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Determination of 5-caffeoylquinic acid (5-CQA) as one of the major classes of chlorogenic acid in commercial tea and coffee samples

Određivanje 5-kafeoilhinske kiseline (5-CQA) kao jedne od najznačajnijih klasa hlorogenske kiseline u komercijalnim uzorcima čaja i kafe

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Abstract

Background/Aim. Tea and coffee are one of the most widely consumed beverages in the world due to their beneficial health effects which are largely associated with their phenolic compounds composition, including chlorogenic acid. The main aim of this study was to determine 5-caffeoylquinic acid (5-CQA), as one of the major classes of chlorogenic acid, in various commercial tea and coffee samples present at the Serbian market. **Methods.** A high-performance liquid chromatography (HPLC) method for determination of 5-CQA in plant extracts was applied to determine the content of this active compound in commercial tea and coffee samples. Mobile phase was aqueous 1.5% acetic acid – methanol (80:20, v/v) with the flow rate of 0.8 mL/min. Run time was 15 min and column temperature 25°C. The detection was performed at 240 nm. **Results.** The HPLC method was modified and revalidated. The 5-CQA content varied depending on the type of tea (white, green, black tea and mate) and the processing technology. Green tea had the highest 5-CQA content (16 mg/100 mL) among the analyzed tea samples. The content of 5-CQA in coffee samples ranged 0–36.20 mg/g of coffee and 0–46.98 mg/100 mL of beverage, showing that the content varied depending on the type of coffee, coffee processing technology and the formulation. **Conclusion.** The modified and revalidated HPLC method showed a good accuracy, repeatability, selectivity and robustness. The highest amount of 5-CQA was determined in green tea in comparison to white, black and mate tea because the increased oxidation level decreases the amount of 5-CQA. The obtained results for commercial coffee samples indicated that the formulation was the most important factor determining the amount of 5-CQA. It can be concluded that plant material selection, processing conditions and formulation have great influence on the amount of chlorogenic acid (5-CQA) in the final tea and coffee products.

Key words:
tea; coffee; cinnamates; chromatography, liquid; serbia.

Apstrakt

Uvod/Cilj. Čaj i kafa su jedni od najčešće konzumiranih napitaka širom sveta zahvaljujući svom lekovitom dejstvu koje se u velikoj meri pripisuje fenolnim komponentama koje sadrže, uključujući i hlorogensku kiselinu. Glavni cilj istraživanja bio je određivanje 5-kafeoilhine kiseline (5-CQA), kao jedne od najznačajnijih klasa hlorogenske kiseline, u različitim komercijalnim uzorcima čaja i kafe prisutnih u slobodnoj prodaji na tržištu Republike Srbije. **Metode.** Metoda za određivanje 5-CQA u biljnim ekstraktima primenom visokoefikasne tečne hromatografije (HPLC) korišćena je za ispitivanje sadržaja ove aktivne komponente u komercijalnim uzorcima čaja i kafe. Mobilnu fazu predstavljao je vodeni rastvor 1.5% sirćetne kiseline – metanol (80 : 20, v/v) sa protokom od 0,8 mL/min. Vreme analize iznosilo je 15 minuta, a temperatura analitičke kolone 25°C. Detekcija je vršena na 240 nm. **Rezultati.** HPLC metoda je modifikovana i revalidovana. Sadržaj 5-CQA varirao je u zavisnosti od vrste čaja (beli, zeleni, crni i mate čaj) i tehnološkog postupka prerade. Najveći sadržaj 5-CQA ustanovljen je u uzorku zelenog čaja (16 mg/100 mL). Sadržaj 5-CQA u uzorcima kafe kretao se u opsegu 0–36,20 mg/g kafe i 0–46,98 mg/100 mL napitka ukazujući da je sadržaj zavisio od tipa kafe, tehnološkog postupka prerade i formulacije. **Zaključak.** Modifikovana i revalidovana HPLC metoda pokazala je dobru preciznost, ponovljivost, selektivnost i postojanost (robusnost). Najveći sadržaj 5-CQA u uzorcima čajeva je utvrđen u uzorku zelenog čaja u poređenju sa belim, crnim i mate čajem jer sa većim stepenom oksidacije opada sadržaj 5-CQA. Rezultati dobijeni za uzorke kafe ukazivali su da je na količinu 5-CQA najviše uticala formulacija. Može se zaključiti da selekcija biljnog materijala, kao i uslovi tehnološke obrade i formulacija pokazuju značajan efekat na sadržaj hlorogenske kiseline (5-CQA) u finalnim proizvodima čaja i kafe.

Ključne reči:
čaj; kafa; cinamati; hromatografija, tečna; srbija.

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Introduction

Tea and coffee are among the most widely consumed beverages in the world. It is estimated that about 3 million tons of tea is produced and consumed annually, among which 76% to 78% is black, 20% to 22% is green and less than 2% is oolong [1, 2]. Due to its attractive aroma and pleasant taste it has been used for thousands of years as a refreshing drink, while nowadays the scientific interest in this plant is increasing due to its potential beneficial health effects. Herbal teas are widely used for a great number of health problems, as an additional or sometimes only "medicine" given [3, 4]. Different types of tea are gained from the same *Camellia sinensis* (Theaceae) plant, with different production processes: white and green tea (the least processed, both unfermented), oolong tea (partially fermented) and black tea (fully fermented). Another plant originating from South America whose leaves are important source of purine alkaloids, but also chlorogenic acid, is *Ilex paraguariensis*, Aquifoliaceae (Paraguayan tea or mate) [5–7].

Coffee is a bitter drink made from roasted seeds of plants *Coffea* spp., Rubiaceae. This plant grows in over 70 countries, primarily in equatorial South America, Southeast and South Asia and Africa [8]. Brazil is the largest manufacturer and exporter of green coffee seeds making 35% of the world coffee production. There are several types of coffee species used for commertial production, but the most important are *Coffea arabica* and *Coffea canephora* [9]. After harvest, green coffee beans are processed in one of the three ways: dry ('natural' or 'unwashed' coffee), wet ('washed' or 'parchment' coffee) or semidry method [10].

Both tea leaves and coffee seeds have very diverse chemical composition. Various positive health effects of *Camellia sinensis* and *Coffea* spp. are attributed to the presence of purine alkaloids (caffeine, theobromine and theophylline), but also to their phenolic components. Antioxidant, hypoglycemic, antiviral and hepatoprotective activities are some of the potential beneficial properties associated with the chlorogenic acid and other phenolic compounds. Some studies demonstrated the connection between chlorogenic acid and reduced risk of cardiovascular diseases, diabetes mellitus type 2 and Alzheimer`s disease [11–13]. Farah et al. [14] found that the consumption of green coffee significantly reduced blood pressure, improved vasoreactivity and reduced accumulation of adipose tissue and glucose metabolism due to high content of chlorogenic acid (5–12 g/100g. Similar effects are associated with the tea consumption [11].

The major classes of chlorogenic acids are caffeoylquinic acid (CQA), dicaffeoylquinic acid (diCQA) and less frequently, feruloyilquinic acid (FQA). Each class has at least three isomers. The most abundant and responsible for many beneficial health effects is considered to be 5-CQA [12]. It is the most responsible for pigmentation and the flavour of tea and coffee [15]. During tea fermentation, some of the phenolic compounds, including 5-CQA, are oxidized and tea undergoes changes in color, taste, scent and aroma [16]. Also, when it comes to coffee, it degrades by heat into several different compounds which determine the quality of coffee [17]. Beside the processing method, the type of coffee has great effect on the content of 5-CQA in commercial samples (the content is lower in type *Coffea arabica* than in *Coffea canephora*) [18].

There is a general lack of information on the content of 5-CQA, as one of the major antioxidants, but responsible also for many other health benefits, in commercial tea and coffee preparations of domestic and foreign manufacturers available at the Serbian market. Accordingly, the aim of this study was to apply high-performance liquid chromatography (HPLC) method for chlorogenic acid (5-CQA) determination in herbal infusions for quantifying 5-CQA in commercial tea and coffee samples present at the Serbian market. This research will also give the information on approximate chlorogenic acid (5-CQA) intake by consumers which is important due to its beneficial health effects.

Methods

Standard of 5-CQA (≥ 95%) was purchased from Sigma Aldrich (St. Louis, USA), methanol (95%, v/v) from Sigma (Deisenhofen, Germany) and glacial acetic acid from Zorka Pharma a.d. (Šabac, Serbia). Ultra pure water was used for the preparation of solutions (Milli-Q-quality). All solvents and reagents were of an analytical grade unless indicated otherwise.

Tea samples analyzed in this paper are presented in Table 1. Infusions were prepared by adding 200 mL of boiling water to

Table 1

Commercial tea samples used in the study

Sample number	Sample name	Producer	Serial number
1	Aromatized white tea with melon and orange	Fructus d.o.o., Serbia	002 10383 T2 A
2	White tea	Teekanne, Polland	L16CW02:39K CH:401498
3	Earl Grey – aromatized black tea	Teekanne, Polland	L19LW04:02K CH:402560
4	Indian tea – black tea	Vitamin, Horgoš, Serbia	-
5	Black tea	Kirka Pharma, Novi Beograd, Serbia	-
6	Black tea	Welton, Holland	-
7	Black Earl Grey tea	Winston Tea Company LTD, London, UK	CH:877594 L22 KW03:00 S
8	Green tea	Macval Tea d.o.o. Novi Sad, Serbia	-
9	Green tea	Welton, Holland	-
10	Mate tea	Neiner's Gesundheit & Wellness GmBH	9570; L.: 520902305

each tea bag allowing it to infuse for 10 minutes. After cooling, 2 mL of each extract was filtered through 0.45 μm membrane filter directly into the vial, while 20 μL of filtrate was injected into the high performance liquid chromatography (HPLC) system for analysis.

Coffee samples analyzed in our research are shown in Table 2. Infusions were prepared by the same way as described for the tea samples. Sample preparations used in our study are thoroughly presented and described in recent publications performing similar investigations [19, 20]. These extraction methods were chosen due to similarity with a manufacturer's recommendations for the product preparation, so 5-CQA intake could be evaluated more reliably.

HPLC conditions working standard solutions of 5-CQA (0.1001 – 1.001 mg/mL) were injected into the HPLC system and a linear standard curve was constructed by plotting concentrations *versus* peak areas (y = 251.81 ×–0.994). A high value of coefficient of correlation R = 0.999 showed an excellent correlation between concentrations and peak areas. Limit of detection (LD = 0.0127 mg/mL) and limit of quantification (LQ = 0.0373 mg/mL) were also calculated based on the standard deviation of the response and the slope of the calibration curve. Accuracy of this method was tested by comparing the measured and known values of concentrations for standard solutions of 5-CQA. According to the recovery value of 98.74% the method showed acceptable accuracy. Repeatability of the method was tested by analyzing three different concentrations of 5-CQA standards in six repetitions. The relative standard deviations (RSD) ranged from 0.22% to 0.52% for retention time and from 0.03% to 0.38% for peak area, showing good repeatability. By comparing the chromatograms of 5-CQA standards and tea and coffee samples as well as the obtained signal spectrums, the selectivity of the method was evaluated. The chromatograms showed no other signals with the same retention time as the signal deriving from 5-CQA standard, which confirmed high selectivity of the method. Analyzing the effects of slightly changed parameters of the used HPLC conditions, such as different column temperature (± 1°C), flow rate (± 0.05 mL/min) and wavelength of detection (± 3 nm), the robustness of the method was confirmed.

Table 2

Commercial coffee samples used in the study

Sample number	Sample name	Producer	Serial number
1	Jacobs 2 in 1	Kraft foods, Czech Republic	VM26 10052013
2	Jacobs original 3 in 1	Kraft foods, Czech Republic	VM26 22042013
3	Jacobs intense 3 in 1	Kraft foods, Czech Republic	VM26 05042013
4	Grand coffee 3 in1	Droga Kolinska, Slovenia	18.07.13 16:23 WII
5	Mokate coffee drink 3 in 1	Mokate Sp., Polland	21062013A12
6	Doncafe 3 in1	Strauss coffee, Romania	281011011
7	Grand aroma	Grand prom, Serbia	2811M08 18:22
8	Nescafe 3 in 1 classic	Nestle, Romania	128209731 3
9	Nescafe 3 in 1 strong	Nestle, Hungary	100588384 43336638
10	Nescafe 3 in 1 mild	Nestle, Hungary	128009731 9

The stock solution of 5-CQA was prepared by weighing 100.1 mg of the standard substance and dissolving in 10 mL of methanol (95%, v/v). Working solutions were prepared by diluting 0.1, 0.3, 0.6, 0.8 and 1 mL of the stock solution to 10 mL with methanol to obtain different concentrations of 5-CQA (0.1001–1.001 mg/mL). HPLC analysis was performed using modified and revalidated HPLC method for chlorogenic acid (5-CQA) determination in herbal infusions [21] using different detection wavelength (240 nm).

Qualitative and quantitative determination of 5-CQA in commercial tea samples was carried out using an Agilent HP 1100 HPLC-diode array detection (DAD) system equipped with autosampler (Agilent, Waldbronn, Germany). The analytical column was the Zorbax CB-C18 (4.6 × 150 mm, *id*, 5 μm particle size). Mobile phase was aqueous 1.5% acetic acid-methanol (85 : 15) with a flow rate of 0.8 mL/min. Run time was 15 min and column temperature 25°C. The detection was performed at 240 nm.

HPLC method used for the analysis in our study was developed and validated for chlorogenic acid (5-CQA) determination in mate tea extracts obtained by supercritical carbon dioxide extraction [21]. When applied on tea and coffee infusions, this method showed lower selectivity for coffee samples, so the analysis had to be performed with different mobile phase composition and on a different wavelength (240 nm). Therefore, the applied method needed to be revalidated due to changed chromatographic conditions. Procedures used for re-validation of HPLC method for determination of 5-CQA in commercial tea and coffee samples are described in USP 24 [22] and other literature [23–25]. Under determined

The statistical analyses were done by MS Excel for Windows, v. 2007 software and also by ANOVA (Duncan's test, SPSS, version 17). Level $p < 0.05$ was considered statistically significant.

Results

Representative chromatogram of analyzed commertial tea sample (sample No. 6) is presented in Figure 1. The results of 5-CQA content in commercial tea samples obtained by the revalidated HPLC method are shown in Table 3.

The results of 5-CQA content in the examined coffee samples are presented in Table 4 and the representative chromatogram of the coffee sample No. 2 is shown in Figure 2.

Table 3

Content of 5-coffeoylquinic acid (5-CQA) in the examined tea samples

Sample number	Tea type	5-CQA content (mg/100mL)
1	White tea	0.659 ± 0.003^{b}
2		2.160 ± 0.022^{d}
3	Black tea	2.979 ± 0.009^{d}
4		0.939 ± 0.011^{c}
5		0.327 ± 0.023^{a}
6		0.562 ± 0.008^{b}
7	Green tea	6.110 ± 0.012^{e}
8		7.374 ± 0.013^{e}
9		16.020 ± 0.033^{g}
10	Mate	9.700 ± 0.010^{f}

***Data are expressed as average ± standard deviations of triplicate measurements. Statistically significant differences were noted by different superscript letters ($p < 0.05$). Same letters – no statistically significant differences.**

Table 4

Content of 5-coffeoylquinic acid (5-CQA) in coffee samples

Sample number	Sample name	5-CQA content (mg/100 mL)	5-CQA content (mg/g of coffee)
1	Jacobs 2 in 1	-	-
2	Jacobs original 3 in 1	7.446 ± 0.010^{a}	7.299 ± 0.022^{a}
3	Jacobs intense 3 in 1	46.982 ± 0.033^{e}	36.196 ± 0.068^{e}
4	Grand coffee 3 in1	34.940 ± 0.028^{d}	28.757 ± 0.051^{d}
5	Mokate coffee drink 3 in 1	-	-
6	Doncafe 3 in1	10.012 ± 0.009^{b}	8.939 ± 0.012^{a}
7	Grand aroma	22.188 ± 0.012^{c}	8.875 ± 0.010^{a}
8	Nescafe 3 in 1 classic	9.309 ± 0.011^{b}	11.822 ± 0.009^{b}
9	Nescafe 3 in 1 strong	23.074 ± 0.018^{c}	14.906 ± 0.011^{c}
10	Nescafe 3 in 1 mild	11.245 ± 0.009^{b}	27.112 ± 0.031^{d}

***Data are expressed as average ± standard deviations of triplicate measurements. Statistically significant differences were noted by different superscript letters ($p < 0.05$). Same letters – no statistically significant differences.**

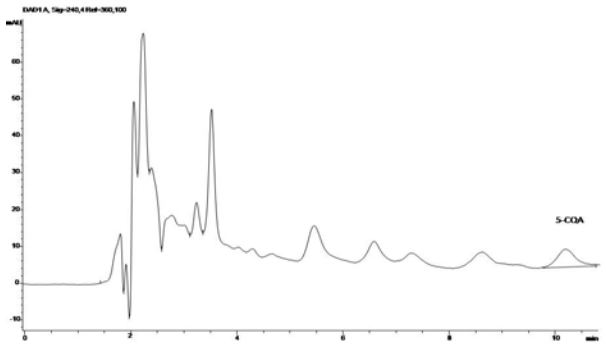

Fig. 1 – High performance liquid chromatography – array detection (HPLC-DAD) chromatogram of the analyzed commercial tea sample (sample number 6).

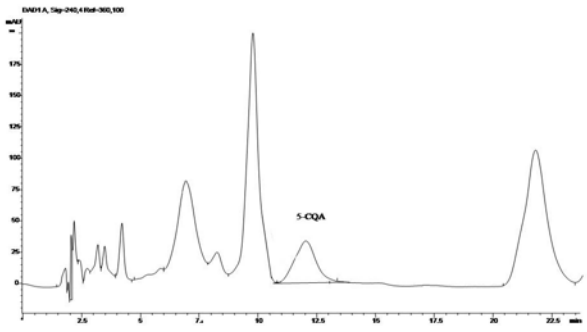

Fig. 2 – Representative high performance liquid chromatography – array detection (HPLC-DAD) chromatogram of the analyzed commercial coffee sample (sample number 2).

Discussion

The results of our study obtained by HPLC analysis of different tea samples showed that the highest amount of 5-CQA was determined in green tea, which was expectable due to its minimal processing during production, where oxidation of phenolic compounds, including chlorogenic acid (5-CQA), was prevented. Differences between the same types of tea, but originating from different producers, were also observed and showed the great effect of the quality of the selected plant material and final formulation. In our study the highest amount of 5-CQA was observed in Welton green tea, which was twice as high as in green tea sample from a domestic producer and many times exceeded the amount of 5-CQA in most of the analyzed samples.

Comparing the results of 5-CQA content in white tea infusions, it was observed that these values were higher than those found in a similar research conducted in Croatia (0.065

– 0.356 mg/100 mL) [26]. The content of 5-CQA in black tea ranged 0.33 – 6.11 mg/100 mL, which was in good agreement with the results obtained in a similar research in UK (6.2 mg/100 mL) [27]. The values for 5-CQA content in green tea were found to be between the results attained in Brazil (0.13 – 0.19 mg/100 mL) [12] and in UK (23.1 mg/100 mL) [27]. In mate tea sample the amount of 5-CQA corresponded to the results obtained in Brazil (5.97 – 12.69 mg/100 mL) [28]. This value was slightly higher than the one established in another research in Brazil (7.5 mg/100 mL) [12] and significantly lower than the result from a recently published study conducted also in Brazil (52.5 mg/100 mL) [29].

These differences can be attributed to different brewing conditions, quality of selected plant material as well as technological processes and formulation [9].

The content of 5-CQA in different coffee samples varied from 0 to 36.20 mg/g of pure coffee (specified on the commercial package). Ky et al. [18] showed that the highest content of 5-CQA was found in samples derived from *Coffea canephora* species, as well as in those processed by the semidry method [18]. The study performed in Brazil in 2008 showed that the 5-CQA content was slightly higher in samples that were treated with semidry method and ranged 31.63 – 48.45 mg/g of coffee than in the samples that were treated with wet method, where the content was from 29.67 to 42.00 mg/g of coffee [30]. Our results also confirmed that coffee species and processing technology of the grain had a notable influence on 5-CQA content.

The concentrations of 5-CQA in our samples ranged 0 – 46.89 mg/100 mL of beverage. Different range of concentrations could be the result of different composition of commercial samples, which is typical for each manufacturer and the product [31]. In a study conducted at the Department of Toxicology at University of California in 2006, 7 commercial coffee samples from the USA were analyzed. The content of 5-CQA ranged from 2.13 to 7.06 mg/g of commercial sample and the content highly depended on the sample composition [32]. Our results were consistent with this study, showing that the formulation of the sample (percentage of coffee) had a major influence on the content of 5-CQA, beside different coffee type and technological processing of the grain. This can be explained by comparing samples number 4 and 10 (Table 4). The mass of the sample number 4 was 18 g (weight of pure coffee was 2.43 g) and the mass of the sample number 10 was 16 g (weight of pure coffee is 0.80 g). Although the content of 5-CQA (mg/g of coffee) was similar, after consumption of sample number 4 higher quantities will enter the human body (commercial sample number 4 contained three times more coffee than sample number 10). Also, lower quality of coffee beans (containing less 5-CQA) can be compensated by making a formulation with greater representation of coffee in relation to other components as shown in Table 4 for samples 9 and 10. Coffee sample number 9 contained significantly less 5-CQA (mg/g of coffee) in comparison to sample number 10, but coffee amount in formulation (3.10 g) was almost four times higher than in sample number 10 (0.80 g). The results showed that the consumption of sample number 9 could lead to two times higher intake of chlorogenic acid (5-CQA).

Conclusion

During this study a HPLC method was modified, revalidated and successfully applied for 5-CQA determination in commercial tea and coffee samples. The method showed good accuracy, repeatability, selectivity and robustness. HPLC analysis of tea samples showed that the highest amount of 5-CQA was obtained in green tea in comparison to white, black and mate tea because the increased oxidation level decreases the amount of 5-CQA. The results of coffee samples analysis indicated that the content of 5-CQA depended on the type of coffee (*Coffea canephora* species contained more 5-CQA), the technological processing of coffee (higher amount was determined in coffee treated with semi-dry process) and the formulation. The obtained results of this study led to a conclusion that adequate plant material selection, processing, brewing conditions and product formulation have the major influence on the amount of chlorogenic acid (5-CQA) in the final tea and coffee products.

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