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Evaluation of a Method To Characterize the Phenolic Profile of Organic and Conventional Tomatoes

Anna Vallverdú-Queralt,[†,‡] Olga Jáuregui,[§] Alexander Medina-Remón,[†,‡] and Rosa Maria Lamuela-Raventós*[,†,‡]

[†]Nutrition and Food Science Department, XaRTA, INSA, Pharmacy School, University of Barcelona, 08028 Barcelona, Spain

[‡]CIBER CB06/03 Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), and RETICS RD06/0045/0003, Instituto de Salud Carlos III, 28029 Madrid Spain

[§]Scientific and Technical Services, University of Barcelona, 08028 Barcelona, Spain

ABSTRACT: The present study aims to compare the phenolic profiles of organic and conventional tomatoes bought in the market. For the quantification and identification of individual polyphenols, liquid chromatography coupled to mass spectrometry in tandem mode (LC-MS/MS) was carried out. Confirmation of the compounds previously identified on the triple-quadrupole was accomplished by injection in the high-resolution system (QToF-MS). In this way, 34 compounds were identified in tomato fruits. Recoveries of targeted polyphenols exceed 78% for conventional and organic tomatoes, respectively. The method intraday precision ranged between 3 and 5%, whereas the interday one was below 12%. Comparing the analyses of tomatoes from conventional and organic production systems demonstrated statistically higher levels ($P < 0.05$) of phenolic compounds in organic tomatoes. This methodology allowed finding differences in the bioactive components of organic and conventional tomatoes not previously reported.

KEYWORDS: *organic tomatoes, conventional tomatoes, polyphenols, LC-ESI-MS/MS, QToF-MS*

INTRODUCTION

The consumption of raw tomato is associated with a decrease in chronic degenerative diseases.[1] Tomatoes contain some valuable bioactive components, including polyphenols.[2−4] The polyphenol content of plants is affected by cultivation, growing conditions, degree of ripeness, and plant variety.[5−9] The nutrient composition of plants, including secondary plant metabolites, may be affected by different production systems, such as organic and conventional.[10,11] These systems differ in the amounts of nutrients applied as fertilizers and in organic matter applied to the soil. The synthesis of secondary plant metabolites, proteins, and soluble solids is influenced by inorganic nitrogen availability. Moreover, organically produced plants have a longer ripening period because of a slower release of the supplied nutrients compared to conventional plants,[12] and as polyphenols are synthesized during the ripening period and the plant synthesizes them as phytoalexins under stress conditions, one may expect a higher content of these compounds in organically grown plants. In previous works made with market tomato-based products, organic ketchups and tomato juices had a significantly higher phenolic content than conventional alternatives.[13,14] However, for these products, there is a lack of information about which tomato varieties have been used and if there are some differences in the technological treatments applied, so it is necessary to perform a study with the same varieties and grown under the same weather conditions, in which the only difference is organic or conventional growth.

Grinder-Pedersen et al.[15] compared conventionally produced diets (CPD) and organically produced diets (OPD) in a human crossover intervention study ($n = 16$) in terms of the intake and excretion of five selected flavonoids and the effect on markers of oxidative defense. The urinary excretion of quercetin and kaempferol was higher ($P < 0.05$) after 22 days of intake of the OPD than of the CPD. Therefore, the food production method not only affected the content of the major flavonoids in foods but also affected urinary flavonoids and markers of oxidation in humans.

Liquid chromatography (LC) coupled to mass spectrometry (MS) with electrospray ionization (ESI) is one of the most powerful tools to analyze phenolic compounds. For this kind of application, liquid chromatography electrospray ionization time-of-flight mass spectrometry (LC-ESI-QToF) allows the exact mass measurements of both MS and MS/MS ions to be achieved, which is essential for the characterization of small molecules.

We carried out a study to find phenolic profile differences between conventional and organic tomatoes in the content of flavonols (kaempferol-3-*O*-rutinoside, rutin, and quercetin), flavanones (naringenin and naringenin-7-*O*-glucoside), flavones (apigenin-7-*O*-glucoside), and hydroxycinnamic acids (ferulic, *p*-coumaric, caffeic, and chlorogenic acids). Therefore, we propose an analytical approach to obtain the phenolic profile of organic and conventional tomatoes. The methodology was optimized and validated. Confirmation of the compounds was accomplished by injection in the high-resolution system (LC-QToF-MS) using accurate mass measurements in MS and MS^2 modes. Moreover, the combination of LC-QToF-MS and

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triple-quadrupole enabled us to identify 34 polyphenols in tomato fruits.

MATERIALS AND METHODS

Standards and Reagents. All samples and standards were handled without exposure to light. Caffeic, *p*-coumaric, and chlorogenic acids, rutin, and quercetin were purchased from Sigma (Madrid, Spain) and naringenin, naringenin-7-*O*-glucoside, apigenin-7-*O*-glucoside, and kaempferol-3-*O*-rutinoside from Extrasynthèse (Genay, France). Hydrochloric acid 35% was from Panreac (Barcelona, Spain) and anhydrous sodium acetate (2 mol/L) from Merck (Darmstadt, Germany). Ethanol and formic acid of HPLC grade were obtained from Scharlau (Barcelona, Spain), and ultrapure water (Milli-Q) was from a Millipore system (Bedford, MA, USA).

Samples: Organic and Conventional Tomatoes. Organic and conventional tomatoes (cv. Daniella) were bought in Barcelona markets over two consecutive years, 2010 and 2011. All conventional and organic tomatoes selected had the same degree of ripeness (4.3−5 °Brix) and were normal-sized (75−80 g). Conventional farms utilize fertilizers containing soluble inorganic nitrogen and other nutrients, which are more directly available to plants. Organic systems emphasize the accumulation of soil organic matter and fertility over time through the use of cover crops, manures, and composts and rely on the activity of a diverse soil ecosystem to make nitrogen (N) and other nutrients available to plants. The amount of N present in cover crops varies from year to year, but, typically, organic plots currently receive between 240 and 260 kg of N ha^{-1} per year in addition to the N fixed by the legume cover crop. Conventional tomatoes usually receive 50 kg ha^{-1} of an N−P−K starter fertilizer and 118 kg ha^{-1} of ammonium nitrate as side dressing.[16] Tomatoes were bought and frozen until the analyses were carried out.

Extraction and Isolation of Phenolic Compounds. Sample treatment was performed by duplicate, in a darkened room with a red safety light to avoid the oxidation of the analytes during the process, following the procedure of Vallverdú-Queralt et al.[5] with some modifications. Tomatoes (skin, flesh, and pips) were blended to obtain a tomato paste containing an average particle size of about 1 mm. Then, 0.5 g of the paste was weighed and homogenized with 4 mL of 80% ethanol in Milli-Q water and centrifuged (4000 rpm at 4 °C) for 20 min. The supernatant was transferred into a flask, and the extraction was repeated on the pellet with the same conditions. Both supernatants were combined and evaporated under nitrogen flow. Finally, the residue was reconstituted with up to 1.5 mL of Milli-Q water containing 0.1% formic acid.

Solid-phase extraction (SPE) of these extracts was carried out following the procedure previously reported[17,18] using Oasis MAX cartridges (30 mg) from Waters. These cartridges were selected because they provided the highest recoveries. First, 1 mL of methanol and subsequently 1 mL of sodium acetate (50 mmol/L, pH 7) were loaded into the cartridges to equilibrate the cartridges; then, 1 mL of each extract was diluted with 1 mL of Milli-Q water and acidified with 34 μL of hydrochloric acid at 35% before being loaded into the cartridges. These were rinsed with 1 mL of 50 mmol/L sodium acetate (pH 7) at 5% of methanol. The polyphenols were eluted with 1800 μL of methanol containing 2% formic acid. The eluted fractions were evaporated under nitrogen flow, and the residue was reconstituted with up to 250 μL of water containing 0.1% formic acid and filtered with 13 mm polytetrafluoroethylene (PTFE) 0.45 μm filters from Waters (Milford, MA, USA) into an insert-amber vial for HPLC analysis. Samples were stored at −20 °C until analysis.

HPLC-ESI-MS/MS Analysis. To evaluate the differences between organic and conventional production systems, target analytes were identified and quantified using HPLC-ESI-MS/MS. An API 3000 (PE Sciex, Concord, ON, Canada) triple-quadrupole mass spectrometer equipped with a Turbo Ionspray source in negative ion mode was used to obtain MS/MS data. Turbo Ionspray source settings were as follows: capillary voltage, −3500 V; nebulizer gas (N_2), 10 arbitrary units (au); curtain gas (N_2), 12 au; collision gas (N_2), 4 au; focusing potential, −200 V; entrance potential, −10 V; drying gas (N_2), heated to 400 °C and introduced to a flow rate of 6000 cm^3/min. The declustering potential and collision energy were optimized for each compound in infusion experiments: individual standard solutions (10 μg/mL) dissolved in 50:50 (v/v) mobile phase were infused at a constant flow rate of 5 μL/min using a model syringe pump (Harvard Apparatus, Holliston, MA, USA). Full-scan data acquisition was performed scanning from *m*/*z* 100 to 800 in profile mode and using a cycle time of 2 s with a step size of 0.1 u and a pause between each scan of 2 ms. To confirm the identity of some compounds, neutral loss scan and precursor ion scan experiments were carried out as described by Vallverdú-Queralt et al.[19]

For quantitative purposes, two MRM transitions were selected for each of them, after having observed their product ion scan spectra. Table 1 shows the two MRM transitions together with the collision energy for polyphenols and internal standard (ethyl gallate). Each polyphenol was identified on the basis of its retention time, two selected MRM transitions, and their relative abundance. Quantitative analysis was performed by means of standard addition method because blank samples were not available.

Table 1. HPLC-MS/MS Parameters for Polyphenols and Internal Standard

analyte	rt[a]	MRM transition[b]	collision energy
chlorogenic acid	3.43	353→191	−20.00
		353→179	
caffeic acid	3.80	179→135	−20.00
		179→107	
p-coumaric acid	5.82	163→119	25.00
		163→145	
ferulic acid	6.00	193→134	−20.00
		193→149	
rutin	8.21	609→300	−50.00
		609→151	
kaempferol-3-*O*-rutinoside	9.18	593→285	−30.00
		593→255	
apigenin-7-*O*-glucoside	9.66	431→269	−20.00
		431→161	
naringenin-7-*O*-glucoside	10.70	433→271	25.00
		433→151	
quercetin	11.93	301→151	−30.00
		301→121	
naringenin	13.03	271→151	−30.00
		271→119	
internal standard: ethyl gallate	8.65	197→169	−20.00

[a]rt, retention time. [b]The first line reports the quantifier MRM transition and the second line the qualifier transition.

The liquid chromatograph was an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, an autosampler, and a column oven set to 30 °C. A Luna C_{18} column 50 × 2.0 mm i.d., 5 μm (Phenomenex, Torrance, CA, USA), was used. Mobile phases consisted of 0.1% formic acid in Milli-Q water (A) and 0.1% formic acid in acetonitrile (B). The injection volume was

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20 μL, and the flow rate was 0.4 mL/min. Separation was carried out in 20 min under the following conditions: 0 min, 5% B; 16 min, 40% B; 17 min, 95% B; 19 min, 95% B; 19.5 min, 5% B. The column was equilibrated for 5 min prior to each analysis.

HPLC-ESI-QToF Analysis. For accurate mass measurements, a HPLC-ESI-QToF was used following the procedure of Vallverdú-Queralt et al.[20] The chromatography was performed on an Agilent 1200 RRLC using the column and the gradient elution described above. Flow rate was 0.4 mL/min, and injection volume was 5 μL. The column was equilibrated for 5 min prior to each analysis. The HPLC system was coupled to a hybrid quadrupole ToF QSTAR Elite (ABSciex). The MS acquisition was performed in negative ionization using IDA between m/z 90 and 1100. MS parameters were as follows: ion spray voltage, −4200 V; declustering potential, −60 V; focusing potential, −190 V; declustering potential 2, −15 V; ion release delay, 6 V; ion release width, 5 V; temperature, 400 °C with curtain gas (N_2), 50 au; auxiliary gas, 50 au; and nebulizer gas (N_2), 50 au. IDA was performed using the following criteria: ions that exceed 5 counts; ion tolerance, 50 mDa; collision energy, fixed at −30 V; dynamic background subtract activated. The QToF was calibrated as recommended by the manufacturer.

The elemental composition of each polyphenol was selected according to the accurate masses and the isotopic pattern (through the Formula Finder feature in Analyst QS 2.0) and searched for in the *Dictionary of Natural Products* (Chapman & Hall/CRC) and the MOTO database (http://appliedbioinformatics.wur.nl/moto). The interpretation of the observed MS/MS spectra in comparison with those found in the literature was the main tool for putative identification of polyphenols.

Validation Method. Validation assays were performed by means of the standard addition method. Therefore, we estimated the unknown amount of the analytes in organic and conventional tomatoes, and we evaluated the linear dynamic range and sensitivity in the analysis in both organic and conventional tomatoes. Recoveries, precisions, limits of detection (LODs), limits of quantitation (LOQs), and stabilities were calculated after determination of the natural levels of each polyphenol in organic and conventional tomatoes.

Standard Addition Method. Analyte quantification was performed by the standard addition method (five points). For this purpose, five aliquots (0.5 g each) of organic tomato and five aliquots (0.5 g each) of conventional tomato were spiked with the same amount of the internal standard (ethyl gallate); four aliquots of each group were then spiked with different concentrations of phenols (between 11.70 and 465.80 μg/g) and submitted to the extraction process. The spiked level was chosen to increase the original content of polyphenols in organic and conventional tomatoes by a factor between 2 and 3.

Recovery and Precision. After a preliminary screening of the phenolic profile in organic and conventional tomatoes, analyte recoveries were assessed by analyzing the percentage of recovery of each polyphenol in conventional and organic tomatoes. The samples were spiked with four different concentrations (50, 100, 150, and 200% of expected value) of each polyphenol before extraction. The spiked samples were extracted by triplicate and analyzed under the previously established optimal conditions. The percentage of recovery was calculated for each polyphenol, whereas the corresponding relative standard deviation (RSD) was representative for intraday precision. Interday precision was estimated as the RSD performed within three different days.

Limits of Detection and Limits of Quantitation. The sensitivity of the method was evaluated determining the LODs and LOQs. With regard to the two MRM transitions, the quantifier transition was used for quantitative purposes, whereas the confirmation transition was used for qualitative analysis and for method limits. The LOD was calculated as the quantity of analyte able to produce a chromatographic peak 3 times higher than the noise of the baseline in a chromatogram (S/N = 3) of a nonfortified sample, after having estimated the endogenous amount. The LOQ was set at 10 times higher than the noise of the baseline in a chromatogram (S/N = 10).[21]

Stability. Three aliquots with four different quantities (50, 100, 150, and 200% of expected value) were used to evaluate the short-term temperature stability at −20 and −80 °C. The aliquots were thawed at room temperature for 6 h (the mean sample preparation time) and then analyzed. To evaluate the stability after successive freeze−thaw cycles, three aliquots of each concentration (50, 100, 150, and 200% of expected value) were stored at −20 and −80 °C for 24 h and then thawed at room temperature up to four times over a 1 week period. Analytes were assessed in each aliquot in a single run at the end of the last freeze−thaw cycle. Aliquots of each concentration for long-term stability were prepared and immediately frozen at −20 and −80 °C until analysis (within 1 year). The concentrations of all the stability samples were compared with the mean of back-calculated values for the standards at each concentration from the first day of long-term stability testing.

RESULTS AND DISCUSSION

It is well-known that the biosynthesis of phenolic compounds in plants is strongly influenced by the cultivar[5] and mode of fertilization.[22] The level of carbon-based secondary metabolites such as phenolic compounds is usually higher in organic plants[23,24] due to their defensive role in plants under stress conditions.[25] Data on the phenolic composition of fruits and vegetables grown either organically or conventionally remain scarce in the literature as these compounds have only recently been considered to be interesting functional microconstituents due to their potential role in the prevention of cardiovascular diseases, degenerative diseases, and cancer.[26] Among papers mentioning total phenolic content, the majority describe a higher phenolic concentration in organically grown fruits or vegetables.[27] Our results are in accordance with these studies because organic tomatoes showed a higher content of polyphenols than conventional tomatoes.

Validation Results. *Standard Addition Method.* Linear regression parameters are reported in Table 2. A good linearity was verified, with all correlation coefficients exceeding 0.9907. Slopes were very similar for curves representing organic and conventional tomatoes, suggesting a comparable matrix effect. LODs and LOQs are also shown in Table 2. The noise level depended on the matrix and, therefore, the same analyte was characterized by different LODs and LOQs in organic and conventional tomatoes. The LODs varied between 0.13 and 0.66 μg/g fresh weight (FW) for conventional tomatoes and between 0.18 and 0.61 μg/g FW for organic tomatoes. The LOQs ranged between 0.43 and 2.20 μg/g FW for conventional tomatoes and between 0.60 and 2.04 μg/g FW for organic tomatoes.

Recovery and Precision. After a preliminary determination of the polyphenol content in organic and conventional tomatoes, Table 3 reports the definitive values of the estimated quantities by the standard addition, recoveries, and precision data in organically and conventionally grown tomatoes. Recoveries ranged between 78 and 98%, and intraday and interday precisions were less than 5 and 11%, respectively, for all of the analytes. For the values analyzed, recovery and precision were between the accepted values of AOAC International.[21]

Stability. Freeze and thaw cycles did not significantly modify the polyphenol concentration at either temperature tested; polyphenol recoveries were between 85 and 98% at −80 °C and between 81 and 97% at −20 °C. For short-term stability, the recoveries of polyphenols were between 80 and 98% at −20 °C and between 78 and 94% when the temperature was −80 °C. Long-term storage at −20 and −80 °C did not affect the polyphenol recoveries. They were between 75 and 91% after

Table 2. Method Validation Parameters: Slopes (m), Correlation Coefficients (R^2) and Limits of Detection (LOD) and Quantification (LOQ)

	conventional tomatoes				organic tomatoes			
analyte	m (10^3)	R^2	LOD (μg/g FW)	LOQ (μg/g FW)	m (10^3)	R^2	LOD (μg/g FW)	LOQ (μg/g FW)
caffeic acid	3.50	0.9933	0.30	0.99	3.30	0.9911	0.22	0.73
chlorogenic acid	4.23	0.9943	0.26	0.88	3.60	0.9907	0.29	0.97
ferulic acid	0.78	0.9958	0.42	1.39	0.43	0.9926	0.53	1.77
p-coumaric acid	4.40	0.9934	0.28	0.94	4.08	0.9934	0.22	0.73
naringenin	2.10	0.9932	0.19	0.63	2.60	0.9918	0.20	0.67
naringenin-7-*O*-glucoside	2.10	0.9934	0.13	0.43	2.35	0.9958	0.18	0.60
rutin	7.30	0.9927	0.21	0.70	7.62	0.9990	0.24	0.80
quercetin	2.90	0.9919	0.66	2.20	2.35	0.9957	0.61	2.04
kaempferol-3-*O*-rutinoside	22.35	0.9957	0.36	1.20	25.30	0.9908	0.33	1.10
apigenin-7-*O*-glucoside	0.82	0.9985	0.23	0.77	0.80	0.9950	0.26	0.86

1 year, at both temperatures. Therefore, the storage conditions used for the assays allowed the phenols to remain stable.

Application of the Method for the Analysis of Conventional and Organic Tomatoes. The results concerning the quantitative determination of the target polyphenols are summarized in Table 3, whereas those related to the screening of polyphenols (hydroxycinnamic acids, flavonols, flavanones, flavones, and their derivatives) are given in Table 4. To avoid the effect of water evaporation and concentration of solids taking place on the quantification, the estimated values were expressed as FW and dry weight (DW).

The main polyphenol in all tomatoes was rutin, followed by naringenin, as reported in other studies.[5,28] Rutin and naringenin concentrations were significantly higher in organically grown tomatoes. Chassy et al.[29] found significantly higher mean levels of soluble solids, flavonoids, total phenolics, and ascorbic acid in organic tomatoes than in their conventional counterparts grown in model plots over a 3 year period. However, in their study, a complete phenolic profile identification was not performed because only flavonoids were identified.

Simple Phenolic Acid Derivatives. Phenolic acids and their derivatives are widely distributed in plants. They are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because of their stable radical intermediates, which prevent the oxidation of various food ingredients.[30]

The examination of the chromatograms in full scan mode on the triple-quadrupole system revealed the presence of ferulic (m/z 193), caffeic (m/z 179), and *p*-coumaric (m/z 163) acids (Table 4). MS^2 of these ions showed the deprotonated molecule $[M - H]^-$ and the typical loss of CO_2, giving $[M - H - 44]^-$ as a characteristic ion. These results were confirmed by injection in the high-resolution system (ToF-MS) using accurate mass measurements and the MS^2 fragmentation patterns.

Absolute differences in the levels of ferulic, caffeic, and *p*-coumaric acid were found in organic and conventional tomatoes (Table 3). Phenolic acids were higher in organic tomatoes, having contents of 34.25 μg/g FW (380.55 μg/g DW) for *p*-coumaric acid and 41.70 μg/g FW (463.33 μg/g DW) for caffeic acid, whereas for conventional tomatoes phenolic acids ranged between 20.59 μg/g FW (257.38 μg/g DW) for *p*-coumaric acid and 22.88 μg/g FW (286.00 μg/g DW) for caffeic acid. We tentatively attributed the higher concentration of phenolic acids in organic tomatoes to mode of fertilization.[22]

Phenolic acid-*O*-hexosides were identified in ToF-MS mode using accurate mass measurements and MS^2 fragmentations (Table 4). Caffeic acid-*O*-hexosides (m/z 341) were detected in all conventional and organic tomatoes. The MS^2 of m/z 341 showed a characteristic fragmentation involving cleavage of the intact sugar $[M - H - 162]^-$ (m/z 179) and the ion corresponding to the loss of CO_2 (m/z 135).

In addition, the analysis in ToF-MS mode revealed the presence of two homovanillic acid-*O*-hexosides (m/z 343) and two coumaric acid-*O*-hexosides (m/z 325). The MS^2 of m/z 343 and 325 showed ions corresponding to the deprotonated molecule $[M - H]^-$, the loss of a hexose $[M - H - 162]^-$, and the loss of the carboxylic group (Table 4).

The analysis in ToF-MS mode also showed the presence of ferulic acid-*O*-hexoside (m/z 355). The MS^2 of m/z 355 showed ions at m/z 193, 178, and 149 corresponding to the loss of a hexose moiety $[M - H - 162]^-$ and the loss of a methyl and acid group from the aglycone (Table 4).

Hydroxycinnamoylquinic Acid Derivatives. Preliminary structure–activity relationship studies of cinnamic acids and derivatives demonstrated the importance of the catechol group in the antiradical efficacy.[31] The examination of chromatograms in full scan mode in the triple-quadrupole system of organic and conventional tomatoes revealed a peak at m/z 353 corresponding to chlorogenic acid (Table 4). Chlorogenic acid was the most abundant hydroxycinnamic acid, ranging from 36.87 μg/g FW (460.88 μg/g DW) in conventional tomatoes to 56.99 μg/g FW (633.22 μg/g DW) in organic tomatoes (Table 3). These results are in line with those reported by Caris-Veyrat et al.,[32] who retrieved significantly higher concentrations of chlorogenic acid from organic tomatoes ($P < 0.05$) in comparison to the conventional variant. These results were confirmed by injection in the ToF-MS using accurate mass measurements and the MS^2 fragmentation patterns.

Neochlorogenic and cryptochlorogenic acid were also present (Table 4). It was possible to differentiate the isomers of chlorogenic acid by their relative intensities in MS^2 spectra according to the method cited by other authors using liquid chromatography–tandem mass spectrometry.[33] Another caffeoylquinic isomer acid was also detected, which might be a stereoisomer of the neochlorogenic acid, chlorogenic acid, or cryptochlorogenic acid as described by other authors.[20,34]

The analysis in ToF-MS mode also showed the presence of two dicaffeoylquinic acid isomers (m/z 515). The MS^2 of m/z 515 showed the ion $[M - H - 162]^-$ (m/z 353, corresponding to the loss of a caffeic acid unit) and the deprotonated quinic acid (m/z 191). Two tricaffeoylquinic acids (m/z 677) were also identified in both varieties of tomatoes. MS^2 experiments of m/z 677 of both compounds revealed characteristic

Table 3. Estimated Quantities and Recoveries (Relative Standard Deviations) of Polyphenols from Organic and Conventional Tomatoes

	conventional tomatoes				organic tomatoes			
analyte	estimated quantity (μg/g FW ± SD[a])	lowest spiked level[b]	recovery[c] ± SD	RSD[d] (%)	estimated quantity (μg/g FW ± SD)	lowest spiked level	recovery ± SD	RSD (%)
caffeic acid	22.88 ± 0.45	46.60	98.40 ± 3.22	3.27	41.70 ± 0.81	93.16	97.04 ± 2.95	3.05
chlorogenic acid	36.87 ± 0.61	69.90	96.23 ± 2.89	3.00	56.99 ± 0.90	116.50	96.12 ± 3.10	3.23
ferulic acid	21.69 ± 0.40	69.90	94.15 ± 4.49	4.77	35.11 ± 0.63	69.90	96.88 ± 4.01	4.15
p-coumaric acid	20.59 ± 0.36	46.60	94.89 ± 2.87	3.02	34.25 ± 0.55	69.90	97.65 ± 2.90	2.97
naringenin	36.46 ± 0.69	116.50	85.11 ± 3.97	4.69	87.38 ± 0.98	163.00	88.10 ± 3.51	3.98
naringenin-7-*O*-glucoside	7.68 ± 0.15	23.30	87.02 ± 3.14	3.58	13.91 ± 0.28	23.30	88.63 ± 3.02	3.41
rutin	119.82 ± 1.49	233.00	93.44 ± 4.72	5.05	272.75 ± 2.98	465.80	95.11 ± 4.51	4.74
quercetin	5.69 ± 0.12	11.70	77.81 ± 3.88	5.00	11.42 ± 0.18	23.30	78.20 ± 3.41	4.36
kaempferol-3-*O*-rutinoside	6.03 ± 0.11	11.70	89.15 ± 3.27	3.67	12.70 ± 0.24	23.30	91.34 ± 3.02	3.31
apigenin-7-*O*-glucoside	28.28 ± 0.63	46.60	91.05 ± 2.65	2.90	31.63 ± 0.52	46.60	93.54 ± 2.87	3.07

[a]SD, standard deviation. [b]The lowest spiked level (50%) was applied for doubling or tripling the endogenous concentration of each compound. [c]Mean recovery of all spiked levels (50, 100, 150, and 200%). [d]RSD, relative standard deviation.

Table 4. Analytes Tentatively Identified in Organic and Conventional Tomatoes

analyte[a]	rt[b]	$[M - H]^-$	*m/z* ions	accurate mass	mDa[c]	MF[d]
caffeic acid-*O*-hexoside 1	1.43	341	179 (100), 135 (30)	341.0877	1.60	$C_{15}H_{18}O_9$
caffeic acid-*O*-hexoside 2	1.54	341	179 (100), 135 (30)	341.0877	0.70	$C_{15}H_{18}O_9$
neochlorogenic acid	1.65	353	191 (100), 179 (70), 135 (30)	353.0877	0.70	$C_{16}H_{18}O_9$
caffeic acid-*O*-hexoside 3	2.16	341	179 (100)	341.0877	2.30	$C_{15}H_{18}O_9$
homovanillic acid-*O*-hexoside 1	2.22	343	343 (100), 137 (70), 109 (40)	343.1034	1.10	$C_{15}H_{20}O_9$
ferulic acid-*O*-hexoside	2.49	355	193 (60), 178 (30), 149 (100)	355.1034	2.50	$C_{16}H_{20}O_9$
homovanillic acid-*O*-hexoside 2	2.82	343	343 (100), 137 (70), 109 (40)	343.1034	2.10	$C_{15}H_{20}O_9$
caffeic acid-*O*-hexoside 4	2.90	341	179 (100), 135 (50)	341.0877	0.70	$C_{15}H_{18}O_9$
coumaric acid-*O*-hexoside 1	2.99	325	163 (85), 119 (100)	325.0928	1.40	$C_{15}H_{18}O_8$
coumaric acid-*O*-hexoside 2	3.26	325	163 (90), 119 (100)	325.0928	0.20	$C_{15}H_{18}O_8$
chlorogenic acid*	3.43	353	191 (100), 179 (5)	353.0877	1.50	$C_{16}H_{18}O_9$
caffeic acid*	3.80	179	135 (100), 107 (40)	179.0349	1.40	$C_9H_8O_4$
cryptochlorogenic acid	4.14	353	191 (70), 173 (100), 135 (20)	353.0877	0.80	$C_{16}H_{18}O_9$
naringenin-*C*-diglycoside	4.96	595	595 (100), 505 (35), 475 (60), 385 (50), 355 (45)	595.1667	0.70	$C_{27}H_{32}O_{15}$
chlorogenic acid isomer	5.15	353	191 (100)	353.0877	1.00	$C_{16}H_{18}O_9$
rutin-*O*-hexoside	5.20	771	771 (100), 609 (80), 300 (20)	771.1989	1.60	$C_{33}H_{40}O_{21}$
p-coumaric acid*	5.82	163	163 (40), 119 (100)	163.0400	0.86	$C_9H_8O_3$
ferulic acid*	6.00	193	193 (20), 178 (70), 149 (20), 134 (100)	193.0506	1.19	$C_{10}H_{10}O_4$
apigenin-*C*-hexoside-hexoside	6.26	593	503 (10), 473 (40), 353 (10)	593.1511	0.30	$C_{27}H_{30}O_{15}$
rutin-*O*-pentoside	7.66	741	741 (100), 609 (90), 300 (20)	741.1883	0.70	$C_{32}H_{38}O_{20}$
apigenin-*C*-hexoside-pentoside	7.78	563	503 (10), 473 (35), 383 (15), 353 (25)	563.1406	0.90	$C_{26}H_{28}O_{14}$
quercetin-*O*-dihexoside	7.90	625	463 (100), 300 (40)	625.1410	1.60	$C_{27}H_{30}O_{17}$
rutin*	8.21	609	609 (100), 300 (40)	609.1460	0.50	$C_{27}H_{30}O_{16}$
naringenin-*C*-hexoside	9.03	433	433 (30), 343 (20), 313 (40)	433.1140	0.60	$C_{21}H_{22}O_{10}$
naringenin-*O*-hexoside	9.12	433	433 (10), 271 (70)	433.1140	0.10	$C_{21}H_{22}O_{10}$
kaempferol-3-*O*-rutinoside*	9.18	593	593 (100), 285 (70), 255 (20)	593.1511	0.70	$C_{27}H_{30}O_{15}$
dicaffeoylquinic acid 1	9.50	515	515 (20), 353 (100), 335 (20), 191 (45), 173 (60)	515.1194	0.10	$C_{25}H_{24}O_{12}$
apigenin-7-*O*-glucoside*	9.66	431	431 (25), 269 (60), 161 (75)	431.0983	0.80	$C_{21}H_{20}O_{10}$
dicaffeoylquinic acid 2	10.25	515	515 (25), 353 (70), 191 (100), 179 (75)	515.1194	1.60	$C_{25}H_{24}O_{12}$
naringenin-7-*O*-glucoside (prunin)*	10.70	433	433 (30), 271 (100), 151 (50)	433.1140	1.10	$C_{21}H_{22}O_{10}$
tricaffeoylquinic acid 1	11.31	677	677 (25), 515 (100), 353 (40), 191 (15)	677.1511	1.40	$C_{34}H_{30}O_{15}$
quercetin*	11.93	301	301 (10), 151 (100)	301.0353	0.40	$C_{15}H_{10}O_7$
tricaffeoylquinic acid 2	12.98	677	677 (55), 515 (100), 353 (20), 173 (15)	677.1511	1.00	$C_{34}H_{30}O_{15}$
naringenin*	13.03	271	151 (100), 119 (70)	271.0611	1.50	$C_{15}H_{12}O_5$

[a]The asterisk (*) indicates comparison with standard. [b]rt, retention time. [c]mDa. millidaltons of error between the mass found and the of each polyphenol. [d]MF, molecular formula.

fragmentations involving cleavage of three caffeoyl moieties for tricaffeoylquinic acid isomers (Table 4).

Flavone Derivatives. The flavone apigenin is known to have a number of biological functions such as possible anti-inflammatory,

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cytotoxic, and free radical scavenging properties. Studies of human malignant cancer cell lines have shown that apigenin inhibits cancer cell growth via apoptosis.[35] The analysis in full scan mode in the triple-quadrupole system showed the presence of apigenin 7-*O*-glucoside (m/z 431). The product ion scan of m/z 431 showed peaks at m/z 269 and 151, which were attributed to the loss of a glucose moiety $[M - H - 162]^-$ and to the ion corresponding to retro-Diels–Alder (RDA) fragmentation, as described by other authors.[36,37] These results were confirmed by injection in the ToF-MS using accurate mass measurements and the MS^2 fragmentation patterns (Table 4). The smallest differences between organic and conventional tomatoes were shown for apigenin-7-*O*-glucoside. Levels of apigenin-7-*O*-glucoside were higher in organic tomatoes than in conventional ones. However, the differences were not so pronounced as in the case of phenolic and hydroxycinnamoylquinic acid derivatives. Organic tomatoes contained 31.63 μg/g FW (351.44 μg/g DW) apigenin-7-*O*-glucoside, whereas conventional tomatoes contained 28.28 μg/g FW (353.50 μg/g DW) (Table 3).

Apigenin-*C*-hexoside-pentoside (m/z 563) and apigenin-*C*-hexoside-hexoside (m/z 593) were tentatively identified in ToF-MS mode in conventional and organic tomatoes. The product ion scan of *C*-diglycosides revealed characteristic losses of 60, 90, and 120 u corresponding to cross-ring cleavages in the sugar unit (Table 4). Apigenin-*C*-hexoside-hexoside and apigenin-*C*-hexoside-pentoside could be distinguished by the presence of the ion $[M - H - 60]^-$ according to the method cited by other authors.[20,38]

Flavanone Derivatives. The chromatograms in the triple-quadrupole revealed the presence of naringenin (m/z 271). It was identified by comparing their retention times with their reference substance. These results were confirmed by injection in the high-resolution system (ToF-MS) using accurate mass measurements and the MS^2 fragmentation patterns (Table 4). Naringenin has been identified as one of the major polyphenols in tomatoes. A recent study demonstrated that subchronic administration of flavanones significantly attenuated the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the decrease in striatal dopamine (DA) concentrations. This suggests that these compounds may be considered as potential candidates for a dietary supplement in the treatment of Parkinson's disease.[39]

Two naringenin-*O*-hexosides (m/z 433) and one naringenin-*C*-hexoside (m/z 433) were tentatively identified in the ToF-MS mode (Table 4). The MS^2 mass spectrum of m/z 433, which has been assigned to *O*-substitution, showed an ion at m/z 271 corresponding to the loss of a hexoside moiety $[M - H - 162]^-$, whereas the product ion scan of naringenin-*C*-hexoside revealed characteristic losses of 90 and 120 u from m/z 433 corresponding to cross-ring cleavages in the sugar unit.

Both naringenin and naringenin-7-*O*-glucoside (prunin) were higher in organic tomatoes (Table 3). Naringenin levels were 36.46 μg/g FW (455.75 μg/g DW) in conventional tomatoes and 87.38 μg/g FW (970.88 μg/g DW) in organic tomatoes, whereas levels of naringenin-7-*O*-glucoside varied from 7.68 μg/g FW (96.00 μg/g DW) in conventional to 13.91 μg/g FW (154.56 μg/g DW) in organic tomatoes.

Moreover, a peak showing m/z 595 could be attributed to naringenin-*C*-diglycoside (Table 4). The MS^2 of this ion displayed losses of 90 and 120 u from m/z 595 and 475, respectively, which confirmed the presence of two hexose (glucose or galactose) units.

Flavonol Derivatives. The examination of the chromatograms in the triple-quadrupole in MS mode of organic and conventional tomatoes revealed the presence of some peaks at m/z 609, 593, and 301 corresponding to rutin, kaempferol-3-*O*-rutinoside, and quercetin, respectively. These results were confirmed by injection in the high-resolution system (ToF-MS) (Table 4). The MS^2 of m/z 609 and 593 showed peaks corresponding to the deprotonated molecule $[M - H]^-$ and the loss of the rutinoside unit, $[M - H - 308]^-$. Flavonol aglycons such as quercetin gave as a characteristic ion the deprotonated molecule $[M - H]^-$ and ions corresponding to RDA fragmentation.

The organic tomatoes had higher levels of rutin, 272.75 μg/g FW (3030.56 μg/g DW), than the conventional ones, 119.82 μg/g FW (1497.75 μg/g DW). Similarly, the rates of quercetin and kaempferol-3-*O*-rutinoside were much lower in the conventionally produced tomatoes (Table 3). Mitchell et al.[40] described results in line with our study, reporting that the mean levels of the flavonoids quercetin and kaempferol were significantly higher ($P < 0.001$) in tomato samples from the organic cropping system than in those produced conventionally. They suggested that there is a significant difference between the two systems in the amount of flavonoids occurring in ripe fruit at harvest. Therefore, different food cultivation methods may result in differences in the content of secondary metabolites such as polyphenol compounds, and such an increase may have health-related effects because polyphenols increase significantly after organic food consumption.

The analysis in the ToF-MS confirmed the presence of rutin-*O*-hexoside (m/z 771) and rutin-*O*-pentoside (m/z 741) in both conventional and organic tomatoes (Table 4). The MS^2 mass spectrum of m/z 771 showed peaks at m/z 609 and 300 corresponding to the loss of a hexoside $[M - H - 162]^-$ and to the radical anion of the aglycone (m/z 300) as described in other studies using a triple-quadrupole mass spectrometer.[19,41] The MS^2 mass spectrum of m/z 741 was assessed, showing peaks at m/z 609 and 300 corresponding to the loss of a pentoside $[M - H - 132]^-$ and to the radical anion of the aglycone (m/z 300).

In addition, quercetin-*O*-dihexoside (m/z 625) was also identified in the ToF-MS (Table 4). The MS^2 of m/z 625 showed ions at m/z 463 and 300 corresponding to the loss of one hexoside unit and two hexoside units, respectively.

A number of studies have addressed the question of whether agricultural chemicals and other agricultural methods including organic farming affect nutrient content. The question is still unresolved. When plants are grown with artificial nutrients, they are supposed to lose their natural defense mechanisms. This may result in reduced disease resistance and diluted contents of minerals, vitamins, and defense-related secondary metabolites, which are considered beneficial for human health. In the present study the growing conditions of tomatoes (conventional versus organic) affected the content of phenolic compounds of these vegetables. The organically produced tomatoes displayed a higher phytochemical concentration than conventionally produced tomatoes. Thus, vegetable and fruit products grown in organic agriculture would be expected to be more health-promoting than those produced conventionally. The LC-MS-MS method was completely validated, providing a sensitive analysis for polyphenol detection and showing satisfactory data for all parameters tested. Good results were obtained with respect to linearity and recovery as well as an excellent level of precision.

AUTHOR INFORMATION

Corresponding Author

*Phone: +34-934034843. Fax: +34-934035931. E-mail: lamuela@ub.edu.

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Notes

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REFERENCES

(1) Borguini, R.; Torres, E. Tomatoes and tomato products as dietary sources of antioxidants. *Food Rev. Int.* **2009**, *25*, 313−325.

(2) Ferreres, F.; Taveira, M.; Pereira, D. M.; Valentao, P.; Andrade, P. B. Tomato (*Lycopersicon esculentum*) seeds: new flavonols and cytotoxic effect. *J. Agric. Food Chem.* **2010**, *58*, 2854−2861.

(3) Ferreres, F.; Taveira, M.; Gil-Izquierdo, A.; Oliveira, L. S.; Teixeira, T.; Valentao, P.; Simoes, N.; Andrade, P. B. High-performance liquid chromatography-diode array detection-electrospray ionization multi-stage mass spectrometric screening of an insect/plant system: the case of *Spodoptera littoralis/Lycopersicon esculentum* phenolics and alkaloids. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 1972−1980.

(4) Taveira, M.; Silva, L. R.; Vale-Silva, L. A.; Pinto, E.; Valentâo, P.; Ferreres, F.; Guedes de Pinho, P.; Andrade, P. B. *Lycopersicon esculentum* seeds: an industrial byproduct as an antimicrobial agent. *J. Agric. Food Chem.* **2010**, *58*, 9529−9536.

(5) Vallverdu-Queralt, A.; Medina-Remon, A.; Martinez-Huelamo, M.; Jauregui, O.; Andres-Lacueva, C.; Lamuela-Raventos, R. M. Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. *J. Agric. Food Chem.* **2011**, *59*, 3994−4001.

(6) Toor, R. K.; Savage, G. P.; Heeb, A. Influence of different types of fertilisers on the major antioxidant components of tomatoes. *J. Food Compos. Anal.* **2006**, *19* (1), 20−27.

(7) Grieb, S. M. D.; Theis, R. P.; Burr, D.; Benardot, D.; Siddiqui, T.; Asal, N. R. Food groups and renal cell carcinoma: results from a case-control study. *J. Am. Diet. Assoc.* **2009**, *109*, 656−667.

(8) Connor, A. M.; Luby, J. J.; Tong, C. B. S.; Finn, C. E.; Hancock, J. F. Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. *J. Am. Soc. Hortic. Sci.* **2002**, *127*, 89−97.

(9) Barrett, D. M.; Weakley, C.; Diaz, J. V.; Watnik, M. Qualitative and nutritional differences in processing tomatoes grown under commercial organic and conventional production systems. *J. Food Sci.* **2007**, *72*, C441−C451.

(10) Williams, C. M. Nutritional quality of organic food: shades of grey or shades of green? *Proc. Nutr. Soc.* **2002**, *61*, 19−24.

(11) Daniel, O.; Meier, M. S.; Schlatter, J.; Frischknecht, P. Selected phenolic compounds in cultivated plants: ecologic functions, health implications, and modulation by pesticides. *Environ. Health Perspect.* **1999**, *107*, 109−114.

(12) Nielsen, S.; Molgaard, J. P.; Lærke, P. E. Growing potatoes, four cultivars for consumption: Bintje, Asva, Nicola and Ukama. SP rapport (Lyngby), 1997.

(13) Vallverdú-Queralt, A.; Medina-Remón, A.; Casals-Ribes, I.; Lamuela-Raventos, R. M. Is there any difference between the phenolic content of organic and conventional tomato juices? *Food Chem.* **2012**, *130*, 222−227.

(14) Vallverdu-Queralt, A.; Medina-Remon, A.; Casals-Ribes, I.; Amat, M.; Lamuela-Raventos, R. M. A metabolomic approach differentiates between conventional and organic ketchups. *J. Agric. Food Chem.* **2011**, *59*, 11703−11710.

(15) Grinder-Pedersen, L.; Rasmussen, S. E.; Bugel, S.; Jorgensen, L. V.; Dragsted, L. O.; Gundersen, V.; Sandstrom, B. Effect of diets based on foods from conventional versus organic production on intake and excretion of flavonoids and markers of antioxidative defense in humans. *J. Agric. Food Chem.* **2003**, *51*, 5671−5676.

(16) Kong, A. Y. Y.; Six, J.; Bryant, D. C.; Denison, R. F.; van Kessel, C. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. *Soil Sci. Soc. Am. J.* **2005**, *69*, 1078−1085.

(17) Vallverdú-Queralt, A.; Medina-Remón, A.; Andres-Lacueva, C.; Lamuela-Raventos, R. M. Changes in phenolic profile and ant ioxidant activity during production of diced tomatoes. *Food Chem.* **2011**, *126*, 1700−1707.

(18) Medina-Remón, A.; Barrionuevo-González, A.; Zamora-Ros, R.; Andres-Lacueva, C.; Estruch, R.; Martínez-González, M. A.; Diez-Espino, J.; Lamuela-Raventos, R. M. Rapid Folin-Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Anal. Chim. Acta* **2009**, *634*, 54−60.

(19) Vallverdú-Queralt, A.; Jáuregui, O.; Medina-Remón, A.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M. Improved characterization of tomato polyphenols using liquid chromatography/electrospray ionization linear ion trap quadrupole Orbitrap mass spectrometry and liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2986−2992.

(20) Vallverdú-Queralt, A.; Jáuregui, O.; Di Lecce, G.; Andrés-Lacueva, C.; Lamuela-Raventos, R. M. Screening of the polyphenol content of tomato-based products through accurate-mass spectrometry (HPLC-ESI-QTOF). *Food Chem.* **2011**, *129*, 877−883.

(21) Horwitz, W. *Official Methods of Analysis of AOAC International (Appendix D and E)*, 17 th ed., revision 2; Washington, DC, 2003; Vol. 2.

(22) Macheix, J.-J.; Feuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990; p 378.

(23) Radi, M.; Mahrouza, M.; Jaouad, A.; Amiot, M. J. Influence of mineral fertilization (NPK) on the quality of apricot fruit (cv. Canino). The effect of the mode of nitrogen supply. *Agronomie* **2003**, *23*, 737−745.

(24) Brandt, K.; Molgaard, J. P. Organic agriculture: does it enhance or reduce the nutritional value of plant foods? *J. Sci. Food Agric.* **2001**, *81*, 924−931.

(25) Dixon, R. A.; Paiva, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085−1097.

(26) Medina-Remon, A.; Zamora-Ros, R.; Rotches-Ribalta, M.; Andres-Lacueva, C.; Martinez-Gonzalez, M. A.; Covas, M. I.; Corella, D.; Salas-Salvado, J.; Gomez-Gracia, E.; Ruiz-Gutierrez, V.; Garcia de la Corte F. J.; Fiol, M.; Pena, M. A.; Saez, G. T.; Ros, E.; Serra-Majem, L.; Pinto, X.; Warnberg, J.; Estruch, R.; Lamuela-Raventos, R. M. Total polyphenol excretion and blood pressure in subjects at high cardiovascular risk. *Nutr. Metab. Cardiovasc. Dis.* **2011**, *21*, 323−331.

(27) Agence Francüaise de Sécurité Sanitaire des Aliments Evaluation des Risques et Bénéfices Nutritionnels et Sanitaires des Aliments Issus de l'Agriculture Biologique, 2003.

(28) Le Gall, G.; Dupont, M. S.; Mellon, F. A.; Davis, A. L.; Collins, G. J.; Verhoeyen, M. E.; Colquhoun, I. J. Characterization and content of flavonoid glycosides in genetically modified tomato (*Lycopersicon esculentum*) fruits. *J. Agric. Food Chem.* **2003**, *51*, 2438−2446.

(29) Chassy, A. W.; Bui, L.; Renaud, E. N. C.; Van Horn, M.; Mitchell, A. E. Three-year comparison of the content of antioxidant microconstituents and several quality characteristics in organic and conventionally managed tomatoes and bell peppers. *J. Agric. Food Chem.* **2006**, *54*, 8244−8252.

(30) Cuvelier, M. E.; Richard, H.; Berset, C. Comparison of the antioxidative activity of some acid-phenols − structure activity relationship. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 324−325.

(31) Silva, F. A. M.; Borges, F.; Guimarães, C.; Lima, J. L. F. C.; Matos, C.; Reis, S. Phenolic acids and derivatives:studies on the

relationship among structure, radical scavenging activity, and physicochemical parameters. *J. Agric. Food Chem.* **2000**, *48*, 2122−2126.

(32) Caris-Veyrat, C.; Amiot, M. J.; Tyssandier, V.; Grasselly, D.; Buret, M.; Mikolajczak, M.; Guilland, J. C.; Bouteloup-Demange, C.; Borel, P. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *J. Agric. Food Chem.* **2004**, *52*, 6503−6509.

(33) Parejo, I.; Jauregui, O.; Sanchez-Rabaneda, F.; Viladomat, F.; Bastida, J.; Codina, C. Separation and characterization of phenolic compounds in fennel (*Foeniculum vulgare*) using liquid chromatography-negative electrospray ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 3679−3687.

(34) Fang, N. B.; Yu, S. G.; Prior, R. L. LC/MS/MS characterization of phenolic constituents in dried plums. *J. Agric. Food Chem.* **2002**, *50*, 3579−3585.

(35) Choi, E.; Kim, G. Apigenin induces apoptosis through a mitochondria/caspase-pathway in human breast cancer MDA-MB-453 cells. *J. Clin. Biochem. Nutr.* **2009**, *44*, 260−265.

(36) Fabre, N.; Rustan, I.; de Hoffmann, E.; Quetin-Leclercq, J. Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 707−715.

(37) Sanchez-Rabaneda, F.; Jauregui, O.; Casals, I.; Andres-Lacueva, C.; Izquierdo-Pulido, M.; Lamuela-Raventos, R. M. Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *J. Mass Spectrom.* **2003**, *38*, 35−42.

(38) Han, J.; Ye, M.; Qiao, X.; Xu, M.; Wang, B. R.; Guo, D. A. Characterization of phenolic compounds in the Chinese herbal drug *Artemisia annua* by liquid chromatography coupled to electrospray ionization mass spectrometry. *J. Pharm. Biomed.* **2008**, *47*, 516−525.

(39) Zbarsky, V.; Datla, K. P.; Parkar, S.; Rai, D. K.; Aruoma, O. I.; Dexter, D. T. Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease. *Free Radical Res.* **2005**, *39*, 1119−1125.

(40) Mitchell, A. E.; Hong, Y. J.; Koh, E.; Barrett, D. M.; Bryant, D. E.; Denison, R. F.; Kaffka, S. Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *J. Agric. Food Chem.* **2007**, *55*, 6154−6159.

(41) Hvattum, E.; Ekeberg, D. Study of the collision-induced radical cleavage of flavonoid glycosides using negative electrospray ionization tandem quadrupole mass spectrometry. *J. Mass Spectrom.* **2003**, *38*, 43−49.