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Assessment of carotenoids in pumpkins after different home cooking conditions

Lucia Maria Jaeger de CARVALHO[1]*, Lara de Azevedo Sarmet Moreira SMIDERLE[1], José Luiz Viana de CARVALHO[2], Flavio de Souza Neves CARDOSO[1], Maria Gabriela Bello KOBLITZ[3]

Abstract

Carotenoids have antioxidant activity, but few are converted by the body into retinol, the active form of vitamin A. Among the 600 carotenoids with pro-vitamin A activity, the most common are α- and β-carotene. These carotenoids are susceptible to degradation (e.g., isomerization and oxidation) during cooking. The aim of this study was to assess the total carotenoid, α- and β-carotene, and 9 and 13-*Z*- β-carotene isomer contents in *C. moschata* after different cooking processes. The raw pumpkin samples contained 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. The samples cooked in boiling water contained 258.50, 184.80, 43.97, 6.80, and 0.77 μg.g^{-1} of total carotenoids, β-carotene, α-carotene, 13-*Z*-β-carotene, and 9-*Z*-β-carotene, respectively. The steamed samples contained 280.77, 202.00, 47.09, 8.23, and 1.247 μg.g^{-1} of total carotenoids, β-carotene, α-carotene,13-*Z*-β-carotene, and 9-*Z*-β-carotene, respectively. The samples cooked with added sugar contained 259.90, 168.80, 45.68, 8.31, and 2.03 μg.g^{-1} of total carotenoid, β-carotene, α-carotene, 13-*Z*- β-carotene, and 9-*Z*- β-carotene, respectively. These results are promising considering that *E*-β-carotene has 100% pro-vitamin A activity. The total carotenoid and carotenoid isomers increased after the cooking methods, most likely as a result of a higher availability induced by the cooking processes.

Keywords: pro-vitamin A; carotenoids; pumpkin landraces; *C. moschata*; pumpkin meals; α-carotene; β-carotene; β-carotene isomers.

1 Introduction

A large number of pumpkin varieties, each of which containing different amounts of carotenoids, are cultivated worldwide (Juna et al., 2006). In Brazil, *C. moschata* cultivars are known to contain high amount of α- and β-carotene. β-carotene has 100% pro-vitamin A activity, and α-carotene has approximately 53% pro-vitamin A activity (Mínguez-Mosquera et al., 2002; Silva & Mercadante, 2002; Boiteux et al, 2007; Rodriguez-Amaya et al., 2008).

High contents of total carotenoids (2120 μg.100 g^{-1}) and β-carotene (1180 μg.100 g^{-1}) have been found in *C. maxima*. However, the highest concentration of total carotenoids (47 μg. g^{-1} of *E*-α-carotene and 235 μg.g^{-1} of *E*- β-carotene) were found in peeled *C. moschata* (Baianinha cultivar) (Azevedo-Meleiro & Rodriguez-Amaya, 2007; Kandlakunta et al., 2008; Kurz et al., 2008).

Carotenoids have antioxidant activity, but few of them are converted into retinol, the active form of vitamin A (Burns et al., 2003; Rodriguez-Amaya & Kimura, 2004; Quirós & Costa, 2006; Dini et al., 2013). Of the 600+ carotenoids with pro-vitamin A activity, the most common are α- and β-carotene. These carotenoids are susceptible to degradation (isomerization and oxidation) during cooking.

In the low-income areas of Northeast Brazil, there is a high prevalence of night blindness caused by vitamin A deficiency. Infants, school-age children, and pregnant women are particularly vulnerable.

Sweet pumpkin preparations (a traditional dessert dish in Brazil that is offered in the School Feeding Program) may be a way of increasing the consumption of pumpkin with high concentrations of pro-vitamin A. Generally, school-age children prefer and consume sweet foods that contain added sugar as opposed to salted foods (boiled or steamed). Therefore, pumpkins cooked with added sugar and consumed as a small dessert may be a viable option for increasing the pro-vitamin A intake in children.

Vitamin A deficiency (VAD) represents one of the major avoidable public health problems in the world and is one of the most important factors that contribute to the high morbidity and mortality rates among children in developing countries (Tomkins, 2000; Kapil & Bhavna, 2002).

Vitamin A deficiency is an avoidable cause of blindness worldwide (Underwood & Arthur, 1996; Gilbert & Foster, 2001; Kello & Gilbert, 2003), especially in underdeveloped countries.

Studies have been conducted by Embrapa Coastal Tablelands in Aracaju and in partnership with other Embrapa centers and university researchers to select adequate and promising landrace pumpkins for the formulation of these sweet pumpkin meals. The study of the landrace pumpkins is part of the "Biofortification in Brazil: Breeding Crops for Better Nutrition - BioFORT" project, which is financially supported by the Embrapa Monsanto Research Fund.

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[1] *Centro de Ciências da Saúde – CCS, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro – UFRJ, Rio de Janeiro, RJ, Brasil, e-mail: luciajaeger@gmail.com*
[2] *Embrapa Agroindústria de Alimentos, Guaratiba, Rio de Janeiro, RJ, Brasil*
[3] *Escola de Nutrição, Universidade Federal do Estado do Rio de Janeiro – UNIRIO, Rio de Janeiro, RJ, Brasil*
**Corresponding author*

The aim of this study was to evaluate the total carotenoid, α- and β-carotene, and 9 and 13-*Z*- β-carotene isomers in landrace pumpkin samples subjected to different cooking methods.

2 Materials and methods

2.1 Raw material

Landrace pumpkins (*C. moschata*) were harvested at Embrapa Coastal Tablelands in Aracaju, Brazil and sent to Rio de Janeiro for the determination of moisture, soluble solids, total carotenoids, α- and β-carotene, and 9 and 13-*Z*- β-carotene isomers. All analyses were performed in triplicate.

2.2 Sample preparation

Figure 1 shows the landrace pumpkin sample preparations according to the cooking method applied.

Sampling

Samples of raw landrace pumpkins were peeled and divided into four parts by two longitudinal cuts (from one end to the opposite end), resulting in four sections (Figure 2). From these four sections, the two sections opposite from each other were discarded, and the remaining two sections were used in the cooking experiments. For the analyses, the sections were fragmented and placed in a vertical mixer (IKA - Ultraturrax model T18 basic) to obtain a homogeneous mass.

Cooking methods

Two landrace pumpkins of *C. moschata* (135.0 ± 0.7 g) were washed, peeled, cut in small pieces, and divided into 4 different groups; the pumpkin pieces were then cooked in boiling water (2:1 for 4 minutes), steamed cooked (5 minutes), or cooked with a 60% sucrose solution in 200 mL of water (for the sweet preparation).

Moisture and soluble solids

The moisture and soluble solids were determined by gravimetry and refractometry, respectively (Association of Official Analytical Chemists, 2005).

2.3 Carotenoid extraction and determination

The total amount of carotenoids in the pumpkin samples was determined using a spectrophotometer (Thermo Scientific Evolution 60) set at 450 nm.

All solvents and chemicals were obtained from commercial sources (Tedia and Merck). The α- and β-carotene standards were purchased from Sigma-Aldrich (Brazil).

To determine the amount of total carotenoids, α- carotene, β-carotene, and its *Z* isomers in the raw and cooked pumpkin samples, approximately 0.300 g of the samples and 3.0 g of celite 454 (Tedia, Ohio, USA) were weighed in a mortar on a digital balance (Bel Engineering, model MA0434/05).

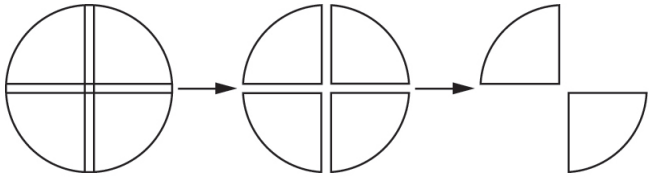

Figure 2. Landrace pumpkins quarterization.

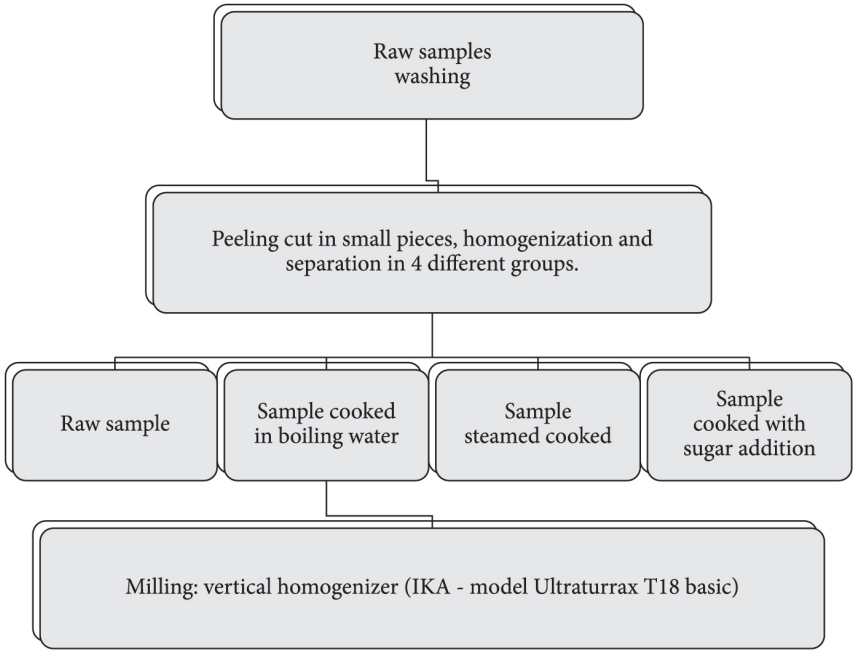

Figure 1. *C. moschata* landrace preparation.

For the carotenoid extraction, 25 mL of acetone was successively added until a paste was obtained. The paste was transferred to a sintered funnel (5 μm) coupled to a 250 mL Buchner flask and filtered under vacuum. This procedure was repeated at least three times until the sample was colorless. The obtained extract was transferred to a 500 mL separatory funnel containing 40 mL of petroleum ether. The acetone was removed through the slow addition of ultrapure water (Milli – Q - Millipore) to prevent the formation of emulsion. The aqueous phase was discarded, and this procedure was repeated four times until no residual solvent remained. The extract was then transferred with a funnel to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate. The final volume was adjusted with petroleum ether, and the samples were analyzed at 450 nm. The total carotenoid content was calculated using the following formula:

$$\text{Carotenoid content (μg/g)} = \frac{A \times V(mL) \times 10^4}{A_{1cm}^{1\%} \times P(g)} \quad (1)$$

where A = absorbance; V = total extract volume; P = sample weight; and $A_{1cm}^{1\%} = 2592$ (i.e., the β-carotene extinction coefficient in petroleum ether).

2.4 Identification and quantification of α-carotene, β-carotene, and β-carotene isomers

Alpha-carotene, β-carotene, and its isomers (*Z*) were analyzed using a high-performance liquid chromatograph (Waters 2695 - Alliance Model, Milford, USA) with a UV/Visible photodiode array detector that scanned between 350 to 600 nm using the Empower software. A C30 column (YCM Carotenoid S-3, 4.6 mm x 250 mm) was purchased from Waters. The HPLC-grade mobile phase solvents were purchased from Tedia (Rio de Janeiro, RJ, Brazil) and consisted of an 8:2 volumetric ratio of methanol to t-butyl methyl ether. The mobile phase flow rate was 0.8 mL/min; 25.0 μL of an ether extract sample was injected. The temperature was set at 30°C, and the analysis lasted 60 minutes. All analyses were conducted in triplicate.

For the identification and quantification of α-carotene, β-carotene, and its *Z* isomers, 2 mL were removed from the carotenoid extract and dried in an amber flask under flowing nitrogen. The sample was diluted in 100 μL of acetone while mixing in a vortex mixer (Genie 2-Scientific Industries) and then transferred to a 2 mL amber flask for high-performance liquid chromatography (HPLC) analysis.

Although the Z isomers have lower pro-vitamin A activity than that of β-carotene, the isomers may be present in the samples in significant proportions relative to the total carotenoids (Clydesdale et al., 1970; Bauernfeind, 1972; Rodriguez-Amaya & Kimura, 2004; Ihl et al., 1998; Dutta et al., 2005; Regal et al., 2014).

The content determination of α-carotene, β-carotene, and its *Z* isomers was performed according to the formula:

$$C(\mu/g) = \frac{A_x \times C_s(\mu/g) \times V(mL)}{A_s \times P(g)} \quad (2)$$

where A_x = carotenoid peak area; C_s = standard concentration; A_s = standard area; V= total extract volume and P = sample weight.

2.5 Statistical analyses

The data were analyzed using ANOVA and Kruskal-Wallis test at a significance level of 0.05. The statistical analyses were performed using the Statistica software version 5.1.

3 Results and discussion

3.1 Moisture and soluble solids

Table 1 shows the values of moisture and soluble solids in the raw and cooked landrace pumpkins (i.e., boiled, steamed, and cooked with added sugar).

As expected, there were significant differences in the moisture and soluble solid contents ($P < 0.05$) between all pumpkin samples before and after the cooking methods.

The pumpkins cooked with added sugar had the lowest moisture content, followed by the raw, steamed, and boiled samples.

The highest soluble solid content was detected in the samples cooked with added sugar. Significant differences ($P < 0.05$) were found between all pumpkin samples analyzed.

Souza et al. (2011) reported higher moisture values (92 g.100 g^{-1}) and lower soluble solids values (9. 4 g.100 g^{-1}) in raw *C. moschata* after subjecting the samples to an osmotic dehydration method. Additionally, Molina Filho et al. (2011), who assessed the moisture sorption isotherms in fresh and blanched *C. moschata*, reported a moisture value of 93.47 g.100 g^{-1}.

However, in our study, the soluble solid content in the raw *C. moschata* was higher than those reported by Russo et al. (2012) in *C. maxima* (6.6 g.100 g^{-1}) and Abou-Zaid et al. (2012) in pumpkin juice (6 g.100 g^{-1}).

Pongjanta et al. (2006) reported moisture and soluble solid contents in fresh pumpkins of 84.32 and 9.27 g.100 g^{-1}, respectively, before processing the pumpkin into powder for bakery purposes.

According to Mayor et al. (2006) and Lee & Lim (2011), the moisture and soluble solid contents in *C. pepo* pumpkin cultivars purchased from a local market in Spain ranged between

Table 1. Moisture (g.100 g^{-1}) and soluble solids (°Brix).

	Raw	Cooked in boiling water	Steamed cooked	Cooked with sugar addition
Moisture	85.29 (± 0.44)[a]	87.96 (± 0.22)[b]	86.59 (± 0.26)[c]	80.43 (± 0.95)[d]
Soluble solids	12.13 (± 0.08)[a]	9.27 (± 0.41)[b]	10.77 (± 0.23)[c]	17.08 (± 0.86)[d]

SD = Standard Deviation. All analyses were carried out in triplicate. Different uppercase letters in the same line means significant differences ($P < 0.05$).

Table 2. Contents of total carotenoid, α and β-carotene, and 9 and 13-*Z*- β-carotene isomers (in wet basis).

Carotenoids (μg.g^{-1})	Raw Sample	Cooked in boiling water	Steamed cooked	Cooked with sugar addition
α-carotene	39.95 (±2.26)^{a}	43.97 (±3.51)^{a,b}	47.09 (±1,87)^{b}	45.68 (±1.38)^{b}
β-carotene	172.20 (±8.16)^{a}	184.80 (±14.91)^{a,b}	202.00 (±12.74)^{b}	168.80 (±8.11)^{a,c}
9-- β-carotene	0.86 (±0.23)^{a}	0.77 (±0,29)^{a}	1.24 (±0.81)^{a}	2.03 (± 1,02)^{a}
13-- β-carotene	3.64 (±1.54)^{a}	6.80 (±1.88)^{a,b}	8.23 (±0.66)^{b}	8.31 (±0.37)^{b}
Total carotenoids	236.10 (± 6.81)^{a}	258.50 (±11.78)^{b}	280.77 (±12.58)^{c}	251.90 (±5.16)^{b}

SD = standard deviation. All analyzes were carried out in triplicate. Different uppercase letters in the same line means significant differences ($P < 0.05$).

95-97 g.100 g^{-1} (wet basis) and 2-4 g.100 g^{-1}, respectively. Abou-Zaid et al. (2012) reported 84.6 g.100 g^{-1} in pumpkin puree.

3.2 Carotenoids in raw and cooked pumpkins

Table 2 shows the total carotenoid, α- and β-carotene, and 9 and 13-*Z*-β-carotene isomers found in the raw pumpkins and after the different home-cooking methods.

The raw samples contained 236.10 μg.g^{-1}, 172.20 μg.g^{-1}, 39.95 μg.g^{-1}, 3.64 μg.g^{-1}, and 0.86 μg.g^{-1} of total carotenoid, β-carotene, α-carotene, 13-*Z*-β-carotene, and 9-*Z*-β-carotene isomers, respectively. Aguilar-Gutiérrez (2009) reported that the total carotenoids in *C. moschata* ranged from 171.9 to 461.9 μg.g^{-1}, and Ramos et al. (2009) reported values ranging from 100.50 to 365.40 μg.g^{-1}. These samples had higher contents of total carotenoids than those of the samples in our study.

Other important sources of β-carotene have been studied. Donado-Pestana et al. (2012) reported that raw biofortified orange-fleshed sweet potatoes (CNPH 1194 cultivar) contained 128.5 μg.g^{-1}, 9.6 μg.g^{-1}, 5.8 μg.g^{-1} of all-*E*-β-carotene, and 13- and 9-*Z*-β-carotene isomers, respectively. These values are lower than those detected in the raw landrace pumpkins of our study.

According to Van Jaarsveld et al. (2006), although the Z-isomers have lower pro-vitamin A activity, the pumpkin samples have high amounts of β-carotene in the Z configuration. On the other hand, in the present study, the 13-Z isomer increased after all cooking methods applied.

Previous studies have shown that heat treatment improves the bioavailability of carotenoids. Cooking practices break down the food matrices and loosen the carotene-binding fibers, leading to nutrient loss; however, the bioavailability and sometimes even the carotene content can increase (Adams & Erdman, 1988; Sungpuag et al., 1999, Fernández-García et al., 2012).

According to De Pee (1996), carotene from fruits, tubers, and pulpy vegetables, such as pumpkin, are considered to be better absorbed than those from dark green leafy vegetables.

The α-carotene content varied from 39.95 μg.g in the raw pumpkin samples to 47.09 μg.g^{-1} in the steamed samples ($P < 0.05$). However, there were no significant differences between the pumpkin samples cooked in boiling water, steamed, or cooked with added sugar.

The β-carotene content in the steamed sample was 202.00 μg.g^{-1}, which was significantly different ($P < 0.05$) from the contents in the other samples. In this study, the β-carotene content was higher than those reported by Ramos et al. (2009) (37.6-63.22 μg.g^{-1}).

The 9-*Z*-β-carotene isomer content did not differ significantly ($P < 0.05$) between all pumpkin samples; it ranged from 0.77 μg.g^{-1} in the steamed cooked sample to 2.03 μg.g^{-1}, in the sample cooked with added sugar.

The 13-*Z*-β-carotene isomer content in the cooked sample was significantly different ($P < 0.05$) from that in the raw sample.

The steamed samples had the highest contents of total carotenoids, β-carotene, and α-carotene.

Additionally, the pumpkin cooked with added sugar had the highest contents of 13- and 9-*Z*- β-carotene.

This result is likely caused by moisture losses during cooking. Another reason could be differences in the biological structures of the landrace samples. Azizah et al. (2009) observed β-carotene retention greater than 100% after cooking pumpkins (*C. moschata*) in boiling water. The authors found a 2- to 4-fold increase in the β-carotene content in the pumpkins after cooking for 2, 4, and 6 minutes.

Generally, the processing of vegetables results in a breakdown of the cellulose plant cell structure and thus improves the bioavailability of carotenoids (Van Het Hof et al., 2000). High contents of total carotenoids were reported by Bengtsson et al. (2008, 2009) in boiled (570.60 μg.g^{-1}) and steamed orange-fleshed sweet potatoes (682.40 μg.g^{-1}).

4 Conclusion

High contents of α- and β-carotene were found in all *C. moschata* pumpkin landraces evaluated. The carotenoids were more bioavailable after the heat treatments. The total carotenoid and β-carotene isomers contents increased according to the cooking methods applied. Pumpkin consumption in Northeast Brazil could be more aggressively promoted to minimize vitamin A deficiency in this geographic area. The results of this study are promising, considering that β-carotene has 100% pro-vitamin A activity. More studies must be carried out with other *C. moschata* pumpkin landraces to identify pumpkins with higher β-carotene contents.

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