Journal of Experimental Agriculture International

28(6): 1-6, 2018; Article no.JEAI.44717
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Change of Main Components and Physiological Functions of Post-fermented Green Tea with Reduced Caffeine

Kieko Saito[1,2*] and Yoriyuki Nakamura[2]

[1]School of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Suruga, Shizuoka, 422-8526, Japan.
[2]Tea Science Center, University of Shizuoka, 52-1 Yada, Suruga, Shizuoka, 422-8526, Japan.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2018/44717
Editor(s):
(1) Dr. Monica Rosa Loizzo, Professor, Department of Pharmacy, Health Sciences and Nutrition, University of Calabria, Italy.
(2) Dr. Biljana Bojovic, Assistant Professor, Faculty of Science, Institute of Biology and Ecology, University of Kragujevac, Republic of Serbia.
(3) Dr. Daniele De Wrachien, Professor, State University of Milan, Italy.
Reviewers:
(1) Alejandra Garcia-Gasca, CIAD, Mexico.
(2) Mohammed Kawser Hossain, Sylhet Agricultural University, Bangladesh.
Complete Peer review History: http://www.sciencedomain.org/review-history/27550

Original Research Article

Received 11 September 2018
Accepted 23 November 2018
Published 03 December 2018

ABSTRACT

We developed a post-fermented tea that contains reduced caffeine and exhibits positive functions to maintain our health. We also analysed the main components and physiological function of the post-fermented tea compared to green tea as the control. The tea leaves with reduced caffeine were fermented with lactic acid bacteria (*Lactobacillus plantarum*) for four weeks. HPLC was employed to analyse the caffeine, catechin and theanine in the infusion of post-fermented tea leaves. The antioxidative activity was analysed spectrophotometrically. The preadipocyte (3T3-L1) differentiation-inhibitory test, which is an index of anti-obesity, was employed. As fermentation progressed, theanine and catechin contents tended to decrease, though caffeine level was unchanged. The infusion of the post-fermented tea exhibited high antioxidative activity during fermentation, while important antioxidant catechin content was decreased. In addition, the preadipocyte differentiation-inhibitory test showed the inhibition effect of the post-fermented tea infusion on adipogenesis in a dose-dependent manner. These results showed that the fermentation

**Corresponding author: E-mail: saitok@u-shizuoka-ken.ac.jp;*

did not affect the antioxidant and anti-adipogenesis activities. Fermentation denatured the structure of leaf tissue, and some novel molecules might have been produced, exhibiting positive effects. These results showed that the post-fermented tea we developed was a unique tea product showing antioxidative activity and adipogenesis inhibitory effect and may prove to be a functional food for our health.

Keywords: Camellia sinensis; green tea; fermentation; reduced caffeine; antioxidative activity; ant-adipogenesis; lactic acid bacteria.

1. INTRODUCTION

Many kinds of functional foods are produced and taken for our health. Tea is an attractive beverage with high palatability produced worldwide. One reason for its popularity is that it exhibits physiological health benefits against conditions such as cancer, obesity, diabetes, arteriosclerosis, neurodegenerative diseases, tooth decay, hepatitis, allergies, and bacterial and viral infection [1]. Traditional fermented foods such as soy sauce, miso and tofu are becoming more popular in Japan, so fermented tea has received much attention as a functional food. Pu-erh which is originally from Yunnan, China, is the most famous fermented tea (also known as post-fermented tea or dark tea) and has undergone microbial fermentation from several months to many years. In the beginning stage of processing Pu-erh tea, freshly plucked tea leaves are pan-fried in order to inactivate the enzymes, which undergo either mould fermentation or ageing maturation. Fermented teas can be divided according to how they are produced. Piled teas, such as the Chinese post-fermented teas, and the Toyama kurocha produced in Japan, are fermented with naturally occurring fungus under relatively dry conditions. Other fermented teas, called pickled teas, are fermented in a wet process with lactic acid bacteria. Pickled teas include Miang from Thailand and Awabancha from Japan [2]. A third category, including the Japanese Goishicha and Ishizuchi-kurocha, is fermented with the piled and pickling methods successively [3,4].

These fermented teas are traditional foods and the contents are unbalanced in some cases and the quality is not uniform because some of them are not using a single microorganism or are made by a method that relies on experience. Also, they contain caffeine whose side effects are a concern. Caffeine, one of the main components of teas, imparts a distinct taste, while exhibiting some side effects, including sleeplessness. Senior citizens, children, and pregnant women should avoid tea despite its known beneficial effects.

In this study, we used green tea leaves that were treated with heated water to reduce caffeine content and manufactured a post-fermented green tea [5]. Further, the main components of the post-fermented green tea with reduced caffeine (post-fermented tea) as well as its anti-oxidative and adipogenesis-inhibitory effect were determined in an effort to elucidate its health benefits.

2. MATERIALS AND METHODS

2.1 Production of Post-fermented Tea

First, to get green tea leaves with lower caffeine (low-caffeine tea), freshly plucked tea leaves (*Camellia sinensis* L. cv. 'Yabukita') were showered with heated water (95℃) for 180 seconds by a low-caffeine tea processing machine [5] and 1 kg of wet low-caffeine tea was mixed with 100 mL of a lactic acid bacteria, *Lactobacillus plantarum* (1.7×10^8 cells/mL). Then, the mixture was packed into an airtight container and stored at 25℃ for 4 weeks under a shaded condition. The leaves were sampled at 3, 7, 14, 21 and 28 days during fermentation.

2.2 Preparation of Post-fermented Tea Infusion

The tea sample was dried at 50℃ for 24 hours, and then milled for 30 seconds to make a powder. 1 g of the powder was added to 100 mL distilled water and heated at 70℃ for 1 hour to get an infusion. The infusion was spun down, and the supernatant was collected followed by filtration with a 0.4-μm membrane to apply to the following experiments.

2.3 Determination of Catechin, Caffeine and Theanine Content

Theanine, catechins and caffeine, the main components of tea, were analysed using a high-performance liquid chromatography (HPLC) (Agilent 1100, Agilent Technologies, Palo Alto, Calif., USA) that was equipped with a C18 column (4.6 i.d. x 150 mm, 5 μm, Tokyo

Chemical Industry Co. Ltd., Tokyo, Japan). The HPLC column was maintained at 30℃ in an oven. The mobile phase for the detection was 0.1 M NaH_2PO_4 buffer/acetonitrile (87:13) at a flow rate of 1.0 ml/min. The reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), and high-performance liquid chromatography (HPLC)-grade reagents were used for the HPLC analysis. Each peak was identified by comparing the UV-Vis spectral characteristics and retention times with those from commercial standards supplied by Wako Pure Chemicals Industry, Ltd., Japan.

2.4 Determination of Anti-oxidative Activity

The stable free radical DPPH (1,1-Diphenyl-2-picryl-hydrazyl, Sigma-Aldrich, St. Louis, MO, USA) was used to estimate the antioxidant activity of the post-fermented tea. 1.5-ml aliquot of DPPH solution (0.1 mM, in 95% ethanol) was mixed with 100 µL of tea infusion. Green tea infusion (*Camellia sinensis* L. cv. 'Yabukita') was used as a control. 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

2.5 Preadipocyte Differentiation-inhibitory Test

Preadipocytes, MC3T3-L1 cells (Japanese Collection of Research Bioresources, Cell Bank, Japan**)**, were used to determine the anti-adipogenesis effect of the post-fermented tea. The 3T3-L1 1cells were cultured and induced to differentiate into adipocytes as described previously [6,7.8]. Briefly, the cells were cultured with α MEM medium (Gibco, Thermo Fisher Scientific, MA , USA), including 10% FBS (Corning Incorporated, NY, USA) for 3 days and changed to induction medium including 10 µg/mL of insulin solution, 2.5 µM of dexamethasone and 0.5 mM of 3-isobutyl-1-methylxantine (Cayman Chemical Company, MI, USA) as the differential factors or the differential factors with samples. Seven days after induction, lipid droplets were observed under a microscope and lipid droplet accumulation in the differentiated cells can be visualised by Oil Red O solution staining (Sigma-Aldrich Co. LLC, MO, USA) [9,10]. To quantify lipid droplets, the stained lipid droplets were extracted with 2-propanol and the extract was measured O.D. at 490 nm photometrically.

2.6 Statistical Analyses

Data are expressed as the mean ± standard error of the mean (SEM). Statistical analyses were performed using Student's *t*-test and one-way analysis of variance (ANOVA). All statistical analyses were performed in a statistical analysis program, JMP ver.13 (SAS Institute Inc., Cary, NC USA).

3. RESULTS AND DISCUSSION

We employed the green tea leaves with reduced caffeine (successfully decreased by 70%) and the same concentration of catechins and theanine in a previous study [5], and conducted fermentation to develop post-fermented tea. *Lactobacillus plantarum,* which was originally derived from a plant, was applied for the fermentation because it is resistant to catechins possessing anti-bacterial activity [11]. During the fermentation in this study, pH of the infusion of the post-fermented tea was gradually decreased, indicating the fermentation was accelerated by lactic acid bacteria in an anaerobic condition (data not shown). pH is an indicator of fermentation, and its gradual decrease shows the favorable progress of lactic acid fermentation.

First, we analyzed the change of contents of caffeine, catechin(s) and theanine, which are well-known as main components of green tea and exhibit the characteristic taste and health benefits. As fermentation progressed, significant change was not seen in the caffeine content for 4 weeks, indicating caffeine was not degraded in the fermentation process and maintained a low concentration (Fig. 1).

Tea catechins consist of epigallocatechin (EGC), catechin, epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG). These catechins seem to be decomposed in the fermentation enzymatically caused by lactic acid bacteria. The content seemed to decrease a little except after 14 days (Fig. 1). Green tea is rich in EGCG, which makes up about 10% of the dry-leaf weight and is the highest amount of catechins. In addition, EGCG is easily decomposed into other catechins with a smaller

molecular weight because of its molecular structure [12]. Therefore, the total amount of catechins was gradually decreased for 7 days. Degradation product of EGCG, such as EGC, seemed to be detected in 14 days, and then the catechins might be decomposed into unidentified molecules by further fermentation.

Theanine was significantly decreased, but after 7days, the content increased. The reason for this increase was unclear, but this result is interesting if theanine might be synthesised by fermentation. Recent research reported that theanine is produced biologically, such as by bacterial enzyme [13,14] in the presence of glutamic acid and ethylamine. Theanine is naturally synthesised from glutamic acid and ethylamine by a synthetic enzyme in the tea roots. In addition, tea leaves contain over 10% glutamic acid, while catechin(s) was degraded to produce ethylamine spontaneously in the process of metabolism [15]. These facts make possible the biosynthesis of theanine in the process of fermentation.

Next, our study showed the physiological function of the post-fermented tea. The antioxidative activity of the infusion of the post-fermented tea was determined as shown in Fig. 2. DPPH is a stable free-radical molecule and used as an indicator of an antioxidant. The infusion of the post-fermented tea was added to DPPH solution and measured spectrophotometrically. The result indicated that no significant difference was seen between the infusion and the control in an anti-oxidative function. The infusion exhibited an anti-oxidative function despite the fermentation period. Kim et al. suggested that fermentation diminished the antioxidant capacity of tea [16], but results obtained from the present study provide that fermentation did not inhibit the antioxidative activity. Fermentation of the green tea with microorganisms resulted in great change in their chemical constituents during the processing of pu-erh tea [17]. Watanabe et al. suggested that catechins in extremely acidic buffer solutions might reversibly combine with each other or with other compounds [18]. The functional molecule in antioxidation was unclear, though, and various phytochemicals caused by biotransformation might affect the activity. Further research will be expected to identify the effective molecules.

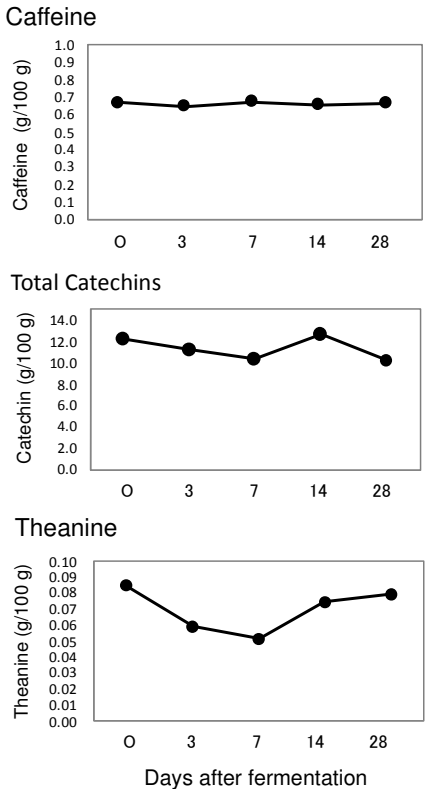

Fig. 1. Change of caffeine, catechin and theanine contents of the infusion of post-fermented green tea with reduced caffeine during fermentation

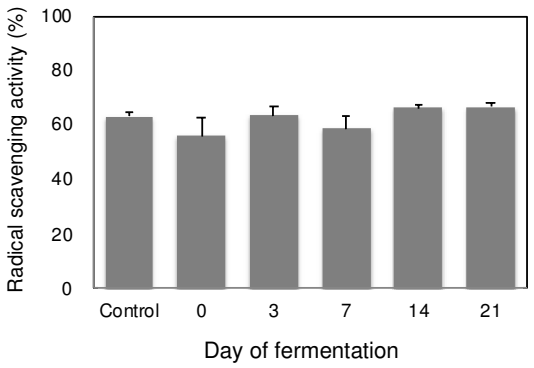

Fig. 2. Anti-oxidative activity of the infusion of post-fermented green tea with reduced caffeine. DPPH radical scavenging activity was analysed spectrophotometrically. Each bar shows the mean ± SEM (n=3)

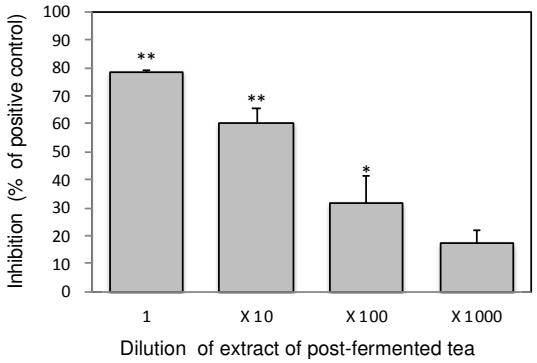

Fig. 3. Inhibitory effect of the infusion of post-fermented green tea with reduced caffeine on adipogenesis. Each bar shows the mean ± SEM (n=3; **p<0.005, *p<0.05)

Next, physiological function study is done with the preadipocyte differentiation-inhibitory test, relating to anti-obesity; obesity has recently been considered to be the cause of metabolic syndrome [19,20]. When preadipocyte differentiates into adipocyte, lipid droplets appear inside of the cell; then, the droplets can be stained with Oil Red O, which makes it possible to visually identify differentiation. In this experiment, the different concentrations of tea infusions fermented for 14 days were added into the preadipocyte culture in the presence of differential factor. As a result, lipid droplets were not observed in high concentration (data not shown). Apparently, the inhibition was in a dose-dependent manner. In adding to the visualisation, stained droplets were dissolved and spectrophotometrically quantified to analyse the effect of the infusions. The result showed that the infusion of the post-fermented tea inhibited adipogenesis in a dose-dependent manner (Fig. 3). Even a weak solution diluted 1000-fold resulted in the inhibitory effect on preadipocyte differentiation, suggesting that the post-fermented tea might exhibit anti-obesity action.

4. CONCLUSION

To prevent the side effects of caffeine and offer a more attractive tea to everyone, we developed a post-fermented tea that contains a low concentration of caffeine. During fermentation, the antioxidative effect was maintained despite catechin degradation, and it exhibited the adipogenesis inhibitory effect as positive functions. This new type of green tea serves not only as a drink but also as a functional food to eat. These results will be helpful for developing processed agricultural products in the future.

ACKNOWLEDGEMENTS

We would like to express the deepest appreciation to M. Ishikawa for collaboration on the early stage of this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Isemura Y, Muramatsu K, Ogura I, Sugiyama K, Yamamoto M, editors. Health Science of Tea; 2002.
2. Okuda S. The world of plant origin lactic acid bacteria, JPN J Lactic Acid Bact. 2002;13:23-36.
3. Miyagawa K, Otsubo F, Katafuchi K. Japanese fermented tea. J Home Econ JPN. 1989;6:545- 551.
4. Tamura A, Kato M, Omori M, Nanba A, Miyagawa K. Characterization of microorganisms in post-heating fermented teas in Japan. J Home Econ JPN. 1994; 45:1095-1101.
5. Saito K, Nakamura Y. Development and properties of green tea with reduced caffeine. J Exp Agri INT. 2017;17:1-6.
6. Kang K, Pariza MW. trans-10, cis-12-Conjugated linoleicacid reduces leptin secretion from 3T3-L 1 adipocytes. Biochem Biophys Res Commun. 2001; 287:377–382.

7. Kim HS, Liang L, Dean RG, et al. Inhibition of preadipocytedifferentiation by myostatin treatment in 3T3-L1 cultures. Biochem Biophys Res Commun. 2001;281:902–906.
8. Bradley RL, Mansfield JP, Maratos-Flier E, et al. Melanin-concentrating hormone activates signaling pathways in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab. 2002;283:E584–E592.
9. Halvorsen YD, Bond A, Sen A, et al. Thiazolidinediones andglucocorticoids synergistically induce differentiation of humanadipose tissue stromal cells: biochemical, cellular, and molecular analysis. Metabolism. 2001;50:407-413.
10. Sen A, Lea-Currie YR, Sujkowska D, et al. Adipogenicpotential of human adipose derived stromal cells from multipledonors is heterogeneous. J Cell Biochem. 2001; 81:312–319.
11. Sanae O. The world of plant origin lactic acid bacteria. JPN J Lactic Acid Bacteria. 2002;13:23-36.
12. Tanaka T, Kouno I. Oxidation of tea catechins: Chemical structures and reaction mechanism. Food Sci Technol Res. 2003;9:128–133.
13. Sasaoka K, Kito M, Onishi Y. Some properties of theanine synthesizing enzyme in teaseedlings. Agric Biol Chem. 1965;29:984–988.
14. Deng WW, Ogita S, Ashihara H. Biosynthesis of theanine (γ-ethylamino-L-glutamic acid) in seedlings of Camellia sinensis. Phytochem Lett. 2008;1:115–119.
15. Konishi S, Takahashi E. Metabolism of theanine in tea seedlings andtransport of the metabolites. Nippon Dojouhiryougaku Zasshi. 1969;40:479-484. In Japanese.
16. Kim Y, Goodner KL, Park JD, Choi J, Talcott ST. Changes in antioxidant phytochemicals and volatile composition of Camellia sinensis by oxidation during tea fermentation. Food Chem. 2011;129: 1331-1342.
17. Zhou Z, Zhang Y, Xu M, Yang C. Puerins A and B, two new 8-Csubstituted flavan-3-ols from pu-er tea. J. Agric. Food Chem. 2005;53:8814-8617.
18. Tu YY, Xia HL, Watanabe N. Changes in catechins during the fermentation of green tea. Appl Biochem Microbiol. 2005;41: 574–577.
19. Reaven GM. Role of insulin resistance in human disease. Diabetes. 1988;37:1595-1607.
20. Fujioka S, Matsuzawa Y. Tokunaga K, Tarui S. Contribution of intra- abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism. 1987;36:54-59.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27550