Full Length Research Paper

Decaffeination of green tea by supercritical carbon dioxide

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Effects of cosolvent, tea particle size, extraction temperature and time on the removal of caffeine and catechins from green tea using supercritical carbon dioxide method were investigated. It showed that ethanol was optimal cosolvent. Water and 50% aqueous ethanol were not suitable for cosolvents because a large percentage of catechins were removed. Based on the orthogonal array design tests, optimal protocol for green tea decaffeination was screened as 10 g of 0.2 - 0.6 mm particle size green tea was extracted using 15 ml ethanol cosolvent at 80°C for 2 h when flow rate and pressure of SC-CO_2 were at 1.5 L min^{-1} and 300 bars, during which 70.2% caffeine and 6.2% catechins were removed, respectively.

Key words: Decaffeinated tea, supercritical fluid extraction, cosolvent, particle size, temperature, caffeine, catechins.

INTRODUCTION

Green tea catechins have received great attention both from scientific community and general public recently due to their health benefits and functionality such as antioxidation, anticancer, and anti-inflammation (Gupta et al., 2002; Kuroda and Hara, 1999; Moyers and Kumar, 2004). Although caffeine can be used medicinally as a cardiac, cerebral and respiratory stimulant as well as a diuretic (Peeling and Dawson, 2007), it has some adverse effects on humans, including sleep deprivation (Hindmarch et al., 2004), abortions and miscarriages (Giannelli et al, 2003). The caffeine in green tea has been a limitation for those who want to avoid it. The limitation brought increase in need for decaffeinated products in the green tea market (Kim et al., 2007; Lee et al., 2009). Thus, it is important to selectively remove caffeine from the green tea while to remain catechins in the green tea for healthy tea production (Kim et al., 2008).

Researchers attempted to selectively remove caffeine from tea or tea extracts by many methods including solvent extraction, column chromatography, hot water treatment and supercritical CO_2 (SC-CO_2) extraction. Many organic solvents such as chloroform and methylene chloride are not accepted owing to their toxicities though they are effective to remove caffeine from tea leaf. Column chromatography can be used to decaffeinate tea extracts but is not fit for decaffeination of tea leaf (Sakanaka, 2003; Ye et al., 2009). Hot water treatment is an inexpensive and safe method to prepare decaffeinated green tea, but it can only be used to treat fresh tea leaf and it could not selectively remove caffeine from dry tea (Liang et al., 2007; Ye et al., 2007). Supercritical CO_2 extraction is environmental but its selectivity depended on many factors such as cosolvent, pressure, temperature, extraction time and moisture content of tea leaf (Park et al., 2007a, 2007b; Lee et al., 2009; Kim et al., 2008). The extraction yield of caffeine increased with an increase in temperature at a constant pressure, and also increased with increasing pressure at a fixed temperature, and that larger particle size yielded the slower extraction rate, when SC-CO_2 extraction was carried out by using 95% (v/v) ethanol as a cosolvent. After supercritical CO_2 extraction, a substantial loss of EGCG, predominant tea catechins, as much as 37.8% of original content was proved to be unavoidable (Park et al., 2007a). Conditions for selective removal of tea caffeine by

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SC-CO_2 need to be further optimized.

Orthogonal array design (OAD) is a fractional factorial design and a series of trials are assigned by orthogonal array. The advantages of OAD include that it may minimize test numbers and time to keep the experimental cost at a minimum level, and that the optimum parameters obtained from the laboratory can be utilized by larger scale of production (Yesilyurt, 2004). It has been adopted in different areas for the optimization of the analytical procedure, including extracting optimal procedure of SC-CO_2 extraction of some polar drugs from spiked matrices and tablets (Bahramifar et al., 2005). In the present paper, an orthogonal design test with four factor and three levels each factor was carried out to optimize the green tea decaffeination conditions of SC-CO_2 extraction.

MATERIALS AND METHODS

Materials

Green tea used was supplied by Qingcheng Tea Co. Ltd., (Qingcheng County, Sichuan, China). The tea was dried in a dryer at 103°C for 4 h to remove the moisture completely and then ground using a EUPA TSK-927S grinder (Cankun Co, Ltd, Shanghai, China) and sifted through sieves with different size meshes (Lantian Co. Ltd., Hangzhou, China) in order to obtain samples with three different size particles (0.2 - 0.6 mm, 0.6 - 1.2 mm and 1.2 - 2.0 mm in diameter). HPLC reference compounds including (-)epigallocatechin gallate (EGCg), (-)epigallocatechin (EGC), (-)epicatechin gallate (ECg), (+)epicatechin (EC), (+)gallocatechin gallate (GCg), (-)gallocatechin (GC), (+)catechin gallate (Cg), (-)catechin (C) and caffeine were Sigma products (Sigma-Aldrich, St. Louis, USA). The other chemical reagents used were of HPLC grade (Jinmei Biotech Corporation, Tianjin, China) except where stated otherwise. The water used in the experiments was purified water prepared by a model D7401 EASYPure II UV Ultrapure system (Barnstead International, Iowa, USA).

Extraction with different cosolvents

A supercritical CO_2 extractor Speed SFE-2 (Applied Separations Allentown Inc., PA, USA) was used in this study. It was equipped with two pumps. The first pump was a master pump (Model SDC-6, Ningbo Scinentz Biotechnology Co. Ltd, China) for circulation of refrigerant in jacket, and the second was a cosolvent pump (Model K-501, Knauer Co. Ltd, Berlin, Germany) for the delivering of cosolvent. Three kinds of cosolvents were tested, that is, AR grade ethanol (Jinmei Biotech Corporation, Tianjin, China), water and a mixture of 50% (v/v) AR grade ethanol and 50% (v/v) water. Fifteen milliliters cosolvent and ten g tea sample (particle size 0.6 - 1.2 mm) were used in each extraction. The pressure, temperature and flow rate of SC-CO_2 were maintained constant at 300 bars, 60°C and 1.5 L min^{-1}, respectively.

Orthogonal array design test

Extraction tests with different cosolvents showed that water and 50% (v/v) aqueous ethanol were not suitable for cosolvents because a large percentage of tea catechins, the antioxidants in tea, were removed during the decaffeination. AR grade ethanol was screened as cosolvent in the subsequent tests. To obtain optimum SC-CO_2 decaffeination conditions, orthogonal array design (OAD) design with four factors and three levels each factor [$L_9 (3)^4$] was carried out on software of the SAS System for Windows version 8.0 (SAS Institute Inc, Cary, NC, USA). The four tested factors were tea particle size, extraction temperature, extraction time and cosolvent volume. The details of the experimental design were shown in Table 1. Ten g tea sample was used in each extraction as above. The tests were carried out at isobaric 300 bars and SC-CO_2 flow rate 1.5 L min^{-1}.

HPLC analysis of caffeine and catechins

Tea samples before or after supercritical CO_2 decaffeination were dried at 103°C for 4 h and ground into particles less than 0.2 mm in diameter. One gram of the ground sample was extracted with 100 mL of 50% (V/V) aqueous ethanol at room temperature for 45 min, and then the extract was centrifuged at 5000 x g and 4°C for 10 min. The supernatant was used for high performance liquid chromatography (HPLC, Model LC20A, Shimadzu Co, Kyoto, Japan) to determine caffeine and catechins (Kim and Liang et al, 2007). The HPLC conditions were as follows: injection volume, 10 μL; 5 μm TC-C_{18} column (4.6 × 250 mm, Agilent Technologies Inc, CA, USA), oven temperature at 28°C, mobile phase A being acetonitrile /acetic acid /water (6/1/193, v/v/v) and mobile phase B acetonitrile /acetic acid /water (60/1/139, v/v/v), linear gradient elution with mobile phase B from 30 - 85% (v) during the early 35 min and holding at 85% mobile phase B for further 5 min at flow rate 1mL min^{-1}, detecting at 280 nm. Caffeine and catechins in the tested sample were determined by comparing retention times and peak heights with those of the corresponding authentic references.

Data statistics

All the tests were carried out in triplicate and the mean values of the triplicate tests were presented. Analysis of variance (ANOVA) and Tukey grouping of the data were

Table 1. Orthogonal design with four factors and three levels.

Treatment	Temperature (°C)	Time (h)	Particle size (mm)	Cosolvent volume (mL)
1	40	1	0.2-0.6	5
2	40	2	0.6-1.2	15
3	40	3	1.2-2.0	30
4	60	1	0.6-1.2	30
5	60	2	1.2-2.0	5
6	60	3	0.2-0.6	15
7	80	1	1.2-2.0	15
8	80	2	0.2-0.6	30
9	80	3	0.6-1.2	5

carried out on software of the SAS System for Windows version 8.0 (SAS Institute Inc, Cary, NC, USA).

RESULT AND ANALYSIS

Effect of different cosolvents on removal of caffeine and catechins

Table 2 showed that the cosolvents had great influence on the decaffeination and removal of catechins of green tea samples. When ethanol was used as cosolvent, 67.39% caffeine and 7.2% total catechins were removed from the green tea. In the case of water as cosolvent, 78.0% caffeine and 67.6% total catechins were removed. The tea sample extracted using 50% aqueous ethanol as cosolvent had the lowest concentrations of caffeine and catechins. It is known that catechins are important health beneficial compounds in tea. It suggests that water and aqueous ethanol is not suitable for cosolvents to do the decaffeination of green tea because of the loss of catechins. Ethanol will be used as cosolvent in the subsequent tests.

Effect of different factors on removal of caffeine

Results of the orthogonal array design tests showed that there were differences in caffeine levels between tea samples decaffeinated by various treatments (Table 3). Caffeine concentrations in the tested samples ranged from 9.99 - 28.43 mg g^{-1}, being 31.4 - 89.3% of un-decaffeinated sample. Treatment No. 8 (temperature 80°C, extraction time 2 h, tea particle size 0.2 - 0.6 mm, ethanol cosolvent 30 mL) had the lowest caffeine concentration, with 68.6% caffeine removed (Tables 2 and 3). Total catechins concentrations remained in the nine treatments were 5.3 - 14.1% less than control, with treatment No. 3 being the lowest and treatment No. 6 the highest. It suggests that the removal of caffeine and extraction selectivity were affected by the SF-CO$_2$

conditions.

Analysis of variance (ANOVA) showed that all tested SF-CO$_2$ conditions including extraction temperature, extraction time, particle size and cosolvent volume had significant influence on the removal of tea caffeine (Table 4). Particle size affected significantly on total catechins concentration but not significantly on the (-) epigallocatechin gallate (EGCG). Concentrations of total catechins and EGCG were not significantly influenced by the other tested factors (Table 4).

Optimization of SF-CO$_2$ decaffeination conditions

Based on the Tukey grouping results (Table 5), optimum SF-CO$_2$ decaffeination conditions were screened as tea particle size 0.2 - 0.6 mm, ethanol cosolvent 15 mL, extraction temperature at 80°C and extraction time 2 h. To confirm the decaffeination efficiency, a test was carried out under the optimum conditions obtained from above screening. Ten g of 0.2 - 0.6 mm particle size tea was extracted with 15 mL ethanol cosolvent at 80 °C for 2 h, with SC- CO$_2$ flow rate 1.5 L min^{-1} and pressure 300 bars and the test was carried out in triplicate. The results showed that the concentration of caffeine in the decaffeinated tea sample was 9.48 ± 0.06 g kg^{-1} and that of total catechins 157.77 ± 0.38 g kg^{-1}, among which EGCG was 89.42 ± 0.03 g kg^{-1}. Figure 1 showed the HPLC profiles of the control and tested samples. According to the results, 70.23% of caffeine was removed while 93.8% of total catechins were remained in the decaffeinated sample.

DISCUSSION

The solubility of solutes in SC-CO$_2$ depends on many factors, including SC-CO$_2$ density, plant material particle size, molecular interactions between solutes and FC-CO$_2$, and also the mass and vapour pressure of the solutes. A small change in the temperature, pressure and

Table 2. Effect of different cosolvents on concentrations of caffeine and catechins in decaffeinated tea samples (mg g⁻¹).

Cosolvent[a]	Caffeine	GC	EGC	EC	C	EGCG	GCG	ECG	CG	Total catechins
A	10.21±1.04	3.45±0.31	31.83±0.98	6.97±0.07	1.31±0.02	88.05±2.36	3.49±0.14	20.73±0.12	0.26±0.01	156.08±3.59
B	7.01±0.06	4.69±0.39	12.66±0.32	2.07±0.03	0.36±0.02	28.01±0.31	0.42±0.01	6.15±0.03	0.15±0.03	54.51±0.32
C	2.30±0.02	1.62±0.12	9.66±0.07	1.52±0.03	0.51±0.02	22.37±0.70	0.33±0.01	4.53±0.04	0.16±0.01	40.71±0.38
Control	31.85±0.62	3.85±0.15	34.44±1.13	7.25±0.21	1.34±0.10	95.33±1.11	3.50±0.03	22.49±0.78	0.26±0.03	168.76±1.66

a. A = 100% ethanol; B = water; C = 50% ethanol + 50% water. Control sample was the green tea before SF-CO_2 extraction.

Table 3. Chemical composition of tested samples[a].

Treatment	Caffeine (mg g⁻¹)	Tea catechins (mg g⁻¹)								
		GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total
1	25.13±0.67B	3.61±0.21A	32.31±0.43 A	1.30±0.06AB	7.20±0.04A	86.73±0.93AB	3.15±0.15ABC	20.93±0.84 A	0.26±0.04A	155.49±1.99AB
2	28.43±0.78AB	3.53±0.39 A	32.21±0.63 A	1.19±0.05AB	6.77±0.24A	86.44±0.95AB	3.07±0.09ABC	20.93±0.75A	0.24±0.08A	154.38±2.64 AB
3	27.71±0.93AB	3.24±0.52 A	29.13±1.59 A	1.09±0.03B	6.26±0.39A	82.30±2.82B	2.78±0.30C	19.49±1.35A	0.18±0.08A	144.47±3.89B
4	25.34±0.58B	3.42±0.66 A	31.08±0.88 A	1.14±0.09AB	6.50±0.23A	85.50±3.60B	2.88±0.18BC	20.09±0.69A	0.19±0.06A	150.81±5.42 AB
5	28.29±1.39AB	3.33±0.57 A	30.85±0.73 A	1.18±0.16AB	6.65±0.74A	87.14±2.41B	2.82±0.09C	19.97±1.27A	0.21±0.08A	152.14±6.08 AB
6	15.80±0.69 C	3.53±0.31 A	32.69±0.14 A	1.35±0.04A	7.23±0.06A	89.66±0.17AB	3.32±0.16AB	21.29±0.33A	0.22±0.04A	159.27±0.31 AB
7	24.23±0.48B	3.28±0.47 A	29.78±0.94 A	1.15±0.08AB	6.59±0.33A	84.99±2.27AB	3.22±0.05ABC	19.91±0.95A	0.18±0.05A	149.10±5.76B
8	9.99±0.18 D	3.33±0.25 A	32.02±0.67 A	1.32±0.01AB	6.95±0.08A	88.50±1.58AB	3.48±0.08A	20.72±0.16A	0.22±0.06A	156.54±2.15 AB
9	25.62±0.24B	3.41±0.08 A	31.61±0.52 A	1.29±0.01AB	6.89±0.03A	86.33±0.43AB	3.14±0.09ABC	20.83±0.47A	0.21±0.07A	153.71±0.82 AB

a Data with different letters in a same column differed significantly at p=0.05

cosolvent near the critical point will lead great changes in the solvent density and vapour pressure of solutes, resulting in changes in the solvation power of the solvent. Although an isobaric increase in the temperature decreases the density of the solvent, it increases the vapour pressure of caffeine (Kopcak and Mohamed, 2005). Increase in temperature decreases SC-CO_2 density and enhance the movement of the solutes and delivery medium (Kuppers, 1992). The sublimation temperature of caffeine is low (178°C) while catechins such as EGCG are not sublimated. It is postulated that the vapour pressure of caffeine is increased while less change in the vapour pressure of catechins happened with the isobaric elevation of temperature. This resulted in the increase in solubility of caffeine in the mixed solvent of SC-CO_2 and ethanol but little changes in the solubility of catechins. It explained that the concentration of caffeine significantly decreased and that of total catechins concentrations were not significantly changed when temperature was lifted (Table 5). The sample particle size is an important variable in extraction because it affects the mass transfer of solute and solvent, and it is directly related to total surface area for contact between sample and solvent (Park et al., 2007a). The concentrations of caffeine and total catechins were significantly affected by particle size of tea (Table 5). As particle size decreased from 1.2 - 2.0 to 0.2 - 0.6 mm, however, caffeine level decreased by 35.5 percent while total catechins decreased by 5.4 percent (Table 5). This suggests that small particle size of tea is advantageous for selective decaffeination.

Table 4. Variance analysis for caffeine, EGCG and total catechins.

Source	DF [a]	Type I SS [b]	Mean square	F [c]	Significant level
Caffeine					
Temperature	2	230.55	115.27	27.51	<.0001
Time	2	33.59	16.79	4.01	0.0363
Particle size	2	557.36	278.68	66.51	<.0001
Cosolvent Volume	2	132.30	66.15	15.79	0.0001
EGCG					
Temperature	2	23.91	11.95	1.10	0.3557
Time	2	13.00	6.50	0.60	0.5616
Particle size	2	56.09	28.04	2.57	0.1043
Cosolvent Volume	2	12.92	6.46	0.59	0.5636
Total catechins					
Temperature	2	31.75	15.87	0.39	0.6795
Time	2	31.53	15.77	0.39	0.6812
Particle size	2	327.87	163.93	4.08	0.0346
Cosolvent Volume	2	70.67	35.33	0.88	0.4323

[a] Degree of freedom.
[b] Type I sum of squares.
[c] F value.

Table 5. Mean comparison between different levels by Tukey grouping[a].

Factors	Levels	Caffeine (mg g^{-1})	Catechins (mg g^{-1})								
			GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total
Temperature (°C)	40	27.09 A	3.46 A	31.21 A	1.19 A	6.74 A	85.16 A	3.00 A	20.45 A	0.23 A	151.45 A
	60	23.14 B	3.43 A	31.54 A	1.22 A	6.79A	87.43 A	3.01 A	20.45 A	0.21 A	154.07 A
	80	19.94 C	3.34 A	31.14 A	1.25 A	6.81 A	86.61 A	3.28 A	20.49 A	0.20 A	153.11 A
Extraction time (h)	1	24.90 A	3.44 A	31.06 A	1.19 A	6.76 A	85.74 A	3.09 A	20.31 A	0.21 A	151.80 A
	2	23.04 B	3.40 A	31.69 A	1.23 A	6.79 A	87.36 A	3.12 A	20.54 A	0.22 A	154.35 A
	3	22.23 B	3.39 A	31.14A	1.24 A	6.80 A	86.10 A	3.08 A	20.54 A	0.20 A	152.48 A
Particle size (mm)	0.2-0.6	16.97 B	3.28 A	29.92 B	1.14 B	6.50 B	84.81 A	2.94 B	20.98 A	0.19 A	148.57 B
	0.6-1.2	26.46 A	3.45 A	31.63 AB	1.21 B	6.72 B	86.09 A	3.03 B	20.62 A	0.22 A	152.96 AB
	1.2-2.0	26.74 A	3.49 A	32.34 A	1.32 A	7.13 A	88.30 A	3.32 A	20.98 A	0.23 A	157.10 A
Cosolvent (ml)	5	26.34A	3.45 A	31.59 A	1.26A	6.91 A	86.73 A	3.04 A	20.58 A	0.23 A	153.78 A
	15	22.82B	3.44 A	31.56 A	1.23 A	6.86 A	87.03 A	3.21 A	20.71 A	0.21 A	154.25 A
	30	21.01B	3.33 A	30.74 A	1.18 A	6.57 A	85.44 A	3.05 A	20.10 A	0.20 A	150.61 A

[a] Mean concentrations were compared between different levels of a same factor group and the data with different letters were significantly different at $p = 0.05$.

Although SC-CO$_2$ is selective for caffeine, the solubility of the polar caffeine in the non-polar SC-CO$_2$ is low, compared to organic solvents like chloroform. Addition of small amount of polar co-solvents like ethanol and water will increase polarity, density and hydrogen bonding capacity of the supercritical mixed solvents (Park et al., 2007a, b). The cosolvent effects in supercritical cosolvent interacts strongly with the solute, resulting in enhancement of solubility of solutes in the polar cosolvent /SC-CO$_2$ mixture (Ting et al., 1993). The interactions include hydrogen bonding and the van der Waals forces such as dispersion forces between solute
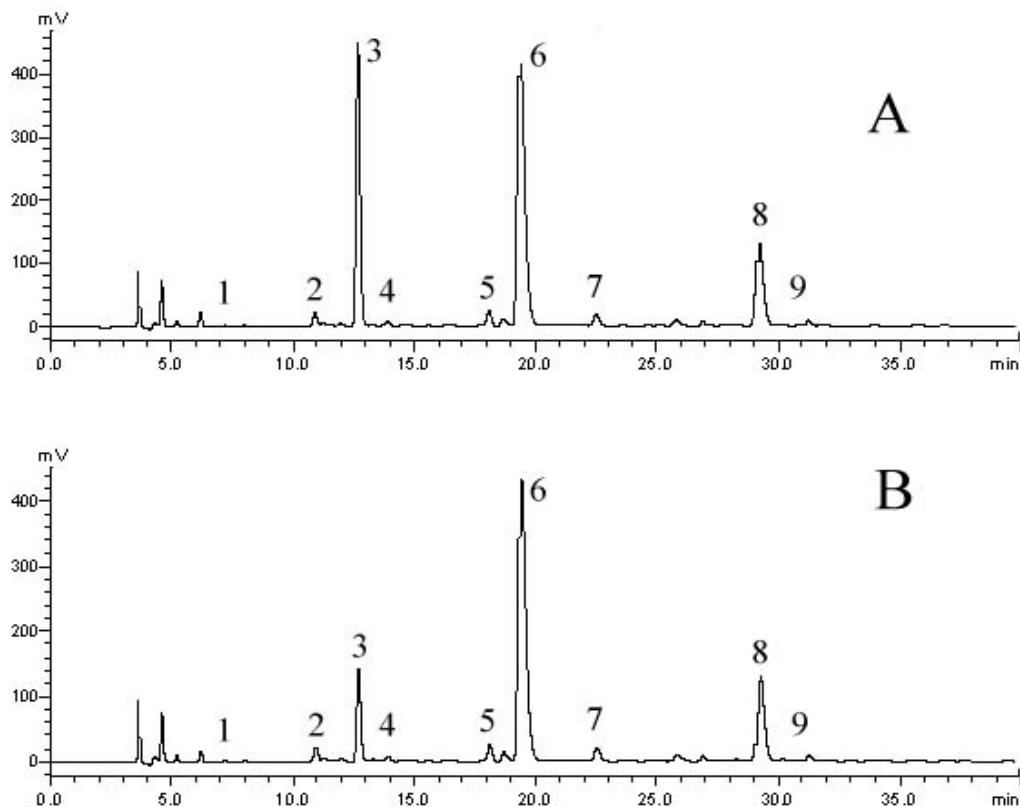
Figure 1. HPLC profiles of control sample (A) and decaffeinated sample (B) 1. GC; 2. EGC; 3. Caffeine; 4. C; 5. EC; 6. EGCG; 7. GCG; 8.ECG; 9.CG.

and solvent molecules. The addition of ethanol and water will strengthen the hydrogen bonding between the mixed solvent and solutes including caffeine and catechins. The hydrogen bonding can take place between hydroxyl groups of hydroxylic derivative solvents and the nitrogen atom on the five membered ring of caffeine and possibly also by similar interaction with the keto groups of the caffeine (Figure 2) (Ghabi and Dhahbi, 1999) as well as between the hydroxyl groups of hydroxylic solvents and the phenol groups of catechins. Figure 2 shows that there are abundant hydroxyl groups on EGCG molecule, a major catechins in green tea. It can be assumed that the hydrogen bonding interactions between solvent and catechins were stronger than that between solvent and caffeine. Hydrogen bonding is an electrostatic attraction between two independent polar molecules. The atoms in the water molecule are arranged with the two H-O bonds at an angle of about 105° and this arrangement results in a polar molecule which provides a net negative charge toward the oxygen end of the V-shaped molecule and net positive charges at the hydrogen ends for hydrogen bonding interaction. Although hydrogen bonding can occur between ethanol molecule and solutes, it is not as effectively as in water since there is only one hydrogen on each ethanol molecule with sufficient positive charge.

In low molecular weight alcohols such as methanol and ethanol, the properties of the polar hydroxyl group dominate the weak intermolecular forces of van der Waals. In higher molecular weight alcohols, the properties of the non-polar hydrocarbon chains dominate and define the solubility. It is known that the London dispersion forces depend on molecular weight and the degree that the molecules are polarized. It is known that the London dispersion forces tend to be stronger between molecules that are easily polarized. HPLC of tea solutions confirmed that caffeine was eluted earlier by polar solvent than EGCG, a major component of tea catechins (Ye et al., 2009). It suggests that caffeine is more polar molecule than EGCG. Table 2 showed that about 67.9% of caffeine and 7.2% of total catechins were removed when pure ethanol was used as cosolvent. However, 78.0% caffeine and 67.6% total catechins were removed when water was used as cosolvent. It may be assumed that solvent-solute interactions differentiated with changes in cosolvent components. The van der Waals forces dominated solvent-solute interactions in the case of ethanol as cosolvent, in which the solubility of caffeine was high. However, hydrogen bonding interactions increased in the case of water or aqueous ethanol solution as cosolvent, in which solubility of
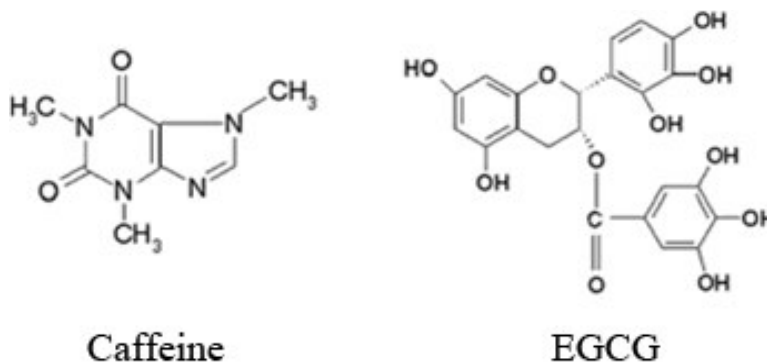
Figure 2. Molecular structures of caffeine and EGCG.

catechins including EGCG lifted because of their abundant hydroxyl groups for hydrogen bonding interactions. The hydrogen bonding interaction between hydrogen on water molecule and nitrogen atom, keto groups of caffeine molecule might also took place in this case, resulting in simultaneous interactions of the hydrogen bonding and the van der Waals forces. That interprets why a large percentage of caffeine and catechins was removed when water or aqueous ethanol was used as cosolvent. Therefore, water is not a suitable cosolvent for decaffeination of green tea because the caffeine was not selectively removed.

Conclusion

Cosolvents had significant influence on selective decaffeination of green tea by SC-CO$_2$ extraction. Owing to strong hydrogen bonding interactions between hydrogen in water and aqueous ethanol solution and hydroxyl groups on catechins molecules, as much as 67.6 - 75.8% of total catechins were removed after decaffeination by SC-CO$_2$ using water or 50% aqueous ethanol as cosolvents. Water and aqueous ethanol are not suitable for cosolvent to decaffeinate green tea by SC-CO$_2$ extraction. The van der Waals forces dominated molecular interactions between ethanol and caffeine in the case of ethanol as cosolvent, resulting in selective decaffeination of green tea. Particle size of tea sample affects the mass transfer of solute and total surface area for contact between solvent and the sample, and smaller particle size is beneficial to selective decaffeination. Increase in isobaric temperature increased the vapour pressure and the movement of caffeine, and 80°C was favorable temperature.

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