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Separation of catechins from green tea using carbon dioxide extraction

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Abstract

This study presents a novel packed-column extractor coupled with an absorption system to improve the quality of green tea essential oils, extracted by using high-pressure carbon dioxide. The effects of various co-solvents on the extract are also examined. In addition, gravimetric measurement and HPLC chromatographic analyses individually determine the amount of essential oil and the concentration of four major catechins. Results show that the mean contents in the extract are 4.4-fold higher by addition of 95% ethanol than by addition of water. The ratio of polyphenols to caffeine is highest in the Soxhlet ethanol extraction.

Keywords: Carbon dioxide; Catechins; Caffeine; Polyphenols; Co-solvent

1. Introduction

Tea is among the most popular beverages consumed worldwide. Water-soluble materials in teas consist of primary polyphenols. Numerous epidemiological and pharmacological studies demonstrate that green tea extract possesses strong antioxidant effects (Lin, Juan, Chen, Liang & Lin, 1996; Roedig-Penman & Gordon, 1997; Tanaka, Kusano & Kouno, 1998; Vinson & Dabbagh, 1998) and antimutagenic activity (Chen, Schell, Ho & Chen, 1998; Leanderson et al., 1997; Otsuka, Ogo, Eto, Asano, Suganuma & Niho, 1998; Paschka, Butler & Young, 1998). According to the previous studies, four polyphenol compounds, epigallocatechin gallate (EGCg), epicatechin gallate (ECg), epigallocatechin (EGC), and epicatechin (EC) are significant antioxidant constituents (Fig. 1). Among these, EGCg is the most luxuriant component in tea extract (Owuor & Obanda, 1998; Price & Spitzer, 1993) and the most potent chemical tested for biological activity (Chen et al.). These polyphenols may account for as much as 30% of the dry weight of fresh tea leaves.

High-performance liquid chromatography is the conventional means of analyzing catechins in tea and other biological constituents (Bronner & Beecher, 1998; Carando, Teissedre & Cabanis, 1998; Dalluge, Nelson, Thomas & Sander, 1998; Goto, Yoshida, Kiso & Nagashima, 1996). Lack and Seidlitz (1993) have investigated decaffeination of coffee and tea using supercritical CO_2. Traditional extraction of caffeine from black tea using an organic solvent has been reported by Davis et al. (1997). However, to our knowledge, no study has separated catechins and caffeine by using supercritical carbon dioxide extraction.

In light of the above discussion, this study presents a novel custom-designed supercritical fluid extraction technique coupled with an absorption system to extract polyphenols from green tea leaf. Controlling the extractive conditions allows us to evaluate the separation of caffeine and polyphenols. A gravimetric method can be applied to determine the amount in the extract. Moreover, HPLC chromatography is performed to quantify four major catechins (TC), along with a spectrophotometer used to measure the amount of total polyphenols (TPP).

2. Materials and methods

Green tea was obtained from a commercial market. Phosphoric acid (85.1%), epicatechin gallate (ECg, 98%), epigallocatechin (EGC, 98%), epigallocatechin

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gallate (EGCg, 95%), epicatechin (EC, 98%), GR grade gallic acid and caffeine were purchased from Sigma Company (United States). All materials were used without further purification. HPLC grade acetonitrile (BDH, UK) was used for mobile phase solvent. Absolute ethanol (99.8%) was obtained from RDH (Germany). Deionized water was obtained from a reverse-osmosis and ion exchange purified water system (Barnstead, UK).

Fig. 2 schematically depicts a high-pressure apparatus used for CO_2 extraction of tea catechins. The whole system consists mainly of a 100 ml-syringe pump, a refrigeration module, a 300 ml extraction vessel, one sight-gauge precipitator, two absorbent vessels, and a wet gas meter. The Isco syringe pump volume can deliver

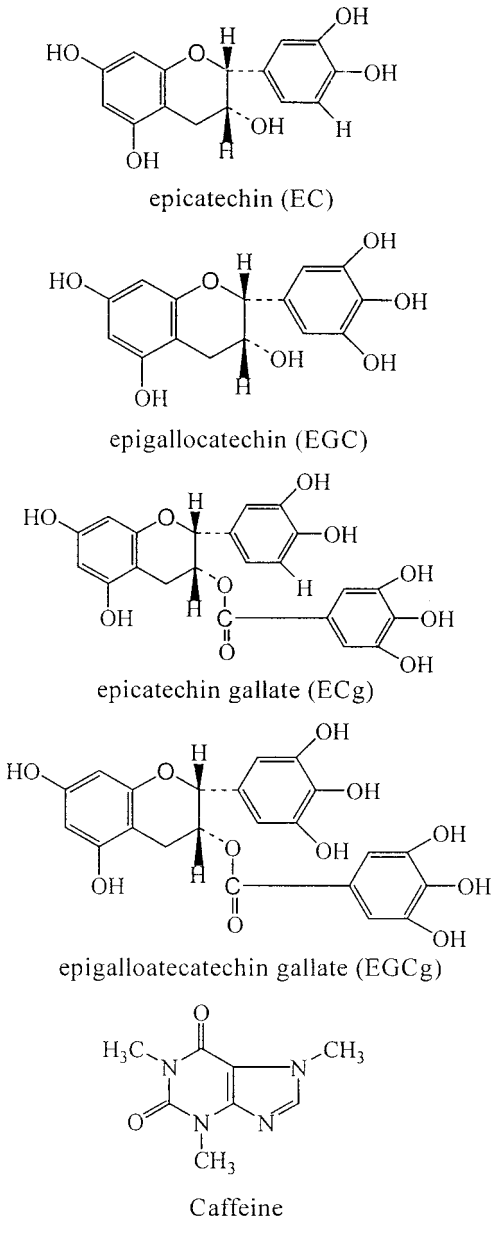


Fig. 1. Structures of four major catechins and caffeine.

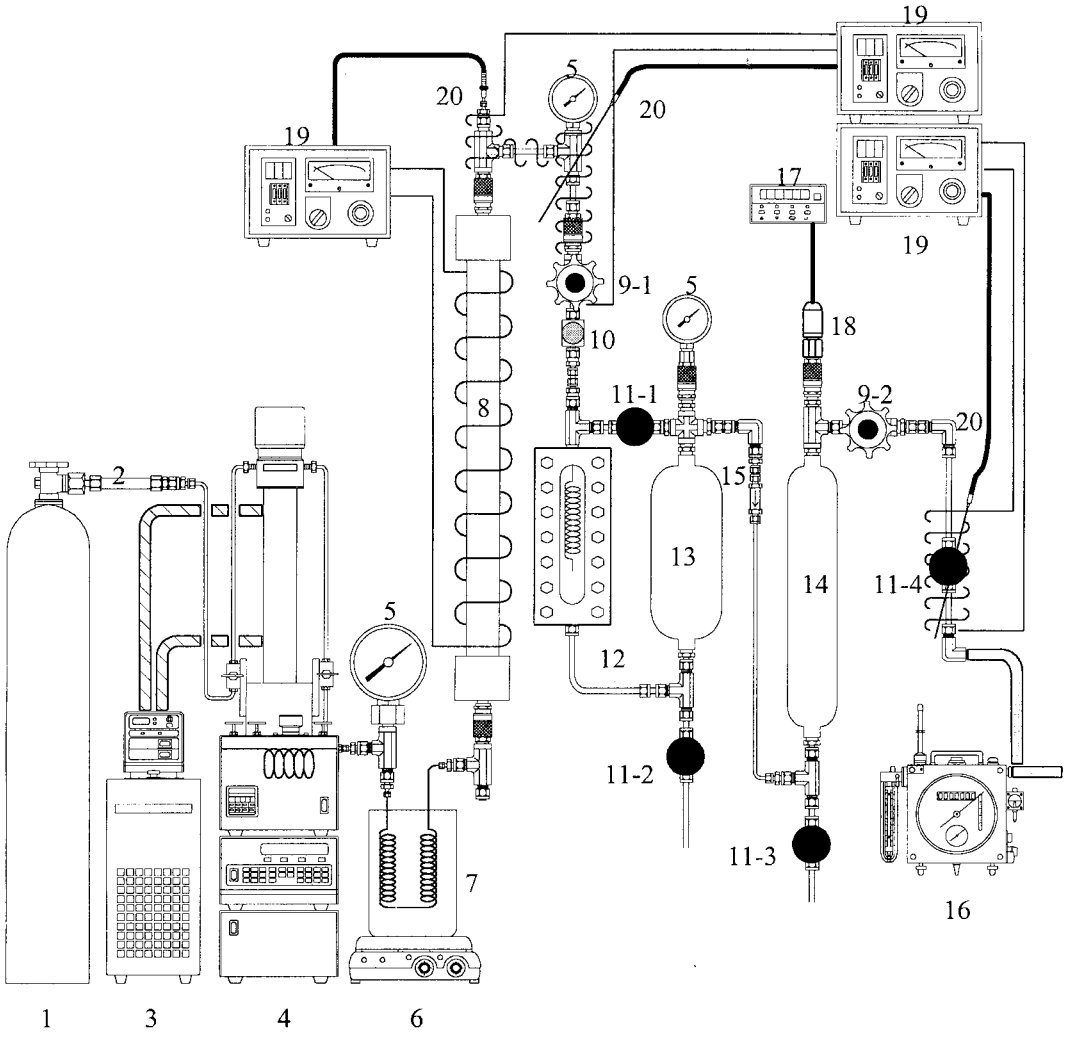
CO_2 at a constant rate ranging from 2.5, 5, and 10 ml/min at 31 MPa and 277 K. Liquid CO_2 was preheated by a coil preheater immersed in a water bath so that CO_2 could attain the desired temperature prior to entering the extraction vessel. A CO_2 preheater was used to maintain temperature above 333 K. The electrically heated extraction vessel was packed with 90 g of tea powders and 90 ml co-solvent. The temperature inside the heated extraction vessel was uniform. The weight fraction of CO_2 (x_{CO_2}) used for each co-solvent run is listed in Table 2. The mixture fluid (90% CO_2 + 10% $EtOH_{aq}$) is still near the supercritical point, as critical temperature is around 333 K. The extractive temperature was measured by one K-type thermocouple and controlled by a proportional-integrator controller. The absorbing system (one precipitator and two absorbent vessels) was filled with 1.4 l of 50% ethanol–water absorbent and maintained at 5.0 MPa and 297 K. The precipitator provides visual assurance that the saturated tea oils-laden supercritical CO_2 comes from the extraction vessel through a 1/16 inch I.D. nozzle, sprayed into and well mixed with the absorbent. In this situation, the absorption pressure was maintained at 5.0 MPa and measured by a digital pressure transducer (Druck, PDCR910). Extraction and absorption pressure were controlled via two back-pressure regulators. 10 ml samples were taken at every 100 l CO_2 volume, as measured by using a wet gas meter.

Stock solutions of four catechins, gallic acid, and caffeine were prepared by dissolving weighed quantities of commercial standards into water. Less concentrated solutions were prepared by diluting with deionized water. The soluble tea extracts from SFE-CO_2 were centrifuged at 5000 rpm for 10 min. The supernatant was taken into a 10 ml syringe and filtered through a 0.45 μm two-phase nylon membrane. A 10-μl volume of the filtered was injected into the HPLC system. Calibration curves were constructed by linear regression of the peak-area ratio versus concentration. The measurement accuracy was within ±5 μg/ml.

The HPLC system consists of a Waters 600E multisolvent delivery pump (Millipore, USA), a Waters 486 tunable absorbance detector (Millipore, USA), and a Waters U6 K 100μl sample injector (Millipore, USA).

Table 1
Gradient elution program for analysis of four catechins, caffeine, and gallic acid

Time (min)	A (%)	B (%)
Initial	95	5
3	90	10
7	90	10
12	70	30
17	60	40
20	60	40



1. CO_2 Cylinder
2. CO_2 Cleanup column
3. Constant temperature circulator
4. High pressure pump
5. Pressure gauge
6. Stirrer/hot plates
7. Preheater
8. Extraction vessel
9-1~9-2. Back pressure regulator
10. Needle valve
11-1~11-4. Metering valve
12. Separator
13. 1st Absorption vessel
14. 2nd Absorption vessel
15. Check valve
16. Wet gas meter
17. Pressure indicator
18. Digital pressure transmitter
19. Temperature controller
20. Thermocouple

Fig. 2. Equipment diagram of CO_2 extraction for polyphenols from green tea.

Table 2
Concentrations of caffeine, four major catechins, and gallic acid in green tea extracts at 31 MPa and 333 K extraction, 5 MPa absorption

Run no.	Solvent	x_{CO_2} (w/w)	Caffeine (ppm)	EGC (ppm)	EGCg (ppm)	ECg (ppm)	EC (ppm)	GA (ppm)	TC/(Caf+GA)	TPP/Caf
1	Standard mixture		61	90	94	54	86	61		
2	CO_2	1.000	218	93	18	ND[a]	ND[a]	ND[a]	0.51	0.73
3	CO_2+H_2O	0.911	223	122	83	8	8	10	0.95	1.17
4	CO_2+18% EtOH(aq)	0.892	394	96	34	3	2	3	0.33	
5	CO_2+70% EtOH(aq)	0.902	1369	96	30	11	16	1	0.11	
6	CO_2+95% EtOH(aq)	0.906	755	290	510	105	90	7	1.32	1.50
7	CO_2+99.8% EtOH(aq)	0.907	880	290	492	89	80	7	1.07	1.28
8	Soxhlet, H_2O		1056	610	1336	202	183	380	1.62	
9	Soxhlet, 95% EtOH		951	1312	2992	459	273	120	4.70	

[a] not detectable.

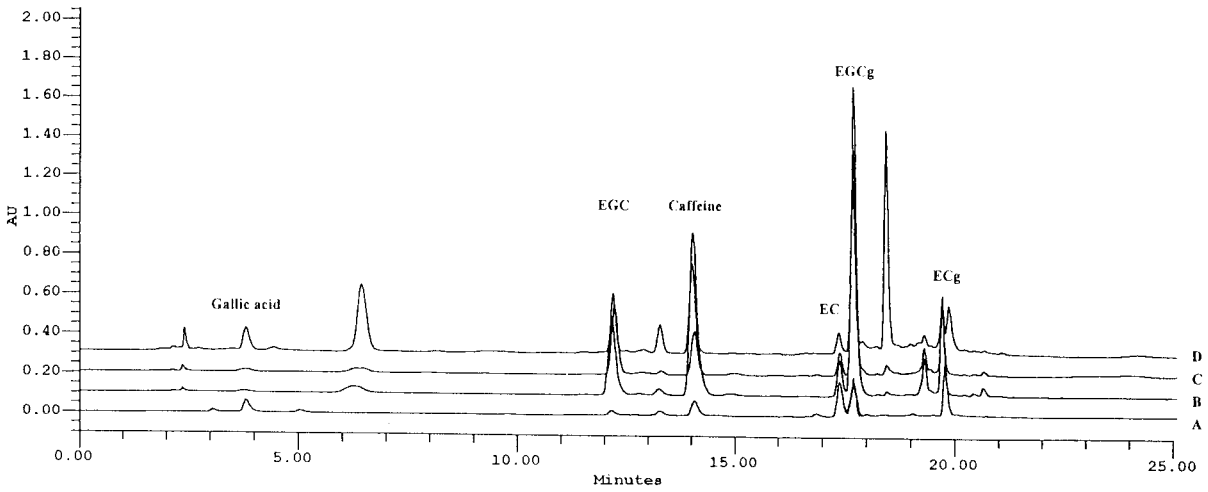


Fig. 3. HPLC chromatograms of green tea extracts. A: standard mixtures; B: extract with CO_2+95% EtOH; C: 95% EtOH extract (Soxhlet); D: H_2O extract (Soxhlet).

The analytical column was a reverse phase Develosil ODS-HG column (150×4.5 mm, Nomura, Japan) equipped with a guard column (10×4 mm, Nomura). The column was thermostatted at 40°C and the flow-rate of the mobile phase was 1 ml/min. UV detection was achieved at a wavelength of 231 nm. A classic Millennium 2010 software was used for manipulation of the pump and data processing. The mobile phase used for analysis was solvent A: 0.05% phosphoric acid solution; solvent B: acetonitrile. Table 1 lists the solvent gradient conditions. A spectrophotometer was used to measure total polyphenols and caffeine, with details outlined in Lin et al. (1996). All extracted samples were dried inside a vacuum oven, operated at 160 mm Hg, 333 K. Finally, a gravimetric measurement was used to obtain the amount of the extract. Standard deviation was within ±10%.

3. Results and discussion

Green tea extracts were analyzed to understand the contents of four catechins, gallic acid, and caffeine by HPLC. Fig. 3 displays the chromatograms of these standard samples. The samples of Soxhlet extracts were diluted four times before analysis. Table 2 summarizes the concentrations of four major catechins (TC), gallic acid (GA), and caffeine (Caf) in the green tea extract and in the standard mixture. Water and 95% ethanol extractions acted as the reference runs at 373 K for 12 h, and were performed in the Soxhlet extractor, with details outlined in Price and Spitzer (1993). Fig. 4 summarizes the effect of ethanol content on the amount of essential oil. The mean content of total essential oils was 4.4-fold higher when adding 95% ethanol than when adding water. Adding 95% ethanol as co-solvent allowed us to extract more essential oils. Also, Soxhlet ethanol extraction produced the largest amount of total polyphenols (TPP) as shown in Table 2. The spectrophotometer was used to measure TPP, which contains at least 10 kinds of catechins. HPLC measured only four important catechins. Therefore, TPP/Caf is larger than TC/(Caf+GA). Numerous investigations have confirmed the feasibility of extracting tea catechins with conventional solvents such as acetonitrile–water (Goto et al., 1996), or hot water (Dalluge et al. 1998; Lin et al., 1996; Price & Spitzer, 1993). Roedig-Penman and Gordon (1997) showed that methanolic tea extract

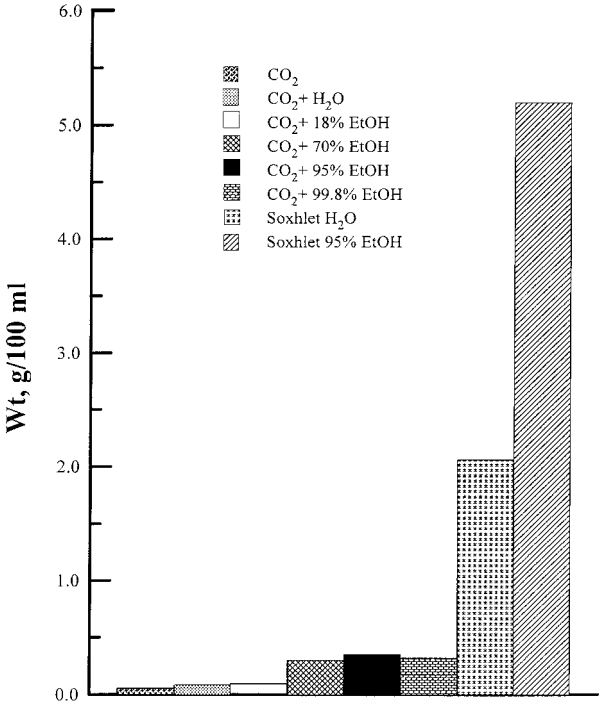


Fig. 4. Effect of ethanol content on the amount of the extract at 31 MPa extraction, 333 K, 5 MPa absorption.

contained higher levels of EGCg and ECg. These studies indicated that the ratio of total catechins to caffeine ranges from 2.5–5.4 (Goto et al.), 1.3 to 5.4 (Lin et al.), 3.3 to 3.7 (Price & Spitzer). Our study indicated that the ratio of TC/(Caf + GA) was 1.3, when 95% ethanol was used as a co-solvent.

4. Conclusion

This study has extracted the essential oil from green tea by using high-pressure carbon dioxide with co-solvent addition. Results show that both the extract and the ratio of TC/(Caf + GA) increase with the ethanol content. Our results further demonstrate that Soxhlet ethanol extraction can produce the largest amount of polyphenols.

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