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Determination of Polyphenolic Profiles by Liquid Chromatography-Electrospray-Tandem Mass Spectrometry for the Authentication of Fruit Extracts

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27 **Abstract**

28 Liquid chromatography-electrospray-tandem mass spectrometry (LC-ESI-
29 MS/MS) was applied to the analysis and authentication of fruit-based products and fruit-
30 based pharmaceutical preparations. A Kinetex C₁₈ reversed-phase column under
31 gradient elution with 0.1 % formic acid aqueous solution and methanol mobile phases
32 was used for the simultaneous determination of 26 polyphenols, allowing an acceptable
33 separation in less than 22 min. Instrumental quality parameters such as limits of
34 detection (LOD, values between 12-14 µg/L for 19 of the 26 analyzed polyphenols),
35 linearity ($r^2 > 0.991$), run-to-run and day-to-day precisions (RSD values lower than 9.9
36 and 13.5 %, respectively), and accuracy (relative errors lower than 8 %) were
37 established. A simple extraction method, consisting of a sample sonication with
38 acetone:water:hydrochloric acid (70:29.9:0.1 v/v/v) and centrifugation, was proposed.
39 Two calibration procedures, external calibration using standards prepared in water and
40 standard addition, were evaluated for polyphenol quantification in several grape and
41 cranberry fruits and processed fruit products. For a 95 % confidence level, no statistical
42 differences were observed between the two calibration methods (p values between 0.06
43 and 0.95), denoting that external calibration was suitable enough for the quantitative
44 analysis of polyphenols in fruit-based products. The proposed LC-ESI-MS/MS method
45 was then applied to the analysis of polyphenols in 23 grape-based and cranberry-based
46 natural products and pharmaceutical preparations. Polyphenolic concentration data was
47 then analyzed by principal component analysis (PCA) to extract information of the most
48 significant profile data contributing to authentication of natural extracts according to
49 their fruit of origin.

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53 **KEYWORDS:** Polyphenols; Natural products; Food characterization; Food Analysis;
54 Liquid chromatography; Mass spectrometry

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60 **1. Introduction**

61 The importance of diet on human health and well-being has been widely
62 recognized all over the world. For instance, USA recommends that people consume at
63 least 2.5 cups of vegetables and 2 cups of fruits daily [1], which is based on a general
64 diet of 2000 kcal per day. In Europe, instead, the traditional Mediterranean diet has
65 formed the basis for food consumption during the past century, originally settled on
66 Mediterranean agronomical, pastoral, and rural archetypes. The regular consumption of
67 fruits and vegetables, rich in antioxidants and bioactive compounds, has been shown to
68 exert an important role in the prevention of many diseases, such as skin pathologies,
69 various types of cancer, cardiovascular disorders, and other age-related degenerative
70 pathologies, besides the general health benefits they provide [2-6].

71 Polyphenols usually are related with characteristic metabolic patterns present in
72 all vegetal tissues, as well as in flowers and fruits. Several thousands of plant
73 polyphenols are known, including a wide variety of molecules that contain at least one
74 aromatic ring with one or more hydroxyl groups in addition to other constituents. They
75 can be divided in several classes, i.e. phenolic acids (hydroxybenzoic acids and
76 hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones,
77 isoflavones, proanthocyanidins (PACs)), stilbenes, and lignans [7]. Phenolic profile is
78 an important indicator of fruit quality because of their contribution to the taste, color
79 and nutritional properties [8]. In addition, these compounds are considered one of the
80 most relevant antioxidants of human diet [9], so over the past ten years food researchers
81 and manufacturers have become increasingly interested in this family of compounds.

82 Berries are an excellent source of polyphenols, especially anthocyanins. The
83 consumption of berry fruits associated with their contribution to improved human health
84 is an issue of considerable interest [10]. Cranberry (*Vaccinium macrocarpon*) and its
85 derived products, including juices and nutraceuticals, have shown some beneficial
86 health effects associated to their polyphenolic content [11]. However, the best known
87 bioactivity of cranberry polyphenols deals with their capacity to inhibit the adhesion of
88 pathogenic bacteria to uroepithelial cells of the urinary tract, thus contributing to the
89 prevention of urinary tract infections [12,13]. The most common polyphenols found in
90 cranberries comprise phenolic and benzoic acids, and flavonoids such as anthocyanins,
91 flavonols, and flavan-3-ols [12]. Recently, many commercial products claiming to be
92 manufactured from cranberry-based extracts have appeared in the market. Some of these
93 products are sold as if they had the same health properties of cranberries, but they do

94 not contain the bioactive polyphenols (i.e. A-type proanthocyanidins among other
95 polyphenols). This fact shows the importance of developing analytical methodologies
96 for the characterization of natural extracts to achieve correct authentication regarding
97 the fruit of origin.

98 Liquid chromatography (LC) with photodiode array (PDA) detection or coupled
99 to mass spectrometry (LC-MS) are among the most common techniques used for the
100 identification, characterization, and determination of polyphenolic compounds in a great
101 variety of plants and fruit-based products [14-21]. High resolution mass spectrometry
102 (HRMS) has also been proposed for the analysis and characterization of polyphenols in
103 fruit products [14,17,21-23]. For instance, Iswaldi *et al.* [22] proposed the use of time-
104 of-flight mass spectrometry (TOF-MS) for the study of the phenolic fraction in
105 cranberry syrup, and Vallverdu-Queralt *et al.* [17] characterized tomato polyphenols by
106 liquid chromatography-electrospray-linear ion trap quadrupole Orbitrap mass
107 spectrometry. Although reversed-phase chromatographic methods are very popular for
108 the determination of low molecular mass flavonoids, a large proportion of this family of
109 compounds in fruits and vegetables consists of highly condensed polymeric
110 proanthocyanidins. Under these circumstances, LC-MS and LC-HRMS play an
111 important role to help in the characterization of PACs in natural extracts [24-26].

112 Characterization and classification of fruit-based products, including some
113 commercial pharmaceutical preparations, can be tackled from the compositional profiles
114 as a source of analytical information. Polyphenolic compounds, as well as other low
115 molecular weight organic acids, alcohols, esters, etc., have been also found to be
116 efficient descriptors of some climatic, agricultural and technological features and, thus,
117 the variability of compounds will strongly depend on the fruit of origin [16,27-29].
118 Therefore, the polyphenolic profile could be a useful platform for reliable
119 discrimination between fruit-based products via chemometric methods such as principal
120 component analysis (PCA). Information recovered mathematically might be essential in
121 order to prevent misuses in the production of commercial fruit-based products with
122 health-promoting properties.

123 This work aims to develop a liquid chromatography-electrospray-tandem mass
124 spectrometry (LC-ESI-MS/MS) method for the identification and determination of
125 polyphenolic profile in fruit-based products and natural extracts. For this purpose, a
126 total of 26 polyphenolic compounds belonging to different families (stilbenes, phenolic
127 acids, and flavonoids) were selected, and a simple sample treatment, consisting of an

128 extraction by sonication with acetone:water:hydrochloric acid (70:29.9:0.1 v/v/v) and
129 centrifugation, was applied [21]. Different kinds of cranberry-based and grape-based
130 samples were analyzed, including fruits, fruit juices, and raisins, as well as commercial
131 cranberry-based products such as pharmaceutical natural extracts, powder capsules,
132 syrup and sachets. Data corresponding to the polyphenolic composition were considered
133 as a source of potential descriptors to be exploited for the authentication of fruit-based
134 products.

135

136 **2. Materials and Methods**

137 **2.1. Chemicals**

138 Unless otherwise stated, all reagents were of analytical grade. Gallic acid,
139 protocatechualdehyde, (+)-catechin hydrate, gentisic acid, p-salicylic acid, chlorogenic
140 acid, caffeic acid, (-)-epicatechin, (-)-epigallocatechin gallate, syringic acid,
141 syringaldehyde, ethyl gallate, umbelliferon, p-coumaric acid, taxifolin, polydatin, ferulic
142 acid, sinapic acid, resveratrol, quercitrin hydrate, fisetin and kaempferol were obtained
143 from Sigma-Aldrich (Steinheim, Germany). Homogentisic acid, protocatechuic acid and
144 vanillic acid were purchased from Fluka (Steinheim, Germany), and quercetin dihydrate
145 from Riedel-de Haën (Seelze, Germany).

146 Formic acid (98-100 %) was provided by Merck (Darmstadt, Germany). LC-MS
147 grade methanol and water were purchased from Sigma-Aldrich.

148 Stock standard solutions of all polyphenols (~1000 mg/L) were prepared in
149 methanol in amber-glass vials. Intermediate working solutions were prepared weekly
150 from these stock standard solutions by appropriate dilution with water. All stock
151 solutions were stored at 4 °C for not more than 1 month.

152

153 **2.2. Instrumentation and methods**

154 Chromatographic separation was performed on an Accela liquid chromatography
155 system (Thermo Fisher Scientific, San José, CA, USA), equipped with a quaternary
156 pump, an autosampler and a column oven. A Kinetex C₁₈ reversed-phase column (100 x
157 4.6 mm, 2.6 µm particles) provided by Phenomenex (Torrance, CA, USA) was used for
158 the proposed method. Gradient separation was created from solvent A (0.1 % formic
159 acid aqueous solution) and solvent B (methanol) as follows: 0-3 min, linear gradient
160 from 5 to 25 % B; 3-6 min, at 25 % B; 6-9 min, from 25 to 37 % B; 9-13 min, at 37 %
161 B; 13-18 min, from 37 to 54 % B; 18-22 min, at 54 % B; 22-26 min, from 54 to 95 % B;

162 26-29 min, at 95 % B; 29-29.15 min, back to initial conditions at 5 % B; and from 29.15
163 to 36 min, at 5 % B. The mobile phase flow rate was 1 mL/min.

164 The mass spectrometer was a TSQ Quantum Ultra AM (Thermo Fisher
165 Scientific) triple quadrupole equipped with heated-electrospray (H-ESI) as ionization
166 source in negative mode. Nitrogen (purity > 99.98 %) was used as a sheath gas, ion
167 sweep gas and auxiliary gas at flow-rates of 65, 0 and 40 a.u. (arbitrary units),
168 respectively. Both H-ESI vaporizer temperature and ion transfer tube temperature were
169 set at 350 °C, and the electrospray voltage at -2.5 kV. Full-scan MS acquisition mode
170 (m/z 50-500) in Q1 (mass resolution of 0.7 m/z FWHM, full width half maximum) with
171 an scan time of 0.5 s was primarily used for characterization and evaluation. Selected
172 reaction monitoring (SRM) acquisition mode (mass resolution of 0.7 m/z FWHM on
173 both Q1 and Q3), with a scan width of 0.5 m/z and a scan time of 0.01 s, was used for
174 quantification purposes by monitoring two SRM transitions. Argon was used as
175 collision gas at 1.0 mTorr and the optimum collision energy (CE) for each transition
176 monitored (quantifier and qualifier) is shown in Table 1. For LC-MS experiments, a 1:1
177 post-column split of the chromatographic eluent, by means of a Valco zero dead volume
178 tee piece, was used.

179 To optimize both the H-ESI source and tandem mass spectrometry working
180 conditions, 5 mg/L stock standard solution of each compound prepared in
181 methanol:water (1:1 v/v) was infused at a flow-rate of 15 μ L/min using the syringe
182 pump integrated in the TSQ instrument, and mixed with 500 μ L/min of a 0.1 % formic
183 acid aqueous solution:methanol (1:1 v/v) mobile phase, by means of a Valco zero dead
184 volume tee piece (Supelco, Gland, Switzerland). Precursor and product ion assignments
185 are also indicated in Table 1.

186

187 **2.3. Sample treatment**

188 Different classes of fruit-based products: two fruit samples (cranberry and
189 grapes), five raisin samples (2 based on cranberry and 3 based on grapes), and six juice
190 samples (3 based on cranberry and 3 based on grapes) from different trademarks were
191 purchased from Barcelona markets. In addition, a total of 10 raw extract materials and
192 commercial cranberry-based pharmaceutical preparations presented as powder capsules,
193 syrup, sachets, and natural extracts were provided by Deiters S.L. Company (Barcelona,
194 Spain).

195 Prior to sample treatment, fruits, raisins and liquid samples (juices and cranberry
196 pharmaceutical syrup) were freeze-dried to achieve a fully lyophilized products with a
197 texture similar to that of natural extracts and commercial pharmaceutical samples
198 (powdered samples). To this end, samples remained 24 h inside a lyophilizer from -80
199 °C to room temperature, and then were kept for 6.5 h at 40 °C.

200 Sample treatment was carried-out following a previously described method with
201 some modifications [21,30]. Briefly, 0.1 g of sample were dispersed in 10 mL of
202 acetone:water:hydrochloric acid (70:29.9:0.1 v:v:v) and sonicated for 30 min. After that,
203 the mixture was centrifugated for 15 min at 3500 rpm, and the extracts were stored at
204 -4 °C until analyzed. Before injection extracts were filtered through 0.45 µm nylon
205 filters (Whatman, Clifton, NJ, USA).

206

207 **2.4. Data analysis**

208 MATLAB (Version 6.5) was used for calculations. Principal component analysis
209 (PCA) was from the PLS-Toolbox (Eigenvector Research Inc., Mason, WA, USA) [31].
210 A detailed description of this method is given elsewhere [32].

211 The data matrix to be treated consisted of concentration values of quantified
212 polyphenols in the different samples under study (see section 2.3). The dimension of the
213 matrix was 23 samples x 26 analytes). Since concentrations of some pharmaceutical
214 samples were 3 orders of magnitude higher than those occurring in the fruit samples
215 (fruit, raisins and juices), normalization pretreatment with respect to the overall
216 polyphenolic concentration was required to provide similar weights to all the samples.
217 The plot of scores showing the distribution of the samples on the principal components
218 (PCs) revealed patterns that may be correlated to sample characteristics, such as source
219 fruit in this case. The study of the distribution of variables from the loading plot
220 provided information dealing with their correlations as well as dependences of
221 polyphenols on fruit product properties.

222

223 **3. Results and discussion**

224 **3.1. Chromatographic separation**

225 The chromatographic separation was carried out with a Kinetex C₁₈ reversed-
226 phase (100 x 4.6 mm, 2.6 µm particles) column and gradient elution with 0.1 % formic
227 acid aqueous solution and methanol mobile phases, as previously established by HPLC
228 with UV absorbance detection [21]. In comparison with the previous HPLC-UV

229 method, a 1:1 post-column split of the chromatographic eluent was applied using a
230 Valco T piece to make compatible chromatographic and MS conditions. As a result, the
231 flow rate of mobile phase entering into the MS instrument was 500 $\mu\text{L min}^{-1}$. Under
232 these conditions, an acceptable chromatographic separation of the 26 polyphenolic
233 compounds was obtained (Figure 1) in less than 22 min. However, several full or partial
234 co-elutions occurred such as those of caffeic acid, epicatechin and epigallocatechin
235 gallate (peaks 10, 11 and 12), taxifolin, polydatin, ferulic acid and sinapic acid (peaks
236 18, 19, 20 and 21), and quercitrin hydrate and fisetin (peaks 23 and 24).

237 However, baseline chromatographic separation is not mandatory because co-
238 elutions can be selectively resolved by MS using the appropriate SRM transitions (see
239 Table 1) if no ion suppression effects were present. To study the ion suppression effect,
240 these co-eluting compounds were analyzed by triplicate with the proposed method both
241 individually and in the corresponding co-eluting mixtures. As an example, Figure 1S
242 (supplementary material) shows the signals obtained for caffeic acid, epicatechin and
243 epigallocatechin gallate compounds (peaks 10, 11 and 12) at a concentration of 500
244 $\mu\text{g/L}$. For all evaluated compounds, analysis of variance (ANOVA) was applied
245 showing that for a 95% confidence level polyphenolic peak signals when analyzed
246 individually and in co-eluting mixtures were not significantly different (p values always
247 higher than 0.05), so no ion-suppression effects were observed.

248

249 **3.2. LC-MS/MS conditions**

250 The liquid chromatographic system was coupled to a triple quadrupole mass
251 spectrometer using an H-ESI source in negative mode. Full scan MS spectra from m/z
252 50-500 were recorded. For all polyphenols under study the base peak was the
253 deprotonated molecule $[\text{M-H}]^-$. Also, neither adducts nor in-source collision-induced
254 dissociation ions were observed in the MS spectra of the compounds at significant
255 intensities except for taxifolin and polydatin which showed in-source fragmentation at
256 relative intensities above 30 % and 50 %, respectively. Thus, the deprotonated molecule
257 was selected as precursor ion for all the studied compounds in tandem MS
258 fragmentation experiments.

259 The fragmentation of these compounds in the triple quadrupole was studied
260 under tandem MS conditions. For the correct product ion assignment, collision energy
261 curves (5-80 eV) were studied. Some similarities were found in the fragmentation of the
262 studied families of polyphenols. For instance, the compounds belonging to the flavonoid

263 family ((+)-catechin, (-)-epicatechin, (-)-epigallocatechin gallate, taxifolin, fisetin,
264 quercetin and kaempferol) presented highly fragmented product ion spectra, with most
265 product ions arising from cross-fragmentation of the aromatic rings in their structures.
266 For this reason, sensitivity for these compounds was expected to be lower than for the
267 rest of the polyphenols studied. Typically, most phenolic acids showed the loss of CO₂
268 in their product ion scan spectra, along with the losses of radical •CH₃ and/or CH₂O
269 when methoxy substituents were present in the aromatic ring of the compounds (as
270 happens with ferulic acid, sinapic acid, syringic acid, vanillic acid and syringaldehyde).
271 Moreover, the phenolate and hydroxyphenolate ions (*m/z* 93 and 108, respectively),
272 which are characteristic of polyphenolic compounds, were encountered in most product
273 ion scan spectra, although they were not always the most intense product ions. Lastly,
274 polydatin lost the glycoside ring to yield the resveratrol deprotonated molecule (*m/z*
275 227) which would then produce product ions resulting from the losses of C₂H₂O. After
276 studying the product ion scan spectra of the compounds, the most intense and
277 characteristic transitions were selected for both quantitative and confirmation purposes.
278 The assignments for the precursor ion and the two most intense product ions for each
279 compound, which were selected as quantifier and qualifier SRM transitions, are given in
280 Table 1, and optimal collision energies for both quantifier and qualifier SRM transitions
281 are also indicated.

282

283 **3.3. Instrumental quality parameters**

284 Instrumental quality parameters of the proposed LC-ESI-MS/MS method under
285 optimal conditions were calculated for the 26 polyphenolic compounds and the figures
286 of merit are given in Table 2. Limits of detection (LODs), based on a signal-to-noise
287 ratio of 3:1, were calculated using standard solutions at low concentration levels, and
288 values down to 12-14 µg/L were achieved for 19 of the 26 studied polyphenols, in the
289 range 26-68 µg/L for 5 polyphenols, and only fisetin and kaempferol compounds
290 showed LODs at around 110 µg/L. Limits of quantification (LOQs), based on a signal-
291 to-noise ratio of 10:1, between 40 and 387 µg/L were obtained.

292 Calibration curves based on peak area at concentrations above LOQ to 100 mg/L
293 were established. Good linearity was observed for all compounds with correlation
294 coefficients (*r*²) higher than 0.991.

295 Run-to-run and day-to-day precisions for compound quantifications were
296 calculated at three concentration levels, low level (LOQ), middle level (500 µg/L), and

297 high level (10 mg/L). For both fisetin and kaempferol, compounds which showed the
298 highest LOQs, only two concentration levels (low and high ones) were evaluated. In
299 order to obtain the run-to-run precision, five replicate determinations for each
300 concentration level were carried out. Day-to-day precision was estimated from 15
301 replicate determinations at each concentration level on 3 non-consecutive days (5
302 replicates each day). For run-to-run precision, relative standard deviations (RSD) values
303 in the ranges 1.2-9.9 %, 1.6-6.8 % and 0.6-8.2 % for low, middle and high concentration
304 levels, respectively, were obtained. In general, good and similar precisions regardless
305 the concentration level evaluated were obtained. Day-to-day precision worsened
306 slightly, but RSD values were lower, in any case, than 13.5 %. As a conclusion, good
307 precision was attained for the proposed method even at LOQ levels.

308 As no reference material is available, accuracy was evaluated at the three
309 concentration levels by comparing spiked with calculated concentrations using external
310 calibration. Results were excellent, with relative prediction errors (%) lower than 8.0 %.

311 The results obtained showed that the proposed LC-MS/MS method was
312 satisfactory in terms of sensitivity, precision and accuracy for the determination of
313 polyphenols.

314

315 **3.4. Determination of polyphenols in fruit-based products and pharmaceutical** 316 **preparations**

317

318 The applicability of the proposed LC-MS/MS method was evaluated in the
319 determination of 26 polyphenols in 23 fruit-based products, including fruits, raisins,
320 juices, cranberry-based raw extract materials and commercial products (syrup, sachets,
321 powder capsules and natural extracts). A simple sample treatment, consisting of an
322 extraction by sonication with acetone:water:hydrochloric acid (70:29.9:0.1 v/v/v) and
323 centrifugation, was performed. As an example, Figure 2 shows the LC-ESI-MS/MS
324 chromatograms obtained for the 14 most abundant polyphenolic compounds found in
325 the analysis of a cranberry pill pharmaceutical sample.

326 Prior to the analysis of fruit-based product samples, two different quantification
327 methods were evaluated: (i) external calibration using standards prepared in water, and
328 (ii) standard addition. For comparison, the analysis of three cranberry and three grape
329 samples with different matrices (fruit, juice and raisin) was carried out by triplicate with
330 both external calibration and standard addition. Results are given in electronic

331 supplementary material Table 1S. Compound identification was based on retention
332 times and ion-ratios between quantifier and qualifier SRM transitions. In general,
333 external calibration provided results similar to those obtained by standard addition. Only
334 slightly differences for some compounds were encountered. Results from the two
335 approaches were compared statistically using a paired t-test. For a 95 % confidence
336 level, the results were not significantly different, with p values higher than 0.05 (see
337 electronic supplementary material Table 1S). Hence, external calibration was suited to
338 tackle the quantitative determination of polyphenols in fruit-based products.

339 Table 3 shows the concentration levels of polyphenols found in the analyzed
340 samples. For data simplification, only results for 9 of the 23 analyzed samples are
341 depicted in the table, together with the concentration range observed for each
342 polyphenol. Umbelliferon, resveratrol and fisetin polyphenolic compounds were not
343 detected in any of the cranberry-based or grape-based analyzed samples. Among the
344 other studied polyphenols, homogentisic acid, gentisic acid, syringaldehyde, ethyl
345 gallate, sinapic acid and kaempferol were neither detected in grape-based samples.
346 Some differences in the polyphenolic compounds detected when comparing natural
347 and/or processed cranberry- and grape-based products were also observed. As an
348 example, Figure 3a compares the concentration level of the 10 most relevant
349 polyphenols detected in cranberry and grape fruits, as well as their juices and raisins. As
350 can be seen, chlorogenic acid, epicatechin, coumaric acid, quercitrin and quercetin are
351 more characteristic polyphenolic compounds in cranberry fruit and fruit-processed
352 products, while other such as gallic acid and catechin tend to be more abundant in grape
353 fruit products. Regarding cranberry pharmaceutical preparations, higher concentrations
354 of some polyphenolic compounds were found in comparison to fruit and related food
355 samples. As an example, Figure 3b compares the concentration level of the 10 most
356 relevant polyphenols detected in these samples. Catechin, chlorogenic acid, epicatechin,
357 epigallocatechin, quercetin and quercitrin were found at very high concentrations levels
358 with catechin, epicatechin and quercitrin being the most abundant ones (with
359 concentrations higher than 3000 mg/kg in some of the samples).

360 The interesting differences observed among concentration levels of polyphenolic
361 compounds suggest that polyphenolic profile derived from LC-ESI-MS/MS analysis
362 could be proposed as a feature well-suited for the authentication of fruit-based products.

363

364 **3.5. Principal component analysis**

365 The polyphenolic concentrations of the samples under study, determined by the
366 proposed LC-ESI-MS/MS method, were analyzed chemometrically to draw relevant
367 patterns dealing with the characteristics of natural and processed products. As the first
368 issue to be considered, polyphenolic contents in the extracts and pharmaceutical
369 preparations were 100- to 1000-fold higher than those occurring in the fruits and related
370 food samples. Hence, normalization pretreatment with respect to the overall
371 polyphenolic concentration was required in order to provide similar influences on the
372 chemometric model to all the samples.

373 Normalized data was treated by PCA and the corresponding results are given in
374 Figure 4. As shown in the plot of scores (Figure 4a), grape and cranberry products
375 appeared in different zones so that PCA was basically able to distinguish among the two
376 fruits of origin. In particular, grape and related samples were located to the top-left part
377 of this graph. In contrast, cranberry samples were mainly spread out on the bottom area.
378 A group of cranberry samples was to the left, close to the area of distribution of grape
379 samples. This finding might indicate that compositions in percentages could be rather
380 similar for the two groups. Conversely, it suggested that there were significant
381 qualitative differences in the compositional profiles of some cranberry products.

382 Regarding the map of loading (Figure 4b), it was found that gallic acid and
383 polydatin were characteristic of grape-related samples so they were present in higher
384 proportions in this class of products. Analytes located to the right part of PC1 (e.g.,
385 sinapic, ferulic, coumaric and chlorogenic acids and quercitrin) were comparatively
386 more abundant in cranberry samples. These results agree with those previously reported
387 in the literature, where these last mentioned compounds are relatively more abundant
388 and available in berry products, although no levels in cranberry were reported [7].
389 Catechin was found to the left on PC1. In fact, it has been reported in grape samples at
390 levels between 30-175 mg/kg [7]. Indeed, catechin could be released from the
391 degradation of polymeric condensed tannins, typically occurring in high amounts in
392 cranberries, so this component might be a potential index of decay processes.

393

394 **4. Conclusions**

395 The results obtained in this work show that the developed LC-ESI-MS/MS
396 method, using a simple external calibration, can be proposed as a suitable method for
397 the determination of polyphenols in fruit-based products and pharmaceutical
398 preparations. LC-ESI-MS/MS showed a good performance, with low limits of detection

399 for most of the studied compounds (down to 12-14 µg/L), and with very good precisions
400 (RSD lower than 13.5 %) and accuracies (relative errors lower than 8.0 %). The method
401 was applied to the analysis of 23 grape-based and cranberry-based products and
402 pharmaceutical preparations after a simple sample extraction procedure consisting of an
403 acetone:water:hydrochloric acid extraction by sonication.

404 Among the 26 polyphenolic compounds analyzed, only three (umbelliferon,
405 resveratrol and fisetin) were not detected in any of the analyzed samples, and other
406 polyphenols such as homogentisic, gentisic and sinapic acids, syringaldehyde, ethyl
407 gallate, and kaempferol were neither detected in grape-based products.

408 Regarding cranberry-based pharmaceutical preparations, extremely higher
409 concentration of some polyphenolic compounds such as catechin, epicatechin and
410 quercitrin were found in comparison to fruit and related food products. The interesting
411 differences observed among concentration levels of some polyphenolic compounds
412 between grape-based and cranberry-based products, as well as between pharmaceutical
413 preparations and related food products, suggest that polyphenolic concentrations
414 determined by LC-ESI-MS/MS could be proposed as a suitable source of potential
415 descriptors to be exploited for the authentication of fruit-based products. Results from
416 PCA proved that such polyphenolic concentration data allowed the analyzed samples to
417 be clustered according to their source fruit.

418

419

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425

426 **References**

427

428 [1] U.S. Department of Agriculture and U.S. Department of Health and Human
429 Services. *Dietary Guidelines for Americans*, 7th ed.; U.S. Government Printing
430 Office: Washington, DC, USA, 2010.

431 [2] Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S,
432 Medina FX, Battino M, Belahsen R, Miranda G, Serra-Majem L (2011)

- 433 Mediterranean Diet Foundation Expert Group. Mediterranean diet pyramid
434 today. *Science and cultural updates. Public Health Nutr.* 14:2274-2284.
- 435 [3] Yin Y, Li W, Son YO, Sun L, Lu J, Kim D, Wang X, Yao H, Wang L,
436 Pratheeshkumar P, Hitron AJ, Luo J, Gao N, Shi X, Zhang Z (2013) Quercitrin
437 protects skin from UVB-induced oxidative damage. *Toxicol. Appl. Pharmacol.*
438 269:89-99.
- 439 [4] Pluemsamran T, Onkoksoong T, Panich U (2012) Caffeic acid and ferulic acid
440 inhibit UVA-induced matrix metalloproteinase-1 through regulation of
441 antioxidant defense system in keratinocyte HaCaT cells. *Photochem. Photobiol.*
442 88:961-968.
- 443 [5] Pastore S, Potapovich A, Lulli D, Fidanza P, Kostyuk V, De Luca C, Mikhalchik
444 E, Korkina L (2012) Plant polyphenols regulate chemokine expression and
445 tissue repair in human keratinocytes through interaction with cytoplasmic and
446 nuclear components of epidermal growth factor receptor (EGFR) system.
447 *Antioxid. Redox. Signal.* 16:314-328.
- 448 [6] Pastore S, Lulli D, Potapovich A, Fidanza P, Kostyuk V, Dellambra E, De Luca
449 C, Maurelli R, Korkina L (2011) Differential modulation of stress-inflammation
450 responses by plant polyphenols in cultured normal human keratinocytes and
451 immortalized HaCaT cells. *J. Dermatol. Sci.* 63:104-114.
- 452 [7] Manach C, Scalbert A, Morand C, Remesy C, Jimenez L (2004) Polyphenols:
453 Food sources and bioavailability. *Am. J. Clin. Nutr.* 79:727-747.
- 454 [8] Cheynier V (2005) Polyphenols in foods are more complex than often thought.
455 *Am. J. Clin. Nutr.* 81:223S-229S.
- 456 [9] El Gharras H (2009) Polyphenols: food sources, properties and applications - a
457 review. *Int. J. Food Sci. Technol.* 44:2512-2518.
- 458 [10] Basu A, Rhone M, Lyons TJ (2010) Berries: emerging impact on cardiovascular
459 health. *Nutrition Reviews* 68:168-177.
- 460 [11] Sanchez-Patan F, Bartolome B, Martin-Alvarez PJ, Anderson M, Howell A,
461 Monagas M (2012) Comprehensive assessment of the quality of commercial
462 cranberry products. Phenolic characterization and in vitro bioactivity. *J. Agric.*
463 *Food Chem.* 60:3396-3408.
- 464 [12] Foo LY, Lu Y, Howell AB, Vorsa N (2000) The structure of cranberry
465 proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated
466 *Escherichia coli* in vitro. *Phytochemistry* 54:173-181.
- 467 [13] Howell AB, Reed JD, Krueger CG, Winterbottom R, Cunningham DG, Leahy M
468 (2005) A-type cranberry proanthocyanidins and uropathogenic bacterial anti-
469 adhesion activity. *Phytochemistry* 66:2281-2291.
- 470 [14] Rodríguez-Medina IC, Segura-Carretero A, Fernández-Gutierrez A (2009) Use
471 of high-performance liquid chromatography with diode array detection coupled
472 to electrospray-Qq-time-of-flight mass spectrometry for the direct

- 473 characterization of the phenolic fraction in organic commercial juices. *J.*
474 *Chromatogr. A* 1216:4736-4744.
- 475 [15] Simirgiotis MJ, Schmeda-Hirschmann G (2010) Determination of phenolic
476 composition and antioxidant activity in fruits, rhizomes and leaves of the white
477 strawberry (*Fragaria chiloensis* spp. *chiloensis* form *chiloensis*) using HPLC-
478 DAD-ESI-MS and free radical quenching techniques. *J. Food Compos. Anal.*
479 23:545-553.
- 480 [16] Zhao Y, Chen P, Lin L, Harnly JM, Yu L, Li Z (2011) Tentative identification,
481 quantitation, and principal component analysis of green pu-erh, green, and white
482 teas using UPLC/DAD/MS. *Food Chem.* 126:1269-1277.
- 483 [17] Vallverdu-Queralt A, Jauregui O, Medina-Remon A, Andres-Lacueva C,
484 Lamuela-Raventos RM (2010) Improved characterization of tomato polyphenols
485 using liquid chromatography/electrospray ionization linear ion trap quadrupole
486 Orbitrap mass spectrometry and liquid chromatography/electrospray ionization
487 tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 24:2986-2992.
- 488 [18] Yang B, Kortensniemi M, Liu P, Karonen M, Salminen JP (2012) Analysis of
489 Hydrolyzable Tannins and Other Phenolic Compounds in Emblic Leafflower
490 (*Phyllanthus emblica* L.) Fruits by High Performance Liquid Chromatography-
491 Electrospray Ionization Mass Spectrometry. *J. Agric. Food Chem.* 60:8672-
492 8683.
- 493 [19] Cortina-Puig M, Gallart-Ayala H, Lacorte S (2012) Liquid chromatography
494 coupled to electrochemical detection and mass spectrometry for the
495 determination of phenolic compounds in food and beverages. *Curr. Anal. Chem.*
496 8:436-455.
- 497 [20] Hammouda H, Cherif JK, Trabelsi-Ayadi M, Baron A, Guyot S (2013) Detailed
498 polyphenol and tannin composition and its variability in Tunisian dates (*Phoenix*
499 *dactylifera* L.) at different maturity stages. *J. Agric. Food Chem.* 61:3252-3263.
- 500 [21] Navarro M, Núñez O, Saurina J, Hernández-Cassou S, Puignou L (2014)
501 Characterization of fruit products by capillary zone electrophoresis and liquid
502 chromatography using the compositional profiles of polyphenols: Application to
503 authentication of natural extracts. *J. Agric. Food Chem.* 62:1038-1046.
- 504 [22] Iswaldi I, Gomez-Caravaca AM, Arraez-Roman D, Uberos J, Lardon M, Segura-
505 Carretero A, Fernández-Gutierrez A (2012) Characterization by high-
506 performance liquid chromatography with diode-array detection coupled to time-
507 of-flight mass spectrometry of the phenolic fraction in a cranberry syrup used to
508 prevent urinary tract diseases, together with a study of its antibacterial activity. *J*
509 *Pharm Biomed Anal* 58:34-41.
- 510 [23] Rockenbach II, Jungfer E, Ritter C, Santiago-Schuebel B, Thiele B, Fett R,
511 Galensa R (2012) Characterization of flavan-3-ols in seeds of grape pomace by
512 CE, HPLC-DAD-MSn and LC-ESI-FTICR-MS. *Food Res. Int.* 48:848-855.
- 513 [24] Li HJ, Deinzer ML (2007) Tandem mass spectrometry for sequencing
514 proanthocyanidins. *Anal. Chem.* 79:1739-1748.

- 515 [25] Hümmer W, Schreier P (2008) Analysis of proanthocyanidins. *Mol. Nutr. Food*
516 *Res.* 52:1381-1398.
- 517 [26] Cote J, Caillet S, Doyon G, Sylvain JF, Lacroix M (2010) Analyzing Cranberry
518 Bioactive Compounds. *Crit. Rev. Food Sci. Nutr.* 50:872-888.
- 519 [27] Saurina J (2009) Characterization of wines through the compositional profiles of
520 biogenic amines and related compounds. focusing on the description of
521 toxicological and organoleptic features. *Red Wine Health*:1-24.
- 522 [28] Saurina J (2010) Characterization of wines using compositional profiles and
523 chemometrics. *TrAC, Trends Anal. Chem.* 29:234-245.
- 524 [29] Serrano-Lourido D, Saurina J, Hernández-Cassou S, Checa A (2012)
525 Classification and characterisation of Spanish red wines according to their
526 appellation of origin based on chromatographic profiles and chemometric data
527 analysis. *Food Chem.* 135:1425-1431.
- 528 [30] Wallace TC, Giusti MM (2010) Extraction and normal-phase HPLC-
529 fluorescence-electrospray MS characterization and quantification of
530 procyanidins in cranberry extracts. *J Food Sci* 75:C690-C696.
- 531 [31] Wise B, Gallager NB (1992) *PLS_Toolbox for use with MATLAB*, version 2.0;
532 Eigenvector Research Inc.; Mason, WA.
- 533 [32] Massart DL, Vandeginste BGM, Buydens LMC, de Jong S, Lewi PJ, Smeyers-
534 Verbeke J (1997) *Handbook of Chemometrics and Qualimetrics*; Elsevier:
535 Amsterdam.
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539 **Figure captions**

540

541 **Fig. 1.** LC-ESI-MS chromatogram for a mixture of the 26 analyzed polyphenols at 500
542 $\mu\text{g/L}$. Peak identification as in Table 1.

543

544 **Fig. 2.** LC-ESI-MS/MS chromatograms of 14 selected polyphenols found in a cranberry
545 pill pharmaceutical sample.

546

547 **Fig. 3.** Concentration levels of 10 selected polyphenols in (a) cranberry and grape fruit,
548 juices and raisins, and (b) cranberry-based pharmaceutical preparations.

549

550 **Fig. 4.** PCA results using normalized concentrations as the analytical data. (a) Scatter
551 plot of scores of PC1 and PC2; Grape samples in green circles, cranberry samples in red
552 circles. F: fruit; J: juice; R: raisin (dried sample); E: extract; S: sachet; P: pill; and Sy:
553 syrup. (b) Scatter plot of loadings of PC1 and PC2. Dashed line indicates the separation
554 among cranberry- and grape-based samples

555

556

557

Table 1. Selected reaction monitoring (SRM) acquisition parameters

Peak	Compound	Precursor ions	Product ion assignment (quantifier/qualifier)	Collision energy (CE, eV)
1	Gallic acid	169.0 [M-H] ⁻	125.1 [M-H-CO ₂] ⁻	15
			79.0 [M-H-C ₂ H ₂ O ₄] ⁻	23
2	Homogentisic acid	167.1 [M-H] ⁻	123.0 [M-H-CO ₂] ⁻	13
			122.1 [M-H-CHO ₂] ^{-*}	23
3	Protocatechuic acid	153.0 [M-H] ⁻	109.0 [M-H-CO ₂] ⁻	16
			108.0 [M-H-CHO ₂] ^{-*}	24
4	Protocatechualdehyde	137.0 [M-H] ⁻	108.0 [M-H-CHO] ^{-*}	22
			92.0 [M-H-CHO ₂] ^{-*}	25
5	(+) -Catechin hydrate	289.1 [M-H] ⁻	245.1 [M-H-C ₂ H ₄ O] ⁻	15
			203.1 [M-H-C ₄ H ₆ O ₂] ⁻	20
6	Gentisic acid	153.1 [M-H] ⁻	108.0 [M-H-CHO ₂] ^{-*}	22
			109.0 [M-H-CO ₂] ⁻	14
7	p-Salicylic acid	137.0 [M-H] ⁻	93.0 [M-H-CO ₂] ⁻	16
			65.0 [M-H-C ₂ O ₃] ⁻	30
8	Chlorogenic acid	353.0 [M-H] ⁻	191.1 [M-H-C ₉ H ₆ O ₃] ⁻	21
			85.0 [M-H-C ₁₂ H ₁₂ O ₇] ⁻	44
9	Vanillic acid	167.1 [M-H] ⁻	152.1 [M-H-CH ₃] ^{-*}	15
			108.0 [M-H-C ₂ H ₃ O ₂] ^{-*}	18
10	Caffeic acid	179.1 [M-H] ⁻	135.1 [M-H-CO ₂] ⁻	16
			134.1 [M-H-CHO ₂] ^{-*}	25
11	(-) -Epicatechin	289.1 [M-H] ⁻	245.1 [M-H-C ₂ H ₄ O] ⁻	16
			203.1 [M-H-C ₄ H ₆ O ₂] ⁻	20
12	(-) -Epigallocatechin gallate	457.0 [M-H] ⁻	169.0 [M-H-C ₁₅ H ₁₂ O ₆] ⁻	19
			125.1 [M-H-C ₁₆ H ₁₂ O ₈] ⁻	39
13	Syringic acid	197.0 [M-H] ⁻	182.1 [M-H-CH ₃] ^{-*}	14
			123.0 [M-H-C ₂ H ₂ O ₃] ⁻	24
14	Syringaldehyde	181.0 [M-H] ⁻	166.0 [M-H-CH ₃] ^{-*}	13
			151.0 [M-H-CH ₂ O] ⁻	19
15	Ethyl gallate	197.0 [M-H] ⁻	124.0 [M-H-C ₃ H ₅ O ₂] ^{-*}	22
			169.0 [M-H-C ₂ H ₄] ⁻	14
16	Umbelliferon	161.0 [M-H] ⁻	133.0 [M-H-CO] ⁻	19
			105.0 [M-H-C ₃ H ₄ O] ⁻	21
17	p-coumaric acid	163.1 [M-H] ⁻	119.1 [M-H-CO ₂] ⁻	16
			93.1 [M-H-C ₃ H ₂ O ₂] ⁻	34
18	Taxifolin	303.0 [M-H] ⁻	285.0 [M-H-H ₂ O] ⁻	13
			175.0 [M-H-C ₆ H ₈ O ₃] ⁻	24
19	Polydatin	389.1 [M-H] ⁻	227.1 [M-H-C ₆ H ₁₀ O ₅] ⁻	20
			185.1 [M-H-C ₈ H ₁₂ O ₆] ⁻	38
20	Ferulic acid	193.1 [M-H] ⁻	134.1 [M-H-C ₂ H ₃ O ₂] ^{-*}	18
			178.1 [M-H-CH ₃] ^{-*}	14
21	Sinapic acid	223.0 [M-H] ⁻	208.0 [M-H-CH ₃] ^{-*}	15
			164.1 [M-H-C ₂ H ₃ O ₂] ^{-*}	18
22	Resveratrol	227.0 [M-H] ⁻	143.1 [M-H-C ₄ H ₄ O] ⁻	27
			185.0 [M-H-C ₂ H ₂ O] ⁻	19
23	Quercitrin hydrate	447.0 [M-H] ⁻	300.1 [M-H-C ₆ H ₁₁ O ₄] ^{-*}	21
			271.0 [M-H-C ₇ H ₁₂ O ₅] ⁻	37
24	Fisetin	285.0 [M-H] ⁻	135.0 [M-H-C ₈ H ₆ O ₃] ⁻	23
			121.1 [M-H-C ₉ H ₄ O ₅] ⁻	27
25	Quercetin dihydrate	301.1 [M-H] ⁻	151.1 [M-H-C ₈ H ₆ O ₃] ⁻	22
			179.0 [M-H-C ₇ H ₆ O ₂] ⁻	18
26	Kaempferol	285.0 [M-H] ⁻	185.0 [M-H-C ₄ H ₄ O ₃] ⁻	25
			117.0 [M-H-C ₇ H ₆ O ₃] ⁻	43

Table 2. Instrumental quality parameters

Peak	Compound	LOD (µg/L)	LOQ (µg/L)	Working range (mg/L)	run-to-run precision, %RSD (n=5)			day-to-day precision, %RSD (n=5x3)			Accuracy ^a (% relative error)
					Low level (LOQ)	Middle level (500 µg/L)	High level	Low level (LOQ)	Middle level (500 µg/L)	High level (10 µg/L)	
1	Gallic acid	13	43	0.043-100	4.8	5.5	3.0	8.3	4.7	4.5	0.6-6.1
2	Homogentisic acid	12	40	0.040-100	4.4	2.5	3.4	5.6	7.7	4.0	1.9-7.8
3	Protocatechuic acid	14	47	0.047-100	4.9	1.6	1.6	9.0	6.2	4.7	1.1-3.5
4	Protocatechualdehyde	14	47	0.047-100	3.7	4.5	2.9	8.7	7.8	3.8	0.5-1.3
5	(+)-Catechin hydrate	13	43	0.043-100	5.9	3.6	1.3	6.2	8.2	4.8	0.2-7.7
6	Gentisic acid	14	47	0.047-100	8.2	5.6	2.8	13.4	8.2	4.9	0.3-2.1
7	p-Salicylic acid	13	43	0.043-100	7.3	2.1	5.2	8.5	6.7	7.2	0.1-4.6
8	Chlorogenic acid	13	43	0.043-100	7.8	3.5	2.5	9.6	6.5	5.8	4.1-5.8
9	Vanillic acid	14	47	0.047-100	9.9	5.0	4.1	12.9	12.5	7.5	0.6-5.1
10	Caffeic acid	31	103	0.103-100	6.8	1.6	1.3	8.8	3.8	3.5	0.4-2.1
11	(-)-Epicatechin	26	87	0.087-100	4.8	1.6	2.1	8.1	5.6	4.1	0.3-4.2
12	(-)-Epigallocatechin gallate	32	107	0.107-100	1.2	2.3	1.7	9.6	4.5	6.2	3.5-5.0
13	Syringic acid	14	47	0.047-100	6.3	5.8	2.1	13.5	7.9	7.4	1.8-8.0
14	Syringaldehyde	13	43	0.043-100	3.2	1.9	3.2	6.8	5.2	5.2	0.4-2.5
15	Ethyl gallate	13	43	0.043-100	6.3	2.6	0.6	7.7	3.5	4.9	0.3-5.6
16	Umbelliferon	13	43	0.043-100	4.0	4.6	3.4	5.4	5.4	3.6	0.1-0.4
17	p-coumaric acid	34	113	0.113-100	9.2	2.3	2.6	8.7	5.6	4.9	1.2-3.3
18	Taxifolin	13	43	0.043-100	6.7	4.5	3.8	8.6	6.3	4.4	1.5-2.4
19	Polydatin	13	43	0.043-100	6.7	2.8	3.2	10.0	7.7	5.4	1.4-2.9
20	Ferulic acid	13	43	0.043-100	4.1	6.8	8.2	7.3	6.1	8.1	1.3-4.8
21	Sinapic acid	14	47	0.047-100	3.4	6.1	1.3	6.3	5.6	4.2	0.1-0.9
22	Resveratrol	68	227	0.227-100	2.8	3.2	1.8	7.2	6.4	3.1	1.2-4.9
23	Quercitrin hydrate	14	47	0.047-100	3.1	3.9	5.1	11.9	7.5	6.7	3.1-5.6
24	Fisetin	116	387	0.387-100	6.6	-	4.0	6.8	-	5.6	3.1-4.2
25	Quercetin dihydrate	39	130	0.130-100	4.9	3.0	3.6	5.3	4.4	3.8	1.1-2.6
26	Kaempferol	111	370	0.370-100	4.4	-	4.7	5.3	-	4.8	0.2-2.8

^a Accuracy range for all evaluated concentration levels

Table 3. Polyphenol concentration levels in cranberry-based and grape-based products^a

Peak	Compound	Cranberry-based samples						Grape-based samples				
		juice 2	syrup	raw extract 1	sachet 1	capsules 1	concentration range	juice 2	juice 3	raisin 2	raisin 3	concentration range
1	Gallic acid	LOD	10.9±0.4	150.4±0.8	10.0±0.6	38.0±0.8	2.2-235.6	15.7±0.8	70.9±1.6	4.4±0.3	6.0±0.2	3.3-99.7
2	Homogentisic acid	nd	nd	6.4±0.2	nd	9.2±0.1	1.2-11.0	nd	nd	nd	nd	nd
3	Protocatechuic acid	22.4±0.9	172.8±1.1	904.8±4.1	230.8±1.8	370.6±2.0	6.5-904.8	8.1±0.2	22.4±0.5	10.6±0.2	LOQ	3.0-22.4
4	Protocatechualdehyde	LOD	LOD	13.7±0.4	LOD	45.5±0.5	1.2-125.2	LOD	LOD	LOD	LOD	1.3-2.6
5	(+)-Catechin hydrate	4.3±0.2	nd	142.8±1.6	13.5±0.2	3363.0±4.6	2.6-7383.3	10.0±0.1	70.5±0.5	nd	7.1±0.1	7.1-109.4
6	Gentisic acid	nd	LOD	16.0±0.3	LOD	LOD	1.1-36.6	nd	nd	nd	nd	nd
7	p-Salicylic acid	nd	LOD	5.6±0.1	nd	LOD	1.5-5.6	nd	nd	nd	nd	5.0
8	Chlorogenic acid	14.0±0.3	88.2±0.8	368.5±1.1	44.7±0.7	22.1±0.6	6.1-368.5	nd	nd	nd	nd	6.8
9	Vanillic acid	nd	16.0±0.4	135.0±0.8	LOD	nd	1.7-135.0	nd	nd	nd	nd	4.2-7.1
10	Caffeic acid	nd	19.6±0.1	248.5±0.8	LOD	16.9±0.1	3.0-248.5	nd	LOD	nd	nd	4.3-5.6
11	(-)-Epicatechin	13.7±0.3	nd	1038.8±3.4	344.8±0.8	3239.6±2.6	1.3-7297.6	2.3±0.3	23.7±0.5	nd	nd	1.3-43.2
12	(-)-Epigallocatechin gallate	nd	nd	nd	nd	128.9±0.1	12.5-1425.7	nd	nd	nd	nd	6.0
13	Syringic acid	nd	LOD	33.6±0.3	LOD	nd	1.6-304.2	8.7±0.2	8.1±0.1	nd	nd	8.1-13.4
14	Syringaldehyde	nd	nd	10.1±0.4	nd	nd	6.4-10.1	nd	nd	nd	nd	nd
15	Ethyl gallate	nd	nd	nd	nd	nd	2.3-303.2	nd	nd	nd	nd	nd
16	Umbelliferon	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
17	p-coumaric acid	14.1±0.7	206.0±0.8	760.4±1.9	LOD	43.3±0.7	1.5-760.4	nd	4.3±0.1	nd	nd	4.3-4.8
18	Taxifolin	nd	nd	273.5±0.7	8.6±0.1	75.3±0.5	3.6-273.5	nd	nd	nd	nd	nd
19	Polydatin	nd	nd	5.4±0.4	nd	8.7±0.7	5.4-16.5	LOD	12.9±0.3	nd	LOD	1.3-12.9
20	Ferulic acid	LOD	33.8±0.7	93.0±1.0	LOD	6.4±0.1	1.2-93.0	nd	LOD	LOD	nd	1.0-1.7
21	Sinapic acid	nd	19.9±0.5	24.1±0.5	LOD	LOD	1.9-50.9	nd	nd	nd	nd	nd
22	Resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
23	Quercitrin hydrate	16.7±0.5	89.1±0.8	1228.6±1.2	nd	24.3±0.6	3.6-1857.5	6.7±0.1	LOD	nd	nd	1.6-6.7
24	Fisetin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
25	Quercetin dihydrate	21.8±0.4	635.1±0.9	3356.1±3.2	91.6±0.1	572.3±1.1	3.9-3526.7	nd	14.8±0.2	LOD	5.6±0.1	3.7-14.8
26	Kaempferol	nd	nd	16.8±0.4	nd	53.2±0.3	16.8-130.0	nd	nd	nd	nd	nd

^a All concentrations are in mg/kg. Quantifications performed by triplicate (n=3); results are expressed as mean of samples analyzed ± standard deviation; nd, not detected.

Figure 1

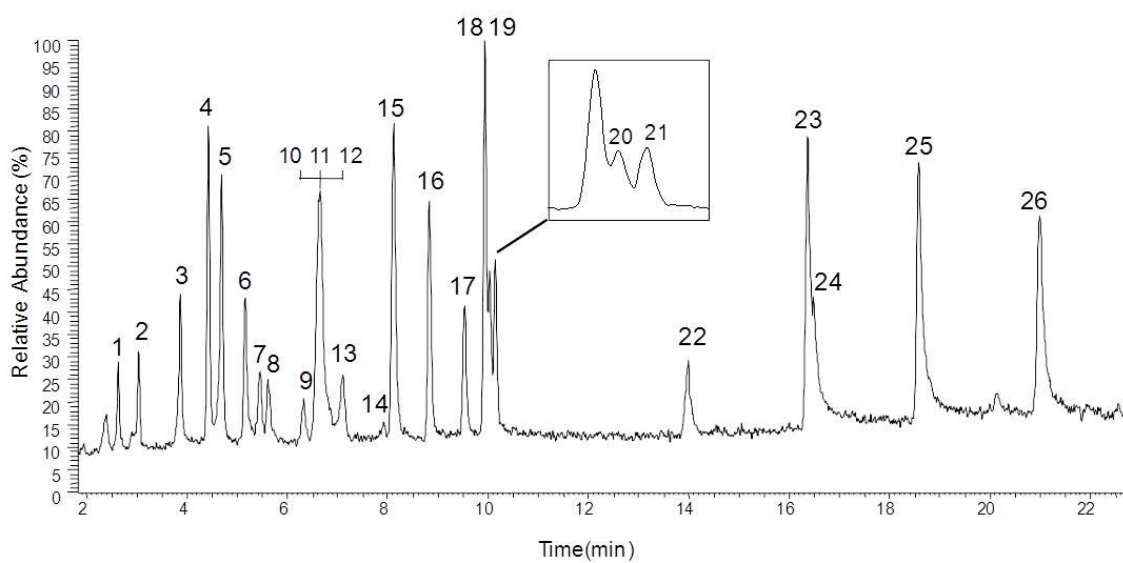


Figure 2

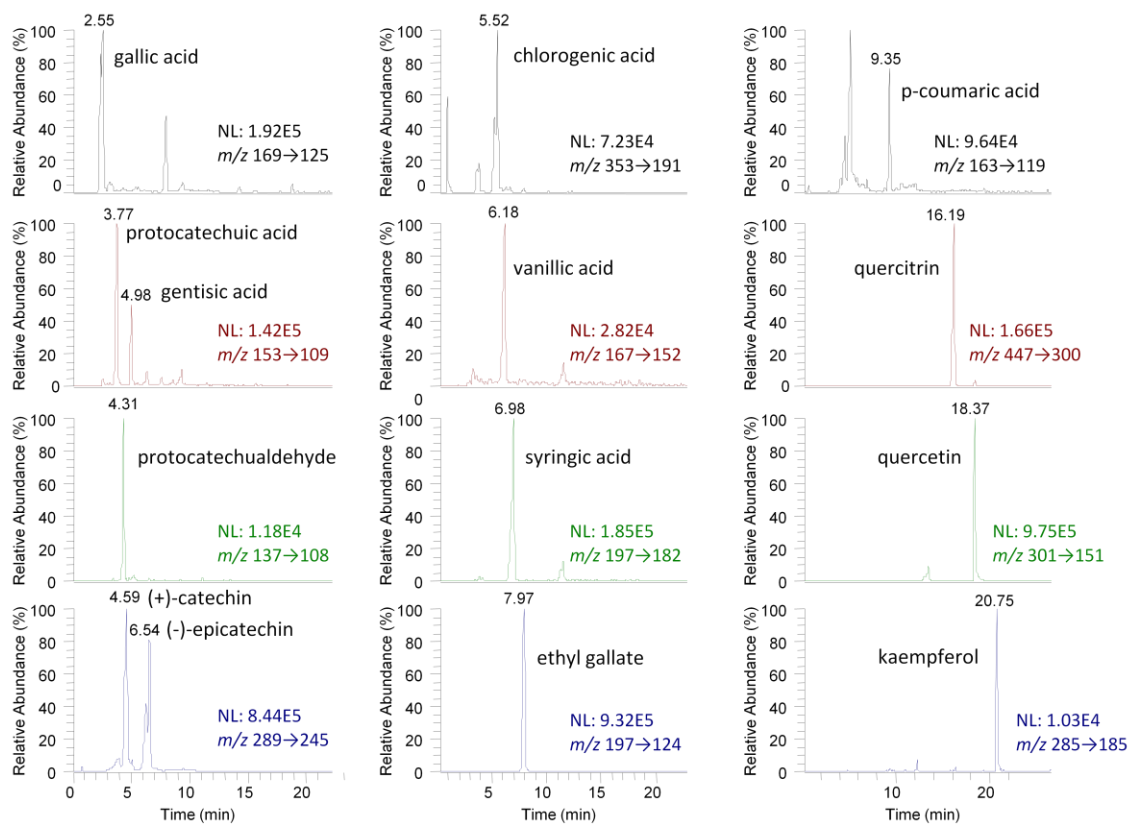


Figure 3

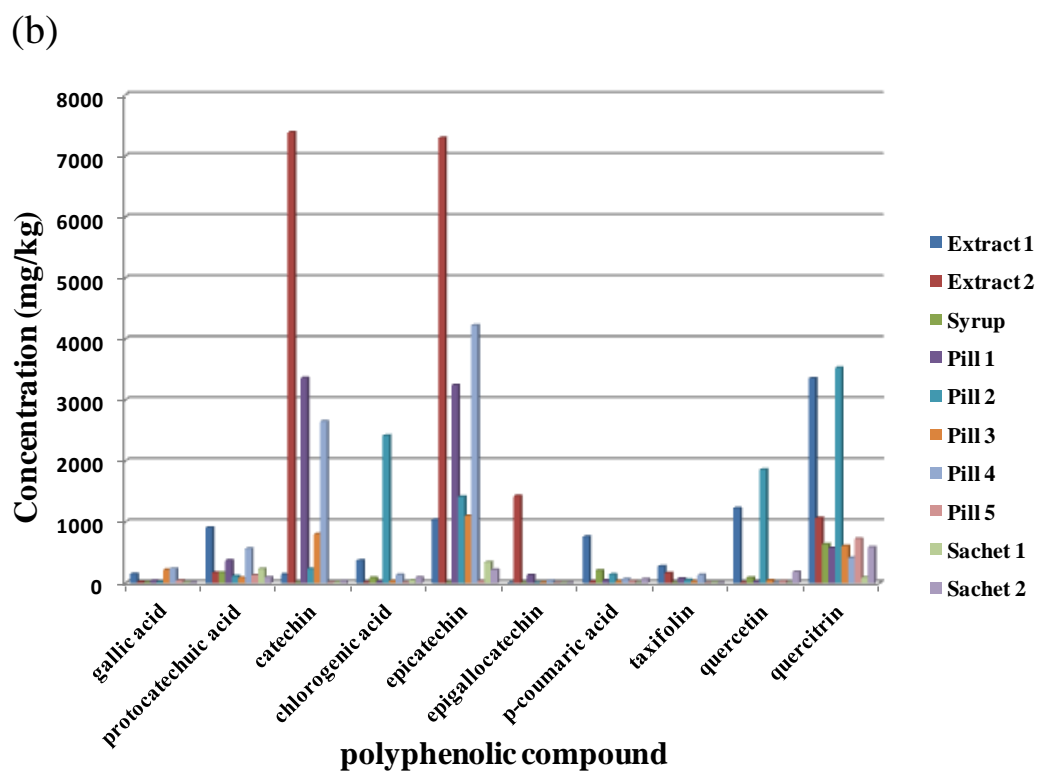
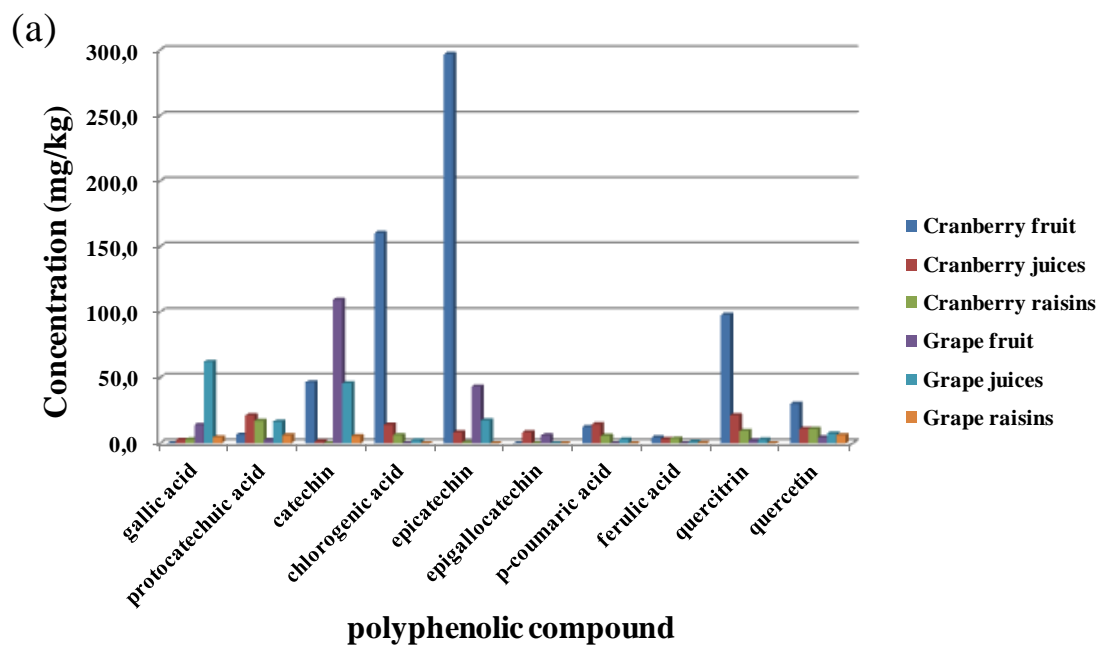


Figure 4

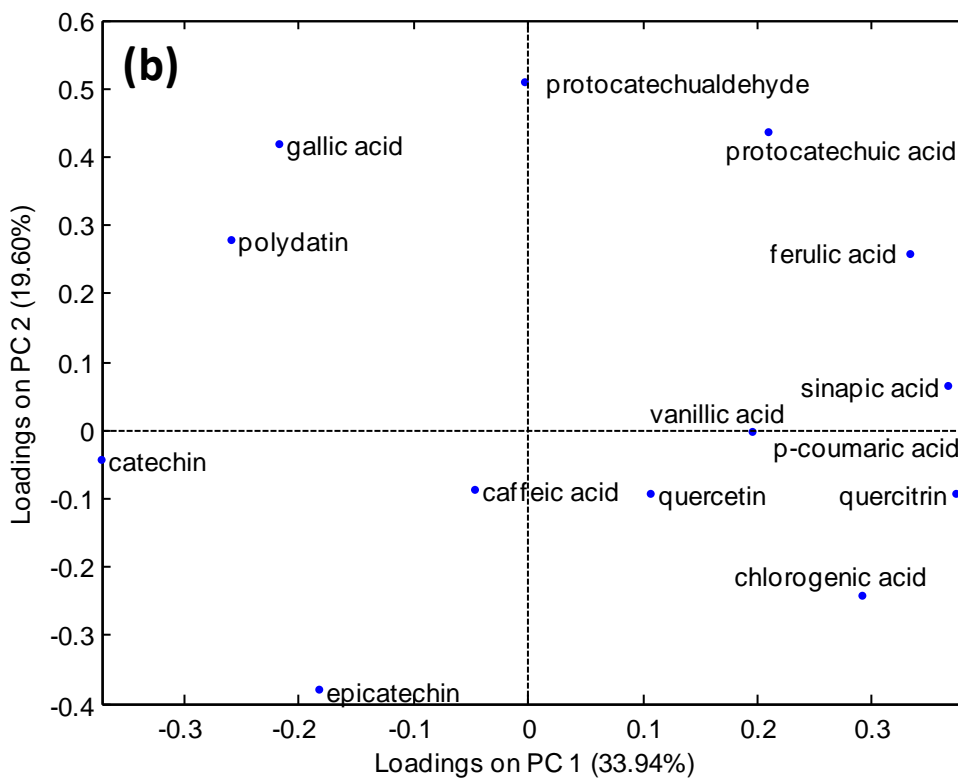
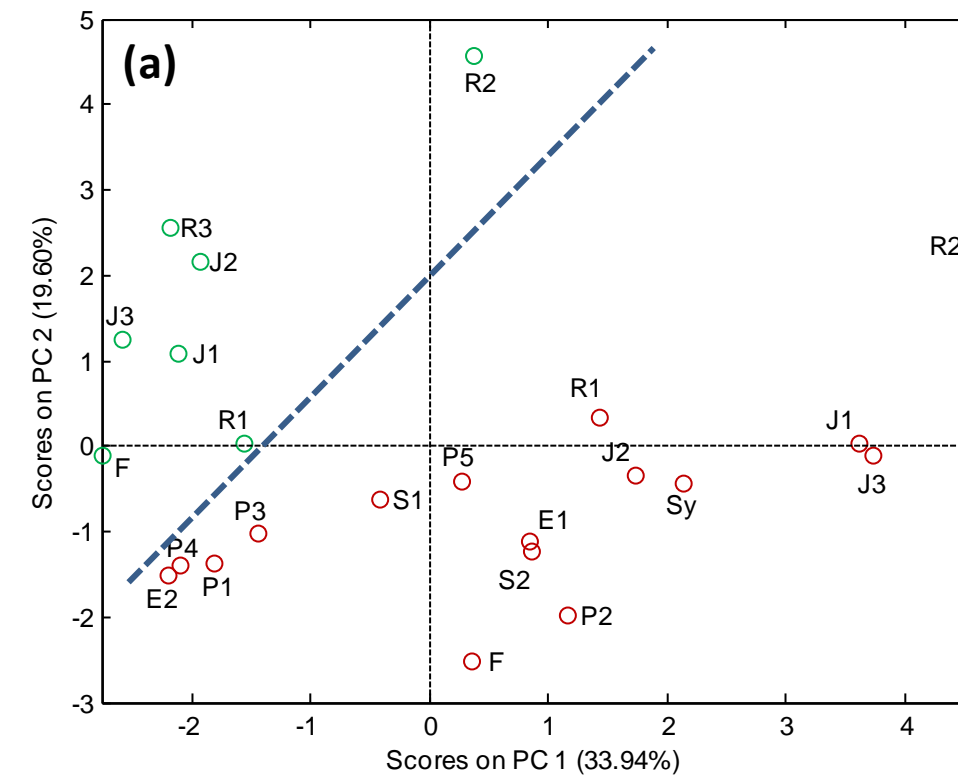


Figure 1S. Ion suppression study for three coeluting polyphenols (caffeic acid, epicatechin and epigallocatechin gallate).

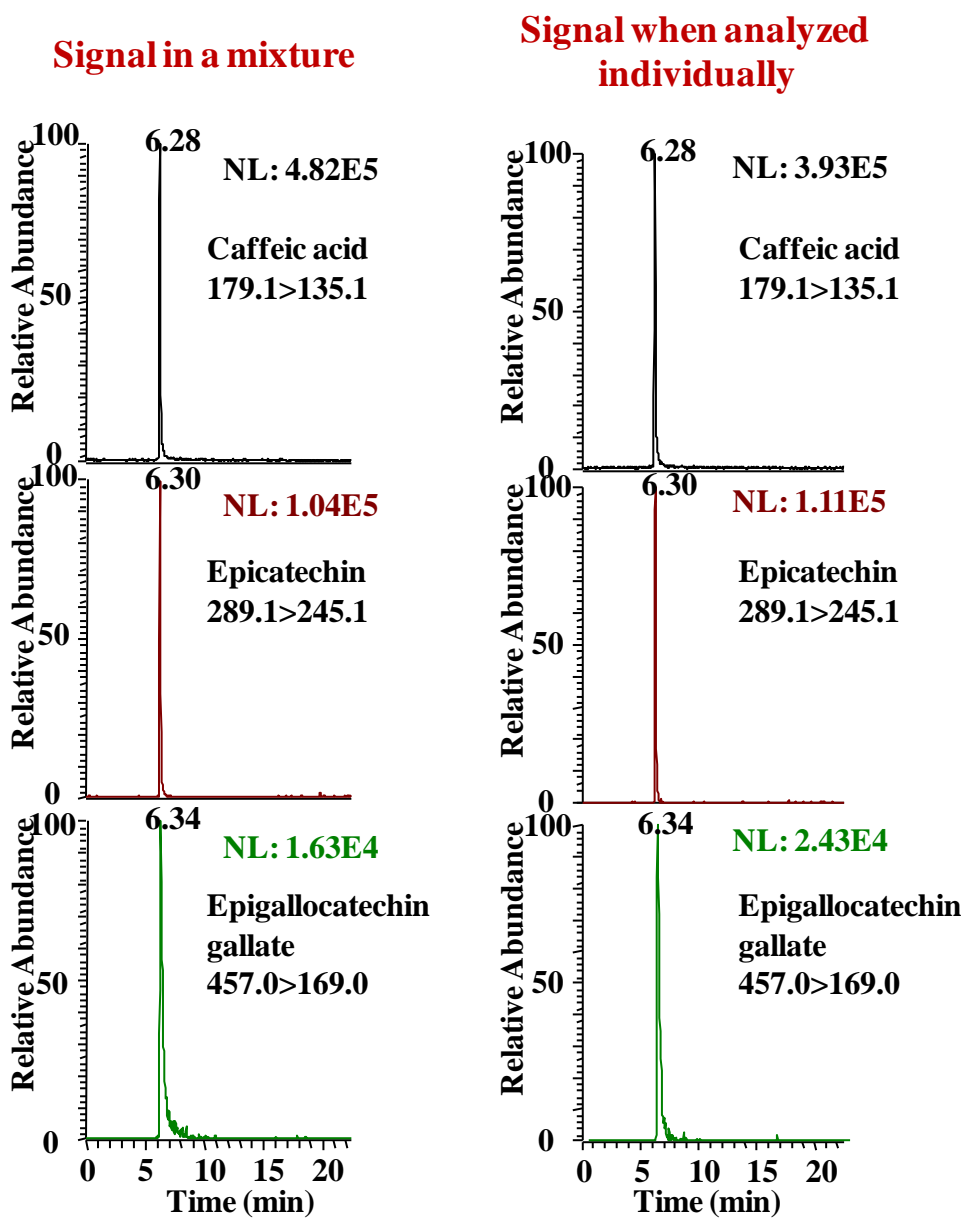


Table 1S. Comparison of calibration procedures for polyphenol quantification in fruit-based products by LC-ESI-MS/MS^a

Peak	Compound	Cranberry fruit		Cranberry juice		Cranberry raisins		Grape fruit		Grape juice 1		Grape raisins	
		EC	SA	EC	SA	EC	SA	EC	SA	EC	SA	EC	SA
1	Gallic acid	nd	nd	LOD	LOD	LOD	LOD	14.0±1.2	12.4±0.2	99.7±4.3	99.5±1.4	LOD	LOD
2	Homogentisic acid	LOD	LOD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	Protocatechuic acid	6.5±0.1	3.9±0.4	26.7±2.0	29.6±0.8	9.4±1.5	11.9±0.8	LOD	LOD	18.7±2.6	16.2±0.2	LOD	LOD
4	Protocatechualdehyde	nd	nd	nd	nd	LOD	LOD	LOD	LOD	LOD	LOD	nd	nd
5	(+)-Catechin hydrate	46.5±2.5	50.4±0.5	nd	nd	nd	nd	109.4±10.8	112.9±10.2	57.0±5.1	54.6±4.4	9.2±0.1	13.4±0.2
6	Gentisic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	p-Salicylic acid	nd	nd	nd	nd	nd	nd	nd	nd	5.0±0.8	3.9±0.9	nd	nd
8	Chlorogenic acid	160.4±5.6	165.2±1.1	14.2±1.3	17.7±1.1	6.3±0.8	8.2±1.1	nd	nd	6.8±0.7	3.7±0.3	nd	nd
9	Vanillic acid	5.1±0.5	4.4±0.4	6.9±0.6	7.2±0.5	LOD	LOD	LOD	LOD	7.1±0.5	4.3±0.2	nd	nd
10	Caffeic acid	4.4±0.2	7.4±0.5	nd	nd	nd	nd	nd	nd	5.6±0.6	7.4±0.2	nd	nd
11	(-)-Epicatechin	296.8±11.7	257.3±0.2	9.3±1.1	8.6±0.2	LOD	LOD	43.2±2.9	37.7±2.3	26.1±0.6	30.6±0.3	nd	nd
12	(-)-Epigallocatechin gallate	nd	nd	nd	nd	nd	nd	6.0±0.4	3.0±0.6	nd	nd	nd	nd
13	Syringic acid	nd	nd	nd	nd	nd	nd	nd	nd	13.4±0.8	17.6±0.3	nd	nd
14	Syringaldehyde	6.4±0.4	4.3±0.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	Ethyl gallate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
16	Umbelliferon	nd	nd	nd	nd	nd	dn	nd	nd	nd	nd	nd	nd
17	p-coumaric acid	12.4±1.2	15.9±0.8	14.7±1.7	18.8±0.8	LOD	LOD	nd	nd	4.6±0.1	9.9±0.7	nd	nd
18	Taxifolin	LOD	LOD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
19	Polydatin	nd	nd	nd	nd	nd	nd	8.9±0.9	9.6±0.7	7.4±0.3	3.4±0.1	nd	nd
20	Ferulic acid	4.3±0.4	5.5±0.6	4.4±0.6	5.5±1.1	nd	nd	nd	nd	LOD	LOD	nd	nd
21	Sinapic acid	LOD	LOD	LOD	LOD	nd	nd	nd	nd	nd	nd	nd	nd
22	Resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
23	Quercitrin hydrate	97.9±4.7	93.1±1.2	23.6±1.8	25.0±1.2	3.6±0.2	7.5±1.2	2.2±0.4	5.0±0.7	nd	nd	nd	nd
24	Fisetin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
25	Quercetin dihydrate	30.0±1.6	26.2±0.4	7.3±0.5	6.6±0.4	14.1±3.3	10.4±0.4	4.3±0.1	4.3±0.1	7.0±0.1	3.8±0.3	9.1±0.3	4.3±0.4
26	Kaempferol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<i>p</i> value ^b		0.38		0.06		0.54		0.73		0.77		0.95	

^a All concentrations are in mg/kg. Quantifications performed by triplicate (n=3); results expressed as concentration mean of samples analyzed ± standard deviation.

EC, external calibration; SA, standard addition; nd, not detected

^b For a 95 % confidence level.