

Evaluation of the antioxidant properties of fruit and flavoured black teas

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Abstract

Objective Antioxidant properties of the water extracts of the commercial bagged fruit and flavoured black teas were evaluated and compared with typical black teas of *C. sinensis*.

Methods Folin–Ciocalteu (FC) assay, cupric ion reducing antioxidant capacity (CUPRAC) and DPPH radical method were used for these purposes. The content of selected flavonoids and phenolic acids was also determined by high-performance liquid chromatography with tandem mass spectrometry in the negative electrospray ionization mode.

Results Flavoured black teas contain significantly higher level of catechins, quercetin, and rutin, while the content of chlorogenic and caffeic acids as well as naringin and hesperidin was higher in fruit teas. Supplementation with these flavonoids could reduce blood glucose. In FC and DPPH assays, the antioxidant properties of studied tea infusion increases in the order: fruit tea < flavoured black tea > premium black tea, while in CUPRAC method, some aromatized teas exhibit the highest antioxidant properties. Tea infusions with nice smell of fruits would also support the human diet with some source of antioxidants.

Keywords Fruit and flavoured teas · Antioxidant properties · Polyphenol content · CUPRAC · DPPH

Introduction

Tea is one of the most widely consumed beverages in the world, next only to water. It is produced from the same

plant *Camelia sinensis* and can be categorized into 3 types, depending on the level of fermentation, i.e., green (unfermented), oolong (partially fermented), and black (fermented) tea. Although this process is often assumed, incorrectly, to be fermentation, which usually implies additives, the more correct term should be oxidation, which means exposure to air while drying [1]. Green tea processing includes a step to inactive oxidizing enzymes, such as polyphenol oxidase, which partially converts monomeric polyphenols (mainly catechins) into dimeric/oligomeric compounds, such as theaflavins and thearubigins, responsible for the characteristic aroma and colour of black tea. Oolong tea is a result of oxidation being stopped somewhere in between that of green and black tea and therefore contains flavonoids that are found in both teas. Not only green and black tea but also various kinds of fruit and flavoured teas are very popular in many European countries. These teas are obtained by the addition of natural aromas and dry fruits or herbs to tea leaves in the last stage of processing before packing. They are popular because of their fragrance, therapeutic applications, and lower content of caffeine, which could inhibit calcium absorption, particularly for older people [2, 3]. One cup of very strong brewed tea (about 45 mg of caffeine) might lead to a loss of 2–3 mg of calcium [4].

Tea polyphenols, especially flavonoids, have attracted considerable interest because of their associated health properties, and these have been summarized in several papers [5–7]. The studies have demonstrated that tea flavonoids possess strong antioxidant activity and metal-chelating properties, thus may protect cells and tissues against reactive oxygen and nitrogen species. A typical cup of green tea of usual strength (1.5% w/v) contains an amount of antioxidant power similar to that found in 100–200 mg of pure vitamin C) [8]. Regular intake of tea

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may therefore improve antioxidant status in vivo and help lower risk of certain types of cancer and coronary heart disease [9].

Considering the increasing interest in the health properties of tea, the present study has been designed to compare the antioxidant properties of different commercially available bagged flavoured black teas and fruit teas (all Lipton brand names). The typical black teas of *C. sinensis* (Yellow label) were used as positive control. Like most branded teas, Lipton teas are a blend selected from many different plantations around the world, from well-known producing countries like China, India and Sri Lanka. Folin–Ciocalteu (FC) assay, cupric ion reducing antioxidant capacity (CUPRAC), and DPPH radical method were used to evaluate the antioxidant properties of tea infusions. The content of selected flavonoids and phenolic acids was also determined by LC–MS in the negative electrospray ionization mode.

Materials and methods

Reagents and apparatus

The commercial standards of flavonoids and polyphenolic acids, Folin–Ciocalteu's reagent, and DPPH (2,2-diphenyl-1-picrylhydrazyl radicals) as well as the rest of the chemicals were purchased from Sigma (Steinheim, Germany). Methanol and acetonitrile were of HPLC gradient grade from Merck (Darmstadt, Germany). Ultrapure water from Milli-Q system (Millipore, Bedford, MA, USA) with a conductivity of 18 MQ was used in all experiments. All solutions were filtered through 0.45- μ m membranes (Millipore) and degassed prior to use.

Spectrophotometric determinations were carried out on a Perkin Elmer model Lambda 20 UV–VIS spectrophotometer with cuvettes of 1 cm length. Spectra were recorded in the range from 220 to 600 nm with 0.2-nm resolution. Data were processed with WinLab software.

Chromatographic analysis was performed with a Shimadzu LC system consisted of binary pumps LC20-AD, degasser DGU-20A5, column oven CTO-20AC, autosampler SIL-20AC, detector UV SPD 20A connected to 3200 QTRAP Mass spectrometer (Applied Biosystem/MDS SCIEX). A MS system equipped with electrospray ionization source (ESI) operated in negative ion mode and a quadrupole mass analyser in a scan mode from 50 to 1500 m/z. ESI conditions were the following: capillary temperature 450 °C, curtain gas at 0.3 MPa, auxiliary gas at 0.3 MPa, negative ionization mode source voltage—4.5 kV. Nitrogen was used as curtain and auxiliary gas.

Compounds were separated on Kinetex™ (Phenomenex) C-18 column (100 \times 2.1 mm, 2.6 μ m) with precolumn at 30 °C; 2 mM formic acid (pH 2.8) as eluent A and acetonitrile as eluent B were used. The mobile phase was delivered at 0.2 mL/min in linear gradient mode: 0–5 min 20% B, 10–15 min 25% B, 20–25 min 30% B, 30–31 min 90% B, 32 min 20% B. Compounds were identified by comparing retention time and *m/z* values obtained by MS and MS² with the mass spectra from standards tested under the same conditions (Table 1). Quantification of compounds was made from the calibration curves obtained in multiple reaction mode (MRM) [10].

Teas and preparation of infusions

All bagged teas were purchased from a local market. They contain, except leaves of *C. sinensis*, also dry fruit and herbs which are visible, as well as some natural aroma. This set of teas was chosen as they are very popular and widely sold. This includes four fruit teas with commercial names: *Caribbean* (containing orange leaves, rosehip, papaya, hibiscus), *Delight citrus* (rosehip, apple, lemon, and grapefruit peels), *Char Ming* (blackcurrant, rosehip, hibiscus), and *Temptation* (rosehip, strawberry, blackberry, raspberry) as well as four flavoured black teas named: *Blue fruit* (bilberry, blackcurrant, blackberry, raspberry), *Forest fruit* (raspberry, cherry, blackberry, redcurrant), *Citrus* (orange, lemon, grapefruit, limon), and *Tropical fruit* (pineapple, grapefruit peels). For comparison, also typical black tea Lipton brand (Yellow label) was studied. The content of tea bags supplied from the market was opened, and tea was weighted; masses were ranging between 1.64 and 2.02 g.

The tea bags were dipped into 200 mL of freshly boiled water for 5 min to represent the typical quantity consumed by tea drinkers. After the infusion time, the bags were removed and the partly turbid solutions were filtered after cooling to room temperature and then analysed.

Antioxidant properties of teas

In Folin–Ciocalteu assay, 1 mL of tea infusion was introduced into test tubes followed by 0.1 mL of FC reagent and 0.9 mL of water. The tubes were allowed to stand for 5 min. At the end of this period, 1 mL of sodium carbonate (7%, w/v) and 0.4 mL of water were added, and 10 more min was allowed for stabilization of the blue colour formed. The absorbance against a reagent blank was measured at 765 nm. The results were expressed as gallic acid equivalent (GAE) in mg/g of dry matter. The determinations were carried out for three independently prepared samples of each type of tea.

Table 1 LC/MS/MS characteristics of studied compounds in negative ion mode

Compound	Retention time, min	Q1 Mass (amu)	Q3 Mass (amu)	DP, V	CE, V
Gallic acid	2.18	169	125	−45	−20
Chlorogenic acid	2.39	353	191	−20	−22
Caffeic acid	2.98	179	135	−10	−24
Syringic acid	3.02	197	182	−20	−18
Rutin	3.55	609	300	−65	−56
<i>p</i> -coumaric	4.14	163	119	−20	−18
Synapic acid	4.48	223	164	−35	−20
Ferulic acid	4.75	193	134	−30	−20
Catechin	5.12	289	109	−45	−34
Naringin	5.49	579	271	−80	−52
Hesperidin	5.75	609	301	−65	−30
Epicatechin	8.20	289	109	−40	−32
Epigallocatechin 3-gallate	9.55	457	169	−35	−22
<i>Trans</i> -cinnamic acid	13.26	146	103	−30	−14
Luteolin	14.46	285	133	−60	−44
Quercetin	14.72	301	151	−40	−30
Naringenin	18.71	271	151	−45	−26
Genistein	19.41	269	133	−75	−52
Apigenin	20.62	269	117	−55	−42
Kaempferol	21.82	285	151	−45	−25
Isorhamnetin	28.86	315	300	−35	−24
Rhamnetin	30.88	315	165	−35	−30

Radical scavenging activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay; 0.1 mL of tea infusion was mixed with 2.4 mL DPPH solution (3×10^{-5} M) in methanol, and immediately, the change of absorbance at 539 nm was recorded over time against the blank. The results were expressed as the percentage of reduction (inhibition) of the DPPH according to expression: $(A_0 - A_t)/A_0 \times 100$, where A_0 is the initial absorbance, and A_t is the absorbance at increasing time t .

For assessing cupric ion reducing ability (CUPRAC), the assay described by Apak et al. [11] was adapted. In a test tube, 1 mL CuCl_2 solution (1.0×10^{-2} M) was mixed with 1 mL of neocuproine alcoholic solution (7.5×10^{-3} M) and 1 mL of 1 M NH_4AC buffer (pH 7), followed by mixing 0.5 mL of tea infusion and 0.6 mL of water. Absorbance against the reagent blank was measured after 30 min at 450 nm. In another sets of experiments, the tube containing sample and reagents was incubated in a water bath at a temperature of 50 °C for 20 min. Then the tube was cooled under running water, and its A_{450} value was measured. The infusions of black teas were diluted tenfold with water to keep the absorbance at 450 nm between 0.2 and 0.4 absorbance units. The calibration curve was built-up with trolox (TR), and the antioxidant activity of the tea infusions was expressed as trolox equivalent (mmol TR/g of dry matter).

Results

HPLC–MS analysis

Examples of the chromatograms of tea infusions by LC/MS/MS are presented in Fig. 1. Peak identity was established by both the retention time compared with that of the standards and the characteristic transitions (precursor and product ion pair). The concentration of some relevant flavonoids and phenolic acids found in the selected tea infusions are presented in Table 2. Gallic, chlorogenic, *p*-coumaric, ferulic and synaptic acid were detected in all studied infusions. Flavoured black tea infusions had significant content of gallic acid (5.59–8.51 mg/L) but did not contain synaptic acid (except detected traces in *Citrus* and *Blue fruit* teas). The level of chlorogenic and caffeic acid was higher in fruit teas. In plants, caffeic acid is formed from *p*-coumaric acid and is transformed later to ferulic acid. The order of hydroxycinnamic acids found in citrus fruits was *p*-coumaric acid > ferulic acid > caffeic acid [12]. This order agrees with the content of these compounds in the studied fruit teas which contain orange leaves (*Caribbean*) and citrus peels (*Delight citrus*). Also these phenolic acids were detected in similar order in black tea aromatized with citrus fruits (*Citrus*) (Table 2). No peaks of *p*-HPA and *trans*-cinnamic acid (except *Caribbean* tea)

were recorded, which confirms data obtained by Mattila et al. [13].

In relation to the catechin content in the analysed samples, flavoured black teas contain much higher level of these compounds in comparison with fruit black teas and even to premium black tea (*Yellow label*). The level of epigallocatechin 3-gallate (EGCG), polyphenol with very high antioxidant activity [14], ranged from 12.3 to 29.8 mg/L in studied flavoured teas. In some fruit tea infusions, epicatechin (*Char Ming*) and EGCG (*Char Ming* and *Temptation*) were not found.

The predominant flavonones in citrus fruits are naringenin and hesperidin [15], and these compounds were detected in studied fruit teas. Particularly, *Delight citrus* tea is very rich in these flavonones and contains 5.81 and 25.2 mg/L of hesperidin and naringin, respectively. Flavone luteolin was detected only in brewed black teas in the range of 0.006–0.010 mg/L. Amongst flavonols, quercetin and traces of myricetin (about 0.014 mg/L) were present only in black tea infusions. Kaempferol as aglycone has not been found in all studied teas, although its presence in the forms of different glycosides was determined in several tea and herbal infusions [16]. The concentration of rutin was much higher in teas of *C. sinensis* than in fruit teas; the highest value of 18.75 mg/L was found in *Yellow label* brand. Differences in flavonoid content per teas serving are the result of tea variety, weight of tea used, and to a lesser extent, brewing technique [17]. It should be mentioned that some flavoured teas, particularly *Blue fruit* and *Forest fruit*, contain also anthocyanins. Apart from their colouring effects in fruits, anthocyanins show ability to prevent lipid oxidation and scavenging activity against various free radicals [18].

Antioxidant properties

The results obtained by Folin–Ciocalteu method varied widely in the studied types of tea. The obtained results for fruit tea infusions are very similar (in the range of 123–141 mg GAE/g dry weight) but much lower than for flavoured black teas (Fig. 2). The highest results were found for *Blue fruit* (361.6 mg GAE/g), while the lowest for *Caribbean* (122.9 mg GAE/g). Typical Lipton black tea (*Yellow label* brand) exhibits the antioxidant properties equals to 420 mg GAE/g and 835.6 mg GAE/200 mL of infusion.

Two set of experiments were conducted using CUPRAC method; in the first assay, absorbance was measured after keeping the mixed components at room temperature for 30 min, while in the second assay, the samples were incubated after reagent addition at 50 °C. The obtained results were expressed in mmol of TR/g of tea as trolox equivalent (TE) and are presented in Fig. 3. As expected,

black tea infusion exhibit higher TE values than studied fruit teas. These teas contain much higher content of quick reacting flavonoids such as catechins, quercetin and gallic acid, which have also the highest antioxidant capacities in CUPRAC assay [11]. Slow reacting antioxidants such as naringenin, sinapic and chlorogenic acids, the most abundant phenolic in the citrus and berry families as well as in some other fruits, needed elevated temperature incubation to complete their oxidation with the copper(II)-neocuproine reagent. In the measurement without incubation, the highest antioxidant capacity of 10.8 mg TR/g was observed for *Forest Fruit* tea, which is aromatized mainly with raspberry and blackberry. After incubation, all TE values increased, amongst fruit teas particularly for *Char Ming* and *Temptation* (fruit teas) as well as for *Tropical fruit* (black flavoured tea).

The percentages of DPPH radical scavenging effect of each tea infusion for 5 and 20 min after addition of reagent are shown in Table 3. As can be seen, black flavoured teas as well as typical black teas of *C. sinensis* (*Yellow label*) exhibit high capacity to scavenging the DPPH radicals already in 5 min. Very similar inhibition of DPPH in the range of 59–68% was observed for that kind of teas, despite the time after mixing the components. The decay slope and the absorbance level reached by the remaining DPPH radicals vary significantly with different types of studied tea infusions (Fig. 4). A fast and remarkable decrease in the absorbance of DPPH was observed during the first few minutes of the reaction for black teas, followed by slow subsequent disappearance of the reagent. This fast step essentially refers to the electron transfer process from B ring (3'-OH and 4'-OH) of flavonoid molecules to DPPH and latter kinetic reflects the remaining activity of the oxidation–degradation products [19]. For flavoured black teas, constant slow decay of DPPH absorbance was observed. The higher antioxidant properties of black teas could be due to the content and composition of major polyphenols (Table 2).

Discussion

Tea provides a dietary source of biologically active compounds, including polyphenols, which help prevent a wide variety of diseases. The antioxidant activity of tea polyphenols is mainly due to their ability to scavenge free radicals and to increased activity of some detoxifying enzymes in small intestine, liver and lungs [2]. Thus, tea consumed throughout the world is believed to be not only a popular beverage but also an antioxidant agent available in everyday life. Flavoured and fruit teas, which are popular because of their fragrance and lower amounts of caffeine, could be a good source of compounds with antioxidant

Fig. 1 Total ion current of MRM of **a** *Delight citrus* and **b** *Tropical fruit* infusions and traces of phenolic compounds in MRM mode. MS/MS conditions described in the text

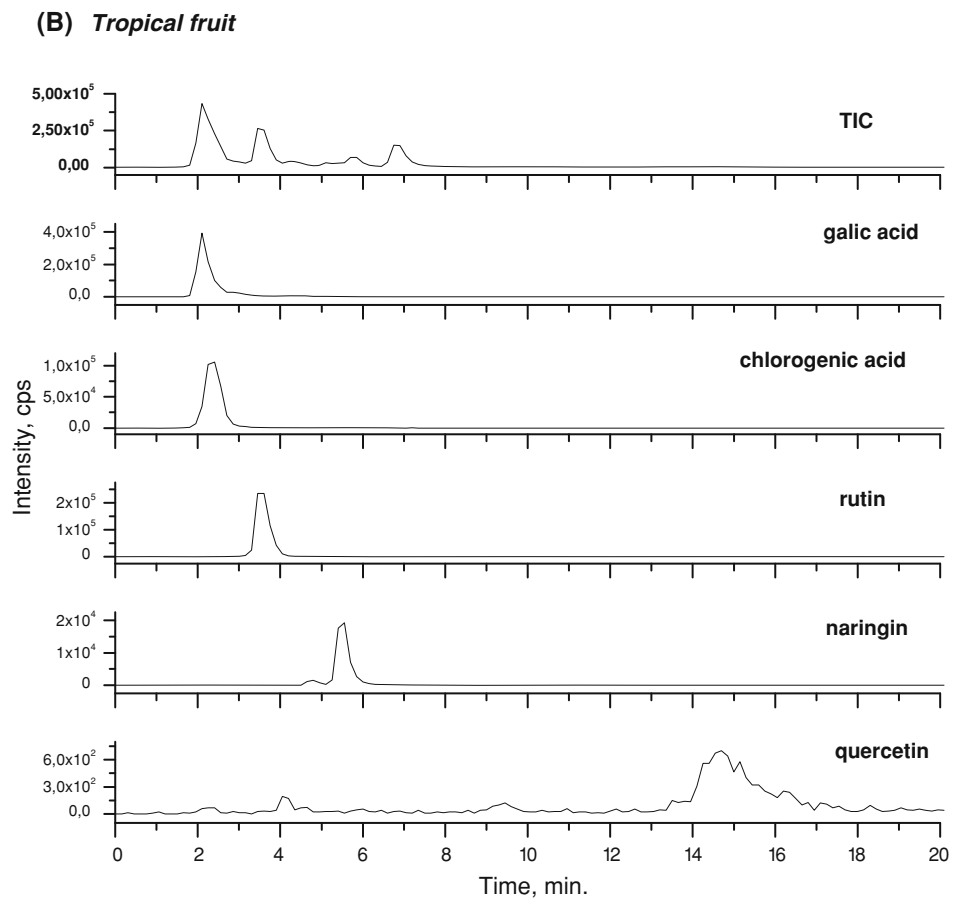
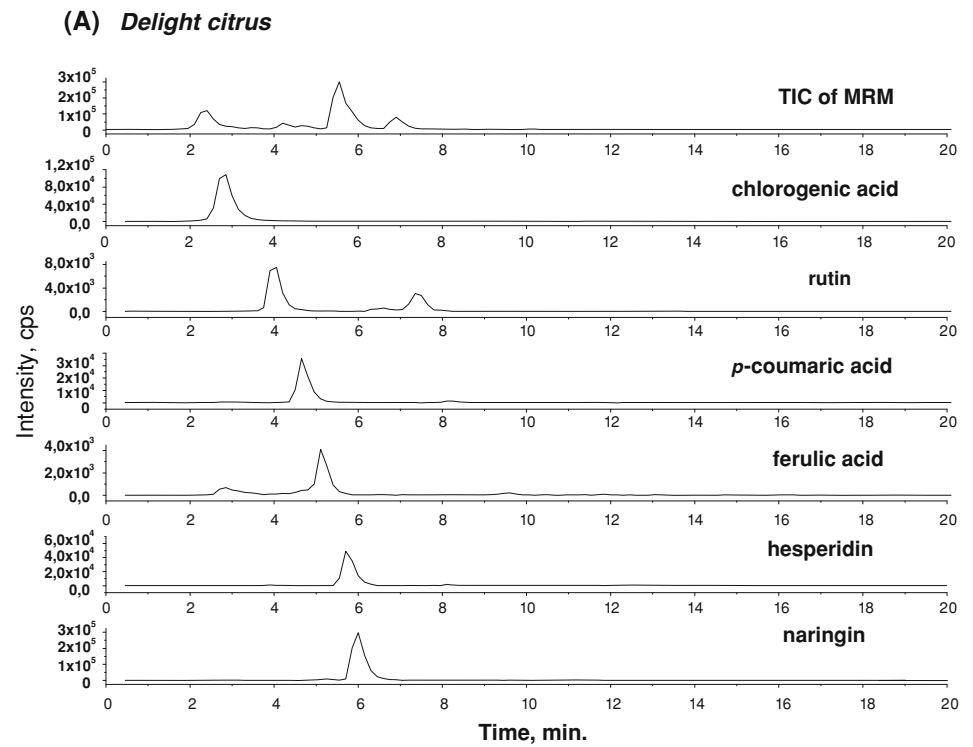
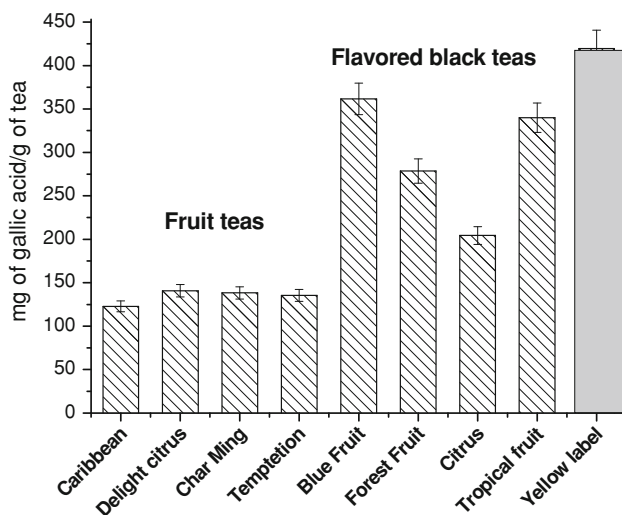


Table 2 Contents of selected flavonoids and phenolic acids in tea infusions expressed in mg/L

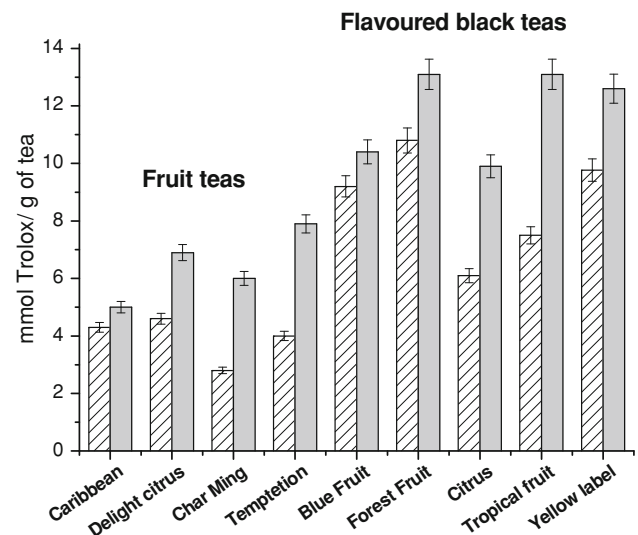
	Caribbean	Delight citrus	Char ming	Temptation	Blue fruits	Forest fruits	Citrus	Tropical fruits	Yellow label	LOD (mg/L)
Rutin	0.18	0.37	1.89	1.16	9.91	9.05	8.59	11.3	18.8	0.001
Quercetin	nd	nd	nd	nd	0.10	0.06	0.04	0.3	0.17	0.03
Naringin	4.99	25.2	nd	nd	0.007	0.009	3.51	1.64	nd	0.001
Rhamnetin	0.005	0.004	0.004	0.004	0.006	0.038	0.002	0.004	0.003	0.001
Luteolin	nd	nd	nd	nd	0.008	nd	0.006	0.010	0.009	0.001
Hesperidin	0.23	5.81	nd	nd	nd	nd	0.69	nd	nd	0.005
Catechin	1.58	0.61	0.55	0.88	12.0	7.97	9.17	15.9	1.71	0.001
Epicatechin	1.87	0.72	nd	1.04	7.01	9.42	10.9	18.8	2.35	0.05
Epigallocatechin 3-gallate	0.63	0.024	nd	nd	12.3	14.9	21.7	29.8	9.47	0.001
Gallic acid	0.049	0.051	0.031	0.007	6.32	5.59	7.94	8.51	8.48	0.0005
Ferulic acid	0.10	0.10	0.032	0.021	0.025	0.025	0.0060	0.036	0.046	0.001
Synapic acid	0.031	0.065	0.050	0.037	0.001	nd	0.010	nd	nd	0.001
Caffeic acid	0.041	0.052	0.076	0.037	0.018	0.020	0.031	0.023	0.020	0.001
Chlorogenic acid	2.44	1.74	2.72	2.44	1.77	1.72	1.60	1.72	1.93	0.001
<i>p</i> -coumaric acid	0.32	0.24	0.069	0.098	0.18	0.22	0.36	0.27	0.49	0.0005

nd not detected, e.g. < LOD

**Fig. 2** Antioxidant capacity of flavoured black and fruit tea infusions measured by Folin–Ciocalteu method

properties. Moreover, they are characterized by the lesser astringent and bitter taste perceived upon black tea consumption. Bitterness of tea is generally ascribed to the combination of catechins, saponins, polyphenols and amino acids [20, 21]. Some flavonoids are very bitter whereas others not, depending on the type of the glycoside chain. Naringin and neohesperidin are very bitter, whereas hesperidin is tasteless [20].

These studies show very marked differences in the level of some major flavonoids and phenolic acids between flavoured black tea and fruit teas. The last type contains,

**Fig. 3** Antioxidant capacities of tea infusions (expressed as mmol TR/g) measured by the CUPRAC method; lined rectangle—without incubation, shaded rectangle—incubation at 50 °C

except leaves of *C. sinensis*, also dry fruit and herbs, which are visible, while natural aroma of different fruits is added to fermented tea leaves for producing flavoured products. Fruit teas are richer in phenolic acids, particularly chlorogenic, ferulic and caffeic acid. The research studies suggest that chlorogenic acid could help lower blood sugar levels and control blood glucose levels in type II diabetes mellitus condition [22, 23]. Caffeic acid functions as a carcinogenic inhibitor and has tumour-shrinking properties [22]. HPLC analysis of *Delight citrus* and *Caribbean* teas showed the

Table 3 Scavenging effect of aqueous extracts from selected teas on DPPH radicals

Tea	Scavenging effect, % ^a	
	5 min	20 min
Caribbean	33.5 ± 1.7	51.9 ± 2.4
Delight citrus	25.1 ± 1.2	39.6 ± 1.9
Char Ming	29.1 ± 1.4	40.3 ± 2.0
Temptation	32.1 ± 1.6	55.0 ± 2.2
Blue fruit	67.6 ± 3.3	68.4 ± 3.4
Forest fruit	63.4 ± 3.1	65.6 ± 3.3
Citrus	58.6 ± 2.9	58.5 ± 2.9
Tropical fruit	64.5 ± 3.1	67.7 ± 3.2
Lipton yellow label	63.3 ± 3.0	63.6 ± 3.0

^a Scavenging effect calculated as $(A_0 - A_t)/A_0 \times 100$, where A_0 is the initial absorbance and A_t is the absorbance at increasing time t

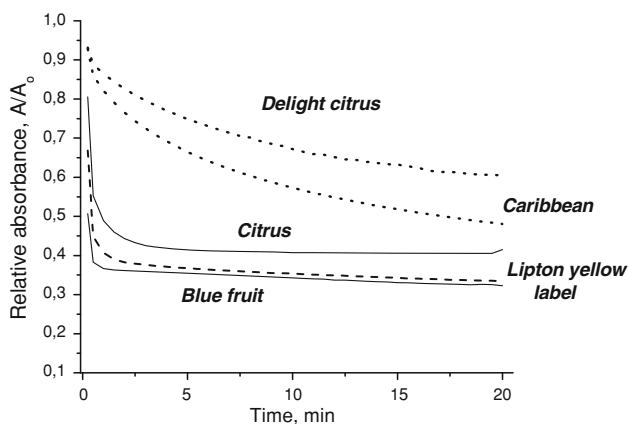


Fig. 4 The kinetic curves of scavenged DPPH by studied tea infusions. *Solid lines*—flavoured black teas, *dotted lines*—fruit teas

predominance of glycosides naringin and hesperidin. Jung et al. [24] found that supplementation with hesperidin and naringin, both citrus bioflavonoids, significantly reduced blood glucose, while the bone and lipid benefits of hesperidin make it an attractive dietary agent for the management of the health of postmenopausal women [25].

Flavoured black teas contain significant content of main catechins, particularly the concentration of EGCG in *Citrus* and *Tropical fruit* teas was high. EGCG is known to be the most active antioxidant catechin [16]. Sing et al. [14] reported that EGCG acts as an angiogenesis inhibitor by modulating protease activity during endothelial morphogenesis. Angiogenesis is a crucial step in the growth and metastasis of cancer.

The methods employed for the evaluation of antioxidant properties of studied tea infusions are based on their reduction properties (FC and CUPRAC) and the ability for scavenging of free radicals (DPPH). Conventional Folin–Ciocalteu method for the determination of so-called total

polyphenols content is based on a non-specific phenol oxidation reaction by the two strong inorganic oxidants (phosphotungstic and phosphomolibdic acids), but this assay gives different responses to different phenolic compounds, depending on chemical structures [26]. Moreover, the FC reagent could simultaneously oxidize several non-phenolic organic compounds as well some inorganic substances to give elevated apparent phenolic content; thus, it can be used for the measurement of total reducing capacity of samples. CUPRAC assay is based on the reduction of Cu(II)-neocuproine complex by the combined action of all antioxidants (reducing agents) in a sample. These two methods have different reduction potentials as well as different kinetics and experimental conditions, thus do not yield the same values for the antioxidant activity. In FC assay, *Yellow label* tea was characterized by the highest antioxidant properties followed by *Forest fruit* and *Tropical fruit* teas, while in CUPRAC method for flavoured black teas, especially *Forest fruit* and *tropical fruit*, the highest TE values was obtained. The DPPH assay showed similar trends for tea infusion to FC and CUPRAC. Flavoured black teas were characterized by the greatest inhibition of DPPH, and the kinetic of this reaction is very fast. It can be seen that some flavoured black teas had the antioxidant properties superior to or comparable with typical black tea.

Conclusion

Evaluation of the antioxidant status of different kind of commercially available teas will promote research on the identification and quantification of active components of these teas that may help protect consumers against free radical damage and oxidative stress-related diseases. In FC and DPPH assays, the antioxidant properties of studied tea infusion increases in the order: fruit tea < flavoured black tea > premium black tea, while in CUPRAC method, some aromatized teas (particularly *Forest fruit*) exhibit the highest antioxidant properties. This is due to the phenolic components present in these tea extracts. Flavoured black teas contain significantly higher level of catechins, quercetin and rutin, while the content of chlorogenic acid, which could help lower blood sugar levels, was higher in fruit teas. *Delight citrus* and *Caribbean* fruit teas seem to be a rich source of naringin and hesperidin. The flavonone naringin has a role in the attenuation of hyperglycaemia and may exert this effect in a manner similar to the drug, metformin [27].

While higher level of phenolic antioxidants is expected to be beneficial for health, this can also lead to more astringent beverage, which may not suit all tastes. However, teas with nice smell of fruits would also support the human diet with some source of antioxidants.

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