JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Selenium on Preservation Quality of Green Tea during Autumn Tea-Processing Season

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Selenium-enriched green tea leaves were prepared by foliar applications of selenium-amended fertilizer during autumn season. The influence of selenium (Se) on preservation quality of tea during 4-month storage was determined. The results showed that the Se and vitamin C contents of green tea were significantly increased by selenium spraying during the autumn tea-producing season. The vitamin C content of Se-enriched green tea was higher and its decline was significantly slower during storage compared to normal green tea. However, there was no significant difference between the contents of chlorophyll and polyphenol of Se-enriched and regular tea. During the first 60 days, the color of green tea extract in Se-enriched green tea and normal tea showed no significant difference. However, the color of green tea extract in Se-enriched green tea was more stable compared with normal tea during the storage period. The sweetness and aroma of extracts of Se-enriched green tea were also significantly higher than that of normal tea and the bitterness of extracts of Se-enriched green tea was significantly lower compared with normal tea. These results showed that selenium application can slow the reduction of tea's major component and thus improve preservation qualities of green tea.

KEYWORDS: **Selenium; foliar spray; green tea; preservation quality**

INTRODUCTION

Tea is one of the most popular beverages in the world. The popularity of tea is increasing in western countries in recent years, owing to its medical care functions such as antioxidative, antitumorigenic, and anticarcinogenic activities (*1*−*3*). However, when fresh green tea is stored for a longer period, the color and quality of fresh green tea tend to decrease. To overcome this, there were many technologies for preserving the quality during storage like freezing, vacuum packaging, nitrogen packaging, and lime-removing moisture packaging (*4*). These methods are costly and not easily adoptable by household consumers. Selenium has received considerable attention as an essential micronutrient for animal and human bodies for anticancer and other physiological functions (*5*−*7*). Selenium is the active site of a large number of enzymes, such as glutathione peroxidase (GSH−Px), which has antioxidant activity in the human body (*8*). The antioxidant capacity of Se was related to its inhibition of lipid peroxidation and promotion of GSH−Px and superperoxide dismutase (SOD) activities in higher plants (*9*, *10*).

It was also reported that the oxidation of vitamin C and polyphenol and decomposition of chlorophyll were responsible for the reduction of green tea quality (*11*). Our previous studies have shown that foliar application of Se-enriched fertilizer not only increased Se, total amino acid, and vitamin C concentrations in tea leaves but also improved sensory quality and antioxidant properties of green tea leaves harvested in early spring (*12*−*17*). However, little attention has been given to its role on preservation of food materials, especially on that of quality of tea preservation (*18*). In the present study we sought to investigate the effect of foliar application of Se on the preservation quality of green tea.

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MATERIALS AND METHODS

Selenium Fertilizer. Selenium fertilizer was prepared as detailed by Hu et al. (*13*, *15*). Lobster waste of 5%, chicken excreta of 10%, silkworm excreta of 5%, pig excreta of 70%, and effective bacterium of 10% (effective bacterium including lactobacillus, yeast, actinomycetes, photosynthesis bacterium, etc.) were mixed to ferment about 2 weeks in a methane-generating pit. The sodium selenate and water were added to mixed fertilizer and well-distributed and the solution then continued to ferment for 4 weeks. The fermented solution was filtered and concentrated in vacuum conditions below 50 °C. The total selenium concentration of fertilizer was 50 g L^{-1} containing 90% of organically bound selenium (data not shown).

Preparation of Green Tea Sample. The experiment was conducted in September 2003 at the Jiangpu Zheluoshang Tea Co., Jiangsu Province. Selenium fertilizer with selenium concentration of 150 mg L^{-1} was foliar-sprayed at a rate of 150 g ha^{-1} on tea leaves with a 25 L sprayer produced by Nanjing Research Institute of Agricultural Mechanization of Ministry of Agriculture China (Nanjing, China). The tea trees were 9 years old when the selenium fertilizer was applied in

10.1021/jf048314j CCC: $30.25
Published on Web 08/16/2005

Table 1. Effect of Application of Selenium (150 g ha^{-1}) on Quality of Green Tea[a]

sample	Se content (mg kg^{-1})	vitamin C (g kg^{-1})	tea polyphenol (g kg^{-1})	chlorophyll (g kg^{-1})	color (absorption value)
regular tea	0.048 ± 0.002[b]	4.46 ± 0.06[b]	206.9 ± 1.4[b]	1.48 ± 0.03[b]	0.61 ± 0.01[b]
Se-enriched tea	5.025 ± 1.303[c]	5.26 ± 0.11[c]	209.9 ± 1.2[b]	1.54 ± 0.01[b]	0.61 ± 0.01[b]

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

this experiment. The experimental area of 1000 m^2 was divided into two plots with each 500 m^2, one for selenium application and the other as a control. After 7 days of foliar spray, new tea shoots containing one leaf and a bud were collected from every tree in the 500 m^2 area. The harvested tea leaves were processed into commercial roasted green tea. Every 100 g tea samples with about 3000 leaves and buds were packed in polypropylene bags according to different treatments and then stored at room temperature, 20−32 °C.

Determination of Preservation Quality. The preservation quality was analyzed in triplicate in terms of vitamin C content, chlorophyll, tea polyphenol (TPP), and the color of green tea extracts on the day of 30, 60, 90, and 120 from the beginning of the storage. Above 100 g of tea samples were ground manually to a fine powder in a mortar before analysis.

Determination of Ascorbic Acid. A sample of 0.250 g of tea powder was extracted with 50 mL of 0.1% (w/v) oxalic acid for 5 min and then filtered with a 0.45 μm membrane made by Agilent Technologies. The concentration of vitamin C in the extracted solution was determined by HPLC (Agilent 1100, purchased from USA). The column used was a Zorbax 5 μm C18, (4.6 × 250 mm made by Agilent Technologies), which was operated at 25 °C. The mobile phase eventually adopted for this study was 0.1% oxalic acid and the flow rate was 1.0 mL min^{-1}. The detection wavelength was 254 nm. The sample injection volume was 20 μL. The concentration standards of vitamin C were 0.1, 0.05, and 0.01 mg mL^{-1} (*19*).

About 0.1−0.2 g of tea powder was extracted with 50 mL of 80% (v/v) acetone for 2 min and filtered in the daylight. The absorption (*A*) values of the extracted solution were measured spectrophotometerically at 30 °C at a wavelength of 663 nm (chlorophyll a) and 645 nm (chlorophyll b) by converting the chlorophyll a and the chlorophyll b into the form of chlorophyll in tea powder, according to the formula $C_a = 12.7 \times A_{663} - 2.59 \times A_{645}$, $C_b = 22.9A_{645} - 4.67A_{663}$, $C_t = C_a + C_b$, the total chlorophyll (mg g^{-1}) = C_t (mg L^{-1}) × 50 mL/(sample weight × 1000) (*20*).

Determination of Polyphenols. Tea powder of 0.5 g was extracted with 50 mL of boiling water and kept in a water bath at 100 °C for 30 min. After cooling, the mixture was filtrated through a piece of middling rate filter paper in a funnel and 2 mL of filtrate was put into 8 mL of distilled water, 10 mL of 3.6 mM ferrous tartrate and 30 mL of 200 mM phosphoric acid buffer solution were added, and the absorption values of the extracted solution were measured at a wavelength of 540 nm using 1 cm cells by 722 visibility spectrophotometer (Shanghai Precise Science Instrument Co., Ltd., Shanghai, China). The tea polyphenol content was calculated according to the following formula: tea polyphenol (%) = $A \times 7.826 \times$ the total test volume (mL) × 100/(1000 × the filtrate volume (mL) × the sample weight). The parameter of 7.826 is equivalent to the content of mg mL^{-1} tea polyphenol when the value of absorption is 1.0.

Determination of Color. Two grams of tea powder was extracted with 100 mL of boiling water and filtered after 5 min for evaluation of the color of tea (*20*). The absorption of tea infusion was measured at 420 nm by 722 visibility spectrophotometer (Shanghai Precise Science Instrument Co., Ltd., Shanghai, China). The flavonoid is responsible for the color of green tea infusion. The higher absorbance at 420 nm indicated high concentration of flavonoid in extracts (*20*).

Assessment of Sensory Quality. Assessment of sensory quality on green tea was determined according to Hu et al. (*13*). Aqueous extraction of tea leaves was carried out under controlled conditions, so as to give the extracts the optimum aroma, taste, and color characteristics. One gram of tea was extracted with 100 mL of boiling distilled water in a cup for 3 min. The extract was filtered through a piece of middling rate filter paper in a funnel and then used for sensory evaluation in the Food Analysis Laboratory. Ten qualified panelists were selected from 20 volunteers by a triplicate pretest in three tea infusions. Questionnaires, based on the method of magnitude estimation of descriptive analysis, were composed of 10 records—the highest intensity was expressed as 10 and the lowest as 0. The results in replicates were statistically analyzed for multiple comparisons using SAS software (Release 8.0).

Determination of Selenium. Selenium concentration in dried green tea was determined by atomic fluorescence spectroscopy (*15*).

Statistical Analysis. One-way analysis of variance was performed on the data using SAS (Release 8.0). Student's *t*-test ($p < 0.05$) was calculated to compare the means for the different samples.

RESULTS AND DISCUSSION

Effect of Application of Selenium on Chemical Quality of Green Tea. The major components in tea during autumn-processing season determined in this experiment are shown in Table 1. The selenium content and vitamin C of green tea were significantly increased by foliar application of selenium when compared with control. However, there was no significant difference in the contents of tea polyphenol and total chlorophyll or the color of tea extracts between the treatments ($P < 0.05$). This is in contrast to the result in our previous studies that selenium can significantly reduce the tea polyphenol content in tea produced in early spring and early summer (*15*, *17*). The present results were negative due to the tea samples being collected in a different tea-producing season, which led to different contents of tea polyphenols and chlorophyll.

In addition, the regular tea harvested in autumn had a higher vitamin C content (4.46 g kg^{-1}) than that produced in early spring and in early summer. However, regular green tea harvested in early spring had the highest tea polyphenol content of 240.92 g kg^{-1} among three kinds of teas, which accounted for its high astringency (*15*).

Effect of Application of Selenium on the Chemical and Sensory Quality of Green Tea during Storage. Vitamin C content in both normal and Se-enriched tea has decreased during storage; the final concentration was higher in Se-enriched green tea (**Figure 1a**). The mechanism of Se in stabilizing vitamin C in green tea is not known; however, this could be due to its involvement in ascorbic acid biosynthesis and enhancement in antioxidant activities (*16*, *17*). In the case of polyphenol, the trend was different. There was no significant difference of polyphenol content between normal and Se-enriched tea (**Figure 1b**). In both cases the polyphenol content gradually decreased up to 90 days of storage and then exhibited an increase. Here, too, the polyphenol content was marginally higher in Se-enriched green tea. The reason for the increase in polyphenol after 90 days may be attributed to the reduction of oxidized polyphenols (*21*).

After storage, Se-enriched tea possessed higher chlorophyll than regular tea, leading to its greener color of tea infusion. However, no marked difference in the contents of chlorophyll was found between these two teas (**Figure 1c**). This result suggested that the application of selenium could reduce the

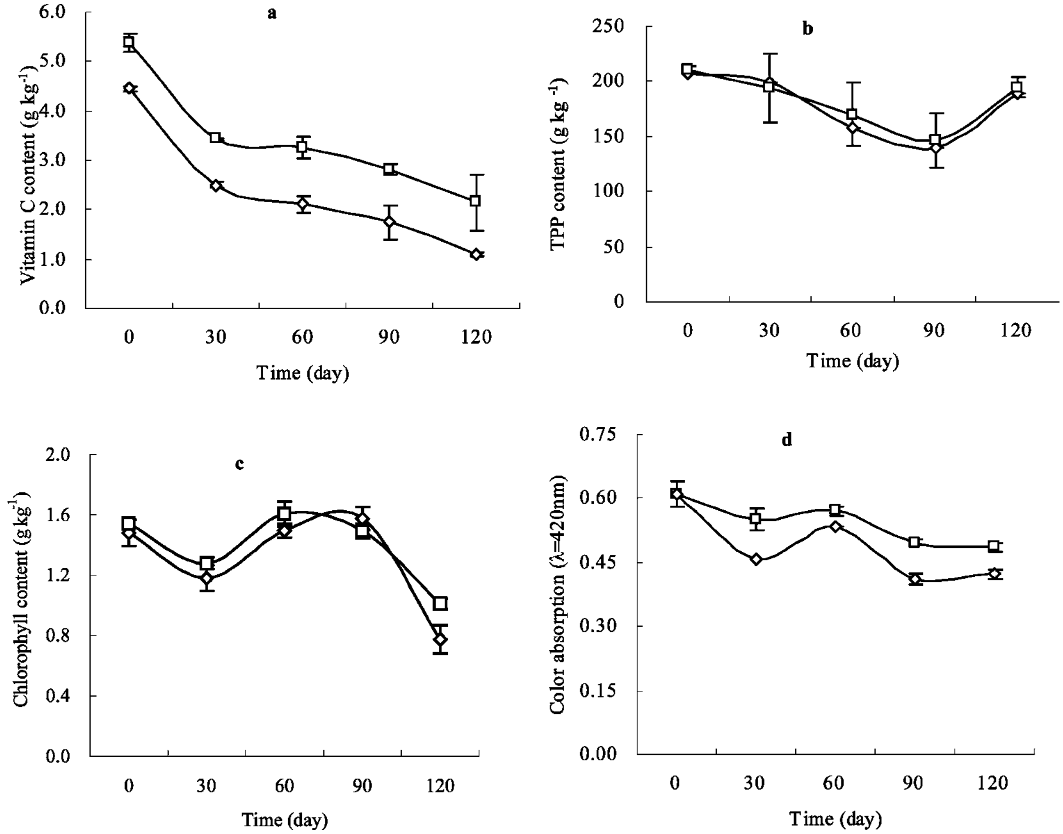

Figure 1. Effect of selenium application (150 g ha^{-1}) on changes in vitamin C (a), total polyphenol (b), chlorophyll content (c), and color (d) of green tea during storage. ◇, Normal tea; □, Se-enriched tea.

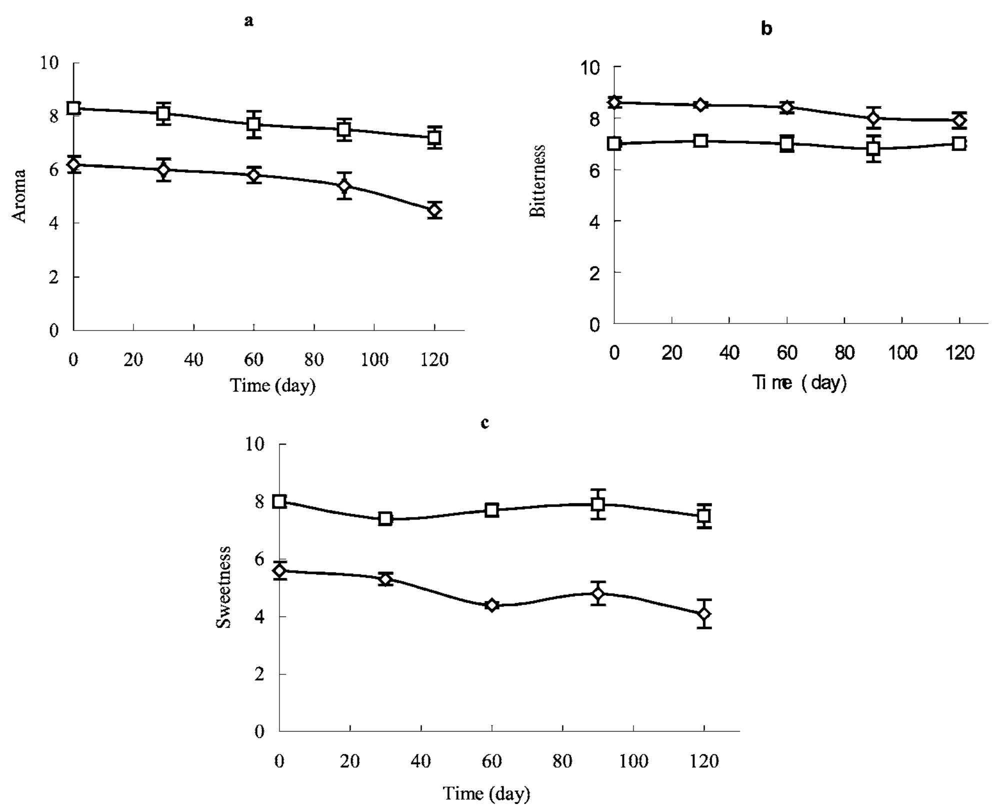

Figure 2. Effect of selenium application (150 g ha^{-1}) on aroma (a), bitterness (b), and sweetness (c) of green tea during storage. ◇, Normal tea; □, Se-enriched tea.

decomposition of chlorophyll and enhance the green tea chemical quality during storage.

The color of tea liquor reflects on its quality. The processing technology has a great influence on the color of tea liquor. There was no significant reduction in the absorption values of normal and Se-enriched tea up to 60 days (**Figure 1d**). After this period the reduction in absorption value was higher in normal tea, indicating that Se-enrichment helps to improve the stability of color for a longer period of storage.

The aroma and sweetness were significantly high and bitterness was significantly low in Se-enriched tea (**Figure 2**). Further, the aroma was more stable in Se-enriched tea (**Figure 2a**). No significant changes in bitterness and sweetness of Se-enriched tea and regular tea were found during storage (**Figure 2b,c**). The sensory quality of green tea during storage was significantly stable in Se-enriched tea.

In summary, our results showed Se-enriched tea had a significantly higher vitamin C content and higher liquor color. The change in aroma of Se-enriched tea was more stable during storage compared to that of regular tea. The results conclusively prove that foliar application of Se can improve the preservation quality of green tea during autumn season.

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Received for review October 11, 2004. Revised manuscript received July 4, 2005. Accepted July 15, 2005. This research was supported by Jiangsu Science and Technology Department under BE2003301, Program for New Century Excellent Talents in University under NCET-04-0501, Program for Excellent Young Teachers under EYTP-2003-355, and Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

JF048314J