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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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Abstract

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

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

1. Introduction

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

Cocoa (*Theobroma cacao*) is known as a rich source of dietary phenolic compounds. Cocoa-derived products such as dark chocolate are widely studied for their beneficial effects ascribed to polyphenols. There is good evidence to suggest that cocoa derived polyphenols may have beneficial effects on cardiovascular disease risk factors (Del Rio et al., 2013). Short-term dark chocolate intake has been shown to reduce blood pressure in hypertensive subjects, to improve endothelial function and insulin resistance as well as to inhibit platelet activation (Del Rio et al., 2013). As reported by Rusconi, & Conti (2010), cocoa beans are characterised by phenolic compounds of the flavan-3-ol group (catechin, epicatechin, gallocatechin and epigallocatechin) comprising oligomeric procyanidins, anthocyanins (cyanidin glycosides) and flavonol glycosides such as quercetin-3-*O*-rutinoside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucuronide and quercetin (Sanbongi et al., 1998). So far, only few studies have investigated the phenolic composition of dark chocolate, focusing on flavan-3-ols as the major class in chocolate phenolic profile (Ortega et al., 2008; Wollgast, & Anklam, 2000a). This lack of information is also due to the great interest addressed to the study of phenolic profile of cocoa, intended as raw material in chocolate production, without considering the impact of processing temperature, microbial fermentation or oxidative phenomena on the phenolics structure during cocoa processing in chocolate production. The majority of published researches were aimed at analyzing the impact of processing on the polyphenol content and antioxidant properties of cocoa more than that of chocolate (Di Mattia, Sacchetti, Mastrocola, & Serafini, 2017; Dorota, Oracz, Sosnowska, & Nebesny, 2016). Concerning this, it was considered purposeful to investigate the comprehensive phenolic profile of commercial dark chocolate (70%), using an un-targeted mass spectrometry approach, in order to fill the gap of information about dark chocolate phenolic composition. Finally, the last task was to evaluate a possible polyphenolic enrichment of dark chocolate recipe, by adding widely studied polyphenol-rich ingredients (Sakura green tea leaves and turmeric powder) in order to obtain potential functional food, which can combine the above-mentioned chocolate properties and those of green tea leaves and turmeric powder (Del Rio et al., 2013; Kunnumakkara et al., 2017).

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

2. Materials and methods

2.1. Materials

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

2.2. Extraction of phenolic compounds

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

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

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

mm, 5 µm particle size, Hamilton Company, Reno, Nevada, USA). The mobile phase consisted of (A) H_2O/formic acid (99:1, v/v) and (B) acetonitrile/formic acid (99:1, v/v). The gradient started at 4% B for 0.5 min then linearly ramped up to 30% B in 60 min. The mobile phase composition was raised up to 100% B in 1 min and maintained for 5 min in order to wash the column before returning to the initial condition. The flow rate was set at 1 mL/min. The chocolate extracts were injected in the amount of 20 µL. After passing to the column, the eluate was split, and 0.3 mL/min was direct to a 6520 accurate-mass Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA). Identification of phenolic compounds in all samples was carried out using full scan, data-dependent MS^2 scanning from m/z 100 to 1700 and selected reaction monitoring. MS operating conditions (negative mode) were: a capillary temperature of 350°C, a dry gas flow rate of 10 L/min, a nebulizer pressure of 35 psi, potential of the ESI source, 3.5 kV.

The quantification of single phenolic compounds was carried out by integrating the area under the peak from the extracted ion chromatograms (EICs). To obtain an accurate quantification the EICs were obtained by centering a narrow mass window (± 5 ppm) on the theoretical *m/z* value of each phenolic compound. For each standard compound, the calibration curve was built using seven concentration points in the range of 0.2-50 ng. Hydroxycinnamic acids, hydroxybenzoic acids, flavan-3-ols and ellagitannins were quantified as *p*-coumaric or ferulic acid, protocatechuic acid, (-)-epicatechin and ellagic acid equivalents, respectively. Flavonols and flavones were quantified as quercetin-3-rutinoside equivalents. Finally, curcuminoids were quantified as curcumin equivalent. Quantitative results were expressed as mg of compounds per 100 g of chocolate. Calibration curve equations, linearity ranges and limit of quantification (LOQ) for the different standards are given in supplementary materials (**Table S1**). Folin-Ciocalteau assay was also performed to quantify the total phenolic compounds as reported by Singleton, Orthofer, & Lamuela-Raventós (1999). The results were expressed as mg of gallic acid per 100 g of chocolate.

2.4. Antioxidant activity assays

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

2.5. Statistic

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

3. Result and discussion

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

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

3.1.1. Flavan-3-ols and derivatives

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

Among the monomeric flavan-3-ols, four compounds had been already reported in dark chocolate (compounds 9.1, 13.2, 15.2 and 19.9) (Wollgast, 2004), seven compounds had been detected in cocoa beans or products but not in dark chocolate (compounds 13.1, 14.1, 15.1, 16.2 , 21.4, 22.6 and 25.3) (D'Souza et al., 2017; Patras et al., 2014), whereas seven compounds were newly identified in both dark chocolate and cocoa beans or products. Gallocatechin-3-*O*-hexoside (*m/z* 467.1270; compound 10.1) and epigallocatechin-3-*O*-hexoside (*m/z* 467.1270; compound 11.1) were tentatively identified since they gave MS^2 major product ion at *m/z* 305, displaying typical hexosyl group loss (162 amu) (Jiang et al., 2013). Otherwise, (epi)catechin-*C*-pentoside isomer (compound 20.3) has been ascribed to the deprotonated ion $[M-H]^-$ ion at *m/z* 421.1223, yielding major MS^2 fragment ions at *m/z* 361 and *m/z* 331, corresponding to the loss of 60 and 90 amu (i.e. *C*-pentosyl moiety) (Hvattum, & Ekeberg, 2003). Compounds 23.6, 26.2 and 27.5, *m/z* 415.1111, were speculated to be isomers of (epi)catechin trihydroxybenzene, since the difference between the precursor ion (*m/z* 415) and its major product ion (*m/z* 289, i.e. (epi)catechin-aglycone) was 126 amu, indicating the typical loss of a trihydroxybenzene moiety and the MS^2 fragmentation spectra showed typical (epi)catechin fragmentation pattern (**Table 1**) with MS^2 fragment ions at *m/z* 245, 205 and 125. The presence of distinctive MS^2 product ions at *m/z* 259 (compounds 23.6 and 26.2; deprotonated aglycone -30 amu, $[Y_0\text{-}2H\text{-}CO]^-$) and *m/z* 261 (compound 27.5; deprotonated aglycone-28 amu, $[Y_0\text{-}CO]^-$) were observed, distinguishing the two different *O*-binding sites, 3-*O* and 7-*O*, respectively (Hvattum, & Ekeberg, 2003). Compounds 27.6 and 34.6 with negative charged $[M\text{-}H]^-$ ion at *m/z* 617.1413 gave product ions in the MS^2 spectra at *m/z* 465 ($[M\text{-}H]^-$ -152 amu, detailing the loss of a galloyl group), 289 ((epi)catechin-aglycone and 465-176 amu; i.e. the loss of a glucuronic acid moiety), 435 (gallate group 3-*O*-binding site) and 261 (glucuronide 7-*O*-binding site), and were tentatively identified as (epi)catechin-*3-O*-gallate-*7-O*-glucuronide (Shrestha et al., 2012). Compounds 18.5 and 28.1 had the same precursor ion $[M\text{-}H]^-$ at *m/z* 613.1138 and their main MS^2 fragment ions at *m/z* 289 ((epi)catechin aglycone), *m/z* 451 ($[M\text{-}H]^-$ - 162 amu, i.e. hexosyl moiety loss) and *m/z* 433 revealing that one sugar is attached to another and not directly to the aglycone describing two hexose sequential losses, as suggested by Hvattum, & Ekeberg (2003). Moreover, the presence of MS^2 product ion at *m/z* 259 identified 3-*O*-glycosylation site. Regarding this, they were tentatively identified as two isomers of (epi)catechin-3-*O*-dihexoside. Finally, three compounds (compounds 28.3, 29.5, 20.6) with deprotonated ions at *m/z* 441.0892 and 457.0829 were only detected in dark chocolate with added Sakura green tea leaves (**Table 1**). They had been classified as catechin-*3-O*-gallate, epicatechin-*3-O*-gallate and epigallocatechin-3-*O*-gallate in comparison with authentic standards (**Table 1**).

Procyanidins are mostly flavan-3,4-diols, found as dimers, trimers or oligomers with epicatechin as the main extension sub-unit. **Table 1** shows six B-type procyanidin dimers (compounds 12.1, 17.1, 18.3, 18.9, 23.1 and 29.3) and four derivative forms (compounds 18.10, 16.3, 18.2 and 18.7). Among these, three glycosidic dimers of procyanidin B-type were newly identified in dark chocolate but already reported in cocoa beans (D'Souza et al., 2017). As reported by Gu, House, Wu, Ou, & Prior (2006) chocolate and cocoa products were also characterized by polymeric procyanidins. Trimers (compounds 13.5, 18.1, 18.8, 20.7, 21.2, 22.5, 23.3 and 29.2), tetramers (compounds 16.1, 19.5, 19.7 and 22.8), pentamers (compounds 19.13, 23.2 and 24.2) and hexamers (compounds 23.8, 24.7 and 26.1) of B-type procyanidins were also found. Compound 12.2 (**Table 1**) with precursor ion at *m/z* 593.1345 was only detected in Sakura green tea dark chocolate and tentatively identified as (epi)catechin-(epi)gallocatechin in accordance to Jiang et al. (2013). A-type 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 MS^2 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

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

3.1.3.1. Flavonols and derivative forms

According to MS and MS/MS data, the elution profile and literature (Andres-Lacueva et al., 2008; Counet, Callemien, & Collin 2006; Ortega et al., 2008; Sanbongi et al., 1998; Wollgast, 2004), four flavonols already reported in chocolate and cocoa beans or products were identified as quercetin at *m/z* 301.0423 (compound 35.1) and its pentoside at *m/z* 433.0832 (compound 33.2) and hexoside at *m/z* 463.0950 (compounds 30.1 and 31.3) derivatives. Concerning flavonols *O*-glycosides, compounds 32.4, 34.3 and 34.5 had been already detected in cocoa but never in dark chocolate (Ortega et al., 2008; Sánchez-Rabaneda et al., 2003) and were tentatively ascribed to kaempferol-3-*O*-hexoside isomers and quercetin-3-*O*-rhamnoside. Two quercetin derivatives, quercetin-7-*O*-rhamnoside-3-*O*-rutinoside and quercetin-7-*O*-hexoside-3-*O*-rutinoside isomers (*m/z* 755.2103 and 771.2042, respectively; compounds 27.4, 25.2 and 26.3) were detected for the first time in dark chocolate and cocoa beans or products by tentatively identification, screening the fragmentation pattern (**Table 1**) (Guimarães et al., 2013; Lin, Chen, & Harnly, 2008). Compound 27.4 with a $[M–H]^-$ deprotonated ion at *m/z* 755.2103 and MS^2 fragment ions at *m/z* 609 (quercetin-3-*O*-rutinoside, by loss of rhamnose moiety, 146 amu), 301 (quercetin-aglycone, underlining the loss of a rutinose moiety, 308 amu) was tentatively identified as quercetin-7-*O*-rhamnoside-3-*O*-rutinoside (Lin et al., 2008). Compound 25.2 and 26.3 with a $[M–H]^-$ precursor ion at *m/z* 771.2042, producing product ions at *m/z* 609 (loss of 162 mass units, a hexosyl-moiety), 463 (quercetin-3-*O*-glucoside, loss of 308 amu, a rutinose moiety) and 301 (quercetin-aglycone), and according to Martini et al. (2017), were tentatively identified as quercetin-7-*O*-hexoside-3-*O*-rutinoside. As far as we know, also 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 chocolate and cocoa in general. Compound 31.4 was speculated to be kaempferol-7-*O*-hexoside, since the difference between the precursor ion (*m/z* 447.1028) and its major fragment ion (*m/z* 285, i.e. kaempferol-aglycone) was 162 amu detailing the loss of a hexosyl-moiety; the presence of the MS^2 ion at *m/z* 257 identified 7-*O*-glycosylation site (Hvattum, & Ekeberg, 2003). According to Mena et al. (2012), compound 18.6 (*m/z* 449.1182) was tentatively identified as dihydrokaempferol-7-*O*-hexoside, borne out by the presence of MS^2 major fragment ions at *m/z* 287 ($[M-H]^-$– 162 amu; characteristic loss of *O*-hexoside) and *m/z* 259, confirming 7-*O*-glycosylation site (Hvattum, & Ekeberg, 2003). Compounds 32.1, 28.2 and 31.2 had deprotonated ions at *m/z* 739.2182 and *m/z* 755.2103 and gave product ions in the MS^2 spectra at *m/z* 593 and 285 characteristic of kaempferol-3-*O*-rutinoside and kaempferol-aglycone (Sánchez-Rabaneda et al., 2003). The negative product ion at *m/z* 593 was formed by the loss of rhamnose, glucose or galactose moiety from the glycosides. The presence of the product ion in MS^2 spectra at *m/z* 257 identified 7-*O*-glycosylation site (Hvattum, & Ekeberg, 2003). The loss of 308 amu is typical of a rutinose moiety, and therefore these compounds were tentatively identified as kaempferol-7-*O*-rhamnoside-3-*O*-rutinoside (compound 32.1) and kaempferol-7-*O*-hexoside-3-*O*-rutinoside isomers (compounds 28.2 and 31.2). Finally, peaks 22.3, 22.4, 23.9, 24.5 and 24.8 were tentatively identified as myricetin-derivative-compounds. All of the compounds gave MS^2 product ion at *m/z* 317, corresponding to myricetin aglycone. Compounds 24.5 and 24.8, identified as myricetin-3-*O*-hexosides, had already been detected in cocoa but never in dark chocolate (Lin et al., 2008). Two myricetin-derivatives (compounds 22.3 and 22.4) have been described for the first time in dark chocolate and cocoa beans or products in this study. The MS^2 spectra of the compound 22.3 (*m/z* 631.1039) was characterized by fragment ions at *m/z* 479 and 317, depicting losses of 152 and 162 amu (i.e. galloyl and hexosyl moiety). This behaviour is indicative of myricetin-3-*O*-(*O*-galloyl)-hexoside (Saldanha, Vilegas, & Dokkedal, 2013). Compound 22.4 showed a deprotonated ion at *m/z* 787.2050, which fragmented in the MS^2 experiments giving product ions at *m/z* 625 and 317, suggesting the presence of a myricetin aglycone, through the loss of 308 amu (rutinose moiety) and the loss of 162 amu (hexose group). Concerning this, the compound was therefore tentatively identified as myricetin-7-*O*-hexoside-3-*O*-rutinoside (Lin et al., 2008). Finally, compound 23.9 (**Table 1**), with negative precursor ion ($[M-H]^-$) at *m/z* 625.1455, was only detected in chocolate with added Sakura green tea leaves **(Table 1**). Basing on its fragmentation pattern (**Table 1**) and according to Lin et al. (2008) it had been tentatively classified as myricetin-3-*O*-rutinoside.

3.1.3.2. Flavones

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

3.1.3.3. Other phenolics

Compounds 29.1 and 34.2, showing the same negative precursor ion $[M\text{-}H]^-$ at *m/z* 451.1103, were tentatively ascribed to cinchonain isomers, already detected in cocoa powder (Cádiz-Gurrea et al., 2014) but never before in dark chocolate. Four glycosidic forms of naringenin (compound 19.3), eriodictyol (compounds 18.4 and 19.2) and phloretin (compound 32.3) were tentatively identified for the first time in dark chocolate and cocoa products and listed in **Table 1**. Compound 19.3 at *m/z* 593.1591, was tentatively identified as naringeni-*C*-hexoside-*7-O*-hexoside isomer, confirmed by the characteristic loss of 120 amu (*C*-glycosylation site) and 162 amu (*O*-glycosylation site) from *m/z* 473 and 413, respectively, which pinpointed the presence of two hexose units attached to the flavonoid aglycone in different positions (Hvattum, & Ekeberg, 2003; Waridel et al., 2001). The 7-*O*-glycosylation site was proved by the presence of MS^2 fragment ion at *m/z* 283. Compound 18.4 displayed deprotonated ion at *m/z* of 449.1169 and showed MS^2 major fragment ions at m/z 287 ($[M–H]^-$ -162; i.e. hexose moiety loss) and *m/z* 259, confirming *7-O*-glycosylation site presence. Based on these data, compound 18.4 was tentatively identified as eriodictyol-*7-O*-hexoside (De Beer at al. 2012). Compound 19.2, characterized by the deprotonated ion at *m/z* 611.1662, gave MS^2 major fragment ions at *m/z* 449, 329 (corresponding to the loss of 120 amu (*C*-hexoside) and 162 amu (*O*-hexoside), respectively), 287 (eriodyctiol-aglycone) and 259 (*7-O*-glycosylation site). Therefore, compound 19.2 was speculated to be eriodictiol-*C*-hexoside-*7-O*-hexoside isomer (De Beer at al. 2012; Hvattum, & Ekeberg, 2003). The negative ionization mode of compound 32.3 exhibited a $[M\text{-}H]^-$ precursor ion at *m/z* 435.1376, with MS^2 product ions at *m/z* 345 and 315, losing 90 and 120 amu, respectively. This fragmentation pattern has been previously described for phloretin-*C*-hexoside isomer (Kazuno, Yanagida, Shindo, & Murayama, 2005).

3.1.4. Ellagitannins

Ellagitannins are known as polymeric structures including different numbers of galloyl and hexahydroxydiphenoyl (HHDP) units esterified with glucose. Three ellagitannins were detected for the first time in cocoa. They were distinguished by their characteristic fragment ion spectra yielding sequential losses of galloyl (152 amu), gallate (170 amu), and HHDP residues (301 amu). Following the ellagitannins fragmentation scheme pattern proposed by Mena et al. (2012), compounds 16.5 (*m/z* 633.0796) and 37.1 (*m/z* 301.0054) can be tentatively identified as HHDP-galloyl-hexose and ellagic acid, respectively. The ellagic acid was also confirmed by comparison with the retention time of the standard and the MS^2 spectrum. Compound 27.1, characterized by the deprotonated ion at *m/z* 615.0723 and MS^2 fragment ions at *m/z* 463, due to the loss of a galloyl group from ($[M–H]^-$-152) and at *m/z* 301, due to the loss of one hexose moiety (162 amu), was tentatively identified as ellagic acid-galloyl-hexoside (Teixeira, Bertoldi, Lajolo, Mariko, & Hassimotto, 2015)

3.1.5. Hydroxybenzoic acids

A total of twenty hydroxybenzoic acids and derivatives were detected in this study. Three of these (compounds 8.4, 9.2 and 9.3) were tentatively identified for the first time in dark chocolate and cocoa beans and products. Whereas 11 compounds (compounds 2.2, 5.1, 7.1, 8.1, 8.3, 9.4, 11.2, 13.4, 16.6, 16.7, 19.11) had been already identified in cocoa but never in dark chocolate (Ortega et al., 2008). Compound 8.4 (*m/z* 315.0793) yielded MS^2 fragment ions at *m/z* 153 and 109, displaying the hexose moiety loss and the presence of protocatechuic-aglycone. It was tentatively identified as protocatechuic acid-4-*O*-hexoside (Martini et al., 2017). Compounds 9.2 and 9.3, *m/z* 359.1073, fragmented in the MS^2 experiments giving major product ions at *m/z* 197, 182 and 153, suggesting the presence of a syringic acid residue. The loss of 162 amu, proved by MS^2 fragment ion at *m/z* 197, prompt us to tentatively identify this compound as syringic acid-4-*O*-hexoside.

3.1.6. Curcuminoids

From the extracts of turmeric dark chocolate sample, we detected the $[M–H]^-$ precursor ions at *m/z* 307.1043, 337.1164, and 367.1257 (compounds 36.1, 36.2 and 36.3). As reported by Jiang, Somogyi, Jacobsen, Timmermann, & Gang (2006), product ions at *m/z* 187 or 217 were the typical fragment ions in the MS^2 spectra of deprotonated $[M–H]^-$ curcuminoids. In comparison to fragmentation pattern proposed, compounds 36.1, 36.2 and 36.3 were tentatively identified as bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively (**Table 1**).

3.2. Phenolic compounds in chocolate

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

3.2.1. Dark chocolate (DC) phenolic profile

As determined by LC-MS/MS experiments, the total phenolic concentration in DC was 787.63 ± 10.90 mg/100 g of chocolate, representing about 30.0% of total phenolic compounds determined with the Folin-Ciocalteau assay (2624.15 ± 112.36 mg/100 g of chocolate). The ABTS radical scavenging and Fe^{3+}-reducing ability of DC (**Figure 4**) were tested (11.00 ± 0.26 and 6.29 ± 0.13

mmol trolox equivalents/100 g of chocolate, respectively) resulting in line with the findings proposed by Batista et al. (2016). As reported by Wollgast & Anklam (2000a), catechins and procyanidins represent more than 90% of phenolic profile of cocoa beans and cocoa-products. We found out that total flavan-3-ols amount in DC was 503.76 ± 8.98 mg/100 g of chocolate representing the 64.0% of total polyphenols identified by MS experiments. Considering monomeric structures, epicatechin and catechin were the major represented flavan-3-ols, whose estimated concentrations were higher than those reported so far (Gu et al., 2006). Epicatechin alone represented the 40.4 % of total flavan-3-ols and the 25.8% of total phenolic identified by MS experiments, resulting the most present compounds in DC. Large amounts of oligomeric structures were also found, displaying a total concentration value of 166.28± 4.13 mg/100 g of chocolate and reaching approximately 33.0% of flavan-3-ols class. Epicatechin has been causally linked to the reported cardiovascular effects observed after the consumption of cocoa (Schroeter et al., 2006). The ingestion of flavanol-rich cocoa in healthy adult males was associated with acute elevations in levels of circulating nitric oxide, an enhanced flow-mediated dilation response of conduit arteries, and an augmented microcirculation in humans and the results were repeatable with pure epicatechin intake (70 mg/day; equivalent to 35-40 g of DC). Indeed, elderly men with a median epicatechin intake of 22 mg/day (equivalent to 10-15 g of DC) had a 38% lower risk of cardiovascular disease mortality than that of subjects with a median intake of 8 mg/day (Dower, Geleijnse, Hollman, Soedamah-Muthu, & Kromhout, 2016).

The hydroxycinnamic acids made up about 20.6% of DC phenolic profile, among these ferulic acid, di-hydroxycinnamic aspartate and coumaroyl aspartate were the main hydroxycinnamic acids detected in DC. The largest contribution was given by ferulic acid, with a concentration of 61.23 ± 3.74 mg/100 g of chocolate. Among the *N*-phenylpropenoyl-L-amino acids, clovamide or caffeoyl-tyrosine, described for the first time in cocoa by Sanbongi et al. (1998), was the main representative 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-Ciocalteau 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 epigallocatechin gallate, showing a remarkable concentration value of 33.54 ± 2.16 mg/100 g of chocolate. The hydroxycinnamic acids were still the second most representative class of phenolic profile in GTDC, explaining about 15.3% of GTDC phenolic profile (**Figure 2**). Ellagitannins showed a significant content increasing in GTDC respect to DC (89.12 ± 1.50 mg/100 g of chocolate, *P value* <0.001) with an incidence rate of 8.6%. These results may confirm a possible polyphenols enrichment of dark chocolate profile which can lead to a potential combination of the positive health effects and properties derived from both chocolate and green tea. LC-MS experiments showed that GTDC contained 49% more epicatechin and 43% more flavan-3-ols than DC. This can result in a lower intake to achieve the same biological effects. This seems a promising way to maximise the potential beneficial effect of epicatechin consumption, contemporaneously reducing the amount of sugars and calories introduced with chocolate.

3.2.3. Turmeric dark chocolate (TDC) phenolic profile

The TDC phenolic amount showed a significant increase (*P value* <0.001) respect to that of DC which recorded a total concentration value of 1094.03 ± 10.15 mg/100 g of chocolate (**Figure 3**), representing about 36% of total phenolic compounds assayed with the Folin-Ciocalteau method (3043.81 ± 294.64 mg/100 g of chocolate). Despite that, single phenolic classes did not show a significant and remarkable increase respect to those of DC. This higher concentration can be ascribed to turmeric powder contribution as well as the related curcuminoids, which accounted for about 25% of TDC total phenolic profile, displaying a concentration value of 272.73 ± 2.58 mg/100 g of chocolate (**Figure 3**). ABTS radical scavenging ability and ferric-reducing power were tested, resulting in 12.30 ± 0.27 and 10.57 ± 0.2 mmol trolox equivalents/100 g of chocolate, respectively (**Figure 4**). Bisdemethoxycurcumin was the most concentrated curcuminoid (115.55 ± 2.16 mg/100 g of chocolate), followed by demethoxycurcumin (82.64 ± 1.33 mg/100 g of chocolate) which are considered to be curcumin natural analogues and were reported to have a similar biological activity to curcumin itself (Kocaadam, & Şanlier, 2017). Curcumin was found at the lowest concentration of 74.55 ± 0.47 mg/100 g of chocolate. Normally, curcumin is present at a concentration higher or similar to the demethoxylated analogue (Jayaprakasha, Rhao, & Sakariah, 2002). Since the phenolic composition of spices (and of vegetable food in general) is greatly variable depending on the cultivar and agro-climatic factors (such as growing, harvesting time, seasonal variability) as well as technological processes, it is plausible that different turmeric powder preparation had different phenolic composition. Moreover, in the case of dark chocolate enriched with turmeric powder a possible food matrix effect should be considered since some macromolecules such as proteins and polysaccharide may interact with curcuminoids reducing their extractability. It is important also to note that only free and extractable phenolic compounds were considered and analysed in this study. Curcuminoids are widely known for their healthy properties such as anti-inflammatory, antioxidant, antimicrobial, anticoagulant, anticancer and antimutagenic properties (Kocaadam, & Şanlier, 2017; Kunnumakkara et al., 2017). To date, over 100 different clinical trials have been successfully carried out, showing their safety, tolerability and effectiveness against several chronic diseases in humans such as various types of cancers, diabetes, obesity, cardiovascular and neurological diseases (Kunnumakkara et al., 2017). Finally, the synergistic behaviour displayed by curcuminoids with other nutraceuticals such as catechins and quercetin, resulting an increased effect against oxidative stress in normal healthy adults, was demonstrated (Dominiak, McKinney, Heilbrun, & Sarkar, 2010). Therefore, an enhanced and strengthened health effect because of the union of polyphenol-rich sources, combining the positive effects of dark chocolate phenolics and turmeric curcuminoids, can be speculated.

4. Conclusions

Literature provides a lot of information about cocoa polyphenols and properties, but there is still a big gap about the phenolic composition of chocolate. Few studies investigated the phenolic composition of dark chocolate, focusing on flavan-3-ols as the major class in chocolate phenolic profile. The purpose of this study was to overcome this lack of information, providing an accurate and comprehensive characterisation of the phenolic profile of dark chocolate (70%). The quantitative metabolomics approach used in this study allowed a tentative identification of 158 individual phenolic compounds in dark chocolate. Among the detected compounds, 67 have been reported for the first time in dark chocolate, 38 of whom were identified for the first time in chocolate, cocoa beans and cocoa products. This characterization extends the current knowledge on the phytochemistry of dark chocolate and is, to our knowledge, the broadest profiling of its phenolic compounds to date.

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

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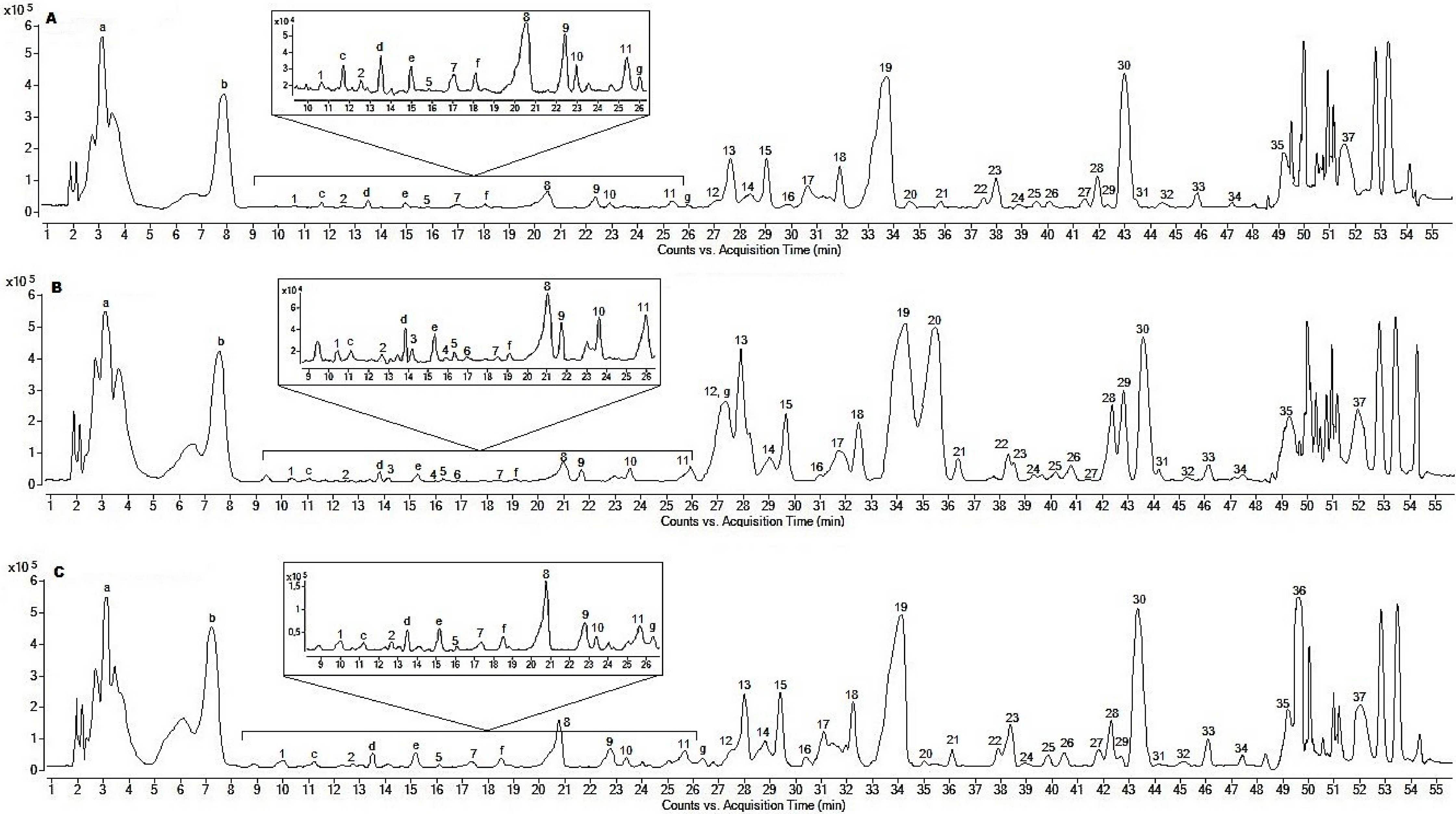
Figure captions

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 ± SD. Values in the same graph with different lowercase letter are significantly different ($P < 0.05$).


A
x10 5
a
b
c
d
e
f
g
1 2 5 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 37
x10 4
Counts vs. Acquisition Time (min)
B
x10 5
12, g
1 2 3 4 5 6 7 8 9 10 11 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 37
x10 4
Counts vs. Acquisition Time (min)
C
x10 5
1 2 5 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
x10 5
Counts vs. Acquisition Time (min)

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

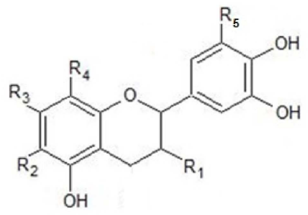
C	**Protocatechuic acid-4-*O*-hexoside**	**Syringic acid-4-*O*-hexoside isomer**	**Di-hydro-coumaric acid**	**Di-hydro-caffeic acid**	**Ferulic acid-4-*O*-pentoside**
R1	-COOH	-COOH	-CH_2-CH_2-COOH	-CH_2-CH_2-COOH	-CH=CH-COOH
R2	-OH	-OCH_3	-H	-OH	-OCH_3
R3	-*O*-hex	-*O*-hex	-OH	-OH	-*O*-pent
R4	-H	-OCH_3	-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

A



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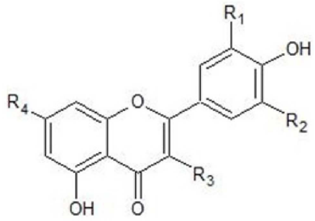

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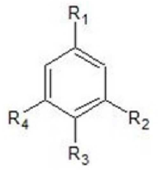
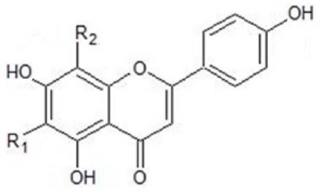


D



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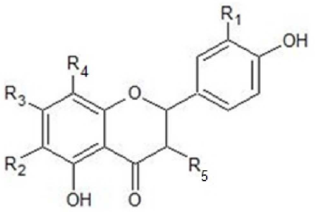
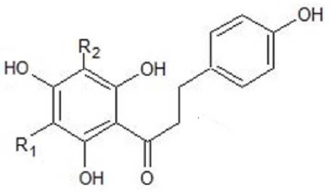
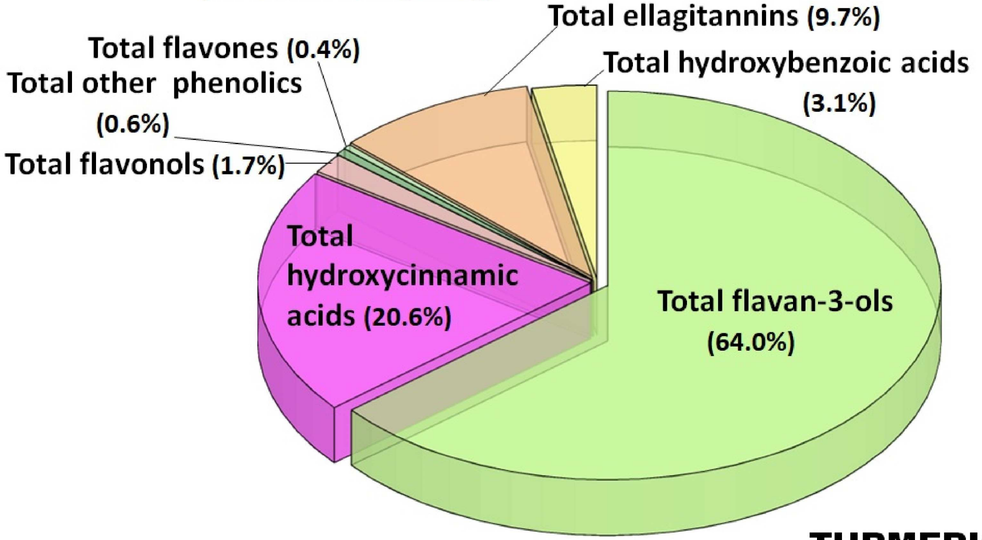


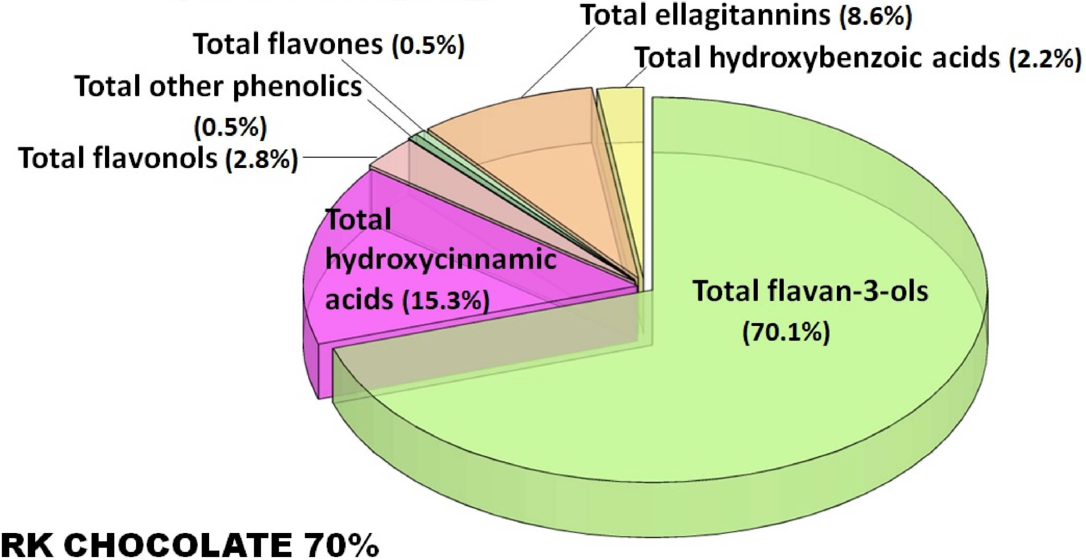
F



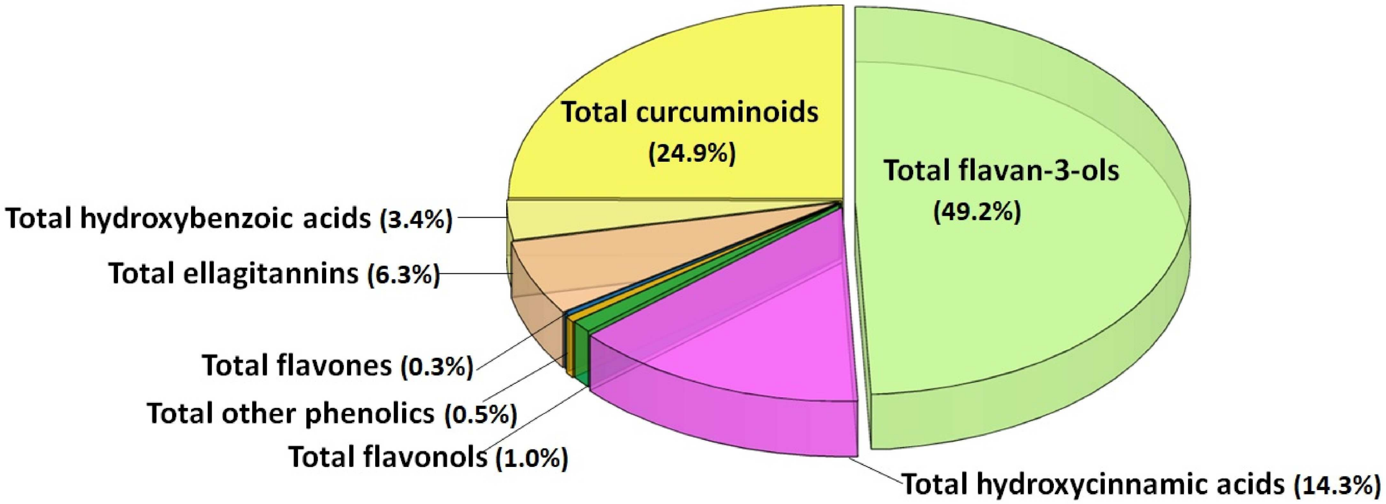
DARK CHOCOLATE 70%
(787.6 ± 10.9 mg/100g)



GREEN TEA DARK CHOCOLATE 70%
(1035.4 ± 14.8 mg/100g)



TURMERIC DARK CHOCOLATE 70%
(1094.0 ± 10.1 mg/100g)



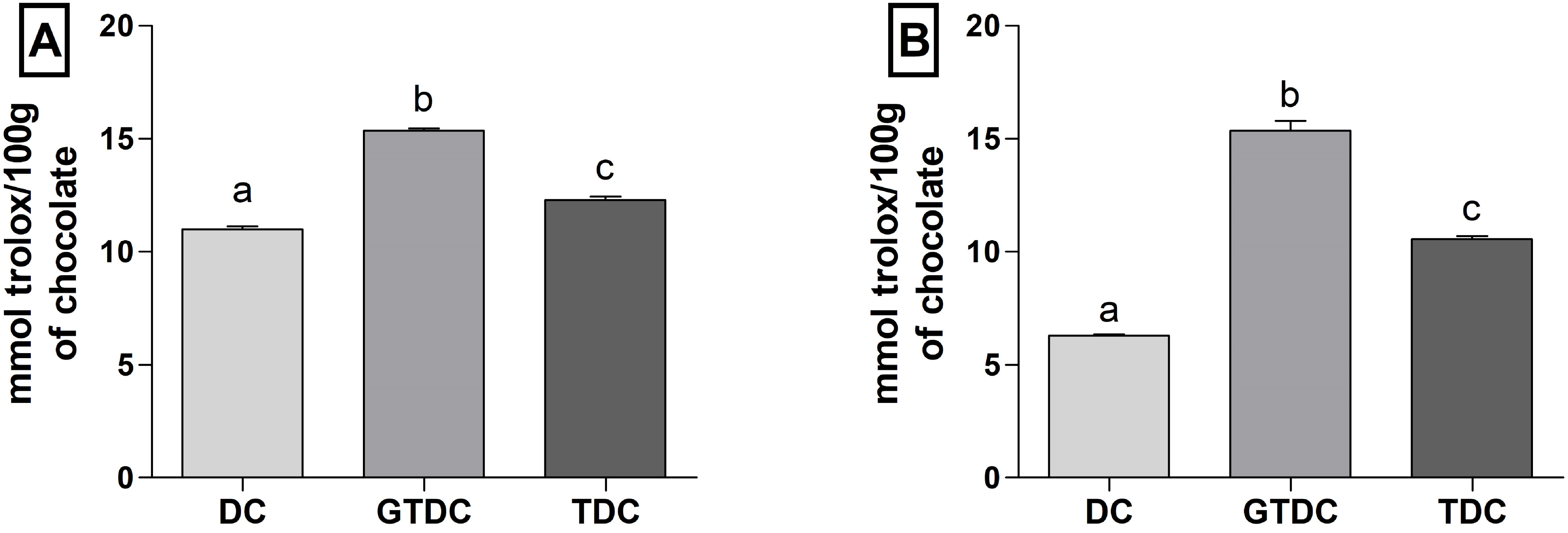

A
mmol trolox/100g of chocolate
20
15
10
5
0
a
b
c
DC
GTDC
TDC
B
mmol trolox/100g of chocolate
20
15
10
5
0
a
b
c
DC
GTDC
TDC

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

Peak	**Compound**		**Formula**	**Calc. *(m/z)***	**Exp. *(m/z)***	**Error (ppm)**	**MS^2 ion fragments *(m/z)* (relative abundance in %)**
1	1.1	Coumaric acid[s]	$C_9H_8O_3$	163.0473	163.0466	4.54	119.0492 (100), 93.0435 (5)
2	2.1	Di-hydro-caffeic acid	$C_9H_{10}O_4$	181.0579	181.0575	2.25	137.0234 (100)
	2.2	Hydroxybenzoic acid isomer	$C_7H_6O_3$	137.0317	137.0316	0.09	93.0315 (100)
3	3.1	Gallic acid[a,s]	$C_7H_6O_5$	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-*O*-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	$C_7H_6O_3$	137.0317	137.0316	0.09	93.0390 (100)
8	8.1	Vanillic acid-4-*O*-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	$C_{15}H_{14}N_2O_9$	365.0699	365.0690	2.54	167.0319 (100), 123.0463 (14)
	8.3	Vanillic acid isomer	$C_8H_8O_4$	167.0423	167.0417	3.33	151.0067 (100), 123.0479 (24)
	8.4	Protocatechuic acid-4-*O*-hexoside	$C_{13}H_{16}O_9$	315.0794	315.0793	0.42	153.0183, 109.0270
	8.5	Di-hydroxycinnamic acid isomer	$C_9H_8O_4$	179.0423	179.0431	-4.67	163.0253 (5), 135.0475 (100)
	8.6	Protocatechuic acid[s]	$C_7H_6O_4$	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-*O*-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-*O*-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	$C_9H_{10}O_5$	197.0528	197.0532	-0.38	182.0320 (100), 167.0073 (30), 153.0246 (13)
10	10.1	Gallocatechin-3-*O*-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	$C_9H_8O_4$	179.0423	179.0431	-4.67	163.0348 (10), 135.0426 (100)
11	11.1	Epigallocatechin-3-*O*-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	$C_7H_6O_3$	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-*O*-Coumaroylquinic acid *cis*[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-*O*-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]	$C_9H_8O_4$	179.0423	179.0431	-4.67	163.0244 (8), 135.0398 (100)
	13.4	Hydroxybenzoic acid isomer	$C_7H_6O_3$	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-*C*-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-*O*-Coumaroylquinic acid *trans*[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-*O*-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-*C*-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	$C_8H_8O_3$	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	$C_8H_8O_4$	167.0423	167.0417	3.33	151.0050 (30), 123.0471 (100)
	16.7	Vanillic acid-4-*O*-hexoside isomer	$C_{14}H_{18}O_9$	329.0951	329.0967	-4.90	167.0473 (100), 123.0524 (6)
	16.8	5-*O*-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-*O*-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-*O*-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-*O*-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-*O*-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	$C_9H_8O_4$	179.0423	179.0431	-4.67	163.0337 (11), 135.0453 (100)
	19.2	Eriodictyol-*C*-hexoside-7-*O*-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-*C*-hexoside-7-*O*-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-*O*-Coumaroylquinic acid *cis*[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-*C*-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	$C_9H_8O_4$	179.0423	179.0431	-4.67	163.0277 (10), 135.0421 (100)
	19.11	Hydroxybenzoic acid isomer	$C_7H_6O_3$	137.0317	137.0316	0.09	93.0336 (100)
	19.12	Apigenin-6,8-di-*C*-hexoside isomer	$C_{27}H_{30}O_{15}$	593.1585	593.1591	-1.06	473.0960 (100), 383.0630 (61), 353.0540 (54), 149.0.316 (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-*O*-Coumaroylquinic acid *trans*[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-*C*-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-*O*-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),

							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	$C_{15}H_{14}O_9S$	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	$C_9H_{10}O_3$	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),

		tyrosine) isomer					161.0339 (43), 135.0517 (54)
	23.6	(Epi)catechin-3-*O*-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-*O*-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-*O*-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-*O*-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-*C*-hexoside-2''-*O*-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-*O*-hexoside-3-*O*-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-*O*-sulphate isomer	$C_{15}H_{14}O_9S$	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-*O*-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-*O*-hexoside-3-*O*-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),

		hexoside					300.0047 (46), 229.0317 (11)
	27.2	Ferulic acid-4-*O*-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-*C*-hexoside-2''-*O*-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-*O*-rhamnoside-3-*O*-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-*O*-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-*O*-gallate-7-*O*-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-*C*-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-*O*-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-*O*-hexoside-3-*O*-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-*O*-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-*O*-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-*O*-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-*O*-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-*O*-hexoside-3-*O*-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-*O*-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),

							179.0048 (5), 151.0086 (5)
	31.4	Kaempferol-7-*O*-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-*O*-rhamnoside-3-*O*-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-*C*-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-*O*-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-*O*-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-*O*-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-*O*-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-*O*-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-*O*-gallate-7-*O*-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. **s** 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 ± standard deviation of triplicate determination.

Compound		Dark chocolate	Green tea dark chocolate	Turmeric dark chocolate
		<u>Flavan-3-ols</u>		
15.2	Catechin	66.20 ± 1.99 ^{a}	69.62 ± 5.28 ^{a}	71.13 ± 2.15 ^{a}
19.9	Epicatechin	203.29 ± 10.68 ^{a}	303.69 ± 11.65 ^{b}	218.43 ± 8.08 ^{a}
9.1	Gallocatechin	1.88 ± 0.10 ^{a}	2.09 ± 0.14 ^{a}	< l.o.q. ^{b}
13.2	Epigallocatechin	15.93 ± 0.10 ^{a}	29.76 ± 1.74 ^{b}	15.11 ± 0.08 ^{a}
22.6	(Epi)catechin-*O*-sulphate isomer	2.06 ± 0.13 ^{a}	1.73 ± 0.20 ^{a}	3.07 ± 0.31 ^{b}
25.3	(Epi)catechin-*O*-sulphate isomer	7.15 ± 0.35 ^{a}	7.16 ± 0.12 ^{a}	10.23 ± 0.83 ^{b}
23.6	(Epi)catechin-3-*O*-trihydroxybenzene	4.92 ± 0.50 ^{a}	4.66 ± 0.10 ^{b}	5.94 ± 0.56 ^{ab}
26.2	(Epi)catechin-3-*O*- trihydroxybenzene	3.61 ± 0.06 ^{a}	4.58 ± 0.26 ^{b}	4.84 ± 0.42 ^{b}
27.5	(Epi)catechin-7-*O*-trihydroxybenzene	2.60 ± 0.15 ^{a}	3.83 ± 0.56 ^{b}	3.39 ± 0.14 ^{ab}
20.3	(Epi)catechin-*C*-pentoside isomer	0.42 ± 0.05 ^{a}	0.30 ± 0.07 ^{a}	0.83 ± 0.11 ^{a}
28.3	Catechin-3-*O*-gallate *	n.d.	< l.o.q. ^{a}	n.d.
29.5	Epicatechin-3-*O*-gallate *	n.d.	9.12 ± 0.13 ^{a}	n.d.
13.1	(Epi)catechin-3-*O*-hexoside isomer	12.64 ± 0.20 ^{a}	9.78 ± 1.44 ^{a}	13.51 ± 0.82 ^{a}
14.1	(Epi)catechin-*C*-hexoside isomer	1.91 ± 0.04 ^{a}	1.62 ± 0.10 ^{a}	1.32 ± 0.19 ^{a}
15.1	(Epi)catechin-7-*O*-hexoside	4.11 ± 0.29 ^{a}	4.26 ± 0.30 ^{a}	5.24 ± 0.43 ^{a}
16.2	(Epi)catechin-*C*-hexoside isomer	4.09 ± 0.21 ^{a}	3.48 ± 0.80 ^{a}	3.72 ± 0.01 ^{a}
21.4	(Epi)catechin-3-*O*-hexoside isomer	1.36 ± 0.21 ^{a}	1.81 ± 0.22 ^{a}	1.48 ± 0.08 ^{a}
20.6	Epigallocatechin-3-*O*-gallate *	n.d.	33.54 ± 2.16 ^{a}	n.d.
10.1	Gallocatechin-3-*O*-hexoside	0.09 ± 0.01 ^{a}	0.06 ± 0.01 ^{a}	0.15 ± 0.01 ^{a}
11.1	Epigallocatechin-3-*O*-hexoside	0.09 ± 0.01 ^{a}	0.07 ± 0.01 ^{a}	0.17 ± 0.01 ^{a}
34.1	Procyanidin dimer A type	1.10 ± 0.06 ^{a}	1.31 ± 0.08 ^{a}	1.21 ± 0.07 ^{a}
12.1	Procyanidin dimer B type isomer	8.65 ± 0.84 ^{a}	8.39 ± 0.34 ^{a}	10.59 ± 1.48 ^{a}
17.1	Procyanidin dimer B type isomer	3.05 ± 0.22 ^{a}	2.69 ± 0.14 ^{a}	3.62 ± 0.19 ^{a}
18.3	Procyanidin dimer B type isomer	37.23 ± 3.44 ^{a}	52.45 ± 5.44 ^{b}	35.12 ± 0.04 ^{a}
18.9	Procyanidin dimer B type isomer	34.08 ± 1.98 ^{a}	44.99 ± 1.15 ^{b}	38.25 ± 2.76 ^{a}
23.1	Procyanidin dimer B type isomer	5.32 ± 0.30 ^{a}	6.66 ± 0.64 ^{a}	6.93 ± 0.74 ^{a}
23.3	Procyanidin dimer B type isomer	10.17 ± 0.13 ^{a}	12.35 ± 0.35 ^{a}	13.12 ± 0.89 ^{a}
12.2	(Epi)catechin-(Epi)gallocatechin *	n.d.	0.12 ± 0.01 ^{a}	n.d.
18.5	(Epi)catechin-3-*O*-dihexoside isomer	0.66 ± 0.04 ^{a}	0.73 ± 0.09 ^{a}	0.44 ± 0.03 ^{a}
28.1	(Epi)catechin-3-*O*-dihexoside isomer	1.65 ± 0.02 ^{a}	1.04 ± 0.51 ^{a}	1.34 ± 0.03 ^{a}
27.6	(Epi)catechin-3-*O*-gallate-7-*O*-glucuronide isomer	0.33 ± 0.01 ^{a}	0.18 ± 0.01 ^{a}	0.24 ± 0.03 ^{a}
34.6	(Epi)catechin-3-*O*- gallate-7-*O*-glucuronide isomer	0.31 ± 0.02 ^{a}	0.14 ± 0.01 ^{a}	0.23 ± 0.02 ^{a}
20.5	(Epi)catechin derivative isomer	1.16 ± 0.04 ^{a}	0.93 ± 0.10 ^{a}	1.30 ± 0.14 ^{a}
21.1	(Epi)catechin derivative isomer	1.02 ± 0.04 ^{a}	1.06 ± 0.04 ^{a}	0.77 ± 0.05 ^{a}
23.7	Procyanidin dimer A type pentoside isomer	1.42 ± 0.01 ^{a}	1.29 ± 0.24 ^{a}	1.68 ± 0.04 ^{a}
24.3	Procyanidin dimer A type pentoside isomer	1.09 ± 0.01 ^{a}	1.17 ± 0.08 ^{a}	1.04 ± 0.04 ^{a}
31.5	Procyanidin dimer A type pentoside isomer	1.75 ± 0.03 ^{a}	2.24 ± 0.21 ^{b}	1.82 ± 0.11 ^{ab}
32.2	Procyanidin dimer A type pentoside	1.47 ± 0.11 ^{a}	2.74 ± 0.11 ^{b}	1.20 ± 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}
	Total flavan-3-ols	**503.76 ± 8.98** ^{a}	**726.03 ± 14.53** ^{b}	**538.71 ± 8.99** ^{c}
	Hydroxycinnamic acids			
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-*O*-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 *cis* *	n.d.	1.51 ± 0.01 ^{a}	n.d.
14.3	3-Coumaroylquinic acid *trans* *	n.d.	0.68 ± 0.01 ^{a}	n.d.
19.4	4-Coumaroylquinic acid *cis* *	n.d.	2.05 ± 0.10 ^{a}	n.d.
20.2	4-Coumaroylquinic acid *trans* *	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}
	Total hydroxycinnamic acids	**161.95 ± 4.80** ^{a}	**157.98 ± 2.38** ^{a}	**156.51 ± 1.62** ^{a}
		Flavonols		
35.1	Quercetin	1.08 ± 0.01 ^{a}	1.62 ± 0.03 ^{b}	1.37 ± 0.01 ^{a}
33.2	Quercetin-3-*O*-pentoside	3.31 ± 0.21 ^{a}	2.80 ± 0.14 ^{a}	4.15 ± 0.40 ^{b}
31.4	Kaempferol-7-*O*-hexoside	0.14 ± 0.01 ^{a}	0.24 ± 0.01 ^{b}	0.15 ± 0.01 ^{a}
32.4	Kaempferol-3-*O*-galactoside	0.04 ± 0.01 ^{a}	0.28 ± 0.01 ^{b}	< l.o.q. ^{c}
34.3	Kaempferol-3-*O*-glucoside	0.05 ± 0.01 ^{a}	0.37 ± 0.03 ^{b}	< l.o.q. ^{c}
34.5	Quercetin-3-*O*-rhamnoside	0.05 ± 0.01 ^{a}	0.50 ± 0.02 ^{b}	< l.o.q. ^{c}
18.6	Dihydro-kaempferol-7-*O*-hexoside	0.25 ± 0.01 ^{a}	0.21 ± 0.01 ^{a}	0.23 ± 0.01 ^{a}
30.1	Quercetin-3-*O*-galactoside	0.49 ± 0.01 ^{a}	1.67 ± 0.01 ^{b}	2.44 ± 0.04 ^{c}
31.3	Quercetin-3-*O*-glucoside	1.96 ± 0.02 ^{a}	2.29 ± 0.07 ^{a}	3.05 ± 0.27 ^{b}
24.5	Myricetin-3-*O*-galattoside	0.30 ± 0.01 ^{a}	2.35 ± 0.04 ^{b}	< l.o.q. ^{c}
24.8	Myricetin-3-*O*-glucoside	0.46 ± 0.01 ^{a}	1.84 ± 0.14 ^{b}	< l.o.q. ^{c}
33.1	Kaempferol-3-*O*-rutinoside	0.26 ± 0.01 ^{a}	0.65 ± 0.02 ^{b}	< l.o.q. ^{c}
28.4	Quercetin-3-*O*-rutinoside *	n.d.	4.20 ± 0.19 ^{a}	n.d.
23.9	Myricetin-3-*O*-rutinoside *	n.d.	0.58 ± 0.01 ^{a}	n.d.
22.3	Myricetin-3-*O*-(*O*-galloyl) hexoside	0.11 ± 0.01 ^{a}	0.62 ± 0.02 ^{b}	< l.o.q. ^{c}
31.1	Kaempferol-7-*O*-rhamnoside-3-*O*-rutinoside	0.03 ± 0.01 ^{a}	0.09 ± 0.01 ^{a}	< l.o.q. ^{c}
27.4	Quercetin-7-*O*-rhamnoside-3-*O*-rutinoside	0.14 ± 0.01 ^{a}	0.20 ± 0.02 ^{a}	< l.o.q. ^{b}
28.2	Kaempferol-7-*O*-hexoside-3-*O*-rutinoside isomer	0.57 ± 0.01 ^{a}	1.27 ± 0.02 ^{b}	< l.o.q. ^{c}
31.2	Kaempferol-7-*O*-hexoside-3-*O*-rutinoside isomer	0.44 ± 0.01 ^{a}	1.38 ± 0.01 ^{b}	< l.o.q. ^{c}
25.2	Quercetin-7-*O*-hexoside-3-*O*-rutinoside isomer	1.12 ± 0.01 ^{a}	2.24 ± 0.01 ^{b}	< l.o.q. ^{c}
26.3	Quercetin-7-*O*-hexoside-3-*O*-rutinoside	2.09 ± 0.01 ^{a}	3.65 ± 0.26 ^{b}	< l.o.q. ^{c}

	isomer			
22.4	Myricetin-7-*O*-hexoside-3-*O*-rutinoside	0.12 ± 0.01 ^{a}	0.23 ± 0.02 ^{b}	< l.o.q. ^{c}
	Total flavonols	**13.01 ± 0.21 ^{a}**	**29.28 ± 0.39 ^{b}**	**11.40 ± 0.48 ^{a}**
	Other phenolics			
16.4	Vanillin	1.33 ± 0.05 ^{a}	1.81 ± 0.11 ^{b}	1.85 ± 0.08 ^{b}
32.3	Phloretin-*C*-hexoside isomer	0.15 ± 0.01 ^{a}	0.16 ± 0.01 ^{a}	0.63 ± 0.03 ^{b}
18.4	Eriodictyol-7-*O*-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-*C*-hexoside-7-*O*-hexoside isomer	0.23 ± 0.01 ^{a}	0.63 ± 0.02 ^{b}	< l.o.q. ^{c}
19.2	Eriodictyol-*C*-hexoside-7-*O*-hexoside isomer	0.15 ± 0.01 ^{a}	0.28 ± 0.01 ^{b}	n.d.
	Total other phenolics	**4.54 ± 0.69 ^{a}**	**4.99 ± 0.11 ^{a}**	**5.85 ± 0.10 ^{b}**
	Flavones			
27.7	Apigenin-*C*-hexoside isomer	0.10 ± 0.01 ^{a}	0.42 ± 0.01 ^{b}	0.19 ± 0.01 ^{a}
22.10	Apigenin-*C*-hexoside-*C*-pentoside isomer	0.95 ± 0.01 ^{a}	2.11 ± 0.07 ^{b}	0.83 ± 0.03 ^{a}
27.3	Apigenin-*C*-hexoside-2''-*O*-rhamnoside isomer	1.31 ± 0.32 ^{ab}	0.89 ± 0.13 ^{a}	1.71 ± 0.43 ^{b}
19.8	Apigenin-6,8-*di*-*C*-hexoside isomer	0.17 ± 0.01 ^{a}	0.47 ± 0.01 ^{b}	0.23 ± 0.01 ^{a}
19.12	Apigenin-6,8-*di*-*C*-hexoside isomer	0.20 ± 0.01 ^{a}	0.54 ± 0.01 ^{b}	0.33 ± 0.01 ^{a}
25.1	Apigenin-*C*-hexoside-2''-*O*-hexoside isomer	0.25 ± 0.01 ^{a}	0.47 ± 0.01 ^{b}	n.d.
	Total flavones	**2.98 ± 0.32 ^{a}**	**4.90 ± 0.15 ^{b}**	**3.29 ± 0.43 ^{a}**
	Ellagitannins			
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}
	Total ellagitannins	**76.60 ± 3.78 ^{a}**	**89.12 ± 1.50 ^{b}**	**68.70 ± 3.37 ^{a}**
	Hydroxybenzoic acids			
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-*O*-hexoside	0.10 ± 0.01 ^{a}	0.31 ± 0.03 ^{b}	0.19 ± 0.01 ^{a}
5.1	Vanillic acid-4-*O*-hexoside isomer	0.25 ± 0.01 ^{a}	0.20 ± 0.02 ^{a}	0.30 ± 0.01 ^{a}
8.1	Vanillic acid-4-*O*-hexoside isomer	1.77 ± 0.06 ^{a}	1.67 ± 0.04 ^{a}	2.63 ± 0.01 ^{b}
16.7	Vanillic acid-4-*O*-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-*O*-hexoside isomer	0.94 ± 0.08 ^{a}	0.81 ± 0.04 ^{a}	1.07 ± 0.11 ^{a}
9.3	Syringic acid-4-*O*-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}
	Total hydroxybenzoic acids	**24.80 ± 0.33** ^{a}	**23.05 ± 0.20** ^{a}	**36.82 ± 1.08** ^{b}
	Curcuminoids			
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}
	Total curcuminoids	**n.d.**	**n.d.**	**272.73 ± 2.58** ^{a}
	Total phenolic compounds	**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**.