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REVIEW

Health Functions of Compounds Extracted in Cold-water Brewed Green Tea from *Camellia Sinensis* L.

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Abstract

Green tea from *Camellia sinensis* L. has different tastes and physiological functions according to the temperature of water used when brewing the tea leaves. Green tea brewed with boiled water (especially "Sencha") has a strong astringent taste as epigallocatechin gallate (EGCG) and caffeine are extracted with hot or boiled water. These compounds elicit strong astringency and bitterness, and were previously considered the principal functional ingredients in green tea. In contrast, cold-water brewed "Sencha" has much less bitterness and astringency than hot-water brewed "Sencha" because EGCG and caffeine are difficult to extract in cold water. Therefore, the tastes of amino acids predominate in cold-water brewed "Sencha" because they are easily extracted in cold water. The main functional components of cold-water brewed "Sencha" are epigallocatechin (EGC) and theanine, which are easily extracted in cold water. The functions of EGC have not attracted much attention thus far. However, it was recently found that EGC has an immune-enhancing effect and theanine has a psychosocial stress-reducing effect. These effects of EGC and theanine were inhibited by EGCG and caffeine; therefore, to obtain these effects, green tea needs to be brewed with cold water.

Discipline: Food
Additional key words: epigallocatechin, health benefit, sencha, theanine

Introduction

Tea from the plant *Camellia sinensis* L. is one of the most popular beverages consumed worldwide in its green, oolong or black forms. It contains many compounds such as catechins, caffeine, theanine, and flavonols, some of which have been shown to reduce the risk of a variety of diseases such as cardiovascular disease, cancer, diabetes, respiratory disease, and cognitive decline (Mukhtar & Ahmad 2000, Chacko et al. 2010, Saito et al. 2015, Driscoll et al. 2016). It has been thought that epigallocatechin gallate (EGCG) and caffeine are the principal functional ingredients in green tea (Chowdhury et al. 2016, Nehlig et al. 1992). In Japan, fresh harvested tea leaves are steamed to deactivate polyphenol oxidase, and then dried while being rolled and crumpled (Fig. 1). The most characteristic process during green tea production (Fig. 2) is the inactivation of polyphenol oxidase that catalyzes the polymerization of catechins (Tanaka et al. 2003), resulting in higher concentrations of catechins remaining in green tea leaves. Incidentally, black and oolong tea are made using oxidized tea leaves, and fully oxidized black tea mainly contains such catechin polymers as theaflavins and thearubigins. Among green teas, "Sencha" in particular is dried while being rolled and crumpled — one of its key characteristics — and the crumpled leaves easily exude their ingredients into water. Therefore, "Sencha" is brewed in water of various temperatures (cold to hot) and for a relatively short time. The compositions of the ingredients in tea infusions are influenced by the water temperature. The boiling-water infusion of "Sencha" has a strong astringent taste. Conversely, the tastes of amino acids predominate from cold-water infusion. Japanese green tea "Sencha" is enjoyed for each of these tastes that differ based on water temperature. However, recent studies have found other differences resulting from the water temperature in addition to taste.

Differences in tea ingredients brewed at different water temperatures

Epigallocatechin (EGC) is easily extracted in cold water. In contrast, EGCG and caffeine are difficult to

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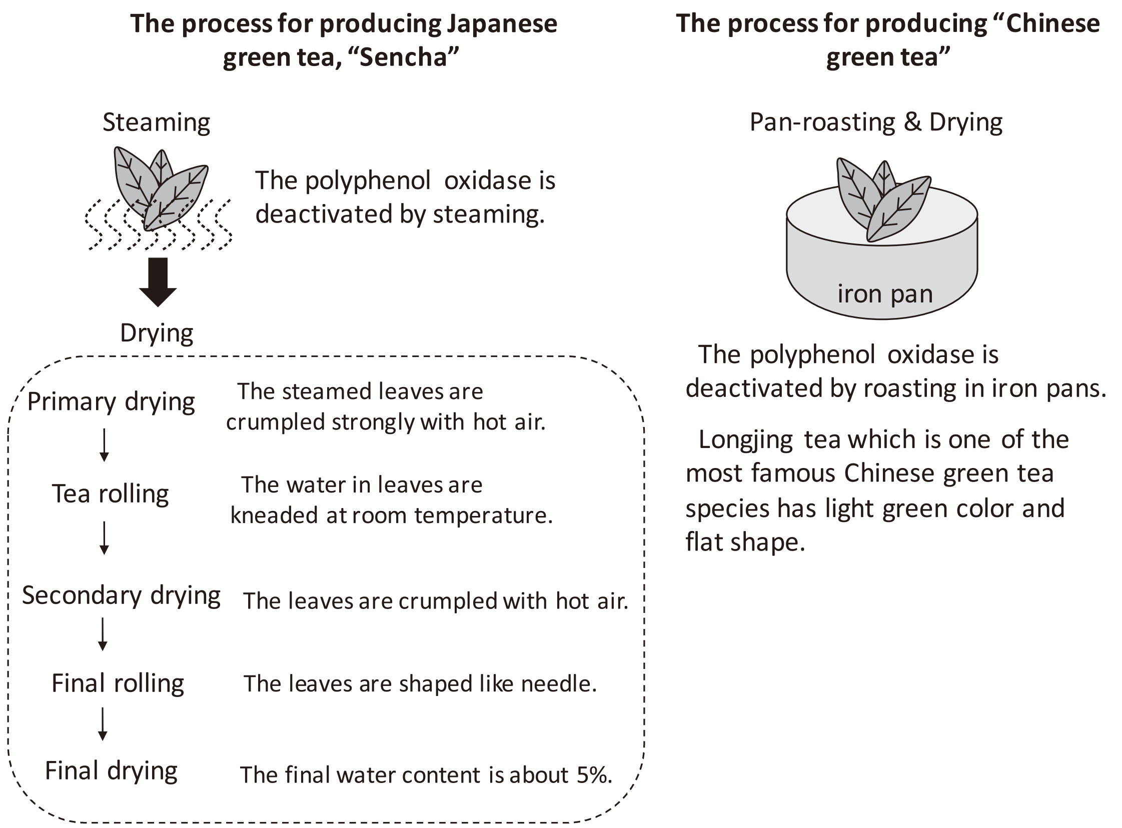

Fig. 1. The difference in the process of producing Japanese green tea and Chinese green tea

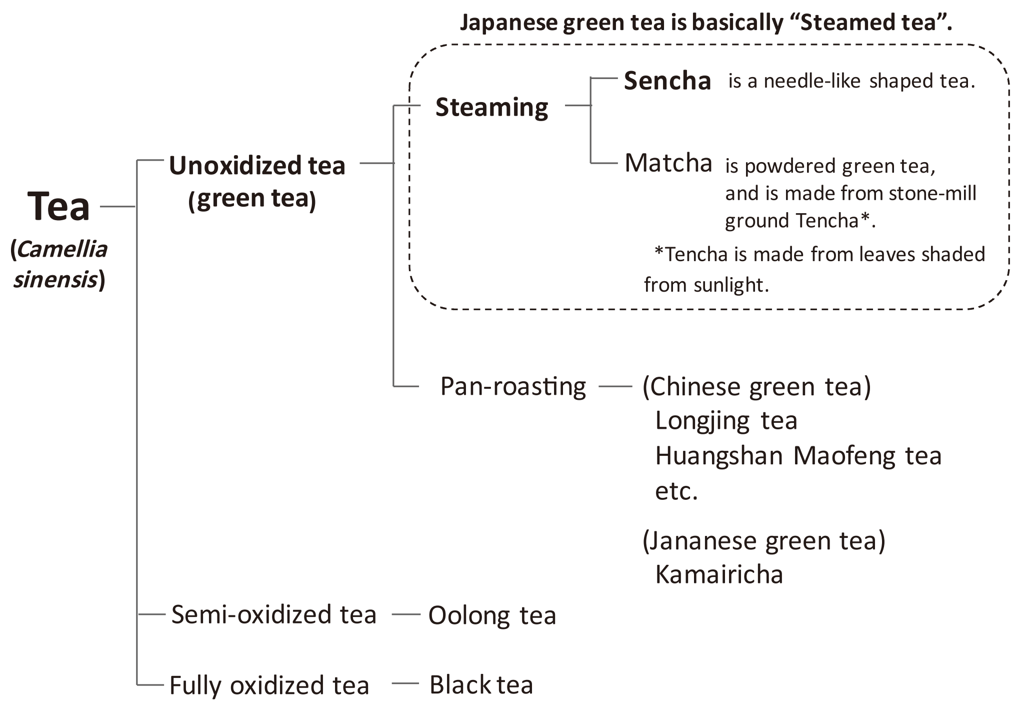

Fig. 2. The types of tea by oxidation

extract in cold water, especially under 10°C. When cold water (around 10°C) was used, the levels of EGC, EGCG, and caffeine after infusion for 1 h were reduced to about 70%, 20%, and 50%, respectively, of those obtained with hot-water infusion at 80°C for 2 min. (Monobe et al. 2013) (Fig. 3A). Further extraction did not affect these concentrations. Moreover, when iced water (0.5°C) was used, caffeine in the infusion was under 20% of that in hot-water infusion (Monobe et al. 2016) (Fig. 3B). After iced-water infusion for 1 h, theanine, glutamic acid, and aspartic acid, which are major amino acid constituents, were extracted at a level of about 80% of that obtained with hot-water infusion (Fig. 4). In summary, EGCG and caffeine can be extracted with hot or boiling water, and both elicit strong astringency; therefore, boiling-water brewed "Sencha" has a strong astringent taste (Hayashi et al. 2006, Kubo et al. 2014). Conversely, the tastes of amino acids predominate in cold-water brewed "Sencha" because they are easily extracted in cold water (Fukushiyama et al. 1999, Kubo et al. 2014, Monobe et al. 2016).

Immune-enhancing effect of cold-water brewed green tea

In the living body, phagocytic cells, which play a vital role in innate immunity, digest antigens such as pathogens, and parts of the antigens are presented on naive T helper lymphocytes (Thp). Thp cells are differentiated into helper T lymphocytes of the Th1 or Th2 type. Th1 lymphocytes activate cytotoxic T lymphocytes that produce cytokine, and Th2 lymphocytes activate B lymphocytes that produce immunoglobulins (Fig. 5). For example, the activation of mucosal B lymphocytes induces the production of immunoglobulin A (IgA), which is the major immunoglobulin in external secretions such as saliva, nasal secretions, and digestive tract mucus. Recently, it was clarified that the activation of transient receptor potential melastatin 2 (TRPM2) is important as a mechanism for macrophage activation (Kashio et al. 2012). TRPM2 is thermosensitive and usually not activated at physiological body temperatures (Fig. 6).

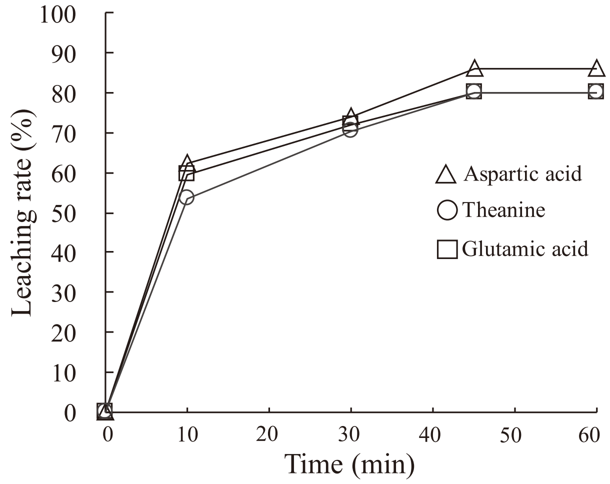

Fig. 4. The leaching rate of theanine, glutamic acid, and aspartic acid after cold-water brewing as compared with after hot-water brewing.
Tea infusions were prepared by extracting green tea (Sencha leaves) in cold water (0.5°C). The leaching rate after 2 min. at 80°C was set at 100%.

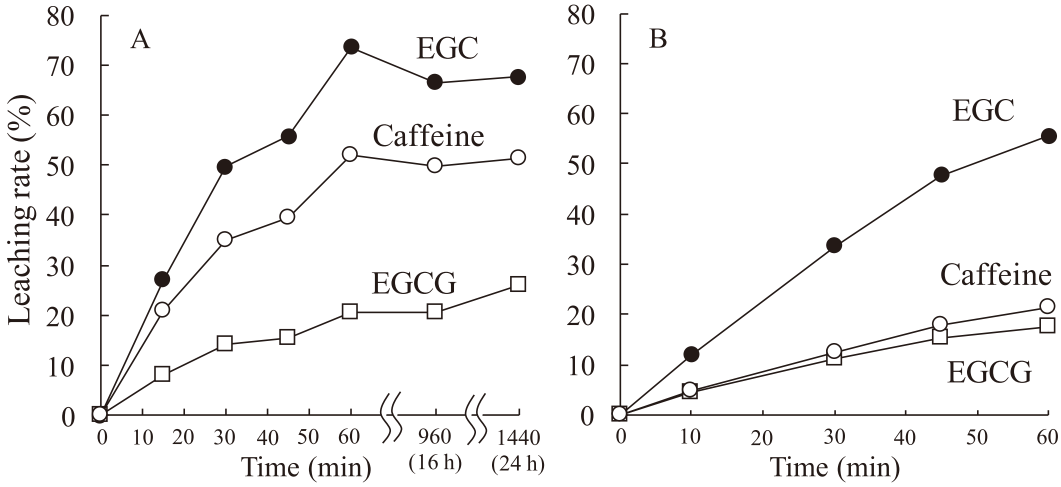

Fig. 3. The leaching rate of EGC, EGCG, and caffeine after cold-water brewing compared with after hot-water brewing.
Tea infusions were prepared by extracting green tea (Sencha leaves) in cold water (A: 10°C, B: 0.5°C) or hot water (80°C, 2 min.). The leaching rate after 2 min. at 80°C was set at 100%.

TRPM2 activation is regulated by redox signals such as hydrogen peroxide (H_2O_2) that enable channel activity at physiological body temperatures (Kashio et al. 2012). EGC activates macrophage phagocytosis through TRPM2 activated by H_2O_2 produced from EGC (Monobe et al. 2014), and induces immunoglobulin production (Monobe et al. 2010, Monobe et al. 2012). On the other hand, although EGCG also activates macrophage phagocytosis through TRPM2, EGCG inhibits the Toll-like receptor (TLR)-signaling pathway, which plays an important role in the regulation of antigen presentation through a 67-kDa laminin receptor (67LR) (Hong et al. 2010, Byun et al. 2011), which is anti-inflammatory action that inhibits macrophage action. Hot-water brewed green tea includes an equal content of EGC and EGCG; therefore, the effect of EGC is offset by EGCG (Monobe et al. 2010, Monobe et al. 2012, Monobe et al. 2014). In contrast, cold-water brewed green tea reduces the elution of EGCG, thus making EGC, which mediates the immune-enhancing effect, the major catechin.

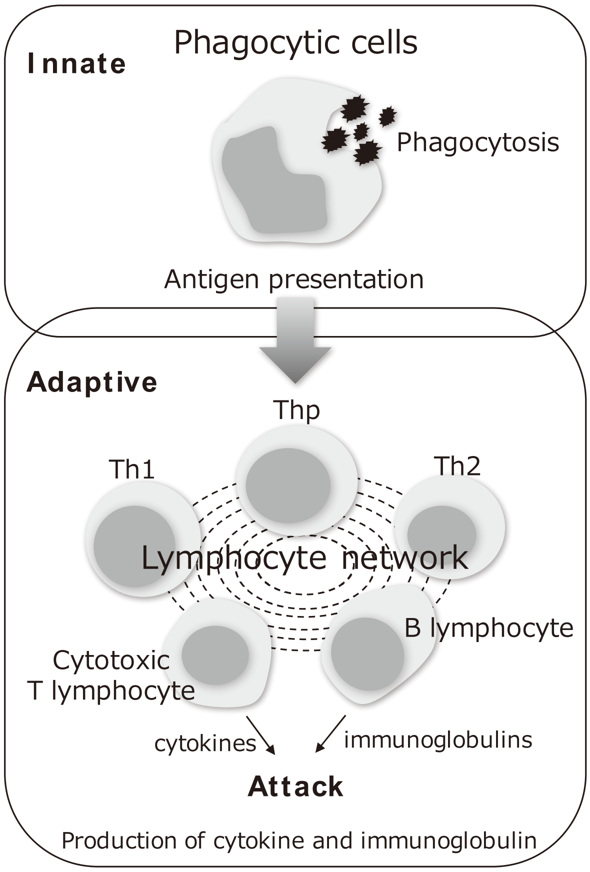

Fig. 5. Innate and adaptive immune system
In the living body, phagocytic cells, which play a vital role in innate immunity, digest antigens such as pathogens, and parts of the antigens are presented on naive T helper lymphocytes (Thp). Thp are differentiated into helper T lymphocytes of the Th1 or Th2 type. Th1 lymphocytes activate cytotoxic T lymphocytes that produce cytokine, and Th2 lymphocytes activate B lymphocytes that produce immunoglobulins.
Innate: Innate immunity is a nonspecific defense system.
Adaptive: Adaptive immunity is a specific defense system.

Anti-psychosocial stress effect of cold-brewed green tea

Theanine is an amino acid analogue of L-glutamic acid. It is well-known that the oral intake of L-theanine causes a feeling of relaxation (Kobayashi et al. 1998) and relieves psychosocial stress (Unno et al. 2013). Here, psychosocial stress was related to personal relationship problems with partners, friends, relatives, managers, employees, etc. Unno et al. examined whether the ingestion of green tea prevented psychological stress (Unno 2011, Unno et al. 2013, Unno et al. 2016), which they evaluated using a unique mouse model of psychological stress evoked by confrontational housing. Two male mice were housed in the same cage separated by a partition to establish a territorial imperative. Then, the partition was removed and the mice were co-housed confrontationally (Fig. 7). The two male mice felt stressed. Mice began to die earlier in confrontational housing than in group-housed control mice; however, the consumption of purified theanine (20 µg/mL, 5-6 mg/kg) suppressed the shorted lifespan. Recently, however, it was reported that the psychosocial stress-reducing effect of theanine was cancelled by EGCG and caffeine (Unno et al. 2016). Theanine is extracted relatively easily in iced water (Monobe et al. 2016). Conversely, caffeine and EGCG are difficult to extract in iced water (Fig. 3B). Caffeine and EGCG levels can be decreased (Monobe et al. 2016), consequently reducing their interference effect.

Conclusion

EGC and theanine are the main components in cold-brewed green tea. The immune-enhancing effect of EGC and the psychosocial stress-reducing effect of theanine are not obtained from EGCG and caffeine, which are the main components in boiled or hot-water brewed green tea. In particular, the original taste of "Sencha" can be brought out by cold- or warm-water brewing, but never by boiled water. For example, besides EGC and theanine, flavonol glycosides are also a principal component in cold-water brewed "Sencha" (Monobe et al. 2015). The other components released after cold-water brewing may also have activities that are not exhibited in the presence of EGCG and caffeine, namely in boiling- or hot-water brewed green tea. Therefore, we need to further examine the interaction of ingredients in green tea in order to elucidate the effective compounds.

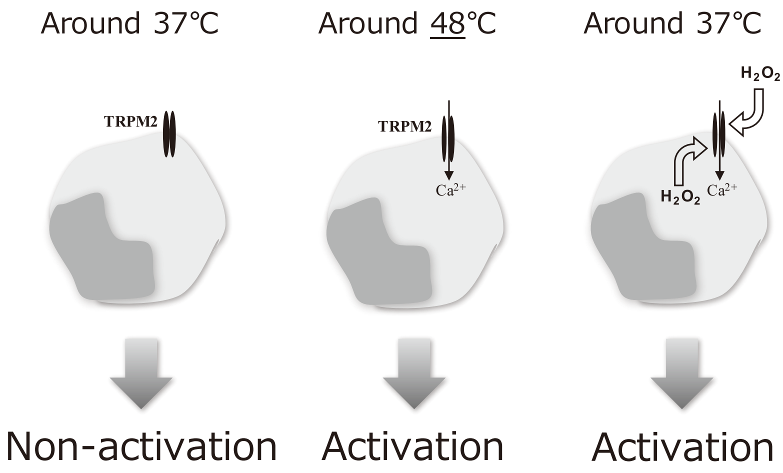

Fig. 6. Macrophage activation through the TRPM2 channel
TRPM2 is a thermosensitive protein and usually not activated at physiological body temperatures. Macrophages, which engulf microbes, release hydrogen peroxide (H_2O_2) and superoxide to kill the microbes. Endogenous or exogenous H_2O_2 enables TRPM2 channel activity at physiological body temperature (around 37°C).

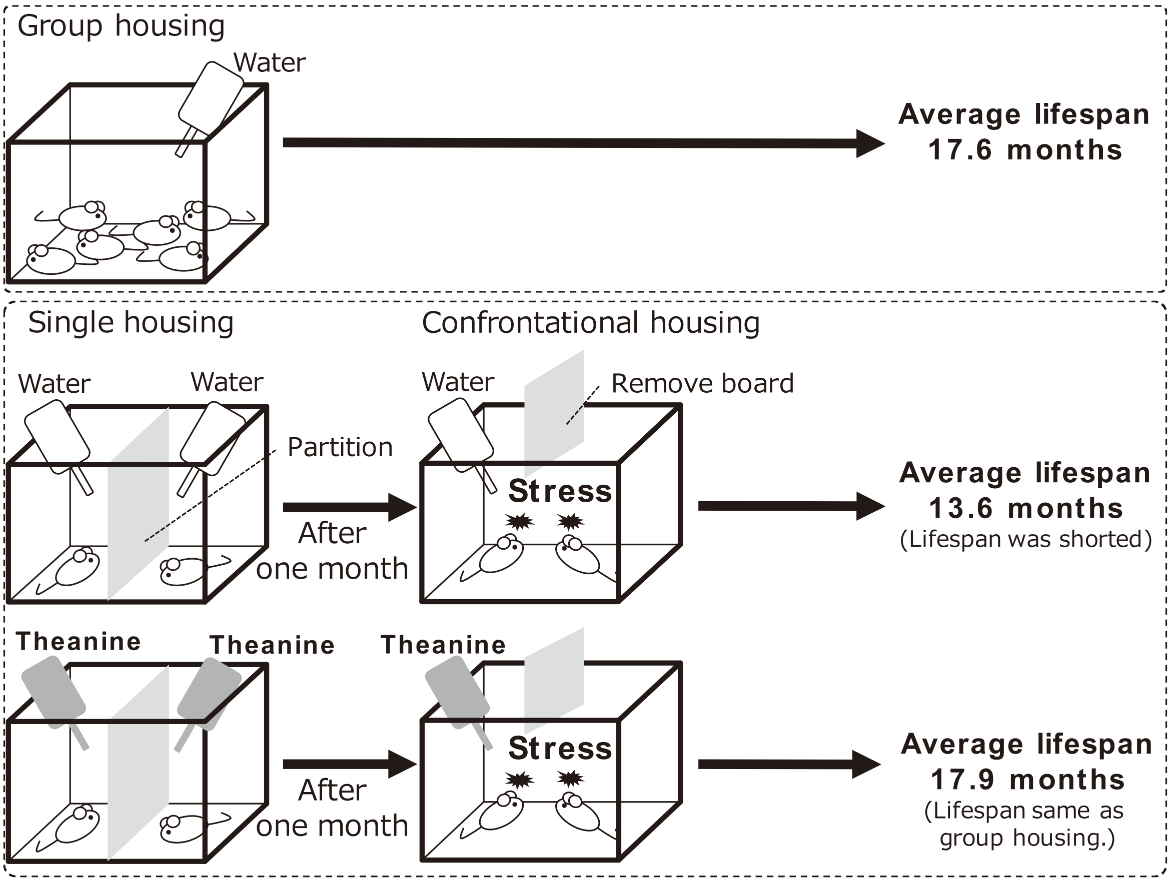

Fig. 7. The effect of confrontational housing and theanine consumption
Mice began to die earlier with confrontational housing than in group-housed control mice. The consumption of purified theanine (20 µg/mL, 5-6 mg/kg) suppressed the shorted lifespan.

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