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Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used

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ABSTRACT

The efficiencies of different solvents in the extraction of phenolics from bagged and loose leaves of white and green tea, after different extraction times, as well as the antioxidative capacity of the obtained extracts, were investigated. The developed HPLC method has the potential to separate and determinate 17 phenolics widely distributed in plants, but in investigated tea extracts only four catechins and traces of three flavonols and one flavone were separated and detected based on comparison with authentic standards. The extraction efficiency of phenolics depended strongly on the time of extraction and the solvents used. The extraction of catechins from green tea was significantly affected by the form (bagged or loose) of the tea, whereas this effect was shown not to be statistically significant for white tea. Green tea was a richer source of phenolics than was white tea. The extraction of phenolics from white tea by water could be accelerated by the addition of lemon juice. Aqueous ethanol (40%) was most effective in the prolonged extraction of catechins. The antioxidative capacity of the investigated tea extracts correlated with their phenolic content.

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1. Introduction

Phenolics are secondary plant metabolites that are involved in a wide range of specialized physiological functions. They appear to be very important for the normal growth, development and defence mechanisms of plants (Rusak, Krajačić, & Pleše, 1997). These compounds are capable of modulating the activity of many enzymes (Di Carlo, Mascolo, Izzo, & Capasso, 1999), suggesting their involvement in biochemical and physiological processes, not only in plants, but also in animals and humans. The major phenolics present in tea (*Camellia sinensis)* leaves are flavan-3-ols (also known as catechins), which constitute up to 30% of their dry weight. Depending on the stereochemical configuration of the 3′,4′-dihydroxyphenyl and hydroxyl groups at the 2- and 3-positions of the C-ring, catechins can exist as two isomers: *trans*-catechins and *cis*-epicatechins. Each of them exists as two optical isomers: (+)- catechin and (−)-catechin and (−)-epicatechin and (+)-epicatechin, respectively. (−)-Catechin can be modified by esterification with gallic acid to form (−)-catechin-3-gallate, (−)-epicatechin-3-gallate, (−)-epigallocatechin-3-gallate and (−)-gallocatechin-3-gallate (Friedman et al., 2005). Although catechins are the dominant phenolic compounds (Kilmartin & Hsu, 2003), various flavonols (up to 4%) and flavones (in traces) are also present in the tea leaves. The main flavonols in tea are conjugates of quercetin and kaempferol with conjugating moiety varying from mono- to di- and triglycosides (Del Rio et al., 2004). Other related compounds found in tea are gallic, coumaric and caffeic acids, as well as the purine alkaloids, theobromine and caffeine.

Tea, known as the most popular beverage in the East, arouses great interest among scientists due to its beneficial health effects. Tea flavonoid consumption has been linked to lower incidences of chronic diseases such as cardiovascular disease and cancer. It has been shown, in different cell lines and animal models, that tea flavonoids inhibit cell proliferation, induce cell cycle arrest and apoptosis, stimulate angiogenesis and affect cell signalling pathways (Henning et al., 2005). The health benefits associated with tea consumption have been attributed in part to the antioxidant and free radical-scavenging activity of the most abundant tea flavonols. Tea is generally consumed in the form of green, black, oolong or white tea. To produce green tea, the young leaves are rolled and steamed to minimize oxidation. In the production of black tea, after the leaves are rolled, which disrupts cellular compartmentation and brings phenolic compounds into contact with polyphenol oxidases, the young *C. sinensis* leaves undergo oxidation (referred to as fermentation) for 90–120 min. During this period, catechins are converted to complex condensation products, the theaflavins and their polymers, thearubigins.

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Oolong tea is produced with a shorter fermentation period than black tea and has a taste and colour somewhere between green and black teas (Del Rio et al., 2004). White tea is prepared from very young tea leaves or buds covered with tiny, silvery hairs, which are harvested only once a year in the early spring. White tea is steamed and dried immediately after picking to prevent oxidation, giving it a light, delicate taste. In spite of numerous data about the phenolic constituents, antioxidant activity and ameliorating effects of green and black tea on human health, little is known in this sense about white tea, which is the rarest and the least processed tea.

All analytical methods for quantifying the biologically active compounds present in tea leaves involve extraction, separation and analysis. Various extraction conditions and analysis methods have been used, resulting in a wide variation in the measured concentrations of tea compounds. Qualitative analysis of these compounds has typically involved the use of a variety of high-performance liquid chromatography (HPLC) systems, with absorbance or diode array detection, in which each system is tailored to the separation of only a limited number of the many phenolic compounds in tea (Del Rio et al., 2004).

The main objectives of this study were (1) to determine the effects of different extraction conditions, solvents and forms of tea (bagged or loose leaf) used on the quantitative and qualitative content of phenolics in tea infusions; (2) to establish possible correlations between the antioxidant capacity of tea infusions and the extraction methods and forms of tea and solvents used; (3) to compare the qualitative and quantitative phenolic contents and antioxidant capacity of green tea infusions with those of white tea; (4) to validate a new HPLC method for the analysis of tea flavanols (catechins), flavonols, flavones and phenolic acids in a single run.

2. Materials and methods

2.1. Chemicals

The formic acid, sodium carbonate, hydrochloric acid, ferric chloride hexahydrate, ammonium peroxodisulphate and Folin-Ciocalteu reagent were of analytical grade and supplied by Kemika (Zagreb, Croatia). The methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker (Deventer, Netherlands). The formaldehyde was obtained from Alkaloid (Skopje, Macedonia). 2,2′-Azinobis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylcroman-2-carboxyl acid (trolox), 2,4,6- tripyridyl-S-triazine (TPTZ), as well as (−)-epigallocatechin-3-gallate, (−)-epicatechin-3-gallate, (−)-epicatechin, (−)-gallocatechin-3-gallate, (−)-gallocatechin, (+)-catechin, gallic acid, caffeic acid, genistein, naringenin, pinocembrin and quercetin, were supplied from Sigma–Aldrich (Steinheim, Germany). The chrysin, coumaric acid, daidzein, galangin, isorhamnetin, kaempferol, luteolin and myricetin were purchased from Fluka (Buchs, Switzerland).

2.2. Preparation of tea extracts

The white (Pai Mu Tan Superior) and the green (Long Jing) teas in bagged and loose leaf form were analysed. Tea samples were purchased at a special market – House of tea (Zagreb, Croatia). The samples (2 g) were infused in 200 ml of: (a) distilled water (80 °C), (b) distilled water (80 °C) with 5 ml of freshly squeezed lemon juice, and (c) aqueous ethanol (10%, 40% and 70%). Extraction with aqueous ethanol was carried out by pouring the required amount of water (80 °C) over the tea samples and then adding absolute ethanol at room temperature up to 200 ml to obtain the required concentration of ethanol. After extraction (5, 15 or 30 min), the infusions were filtered through a tea strainer.

2.3. Hydrolysis of tea extracts

A mixture of 1 ml of filtered tea extract and 4 ml of hydrochloric acid (2 M) was boiled in a water bath for 30 min. After cooling, the mixture was extracted three times with diethyether (4 + 4 + 3 ml). The ethereal phases were collected and evaporated. Residue was dissolved in 1 ml of 96% ethanol, filtered through the nylon filter (0.22 μm) and stored at −20 °C.

2.4. Determination of total phenols and flavonoids

Total polyphenol content (TP) was determined spectrophotometrically using Folin–Ciocalteu's reagent according to a modified method of Lachman, Hosnedl, Pivec, and Orsak (1998). The method is based on the reduction of phosphotungstic acid ($H_3/P[W_3O_{10}]_4$ in alkaline solution to phosphotungstic blue (based on $WO_2 \cdot nWO_3$). The absorbance of formed phosphotungstic blue is proportional to the number of aromatic phenolic groups and is used for their determination, expressed with gallic acid as the calibrant (Singleton, Orthofer, & Lamuela-Raventos, 1999). To determine the content of flavonoids (TF) these compounds are precipitated using formaldehyde, which reacts with C-6 or C-8 on 5,7-dihydroxy flavonoids to form methyl derivates that further react with other flavonoid compounds also at positions C-6 and C-8. The condensed products of these reactions are removed by filtration and the remaining non-flavonoid phenols are determined as previously described (Kramling & Singleton, 1969).

Briefly, 0.5 ml of the sample was pipetted into a 50 ml volumetric flask containing 2.5 ml of Folin–Ciocalteu's reagent, 30 ml of distilled water and 7.5 ml of 20% Na_2CO_3, and the volume was made up with distilled water. During the oxidation of phenolic compounds, phosphomolybdic and phosphotungstic acid, contained in the Folin–Ciocalteu's reagent, were reduced to blue-coloured molybdenum and tungsten oxides. After two hours, the absorbance of blue colouration was measured at 765 nm against a blank sample. Gallic acid was used as the standard and the results expressed as mg/l of gallic acid equivalents (GAE). Flavonid content was calculated as the difference between total phenol and non-flavonoid contents (Kramling & Singleton, 1969). All measurements were performed in triplicate.

2.5. Determination of ferric reducing/antioxidant power (FRAP assay)

The ferric reducing/antioxidant power (FRAP) assay was carried out according to Benzie and Strain (1996). The FRAP assay is based on the reduction of the Fe^{3+}-2,4,6-tripyridyl-S-triazine complex to the ferrous form (Fe^{2+}) and the intensity of the reaction is monitored by measuring the change of absorption at 593 nm. A free radical ABTS scavenging method reported by Re et al. (1999) was used to measure the total antioxidant capacity of tea extracts. The blue colour released due to the formation of ABTS free radicals ($ABTS^{*+}$) after mixing ABTS stock solution and potassium persulfate is sensitive to the presence of antioxidants. The discolouration following the sample addition indicates that ABTS radical cations were quenched or reduced by the antioxidants in the sample (Pellegrini et al., 2003).

A FRAP reagent was prepared by mixing acetic buffer, TPTZ and $FeCl_3 \times 6 \cdot H_2O$ (20 mM water solution) at a ratio of 10:1:1. Briefly, to a volume of 200 μl of tea extract, 3.8 ml of FRAP reagent were added. After 4 min, the absorbance of blue colouration was measured against a blank sample. A standard curve was prepared using different concentrations (100–1200 μM) of Fe^{2+}. All measurements were performed in triplicate.

2.6. Determination of free radical-scavenging ability

The free radical-scavenging activity of tea extracts was determined by ABTS radical cation ($ABTS^{*+}$) decolourization assay (Re et al., 1999). $ABTS^{*+}$ was produced by mixing 5 ml of ABTS water stock solution (7 mM) with 88 μl of potassium persulfate (140 mM). Before use, the mixture was incubated at room temperature in the dark for 12–16 h. Freshly-prepared $ABTS^{*+}$ working solution ($ABTS^{*+}$ stock solution diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm) was used. To a volume of 2 ml of $ABTS^{*+}$ working solution, 100 μl of tea extract were added and, after one minute of incubation, the absorbance was measured at 374 nm. Ethanol, as a solvent blank, was run in each assay. All measurements were performed in triplicate and expressed as mmol/l of trolox.

2.7. HPLC analysis of phenolics

We performed qualitative and quantitative HPLC analyses of the catechins, (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatehin gallate (GCG) and (−)-epicatechin gallate (ECG), the flavonols: quercetin, kaempferol, myricetin, galangin and isorhamnetin, the flavones: luteolin and chrysin, the flavanones: pinocembrin and naringenin, and the isoflavones, daidzein and genistein, as well as caffeic and coumaric acid. An HPLC system (Agilent 1100 Series) equipped with a quaternary pump, multiwave UV/Vis detector, autosampler, fraction collector and 5 μm Zorbax RX-C18 (250 × 4.6 mm, Agilent Technologies) column was used. Injection volume was 10 μl and the constant flow rate was 1.0 ml/min. Phenolic compounds were identified by UV/Vis spectroscopy and by HPLC chromatography with authentic standards. The multiwave UV/Vis detector was set at 268 nm, 280 nm, 374 nm, 310 nm and 350 nm. A three-solvent gradient elution was performed. The solvent compositions used were (A), water–ACN–formic acid (94:5:1 v/v), (B), water–ACN–methanol–formic acid (50:24.5:24.5:1 v/v) and (C), ACN–formic acid (99:1 v/v). Prior to each run, the system was equilibrated to 90/10/0 (A/B/C). The solvent composition changed according to the following gradient: 90/10/0 at 0 min, 70/30/0 at 10 min, 0/100/0 at 20 min, 0/0/100 at 36 min and 0/0/100 at 41 min. Concentrations of investigated phenolics were determined, based on the chromatographic data of the standards. The calibration curves (peak area vs. concentration) for individual compounds were obtained for a wide concentration range.

2.8. Statistical analysis

All measurements and analyses were carried out in triplicate. The results were analysed statistically using the Statistica 6.0 program to determine the average value and standard error. Variance analysis, with a significance level of $\alpha = 0.05\%$ was performed to determine the effect of the solvent, the time of extraction, and the form of tea on the content of extracted polyphenols. Correlation analysis was also run with the same statistical package.

3. Results and discussion

The efficiencies of different solvents (water, mixture of water and lemon juice, 10%, 40% and 70% ethanol) in the extraction of total polyphenols and flavonoids from leaves of white and green tea (bagged and loose form) after different extraction times (5, 15, 30 min) and the antioxidative capacity of the obtained extracts were investigated. Qualitative and quantitative HPLC analyses of the phenolics in these extracts were also performed.

White tea is composed of only the youngest spring buds and immature leaves, so it represents a rare and high-priced type of tea, almost unknown outside of Asia. This has resulted in a low number of studies on chemical composition of white tea and its benefits for human health. In the past two decades, only a few papers dealing with the white tea have been published, compared to a huge number of articles on green tea.

The contents of TP and TF in white and green tea extracts obtained in our study, by the extraction of bagged and loose leaf forms using different extraction solvents at different extraction times, are presented in Figs. 1 and 2. Our results confirmed previously published reports (Cheong, Park, Kang, Ko, & Seo, 2005; Lachman, Hosnedl, Pivec, & Orsak, 2003) that the total content of polyphenols in tea extracts correlates with extraction time and reaches its maximum after 30 min of extraction, but it is plausible that the efficiency of tea extraction, especially of white tea, could be further increased by longer extraction time. Literature data confirm this presumption for green tea (Cheong et al., 2005) but there are no data about effects of different extraction conditions on extraction of bioactive compounds from white tea. Our investigations were focussed on shorter times of extraction (up to 30 min) to simulate extraction conditions usually used for extraction of tea at home. Depending on the time of extraction and the solvent used, TP ranged from 759 to 2377 mg/l as GAE in green tea and from 423 to 2141 mg/l as GAE in white tea. The contents of TF in green and white tea extracts significantly depend on time of extraction and varied from 431 to 1768 and from 218 to 1786 mg/l as GAE, respectively. These results indicate that green tea is a richer source of phenolics than is white tea, but the extraction efficiency of these compounds is highly dependent on the time of extraction and the solvents used. We showed that the extraction of TP and TF from white tea leaves is much slower than the extraction of the same compounds from green tea leaves and that this effect depends on the solvent used. The highest content of TP in extracts of both studied teas was established after 30 min of extraction, but green tea extracts reached a significantly higher TP content, especially flavonoids, in the first 5 min of extraction than did white tea extracts, in the same extraction time. This effect

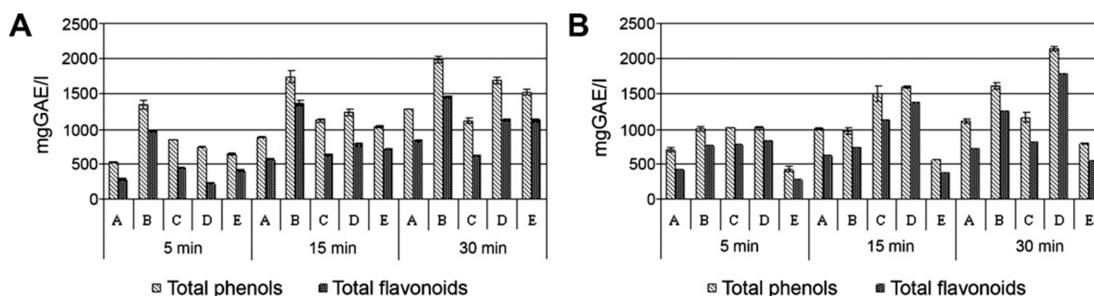

Fig. 1. Total phenol and flavonoid contents in extracts of loose (A) and bagged (B) leaf forms of white tea obtained by different solvents (A = water; B = water + lemon juice; C = 10% ethanol; D = 40% ethanol; E = 70% ethanol) after 5, 15 and 30 min of extraction. Results are expressed as mg GAE/l ± SD.

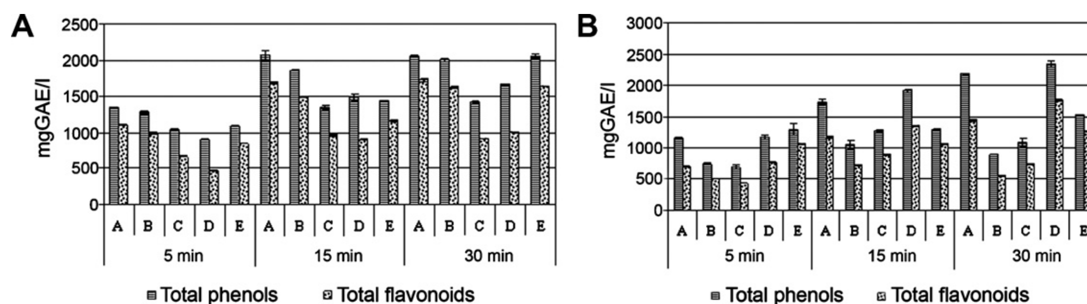

Fig. 2. Total phenol and flavonoid contents in extracts of loose (A) and bagged (B) leaf forms of green tea obtained by different solvents (A = water; B = water + lemon juice; C = 10% ethanol; D = 40% ethanol; E = 70% ethanol) after 5, 15 and 30 min of extraction. Results are expressed as mg GAE/l ± SD.

was observed for all solvents used (water, 10%, 40% and 70% ethanol) except for the mixture of water and lemon juice, which was the most effective solvent in the extraction of phenolics of white tea in the first 5 min of extraction. This indicates that the extraction of phenolic compounds from white tea leaves by water could be accelerated by the addition of lemon juice. We supposed that the acceleration of polyphenol extraction from white tea after the addition of lemon juice could be due to the change in pH of the extraction solvent. Our presumption is based on the findings of Friedman and Jürgens (2000). They pointed out that the change in pH could have an influence on the migration kinetic of catechins, since the presence of OH groups on catechins implies a susceptibility to the effect of pH and thus, an ionization of the molecule. Our results also confirmed previously published data (Liang & Xu, 2001) that the solids yield of extraction increased when tea was extracted at lower pH. White tea is prepared from very young tea leaves or buds covered with tiny, silvery hairs and we supposed that the migration kinetic of hydrophilic catechins in white tea could also be affected by trichomes, the cell walls of which are covered with a lipophilic cuticle.

Aqueous ethanol (40%) was shown to be the most effective extraction solvent in prolonged extraction (30 min) for both teas in bagged form. On the other hand, water and water-lemon juice mixture were the most efficient solvents for polyphenol extraction from loose and bagged forms of green, as well as white tea, in the first 5 min of extraction. There was no significant difference in phenolic content between white and green tea extracts after 5 min of extraction (usually the recommended infusion time for tea preparation) if a water-lemon mixture was used for the extraction of white tea and water for the extraction of green tea.

Water, water-lemon and ethanol (10%) green tea extracts of loose leaves have been shown to have significantly higher contents of TP and especially of TF than have the same extracts obtained from bagged tea. A higher concentration of ethanol in the extraction solvent (40%) caused a higher yield of TP and TF from bagged green tea leaves than from loose leaves, especially after prolonged extraction (30 min). Our results showed that there were some differences in the efficiency of 70% ethanol extraction of TP and TF from loose and bagged green tea leaves which were dependent on extraction time. In the first 5 min of extraction, 70% ethanol was more efficient in extraction of TP and TF from bagged green tea leaves while, after 30 min of extraction, this solvent was more effective in the extraction of these compounds from loose green tea leaves. The extraction patterns of loose and bagged white tea leaves were also shown to be dependent on extraction time and solvents used, but they differed from those of green tea. Unlike green tea extracts, the contents of TP and TF in water and 10% ethanol extracts of bagged white tea leaves were higher (after 15 min of extraction) or almost equal (after 30 min of extraction) than those of loose white tea leaves. In general, water-lemon juice was the most effective solvent used, except for extraction of bagged green tea leaves, where ethanol was shown to be the most effective extraction solvent (Figs. 1 and 2).

Our quantitative HPLC analysis of catechins, the dominant phenolics in tea leaves, also confirmed the fact that green tea is a richer source of phenolics than is white tea. The concentrations of all of the investigated catechins (EGC, EGCG, GCG, ECG) were significantly higher in green tea leaves than in those of white tea. (−)-epicatechin (EC) was also detected in both teas investigated, but it was overlapped with a peak of EGCG and could not be quantitatively determined. HPLC analysis confirmed that 40% ethanol was most effective, among the solvents tested, for the prolonged extraction (30 min) of catechins, especially in the extraction of EGCG, the dominant catechin in tea leaves (Table 1). Some other phenolic compounds, not only the catechins, could also contribute to the antioxidant capacity of tea extracts. Among the other phenolics investigated (quercetin, kaempferol, myricetin, galangin, isorhamnetin, luteolin, chrysin, pinocembrin, naringenin, daidzein, genistein, caffeic and coumaric acid), only myricetin, quercetin, luteolin and kaempferol were detected, in traces, in most of the investigated extracts. The concentrations of myricetin, quercetin and kaempferol were significantly higher (110, 280 and 310 μg/g of dry tea, respectively) in the 40% ethanolic green tea extract obtained after 30 min of extraction and after hydrolysis, indicating that these compounds are predominantly present in tea leaves in glycolsylated form.

Our analysis revealed that the extraction efficiency of catechins from green tea was significantly affected by the form (bagged or loose) of tea used, whereas this effect was not shown to be statistically significant for white tea. It was shown that extraction of these compounds from loose green tea leaves was more effective than was extraction from bagged green tea leaves.

Various extraction conditions and analysis methods have been used in previous studies, resulting in a variety of measured concentrations of tea compounds. Lee and Ong (2000) measured 4 catechins and theaflavins in 8 teas sold in Singapore using HPLC. The mobile phase used consisted of acetonitrile/trifluoroacetic acid. Lin, Tsai, Tsay, and Lin (2003) used an isocratic HPLC procedure, with a mobile phase consisting of ethanol/water/formic acid, to determine caffeine and 5 catechins in 31 Taiwanese tea leaves and flowers. Khokhar and Magnusdottir (2002) used HPLC with acetonitrile as the eluent to determine the content of 5 catechins and caffeine in 4 black, 3 green and 6 fruit teas. Cabrera, Gimenez, and Lopez (2003) used an HPLC method to measure the levels of 4 catechins and caffeine in 15 black, green and oolong teas sold in Spain. Friedman et al. (2005) have developed an HPLC method to analyse 13 compounds from 77 commercial teas in a single run using acetonitrile/potassium dihydrogen phosphate as the mobile phase. In this study 17 phenolics in water and aqueous ethanol extracts of white and green teas were analysed in a single run. To this end the HPLC–UV detection method was adapted, after extensive experimentation, to optimize the analysis, from previously

Table 1
Contents of 4 catechins [(−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatehin gallate (GCG), (−)-epicatechin gallate (ECG)] in green and white teas extracted with water, water + lemon juice, 10%, 40% and 70% ethanol/water solution

Solvent	Time[a]	Green tea (bagged leaf form)				Green tea (loose leaf form)				White tea (bagged leaf form)				White tea (loose leaf form)			
		EGC	EGCG	GCG	ECG	EGC	EGCG	GCG	ECG	EGC	EGCG	GCG	ECG	EGC	EGCG	GCG	ECG
Water	5	32.8 ± 0.9●	70.9 ± 0.8■*	4.9 ± 0.4□	14.9 ± 0.06○	42.3 ± 0.4●	88.2 ± 1.4■	6.3 ± 0.4□*	18.4 ± 0.05○	19.4 ± 0.6	46.0 ± 1.02	1.2 ± 0.12*	9.2 ± 0.2	17.9 ± 0.5	38.9 ± 1.82	1 ± 0.052*	7.5 ± 0.1
	15	36.6 ± 0.7●	88.3 ± 1.2■*	5.8 ± 0.4□	18.9 ± 0.1○	64.5 ± 0.8●	121 ± 1.3■	8.6 ± 0.1□*	25.1 ± 0.1○	31.6 ± 0.8	65.2 ± 0.9	2.0 ± 0.1*	13.6 ± 0.2	26.3 ± 0.4	56.5 ± 0.9	2.0 ± 0.2*	11.4 ± 0.3
	30	39.2 ± 0.7●	82.1 ± 1.5■*	5.9 ± 0.2□	16.6 ± 0.1○	58.0 ± 0.9●	118 ± 1.1■	8.5 ± 0.1□*	24.5 ± 0.1○	36.2 ± 0.8	69.3 ± 1.2	2.5 ± 0.2*	14.2 ± 0.1	38.9 ± 0.9	66.8 ± 1.5	3.3 ± 0.2*	13.8 ± 0.1
Water + lemon juice	5	23.6 ± 0.8	54.3 ± 0.7	3.5 ± 0.3	10.6 ± 0.2	25.5 ± 0.6	56.5 ± 1.2	3.9 ± 0.05	11.4 ± 0.2	20.9 ± 0.7	49.0 ± 1.4	1.0 ± 0.05*	9.8 ± 0.5	24.0 ± 0.4	52.6 ± 1.2	1.1 ± 0.1*	10.7 ± 0.2
	15	28.6 ± 0.8	73.8 ± 1.3	4.6 ± 0.3	15.6 ± 0.2	29.0 ± 0.6	72.4 ± 3.1	4.3 ± 0.3	14.8 ± 0.2	22.8 ± 0.5	50.4 ± 1.6	1.8 ± 0.05*	10.3 ± 0.5	21.3 ± 0.1	53.8 ± 1.5	1.1 ± 0.1*	10.8 ± 0.3
	30	37.8 ± 0.4	87.9 ± 0.8	5.6 ± 0.6	17.3 ± 0.1	47.7 ± 0.8	100 ± 0.5	6.6 ± 0.2	19.8 ± 0.6	33.9 ± 0.9	77.5 ± 1.4	2.3 ± 0.1*	15.7 ± 0.3	29.0 ± 0.7	74.3 ± 1.5	1.4 ± 0.05*	15.3 ± 0.7
10% ethanol	5	38.8 ± 0.4	85.1 ± 2.2*	5.8 ± 0.2	17.7 ± 0.4	52.7 ± 0.6	130 ± 2.7*	8.2 ± 0.1	27.6 ± 0.3	22.8 ± 1.1	69.5 ± 3.2*	1.1 ± 0.04□	14.7 ± 0.6	28.2 ± 1.0	91.9 ± 3.4*	1.6 ± 0.05□	20.8 ± 0.5
	15	60.2 ± 0.6	153 ± 1.4*	9.1 ± 0.1	33.1 ± 0.5	65.3 ± 0.4	155 ± 1.7*	9.8 ± 0.5	34.7 ± 0.5	32.0 ± 0.9	105 ± 2.7*	1.7 ± 0.1□	23.2 ± 0.8	33.5 ± 0.8	115 ± 2.1*	2.1 ± 0.2□	26.6 ± 0.5
	30	71.2 ± 0.6	149 ± 1.4*	9.6 ± 0.4	30.8 ± 0.8	68.7 ± 0.8	160 ± 2.2*	10.3 ± 0.4	36.2 ± 0.5	35.1 ± 1.3	81.0 ± 2.2*	2.6 ± 0.1□	17.0 ± 0.6	32.0 ± 0.2	111 ± 0.9*	3.0 ± 0.1□	26.1 ± 0.7
40% ethanol	5	33.6 ± 0.4	94.5 ± 1.6■	6.0 ± 0.3*	20.6 ± 0.9○	38.5 ± 0.2	115 ± 1.2■	6.8 ± 0.05*	26.4 ± 0.4	20.2 ± 0.4	68.8 ± 3.1	0.8 ± 0.1	15.9 ± 0.3	22.5 ± 0.4	72.1 ± 2.9	0.9 ± 0.1	15.8 ± 0.3
	15	51.8 ± 0.4	155 ± 2.4■	8.7 ± 0.3*	35.8 ± 1.4○	56.1 ± 0.2	180 ± 0.8■	9.7 ± 0.2*	41.7 ± 0.4	29.3 ± 0.4	116 ± 1.7	1.5 ± 0.2	27.5 ± 0.7	34.3 ± 0.6	129 ± 2.2	2.0 ± 0.1	29.4 ± 0.3
	30	59.6 ± 0.1	187 ± 1.8■	10.2 ± 0.4*	43.1 ± 1.7○	68.7 ± 0.4	205 ± 3.4■	11.9 ± 0.2*	48.3 ± 0.9	42.3 ± 0.6	154 ± 2.2	2.0 ± 0.2	36.6 ± 0.7	36.6 ± 0.3	144 ± 4.2	2.5 ± 0.4	34.3 ± 0.5
70% ethanol	5	27.8 ± 0.3	98.7 ± 2.3*	5.3 ± 0.2□	23.6 ± 1.9	27.8 ± 0.6	93.5 ± 1.7*	5.5 ± 0.4□	21.7 ± 1.2*	12.9 ± 0.2	40.2 ± 1.1	0.5 ± 0.05	9.6 ± 0.3	14.4 ± 0.5	44.9 ± 1.3	0.5 ± 0.04*	10.6 ± 0.4
	15	36.2 ± 0.2	109 ± 2.8*	6.1 ± 0.4□	25.2 ± 1.7	39.3 ± 0.8	119 ± 1.9*	6.7 ± 0.2□	29.2 ± 1.3*	21.3 ± 0.3	63.9 ± 2.6	0.6 ± 0.1	15.2 ± 0.3	26.3 ± 0.8	80.5 ± 2.7	0.8 ± 0.05*	18.9 ± 0.2
	30	42.4 ± 0.8	120 ± 4.6*	7.3 ± 0.4□	28.0 ± 1.6	52.3 ± 1.2	169 ± 2.9*	9.5 ± 0.6□	40.5 ± 1.3*	24.8 ± 0.5	90.2 ± 1.7	0.9 ± 0.05	22.3 ± 0.4	31.3 ± 0.7	113 ± 3.5	1.5 ± 0.1*	26.9 ± 0.6

All investigated samples contained only traces of myricetin, luteolin, quercetin and kaempherol while other investigated substances (caffeic acid, coumaric acid, daidzein, naringenin, genistein, isorhamnetin, pinocembrin, chrysin and galangin) were not detected.
Values are expressed as means in mg/g ± SD ($n = 3$) of dry tea.
The same symbols (●, ■, □, ○) denote the catechin, whose content is significantly ($p < 0.05$) affected by the form of tea extracted by specific solvent.
* denotes the catechin, whose content is not significantly ($p < 0.05$) affected by time of extraction with the specific solvent.
[a] Time in minutes.

described procedures (Dalluge, Nelson, Brown Thomas, & Sander, 1998; Friedman et al., 2005; Neilson, Green, Wood, & Ferruzzi, 2006). Using a 5 μm Zorbax RX-C18 (250 × 4.6 mm, Agilent Technologies) column and a three-solvent gradient elution profile, 17 phenolic compounds were separated (see Section 2). The elution positions of a mixture of the 17 standards and chromatograms of representative extracts of green and white teas with the highest content of polyphenols (40% ethanol, 30 min of extraction) are shown in Fig. 3A–C. The developed HPLC method allows simultaneous separation and quantification of 17 phenolic compounds, including catechins, flavonols, flavones and phenolic acids. Although only four chatechins, three flavonols and one flavone were detected in investigated tea leaves, this method could be applied for analysis of all of these bioactive compounds in other samples. Moreover, using this method, luteolin and quercetin were successfully separated by HPLC without the addition of tetrahydrofuran in the mobile phase, which was postulated to be necessary for separation of these two compounds (Wang & Huang, 2004).

In general, tea leaves contain high amounts of polyphenols, among which catechins prevail, and these compounds are the best investigated among all substances present in tea. In comparison with catechins, little is known about other phenolic compounds

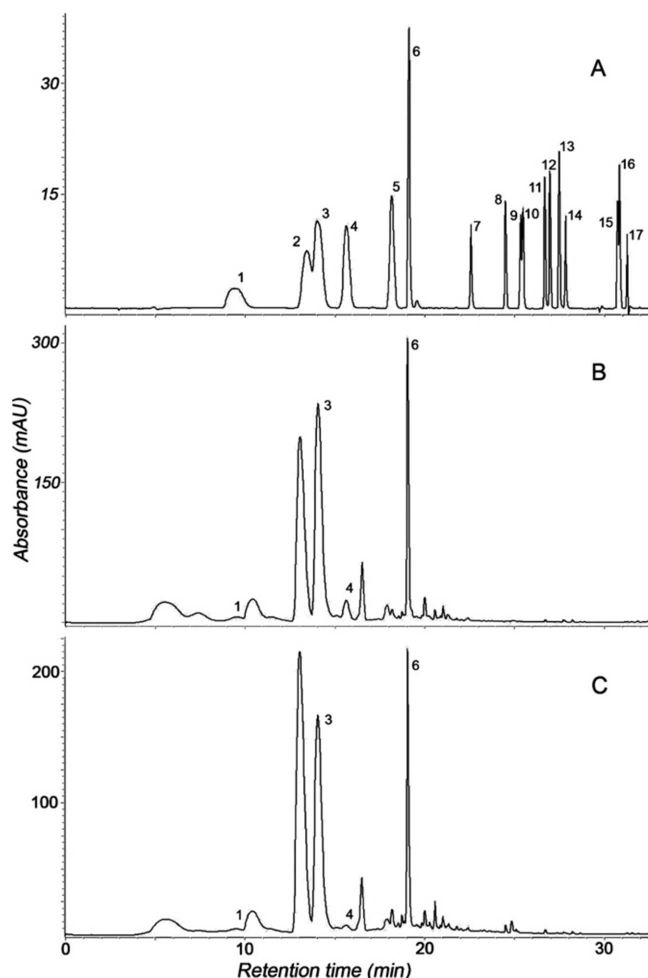

Fig. 3. HPLC chromatograms of a mixture of phenolic standards which were investigated (A), representative extracts of green (B) and white teas (C) obtained using a gradient elution system (see Section 2) by UV detection at 280 nm. 1 (−)-epigallocatechin; 2 caffeic acid; 3 (−)-epigallocatechin gallate; 4 (−)-gallocatechin gallate; 5 coumaric acid; 6 (−)-epicatechin gallate; 7 myricetin; 8 daidzein; 9 quercetin; 10, luteolin; 11 naringenin; 12 genistein; 13 kaempherol; 14 isorhamnetin; 15 pinocembrin; 16 chrysin; 17 galangin.

Table 2
Antioxidant capacity of extracts of loose and bagged leaf forms of white tea obtained by different solvents (*A* = water; *B* = water + lemon juice; *C* = 10% ethanol; *D* = 40% ethanol; *E* = 70% ethanol) after 5, 15 and 30 min of extraction

	Loose leaf form						Bagged leaf form					
	5 min		15 min		30 min		5 min		15 min		30 min	
	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}
A	3.63 ± 0.19	4.02 ± 0.03	5.81 ± 0.16	8.23 ± 0.23	7.56 ± 0.04	10.8 ± 0.07	5.19 ± 0.03	6.29 ± 0.07	6.14 ± 0.33	9.07 ± 0.12	6.73 ± 0.16	10.1 ± 0.32
B	8.27 ± 0.09	15.2 ± 2.90	9.59 ± 0.59	16.3 ± 0.41	10.9 ± 0.04	17.9 ± 0.18	6.64 ± 0.04	8.73 ± 0.28	5.75 ± 0.25	7.14 ± 0.23	8.23 ± 0.41	11.2 ± 0.09
C	5.95 ± 0.14	10.1 ± 0.13	6.73 ± 0.22	12.8 ± 0.36	7.41 ± 0.14	12.9 ± 0.46	6.41 ± 0.27	9.34 ± 0.16	7.64 ± 0.21	11.3 ± 0.11	6.10 ± 0.24	9.02 ± 0.03
D	4.59 ± 0.09	7.91 ± 0.23	8.29 ± 0.07	14.4 ± 0.32	9.78 ± 0.01	17.3 ± 0.61	5.77 ± 0.27	8.45 ± 0.50	8.92 ± 0.12	12.3 ± 0.39	10.3 ± 0.05	16.1 ± 0.45
E	3.45 ± 0.02	4.84 ± 0.17	6.19 ± 0.05	8.30 ± 0.23	8.99 ± 0.06	16.7 ± 0.59	3.86 ± 0.09	5.25 ± 0.01	5.12 ± 0.08	7.56 ± 0.07	5.86 ± 0.23	7.45 ± 0.40

The results are expressed as averages of three independent measurements ± SD.
A significant linear correlation was confirmed between antioxidant capacity (obtained by ABTS and FRAP assays) and the total phenolic content of the extracts of loose (r_{ABTS} = 0.96, r_{FRAP} = 0.88) and bagged (r_{ABTS} = 0.94, r_{FRAP} = 0.92) white tea.

Table 3
Antioxidant capacity of extracts of loose and bagged leaf forms of green tea obtained by different solvents (A = water; B = water + lemon juice; C = 10% ethanol; D = 40% ethanol; E = 70% ethanol) after 5, 15 and 30 min of extraction

	Loose leaf form						Bagged leaf form					
	5 min		15 min		30 min		5 min		15 min		30 min	
	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}	mmol/l trolox	mmol/l Fe^{2+}
A	5.68 ± 0.19	5.73 ± 0.28	10.1 ± 0.36	14.9 ± 0.09	9.77 ± 0.50	16.3 ± 0.03	4.59 ± 0.27	5.70 ± 0.02	10.7 ± 0.32	13.3 ± 0.84	14.3 ± 0.22	16.0 ± 0.11
B	5.91 ± 0.59	6.93 ± 0.16	8.82 ± 0.13	11.5 ± 0.14	10.5 ± 0.32	15.6 ± 0.19	1.11 ± 0.31	4.45 ± 0.10	2.95 ± 0.41	6.18 ± 0.18	2.41 ± 0.18	5.41 ± 0.18
C	4.82 ± 0.56	5.14 ± 0.09	5.48 ± 0.07	5.48 ± 0.21	6.55 ± 0.14	10.3 ± 0.27	1.05 ± 0.22	6.00 ± 0.32	5.73 ± 0.05	8.95 ± 0.27	3.77 ± 0.22	6.14 ± 0.23
D	3.95 ± 0.41	2.45 ± 0.13	6.05 ± 0.13	9.43 ± 0.34	8.05 ± 0.40	13.4 ± 0.05	4.27 ± 0.09	6.68 ± 0.32	11.4 ± 0.14	16.1 ± 0.27	14.5 ± 0.20	19.0 ± 0.05
E	6.31 ± 0.27	6.31 ± 0.17	8.18 ± 0.22	11.8 ± 0.07	10.3 ± 0.09	16.6 ± 0.11	6.85 ± 0.13	9.14 ± 0.19	8.15 ± 0.22	10.3 ± 0.02	8.47 ± 0.11	11.5 ± 0.14

The results are expressed as averages of three independent measurements ± SD.
A significant linear correlation was confirmed between antioxidant capacity (obtained by ABTS and FRAP assays) and the total phenolic content of the extracts of loose (r_{ABTS} = 0.90, r_{FRAP} = 0.89) and bagged (r_{ABTS} = 0.97, r_{FRAP} = 0.94) green tea.

in tea leaves. Therefore a wide spectrum of flavonoids (flavonols, flavones, flavanones, isoflavones) and phenolic acids, which are widely distributed in plants, was included in our investigation of bioactive constituents in tea leaves.

Both methods used (FRAP and ABTS assays) revealed significant antioxidative capacities of investigated tea extracts (Tables 2 and 3). Since the methods used to measure antioxidant activity are extremely dependent on the reaction conditions and the substrates or products, not all methods yield the same values for the activity (Fukumoto & Mazza, 2000). In order to obtain the most relevant data about the antioxidant capacity of tea extracts, two different methods were used in this study. In general, the extracts of green tea have shown higher free radical-scavenging ability. Both methods used confirmed that the highest antioxidant activity was in the 40% ethanol extract of bagged green tea leaves after 30 min of extraction (14.5 mg/l trolox, 18.9 mmol/l $FeSO_4 \times 7 \cdot H_2O$), whereas, the lowest antioxidant activity was exhibited by the 70% ethanol extract of loose white tea leaves (3.45 mmol/l trolox, 4.84 mmol/l $FeSO_4 \times 7 \cdot H_2O$) after 5 min of extraction. The values of the antioxidant capacity of the extracts obtained by both FRAP and ABTS assays were in accordance with the contents of TP and TF. A significant linear correlation was confirmed between the TP content and the antioxidant capacity of the extracts of loose (r_{ABTS} = 0.96, r_{FRAP} = 0.88) and bagged (r_{ABTS} = 0.94, r_{FRAP} = 0.92) white tea, as well as between the TP content and antioxidant capacity of the extracts of loose (r_{ABTS} = 0.90, r_{FRAP} = 0.89) and bagged (r_{ABTS} = 0.97, r_{FRAP} = 0.94) green tea.

In many studies published so far, a plethora of solvents, temperatures and extraction times have been used to extract compounds from tea leaves. Therefore, comparison of data from studies using different extraction methods may not always be justified. There is a need to standardize extraction/analysis methods and units of measurement, preferably extraction with boiling water for 5 min to simulate the home use of teas, analysis by HPLC and units that report the results in mg/g of original weight of tea or as mg/l of tea infusions (Friedman et al., 2005). Although tea is generally consumed after water infusion, isolated tea extracts and individual tea compounds are widely used to prepare dietary supplements. Detailed knowledge of the composition of tea extracts will allow consumers, researchers and producers of dietary supplements to select teas and extracts with the highest content of bioactive substances. The notion that dietary supplements may provide good protection against many human diseases, including cancer and cardiovascular diseases, has received significant support from a number of studies. Most recently, the relationship between tea consumption and the prevention of certain forms of human cancer has received a great deal of attention, although epidemiological studies concerning the effect of tea consumption on human cancer risk have been inconsistent (Dalluge et al., 1998).

4. Conclusions

Green tea is a richer source of phenolics than is white tea, but the extraction efficiency of these compounds strongly depends on the time of extraction and the solvents used. The extraction of total polyphenols and flavonoids from white tea leaves is much slower than is the extraction of the same compounds from green tea leaves but the extraction of these phenolics from white tea by water could be accelerated by addition of lemon juice. In this case, there is no significant difference in phenolic content between white and green tea extracts after 5 min of extraction (usually the recommended infusion time for tea preparation). HPLC analysis of catechins, which are the dominant phenolics in tea leaves, confirmed the fact that green tea is a richer source of

phenolics than is white tea. This analysis also confirmed that 40% ethanol is the most effective among the solvents tested in the prolonged extraction of catechins, especially in the extraction of EGCG, the dominant catechin in tea leaves. The extraction efficiency of catechins from green tea was significantly affected by the form (bagged or loose) of tea used, whereas this effect was shown to be not statistically significant for white tea. The extraction of these compounds from loose green tea leaves was more effective than was extraction from bagged tea leaves. In general, the extracts of green tea show a higher free radical-scavenging ability. A statistical linear correlation was confirmed between the total polyphenol content and the antioxidant capacity of the extracts of both teas investigated.

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