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ARTICLE

Thermal Stability of 5-*o*-Caffeoylquinic Acid in Aqueous Solutions at Different Heating Conditions

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Chlorogenic acid is a naturally occurring phenolic compound found in all higher plants. This component, being the ester of caffeic acid with quinic acid, is an important biosynthetic intermediate and plays an important role in the plant's response to stress. Potential uses of chlorogenic acid are suggested in pharmaceuticals, foodstuffs, feed additives, and cosmetics due to its recently discovered biomedical activity. This finding caused new interest in chlorogenic acid properties, its isomers, and its natural occurrence. It has been found that as many as nine compounds (chlorogenic acid derivatives and its reaction product with water) can be formed from 5-*o*-caffeoylquinic acid during the heating of its water solution. Three of them, two hydroxylated 5-*o*-caffeoylquinic acid derivatives and 4,5-dicaffeoylquinic acid, have been not reported, yet. The amount of each formed component depends on the heating time and temperature. The presented results are important for researchers investigating plant metabolism and looking for new plant components. The transformation product can be mistakenly treated as a new component, not found before in the examined plant, or can be a cause of erroneous quantitative estimations of plant composition.

KEYWORDS: Chlorogenic acid; 5-*o*-caffeoylquinic acid thermal stability; 5-*o*-caffeoylquinic acid isomerization; 5-*o*-caffeoylquinic acid transformation; extraction

INTRODUCTION

Analysis of plant constituents involves the application of the sample preparation method allowing for full isolation of the analyzed substances from the plant matrix. A broad range of sample preparation techniques (e.g., extraction to gas, liquid, or solid phase; distillation; dispersion; membrane filtration; centrifugation; precipitation) are currently used for this purpose (*1*−*3*). Most approaches to plant material analysis mainly involve the application of liquid extraction methods such as Soxhlet extraction, percolation, maceration, and extraction under reflux (*4*, *5*). In an attempt to improve the extraction process and reduce or eliminate the drawbacks of the mentioned classical extraction methods (long extraction time, relatively high solvent consumption, and often unsatisfactory reproducibility), innovative extraction methods such as microwave-, ultrasound-, and pressure-assisted extraction have been recently developed and introduced (*6*, *7*). Pressurized liquid extraction (PLE) draws special attention of investigators due to its high extraction efficiency (*8*, *9*) because variations of temperature and pressure during the PLE process influence the solubility of the compounds. Furthermore, PLE, due to the high pressure of the extraction process, allows for using an extractant at a temperature above its normal boiling point and, as a consequence, for removing the analytes efficiently and quickly from various matrices.

Chlorogenic acid is a naturally occurring phenolic compound found in all higher plants (*10*, *11*). Structurally, it is the ester of caffeic acid with quinic acid. In plants, isomerization of chlorogenic acid has been reported with three isomerizations of quinic acid in positions 3 (3-*o*-caffeoylquinic acid), 4 (4-*o*-caffeoylquinic acid), and 5 (5-*o*-caffeoylquinic acid). Isomerization at position 1 is known from the literature (*12*); however, as a plant constituent it has not been reported yet.

Chlorogenic acid is an important biosynthetic intermediate, for example, in lignin biosynthesis (*13*), and plays an important role in the plant's response to stress. It is claimed to have antiviral (*14*), antibacterial (*15*), and antifungal (*16*) effects combined with relatively low toxicity and side effects. It is also an antioxidant and an inhibitor of the tumor-promoting activity of phorbol esters (*17*). As antioxidant it might contribute to the prevention of type 2 diabetes mellitus (*18*) and cardiovascular diseases. In vivo studies on animal subjects have demonstrated that the administration of chlorogenic acid lessens the hyperglycemic peak resulting from the glycogenolysis brought about by administering glucagon, a hyperglycemic hormone. The studies also confirmed a reduction in glucose levels in blood and an increase in the intrahepatic concentration of glucose-6-phosphate and of glucagon (*19*). Potential uses of chlorogenic acid are also suggested in pharmaceuticals, foodstuffs, feed additives, and cosmetics. Hence, research interest in chlorogenic acid properties, its isomers, and its natural occurrence has been growing.

As previously reported (*20*), superheated water steam in the temperature range of 125−225 °C causes chlorogenic acid isomerization, transforming 5-*o*-caffeoylquinic acid into 3- and 4-*o*-caffeoylquinic acids. The question arises as to 5-*o*-caffeoylquinic acid thermal stability during its extraction by water. The present

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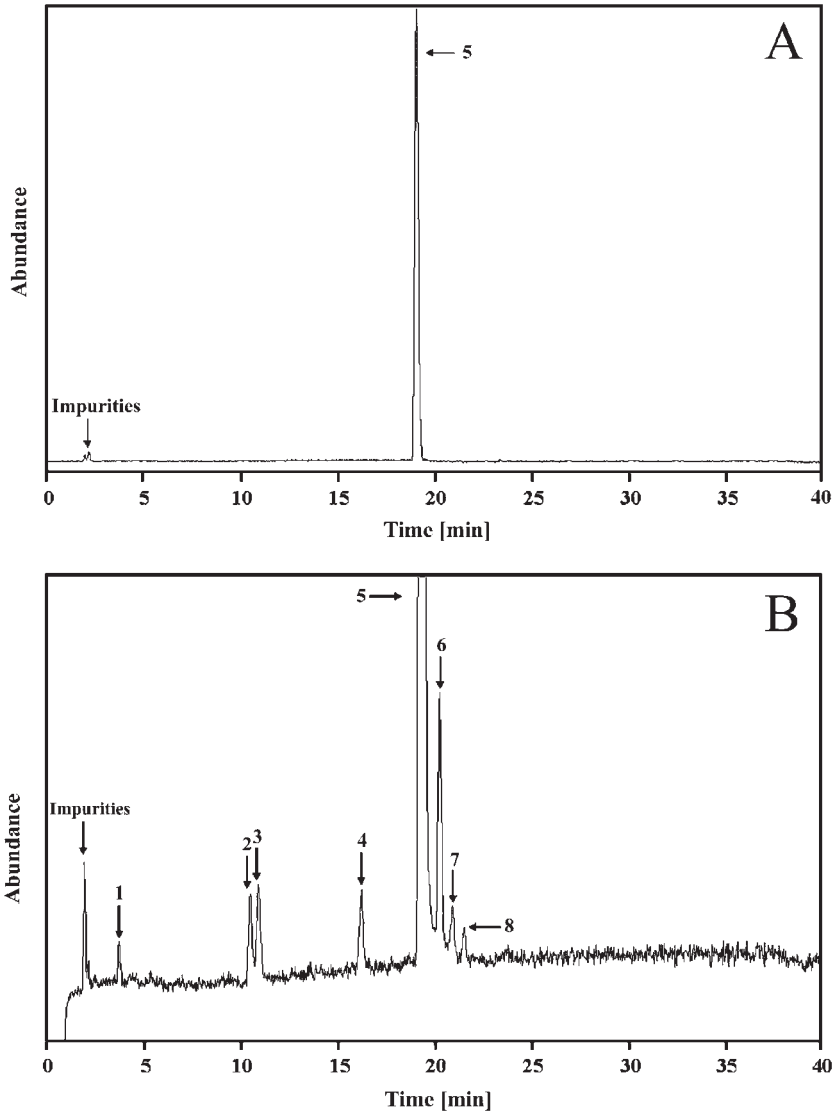

Figure 1. Chromatograms of 5-*o*-caffeoylquinic acid water solution (**A**) and 5-*o*-caffeoylquinic acid water solution after 5 h of reflux (**B**). Peak numbers correspond with compound numbers in **Figure 2**.

study investigated the transformation processes during its heating with water, under both reflux and higher pressure, using PLE. Chlorogenic acid is a very common plant component; hence, the presented results may be valuable for researchers examining many plant materials in which chlorogenic acid derivatives formed during extraction can be mistakenly attributed to the examined plant as its real components.

MATERIALS AND METHODS

Materials. Acetonitrile (HPLC) was purchased from the Polish Chemical Plant, POCh (Gliwice, Poland); formic acid was from Sigma Aldrich (Seelze, Germany); and chlorogenic acid was from Loba-Chemie Austranal Praparate (Austria). Water was purified on a Milli-Q system from Millipore (Millipore, Bedford, MA). Neutral glass (fraction 0.4−0.6 mm) was used as a dispersing agent in the PLE cell.

Sample Preparation. Investigations of the 5-*o*-caffeoylquinic acid transformation process were performed by heating 5-*o*-caffeoylquinic acid water solution under reflux and by extracting this compound in PLE conditions from PLE vials. The heating of the water acid solution under reflux was carried out using two sources of heating: a cart heater and a microwave oven.

Heating under Reflux. The glass equipment for heating of chlorogenic acid under reflux was composed of a boiling flask (50 mL) and a small condenser. The heated water solvents of 5-*o*-caffeoylquinic acid contained 10 mg of 5-*o*-caffeoylquinic acid in 30 mL of water. The acid portions were inserted to the already boiling water. During classical heating (cart heater), individual solutions of 5-*o*-caffeoylquinic acid were heated for 10 min or 1, 3, or 5 h. Various radiation energies (200, 400, and 600 W) at a constant time of 10 min were applied when the microwave oven (Plazmatronika, Wroclaw, Poland) was used for heating. After heating, the solvents were transferred into a volumetric flask (50 mL), filled with water to the flask volume, and subjected to HPLC analysis.

PLE. PLE was performed with a Dionex instrument (Dionex Corp., Sunnyvale, CA). Chlorogenic acid (10 mg) was mixed with neutral glass to reduce the solvent volume used for the extraction, placed into a 22 mL stainless steel extraction cell, and subjected to the extraction procedure.

In the investigation of the influence of temperature (100, 135, 150, 170, and 200 °C) on 5-*o*-caffeoylquinic acid transformation, the static extraction time was 10 min and the static extraction pressure was 60 bar. The influence of extraction time (5, 10, and 20 min) on 5-*o*-caffeoylquinic acid transformation was examined at 100 and 150 °C and at a constant extraction pressure of 60 bar.

The preset default PLE procedure was as follows: solvent flush volume, 100% of the extraction cell volume; purg, 60 s using pressurized nitrogen (10 bar) and collection in 60 mL glass vials with Teflon-coated rubber caps. The system was washed with the extraction solvent between the runs. The obtained extracts (volumes of ca. 35 mL) were transferred into volumetric flasks (50 mL), filled with water to their volume, and subjected to HPLC analysis.

HPLC Measurements. The chromatographic measurements were performed using an LC-ESI/IT/MS system equipped with a diode array detector, all from Finnigan (ThermoElectron Corp., San Jose, CA). The column used was a 100 mm × 4.6 mm i.d., 3 μm, Gemini C18 (Phenomenex, Torrance, CA). Chromatographic separation was 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 30 min, next from 35 to 100% B for 5 min, and finally isocratic elution followed (100% B) by 5 min. The total run time was 40 min at a mobile phase flow rate of 0.4 mL/min.

During the course of each run, PDA spectra in the range of 200−600 nm and MS spectra in the range of m/z 100−1000 were collected continuously. The column effluent was ionized by electrospray (ESI). The ESI needle potential was 4.5 kV in the negative ionization mode. To identify the chlorogenic acid isomers and the chlorogenic acid transformation products, the functions of secondary (MS^2) ion fragmentation were applied. The collision energy for each examined compounds was chosen individually. To identify dicaffeoylquinic acid, single ion monitoring (SIM) function and MS^2 were used.

To identify hydroxylated 5-*o*-caffeoylquinic acid derivatives in examined samples, LC-ESI/TOF/MS analysis was additionally performed. LC-ESI/TOF/MS analysis was carried out on an Agilent (Agilent Technologies, Palo Alto, CA) liquid chromatograph system. The MS analysis was performed on an orthogonal TOF/MS equipped with an electrospray interface (Agilent Technologies). A negative mode using full scan mode in TOF/MS analysis was applied, and the mass range was set at 150−550 Da. Chromatographic separation was performed using the same chromatographic column and the same gradient elution as described for LC-ESI/IT/MS analysis. Experimental molecular weights of the hydroxylated 5-*o*-caffeoylquinic acid derivatives, established by LC-ESI/TOF/MS analysis, were 371.0990 and 371.0971 Da, whereas the calculated molecular weights of these compounds equal 371.0978 Da. Data structure for the hydroxylated 5-*o*-caffeoylquinic acid derivatives should be treated as tentative due to the differences in their experimental and theoretical molecular weights of −3.2 and 1.9 ppm, respectively.

RESULTS AND DISCUSSION

Investigations of the 5-*o*-caffeoylquinic acid thermal stability in water were performed both by heating a water solution of 5-*o*-caffeoylquinic acid under reflux and by extracting this compound in PLE conditions from PLE vials. Ten milligram portions of 5-*o*-caffeoylquinic acid were used in all experiments because a 10 mg portion of the examined acid is more or less equivalent to the amount of the compound in a 0.5 g sample of many plants, that is, a plant sample mass typically used in the PLE process.

Figure 1 presents the chromatograms of the 5-*o*-caffeoylquinic acid water solution (**Figure 1A**) and of the 5-*o*-caffeoylquinic acid water solution heated under reflux for 5 h (**Figure 1B**). The last sample imitates the chlorogenic acid extract obtained for this compound during its water extraction under reflux. As shown in **Figure 1B**, the 5-*o*-caffeoylquinic acid water solution heated under reflux contains, besides the parent substance, seven additional compounds formed as a result of the 5-*o*-caffeoylquinic acid transformation and degradation. Five of them (quinic acid, peak 1; *trans* 3-*o*-caffeoylquinic, peak 4; *trans* 4-*o*-caffeoylquinic, peak 6; caffeic acid, peak 7; *cis* 5-*o*-caffeoylquinic, peak 8) were identified and confirmed on the basis of the retention data of their standards and of the PDA, MS^2 (*21*, *22*), and HRMS data. The MS^n and HRMS data for the mentioned compounds are collected in **Table 1**. The chemical structures of all identified compounds are presented in **Figure 2**.

Two other compounds (peaks 2 and 3 in **Figure 1B**) can be identified as hydroxylated chlorogenic acid derivatives, namely, (1*S*,3*R*,4*R*,5*R*)-5-[3-(3,4-dihydroxyphenyl)-2-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid (**2**) (**Figure 2**) and (1*S*,3*R*,4*R*,5*R*)-5-[3-(3,4-dihydroxyphenyl)-3-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid (**3**) (**Figure 2**). Such identification is suggested by the following arguments. The molecular weights of the compounds represented by peaks 2 and 3 are almost the same (**Table 1**) and exceed chlorogenic acid's molecular weight by 18.0107 and 18.0088 Da, respectively, which correspond to the molecular weight of water, 18.0106 Da. The addition of the water molecule to the double bond in the chlorogenic acid molecule should result in the formation of similar amounts of two hydroxylated chlorogenic acid derivatives with similar physicochemical properties. The retention times and peak heights of the considered compounds are very close, indicating the similarity of their physicochemical properties and the similarity of their formed amounts, although peak 3 is a little higher than peak 2 as it probably corresponds to a hydroxylated chlorogenic acid derivative of **3** (**Figure 2**). This assumption results from the literature (*23*) reporting a privileged location of positive charge on a carbon neighboring a phenyl group when a carbocation is formed (the intermediate stage in water attachment to double bond). Moreover, the introduction of the OH group to any molecule increases its polarity, which results in a diminution of its retention in the RP chromatographic system. The retention times of the considered compounds are shorter than the 5-*o*-caffeoylquinic acid retention time, indicating their higher polarity as compared with the polarity of their precursor. All of the above facts support the correctness of the identification. MS^n spectra of peaks 2 and 3 are less helpful in differentiating hydroxylated chlorogenic acid derivatives as both derivatives are fragmented to ions of very similar mass and intensity (**Table 1**).

Table 1. Negative Ion MS^2 Data for 5-*O*-Caffeoylquinic Acid and Products of Its Transformation

	MS^1	MS^2		
		base peak	secondary peak	
compd no. from **Figure 2**	parent ion m/z	m/z	m/z	intensity (%)
1	191.1	127.0	85.2	3.6
			93.2	45.9
			173.1	41.8
2	371.2	352.9	179.2	1.4
			191.1	40.3
3	371.1	352.9	179.1	0.7
			191.2	36.8
4	352.9	191.1	135.4	14.1
			173.5	0.8
			179.2	89.5
5	352.8	191.1	179.1	4.3
6	352.7	179.0	135.3	8.9
			173.1	88.3
			191.1	14.4
7	179.1	135.3		
8	352.9	191.1	179.1	2.0
9	515.1	352.9		

The experimental results presented in **Figure 1B** confirm the reported transformation of 5-*o*-caffeoylquinic acid to 3- and 4-*o*-caffeoylquinic acids (*20*, *24*). They also show that, besides 3- and 4-*o*-caffeoylquinic acids, other compounds appear in the 5-*o*-caffeoylquinic acid water solution during its heating under reflux: quinic and caffeic acids, hydroxylated chlorogenic acid derivatives, and *cis*-5-*o*-caffeoylquinic acid. The presence of quinic and caffeic acids in water solution is not surprising as 5-*o*-caffeoylquinic acid is the ester composed of the mentioned acids. According to Clifford et al. (*25*), UV exposure of *trans*-5-*o*-caffeoylquinic acid water and alcoholic/water solutions leads to the formation of *cis*-5-*o*-caffeoylquinic acid. The data from **Figure 1B** show that the same transformation also occurs during the heating of a chlorogenic acid water solution. The formation of hydroxylated chlorogenic acid

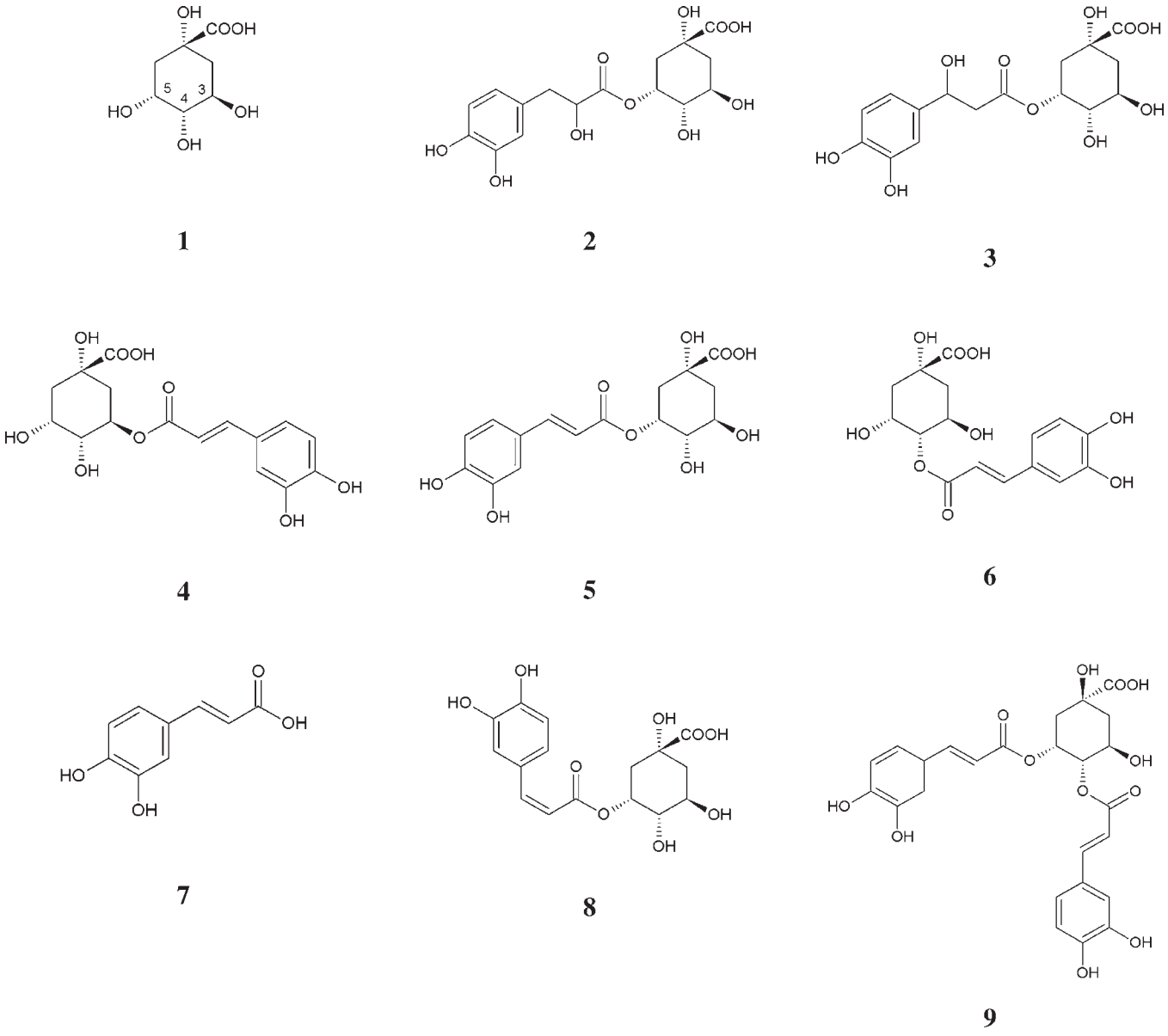

Figure 2. Molecular structures of 5-*o*-caffeoylquinic and of its transformation products: **1**, quinic acid; **2**, (1*S*,3*R*,4*R*,5*R*)-5-[3-(3,4-dihydroxyphenyl)-2-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid; **3**, (1*S*,3*R*,4*R*,5*R*)-5-[3-(3,4-dihydroxyphenyl)-3-hydroxypropanoyl]-1,4,5-trihydroxycyclohexanecarboxylic acid; **4**, *trans* 3-*o*-caffeoylquinic acid; **5**, *trans* 5-*o*-caffeoylquinic acid; **6**, *trans* 4-*o*-caffeoylquinic acid; **7**, caffeic acid; **8**, *cis*-5-*o*-caffeoylquinic acid; **9**, 4,5-dicaffeoylquinic acid.

derivatives in 5-*o*-caffeoylquinic acid water solution was not reported and seems to be worth special notice. It cannot be excluded that *cis*-5-*o*-caffeoylquinic acid results from dehydration of hydroxylated chlorogenic acid derivatives.

The influence of the heating time on the amount of individual components formed at the boiling water temperature under atmospheric pressure is illustrated in **Figure 3A**. Due to the lack of standards of hydroxylated chlorogenic acid derivatives (**2** and **3** in **Figure 2**), their amounts were calculated using the calibration curve for 5-*o*-caffeoylquinic acid. As shown, the increase of heating time causes a gradual increase of the amount of all the transformation products except hydroxylated chlorogenic acid derivatives, for which a quick increase followed by a slow decrease of their amount versus time is observed. This slow decrease of the amount of the hydroxylated chlorogenic acid derivatives (at time above 3 h) is accompanied by a simultaneous distinct increase in the *cis*-5-*o*-caffeoylquinic acid formation. This contrast is understandable if one takes into account that the *cis* form is created from hydroxylated chlorogenic acid derivatives as a result of their dehydration (*23*). The results in **Figure 3A** additionally show that the formation of 4-*o*-caffeoylquinic acid is much more predominant than that of 3-*o*-caffeoylquinic acid. This phenomenon is known from the literature (*24*) and can be explained by the higher thermodynamic stability (possibly due to intramolecular hydrogen bond contacts) of 4-*o*-caffeoylquinic acid.

The results presented in **Figure 3A** prove that even short heating of 5-*o*-caffeoylquinic acid water solution causes the acid degradation and transformation. This fact is partly confirmed by De Maria et al. (*26*), showing a small decrease of 5-*o*-caffeoylquinic acid amount in boiling water. It has to be stressed, however, that these experiments refer to plant materials and 5-*o*-caffeoylquinic acid model solutions containing smaller amounts of 5-*o*-caffeoylquinic acid (*26*) than those used in this study. The chlorogenic acid stability can be connected with the effect of the initial 5-*o*-caffeoylquinic acid concentration on pH of the acid solution and on transformation reaction rates.

The results in **Figure 3A** were obtained using the classical extraction process under reflux in which an outer electric heater (a cart heater) was applied to heat the boiling flask. This source of heating does not guarantee the homogeneity of temperature in the extractor. Moreover, using a cart heater, extra time is needed to heat up the extractor to the boiling temperature of the extractant, which prolongs the entire extraction procedure. The simplest way to overcome these obstacles is the application of a microwave oven as a heating source. Microwave-assisted extraction has been successfully employed in recent years as it shortens extraction time, reduces solvent consumption, prevents pollution, and lowers sample preparation costs (*27*, *28*). **Figure 3B** presents the influence of microwave power on the 5-*o*-caffeoylquinic acid transformation in water solution after 10 min of exposure. Five additional compounds appear in the extract. The growth of their amounts with microwave power increase is visible, but the increase is less pronounced for quinic and caffeic acids. Interestingly, these two compounds are not detected in the 5-*o*-caffeoylquinic acid water solution heated for 10 min in a classical way (**Figure 3A**). From a comparison of the amounts of the components in 5-*o*-caffeoylquinic acid water solutions after their 10 min of classical (**Figure 3A**) and microwave (**Figure 3B**) heating, it can be said that microwave heating favors 5-*o*-caffeoylquinic acid transformation. The absence of *cis*-5-*o*-caffeoylquinic acid in the water extract heated by microwave exposure differentiates this extract from the extract heated in the classical way.

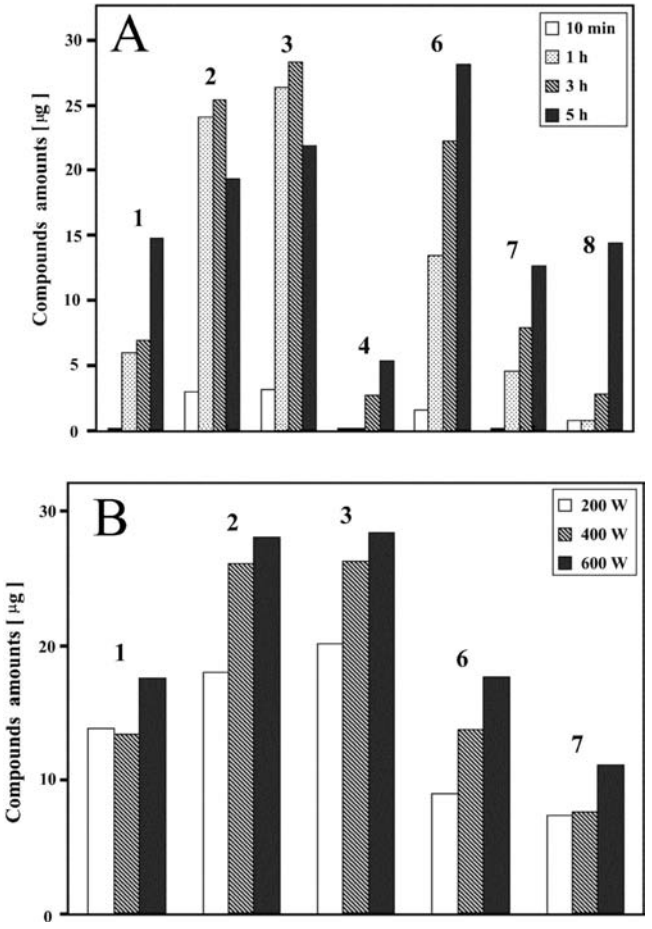

Figure 3. Influence of heating time (**A**) and/or microwave energy (**B**) on the amounts of 5-*o*-caffeoylquinic acid transformation products. Numbers at bars specify the corresponding compounds from **Figure 2**.

The influence of high extraction temperature (> 100 °C) on the amount of individual components formed during 5-*o*-caffeoylquinic acid water extraction was examined in the PLE equipment at constant extraction time and pressure of 10 min and 60 bar, respectively. As shown from the chromatographic analysis, these high-temperature extracts contain one substance more than the extracts obtained under reflux (peak 9, **Figure 4**), which shows an example chromatogram corresponding to the 5-*o*-caffeoylquinic acid extract obtained at 170 °C. As reported by Clifford et al. (*22*) and MS^n data shown in **Table 1** peak 9 corresponds to 4,5-dicaffeoylquinic acid (**9** in **Figure 2**). The presence of other isomers of dicaffeoylquinic acid cannot be excluded in the heated chlorogenic acid water solution; however, they are not observed by LC-MS, due to their very low concentration. Probably 4,5-dicaffeoylquinic acid exhibits a higher thermodynamic stability than other dicaffeoylquinic acid isomers and its formation is much greater.

The chromatogram presented in **Figure 4** additionally shows that 3- and 4-*o*-caffeoylquinic acids are the main transformation products created during 5-*o*-caffeoylquinic acid extraction in PLE conditions. The influence of temperature on the amount of 3- and 4-*o*-caffeoylquinic acids formed during the PLE process is illustrated in **Figure 5A**. As shown, the temperature increase leads to

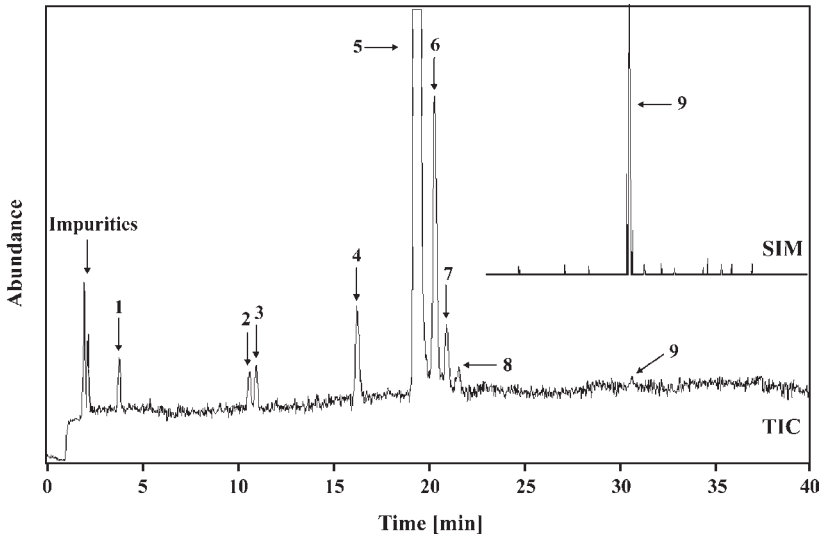

Figure 4. Exemplary chromatogram of the 5-*o*-caffeoylquinic acid water extract obtained in PLE (extraction temperature, 150 °C; extraction time, 10 min; extraction pressure, 60 bar). Peak number corresponds with compound number in **Figure 2**.

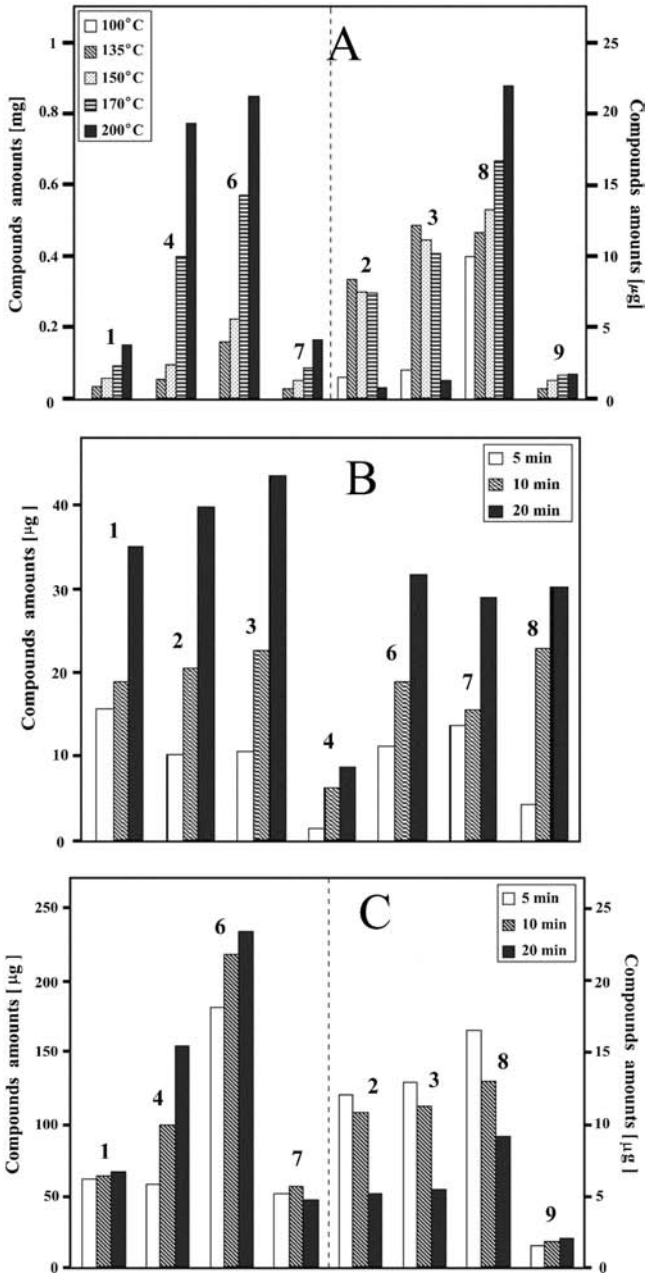

Figure 5. Influence of extraction temperature at constant extraction time 10 min (**A**) and/or extraction time at both extraction temperatures, 100 °C (**B**) and 150 °C (**C**), on the amounts of 5-*o*-caffeoylquinic acid transformation products obtained in PLE. Extraction pressure in all experiments equaled 60 bar. Numbers at bars specify the corresponding compounds from **Figure 2**.

the increase of the amount of 3- and 4-*o*-caffeoylquinic acids. The increase in the amount of both isomers is more pronounced at temperatures exceeding 150 °C. At 200 °C >15% of 5-*o*-caffeoylquinic acid is transformed into 3- and 4-*o*-caffeoylquinic acids. It is worth mentioning that 4-*o*-caffeoylquinic acid formation during high-temperature water extraction is also greater in relation to the 3-*o*-caffeoylquinic acid, yet not so high as in the temperature of boiling water under atmospheric pressure (**Figures 3A** and **5A**).

The amount of other substances present in high-temperature extracts gradually increases with temperature increase (caffeic acid, *cis*-5-*o*-caffeoylquinic acid, and 4,5-dicaffeoylquinic acid) or initially increases and then decreases (hydroxylated chlorogenic acid derivatives). However, the concentration changes resulting from the extraction temperature rise for these substances are not as high as for 3- and 4-*o*-caffeoylquinic acids. The characteristic feature of the high-temperature extraction process is the formation of 4,5-dicaffeoylquinic acid. It is not observed in water solvents heated at the temperature of water boiling under atmospheric pressure. In PLE extracts this high molecular weight compound is formed in trace amount, which precludes an unequivocal determination of caffeic acid molecule position at the 5-*o*-caffeoylquinic acid molecule. Due to the lack of 4,5-dicaffeoylquinic acid standard, the amount of the compound was calculated by relating its chromatographic responses to the calibration curve for 5-*o*-caffeoylquinic acid.

The influence of extraction time on the amounts of the components formed in PLE conditions at 100 and 150 °C are illustrated in **Figure 5B**,**C**, respectively. These experiments were performed in the range of time typically applied for PLE of plant material. As appears from **Figure 5B**, the increase of PLE extraction time at 100 °C results in a greater or lesser increase of the amount of the formed components. Such a trend is analogous with that showing the influence of heating time on the amount of individual component formed at the temperature of boiling water under atmospheric pressure (**Figure 3A**). The influence of extraction time on the amount of the formed compounds at 150 °C is more complex. For 3- and 4-*o*-caffeoylquinic acids, quinic acid, and 4,5-dicaffeoylquinic acid, the increase of their amount with extraction time increase is observed, yet it is insignificant. In the case of hydroxylated chlorogenic acid derivatives and *cis* 5-*o*-caffeoylquinic acid, PLE time increase causes the decrease of their amount. It can be explained by their lower thermal stability.

Application of increased pressure in the PLE process not only enables the use of an extractant at a temperature above its normal boiling point but also enhances sample matrix penetration by the extracting solvent. The latter advantage of the PLE process is especially important when hard plant matrices, such as grain and corn, are extracted. The PLE results presented above were obtained at the constant pressure of 60 bar. An additional investigation revealed that there were no essential differences in the quantitative and qualitative compositions of PLE extracts of 5-*o*-caffeoylquinic acid in the extraction pressure range of 30−200 bar.

Sample preparation is the crucial step in the procedure of chemical analysis of plant material. Liquid extraction is currently applied most frequently as a sample preparation procedure for plant analysis. The results of this study prove that 5-*o*-caffeoylquinic acid, during its water extraction, not only isomerizes to 3- and 4-*o*-caffeoylquinic acids as previously reported, describing the effect of superheated steam on 5-*o*-caffeoylquinic acid (*20*) or the influence of pH on its transformation (*24*), but also undergoes other transformations such as isomerization, esterification, hydrolysis, and reaction with water, namely, addition of the elements of water. The transformation products can be mistakenly treated as new components, not found before in the examined plant, or can be a cause of erroneous quantitative estimations in plant composition when some or all components forming from 5-*o*-caffeoylquinic acid during its water extraction really exist in the examined plant and the 5-*o*-caffeoylquinic acid transformation process only increases their amounts. In this context, the presented results are important for researchers investigating plant metabolism and looking for new plant components.

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