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Extraction Behavior of Caffeine and EGCG from Green and Black Tea

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Abstract A dipping method was developed to extract the catechins (EGCG) and alkaloids (caffeine) from green tea (Korea) and black tea (Sri Lanka). The effects of the solvent composition (water vs. ethanol), extraction time, temperatures, and solvent pH on the amount of catechins (EGCG) and alkaloids (caffeine) extracted from green and black tea were investigated. Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to analyze the catechins (EGCG) and alkaloids (caffeine) extracted. The content of EGCG and caffeine in green tea extracts was in the range of 2.04~0.30 and 10.22~0.85 mg/g, respectively. The amount of EGCG and caffeine in black tea extracts was in the range of 0.32~0.24 and 5.26~1.01 mg/g, respectively. The amount of caffeine extracted from green and black tea was greater than the amount of EGCG. Pure water is the best solvent for extracting EGCG and caffeine from green tea. The amount of caffeine extracted from green and black tea increased as the temperature, extraction time, and hydrogen ion concentration of the solvent increased. Although the amount of EGCG extracted from green tea increased as the temperature increased, the amount of EGCG extracted from black tea was not affected by temperature. The extraction of EGCG from both green and black tea was not affected by the hydrogen ion concentration of the solvent. © KSBB

Keywords: extraction, caffeine, EGCG, green tea, black tea, HPLC

Tea, the most popular beverage in the East, has attracted much interest from scientists due to its beneficial health effects [1-6]. Plant extracts have been widely used as topical applications for wound-healing, anti-aging, and disease treatments. Teas are classified into three major categories based on how they are manufactured as follows: (a) unfermented green tea, (b) partially fermented Oolong tea, and (c) fully fermented black tea [7,8]. Catechins (EGCG) and alkaloids (caffeine), the main active ingredients in green tea, have been proven to have a variety of physiological functions, such as protecting against cancers of the duodenum, colon, skin, lung, breast, esophagus, pancreas, and prostate.

Catechins (EGCG) and alkaloids (caffeine) also show some anti-mutagenic, anti-carcinogenic, and anti-clastogenic effects [9-12]. EGCG and caffeine are usually isolated by extraction with organic solvents, and the extraction conditions (solvent, temperature, duration of extraction, pH, and composition ratio of solvent to material) can have a variety of effects on the extraction efficiency of EGCG and caffeine [13].

In this paper, we studied the extraction behavior of catechins (EGCG) and alkaloids (caffeine) using green tea (Korea) and black tea (Sri Lanka) in order to obtain basic data on the process for extracting both materials. The effect of different extraction configurations on the extraction efficiency of catechins (EGCG) and alkaloids (caffeine) from green tea leaves and black tea leaves was investigated using various aqueous and pure solvents (ethanol composition of 40, 60, 80 vol%, pure ethanol, and water), times (10, 20, 30, 60, 120, and 180 min), temperatures (5, 15, 25, 60, and 90°C), and values of pH (3, 5, 6.2, 7, and 9) for the extraction solution. The analytical HPLC system was utilized to identify the catechins (EGCG) and alkaloids (caffeine) in the extracted solution [14].

The green tea used in this experiment was cultivated at Bosung (Chonnam, Korea, 2006), and the black tea (Sri Lanka) was purchased from a domestic market (Green Tea Village, Chonnam, Korea, 2006). The standard catechins (EGCG) and alkaloids (caffeine) were obtained from Sigma Co. (USA). Extra-pure grade solvents, ethanol, methanol, acetonitrile, and ethyl acetate, were purchased from J. T. Baker (Phillipsburg, NJ, USA). The twice distilled water was filtered by a pump (Division of Millipore, Waters, Milford, MA, USA) and filter (FH-0.2 μm, Waters, Milford, MA, USA). Fig. 1 shows the structure of the catechins (EGCG) and alkaloids (caffeine) investigated.

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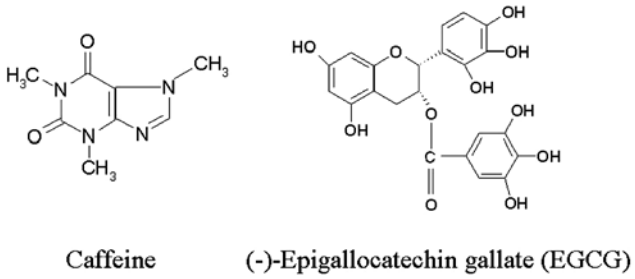

Fig. 1. Structure of EGCG and caffeine.

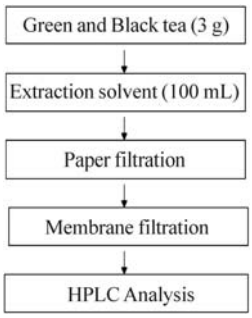

Fig. 2. Extraction and purification procedure.

Samples were prepared by dissolving 2 mg of the standard chemicals [catechins (EGCG) and alkaloids (caffeine)] in 4 mL of ethanol and adjusting the concentration of the isoflavones to 500 ppm.

The HPLC system used for analysis consisted of a 426 HPLC pump (Alltech Co.), a 486 detector (M 7200 Absorbance Detector, Young-In Scientific Co.), and a Reodyne injection valve (20 μL sample loop). An Autochro-WIN data acquisition device (ver. 1.42, Young-In Scientific Co.) connected to a PC was used to collect data. Sufficient time was allowed for the stabilization of the column and detector signal after each injection. The mobile phases of water, methanol, acetonitrile, and acetic acid were experimented. The chromatographic column used in this experiment was a commercial analytical column, RS-tech (0.46 × 25 cm, 5 μm, C_{18}, Daejeon, Korea). The injection volume was 20 μL, and the flow rate of the mobile phase was 1.0 mL/min. The wavelength of the UV detector was fixed at 280 nm.

We prepared samples of dry powder by grinding and sieving (< 30 μm) green and black tea containing the catechins (EGCG) and alkaloids (caffeine). Three grams of green tea powder and black tea powder were loaded in 100 mL of an aqueous ethanol solution (40, 60, and 80%, v/v), pure water and pure ethanol, respectively. Catechins (EGCG) and alkaloids (caffeine) were extracted from each sample by the dipping method at various extraction times (10, 20, 30, 60, 120, and 180 min), temperatures (5, 15, 25, 60, and 90°C) and values of solvent pH (3, 5, 6.2, 7, and 9). The extraction solution was filtered through a 0.2-μm membrane filter (Waters, Milford, MA, USA) prior to HPLC analysis. Fig. 2 shows the schematic diagram of the extraction and purification of catechins (EGCG) and alkaloids (caffeine) from green and black tea.

In order to calculate the amount of catechins (EGCG) and alkaloids (caffeine) extracted from the green and black tea, a calibration curve was drawn using the peak areas method. The standard solution concentration was fixed at 0.5 mg/mL, and various sample volumes (5 to 20 μL) were injected into the HPLC system. The peak areas for the sample of each concentration were obtained, and linear regression analysis was applied to the calibration curve. As a result, the linear regression equations were $Y = 9.1 \times 10^{-7}X$ and $Y = 6.0 \times 10^{-6}X$, with a correlation coefficient (r^2) of more than 0.98 for EGCG and caffeine, respectively. In the equations, Y is the amount (μg) of EGCG and caffeine and X is the peak area (mV × sec). The various experimental variables were analyzed using a commercially available C_{18} column. The mobile phases were composed of water with acetic acid and acetonitrile on isocratic mode. The mobile phase was a composition from water/acetonitrile (87/13, v/v) to acetic acid 0.1% over a run time of 30 min.

Table 1. Amount of EGCG and caffeine extracted from green and black tea

Solvent composition (ethanol, %)	Green tea (mg/g)		Black tea (mg/g)	
	Caffeine	EGCG	Caffeine	EGCG
0	7.68	0.90	1.87	0.31
40	7.47	0.86	–	–
60	6.85	0.73	–	–
80	6.07	0.44	–	–
100	0.85	0.30	–	–

We investigated the extraction efficency of the catechins (EGCG) and alkaloids (caffeine) from the green and black tea using different extraction conditions. In this study, the effects of solvent composition (ethanol 0, 40, 60, 80, and 100 vol%), extraction time (10, 20, 30, 60, 120, and 180 min), temperature (5, 15, 25, 60, and 90°C) and solution pH (3, 5, 6.2, 7, and 9) on the amount of catechins (EGCG) and alkaloids (caffeine) extracted from green and black tea were investigated.

Table 1 shows the content of EGCG and caffeine in the green and black tea extracts obtained using various solvent compositions when the temperature (25°C) and extraction time (60 min) remained constant. The amount of EGCG and caffeine in green tea extracts was in the range of 0.90~0.30 and 7.68~0.85 mg/g, respectively, and that in black tea obtained using pure water was 0.31 and 1.87 mg/g, respectively. It can be seen that the amount of EGCG and caffeine extracted from green tea was greater than that extracted from black tea. The amount of EGCG and caffeine in green tea was greater than that in black tea [3].

In addition, the amount of caffeine extracted from green and black tea was greater than the amount of EGCG. This is due to the difference in water solubility and molecular weight between EGCG and caffeine. The water solubility of caffeine is 21.7 g/L, while that of EGCG is about 521.7 g/L.

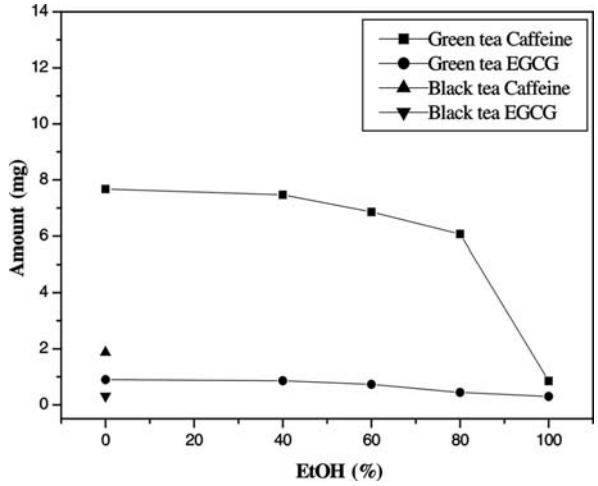

Fig. 3. Effect of solvent composition on extraction of EGCG and caffeine.

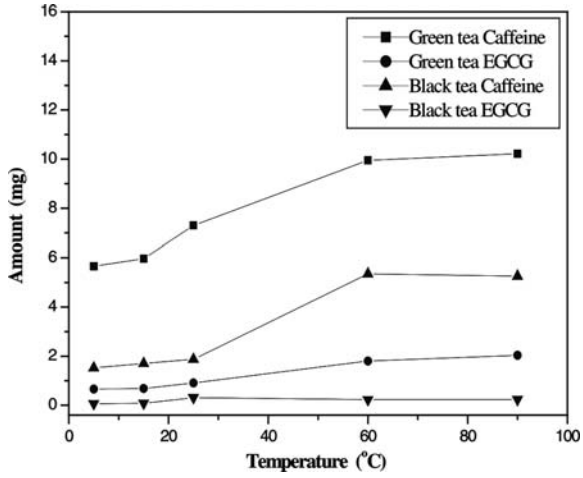

Fig. 5. Effect of temperature on extraction of EGCG and caffeine.

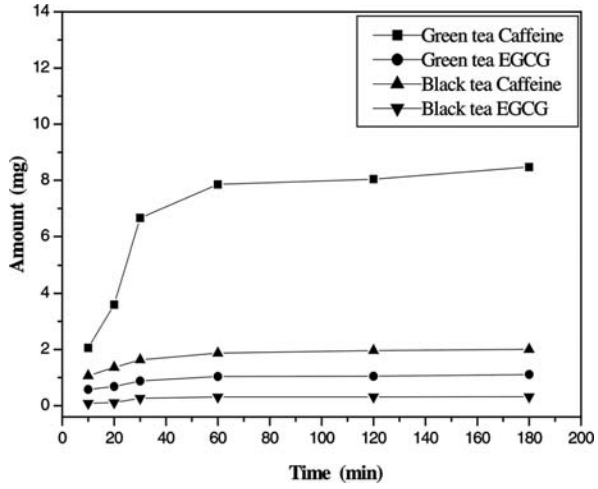

Fig. 4. Effect of extraction time on extraction of EGCG and caffeine.

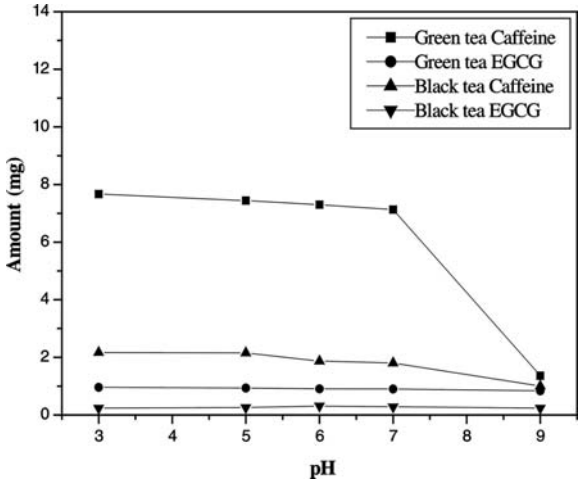

Fig. 6. Effect of pH on extraction of EGCG and caffeine.

The molecular weight of caffeine is 194.2, while that of EGCG is 458.4. When the tea leaf was extracted in an aqueous solution, caffeine could diffuse and dissolve into the aqueous solvent with greater ease than EGCG due to its higher solubility in water and smaller molecular weight [15].

Fig. 3 shows that the amount of EGCG and caffeine extracted from green tea increased as the ethanol content of the extraction solvent decreased. This may be due to the difference in the solubility of EGCG and caffeine between water and ethanol. The solubilities of EGCG and caffeine are higher in water than in ethanol.

Fig. 4 shows the content of EGCG and caffeine in green and black tea extracts obtained using various extraction times at constant temperature (25°C). It can be seen that the amount of EGCG and caffeine extracted from green and black tea increased as the extraction time increased, but it remained constant when the extraction time was greater than 60 min.

Fig. 5 shows the amount of EGCG and caffeine in green and black tea extracts obtained at various temperatures when the extraction time was held constant (60 min). The amount of caffeine extracted from green and black tea increased with increasing temperature because the solubilities of EGCG and caffeine increase with increasing temperature. The amount of EGCG extracted from black tea remained nearly constant when the temperature was more than 25°C because there was a very small amount of EGCG.

Fig. 6 shows the amount of EGCG and caffeine in green and black tea extracts obtained with an aqueous solution at various values of pH and under a constant temperature (25°C) and extraction time (60 min). The amount of caffeine extracted from green and black tea slightly decreased as the

pH of the aqueous solution increased, but it sharply decreased when the pH was more than 7. In addition, the amount of EGCG extracted from green and black tea was nearly constant when the pH was in the range of 3~9. This may be caused by the isomerization of caffeine due to the addition of OH^- in order to increase the pH of the aqueous solution [16].

Based on the above discussion, the optimal extraction conditions were determined to be the following: extraction solvent, pure water; extraction time, 60 min; extraction temperature, 60°C, and solution pH 7.

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