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Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study

Lucia Maria Jaeger de Carvalho [a,*], Patrícia Barros Gomes [a], Ronoel Luiz de Oliveira Godoy [b], Sidney Pacheco [b], Pedro Henrique Fernandes do Monte [a], José Luiz Viana de Carvalho [b], Marília Regini Nutti [b], Ana Cristina Lima Neves [a], Ana Carolina Rodrigues Alves Vieira [a], Semíramis Rabelo Ramalho Ramos [c]

[a] Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373, CCS, Bloco K, Sala KI-38, Ilha do Fundão, 21941902, Rio de Janeiro, RJ, Brazil
[b] Embrapa Food Technology, Rio de Janeiro, RJ, Brazil
[c] Embrapa Coastal Tablelands, TE, Brazil

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ABSTRACT

Landrace pumpkins occur in nature and their potential as source of pro-vitamin A may be investigated in order to be used in conventional plant breeding or biofortification programs, aiming to increase the total carotenoids and β-carotene contents. The objective of the study was to determine the total carotenoid, α-carotene, β-carotene and its isomers and contents in two landrace samples (A and B) of raw pumpkins (Cucurbita moschata) to verify its seed production potential. High Performance Liquid Chromatography and UV/Visible spectrophotometry were used to determine α-carotene, β-carotene and its isomers, and total carotenoid contents, respectively. All analyses were carried out in triplicate. The results showed mean total carotenoid contents of 404.98 in sample A, and 234.21 μg/g in sample B. The α-carotene contents varied from 67.06 to 72.99 μg/g in samples A and B, respectively. All E-β-carotene was the most abundant isomer found varying from 244.22 to 141.95 μg/g in samples A and B, respectively. The 9 and 13-Z-β-carotene isomers were still found in low concentrations in both analyzed landrace samples. The content of β-carotene in raw sample A showed to be promising for the production of seeds for cultivation and consumption.

1. Introduction

The populations of underdeveloped and developing countries, such as Brazil, commonly suffer undernourishment and so-called hidden hunger, which can cause diseases from both caloric/proteic and micronutrients deficiencies. Vitamin A deficiency constitutes a public health problem and affects mainly children and women. Interest in raw materials of vegetal origin that contain high levels of carotenoids with provitamin A activity, has increased substantially in recent years. Some cultivars of pumpkin (*Cucurbita*) staining intense yellow to orange have revealed high levels of carotenoids, mainly β and α-carotene (Arima & Rodriguez-Amaya, 1988; Azevedo-Meleiro & Rodriguez-Amaya, 2007; Nestel, Bouis, Meenakshi, & Pfeiffer, 2006).

In Brazil, there is a large variability in species of pumpkin for cultivation and consumption, mainly in the states of Sergipe, Maranhão, Pernambuco and Bahia, but all other Brazilian states cultivate and use it for human consumption. Its orange pulp may contain promising levels of carotenoids, especially β-carotene, essential micronutrients for human metabolism.

To minimize nutritional problems, especially those arising from vitamin A deficiency in children and women of needy areas in Brazil, Embrapa Coastal Tablelands, Aracaju, Embrapa research centers, in partnership with other universities, have started new projects to screen local pumpkin landraces to gain information about their carotenoid content for conventional breeding purposes.

The pumpkin (*Cucurbita moschata* Duch) of the Cucurbitaceae family is widely grown and consumed in many countries around the world (Juna, Leeb, Songc, & Kima, 2006). Some varieties such as *C. moschata*, *C. maxima* and *C. pepo*, with colors ranging from intense yellow to orange, have revealed high levels of carotenoids, mainly α and β-carotene, β-criptoxanthina, lutein and zeaxanthin (Boiteux et al., 2007; Rodriguez-Amaya, Kimura, Godoy, & Amaya-Farfan, 2008).

With respect to the research and development of biofortified foods, Brazil is unique among countries. For example, Brazil is the only country where eight different crops are studied at the same time, namely, pumpkin, rice, sweet potatoes, beans, cowpeas, cassava, maize, and wheat (Nutti, 2011). Among the eight crops studied in Brazil's biofortification network, the pumpkin has increased in importance owing to its potentially high content of β-carotene, the

* Corresponding author at: Rua Silva Teles, 30A, apto. 205, Zipcode: 20541110, Rio de Janeiro, Brazil. Tel.: +55 21 2562 6602, +55 21 25721163.
E-mail address: luciajaeger@gmail.com (L.M.J. de Carvalho).

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precursor of vitamin A. This nutrient is a possible supplement to the diet of the populations in the areas of Brazil where vitamin A deficiency is a serious public health concern, mainly for children and women.

The objective of this preliminary study on pumpkin landraces was to determine the total amount of carotenoids, α-carotene and β-carotene and its isomer content, in two samples (A and B) of raw pumpkins (*C. moschata* Duchesne).

2. Material and methods

2.1. Material

The pumpkin samples were cultivated at Embrapa Coastal Tablelands, Aracaju, Brazil. They were harvested 4 months after the planting (June 2009). Landraces A and B presented better agronomic performance, and five samples were selected and sent, by air, to the Laboratory of Food Technology, Universidade Federal do Rio de Janeiro and to Embrapa Food Technology, Rio de Janeiro, for analysis.

2.2. Sampling

Ten samples of raw pumpkin (five from A and five from B) were peeled, and each one was divided into four parts by two longitudinal cuts from one end to the opposite. Of these four sections, two sections (opposite from each other) were discarded, and the remaining two, in preparation for analysis, were fragmented and placed in a vertical mixer (IKA — Ultraturrax model T18 basic) to obtain a homogeneous mass (Rodriguez-Amaya & Kimura, 2004).

2.3. Instrumental analyses

The total amount of carotenoids was determined using a spectrophotometer (Specord 210, model Analytikjena), at 450 nm. Alpha and β-carotene and its isomers (*E* and *Z*) were analyzed with a high-performance liquid chromatography (Waters 2695 — Alliance Model, Milford, USA) UV/Visible photodiode array detector and scanned between 350 to 600 nm using Empower software. A C_{30} column (YCM Carotenoid S-3, 4.6 mm × 250 mm reversed phase) was purchased from Waters. The mobile phase HPLC grade solvents were purchased from Tedia (Rio de Janeiro, RJ, Brazil) and consisted of 8: 2 (methanol: *t*-butyl methyl ether, v:v). The mobile phase flow rate was 0.8 mL/min, and 25 μL of an ether extract sample was injected. Analysis temperature was 30 °C with total analysis time of 60 min (Rodriguez-Amaya & Kimura, 2004). All analyses were performed in triplicate.

All the solvents and chemicals were obtained from commercial sources (Sigma and Merck). The carotenoid standards were obtained from Sigma-Aldrich.

2.4. Carotenoids extraction and total content

To determine the total amount of carotenoids, β-carotene and its *Z* and *E* isomer content, approximately 15 g of the samples, plus 3 g of celite 454 (Tedia, Ohio, USA) were weighed in a mortar on a digital balance (Bel Engineering, model MA0434/05). For the carotenoid extraction, successive additions of 25 mL of acetone were made to obtain a paste, which was transferred into a sintered funnel (5 μm) coupled to a 250 mL Buchner flask and filtered under vacuum. This procedure was repeated three times or until the sample became colorless. The extract obtained 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 emulsion formation. The aqueous phase was discarded. This procedure was repeated four times until no residual solvent remained. Then, the extract was transferred through a funnel to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate. The volume was made up by petroleum ether, and the samples were read at 450 nm. The total carotenoid content was calculated using the following formula:

$$\text{Carotenoids content}(\mu g/g) = \frac{A \times V(mL) \times 10^4}{A^{1\%}_{1cm} \times P(g)}$$

where A = Absorbance; V = Total extract volume; P = sample weight; $A^{1\%}_{1cm} = 2592$ (β-carotene Extinction Coefficient in petroleum ether).

2.5. Identification and quantification of α-carotene, β-carotene and its isomers

The *cis* (*Z*) isomers were quantified because they present smaller pro-vitamin A activity (Bauernfeind, 1972; Clydesdale, Fleischman, & Franz, 1970; Ihl, Monslaves, & Bifani, 1998 in Dutta, Raychaudhuri, & Chahraborty, 2005; Rodriguez-Amaya & Kimura, 2004) and may be present in the samples in significant proportions compared to the total carotenoid content.

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

Determination of α-carotene, β-carotene and its *E* and *Z* isomers was made according to the formula:

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

where: A_x = Carotenoid peak area; C_s = Standard concentration; A_s = Standard area; V = Total extract volume and P = Sample weight.

2.6. Statistical analysis

Each sample of the two landraces (5 of sample A and 5 of sample B) was extracted in triplicate. All data was reported as the mean ± standard error of triplicate determinations, analyzed using one-way analysis of variance (ANOVA) with significant differences between means determined at $p<0.05$ and measured with Duncan's multiple range tests using the Statistical Package for Social Science Research version 14 (SPSS).

Table 1
Total carotenoid, α-carotene, and All—*E*-β-carotene and 9 and 13-*Z* isomers of landrace pumpkin samples.

Landrace samples	Total carotenoid*	All — *E*-β-carotene*	9-*Z*-β-carotene*	13-*Z*-β-carotene*	α-carotene*
A	404.98[a]	244.22[a]	2.34[a]	3.67[a]	67.06[a]
	±17.37	±9.63	±0.35	±0.32	±0.33
B	234.21[b]	141.95[b]	0.97[b]	1.84[b]	72.99[b]
	±1.01	±0.39	±0.	±0.14	±0.11

± = Standard Deviation, and * mean of triplicates of the five landrace pumpkins (A and B samples).
[a,b] Variation in the letters between samples indicates significance difference at 5% level ($P<0.05$) utilizing Duncan's test.

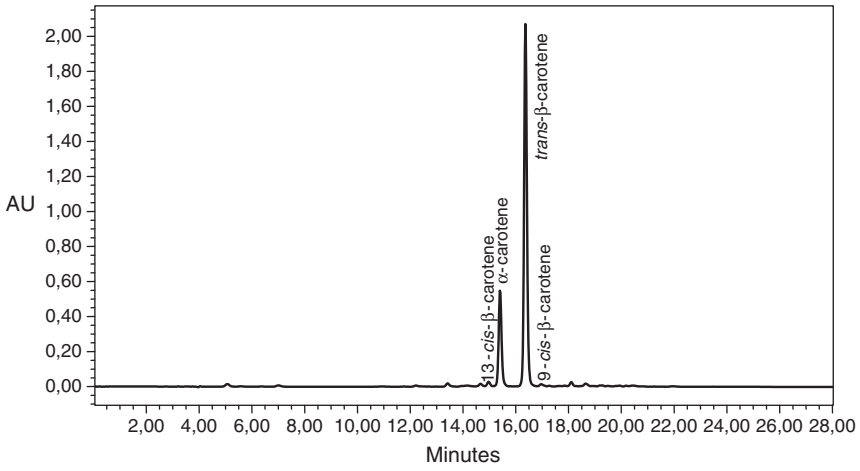

Fig. 1. Chromatogram of the α-carotene, β-carotene and cis-β-carotene isomers of landrace A.

3. Results and discussion

In Table 1, the total carotenoid content, α-carotene, β-carotene and the 9 and 13 *Z*-β-carotene isomers, from the analyzed pumpkin landraces can be observed.

The results showed a mean total carotenoid content of 404.98 μg/g in landrace sample A and 234.21 μg/g in landrace sample B. These values are similar to those found by Ramos et al. (2009a) in the pumpkin (*C. moschata*), which ranged from 100.50 to 365.40 μg/g. This cultivar is commonly found in Northeastern Brazil. However, these pumpkins are also cultivated by Southern farmers (*C. moschata*) and have values of carotenoid content higher than the average reported by other authors (Ramos et al., 2009b).

On the other hand, a previous study evaluating twenty-two cultivars of *C. moschata* reported a total carotenoid content ranging from 7.02 μg/g to 138.56 μg/g (Assis et al., 2010, as well as Azizah, Wee, Azizah, & Azizah, 2009).

The yellow pumpkin (*C. maxima*) showed total carotenoid content levels of 2120 μg/100 g and β-carotene 1180 μg/100 g in an evaluation study of these micronutrients in vegetables, spices and condiments (Kandlakunta, Rajendran, & Thingnganing, 2008).

The α-carotene content varied from 67.06 to 72.99 μg/g in landrace samples A and B, respectively, superior results to those found in *C. moschata* pulp, cultivar *Baianinha*, peeled (47 μg/g) by Rodriguez-Amaya et al. (2008) and by Kurz, Carle, and Schieber (2008) in *C .moschata* grown in Germany (10.60 e 0.58 μg/g de α-carotene).

Total *E*-β-carotene content varied from 244.22 μg/g to 141.95 μg/g in samples A and B, respectively; *E*-β-carotene was the most abundant in both landrace samples.

Considering that *E*-β-carotene has 100% pro-vitamin A activity, these results are promising. Previous studies have reported levels of 235 μg/g of β-carotene in *C. moschata* pulp, cultivar *Baianinha*, peeled (Rodriguez-Amaya et al., 2008), 7 mg/100 g of β-carotene in *C. Moschata,* cultivar *Long Island Cheese* and 3.5 mg/100 g of β-carotene in a cross between *C. maxima*×*C. moschata* (cultivar *Buto Tetsuko*) grown in Austria (Murkovic, Mulleder, & Neunteufl, 2002). Seo, Burri, Quan, and Neidlinger (2005) evaluated the carotenoid content of the pumpkin (*C. moschata)* and found that *E*-β-carotene was the most abundant isomer, followed by α-carotene.

As expected, low *Z*-β-carotene isomer content was observed. In the samples of *C. moschata* analyzed in this study, levels of 9-*Z*-β-carotene were 2.34 (sample A) and 0.97 μg/g (sample B), respectively. Similarly, levels of 13-*Z*-β-carotene were relatively negligible when compared to levels of α and β-carotene, ranging from 3.67 (sample A) to 1.84 μg/g (sample B).

The chromatograms of α-carotene, β-carotene and its 9 and 13-*Z*-isomers in the A and B samples of the landraces, respectively, can be observed in Figs. 1 and 2.

Reduced levels of the isomers 9 and 13-*Z*-β-carotene ranged from 0.28 to 1.61, and 0.22 to 1.24 μg/g, respectively, were also found in yellow bitter cassava roots (*Manihot esculenta*) (Oliveira, Carvalho, Nutti, Carvalho, & Fukuda, 2010). In the presence of two isomers, 9

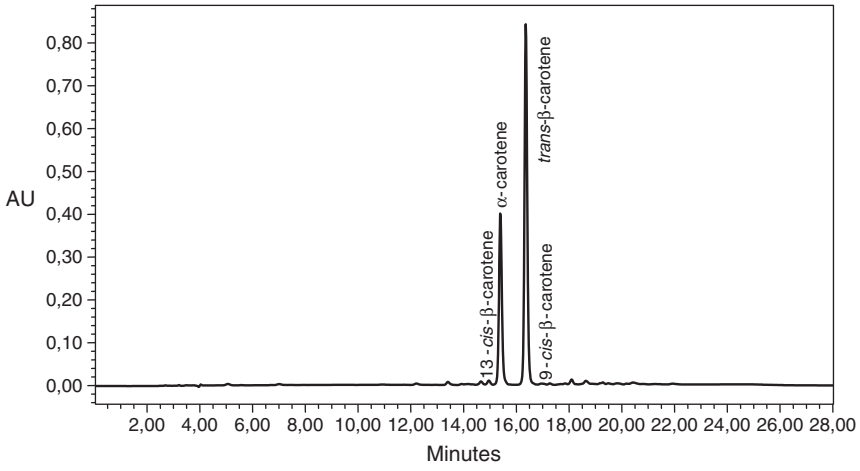

Fig. 2. Chromatogram of the α-carotene, β-carotene and cis-β-carotene isomers of landrace B.

and 13-*Z*-β-carotene, low pro-vitamin A occurs in various matrices, but usually their levels are not very high, compared to the *E*-isomer β-carotene.

Studies reviewed by Rodriguez-Amaya, Nutti, and Carvalho (2011) indicate that the carotenoid compositions of sweet potato roots, cassava roots and corn kernels vary widely between varieties and crops. This preliminary study in Brazilian pumpkin landraces for the quantification and identification of their carotenoid composition can be considered as an important preliminary step towards the use of these landraces in conventional breeding of this crop. Raising the pro-vitamin A contents of the pumpkin may be a goal to pursue, especially by the Biofortification Project in Brazil, considering the loss of carotenoids during the processing and storage of the flour produced from staple foods, such as cassava and sweet potato, that has been reported and the influencing factors that have been pointed out (Chávez et al., 2007; Van Jaarsveld et al., 2000). The study and identification of the carotenoid content in pumpkin landraces are of importance not only to Brazil, but also to Venezuela, El Salvador, and Mexico, among other countries, in order to have a local source of foods with a high carotenoid content. Additionally, the possibility for the breeding of these foods should be investigated.

4. Conclusions

This study showed that sample A of landrace *C. moschata* contained a high content of α and β-carotene and thus can be considered a promising source for the production of seeds for cultivation and consumption.

However, additional studies with different pumpkin landraces are being conducted to determine which ones have high carotenoid content.

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References

Arima, H. K., & Rodriguez-Amaya, D. B. (1988). Carotenoid composition and Vitamim A value of commercial Brazilian squashes and pumpkins. *Journal of Micronutrient Analysis*, *4*, 177–191.

Azevedo-Meleiro, C. H., & Rodriguez-Amaya, D. B. (2007). Quantitative and qualitative differences in carotenoids composition among *Cucurbita moschata, Cucurbita maxima* and *Cucurbita pepo*. *Journal of Agricultural and Food Chemistry*, *55*, 4027–4033.

Azizah, A. H., Wee, K. C., Azizah, O., & Azizah, M. (2009). Effect of boiling and stir frying on total phenolics, carotenoids and radical scavenging activity of pumpkin (*Cucurbita moschata*). *International Food Research Journal*, *16*, 45–51.

Bauernfeind, J. C. (1972). Carotenoid vitamin A precursors and analogues in foods and feeds. *Journal of Agricultural and Food Chemistry*, *20*(3), 456–473.

Boiteux, L. S., Nascimento, W. M., Fonseca, M. E. N., Lana, M. M., Reis, A., Mendonça, J. L., et al. (2007). 'Brasileirinha': cultivar de abóbora (*Cucurbita moschata*) de frutos bicolores com valor ornamental e aptidão para consumo verde. *Horticultura Brasileira*, *25*, 103–106.

Chávez, A. L., Sánchez, T., Rodriguez-Amaya, D. B., Nestel, P., Tohme, J., & Ishitani, M. (2007). Retention of carotenoids in cassava roots submitted to different processing methods. *Journal of the Science of Food and Agriculture*, *87*, 388–393.

Clydesdale, F. M., Fleischman, D. L., & Franz, F. L. (1970). Maintenance of color in processed green vegetables. *Journal of Food Production and Development*, *4*, 127–130.

Dutta, D., Raychaudhuri, U., & Chahraborty, R. (2005). Retention of ß-carotene in frozen carrots under varying conditions of temperature and time of storage. *African Journal of Biotechnology*, *4*, 103-103.

Ihl, M., Monslaves, M., & Bifani, V. (1998). Chlorophylase inactivation as a measure of blanching efficiency and color retention of artichokes (*Cynara scolyus* L.). *LWT - Food Science and Technology*, *31*, 1–56.

Juna, H., Leeb, C. H., Songc, G. S., & Kima, Y. S. (2006). Characterization of the pectic polysaccharides from pumpkin peel. *LWT - Food Science and Technology*, *39*, 554–561.

Kandlakunta, B., Rajendran, A., & Thingnganing, L. (2008). Carotene content of some common (cereals, pulses, vegetables, sp ices and condiments) and unconventional sources of plant origin. *Food Chemistry*, *106*, 85–89.

Kurz, C., Carle, R., & Schieber, A. (2008). HPLC-DAD-MSn characterization of carotenoids from apricots and pumpkins for the evaluation of fruit product authenticity. *Food Chemistry*, *110*, 522–530.

Murkovic, M., Mulleder, U., & Neunteufl, H. (2002). Carotenoid content in different varieties of pumpkins. *Journal of Food Composition and Analysis*, *15*, 633–638.

Nestel, P., Bouis, H. E., Meenakshi, J. V., & Pfeiffer, W. (2006). Biofortification of staple food crops. *Journal of Nutrition*, *136*, 1064–1067.

Oliveira, A. R. G., Carvalho, L. M. J., Nutti, R. M., Carvalho, J. L. V., & Fukuda, W. G. (2010). Assessment and degradation study of total carotenoids and ß-carotene in bitter yellow cassava (*Manihot esculenta* Crantz) varieties. *African Journal of Food Science*, *4*(4), 148–155 (April).

Ramos, S. R. R., Carvalho, H. W. L., Oliveira, I. R., Anjos, J. L., Queiróz, M. A., Lima, M. A. C., et al. (2009). Identificação de acessos locais de abóbora com elevados teores de carotenóides totais e características agronômicas e comerciais. *3ª Reunião Anual de Biofortificação no Brasil. 31 de maio a 05 de junho de 2009. Aracaju, Sergipe, Brazil*.

Ramos, S. R. R., Carvalho, H. W. L., Queiroz, M. A., Santos, E. D., Silva, H. M., Trindade, B. C., et al. (2009). Avaliação preliminar de acessos locais de abóbora para teores de carotenóides totais e sólidos solúveis. *3ª Reunião Anual de Biofortificação no Brasil. 31 de maio a 05 de junho de 2009. Aracaju, Sergipe, Brazil*.

Rodriguez-Amaya, D. B., & Kimura, M. (2004). *HarvestPlus handbook for carotenoids analysis* (first ed.). Washington, DC and Cali: IFPRI and CIAT (Chapther 2), 58 pp..

Rodriguez-Amaya, D. B., Kimura, M., Godoy, H. T., & Amaya-Farfan, J. (2008). Updated Brazilian database on food carotenoids: Factors affecting carotenoids composition. *Journal of Food Composition and Analysis*, *21*, 445–463.

Rodriguez-Amaya, D. B., Nutti, M. R., & Carvalho, J. L. V. (2011). Carotenoids of sweetpotato, cassava and corn and their application in the fortification of flour and bread. In V. R. Preedy, R. R. Watson, & V. B. Patel (Eds.), *Flour and breads and their fortification in health and disease prevention* (pp. 301–311). London, Burlington, San Diego: Academic Press, Elsevier.

Seo, J. S., Burri, B. J., Quan, Z., & Neidlinger, T. R. (2005). Extraction and chromatography of carotenoids from pumpkin. *Journal of Chromatography. A*, *1073*, 371–375.

Van Jaarsveld, P. J., Marais, D. W., Harmse, E., Laurie, S. M., Nestel, P., & Rodriguez-Amaya, D. B. (2000). Beta-carotene content ofsun-dried and oven-dried chips of orange-fleshed sweetpotato. *Book of abstracts. XXII IVACG Meeting, Lima, Peru*.

Web references

Assis, J. G. A., Ramos Neto, D. C., Druzian, J. I., Souza, C. O., Aragão, C. A., & Queiroz, M. A. (2010). Identificação de acessos de abóbora (*Cucurbita moschata*) com altos teores de carotenóides. http://www.abhhorticultura.com.br/eventosx/trabalhos/ev_1/CURC11.PDF Acessed in june 5, 2010.

Nutti, M. R. (2011). Biofortification in Brazil: A sustainable way to improve nutrition and health. http://abstracts.acs.org/chem/241nm/program/view.php Acessed in july, 16, 2011.