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Effects of Temperature and Water Steeping Duration on Antioxidant Activity and Caffeine Content of Tea

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Abstract

Tea (*Camellia sinensis* L.) is a popular beverage. This study inspects the effects of temperature (90°C, 4°C) and steeping duration (6 min, 24 hrs) on antioxidant activity and caffeine content prepared via different fermentation of tea. The total phenolic content of green tea prepared with cold water steeping is significantly higher compared to any other tea prepared in hot or cold steeping treatments. The caffeine content of tea prepared with hot water steeping is significantly higher than that with cold water steeping treatment. The treatment of tea prepared with cold water steeping, as shown in this study, provides a sound basis for future exploration concerning the beneficial effects of tea beverage.

Keyword: cold water steeping tea, antioxidant capacity, caffeine
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Introduction

Tea (*Camellia sinensis* L.) is widely grown in Asia, Africa and South America and is a popular beverage. Tea is traditionally classified based on the degree or period of oxidation/fermentation in its leaves [1]. The degree of processing and oxidation/fermentation determines whether the tea becomes a green, oolong or black variety. Inhibiting interactions of the enzymes and the catechins produces green tea (non-fermented tea), allowing the tea to remain green in color. Oolong tea (partially fermented tea) is a partially fermented product and produced through partial oxidation of catechins. Black tea (fully fermented tea) is produced by extended fermentation of tea leaves,

producing polymeric polyphenols, such as thearubigins in addition to some theaflavins [2].

Reactive oxygen species (ROS) are generally reactive molecules or radical species, including hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), superoxide anion radical ($O_2^{\bullet-}$), and peroxyl radical ($ROO^{\bullet}$), which may subsequently oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases.

One of the main characteristics of an antioxidant is its ability to trap free radicals. It also has been reported, for the antioxidant activity of phenolic compounds used in traditional medicinal plants, a positive correlation was observed between high phenolic content and strong antioxidant activity [3]. Antioxidant activity can be measured by radical scavenging ability and reducing power. The reducing power of a compound is expressed as its capacity to donate electrons by reacting with unstable free radical species, convert them into more stable metabolites, and terminate the radical chain reactions [4].

Polyphenols are normally polyhydroxy phenolic complexes and secondary metabolites in plants. These compounds possess an aromatic ring bearing one or more hydroxyl groups, and their structures may vary from that of a simple phenolic molecule to that of a complex high-molecular weight polymer [5]. Tea leaves contain 10-30% (dry leaf weight) of polyphenols. Tea polyphenols are natural antioxidants [6] and considered to be responsible for the anticarcinogenic and antimutagenic properties of tea in addition to their protective roles against cardiovascular diseases [7]. The riboflavin photo-reactions generating $O_2^{\bullet-}$ enhance the levels of DNA cleavage, but the green tea extract can scavenge the $O_2^{\bullet-}$ and in turn protect the integrity of super-coiled plasmid DNA under riboflavin photo-chemical treatment [8].

The beneficial effects in hot or cold water steeping methods of preparing tea beverage vary worldwide. Recently, cold water (4 or 25°C) steeping is popular for making tea infusion in Taiwan. For cold water preparation, tea leaves steeped at 25 °C water for at least for 2 hrs or in 4°C water over 4 hrs generally contain lower amount of caffeine, less bitter taste and more aroma, and would not influence sleep comparatively [9]. Usually, tea is steeped in cold water in a refrigerator (4°C) for overnight, and this is a popular and simple way for making tea beverage. Venditti *et al.* (2010) using green tea, oolong tea, black tea and white tea steeped in hot water (90°C) or cold water (room temperature), suggested that the total phenolic contents and antioxidant activity were of no significant differences among hot or cold teas, except in the case of white tea, where significantly higher levels of phenolic content and antioxidant activity were obtained after cold water steeping [10].

The aim of this study was to inspect the effects of temperatures and steeping duration on caffeine contents and antioxidant activity of tea achieved via different fermentation tea by examining their scavenging ability against DPPH radical, reducing power and total phenolic contents.

Materials and Methods

Tea extract

Green tea (Pi Lo Chun), partially fermented tea (Oolong tea) and black tea (Red Jade black tea) were purchased from Ten-Ren Tea Co. (Taipei, Taiwan). Tea was extracted by hot water and cold water treatments. One-gram tea and 50 mL hot water (90°C) were put into a glass beaker for 6 min, while in the other treatment tea was extracted by ultra-pure water in a 4°C refrigerator for 24 hrs. In table 1, the symbols of different treatments of tea extraction used in this study are shown. The extracted tea was filtered through an Advantec No.1 qualitative filter paper and quantitated to 50 mL and stored at -20°C.

Table 1. The symbols of steeping treatment of tea

Tea	Hot water	Cold water
Green tea	HG	CG
Oolong tea	HO	CO
Black tea	HB	CB

Chemicals

Nitro blue tetrazolium (NBT) was purchased from Bio Basic Inc. (Markham Ontario, Canada). DPPH, ferric chloride, Folin-Ciocalteu reagent, L-methionine, monopotassium phosphate, potassium ferricyanide, potassium dihydrogen phosphate, sodium carbonate and trichloroacetic acid were obtained from Sigma-Aldrich (St. Louis, MO). Ultra-pure water prepared from the Milli-Q system was used as a solvent in this study.

Assay of total phenolic contents

The total phenolic contents of tea were determined by a modified Folin-Ciocalteu

method [11]. The tea extract (250 µL) was mixed with 1 N Folin-Ciocalteu reagent (250 µL), and incubated for 5 min. Then, the solution was mixed with the 0.5 mL of 20% sodium carbonate (Na_2CO_3) and 4 mL water and incubated for 25 min at room temperature. The mixture was then centrifuged at 4°C and 5,000 rpm for 10 min. The supernatant was measured at 730 nm (PerkinElmer Lambda35 UV/Vis spectrometer). The contents of total phenolics were determined as a gallic acid equivalent (GAE) in mg per gram of tea extract.

Determination of reducing power activity

The reducing power of tea extracts was determined by the method of Oyaizu [12]. The extract solution (200 µL) was mixed with of 0.2 M phosphate buffer (200 µL, pH 6.6), 1% potassium ferricyanide (200 µL). The mixture was incubated at 50°C for 20 min. Ten-percent trichloroacetic acid (200 µL) was added to the mixture, followed by centrifugation at 3,000 rpm for 10 min. The supernatant (500 µL) was mixed with ultra-pure water (500 µL) and 0.1% ferric chloride (100 µL), and incubated for additional 10 min. The supernatant was then measured at 730 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

Capacity of scavenging DPPH radical

The DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radical species. Each extract sample was mixed with 160 µM DPPH in methanol. After incubation for 20 min at room temperature in the dark, the absorbance was read at 517 nm [13]. The inhibitory percentage of DPPH (% scavenging activity) was calculated according to the equation shown as follows. The IC_{50} is defined as the equivalent concentration of tea extract that is able to remove 50% of the DPPH radicals.

$$\text{Scavenging DPPH radical effect (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100\% \ .$$

Determination of caffeine

The caffeine content of tea extract was determined by Chinese National Standard method (CNS 2009) [14]. Chromatographic analysis was performed as follows. Different reaction solutions were separated via a Mightysil RP-18 GP Aqua column (5 µm, 4.6 mm id × 250 mm, Kanto Chemical Co., Tokyo, Japan) at 25°C within an HPLC system, equipped with a pump (Hitachi-L2130) and a photodiode-array detector (Hitachi-L2450). The photodiode-array detector was used to detect caffeine, catehin and gallic acid was at 280 nm. The mobile phase was prepared with 30% (v/v) methanol solution (in water). Each sample of a 20 µL was subsequently injected at a flow rate of 1.0 mL/min at 25°C with a Colbox column oven (Hipoint, Kaohsiung, Taiwan).

Statistical analysis

The results are expressed as mean ± standard deviation (SD) of three separate experiments. A homoscedastic two-sample *t*-test was employed to assess whether the two sets of measurements differed, and values of $P < 0.05$ were considered to be significant.

Results

Antioxidant activity of tea extract

The antioxidant activity of tea was investigated according to their DPPH radical scavenging activity, reducing power and total phenolic contents. The total phenolic contents were expressed as gallic acid equivalents (GAE). As shown in Figure. 1, the total phenolic contents of green tea prepared with cold water steeping is significantly higher than those with hot water steeping treatment. The total phenolic contents of black tea prepared with cold water steeping is lower than hot water steeping treatment as shown in Figure. 1.

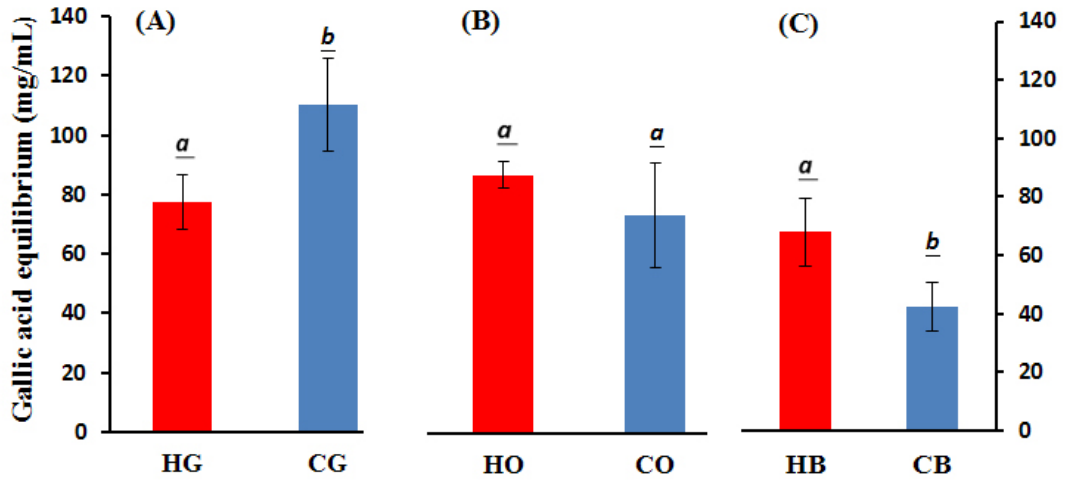

Figure 1. The total phenolic contents of hot or cold water steeping tea with different differences ($p < 0.05$) between groups are indicated by different letters above the bar.

The IC_{50} of scavenging DPPH radical of tea with different fermentation are shown in Figure 2. The IC_{50} of scavenging DPPH radical under hot or cold water steeping treatments are both insignificant. The exception is with black tea, where the IC_{50} of cold water steeping treatment is higher than that of the hot one.

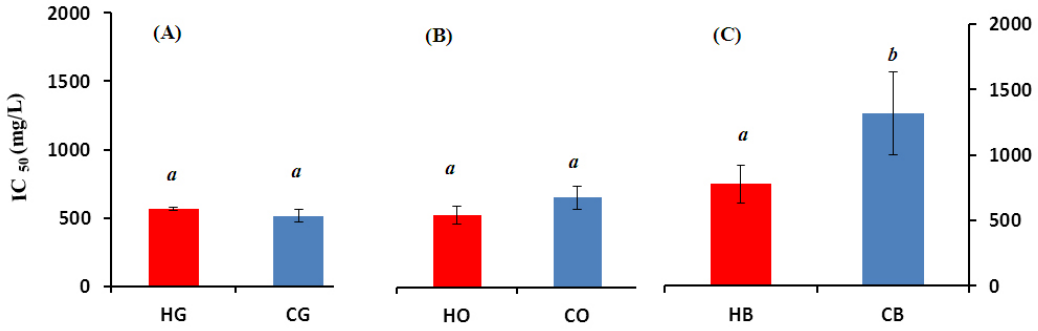

Figure 2. The capacity of scavenging DPPH radical of hot or cold water steeping tea with different fermentation. IC_{50} of tea scavenging DPPH radical are shown. Data were represented by mean ± SD, where n = 3. Statistical differences ($p < 0.05$) between groups are indicated by different letters above the bar.

In the reducing power assay, the antioxidant activity of the samples was measured by formation of ferrous products monitored at 700 nm with increased absorbance indicating an enhanced reducing power. The slope of calibration was measured by the absorbance of reducing power with increased absorbance, indicating a stronger reducing power.

As shown in Figure 3, the reducing power capacities of tea under hot water steeping are higher than those under cold water steeping treatments. The exception is green tea, where the reducing power under cold water steeping and hot water steeping treatments are both insignificant.

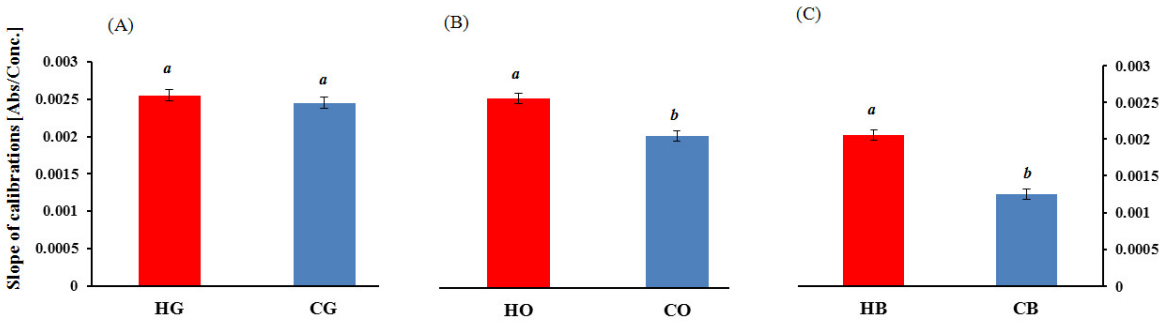

Figure 3. The capacity of reducing power of hot or cold water steeping of tea with different fermentation. Data were represented by mean ± SD, where n = 3. Statistical differences ($p < 0.05$) between groups are indicated by different letters above the bar.

Determination of caffeine

The chromatograms of 100 μg/g aqueous caffeine, catechin and gallic acid are shown in Figure 4. As shown in Figure 4, peaks of gallic acid, catechin and caffeine are eluted at 5.1, 5.8 and 9.4 min, respectively.

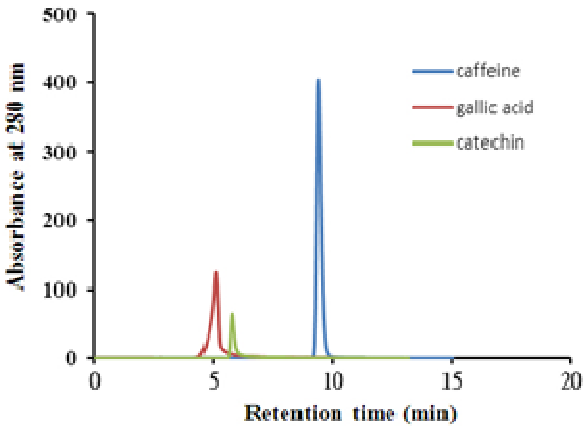

Figure 4. The chromatograms of caffeine, gallic acid and catechin.

The caffeine contents of tea extracts under hot or cold water steeping treatment were investigated. As shown in Figure 5, the contents of caffeine of tea prepared with cold water steeping is lower than those with hot water steeping treatment.

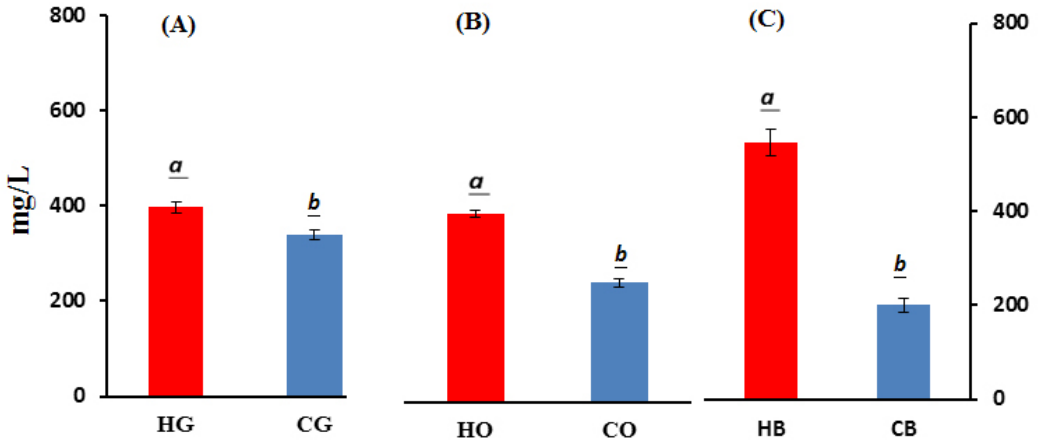

Figure 5. The caffeine contents of hot or cold water steeping of tea with different fermentation. Data were represented by mean ± SD, where n = 3. Statistical differences ($p < 0.05$) between groups are indicated by different letters above the bar.

The catechin and gallic acid contents of green tea extract under hot or cold water steeping were investigated. As shown in Figure 6, the contents of catechin and gallic acid of green tea prepared with cold steeping are more than those with hot water steeping treatment. The catechin and gallic acid are 1.7 and 1.2-fold higher in tea

under cold water steeping compared to those in hot water steeping treatment, as shown in Figure 6.

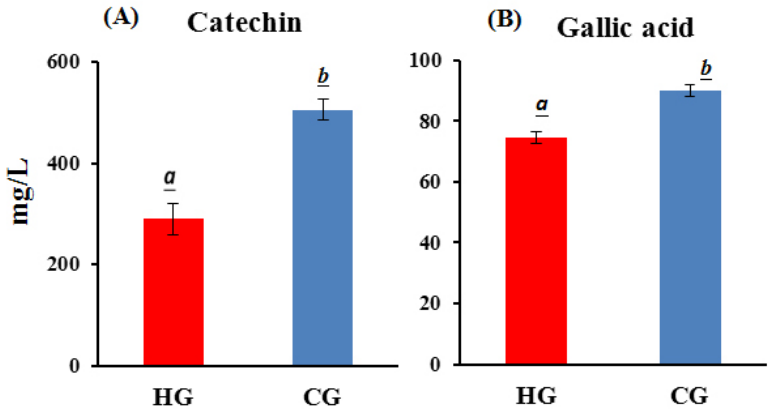

Figure 6. The catechin and gallic acid contents of hot or cold water steeping on green tea. Data were represented by mean ± SD deviation, where n = 3. Statistical differences ($p < 0.05$) between groups are indicated by different letters above the bar.

Discussion

In this study, the results show that the total phenolic contents of green tea prepared with cold water steeping are higher than those of any other teas prepared in hot or cold steeping treatments, suggesting that some phenolic compounds in green tea might not be heat resistant and degraded at higher temperatures. Catechins are a class of phenolic poly flavonoids. The cross-linkage between catechin and bicarboxylic acid can be observed in alkaline solutions when the reaction temperature is increased [15]. Catechins are chemically unstable [16]. In solution, catechins readily undergo oxidation, involving the loss of hydrogen atoms, generation of a semiquinone radical intermediate and formation of oxidized quinone products [17,18].

Catechins are the largest part of the polyphenols found in green tea and are effective free radical scavengers [19]. The total phenolic content of green tea prepared with cold water steeping is higher than those prepared in hot water steeping treatment. Shimizu *et al.* (1988) reported on the hypoglycemic activity of Japanese green tea with cold, warm and hot water extract in normal and streptozotocin-induced diabetic rats with the green tea cold water extract exhibiting the best hypoglycemic activity [20]. The hypoglycemic component in normal rats was identified as a polysaccharide and was not heat resistant. In this study, green tea prepared with cold water steeping (4°C, 12 hrs) has the highest phenolic content and antioxidant

activity. The results presented here could provide a useful supportive evidence for green tea prepared with cold water steeping treatment.

Acknowledgments

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References

[1] Cabrera C, Gimenez R, Lopez C: Determination of tea components with antioxidant activity. J Agric Food Chem 2003, 51: 4427-4435.

[2] Shitandi AA, Ngure FM, Mahungu SM: Tea Processing and its impact on catechins, theaflavin and thearubigin formation imprint. In P. V.R. (Ed.), Tea in health and disease prevention 2013, 193-205, Academic Press.

[3] Cai Y, Luo Q, Sun M, Corke H: Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004, 74: 2157-2184.

[4] Yen GC, Chen HY: Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 1995, 43: 27-32.

[5] Balasundram N, Sundram K, Samman S: Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem 2006, 99: 191-203.

[6] Tanizawa H, Toda S, Sazuka Y, Taniyama T, Hayashi T, Arichi S, Takino Y: Natural antioxidants. I. Antioxidative components of tea leaf (*Thea sinensis* L.). Chem Pharm Bull (Tokyo) 1984, 32: 2011-2014.

[7] Farhoosh R, Golmovahhed G, Mohammad HHK: Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). Food Chem 2007, 100: 231-236.

[8] Liang JY, Yuann JMP, Cheng CW, Jian HL, Lin CC, Chen LY: Blue light induced free radicals from riboflavin on *E. coli* DNA damage. J. Photochem Photobiol B 2013, 119: 60–64.

[9] Yang DJ, Hwang LS, Lin JT: Effects of different steeping methods and storage on caffeine, catechins and gallic acid in bag tea infusions. J Chromatogr A 2007, 13;1156:

312-320.

[10] Venditti E, Bacchetti T, Tiano L, Carloni P, Greci L, Damiani E: Hot vs. cold water steeping of different teas: Do they affect antioxidant activity? Food Chem 2010, 119: 1597–1604.

[11] Gahler S, Otto K, Bohm V: Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. J Agric Food Chem 2003, 51: 7962-7968.

[12] Oyaizu M: Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986, 44: 307-315.

[13] Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY, Lee MH: Free radical-scavenging activity of Taiwanese native plants. Phytomedicine 2003, 10: 170-175.

[14] CNS Method of test for caffeine content in foods. Chinese National Standard Method 9432 N6174, 2009.

[15] Chen LY, Cheng CW, Liang JY: Effect of esterification condensation on the Folin-Ciocalteu method for the quantitative measurement of total phenols. Food Chem 2015, 170: 10-15.

[16] Dube A, Ken N, Nicolazzo J, Larson I: Effective use of reducing agents and nanoparticle encapsulation in stabilizing catechins in alkaline solution. Food Chemistry 2010, 122: 662-667.

[17] Janeiro P, Oliveira BAM: Catechin electrochemical oxidation mechanisms. Analytica Chimica Acta 2004, 518: 109-115.

[18] Mochizuki M, Yamazaki S, Kano K, Ikeda T: Kinetic analysis and mechanistic aspects of autoxidation of catechins. Biochim Biophys Acta 2002, 1569: 35-44.

[19] Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C: Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch Biochem Biophys 1995, 322: 339-346.

[20] Shimizu M, Wada S, Hayashi T, Arisawa M, Ikegaya K, Ogaku S, Yano , Morita N: Studies on hypoglycemic constituents of Japanese tea. Yakugaku Zasshi 1988, 108: 964-970.

溫度與浸泡時間對茶湯抗氧化活性與其咖啡因含量的影響

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中文摘要

茶是廣受歡迎的飲品。本研究使用不同溫度(90°C，4°C)及不同的浸泡時間(6 分鐘, 24 小時)的沖泡方式，探討不同發酵茶葉沖泡後的抗氧化活性及咖啡因的變化。冷泡綠茶的多酚類含量明顯的比熱泡的綠茶及其他種茶類的處理為高。熱泡茶的咖啡因含量高於冷泡茶。本研究顯示冷泡綠茶之製備為未來茶類飲料健康效應提供良好基礎。

關鍵字：冷泡茶，抗氧化活性，咖啡因
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