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Separation of major catechins from green tea by ultrahigh pressure extraction

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ABSTRACT

This study presents a novel extraction technique, ultrahigh pressure extraction, to obtain major catechins from green tea leaves. The effects of various high pressure level (100, 200, 300, 400, 500, 600 MPa) on the extract are examined. HPLC chromatographic analyses determine the concentration of four major catechins and caffeine. The extraction yields of active ingredients with ultrahigh pressure extraction (400 MPa pressure) for only 15 min were given the same as those of organic solvent extraction for 2 h. These excellent results for the ultrahigh pressure extraction are promising for the future separation of active ingredients from traditional Chinese herbal medicine.

1. Introduction

Tea is the most widely consumed drink in the world and it has been used as a daily beverage and crude medicine in China and Japan for thousands of years. Numerous epidemiological and pharmacological studies demonstrate that green tea extract possesses strong antioxidant effect (Tanaka et al., 1998; Vinson and Dabbagh, 1998) and antimutagenic activity (Otsuka et al., 1998). According to the previous studies, in general, fresh green tea leaves contain 36% polyphenols, among which catechins prevail. Catechins are divided into four primary compounds (Fig. 1) (Amra et al., 2006), epigallocatechin gallate (EGCg), epicatechin gallate (ECg), epigallo catechin (EGC) and epicatechin (EC). Among these, EGCg is the most luxuriant component in tea extract (48–55% of total polyphenols) (Ho et al., 1997) and the most potent chemical tested for biological activity (Owuor and Obanda, 1998).

The ultrahigh pressure technique, as identified by US FDA (2000), which ranges from 100 to 800 MPa, has been widely used in ceramics, graphite, casting industry, pharmaceutics, metallurgy, plastic making, civil engineering and food industry (US FDA, 2000). Mass transport phenomena can be enhanced by changes in concentration gradients, diffusion coefficients or boundary layer (Liang, 1993). Ultrahigh pressure technique is a novel method to enhance mass transport phenomena (Rastogi et al., 2003). Higher caffeine extraction yields from coffee and a higher carotenoid content in tomato puree have been demonstrated when extractions were assisted by ultrahigh pressure technique (Knorr, 1993; Sanchez-Moreno et al., 2004), which had exhibited excellent advantages in natural product extraction field. Studies showed that ultrahigh pressure technique could shorten processing time, obtain higher extraction yields, and had no negative effect on the activity and structure of bioactive components. Above all, this extraction technique could be operated at room temperature. Recently it had been successfully used for extraction of lycopene from tomato paste waste (Jun, 2006), ginsenoside from Korean red ginseng (Kim et al., 2007), anthocyanins from grape skins (Corrales et al., 2008, 2009), and corilagin from longan fruit pericarp (Nagendra Prasad et al., 2010). Many efforts successfully separated tea catechins using supercritical CO_2 (Chang et al., 2000), soxhlet extraction (Price and Spitzer, 1993) and organic solvent extraction (Ho and Jin, 2006). However, to our knowledge, no study has been carried on separating catechins using ultrahigh pressure extraction.

In light of the above discussion, this study presents a novel extraction technique, ultrahigh pressure extraction, which was used to obtain catechins from green tea leaves. Controlling the extractive conditions allows us to evaluate the separation of catechins. HPLC chromatography is performed to quantify four major catechins and caffeine. In this study, the major objective was to build up ultrahigh pressure extraction method to extract catechins from green tea leaves, and then compared it with the organic solvent extraction. This work could help better utilize green tea leaves to obtain catechins as a readily accessible source of bioactive compounds for pharmaceutical usage.

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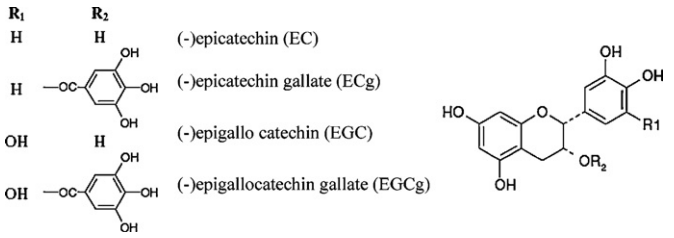

Fig. 1. Chemical structures of four major catechins.

2. Materials and methods

2.1. Plant materials and chemicals

Dry green tea leaves (*Thea sinensis* L.) (Place of origin: Hangzhou, China) were purchased from a local market.

Phosphoric acid (85.1%), epicatechin gallate (ECg, 98%), epigallocatechin (EGC, 98%), epigallocatechin 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 (Sigma, United States) was used for mobile phase solvent. Ethanol used in the experimental work was analytical reagent grade chemicals (Beijing Chemical Reagents Company, Beijing, China). Deionized water was obtained from a reverse osmosis and ion exchange purified water system (Barnstead, UK).

The HPLC system used was a LC-10AT vp HPLC system with a SPD-M10A vp diode-array detector (Shimadzu, Japan) and a Diamonsil^{TM} C18 column (250 mm × 4.6 mm, 5 μm, Dikma, Beijing, China) at 25 °C.

The ultrahigh pressure isostatic apparatus was purchased from Chengdu Suohaipu Super high Pressure Machine Co. Ltd. (Chengdu, China). Effective volume of vessel: 5 L; maximal working pressure: 600 MPa; inner diameter: 200 mm; pressure transmitting media: mixture of transformer oil and kerosene.

2.2. Ultrahigh pressure extraction

The green tea leaves were dried in vacuum at 60 °C for 6 h, then pulverized and sieved. Twenty grams sample of green tea leaves powder through 40 mesh screen were mixed with 400 mL of 50% (v/v) ethanol solvent (ratio 20 mL:1 g) and placed into a sterile polyethylene bag. The bag was sealed after eliminating air from the inside and placed into a hydrostatic pressure vessel. After processed in the ultrahigh pressure apparatus for 15 min (high pressure level: 100, 200, 300, 400, 500, 600 MPa) at room temperature, the mixture was filtered through a syringe filter. The extraction solution was centrifuged at a speed of 4000 rpm for 10 min, and the supernatant was collected, evaporated under vacuum at 40 °C, and stored at 4 °C in refrigerator for subsequent HPLC determination.

2.3. Organic solvent extraction

Green tea leaves ethanol extracts were boiled (20 g of green tea leaves powder, mixed with 400 mL of 50% (v/v) ethanol solvent (ratio 20 ml: 1 g)) at boiling point, about 85 °C for 2 h (super-boiling of the solution did not occur). The extraction mixture was constantly stirred with a magnetic stirrer. After 2 h of extraction, the extraction mixture was cooled. Then, the extracts were filtered through a syringe filter. The extraction solution was centrifuged at a speed of 4000 rpm for 10 min, and the supernatant was collected, evaporated under vacuum at 40 °C, and stored at 4 °C in refrigerator for subsequent HPLC determination (Ho and Jin, 2006).

2.4. Major catechins determination

The four major catechins and caffeine in the extracts were determined using the HPLC procedure of Yinzhe et al. (2006). 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 were centrifuged at 4000 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 mobile phase applied was the binary system of A (0.05% phosphoric acid solution) and B (acetonitrile) from 90:10 (A:B, %) to 70:30 (A:B, %) in a linear gradient time of 30 min at a flow rate of 1 ml/min. The injection volume was 10 μl and UV wavelength was set at 280 nm.

2.5. Statistical analysis

All experiments were done in triplicate and data in tables and figures represent mean values ± standard deviation ($n = 3$). Results were evaluated for statistical significance using one-way ANOVA by the SPSS Statistic Method (Version 11.5). The confidence level for statistical significance was set at a probability value of 0.05.

3. Results and discussion

The factors that may influence the ultrahigh pressure extraction are the pressure level, pressure holding time, liquid/solid ratio, different solvent, time to achieve treatment pressure, decompression time, product initial temperature, product pH, solvent concentration and so on (Knorr, 1993; Sanchez-Moreno et al., 2004). In order to more conveniently and clearly explore the effect of ultrahigh pressure on the extraction of active ingredients from green tea, the pressure level was selected as the critical extraction factor. Other extraction factors, such as ethanol concentration and solid/liquid

Table 1
Concentrations of caffeine (Caf), four major catechins (TC) and gallic acid (GA) in tea extracts.

Pressure (MPa)	Caffeine (mg/kg)	EGC (mg/kg)	EGCg (mg/kg)	ECg (mg/kg)	EC (mg/kg)	GA (mg/kg)	TC/Caf
100	530 ± 6.4 a	748 ± 5.2 e	2399 ± 6.3 i	380 ± 4.2 m	210 ± 3.4 r	49 ± 2.5 v	7.05
200	640 ± 4.3 b	820 ± 6.3 f	2589 ± 4.6 j	417 ± 4.3 n	249 ± 3.4 s	71 ± 3.4 w	6.36
300	768 ± 8.2 c	1080 ± 4.3 g	2720 ± 6.4 k	458 ± 3.2 o	268 ± 2.4 t	98 ± 2.4 x	5.89
400	880 ± 5.2 d	1298 ± 8.3 h	2890 ± 6.3 l	480 ± 6 p	280 ± 4.2 u	130 ± 4 y	5.63
500	876 ± 8.1 d	1305 ± 10.1 h	2901 ± 8.1 l	483 ± 4.3 p	278 ± 3.1 u	128 ± 3.4 y	5.67
600	883 ± 4.3 d	1301 ± 7.2 h	2894 ± 5.3 l	479 ± 7 p	282 ± 4.3 u	132 ± 4.1 y	5.62
Organic solvent extraction	885 ± 7.2 d	1310 ± 9.2 h	2885 ± 4.6 l	483 ± 3.4 p	284 ± 5.2 u	129 ± 2.3 y	5.60

Values are means ± standard deviations of triplicate measurement. For different extraction methods, means in every column with different letters were significantly different ($P < 0.05$, Student's t-test). Organic solvent extraction: 20 g of green tea leaves powder, 400 mL of 50% (v/v) ethanol solvent, at boiling point about 85 °C for 2 h. Ultrahigh pressure extraction: 20 g of green tea leaves powder, 400 mL of 50% (v/v) ethanol solvent, extraction for 15 min.

ratio were the same as those used for the organic solvent extraction.

Green tea extracts were analyzed to understand the contents of four catechins, gallic acid, and caffeine by HPLC. Table 1 summarizes the concentrations of four major catechins (TC), gallic acid (GA) and caffeine (Caf) in the green tea extract. Organic solvent extraction, 50% (v/v) ethanol extractions, acted as reference runs at boiling points about 85 °C for 2 h, with the details outlined in Row and Jin (2006).

Table 1 shows that the concentrations of four major catechins (TC), gallic acid (GA) and caffeine (Caf) in the green tea extract were influenced by pressure level. The extraction yields of active ingredients with ultrahigh pressure extraction (400 MPa pressure) for only 15 min were given the same as those of organic solvent extraction for 2 h, therefore it is obvious that pressure is useful for improving the concentrations of major catechins in tea extracts.

According to Le Chatelier's theory (Chen et al., 2005), the volume of system tends to be reduced during the pressure-promoting period. In this process, the extracting solvent comes into cells to integrate with bioactive components. Besides, the pressurized cells show increased permeability (Yan, 2002). The higher the hydrostatic pressure is, the more solvent can enter cells and the more compounds can permeate out to the solvent. The equilibrium of solvent concentration between inner and outer of cells would be established during the pressure holding period. Butz et al. (1994) reported that pressures of 100 MPa were enough to cause rupture of intracellular vacuoles and plant cell walls in onions. Furthermore, in the extraction process with high pressure, the solubility of extracts is improved as the pressure increases (Noble, 1988; Richard, 1992). So increasing pressure could increase the concentrations of major catechins in tea extracts. These results are in accordance with studies of Sanchez-Moreno et al. (2004), Jun (2006) and Nagendra Prasad et al. (2010).

Numerous investigations have confirmed the feasibility of extracting tea catechins with conventional solvents such as acetonitrile-water (Goto et al., 1996), or hot water (Lin et al., 1996; Price and Spitzer, 1993). Roedig-Penman and Gordon (1997) showed that methanolic tea extract contained higher levels of EGCg and ECg. These studies indicated that the ratio of total catechins to caffeine ranges from 2.5 to 5.4 (Goto et al., 1996), 1.3 to 5.4 (Lin et al., 1996), 3.3 to 3.7 (Price and Spitzer, 1993). Our results indicated that the ratio of TC/Caf was 5.62–7.05 and 5.6, for ultrahigh pressure extraction and organic solvent extraction, respectively. Because tea composition varies with climate, season, tea variety, and age of the leaf.

4. Conclusion

Ultrahigh pressure extraction, as a new extraction method, is used to extract major catechins from green tea. Comparing with organic solvent extraction, ultrahigh pressure extraction has excellent advantages, such as shorter extraction time, higher yield, lower energy consumption, eco-friendly and so on. Ultrahigh pressure extraction is expected to offer a new way for the production and analyses of the plant extractions, and the modernization of pharmaceutical engineering of traditional Chinese herbal medicine.

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