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Qualitative and Quantitative Differences in Carotenoid Composition among *Cucurbita moschata*, *Cucurbita maxima*, and *Cucurbita pepo*

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Squashes and pumpkins are important dietary sources of carotenoids worldwide. The carotenoid composition has been determined, but reported data have been highly variable, both qualitatively and quantitatively. In the present work, the carotenoid composition of squashes and pumpkins currently marketed in Campinas, Brazil, were determined by HPLC-DAD, complemented by HPLC-MS for identification. *Cucurbita moschata* 'Menina Brasileira' and *C. moschata* 'Goianinha' had similar profiles, with β-carotene and α-carotene as the major carotenoids. The hybrid 'Tetsukabuto' resembled the *Cucurbita pepo* 'Mogango', lutein and β-carotene being the principal carotenoids. *Cucurbita maxima* 'Exposição' had a different profile, with the predominance of violaxanthin, followed by β-carotene and lutein. Combining data from the current study with those in the literature, profiles for the *Cucurbita* species could be observed. The principal carotenoids in *C. moschata* were β-carotene and α-carotene, whlereas lutein and β-carotene dominate in *C. maxima* and *C. pepo*. It appears that hydroxylation is a control point in carotenoid biosynthesis.

KEYWORDS: Carotenoids; *Cucurbita moschata*; *Cucurbita maxima; Cucurbita pepo*; compositional variation; carotenoid profile

INTRODUCTION

Native to tropical and subtropical America, squashes and pumpkins (*Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*) are important food sources of carotenoids worldwide. They are rich in carotenoids and are widely available year-round. They have good postharvest quality, allowing storage at ambient conditions during several months.

The carotenoids of squashes and pumpkins have been investigated for some time, but the studies were mostly limited to provitamin A carotenoids, principally α-carotene and β-carotene, and sometimes β-cryptoxanthin (*1*−*7*). Godoy and Rodriguez-Amaya (*8*) and Ben-Amotz and Fishler (*9*) determined only the provitamins A, but quantified the *E*/*Z*-isomers separately. With more recent roles attributed to carotenoids in terms of human health, that is, reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataracts, and macular degeneration, not related to the provitamin A activity, the importance of determining the vitamin A-inactive carotenoids became evident.

Lee et al. (*10*) quantified nine carotenes (i.e., hydrocarbon carotenoids) in six cultivars of winter squash. Only β-carotene showed appreciable concentrations in the winter squash, whereas the other carotenes were determined at <1 μg/g. Arima and Rodriguez-Amaya (*11*) quantified 14 carotenoids in *C. moschata* 'Menina Brasileira" of which β-carotene and α-carotene were the principal carotenoids. In *C. maxima* 'Exposição' β-carotene, lutein, and violaxanthin were the major carotenoids of 15 carotenoids identified. Lutein and β-carotene predominated over 17 carotenoids in the hybrid 'Tetsukaboto'. Arima and Rodriguez-Amaya (*12*) also determined 19 carotenoids in *C. moschata* 'Baianhinha', β-carotene and α-carotene being the principal carotenoids. In *C. maxima* 'Jerimum Caboclo', lutein and β-carotene were the major carotenoids of 11 identified. In spite of the large number of carotenoids encountered, only two to four carotenoids in each cultivar showed appreciable levels, whereas the other carotenoids presented trace or very small amounts (<3 μg/g) These three studies were all done by open column chromatography and spectrophotometric measurement.

Matsuno et al. (*13*) isolated and elucidated the structures of two new carotenoids in *C. maxima*, called cucurbitaxanthin A and cucurbitaxanthin B, employing nuclear magnetic resonance and mass spectrometry. Khachik and Beecher (*14*), using high-performance liquid chromatography (HPLC) with a C_{18} column and a combination of isocratic and gradient elution, separated and identified carotenoids and carotenoid esters in acorn squash (*C. pepo*) and in three varieties of baby food squash (*C. maxima*). The chromatographic pattern differed markedly among the varieties. Although complex mixtures of esters were observed, only eight carotenoids were found. Lutein predomi-

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nated in two varieties of *C. maxima*, whereas α-carotene and β-carotene were the major carotenoids of another variety of this same species. *C. pepo* had β-carotene as principal carotenoid. Ten carotenoids were identified in an Argentinian squash (*C. moschata*), but only β-carotene, α-carotene, and lutein were quantified (*15*). Murkovic et al. (*16*) analyzed a wide range of squashes and pumpkins commercially available in Austria and found the carotenoid concentrations ranged from 0.6 to 74 μg/g for β-carotene, from 0 to 75 μg/g for α-carotene, and from 0 to 170 μg/g for lutein.

Considering the substantial qualitative and quantitative variation reported for carotenoid composition of squashes and pumpkins, more data are needed. It is imperative to ensure carotenoids are conclusively identified and that the quantitative variations observed are natural, and not from analytical variability. The present work had a twofold objective: (a) determine the carotenoid concentrations in squashes and pumpkins currently marketed in Brazil; and (b) verify the possible existence of carotenoid profiles in *Cucurbita* species. Two of the Brazilian Cucurbitaceae had been previously analyzed with an open column chromatographic method (*11*). They were reanalyzed in this work, using modern methods, along with three others that were not previously studied.

MATERIALS AND METHODS

Sample Collection and Preparation. The following *Cucurbita* fruits were analyzed: *C. moschata* cultivars 'Menina Brasileira' and 'Goianinha', *C. maxima* 'Exposição', *C. pepo* 'Mogango', and *C. maxima* × *C. moschata* hybrid 'Tetsukabuto'. All samples were purchased from supermarkets in Campinas, SP, Brazil. For each variety, five samples of mature squash or pumpkin were taken at random at different times during the second semester of 2001 to the first semester of 2002 and analyzed in duplicate. *C. moschata* 'Menina Brasileira' is 50−71 cm long, with an 18−27 cm transverse diameter in the cylindrical portion and a 26−32 cm transverse diameter in the bulbous portion, and weighs 6.6−10.7 kg. It has a cream or light orange exterior with large dark green longitudinal stripes, a smooth surface, and dark orange pulp, and it is cylindrical, slightly curved with an enlarged bulb-like section at the blossom end. *C. moschata* 'Goianinha' is 24−30 cm long, with a 10−14 cm transverse diameter in the cylindrical portion and a 15−18 cm transverse diameter in the bulbous portion, weighing 1.1−1.3 kg. It has a dark green exterior blotched with cream-orange streaks and orange pulp. It is pear-shaped, sometimes elongated with a slight bulbous portion. *C. maxima* 'Exposição' weighs 2.3−3.3 kg and has a 10−16 longitudinal diameter and a 20−30 transverse diameter. It has a dark orange exterior and pulp and a smooth surface with prominent ribbing, and it is spherical but flattened at both stem and blossom ends. The hybrid 'Tetsukabuto' has a 17−20 cm longitudinal diameter and a 19−22 cm transverse diameter, and it weighs 2.2−2.7 kg. It is spherical and has a dark green exterior, a rough surface, a thick peel, and orange pulp. *C. pepo* 'Mogango' has an 18−20 cm longitudinal diameter and a 19−24 cm transverse diameter and weighs 1.8−2.5 kg. It is oval and prominently grooved and has a rough surface, thick peel, dark green exterior, and yellow pulp.

For sample preparation, *C. moschata* 'Menina Brasileira' was quartered longitudinally and transversally and opposite sections from the cylindrical and bulbous portions were taken. The *C. maxima* 'Exposição' was quartered longitudinally, and opposite sections were taken. The whole fruit was used for the other varieties. For each sample, peel and seeds were removed, and the flesh was cut into small pieces by hand and homogenized in a food multiprocessor (Walita, Brazil). Twenty grams of the homogenized samples of *C. pepo* 'Mogango' and 10 g of the other varieties were weighed for analysis.

Carotenoid Analysis. The carotenoids were determined according to the method of Kimura and Rodriguez-Amaya (*17*), adapted to squash and pumpkin samples in terms of weight of analytical samples, volumes of solvents and reagents, number of times extraction was carried out (until the residue was colorless), and chromatographic conditions. This method, without the saponification step, was validated with a certified reference material (CRM 485, lyophilized vegetable mix); *z* scores of ≤1 were obtained for those carotenoids with certified values and total uncertainty (*all-E*- and total α-carotene, *all-E*- and total β-carotene, total lutein), attesting to excellent method performance (*18*). Our saponification procedure was evaluated previously (*19*).

The method consisted of extraction with cold acetone (four to five times), partition to petroleum ether, overnight saponification with 10% methanolic KOH, washing, concentration in a rotary evaporator, and evaporation of the solvent to dryness under nitrogen. Prior to injection, the carotenoids were dissolved in LC grade acetone. All of the necessary precautions were taken to avoid alterations or losses of the carotenoids and other errors during analysis (*20*).

The LC system consisted of a Waters separation module, model 2690 (Milford, MA), equipped with a quaternary pump, an autosampler injector, a degasser, a photodiode array (DAD model 996), and electron impact mass (Waters Integrity System with Thermabeam interface) detectors, controlled by a Millenium workstation (version 2010). Detection with DAD was at the wavelengths of maximum absorption (max plot).

The column was monomeric C_{18} Spherisorb ODS2, 3 μm, 4.6 × 150 mm. The mobile phase consisted of acetonitrile (containing 0.05% of triethylamine), methanol, and ethyl acetate, used at a flow rate of 0.5 mL/min. For *C. moschata* 'Menina Brasileira', a concave gradient (curve 10) was applied from 95:5:0 to 60:20:20 in 20 min, maintaining this proportion until the end of the run. For the other samples, which were rich in xanthophylls, the gradient started from 98:2:0, which was also altered to 60:20:20 in 20 min. Reequilibration in both cases took 15 min.

Quantification was by external standardization. α-Carotene standard was isolated from carrot, and those of the other carotenoids were isolated from a leafy vegetable (roquette or water cress) by open column chromatography on MgO:Hyflosupercel (1:1, activated for 2 h at 110 °C) packed to a height of 20 cm in a 2.5 cm i.d. × 30 cm glass column. This column was developed with increasing amounts of ethyl ether and acetone in petroleum ether (*17*, *20*); the purity of the carotenoid isolates was monitored by HPLC. The mean purity of the standards was 94% for neoxanthin, 98% for violaxanthin, 96% for lutein, 97% α-carotene, and 92% for β-carotene. The concentrations of the standard solutions were corrected accordingly.

For LC-MS, the expansion region and nebulizer temperatures were 80 and 90 °C, respectively. The ionizing voltage was 70 eV, and the temperature of the ion source was 210 °C. The *m*/*z* range was 150−650. Interpretation of the mass spectra was done according to Enzell and Back (*21*).

The wide variation in the concentrations of the carotenoids in any given squash or pumpkin made fractionation necessary prior to LC-MS so that spectra could be obtained for the minor carotenoids. For this purpose, open column chromatography as cited above was employed. The carotenes were separated as the first fraction and the monohydroxy carotenoids as the second fraction. For the dihydroxy carotenoids, because violaxanthin eluted before lutein from the column and lutein was usually in much higher amount, violaxanthin was collected as the third fraction, lutein the fourth, and neoxanthin the fifth.

Chemical tests to verify the type and position of the substituents in the xanthophylls were also carried out (*20*, *22*, *23*). These were acetylation with acetic anhydride of secondary hydroxyl groups, methylation with acidified methanol of allylic hydroxyl groups, epoxide-furanoxide rearrangement (5,6- to 5,8-epoxide) with dilute HCl, and iodine-catalyzed *E*/*Z*-isomerization. The carotenoids submitted to the chemical reactions were isolated by open column chromatography with MgO:Hyflosupercel as in the isolation of standards.

Statistical Methods. Principal component analysis (PCA) was used to investigate the correlation structure among the three principal carotenoids (α-carotene, β-carotene, lutein) that dictated a different profile for each species. On the basis of the coordinates generated for each *Curcurbita*, the cluster analysis (CA) was applied to consolidate the groups evidenced by the visual inspection provided by the PCA map, using hierarchical classification (*24*).

Table 1. Identifying Characteristics of the Carotenoids of Squashes and Pumpkins

peak	t_R time (min)	identification	λ_{max}[a] (nm)	λ_{max}[b] (nm)	response to chemical tests
1	8.1	neoxanthin	415, 439, 467	414, 438, 466	positive to *all-E* form; positive to 5,6-epoxide test (1 group); positive to acetylation (3 OH)
2	11.3	violaxanthin	417, 441, 470	416, 440, 468	positive to *all-E* form; positive to 5,6-epoxide test (2 groups); positive to acetylation (2 OH)
3	14.4	lutein	423, 447, 475	421, 443, 472	positive to *all-E* form; positive to acetylation (2 OH) positive to methylation (1 allylic OH)
4	16.3	zeaxanthin	(428), 454, 480	(425), 448, 476	positive to *all-E* form; positive to acetylation (2 OH)
5	26.8	α-cryptoxanthin	425, 448, 476	422, 444, 472	positive to *all-E* form; positive to acetylation (1 OH groups); positive to methylation (1 allylic OH)
6	31.6	β-carotene-5,6-epoxide	425, 449, 477	423, 441, 470	positive to *all-E* form; positive to 5,6-epoxide (1 group)
7	37.5	ζ-carotene	378, 400, 425	376, 397, 421	positive to *all-E* form
8	40.1	α-carotene	425, 448, 476	422, 444, 472	positive to *all-E* form
9	42.1	β-carotene	(428), 454, 480	(424), 448, 476	positive to *all-E* form
10	44.5	(13*Z*)-β-carotene	340, (423), 449, 474		

[a] λ_{max} (nm) in the mobile phase, obtained by DAD. [b] λ_{max} (nm) in petroleum ether.

RESULTS AND DISCUSSION

Carotenoid Composition. The parameters used to conclusively identify the carotenoids are presented in **Table 1**, which has the wavelengths of maximum absorption (λ_{max} and %III/II) and results of the chemical reactions. **Table 2** has the molecular ions and characteristic mass fragments.

For the carotenoid identified as neoxanthin (5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′-triol), the visible absorption spectrum (λ_{max} and spectral fine structure) reflected the chromophore with eight conjugated double bonds and an allene group in the polyene chain. The presence of three hydroxyl groups and one 5,6-epoxide, indicated initially by the chromatographic behavior, was confirmed by the positive response to acetylation and the 5,6-epoxide test (hypsochromic shift of 20 nm, corresponding to one epoxide at the 5,6-position). The mass spectrum exhibited a molecular ion at *m*/*z* 600, which is consistent with $C_{40}H_{56}O_4$, and characteristic fragments at *m*/*z* 520 $[M - 80]^+$, representing the loss of one epoxide group, at *m*/*z* 419 $[M - 181]^+$, and at *m*/*z* 172, corresponding to a β-ring with a 5,6-epoxy and a hydroxyl substituent. Those at *m*/*z* 352, 221, and 181 referred to fragments with the β-ring containing epoxy and hydroxyl substituents with part of the lateral polyene chain resulting from cleavage at the C-12,13, C-10′,11′, and C-8′,9′ bonds, respectively.

Violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol) had a visible spectrum typical of a carotenoid with nine conjugated double bonds in the polyene chain. Acetylation and the chromatographic behavior demonstrated the presence of two hydroxyl groups, whereas the epoxide−furanoxide rearrangement (hypsochromic shift of 40 nm) proved the presence of two epoxide groups at the 5,6- and 5′,6′-positions. The mass spectrum displayed a molecular ion at *m*/*z* 600 ($C_{40}H_{56}O_4$) and fragments at *m*/*z* 520 $[M - 80]^+$, reflecting the loss of one epoxide group, and at *m*/*z* 502 $[M - 80 - 18]^+$, due to losses of one epoxide and one hydroxyl group. As with neoxanthin, the peaks at *m*/*z* 352, 221, 181, and 172 indicated that the epoxy substituent was in a ring with a hydroxyl group.

The visible spectrum of lutein (β,ε-carotene-3,3′-diol), with λ_{max} values slightly higher than those of violaxanthin and less fine structure, was consistent with a carotenoid having 9 of 10 conjugated double bonds in the polyene chain and 1 in a ring. The presence of two secondary hydroxyls was shown by the chromatographic behavior and the positive reaction to acetylation and the allylic position of one of them by methylation. The mass spectrum showed the molecular ion at *m*/*z* 568 ($C_{40}H_{56}O_2$) and fragments at *m*/*z* 550 $[M - 18]^+$, corresponding to the loss of one molecule of water, at *m*/*z* 415 $[M - 153]^+$, and at *m*/*z* 476 $[M - 92]^+$, due to the elimination of toluene. The peaks at *m*/*z* 193 and 153 were consistent with fragments having a hydroxyl group in a ring, cleaved at the C-9,10 and C-7,8 bonds, respectively.

Table 2. Molecular Ions and Typical Fragments of the Carotenoids of Squashes and Pumpkins

carotenoid	molecular ion (*m*/*z*)	typical fragments
neoxanthin	600	520, 419, 352, 221, 181, 172
violaxanthin	600	520, 502, 352, 221, 181, 172
lutein	568	550, 476, 415, 193, 153
zeaxanthin	568	
α-cryptoxanthin	552	153
ζ-carotene	540	403, 335
α-carotene	536	444
β-carotene	536	444, 430

Zeaxanthin (β,β-carotene-3,3′-diol) presented a visible spectrum with λ_{max} values higher than those of lutein and less definition of the peaks, commensurate with a chromophore of 11 conjugated double bonds, 2 of which were situated in rings. Acetylation confirmed the presence of the hydroxy groups, the non-allylic position of which was shown by the negative response to methylation. Because of the very low amount, only the molecular ion at *m*/*z* 568 ($C_{40}H_{56}O_2$) was seen in the mass spectrum.

α-Cryptoxanthin (β,ε-caroten-3′-ol), having the same chromophore as lutein, had the same visible spectrum. The existence of one allylic hydroxy substituent was demonstrated by the positive reaction to acetylation and methylation. The molecular ion was at *m*/*z* 552 ($C_{40}H_{56}O$); the peak at *m*/*z* 153 indicated the cleavage of a ring with a hydroxyl group at the C-7,8 bond.

Not having functional groups, diagnostic chemical reactions are not done with carotenes, and characteristic fragmentations of end groups with substituents are not found in the mass spectra; thus, identification is based mainly on the chromatographic behavior, the λ_{max} and fine structure of the visible spectrum, and the molecular ion as shown in the mass spectrum.

ζ-Carotene (7,8,7′,8′-tetrahydro-ψ,ψ-carotene and 7,8,11,12-tetrahydro-ψ,ψ-carotene) had a visible spectrum characteristic of an acyclic carotenoid of seven conjugated double bonds in the polyene chain. The mass spectrum displayed a molecular ion at *m*/*z* 540 ($C_{40}H_{60}$) and fragments at *m*/*z* 403 $[M - 137]^+$

Table 3. Concentrations of the Principal Carotenoids of Mature Squashes and Pumpkins

vegetable	concentration[a] (μg/g)				
	β-carotene	α-carotene	lutein	violaxanthin	neoxanthin
C. moschata 'Menina Brasileira'	66.7 ± 9.1	26.8 ± 5.1	17.4 ± 3.5	ND[b]	7.8 ± 2.1
C. moschata 'Goianinha'	56.7 ± 7.6	23.8 ± 3.3	18.3 ± 5.0	Tr[c]	6.3 ± 1.7
C. maxima 'Exposição'	15.4 ± 4.2	ND	10.7 ± 3.9	20.6 ± 3.3	9.8 ± 1.9
C. maxima × *C. moschata* hybrid 'Tetsukabuto'	30.5 ± 5.4	Tr	56.6 ± 9.7	21.9 ± 5.0	14.4 ± 3.0
C. pepo 'Mogango'	5.4 ± 1.6	ND	9.8 ± 2.9	6.9 ± 2.2	3.6 ± 1.4

[a] Mean and standard deviation of five samples collected at different times for each variety. [b] Not detected. [c] Trace (<0.2 μg/g).

and 335 $[M - 205]^+$. The first fragment corresponds to the loss of the 7,8-dihydro-ψ end group of symmetrical ζ-carotene. The second fragment results from bis-allylic cleavage at a C-11,-12 single bond of an acyclic carotene (*21*) and is indicative of asymmetric ζ-carotene (*25*). Thus, the ζ-carotene identified in this study appears to be a mixture of symmetric and asymmetric ζ-carotene.

Having the same chromophores, α-carotene (β,ϵ-carotene) and β-carotene (β,β-carotene) had absorption spectra resembling those of lutein and zeaxanthin, respectively. Both had mass spectra with molecular ions at m/z 536 ($C_{40}H_{56}$) and a fragment at m/z 444 $[M - 92]^+$ due to the elimination of toluene. β-Carotene also showed a fragment at 430 $[M - 106]^+$ due to the elimination of *m*-xylene.

β-Carotene-5,6-epoxide (5,6-epoxy-5,6-dihydro-β,β-carotene) was encountered in limited concentrations, so a mass spectrum could not be taken. It was identified by the chromatographic behavior, the visible spectrum reflecting 10 conjugated double bonds (9 in the chain and 1 in a β-ring) and 5,6- to 5,8-epoxide rearrangement (hypsochromic shift of 20 nm). *Z*-β-carotene appeared as the last peak in the LC chromatogram and was identified by the λ_{max} values lower than those of β-carotene and the *cis* peak at 341 with a $\%A_B/A_{II} = 45$ as (13*Z*)-β-carotene (*26*). All of the other carotenoids identified and quantified in this study had the *all-E* configuration.

Because of inconclusive or incorrect identifications in the literature, Pfander et al. (*27*) and Schiedt and Liaaen-Jensen (*28*) recommended that the following minimum criteria for identification be fulfilled: (a) the visible (or ultraviolet for shorter chromophores) absorption spectrum (λ_{max} and fine structure) in at least two different solvents must be in agreement with the chromophore suggested; (b) chromatographic properties must be identical in at least two systems, preferably TLC (R_F) and HPLC (t_R), and cochromatography with an authentic sample should be demonstrated; and (c) a mass spectrum should be obtained, which allows at least the confirmation of the molecular mass. The present paper fulfills these suggested criteria. Additionally, specific group chemical reactions were carried out. These reactions can take the place of mass spectrometry in identifying xanthophylls (*29*).

Marked qualitative and quantitative differences could be observed in the five fruit vegetables analyzed (**Table 3**). In *C. moschata* 'Menina Brasileira' and 'Goianinha', there was a predominance of β-carotene and α-carotene. Lutein and neoxanthin were the third and fourth major carotenoids, respectively. Violaxanthin was either undetected or detected in only trace

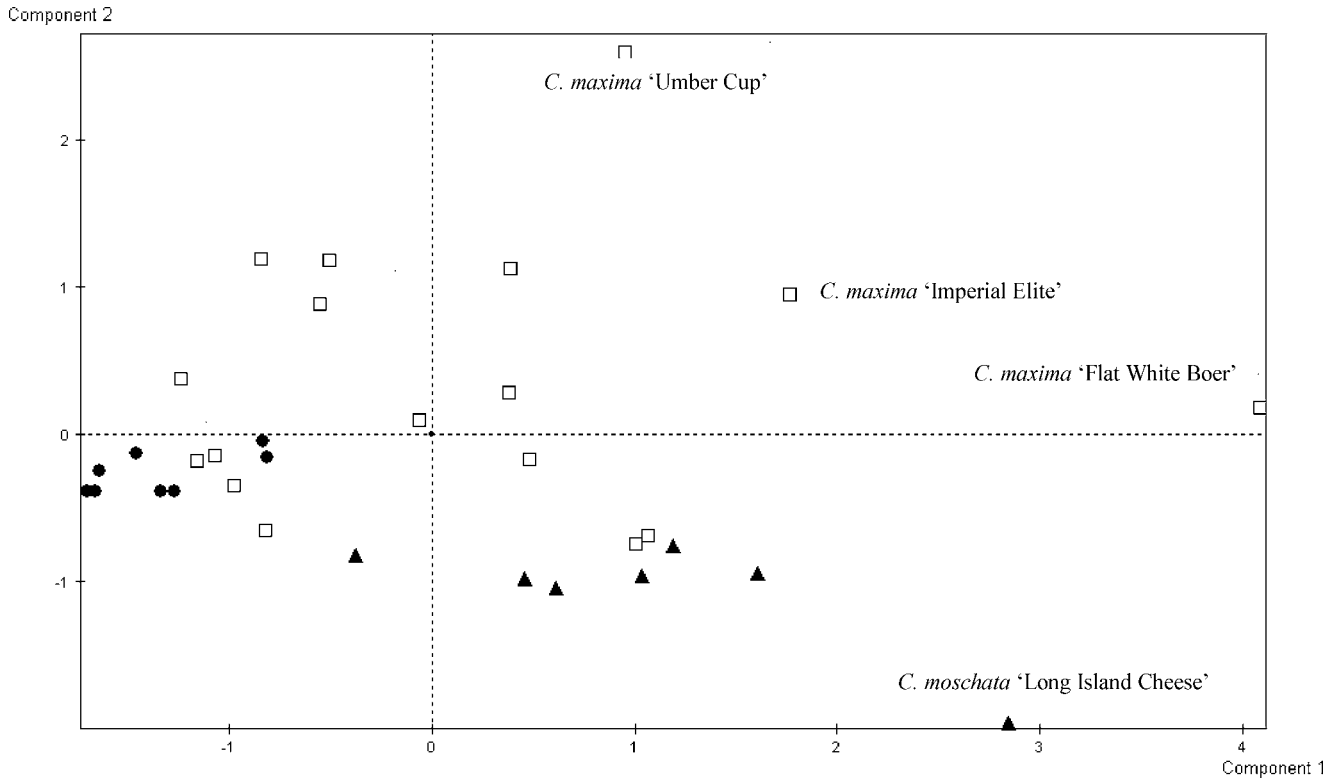

Figure 1. PCA map for α-carotene, β-carotene, and lutein of mature squashes and pumpkins: (▲) *C. moschata*; (□) *C. maxima*; (●) *C. pepo*. Named points are those of cultivars not having any found profile.

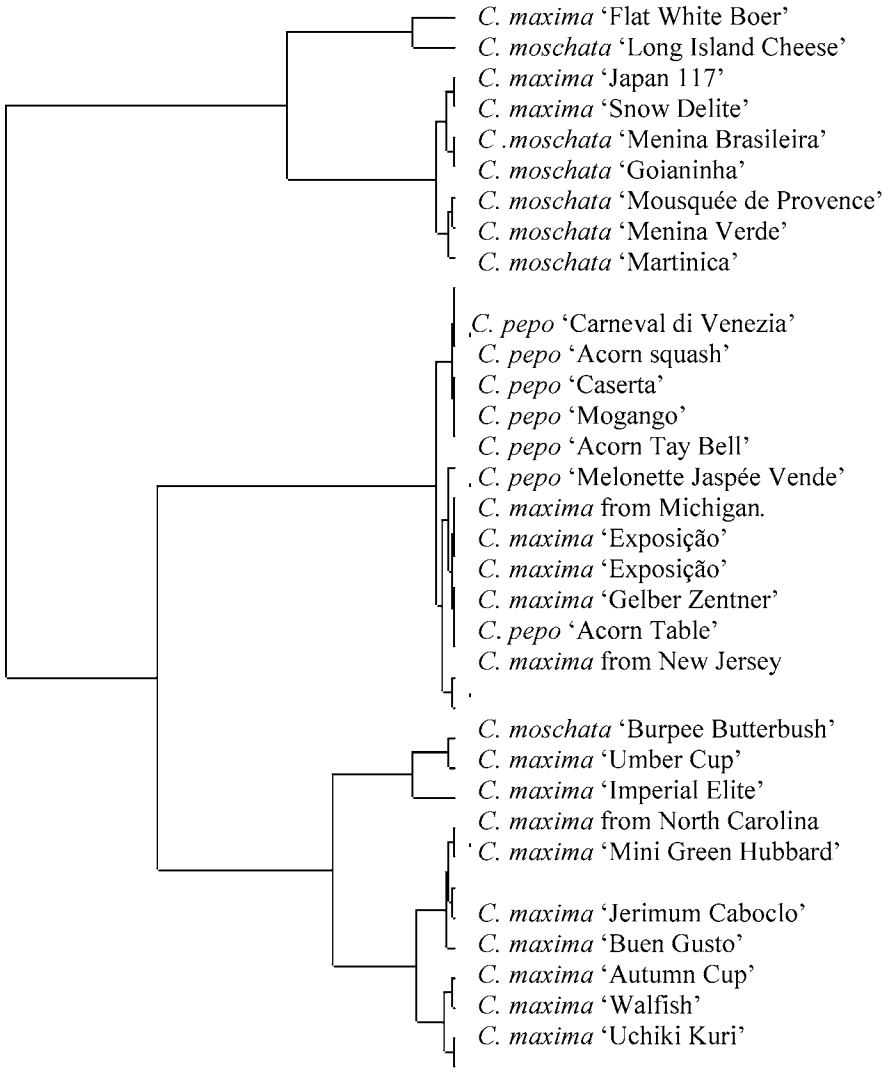

Figure 2. Dendogram provided for carotenoid profile. Three groups are evidenced.

amount. Only in these two Cucurbitaceae was ξ-carotene found. Xanthophylls predominated in the other vegetables, lutein being the principal carotenoid in the hybrid 'Tetsukabuto' and in *C. pepo* 'Mogango' and violaxanthin in *C. maxima* 'Exposição'. In the hybrid 'Tetsukabuto', β-carotene was the second major carotenoid, followed by violaxanthin and neoxanthin. In *C. pepo* 'Mogango', violaxanthin surpassed β-carotene. In *C. maxima* 'Exposição', violaxanthin was followed by β-carotene, lutein, and neoxanthin.

Squashes and pumpkins could be harvested over a wide range of maturity and can be stored for a long period; thus, a substantial variation in the carotenoid levels of these fruit vegetables offered for sale can be expected. It is well documented that enhanced carotenoid biosynthesis accompanies maturing or ripening of carotenogenic fruits and fruit vegetables, the carotenoids increasing markedly in number and quantity (*30*, *31*). In *C. moschata* 'Menina Brasileira', the mean total carotenoid content increased from 5.4 μg/g in the immature vegetable to 79.6 μg/g in the mature vegetable (*11*). Although lutein appeared to be the principal carotenoid at the immature stage, β-carotene and α-carotene predominated at the mature stage. It is also known that carotenogenesis continues after harvest provided the fruit or vegetable is kept intact (*30*, *31*). Thus, although the absolute concentrations differ, the carotenoid pattern is the same for mature fruits and vegetables, whether they mature on the tree or are harvested but kept intact.

Compared with the previous work on Brazilian squashes and pumpkins (*11*), the samples analyzed in the present study appeared to be more matured and of more uniform maturity, as reflected in the results. Although the mean values for β-carotene (39.3 vs 66.7 μg/g) and α-carotene (23.0 vs 26.8 μg/g) of mature *C. moschata* 'Menina Brasileira' were lower in the previous work, the current values fall within the wide ranges obtained then (14.1−79.3 μg/g for β-carotene and 8.3−42.3 μg/g for α-carotene). The present mean β-carotene concentration (15.4 μg/g) of mature *C. maxima* 'Exposição' is slightly lower than the previous value (16.6 μg/g) but also falls within the previous range (3.1−28.0 μg/g). The lutein levels (10.2 vs 10.7 μg/g) are comparable in the cultivar 'Exposição' but much lower in the previous work for the cultivar 'Menina Brasileira' (3.3 vs 17.4 μg/g). There is an analytical component in the lutein difference as the present procedure has been improved to minimize losses of lutein during saponification and the subsequent washing. Moreover, both violaxanthin and neoxanthin are appreciably lost during open column chromatography, explaining the much higher concentrations of these carotenoids in the current paper. To separate the large number of carotenoids identified in the previous paper, fractions obtained from the first

column were rechromatographed in a second column, increasing the loss of the labile violaxanthin and neoxanthin.

Carotenoid Profiles. In spite of the pronounced qualitative and quantitative variation in the carotenoid composition of mature squashes and pumpkins, when the results of the present study and our previous work (*11*, *12*) and those of Murkovic et al. (*16*) and Khachik and Beecher (*14*) were combined, a pattern appeared to emerge, based on the principal carotenoids, β-carotene, α-carotene, and lutein. Unfortunately, another major carotenoid, violaxanthin, could not be considered because it was underestimated in our previous work and was not determined by Murkovic et al. (*16*).

Figure 1 provides the PCA map with all of the samples represented according to their carotenoid profile, the horizontal axis representing α- and β-carotene (component 1) and the vertical axis lutein (component 2). The first principal component explains 59% of the variability and the second principal component, 30%. By visual inspection, *C. moschata* and *C. pepo* form well-defined groups, the right-bottom quadrant being *C. moschata* dominated and the left-bottom quadrant *C. pepo* dominated. The upper quadrants are dominated by *C. maxima*, with samples spread out in that map area. This map shows that *C. maxima* varies a lot in the α-carotene and β-carotene concentrations, but is homogeneous in lutein, always with higher values than the other species. *C. pepo* and *C. moschata*, also characterized by a homogeneous level in lutein, have less variability toward α- and β-carotene. It can be noted that some points of *C. maxima*, the species with the highest number of varieties/cultivars in the map, are mixed with those of *C. moschata* and *C. pepo*. In three samples of *C. maxima* (cultivar unspecified) from three U.S. sites analyzed by Khachik and Beecher (*14*), for example, two had the general pattern of this species with the predominance of lutein and β-carotene and no detectable α-carotene, but the third sample had α-carotene second to β-carotene with a low lutein content, a pattern typical of *C. moschata*.

Three cultivars of *C. maxima* are specified in the figure because their profiles differ from those found in the other samples: 'Umber Cup', with a very high lutein content; 'Imperial Elite', with almost equal amounts of β-carotene and lutein; and 'Flat White Boer', with equal amounts of α-carotene and lutein, both surpassing β-carotene. *C. moschata* 'Long Island Cheese', although having the qualitative pattern of this species with β- and α-carotene predominating, also falls out of the found patterns because of its very low lutein content. All of these cultivars were analyzed by Murkovic et al. (*16*) and could therefore not be attributed to analytical variability.

The dendogram generated by the cluster analysis (**Figure 2**) provides evidence of three groups. *C. moschata* predominates in the first group and *C. maxima* in the third group. *C. pepo* also predominates but to a lesser extent in group 2, although all cultivars of this species are in this group. A few cultivars differ from the general pattern of its species. This can be explained by the fact that aside from genetic influence, the quantitative carotenoid composition is also affected by environmental factors such as the climate and the stage of maturity. The qualitative composition, however, is more genetically controlled. Thus, all *C. moschata* varieties have the same pattern, with the major carotenoids being β-carotene and α-carotene. The variety 'Baianinha' (*12*) was not included in the statistical analysis because the β-carotene concentration was very high (235 μg/g). Nevertheless, this variety also has β-carotene as the predominating carotenoid, followed by α-carotene. In *C. maxima*, the predominating pattern has lutein and β-carotene as the major carotenoids. The variety 'Hyvita', analyzed by Murkovic et al. (*16*), was not included in the statistical analysis because of its very high lutein concentration (170 μg/g). Nevertheless, it has this pattern. The quantitative order of these two carotenoids in *C. maxima* is not constant, with lutein predominating over β-carotene in about half of the varieties analyzed and the reverse order in the other half. Profiling of this species may be improved by the inclusion of violaxanthin, which is the principal carotenoid of the *C. maxima* variety analyzed in the present study. However, violaxanthin is highly labile and can be partially or completely lost during analysis. In *C. pepo*, the prevailing pattern also has β-carotene followed by lutein as the principal carotenoids, although a couple of varieties have lutein surpassing β-carotene. *C. pepo* and *C. maxima* can be differentiated by the presence of violaxanthin as the predominating carotenoid of *C. maxima*, as stated above. Violaxanthin should be quantified in these species.

Cunningham (*32*) suggested the branching step (i.e., cyclization to α-carotene and β-carotene) as a control point of carotenoid biosynthesis. The data on squashes and pumpkins indicate that hydroxylation is another control point. In *C. moschata*, it appears that hydroxylation is inhibited in both branches, leading to the accumulation of α-carotene and β-carotene. In *C. pepo*, hydroxylation of β-carotene is inhibited but hydroxylation of α-carotene is uninhibited, resulting in the accumulation of lutein, with α-carotene virtually disappearing. Hydroxylation of both branches is not inhibited in *C. maxima*, but unlike lutein, the zeaxanthin formed from β-carotene does not accumulate because it is epoxidized to violaxanthin.

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