Article

Relationship between Total Phenolic Content, Antioxidant Capacity, Fe and Cu Content from Tea Plant Samples at Different Brewing Times

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Abstract: The purpose of this study was to investigate the antioxidant capacity of different tea plant: mint, linden, chamomile, St. John's wort, green and black tea in relation to total phenolic content. The antioxidant capacity of the tea infusions at different brewing times was determined using DPPH assay while the total phenolic content (TPC) was assessed using the modified Folin-Ciocalteu method. The results showed that there were significant statistical differences in antioxidant capacity depending on infusion time, according to one-way ANOVA analysis. Leaves used as components of tea infusions were analyzed by FAAS for their content of iron and copper in the dry product and in the infusion. The correlation between TPC and DPPH capacity of tea plant infusions was evaluated by Pearson correlation matrix. Total phenolics compounds content was positively and significantly correlated with DPPH capacity for all infusions time. Significant correlation was observed between TPC and the copper concentration ($p < 0.05$). Consequently, the correlations between the physicochemical parameters, TPC, DPPH capacity, Fe and Cu content suggested that the TPC may be a good indicator of the DPPH capacity in the tea infusions and also, suggested the influence of antioxidant compounds on mineral bioavailability.

Keywords: TPC; DPPH capacity; trace elements; tea plant infusion

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1. Introduction

Tea is a well-known aromatic drink highly appreciated due to the benefic effect that it has for the human organism and sensory attributes [1–4]. Tea also has powerful antioxidant characteristics which combats the free oxygen radicals due to the presence of flavonoids [5]. Therefore, the presence of polyphenols in tea is a quality indicator [6]. Also, moisture and water extract of tea are parameters used in quality control of tea. Chemical compounds like: polyphenols, sugars, minerals, alkaloids affect the flavor of tea [7]. Moisture content is an important parameter to monitor at different stages in the production of tea [8]. High moisture contents (above 6.5%) are extremely detrimental to tea quality [9].

The most represented categories of tea are: green tea, yellow tea, white tea, black tea, wulong tea and fermented tea. So, the antioxidant activities among them are dissimilar. In the extraction process of phenolic compounds from tea the temperature and time need to be taken into account [10–12]. The preparation conditions of tea infusions are important to extract as many antioxidant compounds as possible. Therefore, the antioxidant capacity of tea is correlated with the preparation conditions, infusion time and temperature, particle size of tea leaves [13,14]. The polyphenols can be quantified by various methods, a commonly used method is the Folin-Ciocalteu assay [15,16]. Several analytical methods have been developed for determining the antioxidant capacity of plants

and plant extracts based on the ability of an antioxidant to quench free radicals. DPPH (1,1-diphenyl-2-picrylhydrazyl) is commonly used to evaluate the free radical capacity of various antioxidants [17–20].

Essential minerals are other important compounds in tea [21,22]. Trace elements are present in tea due to their natural existence in soil and water or to emissions from human activities. The physicochemical properties of these elements can have both beneficial and harmful effects on human health. Some metals, such as zinc, iron, and copper are essential for basic processes in the human body and only at high concentrations become toxic, while lead and cadmium are exclusively toxic at low concentrations and have no beneficial properties. Copper contamination in tea leaves is still a problem in tea production and measures should be taken to ensure food safety from Cu contamination [23]. Therefore, the determination of trace elements in tea and tea infusion is of great value in terms of tea quality control [24–27]. Moreover, the potential health risk assessment was evaluated [28].

The objectives of this study were: (i) to evaluate the antioxidant capacity DPPH of teas prepared from commercial mint, linden, chamomile, St. John's wort, green and black tea leaves and teabags in relation to their phenolic profile, reflected by total phenolic content (TPC); (ii) to find out whether there are significant differences in antioxidant capacity as well as in total phenolic content, depending on infusion time; (iii) to quantify the moisture and water extract used in international markets as indicators of tea quality; (iv) to determine Fe and Cu content in tea leaves and teabags by flame atomic absorption spectrometry (FAAS). Furthermore, transfer rates of Fe and Cu from studied tea plant infusions were carried out.

2. Materials and Methods

2.1. Samples

Six different species of tea (leaves or bag): mint, linden, chamomile, St. John's wort, green and black tea (provided from the same brand available in the market) were used. More, the mint and linden leaf from private manufacturer were analyzed also.

2.2. Reagents

Gallic acid was purchased from Fluka (Buchs, Switzerland) and Folin-Ciocalteu reagent from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent was diluted 1:2 (V:V) with distilled water. Gallic acid (standard phenolic compound) $2 \times 10^{-2} \text{ mol} \cdot \text{L}^{-1}$ was prepared by dissolving 0.376 g gallic acid in 100 mL ethanol.

All metal stock solutions (1000 mg/L) were prepared by dissolving the appropriate amounts of the spectrally pure metals in dilute acids (HNO_3) 1:1 and then diluting with deionized water. The working solutions were prepared by diluting the stock solutions to appropriate volumes. The nitric acid 65% and hydrogen peroxide 25% solutions used were of ultrapure grade, and were purchased from Merck.

All other reagents used in the study were of analytical grade and were procured locally.

2.3. Sample Preparation

Tea infusions and boiled brew were prepared from each plant. Tea infusions were prepared as follows: two grams of tea leaves (or tea bags) were added to 200 mL of hot ultrapure water (100 °C). The ultrapure water was used to avoid any contamination with other minerals. Aliquots of it were taken at different times of infusion varying from 2 to 15 min. The boiled brew was prepared by adding the plant material to boiling water and boiled for 5 min. After each infusion time period (or 5 min boiling), the tea was immediately cooled to room temperature and filtered through filter paper (Whatman no: 1). Each sample was prepared in triplicate and each was analyzed thrice.

In order to determine trace elements concentrations (Fe and Cu), the raw material: tea leaves or teabag (0.5–0.9 g of each dry sample) were accurately weight and transferred to a digestion vessel containing 8 mL HNO_3 65% and 10 mL H_2O_2 25%. Sample decomposition was performed at a maximum temperature of 150 °C in a Digesdhal apparatus (provided by Hach Company). The digestion procedure was performed in triplicate for all tea samples.

Blind digestion was prepared in the same manner without adding the tea sample to the digestion vessels. The digested samples were then diluted to 50 mL with ultrapure water and stored in sealed polyethylene bottles until analysis.

2.4. Physicochemical Analysis of Tea Plant Samples

The moisture content of tea samples was measured using a vacuum oven by following the international standard method [29].

The water extract of tea samples was determined as follows: tea infusion (50 mL) was placed in a weighed evaporating dish and then was evaporated to dryness over a sand bath. The residue (tea extract) in the dish was dried completely for 5 h until the weight of the dish with extract was constant. The water extract was calculated using the following equation:

$$\text{Water extract (\%)} = (D_1 - D_0) \times V_0 \times 100 / V_1 / W \quad (1)$$

where: D_1 is the weight of dry tea extract with the dish; D_0 is the weight of the dish; V_0 is the total volume of the tea solution (250 mL); V_1 is the volume used for the measurement (50 mL); W is the dry weight of the tea sample (2 g).

2.5. Determination of Total Phenolic Content

The total phenolic compound content (TPC) was assessed following the modified Folin-Ciocalteu method [30]. Succinctly, 1 mL of 1:2 (*v/v*) Folin-Ciocalteu, 1 mL ethanol, 1 mL sodium carbonate solution 20% and 1 mL tea extract after infusion were mixed. The solution was mixed vigorously, then was allowed to react for 10 min at room temperature and made up to the mark with distilled water. The absorbance was measured at 675 nm with an UV-VIS spectrophotometer (Jasco 550), after 30 min incubation at room temperature. Gallic acid standard calibration curve was obtained in a linear fit ($R = 0.9955$). The linearity of this method was evaluated with standard solutions covering the range between 37.5 and 225 mg L⁻¹. Total phenolic compound content was expressed as mg gallic acid equivalents per L of infusion.

2.6. Determination of Antioxidant Capacity by DPPH Assay

The DPPH assay is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts [31,32].

The antioxidant activity of methanolic extracts was evaluated by DPPH radical-scavenging method, based on the reduction of stable 2,2-diphenyl-1-picrylhydrazyl radical [33]. A quantity of 0.0394 g 2,2-diphenyl-1-picrylhydrazyl was dissolved in 10 mL methanol to prepare 1 mM DPPH stock solution. The radical stock solution was prepared fresh daily, stored in a lightproof container (to avoid light as DPPH is photosensitive and easily oxidized) and kept in the dark at 4 °C between measurements.

An aliquot of 1 mL tea infusion or 1.0 mM ascorbic acid in methanol was used to react with 2 mL of 1 mM DPPH solution. Then it was incubated at room temperature and in a dark place for 30 min. A UV-VIS spectrophotometer (Jasco 550) was used to measure the absorbance at 517 nm. Ascorbic acid was used as a positive control to compare scavenging capacity of the sample. Antioxidants when interacting with DPPH, neutralize its free radical character and the color of the reaction changes from purple to yellow. The determinations were performed in triplicate.

Antioxidant activity was calculated by the percentage of inhibition of DPPH radical using the following equation:

$$\% \text{ inhibition of DPPH radical} = [(A_0 - A_1) / A_0] \times 100 \quad (2)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.7. Determination of Trace Elements (Fe and Cu)

For determination of Fe and Cu from tea plant samples was used a Shimadzu atomic absorption spectrometer (AA 6500) equipped with air-acetylene flame. Acetylene with a purity of 99.99% at a flow rate of 1.8–2.0 L/min was used as the fuel gas and carrier gas for aerosol introduction. The wavelengths used for the determination of analytes were 324.7 nm for Cu and 248.3 nm for Fe. The calibration standard curves provided the basis to quantify the metal contents. Concentration of working standards ranged from 0.010 to 1.200 ppm for Cu and from 0.020 to 4.000 ppm for Fe. The analyses were carried out in triplicate and the mean values are given.

2.8. Transition Rate

The transition rate T for the transfer of Fe and Cu from the raw material to the infusion was calculated using the following equation:

$$T = C_2 \times 100 \times v / C_1 \times m \quad (3)$$

where:

T is transition rate in (%); C_2 is the element content in the brew ($\mu\text{g/L}$); C_1 is the element content in the raw material (mg/kg); v is the volume for preparing the infusion ($v = 0.2 \text{ L}$); m is the amount of raw material for preparing the infusion ($m = 2 \text{ g}$).

2.9. Statistical Analysis

Statistical analysis was carried out using one-way method ANOVA and Tuckey's test. Correlations between physicochemical parameters, TPC, DPPH capacity, Fe and Cu content were determined using Pearson's correlation coefficient (r).

3. Results

Data regarding physicochemical analysis of tea plant samples are presented in Table 1.

Table 1. Physicochemical analysis of tea samples.

Type of Tea	Moisture %	Water Extract %
Green teabag	7.25	59.25
Black teabag	3.18	41.18
Mint teabag	6.08	52.08
Mint leaves	5.25	35.25
Linden teabag	7.30	47.30
Linden leaves	6.50	37.72
Chamomile leaves	4.11	54.14
St. John's wort leaves	5.33	49.33

The antioxidant capacity of tea plant infusions and boiled brew was evaluated using DPPH. The effect of time brewing conditions on the total antioxidant capacity of the tea samples was also evaluated. Tests were conducted at 2, 5, 7, 10 and 15 min of heating time. Table 2 presents the data for DPPH (% inhibition) and total phenolic compounds content expressed as mg gallic acid (GAE)/L achieved from the analyzed tea plant samples.

Table 2. Evolution of antioxidant capacity and total phenolic compounds content ($\bar{x} \pm SD$) of tea plant infusions.

Tea Sample	Infusion Time					Boiled Brew
	2	5	7	10	15	5
Antioxidant capacity TPC (mg GAE/L)						
Green teabag	50.02 $\pm$ 0.51	53.08 $\pm$ 0.57	48.36 $\pm$ 0.49	47.52 $\pm$ 0.47	51.13 $\pm$ 0.51	58.36 $\pm$ 0.71
Black teabag	46.69 $\pm$ 0.43	45.86 $\pm$ 0.44	46.41 $\pm$ 0.38	49.47 $\pm$ 0.55	49.19 $\pm$ 0.53	56.41 $\pm$ 0.63
Mint teabag	42.25 $\pm$ 0.45	28.63 $\pm$ 0.21	30.86 $\pm$ 0.29	26.97 $\pm$ 0.25	31.69 $\pm$ 0.41	49.75 $\pm$ 0.34
Mint leaves	48.91 $\pm$ 0.52	5.30 $\pm$ 0.20	3.91 $\pm$ 0.19	0.50 $\pm$ 0.54	0.30 $\pm$ 0.11	49.19 $\pm$ 0.33
Linden teabag	27.52 $\pm$ 0.22	26.41 $\pm$ 0.25	30.58 $\pm$ 0.23	20.86 $\pm$ 0.19	30.02 $\pm$ 0.21	49.75 $\pm$ 0.32
Linden leaves	23.36 $\pm$ 0.24	0.25 $\pm$ 0.11	6.13 $\pm$ 0.18	13.63 $\pm$ 0.23	0.27 $\pm$ 0.17	49.47 $\pm$ 0.42
Chamomile leaves	38.36 $\pm$ 0.33	27.25 $\pm$ 0.22	30.58 $\pm$ 0.29	25.58 $\pm$ 0.22	29.19 $\pm$ 0.25	47.25 $\pm$ 0.37
St. John's wort leaves	46.41 $\pm$ 0.39	45.02 $\pm$ 0.31	47.80 $\pm$ 0.46	45 $\pm$ 0.34	44.69 $\pm$ 0.57	50.86 $\pm$ 0.41
Antioxidant capacity DPPH (%) inhibition						
Green teabag	91 $\pm$ 0.81	89 $\pm$ 0.90	69 $\pm$ 0.68	100 $\pm$ 0.88	74 $\pm$ 0.76	70 $\pm$ 0.72
Black teabag	91 $\pm$ 0.77	87 $\pm$ 0.76	83 $\pm$ 0.67	86 $\pm$ 0.71	62 $\pm$ 0.65	69 $\pm$ 0.75
Mint teabag	89 $\pm$ 0.74	84 $\pm$ 0.74	17 $\pm$ 0.21	75 $\pm$ 0.54	10 $\pm$ 0.15	79 $\pm$ 0.84
Mint leaves	85 $\pm$ 0.79	20 $\pm$ 0.18	15 $\pm$ 0.23	18 $\pm$ 0.17	13 $\pm$ 0.19	79 $\pm$ 0.87
Linden teabag	64 $\pm$ 0.69	81 $\pm$ 0.58	72 $\pm$ 0.51	40 $\pm$ 0.43	30 $\pm$ 0.30	79 $\pm$ 0.84
Linden leaves	66 $\pm$ 0.63	30 $\pm$ 0.26	26 $\pm$ 0.33	24 $\pm$ 0.33	18 $\pm$ 0.22	80 $\pm$ 0.77
Chamomile leaves	87 $\pm$ 0.67	64 $\pm$ 0.62	56 $\pm$ 0.58	42 $\pm$ 0.34	16 $\pm$ 0.20	84 $\pm$ 1.01
St. John's wort leaves	88 $\pm$ 0.75	79 $\pm$ 0.64	85 $\pm$ 0.73	83 $\pm$ 0.79	18 $\pm$ 0.29	82 $\pm$ 0.76

The analysis of trace elements in tea leaves is necessary to control the quality of tea because agricultural and climatic characteristics, mostly related to soil conditions, as well as the processing manufacturers of the tea, influence the composition [34,35]. The most plentiful trace elements in teas are Fe and Cu [36].

The content of each element (Fe and Cu) was measured in the dry material (mg/kg) and in the corresponding infusion (2 min of infusion time) ($\mu\text{g/L}$). Fe and Cu content and the transition rates (%) are given in Table 3.

Table 3. Fe and Cu concentrations in raw materials ($\bar{x} \pm SD$) R (mg/kg), infusions ($\bar{x} \pm SD$) I ($\mu\text{g/L}$) and transition rates T (%).

Type of Tea	Fe			Cu		
	R	I	T	R	I	T
Green teabag	3.56 $\pm$ 0.08	2.79 $\pm$ 0.04	7.83	5.14 $\pm$ 0.18	50.6 $\pm$ 0.74	98.44
Black teabag	4.17 $\pm$ 0.23	6.36 $\pm$ 0.28	15.25	5.40 $\pm$ 0.20	66.2 $\pm$ 0.65	122.59
Mint teabag	2.7 $\pm$ 0.14	0.56 $\pm$ 0.03	2.07	17.1 $\pm$ 0.30	55.3 $\pm$ 0.55	32.33
Mint leaves	3.43 $\pm$ 0.10	2.23 $\pm$ 0.07	6.50	17.02 $\pm$ 0.29	104.8 $\pm$ 1.03	61.57
Linden teabag	3.07 $\pm$ 0.09	1.5 $\pm$ 0.16	1.62	35.83 $\pm$ 0.22	37.6 $\pm$ 0.33	10.49
Linden leaves	2.01 $\pm$ 0.13	0.67 $\pm$ 0.08	3.33	25.72 $\pm$ 0.23	13.2 $\pm$ 0.18	5.13
Chamomile leaves	2.10 $\pm$ 0.15	1.22 $\pm$ 0.12	5.80	32.68 $\pm$ 0.19	40.58 $\pm$ 0.25	12.41
St. John's wort leaves	1.92 $\pm$ 0.19	1.45 $\pm$ 0.17	7.55	36.29 $\pm$ 0.24	54.52 $\pm$ 0.32	15.02

4. Discussion

Moisture content is an important quality parameter of tea and in this study, ranged from 3.18 to 7.30%. Adnan et al. [1] stated that for better quality of product moisture content should be controlled between 2.5–6.5%. This result means that the moisture content of green teabag and linden teabag is high and has negative effects on the shelf life of the tea product. The researchers suggested that important factors are the packaging material and weight of teabags to maintain constant moisture content during storage of commercial tea samples.

In this study the water extract of tea samples ranged from 35.25 to 59.25% (Table 1). Water extract in tea should not be less than 32% of dry weight [37,38]. Table 1 shows that all values obtained fall within the limits of the standard. Also, the values of the water extract

obtained in this study for green tea and black tea are comparable to those in the literature (32.9–58.6%) [1]. In mint and linden teabags, the water extract was higher than in mint and linden leaves. This may be due to differences in manufacture (mint and linden leaves are provided by the private manufactures), resulting in different degrees of degradation of the tea components [9].

An analysis of variance (one way ANOVA) yielded no significant variation among TPC with different brewing time, $F = 0.66$, $p = 0.62 > 0.05$. In the case of DPPH significant differences can be observed, $F = 4.54$, $p = 0.004 < 0.05$. According Tukey's test DPPH data were divided into five groups and the results are presented in Table 4. Tea infused for 2 min generally produced the highest DPPH values, except for green teabag and linden teabag (Table 2). It could be mentioned that antioxidant capacity of green tea samples increased up to the maximum value with 10 min infusion time, similarly to Kelebek research [12].

Table 4. DPPH results according Tukey's test.

HSD _{0.5} = 36.77				
	M1	M2	M3	M4
M2	15.88			
M3	29.75	13.88		
M4	24.13	8.25	5.63	
M5	52.50 *	36.63	22.75	28.38

* indicates a significant result.

The infusion treatments also produced statistically significant differences in the total phenolic content of the tea. The highest TPC values were observed in boiled brew (Table 2). In general, a decrease in total phenolic content was observed in all tea plant infusions between 2 min to 5 min infusion time, followed by an increase in the value at 7 min infusion time. When boiled water is added over tea leaves, the diffusion of phenolic compounds takes place in a rapid manner because these compounds do not exist in the ultrapure water used to make tea [39]. For the black teabag, mint teabag and linden teabag samples the total phenolic compounds content increased at 7 min, then decreased at 10 min followed by an increase again at 15 min, while for other samples like St. John's wort leaves, the total phenolic content decreased after 7 min of infusion and remained low. Mint leaves and linden leaves presented different behaviors related to the origin (produced by private manufacturer) as these were exposed to climate and soil variations along with the processing. An increased infusion time, at 100 °C leads to phenolic compounds degradation [40]. Also, temperature has an important role in TPC increase and decrease in tea infusions [11,13].

Cu concentration in tea plant infusions was in the range of 5.14 to 36.29 µg/L, while Fe content in tea infusions was low (Table 3), confirming its minimal solubility in tea infusions due to chelation with tea polyphenols [24]. Infusions prepared from black teabag contained the largest amount of Fe (6.36 µg/L), while in mint and linden infusions level of Fe was very low (0.56–0.67 µg/L). Iron concentrations in tea infusion were low, according to literature studies [41–43]. Also, the amount of Cu was higher in black teabag than green teabag infusions. Usually, black teas contain higher levels of trace elements than other teas due to fermentation, maturation and storage [42].

The lowest amount of Cu was detected in the infusion of linden leaves and the highest one, in the infusion of mint leaves. Large amounts of Cu have passed to the infusions of chamomile and St. John's wort leaves. Among the different teas, linden leaves infusion had the lowest Fe and Cu concentrations.

In our study, the transition rates for Cu varies between 5.13% and 122.59%. Also, the highest transition rates were reported for black teabag infusion (122.59%) and green teabag infusion (98.44%). Schulzi et al. [44] found lower transition rate (16.6–92.2%) in tea infusions, but these authors analyzed herbal and fruit infusions. These authors pointed out that the transition of copper could be influenced by matrix characteristics.

Iron transition rates for tea infusions were lower than for copper and ranged in a narrow range: from 1.62% for linden teabag infusion to 15.25% for black teabag infusion. But copper is not only a natural component of the tea plant, it is also used in plant disease management, being essential in organic farming [45].

Cu concentration in the digested tea plant samples varied from 5.14 to 35.83 mg/Kg. Similar quantities of Cu were also indicated by other authors [23,46,47]. The highest Fe content among the digested samples was found in black tea (4.17 mg/kg), followed by green tea (3.56 mg/kg) and mint leaves (3.43 mg/kg).

The relationship between total phenolic content (TPC) and antioxidant capacity (DPPH capacity) of boiled brew and tea plant infusions was evaluated by Pearson correlation matrix. Total phenolics compounds content was positively and significantly correlated with DPPH capacity for all infusions time (Table 5). No correlation between TPC and DPPH capacity was observed for boiled brew samples. In case of mint and linden leaves infusions, produced by private manufacturer, was observed the strongest positive correlation between TPC and DPPH capacity (Table 6). Also, high correlations between TPC and DPPH capacity of tea infusions were found by Rodriguez Vaquero et al. [48]. The increase in total phenolic content might be related to the increased antioxidant capacity of tea infusions as measured by DPPH assay (Table 2). Similarly, findings were reported by Fu et al. [49]. Thus, the levels of TPC makes tea a good dietary source of natural antioxidants [50].

Table 5. Pearson correlation coefficients of TPC and DPPH at different times of infusion.

Pearson Correlation Coefficient (r)						
DPPH						
TPC	2 min infusion	5 min infusion	7 min infusion	10 min infusion	15 min infusion	5 min boiled brew
	0.9156 **	0.8297 *	0.8224 *	0.9370 **	0.6490	−0.9384

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

Table 6. Pearson correlation coefficients of TPC and DPPH of tea plant infusions.

Pearson Correlation Coefficient (r)		
Tea Plant		DPPH
TPC	Green teabag	−0.0964
	Black teabag	−0.5534
	Mint teabag	0.2164
	Mint leaves	0.9956 **
	Linden teabag	0.1964
	Linden leaves	0.8111 *
	Chamomile leaves	0.6416
	St. John's wort leaves	0.6003

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

The tea infusions, boiled brew and digested samples differed significantly, in respect of Cu (*t* test, $\alpha = 0.05$).

A possible relationship between total phenolic content, antioxidant capacity of tea plant samples and their Fe, Cu content was tested. Our results show that there were many correlations (positive, negative, weak) between either TPC or DPPH capacity and studied trace elements in tea infusions, boiled brew and digested samples.

Applying Pearson's correlation between the total phenolic content and Fe, respectively Cu concentrations in tea infusions (2 min of infusion time) were observed positive correlations: Fe ($r = 0.4684$) and Cu (0.7664). Statistically, a significant correlation was observed between TPC and the copper concentration ($p < 0.05$) and higher content of Cu is related with higher polyphenol content in the tea infusions. This finding is in agreement with the study of Theuma and Attard [15].

Additionally, the DPPH capacity was positively correlated with Fe for all tea infusions studied: moderate correlations for 2, 7, 10 min infusion, weak correlation for 5 min infusion,

and significant correlation for 10 min infusion (Table 7). Negatively correlation with Fe was observed for the boiled brew samples (Table 7). Regarding Cu, we obtained positive correlation between DPPH capacity and Cu (Table 8), but very weak correlation for 10 and 15 min infusions, while negatively correlation between DPPH capacity and Cu in 5, 7 min infusions and boiled brew samples were found.

Table 7. Correlation coefficient of antioxidant activity and Fe in studied tea plant infusions.

Pearson Correlation Coefficient (r)						
Fe						
	2 min infusion	5 min infusion	7 min infusion	10 min infusion	15 min infusion	5 min boiled brew
TPC	0.4684	0.4808	0.4306	0.5131	0.4706	0.7108
DPPH	0.4098	0.3032	0.5017	0.4258	0.7291 *	−0.7999

* Correlation is significant at the 0.05 level.

Table 8. Correlation coefficient of antioxidant activity and Cu in studied tea plant infusions.

Pearson Correlation Coefficient (r)						
Cu						
	2 min infusion	5 min infusion	7 min infusion	10 min infusion	15 min infusion	5 min boiled brew
TPC	0.7664 *	0.0476	−0.0664	−0.1391	−0.0318	0.1178
DPPH	0.5544	−0.1754	−0.1667	0.0181	0.0053	−0.2107

* Correlation is significant at the 0.05 level.

For the digested leaves no correlations were observed between TPC respectively DPPH capacity and Cu. TPC respectively DPPH capacity had positively correlations with Fe (Table 9). Moderate correlation for 2 min infusion ($r = 0.4686$), weak correlations for 5, 7, 10 and 15 min infusions and relative high correlation for boiled brew samples ($r = 0.7866$) were observed between TPC and Fe. DPPH capacity showed generally weak correlations with Fe in tea infusions (Table 9) and negatively correlation in boiled brew. However, no statistically significant correlation was found between these pairs, indicating that Fe and Cu content from raw materials could not be liable for the antioxidant action of tea, but other factors are obviously involved.

Table 9. Correlation coefficient of antioxidant activity and Fe in digested leaves.

Pearson Correlation Coefficient (r)						
Fe						
	2 min infusion	5 min infusion	7 min infusion	10 min infusion	15 min infusion	5 min boiled brew
TPC	0.4686	0.3249	0.2210	0.2185	0.3012	0.7866
DPPH	0.2968	0.2401	0.1720	0.3012	0.6994	−0.8580

Pearson correlation was also used, to identify the relationship between the concentrations of trace elements and tea physicochemical parameters. From the calculated matrix negative correlation of Fe and Cu content was observed with moisture and water extract for all tea samples. Similar, negative correlation was found between moisture and either TPC or DPPH capacity.

The water extract displayed positive correlation with TPC or DPPH capacity for all infusions. In these cases, the correlation coefficients were below 0.69, but there was no statistically significant correlation, except the correlation observed between water extract and DPPH capacity for 5 min infusion time ($r = 0.7246$, $p < 0.05$). So, there is inconclusive evidence about the significance of the association between these pairs.

5. Conclusions

The total polyphenol content, moisture, water extract, the antioxidant activity, Fe and Cu content are parameters of quality for tea. The results showed that there were significant statistical differences in antioxidant capacity depending on infusion time, according to one-way ANOVA analysis. A correlation study conducted between different groups of the total polyphenol content and antioxidant capacity, and between total polyphenol content and Cu content, showed that the higher the polyphenol content, the higher the antioxidant capacity and also, higher polyphenol content corresponds to higher copper content in tea infusions. Also, the results showed a variation in percentage transfer of Fe and Cu from the tea leaves or teabag to the tea infusion. Correlation between total phenolic content, antioxidant capacity and Fe and Cu content in digested tea leaves demonstrated that these trace elements content from tea leaves could not be liable for the antioxidant action of tea, but obviously other factors are involved. According to the obtained results, the studied tea plant infusions are of good quality, being a good dietary source of natural antioxidants.

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