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Extraction behaviors of caffeine and chlorophylls in supercritical decaffeination of green tea leaves

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ABSTRACT

The decaffeination of green tea using supercritical carbon dioxide (SC-CO_2) was optimized by response surface methodology (RSM) for the maximal removal of caffeine, and the coextration of chlorophylls was also monitored during decaffeination. The experimental conditions for the SC-CO_2 extraction of caffeine were set up according to the Box-Behnken design of RSM. The relationships between the extraction yield of caffeine and various parameters used for the SC-CO_2 extraction such as pressure, temperature and concentration of ethanol were studied at a fixed CO_2 flow rate. The extraction yields of caffeine and total chlorophyll were significantly influenced by extraction pressure, temperature and concentration of cosolvent, and their extraction yields behaved almost in parallel at different extraction conditions that were obtained by varying pressure, temperature and ethanol cosolvent concentration. At the optimal decaffeination conditions such as 3.0 g of 95% (v/v) ethanol cosolvent per 100 g of CO_2, 23 MPa, 63 °C and an extraction duration of 120 min for 10 g of green tea leaves, the extraction yields for caffeine and catechins were 96.60% (w/w) and 40.61% (w/w), respectively, and the substantial coextraction of total chlorophyll (43.09% of the total amount) was also observed during the decaffeination process.

1. Introduction

Green tea (*Camellia sinensis*) is currently one of the most commonly consumed beverages worldwide (Yang, Lambert, Ju, Lu, & Sang, 2007). Polyphenols and caffeine are important components of green tea due to their unique flavors and biological effects. Specifically, catechins, the main component of polyphenols, are known to have beneficial health effects on humans such as anti-cancer (Yang et al., 2007), anti-inflammatory (de Mejia, Ramirez-Mares, & Puangpraphant, 2009), and antibiotic (Osterburg, Gardner, Hyon, Neely, & Babcock, 2009).

Caffeine is a kind of alkaloid and present in various species of plant including green tea, coffee and cacao, and it is known to exhibit the effects of health benefit such as Alzheimer's disease prevention (Eskelinen & Kivipelto, 2010) and anticancer (Kang et al., 2010). However, the intake of excessive caffeine has been reported to cause health problems in humans (Hindmarch et al., 2000). For example, daily consumption of greater than a certain amount of caffeine has been shown to be adverse for pregnant women (Boylan et al., 2008), infants and children (Müller-Vahl, Buddensiek, Geomelas, & Emrich, 2008). Accordingly, many countries have already recommended limitations on the daily intake of caffeine in foods depending on gender, age and body weight (Anonymous, 2003, 2011). For example, UK has limited the daily caffeine intake to 200 mg for pregnant women (Anonymous, 2008), and a warning label of high caffeine content is required for drinks containing caffeine higher than 150 mg/L in EU since July 2004 (Anonymous, 2002). Therefore, decaffeinated products need to be available as an option for consumers who are vulnerable to high caffeine foods. Therefore, many efforts have been made to remove or reduce caffeine from foods such as coffee (Ogita, Uefuji, Yamaguchi, Koizumi, & Sano, 2003), guarana (Saldaña, Zetzl, Mohamed, & Brunner, 2002) and black tea (Vitzthum & Hubert, 1979).

Initially, methods employing organic solvents such as trichloroethylene and dichloromethane were commonly used to remove caffeine from coffee and other plant materials, but these methods were banned after they were found to cause cancers (Lavin, Jacobson, & DeSesso, 2000). One alternative to organic solvents used in decaffeination processes is supercritical carbon dioxide (SC-CO_2), which is a fluid that exists beyond the critical pressure (7.38 MPa) and temperature (31.1 °C) of CO_2. Indeed, methods employing SC-CO_2 have been applied to the decaffeination of coffee (Zosel, 1974) and black tea on a commercial scale (Vitzthum & Hubert, 1979). It has also been reported that SC-CO_2 effectively removed caffeine from green tea in the presence of a polar cosolvent (Park et al., 2007a). Despite many advantages of SC-CO_2, such as low CO_2 toxicity, no residual solvent and effective caffeine removal, it leads to the coextraction of a considerable amount of

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chlorophylls when used to decaffeinate green tea, which can result in undesirable effects on the appearance, aroma and tastes of the final product (Lee, Lee, Kim, & Kim, 2009). The extraction of chlorophylls from natural matrices such as marjoram (Vági, Simándi, Daood, Deák, & Sawinsky, 2002), microalgae (Macías-Sánchez et al., 2007) and stinging nettle leaves (Sovová, Sajfrtová, Bártlová, & Opletal, 2004) by SC-CO_2 has been evaluated.

Chlorophylls, which are generally composed of chlorophyll *a* and *b* in a ratio of 3:1, are the most common green pigments in plant leaves (Schwartz & Lorenzo, 1990). Chlorophylls provide natural colors and beneficial health effects (Nakamura, Murakami, Koshimizu, & Ohigashi, 1996). In addition, these natural pigments are widely used in a variety of industrial products such as soft drinks, ice cream, ink and soap. As a result, there have been many attempts to minimize the degradation of chlorophylls during the storage and processing of foods and plant materials (Schwartz & Lorenzo, 1990). However, the coextraction of chlorophylls with caffeine is not avoidable during SC-CO_2 decaffeination of green tea (Park et al., 2007a). The primary objective of this study was to optimize the supercritical decaffeination process to maximize the removal of caffeine from green tea by response surface methodology (RSM), but the amount of coextracted chlorophylls was also monitored at each decaffeination condition.

2. Materials and methods

2.1. Materials

Green tea in the form of dry processed leaves was provided by Nokchawon (Seoul, Korea) in September 2006 and stored at −80 °C in a freezer until use. The green tea leaves were ground using a cutting mill (IKA, Staufen, Germany) and then sieved through screens with mesh sizes of 125 and 425 μm (Chung Gye Sang Gong Sa, Seoul, Korea). After sieving, the mean particle sizes of the ground green tea leaves were determined to be 236.5 ± 30.1 μm using a 780 Accusizer particle size analyzer (Particle Sizing System, Santa Barbara, CA, USA).

2.2. Supercritical CO_2 extraction

The supercritical fluid extraction (SFE) system (Ilshin Autoclave, Daejeon, Korea) shown in Fig. 1 was used in this study. The entire system, including the 100 mL extraction vessel, was constructed of stainless steel. To conduct the extraction, the SFE was heated to the designated temperature, after which 10 g of ground green tea leaves that were soaked with a certain amount of a cosolvent were loaded. The pressure was monitored continuously throughout the extraction and controlled using a back pressure regulator (BPR). To determine the flow rate of CO_2 during the extraction, the weight of the CO_2 tank was monitored using an electronic balance (CAS, Seoul, Korea). The extraction was conducted for 60 min, after which the extraction vessel was depressurized. A detailed description of the startup and shutdown of the extraction has been provided in previously conducted studies (Park et al., 2007a). The high-purity CO_2 (99.5%) used in the SFE system was obtained from Daehan Specialty Gases (Seoul, Korea).

2.3. Analysis of caffeine, catechins and chlorophylls

The caffeine and catechin contents were analyzed by high performance liquid chromatography (HPLC; Agilent 1100, Agilent Technologies, Waldbronn, Germany) as described elsewhere (Park et al., 2007a). Briefly, dried green tea was extracted with 30% (v/v) ethanol solution at 35 °C, after which the extract was centrifuged, filtered and subjected to HPLC analysis. Quantitative analyses were conducted using authentic standards such as caffeine, epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), which were purchased from Sigma (St. Louis, MO, USA).

The methods described by Wang, Park, Chung, Baik, and Park (2004) and Arnon (1949) were used for analysis and quantification of the total chlorophyll in the green tea. Briefly, 200 mg of dry green tea leaves were extracted in a flask with 50 mL of 85% (v/v) acetone solution for 30 min. After the acetone extraction, green tea leaves were filtered into a volumetric flask using a filter paper (110 mm, No. 2, Whatman, Brentford, UK), after which 85% (v/v) acetone solution was added to give a final volume of 50 mL. The total chlorophyll contents were analyzed by UV–Visible spectrophotometry (Dong-il Shimadzu, Seoul, Korea), after which the absorbance was converted to total chlorophyll using Eq. (1):

$$\text{Total chlorophyll}(\text{mg/L}) = 2.09 \times A_{645} + 8.02 \times A_{663} \quad (1)$$

where A_{645} is the absorbance at 645 nm and A_{663} is the absorbance at 663 nm. The contents of caffeine, catechins and total chlorophyll in the green tea used in this study are shown in Table 1. The contents of caffeine, EGCG and total chlorophyll on dry basis were 23.35, 81.69 and 2.96 mg/g, respectively. The major catechin was

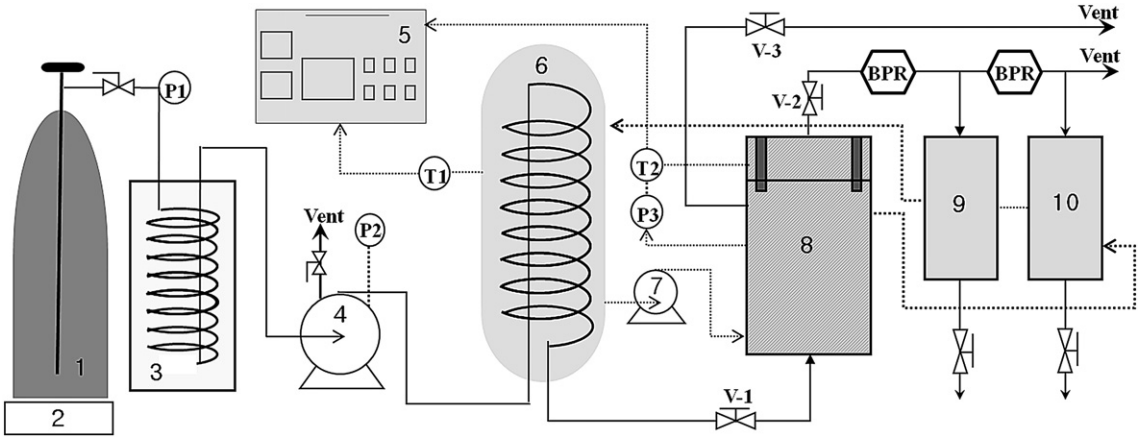

Fig. 1. Schematic diagram of the laboratory-scale supercritical CO_2 extraction system: 1, CO_2 cylinder; 2, electronic balance; 3, chiller; 4, CO_2 pump; 5, controller; 6, heating bath; 7, circulation pump; 8, extractor; 9, separator 1; 10, separator 2; V1, valve 1, V2, valve 2; BPR, back pressure regulator;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