Chapter 11

Carotenoids in Yellow Sweet Potatoes, Pumpkins and Yellow Sweet Cassava

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Abstract

Carotenoids are the most widespread pigments in nature, extremely important for human health, but are highly unstable molecules especially when exposed to light, oxygen and heat. Many authors report the carotenoid's importance, mainly its pro-vitamin A (α- and β-carotene) and, additionally, the antioxidant capacity of some of them. Currently, more than 600 carotenoids are known and characterized by their chemical structures. In vegetables, common pro-vitamin A carotenoids include β-carotene and its 9, 13 and 15 isomers, α-carotene and β-cryptoxanthin. Other common carotenoids such as lycopene, lutein and zeaxanthin do not have pro-vitamin A activity but serve as natural antioxidants. They are found in many fruits and vegetables such as carrots, yellow sweet potatoes, yellow sweet cassava and pumpkins. Normally, in these plant materials, the β-carotene is the most abundant. It is still used as natural food coloring, which is not very expensive, since enough 3–5 g of β-carotene is used to impart a yellow color characteristic of a ton of margarine. There is also a description of its importance in the formation of compounds responsible for flavors that are of interest fragrance and food industries. The purpose of this chapter is to report the presence of pro-vitamin A carotenoids, mainly the β-carotene in pumpkins, yellow sweet potato and yellow sweet and bitter cassava.

Keywords: carotenoids, cassava, yellow sweet potato, pumpkin, β-carotene

1. Introduction

Carotenoids are the most widespread pigments in nature, extremely important for human health, but are highly unstable molecules especially when exposed to light, oxygen and heat. Many authors report the carotenoid's importance, mainly its pro-vitamin A (α- and β-carotene) and, additionally, the antioxidant capacity of some of them. Currently, more than 600 carotenoids are known and characterized by their chemical structures [1–4]. In vegetables and fruits, common pro-vitamin A carotenoids include β-carotene, α-carotene and β-cryptoxanthin [2, 5]. Other carotenoids such as lycopene are rich in tomatoes. Lutein and zeaxanthin had no pro-vitamin A activity but act as natural antioxidants and are the yellow pigments of the human retinal macula [6–9] and are believed to be responsible for the ophthalmological protective effect of carotenoids, acting as both antioxidants and high-energy blue light filters. In Ref. [10], like spinach, sour cream, corn and egg, lutein and zeaxanthin are the yellow pigments of the human retinal macula [7–9] and are believed to be responsible for the ophthalmological protective effect of carotenoids, acting as both antioxidants and high-energy blue light filters [10, 11]. Carotenoids are found in many fruits and vegetables, such as carrots, yellow sweet potatoes, yellow sweet cassava and pumpkins, among other plant materials. The purpose of this chapter is to make an overview on some aspects of the carotenoids such as sources of pro-vitamin A, functional properties and activities in yellow sweet potatoes, yellow sweet and bitter cassavas and pumpkins.

2. Carotenoids

Nowadays, there is a new approach on the prevention of some pro-vtamin A diseases caused by provitamin A deficiencies, at low costs, mainly the β-carotene. The groups affected by nighblindness, corneal scarring, blindness, measles and, increased mortality in infants (0–12 months), an children (1–6 years) [3] as well as the pregnant and lactating women (blindness) in the underdeveloped and in developed countries or regions around the world.

Years ago, carotenoids like β-carotene were and still is used as natural food colorings, since enough 3–5 g of β-carotene is used to impart a yellow color characteristic in margarines. There is also a description of its importance in the formation of compounds responsible for flavors that are of interest in fragrance and food industries. Besides the food industry, there is the incipient interest of the pharmaceutical industry for its nutritional and functional properties, such as vitamin A precursors and the antioxidant activity [1, 4]. The isomerization is one of the best known chemical properties of carotenoids. Some *cis* (Z) forms can be naturally occurring, as in the case of the first carotenoid biosynthetic, phytoene and phytofluene route, there are 15 predominantly in the *cis (Z)* configuration. Few *cis* forms were found naturally because the *cis* double bond of the presence creates steric hindrance between neighbor groups, making it less stable molecule. Thus, most carotenoids exist in nature in the *trans* configuration, which is more thermodynamically stable [12]. In case of carotenoids, the *cis (Z)* and *trans (E)* designations are determined by the arrangement of substituents of the C=C double bond. Thus, if the substituents are on the same side of the axis, C=C double bond is called *cis*, and

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if the substituents are on opposite sides of the axis, C=C double bond is called *trans* [12]. The β-carotene naturally occurs in all *trans* (*E*) forms, which is thermodynamically more stable and less soluble as said previously. However, the occurrence of *cis* isomer has been reported, frequently, estimated on the theory, that there are 272 possible isomers of β-carotene, and only 12 were already detected. Among them, easily formed are the *cis* 9, *cis* 13 and *cis*-15. The carotenoids from plant materials contribute with approximately 68% of vitamin A diet globally, and 82% in developed countries. One benefit of pro-vitamin A carotenoids is that they are only converted into vitamin A when the body needs, therefore avoiding its accumulation. On the other hand, several factors influence on its absorption and utilization, such as the type and physical form in the diet, fat intake, vitamin E, fiber contents and the existence of certain diseases and parasitic infections [13]. There are over 600 known carotenoids, some of these compounds are pro-vitamin A and other has little or no vitamin A activity [14].

Carotenoids from vegetables account for 80–85% of dietary vitamin A supply, and their role as a source of pro-vitamin A has attracted great interest due also to the antioxidant potential effect [15].

The biofortification can be an approach to minimize the pro-vitamin A deficiencies and defined as the enrichment of staple crops with essential micronutrients. At present, it is one of the strategies used to alleviate vitamin A deficiency (VAD) by breeding staple crops with β-carotene. Staple crops that have been successfully biofortified with β-carotene under the Harvest*Plus* program are cassava, maize (corn) and sweet potato [16].

Recently, β-apocarotenoids (cleavage products of β-carotene formed by chemical and enzymatic oxidations) were identified and quantified in cantaloupe melons and orange-fleshed honeydew but not in biofortified foods. Biofortified cassava was evaluated; however, there are no detailed analyses of these compounds in biofortified foods and little is known about their bioavailability and intestinal absorption and kinetics of cell uptake and metabolism of β-apocarotenoids. The β-apocarotenoids in roots of non-biofortified cassava varieties were lower than those of biofortified and were hypothesized that these compounds are directly absorbed from the diet similarly to β-carotene (Caco2 cells) [17]. Two unidentified metabolites (X and Y) of β-apo-8′-carotenal. The cellular uptake of β-apo-13-carotenone was rapid, and this compound was extensively degraded over the time. Understanding the mechanisms of absorption and metabolism of β-apocarotenoids relative to their quantities in foods is critical in exploring the functions of these metabolites, some of which have been shown to be potent antagonists of vitamin A.

Hess,Thurnham and Hurrel (2005) [18] reported some studies about the influence of provitamin A carotenoids on status of iron, zinc and vitamin A, considering the effect of β-carotene on vitamin A, requirements by consumption of plant foods, link between vitamin A deficiency and, iron, the possible interactions between vitamin A and, iron metabolism, the link between vitamin A and zinc and, some interventions studies as well the knowledge gap and suggestions for future research. The bioavailability of these micronutrients and their deficiencies in the developed countries in infants, children, and pregnant and lactating women were studied. Another function of some carotenoids like the β-carotene is the protective ability of these pigments that act as antioxidants, acting in preventing peroxidation. Antioxidants are classified into two categories: chain-breaking antioxidants that interfere with the propagation step and preventive antioxidants that interfere with the process initiation step [1]. At high concentrations of O_2, there is a reduction in the antioxidant activity of β-carotene observed in studies conducted in pulmonary tissues. Since peripheral tissues, the efficiency of carotenoids can be greater because the oxygen pressure is lower [19]. The *Z* isomers of pro-vitamin A have long been known as fitted with vitamin A activity lower than the *E* isomer (*trans*) matching [20]. Furthermore, (*all-E*)-β-carotene was absorbed preferentially to (9-*Z)*-β-carotene in humans [21–23]. The analyses for quantification of total carotenoids are very well described in Ref. [2], using UV/vis spectrophotometry at 450 nm, acetone for extraction and the high-performance liquid chromatography (HPLC) using petroleum ether for their identification. On the other hand, in pro-vitamin A carotenoids, the factors that determine a good antioxidant capacity are as follows: the presence of an electron donor substituent or the hydrogen to radical, depending on their reduction potential; the radical shift capacity formed in its structure and the ability to chelate transition metals involved in oxidative and access to the site of action process, depending on the hydrophilicity or lipophilicity and its partition coefficient [24]. The chemical characteristics of the antioxidants include solubility, regenerative ability, the structure/activity and bioavailability, which are important factors when considering the role of these compounds in human health [25]. In Ref. [26], it was reported that interactions between structurally different compounds and that have variable antioxidant activity promote additional protection against oxidative stress. The antioxidants maybe classified as natural or synthetic. The second antioxidants are widely used in industry, being the most used butylated hydroxyanisole (BHA), butyl hydroxytoluene (BHT), tertiary butyl hydroxyquinone (TBHQ) and propyl gallate (PG). Your choice and concentration vary depending on the food to be used [27–29]. However, due to their potential risks to human health (carcinogenc effects), has increased the interest in research of natural antioxidants [30] present in raw plant materials, processed or not, such as fruit and vegetables, such as tocopherols, ascorbic acid, carotenoids and phenolic compounds [29]. In determination of antioxidant activity of food, in addition to informing its antioxidant potential before ingestion, it is important to assess food's protection against oxidation and deterioration reactions that can lead to decreased quality and its nutritional value [31]. Different methods of measurement of capacity / antioxidant activity of substances and foods such as DPPH (α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging, ORAC (Oxygen Radical Absorbance Capacity) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). These are shown necessary because of the difficulty of measuring each compound separately and the interactions between the different antioxidants in the system [31–33].

2.1. Sweet and bitter yellow cassava

Cassava (*Manihot esculenta*, Crantz) belongs to the *Euphorbiaceae* family, originated from South America where it was cultivated by the Indians who were responsible for its dissemination in almost all over America. In African, Latin American and Asian continents, it is still one of the main caloric foods to nearly 500 million people, mainly in underdeveloped countries [34, 35]. The variability in total carotenoids and β-carotene and isomers, in 12 varieties of raw yellow bitter cassava roots as well as the degradation in five varieties after the flour process to observe the heat treatment effect on carotenoids degradation, revealed that total carotenoids varied according to the variety, with the β-carotene as the most abundant. At the same time, some varieties presented expressive contents of 13 and 9-*cis*-β-carotene isomers. The total degradation of total carotenoids in the flour, after 19th day of storage, showed the necessity of optimizing the drying process to minimize this loss in order to minimize the deficiency in the Brazilian low-income populations [36]. Studies were conducted in seven raw and cooked roots of yellow sweet cassava to identify total carotenoids, *α* and β-carotene and its isomers in new varieties that could contribute in the nutritional quality improvement in the populations with malnutrition problems situated in the tropics and, particularly, in the Brazilian Northeast, where the cassava is almost the one of the main cultivations and sometimes the only nutrient source. The *trans*-β-carotene was predominant; however, isomers 13 and 9-*cis* were found in significant quantities compared to the total carotenoids content. However, there was no cooking style that stood out regarding carotenoids retention. Total carotenoids varied in raw roots from 14.15 to 2.64 $\mu g\,g^{-1}$, and total β-carotene from 10.32 to 1.99 $\mu g\,g^{-1}$. The highest content was the all-*E*-β-carotene (4.55 $\mu g\,g^{-1}$). The highest retention % of total carotenoids was found in two varieties (99.49%) and, in total β-carotene (94.31%) were both after cooking. Carotenoids' variability presented the individual potential of the varieties, in the retention prevailed the heat effect in each cooking style applied. However, no cooking style provided a higher retention of total carotenoids or β-carotene uniformly, in all the varieties, with behavior of each variety of sweet yellow cassava roots prevailing in the cooking style. This evaluation showed differences in behaviors that can be attributed to the total carotenoids that were initially found. Differences were found in the cooking styles among the cooking styles regarding total carotenoid and β-carotene in real retention percentage, suggesting and this retention was high for β-carotene [37]. Another aspect that needs to be point out is the storage of cassava roots after harvest for providing important and fundamental information to plant breeding programs aimed at improving cassava storage root nutritional quality. In Ref. [38], Among the 23 cassava landraces with different types of storage, root color and diverse carotenoid types and profiles, the landrace Cas51 (pink color) had low LYCb transcript abundance, whereas landrace Cas64 (intense yellow storage root) had decreased HYb transcript abundance. Lycopene and total β-carotene increased in landraces Cas51 and Cas64, respectively [38]. Thirteen cassava accessions from Brazilian Northeast in two crops were evaluated by many characters. There were accessions identified with potential use as parents in plant breeding to increment of β-carotene BGMC 1221, BGMC 1223 and BGMC 1224 and lycopene BGMC 1222 and BGMC 1223 contents in storage roots [39]. In Ref. [40], the study reported an allelic polymorphism that, in one of the two expressed phytoene synthase (PSY) genes, is capable of enhancing the flux of carbon through carotenogenesis, leading to the accumulation of colored pro-vitamin A carotenoids in storage roots.

2.2. The yellow-flesh sweet potato (*Ipomoea batatas* (L) Lam.)

Ipomoea batatas belongs to the Convolvulaceae family, genus Ipomoea with 50 genera and more than 1000 species. However, it is the most important species and, sometimes, the only staple food crop. The varieties of potatoes with white or pale yellow flesh are less sweet and moist than those with red, pink or orange flesh [41] and are native to the tropical regions in the Americas and Africa [42]. American types with pink yellow flesh contain as high as 5.4–7.2 mg 100 g^{-1} of β-carotene but higher contents. Additionally, more than a dozen African vegetables, this was the richest in folate (1.93–1.96 mg g^{-1}) [43]. Some cultivars are developed by biofortification program as the yellow/orange-flesh sweet potatoes like the *Beauregard* cultivar in Brazil, with intense orange pulp, because of its high content of β-carotene [16] and, among many studies on it, its antioxidant capacity [44]. The β-carotene is one of the carotenoids with higher pro-vitamin A activity, which is the largest source of vitamin A and its derivatives in the human diet. It is also the most active carotenoid, comprising 15–30% of all serum carotenoids [45]. Because of its high combined structure, the carotenoids are susceptible to degradation by light, oxidation by heat, acid or alkaline pH and the presence of metal ion. They can be hydrophobic, lipophilic, insoluble in water and soluble in solvents such as acetone, alcohol and chloroform. Of more than 600 known carotenoids, only about 50 have pro-vitamin A activity and are antioxidants [46, 47]. On the other hand, studies on the profiles of phenolics, carotenoids and antioxidant capacities of raw and cooked white, yellow, orange, light purple and deep purple sweet potato varieties, grown in Guilin (China), revealed higher anthocyanin contents and antioxidant capacities in purple sweet potato species and higher carotenoid contents in yellow and orange sweet potato. All cooked sweet potatoes exhibited significantly ($p < 0.05$) lower TPC, MAC, TCC, DPPH and Fluorescence recovery after photobleaching (FRAP) values compared to the respective raw samples. Steaming samples showed good results in retention of Total Phenolic Compounds, roasting for keeping anthocyanins, and boiling best preserve the carotenoids [48]. Various types of orange sweet potato (*Ipomoea batatas*) are grown in Brazil and in the world having different shapes and sizes and especially differentiated carotenoid contents of pro-vitamin A. The total carotenoid and β-carotene as well as its isomers 9:13—cis (*Z*) of β-carotene from two cultivars of orange sweet potato: an organic cultivar called 'carrot', and the *Beauregard* sweet potato Beauregard showed the highest β-carotene content among the studied samples being a good source of provitamin A to be cultivated and consumed, mainly, in the areas of low-income populations and where the deficiency of vitamin A is common among children [16, 44]. *The Beauregard* is a biofortified American cultivar with intense orange pulp because of its high β-carotene content. The effect of the drying treatment on the β-carotene and total carotenoid of this cultivar dried at 40°C for 5 h, 50°C for 2 h and at 60°C for 1 h showed total carotenoids, in mg kg^{-1}, of 129.85 in raw samples; 124.26 in bleached samples; 760.65 (40°C); 769.76 (50°C) and 832.40 (60°C), respectively. The results found by Baganha et al. (2016) for total carotenoids, in $mg.kg^{-1}$, were 129.85 ± 2.47 in sweet potatoes raw samples; 124.26 ± 3.40 in bleached samples; 760.65 ± 1.45 (40 °C); 769.76 ± 4.43 (50 °C) and 832.40 ± 6.02 (60 °C), respectively. The mean values for β-carotene (mg.kg-1) were 107.93 ± 0.66 (raw); 97.71 ± 4.13 (bleached); 660.08 ± 11.65 (40 °C); 677.03 ± 9.45 (50 °C) and 736.21 ± 3.46 (60 °C), respectively. Drying at 60°C for 1 h showed the highest retention of total carotenoids and β-carotene, indicating that the shortest time of exposure to heat had a greater influence than the higher temperature [44]. In another study, in India, 15 genotypes of exotic and indigenous orange-flesh sweet potatoes cooked were evaluated after cooking process. The β-carotene contents ranged from 28.80 to 97.40 μg g^{-1}, and its retention after cooked varied from 76. 90 to 87.76% [49]. Ten sweet potato clones with different orange flesh color were processed in an oven-drying, boiling, sun-drying and frying. The carotenoids retention depended on the process applied. The highest retentions of total carotenoids and β-carotene were observed in oven-drying (90–91% and 89–96%) followed by boiling (85–90% and 84–90%) and frying (77–85% and 72–86%), and the lowest in both micronutrients were found in the sun-drying method (63–73%) and β-carotene (63–73%) [50, 51]. The extraction step is very important in β-carotene from sweet potato. According to the reference, the best

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solvent and time of extraction were observed using 91.1% of acetone and 19.6 min of extraction and 278.1 μg g^{-1} of β-carotene in the variety CYY95-26 and small amounts of the isomers 9 and 13-cis [52]. Recently, the β-carotene of four sweet potato varieties from Tanzania (*Jewel*, *Karoti dar*, *Kabode* and *Ejumula*) with different intensities of orange flesh color was evaluated. Sweet potatoes were blanched and boiled. There was a threefold reduction in β-carotene content when fresh samples were dried. Boiling results in more retention of β-carotene than blanching in sweet potatoes. The fresh dried had significantly low β-carotene content and low retention on storage compared to boiled and blanched chips, and blanched cowpea leaves retained more β-carotene after 6 months of storage at room temperature [53].

2.3. Pumpkin (*Cucurbita*)

A large number of pumpkin varieties (Cucurbitaceae), each of which containing different amounts of carotenoids, are cultivated worldwide [54]. In Brazil, *C. moschata* cultivars are known to contain high amount of α- and β-carotene. β-carotene has almost 100% pro-vitamin A activity, and α-carotene has approximately 53% pro-vitamin A activity [11, 55–57]. Some varieties such as *C. moschata*, *C. maxima* and *C. pepo*, with color ranging from intense yellow to orange, have revealed high levels of carotenoids, particularly, α and β-carotene, β-criptoxanthin, lutein and zeaxanthin [11]. The orange-fleshed pumpkins (*C. moschata*) normally present high levels of carotenoids mainly β- and α-carotene as well the 9, 13 and 15-β-carotene isomers. Inspite of the low bioaccessibility and bioavailability of the pumpkin carotenoids, its high contents after the cooking styles can still offer adequate daily dietary. On the other hand, the drying process usually can affect the levels of these micronutrients. In Ref. [6], the carotenoid content within pumpkin and squash measured by HPLC and with colorimeter L*a*b* color space values was correlated, and a range of colors and carotenoid types and concentrations within pumpkins and squash was found as well as strong correlations between colorimetric values and carotenoid content were identified. The authors suggested that the genetic variations should make it possible to increase the nutritional value through crossing and selection from within and among the different types with high levels of carotenoids. The α- and β-carotene) of pumpkin flours were evaluated using an oven with air circulation and finally milled in temperatures at 45 and 50°C. Pumpkins are cut into slices, blanched at 90°C for 3 min and dried. The drying process at 45°C spent 132 h (5.5 days) was longer compared with sliced pumpkins dried at 50 (48 hours). In raw pumpkins, total carotenoids were 442.56 μg g^{-1}, α-carotene was 110.87 μg g^{-1}, and β-carotene was 297.37 μg g^{-1}. In flours dried at 45 and 50°C, the total carotenoids were 1892.98 and 1668.43 μg g^{-1}, respectively. Flours presented high contents of carotenoids, as expected, since their moistures were very low (9.17 and 7.83 g 100^{-1}). The flour dried at 45°C preserved 95% of the α-carotene and 83% of the β-carotene compared to the flour dried at 50°C. The isomers 9 and 13-*Z*- of the β-carotene were present in small percentages in both flours. The results showed to be promising by the fact that the use of these flours in meals in scholar-age children can increase the dietary intake of pro-vitamin A minimizing the vitamin A deficiencies in underdeveloped countries [58]. As wrote previously, some carotenoids are rich in β-carotene, but few are converted by the body into retinol, the active form of vitamin A. These carotenoids are susceptible to degradation (e.g., isomerization and oxidation) during cooking. Total carotenoid, α- and β-carotene, and 9 and 13-*Z*-β-carotene isomer contents in *C. moschata* after different cooking styles were evaluated. The raw pumpkin presented 236.10, 172.20, 39.95, 3.64 and 0.8610 μg g^{-1} of total carotenoids, β-carotene, α-carotene, 13-*cis*-β-carotene and 9-*Z*-β-carotene, respectively. Samples cooked these total carotenoids in boiling water were 258.50, 184.80, 43.97, 6.80 and 0.77 μg g^{-1}, respectively. Steamed samples revealed 280.77, 202.00, 47.09, 8.23 and 1.247 μg g^{-1}, respectively. Since almost 100% of β-carotene is converted into vitamin A, these results are promising. All carotenoids increased after the cooking methods, most likely of a higher availability induced by the cooking style [59]. The carotenoids should be more bioavailable after the heat treatments. The total carotenoid and β-carotene isomers contents, normally, may increase according to the cooking styles applied. Pumpkin consumption in Northeast Brazil could be more, aggressively, promoted to minimize vitamin A deficiency in this geographic area. Landrace pumpkins occur in nature, and their potential as source of pro-vitamin A, were investigated, in order to be used in conventional plant breeding or biofortification programs, aiming to increase the total carotenoids and β-carotene contents. The total carotenoid, α-carotene, β-carotene and its isomers in two raw landraces pumpkins (*C. moschata*) (A and B) were evaluated to verify its seed production potential. Total carotenoid content of 404.98 (A) and 234.21 μg g^{-1} (B), respectively, were found. The best value for α-carotene contents 72.99 μg g^{-1}. All *E*-β-carotene was the most abundant micronutrient varying from 244.22 to 141.95 μg g^{-1} in both samples. The 9 and 13-Z-carotene isomers were still found in low concentrations. The best β-carotene content in raw sample (A) revealed to be promising for the production of seeds for cultivation and consumption [37]. Recently, the retention of pro-vitamin A carotenoids in the pulp from orange-fleshed pumpkin that was briefly steamed or boiled in either water or water containing 60% sucrose in five genotypes grown in Brazil was investigated and their bioaccessibility in cooked pulp was also determined by *in vitro* digestion and confirmed with Caco-2. Genotypes varied from 209 to 658 μg g^{-1} in pro-vitamin A carotenoids. The retention after cooking was more than 78%. The bioaccessibility of β- and α-carotene was <4%, which showed high variability, affected by food matrix and cooking. One genotype has the potential to provide more than 40% required for children 4–8 years of age per 100 g serving. Pumpkin (*Cucurbita moschata*) is a food crop targeted for enrichment with pro-vitamin A carotenoids [60].

Thus, studies on how the pro-vitamin A carotenoids are assimilated by the human organism, mainly in pumpkins, are relevant and necessary, although, since β-carotene and α-carotene in the pumpkin are poorly bioavailable, these levels are high and supply the daily necessities without the amount of daily food being increased.

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