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Comprehensive evaluation of phenolic profile in dark chocolate and dark chocolate enriched with Sakura green tea leaves or turmeric powder

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1 **Abstract**

2 Recently, a huge number of studies have confirmed the important role of chocolate polyphenols in
3 human health, underlining its beneficial effects especially in the treatment of cardiovascular
4 diseases. However, a thorough evaluation of chocolate phenolic profile is still lacking. This study
5 aimed at a comprehensive characterisation of dark chocolate phenolic profile, using non-targeted
6 mass spectrometry identification. This approach allowed a tentative identification of 158 individual
7 phenolic compounds: 67 were newly detected in dark chocolate, among these 38 were observed for
8 the first time in chocolate as well as in cocoa beans or products. Ellagitannins, which have never
9 been reported in cocoa or chocolate, represented about the 10% of the phenolic profile of dark
10 chocolate. The enrichment of dark chocolate with Sakura green tea leaves or turmeric powder
11 influenced and modified the phenolic profile, resulting in a phenolic concentration increase. In this
12 way, this functional chocolate might maximize the beneficial effect of chocolate consumption,
13 combining the positive health effects of chocolate, turmeric and green tea and, at the same time,
14 reducing the amount of sugars and calories introduced with chocolate.

15

16 **Keywords:** epicatechin, curcuminoids, ellagitannins, mass spectrometry, polyphenols,
17 metabolomics, functional foods

18 **1. Introduction**

19 Western lifestyle built-around a highly refined diet rich in saturated fat and sugars but low in
20 complex plant carbohydrates, phytochemicals and vitamins is a hot research topic in the field of
21 nutrition. It is widely known that diet is the cause of many pathogenic age-related conditions. The
22 intake of certain dietary components is plays an essential role in the prevention or management of
23 these diseases (Del Rio et al., 2013). Increasing interest has pointed to naturally occurring
24 compounds, which have been considered non-nutritive for a long time. Polyphenols are a
25 representative class of these compounds and can be summarised into several groups, i.e.
26 hydroxybenzoic acids, hydroxycinnamic acids, flavan-3-ols, flavonols, flavones, flavanones,
27 isoflavones, anthocyanins, ellagitannins, stilbenes, and lignan. They occur in all fruits, vegetables,
28 nuts, seeds, flowers, bark, beverages and processed food. As reviewed by Wollgast, & Anklam
29 (2000a; 2000b) polyphenols are characterised by several beneficial effects including anti-
30 carcinogenic, anti-atherogenic, anti-inflammatory, immunomodulating and vasodilatory activities.
31 They can exert their protective effects through several mechanisms such as plasma cholesterol
32 reduction, modulation of lipid and lipoprotein metabolism, modulation of enzymes (phase I and
33 phase II) and apoptosis as well as their activity against reactive oxygen species (Del Rio et al.,
34 2013).

35 Cocoa (*Theobroma cacao*) is known as a rich source of dietary phenolic compounds. Cocoa-derived
36 products such as dark chocolate are widely studied for their beneficial effects ascribed to
37 polyphenols. There is good evidence to suggest that cocoa derived polyphenols may have beneficial
38 effects on cardiovascular disease risk factors (Del Rio et al., 2013). Short-term dark chocolate
39 intake has been shown to reduce blood pressure in hypertensive subjects, to improve endothelial
40 function and insulin resistance as well as to inhibit platelet activation (Del Rio et al., 2013). As
41 reported by Rusconi, & Conti (2010), cocoa beans are characterised by phenolic compounds of the
42 flavan-3-ol group (catechin, epicatechin, galocatechin and epigallocatechin) comprising oligomeric

43 procyanidins, anthocyanins (cyanidin glycosides) and flavonol glycosides such as quercetin-3-*O*-
44 rutinoside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucuronide and
45 quercetin (Sanbongi et al., 1998). So far, only few studies have investigated the phenolic
46 composition of dark chocolate, focusing on flavan-3-ols as the major class in chocolate phenolic
47 profile (Ortega et al., 2008; Wollgast, & Anklam, 2000a). This lack of information is also due to the
48 great interest addressed to the study of phenolic profile of cocoa, intended as raw material in
49 chocolate production, without considering the impact of processing temperature, microbial
50 fermentation or oxidative phenomena on the phenolics structure during cocoa processing in
51 chocolate production. The majority of published researches were aimed at analyzing the impact of
52 processing on the polyphenol content and antioxidant properties of cocoa more than that of
53 chocolate (Di Mattia, Sacchetti, Mastrocola, & Serafini, 2017; Dorota, Oracz, Sosnowska, &
54 Nebesny, 2016). Concerning this, it was considered purposeful to investigate the comprehensive
55 phenolic profile of commercial dark chocolate (70%), using an un-targeted mass spectrometry
56 approach, in order to fill the gap of information about dark chocolate phenolic composition. Finally,
57 the last task was to evaluate a possible polyphenolic enrichment of dark chocolate recipe, by adding
58 widely studied polyphenol-rich ingredients (Sakura green tea leaves and turmeric powder) in order
59 to obtain potential functional food, which can combine the above-mentioned chocolate properties
60 and those of green tea leaves and turmeric powder (Del Rio et al., 2013; Kunnumakkara et al.,
61 2017).

62 Therefore, the aim of the present study was to identify, quantify and compare phenolic compounds
63 from three different types of dark chocolate using liquid chromatography-electrospray ionization
64 mass spectrometry (LC-ESI-QTOF-MS/MS).

65 **2. Materials and methods**

66 **2.1. Materials**

67 Phenolic compounds standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox),
68 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tri(2-pyridyl)-S-triazine
69 (TPTZ), Folin-Ciocalteu phenol reagent were purchased from Sigma (Milan, Italy). Methanol and
70 formic acid were obtained from Carlo Erba (Milan, Italy). Three different types of chocolate (dark
71 70% cocoa (DC), dark 70% cocoa and 8% turmeric (TDC), dark 70% cocoa and 2% Sakura green
72 tea (GTDC)) were bought from a local shop in Modena (Italy).

74 **2.2. Extraction of phenolic compounds**

75 Polyphenols were extracted as reported in Martini, Conte, & Tagliazucchi (2017) with minor
76 modifications. Ten grams of chocolate were melted at 50°C for 10 minutes and homogenized with
77 20 mL of water/methanol/formic acid solution (28:70:2, v/v/v). The mixtures were stirred and
78 maintained at 37°C for 30 minutes. The homogenates were centrifuged (5000 rpm, 10 min, 4°C),
79 after that the floating cocoa butter layers were removed and the supernatants collected. Pellets were
80 then used for a second extraction step with acetone. Each pellet was added with 20 mL of acetone,
81 kept in agitation at 37°C, for 30 minutes and then centrifuged for 20 minutes at 5000 rpm, 4°C. The
82 supernatants were collected. Both methanol and acetone extractions were performed twice. The
83 methanolic and acetone extracts were diluted 8 and 2 times, respectively, using MilliQ water and
84 further used for the MS analysis.

86 **2.3. Identification and quantification of phenolic compounds by liquid chromatography mass 87 spectrometry (LC-ESI-QTOF-MS/MS)**

88 Chocolate methanolic and acetone extracts were analysed on Agilent HPLC 1200 Infinity (Agilent
89 Technologies, Santa Clara, CA) equipped with a C18 column (HxSil C18 Reversed phase, 250×4.6

90 mm, 5 μ m particle size, Hamilton Company, Reno, Nevada, USA). The mobile phase consisted of
91 (A) H₂O/formic acid (99:1, v/v) and (B) acetonitrile/formic acid (99:1, v/v). The gradient started at
92 4% B for 0.5 min then linearly ramped up to 30% B in 60 min. The mobile phase composition was
93 raised up to 100% B in 1 min and maintained for 5 min in order to wash the column before
94 returning to the initial condition. The flow rate was set at 1 mL/min. The chocolate extracts were
95 injected in the amount of 20 μ L. After passing to the column, the eluate was split, and 0.3 mL/min
96 was direct to a 6520 accurate-mass Q-TOF mass spectrometer (Agilent Technologies, Santa Clara,
97 CA). Identification of phenolic compounds in all samples was carried out using full scan, data-
98 dependent MS² scanning from m/z 100 to 1700 and selected reaction monitoring. MS operating
99 conditions (negative mode) were: a capillary temperature of 350°C, a dry gas flow rate of 10 L/min,
100 a nebulizer pressure of 35 psi, potential of the ESI source, 3.5 kV.

101 The quantification of single phenolic compounds was carried out by integrating the area under the
102 peak from the extracted ion chromatograms (EICs). To obtain an accurate quantification the EICs
103 were obtained by centering a narrow mass window (\pm 5 ppm) on the theoretical m/z value of each
104 phenolic compound. For each standard compound, the calibration curve was built using seven
105 concentration points in the range of 0.2-50 ng. Hydroxycinnamic acids, hydroxybenzoic acids,
106 flavan-3-ols and ellagitannins were quantified as *p*-coumaric or ferulic acid, protocatechuic acid, (-
107)-epicatechin and ellagic acid equivalents, respectively. Flavonols and flavones were quantified as
108 quercetin-3-rutinoside equivalents. Finally, curcuminoids were quantified as curcumin equivalent.

109 Quantitative results were expressed as mg of compounds per 100 g of chocolate. Calibration curve
110 equations, linearity ranges and limit of quantification (LOQ) for the different standards are given in
111 supplementary materials (**Table S1**). Folin-Ciocalteu assay was also performed to quantify the
112 total phenolic compounds as reported by Singleton, Orthofer, & Lamuela-Raventós (1999). The
113 results were expressed as mg of gallic acid per 100 g of chocolate.

114

115 **2.4. Antioxidant activity assays**

116 The antioxidant properties of chocolate were evaluated performing two different assays. The ABTS
117 (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and ferric reducing power (FRAP) assays
118 were performed according to the protocols described by Re et al. (1999) and Benzie, & Strain
119 (1996), respectively. The ABTS scavenging capacity and FRAP value were expressed as mmol of
120 trolox equivalent per 100 g of chocolate, by means of a calibration curve obtained with Trolox 50-
121 500 µmol/L, in the same assay conditions. The absorbances were read using a Jasco V-550 UV/Vis
122 spectrophotometer (Orlando, FL, USA).

123

124 **2.5. Statistic**

125 All data are presented as mean \pm SD for three replicates for each prepared sample. One-way
126 analysis of variance (one-way ANOVA) with Tukey's post-hoc test was applied using Graph Pad
127 prism 6.0 (GraphPad software, San Diego, CA, U.S.A.). The differences were considered
128 significant with $P < 0.05$.

129 3. Result and discussion

130 3.1. *Non-targeted LC-MS profiling of phenolic compounds in different types of dark chocolate*

131 This study aimed to identify and quantify the phenolic profile and content of three different types of
132 dark chocolate (dark chocolate 70% cocoa, dark chocolate 70% cocoa and 8% turmeric, dark
133 chocolate 70% cocoa and 2% Sakura green tea). The phytochemical composition focused on the
134 phenolic fraction, was investigated using a non-targeted procedure through LC-ESI-MS/MS
135 experiments, representative base peak chromatograms (BPCs) are shown in **Figure 1**. Within the 37
136 resolved peaks, 158 individual phenolic compounds were tentatively identified. Among them, 67
137 were firstly identified in dark chocolate and of these 38 were identified for the first time in
138 chocolate, cocoa beans and cocoa products. The structure of the newly identified phenolic
139 compounds is depicted in **Figure 2**. Peaks annotated with letters from **a** to **g** in **Figure 1** did not
140 contain phenolic compounds and were not further investigated in this study. Two additional non-
141 phenolic compounds were recognised in peaks **28** and **30** and identified as 12-hydroxy jasmonic
142 acid sulphate as already described in raw fermented cocoa beans by Patras, Milev, Vrancken, &
143 Kuhnert (2014). The description of the non-phenolic compounds is reported in supplementary
144 material (**Table S2**). **Table 1**, instead, shows mass spectrum data along with peak assignments and
145 retention time for the identified phenolic compounds. A total of 16 compounds were identified by
146 comparison with authentic standards. The remaining compounds were tentatively identified based
147 on the interpretation of their fragmentation patterns obtained from MS² experiments and by
148 comparison with literature. The description of the MS fragmentation pattern of phenolic compounds
149 already identified in cocoa beans or products will not be further described. All identified
150 compounds were found in the methanol extract. The subsequent extraction of the pellet with
151 acetone did not resulted in the recovery of new compounds. The acetone extract just contained low
152 amount of the same compounds found in methanol extract (data not shown).

153

154

155 **3.1.1. Flavan-3-ols and derivatives**

156 The high-resolution mass-spectrometry method used in this study enabled the characterization of 72
157 flavan-3-ol derivatives. According to their chemical structures, this group may be divided into
158 monomeric forms, A-type, and B-type oligomeric forms.

159 Among the monomeric flavan-3-ols, four compounds had been already reported in dark chocolate
160 (compounds 9.1, 13.2, 15.2 and 19.9) (Wollgast, 2004), seven compounds had been detected in
161 cocoa beans or products but not in dark chocolate (compounds 13.1, 14.1, 15.1, 16.2 , 21.4, 22.6
162 and 25.3) (D'Souza et al., 2017; Patras et al., 2014), whereas seven compounds were newly
163 identified in both dark chocolate and cocoa beans or products. Gallocatechin-3-*O*-hexoside (m/z
164 467.1270; compound 10.1) and epigallocatechin-3-*O*-hexoside (m/z 467.1270; compound 11.1)
165 were tentatively identified since they gave MS² major product ion at m/z 305, displaying typical
166 hexosyl group loss (162 amu) (Jiang et al., 2013). Otherwise, (epi)catechin-*C*-pentoside isomer
167 (compound 20.3) has been ascribed to the deprotonated ion [M-H]⁻ ion at m/z 421.1223, yielding
168 major MS² fragment ions at m/z 361 and m/z 331, corresponding to the loss of 60 and 90 amu (i.e.
169 *C*-pentosyl moiety) (Hvattum, & Ekeberg, 2003). Compounds 23.6, 26.2 and 27.5, m/z 415.1111,
170 were speculated to be isomers of (epi)catechin trihydroxybenzene, since the difference between the
171 precursor ion (m/z 415) and its major product ion (m/z 289, i.e. (epi)catechin-aglycone) was 126
172 amu, indicating the typical loss of a trihydroxybenzene moiety and the MS² fragmentation spectra
173 showed typical (epi)catechin fragmentation pattern (**Table 1**) with MS² fragment ions at m/z 245,
174 205 and 125. The presence of distinctive MS² product ions at m/z 259 (compounds 23.6 and 26.2;
175 deprotonated aglycone -30 amu, [Y₀-2H-CO]⁻) and m/z 261 (compound 27.5; deprotonated
176 aglycone-28 amu, [Y₀-CO]⁻) were observed, distinguishing the two different *O*-binding sites, 3-*O*
177 and 7-*O*, respectively (Hvattum, & Ekeberg, 2003). Compounds 27.6 and 34.6 with negative
178 charged [M-H]⁻ ion at m/z 617.1413 gave product ions in the MS² spectra at m/z 465 ([M-H]⁻ -152

179 amu, detailing the loss of a galloyl group), 289 ((epi)catechin-aglycone and 465-176 amu; i.e. the
 180 loss of a glucuronic acid moiety), 435 (gallate group 3-*O*-binding site) and 261 (glucuronide 7-*O*-
 181 binding site), and were tentatively identified as (epi)catechin-3-*O*-gallate-7-*O*-glucuronide
 182 (Shrestha et al., 2012). Compounds 18.5 and 28.1 had the same precursor ion $[M-H]^-$ at m/z
 183 613.1138 and their main MS² fragment ions at m/z 289 ((epi)catechin aglycone), m/z 451 ($[M-H]^-$ -
 184 162 amu, i.e. hexosyl moiety loss) and m/z 433 revealing that one sugar is attached to another and
 185 not directly to the aglycone describing two hexose sequential losses, as suggested by Hvattum, &
 186 Ekeberg (2003). Moreover, the presence of MS² product ion at m/z 259 identified 3-*O*-glycosylation
 187 site. Regarding this, they were tentatively identified as two isomers of (epi)catechin-3-*O*-
 188 dihexoside. Finally, three compounds (compounds 28.3, 29.5, 20.6) with deprotonated ions at m/z
 189 441.0892 and 457.0829 were only detected in dark chocolate with added Sakura green tea leaves
 190 (**Table 1**). They had been classified as catechin-3-*O*-gallate, epicatechin-3-*O*-gallate and
 191 epigallocatechin-3-*O*-gallate in comparison with authentic standards (**Table 1**).
 192 Procyanidins are mostly flavan-3,4-diols, found as dimers, trimers or oligomers with epicatechin as
 193 the main extension sub-unit. **Table 1** shows six B-type procyanidin dimers (compounds 12.1, 17.1,
 194 18.3, 18.9, 23.1 and 29.3) and four derivative forms (compounds 18.10, 16.3, 18.2 and 18.7).
 195 Among these, three glycosidic dimers of procyanidin B-type were newly identified in dark
 196 chocolate but already reported in cocoa beans (D'Souza et al., 2017). As reported by Gu, House,
 197 Wu, Ou, & Prior (2006) chocolate and cocoa products were also characterized by polymeric
 198 procyanidins. Trimers (compounds 13.5, 18.1, 18.8, 20.7, 21.2, 22.5, 23.3 and 29.2), tetramers
 199 (compounds 16.1, 19.5, 19.7 and 22.8), pentamers (compounds 19.13, 23.2 and 24.2) and hexamers
 200 (compounds 23.8, 24.7 and 26.1) of B-type procyanidins were also found. Compound 12.2 (**Table**
 201 **1**) with precursor ion at m/z 593.1345 was only detected in Sakura green tea dark chocolate and
 202 tentatively identified as (epi)catechin-(epi)gallocatechin in accordance to Jiang et al. (2013). A-type
 203 linkage is a less common feature in procyanidins, however A-type procyanidins and their

glycosides have been already isolated from chocolate and cocoa (Hatano et al. 2002). Dimeric (compounds 21.3, 22.2, 23.7, 24.3, 30.2, 31.1, 31.5, 32.2 and 34.1), trimeric (compounds 20.8, 24.4 and 24.9), tetrameric (compounds 19.6, 22.1, 22.7 and 24.1) and hexameric (compound 22.9) structures of A-type procyanidins were also found and listed in **Table 1**.

3.1.2 *Hydroxycinnamic acids*

A total of 25 hydroxycinnamic acids were tentatively identified. Among these, three compounds (compounds 2.1, 23.4 and 27.2) had never been identified in dark chocolate and cocoa beans or products, whereas compound 20.4 had been already detected in cocoa but never in dark chocolate (Stark, & Hofmann, 2005). According to Bauer, Harbaum-Piayda, & Schwarz (2012), the precursor ion at m/z 325.1004 can be tentatively classified as ferulic acid-4-*O*-pentoside, which MS² yielded a major fragment ion at m/z 193, corresponding to the loss of pentose ($[M-H] - 132$ amu). Using the fragmentation pattern and literature comparison, di-hydro-caffeic acid (compound 2.1) and di-hydro-coumaric acid (compound 23.4) were tentatively ascribed to deprotonated ions 181.0575 and 165.0470, respectively (Bresciani et al., 2017). These two compounds can be originated from the microbial metabolism during cocoa beans fermentation. Finally, compounds 12.3, 14.3, 19.4 and 20.2 (**Table 1**), with negative charged ion ($[M-H]^-$) at m/z 337.1006, were only detected in chocolate with added Sakura green tea leaves (**Table 1**). Based on their fragmentation pattern, elution profile and in comparison with the scheme proposed by Clifford, Johnston, Knight, & Kuhnert (2003), they had been tentatively classified as coumaroyl-quinic acids (Martini et al., 2017).

3.1.3. *Flavonols, flavones and other phenolics*

227 A tentative characterization of 22 flavonols, 6 flavones and 7 other phenolics has been enabled
228 thanks to LC-ESI-MS/MS experiments. Among these, 15 compounds were identified for the first
229 time in chocolate and cocoa beans or products.

230

231 **3.1.3.1. Flavonols and derivative forms**

232 According to MS and MS/MS data, the elution profile and literature (Andres-Lacueva et al., 2008;
233 Counet, Callemien, & Collin 2006; Ortega et al., 2008; Sanbongi et al., 1998; Wollgast, 2004), four
234 flavonols already reported in chocolate and cocoa beans or products were identified as quercetin at
235 m/z 301.0423 (compound 35.1) and its pentoside at m/z 433.0832 (compound 33.2) and hexoside at
236 m/z 463.0950 (compounds 30.1 and 31.3) derivatives. Concerning flavonols *O*-glycosides,
237 compounds 32.4, 34.3 and 34.5 had been already detected in cocoa but never in dark chocolate
238 (Ortega et al., 2008; Sánchez-Rabaneda et al., 2003) and were tentatively ascribed to kaempferol-3-
239 *O*-hexoside isomers and quercetin-3-*O*-rhamnoside. Two quercetin derivatives, quercetin-7-*O*-
240 rhamnoside-3-*O*-rutinoside and quercetin-7-*O*-hexoside-3-*O*-rutinoside isomers (m/z 755.2103 and
241 771.2042, respectively; compounds 27.4, 25.2 and 26.3) were detected for the first time in dark
242 chocolate and cocoa beans or products by tentative identification, screening the fragmentation
243 pattern (**Table 1**) (Guimarães et al., 2013; Lin, Chen, & Harnly, 2008). Compound 27.4 with a [M-
244 H]⁻ deprotonated ion at m/z 755.2103 and MS² fragment ions at m/z 609 (quercetin-3-*O*-rutinoside,
245 by loss of rhamnose moiety, 146 amu), 301 (quercetin-aglycone, underlining the loss of a rutinose
246 moiety, 308 amu) was tentatively identified as quercetin-7-*O*-rhamnoside-3-*O*-rutinoside (Lin et al.,
247 2008). Compound 25.2 and 26.3 with a [M-H]⁻ precursor ion at m/z 771.2042, producing product
248 ions at m/z 609 (loss of 162 mass units, a hexosyl-moiety), 463 (quercetin-3-*O*-glucoside, loss of
249 308 amu, a rutinose moiety) and 301 (quercetin-aglycone), and according to Martini et al. (2017),
250 were tentatively identified as quercetin-7-*O*-hexoside-3-*O*-rutinoside. As far as we know, also
251 compounds 31.4, 18.6, 32.1, 28.2, 31.2, 22.3 and 22.4 have been described for the first time in dark

252 chocolate and cocoa in general. Compound 31.4 was speculated to be kaempferol-7-*O*-hexoside,
 253 since the difference between the precursor ion (m/z 447.1028) and its major fragment ion (m/z 285,
 254 i.e. kaempferol-aglycone) was 162 amu detailing the loss of a hexosyl-moiety; the presence of the
 255 MS² ion at m/z 257 identified 7-*O*-glycosylation site (Hvattum, & Ekeberg, 2003). According to
 256 Mena et al. (2012), compound 18.6 (m/z 449.1182) was tentatively identified as dihydrokaempferol-
 257 7-*O*-hexoside, borne out by the presence of MS² major fragment ions at m/z 287 ([M-H]⁻ - 162 amu;
 258 characteristic loss of *O*-hexoside) and m/z 259, confirming 7-*O*-glycosylation site (Hvattum, &
 259 Ekeberg, 2003). Compounds 32.1, 28.2 and 31.2 had deprotonated ions at m/z 739.2182 and m/z
 260 755.2103 and gave product ions in the MS² spectra at m/z 593 and 285 characteristic of kaempferol-
 261 3-*O*-rutinoside and kaempferol-aglycone (Sánchez-Rabaneda et al., 2003). The negative product ion
 262 at m/z 593 was formed by the loss of rhamnose, glucose or galactose moiety from the glycosides.
 263 The presence of the product ion in MS² spectra at m/z 257 identified 7-*O*-glycosylation site
 264 (Hvattum, & Ekeberg, 2003). The loss of 308 amu is typical of a rutinose moiety, and therefore
 265 these compounds were tentatively identified as kaempferol-7-*O*-rhamnoside-3-*O*-rutinoside
 266 (compound 32.1) and kaempferol-7-*O*-hexoside-3-*O*-rutinoside isomers (compounds 28.2 and
 267 31.2). Finally, peaks 22.3, 22.4, 23.9, 24.5 and 24.8 were tentatively identified as myricetin-
 268 derivative-compounds. All of the compounds gave MS² product ion at m/z 317, corresponding to
 269 myricetin aglycone. Compounds 24.5 and 24.8, identified as myricetin-3-*O*-hexosides, had already
 270 been detected in cocoa but never in dark chocolate (Lin et al., 2008). Two myricetin-derivatives
 271 (compounds 22.3 and 22.4) have been described for the first time in dark chocolate and cocoa beans
 272 or products in this study. The MS² spectra of the compound 22.3 (m/z 631.1039) was characterized
 273 by fragment ions at m/z 479 and 317, depicting losses of 152 and 162 amu (i.e. galloyl and hexosyl
 274 moiety). This behaviour is indicative of myricetin-3-*O*-(*O*-galloyl)-hexoside (Saldanha, Vilegas, &
 275 Dokkedal, 2013). Compound 22.4 showed a deprotonated ion at m/z 787.2050, which fragmented in
 276 the MS² experiments giving product ions at m/z 625 and 317, suggesting the presence of a myricetin

277 aglycone, through the loss of 308 amu (rutinose moiety) and the loss of 162 amu (hexose group).
278 Concerning this, the compound was therefore tentatively identified as myricetin-7-*O*-hexoside-3-*O*-
279 rutinoside (Lin et al., 2008). Finally, compound 23.9 (**Table 1**), with negative precursor ion ([M-H]⁻)
280 at m/z 625.1455, was only detected in chocolate with added Sakura green tea leaves (**Table 1**).
281 Basing on its fragmentation pattern (**Table 1**) and according to Lin et al. (2008) it had been
282 tentatively classified as myricetin-3-*O*-rutinoside.

283

284 3.1.3.2. Flavones

285 Six glycosylated apigenins were detected in dark chocolate (**Table 1**); among these, five apigenins
286 (compounds 19.8, 19.12, 22.10, 25.1 and 27.3) were newly detected in this study. Compound 27.3
287 was tentatively associated to apigenin-*C*-hexoside-2''-*O*-rhamnoside isomer, m/z 577.1617, whose
288 MS² spectrum gave main fragment ions at m/z 457, 413 and 293, arising from the loss of 120 amu
289 (suggesting 1-2 linking between rhamnosyl-glucosyl group), 164 amu (rhamnosyl group), and 284
290 amu (164 plus 120 amu, i.e. rhamnose-glucosyl residue) (Dou, Lee, Tzen, & Lee, 2007; Hvattum, &
291 Ekeberg, 2003; Waridel et al., 2001). The next two isomers (compounds 19.8 and 19.12) at m/z
292 593.1591 were tentatively identified as *C*-diglycosylated apigenins; according to Jiang et al. (2013)
293 they were pinpointed as apigenin-6,8-di-*C*-glucoside isomers. Compound 25.1 generated the same
294 deprotonated ion at m/z 593.1591 and MS² fragment ions at m/z 473, 413 and 293 corresponding to
295 the loss of 120 amu (suggesting 1-2 linking between two glucosyl groups), 180 amu (glucosyl
296 moiety) and 300 amu (a glucose-glucosyl residue). Thus, compound 25.1 was speculated to be
297 apigenin-*C*-hexoside-2''-*O*-hexoside isomer (Hvattum, & Ekeberg, 2003; Dou et al., 2007). Finally,
298 following the scheme proposed by Lin et al. (2008) for the negative precursor ion [M-H]⁻ m/z
299 563.1462 and considering the losses of 90 amu (MS² fragment at m/z 473) and 120 amu (MS²
300 fragment at m/z 443) proving the existence of *C*-pentosyl- and *C*-hexosyl groups, compound 22.10
301 was tentatively identified as apigenin-*C*-hexoside-*C*-pentoside isomer.

302

303 **3.1.3.3. Other phenolics**

304 Compounds 29.1 and 34.2, showing the same negative precursor ion $[M-H]^-$ at m/z 451.1103, were
305 tentatively ascribed to cinchonain isomers, already detected in cocoa powder (Cádiz-Gurrea et al.,
306 2014) but never before in dark chocolate. Four glycosidic forms of naringenin (compound 19.3),
307 eriodictyol (compounds 18.4 and 19.2) and phloretin (compound 32.3) were tentatively identified
308 for the first time in dark chocolate and cocoa products and listed in **Table 1**. Compound 19.3 at m/z
309 593.1591, was tentatively identified as naringeni-*C*-hexoside-7-*O*-hexoside isomer, confirmed by
310 the characteristic loss of 120 amu (*C*-glycosylation site) and 162 amu (*O*-glycosylation site)
311 from m/z 473 and 413, respectively, which pinpointed the presence of two hexose units attached to
312 the flavonoid aglycone in different positions (Hvattum, & Ekeberg, 2003; Waridel et al., 2001). The
313 7-*O*-glycosylation site was proved by the presence of MS² fragment ion at m/z 283. Compound 18.4
314 displayed deprotonated ion at m/z of 449.1169 and showed MS² major fragment ions at m/z 287
315 ($[M-H]^- - 162$; i.e. hexose moiety loss) and m/z 259, confirming 7-*O*-glycosylation site presence.
316 Based on these data, compound 18.4 was tentatively identified as eriodictyol-7-*O*-hexoside (De
317 Beer et al. 2012). Compound 19.2, characterized by the deprotonated ion at m/z 611.1662, gave
318 MS² major fragment ions at m/z 449, 329 (corresponding to the loss of 120 amu (*C*-hexoside) and
319 162 amu (*O*-hexoside), respectively), 287 (eriodictyol-aglycone) and 259 (7-*O*-glycosylation site).
320 Therefore, compound 19.2 was speculated to be eriodictyol-*C*-hexoside-7-*O*-hexoside isomer (De
321 Beer et al. 2012; Hvattum, & Ekeberg, 2003). The negative ionization mode of compound 32.3
322 exhibited a $[M-H]^-$ precursor ion at m/z 435.1376, with MS² product ions at m/z 345 and 315, losing
323 90 and 120 amu, respectively. This fragmentation pattern has been previously described for
324 phloretin-*C*-hexoside isomer (Kazuno, Yanagida, Shindo, & Murayama, 2005).

325

326 **3.1.4. Ellagitannins**

327 Ellagitannins are known as polymeric structures including different numbers of galloyl and
328 hexahydroxydiphenoyl (HHDP) units esterified with glucose. Three ellagitannins were detected for
329 the first time in cocoa. They were distinguished by their characteristic fragment ion spectra yielding
330 sequential losses of galloyl (152 amu), gallate (170 amu), and HHDP residues (301 amu).
331 Following the ellagitannins fragmentation scheme pattern proposed by Mena et al. (2012),
332 compounds 16.5 (m/z 633.0796) and 37.1 (m/z 301.0054) can be tentatively identified as HHDP-
333 galloyl-hexose and ellagic acid, respectively. The ellagic acid was also confirmed by comparison
334 with the retention time of the standard and the MS² spectrum. Compound 27.1, characterized by the
335 deprotonated ion at m/z 615.0723 and MS² fragment ions at m/z 463, due to the loss of a galloyl
336 group from ([M-H]⁻-152) and at m/z 301, due to the loss of one hexose moiety (162 amu), was
337 tentatively identified as ellagic acid-galloyl-hexoside (Teixeira, Bertoldi, Lajolo, Mariko, &
338 Hassimotto, 2015)

339

340 **3.1.5. Hydroxybenzoic acids**

341 A total of twenty hydroxybenzoic acids and derivatives were detected in this study. Three of these
342 (compounds 8.4, 9.2 and 9.3) were tentatively identified for the first time in dark chocolate and
343 cocoa beans and products. Whereas 11 compounds (compounds 2.2, 5.1, 7.1, 8.1, 8.3, 9.4, 11.2,
344 13.4, 16.6, 16.7, 19.11) had been already identified in cocoa but never in dark chocolate (Ortega et
345 al., 2008). Compound 8.4 (m/z 315.0793) yielded MS² fragment ions at m/z 153 and 109, displaying
346 the hexose moiety loss and the presence of protocatechuic-aglycone. It was tentatively identified as
347 protocatechuic acid-4-*O*-hexoside (Martini et al., 2017). Compounds 9.2 and 9.3, m/z 359.1073,
348 fragmented in the MS² experiments giving major product ions at m/z 197, 182 and 153, suggesting
349 the presence of a syringic acid residue. The loss of 162 amu, proved by MS² fragment ion at m/z
350 197, prompt us to tentatively identify this compound as syringic acid-4-*O*-hexoside.

351

352 3.1.6. *Curcuminoids*

353 From the extracts of turmeric dark chocolate sample, we detected the $[M-H]^-$ precursor ions at m/z
354 307.1043, 337.1164, and 367.1257 (compounds 36.1, 36.2 and 36.3). As reported by Jiang,
355 Somogyi, Jacobsen, Timmermann, & Gang (2006), product ions at m/z 187 or 217 were the typical
356 fragment ions in the MS² spectra of deprotonated $[M-H]^-$ curcuminoids. In comparison to
357 fragmentation pattern proposed, compounds 36.1, 36.2 and 36.3 were tentatively identified as
358 bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively (**Table 1**).

360 3.2. *Phenolic compounds in chocolate*

361 **Table 2** provides information about the amount of the 158 tentatively identified phenolic
362 compounds in the different types of chocolate. In order to quantify the amount of total phenolic
363 compounds in chocolates, seven calibration curves were prepared with the available authentic
364 standards: epicatechin, coumaric and ferulic acids, quercetin-3-*O*-rutinoside, ellagic acid,
365 protocatechuic acid and curcumin. In all cases, the linearity was better than 0.99. The other
366 compounds, for which no commercial standards were available, were tentatively quantified using
367 the standards with similar structural characteristics and considering the functional groups that may
368 affect the ionisation properties. As shown in **Figure 3**, even if flavan-3-ols were the most
369 representative class in each type of chocolate, the phenolic profile is thoroughly influenced by the
370 addition of Sakura green tea or turmeric powder.

372 3.2.1. *Dark chocolate (DC) phenolic profile*

373 As determined by LC-MS/MS experiments, the total phenolic concentration in DC was $787.63 \pm$
374 10.90 mg/100 g of chocolate, representing about 30.0% of total phenolic compounds determined
375 with the Folin-Ciocalteu assay (2624.15 ± 112.36 mg/100 g of chocolate). The ABTS radical
376 scavenging and Fe³⁺-reducing ability of DC (**Figure 4**) were tested (11.00 ± 0.26 and 6.29 ± 0.13

377 mmol trolox equivalents/100 g of chocolate, respectively) resulting in line with the findings
 378 proposed by Batista et al. (2016). As reported by Wollgast & Anklam (2000a), catechins and
 379 procyanidins represent more than 90% of phenolic profile of cocoa beans and cocoa-products. We
 380 found out that total flavan-3-ols amount in DC was 503.76 ± 8.98 mg/100 g of chocolate
 381 representing the 64.0% of total polyphenols identified by MS experiments. Considering monomeric
 382 structures, epicatechin and catechin were the major represented flavan-3-ols, whose estimated
 383 concentrations were higher than those reported so far (Gu et al., 2006). Epicatechin alone
 384 represented the 40.4 % of total flavan-3-ols and the 25.8% of total phenolic identified by MS
 385 experiments, resulting the most present compounds in DC. Large amounts of oligomeric structures
 386 were also found, displaying a total concentration value of 166.28 ± 4.13 mg/100 g of chocolate and
 387 reaching approximately 33.0% of flavan-3-ols class. Epicatechin has been causally linked to the
 388 reported cardiovascular effects observed after the consumption of cocoa (Schroeter et al., 2006).
 389 The ingestion of flavanol-rich cocoa in healthy adult males was associated with acute elevations in
 390 levels of circulating nitric oxide, an enhanced flow-mediated dilation response of conduit arteries,
 391 and an augmented microcirculation in humans and the results were repeatable with pure epicatechin
 392 intake (70 mg/day; equivalent to 35-40 g of DC). Indeed, elderly men with a median epicatechin
 393 intake of 22 mg/day (equivalent to 10-15 g of DC) had a 38% lower risk of cardiovascular disease
 394 mortality than that of subjects with a median intake of 8 mg/day (Dower, Geleijnse,
 395 Hollman, Soedamah-Muthu, & Kromhout, 2016).
 396 The hydroxycinnamic acids made up about 20.6% of DC phenolic profile, among these ferulic acid,
 397 di-hydroxycinnamic aspartate and coumaroyl aspartate were the main hydroxycinnamic acids
 398 detected in DC. The largest contribution was given by ferulic acid, with a concentration of $61.23 \pm$
 399 3.74 mg/100 g of chocolate. Among the *N*-phenylpropenoyl-L-amino acids, clovamide or caffeoyl-
 400 tyrosine, described for the first time in cocoa by Sanbongi et al. (1998), was the main representative
 401 with total concentration of its two isomers of 9.54 ± 0.54 mg/100 g of chocolate. Previous studies

found that clovamide exhibited antiradical properties (Locatelli et al., 2013; Sanbongi et al., 1998), neuroprotective effects (Fallarini et al., 2009) and anti-inflammatory properties (Zeng et al., 2011). Ellagitannins, which were identified for the first time in dark chocolate and cocoa in this study, made up about 10% of DC phenolic profile. The higher amount was ascribed to ellagic acid (56.16 ± 3.58 mg/100 g of chocolate), followed by HHDP-galloyl-hexoside (15.79 ± 1.20 mg/100 g of chocolate). Ellagic acid and ellagitannins can be metabolized by human microbiota in urolithins, which are responsible for the health effects attributed to the consumption of ellagic acid and ellagitannins-rich food (Tomás-Barberán et al., 2017).

3.2.2. *Sakura green tea dark chocolate (GTDC) phenolic profile*

The content of total polyphenolic compounds in GTDC displayed a significant increase (P value <0.001) in respect to that of DC, recording a total concentration value of 1035.45 ± 14.81 mg/100 g of chocolate (**Figure 3**). This value represented the 30.3% of total phenolic compounds determined with the Folin-Ciocalteu assay (3417.81 ± 229.45 mg/100 g of chocolate). The increased phenolic concentration resulted in increased antioxidant properties in comparison with DC, which gave rise to 40% and 144% enhancements of GTDC ABTS radical scavenging and ferric-reducing power, respectively (**Figure 4**). The major phenolics in GTDC were still flavan-3-ols accounting for about 70.1% of total phenolic compounds, displaying a concentration value of 726.03 ± 14.53 mg/100 g of chocolate, significantly different from DC flavan-3-ols content (503.76 ± 8.98 mg/100 g of chocolate, P value <0.001). This flavan-3-ols increase was related to the Sakura green tea leaves enrichment of dark chocolate formulation and was clearly reflected in the significant increase in epicatechin (303.69 ± 11.65 mg/100 g of chocolate, P value <0.001 , detailing about 30% of GTDC phenolic profile), epigallocatechin (29.76 ± 1.74 mg/100 g of chocolate, P value <0.001) and total procyanidins (230.76 ± 15.73 mg/100 g of chocolate, P value <0.001). The Sakura green tea contribution was also confirmed by the presence of typical green tea gallate flavan-3-ols, especially

427 epigallocatechin gallate, showing a remarkable concentration value of 33.54 ± 2.16 mg/100 g of
428 chocolate. The hydroxycinnamic acids were still the second most representative class of phenolic
429 profile in GTDC, explaining about 15.3% of GTDC phenolic profile (**Figure 2**). Ellagitannins
430 showed a significant content increasing in GTDC respect to DC (89.12 ± 1.50 mg/100 g of
431 chocolate, *P value* <0.001) with an incidence rate of 8.6%. These results may confirm a possible
432 polyphenols enrichment of dark chocolate profile which can lead to a potential combination of the
433 positive health effects and properties derived from both chocolate and green tea. LC-MS
434 experiments showed that GTDC contained 49% more epicatechin and 43% more flavan-3-ols than
435 DC. This can result in a lower intake to achieve the same biological effects. This seems a promising
436 way to maximise the potential beneficial effect of epicatechin consumption, contemporaneously
437 reducing the amount of sugars and calories introduced with chocolate.

438

439 **3.2.3. Turmeric dark chocolate (TDC) phenolic profile**

440 The TDC phenolic amount showed a significant increase (*P value* <0.001) respect to that of DC
441 which recorded a total concentration value of 1094.03 ± 10.15 mg/100 g of chocolate (**Figure 3**),
442 representing about 36% of total phenolic compounds assayed with the Folin-Ciocalteu method
443 (3043.81 ± 294.64 mg/100 g of chocolate). Despite that, single phenolic classes did not show a
444 significant and remarkable increase respect to those of DC. This higher concentration can be
445 ascribed to turmeric powder contribution as well as the related curcuminoids, which accounted for
446 about 25% of TDC total phenolic profile, displaying a concentration value of 272.73 ± 2.58 mg/100
447 g of chocolate (**Figure 3**). ABTS radical scavenging ability and ferric-reducing power were tested,
448 resulting in 12.30 ± 0.27 and 10.57 ± 0.2 mmol trolox equivalents/100 g of chocolate, respectively
449 (**Figure 4**). Bisdemethoxycurcumin was the most concentrated curcuminoid (115.55 ± 2.16 mg/100
450 g of chocolate), followed by demethoxycurcumin (82.64 ± 1.33 mg/100 g of chocolate) which are
451 considered to be curcumin natural analogues and were reported to have a similar biological activity

452 to curcumin itself (Kocaadam, & Şanlier, 2017). Curcumin was found at the lowest concentration of
453 74.55 ± 0.47 mg/100 g of chocolate. Normally, curcumin is present at a concentration higher or
454 similar to the demethoxylated analogue (Jayaprakasha, Rhao, & Sakariah, 2002). Since the phenolic
455 composition of spices (and of vegetable food in general) is greatly variable depending on the
456 cultivar and agro-climatic factors (such as growing, harvesting time, seasonal variability) as well as
457 technological processes, it is plausible that different turmeric powder preparation had different
458 phenolic composition. Moreover, in the case of dark chocolate enriched with turmeric powder a
459 possible food matrix effect should be considered since some macromolecules such as proteins and
460 polysaccharide may interact with curcuminoids reducing their extractability. It is important also to
461 note that only free and extractable phenolic compounds were considered and analysed in this study.
462 Curcuminoids are widely known for their healthy properties such as anti-inflammatory, antioxidant,
463 antimicrobial, anticoagulant, anticancer and antimutagenic properties (Kocaadam, & Şanlier, 2017;
464 Kunnumakkara et al., 2017). To date, over 100 different clinical trials have been successfully
465 carried out, showing their safety, tolerability and effectiveness against several chronic diseases in
466 humans such as various types of cancers, diabetes, obesity, cardiovascular and neurological diseases
467 (Kunnumakkara et al., 2017). Finally, the synergistic behaviour displayed by curcuminoids with
468 other nutraceuticals such as catechins and quercetin, resulting an increased effect against oxidative
469 stress in normal healthy adults, was demonstrated (Dominiak, McKinney, Heilbrun, & Sarkar,
470 2010). Therefore, an enhanced and strengthened health effect because of the union of polyphenol-
471 rich sources, combining the positive effects of dark chocolate phenolics and turmeric curcuminoids,
472 can be speculated.

473

474 **4. Conclusions**

475 Literature provides a lot of information about cocoa polyphenols and properties, but there is still a
476 big gap about the phenolic composition of chocolate. Few studies investigated the phenolic

477 composition of dark chocolate, focusing on flavan-3-ols as the major class in chocolate phenolic
478 profile. The purpose of this study was to overcome this lack of information, providing an accurate
479 and comprehensive characterisation of the phenolic profile of dark chocolate (70%). The
480 quantitative metabolomics approach used in this study allowed a tentative identification of 158
481 individual phenolic compounds in dark chocolate. Among the detected compounds, 67 have been
482 reported for the first time in dark chocolate, 38 of whom were identified for the first time in
483 chocolate, cocoa beans and cocoa products. This characterization extends the current knowledge on
484 the phytochemistry of dark chocolate and is, to our knowledge, the broadest profiling of its phenolic
485 compounds to date.

486 Results reported in this study also showed that the addition of Sakura green tea leaves or turmeric
487 powder influenced and modified the phenolic profile of dark chocolate, resulting in a phenolic
488 concentration increase. Mass spectrometry confirmed that this increase was strictly connected to the
489 food matrix, showing typical compounds belonging to green tea and turmeric. In this way, this
490 functional chocolate might maximize the potential beneficial effect of polyphenols-rich food
491 consumption and, at the same time, reducing the amount of sugars and calories introduced with
492 chocolate, resulting in a lower intake to achieve the same biological effects. This work may revise
493 the concept of “optimal” dose of chocolate in the context of a balanced diet, which optimizes the
494 functional properties by avoiding potential side effects, such as high-calorie intake.

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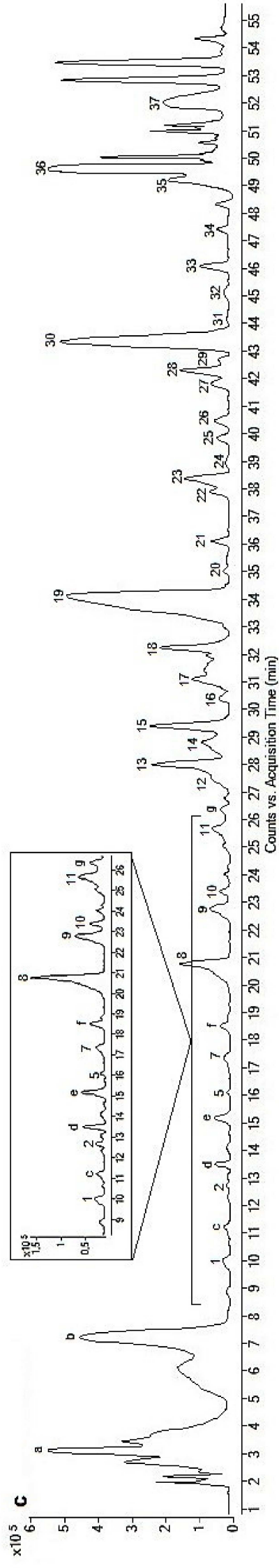
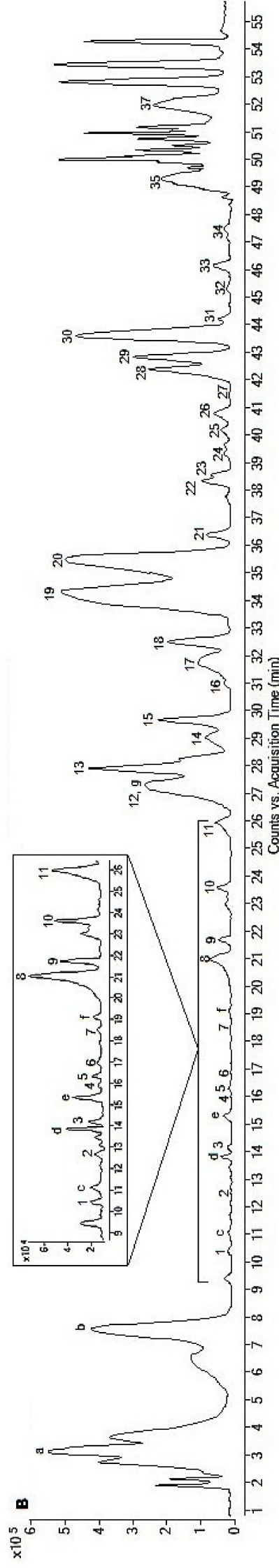
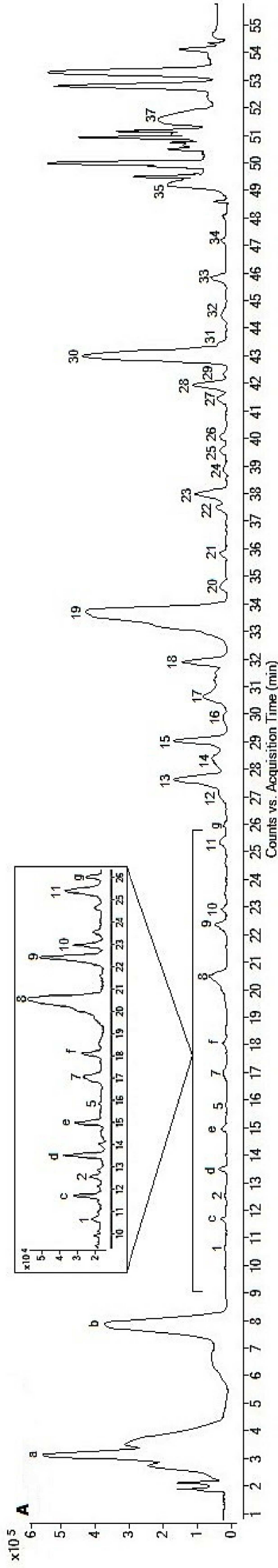
Figure captions

Figure 1. Representative negative ion mode base peak chromatograms (BPCs) of dark chocolate (A) green tea dark chocolate (B) and turmeric dark chocolate (C). The shown BPCs are representative of three independent experiments and represent the profile of the methanol extracts.

Figure 2. Structures of newly identified dark chocolate phenolic compounds. Examples of some newly identified phenolic structures belonging to flavan-3-ols (A), flavonols (B), hydroxybenzoic and hydroxycinnamic acids (C), flavones (D), flavanones (E) and dihydrochalcones (F). Gall: galloyl; gluc: glucuronide; hex: hexoside; pent: pentoside; rham: rhamnoside; rut: rutinoside; trihydroxy: trihydroxybenzene.

Figure 3. Occurrence of phenolic classes in dark chocolates. Global percentage of flavan-3-ols, flavonols, hydroxybenzoic and hydroxycinnamic acids, ellagitannins, flavones and other phenolics in dark chocolate and dark chocolate enriched with Sakura green tea leaves or turmeric powder. In brackets are reported the total amounts of phenolic compounds quantified with mass spectrometry. 70% means the total percentage of cocoa in the dark chocolates.

Figure 4. Antioxidant properties of dark chocolates. Antioxidant capacity (expressed as mmol trolox/100g of chocolate), measured by ABTS (A) and FRAP (B) assays. DC: dark chocolate; GTDC: dark chocolate enriched with Sakura green tea leaves; TDC: dark chocolate enriched with turmeric powder. Each sample was run in triplicate and results are reported as mean values \pm SD. Values in the same graph with different lowercase letter are significantly different ($P < 0.05$).



| A | (Epi)gallocatechin-3-O-hexoside | (Epi)catechin-C-pentoside isomer | (Epi)catechin-3-O-sulphate | (Epi)catechin-3-O-trihydroxybenzene | (Epi)catechin-7-O-trihydroxybenzene | (Epi)catechin-3-O-gallate-7-O-glucuronide | (Epi)catechin-3-O-dihexoside |
|----|---------------------------------|----------------------------------|----------------------------|-------------------------------------|-------------------------------------|---|------------------------------|
| R1 | -O-hex | -OH | -O-SO ₃ | O-trihydroxy | -OH | -O-gall | -O-hex-hex |
| R2 | -H | -pent or -H | -H | -H | -H | -H | -H |
| R3 | -OH | -OH | -OH | -OH | -O-trihydroxy | -O-gluc | -OH |
| R4 | -H | -pent or -H | -H | -H | -H | -H | -H |
| R5 | -OH | -H | -H | -H | -H | -H | -H |

| B | Quercetin-7-O-rhamnoside-3-O-rutinoside | Quercetin-7-O-hexoside-3-O-rutinoside isomer | Kaempferol-7-O-rhamnoside-3-O-rutinoside | Kaempferol-7-O-hexoside-3-O-rutinoside isomer | Myricetin-3-O-(O-galloyl)-hexoside | Myricetin-7-O-hexoside-3-O-rutinoside |
|----|---|--|--|---|------------------------------------|---------------------------------------|
| R1 | -H | -H | -H | -H | -OH | -OH |
| R2 | -OH | -OH | -H | -H | -OH | -OH |
| R3 | -O-rut | -O-rut | -O-rut | -O-rut | -O-gall-hex | -O-rut |
| R4 | -O-rham | -O-hex | -O-rham | -O-hex | -OH | -O-hex |

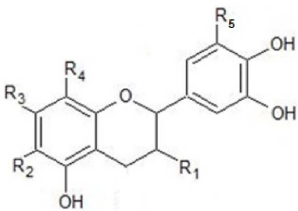
| C | Protocatechuic acid-4-O-hexoside | Syringic acid-4-O-hexoside isomer | Di-hydro-coumaric acid | Di-hydro-cafeic acid | Ferulic acid-4-O-pentoside |
|----|----------------------------------|-----------------------------------|---|---|----------------------------|
| R1 | -COOH | -COOH | -CH ₂ -CH ₂ -COOH | -CH ₂ -CH ₂ -COOH | -CH=CH-COOH |
| R2 | -OH | -OCH ₃ | -H | -OH | -OCH ₃ |
| R3 | -O-hex | -O-hex | -OH | -OH | -O-pent |
| R4 | -H | -OCH ₃ | -H | -H | -H |

| D | Apigenin-C-hexoside-2"-O-rhamnoside isomer | Apigenin-6,8-di-C-hexoside isomer | Apigenin-C-hexoside-2"-O-hexoside isomer | Apigenin-C-hexoside-C-pentoside isomer |
|----|--|-----------------------------------|--|--|
| R1 | -hex-rham or -H | -hex | -hex-hex or -H | -pent or -hex |
| R2 | -hex-rham or -H | -hex | -hex-hex or -H | -pent or -hex |

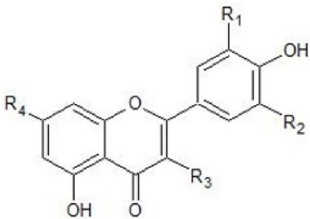
| E | Dihydro-kaempferol-7-O-hexoside | Naringenin-C-hexoside-7-O-hexoside isomer | Eriodictyol-7-O-hexoside | Eriodictyol-C-hexoside-7-O-hexoside isomer |
|----|---------------------------------|---|--------------------------|--|
| R1 | -H | -H | -OH | -OH |
| R2 | -H | -hex or -H | -H | -hex or -H |
| R3 | -O-hex | -O-hex | -O-hex | -O-hex |
| R4 | -H | -hex or -H | -H | -hex or -H |
| R5 | -OH | -hex or -H | -H | -hex or -H |

| F | Phloretin-C-hexoside isomer |
|----|-----------------------------|
| R1 | -hex or -H |
| R2 | -hex or -H |

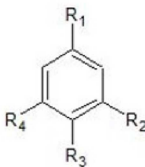
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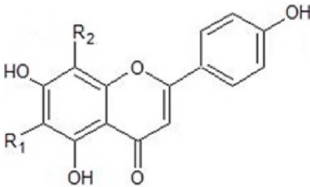
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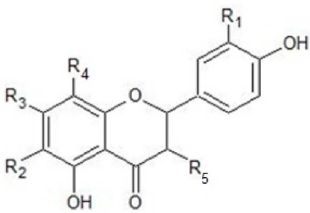
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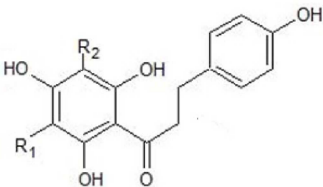
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E

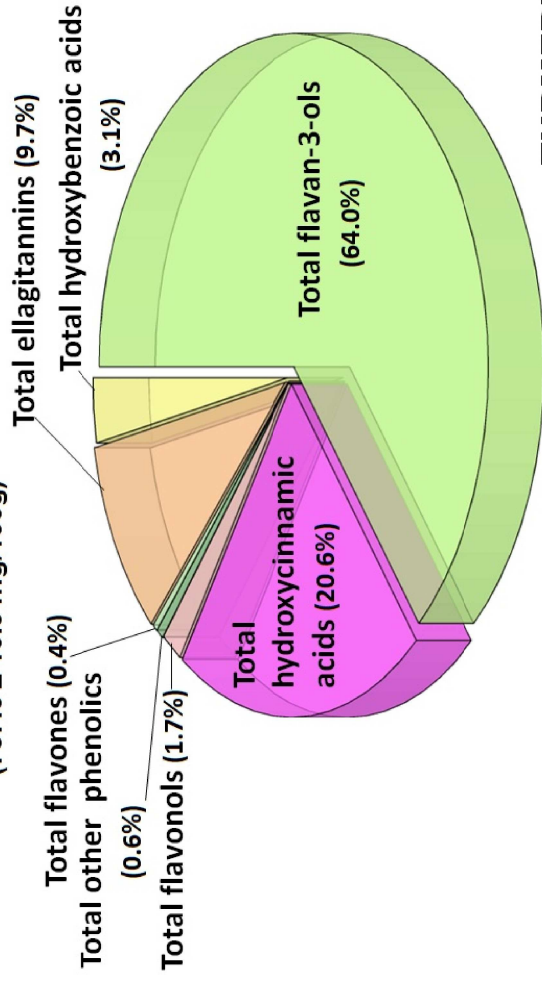


F



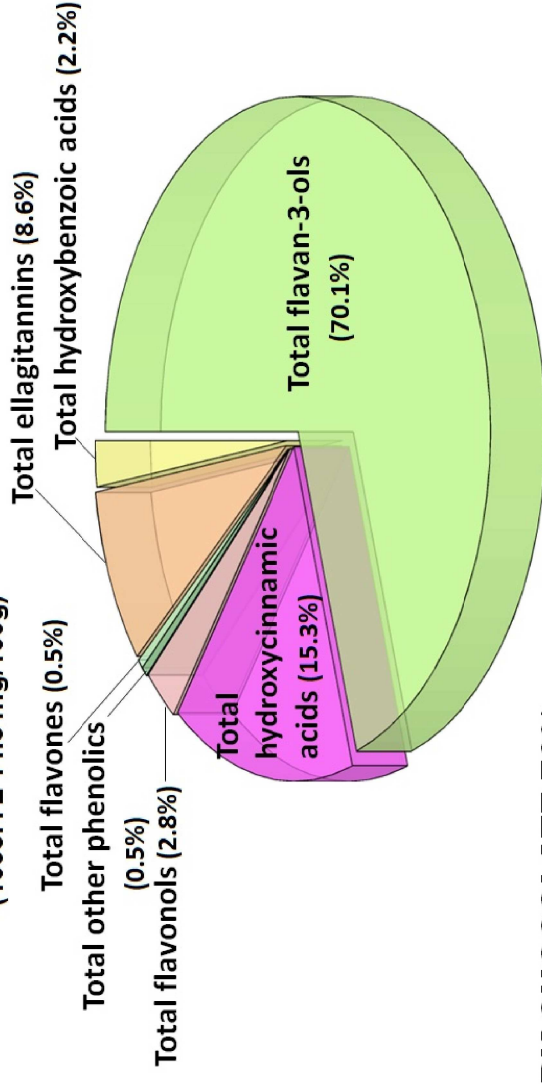
DARK CHOCOLATE 70%

(787.6 ± 10.9 mg/100g)



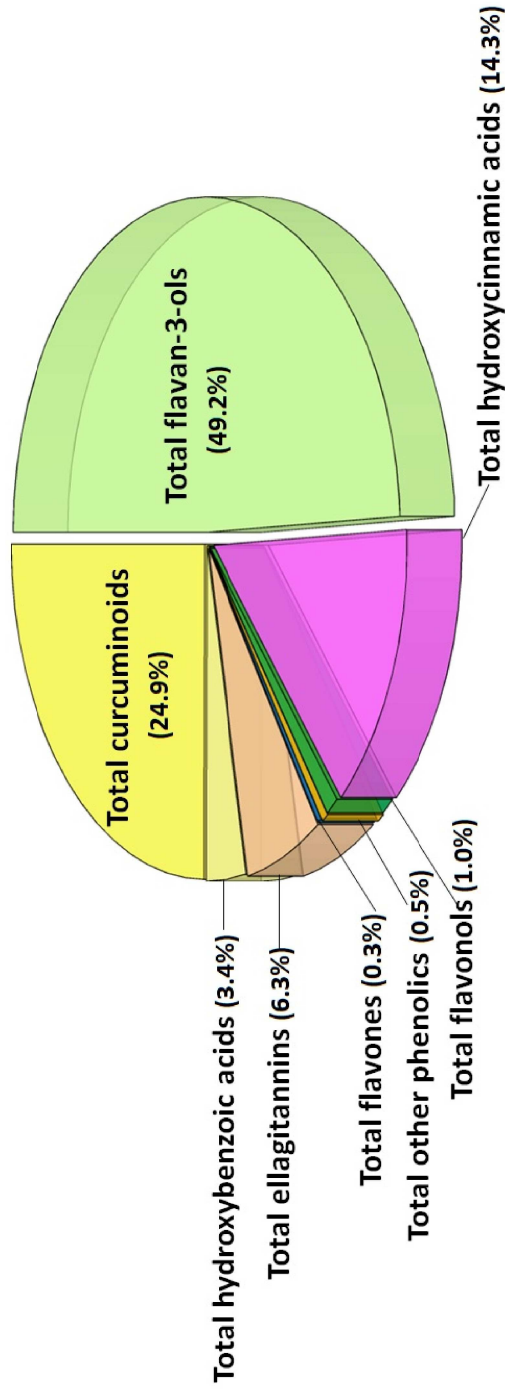
GREEN TEA DARK CHOCOLATE 70%

(1035.4 ± 14.8 mg/100g)



TURMERIC DARK CHOCOLATE 70%

(1094.0 ± 10.1 mg/100g)



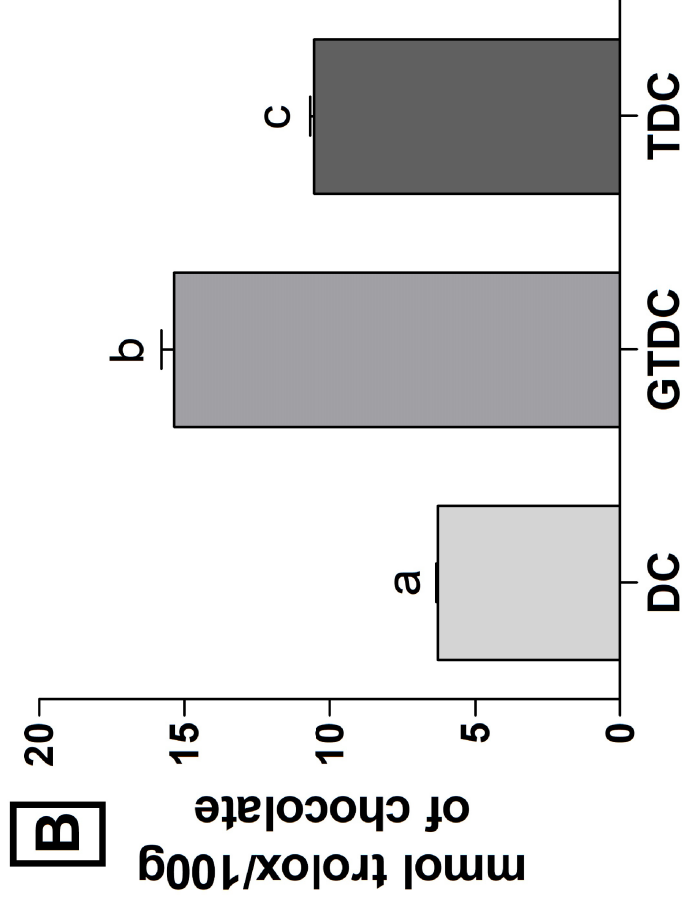
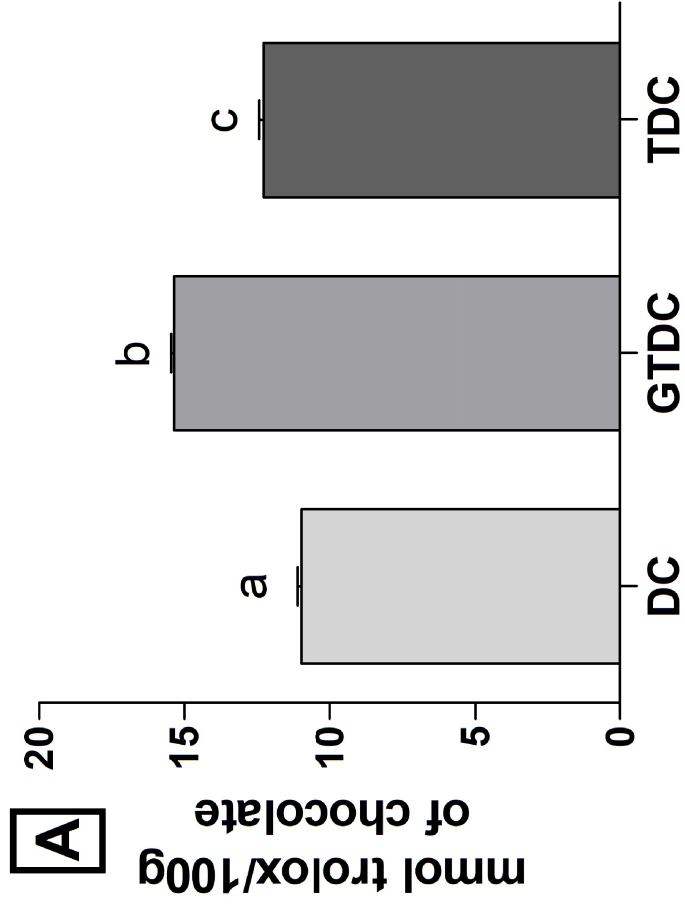


Table 1. Mass spectral data of phenolic compounds identified in different dark chocolates.

| Peak | Compound | Formula | Calc. (<i>m/z</i>) | Exp. (<i>m/z</i>) | Error (ppm) | MS ² ion fragments (<i>m/z</i>) (relative abundance in %) |
|------|--|---|-------------------------|------------------------|----------------|---|
| 1 | 1.1 Coumaric acid ^s | C ₉ H ₈ O ₃ | 163.0473 | 163.0466 | 4.54 | 119.0492 (100), 93.0435 (5) |
| 2 | 2.1 Di-hydro-caffeic acid | C ₉ H ₁₀ O ₄ | 181.0579 | 181.0575 | 2.25 | 137.0234 (100) |
| | 2.2 Hydroxybenzoic acid isomer | C ₇ H ₆ O ₃ | 137.0317 | 137.0316 | 0.09 | 93.0315 (100) |
| 3 | 3.1 Gallic acid ^{a,s} | C ₇ H ₆ O ₅ | 169.0215 | 169.0221 | -3.39 | 125.0287 (100) |
| | 3.2 Galloyl glucose isomer ^a | C ₁₃ H ₁₆ O ₁₀ | 331.0744 | 331.0756 | -3.77 | 169.0194 (100), 125.0252 (29) |
| 4 | 4.1 Galloylquinic acid isomer ^a | C ₁₄ H ₁₆ O ₁₀ | 343.0744 | 343.0730 | 3.91 | 191.0731 (100), 169.0275 (5) |
| 5 | 5.1 Vanillic acid-4- <i>O</i> -hexoside isomer | C ₁₄ H ₁₈ O ₉ | 329.0951 | 329.0967 | -4.90 | 167.0403 (100) |
| 6 | 6.1 Galloylquinic acid isomer ^a | C ₁₄ H ₁₆ O ₁₀ | 343.0744 | 343.0730 | 3.91 | 191.0676 (100), 169.0213 (5) |
| 7 | 7.1 Hydroxybenzoic acid isomer | C ₇ H ₆ O ₃ | 137.0317 | 137.0316 | 0.09 | 93.0390 (100) |
| 8 | 8.1 Vanillic acid-4- <i>O</i> -hexoside isomer | C ₁₄ H ₁₈ O ₉ | 329.0951 | 329.0967 | -4.90 | 167.0341 (100), 123.0494 (25) |
| | 8.2 Vanillic acid derivative | C ₁₅ H ₁₄ N ₂ O ₉ | 365.0699 | 365.0690 | 2.54 | 167.0319 (100), 123.0463 (14) |
| | 8.3 Vanillic acid isomer | C ₈ H ₈ O ₄ | 167.0423 | 167.0417 | 3.33 | 151.0067 (100), 123.0479 (24) |
| | 8.4 Protocatechuic acid-4- <i>O</i> -hexoside | C ₁₃ H ₁₆ O ₉ | 315.0794 | 315.0793 | 0.42 | 153.0183, 109.0270 |
| | 8.5 Di-hydroxycinnamic acid isomer | C ₉ H ₈ O ₄ | 179.0423 | 179.0431 | -4.67 | 163.0253 (5), 135.0475 (100) |
| | 8.6 Protocatechuic acid ^s | C ₇ H ₆ O ₄ | 153.0266 | 153.0267 | -0.09 | 109.0313 (100) |
| 9 | 9.1 Gallocatechin ^s | C ₁₅ H ₁₄ O ₇ | 305.0740 | 305.0733 | 2.13 | 261.0800 (14), 221.0471 (28), 219.0695 (19), 179.0373 (35), 125.0225 (100) |
| | 9.2 Syringic acid-4- <i>O</i> -hexoside isomer | C ₁₅ H ₂₀ O ₁₀ | 359.1056 | 359.1073 | -4.59 | 197.0574 (100), 182.0310 (28), 153.0619 (51) |
| | 9.3 Syringic acid-4- <i>O</i> -hexoside isomer | C ₁₅ H ₂₀ O ₁₀ | 359.1056 | 359.1073 | -4.59 | 197.0463 (100), 182.0212 (18), 153.0541 (22) |
| | 9.4 Syringic acid | C ₉ H ₁₀ O ₅ | 197.0528 | 197.0532 | -0.38 | 182.0320 (100), 167.0073 (30), 153.0246 (13) |
| 10 | 10.1 Gallocatechin-3- <i>O</i> -hexoside | C ₂₁ H ₂₄ O ₁₂ | 467.1268 | 467.1270 | -0.48 | 357.0558 (23), 305.0858 (100), 287.0436 (71), 125.0215 (79) |
| | 10.2 Di-hydroxycinnamic aspartate isomer | C ₁₃ H ₁₃ NO ₇ | 294.0692 | 294.0692 | 0.00 | 276.0682 (13), 250.0946 (3), 232.0868 (5), 206.0683 (3), 179.0190 (16), 132.0395 (100) |
| | 10.3 Di-hydroxycinnamic acid isomer | C ₉ H ₈ O ₄ | 179.0423 | 179.0431 | -4.67 | 163.0348 (10), 135.0426 (100) |
| 11 | 11.1 Epigallocatechin-3- <i>O</i> -hexoside | C ₂₁ H ₂₄ O ₁₂ | 467.1268 | 467.1270 | -0.48 | 357.0588 (19), 305.0885 (100), 287.0722 (75), 125.0232 (83) |
| | 11.2 Hydroxybenzoic acid isomer | C ₇ H ₆ O ₃ | 137.0317 | 137.0316 | 0.09 | 93.0351 (100) |
| 12 | 12.1 Procyanidin dimer B type isomer | C ₃₀ H ₂₆ O ₁₂ | 577.1424 | 577.1411 | 2.29 | 425.0822 (11), 407.0788 (75), 289.0717 (100), 245.0784 (17), 205.0476 (6), 125.0279 (66) |
| | 12.2 (Epi)catechin-(Epi)gallocatechin ^a | C ₃₀ H ₂₆ O ₁₃ | 593.1373 | 593.1345 | 4.78 | 425.1001 (33), 407.0958 (21), 305.0739 (74), 289.0857 (100), 205.0563 (26), 125.0238 (80) |
| | 12.3 3- <i>O</i> -Coumaroylquinic acid <i>cis</i> ^a | C ₁₆ H ₁₈ O ₈ | 337.1002 | 337.1006 | -1.28 | 191.0642 (40), 163.0460 (100), 119.0504 (27) |
| 13 | 13.1 (Epi)catechin-3- <i>O</i> -hexoside isomer | C ₂₁ H ₂₄ O ₁₁ | 451.1319 | 451.1320 | -0.31 | 289.0797 (100), 259.1312 (3), 245.0956 (23), 205.0748 (11), 179.0377 (5) |
| | 13.2 Epigallocatechin ^s | C ₁₅ H ₁₄ O ₇ | 305.0740 | 305.0733 | 2.13 | 261.0883 (19), 221.0467 (19), 219.0688 (28), 179.0355 (42), 125.0219 (100) |
| | 13.3 Caffeic acid ^s | C ₉ H ₈ O ₄ | 179.0423 | 179.0431 | -4.67 | 163.0244 (8), 135.0398 (100) |
| | 13.4 Hydroxybenzoic acid isomer | C ₇ H ₆ O ₃ | 137.0317 | 137.0316 | 0.09 | 93.0328 (100) |

| | | | | | | | |
|----|------|---|---|----------|---------------|-------|---|
| | 13.5 | Procyanidin trimer B type isomer | C ₄₅ H ₃₈ O ₁₈ | 865.2058 | 865.2044 | 1.63 | 577.1764 (5), 425.0757 (10), 407.0839 (42), 289.0634 (64), 287.0617 (38), 245.0573 (14), 125.0264 (100) |
| 14 | 14.1 | (Epi)catechin- <i>C</i> -hexoside isomer | C ₂₁ H ₂₄ O ₁₁ | 451.1319 | 451.1320 | -0.31 | 361.0988 (25), 331.0879 (100) |
| | 14.2 | Di-hydroxycinnamic aspartate isomer | C ₁₃ H ₁₃ NO ₇ | 294.0692 | 294.0692 | 0.00 | 276.0573 (2), 250.0744 (2), 232.0617 (1), 206.0771 (2), 179.0318 (7), 132.0256 (100) |
| | 14.3 | 3- <i>O</i> -Coumaroylquinic acid <i>trans</i> ^a | C ₁₆ H ₁₈ O ₈ | 337.1002 | 337.1006 | -1.28 | 191.0453 (4), 163.0307 (100), 119.0409 (15) |
| 15 | 15.1 | (Epi)catechin-7- <i>O</i> -hexoside | C ₂₁ H ₂₄ O ₁₁ | 451.1319 | 451.1320 | -0.31 | 289.0942 (100), 261.1150 (3), 245.1104 (42), 205.0561 (9), 179.0365 (3) |
| | 15.2 | Catechin ^s | C ₁₅ H ₁₄ O ₆ | 289.0790 | 289.0791 | -0.21 | 245.0878 (100), 205.0554 (38), 179.0372 (31), 125.0247 (40) |
| 16 | 16.1 | Procyanidin tetramer B type isomer | C ₆₀ H ₅₀ O ₂₄ | 576.1346 | 576.1342 (2-) | 0.69 | 425.1088 (5), 407.0948 (15), 289.0840 (100), 287.0653 (6), 245.1158 (43), 125.0282 (77) |
| | 16.2 | (Epi)catechin- <i>C</i> -hexoside isomer | C ₂₁ H ₂₄ O ₁₁ | 451.1319 | 451.1320 | -0.31 | 361.0843 (16), 331.0787 (100) |
| | 16.3 | Procyanidin dimer B type hexoside isomer | C ₃₆ H ₃₆ O ₁₇ | 739.1952 | 739.1915 | 5.00 | 449.1175 (11), 407.0840 (8), 289.0595 (100), 245.0629 (16), 125.0197 (42) |
| | 16.4 | Vanillin | C ₈ H ₈ O ₃ | 151.0473 | 151.0481 | -4.97 | 136.0181 (28), 123.0487 (100), 121.0315 (20), 109.0296 (24), 108.0201 (97) |
| | 16.5 | HHDP-galloyl-hexoside | C ₂₇ H ₂₂ O ₁₈ | 633.0806 | 633.0796 | 1.60 | 463.0687 (18), 301.0102 (100), 275.0315 (11), 257.0196 (4), 229.0331 (4) |
| | 16.6 | Vanillic acid isomer | C ₈ H ₈ O ₄ | 167.0423 | 167.0417 | 3.33 | 151.0050 (30), 123.0471 (100) |
| | 16.7 | Vanillic acid-4- <i>O</i> -hexoside isomer | C ₁₄ H ₁₈ O ₉ | 329.0951 | 329.0967 | -4.90 | 167.0473 (100), 123.0524 (6) |
| | 16.8 | 5- <i>O</i> -Caffeoylquinic acid | C ₁₆ H ₁₈ O ₉ | 353.0951 | 353.0952 | -0.12 | 191.0133 (100), 173.0049 (36) |
| 17 | 17.1 | Procyanidin dimer B type isomer | C ₃₀ H ₂₆ O ₁₂ | 577.1424 | 577.1411 | 2.29 | 425.0867 (30), 407.0750 (61), 289.0641 (100), 245.0792 (20), 205.0497 (25), 125.0158 (72) |
| | 17.2 | 3- <i>O</i> -Caffeoylquinic acid ^s | C ₁₆ H ₁₈ O ₉ | 353.0951 | 353.0952 | -0.12 | 191.0667 (38), 179.0433 (100), 173.0504 (43), 135.0459 (8) |
| 18 | 18.1 | Procyanidin trimer B type isomer | C ₄₅ H ₃₈ O ₁₈ | 865.2058 | 865.2044 | 1.63 | 577.1838 (8), 425.1135 (18), 407.1065 (48), 289.0844 (69), 287.0773 (73), 245.0947 (9), 125.0187 (100) |
| | 18.2 | Procyanidin dimer B type hexoside isomer | C ₃₆ H ₃₆ O ₁₇ | 739.1952 | 739.1915 | 5.00 | 449.1328 (86), 407.1113 (11), 289.0963 (100), 287.0829 (54), 245.0728 (10), 125.0276 (6) |
| | 18.3 | Procyanidin dimer B type isomer | C ₃₀ H ₂₆ O ₁₂ | 577.1424 | 577.1411 | 2.29 | 425.0945 (21), 407.0855 (72), 289.0800 (86), 245.0870 (11), 205.0547 (5), 125.0239 (100) |
| | 18.4 | Eriodictyol-7- <i>O</i> -hexoside | C ₂₁ H ₂₂ O ₁₁ | 449.1162 | 449.1169 | -1.56 | 287.0722 (100), 269.0834 (69), 235.0379 (11), 167.0364 (10), 125.0268 (9) |
| | 18.5 | (Epi)catechin-3- <i>O</i> -dihexoside isomer | C ₂₅ H ₂₆ O ₁₈ | 613.1119 | 613.1138 | -3.07 | 451.1279 (10), 433.0719 (19), 407.0998 (23), 289.0900 (100), 259.0581 (4), 245.0959 (6), 125.0317 (30) |
| | 18.6 | Dihydro-kaempferol-7- <i>O</i> -hexoside | C ₂₁ H ₂₂ O ₁₁ | 449.1162 | 449.1182 | -4.42 | 287.0816 (43), 269.0714 (100), 259.0884 (57), 135.0554 (25) |
| | 18.7 | Procyanidin dimer B type hexoside isomer | C ₃₆ H ₃₆ O ₁₇ | 739.1952 | 739.1915 | 5.00 | 449.1097 (37), 289.0608 (48), 287.0725 (55), 245.0713 (25), 125.0129 (100) |
| | 18.8 | Procyanidin trimer B type | C ₄₅ H ₃₈ O ₁₈ | 865.2058 | 865.2044 | 1.63 | 577.1811 (8), 425.1213 (16), |

| | | | | | | |
|-------|---|--|-----------|---------------|-------|--|
| | isomer | | | | | 407.1120 (34), 289.0958 (50), 287.0598 (26), 245.0738 (16), 125.0292 (100) |
| 18.9 | Procyanidin dimer B type isomer | C ₃₀ H ₂₆ O ₁₂ | 577.1424 | 577.1411 | 2.29 | 425.1060 (8), 407.0974 (55), 289.0850 (100), 245.0920 (10), 205.0600 (3), 125.0251 (45) |
| 18.10 | Procyanidin dimer B type derivative | C ₅₀ H ₃₅ NO ₁₃ | 856.2108 | 856.2104 | 0.51 | 577.1265 (10), 425.0996 (45), 407.0922 (40), 289.0847 (79), 278.0775 (100), 125.0281 (16) |
| 18.11 | Coumaroyl aspartate | C ₁₃ H ₁₃ NO ₆ | 278.0743 | 278.0730 | 4.61 | 260.2382 (2), 234.0819 (14), 216.0670 (11), 190.0861 (10), 172.0810 (2), 163.0414 (56), 119.0481 (100) |
| 19 | 19.1 Di-hydroxycinnamic acid isomer | C ₉ H ₈ O ₄ | 179.0423 | 179.0431 | -4.67 | 163.0337 (11), 135.0453 (100) |
| | 19.2 Eriodictyol- <i>C</i> -hexoside-7- <i>O</i> -hexoside isomer | C ₂₇ H ₃₂ O ₁₆ | 611.1690 | 611.1662 | 4.63 | 449.1540 (41), 329.0991 (92), 287.0956 (17), 269.0810 (10), 235.0310 (5), 167.0427 (59), 149.0208 (100), 125.0245 (10) |
| | 19.3 Naringenin- <i>C</i> -hexoside-7- <i>O</i> -hexoside isomer | C ₂₇ H ₃₀ O ₁₅ | 593.1585 | 593.1591 | -1.86 | 473.0960 (100), 413.0732 (9), 383.0630 (46), 353.0540 (94), 311.0533 (8), 283.0433 (2) |
| | 19.4 4- <i>O</i> -Coumaroylquinic acid <i>cis</i> ^a | C ₁₆ H ₁₈ O ₈ | 337.1002 | 337.1006 | -1.28 | 191.0663 (10), 173.0557 (100), 163.0486 (34), 119.0476 (17) |
| | 19.5 Procyanidin tetramer B type isomer | C ₆₀ H ₅₀ O ₂₄ | 576.1346 | 576.1342 (2-) | 0.69 | 425.1141 (6), 407.0929 (11), 289.0732 (27), 287.0653 (17), 245.1158 (7), 125.0282 (100) |
| | 19.6 Procyanidin tetramer A type isomer | C ₆₀ H ₄₈ O ₂₄ | 1151.2559 | 1151.2548 | 0.97 | 695.2148 (16), 575.1237 (21), 449.0797 (11), 425.0865 (64), 407.0734 (11), 289.0723 (61), 287.0585 (49), 125.0187 (100) |
| | 19.7 Procyanidin tetramer B type isomer | C ₆₀ H ₅₀ O ₂₄ | 576.1346 | 576.1342 (2-) | 0.69 | 425.0893 (37), 407.0788 (30), 289.0752 (100), 287.0605 (55), 245.0874 (5), 125.0205 (95) |
| | 19.8 Apigenin-6,8-di- <i>C</i> -hexoside isomer | C ₂₇ H ₃₀ O ₁₅ | 593.1585 | 593.1591 | -1.06 | 473.1283 (100), 383.0977 (46), 353.0818 (94), 149.0312 (9) |
| | 19.9 Epicatechin ^s | C ₁₅ H ₁₄ O ₆ | 289.0790 | 289.0791 | -0.21 | 245.0877 (100), 205.0549 (34), 179.0368 (26), 125.0255 (34) |
| | 19.10 Di-hydroxycinnamic acid isomer | C ₉ H ₈ O ₄ | 179.0423 | 179.0431 | -4.67 | 163.0277 (10), 135.0421 (100) |
| | 19.11 Hydroxybenzoic acid isomer | C ₇ H ₆ O ₃ | 137.0317 | 137.0316 | 0.09 | 93.0336 (100) |
| | 19.12 Apigenin-6,8-di- <i>C</i> -hexoside isomer | C ₂₇ H ₃₀ O ₁₅ | 593.1585 | 593.1591 | -1.06 | 473.0960 (100), 383.0630 (61), 353.0540 (54), 149.0316 (6) |
| | 19.13 Procyanidin pentamer B type isomer | C ₇₅ H ₆₂ O ₃₀ | 720.1663 | 720.1659 (2-) | 0.56 | 449.0819 (10), 407.0718 (20), 289.0783 (100), 287.0583 (67), 245.0482 (28), 125.0230 (85) |
| 20 | 20.1 Clovamide (caffeoyl-tyrosine) isomer | C ₁₈ H ₁₇ NO ₇ | 358.1005 | 358.0996 | 2.51 | 222.0459 (69), 178.0540 (64), 161.0272 (61), 135.0441 (100) |
| | 20.2 4- <i>O</i> -Coumaroylquinic acid <i>trans</i> ^a | C ₁₆ H ₁₈ O ₈ | 337.1002 | 337.1006 | -1.28 | 191.0553 (6), 173.0441 (100), 163.0369 (23), 119.0478 (2) |
| | 20.3 (Epi)catechin- <i>C</i> -pentoside isomer | C ₂₀ H ₂₂ O ₁₀ | 421.1213 | 421.1223 | -2.38 | 361.1138 (24), 331.1001 (100), 205.0614 (18), 123.0368 (11) |
| | 20.4 Feruloyl aspartate | C ₁₄ H ₁₅ NO ₇ | 308.0849 | 308.0842 | 2.11 | 290.2986 (9), 264.1033 (74), 246.0733 (17), 220.1030 (9), 193.0557 (100), 149.0587 (65) |
| | 20.5 (Epi)catechin derivative isomer | C ₄₃ H ₂₈ O ₁₀ | 703.1682 | 703.1676 | 0.92 | 533.1280 (14), 407.0992 (13), 289.0827 (100), 251.0643 (44), 125.0253 (48) |
| | 20.6 Epigallocatechin-3- <i>O</i> -gallate ^{a,s} | C ₂₂ H ₁₈ O ₁₁ | 457.0849 | 457.0829 | 4.39 | 331.0620 (2), 305.0823 (9), 169.0315 (100), 125.0396 (24) |
| | 20.7 Procyanidin trimer B type isomer | C ₄₅ H ₃₈ O ₁₈ | 865.2058 | 865.2044 | 1.63 | 577.1985 (33), 425.1465 (10), 407.1269 (35), 289.1131 (47), |

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| | | | | | | | 287.0623 (51), 245.0887 (10), 125.0209 (100) |
| | 20.8 | Procyanidin trimer A type | C ₄₅ H ₃₆ O ₁₈ | 863.1902 | 863.1907 | -0.62 | 407.0783 (20), 289.0767 (100), 287.0515 (24), 205.0193 (20), 125.0262 (67) |
| 21 | 21.1 | (Epi)catechin derivative isomer | C ₄₃ H ₂₈ O ₁₀ | 703.1682 | 703.1676 | 0.92 | 533.1227 (15), 407.1080 (69), 289.0865 (99), 251.0703 (38), 245.0918 (32), 125.0245 (100) |
| | 21.2 | Procyanidin trimer B type isomer | C ₄₅ H ₃₈ O ₁₈ | 865.2058 | 865.2044 | 1.63 | 577.1513 (11), 425.0959 (26), 407.0869 (49), 289.0776 (71), 287.0667 (89), 245.0511 (9), 125.0209 (100) |
| | 21.3 | Procyanidin dimer A type hexoside isomer | C ₃₆ H ₃₄ O ₁₇ | 737.1796 | 737.1769 | 3.66 | 611.1895 (44), 539.1341 (67), 449.1191 (100), 407.1020 (13), 289.0952 (28), 287.0778 (4) |
| | 21.4 | (Epi)catechin-3-O-hexoside isomer | C ₂₁ H ₂₄ O ₁₁ | 451.1319 | 451.1320 | -0.31 | 289.0709 (100), 245.0731 (21), 205.0599 (36), 179.0374 (3) |
| 22 | 22.1 | Procyanidin tetramer A type isomer | C ₆₀ H ₄₈ O ₂₄ | 1151.2559 | 1151.2548 | 0.97 | 695.1732 (16), 575.1251 (67), 449.1017 (46), 425.0889 (59), 407.1007 (6), 289.0880 (9), 287.0664 (100), 125.0238 (13) |
| | 22.2 | Procyanidin dimer A type hexoside isomer | C ₃₆ H ₃₄ O ₁₇ | 737.1796 | 737.1769 | 3.66 | 611.2053 (61), 539.1105 (67), 449.1017 (100), 407.1065 (6), 289.0750 (18), 287.0661 (28) |
| | 22.3 | Myricetin-3-O-(O-galloyl)-hexoside | C ₂₈ H ₂₄ O ₁₇ | 631.1013 | 631.1039 | -4.04 | 479.1152 (100), 317.0449 (20), 316.0441 (73), 271.0510 (10), 179.0122 (5) |
| | 22.4 | Myricetin-7-O-hexoside-3-O-rutinoside | C ₃₃ H ₄₀ O ₂₂ | 787.2011 | 787.2050 | -4.92 | 625.1547 (8), 317.0379 (53), 316.0323 (100), 289.0464 (6), 287.0273 (4), 273.0646 (4), 271.0269 (4), 179.0066 (7) |
| | 22.5 | Procyanidin trimer B type isomer | C ₄₅ H ₃₈ O ₁₈ | 865.2058 | 865.2044 | 1.63 | 577.1857 (6), 425.1225 (18), 407.1117 (39), 289.0980 (49), 287.0489 (35), 245.0673 (16), 125.0291 (100) |
| | 22.6 | (Epi)catechin-O-sulphate isomer | C ₁₅ H ₁₄ O ₉ S | 369.0359 | 369.0361 | -0.67 | 289.0763 (100), 245.0704 (53), 205.0674 (8), 137.0302 (16), 125.0271 (3), 79.9372 (2) |
| | 22.7 | Procyanidin tetramer A type isomer | C ₆₀ H ₄₈ O ₂₄ | 1151.2559 | 1151.2548 | 0.97 | 695.1376 (8), 575.1350 (21), 449.0887 (6), 425.1031 (51), 407.0806 (4), 289.0758 (44), 287.0622 (22), 125.0218 (100) |
| | 22.8 | Procyanidin tetramer B type isomer | C ₆₀ H ₅₀ O ₂₄ | 576.1346 | 576.1342 (2-) | 0.69 | 425.1090 (3), 407.1229 (10), 289.0954 (63), 287.0800 (7), 245.0898 (10), 125.0276 (100) |
| | 22.9 | Procyanidin hexamer A type | C ₉₀ H ₇₂ O ₃₆ | 863.1900 | 863.1921 (2-) | -2.43 | 449.1176 (22), 407.1026 (16), 289.1016 (85), 245.0984 (44), 125.0351 (100) |
| | 22.10 | Apigenin-C-hexoside-C-pentoside isomer | C ₂₆ H ₂₈ O ₁₄ | 563.1479 | 563.1462 | 3.02 | 503.1285 (25), 473.1501 (32), 443.1347 (84), 383.1096 (78), 353.0940 (100), 149.0467 (13) |
| 23 | 23.1 | Procyanidin dimer B type isomer | C ₃₀ H ₂₆ O ₁₂ | 577.1424 | 577.1411 | 2.29 | 425.0943 (10), 407.0812 (53), 289.0761 (100), 245.0856 (64), 205.0536 (5), 125.0237 (33) |
| | 23.2 | Procyanidin pentamer B type isomer | C ₇₅ H ₆₂ O ₃₀ | 720.1663 | 720.1659 (2-) | 0.56 | 449.0877 (12), 407.0726 (12), 289.0769 (49), 287.0730 (14), 245.0567 (5), 125.0234 (100) |
| | 23.3 | Procyanidin trimer B type isomer | C ₄₅ H ₃₈ O ₁₈ | 865.2058 | 865.2044 | 1.63 | 577.1938 (20), 425.1202 (18), 407.1051 (52), 289.0944 (35), 287.0653 (66), 245.0623 (14), 125.0276 (100) |
| | 23.4 | Di-hydro-coumaric acid | C ₉ H ₁₀ O ₃ | 165.0477 | 165.0470 | 4.45 | 147.0355 (100), 119.0399 (31) |
| | 23.5 | Clovamide (caffeoyl- | C ₁₈ H ₁₇ NO ₇ | 358.1005 | 358.0996 | 2.51 | 222.0593 (53), 178.0637 (100), |

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| | tyrosine) isomer | | | | | 161.0339 (43), 135.0517 (54) |
| 23.6 | (Epi)catechin-3- <i>O</i> -trihydroxybenzene | C ₂₁ H ₂₀ O ₉ | 415.1107 | 415.1111 | -0.88 | 289.0775 (100), 259.0863 (3), 245.0961 (15), 205.0639 (32), 125.0295 (9) |
| 23.7 | Procyanidin dimer A type pentoside isomer | C ₃₅ H ₃₂ O ₁₆ | 707.1690 | 707.1681 | 1.32 | 581.1333 (21), 539.1166 (67), 449.1031 (100), 407.0918 (8), 289.0797 (13), 287.0638 (7) |
| 23.8 | Procyanidin hexamer B type isomer | C ₉₀ H ₇₄ O ₃₆ | 864.1980 | 864.1963 (2-) | 1.97 | 449.0875 (14), 407.0865 (37), 289.0800 (63), 287.0566 (41), 245.0443 (13), 205.0100 (6), 125.0197 (100) |
| 23.9 | Myricetin-3- <i>O</i> -rutinoside ^a | C ₂₇ H ₃₀ O ₁₇ | 625.1483 | 625.1455 | 4.47 | 317.0572 (28), 316.0519 (100), 287.0203 (5) |
| 24 | 24.1 Procyanidin tetramer A type isomer | C ₆₀ H ₄₈ O ₂₄ | 1151.2559 | 1151.2548 | 0.97 | 695.2385 (2), 575.1613 (34), 449.1279 (19), 425.1263 (12), 407.1210 (14), 289.0922 (42), 287.0837 (71), 125.0338 (100) |
| | 24.2 Procyanidin pentamer B type isomer | C ₇₅ H ₆₂ O ₃₀ | 720.1663 | 720.1659 (2-) | 0.56 | 449.1237 (8), 407.1024 (24), 289.0892 (53), 287.0730 (35), 245.0953 (18), 125.0266 (100) |
| | 24.3 Procyanidin dimer A type pentoside isomer | C ₃₅ H ₃₂ O ₁₆ | 707.1690 | 707.1681 | 1.32 | 581.1413 (48), 539.0950 (30), 449.0884 (100), 407.0789 (16), 289.0629 (21), 287.0685 (5) |
| | 24.4 Procyanidin trimer A type hexoside isomer | C ₅₁ H ₄₈ O ₂₃ | 1025.2430 | 1025.2416 | 1.35 | 407.0897 (16), 289.0675 (100), 285.0442 (22), 125.0300 (6) |
| | 24.5 Myricetin-3- <i>O</i> -galattoside | C ₂₁ H ₂₀ O ₁₃ | 479.0904 | 479.0885 | 3.94 | 317.0394 (27), 316.0316 (100), 287.0228 (5) |
| | 24.6 Mono-deoxyclovamide (caffeoyl-DOPA / coumaroyl-tyrosine) isomer | C ₁₈ H ₁₇ NO ₆ | 342.1056 | 342.1057 | -0.33 | 222.0368 (18), 206.0630 (82), 178.1359 (14), 145.1558 (13), 135.0378 (100), 119.0446 (23) |
| | 24.7 Procyanidin hexamer B type isomer | C ₉₀ H ₇₄ O ₃₆ | 864.1980 | 864.1963 (2-) | 1.97 | 449.0767 (17), 407.0874 (24), 289.0824 (40), 287.0663 (19), 245.0553 (39), 205.0097 (8), 125.0255 (100) |
| | 24.8 Myricetin-3- <i>O</i> -glucoside | C ₂₁ H ₂₀ O ₁₃ | 479.0904 | 479.0885 | 3.94 | 317.0736 (23), 316.0615 (100), 287.0571 (7) |
| | 24.9 Procyanidin trimer A type hexoside isomer | C ₅₁ H ₄₈ O ₂₃ | 1025.2430 | 1025.2416 | 1.35 | 407.1011 (8), 289.0869 (100), 285.0645 (33), 245.1007 (16), 125.0224 (35) |
| 25 | 25.1 Apigenin- <i>C</i> -hexoside-2''- <i>O</i> -hexoside isomer | C ₂₇ H ₃₀ O ₁₅ | 593.1585 | 593.1591 | -1.06 | 473.0871 (10), 413.0978 (63), 311.0682 (12), 293.0528 (100), 149.0416 (4) |
| | 25.2 Quercetin-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside isomer | C ₃₃ H ₄₀ O ₂₁ | 771.2062 | 771.2042 | 2.60 | 609.1837 (4), 463.1192 (3), 301.0584 (100), 300.0492 (60), 273.0559 (3), 271.0419 (8), 255.0513 (3), 179.0098 (5), 151.0028 (3) |
| | 25.3 (Epi)catechin- <i>O</i> -sulphate isomer | C ₁₅ H ₁₄ O ₉ S | 369.0359 | 369.0361 | -0.67 | 289.0755 (100), 245.0713 (33), 205.0686 (10), 137.0321 (58), 125.0288 (7), 79.9361 (11) |
| 26 | 26.1 Procyanidin hexamer B type isomer | C ₉₀ H ₇₄ O ₃₆ | 864.1980 | 864.1963 (2-) | 1.97 | 449.0645 (12), 407.0668 (12), 289.0651 (70), 287.0580 (33), 245.0514 (43), 205.0494 (11), 125.0306 (100) |
| | 26.2 (Epi)catechin-3- <i>O</i> -trihydroxybenzene | C ₂₁ H ₂₀ O ₉ | 415.1107 | 415.1111 | -0.88 | 289.0705 (100), 259.0918 (2), 245.0913 (25), 205.0421 (21), 125.0283 (8) |
| | 26.3 Quercetin-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside isomer | C ₃₃ H ₄₀ O ₂₁ | 771.2062 | 771.2042 | 2.60 | 609.1579 (6), 463.0993 (6), 301.0353 (100), 300.0283 (57), 273.0427 (3), 271.0222 (7), 255.0339 (4), 179.0062 (14), 151.0007 (9) |
| 27 | 27.1 Ellagic acid-galloyl- | C ₂₇ H ₂₀ O ₁₇ | 615.0700 | 615.0723 | 2.04 | 463.0607 (100), 301.0147 (37), |

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| | hexoside | | | | | 300.0047 (46), 229.0317 (11) |
| 27.2 | Ferulic acid-4- <i>O</i> -pentoside | C ₁₅ H ₁₈ O ₈ | 325.1002 | 325.1004 | -0.71 | 221.1495 (21), 193.1510 (100), 178.1361 (68) |
| 27.3 | Apigenin- <i>C</i> -hexoside-2"- <i>O</i> -rhamnoside isomer | C ₂₇ H ₃₀ O ₁₄ | 577.1636 | 577.1617 | 3.21 | 457.1107 (10), 413.0843 (45), 341.0690 (17), 311.0552 (25), 293.0441 (100), 149.0494 (5) |
| 27.4 | Quercetin-7- <i>O</i> -rhamnoside-3- <i>O</i> -rutinoside | C ₃₃ H ₄₀ O ₂₀ | 755.2113 | 755.2103 | 1.31 | 609.1224 (10), 301.0486 (100), 300.0360 (87), 273.0601 (6), 271.0473 (5), 179.0037 (3), 151.0093 (3) |
| 27.5 | (Epi)catechin-7- <i>O</i> -trihydroxybenzene | C ₂₁ H ₂₀ O ₉ | 415.1107 | 415.1111 | -0.88 | 289.0802 (100), 261.0909 (4), 245.0993 (60), 205.0656 (17), 125.0213 (51) |
| 27.6 | (Epi)catechin-3- <i>O</i> -gallate-7- <i>O</i> -glucuronide isomer | C ₂₅ H ₃₀ O ₁₈ | 617.1432 | 617.1413 | 3.10 | 465.0846 (11), 435.1642 (18), 327.0688 (100), 289.0852 (29), 261.0610 (3), 245.0965 (9), 205.0306 (21), 165.0270 (34), 125.0294 (6) |
| 27.7 | Apigenin- <i>C</i> -hexoside isomer | C ₂₁ H ₂₀ O ₁₀ | 431.1056 | 431.1058 | -0.35 | 341.0904 (26), 311.0739 (100), 283.0892 (40), 149.0201 (5), 117.0540 (3) |
| 28 | 28.1 (Epi)catechin-3- <i>O</i> -dihexoside isomer | C ₂₅ H ₂₆ O ₁₈ | 613.1119 | 613.1138 | -3.07 | 451.1227 (11), 433.0755 (5), 407.1000 (56), 289.0864 (71), 259.0650 (3), 245.0938 (10), 125.0257 (100) |
| | 28.2 Kaempferol-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside isomer | C ₃₃ H ₄₀ O ₂₀ | 755.2113 | 755.2103 | 1.31 | 593.1490 (11), 285.0417 (100), 284.0356 (11), 257.0578 (9), 255.0186 (7), 151.0018 (2) |
| | 28.3 Catechin-3- <i>O</i> -gallate ^{a,s} | C ₂₂ H ₁₈ O ₁₀ | 441.0900 | 441.0892 | 1.80 | 331.0496 (4), 289.0773 (42), 259.0667 (6), 245.0862 (10), 169.0148 (100), 125.0227 (19) |
| | 28.4 Quercetin-3- <i>O</i> -rutinoside ^{a,s} | C ₂₇ H ₃₀ O ₁₆ | 609.1534 | 609.1536 | -0.35 | 301.0422 (41), 300.0324 (55), 271.0281 (5), 179.0023 (8), 167.0391 (100), 151.0038 (7) |
| 29 | 29.1 Cinchonain isomer | C ₂₄ H ₂₀ O ₉ | 451.1107 | 451.1103 | 0.96 | 341.0565 (100), 217.0327 (21) |
| | 29.2 Procyanidin trimer B type isomer | C ₄₅ H ₃₈ O ₁₈ | 865.2058 | 865.2044 | 1.63 | 577.1742 (11), 425.1189 (13), 407.1076 (64), 289.0963 (45), 287.0541 (28), 245.0653 (43), 125.0266 (100) |
| | 29.3 Procyanidin dimer B type isomer | C ₃₀ H ₂₆ O ₁₂ | 577.1424 | 577.1411 | 2.29 | 425.0964 (18), 407.0861 (100), 289.0787 (97), 245.0527 (15), 205.0527 (6), 125.0234 (79) |
| | 29.4 Mono-deoxyclovamide (caffeoyl-DOPA / coumaroyl-tyrosine) isomer | C ₁₈ H ₁₇ NO ₆ | 342.1056 | 342.1057 | -0.33 | 222.0469 (35), 206.0538 (36), 178.0531 (78), 145.0297 (27), 135.0478 (100), 119.0507 (14) |
| | 29.5 Epicatechin-3- <i>O</i> -gallate ^{a,s} | C ₂₂ H ₁₈ O ₁₀ | 441.0900 | 441.0892 | 1.80 | 331.0689 (2), 289.0930 (38), 259.0807 (3), 245.0673 (6), 169.0290 (100), 125.0351 (22) |
| 30 | 30.1 Quercetin-3- <i>O</i> -galactoside | C ₂₁ H ₂₀ O ₁₂ | 463.0955 | 463.0950 | 1.03 | 301.0508 (52), 300.0424 (100), 271.0377 (6), 255.0400 (3), 179.0056 (4), 151.0124 (3) |
| | 30.2 Procyanidin dimer A type hexoside isomer | C ₃₆ H ₃₄ O ₁₇ | 737.1796 | 737.1769 | 3.66 | 611.1715 (50), 539.1170 (49), 449.1112 (100), 407.0973 (12), 289.0811 (33), 287.0649 (19) |
| | 30.3 Ferulic acid ^s | C ₁₀ H ₁₀ O ₄ | 193.0579 | 193.0573 | 3.14 | 178.0336 (100), 149.0597 (38) |
| 31 | 31.1 Procyanidin dimer A type hexoside isomer | C ₃₆ H ₃₄ O ₁₇ | 737.1796 | 737.1769 | 3.66 | 611.1492 (29), 539.0970 (32), 449.0885 (100), 407.0748 (16), 289.0705 (24), 287.0577 (31) |
| | 31.2 Kaempferol-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside isomer | C ₃₃ H ₄₀ O ₂₀ | 755.2113 | 755.2103 | 1.31 | 593.1553 (13), 285.0433 (100), 284.0385 (12), 257.0661 (6), 255.0492 (3), 151.0002 (2) |
| | 31.3 Quercetin-3- <i>O</i> -glucoside ^s | C ₂₁ H ₂₀ O ₁₂ | 463.0955 | 463.0950 | 1.03 | 301.0525 (56), 300.0461 (100), 271.0392 (8), 255.0425 (3), |

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|-----------|------|---|---|----------|----------|-------|--|
| | | | | | | | 179.0048 (5), 151.0086 (5) |
| | 31.4 | Kaempferol-7- <i>O</i> -hexoside | C ₂₁ H ₂₀ O ₁₁ | 447.1006 | 447.1028 | 5.00 | 285.0122 (100), 257.0463 (10) |
| | 31.5 | Procyanidin dimer A type pentoside isomer | C ₃₅ H ₃₂ O ₁₆ | 707.1690 | 707.1681 | 1.32 | 581.1482 (49), 539.1210 (86), 449.1154 (100), 407.0421 (12), 289.0778 (30), 287.0569 (13) |
| 32 | 32.1 | Kaempferol-7- <i>O</i> - rhamnoside-3- <i>O</i> -rutinoside | C ₃₃ H ₄₀ O ₁₉ | 739.2164 | 739.2182 | -2.46 | 593.1482 (14), 285.0483 (100), 257.0806 (11), 255.0809 (8) |
| | 32.2 | Procyanidin dimer A type pentoside isomer | C ₃₅ H ₃₂ O ₁₆ | 707.1690 | 707.1681 | 1.32 | 581.1394 (18), 539.1030 (39), 449.0942 (100), 407.0812 (9), 289.0800 (14), 287.0605 (13) |
| | 32.3 | Phloretin- <i>C</i> -hexoside isomer | C ₂₁ H ₂₄ O ₁₀ | 435.1369 | 435.1376 | -1.50 | 345.1005 (24), 315.0928 (100), 285.0967 (25), 167.0404 (14), 137.0580 (14) |
| | 32.4 | Kaempferol-3- <i>O</i> -galactoside | C ₂₁ H ₂₀ O ₁₁ | 447.1006 | 447.1028 | 5.00 | 285.0517 (19), 284.0473 (100), 255.0328 (7), 179.0122 (16), 151.0080 (9) |
| 33 | 33.1 | Kaempferol-3- <i>O</i> -rutinoside | C ₂₇ H ₃₀ O ₁₅ | 593.1585 | 593.1591 | -1.06 | 285.0483 (100), 284.0386 (49), 255.0421 (5), 179.0336 (4), 151.0446 (4) |
| | 33.2 | Quercetin-3- <i>O</i> -pentoside | C ₂₀ H ₁₈ O ₁₁ | 433.0849 | 433.0832 | 3.94 | 301.0474 (21), 300.0406 (100), 271.0364 (6), 255.0390 (2), 179.0059 (2), 151.0101 (3) |
| 34 | 34.1 | Procyanidin dimer A type | C ₃₀ H ₂₄ O ₁₂ | 575.1268 | 575.1274 | -1.08 | 449.1241 (15), 407.1091 (42), 289.0966 (39), 287.0755 (14), 285.0567 (100), 125.0234 (7) |
| | 34.2 | Cinchonain isomer | C ₂₄ H ₂₀ O ₉ | 451.1107 | 451.1103 | 0.96 | 341.0921 (100), 217.0270 (18) |
| | 34.3 | Kaempferol-3- <i>O</i> -glucoside | C ₂₁ H ₂₀ O ₁₁ | 447.1006 | 447.1028 | 5.00 | 285.0462 (51), 284.0395 (100), 255.0390 (4), 179.0704 (12), 151.0920 (11) |
| | 34.4 | Di-deoxyclovamide (coumaroyl-DOPA) | C ₁₈ H ₁₇ NO ₅ | 326.1107 | 326.1109 | -0.70 | 282.1414 (100), 206.0625 (28), 163.0502 (32), 147.0530 (22), 134.0671 (4), 119.0536 (63) |
| | 34.5 | Quercetin-3- <i>O</i> -rhamnoside | C ₂₁ H ₂₀ O ₁₁ | 447.1006 | 447.1028 | 5.00 | 301.0206 (90), 300.0135 (100), 271.0102 (10) |
| | 34.6 | (Epi)catechin-3- <i>O</i> -gallate-7- <i>O</i> -glucuronide isomer | C ₂₅ H ₃₀ O ₁₈ | 617.1432 | 617.1413 | 3.10 | 465.1064 (5), 435.1628 (11), 327.0704 (100), 289.0874 (25), 261.0623 (3), 245.0900 (26), 205.0379 (17), 165.0285 (78), 125.0294 (14) |
| 35 | 35.1 | Quercetin ^s | C ₁₅ H ₁₀ O ₇ | 301.0427 | 301.0423 | 1.17 | 179.0118 (100), 151.0087 (65) |
| 36 | 36.1 | Bisdemethoxycurcumin ^b | C ₁₉ H ₁₆ O ₄ | 307.1049 | 307.1043 | 1.81 | 187.0351 (36), 145.0249 (14), 143.0462 (65), 119.0470 (100) |
| | 36.2 | Demethoxycurcumin ^b | C ₂₀ H ₁₈ O ₅ | 337.1154 | 337.1164 | -2.89 | 217.0450 (33), 173.0556 (42), 149.0563 (32), 119.0466 (100) |
| | 36.3 | Curcumin ^{b,s} | C ₂₁ H ₂₀ O ₆ | 367.1260 | 367.1257 | 0.78 | 217.0426 (38), 175.0331 (50), 173.0540 (57), 149.0547 (100), 134.0316 (48) |
| 37 | 37.1 | Ellagic acid ^s | C ₁₄ H ₆ O ₈ | 301.0063 | 301.0054 | 2.87 | 257.0162 (100), 229.0307 (61) |

a and **b** are referred to the compounds detected only in Sakura green tea dark chocolate or turmeric dark chocolate, respectively, whereas **s** means identification by comparison with authentic standard. **g** is referred to the compounds detected with authentic standards.

Table 2. Quantitative results (mg/100 g of chocolate) for phenolic compounds identified in the different types of chocolate. Values represent means \pm standard deviation of triplicate determination.

| Compound | | Dark chocolate | Green tea dark chocolate | Turmeric dark chocolate |
|---------------------|---|---------------------------------|---------------------------------|--------------------------------|
| <i>Flavan-3-ols</i> | | | | |
| 15.2 | Catechin | 66.20 \pm 1.99 ^a | 69.62 \pm 5.28 ^a | 71.13 \pm 2.15 ^a |
| 19.9 | Epicatechin | 203.29 \pm 10.68 ^a | 303.69 \pm 11.65 ^b | 218.43 \pm 8.08 ^a |
| 9.1 | Gallocatechin | 1.88 \pm 0.10 ^a | 2.09 \pm 0.14 ^a | < l.o.q. ^b |
| 13.2 | Epigallocatechin | 15.93 \pm 0.10 ^a | 29.76 \pm 1.74 ^b | 15.11 \pm 0.08 ^a |
| 22.6 | (Epi)catechin- <i>O</i> -sulphate isomer | 2.06 \pm 0.13 ^a | 1.73 \pm 0.20 ^a | 3.07 \pm 0.31 ^b |
| 25.3 | (Epi)catechin- <i>O</i> -sulphate isomer | 7.15 \pm 0.35 ^a | 7.16 \pm 0.12 ^a | 10.23 \pm 0.83 ^b |
| 23.6 | (Epi)catechin-3- <i>O</i> -trihydroxybenzene | 4.92 \pm 0.50 ^a | 4.66 \pm 0.10 ^b | 5.94 \pm 0.56 ^{ab} |
| 26.2 | (Epi)catechin-3- <i>O</i> - trihydroxybenzene | 3.61 \pm 0.06 ^a | 4.58 \pm 0.26 ^b | 4.84 \pm 0.42 ^b |
| 27.5 | (Epi)catechin-7- <i>O</i> -trihydroxybenzene | 2.60 \pm 0.15 ^a | 3.83 \pm 0.56 ^b | 3.39 \pm 0.14 ^{ab} |
| 20.3 | (Epi)catechin- <i>C</i> -pentoside isomer | 0.42 \pm 0.05 ^a | 0.30 \pm 0.07 ^a | 0.83 \pm 0.11 ^a |
| 28.3 | Catechin-3- <i>O</i> -gallate* | n.d. | < l.o.q. ^a | n.d. |
| 29.5 | Epicatechin-3- <i>O</i> -gallate * | n.d. | 9.12 \pm 0.13 ^a | n.d. |
| 13.1 | (Epi)catechin-3- <i>O</i> -hexoside isomer | 12.64 \pm 0.20 ^a | 9.78 \pm 1.44 ^a | 13.51 \pm 0.82 ^a |
| 14.1 | (Epi)catechin- <i>C</i> -hexoside isomer | 1.91 \pm 0.04 ^a | 1.62 \pm 0.10 ^a | 1.32 \pm 0.19 ^a |
| 15.1 | (Epi)catechin-7- <i>O</i> -hexoside | 4.11 \pm 0.29 ^a | 4.26 \pm 0.30 ^a | 5.24 \pm 0.43 ^a |
| 16.2 | (Epi)catechin- <i>C</i> -hexoside isomer | 4.09 \pm 0.21 ^a | 3.48 \pm 0.80 ^a | 3.72 \pm 0.01 ^a |
| 21.4 | (Epi)catechin-3- <i>O</i> -hexoside isomer | 1.36 \pm 0.21 ^a | 1.81 \pm 0.22 ^a | 1.48 \pm 0.08 ^a |
| 20.6 | Epigallocatechin-3- <i>O</i> -gallate * | n.d. | 33.54 \pm 2.16 ^a | n.d. |
| 10.1 | Gallocatechin-3- <i>O</i> -hexoside | 0.09 \pm 0.01 ^a | 0.06 \pm 0.01 ^a | 0.15 \pm 0.01 ^a |
| 11.1 | Epigallocatechin-3- <i>O</i> -hexoside | 0.09 \pm 0.01 ^a | 0.07 \pm 0.01 ^a | 0.17 \pm 0.01 ^a |
| 34.1 | Procyanidin dimer A type | 1.10 \pm 0.06 ^a | 1.31 \pm 0.08 ^a | 1.21 \pm 0.07 ^a |
| 12.1 | Procyanidin dimer B type isomer | 8.65 \pm 0.84 ^a | 8.39 \pm 0.34 ^a | 10.59 \pm 1.48 ^a |
| 17.1 | Procyanidin dimer B type isomer | 3.05 \pm 0.22 ^a | 2.69 \pm 0.14 ^a | 3.62 \pm 0.19 ^a |
| 18.3 | Procyanidin dimer B type isomer | 37.23 \pm 3.44 ^a | 52.45 \pm 5.44 ^b | 35.12 \pm 0.04 ^a |
| 18.9 | Procyanidin dimer B type isomer | 34.08 \pm 1.98 ^a | 44.99 \pm 1.15 ^b | 38.25 \pm 2.76 ^a |
| 23.1 | Procyanidin dimer B type isomer | 5.32 \pm 0.30 ^a | 6.66 \pm 0.64 ^a | 6.93 \pm 0.74 ^a |
| 23.3 | Procyanidin dimer B type isomer | 10.17 \pm 0.13 ^a | 12.35 \pm 0.35 ^a | 13.12 \pm 0.89 ^a |
| 12.2 | (Epi)catechin-(Epi)gallocatechin * | n.d. | 0.12 \pm 0.01 ^a | n.d. |
| 18.5 | (Epi)catechin-3- <i>O</i> -dihexoside isomer | 0.66 \pm 0.04 ^a | 0.73 \pm 0.09 ^a | 0.44 \pm 0.03 ^a |
| 28.1 | (Epi)catechin-3- <i>O</i> -dihexoside isomer | 1.65 \pm 0.02 ^a | 1.04 \pm 0.51 ^a | 1.34 \pm 0.03 ^a |
| 27.6 | (Epi)catechin-3- <i>O</i> -gallate-7- <i>O</i> -glucuronide isomer | 0.33 \pm 0.01 ^a | 0.18 \pm 0.01 ^a | 0.24 \pm 0.03 ^a |
| 34.6 | (Epi)catechin-3- <i>O</i> - gallate-7- <i>O</i> -glucuronide isomer | 0.31 \pm 0.02 ^a | 0.14 \pm 0.01 ^a | 0.23 \pm 0.02 ^a |
| 20.5 | (Epi)catechin derivative isomer | 1.16 \pm 0.04 ^a | 0.93 \pm 0.10 ^a | 1.30 \pm 0.14 ^a |
| 21.1 | (Epi)catechin derivative isomer | 1.02 \pm 0.04 ^a | 1.06 \pm 0.04 ^a | 0.77 \pm 0.05 ^a |
| 23.7 | Procyanidin dimer A type pentoside isomer | 1.42 \pm 0.01 ^a | 1.29 \pm 0.24 ^a | 1.68 \pm 0.04 ^a |
| 24.3 | Procyanidin dimer A type pentoside isomer | 1.09 \pm 0.01 ^a | 1.17 \pm 0.08 ^a | 1.04 \pm 0.04 ^a |
| 31.5 | Procyanidin dimer A type pentoside isomer | 1.75 \pm 0.03 ^a | 2.24 \pm 0.21 ^b | 1.82 \pm 0.11 ^{ab} |
| 32.2 | Procyanidin dimer A type pentoside | 1.47 \pm 0.11 ^a | 2.74 \pm 0.11 ^b | 1.20 \pm 0.06 ^a |

| | | | | |
|-------------------------------------|---|-----------------------------------|------------------------------------|-----------------------------------|
| | isomer | | | |
| 21.3 | Procyanidin dimer A type hexoside isomer | 1.48 ± 0.06 ^a | 1.52 ± 0.23 ^a | 1.91 ± 0.07 ^a |
| 22.2 | Procyanidin dimer A type hexoside isomer | 1.01 ± 0.09 ^a | 1.24 ± 0.13 ^a | 1.58 ± 0.18 ^a |
| 30.2 | Procyanidin dimer A type hexoside isomer | 2.06 ± 0.09 ^a | 3.09 ± 0.37 ^b | 2.33 ± 0.05 ^a |
| 31.1 | Procyanidin dimer A type hexoside isomer | 1.47 ± 0.03 ^a | 2.58 ± 0.15 ^b | 2.06 ± 0.03 ^a |
| 16.3 | Procyanidin dimer B type hexoside isomer | 0.80 ± 0.04 ^a | 0.89 ± 0.07 ^a | 1.02 ± 0.10 ^a |
| 18.2 | Procyanidin dimer B type hexoside isomer | 1.04 ± 0.02 ^a | 1.06 ± 0.04 ^a | 1.22 ± 0.04 ^a |
| 18.7 | Procyanidin dimer B type hexoside isomer | 0.51 ± 0.08 ^a | 0.55 ± 0.03 ^a | 0.54 ± 0.04 ^a |
| 18.10 | Procyanidin dimer B type derivative | 1.86 ± 0.13 ^a | 1.88 ± 0.47 ^a | 2.27 ± 0.13 ^a |
| 20.8 | Procyanidin trimer A type | 0.70 ± 0.05 ^a | 0.67 ± 0.04 ^a | 0.97 ± 0.04 ^a |
| 13.5 | Procyanidin trimer B type isomer | 0.93 ± 0.05 ^a | 0.92 ± 0.03 ^a | 1.11 ± 0.11 ^a |
| 18.1 | Procyanidin trimer B type isomer | 4.30 ± 0.31 ^a | 7.13 ± 0.51 ^b | 4.96 ± 0.41 ^a |
| 18.8 | Procyanidin trimer B type isomer | 3.21 ± 0.17 ^a | 4.20 ± 0.25 ^b | 3.65 ± 0.21 ^a |
| 20.7 | Procyanidin trimer B type isomer | 11.02 ± 0.45 ^a | 15.16 ± 0.90 ^b | 11.60 ± 0.64 ^a |
| 21.2 | Procyanidin trimer B type isomer | 8.90 ± 0.17 ^a | 17.49 ± 1.34 ^b | 9.64 ± 0.25 ^a |
| 22.5 | Procyanidin trimer B type isomer | 3.60 ± 0.19 ^a | 3.67 ± 0.18 ^a | 3.50 ± 0.22 ^a |
| 23.3 | Procyanidin trimer B type isomer | 2.05 ± 0.08 ^a | 2.15 ± 0.07 ^a | 2.91 ± 0.15 ^a |
| 29.2 | Procyanidin trimer B type isomer | 1.11 ± 0.03 ^a | 1.68 ± 0.08 ^a | 1.38 ± 0.02 ^a |
| 24.4 | Procyanidin trimer A type hexoside isomer | 0.70 ± 0.06 ^a | 0.99 ± 0.03 ^a | 0.84 ± 0.07 ^a |
| 24.9 | Procyanidin trimer A type hexoside isomer | 0.54 ± 0.02 ^a | 0.97 ± 0.08 ^a | 0.70 ± 0.04 ^a |
| 19.6 | Procyanidin tetramer A type isomer | 1.44 ± 0.01 ^a | 1.38 ± 0.07 ^a | 1.42 ± 0.08 ^a |
| 22.1 | Procyanidin tetramer A type isomer | 2.02 ± 0.13 ^a | 3.68 ± 0.57 ^b | 2.04 ± 0.16 ^a |
| 22.7 | Procyanidin tetramer A type isomer | 2.02 ± 0.03 ^a | 4.04 ± 0.82 ^b | 2.83 ± 0.08 ^a |
| 24.1 | Procyanidin tetramer A type isomer | 0.79 ± 0.07 ^{ab} | 1.11 ± 0.29 ^a | 0.66 ± 0.06 ^b |
| 16.1 | Procyanidin tetramer B type isomer | 0.37 ± 0.01 ^a | 0.37 ± 0.05 ^a | 0.37 ± 0.05 ^a |
| 19.5 | Procyanidin tetramer B type isomer | 1.34 ± 0.04 ^a | 1.81 ± 0.22 ^a | 1.85 ± 0.05 ^a |
| 19.7 | Procyanidin tetramer B type isomer | 0.93 ± 0.03 ^a | 1.82 ± 0.07 ^a | 1.71 ± 0.16 ^a |
| 22.8 | Procyanidin tetramer B type isomer | 1.19 ± 0.08 ^a | 2.58 ± 0.03 ^b | 1.65 ± 0.07 ^a |
| 19.13 | Procyanidin pentamer B type isomer | 0.60 ± 0.02 ^a | 0.55 ± 0.04 ^a | 0.67 ± 0.02 ^a |
| 23.2 | Procyanidin pentamer B type isomer | 0.82 ± 0.04 ^a | 1.55 ± 0.14 ^b | 0.99 ± 0.01 ^a |
| 24.2 | Procyanidin pentamer B type isomer | 1.17 ± 0.08 ^a | 3.47 ± 0.28 ^b | 1.75 ± 0.14 ^a |
| 22.9 | Procyanidin hexamer A type | < l.o.q. | < l.o.q. | < l.o.q. |
| 23.8 | Procyanidin hexamer B type isomer | 0.31 ± 0.01 ^a | 0.98 ± 0.02 ^b | 0.37 ± 0.02 ^a |
| 24.7 | Procyanidin hexamer B type isomer | 0.43 ± 0.04 ^a | 1.35 ± 0.04 ^b | 0.29 ± 0.01 ^a |
| 26.1 | Procyanidin hexamer B type isomer | 0.22 ± 0.02 ^a | 1.85 ± 0.20 ^b | 0.46 ± 0.04 ^a |
| <i>Total flavan-3-ols</i> | | 503.76 ± 8.98 ^a | 726.03 ± 14.53 ^b | 538.71 ± 8.99 ^c |
| <i>Hydroxycinnamic acids</i> | | | | |
| 1.1 | Coumaric acid | 0.34 ± 0.01 ^a | 0.37 ± 0.05 ^a | 0.55 ± 0.01 ^b |
| 23.4 | Di-hydro-coumaric acid | 8.13 ± 0.14 ^a | 4.57 ± 0.08 ^b | 11.18 ± 0.41 ^c |
| 8.5 | Di-hydroxycinnamic acid isomer | 0.92 ± 0.06 ^{ab} | 1.14 ± 0.07 ^a | 2.33 ± 0.03 ^b |
| 10.3 | Di-hydroxycinnamic acid isomer | 0.48 ± 0.02 ^a | 0.36 ± 0.01 ^a | 0.57 ± 0.01 ^b |
| 13.3 | Caffeic acid | 1.08 ± 0.01 ^a | 1.68 ± 0.02 ^b | 1.09 ± 0.02 ^a |

| | | | | |
|---|---|-----------------------------------|-----------------------------------|-----------------------------------|
| 19.1 | Di-hydroxycinnamic acid isomer | 0.48 ± 0.05 ^a | 0.34 ± 0.06 ^a | 0.60 ± 0.04 ^b |
| 19.10 | Di-hydroxycinnamic acid isomer | 0.58 ± 0.04 ^a | 0.63 ± 0.03 ^a | 0.58 ± 0.07 ^a |
| 2.1 | Di-hydro-caffeic acid | 1.88 ± 0.16 ^a | 0.24 ± 0.01 ^b | 0.88 ± 0.04 ^c |
| 30.3 | Ferulic acid | 61.23 ± 3.74 ^a | 58.09 ± 2.33 ^a | 55.30 ± 2.36 ^a |
| 18.11 | Coumaroyl aspartate | 15.72 ± 0.23 ^a | 14.07 ± 0.45 ^a | 16.83 ± 0.17 ^a |
| 10.2 | Di-hydroxycinnamic aspartate isomer | 8.59 ± 0.52 ^a | 6.86 ± 0.16 ^b | 6.92 ± 0.04 ^b |
| 14.2 | Di-hydroxycinnamic aspartate isomer | 35.88 ± 2.89 ^a | 33.48 ± 0.75 ^a | 33.12 ± 1.58 ^a |
| 20.4 | Feruloyl aspartate | 8.18 ± 0.26 ^a | 9.11 ± 0.17 ^a | 8.96 ± 0.41 ^a |
| 27.2 | Ferulic acid-4- <i>O</i> -pentoside | 0.40 ± 0.06 ^a | 0.48 ± 0.01 ^a | 0.47 ± 0.02 ^a |
| 34.4 | Di-deoxyclovamide (Coumaroyl-DOPA) | 4.66 ± 0.21 ^a | 4.75 ± 0.02 ^a | 4.57 ± 0.24 ^a |
| 12.3 | 3-Coumaroylquinic acid <i>cis</i> [*] | n.d. | 1.51 ± 0.01 ^a | n.d. |
| 14.3 | 3-Coumaroylquinic acid <i>trans</i> [*] | n.d. | 0.68 ± 0.01 ^a | n.d. |
| 19.4 | 4-Coumaroylquinic acid <i>cis</i> [*] | n.d. | 2.05 ± 0.10 ^a | n.d. |
| 20.2 | 4-Coumaroylquinic acid <i>trans</i> [*] | n.d. | 3.52 ± 0.09 ^a | n.d. |
| 24.6 | Mono-deoxyclovamide (Caffeoyl-DOPA / Coumaroyl-tyrosine) isomer | 0.58 ± 0.02 ^a | 0.48 ± 0.04 ^a | 0.50 ± 0.01 ^a |
| 29.4 | Mono-deoxyclovamide (Caffeoyl-DOPA / Coumaroyl-tyrosine) isomer | 2.93 ± 0.02 ^a | 2.25 ± 0.10 ^a | 2.11 ± 0.13 ^a |
| 16.8 | 5-Caffeoylquinic acid | 0.23 ± 0.02 ^a | 0.65 ± 0.02 ^b | 0.25 ± 0.03 ^a |
| 17.2 | 3-Caffeoylquinic acid | 0.12 ± 0.02 ^a | 0.31 ± 0.02 ^b | 0.21 ± 0.01 ^c |
| 20.1 | Clovamide (caffeoyl-tyrosine) isomer | 1.81 ± 0.05 ^a | 1.42 ± 0.02 ^a | 1.18 ± 0.07 ^a |
| 23.5 | Clovamide (caffeoyl-tyrosine) isomer | 7.73 ± 0.54 ^a | 8.94 ± 0.36 ^b | 8.31 ± 0.01 ^b |
| <u>Total hydroxycinnamic acids</u> | | 161.95 ± 4.80 ^a | 157.98 ± 2.38 ^a | 156.51 ± 1.62 ^a |
| <u>Flavonols</u> | | | | |
| 35.1 | Quercetin | 1.08 ± 0.01 ^a | 1.62 ± 0.03 ^b | 1.37 ± 0.01 ^a |
| 33.2 | Quercetin-3- <i>O</i> -pentoside | 3.31 ± 0.21 ^a | 2.80 ± 0.14 ^a | 4.15 ± 0.40 ^b |
| 31.4 | Kaempferol-7- <i>O</i> -hexoside | 0.14 ± 0.01 ^a | 0.24 ± 0.01 ^b | 0.15 ± 0.01 ^a |
| 32.4 | Kaempferol-3- <i>O</i> -galactoside | 0.04 ± 0.01 ^a | 0.28 ± 0.01 ^b | < l.o.q. ^c |
| 34.3 | Kaempferol-3- <i>O</i> -glucoside | 0.05 ± 0.01 ^a | 0.37 ± 0.03 ^b | < l.o.q. ^c |
| 34.5 | Quercetin-3- <i>O</i> -rhamnoside | 0.05 ± 0.01 ^a | 0.50 ± 0.02 ^b | < l.o.q. ^c |
| 18.6 | Dihydro-kaempferol-7- <i>O</i> -hexoside | 0.25 ± 0.01 ^a | 0.21 ± 0.01 ^a | 0.23 ± 0.01 ^a |
| 30.1 | Quercetin-3- <i>O</i> -galactoside | 0.49 ± 0.01 ^a | 1.67 ± 0.01 ^b | 2.44 ± 0.04 ^c |
| 31.3 | Quercetin-3- <i>O</i> -glucoside | 1.96 ± 0.02 ^a | 2.29 ± 0.07 ^a | 3.05 ± 0.27 ^b |
| 24.5 | Myricetin-3- <i>O</i> -galattoside | 0.30 ± 0.01 ^a | 2.35 ± 0.04 ^b | < l.o.q. ^c |
| 24.8 | Myricetin-3- <i>O</i> -glucoside | 0.46 ± 0.01 ^a | 1.84 ± 0.14 ^b | < l.o.q. ^c |
| 33.1 | Kaempferol-3- <i>O</i> -rutinoside | 0.26 ± 0.01 ^a | 0.65 ± 0.02 ^b | < l.o.q. ^c |
| 28.4 | Quercetin-3- <i>O</i> -rutinoside [*] | n.d. | 4.20 ± 0.19 ^a | n.d. |
| 23.9 | Myricetin-3- <i>O</i> -rutinoside [*] | n.d. | 0.58 ± 0.01 ^a | n.d. |
| 22.3 | Myricetin-3- <i>O</i> -(<i>O</i> -galloyl) hexoside | 0.11 ± 0.01 ^a | 0.62 ± 0.02 ^b | < l.o.q. ^c |
| 31.1 | Kaempferol-7- <i>O</i> -rhamnoside-3- <i>O</i> -rutinoside | 0.03 ± 0.01 ^a | 0.09 ± 0.01 ^a | < l.o.q. ^c |
| 27.4 | Quercetin-7- <i>O</i> -rhamnoside-3- <i>O</i> -rutinoside | 0.14 ± 0.01 ^a | 0.20 ± 0.02 ^a | < l.o.q. ^b |
| 28.2 | Kaempferol-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside isomer | 0.57 ± 0.01 ^a | 1.27 ± 0.02 ^b | < l.o.q. ^c |
| 31.2 | Kaempferol-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside isomer | 0.44 ± 0.01 ^a | 1.38 ± 0.01 ^b | < l.o.q. ^c |
| 25.2 | Quercetin-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside isomer | 1.12 ± 0.01 ^a | 2.24 ± 0.01 ^b | < l.o.q. ^c |
| 26.3 | Quercetin-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside | 2.09 ± 0.01 ^a | 3.65 ± 0.26 ^b | < l.o.q. ^c |

| | | | | |
|-------------------------------------|---|----------------------------------|----------------------------------|----------------------------------|
| isomer | | | | |
| 22.4 | Myricetin-7- <i>O</i> -hexoside-3- <i>O</i> -rutinoside | 0.12 ± 0.01 ^a | 0.23 ± 0.02 ^b | < l.o.q. ^c |
| <u>Total flavonols</u> | | 13.01 ± 0.21 ^a | 29.28 ± 0.39 ^b | 11.40 ± 0.48 ^a |
| <u>Other phenolics</u> | | | | |
| 16.4 | Vanillin | 1.33 ± 0.05 ^a | 1.81 ± 0.11 ^b | 1.85 ± 0.08 ^b |
| 32.3 | Phloretin- <i>C</i> -hexoside isomer | 0.15 ± 0.01 ^a | 0.16 ± 0.01 ^a | 0.63 ± 0.03 ^b |
| 18.4 | Eriodictyol-7- <i>O</i> -hexoside | 0.10 ± 0.01 ^a | 0.19 ± 0.01 ^b | 0.53 ± 0.03 ^c |
| 29.1 | Cinchonain isomer | 1.81 ± 0.20 ^a | 1.13 ± 0.01 ^a | 2.09 ± 0.03 ^b |
| 34.2 | Cinchonain isomer | 0.77 ± 0.66 ^a | 0.79 ± 0.02 ^a | 0.75 ± 0.02 ^a |
| 19.3 | Naringenin- <i>C</i> -hexoside-7- <i>O</i> -hexoside isomer | 0.23 ± 0.01 ^a | 0.63 ± 0.02 ^b | < l.o.q. ^c |
| 19.2 | Eriodictyol- <i>C</i> -hexoside-7- <i>O</i> -hexoside isomer | 0.15 ± 0.01 ^a | 0.28 ± 0.01 ^b | n.d. |
| <u>Total other phenolics</u> | | 4.54 ± 0.69 ^a | 4.99 ± 0.11 ^a | 5.85 ± 0.10 ^b |
| <u>Flavones</u> | | | | |
| 27.7 | Apigenin- <i>C</i> -hexoside isomer | 0.10 ± 0.01 ^a | 0.42 ± 0.01 ^b | 0.19 ± 0.01 ^a |
| 22.10 | Apigenin- <i>C</i> -hexoside- <i>C</i> -pentoside isomer | 0.95 ± 0.01 ^a | 2.11 ± 0.07 ^b | 0.83 ± 0.03 ^a |
| 27.3 | Apigenin- <i>C</i> -hexoside-2''- <i>O</i> -rhamnoside isomer | 1.31 ± 0.32 ^{ab} | 0.89 ± 0.13 ^a | 1.71 ± 0.43 ^b |
| 19.8 | Apigenin-6,8- <i>di-C</i> -hexoside isomer | 0.17 ± 0.01 ^a | 0.47 ± 0.01 ^b | 0.23 ± 0.01 ^a |
| 19.12 | Apigenin-6,8- <i>di-C</i> -hexoside isomer | 0.20 ± 0.01 ^a | 0.54 ± 0.01 ^b | 0.33 ± 0.01 ^a |
| 25.1 | Apigenin- <i>C</i> -hexoside-2''- <i>O</i> -hexoside isomer | 0.25 ± 0.01 ^a | 0.47 ± 0.01 ^b | n.d. |
| <u>Total flavones</u> | | 2.98 ± 0.32 ^a | 4.90 ± 0.15 ^b | 3.29 ± 0.43 ^a |
| <u>Ellagitannins</u> | | | | |
| 37.1 | Ellagic acid | 56.16 ± 3.58 ^a | 53.44 ± 1.02 ^a | 50.50 ± 3.30 ^a |
| 27.1 | Ellagic acid-galloyl-hexoside | 4.65 ± 0.27 ^a | 11.13 ± 0.43 ^b | 4.09 ± 0.15 ^a |
| 16.5 | HHDP-galloyl-hexose | 15.79 ± 1.20 ^a | 24.65 ± 1.01 ^b | 14.11 ± 0.69 ^a |
| <u>Total ellagitannins</u> | | 76.60 ± 3.78 ^a | 89.12 ± 1.50 ^b | 68.70 ± 3.37 ^a |
| <u>Hydroxybenzoic acids</u> | | | | |
| 2.2 | Hydroxybenzoic acid isomer | 0.21 ± 0.03 ^a | 0.20 ± 0.04 ^a | 0.27 ± 0.02 ^a |
| 7.1 | Hydroxybenzoic acid isomer | 0.13 ± 0.01 ^a | 0.11 ± 0.01 ^a | 0.41 ± 0.02 ^b |
| 11.2 | Hydroxybenzoic acid isomer | 5.47 ± 0.03 ^a | 6.46 ± 0.06 ^{ab} | 7.50 ± 0.47 ^b |
| 13.4 | Hydroxybenzoic acid isomer | 0.31 ± 0.01 ^a | 1.32 ± 0.02 ^b | 0.33 ± 0.02 ^a |
| 19.11 | Hydroxybenzoic acid isomer | 0.62 ± 0.04 ^a | 0.94 ± 0.11 ^b | 0.77 ± 0.03 ^a |
| 8.6 | Protocatechuic acid | 10.76 ± 0.30 ^a | 9.11 ± 0.09 ^a | 18.31 ± 0.97 ^b |
| 8.3 | Vanillic acid isomer | 0.39 ± 0.04 ^a | 0.37 ± 0.01 ^a | 0.45 ± 0.02 ^a |
| 16.6 | Vanillic acid isomer | 0.75 ± 0.13 ^a | 0.55 ± 0.15 ^a | 1.12 ± 0.01 ^b |
| 3.1 | Gallic acid * | n.d. | 0.14 ± 0.01 ^a | n.d. |
| 9.4 | Syringic acid | 0.13 ± 0.02 ^a | 0.26 ± 0.03 ^a | 0.14 ± 0.02 ^a |
| 8.4 | Protocatechuic acid-4- <i>O</i> -hexoside | 0.10 ± 0.01 ^a | 0.31 ± 0.03 ^b | 0.19 ± 0.01 ^a |
| 5.1 | Vanillic acid-4- <i>O</i> -hexoside isomer | 0.25 ± 0.01 ^a | 0.20 ± 0.02 ^a | 0.30 ± 0.01 ^a |
| 8.1 | Vanillic acid-4- <i>O</i> -hexoside isomer | 1.77 ± 0.06 ^a | 1.67 ± 0.04 ^a | 2.63 ± 0.01 ^b |
| 16.7 | Vanillic acid-4- <i>O</i> -hexoside isomer | 1.92 ± 0.07 ^a | 1.81 ± 0.03 ^a | 1.73 ± 0.09 ^a |
| 3.2 | Galloyl glucose isomer * | n.d. | 0.29 ± 0.01 ^a | n.d. |
| 4.1 | Galloylquinic acid isomer * | n.d. | 1.84 ± 0.08 ^a | n.d. |
| 6.1 | Galloylquinic acid isomer * | n.d. | 2.09 ± 0.05 ^a | n.d. |

| | | | | |
|--|--|------------------------------------|-------------------------------------|-------------------------------------|
| 9.2 | Syringic acid-4- <i>O</i> -hexoside isomer | 0.94 ± 0.08 ^a | 0.81 ± 0.04 ^a | 1.07 ± 0.11 ^a |
| 9.3 | Syringic acid-4- <i>O</i> -hexoside isomer | 0.74 ± 0.02 ^a | 0.71 ± 0.04 ^a | 1.04 ± 0.11 ^b |
| 8.2 | Vanillic acid derivative | 0.31 ± 0.01 ^a | 0.49 ± 0.03 ^b | 0.56 ± 0.04 ^b |
| <u>Total hydroxybenzoic acids</u> | | 24.80 ± 0.33 ^a | 23.05 ± 0.20 ^a | 36.82 ± 1.08 ^b |
| <u>Curcuminoids</u> | | | | |
| 36.1 | Bisdemethoxycurcumin ^{**} | n.d. | n.d. | 115.55 ± 2.16 ^a |
| 36.2 | Demethoxycurcumin ^{**} | n.d. | n.d. | 82.64 ± 1.33 ^a |
| 36.3 | Curcumin ^{**} | n.d. | n.d. | 74.55 ± 0.47 ^a |
| <u>Total curcuminoids</u> | | n.d. | n.d. | 272.73 ± 2.58 ^a |
| <u>Total phenolic compounds</u> | | 787.63 ± 10.90 ^a | 1035.45 ± 14.81 ^b | 1094.03 ± 10.15 ^c |

n.d. means not detected; **<l.o.q.** means the compound was detected but it was below the limit of quantification; ^{*} and ^{**} mean the compounds were detected only in green tea dark chocolate or turmeric dark chocolate, respectively. The data represent the sum of the quantities of a specific compound found in the methanol extract and in the subsequent acetone extract.

Different superscript letters within the same row indicate that the values are significantly different ($P < 0.05$).

Flavan-3-ols as well as compounds 29.1 and 34.2 were quantified as epicatechin equivalent.

Hydroxycinnamic acids were quantified as coumaric acid equivalent except compounds 20.4, 27.2 and 30.3 which were quantified as ferulic acid equivalent.

Flavonols, flavones as well as compounds 18.4, 19.2, 19.3 and 32.3 were quantified as quercetin-3-*O*-rutinoside equivalent.

Ellagitannins were quantified as ellagic acid equivalent.

Hydroxybenzoic acids as well as compound 16.4 were quantified as protocatechuic acid equivalent.

Curcuminoids were quantified as curcumin equivalent.

The numbering of the compounds is referred to that used in **Table 1**.