Chapter 5

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Development and Properties of Green Tea with Reduced Caffeine

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ABSTRACT

Caffeine is one of the main components of green tea and has side effects such as sleeplessness. Senior citizens, children, and pregnant woman should avoid tea despite its known beneficial effects. In this study, we developed green tea with reduced caffeine content (low caffeine tea) as a palatable tea that can be offered to everyone. To reduce the tea's caffeine content, we subjected the plucked tea leaves to a hot-water spray process, and successfully produced a low caffeine tea infusion with 30% the caffeine content. The concentrations of other main components, such as catechins and theanine, in the low caffeine tea infusion did not differ from the control. Further, the physiological function of the tea was assessed; the anti-oxidative activity was investigated using a stable free radical and the antilipase activity using an artificial substrate. There were no significant differences between the infusions of low caffeine tea and green tea in anti-oxidative and anti-lipase activities. The results showed that our developed low caffeine tea could be an attractive high quality tea with health benefits for everyone.

Keywords: Camellia sinensis; green tea; reduced caffeine; anti-oxidative activity; anti-lipase activity.

1. INTRODUCTION

Many kinds of tea are produced and consumed worldwide. Tea types, based on processing or harvested leaf development are black (fermented), green (non-fermented) and oolong (semifermented). These major tea types differ in how tea is produced and processed according to the different processes of drying and fermentation that determine its chemical composition [1]. One reason for tea's popularity is that it exhibits various physiological functions, such as improvement of brain function as well as anticancer, anti-obesity, antiallergic and antioxidative activities [2-4]. Green tea (*Camellia sinensis (L.) Kuntze*) contains catechins (8-20%), caffeine (2-4%) and theanine (1-8%) as the main components, with each component imparting a distinct taste [5]. However, caffeine exhibits some side effects, including sleeplessness. Senior citizens, children, and pregnant woman should avoid tea despite its known beneficial effects. Several kinds of decaffeinated green tea have been produced [6] and some have been commercially available. McKay and Blumberg [7] reported a per capita mean consumption of tea in the world of 120 mL/day. Approximately 76 –78% of the tea produced and consumed is black tea, 20 –22% is green tea and less than 2% is oolong tea [8]. However, these products were not popular with consumers because of their altered taste, attributable to the decrease in main ingredients during the manufacturing process, as well as the high cost. As an effective way to remove caffeine from tea leaves, Tsushida and Murai reported that fresh green tea leaves were steamed with boiling water for a

few minutes prior to primary rolling [9]. Hot-water treatment is a simple and economically efficient method to decrease the caffeine content in tea leaves without chemical toxicity. 'Benifuuki' and 'Benihomare' green teas, which exhibit anti-allergic activity, were soaked in hotwater to reduce the caffeine content, and it was demonstrated that the anti-allergic compound was maintained in the processed tea leaves [10,11]. Thus, hot-water treatment might not decrease the physiological function of tea leaves. The maximum caffeine levels are always limited to 4 mg g^{-1} for leaf teas and 10 mg g^{-1} for instant teas [12].

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In this study, a green tea with reduced caffeine content (low caffeine tea) was manufactured using a hot-water spray process. Further, the main components of the low caffeine tea infusion as well as its anti-oxidative and anti-lipase activities were determined in an effort to elucidate its health benefits.

2. MATERIALS AND METHODS

2.1 Reagent

The reagents used in this experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA), and high performance liquid chromatography (HPLC) grade reagents were used for the HPLC analysis.

2.2 Low Caffeine Tea Manufacturing Process

Fresh tea leaves (*Camellia sinensis (L.) Kuntze*) were plucked and automatically sprayed with hot water (95°C, 180 seconds) to reduce the caffeine content of tea leaves [13,14]. A tea processing machine with regulated temperature and shower time and possessing high-performance efficiency and stability was used (Terada Co. Ltd. Shizuoka, Japan). After centrifugal dehydration at 3000 rpm for 1 min, the green tea was prepared through a standard manufacturing process.

2.3 Preparation of Tea Leaf Infusions

Three grams of tea leaves (green tea and low caffeine tea) were infused in 100mL of tap water for 0.5, 1, 2 and 6 hours at room temperature. The infusion was centrifuged for 5 min at 3000 rpm and the supernatant was filtered (0.45 μm filter, Millipore, Merck kGaA, Darmstadt, Germany).

2.4 Determination of Caffeine, Catechin and Theanine Contents

To determine the caffeine, catechin and theanine contents, the tea leaf infusions were applied to a reversed-phase high-performance liquid chromatography (Agilent 1100 series HPLC system, Agilent Technologies, Santa Clara, CA, USA) equipped with a reverse phase C18 column (3 μm particle size, 150 x 4.6 mm i.d.; Shiseido, Kyoto, Japan). The HPLC column was maintained at 30°C in an oven. For detection of compounds, 0.1 M NaH2PO4 buffer/acetonitrile was employed at 87:13 for caffeine and catechin, and 87:5 for theanine as the mobile phase at a flow rate of 1.0 ml/min. Individual peaks were identified by comparing their UV-Vis spectral characteristics and retention times with those of

commercial standards supplied by Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Green tea leaves treated without hot water were used as the control.

2.5 Determination of Anti-oxidative Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich) as a stable free radical was used to determine the anti-oxidative activity of the tea infusions. A 1.5-ml aliquot of DPPH solution (0.1 mM, in 95% ethanol) was mixed with 100 μL of tea infusion. The mixture was shaken vigorously and left to stand for 20 min at room temperature. The absorbance at 517 nm of the DPPH solution was measured using a spectrophotometer (Bio Spec, Shimadzu, Kyoto, Japan). The radical scavenging activity was measured as a decrease in the absorbance of DPPH, indicating anti-oxidative activity, and was calculated using the following equation:

Scavenging activity (%) = [1- (absorbance of sample/absorbance of control)] × 100

2.6 Inhibition of Lipase Activity

Lipase inhibitory activity was determined in the infusions in order to estimate its anti-obesity effect. 4-methylumbelliferyl oleate (4-MUO) was used as a substrate to measure the pancreatic lipase inhibitory activity. The sample solution (25 μL of 3 h infusion) was added to 50 μL of 0.1 mM 4-MUO solution dissolved in a buffer consisting of 66 mM Tris–HCl (pH 7.4), 7 mM NaCl, 3 mM CaCl2, and 2 mM dimethyl sulfoxide (DMSO). These were mixed in a 96-well microplate, and then 25 μL of lipase solution (50 U/mL) was added to initiate the enzyme reaction. After incubation at 37°C for 60 min, the reaction was stopped with 50 μL of 0.1 mM citric acid, and the amount of 4-methylumbelliferone (4-MU) released by lipase was measured using a fluorometric microplate reader (Varioskan, Fisher Scientific, MA, USA) at λex 355 nm and λem 460 nm.

3. RESULTS

We manufactured a high quality low caffeine tea with health benefits for everyone. First, we determined the caffeine, catechin and theanine contents of the low caffeine tea and green tea (control) infusions at various infusion times (Fig.1). The concentrations of each component in both the low caffeine tea and green tea infusions were increased in an infusion time-dependent manner. The caffeine in the low caffeine tea was infused slowly, and the concentration was extremely low compared to the green tea, i.e., the level was decreased to less than one-third that of green tea at 6 h (Fig. 1A). The caffeine content differed significantly between all of the low caffeine and green tea samples.

The concentrations of catechin and theanine were also increased in an infusion time-dependent manner; moreover, there were no significant differences between the low caffeine tea and green tea, except at the 1 h infusion time (Fig. 1B, C). In other words, the catechin content of the 6 h infusion was very similar between the low caffeine tea and the green tea. Catechins mainly include epicatechin gallate (ECG), epigallocatechin gallate (EGCG), epicatechin (EC), catechin (C), and epigallocatechin (EGC). Among catechins, the most highly infused were EGC, followed by EC, EGCG, C, ECG in both the low caffeine tea and the green tea, and there was no difference in the rank order of catechins between the two groups (Fig. 1B). The analysis of theanine revealed the same trend as for catechins, and there were no significant differences between the low caffeine tea and the green tea at the 0.5, 3 and 6 h infusions (Fig. 1C). The results showed that the low caffeine infusion had reduced caffeine content; however, both catechin and theanine levels, as the main components, were maintained. Next, we determined the physiological function of the low caffeine tea. The 3 h infusion was used as the

sample in this experiment, in reference to the result of Fig. 1. Fig. 2 shows the anti-oxidative activity of the low caffeine tea infusion in comparison to the green tea. The stable free radical DPPH was used to determine the radical scavenging activity of the sample. Anti-oxidative activity was indicated by a decrease in DPPH absorbance. Anti-oxidative activity was increased up to 1 h and was maintained at the same level until 6 h; further, the activities of the low caffeine tea and green tea did not significantly differ.

We also determined the anti-obesity function of the low caffeine tea by assessing lipase activity (Table 1). Inhibition of lipase activity did not significantly differ between the low caffeine tea infusions and the green tea infusions.

4. DISCUSSION

As the popularity of green tea has increased recently, caffeine-free green tea options are also being marketed. Taking into account physiological function and taste, we produced a green tea with reduced caffeine content instead of a caffeine-free beverage, and succeeded in reducing the caffeine content by 70%. While caffeine has some side effects, it was reported to enhance the physiological function of catechins through synergistic effects [15-17]. In addition, the combination of L-theanine and caffeine improves brain function in humans [18,19]. It has also been reported that caffeine is necessary for the characteristic taste of tea [20]. Therefore, by reducing the caffeine of green tea instead of completely removing it, the taste and physiological function are maintained, enabling the production of a high quality green tea. The complete removal of caffeine negatively impacts the taste of tea, necessitating the addition of chemicals to improve the quality and taste, and this is a serious issue for tea as a functional food and beverage. We treated fresh tea leaves with a hot water process (95°C, 180 seconds) to produce low caffeine tea; the physiological property of caffeine allows it to be easily eluted by hot water [21]. This is a safe and stable processing method that does not necessitate contamination by chemical substances and resins. From the viewpoint of functionality and taste, it is very important that catechin and theanine levels are maintained as the major components besides caffeine. The total amount of catechins was not reduced in the 6 h infusion compared with the standard green tea beverage, although EGCG, which is the most abundant catechin in tea leaves, was not highly contained in the low caffeine tea infusion. This result is in agreement with a report that, due to their physical properties, EGC is easily dissolved in cold water, while EGCG is difficult to elute [22,21].

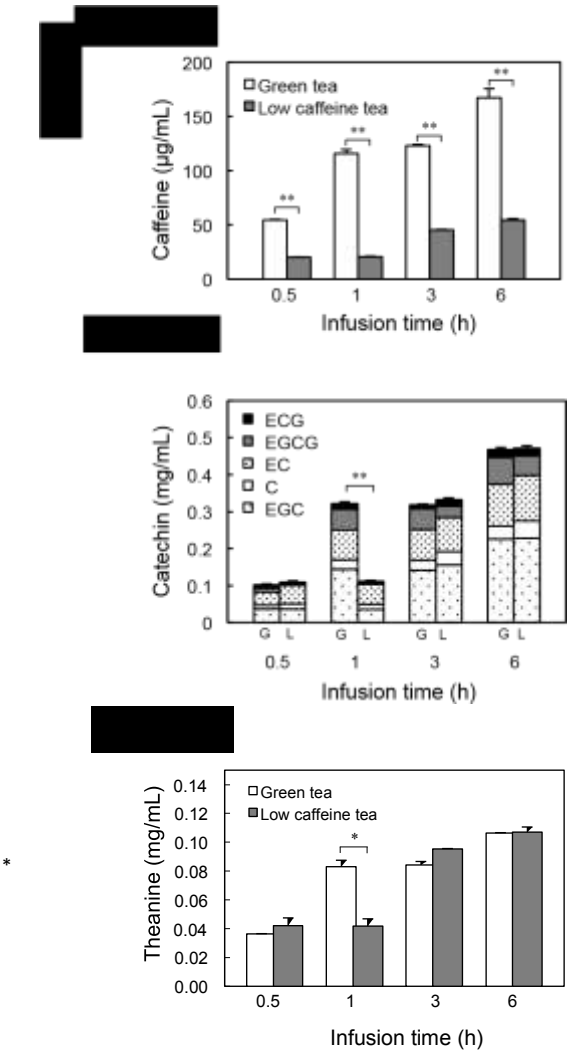

Fig. 1 Quantitative determination of the main components in low caffeine tea and green tea

*Asterisk (*) indicates statistical significance compared with green tea at the same infusion time. G, green tea; L, low caffeine tea. Each bar shows the mean ± SD (n=3, **p<0.005, *p<0.05).*

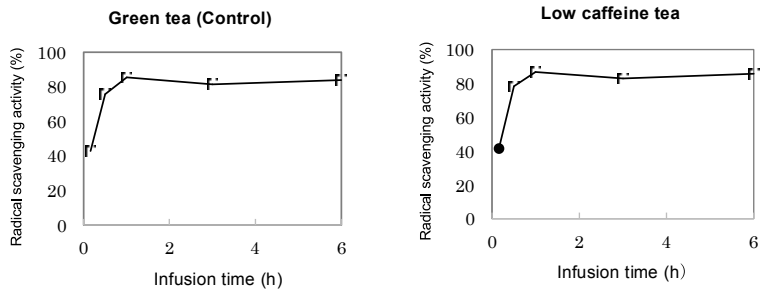

Fig. 2. Comparison of anti-oxidative activity in green tea and low caffeine tea

Table 1. Inhibitory effect of low caffeine tea and green tea on lipase activity

Infusion time (h)	Inhibition (%) of green tea (control)	Inhibition (%) of low caffeine tea	Significance*
0.5	82.06±0.285 (n=3)	83.12±0.393 (n=3)	n.s.
1	86.93±0.377 (n=3)	84.34±0.938 (n=3)	n.s.
3	87.03±0.277 (n=3)	88.19±0.198 (n=3)	n.s.
6	87.67±0.320 (n=3)	88.26±0.025 (n=3)	n.s.

*Low caffeine tea was compared to each control. n.s.: not significant.
Inhibition (%) is normalized to activity in the absence of inhibitor.

Both catechin and theanine levels were much lower in the low caffeine tea than the green tea only for the 1 h infusion, while no differences were seen for the 3 or 6 h infusions. The manufacturing process might have an effect on the elution of compounds from tea leaves, resulting in the significant difference for the 1 h infusion only. Besides, there appeared to be no differences between the low caffeine tea and the green tea in the contents of catechins and theanine. Moreover, the low caffeine tea exhibited the same level of anti-oxidative activity as the green tea at any infusion time, even with the decrease in EGCG as the most abundant anti-oxidant in tea leaves [23]. EGC, which exhibited relatively high anti-oxidative activity, is easily infused in cold water and might be responsible for the antioxidative activity instead of EGCG.

In regards to the inhibitory effect of low caffeine tea on lipase activity, despite the decrease in caffeine content, the low caffeine tea exhibited the same level of lipase activity as the green tea. The role of caffeine in this function is not clear; however, the lipase inhibitory effect might be enhanced by the synergistic interaction between catechin and theanine.

The low caffeine tea with the high-quality components produced in this study is suitable for consumption by everyone, even those avoiding caffeine, and also exhibits the functions of antioxidative and lipase inhibitory activities.

5. CONCLUSION

We reduced the caffeine content of green tea infusion by 70% to avoid the side effect of caffeine using a hot-water spray process. However, both catechin and theanine levels, as the main components, were maintained. The low caffeine tea exhibited the functions of antioxidative and lipase inhibitory activities at the same level as green tea. We developed more reasonable and high-quality low caffeine tea than ever.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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