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Setup of a UHPLC−QqQ-MS Method for the Analysis of Phenolic Compounds in Cherry Tomatoes, Tomato Sauce, and Tomato Juice

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ABSTRACT: The setup of a novel, rapid, and sensitive UHPLC−QqQ-MS method was described for the determination of phenolic compounds in tomatoes and tomato-based products (tomato sauce and juice). Phenolic compounds including hydroxybenzoic and hydroxycinnamic acid derivatives, flavonols, and flavanones were detected, separated, and quantified in a 3 min chromatographic run. The main advantages of the method include high analyte recovery (90.1−115%), low limit of detection (0.008−0.167 mg L^{-1}) and quantification (0.01−0.83 mg L^{-1}), good accuracy (85.6−115%), and precision (<15%). The detection of the phenolic compounds varied according to the physicochemical nature of the extracts, but generally low matrix-dependent suppression/enhancement effects were observed in all three matrices. The possibility to transfer easily the existing HPLC to the fast UHPLC methods is very attractive, and with minor modifications, the methodology described may be applied to the phenolic characterization of a broad range of plant and food matrices.

KEYWORDS: *tomato, tomato sauce, tomato juice, phenolic compounds, UHPLC−QqQ-MS, matrix effects*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill. Solanaceae) is one of the most eaten foodstuffs in Mediterranean countries such as Spain and Italy. The extensive use of tomatoes and their processed products (fried or raw canned tomatoes, tomato sauce, tomato paste, tomato juice) as ingredients in prepared foods such as pizza, pasta, snacks, and a variety of vegetable dishes makes their consumption continuous grow worldwide.[1] Overwhelming evidence from nutritional studies indicates that regular consumption of raw tomatoes and tomato-based products is consistently associated with a reduction in the incidence of chronic-degenerative diseases.[2,3] Together with the most extensively studied bioactive compounds contained in tomato such as vitamins (vitamin C, folates) or carotenoids, the health-promoting effects of its (poly)phenolic constituents is attracting interest, including a potential role in lowering risk of cardiovascular diseases, metabolic alterations, and neurodegeneration, as well as in inhibiting cellular proliferation, modulating enzymatic activities, or signal transduction pathways.[4−11]

Phenolic acids are the most representative phenolic compounds found in tomato and processed products, including hydroxybenzoic (C6−C1) and hydroxycinnamic acids (C6−C3) and their ester conjugates, with chlorogenic acid being one of the most abundant.[12,13] A high content of the chalcone and flavanone forms of naringenin (reported as the most abundant phenolic compound in tomato sauce) and the flavonol rutin has also been described in tomato-based products.[13−16] Also more complex phenolic constituents have been described,[16−18] although quantification may be difficult due to the absence of pure reference standard.

High-performance liquid chromatography (HPLC) is the most commonly used technique for the characterization of the phenolic composition of several food matrices, generally coupled to photo diode array[19,20] or mass spectrometry detection.[18−22] Over the past few years, there has been great interest in developing ultra-HPLC (UHPLC) approaches to speed up and increase the resolving power of the analytical separation process. The UHPLC technology arises from the use of small particle size results in higher plate numbers, as well as faster separations, in accordance with the Van Deemter theory.[23] These effects are due to the fact that (a) the chromatographic efficiency is directly proportional to the ratio of column length and particle diameter, and (b) the mobile phase linear velocity is inversely proportional to the particle diameter.[24,25] Coupled to triple quadrupole mass spectrometry (UHPLC−QqQ-MS) it may offer a powerful tool for the quick and quantitative screening of a large number of phytochemical compounds through multiple reaction monitoring (MRM),[16,26,27] thus there is an interest in transferring previous methods performed in conventional HPLC conditions to UHPLC methods. However, it is crucial to verify the efficiency of the new chromatographic methods and evaluate the possible

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Table 1. Phenolic Compounds Analyzed in Cherry Tomatoes, Tomato Sauce, and Tomato Juice[a]

compounds	t_R (min)	$[M - H]^{-1}$	m/z ions	DP (V)	CE (V)	FP (V)	quantification transition
protocatechuic acid	0.61 ± 0.005	153	109	−40	−20	−150	153 → 109
4-hydroxybenzoic acid	0.83 ± 0.003	137	93	−40	−20	−200	137 → 93
chlorogenic acid	0.90 ± 0.010	353	191, 179	−50	−20	−180	353 → 191
caffeic acid	1.16 ± 0.006	179	135	−40	−20	−170	179 → 135
o-coumaric acid	1.53 ± 0.040	163	119	−40	−25	−150	163 → 119
ethyl gallate (IS)	1.61 ± 0.007	197	169	−60	−25	−200	197 → 169
rutin	1.68 ± 0.005	609	300	−60	−50	−230	609 → 300
ferulic acid	1.73 ± 0.010	193	178, 149, 134	−40	−20	−170	193 → 134
naringenin-7-*O*-glucoside	1.96 ± 0.009	433	271	−50	−30	−280	433 → 271
naringenin	2.13 ± 0.010	271	177, 151, 119	−50	−30	−190	271 → 151

[a] t_R, retention time; DP, declustering potential; CE, collision energy; FP, focusing potential.

matrix effects which may increase the level of random errors.[28,29] In the present work, the setup, validation, and application of a novel, rapid, and sensitive UHPLC−QqQ-MS method for the analysis of phenolic compounds (hydroxybenzoic and hydroxycinnamic acid derivatives, flavonols, and flavanones) in tomato fruits, tomato sauce, and tomato juice is reported.

MATERIALS AND METHODS

Standards and Solvents. The standards were handled without exposure to light. Protocatechuic acid, 3- and 4-hydroxybenzoic acid, chlorogenic acid (5-caffeoylquinic acid), gallic acid, caffeic acid, ferulic acid, *o*-, *m*-, and *p*-coumaric acids, naringenin-7-*O*-glucoside, naringin (naringenin-7-*O*-rhamnoglucoside), and rutin (quercetin-3-*O*-rhamnosyl-glucoside) were purchased from Sigma-Aldrich (St. Louis, MO, United States). Naringenin (4′,5,7-trihydroxyflavanone), and kaempferol-3-*O*-glucoside were supplied by Extrasynthese (Genay, France). HPLC-grade acetonitrile, formic acid, and ethanol were purchased from ScharlauChemie S. A. (Barcelona, Spain), while ultrapure water (Milli-Q) was obtained from a Millipore system (Millipore, Bedford, MA, United States).

Commercial Samples. Unprocessed tomato fruits and commercial tomato products were purchased from a local market (Barcelona, Spain). Cherry-type tomatoes were selected to represent the commonly consumed unprocessed tomatoes,[30] whereas tomato sauce and juice were chosen as representative tomato processed products due to their wide consumption worldwide and the different physicochemical properties of the final products (thus matrix effects), as a consequence of the different manufacturing process (i.e., tomato juice contains about 90% of water and is an intermediate product in the making process of the sauce).[31]

Preparation of Standard and Stock Solutions. Individual stock solutions of the standard phenolic compounds (protocatechuic, 4-hydroxybenzoic, chlorogenic, caffeic, ferulic and *o*-coumaric acid, naringenin-7-*O*-glucoside, naringenin, and rutin) were prepared at a concentration of 1 mg mL^{-1} in 80% methanol. A phenolic pool obtained by mixing the individual standard solutions with acidified water (0.1% formic acid) was used as working solution to spike the cherry tomatoes, tomato sauce, and juice extracts. A solution of ethyl gallate at a final concentration of 400 ng mL^{-1} was prepared in acidified water (0.1% formic acid) and used as an internal standard (IS) to check for the extraction efficiency. All standard solutions were stored at −80 °C.

Sample Extraction. Sample extraction was performed in a darkroom with a red safety light as previously described by our research group,[32,33] with some modifications. Aliquots of cherry tomatoes, tomato juice, and tomato sauce (0.3 g each) were homogenized with a blender over an ice bed with 3 mL of 80% ethanol in acidified Milli-Q water (0.1% formic acid), after the addition of the IS and, eventually, of the phenolic standard pool. The homogenate was sonicated for 5 min and centrifuged at 900*g* for 20 min at 4 °C. The supernatant was collected, and the extraction procedure was repeated. Both supernatants were combined and evaporated to dryness on a sample concentrator (Techne, Duxford, Cambridge, U.K.) at room temperature under a stream of nitrogen gas. The residues were reconstituted up to 1.2 mL of water contained 0.1% formic acid, filtered thought a 0.22 μm polytetrafluoroethylene (PTFE) syringe filters (Waters Corporation, United States), and injected into a UHPLC−QqQ system.

UHPLC System. The UHPLC system consisted of an Acquity UHPLC equipped with a Waters binary pump system (Milford, MA, United States), and a Waters BEH C_{18} column (50 mm × 2.1 mm) packed with 1.7 μm particles. The samples were injected into a 10 μL loop, with a mobile-phase flow rate of 400 μL min^{-1}. Gradient elution was carried out with a solvent system of water/formic acid (99.9:0.1 v/v) as mobile phase A and acetonitrile/formic acid (99.9:0.1 v/v) as mobile phase B; the total run time was 3 min, and the gradient elution was as follows: 0.0−1.1 min, B 5−18%; 1.1−1.8 min, B 18−50%; 1.8−2.4 min, B 50−74%; 2.4−2.5 min, B 74−100%; 2.5−2.7 min, B 100−5%; 2.7−3.0 min, B 5%. All the solvents were filtered through 0.22 μm PTFE filters (Waters Corporation, Milford, MA, United States) prior to use. The column was maintained at 30 °C while the autosampler was thermostated at 4 °C. The system was controlled by Analyst v. 1.4.2 software supplied by Applied Biosystems (Foster City, CA, United States).

Mass Spectrometry Conditions. The UHPLC was coupled online with an API 3000 (ABSciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer equipped with a TurboIonSpray source in negative-ion mode to obtain MS/MS data. TurboIonSpray source settings were as follows: capillary voltage −3500 V; nebulizer gas (N_2) 10 (arbitrary units); curtain gas (N_2) 12 (arbitrary units); collision gas (N_2) 4 (arbitrary units); focusing potential −200 V; entrance potential 10 V; drying gas (N_2) heated to 400 °C and introduced to a flow rate of 8000 cm^3 min^{-1}. The declustering potential (DP), collision energy (CE), and focusing potential (FP) were optimized for each compound by infusion experiments of individual standard solutions (10 μg μL^{-1}) dissolved in a 50:50 (v/v) mobile phase at a constant flow rate of 5 μL min^{-1}, using a model syringe pump (Harvard Apparatus, Holliston, MA, United States). 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 0.002 s; dwell time was set at 0.016 s. To confirm the identity of some compounds, neutral loss scan and product ion scan experiments were carried out. MS/MS product ions were produced by collision-activated dissociation (CAD) of selected precursor ions in the collision cell of the QqQ mass spectrometer and mass analyzed using the instrument's second analyzer. Additional experimental conditions for MS/MS included collision energy (depending on the compound), CAD gas (nitrogen) at 6 (arbitrary units), and scan range, as necessary for the precursor selected. Neutral loss scan of 162 u was done by scanning within the m/z range from 200 to 800 u, and product ion scan experiments were carried out by scanning within 300 and 800 u. In all the experiments, both quadrupoles (Q1 and Q3) were operated at unit resolution.

The phenolic compounds present in cherry tomatoes and in the tomato-based products were detected and quantified by using the

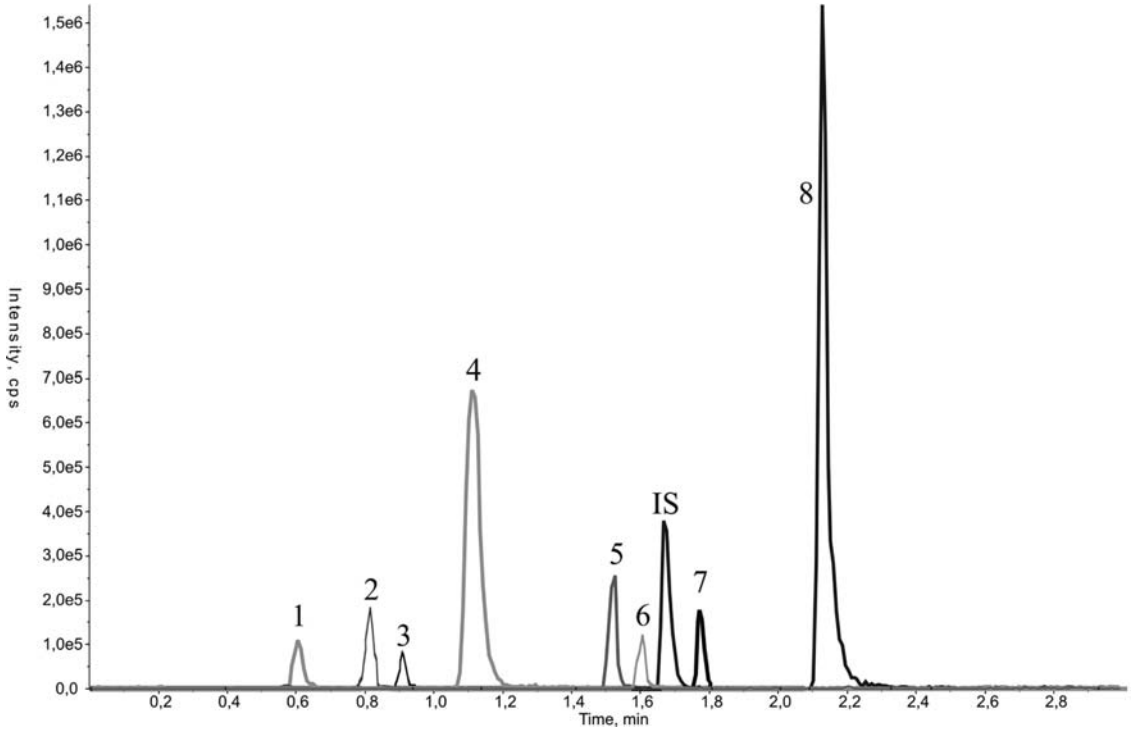

Figure 1. UHPLC chromatogram of spiked and with internal standard tomato juice extract. Peaks: (1) protocatechuic acid, (2) 4-hydroxybenzoic acid, (3) chlorogenic acid, (4) caffeic acid, (5) *o*-coumaric acid, (IS) ethyl gallate, (6) rutin, (7) ferulic acid, and (8) naringenin.

multiple reaction monitoring mode (MRM), tracking the transition of parent and product ions specific to each compound (Table 1).

Method Validation. Quantitative analysis was performed by means of the standard-addition method.[34] In this way, besides estimating the unknown amount of polyphenols occurring in the extracts, it was possible to evaluate sensitivity and linear dynamic range in the different matrices. Recoveries, precision, limits of detection (LODs), and limits of quantitation (LOQs) were calculated after having determined the natural levels of each phenolic compound in the each tomato-based product.

For calibration curve preparation (seven points in duplicate) six of the seven aliquots of tomato, juice, and sauce samples were spiked with different concentrations (50%, 100%, 150%, 250%, 300%, and 400%) of the phenolic standard pool prior to extraction, while the remaining nonspiked aliquot was added with the corresponding volume of Milli-Q water, to maintain the same dilution factor across the samples.

The sensitivity of the method was evaluated by determining the LODs and LOQs. The LOD was calculated as the quantity of analyte able to produce a chromatographic peak three times higher than the noise of the baseline in a chromatogram (S/N = 3) of a nonspiked sample, after having estimated the endogenous amount. The LOQ was set at ten times higher than the noise of the baseline in a chromatogram (S/N = 10). Five replicates were carried out for LOD and LOQ determination.

For analyte recoveries, seven-point calibration curves (0%, 50%, 100%, 150%, 250%, 300%, and 400%) were prepared by spiking tomato and tomato based products (tomato juice and tomato sauce) after and before extraction. Analyte recoveries were determined by subtracting the response obtained from the analyte added and extracted from the matrix, compared to the detector response obtained for the same concentration of the standard added after the extraction. To assess intraday precision and accuracy, six replicates of cherry tomato, tomato sauce, and tomato juice extracts spiked at three different concentration levels (50%, 150%, 300%) were prepared, extracted, and analyzed by UHPLC–QqQ-MS. The procedure was repeated on three different days to determine interday precision. Spiked samples used to assess intra- and interday precision were prepared daily. Accuracy was calculated as the ratio of the mean observed concentration and the known spiked concentration in the matrix and was expressed as [(mean observed concentration)/(added concentration)] × 100. Precision is expressed as the relative standard deviation (% RSD) of all determinations.

Finally, suppression/enhancement effects were also evaluated by calculating the ratio of the peak area of the analytes recorded for the sample spiked with the standards after extraction and the peak area of analytes recorded for the standard solution (expressed in percentage). The described method was fully validated following the criteria of the AOAC International for method validation.[34]

Statistics. The concentration of the phenolic compounds quantified in the extracts was expressed as mean ± SD (μg g^{-1} FW). One-way analysis of variance for mean comparison (SPSS software, Version 17.0 Japan Inc., Tokyo, Japan) was used to assess the observed differences in phenolic content among technical replicates and food matrices. Differences were considered to be statistically significant when the p-value was <0.05.

RESULTS AND DISCUSSION

Until now, HPLC–MS/MS-based methods have been mainly used to study the levels of phenolics in different varieties of tomato,[35] diced tomatoes, and tomato sauces.[36] It was also used to evaluate the effects of storage on phenolic compounds[36] and the effects of pulsed electric fields on tomato polyphenols.[37] The method presented and validated in this work allows quantification of the main phenolic compounds present in tomatoes and tomato-based products (hydroxybenzoic and hydroxycinnamic acid derivatives, flavonols, and flavanones) in a total run time of 3.0 min (Figure 1), thus proposing a useful alternative to conventional HPLC–MS/MS profiling in terms of analysis time, costs, and improved resolution and sensitivity.[1,38,39]

A preliminary screening of the main phenolic compounds present in the dietary matrices in the study was assessed (data not shown). Then, the optimization of the method achieved the best chromatographic (gradient, flow rate, injection volume,

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Table 2. Validation Parameters[a]

compounds	regression eq	linear fit	concn range (mg L^{-1})	LODs (mg L^{-1})	LOQs (mg L^{-1})	recovery (%)
			Cherry Tomato			
protocatechuic acid	$y = 2.1437x + 0.2930$	0.9926	0.04−0.40	0.008	0.020	98.10 ± 7.60
chlorogenic acid	$y = 1.0833x + 1.6091$	0.9957	1.50−12.00	0.045	0.140	99.10 ± 8.70
caffeic acid	$y = 3.1861x + 4.3740$	0.9935	0.50−4.00	0.098	0.390	90.10 ± 3.90
o-coumaric acid	$y = 0.9943x + 1.9596$	0.9901	0.50−4.00	0.034	0.300	96.50 ± 9.80
rutin	$y = 0.1001x + 12.998$	0.9901	14.00−210.00	0.122	0.980	98.60 ± 12.00
ferulic acid	$y = 0.0311x + 0.0724$	0.9907	0.80−6.40	0.129	0.460	110.00 ± 1.90
naringenin-7-*O*-glucoside	$y = 2.4943x + 0.1338$	0.9928	0.10−10.00	0.010	0.080	99.40 ± 10.00
naringenin	$y = 0.4051x + 23.568$	0.9902	12.4−99.20	0.072	0.590	97.80 ± 4.10
			Tomato Sauce			
protocatechuic acid	$y = 1.0273x + 0.5232$	0.9944	0.12−0.96	0.027	0.100	113.00 ± 8.40
chlorogenic acid	$y = 0.6282x + 3.7147$	0.9926	3.80−30.40	0.078	0.260	95.90 ± 13.00
caffeic acid	$y = 1.7994x + 5.2278$	0.9918	0.70−5.60	0.056	0.180	101.00 ± 6.60
o-coumaric acid	$y = 1.0255x + 0.8993$	0.9938	0.50−4.00	0.020	0.300	95.30 ± 14.00
rutin	$y = 0.0820x + 9.3400$	0.9908	11.50 −192.00	0.150	0.830	108.00 ± 12.00
naringenin-7-*O*-glucoside	$y = 0.6687x + 3.3727$	0.9940	1.40−11.20	0.085	0.580	99.20 ± 10.00
naringenin	$y = 0.2047x + 26.325$	0.9915	20.00−220.00	0.154	0.640	105.50 ± 13.00
			Tomato Juice			
protocatechuic acid	$y = 2.2568x + 0.1449$	0.9927	0.20−1.80	0.021	0.120	98.20 ± 3.20
4-hydroxybenzoic acid	$y = 0.5833x + 2.5876$	0.9972	0.30−2.40	0.083	0.260	100.00 ± 6.10
chlorogenic acid	$y = 0.6934x + 0.0605$	0.9900	0.08−0.80	0.031	0.050	101.00 ± 5.60
caffeic acid	$y = 2.5983x + 4.3194$	0.9951	0.40−3.20	0.167	0.310	101.00 ± 6.30
o-coumaric acid	$y = 0.2520x + 0.1709$	0.9922	0.50−10.00	0.087	0.340	99.30 ± 6.20
rutin	$y = 0.2766x + 1.0627$	0.9952	1.50−12.00	0.136	0.650	101.00 ± 0.20
ferulic acid	$y = 0.0345x + 0.0847$	0.9971	1.50−12.00	0.108	0.490	104.00 ± 8.60
naringenin	$y = 0.8636x + 3.7910$	0.9915	1.20−9.60	0.012	0.110	91.40 ± 10.00

[a]Regression equation, linear fit, concentration range, limits of detection (LODs) and quantification (LOQs), and recovery (%) in cherry tomatoes, sauce, and juice extracts.

etc.) and MS conditions parameters (ESI mode, DP, CE, FP, MRM quantitative transition, cycle time, dwell time) for the targeted compounds that were selected during the method optimization phase (Table 1). Finally, the performance validation of the method was carried out by evaluating the quality parameters established for the method validation: linearity, LODs, LOQs, recovery, selectivity, accuracy, precision, and matrix effect (Table 2 and Table 3).

Validation parameters. Linearity and Limits of Detection and Quantification. The linearity of the method was evaluated by analyzing six calibration standards in duplicate over the nominal concentration range (Table 2). A good linearity was obtained, and all correlation coefficients exceeded 0.99. Slopes, which are representative of the method sensitivity, resulted very similar in the different matrices; this suggests that the applied extraction procedure cleaned up the extracts from the various matrices analogously, producing a comparable matrix effect.

The LOD and LOQ values obtained showed a wide range of sensitivity among the different analytes (Table 2). LODs ranged from 0.008 mg L^{-1} to 0.129 mg L^{-1} in cherry tomato, 0.020 to 0.154 mg L^{-1} in tomato sauce, and 0.012 to 0.167 mg L^{-1} in tomato juice. The LOQs varied between 0.02 and 0.98 mg L^{-1} in cherry tomato, 0.10 and 0.83 mg L^{-1} in sauce, and 0.01 and 0.65 mg L^{-1} in juice. The sensitivity of this method represents a significant improvement for most of the analytes when compared to published LC−MS methods in tomatoes and tomato-based products.[14,40,41] Gómez-Romero et al.[40] reported LODs between 0.03 and 1.50 mg L^{-1} and Vallverdú-Queralt et al.[41] higher than 0.0475 mg L^{-1}. The LOD and LOQ obtained are in agreement with data recently proposed in the analysis of low molecular weight polyphenols in several vegetables.[42] Thus, this method offers a strong alternative to conventional HPLC−MS/MS in terms of improved resolution and sensitivity.

Recovery, Accuracy, Precision, and Matrix Effects. The results showed comparable levels of recovery in spiked cherry tomato, and tomato sauce and juice extracts, obtaining values higher than 95% except for caffeic acid, which had recoveries between 90.1 and 94.5% in cherry tomato and juice extracts, respectively. On the other hand, high values of recovery were obtained for protocatechuic acid in tomato sauce extracts (113 ± 8.4) and ferulic acid in cherry tomato (110 ± 1.9) and juice extracts (115 ± 14), respectively (Table 2). Recovery values resulted independent by the applied fortification level. These values are similar to those reported by other authors in tomatoes and tomatoes based products.[13,41]

The precision of the method, expressed as % RSD, met acceptance criteria, since % RSD was lower than 15% at each tested concentration level for intra- and interday precision. These results are in agreement with those reported in a study carried out by Vallverdú-Queralt et al., who reported levels of % RSD lower than 15% in raw tomatoes.[41] The accuracy of phenolic compounds for tomatoes, tomato sauce, and tomato juice ranged between 85.6% and 113%, between 82.8% and 115%, and between 86% and 113%, respectively. These values were acceptable for the low, medium, and high concentration levels (Table 3).

In the evaluation of matrix effects, values lower than 100% indicate that matrix-dependent signal suppression occurs, while values higher than 100% indicate matrix-dependent signal enhancement.[43] The eventual suppression and enhancement

Table 3. Cherry Tomato and Tomato-Based Product Validation: Accuracy and Intraday and Interday Precision[a]

	low concentration (mg L^{-1})						medium concentration (mg L^{-1})						high concentration (mg L^{-1})					
			intraday (% RSD)						intraday (% RSD)						intraday (% RSD)			
analyte	added	accuracy (%)	1	2	3	interday (% RSD)	added	accuracy (%)	1	2	3	interday (% RSD)	added	accuracy (%)	1	2	3	interday (% RSD)
Cherry Tomato																		
protocatechuic acid	0.04	92.50	7.40	6.90	4.90	12.50	0.12	91.60	3.00	4.20	3.70	7.62	0.24	92.90	12.00	5.20	4.10	8.04
chlorogenic acid	1.50	86.60	7.20	0.70	4.70	2.83	4.50	97.10	4.50	1.00	3.10	3.39	9.00	89.10	4.50	1.90	1.50	7.99
caffeic acid	0.50	92.20	3.50	3.70	6.00	2.66	1.50	91.50	3.60	1.20	6.90	9.43	3.00	89.30	3.50	1.10	3.60	8.17
o-coumaric acid	0.50	94.00	2.70	7.30	3.50	3.62	1.50	85.60	3.20	8.60	3.70	12.50	3.00	113.00	4.70	2.20	3.00	3.32
rutin	14.0	89.40	2.90	2.40	5.40	1.18	42.0	85.90	2.20	1.00	5.30	4.94	84.0	87.40	1.80	0.70	3.20	8.43
ferulic acid	0.80	92.70	3.30	2.30	2.40	1.72	2.40	88.10	2.20	9.00	6.00	9.33	4.80	99.90	2.20	3.20	9.20	7.25
naringenin-7-*O*-glucoside	0.10	87.00	7.70	4.40	6.70	6.15	0.30	86.30	6.40	1.30	7.80	8.47	0.60	86.60	13.00	1.60	5.10	7.81
naringenin	12.4	95.50	6.60	13.10	7.40	14.00	37.2	90.00	5.90	2.60	7.10	5.12	74.40	87.90	2.40	3.70	7.10	9.50
Tomato Sauce																		
protocatechuic acid	0.12	114.00	1.80	2.90	2.00	5.86	0.36	112.00	1.30	2.70	2.50	3.37	0.72	82.80	12.00	2.00	6.50	7.30
chlorogenic acid	3.80	101.00	8.40	3.40	4.30	5.87	11.4	98.00	12.00	0.80	5.60	14.90	22.80	96.60	2.00	4.20	5.90	6.44
caffeic acid	0.70	97.40	1.70	1.50	5.10	10.7	2.10	115.00	4.60	3.10	3.40	13.50	4.20	98.20	3.20	1.10	2.40	6.02
o-coumaric acid	0.50	102.00	12.00	7.20	6.20	6.54	1.50	112.00	7.20	3.20	4.40	5.02	3.00	115.00	8.50	1.60	6.10	12.50
rutin	11.5	113.00	9.00	4.90	4.80	8.43	34.5	91.20	10.00	9.50	12.50	8.55	69.00	96.40	12.00	3.10	2.10	5.98
naringenin-7-*O*-glucoside	1.40	92.50	4.20	2.80	4.40	7.82	4.20	97.20	2.00	3.20	6.10	10.00	8.40	92.10	2.70	2.60	9.50	3.96
naringenin	20.0	96.40	7.80	8.70	2.50	7.51	60.0	90.70	1.90	1.10	1.10	1.15	120.00	88.00	2.50	3.00	2.70	12.20
Tomato Juice																		
protocatechuic acid	0.60	96.60	13.00	10.00	5.40	5.29	0.12	109.00	5.20	8.90	1.90	6.02	0.36	90.30	14.00	11.00	3.00	6.41
4-hydroxybenzoic acid	0.30	87.30	4.00	5.00	2.20	8.87	0.90	87.00	14.00	13.00	7.60	7.24	1.80	105.00	12.00	7.20	3.00	2.02
chlorogenic acid	0.08	113.00	10.00	8.10	5.80	11.8	0.24	98.70	13.00	8.10	6.10	3.04	0.48	95.40	11.00	4.90	6.80	4.13
caffeic acid	0.40	94.20	4.60	3.80	3.50	4.39	1.20	111.00	5.20	8.40	5.60	6.91	2.40	108.00	5.60	10.00	6.30	8.87
o-coumaric acid	0.50	99.20	9.10	9.50	4.50	12.10	1.50	86.00	8.10	6.50	8.60	10.10	3.00	88.60	3.50	2.50	5.90	3.48
rutin	1.50	104.00	4.70	11.00	3.10	7.06	4.50	92.80	14.00	5.10	2.50	7.95	90.0	89.20	9.40	4.30	4.20	13.50
ferulic acid	1.50	110.00	13.60	8.90	3.70	4.64	4.50	94.00	3.30	7.60	5.30	1.49	9.00	86.70	14.00	9.20	5.80	4.18
naringenin	1.20	108.00	3.70	11.00	3.50	4.09	3.60	99.70	12.00	4.90	5.60	3.26	7.20	103.00	4.40	11.00	3.90	3.90

[a]Accuracy and intraday precision: 6 replicates of cherry tomato, tomato sauce, and tomato juice extracts spiked at 3 different concentration levels (50%, 150%, 300%). Interday precision: data obtained in 3 different days.

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Table 4. Phenolic Content and Matrix Effect in Cherry Tomato, Sauce, and Juice Extracts[a]

	content (μg g^{-1} FW)			suppression/enhancement effect (%)		
compound	cherry	sauce	juice	cherry	sauce	juice
protocatechuic acid	0.29 a ± 0.00	0.81 a ± 0.00	0.50 a ± 0.00	101.00 a ± 6.70	95.70 a ± 6.30	98.20 a ± 3.20
4-hydroxybenzoic acid			1.48 ± 0.10			100.00 ± 6.10
chlorogenic acid	2.38 b ± 0.20	9.46 a ± 0.80	0.30 b ± 0.00	99.10 a ± 5.30	92.30 b ± 7.00	101.00 a ± 5.60
caffeic acid	2.20 b ± 0.10	4.65 a ± 0.30	2.66 b ± 0.10	100.00 a ± 13.00	96.40 a ± 2.60	101.00 a ± 6.30
o-coumaric acid	3.15 b ± 0.20	1.40 b ± 0.00	7.10 a ± 0.50	101.00 a ± 5.00	101.00 a ± 7.90	99.30 a ± 6.20
rutin	208.00 a ± 13.00	182.00 a ± 9.10	6.15 b ± 0.30	100.00 a ± 9.40	95.30 a ± 10.50	101.00 a ± 0.20
ferulic acid	3.72 a ± 0.10		3.93 a ± 0.20	96.10 b ± 0.10		104.00 a ± 8.60
naringenin-7-*O*-glucoside	nq ±0.00	8.08 a ± 0.50		101.00 a ± 3.30	91.50 b ± 12.00	
naringenin	93.10 a ± 5.70	206.00 a ± 14.00	7.04 b ± 0.50	99.90 a ± 7.50	93.80 b ± 14.00	91.40 b ± 10.00

[a]Data are the mean ± standard deviation (n = 3) for each matrix. Different letters in the rows represent significant differences in concentration (columns 2−4) or matrix effect (columns 5−7) observed among matrices ($p < 0.05$); nq = detected compound although below the LOQ.

effects are generally matrix, analyte, LC−MS/MS method, and ion source dependent.[43] The sources of these effects are so extremely diverse that they cannot be attributed to only one cause, while depending on a synergic effect of all the analytical conditions involved. However, especially in the analysis of complex dietary matrices, matrix components of the extracts may play a relevant role in causing either ion suppression or enhancement effects, in a variable extent depending of the compound elution and physical-chemical properties. As a consequence, the evaluation of matrix effects in the analysis of food item composition is essential for method assessment. And the generalized use of the external standard calibration plot often gives biased results. In our study, the data obtained did not seem to fit a common rule for all compounds. However, as shown in Table 4, no significant matrix-dependent suppression/enhancement effects were generally observed in the three matrices under the proposed LC−MS conditions (ratio between 91.4% and 104%).

Phenolic Composition of the Three Dietary Matrices. The application of the method has allowed the analysis of main phenolic compounds belonging to the hydroxycinnamic acid, flavonol, and flavanone classes. The reported UHPLC−QqQ method could be successfully applied to the phenolic characterization of similar matrices, with minor modifications (gradient elution, flow rate, total chromatographic runtime). The results obtained of the screening of phenolic compounds (hydroxycinnamic acids, flavonols, flavanones, and their derivatives) are schemed in Table 4.

Chromatographic peaks obtained for each run were drawn by data points ranging between 11 (naringenin-7-glucoside) and 14 (chlorogenic acid). The quantitative determination of the target phenolic compounds was carried out selecting the MRM transitions corresponding to the most abundant phenolics in cherry tomato and tomato-based products and was expressed as mean values ± SD of fresh weight (FW) (Table 4). Other principal compounds such as chalconaringenin and quercetin 3-*O*-(2″-*O*-beta-apiofuranosyl-6″-*O*-alpha-rhamnopyranosyl)-beta-glucopyranoside have been found previously in other types of tomato and tomato based-products, but in this study, these last two compounds were not identified and the setup of validation method was performed only for the main and more representative compounds detected in all extracts.

Statistically significant differences in the content of phenolic compounds were found in cherry tomato and tomato-based products.

Hydroxycinnamic Acids. The spectra generated for hydroxycinnamic acids showed the deprotonated molecule $[M - H]^-$, together with additional identifying fragments. A typical loss of CO_2 was observed for protocatechuic, caffeic, ferulic, and *o*-coumaric acids, giving $[M - H - 44]^-$ as a characteristic ion; ferulic acid was also identified by the loss of a methyl group $[M - H - 15]^-$.

In the product ion spectra, chlorogenic acid (m/z 353) gave a fragment at m/z 191 $[M - H - 162]^-$ corresponding to quinic acid. Furthermore, neutral loss experiment of 162 unit confirmed the loss of a caffeic acid $[179 - H_2O]^-$ unit from the chlorogenic acid.[41] The confirmation of chlorogenic acid was performed by matching reference compound.

Flavonols and Flavanones. The peak with mass signals at m/z 609 was attributed to rutin. The product ion scan of m/z 609 showed peaks corresponding to produce a loss of the rutinoside unit, $[M - H - 308]^-$,while the peak at m/z 271 was ascribed to naringenin, that gave as a characteristic ion (m/z 151) corresponding to retro-Diels−Alder fragmentation in the C-ring involving 1,3 scission.

The main phenolic compound in all the samples was rutin, followed by naringenin (Table 4). Naringenin (45%) is reported to be the main flavonoid in tomatoes, followed by quercetin (39%), myricetin (10%), and kaempferol (5%).[41] Other studies report rutin as the major flavonoid in several tomato cultivars.[14,41] In this study, rutin was found at different concentrations ranging from 6.15 μg g^{-1} FW (tomato juice) to 182 μg g^{-1} FW and 208 μg g^{-1} FW (tomato sauce and raw tomatoes, respectively). The same pattern was observed for naringenin (7.04 μg g^{-1} concentration in tomato juice while 93.1 and 206 μg g^{-1} FW respectively in raw fruit and tomato sauce).

Regarding the family of phenolic acids, chlorogenic acid was the main phenolic acid ranging from 0.30 μg g^{-1} (tomato juice) to 9.46 μg g^{-1} (tomato sauce). The differences in other compounds, such as caffeic or ferulic acid, between tomato-based products were less pronounced.

Cherry tomato and sauce extracts showed a higher content of phenolic compounds than tomato juice extracts. Cherry tomatoes showed the highest amount of rutin and *o*-coumaric acid, while the content of caffeic, chlorogenic, naringenin-7-*O*-glucoside, and protocatechuic acid was higher in tomato sauce. The differences in concentration among these tomato-based products may be due to technological processes or due to the high content of water in tomato juice.

As mentioned above, tomato juice is an intermediate product in the processing of tomato paste which contains a higher quantity of water and, thus, the content of phenolic compounds could decrease. Otherwise, thermal processing of tomatoes to

obtained paste involved a number of heating stages which could be expected to have an effect on heat-labile and oxidizable compounds or increasing the bioavailability for certain compounds. The effect of thermal treatment on phenolic compounds is a controversial issue in the literature, with some studies suggesting exposure to heat results in a considerable loss of hydrophilic antioxidants. Crozier et al. found that cooking involving boiling, microwaving, and frying reduced the quercetin content of tomatoes.[44] Similarly, Capanoglu et al. found that rutin decreased after samples were treated in a three-effect evaporator unit, which included heat treatment up to 80 °C.[45] In contrast, other studies have reported an increase in total phenolic content as a result of processing. In experiments carried out by Chang et al., two tomato varieties were air-dried at 80 °C for 2 h and then at 60 °C for 6 h. Analyses showed that the total flavonoid and total phenolic content increased in comparison with fresh tomatoes.[46] Processing also increased the phenolic content in diced tomatoes,[36] and in tomato paste production.[47]

To the best of our knowledge, this paper describes for the first time an easy, fast, and sensitive UHPLC−QqQ-MS method to identify and quantify the most abundant phenolic compounds present in cherry tomato, tomato sauce, and tomato juice extracts under the same conditions. The UHPLC−QqQ-MS method was completely validated and provided a sensitive analysis for phenol detection, showing satisfactory data for all the parameters tested. Good results were obtained with respect to linearity and recovery as well as precision. The often neglected matrix effects were also taken into account, since each matrix can have a variable influence on analyte determination and consequently on the quality of the results. No significant matrix effects (91.4% < %ME < 104%) were observed in tomatoes, tomato sauces and tomato juices under these chromatographic conditions.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Stewart, A. J.; Bozonnet, S.; Mullen, W.; Jenkins, G. I.; Lean, M. E. J.; Crozier, A. Occurrence of Flavonols in Tomatoes and Tomato-Based Products. *J. Agric. Food Chem.* **2000**, *482*, 2663−2669.

(2) Willcox, J. K.; Catignani, G. L.; Lazarus, S. Tomatoes and cardiovascular health. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 1−18.

(3) Thies, F.; Masson, L. F.; Rudd, A.; Vaughan, N.; Tsang, C.; Brittenden, J.; Simpson, W. G.; Duthie, S.; Horgan, G. W.; Duthie, G. Effect of a tomato-rich diet on markers of cardiovascular disease risk in moderately overweight, disease-free, middle-aged adults: a randomized controlled trial. *Am. J. Clin. Nutr.* **2012**, *95*, 1013−1022.

(4) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.

(5) Gao, X.; Cassidy, A.; Schwarzschild, M. A.; Rimm, E. B.; Ascherio, A. Habitual intake of dietary flavonoids and risk of Parkinson disease. *Neurology* **2012**, *78*, 1138−1145.

(6) McCullough, M. L.; Peterson, J. J.; Patel, R.; Jacques, P. F.; Shah, R.; Dwyer, J. T. Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. *Am. J. Clin. Nutr.* **2012**, *95*, 454−464.

(7) Wan, Y.; Vinson, J. A.; Etherton, T. D.; Proch, J.; Lazarus, S. A.; Kris-Etherton, P. M. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *Am. J. Clin. Nutr.* **2001**, *74*, 596−602.

(8) Desch, S.; Schmidt, J.; Kobler, D.; Sonnabend, M.; Eitel, I.; Sareban, M.; Thiele, H. Effect of cocoa products on blood pressure: Systematic review and meta-analysis. *Am. J. Hypertens.* **2010**, *23*, 97−103.

(9) Widlansky, M. E.; Hamburg, N. M.; Anter, E.; Holbrook, M.; Kahn, D. F.; Elliott, J. G.; Vita, J. A. Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. *J. Am. Coll. Nutr.* **2007**, *26*, 95−102.

(10) Erlund, I.; Koli, R.; Alfthan, G.; Marniemi, J.; Puukka, P.; Mustonen, P.; Jula, A. Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. *Am. J. Clin. Nutr.* **2008**, *87*, 323−331.

(11) Zhu, Y.; Ling, W.; Guo, H.; Song, F.; Ye, Q.; Zou, T.; Yang, Y. Anti-inflammatory effect of purified dietary anthocyanin in adults with hypercholesterolemia: A randomized controlled trial. *Nutr. Metab. Cardiovasc.* **2012**, DOI: 10.1016/j.numecd.2012.06.005.

(12) Frusciante, L.; Carli, P.; Ercolano, M. R.; Pernice, R.; Di Matteo, A.; Fogliano, V.; Pellegrini, N. Antioxidant nutritional quality of tomato. *Mol. Nutr. Food Res.* **2007**, *51*, 609−617.

(13) Vallverdu-Queralta, A.; Arranz, S.; Medina-Remón, A.; Casals, I.; Lamuela-Raventós, R. M. Changes in phenolic content of tomato products during storage. *J. Agric. Food Chem.* **2011**, *59*, 9358−9365.

(14) Slimestad, R.; Fossen, T.; Verheul, M. J. The flavonoids of tomatoes. *J. Agric. Food Chem.* **2008**, *56*, 2436−2441.

(15) Vallverdu-Queralta, A.; Jaureguic, O.; Medina-Remón, A.; Lamuela-Raventós, R. M. Evaluation of a method to characterize the phenolic profile of organic and conventional tomatoes. *J. Agric. Food Chem.* **2012**, *60*, 3373−3380.

(16) Moco, S.; Bino, R. J.; Vorst, O.; Verhoeven, H. A.; de Groot, J.; van Beek, T. A.; Vervoort, J.; de Vos, C. H. R. A liquid chromatography-mass spectrometry-based metabolome database for tomato. *Plant Physiol.* **2006**, *141*, 1205−1218.

(17) Iijima, Y.; Suda, K.; Suzuki, T.; Aoki, K.; Shibata, D. Metabolite profiling of chalcones and flavanones in tomato fruit. *J. Jpn. Soc. Hortic. Sci.* **2008**, *77*, 94−102.

(18) Vallverdu-Queralt, A.; Jáuregui, O.; Medina-Remón, A.; Andres-Lacuéva, 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.

(19) Sun, J. Q.; Liang, F.; Bin, Y.; Li, P.; Duan, C. Q. Screening non-colored phenolics in red wines using liquid chromatography/ultraviolet and mass spectrometry/mass spectrometry libraries. *Molecules* **2007**, *12*, 679−693.

(20) Kerem, Z.; Bravdo, B.; Shoseyov, O.; Tugendhaft, Y. Rapid liquid chromatography−ultraviolet determination of organic acids and phenolic compounds in red wine and must. *J. Chromatogr., A* **2004**, *1052*, 211−215.

(21) Sanchez-Rabaneda, F.; Jauregui, O.; Casals, I.; Andrés-Lacueva, C.; Izquierdo-Pulido, M.; Lamuela-Raventós, 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.

(22) Vallverdu-Queralta, A.; Jauregui, O.; Di Lecce, G.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M. Screening of the polyphenol content of tomato-based products through accurate-mass spectrometry (HPLC-ESI-QTOF). *Food Chem.* **2011**, *129*, 877−883.
(23) Guillarme, D.; Nguye, D. T. T.; Rudaz, S.; Veuthey, J.-L. Recent developments in liquid chromatography—impact on qualitative and quantitative performance. *J. Chromatogr., A.* **2007**, *1149*, 20−29.
(24) Leandro, C. C.; Hancock, P.; Fussell, R. J.; Keely, B. J. Comparison of ultra-performance liquid chromatography and high-performance liquid chromatography for the determination of priority pesticides in baby foods by tandem quadrupole mass spectrometry. *J. Chromatogr., A.* **2006**, *1103*, 94−101.
(25) Li, X.; Xiong, Z.; Ying, X.; Cui, L.; Zhu, W.; Li, F. A rapid ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometric method for the qualitative and quantitative analysis of the constituents of the flower of Trollius ledibouri Reichb. *Anal. Chim. Acta* **2006**, *580*, 170−180.
(26) Jaganath, I. B.; Mullen, W.; Edwards, C. A.; Crozier, A. The relative contribution of the small and large intestine to the absorption and metabolism of rutin in man. *Free Radical Res.* **2006**, *40*, 1035−1046.
(27) Xu, H.; Kulkarni, K. H.; Singh, R.; Yang, Z.; Wang, S. W. J.; Tam, V. H.; Hu, M. Disposition of naringenin via glucuronidation pathway is affected by compensating efflux transporters of hydrophilic glucuronides. *Mol. Pharmaceutics* **2009**, *6*, 1703−1715.
(28) Rogatsky, E.; Stein, D. Evaluation of matrix effect and chromatography efficiency: new parameters for validation of method development. *J. Am. Soc. Mass. Spectrom* **2005**, *16*, 1757−1759.
(29) Hajšlová, J.; Zrostlíková, J. Matrix effects in (ultra)trace analysis of pesticide residues in food and biotic matrices. *J. Chromatogr., A* **2003**, *1000*, 181−197.
(30) George, B.; Kaur, C.; Khurdiya, D. S.; Kapoor, H. C. Antioxidants in tomato (Lycopersium esculentum) as a function of genotype. *Food Chem.* **2004**, *84*, 45−51.
(31) Galiacia-Cabrera, R. M. Tomato processing. In *Hanbook of food products manufacturing*; Hui, Y. H., Ed.; Wiley-Interscience: 2007, pp 1091−1120.
(32) Tulipani, S.; Martinez-Huelamo, M.; Rotches-Ribalta, M.; Estruch, R.; Escribano-Ferrer, E.; Andres-Lacueva, C.; Illan, M.; Lamuela-Raventós, R. M. Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: Evidence from a human pilot study. *Food Chem.* **2012**, *130*, 581−590.
(33) Martinez-Huelamo, M.; Tulipani, S.; Torrado, X.; Estruch, R.; Lamuela-Raventós, R. M. Validation of a New LC-MS/MS Method for the Detection and Quantification of Phenolic Metabolites from Tomato Sauce in Biological Samples. *J. Agric. Food Chem.* **2012**, *60*, 4542−4549.
(34) Horwitz, W. *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gaithersburg, MD, 2003; Vols. *1* and *2*.
(35) 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 chemo-taxonomic markers of tomato varieties. *J. Agric. Food Chem.* **2011**, *59*, 3994−4001.
(36) Vallverdu-Queralt, A.; Arranz, S.; Medina-Remon, A.; Casals-Ribes, I.; Lamuela-Raventos, R. M. Changes in phenolic content of tomato products during storage. *J. Agric. Food Chem.* **2011**, *59*, 9358−9365.
(37) Vallverdu-Queralt, A.; Oms-Oliu, G.; Odriozola-Serrano, I.; Lamuela-Raventos, R. M.; Martin-Belloso, O.; Elez-Martinez, P. Effects of pulsed electric fields on the bioactive compound content and antioxidant capacity of tomato fruit. *J. Agric. Food Chem.* **2012**, *60*, 3126−3134.
(38) Martínez-Valverde, I.; Periago, M. J.; Provan, G.; Chesson, A. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). *J. Sci. Food Agric.* **2002**, *82*, 323−330.
(39) Nicoletti, I.; De Rossi, A.; Giovinazzo, G.; Corradini, D. Identification an quantification of stilbenes in fruits of transgenic tomato plants (Lycopersicon sculentum Mill.) by reversed phase HPLC with photodiode array and mass detection. *J. Agric. Food Chem.* **2007**, *55*, 3304−3311.
(40) Gómez-Romero, M.; Segura-Carretero, A.; Fernández-Gutiérrez, A. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry* **2010**, *71*, 1848−1864.
(41) Vallverdu-Queralt, A.; Jauregui, O.; Medina-Remon, A.; Lamuela-Raventos, R. M. Evaluation of a method to characterize the phenolic profile of organic and conventional tomatoes. *J. Agric. Food Chem.* **2012**, *60*, 3373−3380.
(42) Silva, C. L.; Haesen, N.; Câmar, J. S. A new and improved strategy combining a dispersive-solid phase extraction-based multiclass method with ultra high pressure liquid chromatography for analysis of low molecular weight polyphenols in vegetables. *J. Chromatogr., A* **2012**, *1260*, 154−163.
(43) Gosetti, F.; Mazzucco, E.; Zampieri, D.; Gennaro, M. C. Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. *J. Chromatogr., A* **2010**, *1217*, 3929−3937.
(44) Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* **1997**, *45*, 590−595.
(45) Capanoglu, E.; Beekwilder, J.; Boyacioglu, D.; Hall, R.; de Vos, R. Changes in antioxidant and metabolite profiles during production of tomato paste. *J. Agric. Food Chem.* **2008**, *56*, 964−973.
(46) Chang, C. H.; Liu, Y. C. Study on lycopene and antioxidant contents variations in tomatoes under air-drying process. *J. Food Sci.* **2007**, *72*, 532−540.
(47) Vallverdu-Queralt, A.; Medina-Remón, A.; Casals, I.; Waterhouse, A. L.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M. Effect of tomato industrial processing on phenolic profile and hydrophilic antioxidant capacity. *Food Sci. Technol.* **2012**, *47*, 154−160.