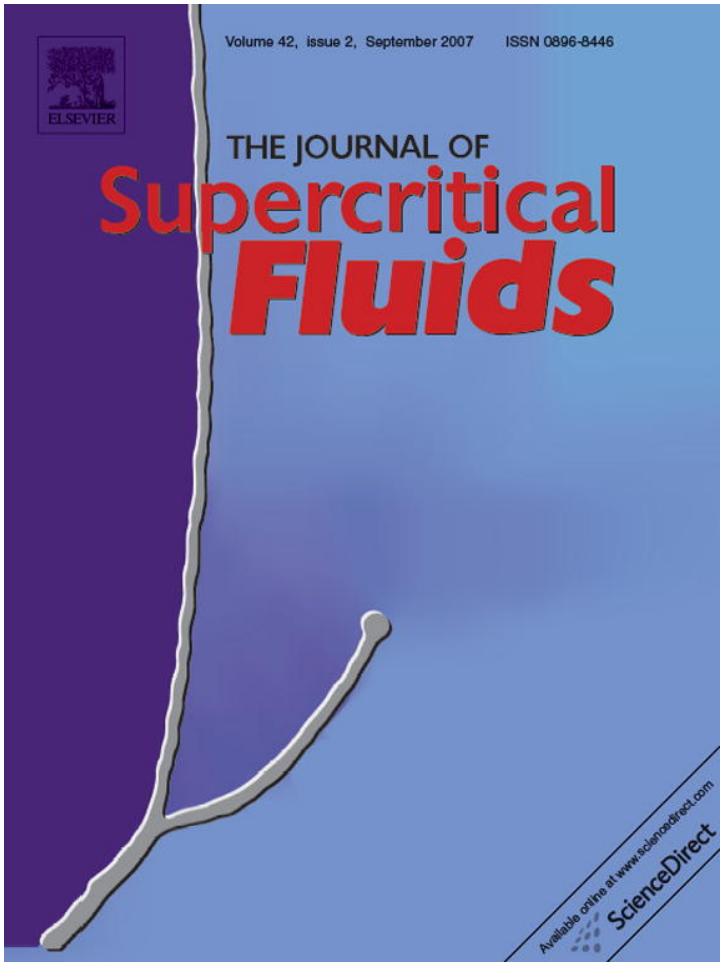

Available online at www.sciencedirect.com
ScienceDirect
J. of Supercritical Fluids 42 (2007) 205–211
THE JOURNAL OF Supercritical Fluids
www.elsevier.com/locate/supflu

# Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide

Hyong Seok Park [a], Hyung-Kyoon Choi [b], Sung Jun Lee [a], Kwon Woo Park [a], Sung-Gil Choi [c], Kyoung Heon Kim [a,*]

[a] *College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea*
[b] *College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea*
[c] *College of Agriculture and Life Science, Department of Food Science and Technology, Gyeonsang National University, Jinju 660-701, Republic of Korea*

Received 29 November 2006; received in revised form 7 February 2007; accepted 6 March 2007

**Abstract**

To remove caffeine from green tea, supercritical $CO_2$ (SC-$CO_2$) extraction using 95% (v/v) ethanol as a modifier was carried out on a laboratory scale in the ranges of 150–300 bar and 50–80 °C. The extraction yield of caffeine and catechins including epigallocatechin gallate (EGCG) increased with an increase in temperature at a constant pressure, and also increased with increasing pressure at a fixed temperature. When the $CO_2$ mass flow rate increased, the total extraction yield of caffeine and catechins also increased, but the extraction efficiency of $CO_2$, which was determined by the amount of the solutes extracted per amount of $CO_2$ used, decreased, possibly due to the negligible effect of external mass transfer resistance around green tea particles and the reduced contact time for SC-$CO_2$ and green tea. The reduction of green tea particle size by grinding also resulted in the enhanced extraction of caffeine and catechins, which indicates the larger particle size yielded the slower extraction rate. These results gave rise to the conclusion that internal mass transfer resistance is predominant over the external mass transfer resistance in the extraction of green tea by SC-$CO_2$ like other herbaceous materials. In addition to the extraction of caffeine, the substantial amount of catechins was also found to be extracted during the decaffeination processes.


*Keywords:* Supercritical carbon dioxide; Green tea; Caffeine; Catechins; Decaffeination

## 1. Introduction

Green tea, processed leaves of the *Camellia sinensis* plant, which has long been used to brew a traditional drink in Asian countries, is now becoming popular all over the world. Green tea is prepared via the processing of the tea leaves by steaming or roasting without fermentation. Depending on whether the green tea leaves are fermented, semi-fermented, or unfermented, the products are categorized into black tea, oolong tea, and green tea, respectively.

A variety of positive health benefits from drinking green tea are strongly associated with catechins, which are composed primarily of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). In particular, the most profound antioxidative activity among catechins is attributed to EGCG, generally the most abundant catechin in green tea. Due to the health-benefiting effects from the catechins in green tea, including antioxidative [1–3], anticancer [4], anti-inflammatory [5,6], antiaging [7], antibiotic, and antiviral effects [8–10], the consumption of green tea is rapidly increasing, as is the consumption of a variety of green tea products, including beverages, ice cream, and cosmetics.

Meanwhile, caffeine, which is contained as another unique component in green tea, is known to exert relatively adverse effects in humans, including sleep deprivation [11], abortions and miscarriages [12,13], and hypersensitivity [14]. Therefore, the intake of caffeine by vulnerable consumers, including pregnant women, infants, and children, has become a major cause of concern.

Considering the adverse effects of caffeine, some efforts have been made to remove caffeine from several caffeine-containing foods, including coffee [15,16], guarana [17], and black tea [18].

* Corresponding author. Tel.: +82 2 3290 3028; fax: +82 2 925 1970.
*E-mail address:* khekim@korea.ac.kr (K.H. Kim).

0896-8446/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.supflu.2007.03.002

The organic solvents used in traditional decaffeination techniques, which include trichloroethylene and dichloromethane, have been banned for the decaffeination of foods due to their potential carcinogenic effects in humans. Therefore, alternatives for effective decaffeination of food materials have been actively considered. Due to the substantial coextraction of health-benefiting polyphenols including catechins, lactine, and chologenic acid during the decaffeination process using various aqueous solvents [19,20] lately there have been reports on more selective methods such as decaffeination using hot water [21] and microbial degradation of caffeine [22]. But some limitations are also found in these methods since the hot water treatment is applicable to green tea leaves only in fresh form, and the microbial degradation of caffeine is too early to apply to food products. Currently, ethyl acetate and supercritical carbon dioxide (SC-$CO_2$) are being employed as alternatives for the removal of caffeine from foods.

Despite the advantages of SC-$CO_2$, only a few attempts have been made to apply SC-$CO_2$ to the processing of green tea or green tea components. Although the equilibrium solubility of catechin and epicatechin in SC-$CO_2$ have been assessed [23,24], it has also been determined that the solubility of catechins in SC-$CO_2$ was too low as a consequence of the low polarity of SC-$CO_2$. The attempt to acquire low caffeine-containing polyphenol oleoresin oil from green tea and oolong tea using SC-$CO_2$ proved infeasible because the extraction of polyphenols was less effective when using SC-$CO_2$ than when using the Soxhlet method [25,26].

Meanwhile, to the best of our knowledge, there have been no reports of process development for producing decaffeinated green tea leaves using SC-$CO_2$. In this study, we focused primarily on the mass transfer effect, especially the effects of particle size, $CO_2$ mass flow rate, and extraction time, on the removal of caffeine from green tea using supercritical $CO_2$.

## 2. Experimental

### 2.1. Materials

Commercial green tea leaves (*Camellia sinensis*) were provided by the Boseong Tea Experimental Station (Boseong, Jeonnam, Korea) in July 2004, and were stored at −70 °C until use. The green tea leaves were ground using a cutting mill (IKA, Staufen, Germany) and were put through sieves (Chung Gye Sang Gong Sa, Seoul, Korea) in order to obtain particle sizes in different ranges. The particle size of ground green tea sieved with the sieves with openings of 125 and 425 μm was determined by the 780 Accusizer particle size analyzer (Particle Sizing System, Santa Barbara, USA), and the ground tea sieved with the sieves of 425 and 710 μm openings was analyzed by the Winner2116 particle size analyzer (Jinan Winner Instrument, Jinan, China).

Authentic standards including caffeine (100%), epicatechin (EC, 97%), epigallocatechin (EGC, 98.3%), epigallocatechin gallate (EGCG, 94.4%), and epicatechin gallate (ECG, 99.5%) were all purchased from Sigma (St. Louis, USA). All solvents used in this study were obtained from TEDIA (Fairfield, USA), and were of HPLC grade. High-purity $CO_2$ (99.5%), employed throughout the supercritical fluid extraction in this study, was obtained from Daehan Specialty Gases (Seoul, Korea).

### 2.2. Supercritical $CO_2$ extraction

Fig. 1 shows the laboratory-scale supercritical fluid extraction (SFE) system (Ilshin Autoclave Co., Daejeon, Korea) equipped with an extraction vessel (16.2 mm × 2.8 mm) of an internal volume of 100 mL and used in the present study. When the temperature of the SFE system reached the designated temperature, 10 g of ground green tea sample were soaked in a certain amount of a cosolvent and loaded into the extraction vessel. After the vessel had been tightly closed, the connecting valves (V-1 and V-2 in Fig. 1) were opened carefully, and the decompression valve (V-3) was closed. Then, the liquid $CO_2$ contained in the siphoned cylinder was pumped through the water bath, the temperature of which was kept constant, into the SC-$CO_2$ vessel, after which the vessel was generally fully pressurized to a designated pressure within 2 min. The valves connecting to the treatment vessel (V-1 and V-2) were then maintained in the "open" position throughout the entirety of the extraction time.

The extraction pressure was monitored continuously and controlled using a back pressure regulator (BPR) connected to the extraction vessel. In order to determine the total mass flow rate of $CO_2$ utilized in the extraction, the weight of the $CO_2$

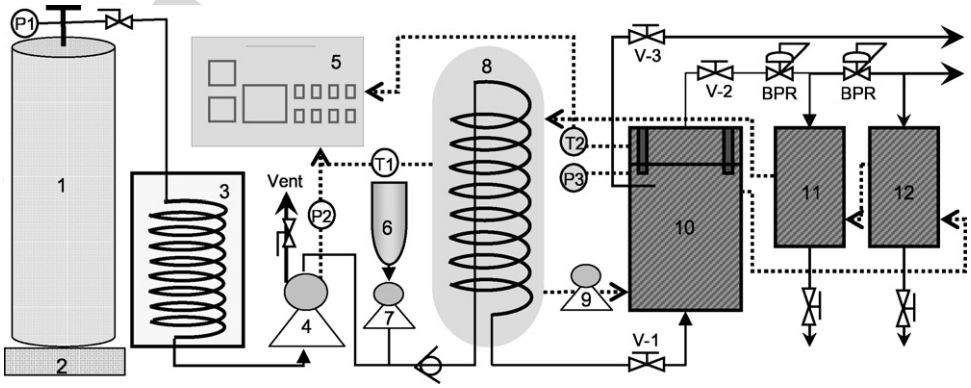


Fig. 1. Schematic diagram of the supercritical $CO_2$ extraction system: 1, $CO_2$ cylinder; 2, electronic balance; 3, chiller; 4, $CO_2$ pump; 5, controller; 6, cosolvent reservoir; 7, cosolvent pump; 8, heating bath; 9, circulation pump; 10, extractor; 11, separator 1; 12, separator 2; V1, valve 1, V2, valve 2; BPR, back pressure regulator; dotted lines, water line; solid lines, $CO_2$ line.

tank on an electronic balance (CAS, Seoul, Korea) was monitored and recorded at a certain time interval. After each 40-min extraction batch was completed, to replenish the green tea with ethanol cosolvent off line, the extraction vessel was depressurized by slowly opening the decompression valve (V-3), and the extracted green tea was then removed and soaked with the same amount of the cosolvent used initially. Then, the green tea leaves were reloaded into the extractor for further SC-$CO_2$ extraction. After the end of the extraction cycle, the extracted green tea was subjected to composition analysis by high-performance liquid chromatography (HPLC).

### 2.3. Composition analysis of green tea

The catechin and caffeine contents of the original green tea prior to extraction and SC-$CO_2$ extracted green tea were analyzed by HPLC after solvent extraction with 30% (v/v) ethanol. The green tea sample subjected to the compositional analysis was dried overnight in a convective drying oven at 105 °C in order to remove all possible moisture and residual solvents. Using the cutting mill, the dried green tea sample was ground to a small enough size to pass through the sieve with an opening of 425 μm, and 200 mg of the samples were extracted in triplicate using 20 mL of 30% (v/v) ethanol solution in a shaking water bath (Biofree, Seoul, Korea) for 30 min at 35 °C and 100 rpm. The extracted slurry was then filtered using a filter paper (110 mm, No. 2, Whatman, Brentford, UK), and the filtrate was centrifuged for 10 min at 13,000 rpm using a microcentrifuge (Hanil, Seoul, Korea). The centrifuged filtrate was then further filtered using a 0.45 μm syringe filter (Hydrophilic PTFE, Advantec, Dublin, USA) prior to HPLC analysis. The analytical procedures above were optimized and validated in a preliminary study by comparing with similar protocols found in other literatures [27,28].

The prepared sample was analyzed for caffeine and catechin contents using an HPLC system (Agilent 1100, Agilent Technologies, Waldbronn, Germany). Twenty microliters of the sample were injected into the HPLC system equipped with an Hypersil ODS column (Hypersil ODS, 5 μm, 4.6 mm × 100 mm, Thermo Electron Corporation, Bellefonte, USA) operated at 20 °C with a gradient elution, as described previously [26], and peaks in the eluent were detected at 280 nm using a UV/Vis detector (Agilent Technologies). The total contents of caffeine and each catechin determined by the 30% (v/v) aqueous ethanol extraction and HPLC analysis as the above were deemed to be 100% of the extractable mass of each component.

## 3. Results and Discussion

### 3.1. Composition of green tea

The caffeine and catechin contents in the green tea leaves used in the study were determined as shown in Table 1. The analysis showed that caffeine was contained in the green tea at levels as high as 41.38 mg/g on a dry weight basis. The most abundant catechin was EGCG, and the contents of catechins were in the order of EGCG, EGC, ECG, and EC, from highest to lowest.

### 3.2. Effects of temperature and pressure

Table 2 shows the effects of extraction temperature and pressure on the extraction yields of caffeine and catechins from green tea with a mean particle size of 543.7 μm in the range of 50–80 °C and 150–300 bar at a $CO_2$ flow rate of 8.5 g/min for 120-min extraction time, where 95% (v/v) ethanol was used as a cosolvent at 4.6 g/100 g of $CO_2$. When extraction pressure was fixed at 300 bar, the extraction yield of caffeine increased with an increase in temperature. The highest removal yield of

Table 1
Composition of green tea used in the experiment

| Component | Caffeine | EGCG | EGC | ECG | EC |
|---|---|---|---|---|---|
| Content (mg/g)[a] | 41.38 ± 0.41 | 145.28 ± 0.89 | 81.52 ± 3.72 | 27.26 ± 0.49 | 6.01 ± 0.14 |

[a] Concentration of each component is milligram(s) of component per gram (dry weight) of green tea.
Each value is expressed as mean ± standard deviation based on triplicate measurements.

Table 2
Effects of temperature and pressure on the removal of caffeine from green tea leaves by using SC-$CO_2$

| Extraction conditions | | Component removal yield based on initial content (%, w/w) | | | | |
|---|---|---|---|---|---|---|
| Temperature (°C) | Pressure(bar) | Caffeine | EGCG | EGC | ECG | EC |
| 50 | 300 | 62.6 | 53.2 | 82.4 | 60.6 | 33.3 |
| 60 | 300 | 81.6 | 57.4 | 82.6 | 66.7 | 38.5 |
| 70 | 300 | 92.8 | 64.2 | 78.2 | 72.5 | 24.0 |
| 80 | 300 | 92.8 | 69.5 | 82.3 | 71.2 | 50.0 |
| 70 | 150 | 77.7 | 50.5 | 62.0 | 54.6 | 20.0 |
| 70 | 200 | 90.5 | 66.7 | 72.3 | 72.6 | 35.5 |
| 70 | 250 | 92.1 | 69.0 | 75.6 | 78.0 | 37.6 |

Extraction was conducted with green tea leaves with a particle size of 543.7 μm at a $CO_2$ flow rate of 8.5 g/min for 120 min, where 95% (v/v) ethanol was used as a cosolvent at 4.6 g/100 g of $CO_2$.

caffeine and EGCG, 92.8% and 69.5%, respectively, resulted at the highest temperature, 80 °C among the tested temperatures. In general, other catechins also showed increased extraction yields with increasing extraction temperature. From 1 g of green tea on a dry weight basis, 38.4 mg of caffeine was removed at 80 °C and 300 bar.

At equilibrium, the solubility of a solute in a supercritical phase is balanced by the effects of supercritical solvent density and vapor pressure of solute. If the density effect dominates, then as the temperature increases, a decrease in solvent density results in reduced dissolving power of the solvent, thus the solubility of the solute eventually decreases. In the case of predominance of the vapor pressure effect, when the temperature increases, the solubility of the solute increases due to an increase in the vapor pressure effect of the solute. The crossover phenomena of solubility caused by the balance of the solvent density and vapor pressure effects were often observed in the SFE of plant materials [29,30]. In this experiment, the extraction of caffeine was enhanced at a higher temperature, likely due to the predominance of the vapor pressure effect over the density effect. EGCG, which is the most health-benefiting catechin in green tea, was also shown to be removed at higher levels at a higher extraction temperature.

Table 2 also shows the effect of pressure on the extraction yields of caffeine and catechins when the extraction was carried out at different pressures in the range of 150–300 bar at 70 °C. As pressure increased, the extraction yield of caffeine and catechins was shown to increase. These results may be attributed to the increased dissolving power of SC-$CO_2$ due to the increased density caused by an increase in pressure at the same temperature. However, except at 150 bar, the extraction yields of caffeine for 200, 250, and 300 bar were similar. When the extraction pressure was over 200 bar, more than 90% of caffeine and over 60% of EGCG were removed. The highest caffeine extraction yield, 92.8%, was obtained at the highest pressure, 300 bar.

### 3.3. Effect of $CO_2$ flow rate

Under the fixed conditions of 70 °C and 300 bar, 10 g of dry green tea ground to a mean particle size of 543.7 μm were extracted with SC-$CO_2$ modified with 4.6 g of 95% (v/v) ethanol per 100 g of $CO_2$ for 120 min at different mass flow rates of $CO_2$. As shown in Table 3, the residence time of $CO_2$ in the extraction vessel was determined to decrease with an increase in the mass flow rate of $CO_2$. The total amounts of caffeine and catechins removed generally increased with increasing $CO_2$ mass flow rate, which indicates that a larger amount of SC-$CO_2$ contacting the green tea at a higher $CO_2$ flow rate brought out larger amounts of caffeine and catechins during the same duration of extraction. When the caffeine and catechins extraction efficiencies were determined based on the total amount of $CO_2$ used for a given time as shown in Table 3, the extraction efficiencies mostly decreased with an increase in $CO_2$ mass flow rate.

These results could be due to the short residence time of $CO_2$ in the green tea-packed extraction vessel, which implies that the external mass transfer resistance around green tea particles is negligible. In other words, for the enhanced extraction of solutes, the use of a more rapid supply of fresh $CO_2$ into the green tea-packed extractor to disrupt or sweep off the possibly existing external film or boundary layer surrounding green tea particles, thereby reducing the external mass transfer resistance, was not necessary in the current stage of the extraction using this experiment. The negligible effect of external mass transfer in SFE has been described elsewhere using herbaceous materials such peppermint leaves [29]. Instead, leaving the SC-$CO_2$ in the extraction vessel longer gave a better extraction yield for a given amount of $CO_2$ since the intraparticle diffusion of SC-$CO_2$ and the solubilization of solutes within micropores would have required a longer contact time between green tea leaves and SC-$CO_2$. At the highest mass flow rate of $CO_2$, 14.5 g/min, 93.2% of the initial level of caffeine was removed, and 209.8 mg of caffeine was extracted per 1 kg of $CO_2$ used. Meanwhile, at the lowest flow rate of $CO_2$, 5.5 g/min, 80.1% of caffeine was removed, and 475.1 mg of caffeine was extracted per 1 kg of $CO_2$ used. In terms of the total extraction rate, a higher $CO_2$ mass flow rate is desirable, but considering the efficiency of $CO_2$ as a solvent, a lower $CO_2$ flow rate is preferred.

When the extraction efficiency of caffeine from the green tea leaves, ranging from 209.8 to 475.1 mg/kg of $CO_2$ at 70 °C, 300 bar, and a $CO_2$ flow rate of 5.5–14.5 g/min for 120 min shown in Table 3, can be compared to those of caffeine from other plants such as coffee beans, 45.2–120.5 mg/kg of $CO_2$ at 50 °C, 103–193 bar, and a $CO_2$ flow rate of 1.51 g/min for 200 min [16]; guarana seeds, 74.3–118.9 mg/kg of $CO_2$ at 40–70 °C, 400 bar, and a $CO_2$ flow rate of 5.7 g/min for 210 min [31]; and mate leaves, 7.2–5.3 mg/kg of $CO_2$ at 40–70 °C, 400 bar, and a $CO_2$ flow rate of 5.7 g/min for 400 min [31], the extraction efficiency

Table 3
Effect of $CO_2$ flow rate on the removal of caffeine from green tea leaves by using SC-$CO_2$

| Extraction conditions | | Component removal yield based on initial content (%, w/w) | | | | | Extraction efficiency(mg component/kg $CO_2$ used) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CO2 flow rate (g/min) | Residence time of $CO_2$ (min) | Caffeine | EGCG | EGC | ECG | EC | Caffeine | EGCG | EGC | ECG | EC |
| 5.5 | 12.90 | 80.1 | 46.0 | 67.7 | 46.0 | 8.9 | 475.1 | 958.4 | 791.9 | 179.8 | 7.7 |
| 8.5 | 8.34 | 92.8 | 64.2 | 78.2 | 72.5 | 24.0 | 356.4 | 865.0 | 591.5 | 183.4 | 13.4 |
| 11.5 | 6.17 | 95.6 | 73.6 | 80.8 | 79.0 | 38.1 | 271.3 | 732.8 | 451.7 | 147.7 | 15.7 |
| 14.5 | 4.89 | 93.2 | 77.0 | 86.5 | 80.9 | 51.2 | 209.8 | 608.1 | 383.6 | 119.9 | 16.8 |

Extraction was conducted with green tea leaves with a mean particle size of 543.7 μm at 300 bar and 70 °C and at a $CO_2$ flow rate of 5.5, 8.5, 11.5, and 14.5 g/min for 120 min, where 95% (v/v) ethanol was used as a cosolvent at 4.6 g/100 g of $CO_2$.

of mate leaves showed much lower values than those of green tea leaves, coffee beans, and guarana seeds.

### 3.4. Effect of particle size of green tea

The particle size of a sample is also an important variable in extraction, as it may affect the mass transfer of solute and solvent, and may also be directly related to total surface area for contact between sample and solvent. The extraction conditions of 70 °C and 300 bar were adopted for investigating the effect of particle size of green tea leaves. Green tea leaves ground to particle sizes (mean ± standard deviation) of 236.5 ± 30.1 and 543.7 ± 51.7 μm, and green tea leaves without grinding in the particle size range of 5.5–17.4 mm were extracted at the above extraction conditions. As shown in Table 4, the extraction yield of caffeine was found to be affected by whether the green tea leaves had been ground or not. Approximately, 15–17% more of the caffeine based on its initial content was extracted when green tea leaves were ground to smaller particle sizes. The smallest particle size sample (236.5 μm) gave the highest caffeine removal yield, 97.3% (w/w) of its initial content. The caffeine removal yield from the green tea leaves with a particle size of 543.7 μm was 95.6%, which was not significantly different from the yield from the sample with the smaller particle size of 236.5 μm. The enhanced extraction for the green tea leaves with a smaller particle size can be explained by the process of solute diffusion in particles. As the particle size increases, the distance that a solute has to travel from inside the particle to the surface of the particle also increases, which results in slower extraction of the solute. Additionally, when the particle size is small, a comparatively large surface area of a particle is available for contact with the solvent. In the current stage of the extraction in this study, the intraparticle mass transfer controls the overall mass transfer rate, which has also been reported in the SFE of essential oils from many herbaceous materials such as rosemary, basil, and majoram [32,33]. In such herbaceous materials, due to the fact that essential oil is located in vacuoles, which is rather difficult to access compared to the leaf surface, the SFE process is controlled by the intraparticle mass transfer.

Meanwhile, the effect of particle size on the extraction yield was similar to that of catechins. In particular, the particle size effect on the extraction yield was strikingly noticeable in the case of EGCG, the major catechin in green tea. While only 68.9 mg of EGCG was obtained from 1 g of dry green tea leaves without grinding, over 100 mg of EGCG was removed from one gram of green tea leaves. The pronounced effect of particle size on EGCG might be attributed to the higher quantity of EGCG (than caffeine) in green tea subject to extraction enhanced by reducing particle size by grinding. Consequently, while the smallest particle was the best for the caffeine removal yield, the largest particle size from no grinding was the best for the preservation of EGCG.

Table 4
Effect of the particle size of green tea leaves on the removal of caffeine from green tea leaves by using SC-$CO_2$

| Particle size | Component removal yield based on initial content (%, w/w) | | | | |
|---|---|---|---|---|---|
| | Caffeine | EGCG | EGC | ECG | EC |
| 236.5 μm | 97.3 | 70.8 | 81.3 | 76.6 | 47.8 |
| 543.7 μm | 95.6 | 73.6 | 80.8 | 79.0 | 38.1 |
| 5.5–17.4 mm | 80.6 | 47.5 | 64.2 | 50.9 | 31.7 |

Extraction was conducted with green tea leaves with a particle size of 236.5 μm, 543.7 μm, and 5.5–17.4 mm at 300 bar and 70 °C and at a $CO_2$ flow rate of 11.5 g/min for 120 min, where 95% (v/v) ethanol was used as a cosolvent at 4.6 g/100 g of $CO_2$.

### 3.5. Effect of extraction time

To monitor the extraction behavior of caffeine and catechins with time, extracted samples were analyzed at 40-min intervals. Fig. 2 shows the time course for the extraction carried out with green tea with a particle size of 236.5 μm at a $CO_2$ flow rate of 11.5 g/min and at 300 bar and 70 °C, in which the cosolvent was 4.6 g of 95% (v/v) ethanol per 100 g of $CO_2$ used. In the initial period of extraction, the extraction rate of caffeine was higher than that in the latter period of extraction. More than 75.7%, 93.9%, and 97.8% of caffeine was extracted after 40, 80, and 120 min extraction, respectively. The pattern of the decrease in the extraction rate as time passed was similarly observed in the extraction of other components, including EGCG. In the early period of extraction, the solvent must only remove easily accessible solutes. However, as extraction time passes, the readily available solutes are depleted, and the solutes located inside the particles then must be transferred to the surface of the particle. It is the internal mass transfer resistance that limits the rate of the extraction process. Therefore, to remove the solutes, more time and $CO_2$ are required as extraction time passes because of the reduction of readily accessible solutes by the solvent with time.

After 200 min of extraction, 41.4 mg/g of caffeine and 145.3 mg/g of EGCG in the initial dry green tea before extraction decreased to 0.0 and 19.1 mg/g, respectively. It is promising that caffeine was reduced to less than 0.1% (w/w) of its initial

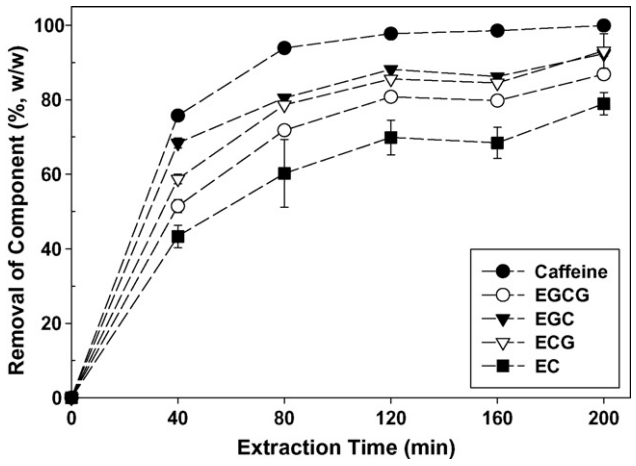


Fig. 2. Removal yield of caffeine and catechins from green tea leaves (% of their initial contents) with time on a laboratory scale (10 g of dry green tea leaves in the 100 mL internal volume of extraction vessel), where the SC-$CO_2$ extraction conditions were a mean particle size of 236.5 μm for the ground green tea, 300 bar, 70 °C, a $CO_2$ flow rate of 11.5 g/min, and 4.6 g of 95% (v/v) ethanol as a cosolvent per 100 g of $CO_2$.

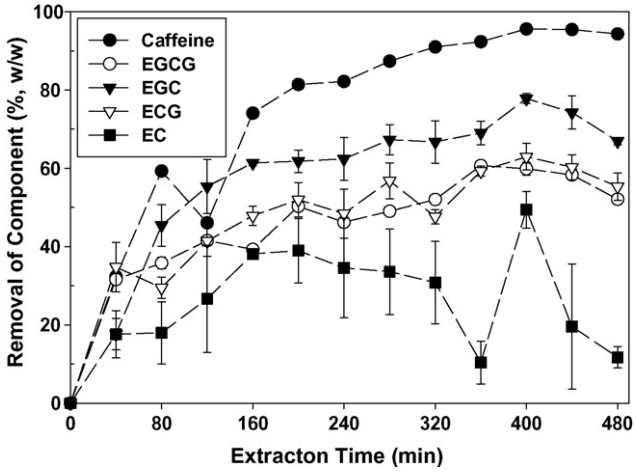


Fig. 3. Removal yield of caffeine and catechins from green tea leaves (% of their initial contents) with time on a laboratory scale (10 g of dry green tea leaves in the 100 mL internal volume of extraction vessel), where the SC-$CO_2$ extraction conditions were a green tea particle size of 5.5–17.4 mm without grinding, 300 bar, 70 °C, a $CO_2$ flow rate of 5.5 g/min, and 4.6 g of 95% (v/v) ethanol as a cosolvent per 100 g of $CO_2$.

content. At the same time, however, the loss of catechins due to extraction was also substantial. For example, EGCG decreased to less than 13.1% (w/w) of its initial content. Therefore, extraction was also carried out with the green tea without grinding having a particle size of 5.5–17.4 mm, which exhibited much less significant loss of EGCG, as shown in Table 4.

Fig. 3 presents the time course for the extraction conducted at the same conditions, including temperature, pressure, and cosolvent content, as in Fig. 2, except for the particle size, 5.5–17.4 mm, of the green tea without grinding and the $CO_2$ flow rate of 5.5 g/min. In general, the experimental data showed large deviations among triplicate samples compared to those in Fig. 2 with the smaller particle size. This is probably due to uneven extraction depending on individual particles and the difficulty of taking representative samples using green tea without grinding.

When using the green tea leaves with the larger particle size (5.5–17.4 mm) in Fig. 3, the extraction rate of caffeine was much slower than that observed when using the smaller particle size (236.5 μm) in Fig. 2. For instance, after 40-min extraction, only 32.3% of caffeine was removed from the larger particle size of green tea (5.5–17.4 mm), whereas 75.7% of caffeine was extracted from the smaller particle size green tea (236.5 μm). After an extraction time of 320 min had elapsed, 91.0% of caffeine was removed, but only 52.0% of EGCG was extracted. When extraction time was further extended to 480 min, the removal yield of caffeine increased to 94.3% of its initial content, but the extraction yield of EGCG remained near 52.1%. When comparing these results with 99.9% caffeine removal and 86.9% EGCG removal using the ground green tea leaves with the smaller particle size of 236.5 μm as shown in Fig. 2, a much less significant loss of EGCG as shown in Fig. 3, is more desirable in terms of maximal preservation of catechins. However, it also must be taken into account that the large particle size caused the slower extraction rate, the greater consumption of $CO_2$, and the longer extraction time, which could be disadvantageous to process economics.

## 4. Conclusions

In the extraction of green tea by SC-$CO_2$ with 95% (v/v) ethanol as a modifier, the extraction yield of caffeine and catechins increased with an increase in pressure and temperature up to 300 bar and 80 °C. As the $CO_2$ mass flow rate increased, the extraction efficiency of $CO_2$, that is the amount of extracted solute based on the amount of $CO_2$ used, decreased probably due to the negligible external mass transfer resistance and the reduced $CO_2$-green tea contact time. The reduction of green tea particle size by grinding resulted in the enhanced extraction rate of caffeine and catechins, indicating the effects of intraparticle mass transfer resistance and the larger contacting area between SC-$CO_2$ and green tea. Therefore, the extraction was found to be rather controlled by the internal mass transfer resistance than the external mass transfer resistance. In the above extraction conditions, the extraction of a substantial quantity of catechins was also observed during the decaffeination process, and thus it requires further process improvements such as more selective extraction toward caffeine than catechins as well as the recovery of EGCG and other catechins from the outlet stream.

## Acknowledgments

This work was supported by the Technology Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea. The authors also acknowledge the support of the BioGreen 21 Program (20050401-034-749-180-02-00), Rural Development Administration, Republic of Korea.

## References

[1] S. Joshi, S.K. Hasan, R. Chandra, M.M. Husain, R.C. Srivastava, Scavenging action of zinc and green tea polyphenol on cisplatin and nickel induced nitric oxide generation and lipid peroxidation in rats, Biomed. Environ. Sci. 17 (2004) 402–409.
[2] Y.J. Cai, L.P. Ma, L.F. Hou, B. Zhou, L. Yang, Z.L. Liu, Antioxidant effects of green tea polyphenols on free radical initiated peroxidation of rat liver microsomes, Chem. Phys. Lipids 120 (2002) 109–117.
[3] Z.Q. Liu, L.P. Ma, B. Zhou, L. Yang, Z.L. Liu, Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein, Chem. Phys. Lipids 106 (2000) 53–63.
[4] S.B. Moyers, N.B. Kumar, Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials, Nutr. Rev. 62 (2004) 204–211.
[5] S. Trompezinski, A. Denis, D. Schmitt, J. Viac, Comparative effects of polyphenols from green tea (EGCG) and soybean (genistein) on VEGF and IL-8 release from normal human keratinocytes stimulated with the proinflammatory cytokine TNF alpha, Arch. Dermatol. Res. 295 (2003) 112–116.
[6] E. Tedeschi, H. Suzuki, M. Menegazzi, Antiinflammatory action of EGCG, the main component of green tea, through STAT-1 inhibition, Ann. N. Y. Acad. Sci. 973 (2002) 435–437.
[7] R. Cooper, D.J. Morre, D.M. Morre, Medicinal benefits of green tea: part I. Review of noncancer health benefits, J. Altern. Complement Med. 11 (2005) 521–528.
[8] P.D. Stapleton, S. Shah, J.C. Anderson, Y. Hara, J.M.T. Hamilton-Miller, P.W. Taylor, Modulation of beta-lactam resistance in *Staphylococcus*

*aureus* by catechins and gallates, Int. J. Antimicrob. Agents 23 (2004) 462–467.
[9] Y. Yanagawa, Y. Yamamoto, Y. Hara, T. Shimamura, A combination effect of epigallocatechin gallate, a major compound of green tea catechins, with antibiotics on *Helicobacter pylori* growth in vitro, Curr. Microbiol. 47 (2003) 244–249.
[10] G. Fassina, A. Buffa, R. Benelli, O.E. Varnier, D.M. Noonan, A. Albini, Polyphenolic antioxidant (−)-epigallocatechin-3-gallate from green tea as a candidate anti-HIV agent, AIDS 16 (2002) 939–941.
[11] I. Hindmarch, U. Rigney, N. Stanley, P. Quinlan, J. Rycroft, J. Lane, A naturalistic investigation of the effects of day-long consumption of tea, coffee and water on alertness, sleep onset and sleep quality, Psychopharmacology 149 (2000) 203–216.
[12] M. Giannelli, P. Doyle, E. Roman, M. Pelerin, C. Hermon, The effect of caffeine consumption and nausea on the risk of miscarriage, Paediatr. Perinat. Epidemiol. 17 (2003) 316–323.
[13] V. Rasch, Cigarette alcohol, and caffeine consumption: risk factors for spontaneous abortion, Acta Obstet. Gynecol. Scand. 82 (2003) 182–188.
[14] J. Bernhisel-Broadbent, Diagnosis and management of food hypersensitivity, Immunol. Allergy Clin. North Am. 19 (1999) 463.
[15] G. Brunner, Extraction of caffeine from coffee with supercritical solvents, in: M. Perrut (Ed.), Proceedings of the 1st International Symposium on Supercritical Fluids, Nice, France, October 17–19, 1988, pp. 691–698.
[16] H. Peker, M.P. Srinvasan, J.M. Smith, B.J. McCoy, Caffeine extraction rates from coffee beans with supercritical carbon dioxide, AIChE J. 38 (1992) 761–770.
[17] C.B. Mehr, R.N. Biswal, J.L. Collins, H.D. Cochran, Supercritical carbon dioxide extraction of caffeine from Guarana, J. Supercrit. Fluids 9 (1996) 185–191.
[18] O. Vitzthum, P. Hubert, Method for the manufacture of caffeine free black tea, (1979) U.S. Patent 4,167,589.
[19] A. Farah, T. De Paulis, D.P. Moreira, L.C. Trugo, P.R. Martin, Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffees, J. Agric. Food Chem. 54 (2006) 374–381.
[20] A. Perva-Uzunalic, M. Skerget, Z. Knez, B. Weinreich, F. Otto, S. Gruner, Extraction of active ingredients from green tea (*Camellia sinensis*): extraction efficiency of major catechins and caffeine, Food Chem. 96 (2006) 597–605.
[21] H.L. Liang, Y.R. Liang, J.J. Dong, J.L. Lu, H.R. Xu, H. Wang, Decaffeination of fresh green tea leaf (*Camellia sinensis*) by hot water treatment, Food Chem. 101 (2007) 1451–1456.
[22] S. Gokulakrishnan, K. Chandraraj, S.N. Gummadi, Microbial and enzymatic methods for the removal of caffeine, Enzyme Microb. Technol. 37 (2005) 225–232.
[23] A. Berna, A. Chafer, J.B. Monton, S. Subirats, High-pressure solubility data of system ethanol (1) plus catechin (2) plus $CO_2$ (3), J. Supercrit. Fluids 20 (2001) 157–162.
[24] A. Chafer, A. Berna, J.B. Monton, R. Munoz, High-pressure solubility data of system ethanol (1) plus epicatechin (2) plus $CO_2$ (3), J. Supercrit. Fluids 24 (2002) 103–109.
[25] C.J. Chang, K.L. Chiu, Y.L. Chen, C.Y. Chang, Separation of catechins from green tea using carbon dioxide extraction, Food Chem. 68 (2000) 109–113.
[26] C.J. Chang, K.L. Chiu, Y.L. Chen, P.W. Yang, Effect of ethanol content on carbon dioxide extraction of polyphenols from tea, J. Food Compos. Anal. 14 (2001) 75–82.
[27] L.P. Wright, N.I.K. Mphangwe, H.E. Nyirenda, Z. Apostolides, Analysis of caffeine and flavan-3-ol composition in the fresh leaf of *Camellia sinensis* for predicting the quality of the black tea produced in Central and Southern Africa, J. Sci. Food Agric. 80 (2000) 1823–1830.
[28] H.F. Wang, G.J. Provan, K. Helliwell, HPLC determination of catechins in tea leaves and tea extracts using relative response factors, Food Chem. 81 (2003) 307–312.
[29] E.L. Copeland, M.N. Clifford, C.M. Williams, Preparation of (−)-epigallocatechin gallate from commercial green tea by caffeine precipitation and solvent partition, Food Chem. 61 (1998) 81–87.
[30] M. Goto, M. Sato, T. Hirose, Extraction of peppermint oil by supercritical carbon dioxide, J. Chem. Eng. Jpn. 26 (1993) 401–407.
[31] M.D.A. Saldana, C. Zetzl, R.S. Mohamed, G. Brunner, Extraction of methylxanthines from guarana seeds, mate leaves, and cocoa beans using supercritical carbon dioxide and ethanol, J. Agric. Food Chem. 50 (2002) 4820–4826.
[32] B.C. Roy, M. Goto, T. Hirose, Extraction of ginger oil with supercritical carbon dioxide: experiments and modeling, Ind. Eng. Chem. Res. 35 (1996) 607–612.
[33] E. Reverchon, Fractional separation of SCF extracts from marjoram leaves: mass transfer and optimization, J. Supercrit. Fluids 5 (1992) 256–261.