

Changes of antioxidant activity and active compounds content in selected teas

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Abstract: Our study tested 45 tea infusions classified into five groups (white, green, red, black, and other teas) for the content of total polyphenols and flavonoids, as well as antioxidant properties, by the FRAP and DPPH methods. We examined these parameters after prolongation of the brewing time from 10 to 30 min and overnight storage. The results showed that the capacity of the teas to bind free radicals was differentiated and the amount of anti-oxidant compounds depended on their nature. In terms of antioxidant activity and total polyphenol content, the tested tea types were ranked in the following order: white > green > black > red > other teas (yerba mate > rooibos). Our experiment demonstrated a positive correlation between the polyphenol content and antioxidant activity of the analyzed teas. Also, the DPPH antiradical efficiency was comparable to their ability to reduce ferric ions. The extended brewing time had a significant effect on the antioxidant activity of the infusions and the polyphenolic compounds analyzed therein. In contrast, storage of the infusions for 24 h at room temperature changed their antioxidant activity and affected the total polyphenol content.

Keywords: Tea infusion, FRAP, DPPH, polyphenols, flavonoids, brewing time

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INTRODUCTION

Tea (*Camellia sinensis* L.) is considered to be the most popular beverage in the world [1, 2]. It has been estimated that the average daily consumption of tea is approximately 120 mL. Poland is among the first three European countries and ten world countries in terms of tea consumption. Of several species commonly referred to as tea, the most important is Chinese tea produced from the *Camellia sinensis* leaves [3]. The chemical composition of tea is highly diverse and may vary within a relatively broad range, depending on many factors (e.g. the position of leaves on the stem, growth conditions, processing methods, or brewing time) [4–6].

In recent years, of great interest has been the therapeutic activity of tea and, in particular, the antioxidant effects of its compounds, e.g. polyphenols and flavonoids [3, 6–10]. Due to differences in processing technology or species composition, each type of tea contains a different combination of biologically active substances, depending on the extraction time and temperature, as well as leaf fineness. Fresh green leaves are rich in isomeric flavan-3-ols (catechins), on average accounting for 30% of their mass, with epigallocatechin

gallate (EGCG) being the most common component. During fermentation, some catechins undergo oxidation and condensation to high molecular weight compounds (3–6% theaflavins and 12–18% thearubigins) responsible for the characteristic flavor and aroma of infusions [4, 5, 9, 8, 11, 12].

Our study is an attempt to estimate the effect of infusions brewing time and storage on the content of bioactive compounds (polyphenols and flavonoids) and antioxidant properties of 45 loose-leaf and bagged teas available on the Polish market.

STUDY OBJECTS AND METHODS

Using the FRAP and DPPH methods, we compared the total content of polyphenols and flavonoids, as well as the antioxidant properties of infusions made from several types of loose-leaf (L) and bagged (E) teas available on the Polish market. Additionally, we assessed the effects of the brewing time and storage of infusions on the total content of polyphenols and antioxidant properties.

The research material consisted of 45 teas produced commercially by leading manufacturers

Table 1 Polyphenols content, flavonoids content, FRAP and DPPH antioxidant activity of tea after 10 min of brewing

Sample number	Polyphenols content (caffeic acid equivalent mg g ⁻¹ dry weight)	FRAP antioxidant activity (equivalent μmol FeSO ₄ g dm ⁻¹)	DPPH antioxidant activity (% scavenging for 1g dry matter)	Flavonoids content, % g dry matter ¹ (calculated as quercetin)
1 BL	39.46	880.96	47.96	0.25
2 BL	83.40	982.64	91.76	0.47
3 BE	73.80	941.42	83.05	0.41
4 BL	71.40	930.43	68.63	0.42
5 BL	37.17	590.45	44.54	0.23
6 BL	81.08	578.28	66.53	0.49
7 BL	65.52	788.70	65.23	0.25
8 BL	57.56	645.41	60.49	0.28
9 BE	64.64	685.45	55.09	0.36
10 BE	75.72	515.12	3.76	0.08
11 BL	71.36	505.96	55.01	0.20
12 BL	32.16	461.53	72.54	0.06
13 BE	97.02	1035.66	57.15	0.39
14 BL	103.53	1317.66	66.01	0.44
15 BL	83.64	1148.91	53.39	0.21
Mean for B	69.17	800.57	59.41	0.30
16 GL	61.41	874.29	60.78	0.31
17 GL	121.50	1304.95	76.05	0.38
18 GE	114.32	1337.54	69.94	0.43
19 GL	78.69	1167.55	67.70	0.40
20 GE	85.60	1183.65	76.71	0.35
21 GE	107.03	899.82	37.38	0.24
22 GL	96.55	748.12	47.84	0.16
23 GL	86.67	379.59	24.35	0.30
24 GE	119.98	1033.55	30.12	0.14
25 GL	67.16	657.03	74.30	0.38
26 GL	126.35	2257.19	93.29	0.53
27 GE	94.55	1451.43	81.27	0.34
28 GL	126.04	1251.54	43.95	0.37
29 GL	114.23	1993.17	85.20	0.46
30 GL	107.69	1944.46	89.89	0.25
Mean for G	100.52	1232.26	63.92	0.34
31 WE	105.69	1388.97	79.16	0.35
32 WE	105.32	1300.64	70.92	0.40
33 WL	87.97	1311.89	64.42	0.23
34 WL	70.84	1400.81	61.26	0.19
35 WE	116.54	1630.40	81.27	0.36
Mean for W	97.27	1406.54	71.40	0.30
36 RE	38.99	676.42	52.24	0.17
37 RE	61.99	606.53	25.77	0.19
38 RL	42.85	707.69	25.34	0.18
39 RL	45.21	534.90	20.97	0.18
40 RL	46.29	622.39	29.08	0.18
Mean for R	47.07	629.59	30.68	0.18
41 RoE	45.81	723.54	54.88	0.14
42 RoE	29.06	603.01	29.62	0.09
43 YML	60.95	997.95	76.71	0.27
44 RoL	41.59	535.05	20.38	0.20
45 YML	75.19	895.75	46.15	0.26
Mean for RoY	50.52	751.06	45.55	0.19
LSD _{0.05}	**	**	**	**

B – black tea; G – green tea; W – white tea; R – red tea; Ro – Rooibos tea; YM – Yerba Mate, L – loose-leaf tea; E – bagged tea
 ***, **, * – significant at $P \leq 0.001$, 0.01 or 0.05

for the Polish market (Bastek Coffee & Tea, Mokate, Unilever Polska, Teekanne Polska, Roger Sp. z o.o., Amber Spark, William's Nature Products, Bio-active Sp. z o.o., Herbapol-Lublin S. A., Posti S.A., Himalaje – Najlepsze herbaty, Tata Global Beverages Polska Sp. z o.o.) and purchased in retail stores. In particular, we experimentally tested 5 white teas (W), 15 green teas (G), 5 red teas (R), 15 black teas (B), and 5 other teas, rooibos and yerba mate (Ro, YM). The names of the teas were assigned digital (chosen randomly in the five groups mentioned above) and letter designations.

Methodology of analyses. One gram of tea was mixed with 100 mL of boiling distilled water, covered, and brewed for 10 min. The extracts were filtered through a medium-sized filter. The infusions were assayed for the total polyphenol content (o-dihydroxyphenols expressed as an equivalent of caffeic acid) using the spectrophotometric method of Singleton and Rossi and the flavonoid content (expressed as an equivalent of quercetin) according to Polish Pharmacopoeia VIII [13, 14].

Furthermore, we determined the antioxidant activity of tea by using the FRAP method of Benzie and Strain (the capacity of reducing 1 mole of Fe (III) to Fe (II) expressed as μmoles of antioxidant compounds in 1 g of raw material) and the modified DPPH Brand-Williams *et al.* method using a free radical of 1,1-diphenyl-2-picrylhydrazyl [15, 16]. The results were presented as % of free radical scavenging.

Brewing time and freshness effects on antioxidant activity and polyphenols. The samples under analysis were brewed for 10 and 30 min and stored at room temperature for 24 h. Next, we assessed their antioxidant activity using the FRAP method and determined the total polyphenol content. All assays for each sample were performed in triplicate.

Statistical analysis. All the data were subjected to variance analysis. The significance of the differences between mean values were verified with Tukey's test ($n = 3$) at a significance level of 95%. Statistica 9.0 (StatSoft) program was employed for the calculations. Additionally, we used Excel to calculate the coefficients of simple correlations between the phytochemical features of the infusions and their antioxidant properties.

RESULTS AND DISCUSSION

The analyzed samples of teas available on the Polish market differed significantly in both the total polyphenol and flavonoid contents and their antioxidant properties evaluated after 10 min brewing (Table 1).

As we can see, the content of flavonoids in the infusions assayed with the methodology provided in FP VIII ranged from 0.06% for sample 12 BL (loose-leaf black tea) to 0.53% for sample 26GL (loose-leaf green tea) [14]. In general, the lowest values of the flavonoid content were found for red teas (0.17–0.19%) and rooibos (0.09–0.2%), whereas the average value in the other

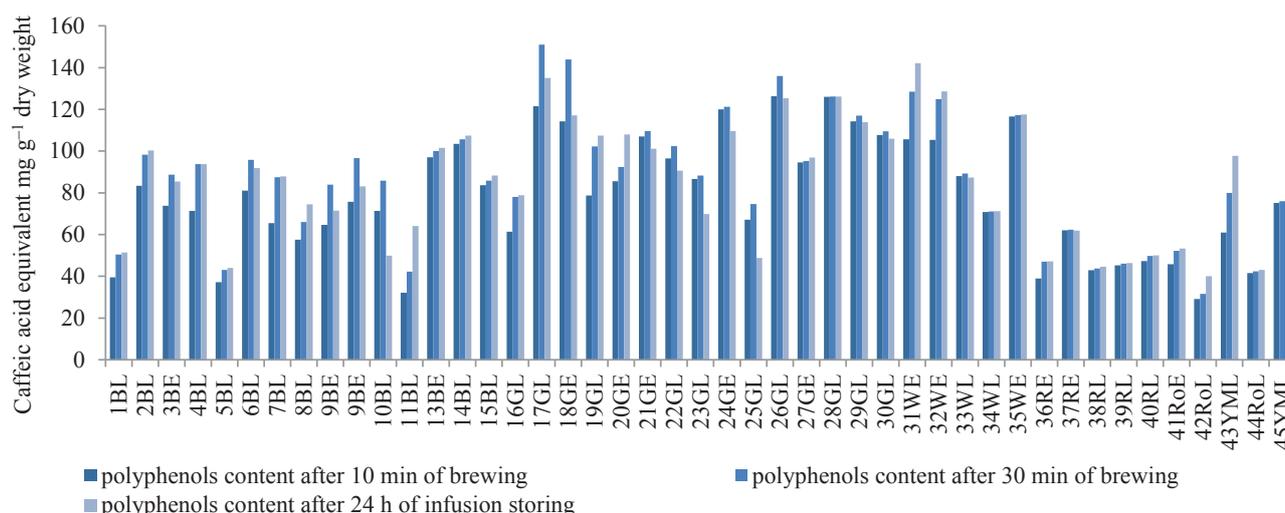


Figure 1 Changes in polyphenol content (caffeic acid equivalent $\text{mg}\cdot\text{g}^{-1}$ dry weight)

types of tea was about 0.3%. The flavonoid content in the analyzed teas highly correlated with their antioxidant activity, determined with the FRAP and DPPH methods ($r^2 = 0.548\text{--}0.666$), and with the polyphenol content ($r^2 = 0.582$). These results were in agreement with those reported by Castiglioni *et al.*, as well as Fernando and Soysa [17, 18].

The total polyphenol content determined with the Folin-Ciocalteu method in the 45 analyzed tea infusions ranged from 29.1 (sample 42 RoE – loose-leaf rooibos tea) to $126.4\text{ mg}\cdot\text{mL}^{-1}$ (sample 26 GL – loose-leaf green tea), expressed as an equivalent of caffeic acid (Table 1, Fig. 1). The values were comparable with those reported by Hilal and Engelhardt [19]. The highest content of polyphenols was found in the green teas (average $100.5\text{ mg}\cdot\text{mL}^{-1}$), just as in the experiments described by McAlpine and Ward, Kiran and Kumar, as well as Shannon *et al.* [20–22]. The white teas were characterized by a slightly lower (mean $97.3\text{ mg}\cdot\text{mL}^{-1}$) content of the active compounds.

On average, the black teas contained $69.2\text{ mg}\cdot\text{mL}^{-1}$ of polyphenols, whereas the red tea as well as rooibos and yerba mate exhibited mean values of 47.1 and $50.5\text{ mg}\cdot\text{mL}^{-1}$, respectively. Similar results were reported by a number of researchers [7, 9, 21, 22]. However, Plust *et al.*, as well as Hilal and Engelhardt, found higher contents thereof in white teas and lower contents in green teas and, especially, in black teas [3, 19]. In our experiment, higher total contents of polyphenols and flavonoids in green tea, compared to white tea, might be due to slight oxidation of white (nonfermented) tea polyphenols during production. In fact, white teas do not undergo the inactivation of enzymes before withering, so enzymes remain active and white tea polyphenols are oxidized slowly [12].

The antioxidant activity (assessed with FRAP and DPPH) and the polyphenol content demonstrated a significant correlation ($r^2 = 0.435\text{--}0.732$) and a consi-

derable diversity of the results. This correlation between the results of the three tests confirmed the validity of the procedure we used for analysis. However, Almajano *et al.* found no linear correlation between the antiradical activity of the analyzed infusions and the content of polyphenolic compounds [9]. They suggested that antiradical performance was influenced not only by the content of polyphenols but also by their quality and presence of other compounds that might enter the infusions during extraction.

A slightly different relationship was discovered by Rusaczonok *et al.* and Castiglioni *et al.*, i.e. a linear correlation between the total polyphenol content, flavonoids content, and antioxidant properties measured with ABST [7, 17]. Similar results were reported by Plust *et al.*, Molan *et al.* and Aldiab, who used the FRAP method [3, 11, 23]. We should emphasize that the available literature describes a variety of methods for determination of antioxidant properties and polyphenolic content of tea infusions. Also, the authors employ different methods of extraction (temperature, time, solvent) while preparing solutions for analyses and express their results in different ways. Therefore, the results are sometimes hardly comparable [1, 9, 11].

The antioxidant activity of the teas measured with the FRAP method was in the range of $379.6\text{--}2257.2\text{ }\mu\text{mol g d.w.}^{-1}$. The highest antioxidant activity was exhibited by the white and green tea samples, while the lowest activity was found for the red teas (over twice as low). A similar trend was observed by Atalay and Erge as well as Shannon *et al.* [12, 22].

On average, the black teas had 35% weaker antioxidant properties than the green teas and a 43% lower Fe ion reduction ability than the white teas. These results were in line with the findings of Aldiab [23]. The antioxidant activity of this group of teas exhibited a wide range of $461.5\text{--}1317.7\text{ }\mu\text{mol g d.w.}^{-1}$, which indicated high variation of tea on the domestic market.

Table 2 Single correlation coefficients between chosen features of teas

	FRAP 10	DPPH 10	FV 10
PL 10	0.732***	0.435**	0.582***
FRAP 10		0.668***	0.548***
DPPH 10			0.666***

PL 10 – polyphenols content after 10 min of brewing; FRAP 10 – FRAP antioxidant activity after 10 min of brewing; DPPH 10 – DPPH antioxidant activity after 10 min of brewing; FV 10 – flavonoids content after 10 min of brewing

***, **, * – significant at $P \leq 0.001$, 0.01 or 0.05

These teas had slightly weaker antioxidant properties than green teas due to the oxidation of catechin derivatives during processing and the presence of less active theaflavins and thearubigins in the infusions [12]. The weaker antioxidant properties and a lower polyphenol content in these tea types were confirmed by other authors [12, 19]. Noteworthy, the loose-leaf white and red teas were characterized by lower activity than the bagged teas, while Plust *et al.* reported an opposite trend for other tea types [3].

Generally, the analyzed red teas had a significantly lower content of polyphenols and exhibited weaker antioxidant activity. This result was probably due to the differences in the manufacturing process, which involves additional drying of tea leaves before they are twisted, leading to slight fermentation of tea. Additional drying may result in an increased loss of active substances. Incomplete fermentation causes polymerization of simple polyphenols, as in black teas, but it does not last long. Another difference is the absence of heat treatment for black tea, which may induce differences in the composition of the pu-erh type teas.

DPPH is a stable free radical that antioxidants can react with, providing it with electrons or hydrogen atoms. As presented in Tables 1 and 2, the value of antioxidant activity determined with the DPPH method was closely related to the results obtained using the FRAP method ($r^2 = 0.668$).

Concurrently, there were substantial differences in the antioxidant activity of the analyzed extracts (from 3.8 to 93.3% radical scavenging). Just as in the study of McAlpine and Ward, the lowest DPPH radical scavenging percentage was found for red tea (20.9–52.2%) and rooibos (20.4–54.9%), while the highest values were reported for white (61.3–81.3%) and green teas (24.4–93.3%) [21]. Similarly, Shannon *et al.* confirmed a higher DPPH radical scavenging capacity of green tea, followed by black tea [20].

The higher antioxidant activity of green tea determined by both methods can be attributed to an epigallocatechin gallate content, showing a greater free radical scavenging capacity than the other catechins [12]. Our study found that the more processed the tea was, the lower its antioxidant capacity. Similar results were reported by Kiran and Kumar, as well as Cleverdon *et al.*

[21, 24]. However, they did not agree with the findings of Fik and Zawislak, possibly due to the application of a different solvent during preparation of infusions and differences in the geographical regions of tea cultivation, harvesting periods, or storage conditions [8].

The most considerable differences between the antioxidant activities of the analyzed extracts were observed for the black teas. The loose-leaf types exhibited higher activity than the bagged teas (Table 1). Additionally, just as in Cleverdon *et al.*, true teas had at least a two-fold greater polyphenol content than the herbal varieties [24]. The weaker free radical scavenging capacity of rooibos tea (originating from *Aspalathus linearis* L.) may result from its chemical composition: unlike *Camellia sinensis* L., it does not contain catechins but aspalathin, isoorientin, orientin, and rutin [24, 25].

Similarly, yerba mate (produced from *Ilex paraguariensis* L.) does not contain catechins but substantial amounts of chlorogenic acid [26]. We found that the antioxidant activity of yerba mate was substantially lower than that reported by Boji *et al.* [26]. However, as emphasized by Komes *et al.*, yerba mate can exhibit low antioxidant activity compared to white and green teas [27].

It seems that the differences between the polyphenol profiles of “true” and herbal or rooibos teas could be the direct cause of the differences in their antioxidant capacity found in our study. We should take into account that the differences observed in these studies can be related to different sample preparation methodologies and use of different brewing times and tea-to-water ratios. What is more, the comparison of results is sometimes difficult due to the lack of uniformity in the properties of green tea, manufacturing and brewing conditions. Leaf age and size, harvesting season, and manufacturing conditions are all important factors that can affect the results [4].

Thus, according to our study, the teas tested with the FRAP and DPPH methods exhibited antioxidant activity in the following order: white teas > green > black > red > other teas (yerba mate > rooibos).

In all the infusions, the total polyphenol content increased (from 6.9% in red teas to 19.7% in black teas) with the infusion time, with higher values noted for bagged teas (Fig. 1). These findings are in line with other studies conducted on different brands of loosely packed and bagged teas. In particular, Armoskaite *et al.* found that longer periods of extraction of green tea (30 min) led to higher quantities of phenolic compounds [2]. Nikniaz *et al.* reported similar results for black teas [10]. However, storing infusions for 24 h at room temperature had a varied effect on the content of these active substances. Increased polyphenol contents were detected in rooibos, yerba mate, red and white teas.

In our study, we found black and green tea samples with increased polyphenol contents (1 BL, 2 BL, 4 BL, 5 BL, 7 BL, 8 BL, 12 BL, 13 BE, 14 BL, 15 BL and

16 GL, 19 GL, 20 GE, 27 GE, 28 GL), while in the other groups, the content declined from 4 to 42%. Similarly, increased quercetin, flavonoids and total polyphenol contents were recorded along with prolonged tea brewing in Molan *et al.*, Castiglioni *et al.*, Fernando and Soysa, as well as Palanivel *et al.* [11, 17, 18, 28]. According to Armoskaite *et al.*, flavonoids (and catechins, their fraction) are basic phenolic compounds in green tea responsible for antioxidant activity [2].

What is more, Saklar *et al.* reported that green tea catechins may be converted from epi forms to non-epi forms due to epimerization reactions at long brewing times [4]. Jin *et al.* proved that concentrations of epicatechins peaked at 10 min, after which they decreased drastically, while levels of non-epicatechins increased steadily for 5 h [5]. However, the antioxidant activity was more dependent on brewing temperature than brewing time. It is worth emphasizing that tea catechins can act as antioxidants by donating hydrogen atoms or by chelating metals, but epicatechins are known to be stronger than their corresponding non-epi isomers [9].

Further, we assessed the Fe ion reduction ability of the infusions brewed for 10 and 30 min (Fig. 2).

In the 45 teas analyzed, both a decline and an increase in antioxidant activity (particularly in red teas and yerba mate) were detected over the prolonged brewing time. These results were in line with Nikniaz *et al.* who reported higher values for bagged teas [10]. The most substantial differences were found among the black teas. In particular, infusions 1 BL, 2 BL, 3 BE, 4 BL, and 5 BL exhibited a decreased Fe ion reduction ability in a range of 4.2–26.6%, whereas the other infusions were characterized by an increased ferric reducing antioxidant power FRAP (from 1.4 to 44.5%). Increased antioxidant activity was detected in the infusions of the other tea types (except for green tea samples 23 GL, 25 GL, and 26 GL).

Previously published studies reported that the antioxidant capacity and total polyphenols in tea extracts correlated with the extraction time [10, 11, 19]. It is worth underlining that according to Armoskaite *et al.* and Pastoriza *et al.*, the time of extraction and antioxidant activity may not always be in direct proportion: longer extraction time results in lower antioxidant activity in some green teas and higher in others [2, 6].

In our study, the FRAP antioxidant activity of the tea infusions stored for 24 h at room temperature varied in a non-uniform manner and the statistical analysis did not confirm the significance of the differences. The magnitude of a decrease or an increase in antioxidant activity was not correlated with the tea type: both were noted in the groups of the same tea types. On average, the black teas exhibited a 14.8% increase in the Fe ion reduction ability. However, some infusions from this group were characterized by a decreased Fe ion reduction ability (samples 10 BE, 13 BE, 14 BL, and 15 BL).

In the other tea groups, we noted a decline in antioxidant activity, particularly in the red and green teas. At the same time, each group comprised some samples with a Fe ion chelating ability that increased throughout storage (samples 16 GL, 17 GL, 18 GE, 19 GL, 20 GE, 23GL of the green teas, samples 31 WL and 32 WL of the white teas, sample 36 RE of the red teas, and samples 41 RoE, 42 RoE, and 43 YML of the teas other than *Camellia sinensis*). According to Komes *et al.*, this variation might be due to a great abundance and variability of tea constituents that participate in various reactions during storage in the presence of oxygen, such as polymerization, or even degradation of some tea compounds [30]. Therefore, as indicated by Jayabalan *et al.*, the qualitative and quantitative composition of tea infusions undergoes change over time with significant differences detectable only after several days of brewing [29].

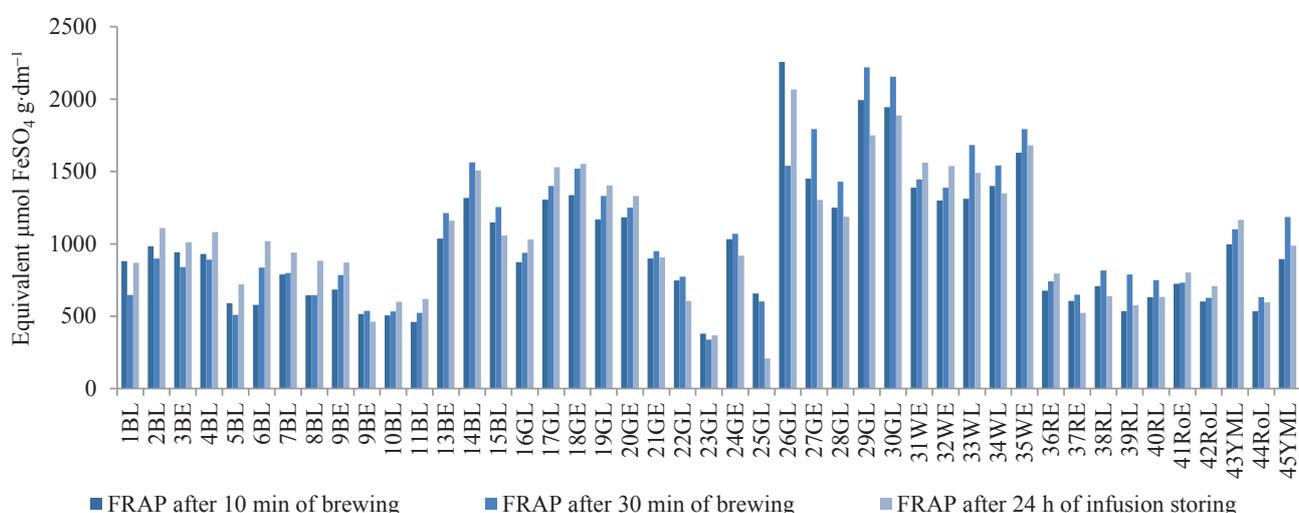


Figure 2 Changes in antioxidant activity of FRAP (Equivalent $\mu\text{mol FeSO}_4 \text{ g}\cdot\text{dm}^{-1}$) in the tested teas

CONCLUSION

Our experiment demonstrated a considerable diversity of teas available on the Polish market, contributing to the variability of active compounds and antioxidant activity of tea infusions. The teas under analysis were characterized by varied free radical scavenging abilities, and the amount of antioxidants depended primarily on the type of tea. The non-fermented teas (white and green) exhibited the highest antioxidant activity (measured with the FRAP and DPPH methods) and total polyphenol content. Weaker antioxidant properties and a lower content of polyphenols were detected in the black teas. The lowest values were found in rooibos and yerba mate, as well as in the red teas.

Our study showed a positive correlation between the polyphenol content and antioxidant activity. In addition, the DPPH antiradical performance of the examined extracts was comparable to their Fe ion chelating ability. The prolonged brewing time had a significant effect on the antioxidant activity of the infusions and polyphenolic compounds contained therein, which was not a linear correlation. Similarly, storage for 24 h at room temperature induced changes in the antioxidant

activity of the infusions and altered their total polyphenol content.

The information contained in our study can be useful for tea consumers in their choice of tea, as well as preparation and storage methods that ensure the best pro-health properties. In addition, the substantial differences in the content of active compounds and antioxidant properties of the examined tea types manufactured by various producers suggest a need for establishing appropriate technological parameters, quality requirements, and systematic control of their composition and properties. Given the fact that there are no standards for teas other than black in Europe, the quality of all types of tea traded on the Polish market should be standardized to ensure similar quality of products and fair market competition.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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