ABSTRACT: Although tomatoes are commonly consumed fresh, over 80% of the consumption of tomatoes is in the form of processed products such as tomato pulp, ketchup, juice and sauce. Research has indicated the potential health benefits of a diet rich in tomatoes and tomato products. The present study was carried out to determine the carotenoid content of fresh tomato, tomato pulp and ketchup by high performance liquid chromatography. The major differences among these products were in the concentration of some of the pigments. Tomato had all-trans-lycopene (1046-1099 µg/g DW), cis-lycopene (125-132 µg/g DW) and all-trans-carotene (45-59 µg/g DW) as principal carotenoids. Tomato pulp and ketchup had all-trans-lycopene (951-999 µg/g DW and 455-476 µg/g DW), all-trans-β-carotene (76-88 DW µg/g and 20-27 DW µg/g) and cis-lycopene (71-83 µg/g DW and 14-25 µg/g DW) as the main pigments, respectively. They also contained other carotenoids in much smaller amounts (lycoxanthin, zeaxanthin, antheraxanthin, lutein, γ-carotene, ζ-carotene and phytofluene).

KEYWORDS: Carotenoids; tomato; tomato pulp; ketchup; HPLC.

INTRODUCTION

Tomato is certainly an important agricultural commodity worldwide. In Brazil, it is one of the most widely consumed foodstuffs, either fresh or processed, and it is the main source of carotenoids for much of the population. Epidemiological studies have shown that increased consumption of tomato and tomato-based products may reduce the risk of certain types of cancer, such as prostate, lung and stomach cancer, and cardiovascular disease.

In a recent study, tomatoes ranked first as a source of lycopene (71.6%), second as a source of vitamin C (12.0%), pro-vitamin A carotenoids (14.6%) and β-carotene (17.2%), and third as a source of vitamin E (6.0%).

One of the major phytochemicals in tomato products to which the anti-carcinogenic function has been attributed is lycopene. Because of presence of long-chain conjugated double bonds, lycopene has been reported to possess antioxidant activity and is superior to lutein or β-carotene. Other quantifiable carotenoids in fresh tomatoes and tomato products such as juice, paste, puree, and sauce include phytoene, phytofluene, ζ-carotene, neurosporene, γ-carotene, and β-carotene, but their concentrations are significantly lower than that of lycopene.

The ability of lycopene to act as a potent antioxidant is thought to be responsible for protecting cells against oxidative damage and thereby decreasing the risk of chronic diseases. In addition to its antioxidant properties, lycopene has also been shown to induce cell to cell communication, and to modulate hormonal and immune systems and other metabolic pathways which may also be responsible for the beneficial effects.

Carotenoids predominantly occur in their all-trans configuration, which is thermodynamically the more stable isomer. Apart from naturally occurring in plants, cis-isomers have been formed as a consequence of food processing and they possess different biological properties. All-trans-lycopene may be converted to its cis configuration during food processing. Several reports have demonstrated that the cis isomers of lycopene are absorbed into the body more easily and play a more important part in biological function than all-trans-lycopene.

The objective of this study was to estimate the carotenoid profiles and carotenoid contents of fresh tomato, tomato pulp and ketchup by HPLC.

MATERIAL AND METHODS

Material

Fresh tomatoes [Lycopersicon esculentum cv. AP 533, (1 kg)], tomato pulp (1.5 kg) and ketchup (1.5 kg)
were supplied by Alimentos Predilecta LTDA (São Lourenço do Turvo Matão, Brazil). Before analysis, fresh tomatoes were homogenized in a Waring blender to obtain a representative sample. Five analyses were carried out in duplicate. To compare tomatoes and their products, the results were expressed on a dry weight basis.

**Industrial Thermal Treatment**

To obtain tomato pulp, fresh tomato was submitted to enzyme inactivation by heating (90°C for 6 min) and concentrated in 3 stages, until the pulp reached 32%Brix. The tomato pulp was sterilized at 105°C for 2-3 min and then cooled to 40°C. In the ketchup manufacturing process, the tomato pulp was heated at 100°C for 5 min. Following addition of condiments, the product was heated at 85°C for 10 min and cooled to 35°C and bottled.

**Methods**

**Extraction of carotenoids**

The carotenoids present in the samples (5 g) were extracted with hexane-acetone (1:1, v/v) with celite in a mechanical Waring blender. The mixture was centrifuged and the supernatant reserved. The residue was further extracted and centrifuged until all color was removed, and the successive supernatants pooled. The pigments in this organic extract were then transferred to petroleum ether, washed with distilled water and concentrated, in a rotary evaporator, at a temperature not exceeding 35°C. Saponification was not carried out, to avoid losses, especially of the more polar carotenoids, and also because HPLC provides enough separation of chlorophyll and carotenoid peaks.\(^{2,19}\)

**Determination of total carotenoids content**

The total carotenoid content was determined in an aliquot of the petroleum ether extract, obtained as described above by measuring the absorbance at 470 nm in a Beckman UV/Vis spectrophotometer DU® 640. Total carotenoid content was calculated as described by Rodriguez-Amaya\(^{22}\) using the lycopene absorption coefficient (\(A_{1%}_{\text{lon}} = 3450\)) in petroleum ether, and expressed in fresh weight (FW) and dry weight (DW) basis.

**HPLC analysis of carotenoids**

Carotenoid pigments were analyzed by RP-HPLC using ternary gradient elution and a Symmetry C\(_{18}\) column (4.6 x 150 mm I.D., 3.5µm) from Waters. The chromatography system was equipped with a Shimadzu LC-10AT VP solvent delivery system and SPD-M 10A VP photodiode array detector (DAD). The mobile phase consisted of acetonitrile:methanol:ethyl acetate containing 0.05% triethylamine flowing at 0.8 mL/min. A gradient was applied from 88:8:4 to 48:26:26 in 25 min, and back to the initial condition (30 min). Volume injection was 20 µL. Detection was at the wavelengths of maximum absorption (max plot). The samples and the solutions were filtered through a 0.22 µm membrane before injection.

The isolation and purification of standards was performed as in Kimura & Rodriguez-Amaya.\(^{14}\) Natural sources, such as tomato (lycopene), carrot (\(\beta\)-carotene), butter collard greens, lettuce and watercress (lutein and zeaxanthin), and passion-fruit juice (\(\zeta\)-carotene) were used to isolate the standards indicated as described in our previous work.\(^{10}\)

**Identification and estimation of carotenoids**

The individually isolated carotenoids in samples were identified by comparison of their HPLC retention times and diode array spectral characteristics with those of the standards and also literature values.\(^6,15,17\)

The identification of cis-carotenoids was based on the UV spectrum which exhibits a new maximum around 330-350 nm (\(cis\)-peak), the intensity of which depends on the localization of the \(cis\)-double bond and is greatest when the double bond is located near or at the center of the chromophore.\(^3\) In addition, a hypsochromic shift in the \(\lambda_{\text{max}}\) is observed in \(cis\)-carotenoid spectra, compared to \(trans\)-isomers.\(^{24}\)

The results are expressed in relative percentage of total peak area taken into account and also in \(\mu g/g\) DW of lycopene.

**RESULTS AND DISCUSSION**

The chromatographic profiles (Figure 1) of extracts of tomato pulp and ketchup are similar to that of tomato. The major differences among them appear to be in the concentration of some carotenoids presented in Table 1.

Because of the predominance of lycopene, the other pigment peaks appeared small. The chromatograms obtained demonstrated that many minor xanthophylls (oxygen-containing carotenoids) were eluted in the region before lycopene while the carotenes (hydrocarbon carotenoids) eluted after it.

In tomato, tomato pulp and ketchup, the carotenes predominate, constituting 86-91 % of the total carotenoids, with very high levels of lycopene (79-88 %), and the xanthophylls make up about 9-14 % of the total.

The data show that the amount of all-\(trans\)-lycopene comprised 82, 74 and 81 % of total carotenoids, while \(cis\)-lycopene represented 6, 6 and 5 %, in tomato, tomato pulp and ketchup, respectively. Thus, lycopene \((cis + trans)\) accounted for 88, 80 and 87 % of the total carotenoid content of tomato, tomato pulp and ketchup, respectively.

Our data were consistent with previous studies. In samples of cherry tomatoes, lycopene was by far the main
FIGURE 1 - HPLC chromatograms of carotenoids in (A) fresh tomato, (B) tomato pulp and (C) ketchup. Chromatographic conditions described in the text. See Table 1 for peak identification.
component of the carotenoid fraction, ranging from 76 to 85 %,
Sass-Kiss et al. reported that lycopene in tomatoes was an excellent source of lycopene, responsible for the dark red appearance of the product.

In fresh tomato, the principal carotenoids present were all-trans-lycopene (1046-1099 µg/g DW), cis-lycopene (125-132 µg/g DW), all-trans-β-carotene (45-59 µg/g DW), all-trans-lycopenan (17-23 µg/g DW), and cis-lutein (14-19 µg/g DW).

Tomato pulp had, mainly, all-trans-lycopene (951-999 µg/g DW), all-trans-β-carotene (76-88 µg/g DW), cis-lycopenan (71-83 µg/g DW), cis-β-carotene (25-29 µg/g DW), and cis-lycopenan (19-23 µg/g DW).

Ketchup had all-trans-lycopenan (455-476 µg/g DW), all-trans-β-carotene (20-27 µg/g DW), cis-lycopenan (14-25 µg/g DW), all-trans-lycopenan (8-12 µg/g DW), and cis-β-carotene (8-11 µg/g DW) as the main pigments.

Therefore, the predominant carotenoid detected in all our samples was lycopene. They also contained β-carotene, lycopenan and lutein, in much smaller amounts. Anteraxanthin, zeaxanthin, γ-carotene, ζ-carotene and phytofluene were also identified in samples. These results were in agreement with others.

The total carotenoid content measured at 470 nm ranges were 1318-1382, 1289-1316 and 547-583 µg/g DW in fresh tomato, tomato pulp and ketchup, respectively.

Table 1 - Chromatographic and spectral characteristics of fresh tomato, tomato pulp and ketchup carotenoids obtained by HPLC.

<table>
<thead>
<tr>
<th>Peak n°</th>
<th>t_R (min)</th>
<th>λ_m (nm) (in line)*</th>
<th>Carotenoids</th>
<th>area %d</th>
<th>µg/g DWb</th>
<th>area %d</th>
<th>µg/g DWb</th>
<th>area %d</th>
<th>µg/g DWb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.68</td>
<td>428 457 487</td>
<td>all-trans-antaraxanthin'</td>
<td>0.6</td>
<td>7.3 ± 1.0</td>
<td>1.2</td>
<td>15.7 ± 1.2</td>
<td>1.0</td>
<td>5.7 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>7.53</td>
<td>426 447 474</td>
<td>all-trans-lutein</td>
<td>0.3</td>
<td>3.3 ± 0.5</td>
<td>0.9</td>
<td>13.1 ± 1.0</td>
<td>0.6</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>8.21</td>
<td>353 423 447 475</td>
<td>cis-lutein</td>
<td>1.4</td>
<td>16.6 ± 2.4</td>
<td>1.5</td>
<td>20.0 ± 1.6</td>
<td>0.4</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>8.83</td>
<td>360 (426) 447 473</td>
<td>cis-zeaxanthin</td>
<td>0.5</td>
<td>6.1 ± 0.9</td>
<td>0.7</td>
<td>9.1 ± 0.7</td>
<td>0.5</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>9.62</td>
<td>369 447 486 503</td>
<td>cis-lycoxanthin</td>
<td>1.1</td>
<td>13.3 ± 1.9</td>
<td>1.6</td>
<td>21.0 ± 1.7</td>
<td>0.9</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>6</td>
<td>10.16</td>
<td>446 472 502</td>
<td>all-trans-lycopene</td>
<td>1.7</td>
<td>19.8 ± 2.8</td>
<td>0.6</td>
<td>8.2 ± 0.6</td>
<td>1.8</td>
<td>10.0 ± 1.6</td>
</tr>
<tr>
<td>7</td>
<td>15.11</td>
<td>456 482 513</td>
<td>all-trans-lycopene</td>
<td>82.4</td>
<td>1122.2 ± 26.6</td>
<td>73.9</td>
<td>974.8 ± 24.0</td>
<td>81.1</td>
<td>465.2 ± 10.5</td>
</tr>
<tr>
<td>8</td>
<td>16.69</td>
<td>361 445 466 500</td>
<td>cis-lycopene</td>
<td>5.7</td>
<td>78.8 ± 13.6</td>
<td>5.9</td>
<td>77.2 ± 6.1</td>
<td>5.4</td>
<td>19.4 ± 5.3</td>
</tr>
<tr>
<td>9</td>
<td>18.11</td>
<td>339 434 462 489</td>
<td>cis-γ-carotene</td>
<td>0.8</td>
<td>9.5 ± 1.4</td>
<td>0.4</td>
<td>5.7 ± 0.5</td>
<td>0.6</td>
<td>6.2 ± 1.0</td>
</tr>
<tr>
<td>10</td>
<td>18.62</td>
<td>435 465 490</td>
<td>all-trans-γ-carotene</td>
<td>0.4</td>
<td>4.9 ± 0.7</td>
<td>1.2</td>
<td>15.4 ± 1.2</td>
<td>0.4</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>19.31</td>
<td>361 380 400 425</td>
<td>cis-ζ-carotene</td>
<td>0.3</td>
<td>3.2 ± 0.5</td>
<td>0.6</td>
<td>8.2 ± 0.6</td>
<td>0.5</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>12</td>
<td>20.28</td>
<td>380 401 426</td>
<td>all-trans-ζ-carotene</td>
<td>0.2</td>
<td>2.2 ± 0.3</td>
<td>0.6</td>
<td>7.8 ± 0.6</td>
<td>0.3</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>13</td>
<td>21.80</td>
<td>(428) 453 479</td>
<td>all-trans-β-carotene</td>
<td>4.4</td>
<td>52.0 ± 7.4</td>
<td>6.2</td>
<td>82.0 ± 6.5</td>
<td>4.2</td>
<td>23.5 ± 3.9</td>
</tr>
<tr>
<td>14</td>
<td>22.43</td>
<td>332 349 368</td>
<td>all-trans-phytofluene</td>
<td>0.5</td>
<td>6.0 ± 0.9</td>
<td>1.3</td>
<td>17.5 ± 1.4</td>
<td>0.9</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>15</td>
<td>23.35</td>
<td>342 (417) 446 472</td>
<td>cis-β-carotene</td>
<td>0.4</td>
<td>4.7 ± 0.7</td>
<td>2.1</td>
<td>27.0 ± 2.1</td>
<td>1.1</td>
<td>9.5 ± 1.6</td>
</tr>
</tbody>
</table>

A gradient mobile phase of acetonitrile:methanol:ethyl acetate was used.

The values (expressed in µg/g of lycopene) are the averages of five determinations in duplicate ± standard deviation.

* Tentative identification.

Expressed in percent of total peak area taken in account.

The total carotenoid content of fresh tomatoes cv. AP533 was higher than those reported by Tavares & Rodriguez-Amaya with cv. Santa Clara (21-62 µg/g FW) due variety differences. The total carotenoid content of ketchup was close to reported by Tavares & Rodriguez-Amaya which was in the ranges 106-139 µg/g FW and, by Ishida & Chapman (59-183 µg/g FW).

The carotenoid profile and carotenoid content (DW) of fresh tomato, tomato pulp and ketchup estimated was relevant because they will allow to us evaluate the effect of thermal process on tomato pulp and ketchup manufacture in following stage of work.

CONCLUSIONS

The results obtained in the present study for tomatoes and their products agree with most of those reported in the literature. In tomato, tomato pulp and ketchup, while lycopene accounted for 88, 80 and 87 % of the total carotenoid content, β-carotene represented 5, 8 and 6 %, respectively. Note once again that the samples having the highest carotenoid contents were fresh tomato followed by tomato pulp. The lowest carotenoid content was verified in ketchup. However, ketchup is an excellent source of lycopene, responsible for the dark red appearance of the product.
ACKNOWLEDGEMENTS

We are grateful to Alimentos Predilecta LTDA for providing all samples.


RESUMO: Embora os tomates sejam comumente consumidos in natura, mais de 80% do seu consumo ocorre na forma de produtos processados como polpa de tomate, catchup, suco e sopa. Estudos têm demonstrado os potenciais benefícios de uma dieta rica em tomates e em seus produtos. O presente trabalho visa determinar o teor de carotenóides em tomate fresco, polpa de tomate e catchup por cromatografia líquida de alta eficiência. A principal diferença decorre da concentração de alguns pigmentos. O tomate apresentou como carotenóides principais o all-trans-licopeno (1046-1099 µg/g BS), cis-licopeno (125-132 µg/g BS) e all-trans-β-caroteno (45-59 µg/g BS). Na polpa de tomate e no catchup, os pigmentos majoritários foram all-trans-licopeno (951-999 µg/g BS e 455-476 µg/g BS), all-trans-β-caroteno (76-88 µg/g BS e 20-27 µg/g BS) e cis-licopeno (71-83 µg/g BS e 14-25 µg/g BS), respectivamente. Estes também outros carotenóides em quantidades menores (lxicoxantina, zeaxantina, anteraxantina, luteína, γ-caroteno, ζ-caroteno e fitoflueno).

PALAVRAS-CHAVE: Carotenóides; tomate; polpa de tomate; catchup; CLAE.

REFERENCES


