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PERCEPTIONS OF CONSUMER FOR PREPARATION AND CONSUMPTION OF TEA AND ANALYSIS OF BIOLOGICALLY ACTIVE COMPOUNDS OF BLACK TEA

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Abstract

The aim of this study was to examine the opinion of tea drinkers about the type and the way the tea is prepared, as well as the knowledge of the biologically active substances contained in the black tea. Finally, the influence of the temperature and the method of preparation of black tea on its colour and content of biologically active compounds was determined. The survey found that black tea was the most consumed with 68.80% of the participants and has been also considered to be the healthiest type of tea (80.40%).Moreover, 39.50% of the respondents consumed tea once a month. Based on the results, tea has usually been prepared for a period of 5 min (74.10%) in boiling water at approximately 90^{0}C (47.50%). In addition, 56.70% of the respondents believe that the black tea contains a high amount of caffeine. As the number of macerations increased, the amount of biologically active substances decreased (total polyphenols 134.93±0.04 mg GAE/mL, tannins 19.98±0.19 %, flavonoids 232.84±0.07 mg QE/g, caffeine 37.33±0.12 mg/mL and antioxidant activity 551.94±0.41 µM Trolox/g in black tea infusions prepared in 90 ^{0}C (5 min) after first maceration and 7.53±0.01 mg GAE/mL, tannins 14.92±0.24 %, flavonoids 55.88±0.18 mg QE/g, caffeine 2.33±0.05 mg/mL and antioxidant activity 122.32±0.17 µM Trolox/g in black tea infusions prepared in 90^{0}C (5 min) after third maceration. The obtained correlation (p>0.01) showed that the total polyphenols were correlated with other biologically active substances tested (tannins, flavonoids, caffeine and antioxidant activity) and L, a* and b* colour parameters of black tea infusions.*

Key words**:** *antioxidant activity, caffeine, flavonoids, polyphenols, tannins*

INTRODUCTION

Tea (*Camellia sinensis*) is a popular plant originating in south-western China. Presently, tea is grown in different parts of the world [17]. Initially, the herb was used as a spice, and later as a drink with many useful benefits [9]. Depending on the level of fermentation (oxidation), the tea is divided into three groups: black tea (fully fermented), green tea (not fermented) and oolong tea (semi-fermented) [28]. The process of making black tea (*Camellia sinensis* (L.) O. Kuntze) involves several operations, such as harvesting, withering, rolling, fermentation and drying. During the process of fermentation, the enzymatic oxidation of the polyphenols found in the tea results in the formation of theaflavin and thearubigins, which provide the characteristic colour and taste of the black tea [23]. Infusions prepared from green and black tea (*Camellia sinensis* (L.) Kuntze) is a popular drink in many countries around the world [14].

The black tea accounts for more than 75% of the total world tea production [26]. It contains naturally occurring biologically active substances such as: polyphenols, alkaloids, pigments [27]. It can inhibit α-amylase and α-glycosidase activity due to the bioactivity of

oxidized catechins [16,20,22]. Numerous studies have shown the relation between the tea utilization and possible disease prevention due to the high content of polyphenols. Polyphenols are secondary metabolites that are classified as flavonoids, phenolic acids, lignans and stilbens. The most commonly used flavonoids are polyphenols and benzo-γ-pyrone derivatives containing a phenolic bond and pyrane bond. They are classified as: flavanols, flavones, flavonols, flavanones, isoflavones and anthocyanidins [26]. In addition, flavanols are made up of compounds such as quercetin, which are of great interest in research into prevention and treatment of important health conditions, such as cardiovascular disease and cancer. Caffeine (1,3,7-trimethylxanthine) is an alkaloid contained in different amounts in the tea. Median caffeine content is in the range of 1-4%, depending on the type of tea [19]. Caffeine, which is contained in large quantities in black tea, is a powerful stimulator of the central nervous system [24]. Tannins are shown with a high molecular weight and they are defined as polyphenolic biomolecules and are found in many plants. They react and precipitate proteins and other organic compounds such as amino acids and alkaloid [15]. Antioxidants are substances that can delay, prevent or remove oxidative damage to specific molecules. They are usually divided into enzyme antioxidants and non-enzyme oxidants in the human body [10]. There are very few studies today examining the impact of the black tea on the oxidative stress [18].

MATERIALS AND METHODS

Materials

The black tea used for the analysis was produced from a Bulgarian Tea Company and was bought from the local markets in Razgrad, Republic of Bulgaria. In regards of the examinations the following chemicals have been used: Folin-Ciocalteau, Gallic acid (3,4,5-Trihydroxybenzoic acid), DPPH (2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl), Trolox ((S)-6-Methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and Quercetin (Sigma-Aldrich, Germany); NaOH, $KMnO_4$, $NaCO_3$, $NaNO_2$, $AlCl_3$, $CHCl_3$, Chloroform (Chimtex Ltd.) and indigo carmine (Fluka Chemie GmbH).

Methods

Preparing of questionnaire

For the purpose of examining the method of preparation, the type of tea used and which biologically active substances the respondents knew, a questionnaire was made available online via the Google platform. A total of 215 people from the district of Razgrad, Republic of Bulgaria, participated in the process of examination. At the beginning, questions were asked in order to be determined the socio-demographic status of the respondents (gender, age and education).

Preparation of black tea infusions

The bag of black tea was placed into a 400 mL Beaker glassware and sprinkle with 300 mL of hot water. The water was boiled at the temperatures of 70°C and 90°C, and the maceration time was exactly 3 and 5 minutes at the appropriate temperatures. After the maceration time had been expired, the tea bag was taken out of the beaker cup, and the final presented the tea obtained after the first maceration at 70°C and 90°C and a maceration time of 3 and 5 minutes, respectively. The same packet of black tea was placed into another Baker at the same temperature for 3 and 5 minutes. The tea obtained was tea obtained after the second maceration. The procedure was repeated again under the same conditions and finally the tea was analyzed for the third maceration. The black tea samples obtained after the first, second and third maceration ware allowed to cool for 30 minutes and then used for analysis.

Biological active compounds in black tea infusions

Method used for the determination of caffeine ad flavonoids in black tea was presented by Zayadi, Rahim and Bakar, (2016) [29] and results were expressed as gram per litre (g/L) and mg of quercetin equivalents per gram (mg QE/g), respectively. Determination of content of tannins in black tea was measured

according to the method described by Atanassova and Christova-Bagdassarian, 2009 [3]. Total polyphenols content (TPC) was measured spectrophotometrically with *Folin-Ciocalteau*. Results was expressed as mg of gallic acid equivalents per millilitre (mg GAE/mL). Antioxidant activity was measured with DPPH radical, according to method as described by Wang and Ryu, 2013 [25], and results were expressed as micromole Trolox per gram (μMTrolox/g). All analyses were performed in quadruplicate.

Colour of black tea infusions

The colour was determined with the Konica Minolta Chroma Meter CR-400, in CIE L*a*b* system. L* is the luminance or lightness component, which ranges from black (L = 0) to white (L = 100), and parameters a* (from green to red) and b* (from blue to yellow). Each colour value was measured in triplicate.

Data Analysis

All results are presented as mean ± SD. Pearson's linear correlation analysis was performed with the software XLSTAT 2017.

RESULTS AND DISCUSSIONS

In Table 1 is presented the socio-demographics status of the correspondents with 73% females and 27 % males. As it can be obtained from the table, 31.20% of the correspondents were in the age group of 21 – 30 years and according to their education 67.44% of the correspondents were with higher education.

Table 1. Socio-demographics status of the correspondents

		Number	%
SEX	male	58	27.00
	female	157	73.00
Age	under 20	23	10.70
	21-30	67	31.20
	31-40	61	28.40
	41-50	43	20.00
	51-60	16	7.40
	over 60	5	2.30
Education	Primary	16	7.44
	Secondary	48	22.33
	Higher	145	67.44
	Ph.D	6	2.79

Source: Own calculation.

From the results demonstrated in the figure 1 it can be obtained that 68.8%, 27.40% and 1.90% of the correspondents drank a black tea, fruit tea and do not drink tea, respectively.

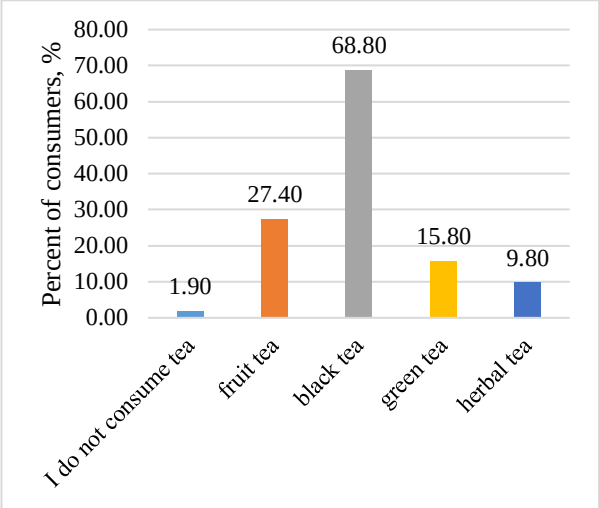

Fig. 1. Answers to the question "Which tea do you prefer for drinking?"
Source: Own results.

The given results in Fig. 2 showed that the respondents used water at a temperature of about 90°C (47.5%) during tea preparation. Additionally, 32.70% of the respondents answered that the water they used for tea was around 100°C. The smallest percentage (2.50%) of the respondents answered that the water used for tea used was around 50°C in the period of tea preparation.

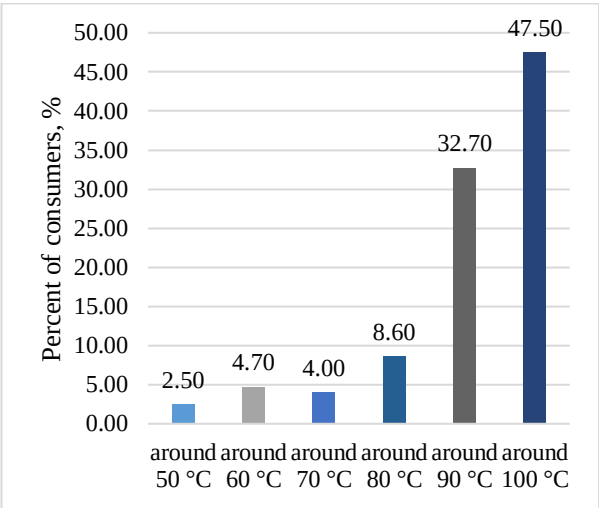

Fig. 2. Answers to the question "At which temperature do you heat the water for the tea preparation?"
Source: Own results.

When asked "When making tea bought from local stores, how many times you use the same tea bag", most of the respondents (68.90%) answered that they use only one bag (Fig. 3). The same tea bag has been used three or more times by 6.40% of the correspondents.

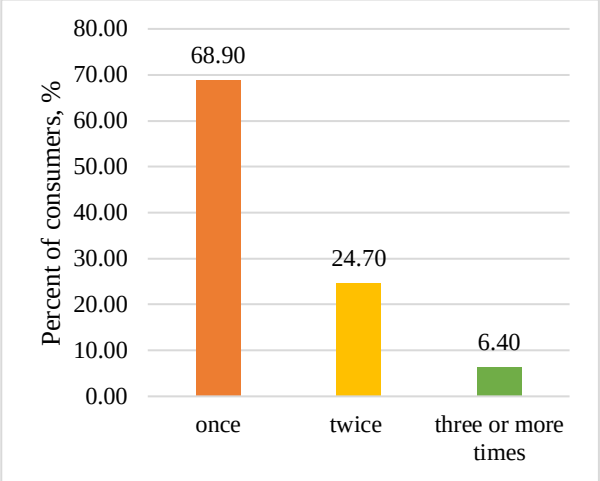

Fig. 3. Answers to the question "When preparing the tea bought from the local markets, how many times you use the same tea bag?"
Source: Own results.

The results presented in Fig. 4 showed that 74.10% of the respondents held the bag for about 5 minutes during the preparation of their tea, and 2.23% of the respondents held the bag for more than 10 minutes.

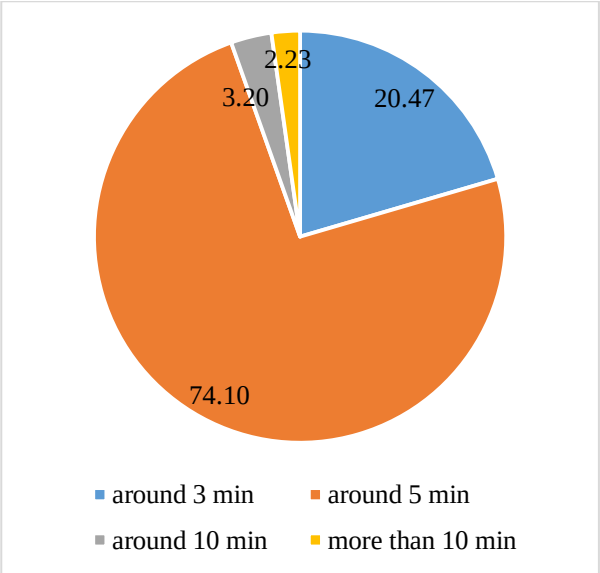

Fig. 4. Answers to the question "How long you keep your tea bag in the hot water for the tea preparation?"
Source: Own results.

It can been obtained from the results presented in Fig. 5 that 39.50% and 25.10% of the correspondents have been consuming tea once per month and once per day, respectively. Infusions prepared from green and black tea (*Camellia sinensis* (L.) Kuntze) is a popular drink in many countries around the world [14].

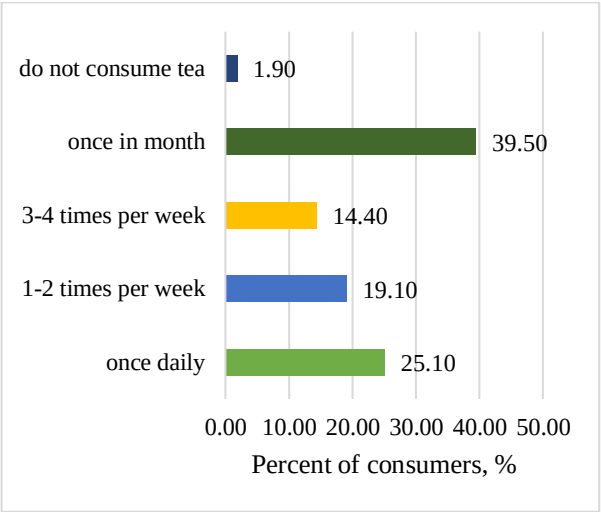

Fig. 5. Answers to the question "How often do you drink tea?"
Source: Own results.

In Fig. 6 there are illustrated the answers of the question "which tea is the healthiest for consuming". Most of the correspondents (80.40%) think that the black tea, and 12.60% believe that the healthiest tea is the green tea.

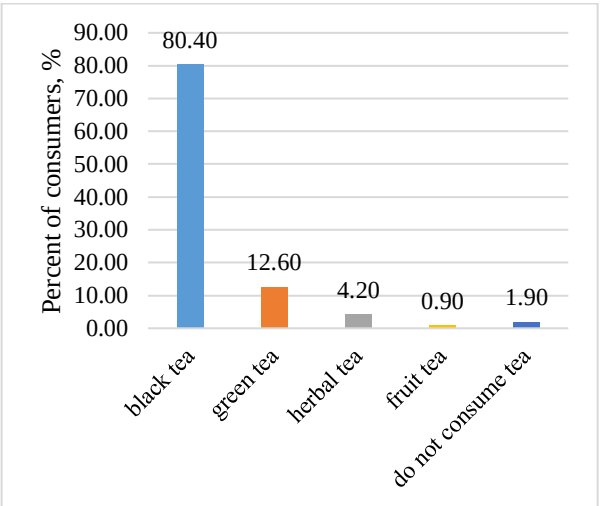

Fig. 6. Answers to the question "Which tea is the healthiest for drinking?"
Source: Own results.

The answers of the following question: Which biologically active components contain in the black tea, are shown in Fig. 7.

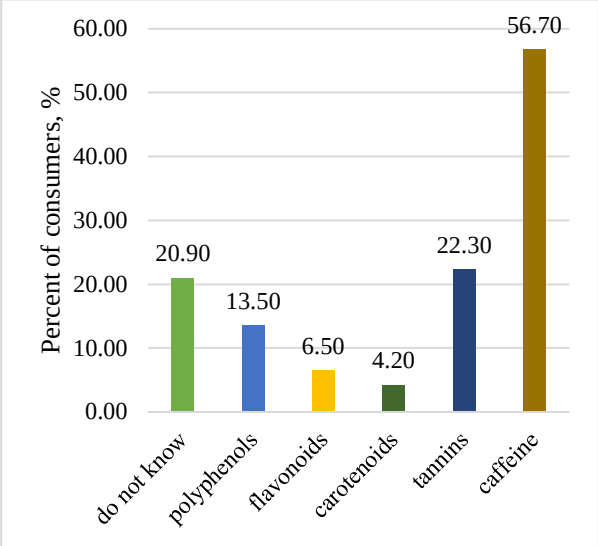

Fig. 7. Answers to the question "Which of the biologically active components you believe contain in the tea?" *One question can have more possible answers
Source: Own results.

On the other hand, the caffeine-containing products have been consumed for a long time because of their pleasant and stimulating effect [13]. High levels of caffeine can irritate the gastrointestinal tract and it is therefore advisable to keep the amount of caffeine in the tea leaves on a low level. Therefore, the determination of caffeine is a very important chemical analysis [19].
Figure 8 shows the amount of caffeine at different temperature (70°C and 90°C), different time (3 and 5 min) and different number (1 to 3) of maceration.
Figure 8 shows that as the number of macerations increased, the amount of caffeine in the black tea infusions decreased. A decrease in caffeine content was also observed when the black tea infusions were prepared at a lower temperature (70°C). After the third maceration at higher temperature (90°C) it was observed that the amount of caffeine was lower (2.33±0.05 mg/mL) compared to the third maceration for 5 minutes at lower temperature at 70°C (6.07±0.10 mg/mL).

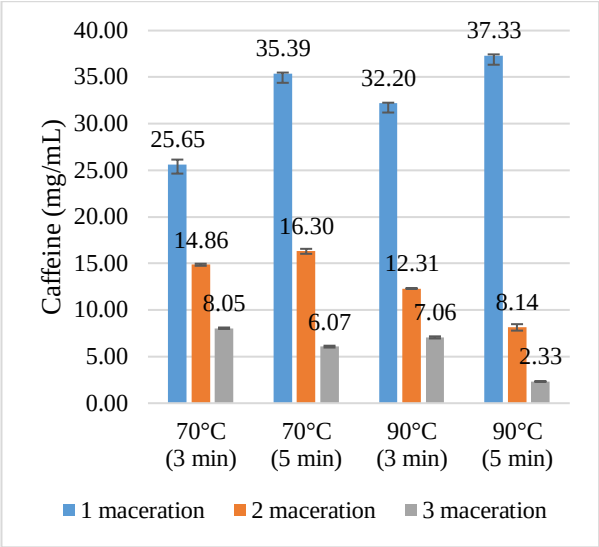

Fig. 8. Caffeine in black tea infusions
Source: Own results.

The results showed that the time of maceration is of great importance for the caffeine content of the tea. Zayadi, Rahim and Bakar, (2016) [29] also made a similar statement in their study. The amount of caffeine determined by Komes et al., (2009) [13] was 3.86%. One previous study by Athayde, Coelho and Schenkel, (2000) [4] have shown that caffeine content is related to the origin, genetics, environment, and the method of the plant preparation (teas), ranging from 24 to 40%.
The polyphenolic compounds have several benzene rings in their composition, each containing at least one hydroxyl group (-OH). These compounds act by inhibiting the activity of the radical that causes side changes [19].
Fig. 9 shows the values obtained for the black tea infusions at different temperatures (70°C and 90°C), at different maceration times (3 and 5 min) and different number of macerations (1 to 3).
The temperature had a great impact ($p>0.01$) on the amount of total polyphenols in black tea infusions (Fig.1). At a temperature of 70°C the amount of total phenols determined was 86.29±0.25 mg GAE/mL, in addition, at a temperature of 90°C it was 134.93±0.04 mg GAE/mL.

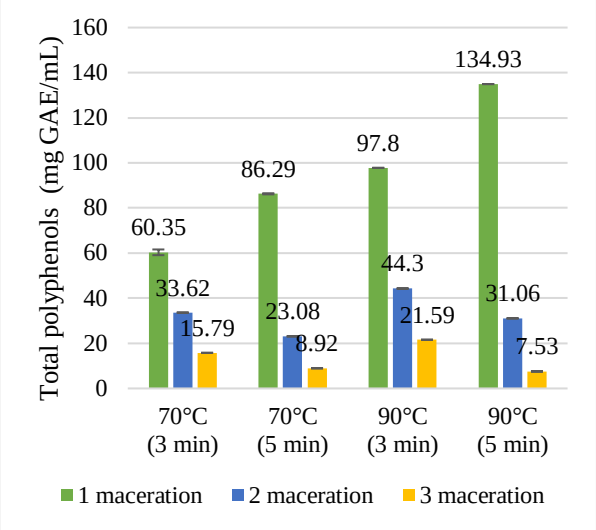

Fig. 9. Total polyphenols in black tea infusions
Source: Own results.

On the contrary, the total amount of polyphenols was also affected by the time for maceration (3 and 5 min), so the highest amount of TPC occurs when the maceration takes place within 5 min at 90°C (134.93±0.04 mg GAE/mL). It can also be seen from the fig. 9 that TPCs decrease with the increase in the number of macerations. Tea leaves are rich in polyphenols that have antioxidant properties. Tea polyphenols create the taste and aroma of beverages [21, 22]. Da Silva Pinto, (2013) [7] showed that the chemical composition of black tea greatly depended on the temperature at which the fermentation of black tea takes place. Choe et al., (2019) [6, 22] when determining total polyphenols in pure tea extracts, found that by increasing the extraction time the amount of TPC increased. Tannins are high molecular weight polyphenolic molecules and can be found in many plant species. Tannins bind and accumulate proteins and other organic compounds such as amino acids and alkaloids [13].

Fig. 10 shows the determined amount of tannins at different temperatures (70 and 90°C), different number of macerations (1 to 3) and different maceration time (3 and 5 min).

From the results presented in Figure 10, it can be observed that the amount of tannin was higher when using a higher maceration temperature (20.22±0.08% at 90°C). It can also be concluded that after the first maceration at both temperatures at 70°C and 90°C the tannin content is the highest (13.28±0.04% and 20.22±0.08%, respectively).

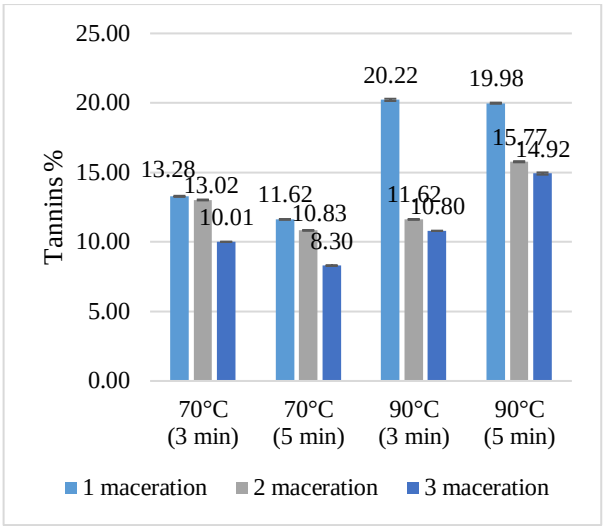

Fig. 10. Tannins in black tea infusions
Source: Own results.

As the number of macerations continues (from the first to the second and finally to the third maceration), the amount of tannin in black tea infusions decreased (form 20.22±0.08% after first maceration and 10.80±0.47% after third maceration). Khasnabis, Rai and Roy, (2015) [12] in their study of different types of tea found that black tea contained more tannins than green tea. The amount of tannins determined in their black tea ranged from 11.76% to 15.14%. Within these limits was the amount of tannins determined in our study when preparing black tea infusions at 70°C. In contrast, Atanassova and Christova-Bagdassarian, (2009) [3] established that green tea had a higher amount of tannin than black tea, 10.23% and 55.89%, respectively. The large differences in tannin content in different tea species may be due to different tea production processes (fermentation), leaf tea aging or differences in climate and soil composition of tea trees [12].

Flavonoids are considered to be the most common, most important, and most widely used singlet group of phenols present in plants. Flavonoids are proven antioxidants. They inhibit lipid oxidation and form complexes with metal ions [1].

Fig. 11 shows the amount of flavonoids determined in black tea, three different tea infusions (1, 2 and 3), different time (3 and 5 min) and different maceration temperature (70 and 90 °C).

It has been noted that the amount of flavonoids was the lowest after the third maceration at both temperatures of 70°C and 90°C (78.54±0.06 mg QE/g and 55.88±0.18 mg QE/g, respectively). The highest amount of flavonoids was determined in the black tea infusions prepared at 90°C after the first maceration (232.84±0.07 mg QE/g) (Fig. 11).

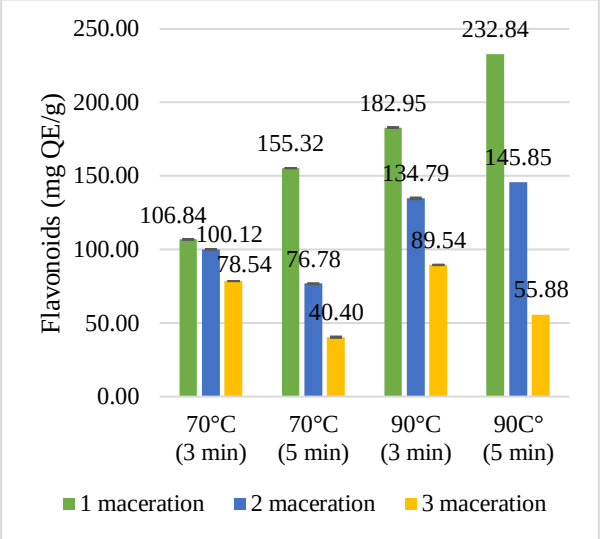

Fig. 11. Flavonoids in black tea infusions
Source: Own results.

Abdeltaif, Sirelkhatim, and Hassan, (2018) [1] in their study determined the amount of total flavonoids in black tea (49.57 mg QE/g). In addition, Mashkouri Najafi, Hamid and Afshin, (2003) [19] presented that black tea contained a higher amount of total flavonoids compared to oolong tea (8.8 mg QE/g total flavonoids in black tea). The different amounts of total flavonoids depend on the method of extraction and the temperature at which the extraction is performed [1].

Antioxidants are substances that can prevent or eliminate oxidative damage to molecules [8]. They are divided into enzymatic antioxidants and non-enzymatic. Exogenous antioxidants are usually non-enzymatic antioxidants. Tea is considered to be a drink with strong antioxidant properties due to its high content of polyphenols [18].

Fig. 12 depicts the antioxidant activity of black tea infusions at different time (3 and 5 min), number (1 to 3), and temperature (70°C and 90°C of maceration).

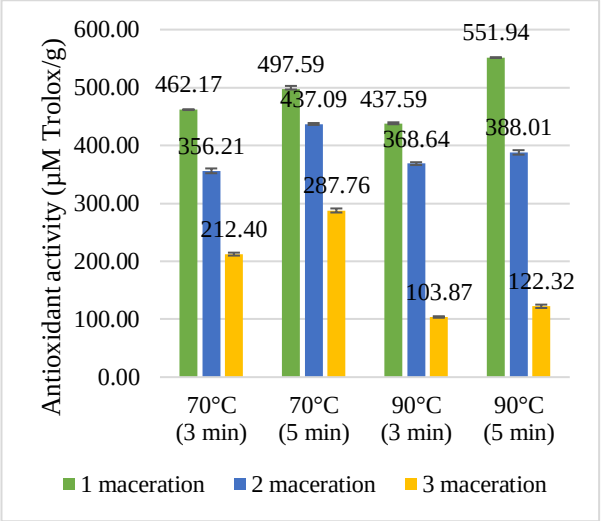

Fig. 12. Antioxidant activity in black tea infusions
Source: Own result

The highest antioxidant activity in the black tea infusions was determined at higher temperature (90°C) during extended maceration (5 min) (551.94±0.41 µM Trolox/g). From the results presented in Fig. 12 it can be seen that with the continuation of maceration the antioxidant activity decreased (form 551.94±0.41 µM Trolox/g after first maceration to 122.32±2.89 µM Trolox/g after third maceration in 90°C in 5 min). The reduction was even greater when the number of macerations was performed at higher temperatures. When comparing the antioxidant activity of green and black tea, previous studies have shown that green tea has a higher antioxidant activity. This is due to the fact that black tea is fermented while green tea is not [2]. Bhuyan et al., (2013) [5, 29] determined the antioxidant activity of black tea produced in different regions of North-East India and found that the antioxidant activity was in the range from 84.50% to 89.03% DPPH.

The colour change of black tea infusions at different temperatures (70°C and 90°C), time (3 and 5 min) and number of maceration (1 to 3) are presented in Table 2.

Table 2. The change of the colour of black tea infusions

Temperature	Time of maceration	Number of maceration	L*	a*	b*
70 °C	3 min	1	21.30±2.26	2.22±0.72	12.81±0.01
		2	24.73±0.04	1.24±0.05	16.43±2.20
		3	25.30±0.42	0.03±0.04	21.33±0.04
	5 min	1	19.81±0.01	2.24±0.06	12.81±0.01
		2	26.53±0.04	1.24±0.05	22.75±0.06
		3	28.25±0.35	-1.13±0.04	27.24±0.06
90 °C	3 min	1	20.91±0.01	2.53±0.04	16.06±0.11
		2	30.21±0.01	0.94±0.06	18.44±0.13
		3	32.08±0.11	-0.73±0.04	21.22±0.33
	5 min	1	18.91±0.01	3.32±0.02	15.63±0.04
		2	30.84±0.05	-1.24±0.05	17.13±0.04
		3	31.73±0.04	-3.92±0.04	28.63±0.04

Source: Own Calculation.

Lightness (L*) was in the range of 19.81±0.01 to 32.08±0.11. From Table 2 it can be observed that as the number of macerations L* increased, the colour of the tea became brighter. Jin et al., (2016) [11] in the study presented that the values for the L* parameter of herbal teas ranged from 46.38 to 99.98. The same authors in green tea for parameter L * set values in the range from 90.36 to 97.21. On the contrary, the b* parameter values in black tea infusions decreased with increasing number of macerations, i.e. the colour of the brewed black tea after the first maceration in all trials was yellow, and with the continuation of the macerations it reduced its intensity and in many of the samples it turns to blue (negative values for b*). The values for parameter a* range from 12.81±0.01 to 28.63±0.04. It has been found that the values for this parameter are higher when the maceration was performed at a higher temperature (90°C). Increasing the values for parameter a* by increasing the number of macerations means that the red colour of the black tea infusions became more intense. In Fig. 13 presents a visual comparison of the color of the black tea infusions at temperature 70°C for 3 and 5 minutes of maceration (a) and at temperature 90°C for 3 and 5 minutes of maceration (b).

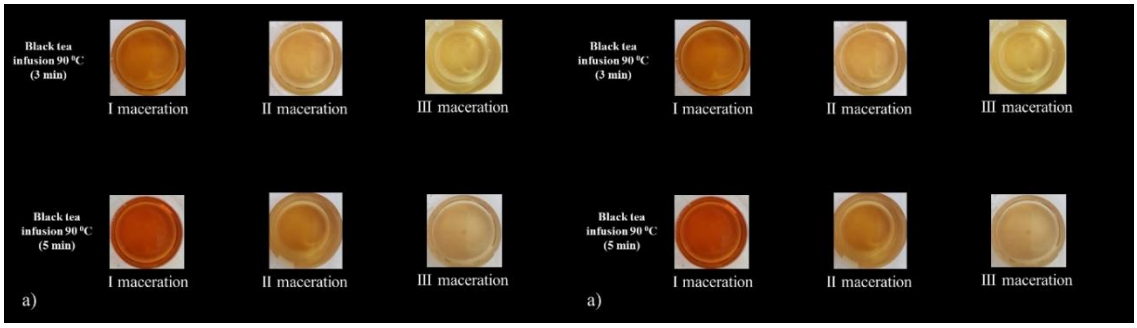

Fig. 13. Visual comparison of the color of the black tea infusions at temperature 70 °C for 3 and 5 minutes of maceration (a) and at temperature 90 °C for 3 and 5 minutes of maceration (b)
Source: Own results in the laboratory.

The correlation between the biologically active substances and the colour are illustrated in Table 3. The table shows that total polyphenols are correlated with all the investigated characteristics (positive correlation with Tannin, Flavonoids, Caffeine and Antioxidant activity and negative correlation with L*and b* parameter). Tannin is in a positive correlation with Caffeine. Flavonoids are in positive correlation with Antioxidant activity and the parameter a* and negative correlation with b* parameter. Caffeine is in positive correlation with antioxidant activity and a* parameter and negative correlation with L*and b* parameter. Antioxidant activity correlated positively with parameter a* and negative correlated with parameter L* and b*. L* value is in a positive correlation with b* value, and negative correlation with a* parameter. a* value correlated negative with b* value.

Table 3. Correlation between the investigated chemical characteristics and the color of black tea infusions

Variables	Total polyphenols	Tannin	Flavonoids	Caffeine	Antioxidant activity	L*	a*
Tannin	0.721*						
Flavonoids	0.928*	0.777*					
Caffeine	0.941*	0.556*	0.799*				
Antioxidant activity	0.766*	0.431	0.705*	0.836*			
L	-0.799*	-0.421	-0.586*	-0.907*	-0.691*		
a	0.653*	0.297	0.562*	0.744*	0.754*	-0.731*	
b	-0.744*	-0.380	-0.809*	-0.739*	-0.692*	0.535*	-0.549*

** $p<0.01$*

Source: Own calculation.

CONCLUSIONS

The survey found that black tea was the most consumed with 68.80% of the participants and has been also considered to be the healthiest type of tea (80.40%). Moreover, 39.50% of the respondents consumed tea once a month. Based on the results, tea has usually been prepared for a period of 5 min (74.10%) in boiling water at approximately 90°C (47.50%). In addition, 56.70% of the respondents believe that the black tea contains a high amount of caffeine. The results from this study have shown that the amount of polyphenols, flavonoids, tannins, caffeine, and antioxidant capacity of black tea infusions depended on the temperature of the maceration, the time and the number of macerations. At higher temperatures (90°C) the amount of biologically active substances was higher compared to the lower temperature (70°C). The number of macerations indicates that after the first maceration the amount of biologically active substances tested decreased significantly. The colour of black tea infusions also depends on the temperature of the maceration, the time and the number of macerations.

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