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Designed Polar Cosolvent-Modified Supercritical CO_2 Removing Caffeine from and Retaining Catechins in Green Tea Powder Using Response Surface Methodology

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This study examines cosolvent-modified supercritical carbon dioxide (SC-CO_2) to remove caffeine from and to retain catechins in green tea powder. The response surface method was adopted to determine the optimal operation conditions in terms of the extraction efficiencies and concentration factors of caffeine and catechins during the extractions. When SC-CO_2 was used at 333 K and 300 bar, 91.5% of the caffeine was removed and 80.8% of catechins were retained in the tea: 3600 g of carbon dioxide was used in the extraction of 4 g of tea soaked with 1 g of water. Under the same extraction conditions, 10 g of water was added to <800 g of carbon dioxide in an extraction that completely removed caffeine (that is, the caffeine extraction efficiency was 100%). The optimal result as predicted by three-factor response surface methodology and supported by experimental data was that in 1.5 h of extraction, 640 g of carbon dioxide at 323 K and 275 bar with the addition of 6 g of water extracted 71.9% of the caffeine while leaving 67.8% of the catechins in 8 g of tea. Experimental data indicated that supercritical carbon dioxide decaffeination increased the concentrations of caffeine in the SC-CO_2 extracts at 353 K.

KEYWORDS: Supercritical carbon dioxide decaffeination; cosolvent addition; caffeine; catechins; extraction efficiency; concentration factor

INTRODUCTION

Tea belongs to one of the *Camellia* families. It is manufactured and sold worldwide, especially in Southeast Asia. On the basis of fermentation, characteristics, and the color of the tea leaves and buds, tea is divided into green tea, black tea, oolong tea, white tea, yellow tea, and black tea. Green tea, which contains the most catechins, is prepared by steaming or roasting without fermentation of the tea leaves. Semifermenting or fully fermenting the leaves reduces the amount of catechins in the tea, which is then referred to as oolong tea or black tea. Nonfermented green tea has various catechins, caffeine, chlorophyll, amino acids, vitamins, theasaponins, and heterochain polysaccharides (*1*).

The primary bioactive components of tea are catechins, which include (+)-gallocatechin and (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (+)-catechin and (−)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin-3-gallate (EGCg), (−)-gallocatechin-3-gallate (GCg), and (−)-epicatechin-3-gallate (ECg) (*2*). These catechins are generally recognized as having desirable biological and physiological effects, including antioxidation (*3*), anti-inflammation (*4*, *5*), antimutagen (*6*), anticancer (*7*–*9*), and endocrine effects (*10*). In particular, EGCg, which is generally the most abundant catechin in green tea, has the greatest antioxidative activity of any catechin (*11*). Catechins EGCg, ECg, EGC, EC, and C have relatively strong antioxidative effects in this order of descending strength (*3*). Mixing catechins may have a synergistic effect (*5*). Unfortunately, EGCg, ECg, EGC, and EC are easily oxidized to GCg, Cg, GC, and C during the heating processing of tea; the health effect of these oxidized products is not well understood yet (*1*).

Another major bioactive component of green tea is caffeine, which is known to have dose-dependent detrimental effects in humans, including causing sleep deprivation, abortions, in-

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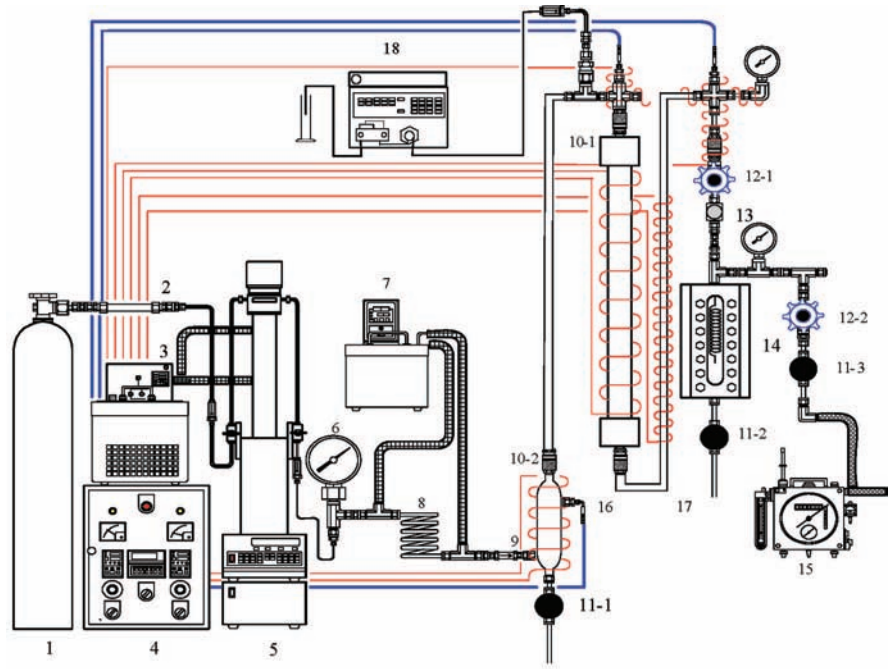


1: CO_2 cylinder **2: CO_2 cleanup column** **3: Cold liquid circulator**
4: Temperature controller **5: High pressure pump** **6: Pressure gauge**
7: Hot liquid circulator **8: Preheater** **9: Check valve**
10-1: Extraction vessel **10-2: Reboiler** **11-1~3: Metering valve,**
12-1~2: Pressure regulator **13: Needle valve** **14: Separator**
15: Wet gas meter **16: Thermocouple** **17: Line connector**
18: Co-solvent pump

Figure 1. Schematic flow diagram of SC-CO_2 decaffeination of tea.

creased heart rate, osteoporosis, and breast cancer (*12*). Giving these adverse effects much research efforts has been performed globally with a view to removing caffeine from caffeine-containing food (*13*). Recently, highly efficient extraction of bioactive compounds from natural plants has become a popular research topic. This extraction can be performed by organic solvent extraction (*14*), adsorptive column separation (*15*, *16*), hot pressurized fluid extraction (*17*, *18*), and supercritical carbon dioxide extraction (*19–21*) among other methods. In particular, carbon dioxide beyond its critical point (73.8 bar and 304 K) has been identified as an ideal green extraction solvent, with high dissolution power, high diffusivity, low viscosity, and low toxicity such that it is not harmful to the environment (*22*). Following supercritical extraction, carbon dioxide can be readily recovered and reused. Among supercritical fluid decaffeination, the ethanol-modified supercritical carbon dioxide extraction of caffeine and catechins from green tea was investigated (*11*); the decaffeination of 8 kg of black tea using 20 kg of supercritical carbon dioxide to which had been added 15% water has been patented (*23*); the caffeine level of instant tea extracted from decaffeinated leaf tea using active carbon has been studied (*24*). Research on the retention of catechins in green tea powder following supercritical carbon dioxide decaffeination is still scarce in the literature. Hence, to determine the proportion of catechins retained in tea after a polar cosolvent-modified supercritical carbon dioxide decaffeination of green tea powder was examined in this study.

MATERIALS AND METHODS

Reagents and Materials. Powdered dry and ground green tea, *Camellia sinensis*, was obtained from the research center at a tea station (Yang-mei branch, Taiwan) and was stored in a cool place before use. The water content of this green tea powder was estimated to be under 5 wt %, as determined using a moisture meter (A&D, AD-4714A). Deionized water was obtained using a Milli-Q reverse osmosis purification system. The 99.95% carbon dioxide (Toyo gas), 99.9% methanol (Mallinckrodt), 99.9% acetonitrile (Merck), 98% TFA (Merck), and 95% ethanol (Tobacco and Liquor Corp.) were purchased from a local supplier and used without further treatment. Authentic standards including 98% caffeine (Sigma), 98% EC (Sigma), 98% EGC (Sigma), 98% ECG (Sigma), 98% EGCG (Sigma), 98% GCG (Sigma), 98% GC (Sigma), 98% C (Sigma), and 98% gallic acid (Sigma) were used herein only for quantification purposes.

Classical Solvent Extraction. In the Soxhlet solvent extraction, 20 g of green tea powder (400, 177, or 74 μm) was loaded into a 250 mL reflux type Soxhlet system and extracted using 250 mL of H_2O and $CHCl_3$ for 2–48 h, respectively. In the stirred solvent extraction, 5 g of green tea powder was extracted using 300 mL of H_2O and EtOH for 1.5 and 3 h, respectively. All of the extracts were collected, filtered using a polytetrafluoroethylene (PTFE) membrane, and weighed. The total amount extracted, the extraction efficiency, and the purity of the catechins and the caffeine in the extracts were then calculated.

Extraction Using Supercritical Carbon Dioxide (SC-CO_2). Figure 1 presents a schematic flow diagram of the SC-CO_2 decaffeination of green tea powder. In the experiments, 8 g of 74 μm (or 177 μm) green tea powder was wetted for 30 min with 2 g of water and then mixed with 50 g of 4 mm o.d. glass beads, before being packed inside a stainless steel tubular extractor (54 cm L $\times$ 2 cm i.d.). A certain quantity

Table 1. Experimental Data on Classical Solvent Extractions of Green Tea Powder[a]

run	solvent	size (μm)	T (K)	t (h)	W_{ext} (g)	TY (%)	W_{CAT} (mg/g)	R_{CAT} (%)	β_{CAT}	W_{CAF} (mg/g)	R_{CAF} (%)	β_{CAF}
Soxhlet Extraction (Soxhlet) of 20 g of Green Tea Powder												
1	H_2O	177	373	2	1.52	7.6	24.71	16.0	2.11	0.99	9.98	1.31
2	H_2O	177	373	4	2.99	15.0	46.24	30.0	2.01	1.96	19.8	1.32
3	H_2O	177	373	8	4.14	20.7	61.41	39.8	1.92	3.17	31.9	1.54
4	H_2O	177	373	12	6.17	30.9	90.16	58.5	1.89	4.94	49.8	1.61
5	H_2O	177	373	24	7.66	38.3	105.25	68.2	1.78	6.06	61.1	1.60
6	H_2O	177	373	48	9.36	46.8	107.73	69.9	1.49	6.10	61.5	1.31
7	$CHCl_3$	74	334	8	1.57	7.9	0.89	0.6	0.07	4.63	46.7	5.95
8	$CHCl_3$	74	334	12	1.67	8.4	0.81	0.5	0.06	7.38	74.4	8.91
9	**$CHCl_3$**	**74**	**334**	**24**	**1.76**	**8.8**	**0.70**	**0.5**	**0.05**	**9.92**	**100.0**	**11.36**
10	$CHCl_3$	74	334	48	1.92	9.6	0.55	0.4	0.04	9.92	100.0	10.4
Stirred Extraction (STW) of 5 g of Green Tea Powder												
1	H_2O	400	373	1.5	1.47	29.4	95.89	62.2	2.12	4.72	47.6	1.62
2	H_2O	177	373	1.5	1.78	35.6	100.29	65.0	1.83	5.01	50.5	1.42
3	H_2O	177	373	3	2.15	43.0	111.48	72.3	1.68	5.68	57.2	1.33
4	EtOH	177	351	1.5	1.63	32.6	56.22	36.5	1.12	0.78	7.9	0.24
5	**H_2O**	**74**	**373**	**1.5**	**1.92**	**38.4**	**154.23**	**100.0**	**2.60**	**7.32**	**73.8**	**1.92**

[a] W_{ext}, weight of the extract (g); W_{CAT}, yield of catechins; W_{CAF}, yield of caffeine; TY, total yield = (W_{ext}/W_{tea}) × 100%; R_{CAT}, catechins extraction efficiency = ($W_{CAT}/W_{CAT,STW}$) × 100%; R_{CAF}, caffeine extraction efficiency = ($W_{CAF}/W_{CAF,Soxhlet}$) × 100%; β_{CAT}, concentration factor of catechins = R_{CAT}/TY; β_{CAF}, concentration factor of caffeine = R_{CAF}/TY; $W_{CAT,STW}$ = 154.23 (mg/g); $W_{CAF,Soxhlet}$ = 9.92 (mg/g).

of glass wool was packed into the two ends of the extractor to prevent the escape of the tea powder from the extractor. Liquid CO_2 from a siphon-tube (1) passed through a cooling bath (3) at 277 K and was then compressed to the desired working pressure using a syringe pump (Isco, 100DX) (5). It was heated to supercritical conditions using a double-pipe heat exchanger (8) and a reboiler (10-2). The cosolvent (EtOH or H_2O) was added to the supercritical CO_2 using a high-pressure pump (18). Gas flowed into an extractor (10-1), came into contact with the green tea powder, and extracted caffeine into the CO_2 phase. One temperature controller (4) and two pressure regulators (12-1, 12-2) were used to control the temperature and pressure. After the extraction, the caffeine- and catechin-laden CO_2 gas was driven into a 130 mL separator (14) by a drop in pressure and then expanded through a spiral-type nozzle. This solute-rich SC-CO_2 solution passed through a heated line connector (17) and relaxed in the separator that was filled with 100 mL of ethanol and maintained at 50 bar and 303 K. The expanded low-pressure CO_2 gas finally passed through a wet gas flow meter (15) and thus returned to ambient conditions. A few precipitates that had washed out from the back-pressure regulators and tube lines were then mixed with the collected extract to prepare them for analysis. At the end of each experiment, the extracted solution was collected and the sample with a volume of 50 mL was weighed, sealed in an opaque bottle, and stored at 277 K before use. The total yield, the extraction efficiency, and the concentration factor of catechins and caffeine in the extracts were then calculated accordingly.

Quantification of Seven Catechins and Caffeine. A HPLC method was adopted to determine the amounts of catechins (GC, EGCg, ECg, EGC, EC, GCG, C), gallic acid, and caffeine in the extracts. This analytical HPLC system consisted of one YMC-C18 (4.6 mm × 250 mm) column, which was equipped with an L-2400 UV detector (Hitachi) and an L-2130 intelligent pump, and was connected to data control interface software (EZ-Chrom Elite version 3.1.3). The column temperature was maintained at 313 K. The UV absorption of the sample was detected at a wavelength of 231 nm, and the injection volume was 20 μL. A two-solvent gradient mobile phase similar to that used by Goto et al. (*25*) was used for the HPLC analysis.

Response Surface Methodology (RSM) Experimental Design. In this study, a three-level, three-factor RSM experimental design with a central composite scheme was employed to find the three-dimensional response of dependent variables on the basis of the change of independent variables. Extraction efficiency and concentration factor of components in the extract were considered as responses. A quadratic polynomial equation including an individual term of mean, linear, and cross-product coefficients was used as the regression model for each response. This model was determined from the F testing and analysis of variance.

RESULTS AND DISCUSSION

Classical Solvent Extraction. Table 1 indicates that the total yield (TY) of the extract, the extracted amount (W_{CAT}, W_{CAF}), the extraction efficiencies (R_{CAT}, R_{CAF}), and the concentration factors (β_{CAT}, β_{CAF}) of catechins and caffeine obtained by several Soxhlet solvent extractions varied with the solvent, the powder particle sizes, and the extraction time. The maximal amount of caffeine in the extract was 9.92 mg/g (mg of caffeine/g of tea), reached in 24 h of the 250 mL Soxhlet $CHCl_3$ extraction of 20 g of green tea powder. This result represents 100% recovery of caffeine from tea.

Table 1 also presents the experimental and calculated data on the stirred solvent extractions with various solvents, powder particle sizes, and extraction times. The maximal amount of catechins in the extract was 154 mg/g (g of catechins/g of tea), obtained by 1.5 h of the 300 mL stirred H_2O extraction of 5 g of green tea powder. This result was regarded as 100% recovery of catechins from tea. The duplicate data obtained in repeated experiments were within 5%.

Initial Caffeine Extraction Rate. Over a short retention period of 6–10 min, SC-CO_2 decaffeination efficiencies were evaluated from the initial rate of the decaffeination, which is an important index of the suitability of the SC-CO_2 conditions. **Table 2** presents the amounts of caffeine that were extracted with SC-CO_2 by varying the extraction temperature and extraction pressure. The data indicated that the pressure dominates the initial caffeine extraction rate, when the temperature is sufficiently high. The pressure ranged from 200 to 300 bar, and the temperature ranged from 313 to 333 K.

Extraction Using Cosolvent-Modified SC-CO_2. Parameters of SC-CO_2 extraction that potentially affect the efficiency include the size of the powder particles, the CO_2 flow rate, the extraction time, the temperature, the pressure, the type of cosolvent, the amount of cosolvent added, and the ratio of CO_2 to tea (SSR). A set of preliminary sensitivity tests was performed as follows: particle sizes ranged from 74 to 177 μm; amounts of CO_2 consumed ranged from 555 to 3600 g; temperatures ranged from 313 to 353 K; pressures ranged from 200 to 300 bar; amounts of cosolvent added ranged from 1 to 32.2 g; SSR values ranged from 69 to 900; and the cosolvent was ethyl alcohol or water. Experimental data obtained in these tests indicated that temperature, pressure, and SSR value are three

Table 2. Initial Caffeine Extraction Rate of SC-CO_2 Extraction of 4 g of Green Tea Powder[a]

run	P (bar)	T (K)	ρ (kg/m^3)	W_{CO2} (kg/L)	W_{CAF} (mg/g)	t (min)	R_{CAF} (%)	initial rate (mg/min)
1	200	313	840	0.16 (89)	0.051	6	0.51	0.034
2	200	323	785	0.16 (86)	0.074	6	0.75	0.049
3	200	333	724	0.16 (90)	0.082	6	0.83	0.055
4	250	313	880	0.16 (91)	0.214	8	2.16	0.107
5	250	323	834	0.17 (94)	0.219	8	2.21	0.110
6	250	333	787	0.17 (96)	0.250	8	2.52	0.125
7	300	313	910	0.18 (102)	0.384	9.5	3.87	0.162
8	300	323	871	0.19 (105)	0.398	9.5	4.01	0.168
9	300	333	830	0.19 (105)	0.420	9.5	4.23	0.177

[a] P,pressure (bar); T,temperature (K); t,extraction time (min); ρ, density of CO_2 (kg/m^3); W_{CO2},weight of CO_2 (g); W_{CAF},yield of caffeine; R_{CAF}, caffeine extraction efficiency $= (W_{CAF}/W_{CAF,Soxhlet}) \times 100\%$; $W_{CAF,Soxhlet}$, amount of caffeine extracted by 24 h of Soxhlet $CHCl_3 = 9.92$ (mg/g); W_{Tea}, weight of tea; initial rate, $(W_{CAF})(W_{Tea})/t$.

Table 3. Experimental Data on Cosolvent-Modified SC-CO_2 Extractions of Green Tea Powder[a]

run	size (μm)	P (bar)	T (K)	P_A/C (g/g)	W_{H2O}/W_{CO2}(g/g)	W_{CAT} (mg/g)	(100 – R_{CAT}) (%)	W_{CAF} (mg/g)	R_{CAF} (%)
					Preaddition of H_2O (4 g of Tea)				
1	177	300	333	1/0	1/759	12.49	91.9	3.98	40.1
2	177	300	333	1/0	1/1476	19.90	87.1	5.25	52.9
3	177	300	333	1/0	1/2220	20.20	86.9	6.39	64.4
4	177	300	333	1/0	1/2928	24.83	83.9	7.51	75.7
5	177	300	333	1/0	1/3636	26.84	82.6	8.50	85.7
6	74	300	333	1/0	1/759	10.95	92.9	4.28	43.1
7	74	300	333	1/0	1/2168	22.83	85.2	6.80	68.5
9	74	300	333	1/0	1/2892	28.69	81.4	7.66	77.2
10	74	300	333	1/0	1/3600	29.61	80.8	9.08	91.5
11	74	300	353	1/0	1/3620	30.54	80.2	9.49	95.6
					Continuous Addition of Cosolvent (8 g of Tea)				
12	74	300	333	1/31.2	32.2/724	75.11	26.7	9.81	98.9
13	74	300	333	1/15.6	16.6/744	113.05	51.3	8.35	84.1
14	74	300	333	1/10	11/735	130.48	15.4	9.92	100
15	74	300	333	1/5	6/766	113.51	26.4	9.66	97.3
16	74	200	313	1/3	4/586	11.88	92.3	4.21	42.4
17	74	200	323	1/3	4/555	23.46	84.8	4.82	48.6
18	74	200	333	1/3	4/562	37.65	75.6	5.94	59.9
19	74	250	313	1/3	4/586	17.13	88.9	5.49	55.3
20	74	250	323	1/3	4/555	39.81	74.2	6.18	62.3
21	74	250	333	1/3	4/562	48.92	68.3	7.14	71.9
22	74	300	313	1/3	4/593	23.46	84.8	6.33	63.8
23	74	300	333	1/3	4/591	70.83	54.1	8.20	82.6
24	74	300	343	1/3	4/573	93.36	39.5	8.68	87.5
25	74	300	353	1/3	4/589	126.23	18.2	9.79	98.6

[a] Run 12–13, EtOH addition; others, H_2O addition; P_A, preaddition of cosolvent; C, continuous addition of cosolvent; W_{H2O}/W_{CO2}, weight fraction of the water to CO_2; W_{CAT}, yield of catechins; W_{CAF}: yield of caffeine; R_{CAF}, caffeine extraction efficiency, i.e., caffeine removing $= (W_{CAF}/W_{CAF,Soxhlet}) \times 100\%$; R_{CAT}, catechins extraction efficiency $= (W_{CAT}/W_{CAT,STW}) \times 100\%$; (100 – R_{CAT}), catechins remaining efficiency (%).

factors that substantially influence the extraction rate of caffeine. **Table 3** presents experimental data on the cosolvent-modified SC-CO_2 decaffeination of green tea powder.

Experimental Water-Modified SC-CO_2 Extraction. Response surface methodology (RSM) with central composite design for three design variables with eight factorial points (duplicate data), six axial points (duplicate data), and one central point (triplicate data) was designed for the SC-CO_2 decaffeination of 8 g of 74 μm green tea powder wetted for 30 min with 2 g of water. The rate of injection of water into carbon dioxide was maintained at 0.56 wt % (1 g of H_2O/180 g of CO_2) at a 12 mL/min flow rate of the liquid CO_2 at 277 K and 70 bar. The total amount of water added was <1.5% of the amount of carbon dioxide. The pressure, temperature, and solvent to solid ratio were three major factors. The caffeine extraction efficiency (R_{CAF}), the catechins remaining efficiency (100 – R_{CAT}), and the concentration factors of caffeine (β_{CAF}) were three main responses. A quadratic polynomial equation including individual terms for mean, linear, and cross-product coefficients was used as the regression model for each response. For these SC-CO_2 extractions, the quadratic regression model was determined from the F testing and analysis of variance (i.e., for R_{CAF}, $R^2 = 0.9542$, SD = 5.52; for 100 – R_{CAT}, $R^2 = 0.945$, SD = 6.43; for β_{CAF}, $R^2 = 0.711$, SD = 2.23). Three-dimensional RSM plots indicated that pressure, temperature, and the SSR value determined the caffeine extraction efficiency (R_{CAF}), the catechins remaining efficiency (100 – R_{CAT}), and the concentration factors of caffeine (β_{CAF}) and catechins (β_{CAT}). **Table 4** presents experimental data on the RSM SC-CO_2 decaffeination at temperatures from 313 to 333 K, at pressures from 200 to 300 bar, and at SSR values from 60 to 100. The effects of three RSM factors on the extractions of caffeine and catechins seem to be of the same order of magnitude. **Figure 2** reveals that the caffeine extraction efficiency reached 97.5% under SC-CO_2 conditions of 333 K, 300 bar, and an SSR value of 100 (run 15 in **Table 4**). **Figure 3** reveals that 97.1% of the catechins remained in the tea at an SC-CO_2 extraction of 313 K, 200 bar, and an SSR value of 60 (run 1 in **Table 4**). **Figure 4** presents the three-dimensional responses of the caffeine concentration factor (β_{CAF}) reaching 13.84 at 326 K, 275 bar, and an SSR value of 80 as predicted using the RSM. In summary, the RSM design for the SC-CO_2 decaffeination at the condition of 326

Table 4. Response Surface Methodology Designed Continuous H_2O Modified SC-CO_2Extraction of 8 g of Green Tea Powder[a]

run	*P* (bar)	*T* (K)	W_{CO2}/W_{Tea} (g/g)	W_{H2O}/W_{CO2} (g/g)	W_{ext} (g)	TY (%)	W_{CAT} (mg/g)	R_{CAT} (%)	β_{CAT}	W_{CAF} (mg/g)	R_{CAF} (%)	β_{CAF}
1	200 (−1)	313 (−1)	480/8 (−1)	5/480	0.338	4.22	4.47	2.9	0.69	2.47	24.9	5.90
					0.335	4.19	4.47	2.9	0.69	2.71	27.3	6.52
2	300 (+1)	313 (−1)	480/8 (−1)	5/480	0.345	4.31	18.97	12.3	2.85	2.68	27.0	6.26
					0.340	4.25	20.36	13.2	3.11	2.82	28.4	6.68
3	250 (0)	323 (0)	480/8 (−1)	5/480	0.388	4.85	26.84	17.4	3.59	5.04	50.8	10.47
					0.381	4.76	28.84	18.7	3.93	5.12	51.6	10.84
4	200 (−1)	333 (+1)	480/8 (−1)	5/480	0.362	4.53	42.26	27.4	6.05	5.25	52.9	11.68
					0.358	4.48	55.21	35.8	7.99	5.17	52.1	11.63
5	300 (+1)	333 (+1)	480/8 (−1)	5/480	0.610	7.63	68.48	44.4	5.82	8.00	80.6	10.56
					0.606	7.58	70.33	45.6	6.02	8.09	81.5	10.75
6	250 (0)	313 (−1)	640/8 (0)	6/640	0.504	6.3	18.66	12.1	1.92	4.23	42.6	6.76
					0.499	6.24	19.90	12.9	2.07	4.35	43.8	7.02
7	200 (−1)	323 (0)	640/8 (0)	6/640	0.557	6.96	32.70	21.2	3.05	4.71	47.5	6.82
					0.554	6.92	28.38	18.4	2.66	4.93	49.7	7.18
8	250 (0)	323 (0)	640/8 (0)	6/640	0.408	5.10	37.32	24.2	4.75	6.40	64.5	12.65
					0.394	4.92	40.72	26.4	5.37	6.32	63.7	12.95
					0.418	5.23	38.87	25.2	4.82	6.39	64.4	12.31
9	300 (+1)	323 (0)	640/8 (0)	6/640	0.378	4.73	56.14	36.4	7.70	8.05	81.1	17.15
					0.370	4.62	57.84	37.5	8.12	8.21	82.7	17.90
10	250 (0)	333 (+1)	640/8 (0)	6/640	0.474	5.92	78.19	50.7	8.56	8.59	86.6	14.63
					0.470	5.88	74.49	48.3	8.21	8.64	87.1	14.81
11	200 (−1)	313 (−1)	800/8 (+1)	7/800	0.560	7.00	38.25	24.8	3.54	4.69	47.3	6.76
					0.551	6.89	39.64	25.7	3.73	5.24	52.8	7.66
12	300 (+1)	313 (−1)	800/8 (+1)	7/800	0.607	7.59	50.59	32.8	4.32	6.78	68.3	9.00
					0.594	7.42	42.72	27.7	3.73	6.73	67.8	9.14
13	250 (0)	323 (0)	800/8 (+1)	7/800	0.562	7.03	104.57	67.8	9.64	8.85	89.2	12.69
					0.559	6.99	114.44	74.2	10.62	8.79	88.6	12.68
14	200 (−1)	333 (+1)	800/8 (+1)	7/800	0.914	11.43	107.19	69.5	6.08	7.80	78.6	6.88
					0.915	11.44	89.76	58.2	5.09	7.63	76.9	6.72
15	300 (+1)	333 (+1)	800/8 (+1)	7/800	0.945	11.81	124.31	80.6	6.82	9.65	97.2	8.23
					0.944	11.8	139.12	90.2	7.64	9.68	97.5	8.26
16	**275**	**323**	**640/8**	**6/640**	**0.391**	**4.89**	**51.21**	**33.2**	**6.79**	**7.33**	**73.9**	**15.11**

[a] W_{CO2}/W_{Tea}, ratio of the CO_2 to tea; W_{H2O}/W_{CO2}, water addition; W_{ext}, weight of the extract; TY, total yield = $(W_{ext}/W_{Tea}) \times 100\%$; W_{CAT}, yield of catechins; W_{CAF}, yield of caffeine; $R_{CAT} = (W_{CAT}/W_{CAT,STW}) \times 100\%$, catechin extraction efficiency; $R_{CAF} = (W_{CAF}/W_{CAF,Soxhlet}) \times 100\%$, caffeine extraction efficiency; $\beta_{CAT} = R_{CAT}$/TY, concentration factor of catechins; $\beta_{CAF} = R_{CAF}$ /TY, concentration factor of caffeine; $W_{CAT,STW} = 154.23$ (mg/g); $W_{CAF,Soxhlet} = 9.92$ (mg/g); run 16, experimental data at the RSM prediction.

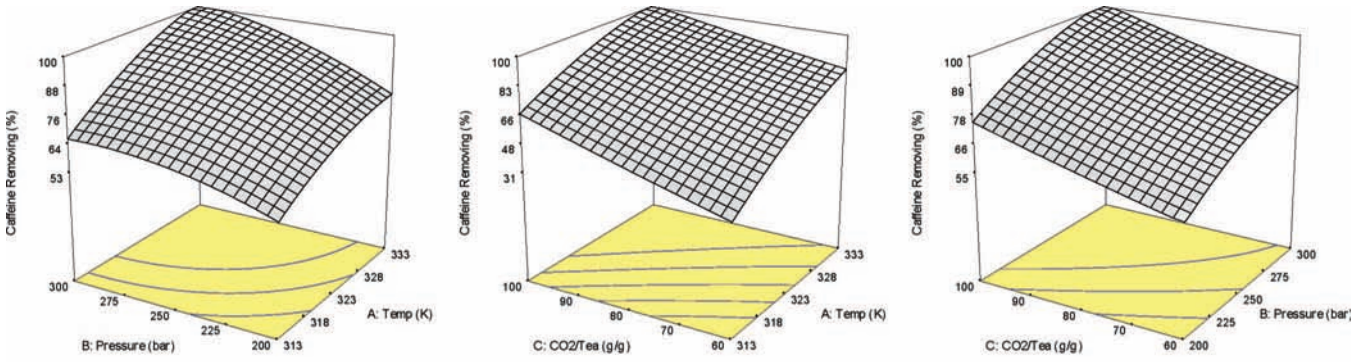


Figure 2. Three-dimensional responded experimental data on caffeine extraction efficiency of SC-CO_2 decaffeination; see run 15 in **Table 4**. (*F* testing: $R^2 = 0.9542$, $SD_{RCAF} = 5.52$.)

K, 275 bar, and an SSR value of 80 predicted favorable amounts of removed caffeine and retained catechins: 71.9% of the caffeine should be removed, and 67.3% catechins should be retained according to the model. Experimental data (run 16 in **Table 4**) on SC-CO_2 decaffeination at 323 K, 275 bar, and an SSR value of 80 removed 73.9% of the caffeine and left 66.8% of the catechins.

Comparison of Data Herein with Published Data. Figure 5 compares our experimental data with those of Klima et al. (*19*). Data herein indicated that 95.6% of the caffeine was removed from tea, whereas 80.2% of the catechins remained, when 3600 g of carbon dioxide was used at 333 K and 300 bar in extraction from 4 g of 74 μm tea powder that had been wetted with 1 g of water. This result is similar to that of Klima et al. (*19*), who used water as the cosolvent. Their addition of the 3.2 kg of water into 200 kg of carbon dioxide at 336 K and 260 bar successfully removed 92.8% of caffeine from 8 kg of black tea. The 300 bar SC-CO_2 decaffeination of 4 g of 74 μm tea powder to which had been added 4 g of water at 333, 343, and 353 K indicated that the proportion of catechins retained increased with the drop in temperature but at the expense of a small drop in the caffeine removal. The SC-CO_2 decaffeination using the response surface methodology design at 326 K and 275 bar was predicted to remove 71.9% of the caffeine and leave 67.3% of the catechins

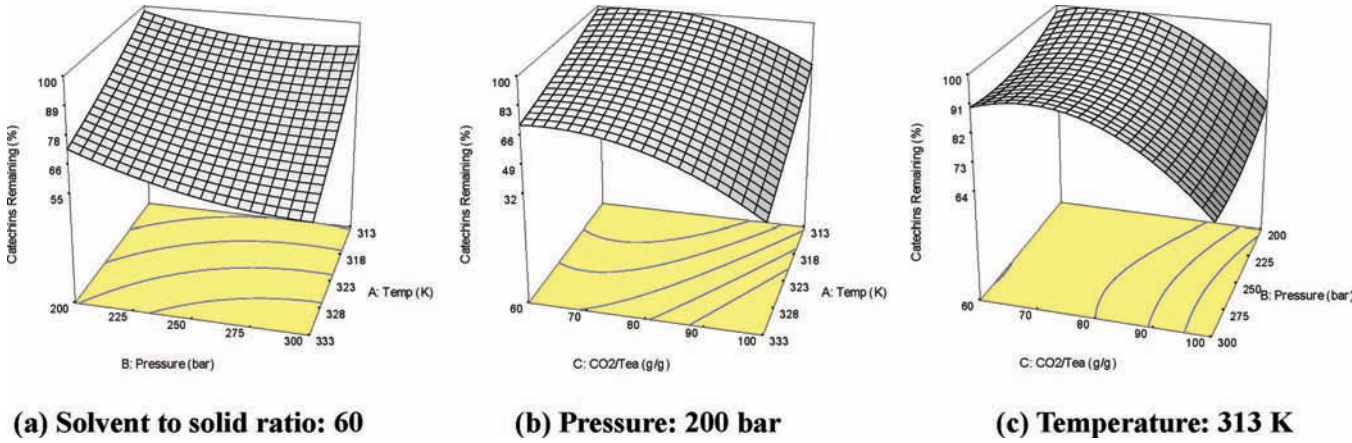


Figure 3. Three-dimensional responded experimental data on catechins remaining efficiency of SC-CO_2 decaffeination; see run 1 in **Table 4**. (*F* testing: $R^2 = 0.945$, $SD_{100\text{-}RCAT} = 6.43$.)

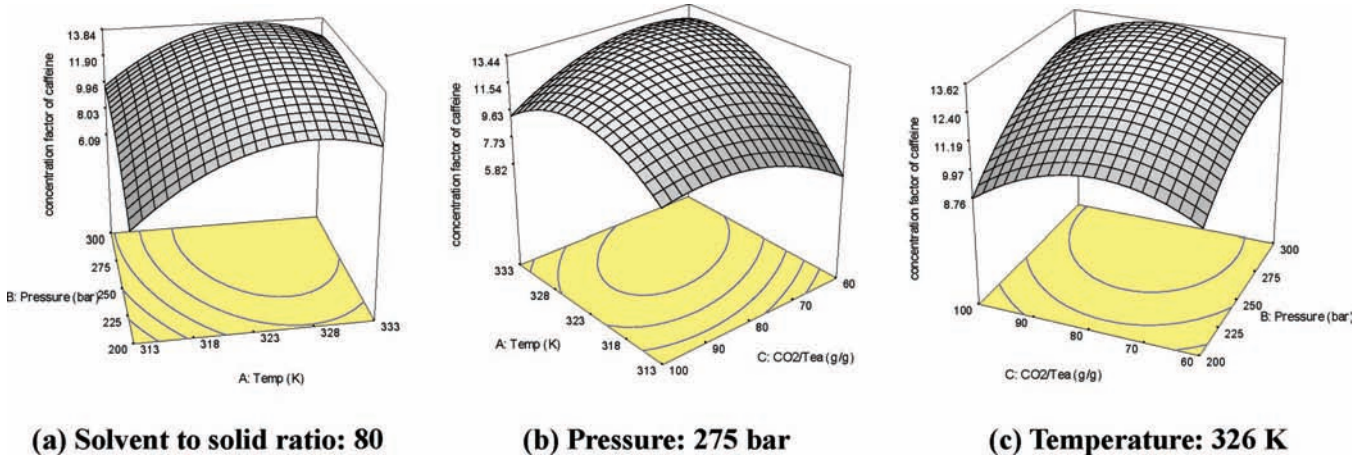
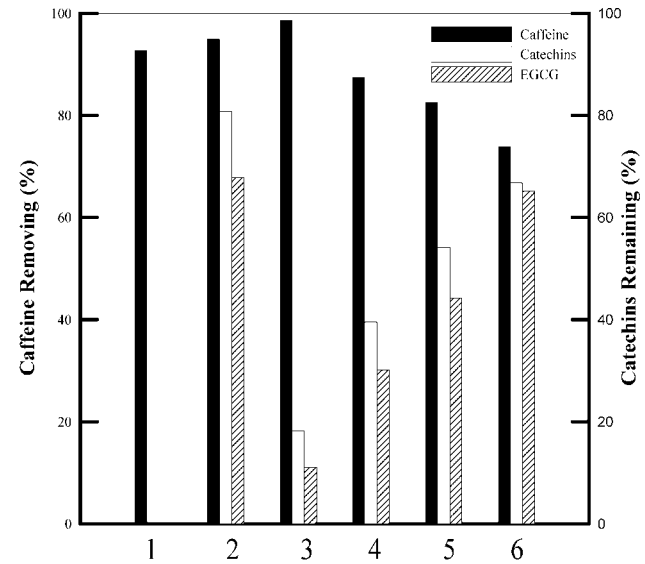


Figure 4. Response surface methodology prediction of concentration factor of caffeine in SC-CO_2 extract. (*F* testing: $R^2 = 0.711$, $SD_{\beta CAF} = 2.23$.)



1~2: pre-addition of water
3~6: continuous addition of water
1: Klima et al. (1990) at 336 K, 260 bar, $W_{CO2}/W_{H2O}/W_{Tea}$: 200,000/3,200/8,000
2: This study at 353 K, 300 bar, $W_{CO2}/W_{H2O}/W_{Tea}$: 3600/1/4
3: This study at 353 K, 300 bar, $W_{CO2}/W_{H2O}/W_{Tea}$: 589/4/4
4: This study at 343 K, 300 bar, $W_{CO2}/W_{H2O}/W_{Tea}$: 573/4/4
5: This study at 333 K, 300 bar, $W_{CO2}/W_{H2O}/W_{Tea}$: 591/4/4
6: This study at 323 K, 275 bar, $W_{CO2}/W_{H2O}/W_{Tea}$: 640/6/8

Figure 5. Removal of caffeine and retention of catechins and EGCg in SC-CO_2 extractions.

(or 64.7% EGCg) in 8 g of tea powder. This prediction was supported by experimental data obtained in 323 K and 275 bar SC-CO_2 decaffeination, which removed 73.9% of the caffeine and left 66.8% of the catechins, according to run 16 in **Table 4**.

This work studied the use of cosolvent-modified top-down flow supercritical carbon dioxide extraction to remove caffeine from food grade green tea powder. The extraction efficiencies of caffeine and catechins and the concentration factors of caffeine and catechins in the extracts were examined. Over 7.5 h of extraction, experimental data obtained using 3600 g of carbon dioxide at 333 K and 300 bar demonstrated that 95.6% of caffeine was removed and 80.2% of the catechins remained in 4 g of 74 μm tea powder that was wetted with 1 g of water. SC-CO_2 extractions by the three-factor response surface method indicated that the pressure, temperature, and ratio of carbon dioxide to tea were almost equally important in determining the removal of caffeine from tea. The RSM results indicated that SC-CO_2 decaffeination at 326 K and 275 bar removed 71.9% of the caffeine and left 67.3% of the catechins. Finally, experimental data revealed that continuously adding 6 g of water to the 640 g of carbon dioxide in extraction from 8 g of tea removed 73.9% of caffeine while leaving 66.8% of the catechins.

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