HPLC–UV AND GC–MS CHARACTERIZATION OF THE FLAVONOL AGLYCONS QUERCETIN, KAEMPFEROL, AND MYRICETIN IN TOMATO PASTES AND OTHER TOMATO-BASED PRODUCTS

Ö. Tokuşoğlu¹,*, M. K. Ünal², and Z. Yıldırım³

¹Celal Bayar University, Akhisar M.Y.O., 45200, Akhisar, Manisa, Turkey
²Ege University, Department of Food Engineering, 35100 Bornova, Izmir, Turkey
³Ege University, Agricultural Faculty, Department of Field Crops, 35100 Bornova, Izmir, Turkey

SUMMARY

The amounts of three flavonoids, quercetin, kaempferol, and myricetin, in tomatoes (Solanum lycopersicum L.) and tomato-based products produced in Turkey has been determined by reversed phase high-performance liquid chromatography (RP-HPLC) with UV detection. The HPLC profiles of five types of tomato, one commercial composite tomato juice, and three types of tomato paste, were obtained after acid hydrolysis and extraction. The presence of the flavonol aglycons was confirmed by gas chromatography with mass spectrometric detection (GC–MS).

Tomatoes and tomato-based products contained primarily quercetin, kaempferol, and the minor flavonol myricetin. The total flavonol aglycon content of different varieties of tomato varied from 3.1 to 10.0 mg kg⁻¹ of fresh weight. Tomato juice and tomato salsa were rich in total flavonols, containing 19.8 mg L⁻¹ and 10.5–13.2 mg kg⁻¹, respectively. The method enabled accurate and reproducible quantitative analysis of these flavonols in tomatoes and tomato-based products.

INTRODUCTION

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin; they are extremely important because of their health effects. It has been predicted that average intake of all flavonoids is several grams per day [1]. They occur in foods as O-glycosides with sugars bound at the C₃ position. Flavonoids with a diphenylpropane skeleton (C₆–C₃–C₆) are known to be antimutagenic and anticarcinogenic [2–5]. They also have
antioxidant properties and inhibit the oxidation of LDL [6–9]; they also have anti-inflammatory and anti-allergic effects [10,11].

Flavonoids consist mainly of flavonols, flavones, catechins, and flavanones [12]. Investigations of the distribution of the major flavonoids in plant foods have been published. High-performance liquid chromatography (HPLC) has been especially widely used for separation and determination of flavonoids in a variety of foods [13–21], including tomatoes and tomato juice [22–25].

Tomatoes are a valuable commodity world-wide. In 1998, tomato production in Turkey was 6.6–7.8 million tons. Tomato salsa exports reached 320 000 tons in 1999 and tomato-based products were valued at $200 million [26].

The objective of this study was analysis of three flavonol aglycons, quercetin, kaempferol, and myricetin, in tomato samples and tomato-based products. In this paper, we suggest use of HPLC and GC–MS for identification and quantification of these flavonoids in five types of tomato, one commercial composite tomato juice, and three types of tomato salsa consumed in Turkey.

MATERIALS AND METHODS

Standards

Flavonol standards quercetin dihydrate, kaempferol, and myricetin were purchased from Sigma (Poole, Dorset, UK), as were tert-butylhydroquinone (TBHQ) and bis(trimethylsilyl)trifluoroacetamide (BSTFA). Methanol (CH$_3$OH; HPLC grade), acetonitrile (CH$_3$CN; HPLC grade), sodium dihydrogen phosphate (NaH$_2$PO$_4$), phosphoric acid (H$_3$PO$_4$), hydrochloric acid (HCl), ethyl acetate (C$_4$H$_8$O$_2$), sodium hydrogen carbonate (NaHCO$_3$), and sodium sulfate (Na$_2$SO$_4$) were purchased from E. Merck (Darmstadt, Germany). Standard stock solutions of the flavonols (500 µg mL$^{-1}$) were prepared in methanol (HPLC grade). After preparation the stock solutions were stored at $-28^\circ$C. Calibration standards (25 µL mL$^{-1}$) in methanol were prepared from these stock solutions. Calibration plots obtained by use of the calibration standards passed through the origin; $R^2$ values were 0.9999. Quantitation of the amounts of the flavonols in tomato and tomato-based samples were determined by use of these plots.
Sampling

Tomato samples (*Solanum lycopersicum* L.) were collected from a growing area in Turkey. Five different types of fresh sample of the same maturity were obtained. The names of the tomato samples were Biga Çanakkale Nun-2048, Biga Çanakkale ES-2111, Bursa Karacabey Rio-Grande, Manisa C-33, and Bornova İzmir organic tomato; all were grown in August 2000. One major brand of tomato juice (“Tad” Company, KOÇ; 395 mL) was purchased from a local market; the production date was August 2000. Two different types of concentrated tomato salsa were purchased from local markets in İzmir and another was produced by the Faculty of Agriculture at Ege University, İzmir. The names of brands were “Tad Tomato Salsa” (“Tad” Company, KOÇ; 430 g, brix 28–30%), “Tamek Tomato Salsa” (“Tamek” Company; 425 g, brix 28–30%), and “Çiftlik Tomato Salsa” (Ege University; 900 g, brix 28–30%).

Four weighed samples from each group were used. The seeds were removed from the tomatoes, the tomatoes were homogenized with a blender, and the fresh juice was obtained (approx. 125 mL). Tomato-based processed products were homogenized with a blender and stored under a N₂ atmosphere before acid hydrolysis.

Extraction and Hydrolysis Conditions

The standard procedure used for acid hydrolysis of quercetin, myricetin, and kaempferol glycosides in tomatoes and tomato-based products has previously been described by Hertog et al. [20]. Acidified methanol (25 mL) containing 1% (v/v) HCl and 0.5 mg mL⁻¹ TBHQ was added to tomato juice (25 mL) or tomato paste (25 g). HCl (1.2 M, 5 mL) was added and the mixture was stirred at 90°C under reflux for 2 h to obtain the aglycons by hydrolysis of the flavonol glycosides. The extract was cooled to room temperature and sonicated for 3 min, to remove oxygen, before injection. The final extract was filtered through a 0.45-µm (Acrodisc) filter then through a 0.5-µm (Acrodisc) filter. The filtrate (10 µL) was injected into the HPLC.

HPLC Conditions

Isocratic RPHPLC analysis of tomatoes and tomato-based products with UV detection was performed by a modification of a method published elsewhere [20]. Compounds were separated on a 250 mm × 4.6 mm i.d., 5-µm particle, Hypersil-ODS column (Phenomenex, CA, USA) with
25:75 (v/v) acetonitrile–pH 2.4 phosphate buffer (25% acetonitrile in 0.025 M NaH₂PO₄) as mobile phase at a flow rate of 1.2 mL min⁻¹.

The HPLC equipment comprised Hewlett–Packard (HP) 1050 ChemStation Software, an HP model 35900 interface unit, an HP 9000 Series 300 computer, and an HP DeskJet 500 Printer. A Waters 486 tunable absorbance detector was operated at 266 nm; detector sensitivity was 0.05 AUFS and the column oven temperature was 30°C. Determinations were performed after three separate extractions of each sample, and each extract was injected in triplicate (n = 3).

Sample Preparation for Gas Chromatography – Mass Spectrometry (GC–MS)

Hydrolysates were extracted with ethyl acetate (1:1, v/v) after acid hydrolysis. Fractionation with NaHCO₃ was performed according to the method described by Sabatier et al. [27]. The ethyl acetate extract was treated with 0.5 M NaHCO₃ (1:1, v/v) three times, to eliminate free phenolic acids. The ethyl acetate extract was evaporated to dryness under a flow of nitrogen and the flavonols were re-dissolved in ethyl acetate. This solution was dried with Na₂SO₄ for 5 min. The dried solution (400 µL) was transferred to a vial, 100 µL bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added, and the vial was heated at 70°C for 15 min.

GC–MS Conditions

GC–MS was performed with Hewlett–Packard (HP) 6890/5973 equipment; conditions modified from a method published by Frankel et al. [28]. Compounds were separated on a 30 m × 0.25 mm capillary column coated with a 0.25 µm film of HP-5-MS (J&W Scientific, Folsom, CA, USA). Samples were injected with a split ratio of 50:1; helium was used as carrier gas at 1.0 mL min⁻¹. The column temperature was maintained at 100°C for 1 min after injection then increased at 10° min⁻¹ to 275°C which was sustained for 20 min. The time required for chromatography of one sample was 40 min.

RESULTS AND DISCUSSION

As is apparent from Fig. 1, quercetin, kaempferol, and myricetin from tomatoes and tomato-based products were perfectly separated by RPHPLC. The quercetin, kaempferol, and myricetin, content of fresh tomatoes is given in Table I. According to our study quercetin levels varied
HPLC chromatogram of flavonol aglycon standards (A), a tomato fruit extract (Biga Çanakkale ES-2111) (B), and a tomato juice extract (C). Mobile phase, 25% acetonitrile–phosphate buffer (pH 2.4), flow rate 1.2 mL min⁻¹; detection was at 266 nm; 0.05 AUFS among different types of fresh tomato (2.7–9.3 mg kg⁻¹) (Table I). Hertog et al. [25] reported that fresh tomato samples in Netherlands contained 4.6–8.2 mg kg⁻¹ hydrolysed quercetin. Crozier et al. [23] reported quercetin levels of 2.2–6.8 µg g⁻¹ fresh weight (Fw) in Dutch tomatoes (variety (var.) Trust), 2.0–8.7 µg g⁻¹ Fw in the equivalent Spanish tomatoes (var. Assun and Daniella), and 4.6–11.2 µg g⁻¹ in Scottish tomatoes (var. Spectra). Stewart et al. [22] reported that Scottish tomatoes (var. Vanessa 2000, Vanessa Beefsteak, 72/47, E27 681, Favorita) contained 1.6–10.9 µg of quercetin g⁻¹. Our findings are in good agreement with these studies.
Table I

The flavonol aglycon content of tomato samples

<table>
<thead>
<tr>
<th>Tomato variety</th>
<th>Myricetin (mg kg⁻¹)</th>
<th>Quercetin (mg kg⁻¹)</th>
<th>Kaempferol (mg kg⁻¹)</th>
<th>Total flavonol aglycons (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biga Çanakkale nun-2048</td>
<td>0.3 ± 0.0</td>
<td>6.2 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>Biga Çanakkale ES-2111</td>
<td>0.2 ± 0.0</td>
<td>3.8 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>4.4 ± 0.0</td>
</tr>
<tr>
<td>Manisa C-33</td>
<td>0.3 ± 0.0</td>
<td>9.3 ± 0.2</td>
<td>0.4 ± 0.0</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td>Bursa Karacabey rio grande</td>
<td>n.d.⁵</td>
<td>3.4 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Bornova izmir organic</td>
<td>0.2 ± 0.0</td>
<td>2.7 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>3.1 ± 0.1</td>
</tr>
</tbody>
</table>

⁵Not detected (<0.2 mg kg⁻¹)

Quercetin levels were higher in tomato salsa samples (9.2–11.7 mg kg⁻¹) than in fresh samples (Table II). Turkish tomato salsa prepared with tomato concentrate and salt by industrial techniques is commonly used in the preparation of many foods. Although no other studies of tomato salsa could be found, Stewart et al. [22] reported quercetin levels of 9.1 µg g⁻¹ in

Table II

The flavonol aglycon content of tomato-based products

<table>
<thead>
<tr>
<th>Tomato product</th>
<th>Brand</th>
<th>Myricetin (mg kg⁻¹)</th>
<th>Quercetin (mg kg⁻¹)</th>
<th>Kaempferol (mg kg⁻¹)</th>
<th>Total flavonol aglycons (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Çiftlik tomato salsa</td>
<td>“Ege University”</td>
<td>0.4 ± 0.1</td>
<td>11.3 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>12.6 ± 0.4</td>
</tr>
<tr>
<td>Tad tomato salsa</td>
<td>“Tad” company, KOÇ</td>
<td>0.3 ± 0.0</td>
<td>9.2 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>10.5 ± 0.0</td>
</tr>
<tr>
<td>Tamek tomato salsa</td>
<td>“Tamek” company</td>
<td>0.4 ± 0.0</td>
<td>11.7 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>13.2 ± 0.3</td>
</tr>
<tr>
<td>Tad tomato juice</td>
<td>“Tad” company, KOÇ</td>
<td>0.5 ± 0.1³</td>
<td>17.6 ± 0.1³</td>
<td>1.7 ± 0.1³</td>
<td>19.8 ± 0.3³</td>
</tr>
</tbody>
</table>

³Mean ± SE (mg L⁻¹ commercial tomato juice, triplicate determination; n = 3)
pasta sauce (Dolmio) and 1.7 µg g$^{-1}$ in canned cherry tomatoes. Stewart et al. [22] determined conjugated and free quercetin level of different varieties of tomato and some processed foods. According to their study most tomato-based products contained significant amounts of free flavonols. Because samples contain both free and conjugated flavonols, the amounts of the latter can be found by subtracting the amount obtained by analysis of the unhydrolysed sample from that determined after acid hydrolysis [22].

In our study, 17.6 mg L$^{-1}$ quercetin was found in tomato juice (Table II). A typical chromatogram obtained from a tomato juice extract is shown in Fig. 1c. Hertog et al. [24] reported that tomato juice contains 13 mg L$^{-1}$ quercetin. Stewart et al. [22] reported that two different types of tomato juice (commercial composite) contained 14.4 µg mL$^{-1}$ and 16.2 µg mL$^{-1}$ quercetin. These levels are in accordance with our results.

We detected kaempferol at lower levels than the major flavonol quercetin. Kaempferol levels were similar in fresh samples (0.2–0.6 mg kg$^{-1}$; Table I) and in tomato juice and tomato salsa samples (1.7 mg L$^{-1}$ and 0.9–1.1 mg kg$^{-1}$, respectively; Table II). Stewart et al. [22] reported that tomato juice (commercial composite) contained 0.7–0.8 µg mL$^{-1}$ kaempferol after hydrolysis, that different varieties of English tomatoes (Favorita, Cherry Belle, 102-Yellow, Flavore, Spectra, Aromato) contained 0.2–0.4 µg g$^{-1}$ (Fw) kaempferol, and that Scotland tomatoes (var. Vanessa 2000, Vanessa Beefsteak, 72/47, E27 681, Favorita) contained 0.2–0.9 µg g$^{-1}$ (Fw) kaempferol. Hertog et al. [25] found low levels of kaempferol in samples collected during the same season (<2 mg kg$^{-1}$ in the fresh edible part). Our findings are in accordance with these studies.

Hertog et al. [24] detected less than 0.5 mg L$^{-1}$ myricetin in tomato juice. In our study the myricetin content of tomato juice (commercial composite) was 0.5 mg L$^{-1}$ (Table II) and myricetin levels in different fresh samples varied from 0.2 to 0.3 mg kg$^{-1}$ (Table I). In tomato salsa samples 0.3–0.4 mg kg$^{-1}$ myricetin was detected (Table II). Fig. 1b shows a typical chromatogram of flavonols from a tomato fruit extract.

Compared with other studies myricetin was found as a minor flavonol aglycon in our samples. It is known that the flavonol content of field-grown fruits is affected by a wide range of environmental conditions, including temperature, soil, light levels, nutritional conditions, and pathogen attack [23,29–32]. Fruit cultivar or variety also affects the level of flavonols in tomatoes [22]. It has been reported that the flavonol aglycon content of some deep red tomato fruits can be correlated with their anthocyanin
content, because the flavonols originate from the same branch of the phenylpropanoid pathway as the anthocyanins [33]. Our suggested isocratic HPLC procedure enabled rapid baseline separation of myricetin ($t_r = 9.4$), quercetin ($t_r = 14.1$), and kaempferol ($t_r = 28.7$ min). The presence of these flavonols in tomatoes, tomato juice, and tomato paste samples was confirmed by GC–MS. The bis(trimethylsilyl)-trifluoroacetamide (BSTFA) derivatives of each phenolic compound and of compounds present in sample extracts were analysed by GC with mass-selective detection. The total ion chromatogram (TIC) obtained from a tomato juice (commercial composite, \textit{TAD}) is shown in Fig. 2 with identified flavonol aglycon peaks numbered (I, II, III). On the basis of the molecular ions and fragmentation patterns of all the samples studied, peaks eluting after 18.08, 25.36, and 29.96 min were tentatively identified as the bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatives of myri-
cetin ($m/z$ 279), kaempferol ($m/z$ 207), and quercetin ($m/z$ 281). Triplicate
samples were analysed to confirm these pattern of peaks in the chromatogram. GC retention times of reference compounds, given to two decimal
places, to indicate the elution sequence of peaks were very close to the
retention times of the flavonol aglycons in samples. The GC retention
time sequence of the two flavonol aglycons kaempferol and quercetin was
also the same as in research published by Greenaway et al. [34] and Sabatier
et al. [27]. Two main flavonols, quercetin and kaempferol, were identified
and one minor flavonol, myricetin, was also detected.

**Fig. 3**
The comparison with mass spectra of BSTFA derivative of quercetin (A), kaempferol
(C) and myricetin (E) reference compounds and mass spectra of BSTFA derivative of
quercetin (B), kaempferol (D) and myricetin (F) in tomato juice (commercial composite,
*TAD*)
Mass spectra of the BSTFA derivatives of quercetin (3,3′,4′,5,7-pentahydroxyflavone), kaempferol (3,5,7-trihydroxy-2-[4-hydroxyphenyl]-4H-1-benzopyran-4-one), and myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone) standards and mass spectra of the BSTFA derivatives of these flavonol aglycons in tomato juice (commercial composite, TAD) were identical, as shown in Fig. 3. The fragmentation patterns of the compounds are given in Table III. By use of mass spectrometry we confirmed the presence of quercetin, kaempferol, and myricetin in all the samples; quercetin was the predominant flavonol in tomato and tomato-based products.

**Table III**

Mass spectral data of bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatives of flavonol aglycons in tomato juice (Tad)

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Compound</th>
<th>Characteristic MS data m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.08</td>
<td>Myricetin standard</td>
<td>M⁺ = 279; 149 (100), 167, 71, 40</td>
</tr>
<tr>
<td>18.08</td>
<td>Compound from extract</td>
<td>M⁺ = 279; 149 (100), 167, 71, 40</td>
</tr>
<tr>
<td>25.36</td>
<td>Kaempferol standard</td>
<td>M⁺ = 207; 73 (100), 281, 200, 55</td>
</tr>
<tr>
<td>25.35</td>
<td>Compound from extract</td>
<td>M⁺ = 207; 73 (100), 281, 200, 55</td>
</tr>
<tr>
<td>29.96</td>
<td>Quercetin standard</td>
<td>M⁺ = 281; 207 (100), 95, 273, 81</td>
</tr>
<tr>
<td>29.97</td>
<td>Compound from extract</td>
<td>M⁺ = 281; 207 (100), 95, 273, 81</td>
</tr>
</tbody>
</table>

The major flavonoid found in fruits and vegetables is quercetin, followed by myricetin. Onion is a principal source of quercetin (347 mg kg⁻¹), cranberries contain extremely high levels of quercetin and myricetin, and turnip tops and strawberries are rich in kaempferol [18–19, 23–25].

Black tea is also a prominent source of the flavonol aglycons (3.89–7.08 mg g⁻¹), especially quercetin (2.22–4.17 mg g⁻¹) in the human diet [14,15].

**CONCLUSIONS**

The tomato spreads and other tomato-based products consumed in Turkey are excellent dietary sources of quercetin and kaempferol. The analytical method proposed for analysis of these compounds enables reproducible and accurate determination of these flavonol aglycons in tomatoes and tomato-based products.
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