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THE INFLUENCE OF ASCORBIC ACID AND HONEY ADDITION ON THE ANTI-OXIDANT PROPERTIES OF FRUIT TEA INFUSIONS: ANTIOXIDANTS IN FRUIT TEA INFUSIONS

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ABSTRACT

The influence of ascorbic acid and honey addition on the total phenol (TP) content and anti-oxidant capacity of ten commercial fruit tea infusions was studied. The Folin-Ciocalteu assay was used to determine the TP content, ferric reducing anti-oxidant power assay for reducing capacity and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt assays for radical scavenging capacity. High pressure liquid chromatography was employed to quantify polyphenolic compounds in infusions. The addition of ascorbic acid (22.5 mg) increases the TP content as well as anti-oxidant capacity of all fruit tea infusions. The addition of honey (1.5 g) to infusions containing ascorbic acid resulted in an average 28% decrease in the TP value, and a significant average (22%) decrease in the DPPH radical scavenging capacity. Chlorogenic acid is the predominant phenolic acid in tested infusions (range: 12.86–91.55 mg/L), while epigallocatechin-gallate is the most concentrated among analyzed catechins (range: 19.26–161.41 mg/L).

PRACTICAL APPLICATIONS

There are no published scientific papers characterizing polyphenolic compounds and anti-oxidant activity of infusions prepared from bagged fruit teas, although these beverages are very popular in Europe and United States where they are often taken with lemon and honey. The results reported in this study indicate that fruit tea infusions are, just like herbal teas, valuable sources of polyphenolic anti-oxidants and that beverages prepared from teas containing a substantial portion of fruit parts exhibit the best anti-oxidant properties.

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Also, we have demonstrated that the addition of ascorbic acid enhances the anti-oxidant capacity of fruit teas while the addition of honey causes a decrease in the total phenol content and may adversely affect the radical scavenging potential of analyzed fruit teas.

INTRODUCTION

Researchers and food manufacturers have become increasingly interested in polyphenols, due to their confirmed anti-oxidant properties, great abundance in human diet and a probable role in the prevention of various pathological conditions associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases (Scalbert *et al.* 2005). In addition, in the past few years, the consumption of tea around the world has increased, mostly encouraged by the recently confirmed positive health benefits of this beverage (Cooper *et al.* 2005a,b). Although green, black, oolong and white teas are most frequently consumed, various types of fruit and herbal teas are also widely appreciated due to their fruity flavor and easy preparation. The positive health effects of tea drinking have mostly been attributed to the anti-oxidant content of tea, including phenolic compounds, vitamins (C, E), carotenoids, etc. (Wiseman *et al.* 1997) that prevent free radical damage (Stähelin *et al.* 1991; Steinberg 1991; Willett 1994). It has been established that flavonoid compounds have a 2–5-fold greater anti-oxidant and free radical scavenging activity on an equimolar basis than vitamins C and E (Vinson *et al.* 1995; Wiseman *et al.* 1997). A lot of attention has been focused on the polyphenolic content of green, white and black teas, in contrast to fruit tea infusions whose chemical composition has not been studied to such an extent.

The way a tea beverage is consumed largely depends on the tradition and local customs. In the United Kingdom, Ireland, Canada and India, tea is consumed with the addition of a substantial amount of milk (Weisburger 1997), while in other countries, it is popular to take tea with lemon. The *in vitro* protective effect of ascorbic acid against oxidative degradation of certain flavonoids, especially anthocyanins, has been observed during processing and storage of juices (Kaack and Austed 1998). Approximately 150 mg of pure ascorbic acid can provide the same anti-oxidant potential as one cup of green tea of usual strength (1.5%) (Benzie and Szeto 1999). Studies in human volunteers have correlated the intake of ascorbic acid and green tea with the inhibiting effects on endogenous formation of N-nitroso compounds (Vermeer *et al.* 1999). Biological activities and anti-oxidant properties have also been attributed to various honey preparations (Aljadi and Kamaruddin 2004; Blasa *et al.* 2006). These findings point to the importance of investigating the anti-oxidant potential of teas with added vitamin C and honey.

The objective of the present study was to evaluate the content and composition of polyphenolic anti-oxidants in some commercially available types of fruit teas, as well as to study the influence of ascorbic acid and honey addition on their anti-oxidant properties.

MATERIALS AND METHODS

Reagents and Apparatus

Except for the Folin-Ciocalteu (FC) reagent (Fluka, Switzerland) and formic acid (Kemika, Croatia), all the chemicals and reagents used in this study were of analytical grade and supplied by Sigma Chemical Co. (St. Louis, MO). Spectrophotometric measurements were performed on a double-beam UV-VIS spectrophotometer Bio-Spec-1601 (Shimadzu Corporation, Kyoto, Japan). High-performance liquid chromatography (HPLC) analysis was performed on a Knauer liquid chromatography system (Berlin, Germany).

Fruit Teas and Honey

Ten of the most widely appreciated and most frequently consumed commercially available fruit teas of different flavors and containing varying amounts of fruit and plant parts (blueberry, peach and tangerine, pink grape, apple and cinnamon, orange, rose hip, forest fruit, apricot, cherry and strawberry) were purchased from the local supermarkets. A detailed composition of selected fruit teas, in bagged form, is given in Table 1.

The monofloral lavender honey (2007) used in this study was purchased from a local store.

Extraction Procedure

In order to simulate the ordinary household preparation conditions, fruit tea infusions were prepared according to the instructions provided on the packaging. All tea extracts were prepared on the day the measurements were performed by infusing 2.0 g fruit tea (one tea bag) with 100 mL deionized water heated to 95C for 8 min. Because fruit teas are usually consumed with the addition of lemon juice and honey, the effect of ascorbic acid and honey on the anti-oxidant capacity of fruit teas was also evaluated. Thus, three different series of tea infusions were analyzed: pure tea (**a** series), tea with added ascorbic acid (**b** series) and tea with added ascorbic acid and honey (**c** series). The optimal amounts of added ascorbic acid (22.5 mg) and forest flower honey (1.5 g) were determined by sensorial evaluation of tea infusions.

TABLE 1.
COMPOSITION (%) AND PRODUCTION DATES OF STUDIED FRUIT TEAS

Fruit tea	FT1	FT2	FT3	FT4	FT5	FT6	FT7	FT8	FT9	FT10
Hibiscus flower	45	28	25	18	18	45	5	6	40	34
Rose hip fruit	11	10	75	10	–	5	3	30	1	18
Apple fruit	25	27.5	–	25	47.3	29	37	38	40.5	28
Other (mostly wild berries and blackberry leaves)	19	34.5	–	47	34.7	21	55	26	18.5	20
Production month/year	11/2007	10/2007	11/2007	10/2007	07/2006	11/2007	07/2007	09/2007	03/2007	11/2007

Total Phenol (TP) Content

The total phenol content of tea infusions was determined using the FC colorimetric method (Singleton and Rossi 1965), with gallic acid as the standard. Absorbance of each tea infusion, as well as the absorbance of the gallic acid standard in concentrations of 0, 50, 100, 150, 250 and 500 mg/L, was determined at 765 nm against the blank (the "0 mL" solution). Three absorbance readings were averaged for each tested infusion and expressed as mg/L gallic acid equivalents (GAE).

Ferric Reducing Anti-Oxidant Power Assay

The ferric reducing anti-oxidant power (FRAP) assay (Benzie and Strain 1999) was used to estimate the reducing capacity potential of tested fruit teas. Working FRAP reagent was prepared daily, containing 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$, 2.5 mL of 10 mM 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) in 40 mM HCl and 25 mL of 300 mM acetate buffer. All measurements were performed as follows: 950 µL of freshly prepared FRAP reagent was added to 50 µL of fruit tea and the mixture was shaken. Reagent blank was prepared by adding 50 µL of water instead of fruit tea. Absorbance readings of tea samples and the reagent blank were taken after 4 min, at 593 nm. The results, obtained from triplicate analyses, were expressed as µM of $FeSO_4 \cdot 7H_2O$, and derived from a calibration curve determined for this standard (100–1,000 µmol/L).

1,1-diphenyl-2-picryl-hydrazyl $(DPPH)^{\cdot}$ Radical Scavenging Assay

Antioxidant capacity of fruit teas was determined using the $DPPH^{\bullet}$ radical scavenging assay described previously (Brand-williams *et al.* 1995). Briefly, 5 µL of fruit tea was added to a volume of 0.094 mM $DPPH^{\bullet}$ radical in methanol up to completing 1 mL. The reaction was carried out in closed eppendorf tubes shaken at 20C. The free radical scavenging capacity using the free radical $DPPH^{\cdot}$ reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at room temperature. The results were expressed as Trolox equivalent anti-oxidant capacity (TEAC) in mmol/L, using a standard curve for Trolox (0–5 mM).

$ABTS^{+}$ Radical Scavenging Assay

The TEAC of fruit teas was also estimated using the $ABTS^{\cdot+}$ radical cation decolorization assay (Re *et al.* 1999). Briefly, adequate stock solutions of ABTS (7 mM) and potassium persulfate (140 mM) in water were prepared, and mixed together to a final concentration of 2.45 mM potassium persulfate. This mixture was left to react overnight (at least 12–16 h) in the dark, at room temperature. On the day of analysis, the $ABTS^{\cdot+}$ solution was diluted with

ethanol to an absorbance of 0.70 (±0.02) at 734 nm. A 20-μL aliquot of the tested fruit teas was added to 2.0 mL of the diluted $ABTS^{\cdot+}$ solution, and the absorbance readings were taken after exactly 6 min. The reagent blank was prepared by adding 20 mL of ethanol instead of the sample. Also, different solutions (0–1 mM) of Trolox were prepared in 96% ethanol, and assayed under the same procedure as the samples. All measurements were performed in triplicate.

HPLC Analysis of Phenolic Compounds

The HPLC separation of polyphenolic compounds from fruit teas was performed using a Knauer liquid chromatography system, equipped with two pumps (K-501), a sample injector (WellChrom Electrical Valve Drive K-6) and variable wavelength UV-VIS Photodiode Array Detector (Knauer). A reversed-phase Nucleodur Sphinx C18 column (150 × 4.6 mm i.d.) was used, with the appropriate Nucleodur Sphinx C18 (5 μm) precolumn from Macherey Nagel (Düren, Germany). The tea samples were filtered through a 0.45 μm filter (Chromafil, Macherey-Nagel, Düren, Germany) prior to HPLC analysis. The flow rate was 1.0 mL/min and the injection volume for all samples was 50 μL. The solvents consisted of 3% formic acid (solvent A) and HPLC grade methanol (solvent B). The elution was performed with a gradient starting at 2% B to reach 32% B at 20 min, 40% B at 30 min and 95% B at 40 min, and isocratic for 5 min. Chromatograms were recorded at 280 nm. Phenolic compounds were identified by comparing the retention times and spectral data with those of authentic standards. The data acquisition and processing were conducted using ChromGate software. All chromatographic measurements were performed at 21C.

Statistical Analysis

All presented numeric values are means of three measurements ± standard deviation (SD). One-way analysis of variance (performed in SigmaStat 3.5) was used to determine whether the differences between measurements among series **a**, **b** and **c** are significant. Differences at $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The most extensively studied food products for their phenol content and anti-oxidant activity are green, white, oolong and black teas (Graham 1992; Harbowy and Balentine 1997; Kilmartin and Hsu 2003), cocoa and cocoa products (Waterhouse *et al.* 1996; Vinson *et al.* 1999; Lee *et al.* 2003), various

types of fruit juices (Gil *et al.* 2000; Van der Sluis *et al.* 2005; Piljac-Žegarac *et al.* 2009) and wine (Kilmartin and Zou 2002; Piljac *et al.* 2004; Piljac *et al.* 2005; Kovačević Ganić *et al.* 2006). However, literature data on the polyphenolic content and anti-oxidant capacity of fruit tea infusions are scarce, despite the fact that fruit teas are widely available and frequently consumed throughout the world.

TP Content and Reducing Capacity of Fruit Tea Infusions

Table 2 presents the TP content in mg/L GAE and FRAP values in mmol/L Fe(II) determined for the three series of fruit tea infusions. The highest TP content in series **a** was determined for *FT3* infusion (1,549.10 mg/L GAE), followed by *FT8* (1,134.55 mg/L GAE) and *FT10* (1,115.60 mg/L GAE). *FT5* exhibited the lowest TP content (323.40 mg/L GAE). There is a 4.8-fold difference between the lowest and the highest ranked tea in terms of the TP content. *FT3* infusion had the highest concentration of phenolic compounds, probably due to its high content of rose hip fruit, as opposed to the other tested teas that contain smaller amounts of different fruit parts (Table 1). Due to limited information available in the literature on the composition of fruit tea infusions, we could compare our results only with those obtained by other authors on green, white, or black teas and fruit juices. In comparison with the TP content of pomegranate juice – 2,566 mg/L (Gil *et al.* 2000), the average TP content of our studied fruit teas was much lower (825.10 mg/L GAE). This is probably due to the fact that fruit juices are produced from fresh fruits, while fruit tea production includes processing steps such as mechanical grinding, drying and other procedures that may result in the degradation of phenolic compounds. The TP content of green (2,083 mg/L GAE) and white teas (2,180 mg/L GAE) (Almajano *et al.* 2008) is approximately 1.3-fold and 1.4-fold higher, respectively, than the TP content of our *FT3* tea.

The FRAP assay results are in accordance with the TP content, indicating that the concentration of phenolic compounds may be a good indicator of the reducing capacity of studied fruit tea infusions. The highest reducing capacity was again observed for *FT3* infusion (11,075.80 μM Fe(II)) and the lowest for *FT5* (2,063.70 μM Fe(II)), yielding a 5.4-fold difference between the two. The average reducing capacity of ten studied fruit tea infusions was 5,339.50 μM Fe(II).

The Impact of Ascorbic Acid and Honey Addition on the TP Content and Reducing Capacity of Fruit Tea Infusions

The addition of ascorbic acid to fruit tea infusions (series **b**), resulted in an increase in the TP content and reducing capacity of all studied fruit tea infusions. In comparison with pure tea infusions, the TP content of fruit teas

TABLE 2.
TOTAL PHENOL CONTENT (TP), EXPRESSED IN MG/L GAE, AND FERRIC REDUCING ANTIOXIDANT POWER (FRAP), EXPRESSED IN MM FE(II), OF STUDIED FRUIT TEA INFUSIONS

Fruit tea	TP			FRAP		
	a	**b**	**c**	**a**	**b**	**c**
FT1	629.05 ± 48.21	1,748.65 ± 169.14	1,410.45 ± 53.57	4,257.80 ± 172.26	13,982.27 ± 206.66	13,870.27 ± 357.69
FT2	741.83 ± 19.52	1,885.68 ± 72.23	1,444.50 ± 47.54	5,187.93 ± 317.69	13,422.73 ± 376.83	13,841.53 ± 99.68
FT3	1,549.10 ± 57.34	2,600.48 ± 19.91	2,274.42 ± 84.41	11,075.80 ± 312.56	19,979.40 ± 198.42	20,286.67 ± 193.08
FT4	611.95 ± 57.69	1,373.63 ± 153.55	1,253.63 ± 46.52	2,986.93 ± 223.59	11,379.07 ± 202.26	11,722.53 ± 221.51
FT5	323.40 ± 34.00	1,421.68 ± 215.42	929.42 ± 40.11	2,063.70 ± 208.96	11,252.00 ± 346.49	9,813.87 ± 113.65
FT6	817.07 ± 54.65	1,925.13 ± 93.44	1,549.92 ± 26.00	5,369.53 ± 120.94	14,545.67 ± 356.54	14,960.13 ± 168.33
FT7	651.70 ± 36.79	2,147.18 ± 166.13	1,384.75 ± 26.70	4,091.67 ± 137.71	13,542.47 ± 181.47	12,753.73 ± 152.56
FT8	1,134.55 ± 106.46	2,282.98 ± 93.44	1,863.95 ± 53.28	6,794.07 ± 186.41	17,431.53 ± 358.57	18,406.00 ± 105.54
FT9	676.78 ± 58.85	1,596.60 ± 250.88	1,350.92 ± 62.13	4,743.33 ± 107.70	13,870.93 ± 235.28	12,938.13 ± 207.27
FT10	1,115.60 ± 62.40	2,438.05 ± 52.33	1,955.30 ± 33.68	6,824.20 ± 241.31	16,160.67 ± 215.24	16,602.47 ± 253.16
Average	825.10	1,942.01	1,541.73	5,339.50	14,556.67	14,519.53

a, Pure tea.
b, Tea with added ascorbic acid (22.5 mg).
c, Tea with added ascorbic acid (22.5 mg) and honey (1.5 g).

with the addition of ascorbic acid was about 2.4-fold higher, while the reducing power was approximately 2.7-fold higher (statistically significant in both cases, $P < 0.05$). The increase in the TP content for series **b** fruit teas may be partly accounted for by poor selectivity of the FC reagent. Namely, the FC reagent also reacts with the added ascorbic acid, resulting in higher absorbance and a higher overall TP content (Singleton and Rossi 1965). Ascorbic acid (vitamin C) is added extensively to a variety of food products as either an anti-oxidant or a vitamin supplement (Kirby *et al.* 1991).

The highest TP content of fruit teas with added ascorbic acid was once again determined for *FT3* infusion (2,600.48 mg/L GAE), which was a logical expectation considering the fact that pure *FT3* initially exhibited the highest TP content. The lowest TP content in series **b** fruit teas was determined for *FT4* infusion (1,373.63 mg/L GAE); a 1.9-fold lower value with regard to *FT3*. As in the case of series **a** infusions, the highest reducing capacity in series **b** was again established for *FT3* infusion (19,979.40 mM Fe(II)), and the lowest for *FT5* (11,252.00 mM Fe(II)).

The addition of honey (1,5 mg) to fruit tea infusions already containing ascorbic acid (22,5 mg) resulted in an average 28%, statistically significant, decrease ($P < 0.05$) in the TP content of fruit tea infusions (series **c**). This is probably due to the interactions between carbohydrates from honey and phenolic compounds from tea, making the hydroxyl groups of polyphenols unavailable for reaction with the FC reagent (Sharma *et al.* 2008). *FT3* infusion with added honey and ascorbic acid once again exhibited the highest TP content (2,274.42 mg/L GAE); a 2.5-fold higher value in comparison to *FT5* (929.42 mg/L GAE).

The observed FRAP values for the series **c** infusions show a different impact of honey addition on the reducing capacity of infusions, in comparison to the TP content. Namely, a slight (~8%) insignificant decrease ($P > 0.05$) in the reducing capacity was observed upon addition of honey to four infusions already containing ascorbic acid (*FT1, FT5, FT7* and *FT9*). Plumb *et al.* (1998) found that there is a requirement for the 3-hydroxyl group in the C ring of catechins to be unblocked for maximum effectiveness of anti-oxidant activity in the aqueous phase. Glycosylation at this position reduces the ability of the B ring hydroxyls to donate hydrogen and, in turn, impairs the anti-oxidant effectiveness of catechins and catechin-containing beverages. In the remaining infusions, a slight increase in the reducing capacity of about 3% was observed. Overall, the addition of honey to fruit tea infusion already containing ascorbic acid did not cause a significant ($P > 0.05$) change in the reducing capacity of tested tea infusions. The highest reducing capacity in series **c** infusions was determined for *FT3* infusion (20,286.67 μM Fe(II)), while the lowest was determined for *FT5* infusion (9,813.87 μM Fe(II)).

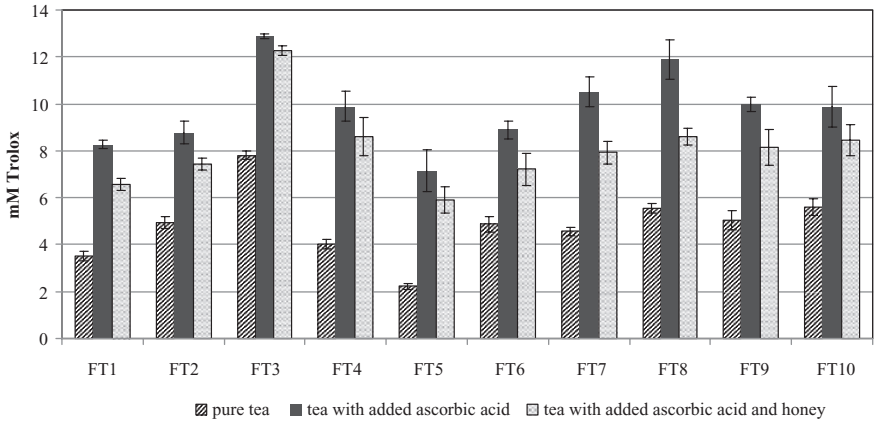

FIG. 1. TROLOX EQUIVALENT ANTI-OXIDANT CAPACITY (TEAC), EXPRESSED IN MM, OF THE THREE SERIES OF FRUIT TEA INFUSIONS OBTAINED IN THE DPPH RADICAL SCAVENGING ASSAY

DPPH· Radical Scavenging Capacity of Fruit Tea Infusions

Figure 1 shows the DPPH· radical scavenging capacities, expressed as mM Trolox, for the three series of analyzed fruit tea infusions. *FT3* infusion (series **a**) is the most efficient DPPH· radical scavenger (7.81 mM Trolox), followed by *FT10* and *FT8* infusions, while *FT5* exhibited the poorest DPPH· radical scavenging capacity (2.23 mM Trolox). The average DPPH· radical scavenging capacity of analyzed infusions amounted to 4.81 mM Trolox, which is comparable to the scavenging capacity of Roiboos tea, 6.8 mM Trolox (Tabata *et al.* 2008).

The Impact of Ascorbic Acid and Honey Addition on the DPPH· Radical Scavenging Capacity of Fruit Tea Infusions

The addition of ascorbic acid caused, on the average, a twofold increase in the DPPH• radical scavenging capacity of tested fruit teas (Fig. 1). This may be explained by the fact that ascorbic acid itself acts as a powerful anti-oxidant and considerably reduces the free DPPH· radical (Villaño *et al.* 2007). Similarly, Murakami *et al.* (2003) evaluated the effect of ascorbic acid and α-tocopherols on the anti-oxidant activity of polyphenols and observed an increase in the DPPH radical scavenging capacity of polyphenols in the presence of ascorbic acid. In the series **b** of fruit tea infusions, *FT3* infusion exhibited the highest DPPH radical scavenging capacity (12.87 mM Trolox), in contrast to *FT5*, which exhibited the lowest DPPH radical scavenging capacity (7.15 mM Trolox).

The addition of honey to fruit tea infusions already containing ascorbic acid resulted in an average 22% decrease (significant, $P < 0.05$) in the DPPH radical scavenging capacity (series **c**). Sharma *et al.* (2008) investigated the effect of milk and sugar addition on the anti-oxidant activity of black tea in the DPPH model system and also observed a decrease in the radical scavenging capacity of black tea upon addition of sugar. Just as in the case of series **a** and series **b** infusions, *FT3* infusion once again exhibited the highest (12.25 mM Trolox), and *FT5* the poorest $DPPH^{\cdot}$ scavenging efficiency (5.90 mM Trolox). In comparison to pure tea infusions (series **a**), a 1.2-fold increase in the $DPPH^{\cdot}$ scavenging capacity was observed in infusions with added ascorbic acid and honey (series **c**).

$ABTS^{\cdot+}$ Radical Scavenging Capacity of Fruit Tea Infusions

On average, 12% higher TEAC values were obtained for tested tea infusions in the ABTS assay (Fig. 2) in comparison to the TEAC values obtained in the DPPH assay (Fig. 1). This observation is in accordance with the finding that $ABTS^{\cdot+}$ radical reacts with lipophilic and hydrophilic compounds, while $DPPH^{\cdot}$ reacts only with lipophilic anti-oxidants (Prior *et al.* 2005). Lee *et al.* (2003) observed up to 30% lower anti-oxidant capacity of cocoa in the $DPPH^{\bullet}$ scavenging system as opposed to the $ABTS^{\bullet+}$ scavenging system. Even though the radical scavenging capacities obtained in the ABTS assay were higher than the ones obtained in the DPPH assay, there were no drastic differences in the

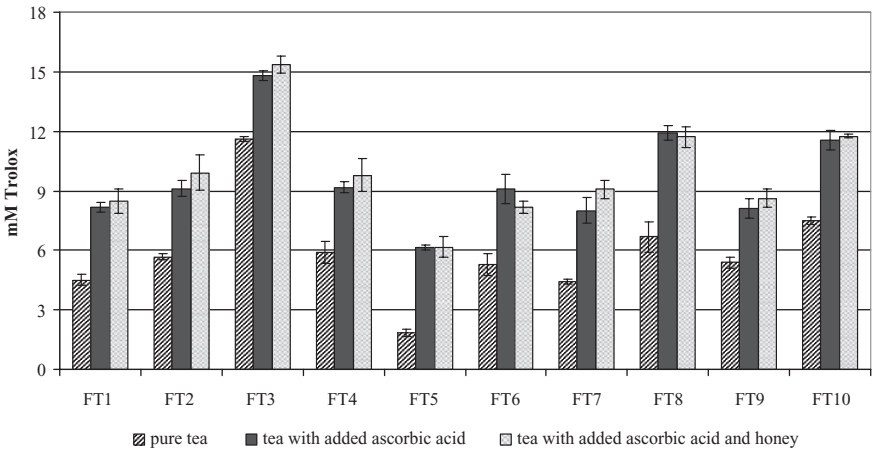

FIG. 2. TROLOX EQUIVALENT ANTI-OXIDANT CAPACITY (TEAC), EXPRESSED IN MM, OF THE THREE SERIES OF FRUIT TEA INFUSIONS OBTAINED IN THE ABTS RADICAL SCAVENGING ASSAY

sequence of TEAC values of analyzed infusions. *FT3* infusion once again exhibited the highest TEAC value (11.63 mM Trolox), followed by *FT10* (7.49 mM Trolox) and *FT8* (6.67 mM Trolox), while *FT5* (1.81 mM Trolox) once again came out at the end of the scale in series **a** fruit teas. Majchrzak *et al.* (2004) found that the anti-oxidant capacity of green tea, as evaluated in the ABTS assay, ranges from 13.3 to 21.6 mM Trolox, which indicates that the scavenging capacity of our *FT3* tea is comparable to green tea.

The Impact of Ascorbic Acid and Honey Addition on the $ABTS^{\cdot+}$ Radical Scavenging Capacity of Fruit Tea Infusions

The addition of ascorbic acid to fruit tea infusions resulted in an average 1.6-fold increase in the TEAC of tea infusions evaluated by the ABTS assay (significant, $P < 0.05$), series **b** in Fig. 2. This may be explained by the earlier mentioned synergistic effect of ascorbic acid on the phenolics present in tea infusions, as well as by the fact that ascorbic acid itself is an excellent $ABTS^{\bullet+}$ radical scavenger (Romay *et al.* 1996). Our findings correspond to the ones of Majchrzak *et al.* (2004), who observed an increase in the ABTS radical scavenging capacity of green and black teas upon the addition of ascorbic acid. Majchrzak *et al.* (2004) observed a linear relationship between the measured anti-oxidant capacity and the amount of added ascorbic acid. In series **b** fruit teas, *FT3* infusion exhibited the highest ABTS radical scavenging efficiency (14.81 mM Trolox), making it a 1.5-fold more potent radical scavenger than *FT5* infusion (6.14 mM Trolox).

The addition of honey to fruit tea infusions already containing ascorbic acid resulted in a more complex situation. In 8/10 analyzed infusions, an increase in the $ABTS^{\bullet+}$ radical scavenging efficiency was observed, while only the radical scavenging efficiency of *FT6* and *FT8* infusions decreased (6%). Based upon the results of the ABTS assay, addition of honey to fruit teas containing ascorbic acid does not significantly ($P < 0.05$) alter their anti-oxidant capacity. The highest radical scavenging capacity in series **c** was once again observed for *FT3* infusion (15.37 mM Trolox), and the lowest for *FT5* infusion (6.16 mM Trolox).

HPLC Analysis of Phenolic Compounds

High performance liquid chromatography analysis of fruit tea infusions was performed to deduce the presence of individual polyphenolic compounds in pure fruit tea infusions (series **a**). A sample chromatogram of 13 standard phenolic compounds is shown in Fig. 3. Considering the complex composition of studied fruit teas, the resulting chromatograms were difficult to interpret, with a large number of unidentified peaks and an elevated base line. Our chromatograms in some cases indicated the presence of sugar moities bound to

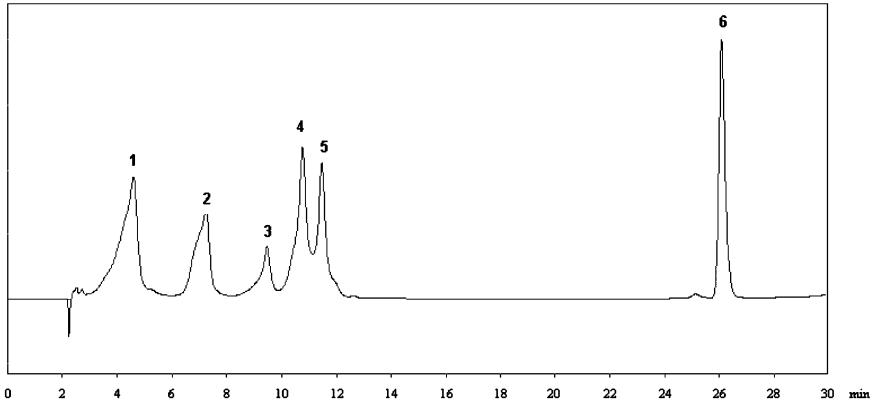

FIG. 3. HPLC CHROMATOGRAM OF SIX STANDARD PHENOLIC COMPOUNDS IDENTIFIED IN FRUIT TEA INFUSIONS: 1. GALLIC ACID, 2. PROTOCATEHUIC ACID, 3. CATECHIN, 4. CHLOROGENIC ACID, 5. EPIGALLOCATECHIN GALLATE, 6. *TRANS*-CINNAMIC ACID

phenolic compounds in the sample, which resulted in shifted retention times and altered UV-spectra in comparison to the corresponding aglycons.

Using the method of internal standard addition, six free phenolic compounds were identified in pure tea infusions, among which the catechins, catechin (C) and epigallocatechin-gallate (EGCG) were the most abundant (Table 3). Catechin was identified in 5 out of 10 fruit teas, ranging from 28.29 mg/L (*FT1*) do 116.19 mg/L (*FT10*). Epigallocatechin-gallate was identified in all tested fruit teas except in *FT7* infusion. The EGCG concentrations ranged from 19.26 mg/L (*FT8*) to 161.41 mg/L (*FT3*). As can be seen, *FT3* and *FT9* infusions contained the highest content of EGCG (161.41 mg/L and 134.57 mg/L, respectively), in contrast to catechin, which was not identified in these infusions. Four different phenolic acids were also identified in our HPLC analysis of fruit tea infusions: gallic acid, protocatehuic acid, chlorogenic acid and *t*-cinnamic acid. While gallic acid was identified in all analyzed fruit tea infusions (ranging from 2.26 mg/L in *FT1* infusion to 15.15 mg/L in *FT10* infusion), protocatehuic acid was identified in only four fruit tea samples. The highest content of protocatehuic acid was identified in *FT1* tea (1.74 mg/L), and the lowest in *FT6* tea (12.85 mg/L). Among the phenolic acids, chlorogenic acid was the most abundant and was identified in all fruit teas. The content of chlorogenic acid varied between 12.86 mg/L in *FT5* infusion to 91.55 mg/L in *FT3* infusion. *Trans*-cinnamic acid was found in five studied fruit teas which contained cinnamon as a flavoring agent. The highest content of *t*-cinnamic acid was determined in *FT4* tea (64.99 mg/L) and the lowest in *FT10* tea (11.78 mg/L).

TABLE 3.
CONCENTRATIONS OF PHENOLIC COMPOUNDS, EXPRESSED IN MG/L, IDENTIFIED IN THE STUDIED FRUIT TEA INFUSIONS (GA-GALLIC ACID, PCA-PROTOCATEHUIC ACID, C-CATECHIN, CHLA-CHLOROGENIC ACID, EGCG-EPIGALLOCATECHIN GALLATE, *T*-CA-*TRANS*-CINNAMIC ACID)

Fruit tea	FT1	FT2	FT3	FT4	FT5	FT6	FT7	FT8	FT9	FT10
Phenolic acids (mg/L)										
GA	2.26	8.31	13.75	5.73	3.82	6.41	4.91	7.23	5.52	15.15
PcA	1.74	–	–	–	9.83	12.85	–	–	10.73	–
ChlA	31.32	48.53	91.55	38.41	12.86	43.34	17.46	28.08	45.51	78.61
t-CA	17.80	–	–	64.99	–	–	29.01	13.62	–	11.78
Catechins (mg/L)										
C	28.29	60.94	–	–	–	83.39	–	68.07	–	116.19
EGCG	53.03	76.77	161.41	61.02	41.95	90.44	–	19.26	134.57	76.37

Standard deviations of all determined concentrations amounted to less than 15%.

All phenolic standards were identified solely in the *FT1* infusion, but in lower amounts compared to the remaining infusions. This observation is in accordance with the previously noted lower TP content as well as poor radical scavenging capacity of *FT1* infusion. Our analysis revealed that *FT3* tea is the richest source of polyphenolic compounds, the most potent radical scavenger and contains the highest content of EGCG and chlorogenic acid.

CONCLUSIONS

Among the 10 analyzed fruit tea infusions *FT3* exhibited the highest TP content and anti-oxidant capacity with respect to all three employed assays, which was attributed to its high content of rose hip fruit. The addition of ascorbic acid (22.5 mg) resulted in an increase in the TP content as well as anti-oxidant capacity of all ten tested fruit tea infusions. The addition of honey (1.5 g) to infusions already containing ascorbic acid resulted in an average 28% decrease in the TP value, and a significant (22% on the average) decrease in the anti-oxidant capacity evaluated by the DPPH assay. In contrary to the initial assumption, the addition of honey to fruit tea infusions already containing ascorbic acid did not cause an increase in the overall anti-oxidant capacity of infusions. The observed decrease in the radical scavenging capacity of series **c** infusions was attributed to glycosylation, which reduces the ability of the B ring hydroxyls to donate hydrogen and, in turn, impairs the anti-oxidant effectiveness of infusions containing honey.

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