

# Retention of Provitamin A Carotenoids in Staple Crops Targeted for Biofortification in Africa: Cassava, Maize, and Sweet Potato

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*HarvestPlus, part of the Consultative Group on International Agriculture research (CGIAR) Program on Agriculture for Nutrition and Health (A4NH) uses conventional plant breeding techniques to develop staple food crops that are rich in micronutrients, a food-based approach to reduce micronutrient malnutrition known as biofortification. The nutritional breeding targets are established based on the food intake of target populations, nutrient losses during storage and processing and bioavailability. This review collates the evidence on the retention of provitamin A carotenoid (pVAC) after processing, cooking, and storing of the staple crops targeted for pVAC biofortification: cassava, maize, and sweet potato. Sun drying was more detrimental to the pVAC levels (27–56% retention) in cassava than shade (59%) or oven (55–91%) drying, while the pVAC retention levels (66–96%) in sweet potato were not significantly different among the various drying methods. Overall, boiling and steaming had higher pVAC retention (80–98%) compared to baking (30–70%) and frying (18–54%). Gari, the most frequently consumed form of cassava in West Africa had the lowest pVAC retention (10–30%). The pVAC retention of maize grain and cassava and sweet potato flour reached levels as low as 20% after 1–4 months of storage and was highly dependent on genotype. Therefore, we recommend that an evaluation of the pVAC degradation rate among different genotypes be performed before a high pVAC crop is promoted.*

**Keywords** Degradation, carotenoids, beta-carotene, yellow cassava, maize, OFSP, biofortification

## INTRODUCTION

Hidden hunger, caused by a lack of micronutrients in the diet, afflicts billions of people. It is caused by a lack of micronutrients in the diet. Fruits, vegetables, and animal products are rich in micronutrients, but these foods are often not available to the poor. Their daily diet consists mostly of a few inexpensive staple foods, such as cassava, maize and sweet potato, which are mostly white in color and devoid of carotenoids. HarvestPlus, part of the Consultative Group on International Agriculture research (CGIAR) Program on Agriculture for

Nutrition and Health (A4NH) uses conventional plant breeding techniques to develop varieties of staple food crops that are rich in micronutrients, a food-based approach to reduce micronutrient malnutrition known as biofortification. Our program is enhancing the levels of iron, zinc, and provitamin A carotenoids (pVAC), precursors of vitamin A, in staple foods without sacrificing essential agronomic qualities, such as high yield and resistance to disease and environmental stress. The contents of pVAC, in particular, are being increased in cassava, maize, and sweet potato to levels that will produce a measurable impact on the nutritional and health status in target African countries where the prevalence of vitamin A deficiency (VAD) is of a public health significance (WHO, 2009).

Vitamin A (retinol) is an essential micronutrient responsible for gene expression, growth, development, visual adaptation to darkness, and certain aspects of the immune system (Rando, 1990; West et al., 1991). An estimated 250 million preschool children and a substantial proportion of pregnant women in developing countries suffer from VAD, which can cause night blindness and increase the risk of child and

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maternal mortality (WHO, 2010). Over two decades ago, vitamin A supplementation was introduced globally as a short-term intervention to reduce VAD for children under five and postpartum women. Complementary food-based approaches have also been explored as a more sustainable and cost-effective strategies (Bouis et al., 2011).

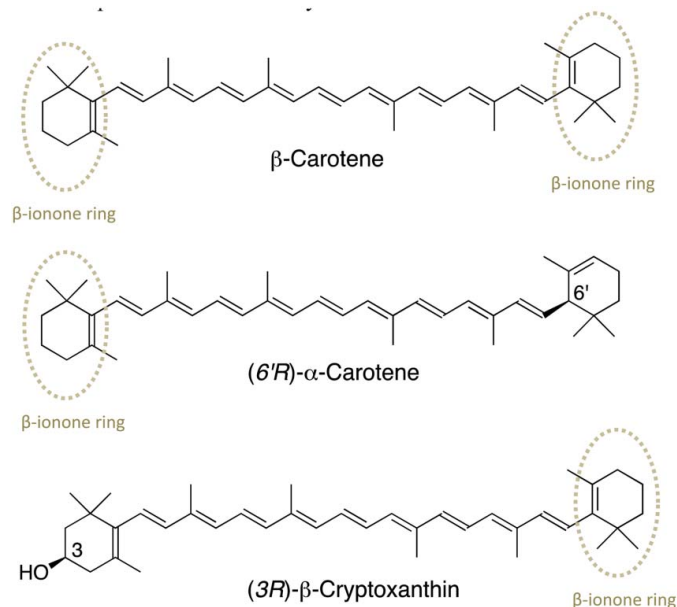
The current target concentration of pVAC for each crop will provide half of the estimated average requirement (EAR) for the target population at average staple food consumption levels. The target levels of pVAC are calculated based on average staple food intake (grams/day, FW), the losses of pVAC with processing, storage, and cooking, and the bioavailability and the retinol equivalency of the ingested pVAC (Hotz & McClafferty, 2007). Studies undertaken to-date by Harvest-Plus collaborators confirmed the high consumption rates of staple crops in rural areas of target African countries with a range of 150–250 g/day among children and 300–400 g/day among women for sweet potato and maize (Hotz et al., 2012a, Hotz et al., 2012b) and 700–900 g/day among women for cassava (unpublished data). Bioconversion rates of  $\beta$ -carotene to retinol were also high, with reported values of 7:1 (Li et al., 2010) and 3:1 (Muzhingi et al., 2011) for biofortified maize as compared to the current 12:1 conversion ratio recommended by the Institute of Medicine (IOM, 2001). However, the highly unsaturated structure of carotenoids makes them susceptible to post harvest degradation by heat, oxygen and light, and evaluation of pVAC retention in cassava, maize, and sweet potato by multiple laboratories has understandably produced varying results, depending on the post-harvest processing and cooking methods and the conditions and duration of storage.

This review critically evaluates the literature on pVAC-rich cassava, maize, and sweet potato, and assesses the findings on pVAC retention during processing, cooking, and storage. The percentage of pVAC retention as total carotenoids and/or  $\beta$ -carotene is presented and, when possible, the summarization of *trans*- to *cis*-isomer configuration is also reported.

## BACKGROUND

### Provitamin A Carotenoids

Carotenoids are usually C40 tetraterpenoids built from eight C5 isoprenoid units in an extensive conjugated double-bond system, which serves as the light-absorbing chromophore responsible for the yellow, orange, or red color in fruits and vegetables. The main condition for provitamin A activity is that the carotenoid must possess at least one  $\beta$ -ionone ring residue (Fig. 1).  $\beta$ -carotene has two  $\beta$ -ionone rings in its structure and is the most widespread of all carotenoids in food. Structurally,  $\beta$ -carotene can generate two molecules of vitamin A (retinol) when centrally cleaved, while  $\alpha$ -carotene and  $\beta$ -cryptoxanthin can generate only one molecule. These three carotenoids are the most commonly found precursors of vitamin A in plant food consumed by humans.



**Figure 1** Chemical structures of provitamin A carotenoids.

### Mechanisms of Carotenoid Degradation

The mechanisms of carotenoid degradation may involve: the reaction of carotenoids with atmospheric oxygen (autooxidation), light (photodegradation), and heat (thermal degradation), as well as degradation by the interactions of carotenoids with singlet oxygen, acid, metals, and free radicals. These mechanisms have been, for the most part, theorized based on a combination of studies in model systems, mostly in organic solutions; however, in food systems, the degradation mechanisms are more complex (Boon et al., 2010). In the intact fruit, vegetable, root/tuber or grain, the carotenoid molecules are less susceptible to degradation because they are protected within tissues through molecular interactions and association into supramolecular proteolipid complexes or crystal formation and the antioxidant regime within living cells. Differences in sequestration and intracellular localization of carotenoids in the tissue may be crucial factors in the susceptibility of these pigments to *trans-cis* isomerization and oxidation (Borsarelli & Mercadante 2009). Additionally, when the crop tissues are disrupted by cutting, chopping, shredding, cooking, or natural aging these physical barriers are affected thus rendering the carotenoids open to exposure to oxygen and oxidizing enzymes (Britton and Khachik, 2009). Given all these possible factors and their interactions, there are still many questions about the exact chain of reactions at the physiological level.

### Isomerization

All-*trans*-carotenoids, the most common form of carotenoids present in foods, can be isomerized to the *cis*-isomer by exposure to direct sunlight. The twisting of the molecule during the isomerization process may lead to an unpaired spin

state, which can react easily with oxygen to form carbon-peroxyl triplet diradicals unavailable for conversion to retinol. The isomerization can occur first, followed by the formation of a diradical, or they may both (isomerization and formation of diradical) occur simultaneously and reversibly (Mordi et al., 1993).

#### *Photodegradation*

The photochemical process may produce isomers and/or degraded products from oxidation or volatile compounds, such as  $\beta$ -ionone. The degraded products are lighter colored because of the resulting shorter chromophore, while in the isomerization process the *cis*-isomer retains most of the properties of the parent carotenoids. Furthermore, photooxidation produces species thought to be carotenoid radical (Mortensen and Skibsted, 1996). The photosensitized transformation of carotenoids has been studied using several sensitizer molecules, such as chlorophylls, iodine, rose Bengal (RB), and methylene blue (MB), and, in general terms, isomerization is the major pathway of reaction (Borsarelli & Mercadante, 2009).

#### *Autooxidation*

Formations of oxidation products with molecular weight lower than  $\beta$ -carotene have been extensively reported. Eccentric cleavage products such as retinal and  $\beta$ -apocarotenals, as well as epoxy carotenoids, were produced by autooxidation of  $\beta$ -carotene (Mordi et al 1993). Bechoff and colleagues investigating the  $\beta$ -carotene degradation in dried sweet potato chips at various temperature, water activity, and oxygen levels, observed that in all cases that,  $\beta$ -carotene submitted to oxidation was degraded into epoxides, apocarotenals, and apocarotenones, which were further oxidized into lighter and volatile compounds, e.g.,  $\beta$ -cyclocitral,  $\beta$ -ionone, 5,6-epoxy- $\beta$ -ionone, and dihydroactinidiolide (DHA) (Bechoff et al., 2010b). The authors suggested that the greater  $\beta$ -carotene degradation rate at lower water was due in particular to autooxidation.

#### *Singlet Oxidation*

Carotenoids can also interact with singlet oxygen present in the food matrix (which is more reactive than the triplet oxygen, present in the air) to yield triplet oxygen and an excited triplet carotenoid. The highly reactive molecules can interact with other free radicals, such as peroxyl or hydroxyl radicals, to generate further oxidative products (Britton G, 1995). Similarly to autooxidation,  $\beta$ -apocarotenals can also be originated from the oxidation of  $\beta$ -carotene with a radical generating reagent and singlet oxygen (McClure and Lieber, 1995 and Stratton et al., 1993).

#### *Thermal Degradation*

The isomerization and degradation of pure  $\beta$ -carotene were evaluated in an oven heated at temperatures between 50°C and

150°C for up to 30 minutes, as well as by reflux heating at 70°C for 140 minutes, using first-order kinetic decay (Chen and Huang 1998). The major isomers formed during heating were 13-*cis*- $\beta$ -carotene, both under oven and reflux heating, while the 13,15-di-*cis*- $\beta$ -carotene was only found at temperatures higher than 120°C. Similar results were obtained by Henry et al., 1998, heating  $\beta$ -carotene at several temperatures formed 13-*cis*- $\beta$ -carotene in higher amounts, followed by 9-*cis*- $\beta$ -carotene. In summary, isomerization is the main reaction that occurs during heating at atmospheric pressure and at temperatures lower than 100°C; the 13-*cis*-isomer is formed at higher rates than 9-*cis*-isomer; formation of oxidation products from  $\beta$ -carotene, such as epoxides and apo-carotenals, as well as di-*cis* isomers occur under higher temperature, longer time, and higher pressure (Mercadante, 2008).

#### *Retention Analysis*

Conditions necessary for isomerization and oxidation of carotenoids exist during preparation, processing, and storage of food, but are also artificially generated during laboratory analysis. Therefore, the inter-laboratorial discrepancies encountered in the literature are usually associated with the analytical practices during the extraction and measurements of the carotenoids and methodology used for calculating retention (true versus apparent retention). In general, errors associated with chromatography are minor, while those from extraction procedures are potentially significant (Howe & Tanumihardjo, 2006).

#### *Carotenoid Extraction*

Good laboratory practices include protection from light during analysis, which can be accomplished by the replacement of the laboratory lights to yellow lights. On the other hand, freeze-drying the samples prior to analysis is innocuous for the pVAC contents of fresh produce. No significant difference in the all-*trans*- $\beta$ -carotene content was observed on seven OFSP samples after freeze-drying (Bengtsson et al., 2008). A major problem in retention studies is the greater efficiency of carotenoid extraction in processed foods compared to unprocessed foods. Processed food crops reporting retention values above 100% can be a result of increased carotenoid extraction efficiency in processed crops compared to raw food, rather than true increases in the carotenoid content, as the enzymes for biosynthesis of carotenoids have already been inactivated during processing (Rodriguez-Amaya, 1997).

#### *Sampling*

One source of variation is the sampling method used, because the same sample cannot be quantified for carotenoids in unprocessed and processed tissue. It is suggested that in the case of cassava and sweet potato, the root be quartered longitudinally and two opposite sections be taken for raw sample

analysis, and the two remaining opposite sections be submitted for processing analysis (Rodriguez-Amaya, 1997; Ceballos et al., 2012).

The use of paired samples in retention studies is highly recommended to avoid any differences in the content of carotenoids in the raw versus the processed sample. Van Jaarsveld et al., 2006 reported a difference in the  $\beta$ -carotene content from 132 to 194  $\mu\text{g/g}$  (FW) among sweet potatoes of the same size from the same harvest batch.

### Calculation of Retention

Nutrient retention in cooked foods may be calculated as either apparent or true retention. True retention measures the proportion of a nutrient remaining in cooked food compared to the amount of that nutrient originally present in a given weight of the food before cooking.

True retention is the recommended method for calculating nutrient retention (Murphy et al., 1975; Rodriguez-Amaya, 1997). The formulas for calculating apparent and true retention are presented in Box 1.

<p><b>Box 1. Calculation of Nutrient Retention in Cooked Foods</b></p> <p><b>True retention</b></p> $\% \text{ TR} = \frac{\text{Nutrient content per g of cooked food} \times \text{g of food after cooking}}{\text{Nutrient content per g of raw food} \times \text{g of food before cooking}} \times 100$ <p><b>Apparent retention</b></p> $\% \text{ AR} = \frac{\text{Nutrient content per g of cooked food (dry basis)}}{\text{Nutrient content per g of raw food (dry basis)}} \times 100$
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Source: Murphy et al., 1975-Comparisons of Methods for Calculating Retentions of Nutrients in Cooked Foods

Apparent retention is defined by calculations based on the nutrient contents of the moisture-free raw and cooked foods. The apparent retention calculation assumes that solids are not lost to a significant extent, for example, by leaching into the steep water, which is later discarded. In cases where solid loss is negligible, some researchers have found that comparable retention results can be obtained by calculating retention on a dry weight basis instead of using the true retention formula (Li et al., 2007; Bengtson et al., 2008).

### METHODOLOGY

A review of available retention literature was performed in English using Pubmed, Google Scholar, and Agricola (USDA, National Agricultural Library). Search terms included: [Provitamin A or Carotenoid], [Retention or Loss], and [Boiling or Drying or Flour or Roasting or Steaming or Frying or

Microwaving]. It also included [Sweet potato] for sweet potato; [Maize or Corn] for maize and [Cassava or Manioc or Yucca] for cassava. Additionally, a number of experts and authors of recent retention publications were contacted for cross-checking the results of the literature review, locating specific articles, and providing unpublished data. A total of 30 studies (maize: 6, cassava: 8, and sweet potato: 16) were included in this review. Information on country of cultivation, cultivar, cooking process, the initial content of  $\beta$ -carotene or total carotenoid, and percentage of retention was extracted from the reports and is presented in table format in the present review. For organizational purposes, this review is presented in sections separated by crop and processing method.

### CASSAVA

Cassava (*Manihot esculenta* Crantz), also known as manioc, mandioca, tapioca, or yuca, is a staple food in West Africa. Total cassava consumption has doubled in Africa from 24 million tons per year to 58 million tons per year, over the last 30 years (FAO, 2012). Nigeria is the largest producer of cassava in the world (FAO, 2008) with about 45 million metric tons and its cassava transformation is the most advanced in Africa (Egesi et al., 2006). According to the data from the Nigeria Food Consumption and Nutrition Survey 2001–2003, cassava consumption is approximately 200–250 g/day among children 4–6 years and 350–400 g/day among women in the southern region of Nigeria (Maziya-Dixon et al., 2007). Although cassava is a good source of carbohydrates and energy, it is a poor source of micronutrients. Given the high prevalence of VAD in the country—29.5% among children less than 5 years old and 13% among women of child-bearing age (Maziya-Dixon et al., 2007)—this root crop is an excellent vehicle for delivery of vitamin A through biofortified varieties with increased levels of pVAC.

Cassava roots can be boiled or steamed for consumption, but are usually processed into flour by oven drying or more laborious traditional West African methods. The processing of cassava can involve: chipping, crushing, milling, slicing or grating the roots, dehydration through pressing and decanting or drying in the sun, which can be followed by fermenting by soaking in water, sieving, and cooking under heat or roasting. In this paper, we examine how much pVAC is retained in cassava after drying, storage, and cooking by traditional West African methods.

**Drying** is used for production of flour and to extend shelf-life of stored cassava. Three drying methods have been investigated for cassava: direct sun-drying, shade-drying, and oven-drying (Table 1). Chavez et al., 2007 evaluated the effect of different drying methods on the true retention of  $\beta$ -carotene in light-yellow cassava roots.  $\beta$ -carotene was the main carotenoid in the three cassava genotypes evaluated, CM 2772–3, MBRA1324, and MCOL2401, with initial levels of 10.4, 7.2, and 13.5  $\mu\text{g/g}$  DW, respectively, representing 93%, 79%, and 62% of total carotenoid (TC). The study was carefully

designed; coarsely chopped chip samples (length 55 mm, width 10 mm, thickness 2 mm) from the same root were used in all three drying methods. To reach a final moisture content of 12% in the cassava chips, the period of drying varied according to the humidity and temperature conditions of each setting (sun: 2–3 days, shade: 6–7 days, oven: 24 hours at 60°C). The highest  $\beta$ -carotene retention was obtained by oven-drying (72%), followed by shade-drying (59%), and sun-drying (38%).

Similar results were reported by Iglesias et al., 1997 when studying the stability of carotene in response to different processing methods. Twenty-eight yellow cassava genotypes with an average initial TC content of 18.90 (range: 7.7 to 46.9)  $\mu\text{g/g}$  DW were evaluated. The average total TC retention was 27% (range: 10–55%) in sun-dried flour and 55% (range: 17–99%) in oven-dried flour. No information on the size of the cassava slices or temperature and period of drying was provided.

In an attempt to evaluate the changes in TC content at each stage of processing cassava, three improved yellow cassava varieties (TMS 94/0006, TMS 01/1235, and TMS 01/1371) were selected and made into flour and chips, either dried in the sun or in the oven (Maziya-Dixon et al., 2008). In this study, the authors collected samples for carotenoid analysis from peeled raw roots (initial carotenoid content) and at each stage of processing of flour (grated cassava mash, pressed grated mash, pulverized sieved mash/cake, and the final product) and chips (grated wet chips, grated wet pressed chips, and the final product). The wet material from flour and chip processing was equally divided to be sun-dried or oven-dried at 45°C for 72 hours. The samples were not weighed before and after each step, so true retention could not be calculated. The unadjusted calculations for the loss of moisture resulted in a false increase in total carotenoid retention, particularly for the chip processing. The original publication presented only the TC content at each stage. Therefore, we calculated the apparent retention presented in Table 1 by applying the following formula:

$$\frac{\text{Total carotenoid content of the final product}}{\text{Total carotenoid content from peeled raw cassava root}} \times 100$$

Another observation was the significant variation in  $\beta$ -carotene retention among different cassava genotypes (Iglesias et al., 1997; Chavez et al., 2007). Iglesias et al., 1997, studying the stability of carotene in response to different processing methods, concluded that genotype is an important determinant of pVAC retention. Chavez et al., 2007 confirmed this finding through an analysis of variance conducted for all processing methods, including drying, storage of cassava as flour and chips, and two cooking processes.

In summary, sun drying produces the lowest rate of pVAC retention in cassava among the methods studied.

Unfortunately, it is the most common method for drying cassava in African countries other than Nigeria where most cassava is processed directly into *gari*. The large drastic reduction in  $\beta$ -carotene retention in sun-drying compared to oven or shade-drying suggests a significant detrimental effect of light on the stability of this pigment (Chavez et al., 2007).

**Blanching.** Postharvest boiling or steaming for a short period of time prior to drying has been shown to improve carotenoid retention in dried foods by inactivating enzymes that can degrade carotenoids (Koca et al., 2005; Ndawula et al., 2004). The  $\beta$ -carotene content of slices of yellow cassava roots (4–5 mm) has been compared after they were blanched only by immersion into boiling water for 1.5 minutes, dried only at 70°C, or were subject to both processes (Nascimento et al., 2007). The  $\beta$ -carotene retention was 84% after blanching only, 91% after drying only, and 79% after blanching and drying. Therefore, blanching did not improve the retention of the carotenoids in cassava. However, after 20 days of storage, the slices that underwent blanching and oven drying retained 83% of the initial amount of  $\beta$ -carotene, compared to only trace amounts found in the samples that were not blanched prior to the drying process, suggesting that blanching may improve carotenoid stability over time. With only one study available, however, more data are needed to determine the effects of blanching on the shelf life of dried cassava, particularly in real-world settings.

**Storage.** Chavez et al., 2007 measured the  $\beta$ -carotene retention during a four-week storage period with yellow cassava flour and chips that had been either oven-dried or sun-dried. Oven-dried cassava initially retained 72% of  $\beta$ -carotene, which decreased to 40% and 32% after two and four weeks of storage at room temperature, respectively. Sun-dried cassava had a lower initial  $\beta$ -carotene retention (38%), which was further decreased to 24% and 18% after two and four weeks of storage, respectively. The  $\beta$ -carotene retention values for cassava chips were very similar to those of cassava flour (Table 1). Although data were not presented, the authors state that  $\beta$ -carotene retention did not vary significantly among the three yellow cassava cultivars during storage.

Even greater losses in TC content has been reported for cassava flour, from five yellow-cassava cultivars, stored at room temperature in the absence of light (Oliveira et al., 2010). The TC retention in cassava flour for all cultivars decreased with storage time, ranging from 24–40% and 11–30% after 5 and 12 days of storage, respectively. At 19 days of storage, only one cultivar had detectable levels of total carotenoids, and none by the 26<sup>th</sup> day of storage. This study showed that the cassava variety with the highest TC content as raw cassava (1751 Bonita: 18.92  $\mu\text{g/g}$ ) had lower retention (1.20  $\mu\text{g/g}$ ) when stored as flour for 12 days compared to another cultivar with lower initial TC levels (1752 Flor do Brasil: 11.63  $\mu\text{g/g}$ ) but higher retention (1.80  $\mu\text{g/g}$ ) after storage.

In an attempt to reduce  $\beta$ -carotene degradation during cassava flour storage, a vacuum was created using plastic bags to

**Table 1** Retention of carotenoids in cassava-effect of drying and storage

Reference	Country	Cultivars	Sample size	Methodology	Time	Temp (°C)	Moisture (%)	Average initial carotenoid content	Retention% average (Min/Max)
<b>Drying</b>									
Iglesias et al., 1997	Colombia	28 genotypes	28 × 2 replications	Oven dried flour (OVF) and sun dried flour (SUF)	NR	NR	NR	Carotene 18.9 µg/g DW	OVF- AR: 55 (17 /99) SUF- AR: 27 (10 /55)
Nascimento et al., 2007	Brazil	NR	NR	Oven dried (OVC) and oven dried blanched chips (BLC)	NR	70	NR	<i>trans</i> - beta-carotene 3.57 µg/g FW	OV-TR: 91 BLC-TR: 79
Chavez et al., 2007	Colombia	CM 2772-3, MBRA1324, MCOL2401	9 (3 cultivars × 3 replications)	Coarse chopped oven dried (OV), Shadow dried (SH), and sun dried (SU)	OV: 24 h SH: 6-7 d SU: 2-3 d	OV: 60 SH: NR SU: NR	12	<i>trans</i> - beta-carotene 10.34 µg/g DW	OV-TR: 72 SH-TR: 59 SU- TR: 38
Maziya-Dixon et al., 2008	Nigeria	TMS 01 /1371, TMS 01 /1412, TMS 01 /1663	3 roots per cultivar	Oven dried flour (OVF) and chips (OVC), sun dried flour (SUF) and chips (SUC)	OVF: 3d OVC: 3 d	OVF: 45 OVC: 45	12	total carotenoids 4.61 µg/g DW	OVF- AR: 67 OVC- AR: 178 SUF- AR: 56 SUC- AR: 249
<b>Storage</b>									
Nascimento et al., 2007	Brazil	NR	NR	Dried slices of blanched (BL) and unblanched (UB) stored in polyethylene bags	UB: 20 d BL1: 20 d BL2: 110 d	30-35	NR	<i>trans</i> - beta-carotene UB: 8.7 µg/g FW BL: 7.8 µg/g FW	UB- trace BL1- TR: 83 BL2- TR:44
Chavez et al., 2007	Colombia	CM2772-3, MBRA1324, MCOL2401	9 (3 cultivars × 3 replications)	Oven dried flour (OVF) and chips (OVC), sun dried flour (SUF) and chips (SUC)	1: 14 d 2: 30 d	Room temp	ND	<i>trans</i> - beta-carotene 10.34 µg/g DW	OVF1- TR: 41 OVF2- TR: 36 SUF1- TR: 22 SUF2- TR: 18 SUC1- TR: 24 SUC2- TR: 18
Oliveira et al., 2010	Brazil	1757, 1783, 1752, 1740, 1751	5 kg of roots per cultivar	Oven dried flour (OVF)	FL: 19 d	Room temp	ND	total carotenoids 12 µg/g DW	OVF- AR: 4 (2 /14)

AR: apparent retention; TR: true retention, OV: oven dried; OVF: oven dried flour; OVC: oven dried chips; SU: sun dried; SUF: sun dried flour; SUC: sun dried chips; SH: shadow dried; BL: blanched; NR: not reported; DW: dry weight; FW: fresh weight.

**Table 2** Retention of carotenoids in cassava-effect of cooking

Reference	Country	Cultivars	Sample size	Methodology	Time	Temp (°C)	Average of initial carotenoid content	Retention % average (min/max)
<b>Boiling or blanched</b>								
Iglesias et al., 1997	Colombia	28 genotypes	28 × 2 replications	Boiled (B)	30 min	NR	Carotene 8.9 µg/g DW	B-AR: 66 (33/98)
Chavez et al., 2007	Colombia	CM2772-3, MBRA1324, MCOL2401	6 (3 cultivars × 2 replications)	Boiled cuts in closed pot (B)	30 min	NR	<i>trans</i> -beta-carotene 10.34 µg/g DW	B-TR: 56 (16/92)
Nascimento et al., 2006	Brazil	NR	5 roots × 2 replications	Boiled cuts (B)	30 min	NR	<i>trans</i> -beta-carotene 1.5 µg/g FW	B-TR: 81 (79/82)
Nascimento et al., 2007	Brazil	NR	NR	Blanched slices (BL)	1.5 min	NR	<i>trans</i> -beta-carotene 3.57 µg/g FW	BL-TR: 84
Thakkar et al., 2009	Nigeria	TMS 01/1371, TMS 01/1412, TMS 01/1663	3 roots per cultivar	Cubes soaked in water prior to boiling (B)	30 min	95	Total beta-carotene 33.6 µg/g DW	B-AR: > 100
Ceballos et al., 2012	Colombia	NR	18 (6 cultivars × 3 replications)	Boiled (B)	30 min	NR	Total carotenoid 32.2 µg/g DW	B-TR: 87 (76/97)
Failla et al., 2012	Puerto Rico	3DXS, 20DXS, and 37DXS	3 roots per cultivar	Boiled (B)	30 min	95	<i>trans</i> -beta-carotene 23.6 µg/g DW	B-AR: 44 (38/52)
<b>Frying</b>								
Nascimento et al., 2006	Brazil	NR	5 roots × 2 replications	Boiled (B) and fried (F)	B: 20 min F: 8 min	NR	<i>trans</i> -beta-carotene 1.3 µg/g FW	B,F-TR: 54 (54/54)
<b>Gari preparation</b>								
Chavez et al., 2007	Colombia	CM2772-3, MBRA1324, MCOL2401	9 (3 cultivars × 3 replications)	Fermented (FE) and roasted (R)	FE: 7 d R: NR	NR	<i>trans</i> -beta-carotene 10.34 µg/g DW	FE,R-TR: 34
Maziyá-Dixon et al., 2008	Nigeria	TMS 94/0006, TMS 01/1235, TMS01/1371	3 roots per cultivar	Fermented (FE) and roasted (R)	FE: 3 d R: 10 min	NR	total carotenoids 4.94 µg/g DW	FE,R-AR: 187
Thakkar et al., 2009	Nigeria	TMS 01/1371, TMS 01/1412, TMS 01/1663	3 roots per cultivar	Fermented (FE) and roasted (R1 and R2)	FE: 3 d R1: 10 min R2: 20 min	FE: NR R1: 165 R2: 195	total beta-carotene 33.6 µg/g DW	FE,R1-AR: 63 FE,R2-AR: 10*
Failla et al., 2012	Puerto Rico	3DXS, 20DXS, and 37DXS	3 roots per cultivar	Fermented (FE) and roasted (R)	FE: 3 d R2: 20 min	R1: 185 R2: 120	<i>trans</i> -beta-carotene 23.6 µg/g DW	AR: 23(16/30)
<b>Fufu preparation</b>								
Thakkar et al., 2009	Nigeria	TMS 01/1371, TMS 01/1412, TMS 01/1663	3 roots per cultivar	Fermented (FE) and roasted (R)	FE: 3 d R: 20 min	FE: NR R: 165	total beta-carotene 33.6 µg/g DW	FE,R-AR: > 100
Maziyá-Dixon et al., 2008	Nigeria	TMS 01/1371, TMS 01/1412, TMS 01/1663	3 roots per cultivar	Fermented (FE) and cooked with water (B)	FE: 5 d	FE: NR	total carotenoids 4.94 µg/g DW	FE,B-AR: 82
Failla et al., 2012	Puerto Rico	3DXS, 20DXS, and 37DXS	3 roots per cultivar	Fermented (FE) and cooked with water (B)	FE: 5 d B: 10 min	B: 100	<i>trans</i> -beta-carotene 23.6 µg/g DW	FE,B-AR: 44(34/54)
<b>Flour</b>								
Oliveira et al., 2010	Brazil	1757, 1783, 1752, 1740, 1751	5 kg of roots per cultivar	Cassava was soaked in water	OVF: 30 min	120–150	total carotenoids 12 µg/g DW	AR: 49 (29/67)
Failla et al., 2012	Puerto Rico	3DXS, 20DXS, and 37DXS	3 roots per cultivar	Non-fermented flour (NFF) and fermented (FE) flour were both dried in oven	FE: 3 d OVF: 24 h	37	<i>trans</i> -beta-carotene 23.6 µg/g DW	NFF-AR: 60(35/78) FE-AR: 48(39/56)

\*Cultivar TMS 01/1371. AR: apparent retention; TR: true retention, F: fried; FE: fermented; B: boiled; R: roasted; NR: not reported; DW: dry weight; FW: fresh weight.

eliminate oxygen prior to storage (Chavez et al., 2007). Storage in a vacuum-packed plastic bag which is semipermeable to air did not seem to make a difference, since cassava flour in vacuum-packed bags displayed only slightly lower (statistical analysis was not performed)  $\beta$ -carotene retention (oven-dried: 32% and sun-dried: 17%) than the samples packed without a vacuum (oven-dried: 36% and sun-dried: 18%). Future research should be conducted with packaging material that is impermeable to air.

### Cooking

**Boiling.** Simply boiling of cassava roots or rapid boiling of fermented cassava paste to prepare fufu is used in the traditional African cooking to reduce the total cyanogen content (Thakkar et al., 2009). Table 2 shows the results from five studies have evaluated the effect of boiling on cassava with the average pVAC retention among cultivars and studies ranging from 56 to 100% (Iglesias et al., 1997, Nascimento et al., 2006, Chavez et al., 2007, and Thakkar et al., 2009 and Ceballos et al., 2012). All studies followed the same protocol—boiling cassava for 30 minutes—with one study reporting to have boiled cassava in a covered pot (Chavez et al., 2007).

A strong genotype effect was observed among the three yellow cassava cultivars boiled for 30 minutes (CM 2772–3, MBRA 1324, and MCOL 2401) by Chavez and collaborators, with  $\beta$ -carotene retention reported as 16, 59, and 92%, respectively (Chavez et al., 2007). To further explore this effect, the study was expanded to include six different genotypes, in which the  $\beta$ -carotene retention ranged from 27 to 73%. Ten years earlier, a study of 28 genotypes using similar boiling conditions found that  $\beta$ -carotene retention ranged from 33 to 98% (Iglesias et al., 1997). In recent years, greater attention has been given to the differences in dry matter content among genotypes and the parts of the root used for the retention studies (Thakkar et al., 2009 and Ceballos et al., 2012).

Thakkar et al., 2009 evaluated the effect of boiling among the TMS 01/1371, TMS 01/1412, and TMS 01/1663 cassava cultivars which initially contained total  $\beta$ -carotene levels of 6.2–7.8  $\mu\text{g/g}$  FW (25.9–40.9  $\mu\text{g/g}$  DW). Due to the difference in the dry matter content before (18–25%) and after (19.1  $\pm$  0.52%) boiling, the authors decided to express the results per unit of dry weight. However, the apparent retention of total  $\beta$ -carotene calculated on a dry weight basis exceeded 100%, proving that the formula of true retention should be used instead. Fresh roots containing lower dry matter (20–30%) tend to maintain their weight after boiling, but the carotenoids tend to leach from the root samples into the water in higher proportion when the dry matter content is low (Ceballos et al., 2012).

The relationship between dry matter content and carotenoid levels in different sections (proximal, central, and distal) of the cassava root has been investigated by Ceballos et al., 2012. The results showed higher concentrations of total

carotenoid in the proximal section of the root (proximal: 34, central: 32.9 and distal: 29.6, expressed as  $\mu\text{g/g}$  DW), similar to the pattern observed for the dry matter content (proximal: 36.3%, central: 34.0% and distal: 32.8%). Although the retention tended to be higher in the proximal and distal sections and lower in the central section, it was not significantly different. On average the TC retention among six cassava cultivars after boiling for 30 minutes was 87% (range: 76–97%). This was the highest reported cassava retention obtained among all studies included in this review. In conclusion, differences among genotypes were highly significant ( $p \leq 0.01$ ) whereas the variety by section of the root interaction was not significant ( $p \geq 0.20$ ).

Lastly, increases in the 13-*cis* (34%) and 15-*cis* (8%) isomers were observed in boiled cassava (Ceballos et al., 2012). The ratio of 13-*cis* to 9-*cis*- $\beta$ -carotene (1.1–1.8) has also been reported to be almost twice in boiled cassava than that in their raw cassava counterpart (0.6–0.7) (Thakkar et al., 2009).

**Frying** is not a common method of preparing cassava for consumption in West Africa. One study evaluated the retention of  $\beta$ -carotene in fried cassava (Nascimento et al., 2006). Cassava roots purchased from the local market in Brazil were boiled for 20 minutes followed by 8-minutes' frying; the *trans*- $\beta$ -carotene retention was 54%. The authors stated that higher levels of epoxy- $\beta$ -carotene were found in the fried cassava, but no data were presented.

**Gari** is the most popular method of cassava processing in sub-Saharan Africa. Gari is a granular flour of varying texture made from cassava roots by peeling, washing, grating, fermenting for approximately four–five days, followed by pressing (to eliminate the water excess), and roasting the pressed cake into granules. Three yellow cassava varieties with an initial  $\beta$ -carotene content of 10.34  $\mu\text{g/g}$  DW were made into gari and 34% (10–58%) of  $\beta$ -carotene was retained (Chavez et al., 2007). No information on the temperature and duration of roasting was provided. Gari made from high  $\beta$ -carotene transgenic cassava roots with average initial  $\beta$ -carotene content of 23.60  $\mu\text{g/g}$  DW showed  $\beta$ -carotene retention of 23% (range: 16–30%) (Failla et al., 2012). In this study, the cassava was fermented for three days and roasted at high temperature (185°C) until granules reached 80°C and continued roasting at lower temperature (120°C) for 20 minutes. However, in another study, roasting cassava at 195°C for 20 minutes preserved approximately 10% of total  $\beta$ -carotene, whereas the retention was increased to 63% by roasting at 165°C for 10 minutes (Thakkar et al., 2009). It appears that the final  $\beta$ -carotene content in gari is a function of the intensity and duration of roasting.

Total carotenoid was measured in raw cassava and after each step of processing cassava into gari in an apparent retention study in Nigeria. The result showed an increase of 187% in the mean total carotenoid in gari compared to the raw cassava (Maziya-Dixon et al., 2008). The authors attributed the increase in TC concentration after processing to unaccounted moisture and soluble solid losses.



It is common practice in Southern Nigeria to add red palm oil during the roasting stage of gari production, which gives the finished gari a light yellow hue and particular taste characteristics. Red palm oil is a rich source of provitamin A containing average 600  $\mu\text{g/g}$  of  $\beta$ -carotene and 230  $\mu\text{g/g}$  of  $\alpha$ -carotene (Trujillo-Quijano et al., 1990). No study in the present review had red palm oil added to gari. The yellow gari described in one of the publications was due to the use of yellow biofortified cassava varieties and not to the addition of red palm oil (Chavez et al., 2007).

**Fufu** is another traditional method of preparing cassava in sub-Saharan Africa (Montagnac et al., 2009). The entire process involves fermentation of the peeled and chopped roots followed by sieving and sedimentation for 24–72 hours or more, the elimination of excess water by means of a hydraulic press leads to an uncooked paste (uncooked fufu) which can be boiled or steamed and pounded into a cooked paste (cooked fufu). In some instances, the grated paste can be submitted to further fermentation (“double fermentation”) prior to boiling or steaming. The effect of fufu preparation on the pVAC retention was evaluated in three studies (Maziya-Dixon et al., 2008; Thakkar et al., 2009; Failla et al., 2012). Calculated apparent retention ranged from 44% in high  $\beta$ -carotene transgenic cassava (Failla et al., 2012) to 82% (Maziya-Dixon et al., 2008) and greater than 100% (Thakkar et al., 2009). Thakkar et al., 2009 found the ratio of 13-*cis* to 9-*cis*  $\beta$ -carotene to be similar in raw cassava and fufu, suggesting that the fermentation and the 10-minutes’ boiling to prepare fufu were not sufficient to induce isomerization.

**Flour.** In Brazil, where 80% of the total cassava root production is used for cassava flour, the retention of total carotenoid was reported as 49% (29–67%) after cassava roots from five varieties with approximately 12  $\mu\text{g/g}$  DW of total carotenoids were processed and oven dried at 120–150°C for 30 minutes (Oliveira et al., 2010). In a recent study with high  $\beta$ -carotene transgenic cassava roots (23.6  $\mu\text{g/g}$  DW of  $\beta$ -carotene), the retention of  $\beta$ -carotene was higher in nonfermented flour 60% (35–78%) compared to the fermented flour 48% (39–56%). In both methods the cassava was dried in the oven at 37°C for 24 hours (Failla et al., 2012).

## MAIZE

The majority of maize produced and consumed in Africa is white maize, which is essentially devoid of carotenoids. In Zambia, maize is by far the most important food crop, with production reaching a record high of 2.85 million tons in 2011/12 (FAOSTAT, 2014). It is mainly produced at the subsistence level for local household consumption, with only about 20% of rural households selling some of their maize after satisfying their household needs. The VAD problem persists in Zambia despite the biannual vitamin A supplementation program and the vitamin A sugar fortification which were introduced in 1992 and in 1998, respectively. The VAD prevalence among

children aged less than five years reported in the past two National Surveys was 66% (NFNC, 1997) and 54% (NFNC, 2003). In our survey conducted in 2009 in two rural districts of Zambia, children showed a 48% prevalence of VAD with infection-adjusted plasma retinol (Hotz et al., 2012a).

This paper examines the retention of pVAC in maize after drying, storage, and traditional cooking methods, mainly porridge in Africa and nixtamalization, a cooking process commonly used in Mesoamerica.

**Drying.** The effect of drying on total carotenoid degradation was evaluated in three yellow dent inbred maize lines (A619, CG102, and CG60) and four high carotenoid lines (HiC-5, HiC-7, HiC-23, and HiC-26) from the breeding program at University of Guelph in Canada (Burt et al., 2010). The maize was dried in the oven either at 90°C for 8 hours or at 25°C (ambient temperature) for 72 hours, and the control samples frozen at –80°C for 48 hours and then lyophilized for another 48 hours. Surprisingly, none of the genotypes exhibited greater carotenoid loss at 90°C than at 25°C with an average of between 55 and 85% retention (Table 3). In an unpublished study the pVAC levels of two genotypes were compared after maize was dried under the sun as grain or cob, but no difference was found between the two genotypes (Natalia Palacios, CIMMYT Global Maize Program, oral communication). More studies are currently being carried out to better understand the effect of drying on the carotenoids retention in maize.

**Storage.** Maize can be stored from one harvest to the next, which is equivalent to 6–12 months, depending on the number of growing seasons per year in any given agro-ecological zone. After six months of storage, the average total carotenoid retention among four inbred lines of maize was reported to be 58% (Weber et al., 1987). The genotype with the lowest initial total carotenoids content (27.4  $\mu\text{g/g}$  DW) showed the highest retention (67%). The degradation of total carotenoids in two genotypes (HiC-7 and HiC-26) was assessed in triplicate samples at 0, 3, 6, and 18 months of storage in the dark at 4°C and 35% relative humidity at the University of Guelph (Burt et al., 2010). Total carotenoids remained constant for the first three months but declined significantly by six months, remaining stable thereafter for an overall total carotenoid loss of 35–40%. In another experiment by the same authors, six genotypes were freeze-dried, fast oven-dried (90°C) or dried at ambient temperature (25°C) and stored for four months at 25°C in the dark. At four months, the total carotenoid retention ranged from 76% for HiC-23 at 90°C to 39% for HiC-5 at 25°C (Burt et al., 2010). In an unpublished study of two maize genotypes in which storage was not performed in dark conditions, 40–70% of the carotenoid content was degraded in the first four months. At four months, there was a significant genotype effect with retention ranging from 45 to 93% according to genotype; the genotype with lower initial  $\beta$ -carotene concentration had the higher retention at four months of storage. However, after 12 months of storage both genotypes displayed 90% degradation at either 22°C or 37°C (Natalia Palacios, CIMMYT, oral communication, unpublished data).

**Table 3** Retention of carotenoids in maize-effect of drying and storage

Reference	Country	Cultivars	Sample size	Methodology	Time	Temp (°C)	Moisture (%)	Average initial carotenoid content	Retention % average, % (min /max)
<b>Drying</b>									
Burt et al., 2010	Canada	Yellow dent inbred maize lines A619, CG102, CG60, and high carotenoid lines: HiC-5, HiC-7, HiC-23, and HiC-26	Triplicate from each genotype	Control (C): lyophilized and stored at -80°C Oven-dried (OV) using two methods	OV1: 8 h OV2: 72 h	OV1: 90 OV2: 25	NR	TC: 70-120 µg/g DW	(55 /85)
<b>Storage</b>									
Weber et al., 1987	US	Maize inbred lines, B84, H51, Oh45, R802A	NR	Storage	6 months	Room temp	NR	27-77 µg/g DW	58 (50 /67)
Burt et al., 2010	Canada	Yellow dent inbred maize lines A619, CG102, CG60, and high carotenoid lines: HiC-5, HiC-7, HiC-23, and HiC-26	Triplicate from each genotype	Control (C): lyophilized and stored at -80°C Oven-dried (OV) at 90°C or 25°C	A) samples of 2 genotypes stored for 18 months; B) 6 genotypes stored for 4 months; All in the dark	A: 4 B: 25	Relative humidity: 35	TC: 70-120 µg/g DW	A: 39-78 B: 60-65

The data just presented seem to support the genotype-based classification proposed by Burt (Burt et al., 2010). The authors categorized the different genotypes into three groups: those with high losses due to drying and low loss due to storage (CG60, HiC-7); those with low losses due to drying, but high loss due to storage (CG102, HiC-5); and the intermediate genotypes with moderate losses due to both drying and storage (A619, HiC-23). The authors concluded that the understanding of the genotype effect has the potential to guide the development of high carotenoid maize inbred lines with good stability during both drying and storage.

### Cooking

**Porridge** preparation from maize involves soaking, milling, and grinding of kernels. This staple food is often consumed as many as three times a day in many settings in Africa, Latin American, and Asia (Rooney & Serna-Saldivar, 2003). In certain African countries such as Benin, Ghana, Nigeria, and Togo, fermentation is also performed prior to boiling in a preparation known as *Ogi* (Jespersen et al., 2003). The effect of fermentation on pVAC degradation was evaluated in high  $\beta$ -carotene maize inbred lines (Li et al., 2007). Maize kernels were soaked in water at room temperature for 24 hours and wet milled into fine flour. The wet flour was fermented at room temperature in the dark for 48 hours (for fermented maize samples only). Fermented and unfermented slurries were heated at 93°C for 9 minutes in order to prepare the porridge. The apparent retention was measured at each stage of porridge preparation and found that the fermentation step prior to cooking reduced the  $\beta$ -carotene degradation during the cooking step. In summary, 7.3% of  $\beta$ -carotene was lost during soaking and milling. Spontaneous fermentation over 48 hours at 30°C in the dark (pH 4) caused an additional 10.2%  $\beta$ -carotene reduction, followed by 6.9% degradation during cooking. The unfermented slurry lost 18% of  $\beta$ -carotene during cooking. Therefore, the cumulative  $\beta$ -carotene loss of 24.5% for the fermented porridge (7.3% soaked/milled + 10.2% fermentation + 6.9% cooked) was almost identical to the 24.8% loss from the unfermented porridge (7.3% soaked/milled + 17.5% cooked). Fermentation does not adversely affect the retention of pVAC carotenoids in porridges prepared with high  $\beta$ -carotene maize.

Another study evaluated the effect of making thick and thin porridge from yellow maize on the level of degradation of individual carotenoids (Muzhingi et al., 2008). Unfortunately, the differences in moisture content and solid loss were not taken into consideration between the raw and cooked products and the concentration of all individual carotenoids were greater in the porridge than the raw uncooked maize flour. The results are shown in Table 4.

**Nixtamalization.** In Central America and Southern Mexico, maize is most commonly prepared as tortilla in a process involving nixtamalization (overnight steeping of maize in

calcium hydroxide solution) followed by partial removal of pericarp and germ, grinding into a thick dough, which is molded by hand or machine into flat cakes that are roasted on an earthenware or metal flat plate (Rooney et al., 1987). Although losses in dry matter, thiamine, riboflavin, niacin, fat, and fiber are known to occur, alkali-cooking can, on the other hand, improve flavor, and the bioavailability of niacin and calcium (Bressani et al., 1990).

The pVAC retention levels observed by De la Parra et al., 2007 after the dough was deep-fried were much lower (18% for the high carotenoid variety, and not detected for the remaining maize varieties) than the 64% average reported by Lozano-Alejo et al., 2007. While time, temperature for the deep-frying, and a number of other variables were similar in both studies, differences that could explain the divergent results include: initial carotenoid levels in the maize, genotype, and time period the maize was lime-cooked prior to soaking. The highest pVAC maize (0.57  $\mu\text{g/g}$  DW) evaluated by De la Parra et al., 2007 had lower carotenoid levels when compared with the lowest pVAC maize (0.70  $\mu\text{g/g}$  DW) evaluated by Lozano-Alejo et al., 2007. The highest pVAC maize evaluated by Lozano-Alejo et al., 2007 had the highest retention (85%), but this was not a trend observed among the other 13 genotypes tested. Lastly, the boiling time of 25–50 minutes prior to steeping used by de la Parra et al., 2007 may have helped to increase the carotenoid degradation. For future analysis, we recommend the continued evaluation of various genotypes with their clear specifications reported, measurement of individual carotenoids at each step of the cooking process (steeping, baking, and frying), and recorded alterations of these processes by time and temperature to gauge their impact on pVAC concentrations—particularly when comparing commercial to household use.

### SWEET POTATO

Sweet potato (*Ipomoea batata* (L.) Lam.) is a major staple crop in sub-Saharan Africa, with more than 100 million metric tons produced per year (FAOSTAT, 2012). The biofortified orange sweet potato varieties have  $\beta$ -carotene content ranging from 30 to 100 ppm, in contrast to the 2 ppm in local varieties of Mozambique and Uganda. The biofortified varieties could help to reduce the high rates of VAD prevalence among children 5–69 months estimated as 69% and 20% in Mozambique and Uganda, estimated as 69% and 20% in children aged 6–59 months, respectively. In fact, it has been estimated that consumption of biofortified orange sweet potato in Uganda could eliminate between 40–66% of the burden of VAD as measured by disability adjust life years (DALYs) (Meenakshi et al., 2010).

Retention studies were undertaken with orange sweet potato varieties that were considered for release in Mozambique (Resisto, MGCL-1, Jonathon, Gaba-Gaba) and Uganda (Ejumula, SPK004, SPK004 /6, and SPK004 /6 /6). The main

**Table 4** Retention of carotenoids in maize-effect of cooking

Reference	Maize cultivar	Cooking style	Time	Temp (°C)	Moisture (%)	Carotenoid retention (%)								
						<i>trans</i> - $\beta$ -carotene	13- <i>cis</i> - $\beta$ -carotene	9- <i>cis</i> - $\beta$ -carotene	$\beta$ -cryptoxanthin	Lutein	Zeaxanthin	ProA**		
Muzhingi et al., 2008***	KUI Synthetic Yellow Maize	Raw*				3.35 ± 0.10	2.08 ± 0.12	0.62 ± 0.04	3.22 ± 0.14	3.96 ± 0.12	32.85 ± 1.17			
		Porridge (thick)	30 min	100			123	138	150	101	130	112		
		Porridge (thin)					113	105	124	89	113	100		
		Boiled	1 h	100			110	119	139	92	115	89		
		Muffin (baked)	25 min	232			27	25	31	51	97	78		
		Raw*					0.05			0.01	0.05	0.06		
		Dough	33 min			56				117	142	129		
		Tortilla (baked)	1 min	280						124	150	125		
		Chips (fried)	1 min	175						6.3	14	163		
		Raw*						0.33		0.19	4.06	3.53	0.43	
De La Parra et al., 2007	White	Raw*												
		Dough	24 min			56								
		Tortilla (baked)	1 min	280										
		Chips (fried)	1 min	175										
		Raw*												
		Dough	53 min			56								
		Tortilla (baked)	1 min	280										
		Chips (fried)	1 min	175										
		Raw*						0.45		0.23	2.45	3.22	0.57	
		Dough	53 min			56								
Lozano-Alejo et al., 2007 (0.7–3.05)	13 genotypes	Raw*												
		Snacks (fried)	1 min	200–210										
		Raw*					14.5 ± 0.5	7.75 ± 0.03	1.20 ± 0.05	1.54 ± 0.10	1.73 ± 0.19	11.71 ± 0.16	9.20 ± 1.08	64 (18–85)
		Wet milled flour	24 h	30		35.2 ± 2.3	94	93	88	90	94	94		
		Wet milled flour, fermented	48 h	30		49.0 ± 1.2	83	84	79	76	76	73		
		Wet milled flour, fermented, and cooked	9 min	93		61.5 ± 0.4	73	88	79	71	67	64		
		Wet milled flour, unfermented and cooked	9 min	93		60.2 ± 0.9	71	98	81	74	71	66		
		Raw*												
		Raw*												
		Li et al., 2007	Kernels resulting from the DeExp x C1.7 cross pollinated with BC Orange	Raw*										
Wet milled flour	24 h			30		35.2 ± 2.3	94	93	88	90	94			
Wet milled flour, fermented	48 h			30		49.0 ± 1.2	83	84	79	76	76			
Wet milled flour, fermented, and cooked	9 min			93		61.5 ± 0.4	73	88	79	71	67	64		
Wet milled flour, unfermented and cooked	9 min			93		60.2 ± 0.9	71	98	81	74	71	66		
Raw*														
Raw*														
Raw*														
Raw*														
Raw*														

\*Uncooked maize is expressed as  $\mu\text{g/g DW}$ .

\*\*Provitamin A content was calculated as the sum of all-*trans*- $\beta$ -carotene plus ( $\beta$ -cryptoxanthin\*0.5).

\*\*\*Cryptoxanthin values are for total ( $\alpha + \beta$ ) cryptoxanthin.

**Table 5** Retention of carotenoids in sweet potato- effect of drying

Reference	Country	Cultivars	Sample size	Methodology	Time	Temp (°C)	Moisture (%)	Average initial carotenoid content	Retention % average
<b>Drying</b>									
Hagenimana et al., 1999	Kenya	32 cultivars	NR	Oven dried (OV)	12 h	65	6–8	Total carotenoids 49 µg/g DM	OV: 70
Nascimento et al., 2007	Brazil	Purchased in local market	NR	Oven dried (OV) and oven dried of blanched slices (BL)	NR	70	NR	<i>trans</i> -beta-carotene 3.57 µg/g FW	OV-TR: 96 BL-TR: 96
Bengtsoon et al., 2008	Uganda	Ejumula and 6 field test varieties		Oven dried (OV), solar dried (SO) sun dried (SU)	OV: 10 h SO: 6–10 h SU: 6–10 h	OV: 57 SO: 45–63 SU: 30–52	OV: 10.8 SU: 6.8 SU: 10	<i>trans</i> -beta-carotene 108 to 314 µg/g	OV: 88 SO: 91 SU: 84
Bechoff et al., 2009	France	Imported from USA	n = 5 roots	Hot air (HA) Solar (SO) Sun (SU)	HA: 2 h SO: 8.5 h SU: 8 h SH: 5 h	HA: 24–45 SO: 27–50 SU: 24–30	HA: 11 SO: 10 SU: 10 SH: 6	<i>trans</i> -beta-carotene 293 µg/g DW	HA: 84 <sup>a</sup> SO: 77 <sup>ab</sup> SU: 66 <sup>b</sup> SH: 79 (chips) SH: 62 (flour) SO: 94–96
Kidmose et al., 2007	Kenya	Variety Zapallo	n = 5 roots	Shade (SH) made into chips (C) and flour from chips (FL)	NR	27–50	8–10	<i>trans</i> -beta-carotene 123 µg/g FW	S-TR: 88 OV1-TR: 64 OV2-TR: 59
Mdziniso et al., 2006	USA	Purchased locally		Solar drying (SO)	NR	27–50	8–10	<i>trans</i> -beta-carotene 972 µg/g DW	(kakamega- Ejumula) T1: (81–92) (98–92) T2: (83–96) (91–95) T3: (87–91) (91–95) TU: (90–93) (89–91) SU: (87–93) (90–95) MGCL01 Resisto <i>Thin chips</i>
Wu et al., 2008	China	Yanshu No. 5	n = 2	Steamed (S) Oven dried 1 (OV1) Oven dried 2 (OV2)	S: 20 min OV1: 5 h OV2: 11 h	50	62.4/32.2	70 µg/g FW	TU: 89.2% 85.4% SU: 95.1% 83.5% SH: 101% 92.3% SU: 95.3% 75.4%
Bechoff et al., 2010a	Uganda	Ejumula and Kakamega on wet and dry weather		Tent dryers: UV resistant (T1) Red resistant (T2) Not resistant (T3) Tunnel dryer (TU) Sun dried (SU)	wet/dry T1: 13 h/8 h T2: 14 h/10 h T3: 13 h/8 h TU: 10 h/6 h SU: 9 h/5 h	wet/dry 27.3/31.2	wet/dry 62.4/32.2	total carotenoids µg/g DW wet/dry 251/306.3 (Ejumula) 75/100.3 (Kakamega)	
Bechoff et al., 2011a	Mozam-bique	MGCL01 and Resisto	n = 5 roots in duplicate	Tunnel dryer (TU) Sun dried (SU) Shade dried (SH)	MGCL01/Resisto <i>Thin chips</i> TU: 25.5 h/26.1 h SU: 23.8 h/25.4 h SH: 26.5 h/50.7 h <i>Thick chips</i> SU: 23.9 h/62.3 h	TU: 26 SU: 22 SH: 22	MGCL01/Resisto <i>Thin chips</i> TU: 6.1/8.1 SU: 7.9/8.5 SH: 10.1/10.1 <i>Thick chips</i> SU: 10.4/9	MGCL01 235.6 µg/g DW Resisto 434.4 µg/g DW	

AR: apparent retention; TR: true retention, OV: oven dried; BL: blanched; UB: unblanched; FL: flour; C: chips; OV: oven dried; SU: sun dried; SO: solar dried; T: tent dried; TU: tunnel dried; SH: shade dried; Z: zipped PE bags; SC: sealed clear bag; SCB: sealed clear PE bag in black PE bag; B: black PE bag; NR: not reported; J: jar; DW: dry weight; FW: fresh weight.

cooking method of orange sweet potato in Mozambique is boiling, while in Uganda involves boiling and steaming; however, four additional cooking methods of orange sweet potato (frying, roasting, microwaving, baking) were evaluated and are included in the review. In both countries, sweet potato is also dried as chips to make sweet potato flour for use in cooking after the harvest season; therefore, extensive studies of  $\beta$ -carotene retention following drying and throughout storage of the dried products were carried out and are presented here (Tables 5 and 6). The retention of sweet potato pVAC was reviewed recently (Boy & Miloff, 2009).

**Sun drying.** The pVAC retention in sweet potato ranged from 66 to 95% after sun drying (Bengtsson et al., 2008, Bechoff et al., 2009, and Bechoff et al., 2010a, Bechoff et al., 2011a). A study conducted with American orange sweet potato reported retentions of 67 and 66% for total carotenoid and  $\beta$ -carotene, respectively, after sweet potato chips were dried for 8 hours under the sun (Bechoff et al., 2009). The fact that the weather was hot (29°C) and dry (39% humidity) allowed the sweet potato chips to dry in a shorter time, where traditionally in sub-Saharan African it would take 2–3 days, resulting in pVAC retention not significantly different from those obtained from solar drying.

In Uganda, seven improved sweet potato roots (variety Ejumula) had an average 84%  $\beta$ -carotene retention after sun-drying, which was not significantly different from the 88% retention from oven-dried samples or the 91% retention from solar-dried samples (Bengtsson et al., 2008). The time required for drying the samples was equivalent among the three methods, 6 to 10 hours for sun- and solar-drying, and 10 hours for oven-drying.

The environmental conditions for sun-drying had a significant effect on the pVAC retention (Bechoff et al., 2010a). Dry weather resulted in significantly higher ( $p < 0.05$ ) carotenoid retention (90 and 95%) compared to wet weather (87 and 93%) in Ugandan sweet potato varieties Kakamega and Ejumula, respectively. This difference may have to do with the shorter sun exposure due to dry weather. During dry weather, the time required to dry the samples was almost half (5 hours) of that required during wet weather (9 hours).

In Mozambique, 95% of total carotenoid was retained after sun-drying for sweet potato variety MGCL01, independent of the thickness of the chips (Bechoff et al., 2011a). Sweet potato variety Resisto showed lower retention, 84% (thin chips) and 75% (thick chips), of total carotenoids after sun-drying. It is important to note that the Resisto variety required a greater time to dry than the MGCL01 variety, which had a higher retention rate.

**Solar drying** is achieved by direct sun radiation on the material covering the solar dryer, reaching internally higher temperatures, and similar or slightly lower humidity as the outside environment. The pVAC retention in sweet potato ranged from 77 to 96% after solar-drying (Mdziniso et al., 2006, Bengtsson et al., 2008, Bechoff et al., 2009, Bechoff et al., 2010a, and Bechoff et al., 2011a). In three studies, the pVAC

retention from solar-drying was not significantly ( $p < 0.05$ ) different from sun-drying (Bengtsson et al., 2008, Bechoff et al., 2009, Bechoff et al., 2010a). In one study, solar-drying showed lower total carotenoid retention in sweet potato variety MGCL01 (89%) compared to sun-drying (95%) (Bechoff et al., 2011a). Mdziniso et al., 2006 only measured the effect of solar-drying on carotenoid retention and, therefore, cannot be compared to sun-drying. The use of UV-resistant polythene material on the solar dryer did not increase carotenoid retention as compared to solar dryer with non-UV resistant materials or sun drying (Bechoff et al., 2010a). The authors concluded that direct sun exposure is as efficacious as solar drying in terms of pVAC retention in sweet potato.

**Hot air drying** was conducted with a wood device that has a heating system composed of a butane gas jet and centrifuge fan to circulate hot air 42°C (24–45°C) through the trays. In 2 hours the sweet potato chips reached 11% moisture and presented a higher retention of total carotenoids (87%) and  $\beta$ -carotene (84%) when compared to sun drying (67 and 66%), but was not significantly different from solar drying (79 and 77%) (Bechoff et al., 2009).

**Shade drying** is used to prevent direct sun exposure during drying with the purpose of reducing or preventing carotenoid degradation. Chips of sweet potato roots variety Zapallo dried outdoors in shade for 5 hours had 79%  $\beta$ -carotene retention, which reduced to 62% after the chips were made into flour (Kidmose et al., 2007). Although 13-*cis*- $\beta$ -carotene was present in all three samples (raw, chips, and flour), 15-*cis*- $\beta$ -carotene was present after the sweet potato was dried as chips and made into flour, and the 9-*cis*- $\beta$ -carotene was present in the flour only.

One comparative analysis of shade-drying with two other methods (solar and sun), shade drying reported higher total carotenoid retention (92–100%) when compared to sun-drying (84–95%) and solar-drying (85–89%) between two sweet potato varieties in Mozambique (Bechoff et al., 2011a).

**Oven drying** of sweet potato roots resulted in average of 85% pVAC retention (range: 70–96%) (Hagenimana et al., 1999; Nascimento et al., 2007; Bengtsson et al., 2008). The  $\beta$ -carotene retention of sweet potato roots variety Ejumula oven-dried at 57°C for 10 hours was reported as 88% (Bengtsson et al., 2008). At higher temperature/time (65°C for 12 hours) of oven-drying the reported total carotenoid retention was 70% (Hagenimana et al., 1999). In addition to the differences in temperature and time, the study by Hagenimana and colleagues measured total carotenoids by spectrophotometer. Lastly, the true retention of  $\beta$ -carotene was 96% for sweet potato and 91% for cassava after these crops were oven-dried at 70°C (time was not reported) (Nascimento et al., 2007). One study was not included due to a pre-steaming step (Wu et al., 2008).

### Storage

Twenty-five sweet potato cultivars from CIP (International Potato Center) were evaluated for the retention of total

Table 6 Retention of Carotenoids in Sweet Potato-Effect of Storage

Reference	Country	Cultivars	Sample size	Methodology	Time	Temp (°C)	Moisture (%)	Average initial carotenoid content	Retention % average
<b>Storage</b>									
Hagenimana et al., 1999	Kenya	25 cultivars	NR	Stored OFSP dried chips	11 months	Room temp	NR	Total carotenoids 49 µg/g DW	AR: 57
Nascimento et al., 2007	Brazil	Purchased locally	Triplicate analysis	Dried slices of blanched (BL) and unblanched (UB) stored in polyethylene bags	UB: 20 d BL1: 20 d BL2: 70 d	30–35	NR	<i>trans</i> -beta-carotene UB: 463 µg/g FW BL: 513 µg/g FW	UB-45 BL1- TR: 80 BL2- TR:49
Bechoff et al., 2010a	Uganda	6 cultivars		OFSP chips stored; Zipped PE bag (Z) Sealed clear PE bag (SC) Black PE bag in black PE bag (SCB) Black PE bag (B)	4 months	Room temp		Total carotenoids Ejumula: 199.8 µg/g DW Kakamega: 52.4 µg/g DW	(Kakamega-Ejumula) Z: (23–23) SC: (35–35) SCB: (34–32) B: (36–29)
Bechoff et al 2010b	United Kingdom	Ejumula	N = 3	Laboratory storage kinetics of carotene in dried chips Experimental data; Stored in Jar in dark (J)-UK Stored in LPDE bags (LPDE)-Uganda	J: 88 d LPED: 125 d	Room temp	NR	<i>trans</i> -beta-carotene J: 181.2 µg/g DW LPDE: 219.6 µg/g DW	J: 41 LPED: 23
Bechoff et al., 2011a	Mozambique	MGCL01 and Resisto	n = 5 roots in duplicate	Stored in traditional bags made of jute and hung inside a house made of mud	31 d 62 d 125 d	25 (20/31)	NA	MGCL01 223.6 µg/g Resisto 366.2 µg/g	(MGCL01-Resisto) 31 d: (61–73) 62 d : (37–52) 125 d: (12–21)
Bechoff et al., 2011b	Uganda	Ejumula	N = 4 roots in duplicate	Pre-treatments Deionized water (W) 0.5% citric acid (CA) 1% ascorbic acid (AA) 1% salt (S) Unsoaked (U) 1% AA, 1% salt (AA&S) 0.5% sodium metabisulfite (SM) 0.5% sodium metabisulfite, 0.5% citric acid (SM&CA) 0.5% citric acid, 1% salt (CA&S)	1 month 2 months 4 months 6 months	-20	NA	Total carotenoid W: 237.7 µg/g CA: 232.2 µg/g CA&S: 254.7 µg/g S: 251.4 µg/g AA&S: 260.4 µg/g U: 269.4 µg/g SM: 250 µg/g AA: 260.8 µg/g SM&CA: 259.6 µg/g	Storage:(1m/2m/4m/6m) W: (52/38/31/17) CA: (57/40/23/16) CA&S: (50/39/25/17) S: (54/47/30/17) AA&S: (53/47/32/18) U: (53/47/39/18) SM: (55/50/37/20) AA: (59/38/37/20) SM&CA: (63/49/32/21)

AR: apparent retention; TR: true retention, OV: oven dried; BL: blanched; UB: unblanched; FL: flour; C: chips; OV: oven dried; SU: sun dried; SO: solar dried; T: tent dried; TU: tunnel dried; SH: shade dried; Z: zipped PE bags; SC: sealed clear bag; SCB: sealed clear PE bag in black PE bag; B: black PE bag; NR: not reported; J: jar; DW: dry weight; FW: fresh weight.

**Table 7** Retention of carotenoids in sweet potato-effect of cooking

Reference	Country	Cultivars	Sample size	Methodology	Time	Temp (°C)	Average of initial carotenoid content	Retention % average (min/max)
<b>Boiling or steaming</b>								
Chandler and Schwartz, 1988	USA	Jewel variety, cured (30°C, 85% humidity) for 7 d	NR	Steamed: starch gelatinization (SG) Steamed: enzyme inactivation (SE) Boiling (B)	SG: 30 min SE: NR	SG: 81 SE: 100	Total beta-carotene 440 µg/g DW	SG: 113 SE: 104
Hagenimana et al., 1999	Kenya	32 cultivars	NR	Boiling (B)	NR	NR	Total carotenoids 49 µg/g DM	B: 80
Van Jaarsveld et al., 2006	South Africa	Resisto	n = 5	Boiled covered with water open pot (BO) Boiled, covered with water, closed pot (BC) Boiled, half covered with water, closed pot (BHC)	BO: 30 min BC: 20 min BHC: 20 min		<i>trans</i> -beta-carotene BO: 156 µg/g FW BC: 173 µg/g FW BHC: 155 µg/g FW	BO-TR: 88 BC-TR: 92 BHC-TR: 83
Nascimento et al., 2006	Brazil	Purchased locally	n = 5	Boiled cuts (B)	20 min	100	<i>trans</i> -beta-carotene 4.8 µg/g FW	BC-TR: 88 (87/88)
Kidmose et al., 2007	Kenya	6 orange and yellow fleshed sweet potato	n = 6	Boiled, covered with water, closed pot (BC)	15-39 min	100	<i>trans</i> -beta-carotene 12 to 108 µg/g FW	BC-TR: 88 (45/126)
Bengtsson et al., 2008	Uganda	Ejumula and 6 field test varieties	n = 28	Boiled (B) immersed in water open pot Steamed (S) by wrapping in banana leave an pot with lid on	B: 20 min S: 30 min	B: S: 93	<i>trans</i> -beta-carotene 108 to 314 µg/g DM	B: 78 S: 77
Wu et al., 2008	China	Yanshu No. 5	n = 2	Sweet potato was covered with water in a electric cooker and boiled with the lid on (BC) *boiling for doneness was about 15 min	10, 20, 30, 40 and 50 min	100	<i>trans</i> -beta-carotene BC: 77 µg/g FW S: 67 µg/g FW	10/20/30/40/50 min BC: 99/92/87/80/68 S: 95/88/69/57/48
Tumuhimbise et al., 2009	Uganda	5 varieties	n = 3	Slices of sweet potato were boiled (B) and steamed (S)	B: 20 min S: 30 min	B: 92 S: 94	<i>trans</i> -beta-carotene 67 to 316 µg/g DM	B: 76 (69-84) S: 75 (66-84)
<b>Frying</b>								
Hagenimana et al., 1999	Kenya	Cultivar Zapallo	NR	Fried Mandazis	NR	65	Total carotenoids 49 µg/g DM	F: 36 (31-43)
Nascimento et al., 2006	Brazil	Purchased locally	n = 5	Boiled for 20 minutes (B) then fried for 8 minutes (F) Deep-frying (F)	B: 20 min F: 8 min F: 10 min	100 160-170	<i>trans</i> -beta-carotene 3 µg/g FW <i>trans</i> -beta-carotene 108 to 314 µg/g DM	B,F-TR: 68 (67/68) F: 78
Bengtsson et al., 2008	Uganda	Ejumula and 6 field test varieties	n = 24	Sweet potato was steamed (S) for 40 minutes then fried (F) in rapeseed oil for 1 minute	S: 40 min F: 1 min		<i>trans</i> -beta-carotene 68 µg/g FW	S,F-TR: 60 (40 min) S,F-TR: 64 (1 min)
Wu et al., 2008	China	Yanshu No. 5	n = 4	Slices of sweet potato were deep-fried on preheated sunflower cooking oil (F)	F: 10 min	170	<i>trans</i> -beta-carotene 67 to 316 µg/g DM	F-TR: 75 (62-88)
Tumuhimbise et al., 2009	Uganda	5 varieties of OFSP	n = 3					

(continued on next page)



**Table 7** Retention of carotenoids in sweet potato-effect of cooking (*Continued*)

Reference	Country	Cultivars	Sample size	Methodology	Time	Temp (°C)	Average of initial carotenoid content	Retention % average (min /max)
Bechoff et al., 2011c	Uganda	OFSP-Ejumula	n = 3	Fried chapatis (CP) and mandazis (MD) made of milled OSP chips (30%) and wheat flour (70%)	F: 1.6 min (0.6–3 min)	143 (116–190)	<i>trans</i> -beta-carotene CP: 30.1 µg/g FW MD: 32.9 µg/g FW	CP:F-TR: 83 (70–97) MD:F-TR: 85 (80–89)
<b>Porridge</b>								
Bechoff et al., 2011c	Uganda	Ejumula	n = 2	Porridge: OSP chips (30%), roasted white-yellow maize (35%) and roasted soybean (35%)	6.2 min (4.3–10.3)	84 (81–89)	<i>trans</i> -beta-carotene 56.4 µg/g FW	TR: 78 (69–93)
<b>Baking / Roasting</b>								
Chandler and Schwartz, 1988	USA	Jewel variety, cured (30°C, 85% humidity) for 7 d		Sweet potatoes were wrapped in aluminum foil and baked (R)	R:80 min	191	Total beta-carotene 440 µg/g DW	R:69
Kidmose et al., 2007	Kenya	6 orange and yellow fleshed sweet potato	n = 6	Roasted until core temperature reached 86°C (R)	R: 30–90 min		<i>trans</i> -beta-carotene 12 to 108 µg/g FW	R-TR: 85 (49/119)
Tumuhimbise et al., 2009	Uganda	5 varieties	n = 3	Slices of sweet potato were baked on preheated electric oven (R)	R:15 min	180	<i>trans</i> -beta-carotene 67 to 316 µg/g DM	R-TR: 65 (54–78)
<b>Microwaving</b>								
Chandler and Schwartz, 1988	USA	Jewel variety, cured (30°C, 85% humidity) for 7d		Sweet potato was micro waved until core temp reached 99°C (M)	M:7 min	6000 W	Total beta-carotene 440 µg/g DW	M:77
Wu et al., 2008	China	Yanshu No. 5	n = 2	Portions of sweet potato were put in plastic microwave bowls without covers (M)	M:10 min M:15 min M:20 min	Medium power	<i>trans</i> -beta-carotene 62 µg/g FW	M-TR: 92 (10 min) M-TR: 67 (15 min) M-TR: 34 (20 min)

B: boiled; BL: blanched; S: steamed; F: fried; BO: boiled, open pot; BC: boiled, closed pot; R: roasted /baked; M: microwaved; S2: blanched strips (2 min); S10: blanched strips (10 min); P: puree; SC: steam, starch, gelatinized; S: steamed; SE: steam, enzyme inactivated; CA: canned; DE: dehydrated, drum-dried; TR: true-retention; NR: not reported; DW: dry weight; FW: fresh weight.

carotenoids after the sweet potato was cut into chips, dried, and stored at room temperature for 11 months (Hagenimana et al., 1999). The total carotenoid content was as low as 2 and as high as 632  $\mu\text{g/g}$  DW that corresponded with a range white, yellow, orange, and purple flesh root colors. The average retention of total carotenoid in dried sweet potato chips was 70%, 64%, and 57% after drying for 3, 6, and 11 months of storage, respectively.

On the other hand, dried sweet potato chips packaged using different packaging materials presented variable total carotenoid retention, ranging from 23 to 36% over a 4-month period of storage at room temperature (Bechoff et al., 2010a) (Table 6). None, however, resulted in statistically different total carotenoid retention. There was also no significant genotypic effect on difference in retention among the six cultivars tested (Ejumula, Kakamega, SPK004/1, SPK004/1/1, SPK004/6 AND SPK004/6/6). The authors noted that none of the cultivars would provide a significant source of vitamin A to the diet after storage for four months even if 100g of finished product were consumed by children.

Another study performed in rural Mozambique examined sweet potato chips dried and stored using traditional methods, by hanging jute bags inside a house constructed from mud (Bechoff et al., 2011a). No effect of the chipping (thin, thick, or sliced by hand) was observed on the total carotenoid retention during storage with overall average retention of carotenoids after one, two, and four months of storage of 67.0%, 44.9%, and 16.3%, respectively. However, a significant effect of sweet potato variety was observed between MGCL01 and Resisto. The total carotenoid retention after 31, 62, and 125 days of storage was 61%, 37%, and 12%, respectively, for MGCL01, and 73%, 52%, and 21%, respectively, for Resisto. The variety Resisto also had a higher initial carotenoid content (366.2  $\mu\text{g/g}$ ) compared to MGCL01 variety (223.6  $\mu\text{g/g}$ ), which makes it a better variety for high carotenoid retention over a period of storage.

Degradation of carotenoids in dried foods has been demonstrated to follow a first-order kinetics. Bechoff et al., 2010b developed a model to predict the thermal degradation of  $\beta$ -carotene in sweet potato chips during storage at temperatures between 10–40°C taking into account oxygen and water activity. The kinetic model showed that the breakdown of *trans*- $\beta$ -carotene followed this first-order kinetic's pattern. The authors tested how well the model predicted the pVAC degradation by storing dried Ejumula sweet potato chips in a laboratory inside of a jar for 88 days at room temperature. The difference between the experimental and the predicted value was 4.3% for total carotenoids and 3.5% for  $\beta$ -carotene. The total carotenoid and  $\beta$ -carotene retention measured after dried sweet potato chips were stored in a jar in the dark for 88 days was 41% and 38%, respectively. When the model was compared to data published in the literature (Bechoff et al., 2010a) where dried sweet potato was stored for 125 days under field conditions in Uganda, the difference between experimental and predicted values for total carotenoids was 9.3%.

Pretreatments to prevent carotenoid degradation in sweet potato have been evaluated (Nascimento et al., 2007 and Bechoff et al., 2011b). Blanching, a pretreatment to inactive enzymes, was carried out with sweet potato slices by immersion into boiling water for 1.5 minutes prior to drying and storage. Blanching sweet potato slices did not reduce the initial levels of  $\beta$ -carotene, as it did with cassava (16% loss), but it enhanced the stability of  $\beta$ -carotene of the dried sweet potato slices when stored at room temperature (Nascimento et al., 2007). After 20 days of storage, 45% of  $\beta$ -carotene was retained in unblanched samples as opposed to 80% retention in blanched samples. Blanched samples reached 50%  $\beta$ -carotene retention only at 70 days of storage. However, in a recent study, blanching of sweet potato for 8–11 minutes showed higher retention, but it was revealed that leaching of solids occurred during blanching and that was responsible for artificially increasing the retention levels (Bechoff et al., 2011b).

Pretreatment of dipping sweet potato chips with chemicals, such as sodium metabisulfite, acids (ascorbic acid, citric acid), and salt, singly or in combination, had little effect on carotenoid preservation after six-month storage (Bechoff et al., 2011b). The authors concluded that even though the pretreatment was effective, the soaking portion had a negative impact on the carotenoid content after drying.

### Cooking

**Boiling** sweet potato for about 20 to 30 minutes had a minor effect on the pVAC degradation as the reported retention of total carotenoids/ $\beta$ -carotene ranged between 80–90% (Hagenimana et al., 1999; Nascimento et al., 2006; van Jaarsveld et al., 2006; Kidmose et al., 2007; Bengtsson et al., 2008; Wu et al., 2008). Boiling sweet potato fully immersed in water in a covered pot for 20 minutes had almost a 10% increase in the pVAC retention (92% versus 83%) compared to when only half of the pot was covered with water (van Jaarsveld et al., 2006). As expected, the period of boiling had a negative effect on the pVAC retention ranging from 99 to 68% if boiled for 10 versus 50 minutes (Wu et al., 2008). The authors demonstrated a strong linear reduction in pVAC content when plotted against boiling time ( $R^2 = 0.97$ ;  $p < 0.001$ ;  $n = 5$ ). The usual boiling time for sweet potato in Mozambique and Uganda is around 20–30 minutes, which is associated with 92–87% retention (Wu et al., 2008). Isomerization of 1% of *trans*- $\beta$ -carotene into 13-*cis*- $\beta$ -carotene occurred during boiling raw sweet potato and increased to about 12% in mashed, boiled sweet potato (van Jaarsveld et al., 2006). The results of the studies are presented in Table 7.

**Steaming** sweet potato for 30 minutes resulted in pVAC retention in the range of 69–77% (Bengtsson et al., 2008, Wu et al., 2008, Tumuhimbise et al., 2009). Wu et al., 2008 reported similar pVAC retention for boiling and steaming until 30 minutes of cooking, at which point steamed sweet potato experienced a 17% greater reduction in pVAC content. Using

a traditional steaming method of slicing sweet potato and wrapping in banana leaves, Bengtsson et al., 2008 documented that after 30 minutes of steaming the pVAC retention levels (77%) were roughly comparable to those in sweet potato boiled for 20 minutes (78% pVAC retention). Banana leaves seemed to have offered no protection against the high heat energy of steam in this time frame.

**Frying** over a short period of time (1 to 10 minutes) was found to be a relatively nondestructive process for pVAC content with an average of 70% retention (Range: 67–78%), Nascimento et al., (2006), Bengtsson et al., (2008) and Wu et al., (2008). Although the pVAC retention did not decrease within a short frying period of 1 to 3 minutes, isomerization did increase significantly ( $p < 0.05$ ) from 2.4 to 15% (Kidmose et al., 2006).

**Roasting** whole sweet potato on a grill was found to be less damaging to the pVAC content (85% retention; range: 49–100%), Kidmose et al., (2007), than baking in the oven (69% retention) (Chandler and Schwartz, 1988). However, isomerization of  $\beta$ -carotene to the *cis* form was 12 and 11% when roasting or baking sweet potato, respectively. A moderate correlation was found between pVAC retention and cooking time ( $R^2 = 0.36$ ;  $p < 0.001$ ).

## THE EFFECT OF PROCESSING AND STORAGE ON CAROTENOID RETENTION

### Drying Effect

The degradation of all-*trans*- $\beta$ -carotene during drying are probably caused by oxidative degradation which is promoted by the presence of oxygen, high temperature, low water activity, and the presence of light, while isomerization appeared to have a minimum effect on degradation during the drying process (Bechoff et al., 2010b). No significant increase in the *cis*-isomers was observed when sweet potato was dried by three different methods: hot-air, solar, and sun-drying (Bechoff et al., 2009). In another study, *cis*-isomers corresponded to only 1% and 3% of total  $\beta$ -carotene content after sweet potato was dried as chips and flour, respectively, with the 13-*cis*- $\beta$ -carotene as the predominant isomer, while only small amounts of 9-*cis*- $\beta$ -carotene were detected in flour (Kidmose et al., 2007). Only when sweet potato had been heated prior to drying did the *cis*-isomers accounted for a large share (29%) of the total  $\beta$ -carotene content (Chandler & Schwartz, 1988).

The higher temperature-short time combination rather than low temperature-longer time resulted in higher pVAC retention in sweet potato (Bengtsson et al., 2008; Bechoff et al., 2009) and maize (Burt et al., 2010). Similar results have been reported for solar-dried mangoes, where the prolonged drying time at lower temperature reflected in a lower retention of all-*trans*- $\beta$ -carotene and an increased isomerization rate (Pott et al., 2003). In this study, mango slices of cultivars Kent and Tommy Atkins dried at 75°C for 3–3.5 hours presented 93 and

69% retention of all-*trans*- $\beta$ -carotene, respectively, compared to a lower retention, 66% and 58%, for cultivars Namdok Mai and Kaew, respectively, when dried for a longer period of time (7–8 hours) at a lower temperature (62°C). Furthermore, the 13-*cis*- $\beta$ -carotene increased by 19% and the 9-*cis*- $\beta$ -carotene was negligible when mango slices of Kent cultivar were dried in the dark; with sun exposure, the formation of 9-*cis*- $\beta$ -carotene increased, representing 64% and 51% of total *cis*-isomers, respectively, for Kaew and Namdok Mai cultivars. Unfortunately, the genotype effect was a confounder in this study and could not be taken into account.

The high variability in the pVAC retention after drying (55–85% for maize, 10–99% for cassava, and 66–96% for sweet potato), was associated not only with the drying method applied, temperature, and duration of exposure to heat but also with the differences in genotype (Clydesdale and Francis, 1976; Britton, 1992). The only physical attribute that has been associated with genotype in the literature has been the differences in the dry matter content. Significant correlations between initial dry matter content and carotenoid losses (Pearson coefficient  $R = -0.518$ ;  $p < 0.05$ ) and between initial total carotenoid content in fresh sweet potato chips and carotenoid losses (Pearson coefficient  $R = 0.589$ ;  $p < 0.05$ ) have been observed (Bechoff et al., 2009). The authors suggested that there is a linkage between both initial dry matter and initial carotenoid content and percentage loss of carotenoid during drying. Bengtsson et al., 2008 suggested that for an equivalent drying time, cultivars with higher moisture content and with higher initial carotenoid content tended to lose more carotenoids during the drying process. Mdziniso et al., 2006, also observed that sweet potato exhibited the least  $\beta$ -carotene loss (5–6% dry basis) whereas carrots exhibited the highest (49–68%), and their respective fresh moisture contents were 76 and 91%.

Overall, shade-drying was not as damaging to the carotenoid content in cassava as drying under the sun and it may be a better option for drying cassava in the rural environment where oven drying is not available (Chavez et al., 2007). On the contrary, drying sweet potato under the sun did not show a significant difference in the pVAC retention levels as compared to solar-drying (Bengtsson et al., 2008; Bechoff et al., 2009). Only one study (Burt et al., 2010) investigated the pVAC retention in maize after drying, showing that high heat (90°C) was not more detrimental than low heat (above 25°C) when maize was dried in the oven. A study with cowpea leaf and mango showed greater  $\beta$ -carotene retention when dried by solar-dryer than under the sun, furthermore, blanching cowpea leaves improved the  $\beta$ -carotene retention by 15% (Ndawula et al., 2004). In cassava, the blanching process reduced the initial levels of  $\beta$ -carotene (84% retention), but not in sweet potato (100% retention) (Nascimento et al., 2007). However, blanched samples showed higher  $\beta$ -carotene retention than unblanched samples during storage for both crops. In a most recent study, although blanched samples of sweet potato had the highest carotenoid content after drying compared to other

pretreatments, e.g., salt, sodium metabisulfite, and ascorbic acid, the authors concluded that blanching artificially increased the level of retention by soluble solids that occurred during the process (Bechoff et al., 2011b). Blanching sweet potato for 2 and 10 minutes has been shown to reduce the solid content by 15 and 28%, respectively (Chandler and Schwartz, 1988).

In conclusion, in rural settings, sweet potato can be dried under the sun with little detriment to the pVAC levels as compared to other drying methods, while shade-drying is recommended for cassava. More research is needed to recommend the best drying method for maize. Therefore, the drying method of choice for better pVAC retention and most cost-effective will depend on the crop and the specific genotype. Future research should investigate the genotype-related elements that influence the higher or lower pVAC retention in those crops during the drying process so that the traits associated with protection against degradation may be included in the biofortification plant-breeding program.

### *Storage Effect*

Storage conditions and duration had a greater negative impact on pVAC retention in cassava, maize, and sweet potato compared to drying and cooking. After one month of storage, cassava flour and chips presented 40 and 20% of the original pVAC levels when oven-dried and sun-dried, respectively (Chavez et al., 2007). Specific maize genotypes stored in the dark for four months showed 60–65% pVAC retention in one study (Burt et al., 2010); and other genotypes stored under subtropic conditions (20°C/20% RH) during a similar period displayed, pVAC retention between 30 and 60% (unpublished data). The case was less dramatic for sweet potato. The Resisto variety on average showed 10% higher pVAC retention (73%, 52%, and 21%) compared to the MGCL01 variety (61%, 37%, and 12%) after one, two, and four months of storage as chips, respectively (Bechoff et al., 2011a). Although at this point it is not clear what makes one variety more resistant to pVAC degradation than another, it is recommended that a retention study be conducted as part of the screening process to select the best biofortified variety for release and that the judgment not be solely based on the high pVAC content. Additionally, because of the intrinsic instability of carotenoids and retinoids, storage practices of pVAC crops should be optimized when they are deployed in target countries.

Autooxidation was the suggested main mechanism of carotenoid degradation during storage of dried sweet potato (Bechoff et al., 2010b). The authors reported that oxygen, in the range studied of 0–21%, had a greater effect on  $\beta$ -carotene degradation than water activity (0.13–0.76) and temperature (10–40°C). Furthermore, lower  $\beta$ -carotene degradation occurred at higher water activities in sweet potato demonstrating that enzymatic degradation is unlikely to occur due to low enzymatic activity in those conditions. It has also been

hypothesized that because the oxidative degradation occurs through a free-radical process that water can have a protective effect on carotenoid stability through its interaction with free-radicals (Haralampu and Karel, 1983). Chen et al., 1996 studying stability of carotenoids in stored carrot juice showed that those stored in glass bottles under light had greater  $\beta$ -carotene degradation than those stored in aluminium foil bags (dark); and the formation of 9-*cis*- $\beta$ -carotene was favored when the carrot juice was stored under light, while 13-*cis*- $\beta$ -carotene was the main isomer when stored in the dark. In this study, higher temperature of storage (35°C vs. 4°C) was also detrimental to carotenoids.

In order to avoid pVAC degradation during storage of sweet potato, Bechoff et al., 2009 conducted experiments by using different types of plastic bags (dark vs. clear, or zipped vs. sealed) to store sweet potato chips. There was no difference among all forms of storage with an average of 30% pVAC retention after four months of storage at room temperature. Most recently, several pretreatments with antioxidants and alkaline solutions were applied prior to drying and although there was some improvement in the pVAC retention it was not maintained after four months of storage (Bechoff et al., 2011b). Chavez et al., 2007 applied vacuum to the bags of cassava flour prior to storage. The cassava flour packed in plastic bags under vacuum resulted in higher losses than the ones packed in plastic bags without vacuum, probably because the plastic bags were not impermeable to oxygen.

In summary, the pVAC losses during storage were considerable in all three crops. Selection based on the genotype through retention studies is the most practical method available, while more research on better understanding the genetic factors, such as the Or cauliflower gene (Li et al., 2012) that makes one variety less susceptible to pVAC degradation is needed as well as studies investigating pretreatments and optimal packaging conditions to decrease pVAC degradation during storage.

### *Cooking Effect*

The average pVAC retention among different cooking methods can be ranked from highest to lowest as: boiling and steaming (80%) > baking (70% for cassava and sweet potato and 30% for maize) > frying (50% for sweet potato, 54% for cassava and 18% for maize) > gari and fufu (30%). Except for frying, which is not typical among the rural African traditional foods, gari and fufu presented the lowest pVAC retention among the cooking processes included in this review. Gari is the most popular form of cassava consumed in West Africa that involves fermentation and roasting (Ezedimna et al., 2008). According to Thakkar et al., 2009, the fermentation step had minimal effect on pVAC retention with 92% of total  $\beta$ -carotene remaining after cassava was fermented for three days; however, high levels of isomerization, mainly 13-*cis*- $\beta$ -carotene, were evident following the roasting step. The

retention was particularly dependent on the temperature/time combination. When cassava was roasted at higher temperature (195°C) for 20 minutes, only 10% of  $\beta$ -carotene was retained. The  $\beta$ -carotene degradation followed a similar pattern whether the biofortified cassava was conventionally bred or genetically modified (Failla et al., 2012). Further retention studies are currently underway with conventionally bred pVAC cassava in Nigeria to investigate the steps that are most critical for carotenoid degradation in the processing of gari and the best method to minimize degradation will be recommended.

As observed with gari, fermentation does not seem to have a dramatic effect on pVAC retention in other fermented products of maize and cassava. Thakkar et al., 2009 reported that fermentation and brief boiling of cassava during the preparation of fufu did not induce isomerization from all-*trans*- $\beta$ -carotene to 13-*cis*- $\beta$ -carotene. The same occurred with maize, despite losses during the fermentation phase, the final product of fermented and unfermented maize porridges did not differ significantly in their  $\beta$ -carotene retention (75%) (Li et al., 2007).

#### **IMPLICATIONS FOR THE BIOFORTIFICATION INTERVENTION PROGRAM**

The overall  $\beta$ -carotene retention in maize stored for four months after harvesting (50%), which may then be wet milled (93%), fermented (90%), and boiled (92%) is approximately 38%. In the biofortification program, the original assumption for retention was 50%, which would provide 50% of the vitamin A estimated average requirement (EAR) for children four to six years of age and women of child bearing age considering the assumptions for maize consumption and  $\beta$ -carotene bioavailability and bioconversion remained constant. However, the biofortified maize has shown higher bioconversion of  $\beta$ -carotene to retinol: 7:1 (Li et al., 2010) and 3:1 (Muzhingi et al., 2011) than the 12:1 originally assumed (IOM, 2001). All things considered, 15  $\mu\text{g/g}$  of pVAC for biofortified maize remains as the biofortification target which will contribute an additional 50% to the vitamin A EAR of women and children. Regarding extreme cooking effects, such as those practiced for production of gari, as little as 20% of the original pVAC will remain when the gari is finally consumed, which may still provide the 50% vitamin A EAR, given that average per capita consumption of cassava by adult women in rural Nigeria and the bioconversion/bioavailability of  $\beta$ -carotene in cassava are about 900g (FW) and 15%, respectively. Cassava, maize and OFSP biofortified with pVACs are expected to be good sources of  $\beta$ -carotene and biofortification a promising food-based intervention for prevention of vitamin A deficiency in women and children in developing countries.

The pVAC retention varied among maize, cassava, and sweet potato, indicating a difference in the protection or exposure of carotenoids to degradation according to the food matrix. Several studies also showed a strong genotype effect on pVAC

retention within the same crop. More research is needed to understand the matrix and genotype effect on the protection of carotenoid degradation from those crops and provide guidance to plant breeders to seek genetic traits associated with these characteristics as well. Therefore, it is necessary to evaluate in more detail the differences among the genotypes to elucidate the reason of the different degradation pattern.

Furthermore, studies evaluating pVAC retention in processed and cooked maize, cassava, and sweet potato can be flawed if raw and cooked samples are not equivalent, extraction efficiency for the raw and cooked samples differ, enzymatic oxidation occurs, and calculation of retention is not corrected for weight changes during cooking. Recommendations for the best practices in retention studies have been compiled in this review.

More recent literature has reported the retention values as both, fresh weight and dry weight (Ceballos et al., 2012). This is a recommendation for future publications as there are different needs and practices within the various scientific research communities.

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