Bioactive compounds and antioxidant capacity of yellow Yinzhen tea affected by different extraction conditions

TEA MIHELJ – ANA BELŠČAK-CVITANOVIĆ – DRAŽENKA KOMES – DUNJA HORŽIĆ – VLASTA TOMAŠIĆ

Summary
Tea is one of the most popular beverages consumed worldwide. Polyphenols in tea contribute to its health benefits possessing high antioxidant, anticarcinogenic and antiallergic activity. The aim of this study was to examine the effect of ethanol concentration, tea forms and storage duration on polyphenols and methylxanthines composition, as well as antioxidant capacity of yellow Yinzhen tea. Total polyphenol, flavonoid concentration and antioxidant capacity were measured spectrofotometrically, while individual catechins and methylxanthines were identified and quantified using high performance liquid chromatography with photodiode array detector (HPLC-PDA). DPPH$^{\bullet}$ (reduction of 1,1-diphenyl-2-picrylhidrazyl), ABTS$^{\bullet+}$ (reaction of 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) and FRAP (reaction with iron(III)tripyridyltriazine) assays were applied to determine the antioxidant capacity. The highest polyphenol concentration and antioxidant capacity were determined in 75% ethanol tea extract stored for 24 h. The application of lower ethanol concentration (50% or less) was the most efficient in individual catechins extraction. The most abundant catechin was epigallocatechin gallate (from 12.60 ± 0.1 mg·l^{-1} to 416.01 ± 2.70 mg·l^{-1}), followed by the epigallocatechin (from 1.59 ± 0.10 mg·l^{-1} to 264.92 ± 2.43 mg·l^{-1}).

Keywords
Yinzhen tea; ethanol; storage duration; polyphenols; methylxantines

Tea is one of the most widely consumed beverages in the world. Its production and consumption grow rapidly, not only because of the health promoting effects, but also due to stimulative effects and desirable sensory properties. The least known type of tea is the minimally fermented version, known as yellow tea. This tea is gradually gaining recognition in Western countries, with the most popular variety of Junshan Yinzhen from Hunan province (China). It is often equalized with green tea because of the very similar antioxidant concentration [1]. However, the slow steaming during processing gives it desired yellowish colour by breaking down and partly oxidizing the chlorophyll in the leaves and removing the grassy taste characteristic of green tea. The aroma of yellow tea is then flowery, fresh, mild and is often described as being somewhere between white tea and green tea.

The chemical composition of tea is very complex, since it contains polyphenols, methylxanthines, amino acids, saccharides, proteins, chlorophyll, volatile compounds, minerals such as manganese and potassium [2], and other undefined compounds. Horžić et al. [3] studied the effect of extraction techniques on yellow tea composition. Although the quantity of polyphenols and methylxanthines varied with the extraction procedure and conditions, yellow tea proved to be a rich source of bioactive compounds, similar to other, extensively examined types of tea [3]. Yellow tea was found to be particularly rich in flavonoids, among which flavan-3-ols are generally the most abundant. Epigallocatechin gallate (EGCG) is the most prominent compound in this group, while cate-

Tea Mihelj, Vlasta Tomašić, Department of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia.
Ana Belščak-Cvitanović, Draženka Komes, Dunja Horžić, Department of Food Processing and Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia.

Correspondence author:
Draženka Komes, tel.: +385 1 4826 250, fax: +385 1 4826 251, e-mail: dkomes@pbf.hr

chin and gallocatechin (GC) are the least abundant. Methylxanthines were also identified and quantified, with caffeine at the highest concentration, and theobromine and theophylline at lower concentrations [3].

Since polyphenols and methylxanthines are the main bioactive compounds in tea, significant effort has been made to find an efficient procedure for their extraction. The composition and nature of polyphenols determine the choice of the extraction conditions [4]. Most extraction techniques manipulate the solvents according to their physical properties in order to reduce the surface tension, increase solubility of the solutes, promote a higher diffusion rate and sometimes to change the solvent polarity. Besides the simple solid-liquid extraction, previous studies reported microwave-assisted extraction [5], ultrasound-assisted extraction [3, 6], or usage of supercritical carbon dioxide [7]. Water has been often used to simulate household brewing conditions for a cup of tea [5, 8]. Moreover, alcoholic solvents such as ethanol [5] and other organic solvents have been used for this purpose. Although alcoholic solvents are not highly selective for polyphenols unlike other organic solvents, the use of ethanol is preferable due to the possible applications of the extracts in food production. Ethanol is a non-toxic, food grade solvent, acceptable in small residual percentages according to good manufacturing practice (GMP). Furthermore, it extracts polyphenols better than water; it can be mixed with water in different ratios and can be much easier evaporated in commercial manufacturing of the extract. Previous studies on green tea showed that the extraction of polyphenols and caffeine from green tea leaves was greatly influenced by the ethanol concentration [5]. Due to the lack of data on yellow tea, the aim of this study was to find the most effective hydroalcoholic ratio in aqueous ethanol mixture as the solvent for the extraction of polyphenols and methylxanthines of yellow Yinzhen tea, depending on the form of tea (loose leaf, bagged, tea infuser) and storage duration.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu's reagent, sodium carbonate, iron (III) chloride hexahydrate and formic acid were supplied by Kemika (Zagreb, Croatia). Iron (II) sulphate heptahydrate was purchased from Gram-mol (Zagreb, Croatia). Nitrogen gas was obtained from Messer Croatia Plin (Zapresic, Croatia). DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhidrazyl), as well as Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), potassium peroxodisulphate and TPTZ (2,4,6-tris(2-pyridyl)-*s*-triazine) were obtained from Fluka (Buchs, Switzerland). ABTS$^{\bullet+}$ (2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)diammonium salt), gallic acid (3,4,5-trihydroxybenzoic acid), (–)-epicatechin (EC), (–)-gallocatechin (GC), (–)-gallocatechin gallate (GCG) (–)-epicatechin gallate (ECG), epigallocatechin (EGC) and theaflavine were obtained from Aldrich (Sigma–Aldrich Chemie, Steinheim, Germany). Formaldehyde (35%), acetic acid and sodium acetate trihydrate were obtained from Alkaloid (Skopje, Macedonia). Hydrochloric acid (37%) and ethanol (96%) were obtained from Carlo Erba Reagents (Cornaredo, Italy). Methanol (HPLC gradient grade) was obtained from J. T. Baker (Deventer, Netherlands).

Sample preparation

Yinzhen tea from Hunan province (China) analysed in this study was purchased in a specialized tea store, House of tea (Zagreb, Croatia). We used tea leaves in loose leaf form, bagged form and in metal infuser to prepare extracts. An amount of 2.0 g of tea leaves was poured with 200 ml of aqueous ethanol (10%, 25%, 50%, 75%) or absolute ethanol, and heated to the boiling temperature of the solvent. Loose leaf tea was stirred occasionally for 3 min and, after that, filtered through a tea strainer. In order to establish the changes of total polyphenol concentration, as well as the composition of biologically active compounds and antioxidant capacity, the prepared extracts were examined after 1 h, 2 h, 4 h, 6 h and 24 h of storage at room temperature.

Determination of total polyphenol concentration and total flavonoid concentration

Total polyphenol concentration (TPC) was measured spectrophotometrically using Folin-Ciocalteu's reagent according to the modified method of Lachman et al. [9]. Briefly, 2.5 ml of Folin-Ciocalteu's reagent, 30 ml distilled water and 7.5 ml 20% Na_2CO_3 were added to 0.5 ml of sample and diluted to 50 ml with distilled water. After 2 h, the absorbance of blue colouration was measured at 765 nm against the blank sample. Total flavonoids were precipitated using formaldehyde and separated from the solution by filtration. The filtrate contained the non-flavonoid polyphenols that were determined by Folin-Ciocalteu method as described previously. The difference between total polyphenol and non-flavonoid concentration was taken as the total flavonoid concentration (TFC). All measurements were performed in triplicate

and expressed as milligrams per litre of gallic acid equivalents (GAE).

HPLC analysis of polyphenol compounds and methylxanthines

HPLC analysis of samples was performed with Varian HPLC system (Varian, Walnut Creek, California, USA) consisting of Pro Star Solvent Delivery System 230, Pro Star 330 photodiode array detector (PDA) and controlled by Star Chromatography Workstation Version 5 software (Varian). Separation was performed using a reversed-phase Pinnacle II-C18 column (Restek, Bellefonte, Pennsylvania, USA; 250 mm × 4.6 mm × 5 μm). The samples were filtered through a 0.45 μm membrane filter (nylon membranes; Supelco, Bellefonte, Pennsylvania, USA). The mobile phases used were 2% formic acid (mobile phase A) and HPLC grade methanol (mobile phase B) at a flow rate of 1 ml·min^{-1}. The elution was performed with a gradient starting at 2% B to reach 32% B at 10 min, 40% B at 20 min and 95% B at 30 min, and becoming isocratic for 5 min. Chromatograms were recorded at 278 nm. PDA detection was performed by recording the absorbance of the eluate between 200 nm and 400 nm, with a resolution of 1.2 nm. Polyphenol compounds and methylxanthines were identified by comparation of the retention times and spectral data with those of authentic standards. All analyses were performed in triplicate.

Determination of antioxidant capacity

Determination of free radical-scavenging ability by the use of DPPH$^\bullet$ radical

The samples were analysed according to the method of BRAND-WILLIAMS et al. [10]. Briefly, a volume of 3.8 ml of methanolic DPPH$^\bullet$ solution, $c_{(DPPH\bullet)}$ = 0.094 mmol·l^{-1}, was added to 200 μl of the diluted sample. Free radical-scavenging capacity of the sample was determined by measuring the absorbance decrease at 517 nm after 30 min of incubation, compared to the blank sample (pure methanol instead of the sample). Antioxidant capacity was expressed as millimoles of Trolox equivalents per litre, using the calibration curve of Trolox (0–1000 μmol·l^{-1}), a water-soluble vitamin E analogue. All determinations were performed in triplicate.

Determination of free radical-scavenging ability by the use of ABTS$^{\bullet+}$ radical cation

The free radical-scavenging activity of tea extracts was also determined using the ABTS$^{\bullet+}$ radical cation decolourization assay [11]. An amount of 20 μl of tea extract was added to 2.0 ml of the ABTS$^{\bullet+}$ radical solution, and the absorbance readings were taken after exactly 6 min against the appropriate reagent blank instead of the sample. The results obtained from triplicate analyses were expressed as Trolox equivalents, and derived from a calibration curve determined for this standard (100–1000 μmol·l^{-1}).

Determination of ferric reducing/antioxidant power

The ferric reducing/antioxidant power (FRAP) assay was carried out according to BENZIE and STRAIN [12]. Briefly, to a volume of 200 μl of tea extract, 3.8 ml of FRAP reagent, iron(III) tripyridyltriazine, was added and, after 4 min, the absorbance of blue colouration was measured against a blank sample. All measurements were performed in triplicate and expressed as millimoles of Fe^{2+} per litre.

Statistical analysis

The results were statistically analysed using Statistica 6.0 software (Statsoft, Hamburg, Germany) to determine the average value, standard error and analysis of variance by two-way ANOVA.

RESULTS AND DISCUSSION

Fig. 1 presents changes of total flavonoid (TFC), total non-flavonoid (TNC) and total polyphenol concentration (TPC, obtained as the sum of TFC and TNC) of Yinzhen tea extracts, after 1 h, 2 h, 4 h, 6 h and 24 h of storage at room temperature. Storage duration and ethanol concentration showed significant influence ($p < 0.05$) on TFC, TNC and TPC. Taking into account all used forms of tea leaves and concentrations of ethanol, TFC, TNC as well as TPC increased during prolonged storage for the majority of extracts, and reached the highest ratio after one-day storage. TPC ranged from 38.56 ± 0.80 mg·l^{-1} (2 h, absolute ethanol) to 2890.91 ± 37.27 mg·l^{-1} (6 h, 75% ethanol) for loose leaf tea, from 65.45 ± 1.33 mg·l^{-1} (6 h, absolute ethanol) to 2691.21 ± 36.03 mg·l^{-1} (24 h, 75% ethanol) for the infuser leaf form, and from 34.09 ± 2.91 mg·l^{-1} (1 h, absolute ethanol) to 2491.01 ± 29.46 mg·l^{-1} (24 h, 75% ethanol) for tea in the bagged leaf form. Depending on the ethanol concentration, TPC of loose leaf form tea during prolonged storage decreased in the following order: 75% ethanol extract > 50% ethanol extract > 10% ethanol extract > 25% ethanol extract > absolute ethanol extract. The ranking of polyphenol concentration during prolonged storage of infuser

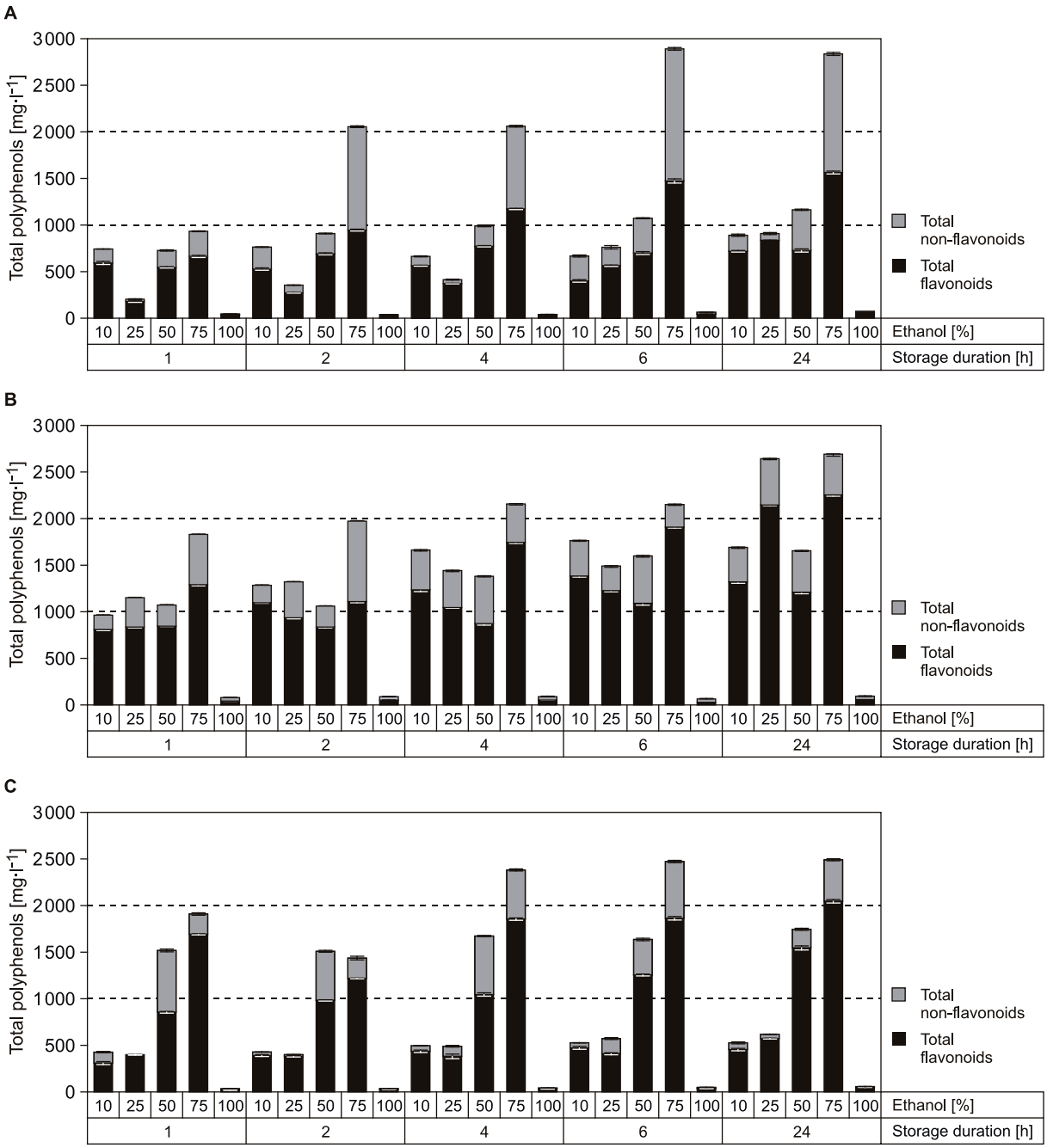

Fig. 1. Changes in total flavonoid, non-flavonoid and total polyphenol concentrations in the extracts from different forms of Yinzhen tea at different ethanol concentrations and storage durations.

A – loose leaf tea, B – infuser tea, C – bagged tea.
Total polyphenol concentration is the sum of flavonoids and non-flavonoids. Concentration of total polyphenols, flavonoids and non-flavonoids is expressed in milligrams of GAE per litre.

leaf form extracts was quite different, but 75% ethanol extracts exhibited the highest values, followed by 10%, 25%, 50% ethanol and absolute ethanol as the solvent with very poor extraction efficiency. Similar to results for other two forms of tea, 75% ethanol was the most efficient and absolute ethanol the least efficient for the extraction of polyphenols from bagged yellow tea, while 50% ethanol was a relatively good solvent for this purpose. The obtained results indicate the selectivity in the extraction of polyphenol compounds depending on the form of tea leaves. Unlike leaves in tea infuser or in a bag, loose leaf form allows "free movement" of leaves that, in combination

with stirring, results in a higher concentration of bioactive compounds in the extract. Additionally, as seen in Fig. 1, flavonoids exerted the main quantitative effect on TPC and, again, 75% ethanol was the most effective solvent for their extraction from yellow tea, among all used tea forms. In our study, TFC of 75% ethanol loose leaf form extract increased from 663.64 ± 3.98 $mg \cdot l^{-1}$ after 1 h to 1563.64 ± 15.72 $mg \cdot l^{-1}$ after 1 day storage. Tea in infuser and bagged leaf form extracted with the same solvent had much higher TFC than of loose leaf (Fig. 1). Among all employed hydro-alcoholic mixtures, absolute ethanol was the least effective solvent for the preparation of flavonoid-rich extracts. Water, ethanol or acetone were studied with some other plants [13, 14]. Wang and Helliwell [15] reported aqueous ethanol as the most effective for flavonoid extraction form tea, which was in accordance with the findings of Horžić et al. revealing that the best extraction performance for yellow tea was achieved with 75% ethanol as the extraction medium [3]. The explanation of our results on TPC and TFC lies in the nature of the solvent. The results were greatly affected by the water: ethanol ratio. Since water is more polar than ethanol, the addition of a certain amount of water improved the extraction efficiency, due to the potential of water to increase the polarity of other solvent in this case ethanol [16]. The second explanation lies in the fact that ethanol, as a less polar solvent, is more efficient in cell walls degradation, that are of non-polar character, and causes the release of polyphenols from cells [17]. The addition of water causes an increase in swelling of the plant material by water, which also enables better contact surface area between ethanol and the matrix. During extraction using aqueous ethanol with the concentration of ethanol lower than 75%, water contributes to increased extraction of other compounds and, as a consequence, a lower concentration of total polyphenols and flavonoids is present in these extracts [18]. In similar studies, the best extraction performance for yellow tea was achieved with the use of an ultrasound probe, or by a combination of 75% ethanol and conventional extraction at high temperatures [3]. Those results are in accordance with the highest TPC, TFC and TNC of 75% ethanol yellow tea extracts obtained in our study.

Catechins (flavan-3-ols) are the major polyphenol constituents of tea and, in this study, (–)-EGCG, (+)-GC, (+)-catechin and (–)-EGC were quantified. Tab. 1, 2 and 3 summarize the concentration of flavan-3-ols and gallic acid in Yinzhen tea extracts depending on solvents, tea form and storage duration. Storage duration did not show a significant ($p > 0.05$) effect on the concentration of majority of flavan-3-ols, but it influenced the concentration of gallic acid ($p < 0.05$). The concentration of the solvent showed the inverse effect, i.e. it significantly affected the concentration of a majority of flavan-3-ols ($p < 0.05$), but not gallic acid concentration ($p > 0.05$). The most abundant catechin during one day storage was EGCG, ranging from 13.27 ± 0.13 $mg \cdot l^{-1}$ to 416.01 ± 2.70 $mg \cdot l^{-1}$ in loose leaf, from 12.60 ± 0.1 $mg \cdot l^{-1}$ to 352.60 ± 2.30 $mg \cdot l^{-1}$ in infuser and from 13.21 ± 0.15 $mg \cdot l^{-1}$ to 351.20 ± 3.30 $mg \cdot l^{-1}$ in bagged tea. The concentration of EGC also varied with the tea form and the solvent, ranging from 1.59 ± 0.10 $mg \cdot l^{-1}$ (absolute ethanol extract of loose leaf form after 2 h of storage) to 264.92 ± 2.43 $mg \cdot l^{-1}$ (25% ethanol extract of loose leaf form after 4 h of storage). Among all used solvents, the highest concentration of EGCG was determined in 50% ethanol extracts of all used tea forms. The EGCG concentration found in 50% ethanol extracts relating to the form of tea decreased in the following order: loose leaf > bagged leaf > infuser leaf form. Solvents with ethanol percentage lower than 50% were superior for the recovery of other identified catechins. This was in accordance with their extremely polar and hydrosoluble nature, pointing again to the importance of the chosen hydroalcoholic solvent ratio. Only the least polar catechin was determined in higher amounts in extracts prepared using 75% ethanol as the solvent, with the exception of bagged leaf form. Absolute ethanol extracts did not contain GC and gallic acid (Tab. 1–3). It was interesting to see that, in the majority of tested extracts, the expected increase of catechins proportionally with the prolonged storage was not observed (as it was the case for TPC and TFC, Fig. 1) and, additionally, fluctuations of their values, or even the highest concentration after some shorter period of storage were detected. This could be explained by the great abundance and variability of all tea constituents, which participate in various reactions during storage of tea extracts, such as polymerization or degradation of some compounds [8]. Moreover, the diverse solvent efficiency in extracting total polyphenols (Fig. 1) and individual catechins (Tab. 1–3) was also observed. These results can be explained by the chemical nature of plant polyphenols, which ranges from simple to highly polymerized substances, as complexes with saccharides, proteins and other plant components, or some high molecular weight polyphenols and their complexes that are insoluble. Also, the selectivity of Folin-Ciocalteu's reagent is small [19] and causes its reactions not only with polyphenols but

Tab. 1. Concentration of gallic acid and flavan-3-ols in loose leaf form extracts of Yinzhen tea at different ethanol concentrations and storage durations.

Storage duration [h]	Ethanol concentration [%]	GA [mg·l^{-1}]	GC [mg·l^{-1}]	EGC [mg·l^{-1}]	EGCG [mg·l^{-1}]	C [mg·l^{-1}]
1	10	5.52 ± 0.51^{a}	77.40 ± 0.90^{A}	139.11 ± 1.51^{B}	345.26 ± 2.01^{C}	32.11 ± 0.11
	25	11.72 ± 0.36^{a}	55.85 ± 1.08^{A}	106.20 ± 1.50^{B}	334.38 ± 2.32^{C}	26.33 ± 0.59
	50	7.34 ± 0.23^{a}	31.42 ± 0.69^{A}	128.69 ± 1.09^{B}	416.01 ± 2.70^{C}	43.35 ± 0.90
	75	nd	21.09 ± 0.37^{A}	59.31 ± 0.59^{B}	304.62 ± 2.27^{C}	54.53 ± 0.70
	100	nd	nd	12.54 ± 0.10^{B}	59.62 ± 1.10^{C}	20.17 ± 0.29
2	10	4.89 ± 0.30^{a}	71.23 ± 0.75^{A}	125.26 ± 0.41^{B}	339.73 ± 3.15^{C}	35.07 ± 0.11
	25	11.43 ± 0.12^{a}	66.94 ± 0.62^{A}	113.97 ± 1.11^{B}	327.31 ± 1.83^{C}	30.93 ± 0.35
	50	7.35 ± 0.28^{a}	30.48 ± 0.61^{A}	130.52 ± 2.33^{B}	407.62 ± 2.91^{C}	39.05 ± 0.62
	75	nd	22.48 ± 0.43^{A}	60.51 ± 0.31^{B}	225.52 ± 2.80^{C}	40.47 ± 1.59
	100	nd	nd	1.59 ± 0.10^{B}	53.95 ± 0.92^{C}	8.61 ± 0.13
4	10	4.74 ± 0.19^{a}	82.25 ± 0.67^{A}	129.94 ± 2.7^{B}	333.75 ± 2.00^{C}	23.44 ± 0.21
	25	11.51 ± 0.89^{a}	71.61 ± 0.45^{A}	264.92 ± 2.3^{B}	342.75 ± 0.71^{C}	27.08 ± 0.11
	50	7.38 ± 0.35^{a}	29.01 ± 0.34^{A}	135.00 ± 1.2^{B}	399.56 ± 2.45^{C}	40.71 ± 0.62
	75	nd	19.07 ± 0.12^{A}	50.23 ± 0.9^{B}	241.87 ± 1.55^{C}	50.93 ± 0.90
	100	nd	nd	nd	44.77 ± 0.63^{C}	13.45 ± 0.32
6	10	6.03 ± 0.30^{a}	88.14 ± 0.83^{A}	144.36 ± 2.82^{B}	345.89 ± 2.31^{C}	30.89 ± 0.87
	25	12.12 ± 0.89^{a}	72.96 ± 1.32^{A}	150.76 ± 1.15^{B}	344.35 ± 3.13^{C}	36.64± 0.42
	50	7.33 ± 0.65^{a}	25.15 ± 0.72^{A}	132.01 ± 1.91^{B}	401.31 ± 1.65^{C}	36.22 ± 0.12
	75	nd	21.40 ± 0.63^{A}	73.72 ± 1.11^{B}	220.67 ± 1.08^{C}	58.49 ± 0.99
	100	nd	nd	1.90 ± 0.20^{B}	58.41 ± 0.60^{C}	86.93 ± 1.31
24	10	9.56 ± 0.58^{a}	70.85 ± 0.92^{A}	158.18 ± 1.90^{B}	366.40 ± 3.61^{C}	32.09 ± 0.32
	25	38.10 ± 0.41^{a}	nd	165.07 ± 3.04^{B}	344.39 ± 2.31^{C}	47.71± 0.42
	50	7.38 ± 0.24^{a}	28.52 ± 0.31^{A}	116.75 ± 0.81^{B}	364.58 ± 2.70^{C}	33.22± 0.45
	75	nd	20.63 ± 0.12^{A}	37.17 ± 0.32^{B}	223.57 ± 1.55^{C}	40.10 ± 0.63
	100	nd	nd	nd	13.27 ± 0.13^{C}	nd

GA – gallic acid, Flavan-3-ols denoted as: GC – (+)-gallocatechin, EGC – (–)-epigallocatechin, EGCG – (–)-epigallocatechin-3-gallate, C – (+)-catechin, nd – not detected.
The same lowercase letter (a) denotes the concentrations of biologically active compounds, which were significantly ($p < 0.05$) influenced only by the storage duration. The same uppercase letters (A–C) denote the concentrations of biologically active compounds, which were significantly ($p < 0.05$) influenced only by the ethanol concentration.

also with other reducing compounds such as carotenoids, amino acids, saccharides and vitamin C [20]. The presence of polymerized polyphenol compounds that can interfere with the determination of polyphenol compounds also affect the TPC and TFC determination and, therefore, result in over-estimation of their concentration when compared to individual polyphenols.

Methylxanthines are also important biologically active compounds of tea, with caffeine (CF), theobromine (TB) and theophylline (TP) as constituents. Extraction methods were previously studied [3], pointing to the conventional extraction as the most effective for yellow tea methylxanthines. In our study, Fig. 2 presents the changes in the concentration of methylxanthines in conventionally extracted Yinzhen tea during 24 h of storage at room temperature, depending on the solvent used as well as on the form of tea. Unlike ethanol concentration ($p < 0.05$), storage duration did not show a significant effect on the concentration of methylxanthines ($p > 0.05$) in tea extracts. CF was quantitatively the most abundant methylxanthine in all tea extracts, ranging from 11.40 ± 0.09 mg·l^{-1} (absolute ethanol extract of bagged tea after 4 h of storage) to 473.89 ± 2.80 mg·l^{-1} (25% ethanol extract of loose leaf form after 6 h of storage). The extraction form of tea leaves and the solvent selectively affected the extraction of CF, whose concentration generally increased during storage (with

Tab. 2. Concentration of gallic acid and flavan-3-ols in infuser leaf form extracts of Yinzhen tea at different ethanol concentrations and storage durations.

Storage duration [h]	Ethanol concentration [%]	GA [mg·l^{-1}]	GC [mg·l^{-1}]	EGC [mg·l^{-1}]	EGCG [mg·l^{-1}]	C [mg·l^{-1}]
1	10	1.87 ± 0.12^{b}	71.17 ± 0.72^{D}	117.32 ± 1.50^{E}	243.97 ± 1.32^{F}	19.01 ± 0.15^{G}
	25	8.41 ± 0.65^{b}	55.12 ± 0.91^{D}	104.08 ± 1.59^{E}	291.37 ± 2.71^{F}	30.80 ± 0.32^{G}
	50	1.85 ± 0.36^{b}	29.66 ± 0.45^{D}	94.09 ± 1.25^{E}	303.73 ± 2.56^{F}	31.21 ± 0.81^{G}
	75	27.82 ± 0.98^{b}	17.54 ± 0.32^{D}	86.20 ± 0.78^{E}	266.86 ± 2.87^{F}	nd
	100	nd	nd	2.90 ± 0.10^{E}	21.94± 0.08^{F}	nd
2	10	1.84 ± 0.10^{b}	60.11 ± 1.21^{D}	126.34 ± 1.21^{E}	247.30 ± 1.9^{F}	18.38 ± 0.14^{G}
	25	5.29 ± 0.84^{b}	59.20 ± 0.67^{D}	95.99 ± 1.10^{E}	285.66 ± 1.3^{F}	28.74 ± 0.62^{G}
	50	1.88 ± 0.10^{b}	27.84 ± 0.36^{D}	126.50 ± 1.23^{E}	331.32 ± 2.1^{F}	28.33 ± 0.83^{G}
	75	27.20 ± 0.56^{b}	19.81 ± 0.09^{D}	77.47 ± 0.81^{E}	234.53 ± 1.4^{F}	61.57 ± 0.80^{G}
	100	nd	nd	2.28 ± 0.05^{E}	12.60 ± 0.1^{F}	nd
4	10	2.34 ± 0.56^{b}	71.69 ± 0.23^{D}	120.51 ± 1.33^{E}	236.92 ± 2.70^{F}	17.13 ± 0.30^{G}
	25	5.20 ± 0.55^{b}	33.34± 0.34^{D}	91.42 ± 1.11^{E}	272.40 ± 1.52^{F}	26.48 ± 0.61^{G}
	50	2.03 ± 0.54^{b}	30.25 ± 0.67^{D}	137.44 ± 3.44^{E}	335.37 ± 2.05^{F}	32.99 ± 0.33^{G}
	75	26.68 ± 0.25^{b}	20.49 ± 0.33^{D}	88.34 ± 0.40^{E}	250.88 ± 1.15^{F}	47.08 ± 0.41^{G}
	100	nd	nd	2.45 ± 0.10^{E}	14.60 ± 0.13^{F}	nd
6	10	2.79 ± 0.25^{b}	50.44 ± 0.72^{D}	131.68 ± 1.89^{E}	250.17 ± 2.21^{F}	18.64 ± 0.33^{G}
	25	2.69 ± 0.25^{b}	40.14 ± 0.71^{D}	97.49 ± 0.79^{E}	271.86 ± 1.90^{F}	26.37 ± 0.10^{G}
	50	2.27 ± 0.82^{b}	31.81 ± 0.22^{D}	157.43 ± 1.23^{E}	352.60 ± 2.30^{F}	41.69 ± 0.60^{G}
	75	25.63 ± 0.38^{b}	19.37 ± 0.43^{D}	92.42 ± 0.44^{E}	270.73 ± 2.71^{F}	nd
	100	nd	nd	2.47 ± 0.09^{E}	19.56 ± 0.12^{F}	nd
24	10	1.81 ± 0.20^{b}	56.06 ± 0.41^{D}	115.37 ± 1.53^{E}	229.49 ± 2.30^{F}	19.94 ± 0.09^{G}
	25	0.18 ± 0.36^{b}	46.94 ± 0.42^{D}	103.55 ± 1.31^{E}	271.32 ± 1.90^{F}	26.26 ± 0.15^{G}
	50	3.05 ± 0.41^{b}	32.50 ± 0.85^{D}	92.13 ± 0.85^{E}	313.93 ± 3.34^{F}	30.13 ± 0.86^{G}
	75	0.40 ± 0.05^{b}	20.44 ± 0.50^{D}	61.90 ± 0.41^{E}	263.86 ± 2.61^{F}	43.22 ± 0.90^{G}
	100	nd	nd	nd	18.67 ± 0.10^{F}	nd

GA – gallic acid, Flavan-3-ols denoted as: GC – (+)-gallocatechin, EGC – (–)-epigallocatechin, EGCG – (–)-epigallocatechin-3-gallate, C – (+)-catechin, nd – not detected.
The same lowercase letter (b) denotes the concentrations of biologically active compounds, which were significantly ($p < 0.05$) influenced only by the storage duration. The same uppercase letters (D–G) denote the concentrations of biologically active compounds, which were significantly ($p < 0.05$) influenced only by the ethanol concentration.

minor fluctuations). When compared to CF, tea extracts contained relatively low concentrations of TB and TP, or they were not detected in samples. Absolute ethanol showed the poorest efficiency to extract methylxanthines. As can be seen from the results (Fig. 2), the higher water concentration was preferable in ethanol/water mixtures for the extraction of tea methylxanthines, in particular for TB and TF, regardless of tea form. Similar results were obtained in a study of Horžić et al. [3], and could be explained by the specific solubility of these compounds. It is known that most of pure methylxanthines are not readily soluble in water, but they dissolve more or less readily in alcohol, some non-polar solvents such as ether or chloroform. The exception is pure CF, which is soluble in hot water and in polar aprotic solvents, such as acetone or dimethylformamide. Moreover, methylxanthines also occur naturally in the forms of salts, which are mostly quite soluble in water, and fairly so in alcohol. To conclude, pure CF and probably, to some minor extent its salt form, as well as TB and TP in their salt forms, might be contained in Yinzhen tea leaves. In this form, they are more soluble in water and, as a consequence, they are found at higher concentrations in extracts with lower ethanol percentage.

Although various mechanisms have been proposed for the beneficial effects of tea in different models of chronic disease, the radical-scavenging

Tab. 3. Concentration of gallic acid and flavan-3-ols in bagged form extracts of Yinzhen tea at different ethanol concentrations and storage durations.

Storage duration [h]	Ethanol concentration [%]	GA [mg·l^{-1}]	GC [mg·l^{-1}]	EGC [mg·l^{-1}]	EGCG [mg·l^{-1}]	C [mg·l^{-1}]
1	10	2.42 ± 0.23^{c}	55.98 ± 0.73^{H}	122.19 ± 2.35	286.24 ± 2.82^{I}	25.33 ± 0.44^{J}
	25	6.12 ± 0.72^{c}	54.12 ± 0.41^{H}	110.57 ± 1.51	299.66 ± 3.21^{I}	23.90 ± 0.40^{J}
	50	4.12 ± 0.51^{c}	28.93 ± 0.60^{H}	137.96 ± 1.78	349.30 ± 2.33^{I}	40.16 ± 0.55^{J}
	75	3.54 ± 0.50^{c}	18.47 ± 0.12^{H}	65.68 ± 0.79	204.34 ± 1.52^{I}	13.23 ± 0.12^{J}
	100	nd	nd	13.75 ± 0.15	nd	nd
2	10	3.07 ± 0.22^{c}	63.29 ± 0.42^{H}	130.26 ± 2.81	296.89 ± 1.91^{I}	25.81 ± 0.33^{J}
	25	6.28 ± 0.32^{c}	43.05 ± 0.75^{H}	105.39 ± 1.30	302.48 ± 2.30^{I}	24.38 ± 0.59^{J}
	50	5.01 ± 0.23^{c}	29.47 ± 0.32^{H}	146.69 ± 2.31	351.20 ± 3.30^{I}	45.81 ± 0.64^{J}
	75	3.90 ± 0.54^{c}	19.85 ± 0.13^{H}	66.23 ± 0.42	259.46 ± 2.24^{I}	15.02 ± 0.10^{J}
	100	nd	nd	4.13 ± 0.23	13.21 ± 0.15^{I}	nd
4	10	2.62 ± 0.45^{c}	62.85 ± 0.90^{H}	129.24 ± 1.52	293.25 ± 3.33^{I}	26.81 ± 0.44^{J}
	25	4.87 ± 0.25^{c}	48.24 ± 0.60^{H}	100.92 ± 1.54	246.03 ± 0.52^{I}	18.02 ± 0.37^{J}
	50	3.94 ± 0.12^{c}	32.34 ± 0.82^{H}	130.57 ± 1.11	337.52 ± 3.13^{I}	39.10 ± 0.22^{J}
	75	5.25 ± 0.23^{c}	21.70 ± 0.13^{H}	58.10 ± 1.82	256.86 ± 1.52^{I}	17.71 ± 0.13^{J}
	100	nd	nd	3.17 ± 0.13	15.68 ± 0.67^{I}	15.79 ± 0.65^{J}
6	10	3.82 ± 0.65^{c}	63.15 ± 0.70^{H}	136.03 ± 2.11	301.70 ± 1.71^{I}	21.82 ± 0.30^{J}
	25	5.21 ± 0.41^{c}	53.69 ± 0.78^{H}	112.46 ± 1.67	256.15 ± 2.31^{I}	22.75 ± 0.34^{J}
	50	1.12 ± 0.10^{c}	30.48 ± 0.11^{H}	124.29 ± 1.81	340.28 ± 2.80^{I}	35.29 ± 0.56^{J}
	75	8.65 ± 0.54^{c}	20.91 ± 0.34^{H}	50.39 ± 0.62	226.90 ± 3.42^{I}	19.06 ± 0.23^{J}
	100	nd	nd	nd	14.21 ± 0.13^{I}	nd
24	10	3.24 ± 0.20^{c}	54.47 ± 0.33^{H}	122.15 ± 1.12	285.85 ± 1.80^{I}	26.62 ± 0.66^{J}
	25	5.29 ± 0.31^{c}	50.93 ± 0.67^{H}	77.33 ± 0.56	252.60 ± 2.67^{I}	27.29 ± 0.11^{J}
	50	1.24 ± 0.23^{c}	29.45 ± 0.55^{H}	129.08 ± 1.11	348.38 ± 3.78^{I}	34.90 ± 0.42^{J}
	75	10.26 ± 0.56^{c}	20.42 ± 0.11^{H}	9.68 ± 0.15	250.72 ± 1.55^{I}	20.88 ± 0.73^{J}
	100	nd	nd	nd	13.57 ± 0.10^{I}	nd

GA – gallic acid, Flavan-3-ols denoted as: GC – (+)-gallocatechin, EGC – (–)-epigallocatechin, EGCG – (–)-epigallocatechin-3-gallate, C – (+)-catechin, nd – not detected.
The same lowercase letter (c) denotes the concentrations of biologically active compounds, which were significantly ($p < 0.05$) influenced only by the storage duration. The same uppercase letters (H–J) denote the concentrations of biologically active compounds, which were significantly ($p < 0.05$) influenced only by the ethanol concentration.

and antioxidant properties of tea polyphenols are frequently cited as important contributors [21]. Numerous studies have demonstrated that tea catechins and polyphenols are effective scavengers of physiologically relevant reactive oxygen and nitrogen species in vitro [22]. Tab. 4 presents the antioxidant capacities (AC) of all tested ethanol extracts of Yinzhen tea determined using three different assays: $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP. The AC values obtained using these methods were quite different within one tea form, but they followed the same trend as previously determined TPC and TFC. The differences in the results obtained within one tea form could be explained with the interference effect of the solvent and non-antioxidant constituents, for which the revealed ranking in AC assays was: ORAC (oxygen radical antioxidant capacity) > $ABTS^{\bullet+}$ > $DPPH^{\bullet}$ > FRAP [23]. Additionally, these three methods differ in the nature of used substances and their working mechanism. $DPPH^{\bullet}$ is more lipophilic, whilst FRAP is basically a hydrophilic antioxidant assay, not well responding to lipophilic antioxidants [24]. FRAP method, based on outer-sphere e-transfer by a coordinately saturated metal complex, is associated with a divalent-charged chromophore ($Fe(TPTZ)_2^{2+}$) having a greater affinity toward the aqueous phase due to ion-dipole interactions of the chromophore with water molecules of the solvent. On the other hand, $ABTS^{\bullet+}$ assay

is successful in antioxidant capacity estimation of both hydrophilic and lipophilic antioxidants in polar and non-polar solvent media, because it involves univalent-charged chromophore species ($ABTS^{\bullet+}$) capable of being solvated by both water and alcohols as well as by less polar solvent mixtures. $ABTS^{\bullet+}$ assay, as an antioxidant assay method based on H-atom donation from a polyphenol compound, is generally affected to a greater extent by the solvent behaviour, in particular by its polarity and hydrogen bond-accepting ability [24]. To conclude, methods applied to measure AC extremely depend on the reaction conditions and substrates or products. Antioxidant

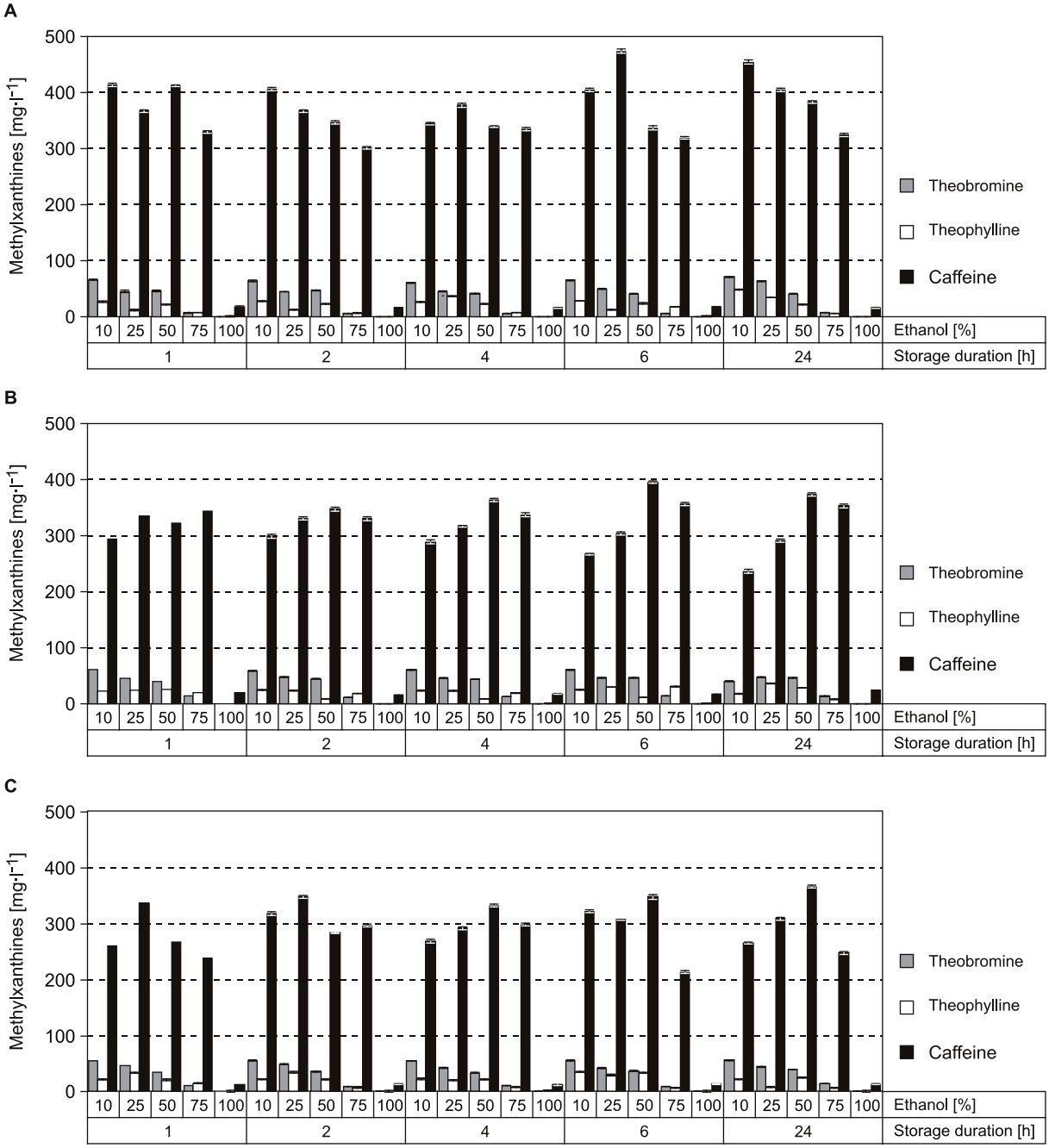

Fig. 2. Concentrations of methylxanthines in extracts from different forms of Yinzhen tea forms with different ethanol concentrations during 24 h of storage.

A – loose leaf, B – infuser, C – bagged.
Concentrations of caffeine, theobromine, and theophylline are expressed in milligrams per litre.

Tab. 4. Antioxidant capacity of the extracts from the loose leaf, bagged and infuser forms of Yinzhen tea at different ethanol concentrations and storage durations.

Storage duration [h]	Ethanol concentration [%]	Loose leaf			Bagged			Infuser		
		DPPH• [mmol·l-1]	ABTS•+ [mmol·l-1]	FRAP [mmol·l-1]	DPPH• [mmol·l-1]	ABTS•+ [mmol·l-1]	FRAP [mmol·l-1]	DPPH• [mmol·l-1]	ABTS•+ [mmol·l-1]	FRAP [mmol·l-1]
1 h	10	13.43 ± 0.28^{a}	7.26 ± 0.05^{b}	7.2 ± 0.11^{A}	12.63 ± 0.12^{E}	8.21 ± 0.06^{c}	11.32 ± 0.09^{F}	11.73 ± 0.13^{B}	10.74 ± 0.16^{C}	11.17 ± 0.07^{D}
	25	10.59 ± 0.27^{a}	5.48 ± 0.09^{b}	2.33 ± 0.08^{A}	8.20 ± 0.05^{E}	5.42 ± 0.03^{c}	8.52 ± 0.05^{F}	13.59 ± 0.07^{B}	11.04 ± 0.25^{C}	11.56 ± 0.15^{D}
	50	9.49 ± 0.07^{a}	7.58 ± 0.10^{b}	7.96 ± 0.10^{A}	20.18 ± 0.11^{E}	12.72 ± 0.17^{c}	15.06 ± 0.12^{F}	19.69 ± 0.13^{B}	9.19 ± 0.14^{C}	11.00 ± 0.05^{D}
	75	12.58 ± 0.07^{a}	7.79 ± 0.13^{b}	11.26 ± 0.11^{A}	22.22 ± 0.04^{E}	10.65 ± 0.19^{c}	16.82 ± 0.04^{F}	22.69 ± 0.06^{B}	10.65 ± 0.20^{C}	21.26 ± 0.10^{D}
	100	1.21 ± 0.05^{a}	0.90 ± 0.02^{b}	1.25 ± 0.10^{A}	0.77 ± 0.06^{E}	0.50 ± 0.05^{c}	0.65 ± 0.03^{F}	0.79 ± 0.05^{B}	2.37 ± 0.09^{C}	0.93 ± 0.04^{D}
2 h	10	13.68 ± 0.27^{a}	8.52 ± 0.03^{b}	13.54 ± 0.14^{A}	14.90 ± 0.07^{E}	8.21 ± 0.16^{c}	14.03 ± 0.10^{F}	13.81 ± 0.12^{B}	11.01 ± 0.16^{C}	12.94 ± 0.10^{D}
	25	12.86 ± 0.19^{a}	9.4 ± 0.24^{b}	10.95 ± 0.07^{A}	8.27 ± 0.04^{E}	5.38 ± 0.07^{c}	8.09 ± 0.04^{F}	13.80 ± 0.05^{B}	11.10 ± 0.13^{C}	14.57 ± 0.10^{D}
	50	12.75 ± 0.10^{a}	9.04 ± 0.10^{b}	11.44 ± 0.11^{A}	24.30 ± 0.13^{E}	15.03 ± 0.12^{c}	15.44 ± 0.10^{F}	20.33 ± 0.07^{B}	10.16 ± 0.12^{C}	11.21 ± 0.14^{D}
	75	13.35 ± 0.24^{a}	9.43 ± 0.23^{b}	14.3 ± 0.03^{A}	22.87 ± 0.02^{E}	11.28 ± 0.10^{c}	16.93 ± 0.13^{F}	23.36 ± 0.11^{B}	11.23 ± 0.15^{C}	22.66 ± 0.17^{D}
	100	1.23 ± 0.03^{a}	1.11 ± 0.08^{b}	1.04 ± 0.07^{A}	0.78 ± 0.07^{E}	0.54 ± 0.09^{c}	0.76 ± 0.07^{F}	0.80 ± 0.07^{B}	2.45 ± 0.10^{C}	0.95 ± 0.04^{D}
4 h	10	16.20 ± 0.06^{a}	9.28 ± 0.07^{b}	14.81 ± 0.05^{A}	14.02 ± 0.10^{E}	8.21 ± 0.04^{c}	12.32 ± 0.03^{F}	18.53 ± 0.15^{B}	10.87 ± 0.07^{C}	11.07 ± 0.12^{D}
	25	15.42 ± 0.06^{a}	10.77 ± 0.19^{b}	12.51 ± 0.05^{A}	11.12 ± 0.06^{E}	5.78 ± 0.02^{c}	8.79 ± 0.14^{F}	16.88 ± 0.17^{B}	10.71 ± 0.20^{C}	13.26 ± 0.09^{D}
	50	19.06 ± 0.26^{a}	10.80 ± 0.07^{b}	16.44 ± 0.09^{A}	27.01 ± 0.16^{E}	16.49 ± 0.06^{c}	15.44 ± 0.13^{F}	22.38 ± 0.15^{B}	18.44 ± 0.20^{C}	18.28 ± 0.11^{D}
	75	18.84 ± 0.21^{a}	11.28 ± 0.06^{b}	18.14 ± 0.08^{A}	26.29 ± 0.21^{E}	16.61 ± 0.12^{c}	24.15 ± 0.10^{F}	25.20 ± 0.13^{B}	18.31 ± 0.25^{C}	28.25 ± 0.05^{D}
	100	1.61 ± 0.05^{a}	1.10 ± 0.05^{b}	1.23 ± 0.03^{A}	0.87 ± 0.07^{E}	0.55 ± 0.03^{c}	0.81 ± 0.08^{F}	0.81 ± 0.07^{B}	2.76 ± 0.01^{C}	0.95 ± 0.06^{D}
6 h	10	13.40 ± 0.07^{a}	12.51 ± 0.21^{b}	15.89 ± 0.18^{A}	16.52 ± 0.11^{E}	8.82 ± 0.16^{c}	14.31 ± 0.15^{F}	16.93 ± 0.08^{B}	11.56 ± 0.10^{C}	13.50 ± 0.12^{D}
	25	12.66 ± 0.12^{a}	7.88 ± 0.10^{b}	12.70 ± 0.16^{A}	12.64 ± 0.11^{E}	8.82 ± 0.09^{c}	10.05 ± 0.09^{F}	14.90 ± 0.10^{B}	11.59 ± 0.09^{C}	12.17 ± 0.09^{D}
	50	13.79 ± 0.09^{a}	8.78 ± 0.07^{b}	16.42 ± 0.23^{A}	26.43 ± 0.12^{E}	12.35 ± 0.17^{c}	16.42 ± 0.08^{F}	23.07 ± 0.05^{B}	16.12 ± 0.16^{C}	18.84 ± 0.07^{D}
	75	19.42 ± 0.20^{a}	10.19 ± 0.12^{b}	17.62 ± 0.14^{A}	29.58 ± 0.18^{E}	16.67 ± 0.12^{c}	26.08 ± 0.08^{F}	25.49 ± 0.09^{B}	19.04 ± 0.18^{C}	29.21 ± 0.20^{D}
	100	1.56 ± 0.05^{a}	1.15 ± 0.03^{b}	1.44 ± 0.04^{A}	0.92 ± 0.01^{E}	1.02 ± 0.03^{c}	0.84 ± 0.09^{F}	0.81 ± 0.02^{B}	4.32 ± 0.04^{C}	0.95 ± 0.10^{D}
24 h	10	15.44 ± 0.10^{a}	10.80 ± 0.10^{b}	20.20 ± 0.10^{A}	17.32 ± 0.11^{E}	8.28 ± 0.19^{c}	15.55 ± 0.10^{F}	20.18 ± 0.06^{B}	11.56 ± 0.21^{C}	11.27 ± 0.17^{D}
	25	21.58 ± 0.10^{a}	13.71 ± 0.10^{b}	17.19 ± 0.09^{A}	19.45 ± 0.12^{E}	7.85 ± 0.09^{c}	12.95 ± 0.18^{F}	24.66 ± 0.11^{B}	11.59 ± 0.04^{C}	19.48 ± 0.15^{D}
	50	14.79 ± 0.08^{a}	13.89 ± 0.12^{b}	23.88 ± 0.10^{A}	29.20 ± 0.09^{E}	17.77 ± 0.16^{c}	23.88 ± 0.14^{F}	26.65 ± 0.15^{B}	16.12 ± 0.14^{C}	20.29 ± 0.13^{D}
	75	23.74 ± 0.14^{a}	14.65 ± 0.12^{b}	25.90 ± 0.13^{A}	30.16 ± 0.08^{E}	23.43 ± 0.12^{c}	30.97 ± 0.17^{F}	26.45 ± 0.22^{B}	19.04 ± 0.08^{C}	36.64 ± 0.18^{D}
	100	1.74 ± 0.04^{a}	1.28 ± 0.07^{b}	1.81 ± 0.06^{A}	1.16 ± 0.05^{E}	1.96 ± 0.02^{c}	0.86 ± 0.06^{F}	0.81 ± 0.08^{B}	3.59 ± 0.07^{C}	0.97 ± 0.04^{D}

Antioxidant capacity obtained by DPPH• and ABTS•+ assays is expressed in millimoles of Trolox, antioxidant capacity obtained by FRAP assay is expressed in millimoles of Fe^{2+}.
The same lowercase letters (a–c) denote the antioxidant capacity, which was significantly ($p < 0.05$) influenced by the ethanol concentration, but not by the storage duration ($p > 0.05$).
The same uppercase letter (A–F) denote the antioxidant capacity, which was significantly ($p < 0.05$) influenced by the storage duration and ethanol concentration.

capacity varies not only with antioxidant structure, its properties or solvent type, but it also depends on solubility and partition coefficient of active species [25], working mechanism based on the method, as well as possible interference of non-antioxidant species. Generally, AC of the majority of tested samples evaluated by all used methods indicated an increase of AC of tea extracts during prolonged storage, with the highest value after one day of storage. According to our results, absolute ethanol extracts exhibited the lowest AC considering all forms of tea during the whole storage period. Equally as for TPC, TFC and individual catechins and methylxanthines, the difference in solvent polarity altered its ability to dissolve a selected group of antioxidant compounds and influenced the concentration of bioactive compounds, as well as AC estimation. In accordance with previous results, the addition of water at low percentages to the solvent improved the antioxidant capacity [26]. As published earlier [3], the most efficient extraction technique in terms of AC of yellow tea varied depending on the assay used. Best results were obtained using conventional extraction with $ABTS^{\bullet+}$ and FRAP assays, and ultrasound bath with $DPPH^{\bullet}$ assay. Considering methods for obtaining AC and ethanol concentration, the highest AC characterized 75% ethanol extracts of yellow tea [3], which was in accordance with our results for the whole period of tea storage. Results of both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays confirmed the highest AC in 75% ethanol extract of bagged tea form after one day storage, while FRAP method revealed the highest AC of 75% ethanol extract of infuser tea form after one day. In addition, minor fluctuations were observed – AC of some tea extracts increased during first 4 h, then it decreased after 6 h, and again increased and reached the highest value after one day of storage. Komes et al. [8] observed a similar phenomenon for green tea extracts. Additionally, the increase in the antioxidant capacity could be the consequence of the strong tendency of polyphenols to undergo polymerization reactions, making more stable polyphenol radical through conjugation and electron delocalization. When the degree of polymerization exceeds a critical value, the higher molecular complexity and steric hindrance reduce the availability of hydroxyl groups in reactions with radicals [27]. In the case of catechin, AC in the aqueous phase increases from monomer to trimer and then decreases from trimer to tetramer [28]. This causes a reduction in the antiradical capacity and may explain the observed fluctuations. Analysis of variance points to mutual significant effect of storage duration and ethanol concentration ($p < 0.05$) on antioxidant capacity of majority of tested tea extracts. The order of AC affected by storage duration is in accordance with TPC and TFC, confirmed by a high linear correlation obtained between the results: $r_{DPPH\bullet/TPC} = 0.847$, $r_{ABTS+\bullet/TPC}=0.821$, $r_{FRAP/TPC}=0.846$, $r_{DPPH\bullet/TFC}=0.873$, $r_{ABTS+\bullet/TFC} = 0.850$, $r_{FRAP/TFC} = 0.868$. This implies that the antioxidant capacity of samples directly related to their polyphenol concentration.

CONCLUSION

Storage duration significantly affected TPC and AC of Yinzhen tea extracts. TPC and TFC, as well as AC of ethanol Yinzhen tea extracts obtained from different leaf forms, increased during one-day storage at room temperature. The highest values were reached after one day of storage, regardless of the tea form or solvent used. The recovery of bioactive compounds was highly dependent on concentration of ethanol in solvent employed for the extraction. The addition of water at low percentages to absolute ethanol improved the polyphenols extraction, as well as AC of the extracts. Extracts obtained with 75% ethanol from all leaf forms were the richest source of polyphenols during prolonged storage. Results also confirmed the selectivity in the extraction of polyphenol compounds depending on the extraction form of tea leaves. Comparison of values linked to 75% ethanol extracts of all tea forms pointed to the highest TPC in loose leaf form of tea. The least effective solvent for all forms of Yinzhen tea was absolute ethanol. A comparison of the results obtained by HPLC analysis showed that the most abundant bioactive constituents of yellow tea were catechins, followed by methylxanthines and gallic acid. EGCG was quantitatively the most abundant catechin and caffeine was the most abundant methylxanthine for this tea type. Solvents with lower ethanol percentage were preferable for extraction of individual catechins and methylxanthines. The highest concentration of EGCG was determined in 50% ethanol extract of loose leaf form, while 25% ethanol extract of loose leaf form after 6 h of storage had the highest caffeine concentration. The antioxidant capacity of the examined extracts correlated with their polyphenol and flavonoid concentration.

Acknowledgments

This work received support from the Ministry of Education, Science and Sport of the Republic of Croatia (Project Nos. 058-0000000-3470 and 098-0982915-2949).

REFERENCES

1. Pettigrew, J. – Richardson, B: The new tea companion. Daniville : Benjamin Press Books, 2nd ed., 2008. ISBN 0979343178.
2. Chuen, L. K. – Sin, L. K: Way of tea (the sublime art of oriental tea drinking). New York : Barron's Educational Series, 2002. ISBN 0764119680.
3. Horžić, D. – Jambrak, A. R. – Belščak-Cvitanović, A. – Komes, D. – Lelas, V.: Comparison of conventional and ultrasound assisted extraction techniques of yellow tea and bioactive composition of obtained extracts. Food and Bioprocess Technology, *5*, 2012, pp. 2858–2870. DOI: 10.1007/s11947-012-0791-z.
4. Naczk, M. – Shahidi, F.: Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis, *41*, 2006, pp. 1523–1542. DOI: 10.1016/j.jpba.2006.04.002.
5. Pan, X. – Niu, G. – Liu, H.: Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. Chemical Engineering and Processing : Process Intensification, *42*, 2003, pp. 129–133. DOI: 10.1016/S0255-2701(02)00037-5.
6. Xia, T. – Shi, S. – Wan, X.: Impact of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusion. Journal of Food Engineering, *74*, 2006, pp. 557–560. DOI: 10.1016/j.jfoodeng.2005.03.043.
7. Park, H. S. – Choi, H.-K. – Lee, S. J. – Park, K. – Choi, S.-G. – Kim, K. H.: Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide. Journal of Supercritical Fluids, *42*, 2007, pp. 205–211. DOI: 10.1016/j.supflu.2007.03.002.
8. Komes, D. – Horzic, D. – Belscak, A. – Ganic, K. K. – Vulic, I.: Green tea preparation and its influence on the content of bioactive compounds. Food Research International, *43*, 2010, pp. 167–176. DOI: 10.1016/j.foodres.2009.09.022.
9. Lachman, J. – Pronek, D. – Hejtmankova, A. – Dudjak, J. – Pivec, V. – Faitova, K: Total polyphenol and main flavonoid antioxidants in different onion *(Allium cepa* L.*)* varieties. Horticultural Science, *30*, 2003, pp 142–147.
10. Brand-Williams, W. – Cuvelier, M. E. – Berset, C.: Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology, *28*, 1995, pp. 25–30. DOI: 10.1016/S0023-6438(95)80008-5.
11. Re, R. – Pellegrini, N. – Proteggente, A. – Pannala, A. – Yang, M. – Rice-Evans, C.: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, *26*, 1999, pp. 1231–1237. DOI: 10.1016/S0891-5849(98)00315-3.
12. Benzie, I. F. – Strain, J. J.: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, *239*, 1996, pp. 70–76.
13. Perva-Uzunalic, A. – Skerget, M. – Knez, Z. – Weinreich, B. – Otto, F. – Grüner, S.: Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. Food Chemistry, *96*, 2006, pp. 597–605. DOI: 10.1016/j.foodchem.2005.03.015.
14. Chavan, U. – Shahidi, F. – Naczk, M.: Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents. Food Chemistry, *75*, 2001, pp. 509–512. DOI: 10.1016/S0308-8146(01)00234-5.
15. Wang H. – Helliwell, K.: Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography. Food Research International, *34*, 2001, pp. 223–227. DOI: 10.1016/S0963-9969(00)00156-3.
16. Shan, B. – Xie, J.-H. – Zhu, J.-H. – Peng, Y.: Ethanol modified supercritical carbon dioxide extraction of flavonoids from *Momordica charantia* L. and its antioxidant activity. Food and Bioproducts Processing, *90*, 2012, pp. 579–587. DOI: 10.1016/j.fbp.2011.09.004
17. Lapornik, B. – Prošek, M. – Golc Wondra, A.: Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering, *71*, 2005, pp. 214–222. DOI: 10.1016/j.jfoodeng.2004.10.036.
18. Spigno, G. – Tramelli, L. – De Faveri, D. M.: Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering, *81*, 2007, pp. 200–208. DOI: 10.1016/j.jfoodeng.2006.10.021.
19. Escarpa, A. – González, M.: Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. Analytica Chimica Acta, *427*, 2001, pp. 119–127. DOI: 10.1016/S0003-2670(00)01188-0.
20. Vinson, J. A. – Su, X. – Zubik, L. – Bose, P.: Phenol antioxidant quantity and quality in foods: fruits. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 5315–5321. DOI: 10.1021/jf0009293.
21. Higdon J. V. – Frei, B.: Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Critical Reviews in Food Science and Nutrition, *43*, 2003, pp. 89–143. DOI: 10.1080/10408690390826464.
22. Frei, B., – Higdon, J. V.: Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. Journal of Nutrition, *133*, 2003, pp. 3275S–3284.
23. Pérez-Jiménez, J. – Saura-Calixto, F.: Effect of solvent and certain food constituents on different antioxidant capacity assays. Food Research International, *39*, 2006, pp. 791–800. DOI: 10.1016/j.foodres.2006.02.003.
24. Apak, R. – Güçlü, K. – Demirata, B. – Özyürek, M. – Çelik, S. E. – Bektaşoğlu, B. – Berker, K. I. – Özyurt, D.: Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules, *12*, 2007, pp. 1496–1547. DOI: 10.3390/12071496.
25. Çelik, S. E. – Özyürek, M. – Güçlü, K. – Apak, R.: Solvent effects on the antioxidant capacity of lipophilic and hydrophilic antioxidants measured by CUPRAC, ABTS/persulphate and FRAP methods. Talanta, *81*, 2010, pp. 1300–1309. DOI: 10.1016/j.talanta.2010.02.025.

26. Trabelsi, N. – Megdiche, W. – Ksouri, R. – Falleh, H. – Oueslati, S. – Soumaya, B. – Hajlaoui, H. – Abdelly, C.: Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. LWT – Food Science and Technology, *43*, 2010, pp. 632–639. DOI: 10.1016/j.lwt.2009.11.003.
27. Pinelo, M. – Manzocco, L. – Nunez, M. J. – Nicoli, M. C.: Interaction among phenols in food fortification: Negative synergism on antioxidant capacity. Journal of Agricultural and Food Chemistry, *52*, 2004, pp. 1177–1180. DOI: 10.1021/jf0350515.
28. Plumb, G. W. – De Pascual-Teresa, S. – Santos-Buelga, C. – Cheynier, V. – Williamson, G.: Antioxidant properties of catechins and proanthocyanidins: effect of polymerisation, galloylation and glycosylation. Free Radical Research, *29*, 1998, pp. 351–358. DOI: 10.1080/10715769800300391.

Received 20 January 2014; 1st revised 24 February 2014; accepted 4 March 2014; published online 6 August 2014.