Carotenoids of tomato and tomato paste: verification of the occurrence of γ-carotene

Carotenóides de tomate e extrato de tomate: verificação da ocorrência de γ-caroteno

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ABSTRACT. Tomato and tomato paste are among the most consumed foodstuffs worldwide. Although their carotenoid compositions have been widely determined, some inconsistencies can be discerned in the literature. In Brazil, γ-carotene was detected in fresh tomato but not in various tomato products, using open column chromatography (OCC). On the other hand, very high amounts of this carotenoid were obtained in American tomato products, using high performance liquid chromatography (HPLC). Thus, this work was carried out to verify if the difference in data was due to natural variation or an artifact of the analysis. In fresh tomato, 11 carotenoids were identified: *trans*-lycopene, phytoene, phytofluene, β-carotene, lutein, 13-*cis*-lycopene, 15-*cis*-lycopene, γ-carotene, *trans*-ζ-carotene, *cis*-ζ-carotene, and neurosporene. In tomato paste, aside from the mentioned carotenoids, *cis*-β-carotene and four unidentified carotenoids were also detected. γ-Carotene was found in comparable concentrations in Brazilian and American tomato pastes, at levels much lower than β-carotene, apparently below the detection limit of OCC. The removal of the peel and the maturity stage of the fresh tomatoes could not explain the loss of γ-carotene in Brazilian tomato pastes, indicating that degradation was involved. The results do not lend support to the high levels of γ-carotene in American tomato products.

KEYWORDS. tomato, tomato products, carotenoids, γ-carotene

INTRODUCTION

In recent years, the marked improvement in the efficiency of high performance liquid chromatographic (HPLC) columns, together with the on-line acquisition of UV-visible spectra with the photodiode array detector, have greatly facilitated the analysis of complex mixtures of carotenoids in foods. With these refinements in analytical instrumentation, conditions are propitious for the clarification of some diverging data in the literature.

Tomato and tomato products are among the most consumed foodstuffs worldwide and are often the major sources of carotenoids for the population. Although widely studied in terms of its carotenoid composition, some inconsistencies in the results reported can be discerned. For example, γ-carotene was found in 10 sample lots of fresh Brazilian tomatoes at 0.7 ± 0.2 μg/g, but not in 39 samples of tomato products (juice, paste, puree and catchup)[9]. On the other hand, substantial amounts of γ-carotene (means of 15 to 100 μg/g), surpassing β-carotene

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(means of 2.3 to 15 μg/g), were found in tomato products in the U.S. (total of 52 samples of tomato soups, tomato juice, whole tomatoes, catchup, spaghetti sauce, paste, puree, and sauce), contributing significantly to the vitamin A value of this products[12].

Based on the data of different laboratories, Gross[4] noted that the total carotenoid content of raw red tomato varied between 90 and 190 μg/g fresh weight. Lycopene, the major pigment, made up to 90% of the total, with phytoene and phytofluene constituting 15-30%. Minor pigments identified were β-carotene, ζ-carotene, γ-carotene, and neurosporene. Data obtained by HPLC methods for ripe tomatoes vary from 3.6 to 17 μg/g β-carotene and 7 to 116 μg/g lycopene[1-3,5-7,10-12]. In tomato products the carotenoid composition will vary depending on the carotenoid composition in the raw material, and the time and severity of the processing treatment that result in varying degrees of degradation.

The importance of reliable carotenoid data cannot be overemphasized, and there is a worldwide effort to this end. Thus, this work was carried out to restudy the qualitative composition of tomato and tomato paste so as to verify if the difference in data is due to natural variation of the samples or to artifacts of the analytical process.

MATERIAL AND METHODS

1. Sample collection and preparation

Fresh tomatoes were purchased from supermarkets in Campinas and analyzed on the same day. Tomato paste of the three commercial brands with the largest sale volume in Brazil were also bought in Campinas. Three national brands of tomato paste from the United States were acquired from grocery stores in Washington, DC.

The fresh tomatoes were homogenized in a Waring blender and 10 to 20 g subsamples were taken for immediate analysis. Since tomato pastes would undergo homogenization during processing, the tomato paste samples were simply mixed and 10 g samples were taken for analysis.

2. HPLC analysis

Carotenoids were extracted with cold acetone, transferred to petroleum ether and concentrated in a rotary evaporator as described by Rodriguez-Amaya[8]. The concentrated extracts were transferred to vials, brought to dryness with N_2, redissolved in 2 mL HPLC grade acetone with ultrasonic agitation, filtered through PTFE filters of 0.22 μm (Millipore) and injected into the HPLC instrument.

A Varian model 9010 ternary solvent system equipped with Waters model 994 photodiode array detector and a Waters model 2690 separation module equipped with Waters model 996 photodiode array detector were used. Separation of the carotenoids was carried out with a polymeric C_{18} Vydac 218 TP 54 (Separations Group) column, 5 μm, 4.6 x 250 mm, using as mobile phase methanol: tetrahydrofuran (with 0.01% butylated hydroxytoluene), (95:5) at a flow rate of 0.8 mL/min. A Spherisorb S5 ODS2 narrow bore column (Waters), 5 μm, 2.0 x 250 mm, was also used with acetonitrile:methanol:ethyl acetate (73:20:7) as mobile phase at a flow rate of 0.25 mL/min. Detection of peaks was done at 450 nm and at the wavelengths of maximum absorption (max plot), and the peak purity was verified through the spectra taken at the ascending and descending slopes and at the maximum by the photodiode array detector.

4. Open column chromatography (OCC)

A glass column, 25 (id) x 300 mm, packed with MgO;Hyflosupercel (1:1) activated at 110°C for 4 hours[8] was used to separate the total extract into three bands corresponding to β-carotene, γ-carotene and lycopene, which were eluted with 12% acetone in petroleum ether, 20% acetone in petroleum ether, and acetone and 10% water in acetone, respectively.

5. Identification of the carotenoids

The carotenes (i.e. hydrocarbon carotenoids) were identified by the combined use of the visible absorption spectra and the chromatographic behavior in TLC, using silica thin layer plates developed with 5% methanol in toluene, HPLC, and OCC. Lutein, the only xanthophyll (i.e. carotenoid containing oxygen substituents) identified, was also submitted to chemical tests to confirm the type and position of the substituents. These were acetylation with acetic anhydride of secondary hydroxyl groups and methylation with acidified methanol of hydroxyl groups in allylic position. The conclusive identification of carotenoids was discussed in detail by Rodriguez-Amaya[8].

RESULTS AND DISCUSSION

1. Identity of the tomato carotenoids

Of the eleven carotenoids identified in fresh tomato, 10 were carotenes. These carotenoids eluted with the solvent front on the silica TLC plates developed with 5% methanol in toluene, before lutein in the MgO:Hyflosupercel column, and after lutein in the HPLC reverse phase column, indicating the absence of oxygen functions. Lycopene, neurosporene, ζ-carotene, and phytofluene exhibited visible absorption spectra with three well defined peaks, reflecting their acyclic structures, at wavelengths commensurate with chromophores of 11, 9, 7 and 5 conjugated double bonds, respectively (Table 1). Phytoene absorbed maximally at 286 nm with shoulders at 276 and 297 nm, consistent with a conjugated double bond system of only three double bonds. Although also having 11 conjugated double bonds, γ-carotene and β-carotene absorbed maximally at wavelengths lower than lycopene and displayed less spectral fine structure because of their monocyclic and dicyclic structures, which put one and two conjugated double bonds, respectively, in β-rings.

Table 1. Wavelengths of maximum absorption (λ_{max}) of the carotenoids of tomato

Carotenoid	λ_{max} nm (petroleum ether)	λ_{max} nm (mobile phase)*
1- lutein	421, 442, 470	425, 446, 475
2 - trans-lycopene	442, 468, 500	446, 473, 504
3 -13-cis-ycopene	-	361, 446, 473, 504
4 -15-cis-lycopene	437, 463, 495	360, 444, 468, 498
5 – neurosporene	416, 438, 467	418, 440, 469
6 - γ-carotene	435, 458, 488	438, 463, 494
7 – cis-ζ-carotene	-	297, 379, 400, 425
8 - ζ-carotene	376, 400, 424	380, 402, 426
9 - β-carotene	(424), 448, 476	(428), 454, 480
10 – phytofluene	-	330, 349, 366
11 – phytoene	-	(276), 286, (297)

*Acetonitrile:methanol:ethyl acetate (73:20:7). 13-*Cis*- lycopene, *cis*-ζ-carotene, phytofluene and phytoene were detected only in HPLC.

The presence of two *cis*-isomers of lycopene and a *cis*-isomer of ζ-carotene was observed only in HPLC, the *cis*-configuration being shown by the wavelengths of maximum absorption lower than those of the respective *trans*-carotenoids and the *cis*-peaks at 361, 360, and 297 nm, respectively. The designation of the 13-*cis*- and 15-*cis*-lycopene was based on the height of the *cis*-peak, which would be higher as the location of the *cis*-double bond approached the center of the molecule.

Compared to β-carotene, lutein absorbed maximally at slightly lower wavelengths, reflecting its chromophore of 10 conjugated double bonds, and had slightly more defined peaks because only one conjugated double bond was in a ring. The presence of two secondary hydroxyl groups was demonstrated by the positive response to acetylation with acetic anhydride, and the allylic position of one of these groups by the positive reaction to methylation with acidified methanol.

2. Occurrence of γ-carotene in Brazilian tomato paste

Typical chromatograms obtained with the Vydac column of Brazilian fresh tomatoes and tomato paste are shown in Figure 1, the carotenoid patterns being similar for the three brands of pastes. The Vydac column had been widely used in carotenoid analysis because of its efficiency. Chromatograms of the carotenoids of fresh tomato, taken at 450 nm, had six defined peaks, which were identified as lutein, β-carotene, γ-carotene, *trans*-lycopene, 13-*cis*-lycopene and 15-c*is*-lycopene. More peaks appeared in the chromatograms of the tomato paste carotenoids and the peak corresponding to γ-carotene appeared distorted. The visible spectra taken at different points of the peak revealed that it was a mixture.

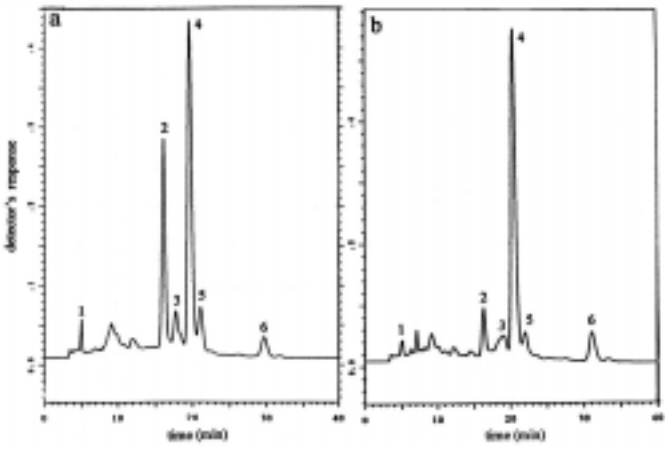

Figure1. HPLC chromatograms of Brazilian fresh tomato (a) and tomato paste (b) extracts obtained with the Vydac column. Chromatographic conditions are described in text. Detection was set at 450 nm. Peak identification: 1. lutein, 2. β-carotene, 3. γ-carotene in fresh tomato and mixture in paste, 4. *trans*-lycopene, 5. 13-*cis*-lycopene and 6. 15-*cis*-lycopene.

Because of the predominance of lycopene, the γ-carotene peak appeared very small. To verify better the occurrence of γ-carotene in tomato paste, preliminary separation on a MgO:Hyflosupercel column was undertaken to separate the β-carotene fraction, the γ-carotene fraction, and the lycopene fraction before HPLC. The chromatograms of the γ-carotene fractions of fresh tomato and tomato paste and the spectra taken at different points of the peak corresponding to γ-carotene are presented in Figure 2. While the purity of the γ-carotene peak of fresh tomato was confirmed, the three spectra resembling each other, that of the tomato paste proved to be γ-carotene mixed with other compounds, probably carotenoid degradation products. If not separated, these compounds would be quantified with γ-carotene, raising its concentration.

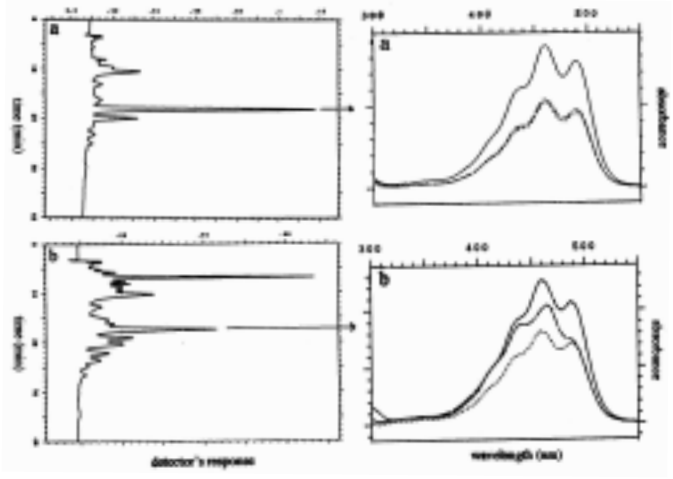

Figure 2. HPLC chromatograms of the γ-carotene fraction obtained with Vydac column and absorption spectra of peak corresponding to γ-carotene of Brazilian fresh tomato (a) and tomato paste (b). Chromatographic conditions are described in text.

The chromatograms obtained with the Vydac column demonstrated that many minor compounds could elute in the region between β-carotene and lycopene. To separate these minor peaks better, other chromatographic conditions were tested.

Better separation was achieved with a monomeric Spherisorb S5 ODS2 narrow bore column, using acetonitrile:methanol:ethyl acetate (73:20:7) as mobile phase (Figure 3). Setting detection at the wavelengths of maximum absorption, 11 carotenoids were identified in fresh tomatoes: lutein, *trans*-lycopene, 13-*cis*-lycopene, 15-*cis*-lycopene, neurosporene, γ-carotene, *cis*-ζ-carotene, *trans*-ζ-carotene, β-carotene, phytofluene, and phytoene. In the tomato paste, *cis*-β-carotene and four other unidentified carotenoids were also detected.

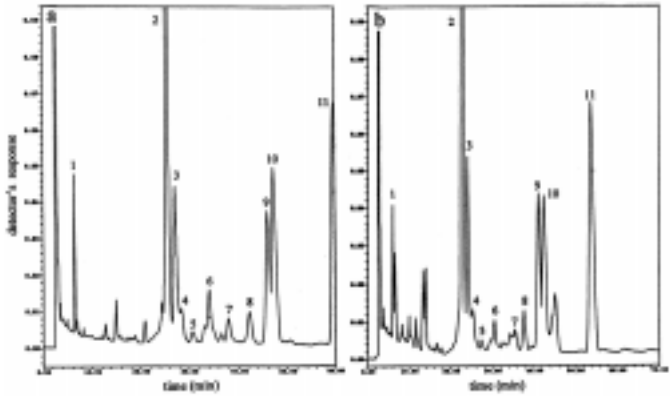

Figure 3. HPLC chromatograms of Brazilian fresh tomato (a) and tomato paste (b) extracts obtained with Spherisorb S5 ODS2 narrow bore column. Chromatographic conditions are described in the text. Detection was set at the wavelength of maximum absorption. Peak identification: 1. lutein, 2. *trans*-lycopene, 3. 13-*cis*-lycopene, 4. 15-*cis*-lycopene, 5. neurosporene, 6. γ-carotene, 7. *cis*-ζ-carotene, 8. *trans*-ζ-carotene, 9. β-carotene, 10. phytofluene, 11. phytoene.

The purity of the γ-carotene peak was confirmed in both the fresh tomato and tomato paste. γ-Carotene was apparently lower in the tomato paste, however. This level was probably below the detection limit of the open column chromatographic method used by Tavares and Rodriguez-Amaya[9], explaining why γ-carotene was not detected in tomato products in this work.

An attempt was also made to explain the unexpected lowering of the γ-carotene content in Brazilian tomato paste. Considering the °Brix reported by Tavares and Rodriguez-Amaya[9], the tomato paste samples analyzed would be about five times more concentrated than the fresh tomato. Thus, significant loss of γ-carotene occurred during the processing of Brazilian tomato paste. This loss could be due to any one or a combination of the following factors: (a) removal of the peel, (b) stage of maturity of the tomatoes used as raw materials, and (c) degradation of the carotenoid during processing.

Comparison of the chromatograms of the pulp and the peel of the same lot of tomatoes (Figure 4) showed that, although β-carotene (peak 9) was much higher in the peel, γ-carotene (peak 6) appeared practically the same in the pulp and the peel.

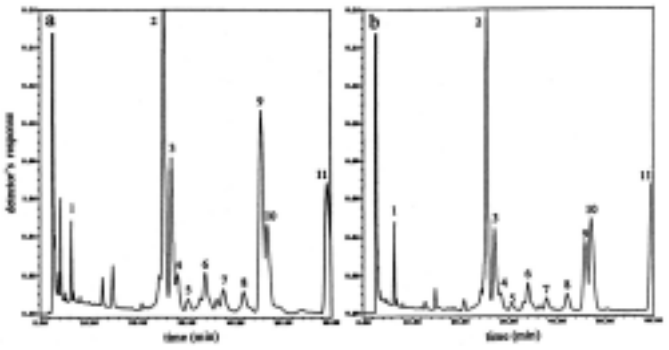

Figure 4. HPLC chromatograms of peel (a) and pulp (b) of fresh tomato extracts. Chromatographic conditions and peak identification are same the figure 3.

Verification of the effect of the stage of maturity showed that with the exception of β-carotene, which was practically the same at the three stages of maturity, all other carotenoids, including γ-carotene, increased from the almost ripe to the overripe stage (Figure 5). The maturity effect, therefore, could not account for the disappearance of γ-carotene in the processed Brazilian tomatoes.

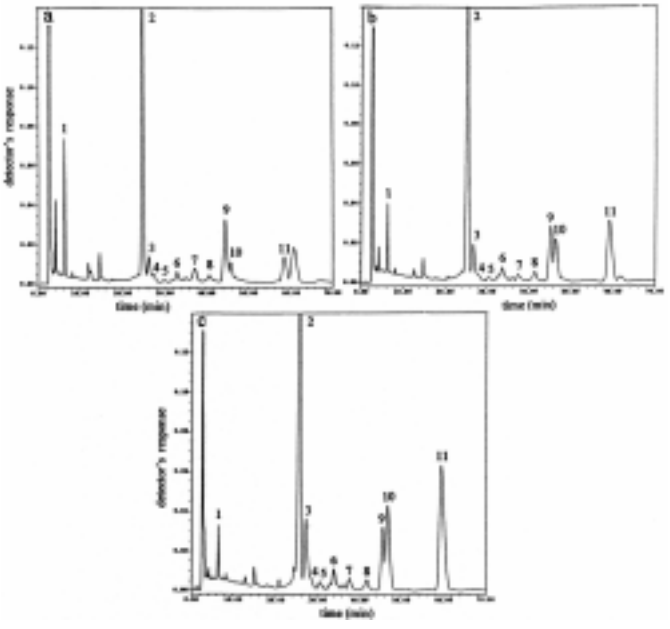

Figure 5. HPLC chromatograms of fresh tomato at three different stages of maturity: almost ripe (a), ripe (b) and overripe (c). Chromatographic conditions and peak identification are same the figure 3.

The major reason for loss of γ-carotene therefore appeared to be degradation during processing. The *trans*-lycopene content of the tomato paste products (means of 158 to 183 μg/g) analyzed by Tavares and Rodriguez-Amaya[9] were in the expected range (about 155 μg/g), calculated from the soluble solids. The β-carotene levels of the pastes (means of 4.3 to 8.7 μg/g) were in the same range as the fresh tomato (5.1 ± 1.1 μg/g), falling short of the expected values (about 25 μg/g). This could be partly explained by the removal of the peel, which appeared to have much higher levels of β-carotene as mentioned earlier. γ-Carotene content in the paste should be about 3.5 μg/g, this small amount being practically lost during processing.

2. Occurrence of γ-carotene in American tomato paste

An attempt was made to find an explanation for the discrepancy in the data obtained by Tonucci et al.[12] and Tavares e Rodriguez-Amaya[9]. The difference in the γ-carotene contents of American and Brazilian tomato pastes, could be due to any one or a combination of the following factors: (a) difference in tomato cultivars used as raw materials, (b) difference in the processing condition, and (c) analytical variation.

Typical chromatograms of the carotenoids of tomato pastes produced in the United States, obtained with the Vydac and Spherisorb column are shown in Figure 6, the three brands showing similar patterns.

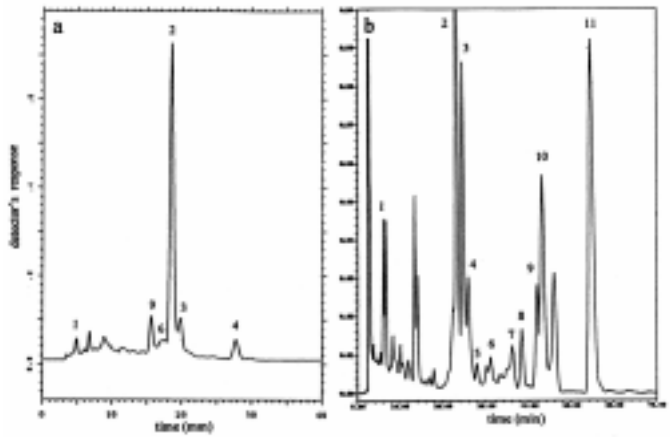

Figure 6. Typical HPLC chromatograms of the carotenoids of American tomato pastes obtained with Vydac 218 TP 54 (a) and Spherisorb S5 ODS2 (narrow bore) columns. Chromatographic conditions are described in the text. Peaks identification are the same as Figure 3.

The major differences in the carotenoids patterns of the tomato paste were: (a) phytoene, *cis*-lycopene, and *cis*-β-carotene were markedly higher in the American tomate paste, and (b) two unidentified peaks, one very close to lutein and the other to γ-carotene were also greater in the American tomato pastes. The γ-carotene peak, however, appeared essentially of same magnitude and evidently smaller than the β-carotene peak in both pastes. Thus, this comparison did not shed any light on the huge difference in the γ-carotene content (99.8 ± 11.5 μg/g vs. not detected) of the American and Brazilian tomato pastes analyzed by Tonucci et al.[12] and Tavares and Rodriguez-Amaya[9]. According to the chromatograms obtained in Figures 1 and 5, the American and Brazilian tomato pastes should have practically the same amounts of γ-carotene. The lycopene level (554.5 ± 4.33 vs. means of 158 to 183 μg/g) was about three times in the American tomato paste while the β-carotene content (12.7 ± 2.4 vs. means of 4.3 to 8.7 μg/g) was slightly higher [9,12].

A closer look at the chromatogram presented by Tonucci et al.[12] revealed that the β-carotene peak was substantially greater than the γ-carotene peak. Even considering that the $A^{1\%}_{1cm}$ of γ-carotene (3100 in petroleum ether) is higher than the of β-carotene (2592 in petroleum ether), and the change in the mobile phase (a gradient was used), the γ-carotene concentration could not be much higher (almost 8 times) than that of β-carotene.

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RIALA6/934

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RESUMO. Tomate e extrato de tomate estão entre os alimentos mais consumidos no mundo. Embora as suas composições de carotenóides tenham sido amplamente determinadas, algumas inconsistências podem ser verificadas na literatura. No Brasil, usando cromatografia em coluna aberta (CCA), o γ-caroteno foi detectado em tomate fresco, mas não em vários produtos de tomate. Por outro lado, altas quantidades deste carotenóide foram relatados em produtos de tomate americanos, usando cromatografia líquida de alta eficiência (CLAE). Assim, este trabalho foi realizado para verificar se a diferença nos dados é devido à variação natural ou um artefato da análise. Em tomate fresco foram identificados *trans*-licopeno, fitoeno, fitoflueno, β-caroteno, luteína, 13-*cis*-licopeno, 15-*cis*-licopeno, γ-caroteno, *trans*-ζ-caroteno, *cis*-ζ-caroteno, e neurosporeno. Em extrato de tomate, além destes carotenóides, foram detectados o *cis*-β-caroteno e quatro carotenóides não identificados. γ-Caroteno foi encontrado em concentrações comparáveis nos extratos de tomate brasileiros e americanos, a níveis muito menores que do β-caroteno, aparentemente abaixo do limite de detecção do CCA. A remoção da pele e o estado de maturação do tomate fresco não justificariam a perda de γ-caroteno nos extratos de tomate brasileiros, indicando o envolvimento da degradação. Os resultados não corroboraram com os altos níveis de γ-caroteno reportados nos produtos de tomate americanos.

Palavras-chave: tomate, produtos de tomate, carotenóides, γ-caroteno.

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