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METHYLXANTHINES AND CATECHINES IN DIFFERENT TEAS (*CAMELLIA SINENSIS* L. KUNTZE) – INFLUENCE ON ANTIOXIDANT PROPERTIES

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ABSTRACT

In general, there are four basic types of tea: green (not fermented), black (fermented), oolong and white tea (partially fermented). The differences among these types are in the processing technology, which is largely reflected in their chemical composition. The most influential factor that significantly affects the quality and quantity of substances (biologically active) is the processing temperature, which causes changes in the composition (isomerization and/or transformation). The present paper focuses on monitoring content of three methylxanthines – alkaloids (caffeine, theophylline and theobromine), and seven flavan-3-ols – catechins ((+)-catechin (C), (-)-catechin-3-gallate (C-3-G), (-)-epicatechin (EC), (-)-epicatechin-3-gallate (EC-3-G), (-)-epigallocatechin-3-gallate (EGC-3-G), (-)-gallocatechin (GC) and (-)-gallocatechin-3-gallate (GC 3-G)), which are characteristic for tea. Attention was also given to the assessment of selected antioxidant parameters using spectrophotometric procedures (ABTS - radical cation decolorization assay and Phosphomolybdenum reducing antioxidant power assay) in relation to the determined substances using RP-HPLC/DAD analysis. Based on the results obtained, it can be concluded that a type of tea clearly affects the quality and quantity of the substances that have a positive impact on the consumer's health, significantly reflected in the levels of antioxidant active substances determined by the spectrophotometric procedures. The highest content of methylxanthin, catechins, polyphenols and antioxidant substances was recorded in the green tea sample GT3. The highest content of flavonoids and phenolic acids was recorded in the Pu-erh tea sample PT 5.

Keywords: methylxantines; catechines; *Camellia sinensis* L.; tea; antioxidants

INTRODUCTION

Tea is the second most widely consumed drink, after water. Its global consumption reached 4.84 million tonnes in 2013 **(FAO, 2015)**. The worldwide popularity of tea is based on several apects and benefits, including therapeutic, refreshing, tasteful and ritual. Its regular and long-term consumption plays a significant role in terms of positive impact on the health of the consumer, which is caused by the presence of a number of biologically active and health-promoting substances **(Sharangi, 2009)**.

The tea plant (*Camellia sinensis* L.) is evergreen plant growing in more than 45 countries worldwide (excluding North America) **(Jeszka-Skowron et al., 2015)**. The biggest producers of dried tea include China, India, Kenya, Sri Lanka, Japan, Taiwan and Nepal **(Marcos et al., 1998; FAO, 2015)**. Global production of black, green and instant tea exceeded 5 million tonnes in 2013 **(FAO, 2015)**. The best conditions for growing tea are in tropical and subtropical areas with sufficient rainfall and well drained and acidic soils. However, it grows also in the alpine zone, which characteristically affects its phytochemical composition. Only the top two leaves and bud is collected in two to three harvests during the growing season. The most valuable is the first harvest (spring). In the dry matter, it contains 25 – 35% of biologically active substances from the polyphenol group **(Almajano et al., 2008)**. There are several types of tea recognized, depending on the technology of the raw tea processing. The most frequently consumed are green (unfermented), black (fermented), oolong and pu-erh tea **(Árvay et al., 2015)**. Recently, the so called "scientific teas" that are specifically bred to increase a content of particular substances came to the fore. Such teas include GABA tea **(Tsai et al., 2008)** that is characterized by high acid γ-aminobutyric acid, which has positive effects on the prevention of diseases of the CNS.

Regular consumption of tea and tea beverages has a significant positive effect on the prevention of various civilization diseases such as high blood pressure **(Chung et al., 2003)**, cardiovascular diseases **(Kuriyama et al., 2015)** tumours **(Yao et al., 2004)**, digestive system cancers **(Nechuta et al., 2012)**. It positively affects cardiovascular

system and lowers level of low density lipids and cholesterol **(Chung et al., 2003)**. Major substances that are present in the tea leaves, as well as the actual drink include polyphenols (flavan-3-ols) that have the highest antioxidant activity of all tea substances **(Nováková et al., 2010)**. Characteristic group of tea substances include also methylxanthines. Their content in dry matter of tea is as follows: caffeine (2.0 – 6.9%), theobromine (0.15 – 0.20%) and theophylline (0.02 – 0.04%) **(Rahim et al., 2014)**.

Teas are generally characterized by a significantly positive biological effect on the consumer's health. They have a high content of broad spectrum of catechins. The most important compounds of this group are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin-3 gallate (EGC) and (-)- epigallocatechin-3-gallate (EGC -3-G) **(Nováková et al., 2010)**. The latter is biologically one the most effective **(Murakami et al., 2013)**.

Qualitative and quantitative determination of the 11 most active biological compounds in 30 samples of different kinds of tea and tea-substitutes was the main objective of this paper. The studied compounds belonging to the group of catechins were (+)-catechin (C), (-)-catechin-3-gallate (C-3-G), (-)-epicatechin (EC), (-)-epicatechin-3-gallate (EC-3-G), (-)-epigallocatechin-3-gallate (EGC-3-G), (-)-gallocatechin (GC) and (-)-gallocatechin-3-gallate (GC 3-G). The studied compounds from the group of methylxanthines were caffeine (CAF), theobromine (TBM) and theophylline (TFL). Analyses were conducted in tea infussions by RP-HPLC-DAD method. The data obtained were statistically processed and evaluated in terms of the total content of the main groups of the studied compounds. We also focused on monitoring antioxidant characteristics of water extracts using ABTS and phosphomolybdenum (PM) method and total amount of polyphenols (TPC), flavonoids (TFC) and phenolic acids (PAC).

MATERIAL AND METHODOLOGY

Materials – samples

The study focused on the qualitative and quantitative determination of seven catechins and three methylxanthines by RP-HPLC-DAD analysis, total content of polyphenols and flavonoids and two antioxidant parameters by spectrophotometry in 30 samples of different kinds of teas and/or tea-substitutes. Their characteristics (name, kind and country of origin) are shown in Table 1.

Chemicals

Single-component standards (theobromine, and theophylline), acetonitrile (HPLC gradient grade), methanol (HPLC grade) and phosphoric acid (ACS grade) were purchased from Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Steiheim, Germany). Blended standard Green Tea Catechin Mix (GTCM) was purchased from Cerriliant company (Cerriliant Corp., RR, Texas, USA). Double deionized water (ddH_2O) was treated (18.2 $M\Omega.cm^{-1}$) in a Simplicity 185 purification system (Millipore SAS, Molsheim, France). Chemicals used for the spectrophotometric analyses were analytical grade and purchased from CENTRALCHEM (Bratislava, Slovakia) and Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Steiheim, Germany).

Preparation of calibration solutions and samples

Single-component standard solutions were prepared by dissolving 5 mg of each compounds (with accuracy to 4 decimal places) in 10 mL of methanol (HPLC grade). Consequently, 100 µL of theobromine and theophylline standards were added to 1 mL GTCM blended standard.

Tea beverages were prepared by extraction of 1 g of dried tea in hot water (85 °C) in a volume of 100 mL for 5 minutes. The tea beverages were afterwards filtered through a Munktell filter paper No. 390 (Munktell & Filtrak, Bärenstein, Germany). After cooling, the filtrates were filtered again through syringe PVDF filters Q-Max (0.22 µm, 25 mm) (Frisenette ApS, Knebel, Denmark) prior to the HPLC analysis.

RP-HPLC-DAD analysis

All studied compounds were determined by HPLC Agilent 1260 (Agilent Technologies, Waldbronn, Germany) with quaternary solvent manager coupled with degasser (G1311B), sample manager (G1329B), column manager (G1316A) and DAD detector (G1315C). All analyses were performed on C18 endcapped column with reverse phase Purosphere® (4 mm x 250 mm x 5 µm) (Merck, KGaA, Darmstadt, Germany). Mobile phases consisted of acetonitrile (A) and 0.1% H_3PO_4 in ddH_2O (v/v) (B). The gradient elution was as follows: 0-1 min isocratic elution (20% A and 80% B), 1 – 5 min linear gradient elution (25% A and 75% B), 5 – 15 min (30% A and 70% B) and 20 – 25 min (40% A and 60% B). Postrun was 3 min. The mobile phase flow was 1 mL min^{-1} and the sample injection was 10 µL. Column thermostat was set to 30 °C and the samples were kept at 4 °C the sampler manager. The detection wavelength was set at 265 nm, with scanning of the spectrum in the range of 210 – 400 nm. The spectral data were collected and processed using Agilent OpenLab ChemStation software for LC 3D Systems.

Total polyphenol content

The total polyphenol content in water extracts of the samples was determined by the methodology of Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. The samples (100 µL) were mixed with 100 µL of the reagent, 1 mL of 20% solution of sodium carbonate and 8.8 mL of deionized water. The samples were left to stand for 30 minutes in the dark and then, absorbance of the samples at 700 nm was measured on a spectrophotometer Jenway 6405 UV/Vis (Cole-Parmer, England). Gallic acid (25 – 250 $mg.L^{-1}$; $R^2 = 0.9978$) was used as the standard and the results were calculated to the gallic acid equivalents (mg $GA.g^{-1}$).

Total flavonoid content

The total flavonoid content was determined by the modified method of **Willett (2002)**. The extract (500 µL) was mixed with 100 µL of 10% ethanol solution of aluminum chloride, 100 µL of sodium acetate (c = 1 mol L-1) and 4.3 mL of deionized water. After 30 minutes of standing in the dark, the absorbance of solutions was

Table 1 Basic characteristics of tea samples.

Name	Abbreviation	Country of origin
Quitou Lu (green)	GT 1	China
Ming Qiah (green)	GT 2	China
Ujitawara (green)	GT 3	Japan
Huang Da Cha (green)	GT 4	China
Huang Ya (green)	GT 5	China
Taimu Shan Bai (green)	GT 6	China
Taimu Shan Shou (green)	GT 7	China
Gan De Benshan (green)	GT 8	China
Quing Bei Huo (green)	GT 9	China
Hojicha Organic (green)	GT 10	Japan
Matcha Organic (green)	GT 11	Japan
Huang Zhi Xiang (green)	GT 12	China
Tonumo Guan Da (green)	GT 13	China
Tie Guan Yin (black)	BT 1	China
Gruzia Ramiz (black)	BT 2	Georgia
Darjeeling 2015 (black)	BT 3	India
Sungma Organic (black)	BT 4	India
Shaanxi Fu (pu-erh)	PT 1	China
Wyzhou Yi Liu (pu-erh)	PT 2	China
Bulang Gu Shu (pu-erh)	PT 3	China
Jin Pai Ban Hou (pu-erh)	PT 4	China
Gua Feng Zhai (pu-erh)	PT 5	China
2014 Kun Lu (pu-erh)	PT 6	China
Nan Jian Tulin (pu-erh)	PT 7	China
Yong De Lao (pu-erh)	PT 8	China
1995 Menghai (pu-erh)	PT 9	China
2008 Mengku (pu-erh)	PT 10	China
Yong De (tea flower)	YD 1	China
Kudingeha (Ku ding cha)	K 1	China
Jiaogulan (5-leaf ginseng)	J 1	China

measured at 415 nm on a spectrophotometer Jenway 6405 UV/Vis (Cole-Parmer, England). Quercetin (1 – 400 mg.L^{-1}, R^2 = 0.9996) was used as a standard and the results were expressed in mg QE.g^{-1}.

Total phenolic acid content

The total content of phenolic acids was determined by the method of **Farmakopea Polska (1999)**. Water extract (0.5 mL) was mixed with 0.5 mL Arnova reagent (10% $NaNO_2$ +10% Na_2MoO_4). Afterwards, 0.5 mL of NaOH with c = 1 mol L^{-1} (w/v) and 0.5 mL of ddH_2O. The total content of phenolic acids was determined by the spectrophotometer Jenway 6405 UV/Vis (Cole-Parmer, England). Caffeic acid (1 – 200 mg L^{-1}, R^2 = 0.9996) was used as a standard and the results were expressed in mg.g^{-1} caffeic acid equivalents.

ABTS radical cation decolorization assay

ABTS radical cation decolorization assay was determined by the method of **Re et al. (1999)** with slight modification. ABTS radical was dissolved in ddH_2O to 7 mM concentration and potassium persulphate added to a concentration of 2.45 mM. The resulted mixture was left to stand in the dark at room temperature overnight before further analysis. The resultant intensely-coloured $ABTS^+$ radical cation was diluted with 0.01 M phosphate buffer saline (PBS), pH 7.00 to give an absorbance value of 0.70 at 734 nm. ABTS solution (2 Ml) was mixed with 098 mL of PBS and 0.02 mL of sample extract. Absorbance was measured spectrophotometrically on Jenway 6405 UV/Vis (Cole-Parmer, England) at time intervals of 6 minutes after addition of sample extract. Trolox (10 – 100 mg.L^{-1}, R^2 = 0.9991) was used as the standard and the results were expressed in mg.g^{-1} Trolox equivalents.

Phosphomolybdenum reducing antioxidant power assay

Reducing power of the extract was determined by the method of **Prieto et al. (1999)**. The mixture of sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), H_2SO_4 (6 mL, 1M), ammonium heptamolybdate (0.4 mL, 0.1 M) and ddH_2O (0.8 mL) was incubated at 90 °C for 120 min. Then rapidly cooled and detected by monitoring absorbance at 700 nm using Jenway 6405 UV/Vis spectrophotometer (Cole-Parmer, England). Trolox (10–100 mg.L^{-1}, R^2 = 0.9980) was used as the standard and the results were expresse in mg g^{-1} Trolox equivalents.

Statistical analysis

All the data obtained were processed and evaluated by basic descriptive statistics (min., max., St. Dev., mean). The results are presented as mean values of four and/or three independent measurements.

RESULTS AND DISCUSSION

Methylxantines and catechines content

All the studied components determined the by the RP-HPLC/DAD process belong to the compounds that are characteristic for tea **(da Silva Pinto, 2013)**. Their content is dependent on many factors **(Sharangia, 2009)**.

The total content of methylxanthines (alkaloids) was on average 17.4 ±9.79 mg.g^{-1} DW in all samples. Relatively high standard deviation can be explained by a wide range of the sample types, but also by the presence of non-tea samples (YD 1, K 1 and J 1), in which the content of methylxanthines was not detected (in the YD 1 and K 1 samples, the content of caffeine wass very low and the contents of theophylline and theobromine were below the detection limit (Table 2).

The highest content of the methylxanthines was recorded in the green tea GT 3 sample (35.0 ±0.07 mg.g^{-1} DW). In general, it can be concluded that the highest concentrations of caffeine as well as the sum of methylxanthines were recorded in the green tea samples (compared to the other kinds of tea). These results are confirmed by the findings of **Bae et al. (2015)** and **Yi et al. (2015)**. Based on the sum of methylxanthines in the individual sample types, there was the following descending order: GT >PT >BT >YD 1 >K 1 >J 1.

All the data obtained are shown in Table 2. Similarly to the methylxanthines, teas are characterized by high content of broad spectrum of flavan-3-ols, commonly known as catechins **(Wang et al., 2011)**. Along with caffeine, they represent a group of substances with the highest content **(da Silva Pinto, 2013)**.

Their content is dependent on several factors (like the content of alkaloids). On average, their content is around 30% in the dry matter of the tea tree leaves 30% **(Balentine et al., 1997)**. The total content of catechins in the studied samples was 15.3 ±17.0 mg.g^{-1} DW (ND – 64.3 mg.g^{-1} DW). As was the case of the alkaloids, the very wide range of the amount of catechins was due to the high number of the sample types. The highest concentration of catechins was recorded in epigallocatechin-3-gallate (EGC-3-G).

The average concentration of catechins was 9.42 ±12.5 mg.g^{-1} DW (ND – 46.6 mg.g^{-1} DW). Content of EGC-3-G represents about 40% of the amount of catechins, which corresponds to the findings of **Bae et al. (2015)** and **Yi et al. (2015)**. The highest concentration of EGC-3-G was recorded in the sample of green tea GT 3. In general, green tea contained the highest concentrations of this substance. Other samples contained lower concentrations of EGC-3-G, which is caused by different levels of fermentation and thus thermal degradation, and/or epimerisation (conversion) of the compound to other forms of flavan-3-ols **(Scholz and Williamson, 2007)**. In terms of total content of catechins, it can be concluded that the GT3 sample had the highest quality from the health point of view. It is confirmed by the fact that it was the only sample that contained all forms of the studied catechins.

Total polyphenol content

Several studies dealing with monitoring the content of

Table 2 Content of methylxanthines and catechines in all samples (mg.g^{-1} DW) (mean ±St.Dev).

Sample	Methylxanthines			Catechines						
	TBM	TFL	CAF	GC	C	EGC-3-G	EC	GC-3-G	EC-3-G	C-3-G
GT 1	2.63 ±0.01	1.47 ±0.00	30.9 ±0.03	ND	0.86 ±0.03	33.9 ±0.05	4.13 ±0.01	1.62 ±0.01	4.12 ±0.03	ND
GT 2	1.59 ±0.01	0.53 ±0.00	27.4 ±0.10	ND	0.64 ±0.43	24.6 ±0.09	2.48 ±0.01	1.60 ±0.01	2.85 ±0.00	ND
GT 3	1.48 ±0.00	1.88 ±0.00	35.0 ±0.07	ND	1.25 ±0.01	46.4 ±0.15	6.57 ±0.07	3.80 ±0.02	5.68 ±0.02	0.26 ±0.02
GT 4	0.98 ±0.01	0.24 ±0.00	16.8 ±0.10	ND	0.42 ±0.28	4.24 ±0.16	0.81 ±0.01	1.24 ±0.01	0.80 ±0.04	ND
GT 5	3.64 ±0.01	0.27 ±0.00	24.0 ±0.06	ND	0.16 ±0.33	25.6 ±0.07	2.05 ±0.01	0.89 ±0.04	4.58 ±0.03	ND
GT 6	1.13 ±0.00	0.32 ±0.00	31.2 ±0.06	ND	ND	20.5 ±0.24	1.46 ±0.04	0.42 ±0.03	3.94 ±0.05	ND
GT 7	0.35 ±0.01	ND	14.8 ±0.02	ND	ND	1.74 ±0.09	ND	ND	0.06 ±0.13	ND
GT 8	0.24 ±0.01	0.63 ±0.00	5.74 ±0.01	ND	ND	3.70 ±0.02	2.24 ±0.01	ND	0.44 ±0.02	ND
GT 9	0.31 ±0.01	0.26 ±0.00	5.49 ±0.02	ND	ND	1.36 ±0.01	0.68 ±0.01	ND	ND	ND
GT 10	0.53 ±0.00	ND	10.4 ±0.02	1.46 ±0.01	0.70 ±0.01	0.76 ±0.01	0.98 ±0.83	0.96 ±0.02	ND	ND
GT 11	0.54 ±0.01	1.11 ±0.00	20.2 ±0.09	ND	0.54 ±0.01	26.3 ±0.35	3.36 ±0.01	1.17 ±0.01	3.31 ±0.01	ND
GT 12	0.68 ±0.02	0.64 ±0.00	14.1 ±0.02	ND	0.15 ±0.31	24.0 ±0.02	2.28 ±0.01	1.77 ±0.01	3.66 ±0.01	ND
GT 13	0.91 ±0.02	0.61 ±0.00	8.96 ±0.01	ND	ND	5.67 ±0.01	1.85 ±0.00	0.85 ±0.01	0.76 ±0.01	ND
BT 1	0.49 ±0.01	ND	10.6 ±0.03	ND	ND	ND	ND	0.37 ±0.03	ND	ND
BT 2	1.24 ±0.01	ND	19.9 ±0.03	ND	ND	ND	ND	0.88 ±0.00	ND	ND
BT 3	1.40 ±0.02	1.01 ±0.00	15.1 ±0.01	1.65 ±0.02	0.81 ±0.01	23.2 ±0.02	3.80 ±0.01	0.88 ±0.01	5.22 ±0.04	ND
BT 4	2.04 ±0.01	ND	14.4 ±0.07	ND	0.58 ±0.01	7.89 ±0.09	1.82 ±0.01	1.71 ±0.01	5.10 ±0.04	ND
PT 1	0.52 ±0.00	0.51 ±0.00	10.7 ±0.01	ND	ND	0.89 ±0.00	1.87 ±0.01	0.40 ±0.01	ND	ND
PT 2	2.04 ±0.01	ND	24.6 ±0.06	ND	ND	ND	0.68 ±0.01	ND	ND	ND
PT 3	2.81 ±0.00	0.30 ±0.11	21.2 ±0.02	ND	1.01 ±0.12	8.03 ±0.04	3.32 ±0.01	0.76 ±0.18	6.47 ±0.18	ND
PT 4	2.52 ±0.01	0.18 ±0.00	18.6 ±0.34	ND	0.48 ±0.00	ND	2.70 ±0.02	ND	ND	ND
PT 5	1.26 ±0.01	0.43 ±0.00	14.6 ±0.09	ND	1.52 ±0.01	7.86 ±0.14	7.04 ±0.02	0.85 ±0.00	6.37 ±0.27	ND
PT 6	3.60 ±0.01	0.57 ±0.00	23.4 ±0.06	ND	1.97 ±0.08	15.9 ±0.06	7.30 ±0.01	1.42 ±0.01	11.9 ±0.01	ND
PT 7	2.52 ±0.00	ND	19.8 ±0.01	ND	ND	ND	0.89 ±0.02	ND	ND	0.45 ±0.00
PT 8	1.70 ±0.03	ND	11.6 ±0.05	ND	ND	ND	ND	ND	ND	ND
PT 9	1.07 ±0.01	ND	8.85 ±0.04	ND	ND	ND	ND	ND	ND	0.26 ±0.02
PT 10	1.80 ±0.00	ND	10.5 ±0.01	ND	ND	ND	ND	ND	ND	ND
YD 1	ND	ND	3.15 ±0.01	ND	ND	ND	ND	ND	ND	ND
K 1	ND	ND	0.71 ±0.00	ND	ND	ND	0.64 ±0.01	ND	ND	ND
J 1	ND	ND	ND	15.8 ±0.13	ND	ND	ND	ND	ND	ND

Note: The results are presented as mean values of four separate measurements of each sample.

biologically active substances with antioxidant effects in different types of teas point to the fact that tea drinks are a major source of polyphenol compounds **(Bae et al., 2015; Wang and Helliwell, 2001)**. The results of polyphenol content are shown in Table 3. The highest concentration of polyphenols was recorded in the green tea sample GT 3 (75.3 ±2.42 mg GAE.g^{-1}). In general, it can be stated that green tea and pu-erh tea had the highest polyphenol content. Our findings are confirmed by results of **Oh et al. (2013)**, who studied antioxidant parameters in leaves of medicinal plants, and/or by **Almajano et al. (2008)**, who observed antimicrobial and antioxidant parameters in 13 samples of tea drinks.

Total flavonoids content

The total content of flavonoids (TFC) varied widely (0.65 – 22.3 mg QE.g^{-1} DW) in all the samples. The highest TFC value was recorded in the sample PT 5, and/or in all Pu-erh teas that had several times higher values compared with the other samples. This fact is caused by changes in the structure of the phenolic compounds during the the processing **(Yi et al., 2015)**.

Total phenolic acids content

Phenolic acids comprise of a large group of substances that are primarily characterized as secondary metabolites of plants. The largest sources of these substances are tea, coffee but also a variety of berries. Their main positive effect on the consumer's health results from many aspects, such as redox processes in metabolism, antimicrobial effects, preventive effect against cancer, etc. **(Halliwell et al., 2012; Hollmann et al., 2011)**.

Our results indicate that the highest content of the phenolic acids was recorded in the samples of Pu-erh tea, particularly in the PT 5 sample (similarly to the content of flavonoids) (42.8 mg ±1.40 CAE.g^{-1}). It is due to the characteristic processing technology and/or transformation processes during the processing.

ABTS radical cation decolorization assay

Determination by ABTS radical is based on the change of the solution colour after the addition of sample extracts. The advantage of this method is sensitive reaction tothe lipophilic and hydrophilic substances with antioxidant properties, therefore its use is broad-range and particularly universal **(Re et al., 1999)**. The content of the antioxidant substances ranged widely in the studied samples (Table 3). The highest average concentration was recorded in green and black teas. The highest value was recorded in the GT 3 sample (33.6 ±0.98 mg TEAC.g^{-1}). Again, it is possible to state that green teas have a high content of substances with antioxidant activity. It is due to, mainly in green teas, the

Table 3 Antioxidant parameters of water extracts of all sample types (mean ±St.Dev).

Sample	ABTS	PM	TPC	TFC	TPA
	mg TEAC.g^{-1}	mg TEAC.g^{-1}	mg GAE.g^{-1}	mg QE.g^{-1}	mg CAE.g^{-1}
GT 1	17.4 ±1.94	288 ±1.92	48.5 ±3.39	1.92 ±0.50	9.67 ±0.16
GT 2	15.6 ±2.13	223 ±1.44	35.9 ±1.18	0.97 ±0.19	7.52 ±0.12
GT 3	33.6 ±0.98	515 ±8.93	75.3 ±2.24	3.77 ±0.29	17.9 ±0.20
GT 4	9.45 ±0.32	173 ±0.73	24.9 ±2.19	2.24 ±0.40	8.26 ±0.23
GT 5	9.34 ±0.61	161 ±6.40	27.6 ±1.03	0.29 ±0.03	5.93 ±0.28
GT 6	6.90 ±0.94	86.1 ±6.00	15.2 ±0.93	0.65 ±0.11	3.03 ±0.14
GT 7	3.61 ±0.73	53.9 ±2.20	13.9 ±1.36	0.78 ±0.19	2.14 ±1.11
GT 8	4.90 ±0.14	56.2 ±2.90	13.2 ±0.77	1.10 ±0.11	1.56 ±0.12
GT 9	5.23 ±0.60	44.8 ±0.96	17.8 ±0.51	0.72 ±0.11	1.46 ±0.08
GT10	5.37 ±0.41	80.0 ±2.65	11.0 ±0.44	0.97 ±0.19	4.05 ±0.08
GT11	26.2 ±0.49	481 ±1.21	29.9 ±0.68	2.24 ±0.29	14.6 ±0.16
GT12	19.1 ±0.53	260 ±10.7	22.6 ±0.89	1.16 ±0.19	11.0 ±0.24
GT13	15.8 ±2.15	239 ±2.94	18.0 ±1.12	1.35 ±0.19	9.04 ±0.16
BT 1	3.63 ±0.20	63.2 ±1.54	13.8 ±0.68	1.86 ±0.29	1.69 ±0.08
BT 2	8.34 ±0.14	76.9 ±2.09	16.7 ±2.22	5.35 ±1.16	5.17 ±0.68
BT 3	18.6 ±1.08	293 ±13.4	24.2 ±1.03	1.03 ±1.11	11.3 ±0.05
BT 4	23.2 ±0.38	381 ±11.7	32.1 ±1.80	5.29 ±0.88	17.9 ±0.21
PT 1	9.36 ±0.49	123 ±4.88	24.7 ±0.93	8.15 ±0.40	8.70 ±0.23
PT 2	7.58 ±0.14	148 ±1.69	29.4 ±3.45	11.7 ±0.40	20.2 ±1.43
PT 3	15.6 ±0.40	234 ±6.56	43.3 ±2.96	14.2 ±0.61	20.7 ±0.42
PT 4	11.9 ±1.56	161 ±7.72	34.9 ±1.56	13.2 ±0.67	19.3 ±0.09
PT 5	26.8 ±1.01	307 ±5.00	63.8 ±0.68	22.3 ±0.38	42.8 ±1.40
PT 6	14.7 ±1.36	199 ±0.48	32.5 ±2.68	13.3 ±2.02	14.5 ±0.73
PT 7	9.72 ±0.17	145 ±2.37	30.3 ±0.68	15.6 ±1.44	19.7 ±0.86
PT 8	5.14 ±0.86	56.4 ±2.94	15.2 ±0.26	7.00 ±1.34	5.38 ±0.31
PT 9	4.78 ±0.72	73.5 ±1.69	17.4 ±1.56	8.46 ±0.22	10.1 ±0.20
PT10	11.5 ±0.18	150 ±1.92	13.7 ±0.89	1.16 ±0.19	14.9 ±0.36
YD 1	7.01 ±1.30	314 ±8.00	12.5 ±0.68	1.29 ±0.29	7.68 ±0.20
K 1	6.92 ±1.24	182 ±1.00	15.6 ±1.36	2.94 ±0.29	24.1 ±0.88
J 1	4.02 ±0.38	114 ±2.54	11.0 ±0.44	4.59 ±0.33	4.47 ±0.59

Note: The results are presented as mean values of three separate measurements of each sample.

absence of fermentation, as well as thermal processes in the tea processing **(Yi et al., 2015)**.

Phosphomolybdenum reducing antioxidant power assay

The principle of the method is based on a reduction of $Mo^{VI+} \rightarrow Mo^{V+}$ and the increase in the content of pentavalent molybdenum is detected and quantified by spectrophotometry. The values of the antioxidant power of the samples varied widely (similarly to the ABTS method). The highest concentration of the antioxidant substances was recorded in the green tea sample (GT 3) (515 ±8.93 mg TEAC.g^{-1}). In contrast to the ABTS method, categorization of the samples by this parameter cannot be explicitly determined. Compared with the results of **Godočíková et al. (2016)**, who studied the antioxidant parameters of the two types of chocolates with different processing technology, it can be concluded that teas are richer in the antioxidantly active substances. The data obtained are shown in Table 3.

CONCLUSION

Tea contains a wide range of biologically active substances of distinct characteristics and chemical nature. Regular and long-term tea consumption thus have a significantly positive impact on the consumers' health. The study focused on monitoring ten characteristic substances belonging to the groups of methylxanthines (alkaloids) and flavan-3-ols (catechins). Especially the second group is typical for tea and contains important health-promoting attributes. Tea contains a wide range of substances providing antioxidant properties, especially green tea, which was confirmed at our study by two spectrophotometric methods.

Based on the results obtained, it can be concluded that the studied parameters are significantly dependent on the type of tea (and/or processing technology). Chemical composition, as well as biologically active substances have a positive effect on the antioxidant properties of tea and therefore provide certain health benefits.

REFERENCES

Almajano, M. P., Carbó, R., Lopéz-Jiménez, J. A., Gordon, M. H. 2008. Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, vol. 108, no. 1, p. 55-63. https://doi.org/10.1016/j.foodchem.2007.10.040

Árvay, J., Hauptvogl, M., Tomáš, J., Harangozo, Ľ. 2015. Determination of mercury, cadmium and lead contents in different tea and teas infusions (*Camellia sinensis* L.). *Potravinarstvo*, vol. 9, no. 1, p. 398-402. https://doi.org/10.5219/510

Bae, I. K., Ham, H. M., Jeong, M. H., Kim, D. H., Kim, H. J. 2015. Simultaneous determination of 15 phenolic compounds, and caffeine in teas and mate using RP-HPLC/UV detection: Method development and optimalization of extraction process. *Food Chemistry*, vol. 172, p. 469-475. https://doi.org/10.1016/j.foodchem.2014.09.050 PMid:25442580

Balentine, D. A., Wiseman, S. A., Bouwens, L. C. 1997. The chemistry of tea flavonoids. *Critical Reviews in Food Science and Nutrition*, vol. 37, no. 8, p. 693-704. https://doi.org/10.1080/10408399709527797 PMid:9447270

da Silva Pinto, M. 2013. Tea: a new perspective on health benefits. *Food Research International*, vol. 53, no. 2, p. 558-567. https://doi.org/10.1016/j.foodres.2013.01.038

FAO. 2015. World tea production and trade Current and future development. Food and Agriculture Organisation. Report. [online] s.a. [cit. 2017-01-17] Available at: http://www.fao.org/3/a-i4480e.pdf.

Farmakopea Polska, V. 1999. 5th ed. Warszawa : PTFarm., p. 880-881. ISBN: 83-88157-04-3.

Godočíková, L., Ivanišová, E., Árvay, J., Petrová, J., Kačániová, M. 2016. The comparison of biological activity of chocolates made by different technological procedures. *Potravinarstvo*, vol. 10, no. 1, p. 316-322. https://doi.org/10.5219/628

Halliwell, B. 2012. Free radical and antioxidants: Updating a personal view. *Nutrition Reviews*, vol. 70, no. 5, p. 257-265. https://doi.org/10.1111/j.1753-4887.2012.00476.x PMid:22537212

Hollman, P. C. H., Cassidy, A., Comte, B., Heinonen, M., Richelle, E. 2011. The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. *Journal of Nutrition*, vol. 141, no. 5, p. 989-1009. https://doi.org/10.3945/jn.110.131490 PMid:21451125

Chung, F. L., Schwartz, J., Herzog, C. R., Yang, Y. M. 2003. Tea and cancer prevention: Studies in animals and humans. *Journal of Nutriion*, vol. 133, no. 10, p. 3268-3274.

Jeszka-Skowron, M., Krawczyk, M., Zgola-Grześkowiak, A. 2015. Determination of antioxidant aktivity, rutin, quercetin, phenolic acids and trace elements in tea infusions: Influence of citric acid addition on extraction of metals. *Journal of Food Composition and Analysis*, vol. 40, p. 70-77. https://doi.org/10.1016/j.jfca.2014.12.015

Kuriyama, S., Shimazu, T., Ohmori, K., Kikuchi, N., Nakaya, N., Nishino, Y., Tsubono, Y., Tsuji, I. 2015. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *The Journal of thr Americam Medical Association*, vol. 296, no. 10, p. 1255-1265.

Marcos, A., Fisher, A., Rea, G., Hill, S. J., 1998. Preliminary study using trace element concentrations and a chemometrics approach to determine geographical origin of tea. *Journal of Analytical Atomic Spectrometry*, vol. 13, p. 521-525. https://doi.org/10.1039/a708658j

Murakami, A. N. N., Amboni, R. D. M. C., Prudencio, E. S., Amante, E. R., Zanotta, L. M., Maraschin, M. 2013. Concentration of phenolic compounds in aqueous mate (Ilex paraguariensis A. St. Hil) extracts through nanofiltration. *Food Chemistry*, vol. 44, no. 10, p. 60-65. https://doi.org/10.1016/j.foodchem.2013.02.119 PMid:23768327

Nechuta, S., Shu, X. O., Li, H. L., Yang, G., Ji, B. T., Xiang, Y. B., Cai, H., Chow, W. H., Gao, Y. T., Zheng, W. 2012. Prospective cohort study of tea consumption and risk of digestive system cancers: results from the Shanghai Women's Health Study. *The American Journal of Clinical Nutrition*, vol. 96, no. 5, p. 1056-1063. https://doi.org/10.3945/ajcn.111.031419 PMid:23053557

Nováková, L., Spáčil, Z., Sifrtová, M., Opletal, L., Solich, P. 2010. Rapid qualitative and quantitative ultra high performance liquid chromatography method for simultaneous analysis of twenty nine common phenolic compounds of various structures. *Talanta*, vol. 80, no. 5, p. 1970-1979. https://doi.org/10.1016/j.talanta.2009.10.056 PMid:20152441

Oh, J., Jo, H., Cho, A. R., Kim, S. J., Han, J. 2013. Antioxidant and antimicrobial activities of various leafy herbal teas. *Food Control*, vol. 31, no. 2, p. 403-409. https://doi.org/10.1016/j.foodcont.2012.10.021

Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, vol. 269, no. 2, p. 337-341. https://doi.org/10.1006/abio.1999.4019 PMid:10222007

Rahim, A. A., Nofrizal, S., Saad, B. 2014. Rapid tea catechins and caffeine determination by HPLC using microwave-assisted extraction and silica monolithic column. *Food Chemistry*, vol. 147, p. 262-268. https://doi.org/10.1016/j.foodchem.2013.09.131 PMid:24206716

Re, R. N., Pellegrini, A., Pannala, M., Yang, R., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, vol. 26, no. 9-10, p. 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Scholz, S., Williamson, G. 2007. Interaction affecting the bioavailability of dietary polyphenols in vivo. *International Journal of Vitamin and Nutrition Research*, vol. 77, no. 3, p. 224-235. https://doi.org/10.1024/0300-9831.77.3.224 PMid:18214024

Sharangi, A. B. 2009. Medicinal and therapeutic potentialities of tea (*Camellia sinensis*, L.) – a review. *Food Research International*, vol. 42, no. 5-6, p. 529-535. https://doi.org/10.1016/j.foodres.2009.01.007

Singleton, V. L., Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Agricultural*, vol. 16, p. 144-158.

Tsai, Y. S., Wang, H. F., Ou, A. S. M. 2008. Biological Functions and Manufacturing of GABA Tea. In Ho et al. *Tea and tea products: Chemistry and health-promoting properties*. United States : CRC Press. 320 p. ISBN-10: 0849380820. https://doi.org/10.1201/9781420008036.ch4

Wang, G., Helliwell, K. 2001. Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography. *Food Research International*, vol. 34, no. 2-3, p. 223-227. https://doi.org/10.1016/S0963-9969(00)00156-3

Wang, H., Chen, L. G., Xu, Y., Zeng, Q. L., Zhang, X. P., Zhao, Q. 2011. Dynamic microwave-assisted extraction coupled on-line with clean-up for determination of caffeine in tea. *LWT – Food Science and Technology*, vol. 44, no. 6, p. 1490-1495. https://doi.org/10.1016/j.lwt.2011.01.015

Willett, W. C. 2002. Balancing life-style and genomics research for disease prevention. *Science*, vol. 292, no. 5568, p. 695-698. https://doi.org/10.1126/science.1071055 PMid:11976443

Yao, L. H., Jiang, Y. M., Shi, J., Tomás-Barberán, F. A., Datta, N., Singanusong, R. 2004. Flavonoids in food and their health benefits. *Plant Food and Human Nutrition*, vol. 59, no. 3, p. 113-122. https://doi.org/10.1007/s11130-004-0049-7 PMid:15678717

Yi, T., Zhu, L., Peng, W. L., He, X. C., Chen, H. L., Li, J., Yu, T., Liang, Z. T, Thao, Z. Z., Chen, H. B. 2015. Comparison of ten major constituents in seven types of processed tea using HPLC-DAD-MS followed by principal component and hierarchical cluster analysis. *LWT – Food Science and Technology*, vol. 62, no. 1, p. 194-201. https://doi.org/10.1016/j.lwt.2015.01.003

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