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THE IMPACT OF ADDITION OF DIFFERENT TEA POWDERS ON THE BIOLOGICAL VALUE OF WHITE CHOCOLATES

Lucia Godočiková[1], Eva Ivanišová[2], Miroslava Kačániová[1,3]*

Address(es): Lucia Godočiková,
[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.
[2]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Technology and Quality of Plant Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.
[3]University of Rzeszów, Faculty of Biology and Agriculture, Department of Bioenergetics and Food Analysis, Aleksandra Zelwerowicza 4 St., 35-601 Rzeszów, Poland.

*Corresponding author: l.godocikova@gmail.com

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ABSTRACT

Chocolate, in addition to green tea and red wine, is considered to be one of the richest sources of bioactive compounds; however, white chocolates are very poor in polyphenolic components. Therefore, this work aimed at the determination and comparison of the addition of different types of tea to increase the content of polyphenols, flavonoids, phenolic acids, and antioxidant activity of white chocolates. In this study, we focused only on white chocolates supplied by Czech chocolate producing company. The content of total polyphenols was evaluated using the Folin-Ciocalteu reagent, the total content of flavonoids was measured using spectrometric assay based on a formation of colored flavonoid-aluminum complex, and the content of total phenolic acids was evaluated using Arnow's reagent. Antioxidant activity was measured by three different assays, which were DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assay and reducing power method. The obtained results showed that plain white chocolate is a poor source of biologically active compounds and also antioxidant activity measured by different methods was found low. However, the addition of various kinds of teas to chocolates enhanced the amount of total polyphenols, flavonoids, and phenolic acids more than two times. This enrichment influenced also the antioxidant activity of the samples positively, which increased several fold. Best results were obtained with the addition of green teas.

Keywords: antioxidant activity, enrichment, polyphenols, tea, white chocolate

INTRODUCTION

Cocoa and chocolate products have been one of the most popular foods for thousands of years. They have been consumed for both energy source and health benefits **(Cerit *et al.*, 2016)**. Main chocolate categories are dark, milk, and white that differs in the content of cocoa solids, milk fat, and cocoa butter. White chocolates vary from milk and dark through the absence of cocoa nibs containing antioxidants, reducing product shelf-life **(Afoakwa *et al.*, 2007)**. During processing, the chocolate composition, in terms of type and amount of each ingredient, plays an essential role in obtaining a high-quality product **(Glicerina *et al.*, 2016)**. The first white chocolate was made in 1930. It was made from sugar, milk powder, and cocoa butter **(Beckett, 2008)**. The popularity of this food appears to mainly associate with its potential to arouse sensory pleasure and positive emotions **(Konar *et al.*, 2016)**.
In the past decade in various parts of the world, there is a growing focus on different foods and beverages that improve or benefit health. The functional foods play an essential role in providing a new type of promising tool with beneficial health effects related to specific components present in the diet **(Rodríguez Furlán *et al.*, 2016)**.
Because chocolate is widely consumed by people of all ages throughout the world, it could be concluded that it is promising bioactive compound carrier **(Konar *et al.*, 2016)**. Preferences of consumers in choosing the foods have changed, especially in the last 20 years. Healthfulness is the primary driver of food purchasing behind taste and price, and the presence of added beneficial components and fortification have at least positive impact on purchasing decisions **(Harwood, 2013)**. Moreover, consumers prefer natural and organic foods or additives, which also needs to be taken into consideration during production. The preference in consumer behaviour and choice has directed scientific researches as well as industrial product development activities **(Konar *et al.*, 2016)**.
It is required to describe the specific functional term for chocolate and confectionery products. Functional confectionery has been defined as 'a confectionery item that has undergone the addition, removal or replacement of standard confectionery ingredients with an ingredient that fulfils a specific physiological function or offers a potential health benefit' **(Pickford and Jardine, 2000)**. A European Union directive simplified previous legislation opening up new possibilities for chocolate makes to try new ingredients, which can be used to create new products beneficial to consumers and industry **(Bolenz *et al.*, 2006)**. This regulation led up the production or improvement of chocolate with functional properties, which can be labelled as standard chocolate. It also speaks of the new freedom chocolate producers have regarding the ingredients of their chocolate. Soluble and insoluble fiber, vitamins and minerals, herbal extracts, and other phytochemicals are the main ingredients, which are used as substitutes or enrichment agents **(Konar *et al.*, 2016)**.
The objective of our study was to assess the positive effect of tea added to the white chocolate and the impact of this addition on the biological value and antioxidant activity of such enriched chocolate. Since white chocolate is one of the most preferred chocolates among children, as well as among a high percentage of adults, and only seen as a sweet treat, it was evaluated for the possible changes after the addition of tea powders. In order to compare the selected parameters, an array of rapid and reliable, widely used spectrophotometric methods were applied.

MATERIAL AND METHODS

Biological material

The chocolate samples evaluated in this study were made and kindly supplied by Czech chocolate-producing company (Hradec Králové, Czech Republic). All samples were made of white chocolate with 40 % cocoa solids, composed of cocoa butter, milk powder cane sugar and enriching powder in this order.

Enrichment ingredients and their addition to plain chocolate are listed in Table 1. The control sample (SC) was plain, without addition on any tea, but with the same amount of cocoa solids.

Table 1 Sample characteristics

Sample	Enrichment	Addition	Cocoa mass
S1	Green tea Darjeeling	4 %	40 %
S2	Green tea Matcha	4 %	40 %
S3	Black tea Earl Grey	4 %	40 %
SC	none	none	40 %

Methods

Sample extracts preparation

All samples were grated into small pieces and then homogenized in a mortar. Step of lipid elimination from samples was not applied due to the possibility of loss in the only cocoa component during the process. Although lipids from chocolates have been removed in most of the studies, there are some studies in the literature which did not eliminate lipids from chocolates **(Cerit *et al.*, 2016)**. Then 0.25 g of homogenized sample was extracted with 20 mL of 80 % ethanol for 2 hours in a shaker (GFL 3005, Germany). After centrifugation at 4000 rpm (Rotofix 32a, Hettich, Germany) for 10 minutes and subsequent filtration, the supernatant was used for measurements. All analyses were done in triplicate.

Determination of total phenolic content

Total polyphenol content was measured using the method of **Singleton and Rossi, (1965)** using Folin-Ciocalteu reagent. Sample extract in volume on 100 µL was mixed with 100 µL of the Folin-Ciocalteu reagent, 1000 µL of 20 % (w/v) sodium carbonate and 8.8 ml of distilled water respectively. After 30 minutes of rest in dark place, the absorbance at 700 nm was measured using spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 250 $mg.L^{-1}$; r^2=0.9978) was used as the standard, and the results were expressed in mg GAE (gallic acid equivalents) in a gram of chocolate.

Determination of total flavonoid content

Content of total flavonoids was determined using the modified method by **Willett, (2002)**. A sample extract of 0.5 mL was added to the 0.1 mL of 10 % (w/v) ethanolic solution of aluminum chloride, 0.1 mL of 1 M sodium acetate and 4.3 mL of distilled water. After 30 minutes of rest in dark place, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.01 – 0.5 $mg.L^{-1}$; r^2=0.9977) was used as the standard and the results were stated as $mg.g^{-1}$ QE (quercetin equivalents).

Determination of total content of phenolic acids

Content of total phenolic acids was determined using the method by **Farmakopea Polska, (1999)**. A 0.5 mL of the extract of each sample was added to the 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnow's reagent (10 % $NaNO_2$ + 10 % Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance was measured at 490 nm by the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 $mg.L^{-1}$, r^2=0.9996) was used as a standard, and the results were stated as $mg.g^{-1}$ caffeic acid equivalents (CAE).

Antioxidant activity

DPPH scavenging activity

Radical scavenging activity of samples was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of **Sánchéz-Moreno *et al.*, (1998)**. The extract (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). After 10 minutes of resting in dark place, the absorbance of the sample extract was measured using the Jenway spectrophotometer (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 100 $mg.L^{-1}$; r^2=0.9881) was used as the standard and the results were stated as $mg.g^{-1}$ Trolox equivalents.

ABTS assay

ABTS radical cation decolorization assay was determined using the method of **Re *et al.*, (1999)** with slight modifications. ABTS (2,2´-azinobis[3ethylbenzthiazoline]- 6-sulfonic acid) was dissolved in distilled water to a concentration of 7 mM, and potassium persulphate was added to a concentration of 2.45 mM. This mixture was then left to stand at laboratory temperature overnight (12~16 h) in the dark place before use. The resultant intensely-coloured ABTS•+ radical cation was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.00 to give an absorbance value of ~0.70 at 734 nm. Two milliliters of ABTS solution were mixed with 0.98 mL of PBS and 0.02 mL of sample extract. Absorbance was measured spectrophotometrically (Jenway 6405 UV/Vis, England), 6 minutes after the addition of sample extract. Trolox (100 – 100 $mg.L^{-1}$; r^2=0.9991) was used as the standard, and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Reducing power

Reducing power of samples was determined by the method of **Oyaizu, (1986)**. One milliliter of sample extract was mixed with 5 mL PBS (phosphate buffer with pH 6.6) and 5 mL of 1 % potassium ferricyanide (w/v). The mixture was stirred thoroughly and heated in a water bath for 20 minutes at 50 °C. After cooling to room temperature, 5 mL of 10 % trichloroacetic acid was added. 5 mL of the mixture was pipetted into the test tube and mixed with 5 mL of distilled water and 1 mL of 0.1 % (w/v) ferric chloride solution. Absorbance was measured at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Reducing power was expressed in $mg.g^{-1}$ Trolox equivalents, using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 100 $mg.L^{-1}$; r^2=0.9974) as the standard and results were expressed in $mg.g^{-1}$ Trolox equivalents.

Statistical analysis

All measurements and analyses were carried out in triplicate. Experimental data were evaluated by basic statistical variability indicators using the Microsoft™ Excel® program. Dependency rate between the tested traits was expressed using the linear correlation analysis.

RESULTS AND DISCUSSION

Total polyphenol content

Polyphenols can be found in a broad spectrum of foodstuffs such as fruit, vegetables, tea, coffee, and red wine. Dark chocolate is shown to have more polyphenols than milk or white chocolate, because it contains more cocoa and polyphenols can be less bioavailable in the presence of milk **(Beckett, 2008)**. According to literature, there is a high positive correlation between the amount of cocoa solids and phenolic compounds **(Cerit *et al.*, 2016)**. Total phenolic contents (TPC) of our white chocolate samples are shown in table 2. It can be seen, that chocolates enriched with green teas (S1 and S2) exhibited very high content of total phenolics, more than four times higher than control.
Todorovic *et al.*, (2015) determined a similar amount of total phenolics in the samples of milk and dark chocolates produced in Serbia similar to our enriched ones. **Komes *et al.*, (2013)** studied the effect of the addition of dried fruits (dried prunes, dried papaya, dried apricots, dried raisins, dried cranberries) on bioactive content of milk and dark chocolates. It was shown that the prunes increased the total phenolic content of dark chocolate and cranberry enhanced the phenolic content of milk chocolate, but white chocolate had not been studied in this case. **Cervellati *et al.*, (2008)** also concluded, that artisan-made chocolate can preserve more biologically active polyphenolic compounds and found the addition of rosemary powder to increase their values even more due to high antioxidant capacity compounds contained in the rosemary. It is uneasy to compare the results, because the concentration of all polyphenols can vary tremendously among cocoa-containing foods, and this can vary depending on the source of the beans, the processing conditions, and how the chocolates are manufactured **(Cooper *et al.*, 2007)**.

Table 2 The amount of total polyphenol (TPC), flavonoid (TFC) and phenolic acids (TPA) content

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	TPA (mg CAE/g)
SC	4,87 ± 0,89	1,05 ± 0,06	4,37 ± 0,38
S1	16,16 ± 1,60	1,89 ± 0,02	8,21 ± 0,53
S2	20,69 ± 7,47	3,81 ± 0,50	8,70 ± 0,33
S3	7,05 ± 0,40	2,42 ± 0,36	8,47 ± 0,46

Legend: GAE – Gallic acid equivalent, QE – quercetin equivalent, CAE – caffeic acid equivalent

Total flavonoid content

Flavonoids belong to an important class of plant pigments that can be naturally found in fruit and vegetables. This group of naturally occurring polyphenolic compounds which cannot be synthesized by humans have many biological properties, acting as antioxidants on biological systems **(Calado *et al.*, 2015)**. Cocoa and dark chocolate have the highest flavanol content of all foods on a per-weight basis and can therefore be seen as a significant contributor to the total dietary intake of flavonoids **(Beckett, 2008)**. Milk and white chocolate exhibit lower flavanol content or even flavanol-free composition, respectively **(Latham *et al.*, 2013)**. Total flavonoid content (TFC) of our samples is shown in table 2. Best results were again obtained in the sample of chocolate flavoured with Matcha tea, sample S2. It is important to note, that the important beneficial

components of tea are the polyphenols, most importantly the flavonoids **(Nibir *et al.*, 2017)**. The percentage of cocoa is not a trustworthy indicator of the flavanol content present in a given product **(Latham *et al.*, 2013)**. To best of our knowledge, up to this day no study has investigated the levels of total flavonoids in white chocolates.

Total phenolic acids content

Phenolic acids are ubiquitous in edible vegetable, fruits, and nuts **(Vinayagam *et al.*, 2016)**. Due to their bioactive properties, phenolic acids are extensively studied and there is evidence of their role in disease prevention **(Heleno *et al.*, 2015)**. They are most abundant in coffee, tea and especially in berries. Recent interest in phenolic acids stems from their potential protective role against oxidative stress, inflammation, diabetes and cancer in experimental studies **(Zamora-Ros *et al.*, 2013)**. Total phenolic acids content (TPA) of our samples is shown in table 2. The total content of phenolic acids in the enriched samples was almost doubled after the addition of tea. There was also found a high positive correlation (r = 0,9956) between the content of total phenolic acids and antioxidant activity measured by DPPH in studied samples. **Lorenzo and Munekata, (2016)** reported, that application of phenolic compounds from green tea is of great interest because the antioxidant state of the products is increased and provides the product with additional antioxidant activity or reduces the unwanted changes of oxidative reactions during food processing or storage. The pure phenolic acids and catechins found in tea are more powerful antioxidants than the vitamins C, E or β-carotene in an in vitro lipoprotein oxidation model **(Bhutia Pemba *et al.*, 2015)**.

Antioxidant activity

Table 3 Antioxidant activity of the samples

Sample	DPPH (mg TE/g)	ABTS (mg TE/g)	RP (mg TE/g)
SC	0.95 ± 0.06	4.25 ± 0.54	1.18 ± 1.81
S1	6.74 ± 0.06	25.21 ± 1.89	11.21 ± 1.12
S2	6.79 ± 0.02	29.00 ± 0.68	17.68 ± 0.51
S3	6.68 ± 0.06	24.94 ± 2.02	13.48 ± 1.66

Legend: TE – Trolox equivalent; RP – reducing power

DPPH scavenging activity

DPPH analysis is most frequently used to determine the antioxidant capacity of foods. As can be seen from table 3, plain white chocolate used as control exhibited only weak antioxidant activity – 0.95 mg TEAC per gram. This can be caused by the reason that the majority of antioxidant capacity in chocolate is due to the non-fat cocoa solids content, which contains important phenolic substances. **Cerit *et al.*, (2016)** also measured low antioxidant activity (9.72 %) of plain white chocolate determined by DPPH method.
On the other hand, enrichment with different types of teas increased their antioxidant activity significantly. **Vertuani *et al.*, (2014)** also reported, that in general, by decreasing the percentage of nonfat cocoa solids, a decrease in the antioxidant capacity associated with a lower content of total polyphenols can be observed. **Cerit *et al.*, (2016)** found, that the addition of cornelian cherry and bee pollen powders provided an increase in antioxidant activity of white chocolates. **Zanchett *et al.*, (2016)** in their study examined the yerba mate extract addition and concluded that enrichment enhanced the amount of phenolic compounds with antioxidant action to the white chocolate, with good sensory acceptability.

ABTS assay

ABTS is a method for the screening of antioxidant activity to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, and carotenoids **(Re *et al.*, 1999)**. ABTS is also one of the most common methods for determining in vitro antioxidant capacity. It is recommended that at least two assays would be combined to provide comprehensive information on the total antioxidant capacity of a foodstuff **(Gülçin, 2012)**.
Results for antioxidant activity measured by this method are shown in table 3. Matcha flavoured chocolate (S2) again achieved the best results. There was also found a high positive correlation ($p \leq 0,05$) between ABTS and DPPH assay results (r = 0,9882) and between ABTS and phenolic acids content (r = 0,9946).
Komes *et al.*, (2013) used this method for studying the effect of dried fruits on the antioxidant activity of chocolates. They concluded that chocolates fortified with dried fruits provided higher antioxidant capacity than plain ones. Namely, various compounds showed synergism in their antioxidant capacity, thus, permitting that mixtures can promote more effective antioxidant responses than when the compounds are applied individually to the substrate. Additionally, it must be considered that the antioxidant activity of a mix is not the sum of the antioxidant activities of each of the components; however, the interactions of the compounds in between might generate synergic or inhibitors effects **(Fernández *et al.*, 2014)**.

Reducing power

The reducing capacity of a compound acts as a significant indicator of its potential antioxidant activity. The reducing power assay is based on the mechanism of electron donating activity, which is the primary mechanism of phenolic antioxidant action **(Aadil *et al.*, 2014)**. Table 3 shows the reducing power of the examined samples. Matcha tea addition (S2) increased the antioxidant activity of chocolate by this assay the best with the result of 17.68 mg TEAC per gram. This may be caused by the higher content of tea in comparison to other enriched samples. To the best of our knowledge, determination of antioxidant activity by this method hasn't been done in chocolates by other authors. **Singh *et al.*, (2015)** used this method for determination of the antioxidant activity of aqueous and ethanolic extract of mint (*Mentha piperita* L.) leaves and expressed the results as absorbance at 700 nm. Results for mint aqueous extract (0.4 ± 0.3 nm) are similar to our Matcha tea flavoured-chocolate ethanolic extract (0.5 ± 0.0 nm), but their ethanolic extract of mint exhibited higher activity with 0.7 ± 0.1 nm as our samples. This could be caused by the interactions of other chocolate components. Despite this, all fortified chocolates exhibited A700 more than 0.3 nm, and when compared to results of **Kim *et al.*, (2013)** we can conclude that functional chocolates can have similar or slightly higher antioxidant activity than broccoli with 0.30 ± 0.0 nm determined in their study.

CONCLUSION

This study investigated the effect of addition of different tea powders on the total polyphenol content and subsequent antioxidant activity. It was determined that plain white chocolate had low content of phenolic substances. Consequently, the antioxidant capacity of plain white chocolate was also low. However, addition of tea powders to the chocolate increased the phenolic compounds amount and antioxidant capacity. Matcha tea powder enrichment was the most efficient one among three powders used in this study. Considering the limited number of research on antioxidant capacity of enriched chocolates, the findings in current study may contribute to literature data. Further studies may be done with adding different types of teas or different powders of plant origin in general to chocolates.

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