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Effect of Selenium on Increasing the Antioxidant Activity of Tea Leaves Harvested during the Early Spring Tea Producing Season

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This research was to determine the effect of foliar application of selenium on increasing the antioxidant activity of tea harvested during the early spring tea producing season using a α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging method and the linoleic acid system. The results showed that the radical scavenging ability of the tea extracts followed this order during the first 60 min: selenium-enriched tea obtained by fertilization with selenate > BHT > selenium-enriched tea obatined by fertilization with selenite > α-tocopherol > regular tea. Se-enriched tea obtained by fertilization with selenate exhibited the highest inhibition percentage of 84.29% at 30 min. Se-enriched tea extracts provided higher hydrogen-donating capabilities than regular tea and contrasts with BHT and α-tocopherol at the concentration of 100 μg of solids/mL of ethanol. There was a little change in the sequence of radical scavenging ability during the later 60 min: Se-enriched tea obtained by fertilization with selenate > Se-enriched tea obtained by fertilization with selenite > BHT > regular tea > α-tocopherol. The individual activity of tea extracts and references measured by the linoleic acid system showed that the tea extracts, BHT, and α-tocopherol manifested almost the same patterns of activity as the DPPH method. Tea enriched in selenium by fertilization with selenate still exhibited the highest inhibition activity of lipid oxidation, whereas α-tocopherol showed the lowest inhibition. The antioxidant activity of Se-enriched green tea harvested during the early spring tea producing season is enhanced compared to regular tea.

KEYWORDS: Selenium; Se-enriched green tea; antioxidant activity

INTRODUCTION

Selenium has received considerable attention as an essential micronutrient for both animals and human beings. It functions in the active site of glutathione peroxidase (GSH-Px) (*1*). Selenium has been reported to be associated with its antioxidant activity, anticancer effect, and other physiological functions (*2*, *3*). A high incidence of cancer and disease is attributed to a lower selenium level in the body. Therefore, the supply of selenium to livestock through forage and to human beings through food has been a practice adopted to prevent selenium deficiencies in several areas (*4*). Selenium is not classified as an essential nutrient for plants, and little attention has been paid to its role in higher plants. However, evidence has indicated that soil or foliar application of selenium can enhance the growth, yield, and quality of crops. Luo reported that the GSH-Px activity in the leaves of cucumber and the yield were significantly increased, whereas the content of malondialdehyde (MDA) decreased, after the soil was fertilized with selenium at 0.026 mg kg^{-1} (*5*). The application of selenocompounds at moderate dose can also strengthen the reduction of root and promote the tillering and yield of rice (*6*). Coffee plants treated with 10 μmol L^{-1} sodium selenite were taller, and their contents of caffeine and soluble sugars were augmented (*7*). Selenium can reduce the toxicity of ultraviolet light on plants to enhance their growth (*8*). Some authors found that the application of selenium to potatoes and garlic increased total and protein amino acid, soluble sugar, DNA, and RNA contents (*9*, *10*).

Tea is one of the most widely consumed beverages. Recently, much attention has been focused on the antioxidant activity, antimutagenic activity, and anticancer effects of green tea (*11*, *12*). Most of these effects have been attributed to the antioxidative and free radical scavenging properties of tea, particularly to its high polyphenolic compound content and microelements (*13*). However, the contents of active components in green tea vary according to geographical areas and seasons. Our previous studies have proved that foliar application of selenium-enriched fertilizer of sodium selenite or sodium selenate could significantly increase selenium and other nutrients contents in green tea harvested during the summer compared to regular tea (*14*, *15*). We also found that Se-enriched green tea harvested during

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the summer possessed a better sensory quality and exhibited higher antioxidant activity (*16*−*18*).

It was of interest to determine whether the antioxidant activity of Se-enriched green tea harvested during the early spring tea producing season is enhanced compared to regular green tea assessed by ferric thiocyanate (FTC) and α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging methods.

MATERIALS AND METHODS

Chemicals. Linoleic acid (99%) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). DPPH and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Butylated hydroxy(toluene) (BHT) and ascorbic acid were purchased from Nanjing Chemical Industry (Nanjing, China). Ammonium thiocyanate and other reagents were of analytic reagent grade.

Preparation of Se-Enriched Green Tea. The experiment was conducted on March 6, 2002, at the Institute of New Century Horticulture of Nanjing, Jangsu Province. The tea garden area of the test district amounted to 120 m^2. The control plants were sprayed with water, treatment 1 plants with fertilizer of sodium selenite, and treatment 2 plants with fertilizer of sodium selenate. Fertilizers of sodium selenite and sodium selenate were respectively dissolved in water at the same selenium content and volume for foliar spray. Se-enriched fertilizer at a concentration of 60 mg of Se L^{-1} and the rate of 75 g ha^{-1} was sprayed on old leaves of tea trees. The tea leaves with one newly growing bud and two young leaves were collected after 13 days of foliar application. The harvested tea leaves were washed with distilled water three times and immediately processed into commercial roasted green tea as detailed by Hu et al. (*16*).

Preparation of Green Tea Extracts. Green tea extracts were prepared by adding 80 mL of boiling distilled water to 1 g of tea leaves in a flask, followed by steeping in the water bath at 75 °C for 30 min. The extracts were filtered in a vacuum and then kept frozen until further use.

Assay of the Chemical Quality and Determination of Total Water-Soluble Solids. The contents of selenium, vitamin C, amino acid, and tea polyphenol (TPP) were determined according to the method of Hu et al. (*16*).

The determination of the total soluble solids of the extracts was conducted according to the von Gadow method with slight modification (*19*). A 50 mL extract was evaporated to dryness by boiling water bath and then further dried at 105 °C until a constant weight was obtained. The difference between two weights was obtained as the content of water-soluble solids.

Standard Green Tea Extracts Solution. A 40 mL extract was concentrated to dryness at 50 °C under vacuum, dissolved in ethanol at the concentration of 100 mg L^{-1}, and then stored in a refrigerator until further use.

Determination of Antioxidant Activity with the DPPH Radical Scavenging Method. The antioxidant activity of green tea extracts, BHT, and α-tocopherol was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (*19*). Ethanol solutions (2 mL) of the tea extracts, BHT, and α-tocopherol were placed in cuvettes, and 2 mL of a 2×10^{-4} mol L^{-1} ethanol solution of DPPH was added. Absorbance measurements commenced immediately. The decrease in absorbance 517 nm was determined continuously at 5 min intervals with a spectrophotometer until the absorbance stabilized (120 min). The inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (*20*).

Determination of Antioxidant Activity with the Ferric Thiocyanate (FTC) Method. Two milliliters of a 200 mg L^{-1} extract, 2 mL of 2.51% (w/v) linoleic acid in ethanol, 4 mL of 0.05 mol L^{-1} of phosphate buffer (pH 7.0), and 2 mL of distilled water were mixed in a vial of 10 mL with a screw cap and then kept in an 40 °C water bath in the dark; 0.1 mL of this mixture was added to 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% (w/v) ammonium thiocyanate. After 5 min, 0.1 mL of 0.02 mol L^{-1} ferrous chloride in 3.5% (v/v) hydrochloric acid was added to the mixture and then kept in an 40 °C water bath in the dark. The absorbance of the mixture was measured every 24 h. The FTC method was described in detail by Kikuzaki (*21*).

Statistics Analysis. One-way analysis of variance was performed on the data using SPSS (release 11.0). Student's *t*−LSD (least significant difference) ($P = 0.05$) was calculated to compare the means of the different samples.

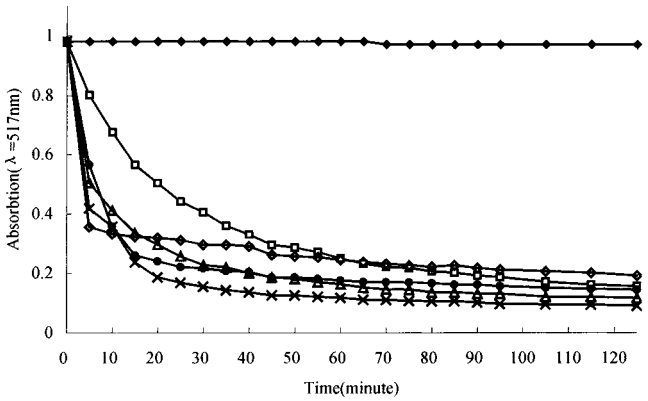

Figure 1. Antioxidant activity of different tea extracts as assessed by the DPPH method compared to BHT and α-tocopherol at 100 μg of soluble solids/mL of ethanol: (∗) blank; (□) regular tea; (△) tea fertilized with sodium selenite; (×) tea fertilized with sodium selenate; (◇) α-tocopherol; (●) BHT.

Table 1. Antioxidant Activity of Different Teas and Contrasts As Assessed with the DPPH Method

sample	inhibition (%) at 16 min	inhibition (%) at 30 min
regular tea	42.34 ± 1.25a	56.67 ± 1.06a
tea fertilized with sodium selenite	65.82 ± 1.52b	76.94 ± 0.57b
tea fertilized with sodium selenate	76.02 ± 1.99c	84.29 ± 0.98c
BHT	73.57 ± 1.11d	78.06 ± 1.22d
α-tocopherol	67.24 ± 1.89be	69.90 ± 0.79e

[a] Values are the means ± standard deviations ($n = 3$). Mean differences followed by the same letter are not significant at the 0.05 level.

RESULTS AND DISCUSSION

Effect of Selenium on the Antioxidant Activity of Tea As Assessed by the DPPH Radical Scavenging Radical Method. The results presented the decrease in absorbance of the DPPH radical due to the scavenging ability of soluble solids in the extracts of the different teas (**Figure 1**). Se-enriched teas were found to possess good DPPH-scavenging activity. All samples showed a rapid decrease in absorbance. However, Se-enriched tea obtained by fertilization with selenate exhibited the fastest rate over the first 10 min and regular tea, the slowest.

The radical scavenging abilities of the tea extracts decreased in the following the order during the first 60 min: tea fertilized with sodium selenate > BHT > tea fertilized with sodium selenite > α-tocopherol > regular tea. Significant differences were found within these groups. α-Tocopherol showed the fastest rate of radical scavenging ability, reaching its peak at 5 min and remaining stable to the end. The inhibition percentage of tea extracts and contrasts are shown in **Table 1**. The higher the inhibition rate, the greater the hydrogen-donating ability and thus the higher the antioxidant activity of the tea extract. It is optimal to calculate the inhibition percentage of antioxidant activity on DPPH at 30 min (*22*, *23*), although Yen and Duh measured the inhibition percentage at 16 min (*20*). In this paper inhibition percentages were determined both at 16 min and at 30 min. Data at two time points followed the same sequence.

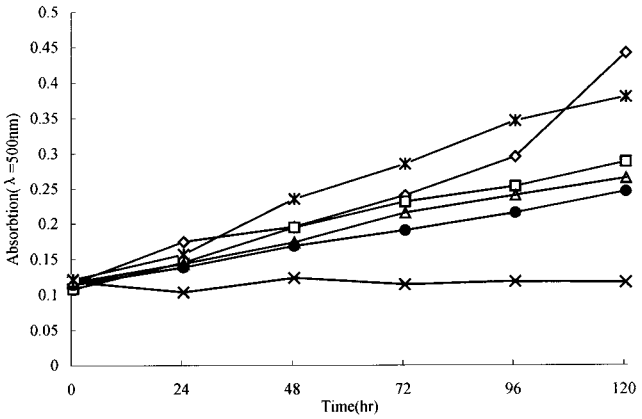

Figure 2. Antioxidant activity of different tea extracts as assessed by linoleic acid compared to BHT and α-tocopherol at 100 μg of soluble solids/mL of ethanol: (∗) blank; (□) regular tea; (△) tea fertilized with sodium selenite; (×) tea fertilized with sodium selenate; (◇) α-tocopherol; (●) BHT.

Table 2. Effect of Selenium on Selenium Content and Chemical Component of Tea Harvested during the Early Spring Tea Producing Season

sample	selenium (mg kg[1])	tea polyphenol (g kg^{-1})	vitamin C (g kg^{-1})
regular tea	0.265 ± 0.066a	240.92 ± 4.71a	2.45 ± 1.51a
tea fertilized with sodium selenite	7.555 ± 0.823b	231.02 ± 3.30b	2.89 ± 0.93b
tea fertilized with sodium selenate	10.610 ± 0.371c	209.35 ± 0.64c	2.58 ± 0.45c

[a] Values are means of three determinations ± standard deviations. Values followed by different letters are different ($P < 0.05$) from one another.

Tea fertilized with selenate provided the highest inhibition percentage of 84.29% at 30 min. On the basis of this analysis, either of the Se-enriched tea extracts exhibited a higher hydrogen-donating capability than the contrasts of BHT and α-tocopherol at the concentration of 100 μg of solids/mL of ethanol. There was a little change in the sequence of radical scavenging ability during the later 60 min: tea fertilized with sodium selenate > tea fertilized with sodium selenite > BHT > regular tea > α-tocopherol. However, no pronounced difference was observed between treatments 1 and 2. BHT and α-tocopherol exhibited lower antioxidant activity than Se-enriched tea extracts, which indicated Se-enriched tea extracts presented higher antioxidant activity than BHT and α-tocopherol.

Antioxidant Activity of Tea Extracts Assessed by the FTC Method. The FTC method was used to measure the amount of peroxide in the initial stages of lipid oxidation. The individual activity of tea extracts and references showed that a low absorbance value indicated a high level of antioxidant activity. All of the tea extracts, BHT, and α-tocopherol manifested almost the same patterns of activity as in the DPPH method (**Figure 2**): tea fertilized with sodium selenate > BHT > tea fertilized with sodium selenite > regular tea > α-tocopherol. Tea fertilized with sodium selenate still had the highest inhibition activity of lipid oxidation, whereas α-tocopherol showed the lowest inhibition. Although the absorption values of treatment 1 were lower than those of regular tea, no notable difference was found between them.

Few studies have been reported about the association between selenium content and antioxidant activity of plants (*24*). In this paper, the relationship between selenium content and the antioxidant activity of tea extract was also studied. The antioxidant activity of Se-enriched green tea harvested during the early spring tea producing season is enhanced compared to regular tea.

In previous studies from other authors, TPP was considered to be the only antioxidant contributing greatly to the potent antioxidant activity of tea. The antioxidant effect of TPP was concentration-dependent (*25*, *26*). In our studies, however, we found that regular tea with a higher content of TPP possessed the lowest antioxidant activity, whereas Se-enriched tea fertilized by selenate with the lowest TPP content showed the highest antioxidant activity (**Table 2**). Therefore, this implied nonphenolic components also play a vital role in enhancing the antioxidant activity of Se-enriched tea (*27*). More studies are needed to clarify the composition of the nonphenolic fraction such as selenocompounds and glycosides in Se-enriched tea and the mechanism of increasing the antioxidant activity.

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