Content of selenoaminoacids and catechins in Chinese green teas

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Abstract

In this study, famous Zhejiang teas were evaluated as a well-advertised source of selenium. The 25 samples from provinces around China and Asia were purchased in Warsaw tea shops. The speciation analysis of selenium as well as the evaluation of catechin content in water tea infusions was performed using hydrophilic interaction liquid chromatography coupled to mass spectrometry (HILIC-MS/MS). It turned out that all of tested samples can be a great source of organic selenium species (no traces of inorganic selenium was found), however, Zhejiang teas did not differ much among others. Such a conclusion can also be drawn when comparing the antioxidant capacity of the tested samples, obtained with the application of four methods (Folin–Ciocalteu method, scavenging of the 2,2-diphenyl-1-picrylhydrazyl radical, hydroxyl radical scavenging and cupric reducing ability assay). What is more, no correlation was found between the selenium content and the antioxidant activity of studied teas. The results obtained for the six samples from Zhejiang Province were very varied, which shows that it is very difficult to interpret the results and compare them with the results of other authors.

Keyword Green tea · Selenoaminoacids · Catechins · Antioxidant properties

Introduction

Tea is the second most widely consumed beverage after water and its popularity is not only due to a great taste but also to its health-promoting properties, especially for green tea [1, 2]. Several studies have confirmed the positive role of green tea in preventing cardiovascular and degenerative diseases, likely due to the antioxidant properties of polyphenolic compounds [3, 4]. The major polyphenols in green tea are flavonoids, the most active of which are catechins and epigallocatechin gallate [5]. These compounds exert their antioxidant and antiradical properties by several mechanisms including direct scavenging of reactive oxygen species, chemical reducing activity, complexation of pro-oxidant metal ions, activation of antioxidant enzymes and inhibition of oxidases [6]. The newest studies proved that green tea catechins inhibit microglial activation which prevents

the development of neurological disorders as well as play a significant role in cancer prevention and therapy [7, 8].

Over the past years, a growing interest in Chinese green tea particularly produced in Ziyang County located in the southwest of Shaanxi Province has been observed. Almost all Chinese tea contain selenium but with different content but Ziyang tea, in addition to health-promoting ingredients found usually in green tea, also contains significantly high content of this element [9–11]. Because of the unique geologic structures, the selenium content in the rock and the soil achieves over there 5.66 mg/kg and 3.98 mg/kg, respectively [12]. Selenium is not classified as an essential nutrient for plants, however, evidence has indicated that soil or foliar application of selenium compounds can enhance the growth, yield and quality of crops [13, 14]. Moreover, selenium is of high importance for many of the body's regulatory and metabolic functions [15, 16]. Epidemiological studies suggested that low Se intake may increase the risk of cardiovascular disease and cancer [17]. However, excessive selenium intake may induce adverse effects; selenium species can interact with glutathione to form reactive selenotrisulfides and generate toxic superoxide and hydrogen peroxide species, thereby oxidizing cell membranes and macromolecules [18]. It should be stressed out that occurring chemical forms of selenium differ from each other in terms of chemical

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properties, environmental effects, biological utilizations, toxicities, and nutrition values and Se organic compounds are less toxic and more bioavailable than inorganic forms [19, 20]. Several plants have the ability to accumulate Se inorganic species from soil and transform them to selenoaminoacids. Selenomethionine is the dominant species in rice, while vegetables from *Allium* and *Brassicaceae* family, such as onion, garlic or broccoli, are better sources of methylselenocysteine [21, 22].

Several studies found that Se-enriched green tea possessed a better sensory quality and exhibited higher antioxidant activity than regular green tea [23–27]. Our research was conducted to compare the content of selenium chemical forms and antioxidant activity of Ziyang green tea available on Polish tea market (sold usually under the name “high Se tea”) with green teas from other regions in China and other Asia countries. Compared to other works on similar topics, these studies were conducted from the point of view of the tea consumer. When selecting tea samples, we relied entirely on the information available on their packaging or information obtained from individual tea houses. The major organic forms of selenium present in plant materials, such as selenomethionine, selenocysteine and methylselenocysteine as well as major polyphenols present in tea, such as catechin, epicatechin and epigallocatechin gallate, were determined in water tea infusions using hydrophilic interaction liquid chromatography coupled with mass spectrometry (HILIC-MS/MS). The antioxidant properties of the tea infusions were examined using Folin-Ciocalteu method (FC), scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydroxyl radical scavenging (OH) and cupric reducing ability (CUPRAC) assays.

Materials and methods

Reagents and apparatus

The commercial standards of sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4), selenomethionine (SeMet), methylselenocysteine (MeSeCys) and selenocysteine (SeCys) as well as catechin (CAT), epicatechin (EPI), epigallocatechin gallate (EGCG) standards were purchased from Sigm-Mercka (Steinheim, Germany). For mobile-phase preparation, MeOH was purchased from Merck (Darmstadt, Germany) and water was obtained from a Mili-Q water purification system (Millipore, Bedford, MA, USA).

Chromatographic analysis was performed with the Shimadzu LC system consisted of binary pumps LC20-AD, degasser DGU-20A5, column oven CTO-20AC, autosampler SIL-20AC and 8030 triple quadrupole Mass Spectrometer (Shimadzu, Japan) equipped with an ESI source operated in negative-ion or positive-ion mode, according to the

determined species. The ESI conditions were as follows: the capillary voltage 4.5 kV, temperature 400 °C, the source gas flow 3 L/min, drying gas flow 10 L/min.

Separation of selenium compounds was performed in HILIC mode. Compounds were separated on Atlantis HILIC (100×2.1, 3 µm) from Waters (Dublin, Ireland). The mobile phase consisted of methanol and 8 mM ammonium acetate pH 7 (85/15, v/v) was delivered at 0.2 ml/min. Selenium compounds were identified by comparing their retention times and m/z values obtained by MS and MS2 with the mass spectra. The optimization of the separation process was described by the author earlier [28].

Samples and brewing processes

All of the analyzed green teas were purchased in Warsaw tea rooms. Each of them had documented origin. Hence, the division of teas into selenized green Chinese teas, regular Chinese green teas and other according to their exact origin. Figure 1 (based on [29]) shows maps indicating the origin of analyzed teas. The samples were marked as follows: selenized green teas from Zhejiang Province: L1 (Lung-Ching), L2 (Lung-Ching), L3 (Lung-Ching), L4 (Lung-Ching), HM (High Mountains), MG (Misty Green); other Chinese green teas: J (Jiangsu Province), S (Xinjiang), F (Fujian), Y1, Y2, Y3 (Yunnan), X1, X2, X3, X4, X5, X6 (Unknown Chinese Province), M (mix of Chinese teas); other non-Chinese teas from Asia: JP1, JP2 (Japan), V (Vietnam), A (Azerbaijan), I (India) and C (Ceylon, Sri Lanka).

To simulate the household brewing process, the samples were prepared using an aqueous extraction procedure. The process was conducted by adding 100 mL of hot distilled water to 2 g of tea leaves and mixing for 1 h. Before the analysis all the extracts was filtered through 0.22 µm PTFE filter (Millipore).

Antioxidant activity

Scavenging of free radicals by the tested tea infusions was evaluated by DPPH assay [30]. Briefly, 0.1 mL of a sample was added to 2.4 mL of methanolic radical solution ($9 \times 10^{-5} \text{ mol L}^{-1}$). The decrease in absorbance was measured 30 min after mixing reagents at 518 nm. The results were expressed as a trolox equivalent (TRE) in µM. Each sample was analyzed in triplicate.

Folin–Ciocalteu assay was performed according to procedure described by Singleton with some changes [31]. In details, 1 mL of sample was mixed with 0.1 mL of FC reagent and 0.9 mL of water. Then, after 5 min, 1 mL of sodium carbonate (7% v/v) and 0.4 mL of water were added. For another 30 min, the mixture was allowed for stabilization and formation of blue color. The absorbance against blank was measured at 765 nm. The obtained results were expressed as gallic

Fig. 1 Geographical origin of tested Chinese tea samples, including the content of selenium in soils. Based on Wang and Gao [29]

acid equivalent (GAE) in mM. Each sample was analyzed in triplicate.

The capacity to reduce cupric ions was determined using CUPRAC method described by Apak [32]. 1 mL of copper chloride ($1 \times 10^{-2} \text{ mol L}^{-1}$) was mixed with 1 mL of methanolic solution of neocuproine ($7.5 \times 10^{-3} \text{ mol L}^{-1}$) and 1 mL of ammonium acetate buffer (1 M, pH 7), followed by adding 0.5 mL of tea extract and 0.6 mL of water. The mixture was then incubated at 50°C in water bath for 20 min. Absorbance against blank was measured at 450 nm. The obtained results were expressed as trolox equivalent (TRE) in μM . Each sample was analyzed in triplicate.

For the hydroxyl radical scavenging activity, the procedure described by Smirnoff and Cumbes was used [33]. The reaction mixture contained 1 mL iron sulfate ($1.5 \times 10^{-3} \text{ mol L}^{-1}$), 0.7 mL hydrogen peroxide ($6 \times 10^{-3} \text{ mol L}^{-1}$), 0.3 mL sodium salicylate ($2 \times 10^{-2} \text{ mol L}^{-1}$) and 1 mL of the extracts. After 1 h incubation at 37°C , the absorbance was measured at 562 nm. The scavenging activity of the extract was calculated as follows:

$$\% \text{ OH} = \frac{1 - (A_1 - A_2)}{A_0} \cdot 100\%$$

where A_0 is an absorbance of the blank (without tea extract), A_1 is the absorbance with the tea extract and A_2 is the absorbance without sodium salicylate.

Statistical analysis

The experimental results were obtained from at least three parallel measurements and are presented as average $\pm$ standard deviation. The significance of differences among means was carried out at 5% probability level using one-way ANOVA and Tukey's test.

Results and discussion

The content of the main selenoaminoacids and catechins in the water extracts of 25 green teas bought in Warsaw tea rooms were compared. The places of origin of tested Chinese green teas are marked in Fig. 1. It should be noted that the analyzed Chinese teas come from provinces in which the soil is rich in selenium to varying degrees, as also shown in Fig. 1. The famous Ziyang teas (samples L1, L2, L3, L4, HM, MG) are well known for high content of selenium, however, samples J and F come from neighbouring provinces, where the soil is also rich in selenium. Another Chinese teas marked as Y (samples Y1, Y2, Y3 from Yunnan Province) and S (Xinjiang) may contain different selenium contents depending on the location of the crop. For six teas, marked as X and M, we know next to nothing about their origin.

Our study is quite a new approach to this subject because the studies regarding Ziyang teas described in the literature have been made by research groups from China, so the tea samples were purchased on the spot. Some of the researchers grow selenized green tea by themselves.

The contents of some selenoaminoacids in the studied green tea water infusions are presented in Table 1. The extract of Ziyang tea L1 had the highest content of methyl-selenocysteine (MeSeCys) followed by X5 and Y1. The concentration of this selenium compound in other Ziyang teas was in range of 2.1–3.04 µg/g, with the exception of HM, which turned out to be the poorest in MeSeCys of the group (1.55 µg/g). Zhang reported the concentration of SeMeCys equals to 1.07 µg/g in the infusion of Se-enriched tea brewed in boiling water for 1 h and 0.98 µg/g in the extract of regular green tea [34]. Selenomethionine (SeMet) was present in all studied tea samples; in Zhejiang green tea, its content was in range from 1.02 µg/g (L1) to 2.05 µg/g (MG). Zhang found

SeMet only in selenized green teas cultivated in Enshi province (1.32 µg/g) [34]. Selenocystine (SeCys) was detected in all studied samples with the exception of Chinese tea X5 and Ceylon tea (C). The concentrations of SeCys in Ziyang teas were below 1 µg/g, with the exception of MG (1.72 µg/g).

Inorganic selenium, selenite and selenate were below limit of detection (0.1 µg/g) in all studied tea samples. These Se species were detected in selenized green tea but plant leaves were sprayed with sodium selenite during cultivation, thus, selenium was delivered not only from soil, but also from the fertilizers [23, 26]. It should be highlighted, that the kind of selenium compound present in fertilizer affects the selenium form and concentration in the final product. Higher concentration of selenium was present in tea fertilized with Na₂SeO₄ (10.61 mg/kg) in comparison to that fertilized with Na₂SeO₃ (7.56 mg/kg), while regular tea leaves contained only 0.121 mg/kg of Se [25]. The same research group reported that also foliar spraying with selenite resulted

Table 1 Selenoaminoacid and catechin contents in the studied green tea infusions

	MeSeCys	SeMet	SeCys	Catechin	Epicatechin	EGCG
Chinese green teas from Zhejiang County						
Lung Ching (L1)	5.13 ± 0.06 ^a	1.02 ± 0.01 ^a	0.432 ± 0.02 ^a	0.180 ± 0.01 ^a	7.31 ± 0.04 ^a	13.2 ± 0.04 ^a
Lung Ching (L2)	2.10 ± 0.01 ^b	1.22 ± 0.02 ^b	0.217 ± 0.01 ^b	13.3 ± 0.02 ^b	4.44 ± 0.09 ^b	12.0 ± 0.03 ^b
Lung Ching L3)	2.58 ± 0.07 ^c	1.52 ± 0.02 ^c	0.179 ± 0.01 ^b	4.57 ± 0.03 ^c	23.5 ± 0.10 ^c	18.4 ± 0.09 ^c
Lung Ching L4)	3.04 ± 0.04 ^d	1.91 ± 0.02 ^d	0.195 ± 0.01 ^b	1.88 ± 0.02 ^d	6.04 ± 0.09 ^d	5.35 ± 0.06 ^d
Misty Green (MG)	2.63 ± 0.01 ^c	2.05 ± 0.03 ^e	1.72 ± 0.02 ^c	4.59 ± 0.01 ^c	28.2 ± 0.07 ^e	15.7 ± 0.01 ^e
High Mountain (HM)	1.55 ± 0.02 ^e	1.61 ± 0.01 ^f	0.787 ± 0.01 ^d	1.61 ± 0.01 ^e	3.85 ± 0.05 ^f	2.00 ± 0.01 ^f
Chinese green teas from other provinces						
Jiangsu (J)	2.31 ± 0.03 ^b	1.06 ± 0.02 ^a	0.560 ± 0.02 ^e	7.43 ± 0.02 ^f	13.3 ± 0.10 ^g	7.17 ± 0.04 ^g
Sinciang (S)	3.91 ± 0.03 ^f	1.07 ± 0.01 ^a	0.367 ± 0.01 ^f	2.51 ± 0.01 ^g	15.5 ± 0.09 ^h	16.5 ± 0.03 ^h
Fujian (F)	2.81 ± 0.08 ^c	2.33 ± 0.01 ^g	1.21 ± 0.01 ^g	0.642 ± 0.02 ^h	3.37 ± 0.01 ^f	3.20 ± 0.01 ⁱ
Yunnan (Y1)	3.62 ± 0.04 ^f	0.369 ± 0.01 ^h	0.254 ± 0.01 ^h	15.6 ± 0.02 ⁱ	67.0 ± 0.30 ⁱ	15.1 ± 0.04 ^j
Yunnan (Y2)	4.47 ± 0.05 ^g	0.214 ± 0.01 ⁱ	0.331 ± 0.02 ⁱ	9.69 ± 0.02 ^j	55.6 ± 0.20 ^j	9.29 ± 0.02 ^k
Yunnan (Y3)	3.04 ± 0.09 ^d	1.12 ± 0.02 ^j	1.02 ± 0.01 ^j	1.04 ± 0.01 ^k	32.4 ± 0.15 ^k	28.7 ± 0.10 ^l
Unknown (X1)	1.33 ± 0.04 ^e	2.29 ± 0.02 ^g	1.20 ± 0.01 ^g	0.807 ± 0.01 ^h	1.47 ± 0.01 ^l	4.22 ± 0.01 ^m
Unknown (X2)	2.92 ± 0.04 ^c	1.67 ± 0.01 ^k	1.08 ± 0.02 ^k	2.56 ± 0.02 ^g	6.31 ± 0.01 ^d	4.01 ± 0.01 ^m
Unknown (X3)	3.03 ± 0.03 ^d	2.29 ± 0.02 ^g	1.14 ± 0.02 ^l	5.00 ± 0.03 ^l	8.61 ± 0.02 ^m	7.13 ± 0.01 ^g
Unknown (X4)	2.45 ± 0.02 ^b	1.48 ± 0.01 ^c	0.400 ± 0.01 ^f	0.166 ± 0.02 ^a	9.28 ± 0.02 ⁿ	7.61 ± 0.01 ^g
Unknown (X5)	4.52 ± 0.07 ^g	0.861 ± 0.02 ^l	< LOD	7.20 ± 0.01 ^m	5.06 ± 0.02 ^o	10.3 ± 0.03 ^o
Unknown (X6)	2.65 ± 0.10 ^c	1.22 ± 0.03 ^b	0.551 ± 0.01 ^e	4.37 ± 0.02 ⁿ	18.9 ± 0.10 ^p	4.66 ± 0.01 ^p
Mixed (M)	2.32 ± 0.04 ^b	2.1 ± 0.01 ^m	2.41 ± 0.02 ⁿ	1.44 ± 0.02 ^c	4.42 ± 0.01 ^b	4.98 ± 0.05 ^p
Other green teas from Asia						
Japan (JP1)	1.40 ± 0.02 ^e	0.297 ± 0.01 ⁿ	0.377 ± 0.01 ^f	8.31 ± 0.03 ^o	14.7 ± 0.20 ^r	3.72 ± 0.02 ^r
Japan (JP2)	1.41 ± 0.01 ^e	0.246 ± 0.02 ⁱ	1.20 ± 0.02 ^g	11.9 ± 0.02 ^p	11.2 ± 0.10 ^s	9.49 ± 0.01 ^k
Vietnam (V)	1.03 ± 0.01 ^h	2.14 ± 0.01 ^m	1.93 ± 0.01 ^o	2.42 ± 0.02 ^g	10.6 ± 0.05 ^t	8.18 ± 0.21 ^s
Azerbaijan (A)	1.30 ± 0.01 ^e	1.02 ± 0.01 ^a	0.715 ± 0.01 ^p	1.61 ± 0.01 ^e	16.4 ± 0.01 ^u	9.94 ± 0.01 ^k
India (I)	3.12 ± 0.04 ^d	1.2 ± 0.01 ^b	0.848 ± 0.01 ^r	4.85 ± 0.02 ^l	10.1 ± 0.01 ^t	7.76 ± 0.02 ^g
Sri Lanka, Ceylon (C)	4.17 ± 0.04 ⁱ	1.45 ± 0.02 ^c	< LOD	3.30 ± 0.01 ^r	7.16 ± 0.01 ^a	1.90 ± 0.01 ^f

Concentrations expressed in µg/g for selenium compounds and in mg/g for catechins in DW of tea. Different letters in each column indicate a difference at a significance level of $p=0.05$

in the Se content lower than in previous work (3.780 mg/g) but higher value (5.895 mg/kg) was obtained using for fertilization organically bound selenium [35]. The authors do not provide a reason for this difference. Literature generally indicates the content of selenium in tea grown with the addition of selenium fertilizers under laboratory conditions, information on the content of this element in samples without fertilization, grown on plantations is not available.

Data regarding the effect of selenium spraying on the total polyphenol content in green teas are inconsistent [23, 25, 27, 35], similarly as for other plant extracts [36–38]. In all of these works, Folin–Ciocalteu (FC) assay was used for the evaluation of total phenolics content. However, several non-phenolic compounds, including proteins, amino acids, thiols and vitamins which are commonly present in plants, also react with FC reagent (mixture of phosphotungstic and phosphomolybdic acids) to form a blue complex [39]. As a consequence, the obtained results depend on the presence of other reductants and they could be overestimated. On the other hand, it is known that the conditions of tea cultivation, type of soil, intensity of solar radiation, temperature, and precipitation affect the polyphenol profile and thus antioxidant capacity of a given extract.

In this work, the contents of major polyphenolic compounds present in green tea (i.e. catechins) were determined using HPLC–MS/MS method for comparison of Ziyang green tea and different green teas from other places in China as well as from other countries in Asia. The obtained results are presented in Table 1. The highest content of catechin was found in sample L2 (13.3 mg/g), while the lowest content was found in X4 (0.166 mg/g) and L1 (0.180 mg/g) samples. High content of this compound was detected in Japanese teas (JP1 and JP2) and two Yunnan teas (Y1 and Y2). The content of catechin in Zhejiang tea extracts varied from 0.18 mg/g (L1) to 4.59 mg/g (MG). He reported the average value of catechin, epicatechin and EGCG in Zhejiang tea as 1.44 mg/g, 6.66 mg/g and 70.55 mg/g, respectively, but for extraction, they employed 50% (v/v) ethanolic solution and heating at 80 °C for 20 min [9]. Our studies with hot water simulate the household brewing process.

The highest content of epigallocatechin gallate (EGCG), the most biologically active catechin, was obtained in Y3 from Yunnan province (28.7 mg/g), followed by sample L3 (18.4 mg/g). On the other hand, the concentration of epicatechin in studied samples is much higher for L3 and MG (23.5 and 28.2 mg/g) or significantly lower, for example, the lowest concentration of epicatechin was obtained in HM (3.85 mg/g). In general, high content of epicatechin was found in non-Chinese teas. For this group of samples, the concentration of this flavonoid was in range from 7.16 to 16.4 mg/g. On the other hand, Vodnar and Socaciu reported the concentration of catechin, epicatechin and EGCG as 2.14, 3.05 and 19.64 mg/g in the water extract of selenized

green tea being originally from Shaanxi China [40]. This highlights the fact that the samples are varied and thus the results.

It was postulated that Se-enriched green tea had higher antioxidant activities in comparison with regular one due to the combination of selenium with tea polyphenols and polysaccharides [25–27]. A number of chemical assays have been developed to measure the radical scavenging capacity, reducing power, chelating ability and other specific attributes of antioxidants in food samples [41, 42]. These methods vary in terms of antioxidant mechanism, redox potential, type of substrate and chemical conditions. Due to the lack of standard quantification method for this purpose, there is the strongly recommendation to use at least two assays which measured different aspects of the antioxidant behaviors to generate a complete antioxidant profile [41].

In this study, the antioxidant properties of all green tea extracts were determined using Folin–Ciocalteu method (FC), scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydroxyl radical scavenging (OH) and cupric reducing ability (CUPRAC) assays. The obtained results are summarized in Table 2. The decrease in the absorbance of DPPH radical due to the scavenging activity of the extracts of selected teas is also presented in Fig. 1S. It was found that Ziyang green tea does not show the highest antioxidant activity in comparison to others. The extracts of teas from Yunnan Province (like Y1 or Y2) were characterized by higher values of Trolox equivalent. Moreover, the results obtained for teas from neighboring provinces (J, S, F) are similar to those obtained for selenium teas from the Ziyang area. The lowest ability to scavenge free radicals was observed for Ceylon (C) tea, followed by Chinese teas marked as X1, X2, X3 and X4 and M. For other Chinese teas, similar results to Ziyang teas were obtained. Molan et al. [27] reported almost similar percentage of DPPH discoloration by the 1% extracts of Chinese green tea with 0.13 mg Se/kg and that with higher in its content (1.4 mg Se/kg)—69.4% and 71.5%, respectively.

Hydroxyl radical scavenging assay was also used to examine the antioxidant activities of teas. The main difference between this assay and the ability to scavenge the DPPH radical is the fact that hydroxyl radicals are found in the living systems, while the 2,2-diphenyl-1-picrylhydrazyl radicals are synthetic. Teas marked as L2 and MG (both originated in Ziyang Province) showed the highest ability to scavenge the hydroxyl radical (99.6% and 99.9%, respectively). A slightly lower value was obtained for a sample from an unknown Chinese province X5. Higher value than 90% were also obtained for L4 (one of selenized teas), Y3 (Yunnan Province) and X6 (unknown origin). Surprisingly, for the other two samples from the Yunnan province, the values were significantly lower than for Y3, suggesting a different location for these crops within one province. The

Table 2 The antioxidant activity of studied green tea infusions

Tea	FC [mg GA·L ⁻¹]	CUPRAC [mmol TR·L ⁻¹]	DPPH [mmol TR·L ⁻¹]	OH radicals [%]
Chinese green teas from Zhejiang County				
Lung Ching (L1)	611 ± 4 ^a	39.8 ± 1.4 ^a	30.6 ± 0.5 ^a	82.1 ± 1.23 ^a
Lung Ching (L2)	543 ± 6 ^b	38.2 ± 0.4 ^a	27.7 ± 0.4 ^b	99.6 ± 1.37 ^b
Lung Ching (L3)	568 ± 3 ^b	39.1 ± 0.8 ^a	30.3 ± 1.1 ^a	58.5 ± 0.986 ^c
Lung Ching (L4)	524 ± 7 ^b	37.9 ± 2.0 ^a	29.9 ± 0.3 ^a	95.8 ± 1.41 ^d
Misty Green (MG)	646 ± 9 ^c	40.1 ± 3.1 ^a	32.5 ± 1.0 ^a	99.9 ± 1.21 ^b
High Mountain (HM)	403 ± 9 ^d	31.6 ± 2.0 ^b	24.8 ± 1.2 ^c	73.9 ± 0.996 ^e
Chinese green teas from other provinces				
Jiangsu (J)	641 ± 6 ^c	41.2 ± 2.1 ^a	30.3 ± 0.7 ^a	59.4 ± 0.872 ^c
Sinciang (S)	630 ± 8 ^c	39.5 ± 1.0 ^a	30.2 ± 0.2 ^a	65.0 ± 0.932 ^f
Fujian (F)	391 ± 3 ^d	31.6 ± 0.9 ^b	23.7 ± 1.1 ^c	64.2 ± 0.865 ^f
Yunnan (Y1)	831 ± 5 ^e	50.9 ± 1.1 ^c	35.7 ± 0.3 ^d	66.7 ± 0.781 ^f
Yunnan (Y2)	679 ± 7 ^f	51.7 ± 1.8 ^c	37.5 ± 0.5 ^d	14.6 ± 0.231 ^g
Yunnan (Y3)	609 ± 6 ^a	39.9 ± 2.2 ^a	29.9 ± 0.6 ^a	93.5 ± 1.13 ^d
Unknown (X1)	442 ± 2 ^g	28.1 ± 0.8 ^b	23.4 ± 0.4 ^c	75.6 ± 0.876 ^e
Unknown (X2)	488 ± 9 ^h	29.5 ± 0.9 ^b	22.4 ± 1.3 ^c	78.1 ± 0.654 ^e
Unknown (X3)	413 ± 3 ^d	30.7 ± 0.7 ^b	23.7 ± 2.1 ^c	76.4 ± 0.591 ^e
Unknown (X4)	433 ± 2 ^g	29.1 ± 0.3 ^b	21.1 ± 0.3 ^c	23.6 ± 0.321 ^h
Unknown (X5)	1010 ± 9 ⁱ	60.8 ± 2.7 ^d	42.7 ± 0.4 ^e	97.6 ± 1.11 ^b
Unknown (X6)	1020 ± 9 ⁱ	55.4 ± 2.1 ^c	37.6 ± 1.1 ^d	93.4 ± 1.12 ^d
Mixed (M)	461 ± 8 ^g	27.7 ± 0.3 ^b	22.2 ± 0.6 ^c	51.2 ± 0.872 ⁱ
Other green teas from Asia				
Japan (JP1)	524 ± 5 ^b	34.8 ± 0.6 ^b	29.2 ± 0.2 ^a	47.2 ± 0.373 ^j
Japan (JP2)	590 ± 7 ^a	36.6 ± 0.5 ^a	28.9 ± 0.3 ^a	32.5 ± 0.245 ^k
Vietnam (V)	549 ± 9 ^b	35.6 ± 0.4 ^b	26.4 ± 0.4 ^b	67.5 ± 0.333 ^f
Azerbaijan (A)	491 ± 8 ^h	31.7 ± 0.4 ^b	26.2 ± 1.3 ^b	86.2 ± 0.527 ^l
India (I)	549 ± 8 ^b	34.9 ± 1.3 ^b	26.0 ± 0.5 ^b	13.0 ± 0.103 ^g
Sri Lanka, Ceylon (C)	457 ± 7 ^g	27.6 ± 0.2 ^b	19.6 ± 0.6 ^f	70.5 ± 0.202 ^m

Different letters in each column indicate a difference at a significance level of $p=0.05$

lowest ability to scavenge the hydroxyl radical was obtained for India tea (13.0%).

The highest reducing activity in CUPRAC assay was obtained for Unknown X6 and Yunnan teas; Y1 and Y2, all of the values were higher than 50 mmol Tr/L. The Zhejiang teas were characterized by middle values ranged from 31.6 (HM) to 40.1 Tr/L (MG). The lowest reducing activity was observed for mixed Chinese tea (M) and Ceylon tea (C). The Folin–Ciocalteu method, also known as the total polyphenol content, was also used. The values obtained for selenized teas were in range from 403 mg GA/L (HM) to 646 mg GA/L (MG). In general, these were middle values. The highest one was obtained for two unknown Chinese teas X5 and X6 (values higher than 100 mg GA/L). It was reported that regular green tea showed higher polyphenol content than selenized one [24, 25]. Xu et al. [35] showed that tea plant enriched with sodium selenite was characterized by lower total polyphenol content than regular green tea, while the same kind of tea but Se-enriched with fertilizer (the authors

did not show which one) contained higher total polyphenols content in comparison to regular green tea. Generally, the effect of chemical stress induced by selenium treatment can enhance the antioxidant activity of plant extracts [38, 43]. However, the opposite results are also reported [44]. The two studied varieties of apples were differently affected: one of them mostly showed a higher antioxidant activity (measured with TEAC) resulting from the biofortification and independently of the selenium form and the level of application, whereas the values for the second was only slightly influenced by the biofortification. Moreover, some selenium compounds and their metabolites can act as antioxidants, pro-oxidants or both depending of specific conditions [45]. The antioxidant properties of selenium compounds in DPPH assay decrease in order: MetSeCys > Se (VI) ≥ SeMet > Se (IV) [46].

Based on the collected data, attempts were made to group the analyzed teas, based on their antioxidant capacities, as shown in Fig. 2. It can be seen that values obtained for

Fig. 2 An attempt to group the results of studies on the antioxidant capacity of teas: **a** Folin-Ciocalteu assay; **b** CUPRAC; **c** DPPH; **d** Hydroxyl radicals scavenging

Zhejiang teas vary and those obtained for neighboring provinces are close. Yunnan teas formed separate group as well as unknown Chinese teas (samples X1-X4). What is interesting that samples marked as X5 and X6 differ from other unknown Chinese teas. This suggests that they could have their origin in other province than the rest. The differences between the J (Jiangsu) and F (Fujian) teas can be surprising, because both of them came from neighboring to Zhejiang provinces, where soil is rich in selenium. However sample J is rather more similar to sample S (Xinjiang) in terms of antioxidant activities. All of them showed some similarities only in OH scavenging activity.

Principal component analysis (PCA) was also performed between each two variables and no correlation between the selenium content and antioxidant activities was found (Fig. 2S). The correlation between the content of selenium, catechins, pH and the antioxidant capacity of the

studied teas was examined also using linear correlation. The obtained values of Pearson's coefficients are presented in the Table 1S. No relationship was found between the content of any of the detected forms of selenium and the antioxidant capacity of the studied teas. The highest value of Pearson's coefficient was obtained for the SeMeSeCys content and antioxidant capacity in FC assay (0.578) and CUPRAC assay (0.559) which suggests a moderate correlation between variables. For the other selenium compounds (SeMet and SeCys), negative values of correlation coefficients between their contents and the antioxidant activity of teas were obtained. This suggests an inverse relationship to the expected one. Sotek et al. [47] found the highest Pearson positive coefficient between selenium content and ABTS radicals scavenging only for a few of the tested medical plants, such as *Centaurea cyanus* (0.656), *Plantago lanceolata* (0.689) and *Majorana hortensis* (0.691). Only for

that first plant, a significant correlation between Se level and the results from FC assay was found (0.626).

No correlation was found also between the selenium content and catechin concentration in the studied infusions. In case of SeMet, negative correlations between its concentration and the EPI and EGCG content were established (-0.656 and -0.564, respectively).

Conclusion

Green tea beverages have been continuously considered as a medicine because of their polyphenols. Additionally, Chinese green teas, particularly produced in Ziyang County located in the southwest of Shaanxi Province of China, are advertised as they are rich in selenium. No wonder that consumers eagerly reach for such a tea, believing in its additional health-promoting properties. However, green tea extracts from other Chinese provinces, also contain considerable amounts of organic selenium forms. For example, teas from Yunnan province (samples Y1-Y3) contain higher content of MeSeCys, which is considered as a selective modulator of the antitumor activity. The content of catechins in studied water tea infusions also varies. HPLC analysis of catechins for quality control and identification of Ziyang green tea was done using 50% ethanol solution for extraction (He et al. 2015). Such conditions provide higher content of all studied polyphenols (due to better solubility) but do not reflect household brewing process.

The demands that Se-enriched green tea extract had higher polyphenols content and higher antioxidant activities in comparison with regular one do not always work. Selenocompounds and other non-phenolic components may play also a vital role in the antioxidant activity of that extract. It should be remembered that selenium compounds can act as antioxidants or pro-oxidants and when the plants were subjected to higher Se concentration, the antioxidant activity may decrease.

A number of difficulties are also encountered when trying to compare the published results. There is no precise data on how the samples of selenium teas were obtained and how they were cultivated (with Se fertilizers or not). In summary, it is worth drinking and enjoying green tea, but it is not worth running around tea houses and looking for selenium green tea.

Compliance with ethical standards

Conflict of interest The Author has no conflict of interest to disclose.

Compliance with ethics requirements This study does not involve any human or animal testing.

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References

1. Namal Senanayake SPJ (2013) Green tea extract: chemistry, antioxidant properties and food applications—a review. *J Funct Foods* 5:1529–1541. <https://doi.org/10.1016/j.jff.2013.08.011>
2. Maiti S, Nazmeen A, Medda N, Paqtra R, Ghosh TK (2019) Flavonoids green tea against oxidant stress and inflammation with related human diseases. *Clin Nutr Exp* 24:1–14. <https://doi.org/10.1016/j.clnex.2018.12.004>
3. Costa C, Tsatsakis A, Manoulakis C, Teodoro M, Briguglio G, Caruso E, Tsoukalas D, Margina D, Dardiotis E, Kouretas D, Fenga C (2017) Current evidence on the effect of dietary polyphenols intake on chronic diseases. *Food Chem Toxicol* 110:286–299. <https://doi.org/10.1016/j.fct.2017.10.023>
4. Mancini E, Beglinger C, Dreweb J, Zanchia D, Undine E, Langa UE (2017) Green tea effects on cognition, mood and human brain function: a systematic review. *Phytomedicine* 34:26–37. <https://doi.org/10.1016/j.phymed.2017.07.008F>
5. Kerio LC, Wachira FN, Wanyoko JK, Rotich MK (2013) Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars. *Food Chem* 136:1405–1413. <https://doi.org/10.1016/j.foodchem.2012.09.066>
6. Grzesik M, Naparło K, Bartosz G, Sadowska-Bartoszyńska I (2014) Antioxidant properties of catechins: comparison with other antioxidants. *Food Chem* 241:480–492. <https://doi.org/10.1016/j.foodchem.2017.08.117>
7. Farkhondeh T, Pourbagher-Shahri AM, Ashrafzadeh M, Folgado SL, Rajabpour-Sanati A, Khazdair MR, Samarghandian S (2020) Green tea catechins inhibit microglial activation which prevents the development of neurological disorders. *Neural Regen Res* 15:1792–1798. <https://doi.org/10.4103/1673-5374.280300>
8. Musial C, Kuban-Jankowska A, Górska-Ponikowska M (2020) Beneficial properties of green tea catechins. *Int J Mol Sci* 21:1744–1755. <https://doi.org/10.3390/ijms21051744>
9. He X, Li J, Zhao W, Liu R, Zhang L, Kong X (2015) Chemical fingerprint analysis for quality control and identification of Ziyang green tea by HPLC. *Food Chem* 171:405–411. <https://doi.org/10.1016/j.foodchem.2014.09.026>
10. Li W, He N, Tian L, Shi X, Yang X (2016) Inhibitory effects of polyphenol-enriched extract from Ziyang tea against human breast cancer MCF-7 cells through reactive oxygen species-dependent mitochondria molecular mechanism. *J Food Drug Anal* 24:527–538. <https://doi.org/10.1016/j.jfda.2016.01.005>
11. Lu L, Chai L, Wang W, Yuan X, Li S, Cao C (2017) A selenium-enriched Ziyang green tea polysaccharide induces bax-dependent mitochondrial apoptosis and inhibits TGF- β 1-simulated collagen expression in human keloid fibroblast via NG2 inactivation. *Biol Trace Elem Res* 176:270–277. <https://doi.org/10.1007/s12011-016-0827-8>

12. Tian H, Ma Z, Chen X, Zhang H, Bao Z, Wei C, Xie S, Wu S (2016) Geochemical characteristics of selenium and its correlation to other elements and minerals in selenium-enriched rocks in Ziyang County, Shaanxi Province, China. *J Earth Sci* 27:763–776. <https://doi.org/10.1007/s12583-016-0700-x>
13. Hu J, Yang F, Chen L, Hu Y, Hu Q (2003) Effect of selenium on increasing the antioxidant activity of tea leaves harvested during early spring tea producing season. *J Agric Food Chem* 51:1081–1084. <https://doi.org/10.1021/jf020940y>
14. Zhang B, Wei Y, Yan S, Shi H, Nie Y, Zou G, Zhang X, Luo L (2019) Characterization of selenium accumulation of different rice genotypes in Chinese natural seleniferous soil. *Plant Soil Environ* 65:15–20. <https://doi.org/10.17221/603/2018-PSE>
15. Kieliszek M, Błażej S (2016) Current knowledge on the importance of selenium in food for living organisms: a review. *Molecules* 21:609–618. <https://doi.org/10.3390/molecules21050609>
16. Tan LC, Nanchaiah YV, van Hullebusch ED, Lens PNJ (2016) Selenium: environmental significance, pollution, and biological treatment technologies. *Biotech Adv* 34:886–907. <https://doi.org/10.1016/j.biotechadv.2016.05.005>
17. Benstoeem C, Andreas Goetzenich A, Kraemer S, Borosch S, William Manzanara W, Hardy G, Christian Stoppe C (2015) Selenium and its supplementation in cardiovascular disease—what do we know? *Nutrients* 7:3094–3118. <https://doi.org/10.3390/nu7053094>
18. Aldosary BM, Sutter ME, Schwartz M, Morgan BW (2012) Case series of selenium toxicity from a nutritional supplement. *J Clin Toxicol* 50:57–64. <https://doi.org/10.3109/15563650.2011.641560>
19. Pyrzynska K (2014) Edible plants enriched with selenium. *J Agric Sci Technol A* 4:627–632. <https://doi.org/10.17265/2161-6256/2014.08.001>
20. Gammelgaard B, Jackson MI, Gabel-Jensen C (2011) Surveying selenium speciation from soil to cell—forms and transformations. *Anal Bioanal Chem* 399:1743–1763. <https://doi.org/10.1007/s00216-010-4212-8>
21. Sun GX, Liu X, Williams PN, Zhu YG (2010) Distribution and translocation of selenium from soil to grain and its speciation in paddy rice (*Oryza sativa* L.). *Environ Sci Technol* 44:6706–6711. <https://doi.org/10.1021/es101843x>
22. Pyrzynska K (2009) Selenium speciation in enriched vegetables. *Food Chem* 114:1183–1191. <https://doi.org/10.1016/j.foodchem.2008.11.026>
23. Hu QH, Xu J, Pan GX (2001) The effect of selenium sprays on green tea quality. *J Sci Food Agric* 81:1387–1390. <https://doi.org/10.1002/jsfa.952>
24. Hu QH, Pan GX, Zhu JC (2001) Effect of selenium on green tea preservation quality and amino acid composition of tea protein. *J Horticult Sci Biotech* 76:344–346. <https://doi.org/10.1080/14620316.2001.11511374>
25. Xu J, Zhu SG, Yang FM, Cheng LC, Hu Y, Hu Q (2003) Effect of selenium on increasing the antioxidant activity of tea leaves harvested during the early spring tea producing season. *J Agricult Food Chem* 51:1081–1084. <https://doi.org/10.1021/jf020940y>
26. Yu F, Sheng J, Xu J, An X, Hu Q (2007) Antioxidant activities of crude tea polyphenols, polysaccharides and proteins of selenium-enriched tea and regular green tea. *Eur Food Res Technol* 225:843–848. <https://doi.org/10.1007/s00217-006-0490-y>
27. Molan AL, Flanagan J, Wei W, Moughan PJ (2009) Selenium-containing green tea has higher antioxidant and prebiotic activities than regular green tea. *Food Chem* 114:829–835. <https://doi.org/10.1016/j.foodchem.2008.10.028>
28. Sentkowska A, Pyrzynska K (2018) Hydrophilic interaction liquid chromatography in the speciation analysis of selenium. *J Chromatogr B* 1074–1075:8–15. <https://doi.org/10.1016/j.jchro.2018.01.006>
29. Wang Z, Gao Y (2001) Biogeochemical cycling of selenium in Chinese environment. *App Geochem* 16:1345–1351. [https://doi.org/10.1016/S0883-2927\(01\)00046-4](https://doi.org/10.1016/S0883-2927(01)00046-4)
30. Pyrzynska K, Pękal A (2013) Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate antioxidant capacity of food samples. *Anal Methods* 55:4288–4295. <https://doi.org/10.1039/C3AY40367J>
31. Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenolics and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 299:152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
32. Apak R, Güclu K, Özyürek M, Karademir SE, Ercag E (2006) The cupric reducing antioxidant capacity and polyphenolic content of some herbal teas. *Int J Food Sci Nutr* 57:292–304. <https://doi.org/10.1080/09637480600798132>
33. Smirnoff N, Cumbes QJ (1987) Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* 28:1057–1060. [https://doi.org/10.1016/0031-9422\(89\)80182-7](https://doi.org/10.1016/0031-9422(89)80182-7)
34. Zhang HJ, Gao PF, Guo XF, Wang H (2013) Simultaneous determination of selenium containing amino acids and their sulphur-analogues in green tea and gynestemma pentaphyllum infusion with high performance liquid chromatography based on fluorescence labelling. *Microchem J* 110:192–197. <https://doi.org/10.1016/j.microc.2013.01.016>
35. Xu J, Zhu SG, Yang FM, Cheng LC, Hu Y, Pan G (2003) The influence of selenium on the antioxidant activity of green tea. *J Sci Food Agric* 83:451–455. <https://doi.org/10.1002/jsfa.1405>
36. Gąsecka M, Młeczek M, Siwulski M, Niedzielski P (2016) Phenolic composition and antioxidant properties of *Pleurotus ostreatus* and *Pleurotus eryngii* enriched with selenium and zinc. *Eur Food Res Technol* 242:723–732. <https://doi.org/10.1007/s00217-015-2580-1>
37. Tian M, Xu X, Liu Y, Xie L, Pan S (2016) Effect of Se treatment on glucosinolate metabolism and health-promoting compounds in the broccoli sprouts of three cultivars. *Food Chem* 190:374–380. <https://doi.org/10.1016/j.foodchem.2015.05.098>
38. Guardado-Felix D, Sarna-Saldivar SO, Cuevas-Rodriguea EO, Jacobo-Velázquez D, Gutiérrez-Urbe JA (2017) Effect of sodium selenite on isoflavonoid contents and antioxidant capacity of chickpea (*Cicer arietinum* L.) sprouts. *Food Chem* 226:69–74. <https://doi.org/10.1016/j.foodchem.2017.01.046>
39. Granato D, Santos JS, Maciel LG, Nunes DS (2016) Chemical perspective and criticism on selected analytical methods used to estimate the total content of phenolic compounds in food matrices. *TrAC Trends Anal Chem* 80:266–279. <https://doi.org/10.1016/j.trac.2016.03.010>
40. Vodnar DC, Socaciu C (2014) Selenium enriched green tea increase stability of *Lactobacillus casei* and *Lactobacillus plantarum* in chitosan coated alginate microcapsules during exposure to simulated gastrointestinal and refrigerated condition. *LWT Food Sci Technol* 57:406–411. <https://doi.org/10.1016/j.lwt.2013.12.043>
41. Apak R, Gorinstein S, Böhm V, Schaich KM, Özyürek MO, Güçlü K (2013) Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). *Pure App Chem* 85:957–998. <https://doi.org/10.1351/PAC-REP-12-07-15>
42. Tan JBL, Lim YY (2015) Critical analysis of current methods for assessing the in vitro antioxidant and antibacterial activity of plant extracts. *Food Chem* 172:814–822. <https://doi.org/10.1016/j.foodchem.2014.09.141>
43. Dai C, Huang J, Cui X, Qui L, Liu C, Guan H, Qu Y, Yang Y (2019) Selenium enhances antioxidant capacity by improving denichine content in *Panax notoginseng*. *Ind Crops Prod* 131:250–256. <https://doi.org/10.1016/j.indcrop.2019.01.058>
44. Groth S, Budke C, Neugart S, Ackermann S, Kappenstein FS, Daum D, Rohn S (2020) Influence of a selenium biofortification

- on antioxidant properties and phenolic compounds of apples (*Malus domestica*). *Antioxidants* 9:187. <https://doi.org/10.3390/antiox9020187>
45. Lee KH, Jeong D (2012) Bimodal actions of selenium essential for antioxidant and toxic pro-oxidant activities: the selenium paradox. *Mol Med Rep* 5:299–304. <https://doi.org/10.3892/mmr.2011.651>
 46. Sentkowska A, Pyrzynska K (2019) Investigation of antioxidant activity of selenium compounds and their mixtures with tea polyphenols. *Mol Biol Rep* 46:3019–3024. <https://doi.org/10.1007/s11033-019-04738-2>
 47. Sotek Z, Białecka B, Pilarczyk B, Drozd R, Pilarczyk R, Tomza-Marciniak A, Kruzhel B, Lysak H, Bąkowska M, Vovk S (2019)

Antioxidant activity and selenium and polyphenols content from selected medicinal plants natives from various areas abundant in selenium (Poland, Lithuania, and western Ukraine). *Processes* 7:878. <https://doi.org/10.3390/pr7120878>

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