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Extraction of Caffeine from Korean Green Tea

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The extraction of caffeine from Korean green tea was performed by various extraction methods such as dipping, stirring, ultrasonic and supercritical CO_2 extraction. The extracts were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC). From the experimental results, the amount of caffeine extracted by supercritical CO_2 extraction using water as co-solvent was 2 times larger than that by all of the different extraction methods applied.

Key Words: Korean green tea, Caffeine, HPLC, Ultrasonic, Supercritical CO_2.

INTRODUCTION

Green tea is consumed in Korea, Japan and China as one of the most popular and traditional non-alcoholic beverages. The pharmaceutical applications of green tea are one of the most interesting and active research areas[1,2]. Usually, the pharmacologically active compounds in medicinal plants are in low concentrations and difficult to extract. Recently, the demand for green tea and black tea has increased because of human health concerns and preferences[3-5]. It is widely used as food, medicine, drink and cosmetics. Green tea leaves contain 10-30 % polyphenols, including catechins, flavonols, flavanones, phenolic acids, glycosides and the aglycones of plant pigments[6,7]. Among the above mentioned components, caffeine, ($C_8H_{10}N_4O_2$, m.w. = 194.19, m.p. 238 ºC) is regarded as the component of green tea and showed many activities. Caffeine belongs to a class of compounds called methylxanthines. Also, caffeine is presented in coffee, tea, cocoa, chocolate[8]. Catechins [(-) epigallocatechin gallate *etc.*] and alkaloids (caffeine *etc.*) have been proven to have a variety of physiological functions, such as those affecting duodenum, colon, skin, lung, breast, esophageal, pancreatic and prostate cancer[4,9]. Typically, caffeine was extracted by aqueous methanol, ethanol or water solvent[10,11]. A modern method used to release the bioactive

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constituents from herbs is ultrasonic enhanced solvent extraction[12]. There are numerous reports on the application of high intensity power ultrasonic in the extraction of useful components from various parts of plants and plants seed[13,14]. The supercritical fluid extraction is a new technique and has been proven to be an alternative method of the traditional extraction. The supercritical fluid extraction has several advantages such as rapid extraction rate, environmental friendly process and selective extraction[15,16].

In this work, we investigated the extraction efficiency of caffeine from Korean green tea using different extraction methods such as dipping, stirring, ultrasonic and supercritical CO_2 extraction. The experimental variables were intensity, pressure, temperature and extraction time. In order to determine caffeine content in extracted solution, high performance liquid chromatography (HPLC) system was utilized.

EXPERIMENTAL

The Korean green tea used in this experiment was cultivated at Bosung (Chonnam, Korea, 2007) and purchased from a domestic market. The standard chemical of alkaloids (caffeine) was obtained from Sigma. Co. (USA). The extra-pure grade solvents, ethanol, methanol, acetonitrile and ethyl acetate were purchased from Baker (Phillipsburg NJ, USA). The double distilled water was filtered by a pump (Division of Millipore, Waters, Milford, MA, USA) and membrane filter (FH- 0.2 μm, Waters, Milford, MA, USA).

Sample preparation: Samples were prepared by dissolving 2 mg standard chemicals alkaloids (caffeine) in 4 mL water and adjusting the concentration of the caffeine to 500 ppm.

The HPLC system for analysis 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). Autochro-WIN (ver. 1.42, Young-In Scientific Co.) connected to a PC was used as a data acquisition system. Sufficient times were allowed for the stabilization of the column and detector signal after each injection. Water, methanol, acetonitrile and acetic acid were experimented as mobile phases. The chromatographic column (0.46 cm × 25 cm, 5 μm, C_{18}, Daejeon, Korea) from RS-tech. 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.

Solvent extraction: 3 g sample of the powder from the Korean green tea was loaded in 100 mL pure water. The effects of variation in extraction methods such as dipping, stirring and ultrasonic were measured. The following experimental variables were applied: ultrasonic frequency of 40 KHz, intensity of 200 and 400 W, temperature of 25 ºC and ultrasonic frequency of 40 KHz for 10, 20, 30, 60, 120 and 180 min. Each extract underwent decompression filtration and decompression concentration to evaporate the solvent. The extraction was applied by stirring from 10-180 min at 1200 rpm. The solution was filtered (0.2 μm) prior to HPLC analysis.

Ultrasonic system: One type of ultrasonic systems was used: a microprocess Controlled Bench-top Ultrosonic Cleaner (frequency 40 KHz, intensity: L 200, H 400 W, dimension 720 L × 450 W × 280 H, main source AC 220V, 6 L, Hwashin Tech. Co. Korea), Model name : Power Sonic 520.

Supercritical CO_2 system: The supercritical fluid apparatus for extracting caffeine contained in Korean green tea is shown in Fig. 1, carbon dioxide was heated and pressed over critical point (304.23 K and 7.68 MPa). Carbon dioxide gas phase was changed to liquid phase by cooling circulator (RBC-11, JEIO Tech., Korea) and pressed into the extractor (50 mm × 500 mm) by liquid pump (Milroyal high pressure pump, Ivyland, USA). Heater for heating of carbon dioxide in the extractor was made from Deapung industry (D-64060). The flow rate and pressure of carbon dioxide were adjusted by regulator (68.94 MPa, TESCOM Co., Minnesota, USA). The amount of carbon dioxide was measured by gas flow-meter (Deahan gas corporation, Seoul, Korea). The extractor loaded 100 g Korean green tea powder. Supercritical CO_2 extraction was performed for 1, 2, 3, 4 and 5 h and the flow rate of carbon dioxide was 20 L/min. The temperature of extractor was kept at 40, 50 and 60 °C, respectively.

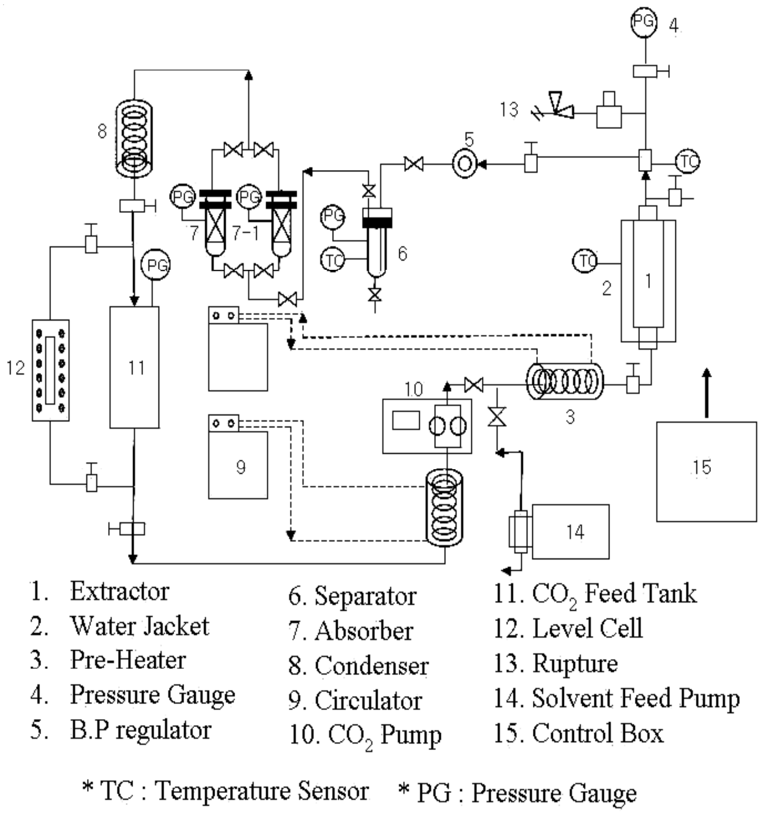

Fig. 1. Apparatus for the supercritical fluid CO_2 extraction of caffeine from Korean green tea

RESULTS AND DISCUSSION

In this work, we investigated the extraction amount of caffeine from Korean green tea using the different extraction methods such as dipping, stirring, ultrasonic and supercritical CO_2 extraction. In order to calculate the amount of caffeine extracted from the Korean green tea, a calibration curve drawn by the area (under the peak) method. First, the solution concentration was 0.5 mg/mL and various sample volumes (5-20 μL) were injected into the HPLC system. The peak areas for the sample of each concentration were obtained and linear regression was applied to the calibration curve. The amount of caffeine extracted in the chromatogram was calculated by regression analysis. The parameters of the calibration curves of the caffeine was $Y = 6.0 \times 10^{-6}$ X. The correlation coefficient (r^2) was higher than 0.98 in each case. Where X means the peak area (mV·s) under the curve in the chromatogram and Y means the amount (mg) of the caffeine according to the injection volume for each standard sample. The various experimental variables were analyzed by a commercially available C_{18} column. The mobile phase was composition from an water/ acetonitrile (87/13, v/v) to acetic acid 0.1 % over 30 min.

Fig. 2 shows the amount of caffeine extracted from Korean green tea using dipping and stirring (1200 rpm) with various extraction time at constant temperature (25 ºC). The caffeine in Korean green tea extracts was obtained about 8 mg/g more than 1 h by stirring, while it was obtained about 8 mg/g at 180 min by dipping. The caffeine extraction by stirring makes more rapid progress than that by dipping because of the decrease in mass transfer resistance of caffeine by stirring.

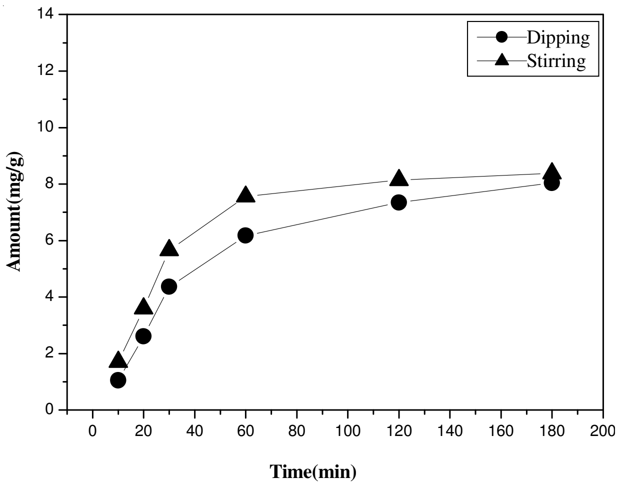

Fig. 2. Amount of caffeine extracted by dipping and stirring (1200 rpm, 25 ºC)

Fig. 3 shows the amount of caffeine extracted from Korean green tea using ultrasonic extraction with various extraction time and intensity at constant temperature (25 ºC). The amount of caffeine in Korean green tea extracts increased

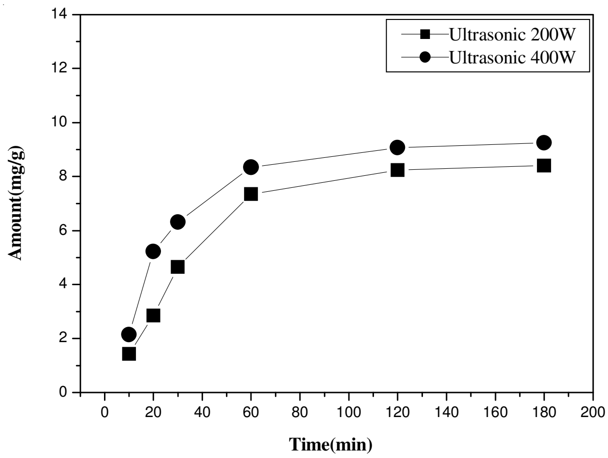

Fig. 3. Amount of caffeine extracted by ultrasonic 25 ºC

with extraction time increasing, but it was constant more than 120 min. The amount of caffeine extracted by ultrasonic at intensity of 400 W was larger than 200 W and it was much larger than that by dipping. This is caused by the mechanical effect of ultrasonic to disrupt plants cell walls. It allows greater penetration of solvent into the green tea matrix and increases the contact surface area between solid and liquid phase. In other words, it facilitated the release of extractable compounds and enhanced mass transport of the solvent into plant cells[13,14].

Fig. 4 shows the amount of caffeine in Korean green tea extracts obtained using supercritical CO_2 with various co-solvent content at optimum condition (pressure of 300 bar, temperature of 60 ºC and extraction time of 180 min). It can be seen that

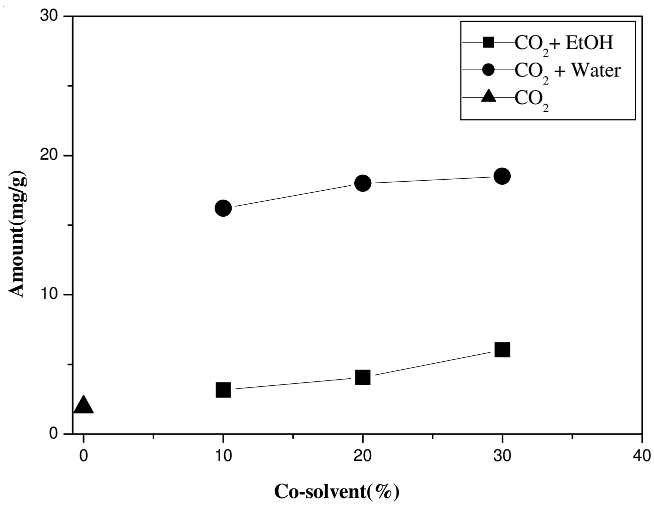

Fig. 4. Amount of caffeine extracted by supercritical CO_2 (pressure: 300 bar, temperature: 60 ºC, extraction time: 180 min)

the amount of caffeine extracted by supercritical CO_2 extraction increased with increasing co-solvent content such as water and ethanol and increased markedly by the addition of water as co-solvent. This may be due to swell of Korean green tea tissue by the addition of water and facilitation of caffeine diffusion out of that[15,16]. Table-1 shows the amount of caffeine in Korean green tea extracts obtained at constant extraction time (180 min) using the different extraction methods such as dipping, stirring, ultrasonic and supercritical CO_2 extraction. The amount of caffeine extracted from Korean green tea increased by stirring or ultrasonic. While the amount of caffeine extracted by ultrasonic was a little larger than that by stirring, the amount of caffeine extracted by supercritical CO_2 extraction using water as co-solvent was 2 times larger than that by all of the different extraction methods applied.

TABLE-1
AMOUNT OF CAFFEINE EXTRACTED BY VARIOUS EXTRACTION METHODS

Extraction method	Condition		Amount of caffeine (mg/g)
Supercritical CO_2	Water 30 %	300 bar, 60 °C	18.5
	EtOH 30%		6.04
Ultrasonic	400 W	40 KHz, 25 °C	9.24
	200 W		8.40
Stirring	1200 rpm	25 °C	8.38
Dipping	–	25 °C	8.04

(Extraction time: 180 min).

Conclusion

The caffeine extraction by stirring makes more rapid progress than that by dipping because of the decrease in mass transfer resistance. The amount of caffeine extracted by ultrasonic at intensity of 400 W was larger than that at intensity of 200 W and was much larger than that by dipping. This is caused by the mechanical effect of ultrasonic, allowing greater penetration of solvent into the green tea matrix and increasing the contact surface area between solid and liquid phase. The amount of caffeine extracted by supercritical CO_2 extraction increased with increasing co-solvent content such as water and ethanol and increased markedly by the addition of water as co-solvent. This may be due to swell of green tea tissue by the addition of water and facilitation of caffeine diffusion out of that. The amount of caffeine extracted by supercritical CO_2 extraction using water as co-solvent was 2 times larger than that by all of the different extraction methods applied. From the experimental results of caffeine extraction by the various methods, the supercritical CO_2 extraction using water as co-solvent was the most efficient extraction method in extracting caffeine from Korean green tea.

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