ORIGINAL ARTICLE



Investigation of antioxidant activity of selenium compounds and their mixtures with tea polyphenols

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Abstract

The antioxidant interactions between selenium species and tea polyphenols were investigated using 1,1-diphenyl-2-pic-ryl-hydrazyl (DPPH) radicals, cupric reducing antioxidant capacity (CUPRAC) and Folin–Ciocalteu (FC) assay. Se(IV) exhibited the lowest antioxidant properties in comparison to other selenium compounds in all assays. The highest reducing power was obtained for SeMet, while the highest ability to scavenging DPPH radicals for MeSeCys. The results obtained experimentally for the mixtures containing selenium species and green or black tea infusion were compared with theoretical values calculated by adding up the effects of both individual components analyzed separately. The results obtained from each assay clearly show that observed effect is not additive. In almost every case the theoretical value of antioxidant capacity was significantly higher from that obtained from the activity of the binary mixture of black tea infusion with selenium compound decreased in the order: SeMet > Se(IV) > Se(VI) > MeSeCys, while for similar mixtures with green tea infusion: MeSeCys > Se(VI) > SeMet ~ Se(IV).

Keywords Selenium compounds · Green tea · Black tea · Antioxidant activity

Abbreviations

BT Black tea
GT Green tea
Se(IV) Selenite
Se(VI) Selenite

MeSeCys Methylselenocysteine SeMet Selenomethionine

Introduction

Selenium is widely known as an essential nutrient, which is linked with some serious conditions like cancer, cardiovascular and inflammatory diseases [34]. It plays an important role in many metabolic pathways such as thyroid hormone metabolism and antioxidant defense systems [39]. However, the knowledge of the total selenium amount is not only important, whereas the content of an appropriate selenium

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species that is present in particular food or dietary supplements is crucial. It is known that organic forms of selenium are less toxic and more bioavailable than its inorganic forms [22]. Some plants exposed to selenium have ability to transform its inorganic forms into bioactive organic compounds. It has important implications for human nutrition and health as the main source of selenium in human beings is their diet [21, 22, 31, 33]. Selenoaminoacids are widely present in different vegetables such as onion, garlic, broccoli (methylselenocysteine) as well as in rice (selenomethionine) [21, 31].

In the recent years a new potential source of selenium has appeared. One geographically specific selenium-enriched Ziyang tea (*Camellia sinensis L.*) is widely distributed in the seleniferous region, in Ziyang County, China [10]. The infusion of that green tea in vitro experiments showed higher antioxidant activities in comparison to the regular one [15, 36]. According to Yu et al. [36] the combination of selenium with tea polyphenols was responsible for the observed effects. However, Se-enriched tea has also significantly higher content of phenolic compounds, which are the most abundant antioxidants in the diet [29]. Some selenium compounds or their metabolites can act as antioxidants, prooxidants or both depending of specific conditions [23, 34, 39]. Selenoaminoacids can also provide antioxidant benefits by acting as a source of Se for synthesis of selenium-dependent



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antioxidants and repair proteins such as gluthathione peroxidases, thioredoxin reductases and methionine sulfoxide [23]. From the other side, Tsopelas et al. [32] reported that among several studied selenium compounds only urea derivatives, such as selenourea and dimethylselenourea, exhibited considerable antioxidant properties according to their oxidation potential. The investigation of combining polyphenol and selenium functionalities and preparation several analogues of polyphenolic acid and phenylselenoethanol was reported by Lin et al. [12]. However, the free radical scavenging profiles of these Se-containing polyphenolic acid esters evaluated using DPPH method were lower than analogous caffeic acid ester.

The aim of this study was to evaluate the antioxidant interactions between selected selenium species and tea polyphenols employing three methods: scavenging of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, cupric reducing antioxidant capacity (CUPRAC) and Folin–Ciocalteu assay. In particular, we have compared the antioxidant capacity of the mixtures containing green tea or black tea polyphenols and selected selenium species with that of the single components measured individually.

Materials and methods

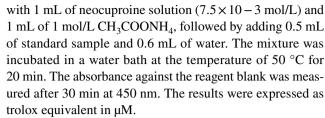
Reagents and samples

The commercial standards of sodium selenate, sodium selenite, selenomethionine, methylselenocysteine as well as the standards of green and black tea infusions were purchased from Merck-Sigma (Steinheim, Germany). Methanol was purchased from Merck (Darmstadt, Germany). Ultra-pure water from Mili-Q system (Milipore, Bedford, MA,USA) with electrical resistivity of $18\,\Omega$ cm $^{-1}$ was used in all experiments. Stock solutions of selenium species, tea standards and their mixtures were prepared in water. Each sample were analyzed in triplicate.

Instrumentation

The DPPH assay was applied to estimate the radical-scavenging ability of green tea extract (Dróżdż, Sentkowska & Pyrzyńska, [2]). 0.1 mL of the appropriate mixture of components or individual compounds was mixed with 2.4 mL of DPPH solution $(9 \times 10^{-5} \text{ mol/L})$ in methanol. The change of absorbance at 518 nm was recorded after 30 min. The antioxidant activity was expressed in terms of EC50 value, i.e. the concentration (in μ M) required to scavenge 50% of DPPH radicals and in terms of trolox equivalent (TEAC).

The cupric ion reducing antioxidant capacity of all extracts was determined according to the method of Özyürek et al. [18]. 1 mL of CuCl₂ solution (0.01 mol/L) was mixed



The Folin–Ciocalteu assay was conducted based on the method described by Sigleton et al. [30] with some changes. 1 mL of a sample solution was mixed with 0.1 mL of FC reagent and 0.9 mL of water. After 5 min, 1 mL of sodium carbonate (7% w/v) and 0.4 mL of water were added and 30 more min was allowed for stabilization of the blue color formed. The absorbance against blank was measured at 765 nm and the results were expressed as gallic acid equivalent (GAE) in mM.

Statistical analysis

The results are from at least three independent experiments and presented as average \pm standard deviation. One-way ANOVA and Turkey test was used to determine the difference of means. Significance was defined at P values < 0.05.

Results and discussion

There are many well-known methods that can be used for study the antioxidant activity [26]. The choice which is appropriate for particular samples should be based on the chemistry of the compounds of interest. Some methods are concerned with electron or radical screening, whereas other assays are focused on reducing ability. In this study three assays differ in the reaction mechanism were used. The antioxidant activity were evaluated using scavenging of a model radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and cupric reducing antioxidant capacity (CUPRAC). The reducing power of the studied compounds was also evaluated by Folin-Ciocalteu (FC) assay. This method is often called "total phenolic content", but FC reagent is not specific for the phenolic compounds as other compounds give elevated apparent phenolic content [1, 4]. The chemistry behind FC assay counts on a single electron transfer of electrons in alkaline medium from phenolic compounds and other reducing species to molybdenum, forming blue complexes that can be monitored spectrophotometrically. It is assumed that the antioxidant activity is equal to the reducing power of the sample.

Antioxidant activity of single selenium species

All measurements were made for the same concentration of the components equals to 10 mg/L. The obtained results



were expressed by TEAC index (Trolox equivalent antioxidant capacity), which expresses antioxidant capacity of a given substance as equivalent to a certain Trolox concentration (for DPPH and CUPRAC assays) and in GAE index (gallic acid equivalent). The solution of α -tocopherol at the same concentration was used for comparison. The results are presented in Table 1. The obtained results in DPPH assay for Se(VI), selenomethinine (SeMet) and methylselenocysteine (MeSeCys) are comparable and only for Se(IV) was significantly lower. α-tocopherol showed higher antioxidant activity in comparison to all studied forms of selenium. The EC50 parameters (the effective concentration necessary to reduce 50% of DPPH radicals) for selenium compounds were also experimentally quantified [24]. Lower value of EC50 parameter means higher antioxidant activity. The results presented in Table 1 confirmed that selenium compounds are weaker antioxidants than α -tocopherol. Se(IV) exhibited the lowest ability for scavenging the DPPH radicals, while the highest one was observed for SeMeCys. The results of one-way ANOVA followed by Turkey's test indicated that TEAC index for other selenium species does not vary significantly (p > 0.05).

CUPRAC method is based on reduction of Cu(II) to Cu(I) at neutral pH by antioxidants present in the sample utilizing copper(II)-neocuproine reagent as the chromogenic oxidant [18]. In this assay only Se(VI) showed higher antioxidant activity than α -tocopherol and it was the highest value from all studied compounds (Table 1). In CUPRAC method, similar to DPPH method, Se(IV) exhibits the lower antioxidant properties in comparison to other selenium compounds. The reducing capacity for selenium compounds increases in the order: Se(IV) < SeMetCys < Se(VI) < SeMet (Table 1). SeMet is easily oxidized and the first appearing degradation product is selenomethionoine selenoxide, which then is converted via the deaminated selenoxide to methane seleninic acid and selenite [7].

The reducing power of studied compounds and their mixtures was also evaluated by Folin–Ciocalteu assay [30]. Taking into consideration that the FC reagent reacts also with several non-phenolic compounds such as reducing sugars, ascorbic acid, some transition metals and reducing amino

Table 1 The antioxidant activities of selenium species and α -tocopherol

Compound	DPPH		CUPRAC	FC
	TEAC (µM)	EC ₅₀ (μM)	$TEAC\ (\mu M)$	GAE (mM)
Se(IV)	65.02 ± 3.2 ^a	1.36 ± 0.07^{a}	8.75 ± 0.43^{a}	0.41 ± 0.020^{a}
Se(VI)	216.0 ± 10.8^{b}	0.98 ± 0.04^{b}	114.2 ± 5.4^{b}	3.93 ± 0.196^{b}
SeMet	215.1 ± 10.1^{b}	1.00 ± 0.04^{b}	$181.0 \pm 9.0^{\circ}$	4.43 ± 0.221^{c}
MetSeCys	223.2 ± 11.0^{b}	0.30 ± 0.02^{c}	109.4 ± 5.4^{b}	2.33 ± 0.116^{d}
α-tocopherol	$294.03 \pm 8.3^{\circ}$	$0.15\pm0.03^{\rm d}$	141.2 ± 7.2^{c}	20.3 ± 1.01^{e}

Concentration of all compounds was 10 mg/L. Values in each column bearing different superscript letters represent significant differences (p < 0.05, ANOVA test)

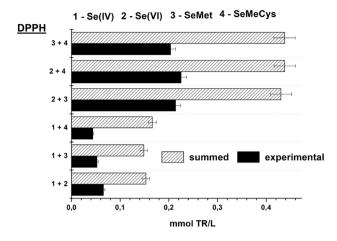
acids, this assay has been denominated as "Folin–Ciocalteau reducing capacity" [9]. As can be seen in Table 1, the values for selenium species are much lower than the value obtained for the same concentration of α -tocopherol. The reducing capacity for selenium compounds were increased in the order: Se IV) < Se(VI) < SeMetCys < SeMet. It can be noticed that Se(VI) exhibits higher reducing power than SeMetCys in FC assay in comparison to the results obtained in CUPRAC method. Although both used assays are based on the redox properties of studied compounds, but their differ in terms of reduction potentials, kinetics and experimental conditions.

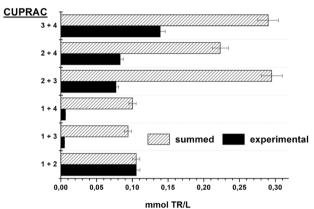
Antioxidant activity of selenium mixtures

Cultivation of plants enriched with selenium could be an effective way of producing selenium rich foodstuffs and thereby increase health benefits. In these samples selenium can exists, depending on the element form used for enrichment, in inorganic species (Se (IV) and Se (VI)) as well as selenoaminoacids (SeMet and MeSeCys), which are the products of inorganic selenium transformation [21, 22]. Thus, the mixture of different selenium forms can exist in the analyzed extracts. In our study the antioxidant activity of binary mixtures containing selenium species were investigated using previously described assays. The results obtained experimentally were compared with the theoretical values calculated by adding up the effects of individual components at the same concentration analyzed separately. This is commonly used method for study the antioxidant interaction between different kind of compounds [5, 19, 25]. Selenium in natural and food samples can exist in different chemical forms. It should be speculated that they can interact which each other. Such interaction can have the impact on antioxidant properties of whole sample that is analyzed. That is why studying the interaction between its different speciation forms is necessary. The obtained results are presented in Fig. 1.

For every analyzed mixtures in all used assays the calculated theoretical value of antioxidant activity was significantly higher than the corresponding value obtained







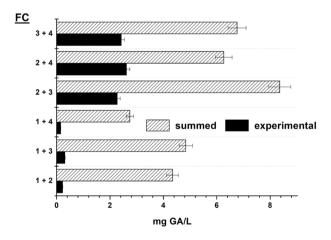


Fig. 1 Antioxidant activity of binary mixtures containing selenium compounds obtained using DPPH, CUPRAC and FC assays. Each compound at concentration 10 mg/L

from experiments. The presence of Se(IV) in the mixtures with SeMet and MeSeCys significantly decreased their antioxidant activity. This negative effect is most visible in CUPRAC and FC assays. Only the value in CUPRAC assay obtained experimentally for the mixture containing both inorganic selenium species and the theoretical value have no significant difference (p>0.05). To the best of our

knowledge there no reports about the results for individual selenium species as well as their mixtures in the antioxidant activity assays used in our studies. More studies are needed in order to clarify the observed effects, particularly a dose response at various concentrations.

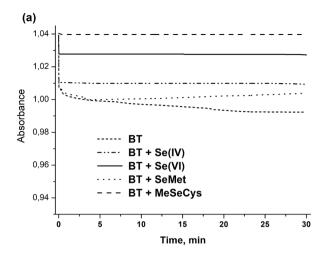
Antioxidant activity of selenium compounds in the mixtures with tea infusions

Recently, the research interest have been focused on Seenriched plants which could be used to supplement the human diet owing to its beneficial effect for human health [21, 22, 33]. Tea is the one of the most widely consumed beverages in the world with high content of polyphenolic compounds [6, 14, 28]. Antioxidative properties of these compounds are manifested particularly by their abilities to scavenge free radicals, inhibit their generation, chelate transition metal ions as well as to increase the activity of enzymes with antioxidative properties [13, 27]. There are some reports that tea supplemented in different ways (Se-enriched fertilizer, foliar spraying) exhibited significantly higher antioxidant activity than regular tea [15, 36–38]. It was reported that Se-enriched tea and regular tea exhibited EC50 values equals to 22.43 ± 0.13 mg/L and 23.65 ± 0.13 mg/L, respectively [36]. According to Molan et al. [15] for Se-enriched tea the DPPH free-radical scavenging percentage was $74.2 \pm 0.85\%$ and the value of $72.4 \pm 0.18\%$ was obtained for regular China tea. The antioxidant activity of the tea extract leaves in DPPH assay decreased in the order: tea obtained by fertilization by Se(VI) > fertilization by Se(IV) > α -tocopherol > regular tea [20]. Information regarding the comparison of phenolic content in these samples, which mainly contributed to the antioxidant activity of tea, are conflicting. Lower [36], higher [15] or similar [38] value have been reported.

On the other hand, there is well known that polyphenols as well as other bioactive compounds could interact between each other, which is clearly visible in the antioxidant activities of their mixtures [3, 8, 11, 20]. Mouly et al. [17] reported that over 300 remedies and mineral supplements are capable of interacting with food components through essential mineral antagonism, induction of inhibition of micronutrient metabolism. It can be predicted that also interactions between the polyphenols and selenium compounds may occur. Thus, in this study the effect of the addition of different selenium species to the samples of green (GT) and black (BT) tea infusions on their antioxidant activity was evaluated. The kinetic curves of scavenged DPPH radicals by BT and GT aqueous extracts and their binary mixtures with appropriate selenium species are presented in Fig. 2.

A fast initial decrease of DPPH absorbance followed by slow subsequent disappearance of this reagent can be observed from the presented plots. According to Villaño





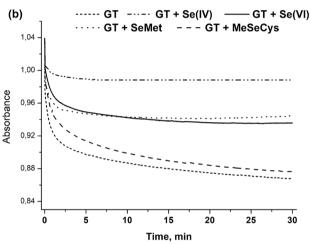


Fig. 2 The kinetic curves of scavenged DPPH radicals by **a** black tea (BT) **b** green tea (GT) infusions and their mixtures with selenium species. Concentration of each Se compounds equals to 10 mg/L

et al. [35], this fast step essentially refers to the electron-transfer process from a phenol molecule or its phenoxide anion to DPPH free radical, while the subsequent decay reflects the remaining activity of the oxidation-degradation products. In every case, the binary mixture of selenium compound and tea polyphenols showed lower antioxidant properties in comparions to alone tea infusion. However, different behaviour of black and green tea extracts in the presence of a given selenium compounds were observed. While MeSeCys decreased the antioxidant activity of BT almost to zero, the presence of this selenoaminoacid in the mixture with GT did not change the plot recorded for black tea infusion alone. The antioxidant activity of the binary mixture of black tea infusion with Se compound decreased in the order:

SeMet > Se(IV) > Se(VI) > MeSeCys, while this order for similar mixtures with green tea infusion was: MeSeCys > Se(VI) > SeMet ~ Se(IV). The results of one-way ANOVA followed by Turkey's test indicated that TEAC index

for BT mixtures with SeMet and Se (IV) does not vary significantly (p > 0.05). On the other hand such mixtures vary significantly when GT instead BT is used (p < 0.05). The same observation was made for the tea itself and its mixture with MeSeCys- the result is not statistically significant (p>0.05)when GT is used. The composition of green and black tea infusions differ from each other in terms of polyphenolic content as well as presence of other substances [14, 27]. The monomeric catechins and their derivatives are the major polyphenols in green tea. Black tea receives substantial oxidation, which results in the polymerization of catechins into theaflavins and thearubigins. There is the possibility that tea polyphenols and Se species form molecular complexes with antioxidant activity different from the activity of individual components. Moreover, these observed effects could also depend on the different ratios of bioactive components in the mixtures as well as the antioxidant mechanisms of these phytochemicals. Motomura et al. [16] found that the relative radical scavenging activity in DPPH assay of selenite-treated red clover extract decreased slightly, up to 0.2 mM of Se(IV), and then decreased strongly (about 79%) but in ryegrass extract no significant difference was observed with the increase of selenite content. Thus, further investigations are necessary to adress the function of phenolic antioxidative activity considering the beneficial effects of these compounds and selenium species. Isobolographic analysis, which take into account of relative dose of components, will be further used in the evaluation of antioxidant interactions.

Conclusions

These studies demonstrate the complex interactions between the different speciation forms of selenium and tea components. These interactions may have a significant influence on the mixture antioxidant activity. These results may be important from the point of view of supplementation of tea trees with selenium. More studies are needed to be performed for better describing the tea-selenium antioxidant interactions.

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Compliance with ethical standards

Competing interests The authors declare that there is no conflict of interest regarding the publication of this paper.

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