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Changes in carotenoids during processing and storage of pumpkin puree

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ABSTRACT

Changes in the contents of carotenoids and their true retentions (% TR) during the production of puree of *Cucurbita moschata* 'Menina Brasileira' and of *Cucurbita maxima* 'Exposição' pumpkins and the stability of such compounds during 180 days of storage were monitored by liquid chromatography coupled with a photodiode array detector. Cooking caused higher losses than commercial sterilisation. High losses of xanthophylls such as lutein and violaxanthin were noted during processing and storage of pumpkin puree. Such losses show the low stability of these compounds. The major carotenoids, pro-vitamin A carotenes, namely, α-carotene and all-*trans*-β-carotene for *C. moschata* 'Menina Brasileira' and all-*trans*-β-carotene for *C. maxima* 'Exposição' obtained high retentions (>75%) after processing. A slight degree of isomerisation of β-carotene was noted in the puree samples, but with low concentrations of *cis*-isomers. Storage for 180 days did not significantly affect ($P \leqslant 0.05$) the concentrations of these carotenoids.

1. Introduction

Pumpkins, which are the fruits of different species of the genus *Cucurbita*, are cultivated worldwide for their pulp and seeds for human nutrition, either for direct consumption or for preparation of other foods such as syrups, jellies, jams, and purees. According to estimations by the Food and Agriculture Organization of the United Nations (FAO), world production of pumpkins in 2007 was over 20 million tons, especially in China, India, Russia, United States, and Egypt (FAOSTAT, 2008).

Pumpkin pulp has large amounts of carotenoids, which are pigments that derive from isoprene and that give flowers, leaves, and fruits a colouration that ranges from yellow to red (Oliver & Palou, 2000). Besides the pro-vitamin A activity of some carotenoids, such as β-carotene, β-cryptoxanthin and α-carotene, studies have also indicated that consumption of carotenoids lowers the risk of degenerative and cardiovascular diseases, cataracts, macular degeneration as well as certain types of carcinomas (Rao & Rao, 2007).

Qualitative and quantitative composition of carotenoids in several plant foods, such as papayas (Wilberg & Rodriguez-Amaya, 1995), mangos (Mercadante, Rodriguez-Amaya, & Britton, 1997), guavas (Wilberg & Rodriguez-Amaya, 1995), carrots (Sant'Ana, Stringheta, Brandão, & Azeredo, 1998), and apricots (Kurz, Carle, & Schieber, 2008), amongst others, have been studied. The variability of the results is high, even within the same species since the composition of carotenoids may also be affected by some factors such as variety, cultivar, maturation stage, geography, climate, harvesting and post-harvesting, as well as the analysis itself (Rodriguez-Amaya, Kimura, Godoy, & Amaya-Farfan, 2008). For example, Assunção and Mercadante (2003) noted higher carotenoid content in cashews cultivated in north-eastern Brazil than those cultivated in south-eastern Brazil, where average temperatures are lower. In the cashews cultivated in north-eastern Brazil, β-cryptoxanthin was the major carotenoid whereas in cashews cultivated in south-eastern Brazil it was β-carotene.

There are several studies on pumpkins that analyse the composition of carotenoids in different species and varieties, showing high concentrations of these compounds in fresh pumpkins (Azevedo-Meleiro & Rodriguez-Amaya, 2007; Kurz et al., 2008; Murkovic, Mulleder, & Neunteufl, 2002). However, there are a number of cultivars, varieties or growing conditions that have not yet been investigated. Moreover, there are few studies about carotenoid composition in industrial products derived from pumpkins.

Since there are double bonds in the carbon chain, carotenoids are susceptible to some reactions such as oxidation and isomerisation (*cis*–trans) during food processing and storage, especially due to light, heat, acids, and oxygen; thus causing loss of colour and reduction of biological activity (Rao & Rao, 2007; Rodriguez-Amaya, 1999). In the case of isomerisation, the trans-isomers are more common and stable in foods while *cis*-isomers are usually formed during food processing (Oliver & Palou, 2000). There are many studies correlating to the processing, packaging, and storage conditions with changes in the composition of carotenoids in many foods (Chen, Peng, & Chen, 1996; Lin & Chen, 2005; Vásquez-Caicedo, Schilling, Carle, & Neidhart, 2007a, 2007b). There are several factors that may affect the stability of these compounds, such as type and

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physical form of the carotenoid, oxygen concentration, presence of metals, exposure to light, severity of heat treatment, food matrix, amongst others (Rodriguez-Amaya, 1999). Hence, the stability of carotenoids in foods varies greatly (Lee & Coates, 2003).

Although there are no official data reported in Brazil, the production of pumpkins is high, mainly amongst small farmers and especially of the *Cucurbita moschata* and *Cucurbita maxima* species. In spite of their nutritional aspects and low production cost, there are few products made from pumpkins on the market and consumption of pumpkins is limited to being in the form of fresh fruits or jam. The pumpkin puree, obtained through commercial sterilisation of pumpkin pulp, is a product with added value and convenience since it can be easily incorporated into preparations, such as breads, pasta and sweets. Moreover, technology for its production is accessible to small and medium-size agro industries.

However, since carotenoids are unstable at high temperatures, studies regarding the consequences of processing (cooking and commercial sterilisation) and storage in the composition of carotenoids in pumpkin puree are important.

Considering what has been mentioned above, the objectives of this study were: (1) evaluate the carotenoid composition in raw *C. moschata* pumpkins of the variety 'Menina Brasileira' and *C. maxima* pumpkins of the variety 'Exposição', both of which are widely cultivated in southern Brazil; (2) investigate the consequences of pumpkin puree processing in the composition of carotenoids; (3) monitor changes that may occur in the concentrations of the major carotenoids in the pumpkin purees during 180 days of storage.

2. Materials and methods

2.1. Materials

Approximately 80 kg of each pumpkin species – *C. moschata* 'Menina Brasileira' and *C. maxima* 'Exposição' – were harvested in different rural units in the municipal districts of Curitibanos (27°16′58″ South, 50°35′04″ West, 987 m altitude) and São Cristóvão do Sul (27°16′00″ South, 50°26′26″ West, 1025 m altitude) (Santa Catarina, Brazil) in 2010 (February–March) and transported to the laboratory in Florianopolis (Santa Catarina, Brazil), where the samples were processed and analysed.

As described by Azevedo-Meleiro and Rodriguez-Amaya (2007), the species *C. moschata* 'Menina Brasileira' has a cream or light orange colour on the outside with large dark green longitudinal stripes, a smooth surface, and orange pulp. Its anatomy can be divided into two parts: a slightly curved cylindrical section and an enlarged bulb-like section at the blossom end. The pumpkins analysed were approximately 45–65 cm long, 15–25 cm transverse diameter in the cylindrical section and 25–35 cm transverse diameter in the bulb-like section, weighing between 5.0 and 10.0 kg. The *C. maxima* 'Exposição' pumpkins have orange coloured outside and pulp, and a smooth surface with prominent ribbing. They have the shape of slightly flattened spheres at both the stem and the blossom ends, weighing from 2.0 to 5.0 kg.

Three batches of purees were produced for each of these two pumpkin species. All analyses were performed in triplicate, with a sample unit from each batch. Acetone, ethyl acetate, acetonitrile, methanol and triethylamine of HPLC grade, purchased from Sigma–Aldrich, Steinheim, Germany, were used in the steps where high performance liquid chromatography was used.

2.2. Pumpkin puree

The fruits were washed with potable water; the parts that had phytopatologies were removed. After that, the fruits were seeded and cut into 4–5 cm slices, which were then steamed in an autoclave (Marte, AVM, Santa Rita do Sapucaí, Brazil) at 100 °C for 20 min (cooking), which was a sufficient length of time for softening the plant tissue.

While the pumpkin slices were still hot, their peel was removed and their remaining pulp was crushed and homogenised in an industrial blender (Metvisa, Brusque, Brazil). The pulp samples were put into 260 ml glass bottles and then heat-treated in an autoclave at 121 °C for 20 min for commercial sterilisation. A headspace was left in all the bottles so that a partial vacuum was generated inside them.

Besides the analysis that was performed on the final product (pumpkin puree), aliquots were removed before and after cooking (raw pumpkin and cooked pumpkin) for analysis of carotenoids. The collection of the aliquots was performed with a special care regarding the uniformity and the quantity of the samples so that they were representative of the batch as a whole. To prevent any modification of carotenoids after collecting the samples, the aliquots were frozen and kept at −20 °C until required for analysis on the following day.

The puree samples were stored in a ventilated environment that was protected from light and had its temperature and relative humidity monitored for 6 months. After specific periods of storage (0, 15, 30, 60, 90, 120, and 180 days), the samples were randomly picked for analysis of the changes in the carotenoids in the pumpkin puree samples.

2.3. Carotenoid analysis

2.3.1. General

The method used for carotenoid analysis was proposed by Kimura and Rodriguez-Amaya (2002) and used by Azevedo-Meleiro and Rodriguez-Amaya (2007) for carotenoid analysis on pumpkins.

The extraction was performed with acetone (previously refrigerated for 2 h) on 10–20 g of sample, using a pestle and mortar until the residue became colourless, and after that the extract was partitioned with petroleum ether. In the case of the *C. maxima* 'Exposição' samples, the extract was submitted to overnight saponification with methanolic KOH (10%, w/v), while in the case of *C. moschata* 'Menina Brasileira', where xanthophylls, which are oxy-carotenes, are in lower concentrations, saponification was not performed in order to minimise the loss which can occur in this step. The extracts were washed with distilled water and concentrated at low pressure in a rotoevaporator (Tecnal, TE-211, Piracicaba, Brazil), always at a temperature below 35 °C and using glass pearl for optimisation of the recovery in the re-dissolving process. In order to avoid errors during the carotenoid analysis, all the necessary precautions were taken as recommended by Rodriguez-Amaya (1999).

The carotenoids were analysed in a liquid chromatograph, consisting of a pump and a degasser (LC-20AT), an autosampler injector (SIL-10 A), a column oven (CTO-20A) and a photodiode array (DAD) (SPD-M20A) controlled by a system controller (CBM-20A), all manufactured by Shimadzu Corporation, Kyoto, Japan. Detection with DAD was at the wavelengths of maximum absorption. Immediately before injection, the samples were redissolved in HPLC grade acetone and filtered with a 0.22 μm PTFE syringe filter. For the stationary phase, a monomeric C_{18} ODS2, 5 μm, 4.6 × 150 mm (Waters Spherisorb®, Wilmington, USA) was used. The mobile phase consisted of acetonitrile (containing 0.05% of triethylamine), methanol and ethyl acetate. A concave gradient was used for *C. moschata* 'Menina Brasileira' from 95:5:0 to 60:20:20 for 20 min, maintaining this proportion until the end of the run. For the *C. maxima* 'Exposição', a gradient from 98:2:0 to 60:20:20 for 20 min was used. Reequilibration took 15 min in both cases. The flow rate was 0.5 ml/min and column temperature was kept at 35 °C.

The identification of the carotenoids was performed considering (a) the combined information from chromatographic parameters (retention time and elution order), (b) UV–visible spectrum

parameters (λ_{max} and spectral fine structure % III/II) compared to standards and to data available in literature, (c) co-chromatography with standards and (d) chemical reactions to verify the type and position of the substituents in the xanthophylls (Pfander, Riesen, & Niggli, 1994; Schiedt & Liaaen-Jense, 1995). The chemical reactions were acetylation of secondary hydroxyl groups with acetic anhydride, methylation of hydroxyl groups in allylic position with acidified methanol, iodine catalysed isomerisation and epoxide-furanoxide rearrangement (5,6-epoxide–5,8-epoxide) with dilute HCl (Rodriguez-Amaya, 1999).

The major carotenoids in each sample were quantified by using calibration curves prepared from standards, and the results were expressed as μg/g of sample. Standard curves were constructed with five different concentrations for each carotenoid, each point in duplicate, with lines passing by origin and coefficients of co-relation greater than or similar to 0.95. Violaxanthin was quantified with the standard curve of lutein, and the *cis*-isomers of β-carotene with the standard curve of all-*trans* isomer (Assunção & Mercadante, 2003). Due to the difficulty of isolating the ζ-carotene standard, its quantification was performed through the standard curve of the all-*trans*-β-carotene.

2.3.2. Isolation and purification of the standards

The standards used in this work were isolated from other plant species, such as carrots and green vegetables, by using open column chromatography (OCC), according to Kimura and Rodriguez-Amaya (2002), with a glass column of 2.5 × 25 cm packed with MgO:Hyflo-supercel (1:1), activated for 2 h at 110 °C and developed with petroleum ether containing varying quantities of acetone and ethyl ether. Concentrations of the standard solutions were determined through a spectrophotometer (Hitachi, U-1800, Tokyo, Japan) and corrected according to their purity through HPLC, considering 90% as minimum purity to be used as standard.

2.3.3. Determination of carotenoid retention during the process

Many studies that propose to investigate retention of carotenoids in processed foods do not take into account the gain or loss of weight during processing through incorporation or through loss of water or water soluble solids. This could cause either underestimation or overestimation of the true retention value. A simple calculation on dry basis may also overestimate the retention of these compounds (De Sá & Rodriguez-Amaya, 2004; Rodriguez-Amaya, 1999).

Therefore, besides the results being expressed as μg/g of sample, they were also presented based on the mass of raw food, multiplying the concentration obtained for the sample by the ratio of the food mass after processing and of the food mass prior to processing. Therefore, true retention (% TR) was calculated by the equation proposed by Murphy, Criner and Gray (1975) cited by De Sá and Rodriguez-Amaya (2004), as follows: % TR = 100 × (nutrient content per g of processed food × g of food after processing)/(nutrient content per g of raw food × g of food before processing).

2.4. Statistical analysis

The results were submitted to analysis of variance (ANOVA) and to Tukey test for any significant differences ($P \leqslant 0.05$). In all the statistical analyses, the ANOVA assumptions, such as independence and normal distribution of the residues and homogeneity of variances, were considered.

3. Results and discussion

The composition of carotenoids in the raw samples, in the cooked samples, and in the *C. moschata* 'Menina Brasileira' and *C. maxima* 'Exposição' pumpkin purees were determined by reverse phase HPLC (Fig. 1). The parameters used for the identification of the peaks are shown in Table 1.

As expected, epoxy-carotenoids and hydroxy-carotenoids, such as violaxanthin and lutein, were the first to elute in the reverse phase column, followed by ζ-carotene, α-carotene, all-*trans*-β-carotene and *cis*-β-carotene, respectively. Peak 3 was not identified. Peaks 4 and 5 showed chromatographic data and UV–visible absorption spectra similar to those described for the carotenoids zeaxanthin and α-cryptoxanthin, respectively, as had already been noted in another study involving the same species of pumpkins (Azevedo-Meleiro & Rodriguez-Amaya, 2007). However, because they are present in low concentrations, it was not possible to obtain isolation by OCC, therefore the spectra were not determined in other solvent systems nor were the necessary reactions of identification carried out, and thus only one indication of the identity of those carotenoids was considered. Other minor peaks were also ignored. Typically, one to four carotenoids are predominant in the pumpkin species, with several other compounds detected in low concentrations or traces. The separation, identification, and quantification of these carotenoids were not the aim of this work; they can be better studied with the use of a mass spectrophotometer (Azevedo-Meleiro & Rodriguez-Amaya, 2004).

The concentration of the major carotenoids identified by HPLC in raw *C. moschata* 'Menina Brasileira' and *C. maxima* 'Exposição' pumpkins are shown in Table 2. The purity of the standard used was of 92% for lutein and 98% for α-carotene e all-*trans*-β-carotene, with coefficient of co-relation of (R^2) of the standard curves of 0.9928, 0.9941 and 0.9933, respectively. Figs. 2 and 3 show the true retention of carotenoids (% TR) during production of *C. moschata* 'Menina Brasileira' and *C. maxima* 'Exposição' pumpkin purees, respectively.

For the *C. moschata* 'Menina Brasileira' samples, the major carotenoids were all-*trans*-β-carotene and α-carotene, with lower amounts of ζ-carotene, violaxanthin and lutein. In the samples of *C. maxima* 'Exposição', the major carotenoid was all-*trans*-β-carotene, with good amounts of violaxanthin and lutein in raw pumpkins.

Although they are still considered interesting when compared with other plant species, concentrations of carotenoids in raw pumpkins are lower than those reported in other studies regarding the same species and varieties of pumpkins. Azevedo-Meleiro and Rodriguez-Amaya (2007) also noted the all-*trans*-β-carotene and α-carotene as the major carotenoids in *C. moschata* 'Menina Brasileira' pumpkins, but with higher concentrations, 66.7 ± 9.1 μg/g to all-*trans*-β-carotene and 26.8 ± 5.1 μg/g to α-carotene. In the *C. maxima* 'Exposição' species, authors noted violaxanthin (20.6 ± 3.3 μg/g) as its major carotenoid. The all-*trans*-β-carotene was the second in concentration, 15.4 ± 4.2 *vs* 13.38 ± 2.25 μg/g detected in this present study, where it was the major carotenoid. Indeed, the concentration ranges cited in literature are wide. Rodriguez-Amaya et al. (2008) detected concentrations of 14–79 μg/g of all-*trans*-β-carotene and 8.3–42 μg/g of α-carotene for *C. moschata* 'Menina Brasileira' pumpkins, and 3.1–28 μg/g of all-*trans*-β-carotene for *C. maxima* 'Exposição' pumpkins. Major qualitative and quantitative differences in carotenoids, even within the same species and variety, can be noted depending on the cultivar, differences in growing environment, such as temperature, nutrient availability, soil, intensity of sunlight, ripening stage, post harvesting, amongst other factors that can significantly affect the biosynthesis and metabolism of carotenoids in vegetables (Cazzonelli & Pogson, 2010; Rodriguez-Amaya, 1999). The studies mentioned above, for example, were conducted with pumpkins harvested in the northeast and southeast regions of Brazil, where the average temperatures are higher than those in the southern region of the country, where the pumpkins used in this study were cultivated.

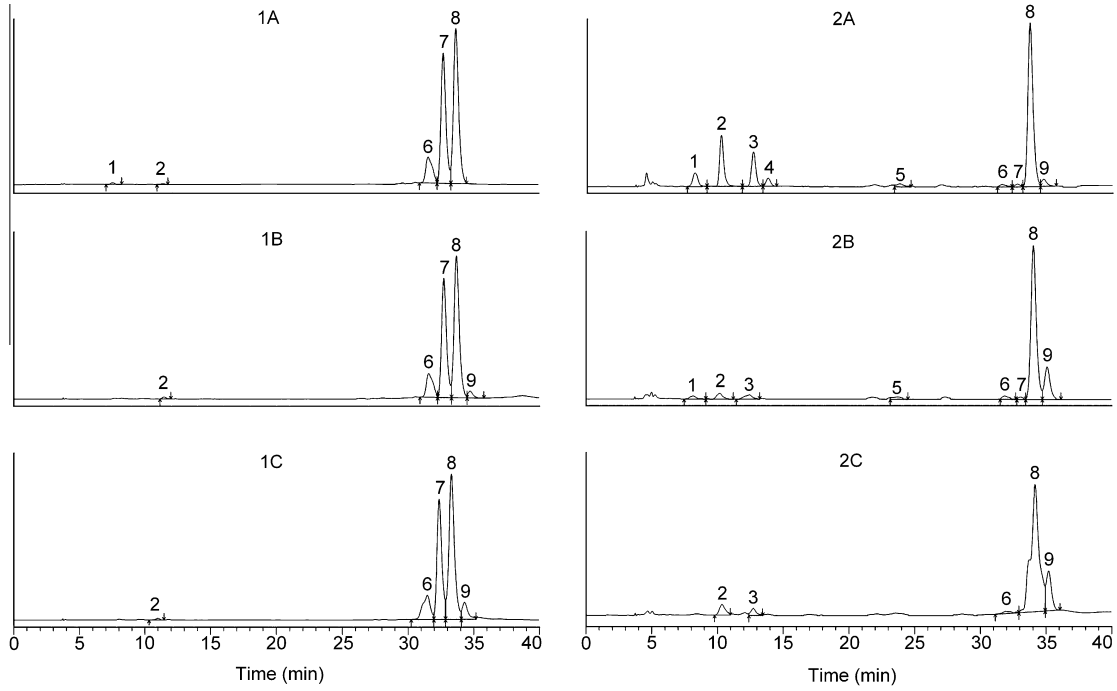

Fig. 1. HPLC chromatograms of carotenoids in raw (A), cooked (B), and puree of (C) *C. moschata* 'Menina Brasileira' (1) and of *C. maxima* 'Exposição' (2). Detection at λ_{max}. Chromatographic conditions cited in the text. Peak characterisation is shown in Table 1. Description: Figure shows the chromatograms obtained in the analyses of carotenoids in the samples of the *C. moschata* 'Menina Brasileira' and *C. maxima* 'Exposition' pumpkins, while raw, after cooked, and in the purees. As the amount of sample injected in the chromatograph varied, the scale was automatically adjusted for the peak of higher absorption, where the values (mAU) were removed from the figure to avoid generating wrong interpretations regarding the concentrations of carotenoids in the samples analysed.

Table 1
Identifying parameters of the carotenoids in raw, cooked, and pumpkin purees.

Peak [a]	Rt (min) [b]	Carotenoid	λ_{max} (nm) in mobile phase	λ_{max} (nm) in petroleum ether	III/II (%)	Response to chemical tests
1	8.1–8.3	Violaxanthin	418, 441, 470	415, 439, 468	97	Positive to all-*trans*, 5,6-epoxide test (2 groups) and acetylation (2 OH)
2	9.5–11.5	Lutein	424, 447, 475	417, 441, 469	55	Positive to all-*trans*, acetylation (2 OH) and methylation (1 allylic OH)
3	10.7–12.8	Not identified	424, 447, 475			
4	13.6–13.9	Zeaxanthin [c]	(427), 453, 479			
5	23.5–23.9	α-Cryptoxanthin [c]	424, 447, 474			
6	30.4–32.1	ζ-Carotene	378, 401, 426	375, 395,420	110	Positive to all-*trans*
7	31.1–32.8	α-Carotene	422, 447, 475	421, 443, 471	50	Positive to all-*trans*
8	32.1–34.4	All-*trans*-β-carotene	(428), 454, 481	(423), 448, 475	24	Positive to all-*trans*
9	33.2–35.4	*cis*-Isomer of β-carotene	(422), 447, 471			

[a] Numbered according to the chromatogram shown in Figs. 1 and 2.
[b] Variation found in 54 chromatographic runs.
[c] Tentative identification.

Table 2
Concentration of the major carotenoids in raw pumpkins.

Carotenoid	Concentration (μg/g)[a]	
	C. moschata 'Menina Brasileira'	*C. maxima* 'Exposição'
Violaxanthin	1.19 ± 0.10	3.08 ± 0.56
Lutein	0.59 ± 0.18	10.43 ± 0.13
ζ-Carotene	4.62 ± 0.13	0.30 ± 0.01
α-Carotene	12.60 ± 1.56	0.43 ± 0.05
All-*trans*-β-carotene	19.45 ± 2.55	13.38 ± 2.25
cis-Isomer of β-carotene	ND[b]	0.55 ± 0.15

[a] Means and standard deviations of triplicate analyses.
[b] Not detected.

Regarding the effect of processing on the carotenoids, in almost all the cases where a decrease in the concentrations was noted during processing, they occurred mainly in the cooking stage. For instance, for the samples of the *C. moschata* 'Menina Brasileira' pumpkins, besides the disappearance of violaxanthin there was also a decrease of 23.7% in ζ-carotene after cooking. Even after cooking, there was a decrease of 17.9% and of 16.9% in α-carotene and all-*trans*-β-carotene, respectively, but the concentrations of these carotenes in cooked pumpkins were not considered significantly different ($P \leqslant 0.05$) from those obtained for raw pumpkins. That is possibly due to variability in the data, considered within the normal limits in carotenoid analysis. Similar losses were observed by Gama and Sylos (2007) after pasteurisation and concentration of orange juices, without the concentration of carotenoids being significantly considered in the statistical tests. There was no significant loss after the commercial sterilisation stage. Concentrations of *cis*-isomers of β-carotene increased slightly after

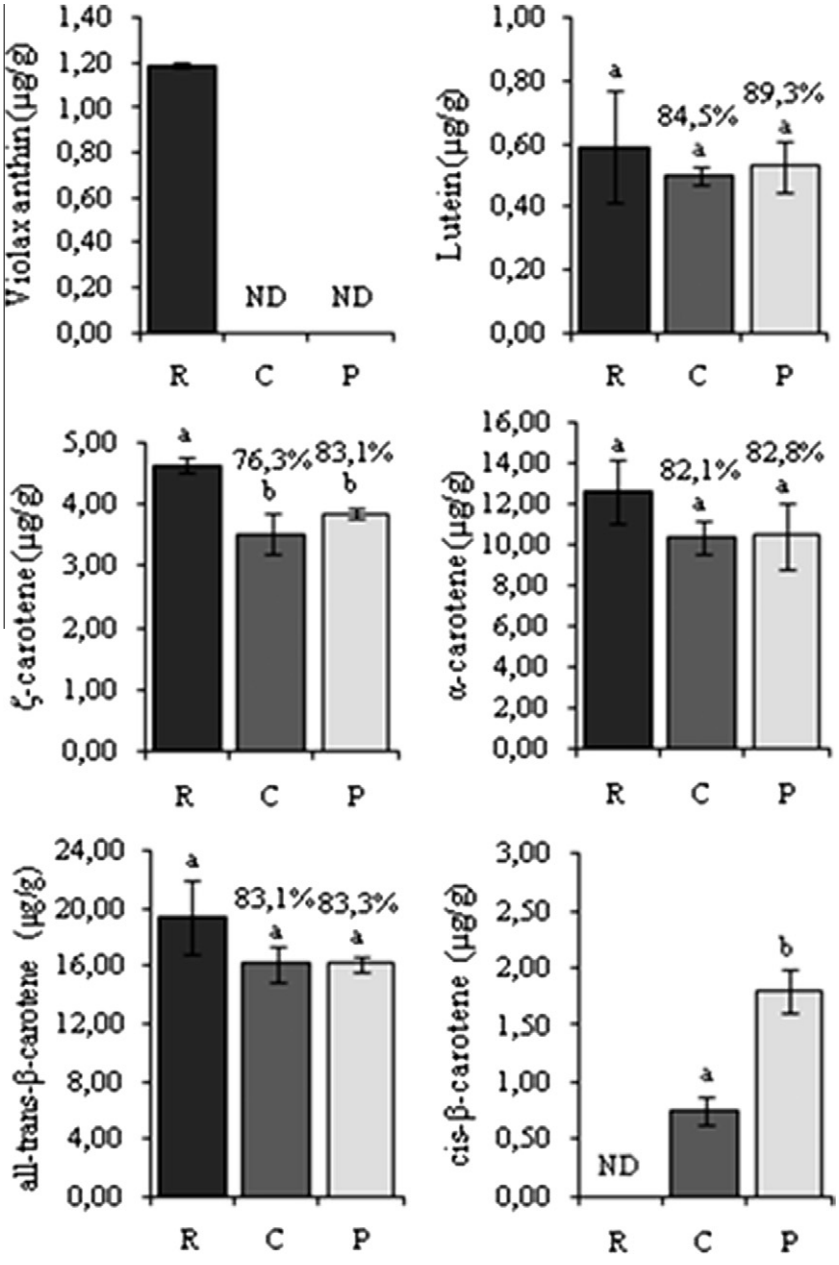

Fig. 2. Changes in carotenoid content and true retention (% TR) during processing of *C. moschata* 'Menina Brasileira' pumpkin puree (R – Raw pumpkin, C – Cooked pumpkin and P – pumpkin puree). Error bars represent standard deviations. ND – not detected. Different letters indicate significant differences amongst the processing steps for each carotenoid ($P \leqslant 0.05$), according to Tukey test. Description: Figure shows more clearly the changes in the concentrations and true retentions (% TR) for each carotenoid, which were calculated and noted during the production of the *C. moschata* 'Menina Brasileira' pumpkin puree, thus including the samples of raw pumpkin (raw), after being cooked (cooked), and after commercial sterilisation (puree).

cooking and commercial sterilisation; still their concentrations in the final products were low.

Similar results were noted with samples of *C. maxima* 'Exposição' pumpkins after cooking, where there were significant reductions of lutein (81.9%) and violaxanthin (72.5%). Violaxanthin was totally absent after commercial sterilisation. Concentrations of α-carotene and ζ-carotene were also affected by processing; however, it is difficult to evaluate the retention of carotenoids which are present in trace or low concentrations (<1 µg/g) (De Sá & Rodriguez-Amaya, 2004). Regarding all-*trans*-β-carotene, losses of 16.1% and of 21.0% was noted after cooking and commercial sterilisation, respectively. However, the all-*trans*-β-carotene concentrations were not considered significantly different from that in the raw sample ($P \leqslant 0.05$) either. Low concentrations of *cis*-isomers of the β-carotene were noted, including in the samples of raw *C. maxima* 'Exposição' pumpkins. In fact, some fruits, such as mangos, have natural *cis*-isomers (Vásquez-Caicedo et al., 2007a). However, since their presence has not been reported in other studies involving carotenoids in pumpkins, it is more likely that this is due to the saponification used in the analysis, which can cause a small percentage of loss and isomerisation (Rodriguez-Amaya, 1999).

In short, the major carotenoids, namely, α-carotene and all-*trans*-β-carotene in *C. moschata* 'Menina Brasileira' pumpkins and the all-*trans*-β-carotene in *C. maxima* 'Exposição' pumpkins, obtained retentions relatively higher after processing (>75%). Similar retentions of carotenes after heat treatment, such as blanching, cooking and sterilisation, have been described elsewhere (De Sá & Rodriguez-Amaya, 2004; Dutta, Dutta, Raychaudhuri, &

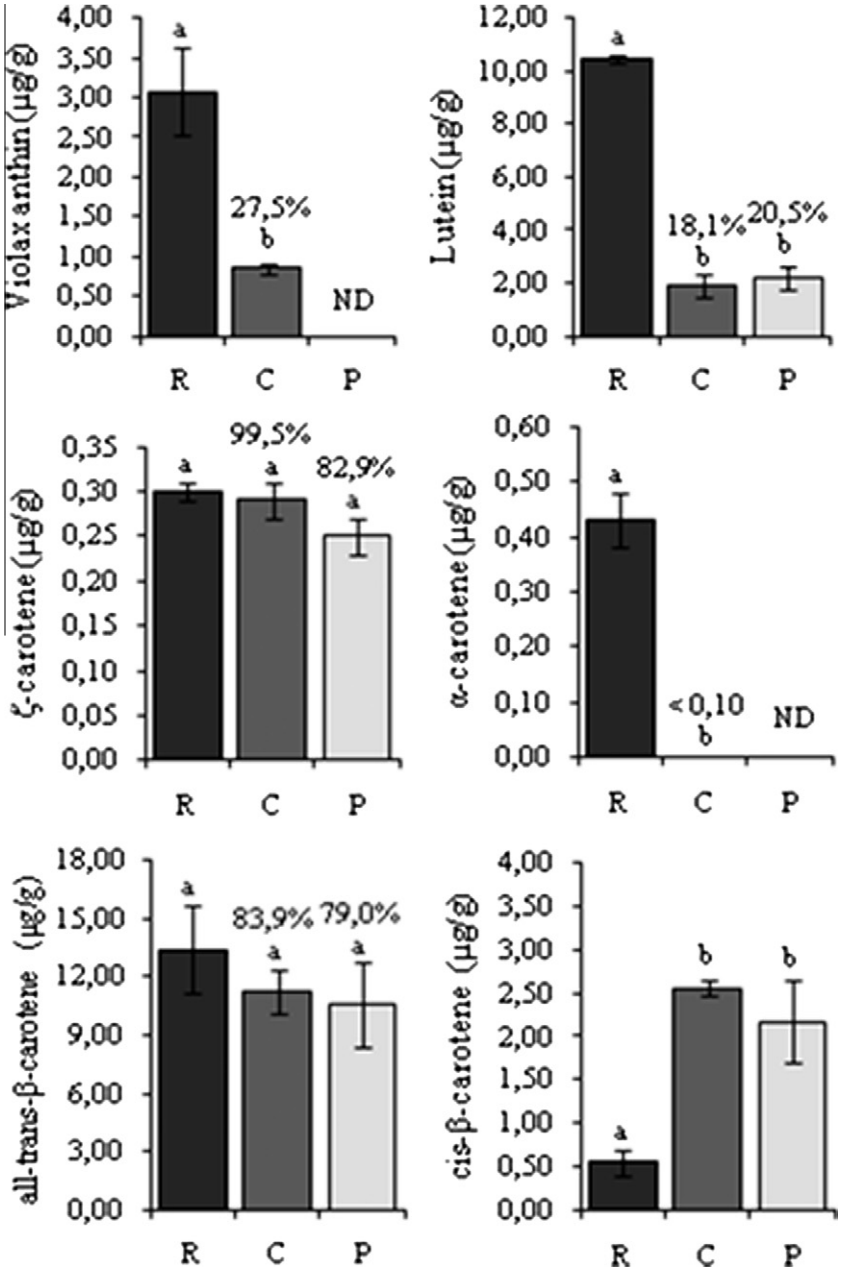

Fig. 3. Changes in carotenoid content and true retention (% TR) during processing of *C. maxima* 'Exposição' pumpkin puree (R – Raw pumpkin, C – Cooked pumpkin and P – pumpkin puree). Error bars represent standard deviations. ND – not detected. Different letters indicate significant differences amongst the processing steps for each carotenoid ($P \leqslant 0.05$), according to Tukey test. Description: Figure shows more clearly the alterations in the concentrations and true retentions (% TR) for each carotenoid, which were calculated as described in Section 2.3.3. and noted during the production of the C. *maxima* 'Exposição' pumpkin puree, thus including the sample of the raw pumpkin (raw), after being cooked (cooked), and after commercial sterilisation (puree).

Chakraborty, 2006; Marx, Stuparic, Schieber, & Carle, 2003; Vásquez-Caicedo et al., 2007a). The stability of cooking and commercial sterilisation is lower for xanthophylls, which is justified due to their structures with the presence of oxygen in the molecules. De Sá and Rodriguez-Amaya (2004) reported high losses of violaxanthin after cooking of leafy green plants. Zepka and Mercadante (2009) also noted disappearance of some xanthophylls during heat treatment of cashew fruits. Similar results were described by Gama and Sylos (2007) in orange juice processing.

Moreover, it is noteworthy that a certain amount of degradation of these pigments can have a positive aspect since some volatile substances, which are important for aroma, derive from the degradation of carotenoid pigments (Lewinsohn et al., 2005).

Besides the effects of processing, changes in the concentrations of the major carotenoids detected in pumpkin purees throughout 180 days of storage were also investigated (Table 3). The average temperature during the storage period was approximately 23 °C and relative humidity of 70%, with values ranging between 15.5 and 27.0 °C and 51% and 82%, respectively. The range in the values noted was as expected because the storage conditions were not controlled. The nonisothermal condition was used to simulate the conditions of the product during its manufacture, distribution, and storage in shops and supermarkets, and also in the consumers' homes (Zanoni et al., 2007).

Due to the difficulty of analysing changes when the concentrations are very low, only the carotenoids with initial concentrations

Table 3
Carotenoid changes in pumpkin purees during storage.

t (days)	Concentration (μg/g)[a]							
	C. moschata 'Menina Brasileira' puree					*C. maxima* 'Exposição' puree		
	Lutein	ζ-Carotene	α-Carotene	β-Carotene	*cis*-β-Carotene	Lutein	β-Carotene	*cis*-β-Carotene
0	0.58 ± 0.08^{a}	4.21 ± 0.09^{a}	11.47 ± 1.76^{a}	17.81 ± 0.56^{a}	1.98 ± 0.21^{a}	2.38 ± 0.49^{a}	11.62 ± 2.39^{a}	2.38 ± 0.52^{a}
15	0.37 ± 0.06^{ab}	4.25 ± 0.44^{a}	11.48 ± 2.24^{a}	17.92 ± 0.69^{a}	1.66 ± 0.18^{a}	2.26 ± 0.29^{ab}	10.40 ± 1.77^{a}	1.84 ± 0.39^{a}
30	0.32 ± 0.08^{ab}	4.01 ± 0.44^{a}	10.93 ± 0.77^{a}	18.03 ± 1.52^{a}	1.51 ± 0.49^{a}	1.67 ± 0.39^{ab}	10.99 ± 1.19^{a}	1.97 ± 0.42^{a}
60	0.29 ± 0.05^{b}	4.16 ± 0.04^{a}	11.60 ± 1.28^{a}	17.11 ± 1.47^{a}	1.66 ± 0.21^{a}	1.74 ± 0.37^{ab}	9.36 ± 1.26^{a}	2.30 ± 0.40^{a}
90	0.20 ± 0.09^{b}	4.13 ± 0.36^{a}	10.78 ± 0.68^{a}	18.43 ± 1.10^{a}	1.57 ± 0.28^{a}	1.16 ± 0.23^{ab}	10.49 ± 0.81^{a}	2.66 ± 0.29^{a}
120	0.20 ± 0.04^{b}	4.18 ± 0.16^{a}	10.87 ± 0.56^{a}	18.12 ± 0.62^{a}	2.00 ± 0.38^{a}	1.06 ± 0.32^{b}	10.58 ± 1.56^{a}	2.60 ± 0.25^{a}
180	0.24 ± 0.11^{b}	4.23 ± 0.13^{a}	11.90 ± 1.1^{a}	17.85 ± 1.12^{a}	1.55 ± 0.21^{a}	1.59 ± 0.43^{ab}	10.03 ± 1.35^{a}	2.46 ± 0.30^{a}

Values in the same column with different superscript letters are significantly different ($P \leqslant 0.05$) according to Tukey test.
[a] Means and standard deviations of triplicate analyses.

of at least 0.50 μg/g were analysed. Therefore, in the samples of *C. moschata* 'Menina Brasileira' pumpkin puree, concentrations of lutein, ζ-carotene, α-carotene, all-*trans*-β-carotene and its *cis*-isomers were evaluated. In the samples of *C. maxima* 'Exposição' pumpkin puree, the concentrations of lutein, all-*trans*-β-carotene and its *cis*-isomers were evaluated. Interestingly, although α-carotene was not detected in *C. maxima* 'Exposição' pumpkin puree on day zero (initial), it was detected in some analyses of the puree samples during their storage, thus suggesting that this carotenoid can continue present in trace quantity (<0.10 μg/g) in puree of this pumpkin species.

A decrease in the concentrations of lutein during storage was noted in both pumpkin purees. As aforementioned, xanthophylls tend to have lower stability in processing and storage because of their chemical structure. No significant alterations were noted in the concentrations of ζ-carotene, α-carotene, all-*trans*-β-carotene and its *cis*-isomers in the puree of *C. moschata* 'Menina Brasileira', and all-*trans*-β-carotene and its *cis*-isomers in the puree of *C. maxima* 'Exposição', throughout all the time of storage, showing the stability of these compounds in the conditions investigated.

The stability of the major carotenoids in the pumpkin purees was expected because the factors that could affect the stability of these compounds were minimised through processing and storage conditions. Heat processing is sufficient for the inactivation of enzymes and micro-organisms which could degrade these compounds. Moreover, there is a partial vacuum situation inside the bottle because oxygen is removed from it and that is important to reduce oxidation reactions. Storage at temperatures lower than 30 °C and protection from light are also important factors for the stability of carotenoids.

Other published studies also detected similar results, with relative stability of carotenoids during food storage, especially provitamin carotene, such as α-carotene and β-carotene, depending on the residual oxygen dissolved in the sample, the incidence of light, and the temperature during storage (Calvo & Santa-María, 2008; Vásquez-Caicedo et al., 2007b).

On the other hand, other studies showed higher losses of carotenoids during processing and/or storage (Chen et al., 1996; Lin & Chen, 2005). In fact, the stability of carotenoids in foods is variable. This happens not only because of extrinsic factors, such as the severity of heat treatment, presence or absence of light, temperature of storage, packaging, amongst others, but also because of the characteristics of the food matrices, such as their chemical composition, the oxygen dissolved in the samples, size of the particles, and the physical state of the carotenoid in the food (Marx et al., 2003; Rodriguez-Amaya, 1999; Vásquez-Caicedo et al., 2007a). For example, while in crystalline form, such as in carrot juice, carotenoids tend to show high stability, whereas in dissolved form, in oil drops, there is a greater potential for occurrence of isomerisation (Marx et al., 2003). Studies regarding the physical form of carotenoids and effects of the food matrix in pumpkin purees could clarify the mechanism of the stability of the carotenoids in this product.

In short, the *C. moschata* 'Menina Brasileira' pumpkins showed good concentrations of α-carotene and all-*trans*-β-carotene, with a lower quantity of ζ-carotene, violaxanthin and lutein, and the *C. maxima* 'Exposição' pumpkins had the all-*trans*-β-carotene as the major carotenoid, with good concentrations of lutein and violaxanthin. The major carotenoids, which in the case of this present study were the pro-vitamin carotenes, had relatively high retentions after the production of the pumpkin purees. A light grade of isomerisation of β-carotene was detected, with low concentrations of *cis*-isomers of β-carotene in both purees. After 180 days of storage, no significant changes in the contents of these compounds were noted. Xanthophylls, as lutein and violaxanthin, were more affected than the carotenes, with significant losses ($P \leqslant 0.05$) during processing and storage of the pumpkin purees. Although these compounds are not precursors of vitamin A, the vitamin A-inactive carotenoids are being increasingly valued due to their action against degenerative and cardiovascular diseases, and certain types of cancers (Azevedo-Meleiro & Rodriguez-Amaya, 2007). New studies which investigate mechanisms of the stability of carotenoids in food matrix of pumpkin puree, the use of antioxidants, or which involve alternative technologies for conventional heat treatment, such as high pressure and the pulsed electric field, are important to improve the retention of these compounds in products such as carrots or pumpkin purees, or other vegetables rich in carotenoids.

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