient from 100 % methanol (plus 1 g ammonium acetate/L) to 100 % methyl tert-butyl ether. Flow rate was 1.0 mL/min and detection was at 453 nm. The numbered peaks were identified as; 1. Lutein, 2. Zeaxanthin, 3. β-Apo-8'-carotenal (internal standard), 4. Zeinoxanthin, 5. β-Cryptoxanthin, 6. α-Carotene, 7. All-trans-β-carotene.

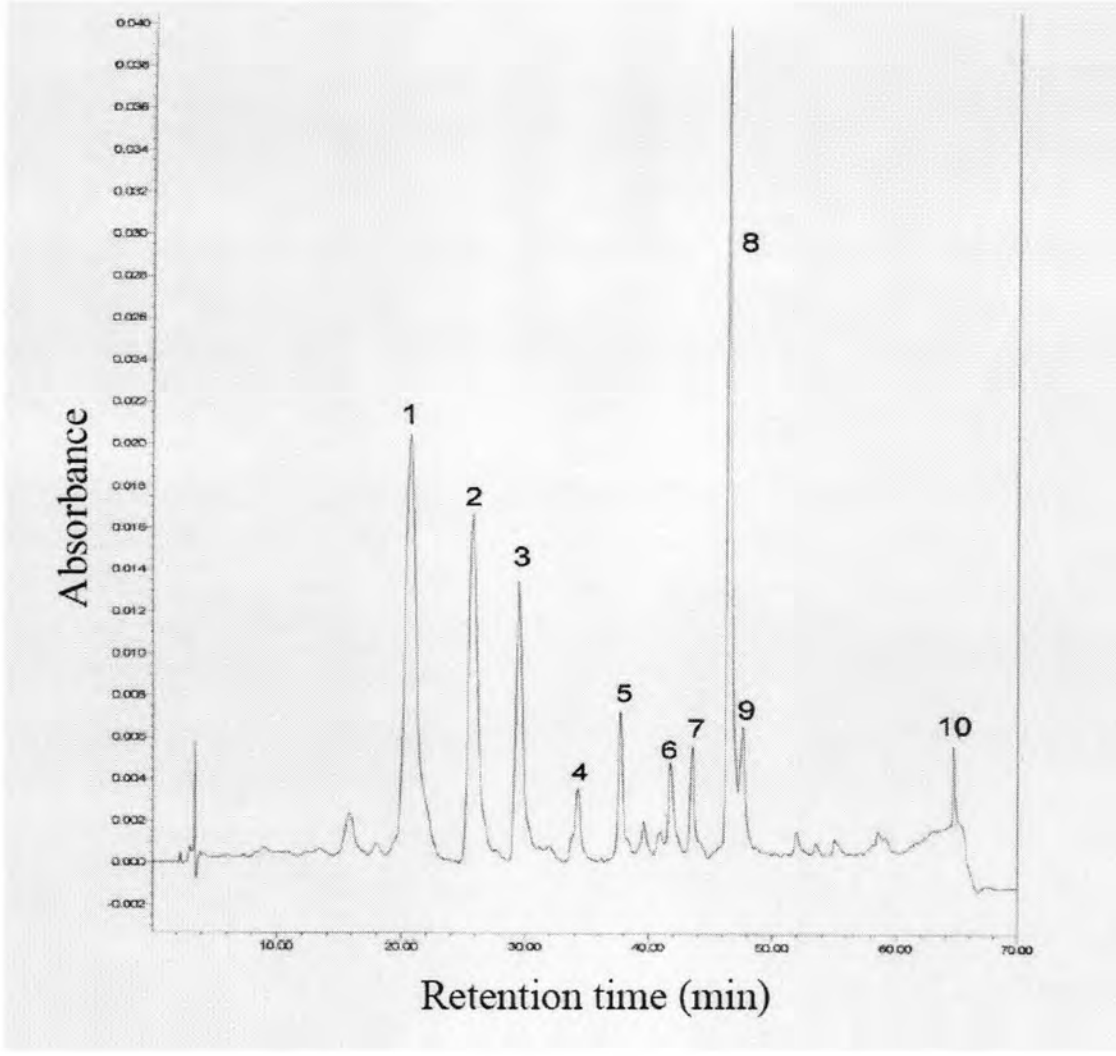

Figure 3. Elution profile of carotenoids in β-carotene-rich maize kernels. Separation was on a C_{30} Carotenoid Column (Waters Chromatography, Milford, MA). The mobile phase was a gradient from 100 % methanol (plus 1 g ammonium acetate/L) to 100 % methyl tert-butyl ether. Flow rate was 1.0 mL/min and detection was at 453 nm. The numbered peaks were identified as; 1. Lutein, 2. Zeaxanthin, 3. β-apo-8'-carotenal (internal standard), 4. Zeinoxanthin, 5. β-Cryptoxanthin, 6. 15-Cis-β-carotene, 7. α-Carotene, 8. All-trans-β-carotene, 9. 9-Cis-β-carotene, 10. Lycopene.

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CHAPTER FOUR

GENERAL CONCLUSIONS

Conclusions

This study investigated the effect of traditional African food processing on carotenoid retention in β-carotene-rich maize and developed a rapid method suitable for quantitative screening of large numbers of maize lines for carotenoid concentrations. There were significant losses of carotenoids during processing. Retention of provitamin A carotenoids in the end product (after soaking, fermentation and cooking) were: 70.3 ± 11.57% for β-cryptoxanthin, 71.7 ± 5.68% for α-carotene and 65.6 ± 7.64% for all-trans-β-carotene. There were significant losses of carotenoids during 40 days of storage of the maize kernels at -20 °C.

In this study, we have been able to establish the percent retention of provitamin A carotenoids and other carotenoids in β-carotene-rich maize during traditional African food processing of maize. This information will be useful to the maize biofortification program and to vitamin A intervention programs.

The rapid HPLC method developed as part of this thesis research was found to be accurate, precise and cost-effective. Due to a substantial decrease in the time required for analysis, the method will be suitable for rapid screening of large numbers of maize varieties for carotenoid concentrations. Thus the method will be useful to facilitate plant breeding to improve the carotenoid contents of staple crops and alleviate the high prevalence of vitamin A deficiency.

Suggestions for future work

Since the traditional African maize processing method studied resulted in significant losses of the carotenoids in the β-carotene-rich maize, it will be important to develop food processing methods that will provide the desirable sensory qualities but will retain the provitamin A carotenoids in the maize. Processing methods requiring shorter steeping and fermentation times may be helpful in retaining carotenoids in the final maize-based product. Maize lines containing higher levels of provitamin A carotenoids should be developed so that the end product after processing will still contain high amounts of provitamin A carotenoids. An acceptability study should be done using carotenoid-rich maize to test differences in taste, smell, texture, color and overall acceptability compared with white maize traditionally favored by African consumers. This should be done in a developing country to encompass the psycho-social environment. The shelf life and retention of carotenoids during storage of the β-carotene-rich maize should also be tested under tropical environmental conditions.

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