

Determining the true content of quercetin and its derivatives in plants employing SSDM and LC–MS analysis

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Abstract Reliable plant analysis is a challenging task due to the physical character and chemical complexity of plant matrices. First of all, it requires the application of a proper sample preparation procedure to fully isolate the analyzed substances from the plant matrix. The high-temperature liquid–solid extraction is commonly applied for this purpose. In the light of recently published results, however, the application of high-temperature extraction for polyphenolics analysis in plants is disputable as it causes their transformation leading to erroneous quantitative estimations of these compounds. Experiments performed on different plants show that the transformation/degradation of quercetin and its glycosides is not induced by sea sand disruption method (SSDM) and prove the method to be most appropriate for the estimation of quercetin and its derivatives in plants. What is more, the application of SSDM in plant analysis allows the researcher, to determine which quercetin derivatives are native plant components and what is their true concentration. In other word, the application of SSDM in plant analysis eliminates errors in the study of plant metabolism involving quercetin and its derivatives.

Keywords Sea sand disruption method · Quercetin derivatives · Rutin transformation · Compound degradation · Plant analysis · Sample preparation

Introduction

Quercetin is one of the most widely distributed polyphenolics in plants. This aglycone compound occurs in fruits, vegetables, leaves and grains, often in the form of glycoside derivatives. Rutin (quercetin-3-*O*-rutinoside), isoquercitrin (quercetin-3-*O*-glucoside) and quercitrin (quercetin-3-*O*-rhamnoside) are the most ubiquitous quercetin glycosides [1]. In view of the antioxidant, anti-inflammatory and anti-cancer properties of quercetin and its glycosides, research interest in the natural occurrence and medical properties of these compounds has been growing [2–4].

Reliable plant analysis is a challenging task due to the physical character and chemical complexity of plant matrices. First of all, it requires the application of a proper sample preparation procedure to fully isolate the analyzed substances from the plant matrix. The high-temperature liquid–solid extraction is commonly applied for this purpose. Yet, the results reported in the literature [5–8] reveal that the high-temperature extraction of polyphenolics with methanol and its water mixtures, i.e. the extractants typically used for the isolation of phenolics from plants, not only causes the hydrolysis of glycosides but also results in the formation of alcoholic derivatives of glycosides and aglycones, and in degradation of the latter. In the light of these findings, the application of high-temperature extraction as a sample preparation technique for polyphenolics analysis in plants is disputable and makes the results obtained for a given plant unreliable. These doubts are justified by the results presented in our earlier work [9] showing that at least 23 compounds are formed from rutin, the most abundant quercetin glycoside, during its extraction under reflux.

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Recently, research work has been focused on sample preparation methods which would limit or even eliminate the degradation/transformation of the analyzed plant constituents. One of such method is the sea sand disruption method (SSDM) combining the homogenization, extraction and purification processes into a single step [8, 10]. There are many examples showing that the effectiveness of this simple, quick and cheap low-temperature method is an alternative not only to the traditional high-temperature solvent extractions (under reflux and in the Soxhlet apparatus) but also to the supported ones (pressurized liquid extraction, supercritical fluid extraction, ultrasound-assisted solvent extraction and microwave-assisted solvent extraction) [10–13].

This paper presents and discusses the results of research work on the application of SSDM for the evaluation of the true content of quercetin and its derivatives in the following plants: flowers of black elder (*Sambucus nigra* L.) and hawthorn (*Crataegus* L.); leaves of green tea, nettle (*Urtica dioica* L.) and yerba maté (*Ilex paraguariensis* A.St.-Hil.); the heartsease herb (*Viola tricolor* Linn.), St John's wort (*Hypericum perforatum* L.), and artichoke (*Cynara cardunculus*) flower buds. The results obtained using SSDM are compared to those revealed by the traditional extraction under reflux.

Materials and methods

Plant material and chemicals

The following plants were used in the experiments: flowers of black elder (*S. nigra* L.) and hawthorn (*Crataegus* L.); leaves of green tea, nettle (*U. dioica* L.) and yerba maté (*I. paraguariensis* A.St.-Hil.); herb of heartsease (*V. tricolor* Linn.) and St John's wort (*H. perforatum* L.), and artichoke (*C. cardunculus*) flower buds. All of them were purchased from a local herbalist's (Lublin, Poland). Before extraction, the plant material was ground, fractionated, and its exactly weighed portions were subjected to the extraction procedure.

Acetonitrile (HPLC), methanol, ethanol (both of analytical grade) were purchased from the Polish Chemical Plant POCh S.A. (Gliwice, Poland). Formic acid, rutin (quercetin-3-*O*-rutinoside), isoquercitrin (quercetin-3-*O*-glucoside), quercitrin (quercetin-3-*O*-rhamnoside) and quercetin were supplied by Sigma-Aldrich (Germany). The sand used as abrasive material in SSDM was a donation from the local glassworks. It was fractionated, leached with 1 M HCl, washed out with distilled water to neutrality, and dried. A 0.2–0.4 mm fraction was applied in the experiments. Water

was purified on the Milli-Q system from Millipore (Millipore, Bedford, MA, USA).

Extraction under reflux

A weighed portion of green tea leaves (2.0 g) or rutin (0.01 g) or quercetin (0.01 g) was heated for 3 h under reflux in methanol(ethanol)/water mixture (75/25 %, v/v). After cooling, the obtained extract was transferred into a 200-mL volumetric flask, filled up to its volume with the extractant mixture and subjected to LC–MS analysis. Extractions under reflux were repeated three times with fresh portions of the material.

Sea sand disruption method

SSDM was performed according to the validated procedure described elsewhere [8, 10]. A 0.2 g sample of ground plant was placed in a glass mortar and mixed with 0.8 g of sand to obtain the most commonly applied mass ratio of 1:4. The mixture, dry or after the application of the same volume (1.0 mL) of methanol or ethanol as dispersing liquids, was blended for 10 min with a glass pestle to obtain a homogenous mixture, the so-called SSDM blend. After homogenization, the SSDM blend was quantitatively transferred to a 5-mL syringe barrel with a paper frit on the bottom. The blend was compressed in the barrel using the syringe plunger and then eluted to a 25-mL calibrated flask using portions of methanol/water or ethanol/water mixtures (75/25 %, v/v). The extraction procedure was repeated five times using fresh portions of plant material.

SSDM of rutin-enriched green tea sample

0.01 g of rutin was added to a 0.2 g sample of ground green tea leaves and mixed with 0.8 g of sand in a glass mortar. The mixture was ground for 10 min, transferred to a 5-mL syringe barrel and eluted to a 25-mL volumetric flask using portions of ethanol/water mixture (75/25 %, v/v).

LC–MS analysis

The chromatographic measurements were taken on a LC–MS system consisting of a UHPLC chromatograph (Ulti-Mate 3000, Dionex, Sunnyvale, CA, USA), a linear trap quadrupole-Orbitrap mass spectrometer (LTQ-Orbitrap Velos from Thermo Fisher Scientific, San Jose, CA) and an ESI source. A Gemini C18 column (4.6 × 100 mm, 3 μm) (Phenomenex, USA) was employed for chromatographic separation performed using gradient elution. Mobile phase A was 25 mM formic acid in water; mobile phase B was

25 mM formic acid in acetonitrile. The gradient program started at 5 % B increasing to 35 % for 60 min, next at 35 % B to 95 % B for 5 min, and ended with isocratic elution (95 % B) lasting 5 min. The total run time was 90 min at the mobile phase flow rate 0.4 mL/min.

In the course of each run, the PDA spectra in the range 200–600 nm and the MS spectra in the range of 100–2000 m/z were collected continuously. The SIM function was used to better visualize the chromatographic separation and to remove the signal from non-examined compounds present in the plant matrix. The time periods and monitored ions were as follows:

0–15 min (197 m/z), 15–16 min (211 m/z), 16–17 min (179 m/z), 17–18 min (305 m/z), 18–19 min (335 m/z), 19–22 min (317 m/z), 22–27 min (331 m/z), 27–29 min (257 m/z), 29–31 min (319 m/z), 31–32 min (345 m/z), 32–37 min (349 m/z), 37–42 min (463 m/z), 42–43 min (609 m/z), 43–45 min (623 m/z), 45–46 min (347 m/z), 46–47 min (447 m/z), 47–51 min (477 m/z), 51–56 min (461 m/z), 56–59 min (475 m/z), 59–63 min (301 m/z), 63–65 min (273 m/z), 65–67 min (361 m/z), 67–71 min (315 m/z), 71–72 min (375 m/z), 72–73 min (287 m/z), 73–90 min (329 m/z).

The column effluent was ionized by electrospray (ESI). ESI was operated in negative polarity modes under the following conditions: spray voltage—3.5 kV; sheath gas—40 arbitrary units; auxiliary gas—10 arbitrary units; sweep gas—10 arbitrary units; capillary temperature—320 °C. Nitrogen (>99.98 %) was employed as sheath, auxiliary and sweep gas. The scan cycle used a full-scan event at the resolution of 60 000.

Due to the lack of standards for methyl/ethyl quercetin derivatives and methyl/ethyl derivatives of quercetin glycosides, the amounts of these compounds were calculated by relating their chromatographic responses to the calibration curves of quercetin and the corresponding quercetin glycosides. For the same reason, the amounts of the low-molecular quercetin derivatives and their methyl derivatives were calculated by relating their chromatographic responses to the calibration curves for quercetin.

Statistical analysis

All data are expressed as the mean of three independent measurements \pm standard deviation. The analysis of variance (ANOVA) and F test were used to assess the influence of experimental factors on the amounts of quercetin and its derivatives. The mean values were considered significantly different when a result of compared parameters differed at $p = 0.05$ significance level. p values were used to check the significance of each Fisher coefficient.

Results and discussion

Green tea extraction under reflux

The exemplary chromatogram of methanol/water (75/25 %, v/v) green tea extract obtained during 3 h heating under reflux is presented in Fig. 1a. It was plotted with the help of the SIM function to show only the concentration zones corresponding to quercetin and its derivatives. As results from the figure, in addition to quercetin (peak 9) and its three main glycoside derivatives—isoquercitrin (peak 5), rutin (peak 6) and quercitrin (peak 8)—the extract contains 16 other compounds, derivatives of quercetin, rutin, isoquercitrin, and quercitrin. They are:

- oxo(2,4,6-trihydroxyphenyl)acetic acid (OTA) (peak 1);
- methyl oxo(2,4,6-trihydroxyphenyl)acetate (Me-OTA) (peak 1');
- 2-[[[(3,4-dihydroxyphenyl)carbonyl]oxy]-4,6-dihydroxybenzoic acid (DDA) (peak 2);
- methyl 2-[[[(3,4-dihydroxyphenyl)carbonyl]oxy]-4,6-dihydroxybenzoate (Me-DDA) (peak 2');
- 2-[carboxy(3,4-dihydroxyphenyl)methoxy]-4,6-dihydroxybenzoic acid (CDA) (peak 3);
- 2-[1-(3,4-dihydroxyphenyl)-2-methoxy-2-oxoethoxy]-4,6-dihydroxybenzoic acid (MeO-CDA) (peak 3');
- 2-[(3,4-dihydroxyphenyl)carbonyl]-2,4,6-trihydroxy-1-benzofuran-3(2*H*)-one (DTB) (peak 4);
- 2-[(3,4-dihydroxyphenyl)carbonyl]-4,6-dihydroxy-2-methoxy-1-benzofuran-3(2*H*)-one (MeO-DTB) (peak 4');
- 2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one (MeO-Isoquercitrin) (peak 5');
- 2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyl-oxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one (MeO-Rutin) (peak 6');
- 3,5-dihydroxy-2-[methoxy(oxo)acetyl]phenyl 3,4-dihydroxybenzoate (DPD) (peak 7);
- 5-hydroxy-3-methoxy-2-[methoxy(oxo)acetyl]phenyl 3,4-dihydroxybenzoate (MeO-DPD) (peak 7');
- 2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2*S*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyl-oxan-2-yl]oxychromen-4-one (MeO-Quercitrin) (peak 8');
- 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one (*iso*-1-MeO-Quercetin) (peak 9'a); (*iso*-2-MeO-Quercetin) (peak 9'b) and (*iso*-3-MeO-Quercetin) (peak 9'c);

Fig. 1 Exemplary chromatograms of methanol/water (75/25 %, v/v) extracts obtained from the green tea leaves (a), solutions of rutin (b) and quercetin (c), all heated under reflux for 3 h. *Peak numbers correspond to compound numbers reported in Table 2*

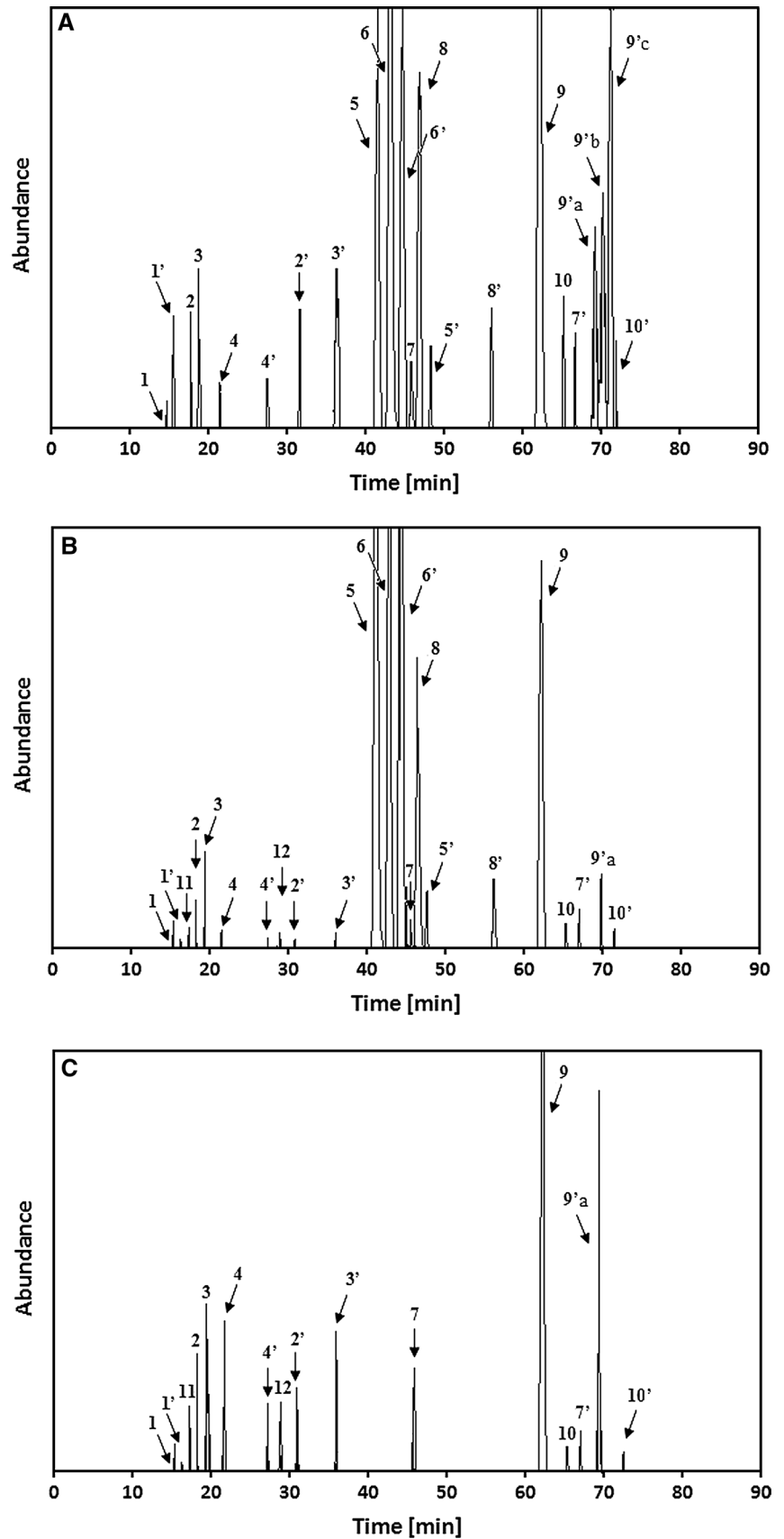


Table 1 Negative ion MSⁿ data for MeO-Quercetin isomers

Peak No.	MS ¹	MS ²			Compound
	Parent ion	Base peak	Secondary peak		
	<i>m/z</i>	<i>m/z</i>	<i>m/z</i>	Intensity (%)	
9'a	315.1	300.1	179.1	27.7	<i>iso</i> -1-MeO-Quercetin
			255.2	19.1	
			283.2	7.9	
9'b	315.1	300.1	179.1	28.1	<i>iso</i> -2-MeO-Quercetin
			255.1	18.7	
			283.2	6.2	
9'c	315.1	300.1	179.1	29.1	<i>iso</i> -3-MeO-Quercetin
			255.2	18.9	
			283.2	7.2	

- 2-(3,4-dihydroxyphenyl)-1-benzofuran-3,4,6-triol (DBT) (peak 10);
- 2-(3,4-dihydroxyphenyl)-3-methoxy-1-benzofuran-4,6-diol (MeO-DBT) (peak 10').

Except for the compounds corresponding to peaks 9'b and 9'c, all others were identified and described earlier in [9]. Three compounds labeled as 9'a, 9'b and 9'c (see Fig. 1a) have the same *m/z* (*m/z* = 315) and UV–Vis spectra. Their MS² spectra are very similar (see Table 1). The analysis of their retention data, MS² and UV–Vis spectra shows that these compounds are structural isomers of methyl derivatives of quercetin (*iso*-1-MeO-Quercetin, *iso*-2-MeO-Quercetin and *iso*-3-MeO-Quercetin).

Comparing the obtained results to those presented in [9], we observed that with the exception of *iso*-2-MeO-Quercetin and *iso*-3-MeO-Quercetin, all other identified compounds were identified as rutin transformation products in the methanol–water rutin solution heated under reflux. It is therefore reasonable to suspect that except for rutin, isoquercitrin and quercitrin, the other quercetin derivatives identified in the green tea extract are not native compounds of the plant, but they are formed during their extraction from quercetin and quercetin glycoside derivatives. To check this supposition and to confirm that *iso*-2-MeO-Quercetin and *iso*-3-MeO-Quercetin are not formed from rutin during its heating under reflux, the extraction process of quercetin and rutin standards was simulated, this time using rutin concentration higher than in [9]. The exemplary chromatograms of rutin and quercetin methanol/water (75/25 %, v/v) solutions heated for 3 h under reflux are presented in Fig. 1b, c, respectively. For easier comparison of the obtained results, the names of all identified compounds, their peaks, structure numbers and shortcuts are collected in Table 2. The chemical structures of all compounds are presented in Fig. 2.

The analysis of the chromatograms presented in Fig. 1a–c and the data listed in Table 2 show that:

- *iso*-2-MeO-Quercetin and *iso*-3-MeO-Quercetin are not formed from quercetin and rutin during heating their solutions under reflux and, in consequence, they can be recognized as native green tea components. This supposition agrees with the literature reports that methyl derivatives of polyphenols can be formed at one of the final biosynthesis stages (in the methylation process catalyzed by methyltransferases) [14, 15];
- the presence of DBD (peak 11) and DBOT (peak 12) in the methanol–water rutin and quercetin extracts and their absence in the green tea extract suggest that they are not native green tea components or that they exist in this plant in very low concentration;
- except for the quercetin glycosides and their methyl derivatives, the rutin standard extract contains exactly the same low-molecular transformation products as those in the quercetin standard extract (OTA, Me-OTA, DBD, DDA, Me-DDA, CDA, MeO-CDA, DTB, MeO-DTB, DBOT, DPD, MeO-DPD, DBT, MeO-DBT). It indicates that these low-molecular rutin transformation products are formed from rutin molecules after their prior hydrolysis to quercetin;
- except for the quercetin glycosides and two isomers of MeO-Quercetin (*iso*-2-MeO-Quercetin and *iso*-3-MeO-Quercetin), all other quercetin derivatives found in the methanol–water green tea extract can be formed from quercetin and its glycosides during their extraction from the plant and, therefore, there is no certainty that they are native green tea components;

In the light of the above, we postulate that the high-temperature extraction with methanolic extractants cannot be used in experiments determining the true content of quercetin and its native derivatives in plants.

SSDM of green tea leaves

Figure 3 presents the exemplary chromatograms of the green tea leaves subjected to the SSDM procedure (Fig. 3a), and the SSDM extracts of rutin and quercetin standards (see Fig. 3b, c, respectively). In these SSDM experiments, methanol was used as a dispersing liquid and a methanol/water mixture (75/25 %, v/v) as an eluent. The lack of peaks corresponding with the quercetin and rutin derivatives in Fig. 3b, c proves that quercetin and rutin do not transform and/or degrade in SSDM and indicates that this sample preparation method can be applied for the analysis of quercetin and its native derivatives in plants. The lower number of the quercetin derivatives in

the SSDM extract of green tea in relation to their number in the green tea extract obtained under reflux (compare Figs. 1a, 3a) additionally supports the conclusion that high-temperature extraction with methanolic extractants promotes the formation of the quercetin derivatives, which are not necessarily native plant components.

Statistical comparison of the data for quercetin derivatives in green tea extracts estimated using SSDM and extraction under reflux

Table 3 collects the concentrations of the quercetin derivatives estimated in green tea leaves using extraction under

reflux and SSDM. Besides methanol/water solution, also ethanol/water solution (75/25 %, v/v) was used in these experiments as extractant for the extraction under reflux and as eluent in the SSDM procedure. Two variants of the SSDM procedure, with and without a dispersing liquid, were applied in the experiments. Methanol and ethanol were used as the dispersing liquids in the first variant of SSDM. In the second, the addition of the dispersing liquid was omitted (the so-called dry (D) SSDM process). These two variants are reflected in the headers of the table. For instance, column Me/Me75 % collects the results obtained using the first variant of the SSDM process, in which methanol was applied as the dispersing liquid and a methanol/

Table 2 Names, shortcuts, and peak or structure numbers of quercetin and its derivatives found in rutin and quercetin methanol/water (75/25 v/v) solutions (RS and QS, respectively) heated for 3 h under reflux

Compounds name	Shortcut	Peak No. ^a	Structure No. ^b	RS	QS
oxo(2,4,6-trihydroxyphenyl)acetic acid	OTA	1	1, 1'	+	+
methyl oxo(2,4,6-trihydroxyphenyl)acetate	Me-OTA	1'		+	+
2-[[[(3,4-dihydroxyphenyl)carbonyl]oxy]-4,6-dihydroxybenzoic acid	DDA	2	2, 2'	+	+
methyl 2-[[[(3,4-dihydroxyphenyl)carbonyl]oxy]-4,6-dihydroxybenzoate	Me-DDA	2'		+	+
2-[carboxy(3,4-dihydroxyphenyl)methoxy]-4,6-dihydroxybenzoic acid	CDA	3	3, 3'	+	+
2-[1-(3,4-dihydroxyphenyl)-2-methoxy-2-oxoethoxy]-4,6-dihydroxybenzoic acid	MeO-CDA	3'		+	+
2-[(3,4-dihydroxyphenyl)carbonyl]-2,4,6-trihydroxy-1-benzofuran-3(2H)-one	DTB	4	4, 4'	+	+
2-[(3,4-dihydroxyphenyl)carbonyl]-4,6-dihydroxy-2-methoxy-1-benzofuran-3(2H)-one	MeO-DTB	4'		+	+
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	Isoquercitrin	5	5, 5'	+	-
2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	MeO-Isoquercitrin	5'		+	-
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methylloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	Rutin	6	6, 6'	+	-
2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methylloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	MeO-Rutin	6'		+	-
3,5-dihydroxy-2-[methoxy(oxo)acetyl]phenyl 3,4-dihydroxybenzoate	DPD	7	7, 7'	+	+
5-hydroxy-3-methoxy-2-[methoxy(oxo)acetyl]phenyl 3,4-dihydroxybenzoate	MeO-DPD	7'		+	+
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methylloxan-2-yl]oxychromen-4-one	Quercitrin	8	8, 8'	+	-
2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methylloxan-2-yl]oxychromen-4-one	MeO-Quercitrin	8'		+	-
2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	Quercetin	9	9, 9'a/b*/c*	+	+
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one	iso-1-MeO-Quercetin	9' a		+	+
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one	iso-2-MeO-Quercetin	9' b		-	-
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one	iso-3-MeO-Quercetin	9' c		-	-
2-(3,4-dihydroxyphenyl)-1-benzofuran-3,4,6-triol	DBT	10	10, 10'	+	+
2-(3,4-dihydroxyphenyl)-3-methoxy-1-benzofuran-4,6-diol	MeO-DBT	10'		+	+
4,6-dihydroxy-1-benzofuran-2,3-dione	DBD	11	11	+	+
7-(3,4-dihydroxyphenyl)bicyclo[4.2.0]octa-1,3,5,7-tetraene-2,4,8-triol	DBOT	12	12	+	+

^a See Fig. 1

^b See Fig. 2

* Tentative structure

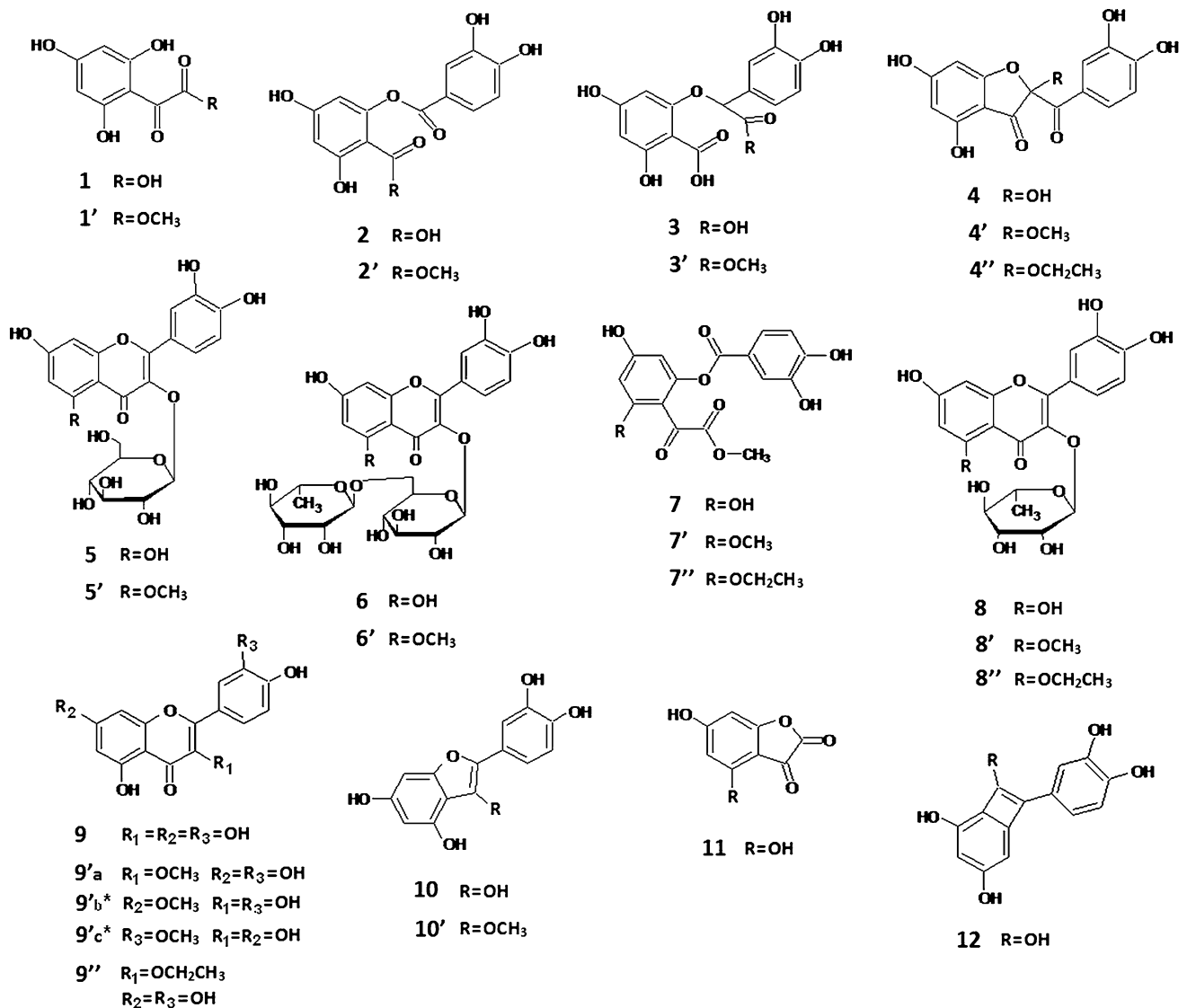


Fig. 2 Molecular structures of all examined compounds: (1) oxo(2,4,6-trihydroxyphenyl)acetic acid (OTA); (1') methyl oxo(2,4,6-trihydroxyphenyl)acetate (Me-OTA); (2) 2-[[3,4-dihydroxyphenyl]carbonyl]oxy]-4,6-dihydroxybenzoic acid (DDA); (2') methyl 2-[[3,4-dihydroxyphenyl]carbonyl]oxy]-4,6-dihydroxybenzoate (Me-DDA); (3) 2-[carboxy(3,4-dihydroxyphenyl)methoxy]-4,6-dihydroxybenzoic acid (CDA); (3') 2-[1-(3,4-dihydroxyphenyl)-2-methoxy-2-oxoethoxy]-4,6-dihydroxybenzoic acid (MeO-CDA); (4) 2-[[3,4-dihydroxyphenyl]carbonyl]-2,4,6-trihydroxy-1-benzofuran-3(2H)-one (DTB); (4') 2-[[3,4-dihydroxyphenyl]carbonyl]-4,6-dihydroxy-2-methoxy-1-benzofuran-3(2H)-one (MeO-DTB); (4'') 2-[[3,4-dihydroxyphenyl]carbonyl]-2-ethoxy-4,6-dihydroxy-1-benzofuran-3(2H)-one (EtO-DTB); (5) 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one (Isoquercitrin); (5') 2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one (MeO-Isoquercitrin); (6) 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one (Rutin); (6') 2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one (MeO-Rutin); (7) 3,5-dihydroxy-2-[methoxy(oxo)acetyl]phenyl 3,4-dihydroxybenzoate (DPD); (7')

5-hydroxy-3-methoxy-2-[methoxy(oxo)acetyl]phenyl 3,4-dihydroxybenzoate (MeO-DPD); (7'') 3-ethoxy-5-hydroxy-2-[methoxy(oxo)acetyl]phenyl-3,4-dihydroxybenzoate (EtO-DPD); (8) 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxychromen-4-one (Quercitrin); (8') 2-(3,4-dihydroxyphenyl)-7-hydroxy-5-methoxy-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxychromen-4-one (MeO-Quercitrin); (8'') 2-(3,4-dihydroxyphenyl)-5-ethoxy-7-hydroxy-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxychromen-4-one (EtO-Quercitrin); (9) 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one (Quercetin); (9'a) 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one (*iso*-1-MeO-Quercetin); (9'b) 2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-methoxy-4H-chromen-4-one (*iso*-2-MeO-Quercetin); (9'c) 3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-chromen-4-one (*iso*-3-MeO-Quercetin); (9'') 2-(3,4-dihydroxyphenyl)-3-ethoxy-5,7-dihydroxychromen-4-one (EtO-Quercetin); (10) 2-(3,4-dihydroxyphenyl)-1-benzofuran-3,4,6-triol (DBT); (10') 2-(3,4-dihydroxyphenyl)-3-methoxy-1-benzofuran-4,6-diol (MeO-DBT); (11) 4,6-dihydroxy-1-benzofuran-2,3-dione (DBD); (12) 7-(3,4-dihydroxyphenyl)bicyclo[4.2.0]octa-1,3,5,7-tetraene-2,4,8-triol (DBOT). The stars indicate the tentative structures. The number of the structure corresponds with the peak number in Figs. 1 and 3. All of the data use the recommended IUPAC numbering system

Fig. 3 Exemplary chromatograms of SSDM extracts from the green tea leaves (a), rutin (b) and quercetin (c)

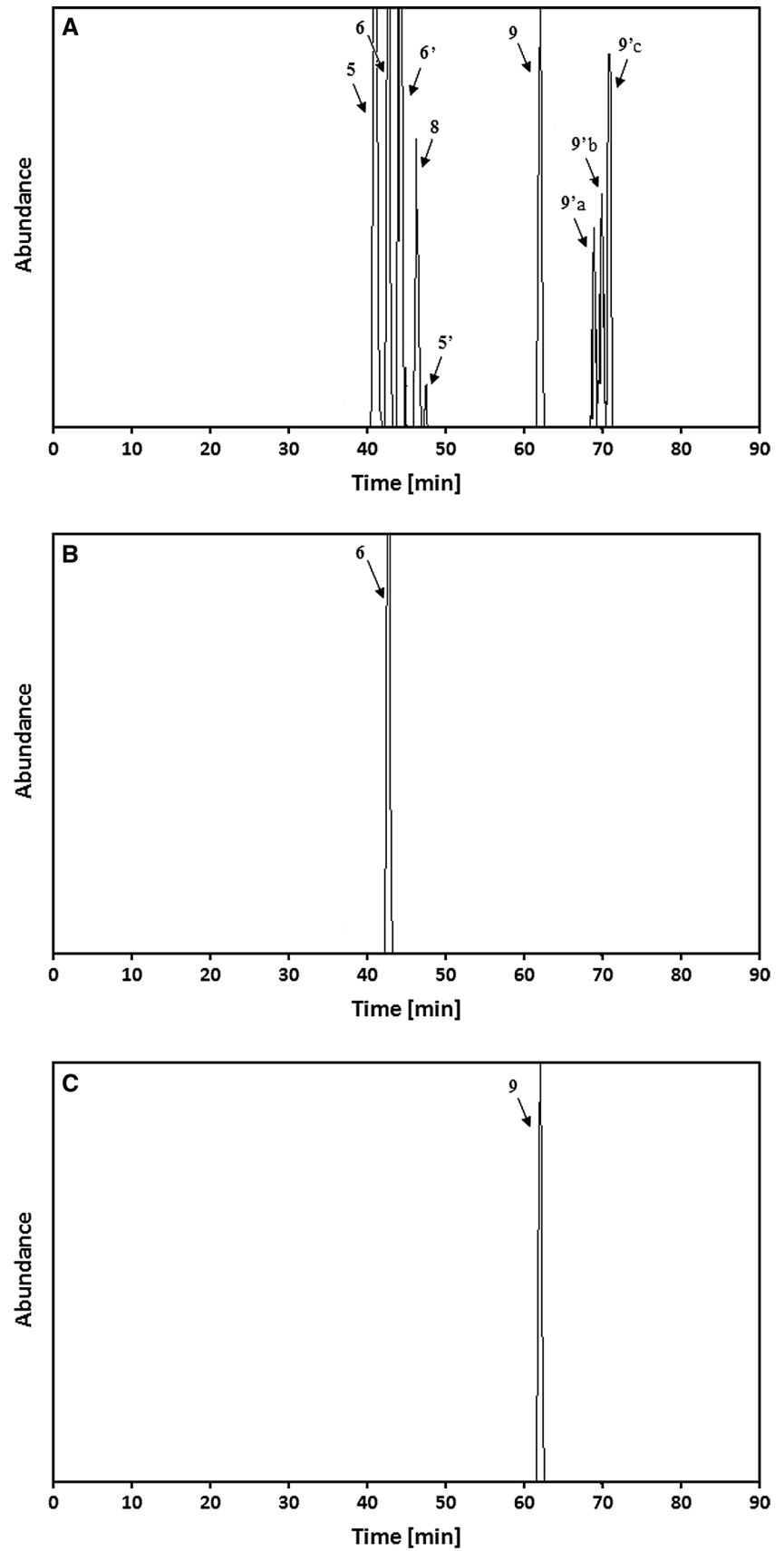


Table 3 Amounts ($\mu\text{g/g}$) of quercetin and its derivatives estimated in green tea leaves by extraction under reflux and SSDM (mean value \pm SD)

Compound No.	Compound	Reflux		SSDM			
		Me75 %	Et75 %	Me/Me75 %	D/Me75 %	Et/Et75 %	D/Et75 %
1	OTA	5.4 \pm 0.9	–	–	–	–	–
1'	Me-OTA	2.2 \pm 0.4	–	–	–	–	–
2	DDA	15.2 \pm 2.0	23.4 \pm 2.9	–	–	–	–
2'	Me-DDA	34.7 \pm 4.1	–	–	–	–	–
3	CDA	46.8 \pm 5.1	12.8 \pm 1.7	–	–	–	–
3'	MeO-CDA	57.2 \pm 5.8	–	–	–	–	–
4	DTB	27.7 \pm 3.6	–	–	–	–	–
4'	MeO-DTB	25.7 \pm 3.4	–	–	–	–	–
4''	EtO-DTB	–	18.2 \pm 2.6	–	–	–	–
5	Isoquercitrin	465.7 \pm 27.5	490.3 \pm 29.9	401.9 \pm 23.7	400.2 \pm 23.4	411.1 \pm 23.6	410.2 \pm 23.8
5'	MeO-Isoquercitrin	16.9 \pm 2.6	9.3 \pm 1.5	7.9 \pm 1.1	7.3 \pm 1.0	9.3 \pm 1.3	9.7 \pm 1.3
6	Rutin	16,113.3 \pm 499.5	16,306.3 \pm 521.8	17,687.1 \pm 512.9	17,680.1 \pm 503.9	18,025.5 \pm 504.7	18,002.0 \pm 486.1
6'	MeO-Rutin	658.3 \pm 39.0	560.7 \pm 33.4	557.9 \pm 31.2	557.1 \pm 31.5	558.9 \pm 29.9	558.6 \pm 30.2
7	DPD	17.2 \pm 2.4	–	–	–	–	–
7'	MeO-DPD	34.4 \pm 4.4	–	–	–	–	–
7''	EtO-DPD	–	31.2 \pm 3.9	–	–	–	–
8	Quercitrin	45.4 \pm 4.9	50.3 \pm 5.2	22.4 \pm 2.9	20.0 \pm 2.6	27.0 \pm 3.3	27.3 \pm 3.6
8'	MeO-Quercitrin	38.1 \pm 4.7	–	–	–	–	–
8''	EtO-Quercitrin	–	19.8 \pm 2.8	–	–	–	–
9	Quercetin	252.2 \pm 17.9	260.2 \pm 18.7	211.1 \pm 11.4	210.6 \pm 14.6	217.7 \pm 14.4	216.3 \pm 15.1
9'a	<i>iso</i> -1-MeO-Quercetin	88.2 \pm 8.9	68.5 \pm 7.4	64.8 \pm 7.5	64.9 \pm 7.7	66.2 \pm 7.4	67.4 \pm 7.7
9'b	<i>iso</i> -2-MeO-Quercetin	107.7 \pm 10.9	81.2 \pm 8.9	78.2 \pm 8.1	77.3 \pm 8.1	79.5 \pm 8.2	80.0 \pm 8.4
9'c	<i>iso</i> -3-MeO-Quercetin	219.2 \pm 17.1	180.2 \pm 14.2	180.0 \pm 12.8	180.3 \pm 12.4	180.2 \pm 12.3	180.2 \pm 11.9
9''	EtO-Quercetin	–	41.2 \pm 5.0	–	–	–	–
10	DBT	18.7 \pm 2.6	26.4 \pm 3.7	–	–	–	–
10'	MeO-DBT	9.8 \pm 1.4	–	–	–	–	–
11	DBD	–	–	–	–	–	–
12	DBOT	–	–	–	–	–	–

Table 4 *F* values and *p* values obtained during variance analysis for the effects of different extraction conditions on the amount of quercetin and its derivatives in green tea leaves: E1—effect of sample preparation method (methanolic/ethanolic extraction under reflux

vs all SSDM types); E2—effect of SSDM type; E3—effect of sample preparation method (methanolic extraction under reflux vs all SSDM types); E4—effect of sample preparation method (ethanolic extraction under reflux vs all SSDM types)

Compound No.	Compound	E1 ^a		E2 ^b		E3 ^c		E4 ^c	
		<i>F</i> value	<i>p</i> value	<i>F</i> value	<i>p</i> value	<i>F</i> value	<i>p</i> value	<i>F</i> value	<i>p</i> value
1	OTA	–	–	–	–	–	–	–	–
1'	Me-OTA	–	–	–	–	–	–	–	–
2	DDA	–	–	–	–	–	–	–	–
2'	Me-DDA	–	–	–	–	–	–	–	–
3	CDA	–	–	–	–	–	–	–	–
3'	MeO-CDA	–	–	–	–	–	–	–	–
4	DTB	–	–	–	–	–	–	–	–
4'	MeO-DTB	–	–	–	–	–	–	–	–
4''	EtO-DTB	–	–	–	–	–	–	–	–
5	Isoquercitrin	6.801	3.16×10^{-03}	0.161	9.20×10^{-01}	1.745	2.16×10^{-01}	6.954	6.05×10^{-03}
5'	MeO-Isoquercitrin	15.235	7.74×10^{-05}	2.726	1.14×10^{-01}	18.406	1.32×10^{-04}	2.087	1.57×10^{-01}
6	Rutin	8.728	1.09×10^{-03}	0.434	7.35×10^{-01}	7.510	4.62×10^{-03}	5.894	1.06×10^{-02}
6'	MeO-Rutin	4.658	1.35×10^{-02}	0.002	$1.00 \times 10 + 00$	5.692	1.18×10^{-02}	0.005	$1.00 \times 10 + 00$
7	DPD	–	–	–	–	–	–	–	–
7'	MeO-DPD	–	–	–	–	–	–	–	–
7''	EtO-DPD	–	–	–	–	–	–	–	–
8	Quercitrin	31.967	1.55×10^{-06}	4.009	5.16×10^{-02}	23.710	4.36×10^{-05}	33.616	8.99×10^{-06}
8'	MeO-Quercitrin	–	–	–	–	–	–	–	–
8''	EtO-Quercitrin	–	–	–	–	–	–	–	–
9	Quercetin	5.778	6.07×10^{-03}	0.184	9.05×10^{-01}	3.852	3.81×10^{-02}	5.443	1.37×10^{-02}
9'a	<i>iso</i> -1-MeO-Quercetin	4.040	2.21×10^{-02}	0.081	9.68×10^{-01}	4.920	1.87×10^{-02}	0.138	9.64×10^{-01}
9'b	<i>iso</i> -2-MeO-Quercetin	5.256	8.71×10^{-03}	0.068	9.75×10^{-01}	6.493	7.65×10^{-03}	0.102	9.79×10^{-01}
9'c	<i>iso</i> -3-MeO-Quercetin	4.136	2.04×10^{-02}	0.000	$1.00 \times 10^{+00}$	5.067	1.71×10^{-02}	0.000	$1.00 \times 10^{+00}$
9''	EtO-Quercetin	–	–	–	–	–	–	–	–
10	DBT	–	–	–	–	–	–	–	–
10'	MeO-DBT	–	–	–	–	–	–	–	–
11	DBD	–	–	–	–	–	–	–	–
12	DBOT	–	–	–	–	–	–	–	–

^a $F_{crit.} = 3.11$

^b $F_{crit.} = 4.07$

^c $F_{crit.} = 3.48$

water mixture (75/25 %, v/v) was used as the eluent. Column D/Et75 % contains the results obtained by the second variant, dry SSDM, in which an ethanol/water mixture (75/25 %, v/v) was used as the eluent. The parameters of the applied SSDM procedure were optimized in preliminary experiments and were as follows: plant to sand mass ratio—1:4; dispersing liquid volume (in the case of the first SSDM variant)—1.0 mL; blending time—10 min, and eluent volume—25 mL.

The importance of the experimental factors determined according to the ANOVA *F* test for the individual compounds is listed in Table 4. The higher the value of Fisher

coefficient (*F* value) and the lower the *p* value, the higher is the significance of the examined parameter [16].

The comparison of the results obtained by SSDM and extraction under reflux for the examined green tea leaves (see Tables 3, 4) indicates that:

- Sample preparation method affects the concentrations of quercetin, its glycosides and their methyl derivatives ($4.040 < F_{exp.} < 31.967$ at $F_{crit.} = 3.11$, see Table 4). The amounts of isoquercitrin, quercitrin, quercetin, their methyl derivatives and the rutin methyl derivative are higher in the green tea extract obtained under reflux,

whereas the rutin amounts are higher in the extract obtained by SSDM.

- The presence of low-molecular quercetin derivatives (OTA, DBD, DDA, CDA, DTB, DPD, DBT) and their methyl derivatives (Me-OTA, Me-DDA, Me-CDA, Me-DTB, MeO-DPD, MeO-DBT) in the methanol/water extract obtained under reflux, and their absence in the extract obtained in SSDM proves that these compounds are not the native green tea components.
- The presence of ethyl quercetin derivatives (EtO-DTB, EtO-DPD, EtO-Quercitrin, EtO-Quercetin) only in the reflux ethanolic extract shows that they are not the native green tea constituents.
- The presence of methyl quercetin derivatives (MeO-Isoquercitrin, *iso*-1-MeO-Quercetin, *iso*-2-MeO-Quercetin, *iso*-3-MeO-Quercetin, MeO-Rutin) in the ethanolic extract obtained under reflux and in the SSDM extracts demonstrates that they are true native plant components. Their greater concentrations in the methanolic reflux

extract than in the ethanolic one additionally proves that the high-temperature extraction with methanol increases their amounts [compare the F values obtained for these compounds in the methanolic extract ($F_{exp.} = 18.406$ for MeO-Isoquercitrin; $4.920 < F_{exp.} < 6.493$ for *iso*-1-MeO-Quercetin, *iso*-2-MeO-Quercetin and *iso*-3-MeO-Quercetin; and $F_{exp.} = 5.692$ for MeO-Rutin at $F_{crit.} = 3.48$) with those obtained in the ethanolic extract ($F_{exp.} = 2.087$ for MeO-Isoquercitrin; $0.0 < F_{exp.} < 0.138$ for *iso*-1-MeO-Quercetin, *iso*-2-MeO-Quercetin and *iso*-3-MeO-Quercetin; and $F_{exp.} = 0.005$ for MeO-Rutin at $F_{crit.} = 3.48$, see Table 4)].

- The variant of the SSDM procedure does not influence the concentration of the native quercetin derivatives ($F_{exp.} \leq F_{crit.}$, see Table 4).

Considering the above, the high-temperature extraction with methanol applied as sample preparation method not only causes the formation of non-native quercetin

Table 5 Amounts ($\mu\text{g/g}$) of quercetin and its derivatives estimated by SSDM for a raw sample of green tea leaves (GTL-1) and for a sample mixed with a known amount of rutin standard (GTL-2) (mean value \pm SD)

Compound No.	Compound	SSDM	
		GTL-1	GTL-2
1	OTA	–	–
1'	Me-OTA	–	–
2	DDA	–	–
2'	Me-DDA	–	–
3	CDA	–	–
3'	MeO-CDA	–	–
4	DTB	–	–
4'	MeO-DTB	–	–
4''	EtO-DTB	–	–
5	Isoquercitrin	412.3 \pm 24.1	408.2 \pm 24.2
5'	MeO-Isoquercitrin	10.2 \pm 1.4	9.8 \pm 1.4
6	Rutin	17,898.3 \pm 486.8	65,342.0 \pm 1378.7
6'	MeO-Rutin	562.4 \pm 31.7	555.1 \pm 29.3
7	DPD	–	–
7'	MeO-DPD	–	–
7''	EtO-DPD	–	–
8	Quercitrin	30.5 \pm 3.9	28.3 \pm 3.7
8'	MeO-Quercitrin	–	–
8''	EtO-Quercitrin	–	–
9	Quercetin	227.5 \pm 16.2	232.6 \pm 16.7
9'a	<i>iso</i> -1-MeO-Quercetin	64.5 \pm 7.7	67.0 \pm 8.1
9'b	<i>iso</i> -2-MeO-Quercetin	81.9 \pm 8.5	80.2 \pm 8.5
9'c	<i>iso</i> -3-MeO-Quercetin	186.1 \pm 12.4	181.1 \pm 11.8
9''	EtO-Quercetin	–	–
10	DBT	–	–
10'	MeO-DBT	–	–
11	DBD	–	–
12	DBOT	–	–

Table 6 *F* values and *p* values obtained during variance analysis for the effect of rutin standard addition on the amount of rutin and rutin derivatives in green tea leaves

Comp. No.	Compound	<i>F</i> value	<i>p</i> value
1	OTA	–	–
1'	Me-OTA	–	–
2	DDA	–	–
2'	Me-DDA	–	–
3	CDA	–	–
3'	MeO-CDA	–	–
4	DTB	–	–
4'	MeO-DTB	–	–
4''	EtO-DTB	–	–
5	Isoquercitrin	0.043	8.45×10^{-01}
5'	MeO-Isoquercitrin	0.178	6.95×10^{-01}
6	Rutin	3158.674	6.00×10^{-07}
6'	MeO-Rutin	0.086	7.84×10^{-01}
7	DPD	–	–
7'	MeO-DPD	–	–
7''	EtO-DPD	–	–
8	Quercitrin	0.480	5.27×10^{-01}
8'	MeO-Quercitrin	–	–
8''	EtO-Quercitrin	–	–
9	Quercetin	0.141	7.27×10^{-01}
9'a	<i>iso</i> -1-MeO-Quercetin	0.151	7.17×10^{-01}
9'b	<i>iso</i> -2-MeO-Quercetin	0.060	8.19×10^{-01}
9'c	<i>iso</i> -3-MeO-Quercetin	0.250	6.43×10^{-01}
9''	EtO-Quercetin	–	–
10	DBT	–	–
10'	MeO-DBT	–	–
11	DBD	–	–
12	DBOT	–	–

$F_{crit.} = 7.71$

derivatives but also leads to a concentration increase of the native constituents.

SSDM of green tea leaves fortified with rutin standard

In order to confirm unequivocally that quercetin and rutin, its main glycoside, do not transform/degrade during SSDM, the amounts of quercetin derivatives estimated by the dry variant of SSDM were compared in two green tea samples. One of them was fortified with a known amount of rutin standard as rutin molecules hydrolyze to quercetin and the same low-molecular transformation products are formed from rutin and quercetin (see Table 2). Ethanol/water mixture (75/25 %, v/v) was applied as the SSDM eluent. The results contained in Tables 5, 6 demonstrate that, except for rutin added to sample GTL-2, the amounts of other quercetin derivatives are similar ($F_{exp.} = 3158.674$

for rutin vs $0.0430 < F_{exp.} < 0.480$ for the other rutin derivatives at $F_{crit.} = 7.71$, see Table 6). The similarity of the Isoquercitrin, MeO-Isoquercitrin, Quercitrin, Quercetin, *iso*-1-MeO-Quercetin, *iso*-2-MeO-Quercetin, *iso*-3-MeO-Quercetin and MeO-Rutin amounts in GTL-1 and GTL-2 samples ($0.0430 < F_{exp.} < 0.480$ at $F_{crit.} = 7.71$, see Table 6) proves that these compounds do not form during the SSDM process and that they are native green tea components.

The results of Tables 3–6 unequivocally demonstrate that SSDM does not transform/degrade quercetin/rutin and their derivatives, and it can be recommended for the estimation of quercetin and its derivatives in plant materials.

Estimation of quercetin and its derivatives in plants by SSDM

Table 7 contains the concentrations of quercetin and its derivatives estimated in a few chosen plants using the dry variant of SSDM and ethanol/water mixture (75/25 %, v/v) as the SSDM eluent. All results were established under optimal SSDM conditions.

The analysis of the results in Table 7 leads to the following conclusions:

- The concentrations of quercetin and its derivatives are different in the examined plants: they are the lowest in nettle and artichoke, and the highest in *V. tricolor* and green tea.
- Of all the quercetin derivatives, rutin is present in the highest amounts in all the examined plants. Yet, the lack of its methyl derivative in yerba mate, Hawthorn and St. John wort is a surprise.
- Although there is proportional correlation between the amounts of quercetin and its methyl derivatives (*iso*-1-MeO-Quercetin, *iso*-2-MeO-Quercetin, *iso*-3-MeO-Quercetin) in *V. tricolor* and green tea, no such correlation is observed for the other quercetin derivatives and other plants.

The conclusions indicate that the types of metabolism involving quercetin and its derivatives are different in the studied plants.

Concluding remarks

The quantitative analysis of plant composition requires complete isolation of the analytes from the plant matrix, which is most often performed by means of high-temperature liquid extraction. Yet, the results presented in the present paper show that high-temperature extraction cannot be applied for the analysis of quercetin and its derivatives in plants as it causes their transformations leading to

Table 7 Amounts ($\mu\text{g/g}$) of quercetin and its derivatives estimated in plants by the dry variant of SSDM (mean value \pm SD)

Comp. No.	Compound	Plant material											
		Heartsease	Green tea	Yerba maté	Black elder	Hawthorn	Nettle	Artichoke	St. John's wort				
1	OTA	-	-	-	-	-	-	-	-	-	-	-	-
1'	Me-OTA	-	-	-	-	-	-	-	-	-	-	-	-
2	DDA	-	-	-	-	-	-	-	-	-	-	-	-
2'	Me-DDA	-	-	-	-	-	-	-	-	-	-	-	-
3	CDA	-	-	-	-	-	-	-	-	-	-	-	-
3'	MeO-CDA	-	-	-	-	-	-	-	-	-	-	-	-
4	DTB	-	-	-	-	-	-	-	-	-	-	-	-
4'	MeO-DTB	-	-	-	-	-	-	-	-	-	-	-	-
4''	EtO-DTB	-	-	-	-	-	-	-	-	-	-	-	-
5	Isoquercitrin	382.6 \pm 22.7	410.2 \pm 23.8	33.0 \pm 4.0	69.7 \pm 7.3	144.8 \pm 11.6	110.2 \pm 9.4	270.2 \pm 18.6	-	-	-	-	-
5'	MeO-Isoquercitrin	7.9 \pm 1.3	9.7 \pm 1.3	4.1 \pm 0.7	2.7 \pm 0.6	16.8 \pm 2.3	19.7 \pm 2.9	45.3 \pm 5.4	-	-	-	-	-
6	Rutin	9045.0 \pm 329.2	18,002.0 \pm 486.1	3594.2 \pm 143.4	52,283.3 \pm 1207.7	8086.0 \pm 317.0	3747.3 \pm 167.9	11,536.8 \pm 417.6	-	-	-	-	-
6'	MeO-Rutin	539.2 \pm 31.7	558.6 \pm 30.2	-	715.3 \pm 29.3	-	1630.6 \pm 82.3	5295.6 \pm 166.3	-	-	-	-	-
7	DPD	-	-	-	-	-	-	-	-	-	-	-	-
7'	MeO-DPD	-	-	-	-	-	-	-	-	-	-	-	-
7''	EtO-DPD	-	-	-	-	-	-	-	-	-	-	-	-
8	Quercitrin	15.4 \pm 2.2	27.3 \pm 3.6	2.8 \pm 0.5	50.0 \pm 6.2	111.2 \pm 9.1	-	602.1 \pm 35.2	-	-	-	-	-
8'	MeO-Quercitrin	-	-	4.5 \pm 0.8	3.5 \pm 0.7	-	-	51.7 \pm 6.2	-	-	-	-	-
8''	EtO-Quercitrin	-	-	-	-	-	-	-	-	-	-	-	-
9	Quercetin	193.6 \pm 13.5	216.3 \pm 15.1	1.0 \pm 0.2	16.7 \pm 2.2	3.8 \pm 0.8	-	7.4 \pm 1.1	-	-	-	-	-
9'a	iso-1-MeO-Quercetin	56.9 \pm 6.4	67.4 \pm 7.7	0.6 \pm 0.1	-	-	-	-	-	-	-	-	-
9'b	iso-2-MeO-Quercetin	78.4 \pm 7.7	80.0 \pm 8.4	-	-	-	-	-	-	-	-	-	-
9'c	iso-3-MeO-Quercetin	148.3 \pm 11.2	180.2 \pm 11.9	-	-	-	-	-	-	-	-	-	-
9''	EtO-Quercetin	-	-	-	-	-	-	-	-	-	-	-	-
10	DBT	-	-	-	-	-	-	-	-	-	-	-	-
10'	MeO-DBT	-	-	-	-	-	-	-	-	-	-	-	-
11	DBD	-	-	-	-	-	-	-	-	-	-	-	-
12	DBOT	-	-	-	-	-	-	-	-	-	-	-	-

erroneous qualitative and quantitative estimations of true plant components.

The performed experiments show that the transformation/degradation of quercetin and its glycosides is not induced by SSDM and prove the method to be most appropriate for the estimation of quercetin and its derivatives in plants. What is more, the application of SSDM in plant analysis allows the researcher, to determine which quercetin derivatives are native plant components and what is their true concentration. In other word, the application of SSDM in plant analysis eliminates errors in the study of plant metabolism involving quercetin and its derivatives.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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