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Variability of Total Carotenoids in *C. moschata* Genotypes

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Nowadays, the vitamin A deficiency (VAD) is still a public health concern, being one of the most important components of the high rate of morbidity and mortality among children in developing countries. There is a high prevalence of night blindness caused by deficiency of vitamin A. Children and pregnant women are particularly affected. Many pumpkins (*C. moschata*) genotypes present a great variability of carotenoid content mainly the beta-carotene. The objective of this work is to evaluate the variability of beta-carotene among pumpkins genotypes. Twenty biofortified pumpkins cultivated at Embrapa Table Coastland and Semiarid were evaluated regarding beta-carotene contents.The analyses were carried out by High Performance Liquid Chromatography. The pumkinks showed a large variability in the contents of total carotenoids varying, in fresh pumpkins from 699.06 (genotype 25) to 124.60 µg/g (genotype 26), from 106.96 (genotype 26) to 655.54 µg/g (genotype 25) in samples cooked in boiling water and 117.55 (genotype 19) and 650.54 µg/g (genotype 25) in steamed cooked, respectively.

1. Introduction

Pumpkins are fruits of different species of *Cucurbita* genus, grown around the world for its pulp, seeds and even the flowers serve for human consumption (Provesi, Dias, Lover, 2011). The Food and Agriculture Organization of the United Nations (FAO) reported an estimated world pumpkins production, in 2007, over 20 million tonnes, especially in China, India, Russia, United States, and Egypt (FAOSTAT, 2008).
Although there are no official data in Brazil, the pumpkins production is high, especially of the species *Cucurbita moschata* and *Cucurbita maxima*. The great majority of pumpkins are harvested and consumed when fruit is mature (Paris,1994). In several regions of the country, *C. moschata* are known to contain high carotenoids contents with antioxidant activity (lutein) and pro- vitamin A activity (α and β-carotene) (Rodriguez- Amaya et al., 2008). The β -carotene has 100 % pro- vitamin A activity and α -carotene around 53 % (Boiteux et al., 2007). The processing of food can increase the bioavailability of carotenoids and functional foods have prepared based on it (Mínguez - Mosquera et al., 2002). Khachik, Goli, Beecher (1992) reported that vegetables, after to be cooked, increased the provitamin A carotenoids in relation to the fresh ones. However, it should be noted that the heat treatment as boilingand steam among others promotes carotenoids isomerization in food (trans to cis isomerization), and the degree of isomerization is directly related to the heat intensity and duration (Rock et al., 1998). Nutritionally, the differentiation between the cis and trans isomers of provitamin is important because the cis form exhibits less potential of provitamin A (Rodriguez-Amaya et al., 2008.). Lucia et al (2007) evaluated the effect of processing in some vegetables on the bioavailability of carotenoids concluding that the heat treatment can increase it, and it is possible that, even with significant losses after processing, the remaining carotenoids are better absorbed. On the other hand, vitamin A deficiency (VAD) is still a major public health concern in developing countries, including Brazil one of the most important components of the high morbidity and mortality among children (Zuorro, 2014). Underwood, Arthur

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(1996) reported the contribution of vitamin A in public health. The benefits include not only improved health and welfare for individuals and their families, but also improved chances of survival for an estimated 254 million children.For example, there is a high prevalence of night blindness caused by vitamin deficiency A (Tomkins, 2000). High carotenoids content were found, in *C. moschata*, cv Baianinha (Kandlakunta et al., 2008; Kurz et al., 2008). Azevedo-Meleiro and Rodrigues-Amaya (2007) evaluated the pro-vitamin A carotenoids of *C. pepo*, *C. moschata* and *C. maxima.* The α and β-carotene were most abundant in *C.moschata*, *C.pepo* in lutein and β-carotene and, *C. maxima* in violaxanthin, followed by β-carotene and lutein. The study of pumpkin *C. moschata* genotypes is part of the project "Biofortification in Brazil: Creating cultures for better nutrition – BioFORT", which is funded by Embrapa Monsanto Research Fund. The evaluation of brazilian *C. moschata* genotypes fresh and cooked will provide data on the carotenoid contents and to be recommended for children diet, minimizing nutritional deficiencies of Vitamin A.

2. Material and methods

2.1 Material

Twenty genotypes of *C. moschata* were cultivated in two different experimental locations: Petrolina, state of Pernambuco (samples 3 to 14 and, 21 to 26), Brazil and, at EMBRAPA Table Coastlands, Aracaju, state of Sergipe (samples 15, 16, 17, 18, 19, 20 and 27), Brazil. After maturity of the fruits, 20 pumpkins were used for experiments and analysis. The pumpkins were sent by plane to the Laboratory of Technology and Instrumental Analysis of the Faculty of Pharmacy, Rio de Janeiro Federal University, Rio de Janeiro, Brazil for experiments and analyses.

2.2 Sampling

Samples of fresh pumpkin were weighted, peeled and divided into four parts by two longitudinal cuts, from one end to the opposite, resulting in four sections (Figure 1). These four sections, two opposite each other, were discarded and the remaining two were used for analysis and were fragmented and placed in a vertical mixer (IKA - Ultraturrax model T18 basic) to obtain a homogeneous mass (Rodriguez-Amaya, & Kimura, 2004).

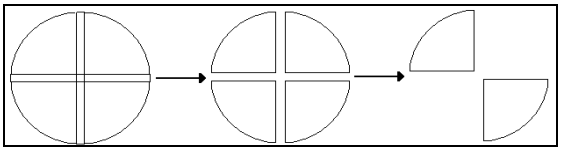

Figure 1. Quarterization (Rodriguez Amaya, Kimura, 2004).

2.3 Methods

2.3.1 Cooking styles

The twenty pumpkin genotypes samples were washed, peeled, cut in small pieces, and divided into 2 different groups. The pumpkin pieces were then, cooked in two different styles: cooked in boiling water (2:1 for 4 minutes) and steamed cooked (five minutes). Additionally, genotypes 15, 16 and 17 were still cooked with cowpea beans (1:1) to verify the carotenoids content and iron and zinc after cooked in boiling water since people from northeast of Brazil still eat pumpkin cooked with cowpea.

2.3.2 Soluble solids

The soluble solids were determined using an Abbé refractometer, AR12, Scmidt & Haensch, Germany (AOAC, 2005).

2.3.3 Carotenoids extraction and determination

The total carotenoids in pumpkin genotypes were determined using a spectrophotometer (Thermo Scientific Evolution 60) set at 450 nm. To determine the total carotenoids in the fresh and cooked pumpkin genotypes, approximately 0.3 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). For the carotenoids extraction, 25 mL of acetone were, 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 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 equation 1:

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

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

2.3.4 - Statistical analyses

The data were analyzed using ANOVA and Tukey tests 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 Soluble solids and weight

The soluble solids contents varied from 4.97 to 17.20 º Brix and, 13 genotypes presented values above 9 º Brix showing a large variability among the samples and, significant differences ($P < 0.05$) were found. XinZheng et al. (2009) reported soluble solids in *C. moschata* cultivars ranging from 3.3 to 12.7%. Zinash, Workneh & Woldetsadik (2013) still reported a great variation in soluble solids contents in pumpkins cultivars (4.10 to 10.03 ºBrix) for fresh fruit quality assessment. On the other hand, Gajewski et al. (2008) found in the cultivar C. moschata variety "Zemcuzina", 6.6 º Brix and Valenzuela-Jacobo et al. (2011) 6.42 Brix in *C. moschata* "Cehualca". The weight of the pumpkins genotypes ranged from 1,464 g to 7,824 g. However, the pumpkins genotypes did not show correlation between soluble solids *versus* weight (Table 1).

Table 1. Soluble solids and weight of the 20 fresh C. moschata genotypes

Identification	Soluble solids (g/100g)	Weight (g)
3*	10.12	6.685
6*	04.97	5.702
8*	10.07	7.824
9*	08.43	4.240
10*	10.33	5.660
11*	09.87	5.692
14*	10.50	3.858
15*	09.00	4.812
16*	10.10	4.075
17*	09.83	5.356
18*	10.16	4.840
19*	05.83	3.930
20*	05.08	4.162
21**	12.75	1.738
22**	08.10	2.146
23**	07.50	1.464
24**	10.66	2.104
25**	11.00	1.618
26**	17.20	2.042
27*	10.50	5.736

* Genotypes from experimental location at Embrapa Table Coastlands, Aracaju, Sergipe, Brazil.
** Genotypes from experimental location at Embrapa Semiarid, Petrolina, Pernambuco, Brazil.

3.2 Total carotenoids in fresh *C. moschata* genotypes

Among genotypes studied, significant differences ($P \leq 0.05$) were observed for total carotenoids suggesting that a genetic variation in carotenoid accumulation is present. Itle and Kabelka (2009) observed the same

differences evaluating twenty *Cucurbita* spp. genotypes. They correlated the L (lightness or darkness), a (color direction in red or green) and, b (color direction in yellow or blue) color space values with the carotenoid contents. According to them these close associations will also assure that breeding for enhanced carotenoid content within pumpkins can be achieved using an easy-to-use and, inexpensive method. Gajewski et al. (2008) found in the cultivar *C. moschata* variety "Zemcuzina", ex Poir, total carotenoids contents of 64.5 $\mu g.g^{-1}$ in fresh samples. In our study the total carotenoids contents in fresh pumpkin genotypes varied from 699.06 (genotype 25) to 124.60 $\mu g.g^{-1}$ (genotype 26). Significant differences in the total carotenoids contents among all fresh samples ($P < 0.05$) were observed. Carvalho et al. (2012) found total carotenoids in two fresh *C. moschata* genotypes varying from 234.21 to 404.98 $\mu g.g^{-1}$. These values are similar to those found by Ramos et al. (2009) in pumpkin (*C. moschata*) genotype, which ranged from 100.50 to 365.40 $\mu g.g^{-1}$. On the other hand, forty-eight accesses of *C. moschata* from Embrapa Semiarid bank were evaluate by Souza et al. (2012) and the total carotenoids, in β-carotene, in samples were lower than our results 11.81 to 290.62 $\mu g.g^{-1}$.

3.3 Total carotenoids in cooked *C. moschata* genotypes

The total carotenoid contents in boiled pumpkin genotypes ranged from 655.54 (genotype 25) to 106.96 $\mu g.g^{-1}$ (genotype 26) and, in steamed cooked pumpkins varied from 133.63 (genotype 6) to 650.54 $\mu g.g^{-1}$ (genotype 25). Samples cooked with cowpea beans showed the lowest contents among all (83.60 $\mu g.g^{-1}$ – genotype 17 to 117.04 $\mu g.g^{-1}$ – genotype 18), as expected. By comparing the initial total carotenoid contents with α-carotene in both cooking styles, the genotype 25 presented the lowest percentage (11.57 % - 75.85 $\mu g.g^{-1}$ - boiled and 11.48 % - 74.71 $\mu g.g^{-1}$ - steamed). Eight cooked pumpkin genotypes showed significant differences ($P < 0.05$) in total carotenoids between the two cooking styles (boiled *versus* steamed). Comparing the total carotenoids between fresh and boiled pumpkins genotypes it was observed that 17 of them did not revealed significant differences ($P < 0.05$). On the other hand, 12 steamed cooked pumpkins showed significant differences ($P < 0.05$) compared to fresh samples.

Table 2. Total carotenoids in pumpkin genotypes of C. moschata cooked in boiling water, steamed and cooked with cowpea beans.

	Total carotenoids		
Identification	Cowpea	Boiled	Steamed
3	-	218.77 ^{c,d,e,f}	242,80 ^{c,d}
6	-	124.20 ^{a,b,c}	133.63 ^{a}
8	-	32.66 ^{g,h}	257,93 ^{d}
9	-	224.02 ^{d,e,f}	239.23 ^{c,d}
10	-	149,23 ^{a,b,c,d}	154,60 ^{a}
11	-	428,20 ^{i,j}	492.76 ^{f}
14	-	325,49 ^{g,h}	340.85 ^{e}
15	-	308,03 ^{f,g,h}	356,80 ^{e}
16	110.02 ^{a}	237.92 ^{d,e,f,g}	211.75 ^{b,c,d}
17	83.60 ^{a}	206.62 ^{b,c,d,e}	224.71 ^{b,c,d}
18	117.04 ^{a}	198.47 ^{a,b,c,d,e}	148.49 ^{a}
19	-	117.55 ^{a,b}	177.93 ^{a,b}
20	-	166.90 ^{a,b,c,d,e}	159.42 ^{a}
21	-	343.76 ^{h,i}	458.25 ^{f}
22	-	151.29 ^{a,b,c,d}	152.50 ^{a}
23	-	178.71 ^{a,b,c,d,e}	208.68 ^{b,c}
24	-	485.67 ^{j}	502.08 ^{f}
25	-	655.54 ^{k}	650.54 ^{g}
26	-	106.96 ^{a}	140.83 ^{a}
27	-	261.38 ^{e,f,g,h}	332.96 ^{e}

Different letters in the same column means significant difference at 0.05%.

Is well known that β-carotene isomers, mainly the 13 and 9-*Z*- β-carotene, are formed by isomerization or decomposition after heat treatments as boiling and steaming, light exposure, singlet oxygen, enzymes, free radicals and high oxygen concentrations (Rios et al., 2009). Provesi et al., (2011) evaluated the carotenoids losses in *C.moschata* and *C. maxima* purees as well as the β-carotene isomers after during 180 days. They observed a slight degree of isomerisation of β-carotene in the puree samples, but with low concentrations of cis-isomers. Okpalamma et al., (2013) studying *T. occidentalis* (fluted pumpkin) leaves observed that the lutein and β-carotene concentrations were much higher than typical contents in conventional edible leafy vegetables. Meals prepared with pumpkin cooked with cowpea beans presented zinc contents ranging from

11.52 to 13.87 and iron from 13.69 to 15.68 mg.kg^{-1}. Comparing the iron and zinc contents in the other preparations (boiled and steamed), as expected, these contents were highest.

4, Conclusions

Taking into account that, usually, the proportion of all-trans-β-carotene,is the most abundant carotenoid found in *C.moschata* genotypes, varying from 37-85% of the total carotenoid content in the cultivars tested in our study, they are promising for consumption, corresponding of the 101 % of DRI (intake) for children of preschool age (1-3 y) in a daily serving of 120g of food, considering the minimum concentration of total carotenoids found in the samples. The *C. moschata* genotypes can be recommended for cultivation given its high levels of total carotenoids.

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References

Azevedo-Meleiro C.H., Rodriguez-Amaya D.B., 2007, Qualitative and quantitative differences in carotenoid composition among *Cucurbita moschata*, *Cucurbita maxima*, and *Cucurbita pepo*, J. Agric. Food Chem. 55, 4027 - 4033.

Boiteux L. S., Nascimento W. M., Fonseca M. E. N., Lana M. M., Reis A., Mendonça J. L., Lopes J. F.,Reifschneiderf J. B, 2007, "Brasileirinha": pumpkin cultivar (*Cucurbita moschata*) of bicolor frui with ornamental value and suitability for green consumption. ('Brasileirinha: cultivar de abóbora (*Cucurbita moschata*) de frutos bicolores com valor ornamental e aptidão para consumo verde). Hortic. Brasil. 25, 103-106.

Carvalho L.M.J., Gomes P.B., Godoy L.O., Pacheco S., Monte P.H.F., Carvalho J.L.V, Nutti M.R., Neves A.C.L., Vieira A.C.R.A., Ramos S.R., 2012, Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. Food. Res. Intern. 47(2), 337 340.

Della Lucia C.M., Campos F.M., Mata G.F.C., San'Ana H.M.P., 2008, Control of carotenoid loss in vegetables prepared in a hospital foodservice. Ciênc. & Saúde Coletiva 13(5),1627 - 1636.

FAO- Food and agriculture organization of de united nations. Statistical Database – FAOSTAT, 2008. Accessed in 20.01.2011.

Gajewski M., Radazanoswski J., Danielcenko H., Jariene E., Cerniawskiene J, 2008, Quality of pumpkin in relation to sensory characterisitics. Not. Botan. Hort. Agrobotan. Cluj- Napoca. 36(1), 73 – 79.

Itle R. A., Kabelka E. A, 2009, Correlation between L*a*b* color space values and carotenoid content inpumpkins and squash (Cucurbita spp.). Hort. Sci. 44(3), 633-637.

Jacobo - Valenzuela N., Zazueta Morales J.L., Galegos-Infante J.A., Aguilar-Gutierrez F., CamachoHernandéz I.L., Rocha-Guzman N.E., Gonzalez-Laredo R.F. Chemical and physico-chemical characterization of winter squash (Cucurbita moschata D.). Not. Botan. Horti. Agrobotan. Cluj Napoca.39(1), 34 - 40, 2011.

Khachik F., Beecher G.R., Goli M.B., Lusby W.R., Smith J.C. Jr., 1992, Separation and identification of carotenoids and their oxidation products in the extracts of human plasma. Anal Chem. , 15, 64(18), 2111 - 2122.

Kandlakunta B., Rajendran A., Thingnganing L., 2008, Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. Food Chem., 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 Chem. 110(2), 522-530.

Mínguez-Mosquera M. I., Hornero-Méndez D., Pérez-Gálvez A., 2002, Carotenoids and provitamin A in functional foods. In W. J. Hurst (Ed.), Methods of analysis for functional foods and nutraceuticals, 1, 101-158). Washington: CRC Press.

Okpalamma F., Ojimelukwe P.C., Mazi E.A., 2013, Post harvest storage and processing changes in carotenoids and micronutrients in fluted pumpkin (*Telferia occidentalis* Hook F.). IOSR J. Agric. Veterin. Sci., 6(4), 34-39.

Paris H. S., 1994, Genetic analysis and breeding of pumpkins and squash for high carotene content. Vegetables and Vegetable Products: Modern Methods Plant Anal. 16, 93-115.

Provesi J.G., Dias C.O., Amante E.R., 2011, Changes in carotenoids during processing and storage of pumpkin puree. Food Chem.128, 195-202.

Rios, A.O., Antunes, L.M.G., 2009. Carotenoids protection against free radicals generated in the treatment of cancer with cisplatin. (Proteção de carotenoides contra radicais livres gerados no tratamento de câncer com cisplatina). Rev. Aliment. e Nutri., 20, 342-350.

Rock C.L., Lovaldo J.L., Emenhiser C., Ruffin M.T., Flatt S.W., Schwartz S.J., 1998, Bioavailability of β carotene is lower in raw than in processed carrots and spinach in women. J. Nutr. 128, 913-916.

Ramos S.R.R., Carvalho H.W.L., Oliveira I.R., Anjos J.L., Queiróz M.A., Lima M.A.C., 2009, Identification of pumpkin local accesses with high levels of carotenoids and agronomic and comercial characteristics (Identificação de acessos locais de abóbora com elevados teores de carotenoides totais e características agonômicas e comerciais). 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. HarvestPlus handbook for carotenoids analysis. Washington, DC and Cali: IFPRI and CIAT, 58p. HarvestPlus Technical Monograph, 2, 2004.

Rodriguez-Amaya D. B.; Kimura, M.; Amaya-Farfan, J. Brazilian sources of carotenoids. (Fontes brasileiras de carotenoids). Brasília, DF: Ministério do Meio Ambiente, 2008.

Souza C.O., Menezes J.D.S., Ramos Neto D.C., Assis J. G. A., Silva S. R., Druzian J. I., 2012, Total carotenoids and vitamin A of cucurbits from germoplasma bank of Embrapa Semiarid. Ciên. Rural, Santa Maria, on line.

Tomkins A., 2000, Malnutrition, morbidity and mortality in children and their mothers. Proceedings of Nutrit. Soc., 59(1), 135-146.

Underwood B. A., Arthur P., 1996, The contribution of vitamin A to public health. The FASEB J. 10(9), 1040-1048.

Zinash A., Workneh T.S., Woldetsadik K., 2013, Effect of accessions on the chemical quality of fresh pumpkin. Afric. J. Biotechnol., 12(51), 7092-7098.

Zuorro A., Lavecchia, R., Medici F. Piga, L., 2014, Use of cell wall degrading enzymes for the production of high-quality functional products from tomato processing waste. Chem. Engin. Transact., 38, 355-360.