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DOES THE ADDITION OF SPICES CHANGE THE CONTENT OF FLUORIDE AND ANTIOXIDANTS IN BLACK TEA INFUSIONS?*

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ABSTRACT

Black tea and spices are a valuable source of natural bioactive ingredients. One of the factors affecting the antioxidant status and the content of bioactive compounds in tea infusions is their enrichment with various additives and consumption in the form of blends and chemical composition. Additives to teas are often herbs, flowers and spices. The aim of the study was to determine the content of polyphenols, fluoride and antioxidant potential in infusions and to examine the correlation between these parameters. In addition, the effect of added spices on the above parameters was verified. The research material consisted of infusions of black tea with the addition of spices prepared at 80°C. The content of polyphenols and antioxidant activity were determined by the spectrophotometric method. Fluoride concentrations were measured by the potentiometric method. The highest content of polyphenols occurred in tea with cloves (345.49 ppm), while the lowest was in the infusion with cardamom (343.59 ppm). The strongest antioxidant properties distinguished the infusion with cloves (79.11% of DPPH inhibition), and the weakest ones were determined in the infusion with turmeric (72.7% of DPPH inhibition). The highest content of fluoride was in the tea with turmeric (0.809 mg/L), while the lowest one appeared in the infusion with cloves (0.358 mg L⁻¹). The study indicates that the spices and fluoride content affect the antioxidant properties of infusions and their content of polyphenols. High fluoride levels decrease the antioxidant capacity of tea infusions.

Keywords: tea drink, additives, antioxidant activity, fluorine.

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INTRODUCTION

Research conducted in recent years implicates relationships between the consumption of foods rich in antioxidants and the incidence of many diseases, including the so-called civilization diseases. A proper diet containing raw materials and products with high antioxidant potential has a positive effect on the blood lipid profile, lower blood pressure and the overall cardiac and anti-cancer prevention (MILLER et al. 2008, KŁYSZ, CZŁONKOWSKA 2013). In this regard, special attention is paid to exploration and investigations into natural resources of antioxidants, especially of plant origin. Teas are noteworthy among the many raw antioxidant materials because of their content of polyphenols. Tea (*Camelia sinensis* L.) is one of the world's most popular beverages (MIAZGA-SŁAWIŃSKA, GRZEGORCZYK 2014). It is estimated that an average Polish person drinks about 50 cups of tea infusions monthly (RUSINEK-PRYSTUPA, SAMOLIŃSKA 2013). This is dictated mainly by socio-cultural factors and, to a lesser extent, the awareness of tea being healthful. Tea leaves contain antioxidant compounds, carbohydrates, proteins, lignins, minerals, pectins, vitamins, amino acids, fatty acids, organic acids, pigments and volatiles (CABRERA et al. 2006, MUSHTAG 2014). Tea leaves are abundant in polyphenol compounds (SHARMA et al. 2007), which play a role in the inhibition of the generation of free radicals, chelation of transition metal ions and free radical scavenging. They are important in reducing oxidative stress (CZAJKA 2006, ŁUSZCZEWSKI et al. 2007), which is a risk factor of cardiovascular diseases, Alzheimer's disease, Parkinson's disease, senile dementia or stroke (ŁUSZCZEWSKI et al. 2007, MILLER et al. 2008). It has been shown that infusions of various types of tea, mainly owing to the presence of polyphenols, have antioxidant, anti-tumor and anti-bacterial activity and they support the immune system (CABRERA et al. 2006, KŁYSZ, CZŁONKOWSKA 2013, MICHALAK et al. 2014, MUSHTAG 2014).

Antioxidant potential is not a constant value; it depends on the origin of plant material (geographic region, type of soil, fertilization, etc.), and on how it is stored and processed (CABRERA et al. 2005). Additionally the type and amount of additives that are used for the recently popular tea blends may be of great importance. In a ready mix of tea beverages you can find herbs, flowers and spices, which improve their organoleptic qualities and, more importantly, health benefits. Tea beverages are also a dietary source of minerals (CABRERA et al. 2005, RUSINEK-PRYSTUPA, SAMOLIŃSKA 2013), including fluoride (WARREN et al. 1996, TOKALIOĞLU et al. 2004, ŁUKOMSKA et al. 2015), which increases the induction of free radicals and can affect to the antioxidant potential of tea beverages.

The antioxidant mechanism of polyphenols arises from their ability to scavenge reactive oxygen species and to chelate metal ions. They may also function indirectly as an antioxidant for inhibition of transcription factors of redox reactions, nuclear factor-kB and inhibition of "pro-oxidant" enzymes,

such as cyclooxygenase, lipoxygenase and xanthine oxidase. Additionally they affect the activity of the antioxidant enzymes, such as glutathione S-transferase and superoxide dismutase. The major group of antioxidants in tea are flavonoids and catechins, which appear to be digested, absorbed and metabolised by the body, which further increases their digestibility and health promoting properties (AUCAMP et al. 1997, CHOW, HAKIM 2011, MAIZURA et al. 2011).

There are no studies investigating the relationship between the antioxidant content and fluorine content in tea infusions, hence the aim of the study was to determine the content of polyphenols, content of fluoride and antioxidant potential in infusions with and without added spices and to examine the correlation between these parameters.

MATERIAL AND METHODS

Preparation of tea infusions

Black tea (2.0 g) from a supermarket in Szczecin, Poland (origin: Sri Lanka, kind: leaves) and dry shredded spices (0.5 g): cloves, cinnamon, turmeric, ginger, cardamom, marketed by a leading national producer, were weighed to conical flasks and poured over with 200.0 mL of distilled water at 80°C (2.0 g of black tea and 200.0 mL of distilled water were used in the case of “tea without spice”). Next, each flask was shaken (10 min, 180 rpm) at room temperature and its contents filtered. All infusions were prepared in triplicate. The antioxidant activity and polyphenol content were determined in infusions using a UV-Vis spectrophotometer Agilent 8453, while fluoride concentrations were measured by the potentiometric method.

Most producers of tea with spices do not give the percentage of spices in the composition. However, some labels provided the information that the proportion of different spices ranged between 1% and 20%. For the purpose of this work, we decided to add the greatest amount. In addition, it was organoleptically confirmed that the amount of spices used in infusions did not affect adversely their taste and smell.

Determination of antioxidant activity by DPPH method

96% ethanol, 1.0 mL of 0.3 mM ethanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) and 0.1 mL of the test sample were introduced into the vial in the v/v ratio 29:10:1 and placed for 30 min in a dark place. During that time, the so-called A_0 solution was prepared by mixing 96% ethanol and 0.3 mM solution of DPPH in the v/v ratio 3:1. As a reference solution, 96% ethanol was used. The vial contents were mixed gently and the spectral absorbance was immediately measured at 518 nm. All assays were performed in triplicate.

Antioxidant activity was expressed by the percentage of DPPH inhibition, using the formula:

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \cdot 100,$$

where:

A_0 – absorbance of DPPH solution without the tested sample,

A_s – absorbance of DPPH solution with the tested sample.

Determination of the content of polyphenols

Determination of polyphenols was performed according ISO 14502-1 using Folin - Ciocalteu reagent. 5.0 mL of a 10% Folin - Ciocalteu solution and 1.0 mL of the test sample were successively introduced into a vial. The sample was shaken vigorously and after 5 min 4.0 mL of 7.5% Na_2CO_3 solution was added. The prepared solution was incubated for 60 min. at room temperature. The reference solution was prepared in the same way but distilled water was added instead of the tested sample. Absorbance at 765 nm was measured. All assays were performed in triplicate. The content of polyphenols was determined from the calibration curve using gallic acid as the reference standard. The results are shown in ppm gallic acid, i.e. mg of gallic acid in 100 mL of infusion ($\text{mg GAE } 100 \text{ mL}^{-1}$).

Determination of fluoride content

Fluoride concentrations in individual samples were measured by the potentiometric method with a fluoride ion-selective electrode (Orion 9409 BN, Thermo Scientific, USA). 1.0 mL of sample and 1.0 mL of TISAB II were transferred to a plastic tube. The potential of each sample was measured before and after the addition of an appropriate fluoride standard with known concentrations: 0.1, 1.0, 10.0 mgL^{-1} (Orion Company, USA) and the fluoride content in samples was calculated based on the difference of potentials (ŁUKOMSKA et al. 2015).

Statistical analysis

Statistical analysis was performed using Statsoft Statistica 12.5. In all the experiments, three samples were analyzed and all the assays were carried out at least in triplicate. The results are expressed as mean values and standard deviation (SD). For fluoride content, the non-parametric *U* Mann-Whitney test was used. The percentage of DPPH inhibition and the content of polyphenols were submitted to one-way analysis of variance (ANOVA), the Tukey *post-hoc* test. Correlation analysis was performed with the Spearman coefficient. Differences were considered significant at $p \leq 0.05$.

RESULT AND DISCUSSION

The results reported in this article have shown how spices (cloves, ginger, cinnamon, turmeric, cardamom) affect the overall content of polyphenols and the antioxidant properties of black tea infusions. The results of the determination of the polyphenolic content, fluoride content and antioxidant activity (% of DPPH inhibition) from the tea infusions are presented in Table 1, and the results showing statistical significance are contained in Table 2.

Table 1

Antioxidant activity, polyphenol content and fluoride content in tea infusions

Black tea infusions	Polyphenol content (ppm of gallic acid)	DPPH inhibition (%)	Fluoride content (mg L ⁻¹)
Without spices	345.2 ± 0.037	78.12 ± 0.557	0.488 ± 0.100
With ginger	345.3 ± 0.163	74.95 ± 0.021	0.545 ± 0.041
With cinnamon	345.3 ± 0.107	78.81 ± 0.041	0.400 ± 0.052
With turmeric	344.8 ± 0.122	72.70 ± 0.701	0.809 ± 0.683
With cardamon	343.5 ± 1.765	77.76 ± 0.401	0.376 ± 0.027
With cloves	345.4 ± 0.066	79.11 ± 0.508	0.358 ± 0.021

* means ± standard deviation (SD)

Table 2

Antioxidant activity, polyphenol content and fluoride content in tea infusions (significance of differences)

Tea infusion	Polyphenols content	Antioxidant activity	Fluoride content
Tea <i>vs</i> tea with ginger	ns	*	ns
Tea <i>vs</i> tea with cinnamon	ns	ns	ns
Tea <i>vs</i> tea with turmeric	ns	*	ns
Tea <i>vs</i> tea with cardamon	*	ns	*
Tea <i>vs</i> tea with cloves	ns	*	*
Tea with ginger <i>vs</i> tea with cinnamon	ns	*	*
Tea with ginger <i>vs</i> tea with turmeric	ns	*	ns
Tea with ginger <i>vs</i> tea with cardamon	*	*	*
Tea with ginger <i>vs</i> tea with cloves	ns	*	*
Tea with cinnamon <i>vs</i> tea with turmeric	ns	*	ns
Tea with cinnamon <i>vs</i> tea with cardamon	*	*	ns
Tea with cinnamon <i>vs</i> tea with cloves	ns	ns	ns
Tea with turmeric <i>vs</i> tea with cardamon	ns	*	ns
Tea with turmeric <i>vs</i> tea with cloves	ns	*	ns
Tea with cardamon <i>vs</i> tea with cloves	*	*	ns

* indicates significant differences at $p \leq 0.05$, ns – not significant

Correlation coefficient (r) between antioxidant activity and polyphenol content and fluoride content

Correlation coefficient (r) between:			
Black tea infusion	antioxidant activity and polyphenol content	antioxidant activity and fluoride content	polyphenol content and fluoride content
Without spices	-0.925*	-0.119	0.193
With ginger	0.000	-0.239	0.250
With turmeric	-0.600	0.358	-0.478
With cinnamon	0.676	-0.358	0.369
With cloves	0.617	0.000	-0.670
With cardamon	-0.257	0.358	0.478

* denotes significance at $p \leq 0.05$

Correlation coefficients (r) between antioxidant activity and polyphenolic content and fluoride content are presented in Table 3.

Black tea is a popular beverage, consumed by 2/3 of the world's population (WOLSKA et al. 2016). Health benefits of this brew are determined by the chemical composition. The main group of biologically active compounds are polyphenols, especially flavonoids and phenolic acids, which are thought to contribute to the health benefits of tea (CHOW, HAKIM 2011). Several *in vitro* and *in vivo* studies have shown that polyphenols present in tea have antioxidant properties (FREI, HIGDON 2003, VINSON et al. 2004, GRAMZA, KORCZAK 2005, GRAMZA et al. 2005, AKRAM et al. 2012) stronger than vitamin C, tocopherol or carotene (BHARAT et al. 2003). Tea beverages are also a rich source of fluoride in our diet (WARREN et al. 1996, TOKALIOĞLU et al. 2004, ŁUKOMSKA et al. 2015). Spices have been widely used to modify the flavours of foods and beverages. Depending on a spice added to tea, properties of ready-to-consume infusion may change.

In the present study, it has been shown that the content of polyphenols in the tea infusions with various additives was similar and ranged from 343.59 to 345.49 ppm (Table 1). The highest content of polyphenols (345.49 ppm) has been demonstrated in the infusion with the addition of cloves, and the lowest one appeared when cardamom had been added (343.59 ppm). Regarding the determination of polyphenols in the infusion with the addition of cardamom, statistically significant differences were observed between this tea and each other tested tea with the exception of tea spiced with turmeric (Table 2). The content of polyphenols in natural raw plant materials varies depending on a plant species and method of preparation. In a study examining the antioxidant potential of various herbal teas, water extract of black tea contained the greater amount of polyphenols (1.430 mg catechin equivalents L^{-1} tea) of all tested raw materials (SHARANGI 2009).

The highest potential of tea with cloves may be associated with the high antioxidant potential of this spice alone, which according to available literature is significantly higher than the potential of tea leaves. In a study which examined 100 richest dietary sources of polyphenols, cloves had also been classified as the richest source of polyphenols (15 000 mg 100 g⁻¹), followed by dried ginger (202 mg), black tea non-alcoholic beverages (90 mg) and cinnamon (48 mg) (PEREZ-JIMENEZ et al. 2010). Total polyphenol content (TPC) evaluated in extracts of ginger was in the order of 132.0 mg gallic acid 100 g⁻¹, and 67.9 mg gallic acid 100 g⁻¹ in turmeric extract (BÜYÜKBALCI, NEHIR 2008).

Consumption of tea can contribute significantly to the total fluoride intake in a diet because it is very rich in fluoride; tea plants absorb this mineral from the soil and accumulate it in leaves (NOWAK, KLIMOWICZ 2013). Young leaves have the smallest content of fluorides and are usually used to make yellow, white and green tea (YUWONO 2005). Fluorides in tea could be beneficial for the prevention of dental caries; on the other hand, excessive intake of this element may lead to enamel fluorosis and increase oxidative stress (NOWAK, KLIMOWICZ 2013). In the present study, the highest content of fluoride was in the tea with turmeric (0.809 mg L⁻¹), while the lowest one was in the infusion with cloves (0.358 mg L⁻¹) – Table 1. Significant differences between the tested groups were observed in black tea compared to cardamom, cloves and cinnamon tea and ginger tea infusion compared to tea with cardamom and tea with cloves (Table 2). Similar studies have reported fluoride levels of 0.95 to 4.73 mg L⁻¹ in black teas infusion (NOWAK, KLIMOWICZ 2013).

The infusions with cinnamon, cardamom, cloves contained less fluoride than the tea without spices (Table 1). It can be assumed that during the preparation of infusion fluorine is incorporated in an insoluble form and is therefore retained on a filter during filtration.

The composition of plant materials, especially the fluoride content, is one of the factors determining the quality and characteristics of black tea (GUNEY et al. 2007). In the case of infusions, another factor determining the health properties is a method of preparation, and especially the brewing time and the mixing of tea leaves with various additives in the form of fruits, flowers or spices. Tea beverages are the main source of fluoride in our diet (WARREN et al. 1996, TOKALIOĞLU et al. 2004, ŁUKOMSKA et al. 2015) and spices added to tea affect the concentration of this element in the infusion. Fluoride is an element that increases the induction of free radicals and can affect to the antioxidant potential of tea beverages. A rich source of bioactive compound can reduce the negative effect of fluoride. Many *in vivo* studies have confirmed protective effects of nonenzymatic antioxidant compounds, such as vitamins and nonvitamin oxidants, against the consequences of exposure to fluoride (HE et al. 2009, INKIELEWICZ-STEPNIAK, CZARNOWSKI 2010, STAWIARSKA-PIĘTA et al. 2012, STAWIARSKA-PIĘTA et al. 2015).

Our research has shown that the highest antioxidant activity appeared in infusions with the addition of cloves and cinnamon (79.11% and 78.81% of

DPPH inhibition, respectively), while the lowest one was in tea with turmeric (72.70% of DPPH inhibition) – Table 1. The leantioxidant potential of tea infusion without additives reached 78.12% (Table 1). Significant differences between the tested groups were observed in all groups except of black tea and tea with cinnamon or tea with cardamom and tea with cinnamon compared to tea with cloves (Table 2). In the light of the available literature it can be concluded that the antioxidant activity of tea is high but it depends on many factors (VINSON et al. 2004, ZERABRUK et al. 2010, AKRAM et al. 2012). In our study, the percentage of DPPH inhibition of tea infusion without additives was evaluated at around 78%, which is consistent with the results of other authors. In the study of ŚMIECHOWSKA et al. (2013), forty-five black teas of different origins and brewing times were investigated. The noted percentage of inhibition varied from 14 to 85% and was achieved by longer brewing times, which probably make more water-soluble flavanols enter the solution, resulting in a higher antioxidant activity of infusions. It is important to choose such raw materials as additives to tea that increase its antioxidant potential.

Natural antioxidants present in herbs and spices are responsible for preventing or inhibiting oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. The presence of these compounds in raw materials can affect their antioxidant properties significantly. The study carried out on 54 patients who received 100 mg of cinnamon 30 ml⁻¹ of tea daily for 2 weeks showed an increased total antioxidant activity and decreased lipid peroxidation levels in their blood in comparison with the control group (GRAMZA et al. 2005). Tea with cinnamon showed a constant percentage of DPPH inhibition of about 80%, independent of the concentrations used, while tea with ginger showed the DPPH inhibition percentage in a range from 88 to 69%, depending on the concentration used. Out of all spices added to tea that have been tested thus far, cinnamon shows the highest antioxidant effect (RANJBAR et al. 2006, OCHANDA et al. 2015), which is confirmed by our research.

The antioxidant activity and composition of tea depend on various factors, including the climatic conditions of cultivation, harvest time, degree of fermentation or degree of fineness of raw material (OWUORA et al. 2006). Additional elements from the environment accumulate in plants and may affect the inhibition of antioxidant enzymes. Many authors have demonstrated that tea having higher antioxidant activity has a lower content of impurities (heavy metals, pesticide residues) (DMOWSKI, ŚMIECHOWSKA 2010, KUMAR et al. 2011, LADDI et al. 2012).

The statistically significant relationship between the polyphenolic content and antioxidant activity ($r = -0,925$) was found only in the tea infusion without spices (Table 3). Negative correlation between the content of polyphenols and antioxidant activity can be attributed to the content of compounds of non-antioxidant action or binding to the insoluble complexes that are removed by filtration. In these infusions, the presence of catechins, which

belong to the phenolic compounds, in tea leaves is of particular importance. Tannins form insoluble sediment with certain substances, including toxic alkaloids or heavy metal salts. Positive but not statistically significant correlation was observed in the infusions of tea with cloves ($r = 0.61$) and cinnamon ($r = 0.67$) – Table 3. Negative correlation between the content of polyphenols and fluoride content was demonstrated in the infusions with cloves ($r = -0.67$) and turmeric ($r = -0.47$) – Table 3. In pure tea infusions, statistically significant positive correlations were found ($r = 0.578$) between the polyphenol content and antioxidant activity and negative ones ($r = -0.399$) between the antioxidant activity and fluoride content in infusions.

CONCLUSIONS

Antioxidant activity is an important determinant of the health quality of black tea infusions, and additives used to infusions prepared from tea leaves have an impact on the composition of the infusion and its properties. Spices and herbs contain free radical scavengers like polyphenols, but not all of the compounds included in the polyphenols have antioxidant properties and mutual interactions between some of them may reduce the health-promoting effect of the infusion. Therefore, when antioxidant properties of tea infusion should be described by the DPPH inhibition percentage as a factor rather than by the content of polyphenols. Additionally, a high fluoride level decreases the antioxidant capacity of tea infusions.

This study recommends cloves and cinnamon as the best spices, since they exhibited high antioxidant activity and can be a valuable source of antioxidants in daily diet.

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