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Comparison of phenolic content and antioxidant activity of matcha, green leaf and white leaf tea infusions

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Abstract

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Tea infusion, as a valuable source of bioactive compounds, substantially contributes to daily polyphenols intake. Among green teas, matcha, which is grounded Tencha tea grown under shaded conditions, gains popularity not only as an infusion but also as an additive to food products. This paper compares the antioxidant activity of matcha, green leaf and white leaf tea and assesses the effect of water temperature on extraction of phenolic compounds. Moreover, based on the results, the contribution to daily polyphenol intake of infusions was calculated. The samples were brewed with water at 60°C, 75°C and 90°C. The temperature of water significantly ($p < 0.0001$) impacted the properties of infusions. Regardless the brewing conditions, matcha infusions were found to contain significantly higher total phenolic content and exhibited stronger ferric reducing antioxidant power compared to green and white leaf tea infusions. Our experiment showed that 172 to 196 ml of matcha infusion depending on the water temperature can provide a sufficient amount of polyphenols to fulfill daily polyphenol intake from tea for the typical Polish consumer, which suggests that matcha is a suitable ingredient in a healthy diet.

Keywords: matcha; antioxidant activity; daily polyphenols intake; tea infusion

Introduction

Tea infusion is a common beverage consumed worldwide, while tea leaves and their extracts are ingredients of many food products and are becoming a popular component in a wide range of beauty products and cosmetics. World tea production and consumption has increased annually by 4.4% and 4.5% over the last decade, to reach 5.7 and 5.5 million tones, respectively, in 2016 (FAO, 2018). The massive expansion is a result of consumer health consciousness, rapid development of herbal tea beverages and public interest in organic, specialized premium teas in countries which do not have longstanding tradition of drinking tea. In the upcoming years, the increase is expected not only in Asia, where the consumption of tea is the highest, but also in Europe and North America (FAO, 2018). Poland, in comparison to the Mediterranean countries, presents high consumption with a daily mean intake of 525 ml. Around 97% of the Polish society consume at least one cup per day, which makes tea a relevant contributor to the polyphenol intake in Poland (Grosso et al., 2014).

Scientific literature is evidence that green tea (*Camellia sinensis* L.), thanks to the bioactive constituents including caffeine, L-theanine, polyphenols/flavonoids, and has many pharmacological and physiological functions. It possesses many benefits such as antimutagenic, anticarcinogenic, hypocholesterolomic, antibacterial, antioxidative and antiallergenic properties that may help in treating various disorders of humans (Cooper, 2011; Aman et al., 2013; Saeed et al., 2017). Varied geographical origin, growth conditions and processing of tea leaves results in characteristic tea types. White tea is made mainly from newly grown buds and young leaves with tiny, silvery hairs not exposed to sunlight to prevent chlorophyll production. It is the least processed type of tea, considered as a non-fermented type, however, slight fermentation occurs since the processing lacks the step of enzyme deactivation, which results in occurrence of some compound characteristics for black tea (Kosińska & Andlauer, 2014). Green tea is a non-fermented type with mainly flavan-3-ols (catechins, proanthocyanidins, theaflavins) contributing to the antioxidant capacity and shaping of sensory properties. The initial heating of the fresh leaves kills the enzyme polyphenol oxidase that prevents the conversion of the flavanols into the dark polyphenolic compounds typical for black tea. The final form of green tea (whole leaf, crushed or powdered) depends on the particular variant being produced (Cabrera et al., 2006).

Among green teas, authentic matcha, which is the powdered form of Tencha, seems to be unique. The organoleptic properties of infusions owe to shade-growing and harvesting stages including hand-picking, steaming, air drying, destemming and then grounding the leaves on granite wheels (Farooq & Sehgal, 2018). Shade treatment prior to harvest increases the amino acid (especially L-theanine) and caffeine contents and decreases the catechin content (Ku et al., 2010; Horie et al., 2017). Thanks to matcha's health promoting properties, it's used as regular tea, which contributes to 56% share of the global matcha market revenue, but also is a component of matcha beverages, food or personal care products (Grand View Research, 2017).

Apart from tea type, the conditions of preparing the infusion are of great importance to obtain the benefits from tea. Many studies measured the effect of water temperature, water quality, water-to-tea ratio, tea particle size, infusion time, stirring or dosage form, i.e., loose-leaf tea versus tea bag on efficiency of the extraction of antioxidants from tea (Vuong et al., 2011). As there is still little research about matcha infusions prepared in domestic conditions in comparison with other tea types, the main aim of this study was to assess the effect of water temperature on extraction of phenolic compounds (PC) and antioxidant activity of matcha, green leaf and white leaf tea. Moreover, the authors compared the infusions in regard to their contribution to daily polyphenol intake.

Materials and Methods

Fresh loose green leaf teas (Vietnam Green and Sencha) and white leaf teas (Mao feng and White dragon) were obtained from a local teahouse in Poznan, Poland. Matchas (Uji and Bio) were purchased in Tokyo, Japan (Table 1). Folin-Ciocalteu reagent, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 3,4,5-trihydroxybenzoic acid (gallic acid, GAE) and, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were acquired from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid, acetic acid, sodium carbonate, sodium acetate and, ferric chloride were purchased from POCH (Gliwice, Poland).

Infusions preparation

An aqueous infusion was freshly prepared in duplicate. A total of 2 g of leaves/powder were brewed for 3 min in glass containers using 100 mL of distilled water at 60°C, 75°C and 90°C. During extraction stirring with magnetic stirring was used both to mimic a typical matcha tea ceremony and to assure equal extraction. Later on, the samples were centrifuged at 3000 rpm for 5 minutes. The samples were diluted with distilled water prior to the analysis.

Total phenolic content measurement (TPC)

Folin – Ciocalteu method (Singleton and Rossi, 1965) was applied to measure total phenolic content (TPC) of infusions with use of 48-well micro plate. Firstly, 10 μL of diluted tea infusion was mixed with 50 μL of Folin-Ciocalteu reagent and 290 μL of water, stirred and left for 3 min. Later on, 150 μL of Na_2CO_3 (20%) and 500 μL of water was added and stirred. After 2-hours of incubation at 20°C with no light, absorbance was measured at 725 nm with UV-Spectrophotometer (Spectronic Genesis 2). A standard curve was prepared using various gallic acid (GAE) concentrations (y. The results of TPC were expressed in mg of gallic acid equivalent (GAE) / L of infusion. All measurements were performed in triplicate.

Antioxidant activity (AA)

Antioxidant activity of tea infusions was measured with the ferric reducing antioxidant power method (FRAP) (Ben-

Table 1. Tea samples and abbreviations

Tea type	Name	Abb.	Origin	Purchased in
Matcha	Uji	UJI	Japan	Japan
	Bio	BIO	Japan	Japan
Green leaf	Vietnam Green	VGR	Vietnam	Poland
	Sencha	SEN	China	Poland
White leaf	Mao feng	MFE	China	Poland
	White dragon (Bai long)	WDR	China	Poland

zie & Strain, 1996). Reaction mixture was freshly prepared by mixing 25 mL of acetic buffer (300 mmol/L), 2.5 mL of TPTZ (10 mmol/L) and 2.5 mL of $FeCl_3*6H_2O$ (20 mmol/L). 25 µL of diluted tea infusion was mixed with 975 µL of reaction mixture in a 48-well micro plate. After 8-minute incubation at 20°C, absorbance was measured at 593 nm with UV-Spectrophotometer (Spectronic Genesis 2). A calibration curve was obtained using Trolox solutions (y). The results were reported in mmol Trolox/L infusion. All measurements were performed in triplicate.

Polyphenols intake calculations

Each tea type contribution to daily polyphenols intake (DPI) was calculated basing on average serving amount and expressed in %. Moreover, taking into consideration results from previous studies (Zujko et al., 2012; Witkowska et al., 2017), the amount of mL of each infusion needed to fulfill the DPI was determined.

Statistical analysis

Data was expressed as the mean values ± standard deviation. Analysis of variance (ANOVA) was carried out using Statistica 13.3 (StatSoftPolska) to determine significant differences ($p<0.05$) between mean values, depending on the tea type and water temperature. Additionally, if significant differences were detected between samples, a post hoc analysis (Tukey's test) was performed. Correlation between the antioxidant activity and total phenolic content was calculated using Pearson's correlation.

Results and Discussion

Total phenolic content

Total phenolic content of matcha, green leaf and white leaf tea infusions was significantly influenced by the tea type ($F(5) = 638.12$, $p < 0.001$) and temperature of water used for extraction ($F(2) = 106.44$, $p < 0.001$). TPC values ranged from 487.5 to 2358.6 mg GAE/L with the highest value for matcha BIO extracted with water at 75°C (Table 2).

Among all the samples, both matchas had significant ($p<0.05$), almost twofold, higher TPC than white and green tea samples, regardless the water temperature used for extraction. BIO sample in comparison to UJI showed significantly higher phenolic content for all extraction conditions ($p<0.05$). Taking into consideration the form of teas, the results of our study are in agreement with the findings of Fujioka et al. (2016) that compared the whole leaf tea and powdered tea from the same tea leaves and indicated higher total polyphenol concentration in the infusion from powdered tea. They suggested that powdering process with the

Table 2. Total phenolic content of tea samples expressed as mg GAE/L infusion

Tea type		Water temperature, °C		
		60	75	90
Matcha	BIO	2083.0 $^{a, E}$ (±144.0)	2358.6 $^{b, E}$ (±94.8)	2069.4 $^{a, E}$ (±17.4)
	UJI	1553.5 $^{a, D}$ (±93.913)	1643.2 $^{a, D}$ (±59.451)	1695.4 $^{a, D}$ (±74.899)
Green leaf	VGR	906.8 $^{b, C}$ (±68.9)	1241.2 $^{a, C}$ (±88.8)	1399.9 $^{a, C}$ (±144.0)
	SEN	487.5 $^{a, A}$ (±20.7)	636.0 $^{b, A}$ (±49.5)	816.2 $^{c, A}$ (±21.4)
White leaf	MFE	817.7 $^{a, BC}$ (±46.9)	1006.9 $^{b, B}$ (±25.4)	1187.1 $^{c, B}$ (±113.7)
	WDR	632.9 $^{a, AB}$ (±89.0)	985.4 $^{b, B}$ (±33.0)	1184.6 $^{c, B}$ (±65.2)

a, b, c – different lower-case letters indicate significant differences ($p < 0.05$) between TPC for one tea type extracted with water at different temperatures (in rows); A, B, C – different upper-case letters indicate significant differences ($p < 0.05$) between TPC for different tea samples extracted with water at the same temperature (in columns)

ceramic mill promote the extraction of epigallocatechin gallate thanks to the lower particle size with torn shapes. Similarly, in the Castiglioni et al. (2015) study, TPC level of white and green tea infusions were always significantly higher for those obtained from milled leaves than from whole leaves. In both studies not original matcha (shade-grown) was investigated, but the process of powdering improved the extraction of bioactive compounds from the leaves.

The combination of cultivation method, time of harvest and origin seems to have cumulated impact on phenolic content and metabolome of teas. The study of Ku et al. (2010) found that TPC of Korean green tea in comparison to Korean tencha (ungrounded matcha) was higher for samples obtained in July, but did not statistically differ for samples harvested in April. Moreover, shading treatment influenced the chemical composition of tea infusion, by lowering the content of epigallocatechin and epicatechin and increasing the content of gallocatechin in tencha in comparison to green tea. However, tencha exhibited higher level of flavonoid derivatives than green teas. On the contrary, in case of Japanese tea samples, Koch et al. (2017) indicated significant similarities between green Sencha and green matcha in terms of catechin content and TPC. In our study samples originated from different countries, so direct comparison is not possible, but Japanese powdered samples showed higher TPC than Vietnamese and Chinese green and white whole leaf teas.

High TPC of matcha samples might be linked with high level of L-theanine. In the work of Tadesee et al. (2015) the Ethiopian green tea showed the highest content of L-theanine, catechins and TPC among analyzed samples. The correlation between the concentration of catechins content and the phenolic content measured by the F-C method in green teas was rather low (Koch et al 2017), which results from Folin-Ciocalteu reagent being reactive towards various compound classes, not only phenols (Everette et al., 2010). As the content of L-theanine is usually much higher in matchas than other teas (Horie et al., 2017), consequently, high TPC of Ethiopian green tea might have resulted from high L-theanine level, which also may support our findings.

The comparison of phenolic content in white and green leaf tea infusions gives ambiguous findings. The highest TPC exhibited VGR sample, while the lowest green tea SEN with white teas on moderate level. Some authors reported that green tea is a richer polyphenol source than white tea (Rusak et al., 2008; Castiglioni et al., 2015; Pérez-Burillo et al., 2018), the others conversely (Hilal & Engelhardt, 2007; Santana-Rios et al., 2001). In other works, the TPC was similar for the green tea and white tea (Almajano et al., 2008). TPC in both green and white tea is highly variable and might be influenced by several factors associated with growth conditions, origins and processing steps (Kosińska & Andlauer, 2014).

Water temperature affected the extraction of phenolics from teas to a various extent. In case of UJI sample, water temperature had no impact on phenolics extraction yield, while for the BIO sample water at 75 °C seemed to maximize PC extraction. For white and green teas, significant impact of temperature of water used for extraction was observed ($p<0.05$). Water at 90°C more efficiently extracted phenolic compounds than water at 75°C and 60°C. Same outcomes were observed in Pérez-Burillo et al. (2018) study where the extraction of phenolics in white teas gradually grew in a linear manner with water temperature. Using water at higher temperatures usually increases the rate and amount of PCs that can be extracted (Ziaedini et al., 2010). Nevertheless, phenolic compounds are also thermo-sensitive, and their antioxidant activity and bioactivity may be compromised if their chemical structure degrades during extraction.

Antioxidant activity

Antioxidant activity of tea infusions measured by FRAP assay significantly varied taking into consideration the tea type ($F(5) = 2278.56$, $p < 0.001$) and water temperature ($F(2) = 126.79$, $p < 0.001$). The reducing power ranged from the lowest (2.27 mmolTrolox/L) for SEN infusion extracted at 60°C to the highest (14.5 mmolTrolox/L) for BIO infusion prepared at 90°C (Table 3). Both matchas showed statistically the highest antioxidant activity for all water conditions. BIO revealed higher reducing power than UJI which is in line with results of TPC.

Table 3. Ferric reducing antioxidant power of tea samples expressed as mmol Trolox equivalent/L infusion

Tea type		Water temperature, °C		
		60	75	90
Matcha	BIO	13.37 $^{a, D}$ (±0.71)	13.15 $^{a, D}$ (±0.33)	14.50 $^{b, E}$ (±0.17)
	UJI	9.65 $^{b, C}$ (±0.33)	10.88 $^{c, C}$ (±0.25)	8.24 $^{a, D}$ (±0.44)
Green leaf	VGR	3.57 $^{a, B}$ (±0.16)	4.67 $^{b, B}$ (±0.60)	4.22 $^{ab, B}$ (±0.31)
	SEN	2.27 $^{a, A}$ (±0.13)	2.99 $^{b, A}$ (±0.29)	3.53 $^{c, A}$ (±0.16)
White leaf	MFE	3.93 $^{a, B}$ (±0.14)	5.41 $^{b, B}$ (±0.14)	7.18 $^{c, C}$ (±0.11)
	WDR	3.64 $^{a, B}$ (±0.17)	5.19 $^{b, B}$ (±0.13)	6.75 $^{c, C}$ (±0.32)

a, b, c – different lower-case letters indicate significant differences ($p<0.05$) between AC for one tea type extracted with water at different temperatures (in rows); A, B, C – different upper-case letters indicate significant differences ($p < 0.05$) between AC for different tea samples extracted with water at same temperature (in columns)

Among green tea infusions, sample VGR from Vietnam showed higher antioxidant capacity than SEN form China, irrespective of extraction conditions. The highest AA of VGR infusion was obtained with water at 75°C which was statistically equal to antioxidant activity received for infusion prepared with water at 90°C. In case of SEN sample, the AA values increased with the rising temperature of water with the highest AA achieved for infusion obtained with water at 90°C.

In general, the average reducing power of studied white tea infusions was 1.5 times higher than of green leaf tea infusions. However, some studies of other authors exhibited contrary results (Almajano et al., 2008; Castiglioni et al., 2015). Both white teas showed statistically similar reducing power ($p>0.05$) for each extraction conditions. The reducing power grew with the increase of water temperature which was also found in white teas by other authors (Pérez-Burillo et al., 2018). This trend was not observed in case of matchas. UJI sample extracted with water at 75°C showed the highest antioxidant activity, while extraction with water at 90°C drastically decreased AA, even more than the results obtained for tea prepared with water of 60°C. BIO infusion gave the highest antioxidant activity in case of using water at 90°C, while samples prepared with water at 60°C and 75°C exhibited reducing power on the same level. Likewise, in Castiglioni's et al. (2015) work the influence of water temperature (70°C and 90°C) on antioxidant activity varied – for some samples an increase of AA with the increase of water temperature was observed, while in others the AA was at stable level.

In this study, a strong and, positive correlation was found between the AA and TPC results (r= 0.935), thus suggesting the high contribution of the extracts' PC to their reducing power (Figure 1).

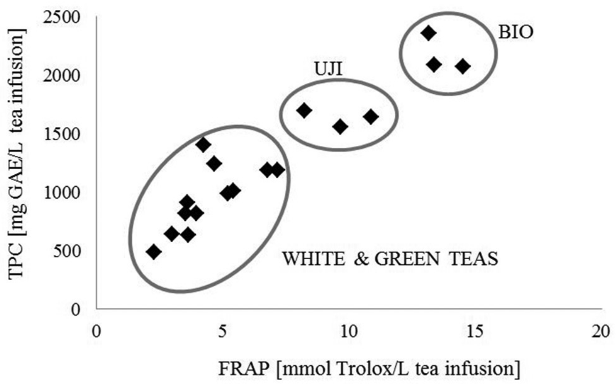

Fig. 1. Relation of the antioxidant activity and total polyphenols content

Daily polyphenols intake

Based on the results of our study, the contribution of matcha, green leaf and white leaf tea consumption to the daily polyphenols intake (DPI) was calculated. In Poland (research based on 10477 consumers) the average daily polyphenols consumption amounted to 1756.5 ± 695.8 mg (Grosso et al., 2014). The serving of 150 mL of studied tea infusion provided on average 104.6-306.1 mg gallic acid equivalents, which contributes to 6.0-17.4% of the daily polyphenol intake (Table 4). The results are comparable with calculations for DPI from the consumption of green and white tea in Spain (Pérez-Burillo et al., 2018).

Previous studies showed that non-alcoholic beverages including tea and coffee were the best sources of polyphenols in daily diet. Tea consumption contributed in 21.3% and 18.9% to DPI in case of Polish postmenopausal women with or without cardiovascular disease (Witkowska et al., 2017) and in around one-fifth in case of Polish consumers in WOBASZ studies (Zujko et al., 2012). Basing on that data, it was presumed that 20% of DPI is covered by consumed tea. The calculations allowed noticing that only 172 to 196 ml of matcha infusion, depending on the water temperature, provided a sufficient amount of polyphenols to fulfill daily polyphenol intake. In case of green tea twice as much infusion was needed to fulfill DPI, but still the amount was low as Poles are rather heavy tea drinkers (Zujko et al., 2012; Grosso et al., 2014).

Conclusions

Recently the various types of tea are becoming more accessible around the world. Thanks to their proved health benefits, the application not only in food products is rising and more research is needed to reveal and compare the properties of lesser known types. In this study, six samples representing matcha, green leaf and white leaf tea types were tested and the impact of brewing temperature on their total phenolic content and antioxidant activity was assessed. It was found that matchas' infusions had significantly more phenolic compounds and much higher ferric reducing antioxidant power than green and white teas regardless the temperature of extraction. Green tea SEN showed the lowest TPC and reducing properties among samples and on average the AC of green teas was 1.5 times lower than white leaf teas. The optimal temperature for extraction depended on the sample, which failed to draw unambiguous conclusions for each tea type. However, in almost all cases, the lowest tested temperature (60°C) was the least suitable. The further study may consider more samples from each tea type, especially matcha type, as it is commonly recommended to brew the matcha with water at not more than 80°C, which was not confirmed in our study. The research affirmed that taking into consideration the daily polyphenols' intake requirements tea infusions may successfully supplement the diet with health-pro-

Table 4. Contribution of tea consumption to the daily polyphenols intake in the Polish diet

Tea type	Water extraction temperature, °C	Average TPC, mg GAE/L infusion	Average TPC per serving, mg/150 mL	Contribution to DPI (%) of a serving	mL needed to fulfill DPI from tea
Matcha	60	1788.8	268.3	15.3	196
	75	2040.6	306.1	17.4	172
	90	1882.4	282.4	16.1	187
Green leaf	60	697.2	104.6	6.0	504
	75	938.6	140.8	8.0	374
	90	1066.3	159.9	9.1	329
White leaf	60	725.3	108.8	6.2	484
	75	997.3	149.6	8.5	352
	90	1185.7	177.9	10.1	296

moting compounds. Among tested samples, matcha infusions provided almost twofold more polyphenols than other samples, which makes them a healthy non-alcoholic alternative for daily consumption.

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