A Review on Selenium-enriched Green Tea: Fortification Methods, Biological Activities and Application Prospect
(Kajian Mengenai Teh Hijau Diperkaya Selenium: Kaedah Penguatan, Aktiviti Biologi dan Prospek Penggunaan)

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ABSTRACT
Selenium (Se) has been recognized as an essential nutrient for humans. Plant foods are the predominant source of selenium and majority of dietary selenium is absorbed depending on the type of food consumed. Nowadays, green tea is becoming increasingly popular for its prominent health benefits, including the ability to supplement selenium in organically bound, natural food form. The selenium content of Se-enriched green tea is influenced by the selenium level of local soils in which it is grown. However, selenium content of plants can also be improved by artificial fortification methods. In this review, the chemical speciation and biological functions of selenium, fortification methods, biological activities and nutraceutical applications of Se-enriched green tea are discussed. This review provides insights into the current research and the importance of Se-enriched green tea in the enrichment of human nutrition and health.

Keywords: Biological activities; fortification; green tea; nutrition and health; selenium

INTRODUCTION
Green tea is one of the popular natural health drinks that contain variety of bioactive compounds, such as tea polyphenols, caffeine, tea pigment, tea polysaccharides, tea saponin, theanine and other functional components (Narotzki et al. 2012). Some reports in recent years showed that green tea non-nutrient bioactive compounds have antioxidant, anticancer, antiobesity and other pharmacological functions, making it an excellent candidate for nutraceutical applications (Basu et al. 2013; Johnson et al. 2012; Perumalla & Hettiarachchy 2011; Rains et al. 2011). Selenium is an essential nutrient element for human health. It may reduce the incidence of myocardial infarction, Keshan disease, liver damage, cancer and other diseases (Navarro-Alarcon & Lopez-Martinez 2000). If the dietary intake of selenium is not enough, it can seriously affect human health, such as a decrease in glutathione peroxidase function, thereby resulting in oxidative damage to many organs; skeletal myopathy and cardiomyopathy (Ishihara et al. 1999); symptoms of hypothyroidism, male infertility (http://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/) and Keshan disease (Mistry et al. 2012). Selenium is a constituent of more than two dozen selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis and protection from oxidative damage and infection. Selenium has received considerable attention for its potential role as a chemopreventative agent. Tea plants possess the ability to retain high levels of selenium than others (Molan et al. 2009). The chemical speciation of selenium in green tea is mostly organic and in its organic form it is more beneficial to the human body compared with inorganic selenium (Molan et al. 2009). This review aimed mainly to introduce the chemical speciation and biological role of selenium, selenium fortification methods and nutraceutical functions of Se-enriched green tea. In addition, current research status and future prospects of Se-enriched tea are discussed.
Selenium mostly exists in metal sulfide ores. Swedish chemist Jons Jacob Berzelius first discovered the selenium as a byproduct in the manufacture of sulfuric acid in 1817 and thus it has long been considered to be a toxic element until Schwarz and Foltz in 1957 demonstrated that selenium was the essential element in animal nutrition (Liao et al. 2008). Rotruck et al. (1973) discovered that selenium was the component of glutathione peroxidase in animals and humans (Satoh et al. 1992). Two years later, Awasthi et al. (1975) made it clear that selenium was an essential trace element to humans (Fakih et al. 2005). Since then, the study on the relationship between selenium and human disease has received extensive attention in various fields of biology, medicine, chemistry and other science aspects.

Natural forms of selenium are usually divided into two categories, inorganic and organic, depending on their chemical composition. The inorganic forms mainly include elemental selenium, selenium metal compound, selenate and selenite. While organic selenium compounds present in living organisms are mostly selenomethionine and selenocysteine (Lyons et al. 2007). These selenium containing amino acids are the predominant source of selenium for humans through dietary sources, especially the selenomethionine which comes from plants, while the selenium mainly exists in the form of selenocysteine in most of the mammals (Ip et al. 2000). In recent years, preparation of biologically active nano-selenium particles (Nano-Se) with zero valence state has been achieved with the advancement of nanotechnology. It has been shown that the Nano-Se exhibit lower toxicity and higher biological activity compared with inorganic and organic selenium compounds (Zhang & Spallholz 2011). Zhang et al. (2005) reported that the Nano-Se was more potent than selenite in the induction of glutathione S-transferase and a short-term high dose selenite exposure has been shown to cause more pronounced oxidative stress, greater liver injury and prominent retardation of growth in mice as compared with Nano-Se.

Selenium has antimutation and antitumor effects. Research showed that it has been associated with lower incidence of different cancers such as liver cancer, colon cancer, breast cancer and skin cancer (El-Bayoumy 2001; Thirunavukkarasu & Sakthisekaran 2003). Epidemiological studies have suggested an inverse association between selenium status and the risk of colorectal, prostate, lung, bladder, skin, esophageal, and gastric cancers (Dennert et al. 2011). An investigation by Schrauer (2000) from 17 regions in 27 countries also found a significantly negative correlation between the content of selenium intake, the levels of selenium in blood and the incidence of lung cancer, prostate cancer, ovarian cancer and leukemia. In addition, selenium is antagonistic to heavy metals and enhances immune response. Selenium deficiency is associated with impaired bone metabolism and osteopenia. Selenoproteins help prevent the oxidative modification of lipids, reducing inflammation and preventing platelets from aggregating (Rayman 2012). Selenium concentration is higher in the thyroid gland than in any other organ in the body, and, like iodine, selenium has important functions in thyroid hormone synthesis and metabolism.

**Selenium Levels in Green Tea and Fortification Methods**

**NORMAL LEVELS OF SELENIUM**

The content of selenium is generally not high (about 0.1 μg/g) in green tea, while the average content can be 1.4 μg/g in teas grown in areas with high-selenium soil, and the levels can reach up to a maximum of 6.5 μg/g. The selenium content of tea trees has a direct relationship with plant age and storage site, especially the selenium content of old green tea leaves which is relatively high, followed by shell > branch > young leaves > seeds (Xu et al. 1996). This indicates that the selenium content increases as new leaf blade grows older. Xu et al. (1996) also reported the pattern of selenium distribution in green tea plant (root > two-year-old branch > new leaves), and the levels as well as distribution were tested by adding selenium fertilizer to soil. In addition, the harvesting season of green tea leaves can also influence their selenium content. The selenium content of fresh leaves is relatively high in the spring > autumn > summer and such variations are statistically significant (Zheng & Sha 1996).

About 80% of the selenium in green tea exists in organic form (Table 1). Zhong and Liu (1992) reported that the selenium-containing proteins accounted for about 79.25% of the total, which included the water-soluble proteins nearly of 2.5% and the selenium in tea polyphenols, polysaccharides (including pectic fractions) were 1.22% and 0.88%, respectively (Table 2). The small-molecule selenium that accounted for 18.65% of the total selenium was the major source of water-soluble selenium, while the inorganic form did not exceed 16% of the total selenium. Du et al. (1991) studied the dynamic distribution of accumulated selenium in tea plants and found that the selenium-containing proteins accounted for 68.8, 72.5 and 84.4% of the total selenium in roots, stems and leaves, respectively. It shows that selenium accumulates predominantly in protein-bound form in tea plants. However, the non selenium-containing proteins only account for 31.2, 27.5 and 15.6% in the root, stems and leaves, respectively.

**Selenium Fortification**

Almost all the varieties of green tea contain selenium, but their selenium contents vary greatly, ranging from 0.017 to 6.590 μg/g. At present, the selenium content of Se-enriched green tea is not classified according to unified international standards. In China, the division on the standards for selenium content in tea has suggested certain guidelines, based on results of investigation, as follows: ≥5.00 μg/g for high-selenium tea, <5.00 ~ ≥0.35 μg/g for...
medium high, <0.35 ~ ≥0.10 μg/g for medium and <0.10 μg/g for low (Chen et al. 1991). Natural Se-enriched tea is mainly produced in some regions where high selenium content exists in soil, whereas in low selenium containing areas enrichment can be achieved through applying selenium-containing fertilizer or by spraying selenate or selenite onto tea leaf surface. At present, the soils of most countries are lacking sufficient selenium. Figure 1 shows the correlation between the selenium concentrations in soil and dietary intake of selenium in different countries. It has been reported that selenium from soil enters into humans predominantly via food consumption. Therefore, at present, applying selenium-containing fertilizer to tea garden soils is the most preferred method of production of Se-enriched green tea. Selenium fertilizer also has obvious effect on promoting the selenium content of green tea. It is generally used at a dose of 0.25 kg/hm² and the selenium content of green tea can be increased to 0.15 ~ 0.25 μg/g accordingly, which can fulfill the quality standard requirements of Se-enriched tea in China (Hu & Ding 1998). While the method by spraying selenate or selenite onto tea leaf surface also can increase the selenium content of green tea. It can rapidly improve the selenium content in tea. Figure 2 shows the effect of fortification methods on selenium contents of Se-enriched green tea, which clearly indicates that both methods can effectively enhanced the selenium content of green tea. In general, the selenium content of green tea is increased with the increasing concentration of spraying selenium fertilizer. It has been reported that spraying selenium at dosage level of 50 ~ 100 g/hm² has increased the selenium content of green tea up to 0.32 ~ 1.45 μg/g after 8 ~ 26 days (Tao et al. 2007). There was no significant difference observed between Se-enriched green tea produced by fortification methods and natural Se-enriched tea in terms of chemical composition (Hu et al. 1999b).

**EFFECT OF SELENIUM FORTIFICATION ON THE QUALITY OF GREEN TEA**

The selenium fortification of green tea can improve not only its selenium content, but also enhances the total quality. Selenium has significant positive effect on the preservation quality of green tea and such improvement was especially noted by selenium spraying during the autumn tea-producing season; and it may be partially due to slowdown in the oxidation of major components of tea in presence of high selenium levels (Huang et al. 2005), besides seasonal effect. In addition, selenium in Se-enriched tea can effectively prevent the oxidative browning and can also effectively suppress the oxidation of vitamin C in tea during storage (Hu et al. 2000). Some reports showed that the yield and quality of green tea from plants grown in low selenium soil can be improved by foliar spraying of selenium fertilizer and the free amino acid content was also found to be significantly higher than ordinary green tea (Hu et al. 2001; Huang et al. 2005; Xu et al. 2003a).

**BIOLOGICAL ACTIVITIES OF SE-ENRICHED GREEN TEA**

**ANTIOXIDANT ACTIVITIES**

Se-enriched green tea has been recognized as a functional food. The polyphenols and pigments in green tea have strong antioxidant effect, while selenium is an important component of glutathione peroxidase, and it shows synergistic effect with vitamin E in enhancing the antioxidant capacity in vivo. It was reported that Se-enriched tea could significantly increase the selenium content in blood and liver of rats, decrease the content of malondialdehyde, improve the activity of superoxide dismutase in blood (Hu et al. 2001). The Se-enriched tea also has the ability to reduce lipid peroxidation and scavenge free radicals and is more effective than sodium.

<table>
<thead>
<tr>
<th>Valence state</th>
<th>Water soluble Se (%)</th>
<th>Total Se (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se&lt;sup&gt;4+&lt;/sup&gt;</td>
<td>-</td>
<td>3.89 ~ 14.17</td>
</tr>
<tr>
<td>Water soluble Se&lt;sup&gt;4+&lt;/sup&gt;</td>
<td>11.49 ~ 46.53</td>
<td>-</td>
</tr>
<tr>
<td>Se&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>-</td>
<td>87.13 ~ 96.11</td>
</tr>
<tr>
<td>Water soluble Se&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>53.47 ~ 88.51</td>
<td>13.44 ~ 19.24</td>
</tr>
</tbody>
</table>

**TABLE 2. Levels of selenium-containing compounds in Se-enriched green tea**

(modified after Hu et al. 1999b; Weng & Bai 2005; Zhong & Liu 1992)

<table>
<thead>
<tr>
<th>Type</th>
<th>Components</th>
<th>Contents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic selenium</td>
<td>Se-polyphenols</td>
<td>0.89 ~ 1.22</td>
</tr>
<tr>
<td></td>
<td>Se-proteins</td>
<td>60.12 ~ 79.25</td>
</tr>
<tr>
<td></td>
<td>Water soluble Se-proteins</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>Se-polysaccharides</td>
<td>0.88 ~ 18.06</td>
</tr>
<tr>
<td>Inorganic selenium</td>
<td>-</td>
<td>8.0</td>
</tr>
</tbody>
</table>

**TABLE 1. Levels of selenium (valence state) in Se-enriched green tea**

(modified after Weng & Bai 2005; Zhong & Liu 1992)
selenite. Tan et al. (2004) reported that Se-enriched tea was more able to scavenge free radicals in vivo, reduce membrane lipid peroxidation and enhance the antioxidant capacity of the body. The selenium in Se-enriched tea could significantly improve the antioxidant benefits of green tea. A significant enhancement especially in the aspects of inhibition of linoleic acid oxidation, scavenging free radicals and lipid oxidation was observed with Se-enriched tea compared with ordinary tea (Xu et al. 2003a, 2003b). Comparing the antioxidant activity of different components in Se-enriched green tea with ordinary green tea, the free radical scavenging capacities of crude tea polyphenols and crude tea polysaccharide in Se-enriched green tea were significantly higher than ordinary green tea (Yu et al. 2007). Molan et al. (2009) also reported that Se-enriched green tea possesses higher antioxidant capacity than regular green tea.

ANTITUMOR EFFECT
Selenium has been shown to prevent certain cancers, inhibit the growth of tumor cells and promotes their differentiation; inhibit the division of malignant cells and promotes reversion of malignant phenotypes. It shows inhibitory effect at the different stages of tumorigenesis, i.e. initiation, promotion and proliferation. There are obvious inhibitory effects on liver cancer, gastric cancer and other digestive cancers. There are lower incidence rates of cancer in some selenium high areas than the low selenium or selenium deficient areas (Fleet 1997). Li et al. (2008) studied the antitumor effect of the ethanolic extracts of Se-enriched green tea. They concluded that the ethanolic extracts exerted significant inhibitory effect on human lung cancer A549 cells, HepG2 liver cancer cells and HeLa cervical carcinoma cells and treatments also showed a dose-effect relationship. Xu et al. (2007) had investigated the anticarcinogenic activity of Se-enriched green tea extracts in vivo. They hypothesized that the antitumor activities of Se-enriched green tea may be attributed to the oxidative stress induced by selenium and green tea components in a suitable selenium supplementation pathway and are independent of the function of glutathione peroxidase (GPx) in the cellular antioxidant systems. Hu et al. (2010) researched the synergistic antitumor effect of selenium and green tea using rat colon cancer models. Compared with selenium and green tea as
separate constituents, combination of both in the form of selenium-enriched green tea has significantly improved the chemopreventive effect on colon cancer cells. These results suggested that Se-enriched green tea may someday become an important dietary supplement for the prevention and treatment of tumors.

ANTIMUTAGENIC EFFECT
As two separate entities, both selenium and green tea have been reported to exhibit cancer chemopreventive and antitumor properties. Furthermore, they also displayed antimutagenic effect against cancer cells. However, Amantana et al. (2002) compared the antimutagenic activities of Se-enriched green tea and regular green tea, the results demonstrated that Se-enriched green tea has significantly greater antimutagenic activity than regular green tea. Li et al. (2009) evaluated the anticlastogenic effect of micrometer powder of Se-enriched green tea by using a chromosomal aberration assay in mouse testicular cells. The results indicated that Se-enriched green tea not only prevent the chromosomal aberrations induced by mitomycin C in mouse spermatocytes, but can also enhance the glutathione peroxidase and superoxide dismutase activity in blood serum and liver. However, the present findings demonstrated that the antimutagenic potential of Se-enriched green tea could not be solely related to the enhancement of antioxidative enzymes glutathione peroxidase and superoxide dismutase (Xu et al. 2008). Therefore, it is worthy of further research on the antimutagenic mechanism of Se-enriched green tea.

PREBIOTIC PROPERTIES
Prebiotics are dietary ingredients that intended to stimulate the growth of probiotic bacteria, especially the intestinal microbiota in humans (Grizard & Barthomeuf 1999). It has been demonstrated that prebiotics as the dietary components can increase the proliferation of beneficial bacteria that are naturally present in the intestine. Some reports showed that Se-enriched green tea has good prebiotic activity and found to be significantly more effective than regular green tea at increasing the growth of lactobacilli and bifidobacteria under in vitro and ex vivo conditions (Molan 2013; Molan et al. 2009). Similar effect was detected using animal model where orally administered aqueous extracts from Se-enriched green tea and regular green tea have shown the positive effect on population size of beneficial bacteria and negative impact on the viability of some undesirable bacteria in the cecum of rats. The result also showed that there was a significant increase in the number of beneficial bacteria in rats by gavage feeding Se-enriched green tea, while the group fed with regular green tea showed a slight but non-significant increase in the population of bifidobacteria and lactobacilli (Molan et al. 2010). It has been identified that Se-enriched green tea is as a good prebiotic that could significantly promote the growth of friendly bacteria and decrease the numbers of undesirable bacteria in the cecum of rats.

SAFETY OF SE-ENRICHED GREEN TEA
Though selenium is an essential nutrient to human health, it is required only in small amounts (Thomson 2004). Selenium is toxic and could cause selenium poisoning in humans and animals when intake exceeds the safe and adequate level (Hartikainen 2005). The recommended dietary allowance (RDA) for selenium, both men and women, is 55 μg (0.7 μmol)/day (http://www.nap.edu). The major forms of selenium in the diet are highly bioavailable. The Tolerable Upper Intake Level (UL) for adults is set at 400 μg (5.1 μmol)/day based on selenosis as the adverse effect. Polyphenol epigallocatechin-3-gallate (EGCG) is the major functional component in green tea. Although several reports have suggested the enhancement of biological effects in animal models on using the Se-enriched green tea, a recent report showed that the growth suppression in mice due to sodium selenite cannot be prevented by the co-administration of pharmacological levels of EGCG and this combination, in turn, aggravates the toxicity (Sun et al. 2013). The mechanism of lethal toxicity appeared to be due to the suppression of a selenite-induced adaptive response by EGCG, not from enhanced selenium accumulation (Sun et al. 2013). However, EGCG has been reported to ameliorate the toxicity of some agents. These contradictory effects indicate that there must be a need for caution regarding toxicity in the development of new health promoting products using selenium and green tea catechins. Therefore, determination of possible adverse interactions between selenium and green tea polyphenols/bioactives is worth to study further for the better formulation of Se-enriched green tea.

DEVELOPMENT AND APPLICATION OF SE-ENRICHED GREEN TEA

THE STANDARDS OF SE-ENRICHED GREEN TEA
The biological effect of selenium in humans is closely associated with its intake dose. In a certain dose range, selenium is beneficial to human body; however it will produce toxic side effects when exceeding the domain value. At present, the daily dietary standards for selenium are not uniform among different countries. Therefore, it is very necessary to establish regional standards for Se-enriched green tea on the basis of selenium content in local green tea as well as the levels of dietary selenium intake. Furthermore, readily bioavailable form of selenium in Se-enriched green tea is the water-soluble selenium in aqueous environment. The leaching rate of selenium in tea infusions is closely related to the selenium content, degree of tenderness of tea leaf, degree of oxidation, type of tea and brewing methods. Therefore, it is necessary to consider the difference in leaching rates and ways of utilization when making the standard for Se-enriched green tea. In addition, the selenium content of natural Se-enriched green tea greatly varies due to the differences in selenium levels of soils where they have grown. Therefore it is also
one of the problems for the formulation of Se-enriched tea standards. In recent years, artificial enrichment or fortification methods have been quickly developed and applied because of their effectiveness in controlling the selenium level of green tea and improving the stability of selenium content and therefore have obvious application advantages.

APPLICATION OF SE-ENRICHED GREEN TEA
In recent years, with the rapid development of functional food industry, Se-enriched green tea as a kind of functional beverage is gaining popularity around the world, especially in some Asian countries. At present, Se-enriched green tea is increasingly being produced in China and is well regarded as a healthy beverage due to its rich bioactive components. Some new food products which are fortified with Se-enriched green tea such as green tea-flavored cola, tea oral liquid, tea wines, tea cakes and tea crisp soft drinks have already emerged in the market place and welcomed by consumers (Wo & Bai 2005).

Recently, some new food processing technologies have been adopted for the green tea processing such as superfine processing technology. Tea powder with particle size less than 76 mm can be obtained by grinding tea leaves using superfine processing technology (Hu et al. 2012). Ultrafine grinding technology can effectively improve the bioavailability of selenium in Se-enriched green tea as it contains a lot of water insoluble selenium-bound protein. It helps keep the native state of fine structure and chemical composition of green tea (Li et al. 2009). Meanwhile, tea leaves morphological features experience great change during grinding process and results in a narrow and uniform particle size distribution powder, possessing the capacity for diversified applicability in foods (Li et al. 2012). Nowadays Se-enriched green tea powder is widely used in drinks and foods. With the in-depth study on aspects of nutrition and health benefits of Se-enriched green tea, more innovative functional food products based on Se-enriched green tea will emerge and play a dominant role in green tea markets.

CONCLUSION
It can be concluded that the artificial fortification is a better way to improve selenium levels of green tea. Foliar spraying is a fast method for selenium enrichment, and green tea plants can convert inorganic selenium on the surface of leaves into organic selenium, however, the inorganic selenium residue from excessive spraying may affect the safety of products. Selenium fortification through the fertilizer-soil-plant route is a relatively safe method for obtaining Se-enriched green tea, but the enrichment efficiency is relatively low. Therefore, there is a need of further research on the absorption and transformation pathways of selenium in green tea plants.

At present, the reported levels of selenium content in Se-enriched green tea represent the total content rather than organically bound selenium fraction; however, the form in which selenium is absorbed by human body is organic selenium. Therefore, while formulating the standards for selenium content in green tea and during safety evaluation, the organic selenium fraction should also be considered besides the total content. Product diversification is the future development direction of Se-enriched green tea market. Advanced technologies like ultra-fine powder grinding could improve the product innovation and therefore there is a need for timely adoption of advanced tea processing technologies by the industry. With further scientific evidences on potential health benefits of Se-enriched green tea, this functional food market will flourish in the future.

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