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Ultrahigh pressure extraction as a tool to improve the antioxidant activities of green tea extracts

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ABSTRACT

In this study, the effect of ultrahigh pressure extraction at pressures of 150 MPa, 250 MPa, 350 MPa and 450 MPa on the total phenolic contents, the extraction yields and the antioxidant activities of green tea were investigated. The antioxidant activities of these extracts were analyzed using DPPH radical scavenging activity and total antioxidant capacity. The results showed that the phenolic contents and the antioxidant activities of extracts were greatly influenced by high pressure. The total phenolic contents and the antioxidant activities of ultrahigh pressure extraction at 450 MPa were higher than those of other ultrahigh pressure extraction and conventional extraction. The high content of phenolic compounds in the green tea leaves could account for the antioxidant activity. This study indicated that this new technology can benefit the food and pharmaceutical industries.

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1. Introduction

Tea is one of popular drinks in the world. The consumption of green tea is especially popular in Asia, mainly for its health benefits (Balentine, Wiseman, & Bouwens, 1997). Green tea is reported to reduce serum cholesterol levels and inhibit hypertension, mutagenesis and tumorigenesis in several experiments *in vitro* and *in vivo* (Hodgson, Puddey, Burke, Beilin, & Jordan, 1999; Yokozawa, Nakagawa, & Kitani, 2002). Many studies have shown that polyphenolic compounds extracted from green tea leaves are good antioxidants against lipid peroxidation in phospholipid bilayers and biological systems, against tumorigenesis and DNA damage (Jankun, Selman, Swiercz, & Skrzypczak-Jankun, 1997; Terao, Piskula, & Yao, 1994). The phenolics are secondary plant metabolites that are involved in a wide range of specialized physiological functions. They appear to be very important for the normal growth, development and defense mechanisms of plants (Rusak, Krajačić, & Pleše, 1997).

Ultrahigh pressure extraction (UPE), which usually works under the pressure ranging from 100 MPa to 600 MPa, has been recognized as an environment-friendly technology by the U.S. Food and Drug Administration, and is extensively applied in pharmaceutical and food industry (Nagendra, Bao, Mouming, Neungnapa, & Yueming, 2009). High pressure can enhance the mass transfer rate, increase solvent permeability in cells as well as secondary metabolite diffusion (Ahmed & Ramaswamy, 2006; Dornenburg & Knorr, 1993). Studies showed that UPE technique could shorten processing time, obtain higher extraction yield, and had no negative effect on the activity and structure of bio-active components (Corrales, Avelina, Peter, & Bernhard, 2009; Shin et al., 2010; Xi, 2006). Above all, this extraction technique could be operated at ambient temperature (Nagendra et al., 2010; Xi et al., 2010). Recently it had been successfully used for extraction of anthocyanins from grape skins (Corrales et al., 2009), ginsenosides from Korean panax ginseng powder (Shin et al., 2010), biologically active compounds from green tea leaves (Xi et al., 2010) and corilagin from longan fruit pericarp (Nagendra et al., 2010) and so on.

The main objective of this study was to investigate the influences of UPE at 150 MPa, 250 MPa, 350 MPa and 450 MPa on the phenolic contents, the extraction yields and the antioxidant activities of green tea. The UPE were compared to those of conventional extraction (CE) to confirm the advantages of UPE.

2. Materials and methods

2.1. Materials

The fresh green tea leaves (*Thea sinensis* L.) (place of origin: Hangzhou, China) were purchased from a local market. They were then dried for 24 h using a hot air oven at 50 °C, and finally powdered and sieved to produce samples with 60 mesh.

The ethanol used in the experimental work was analytical reagent grade chemicals (Beijing Chemical Reagents Company, Beijing, China). α, α-diphenyl-b-picrylhydrazyl (DPPH) and α-tocopherol were purchased from Sigma-Aldrich Chemical Co. (Sigma, USA). Folin–Ciocalteau reagent and other chemicals for analysis of tea polyphenols were also from Beijing Chemical Reagents Company (analytical grade, Beijing, China). Gallic acid, pharmaceutical grade standard, was purchased from the

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National Institute for Control of Pharmaceutical and Biological Products (China). Other reagents were of analytical grade and purchased from Chengdu Chemical Industry (Chengdu, China). The spectrophotometer (751-GW) was from Shanghai Analytical Instrument Overall Factory (Shanghai, China).

The ultrahigh pressure 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: water and glycol (20/80, v/v). Fig. 1 schematically depicts an ultrahigh pressure extraction system (Chen, Meng, Zhang, & Liu, 2009).

2.2. Ultrahigh pressure extraction

The dried green tea powder (10 g) was mixed with 200 ml of 50% ethanol, and then placed into a sterile polyethylene bag. The bag was sealed after eliminating air from the inside and placed into the pressure vessel, and then extracted for 5 min at pressures of 150 MPa, 250 MPa, 350 MPa or 450 MPa at ambient temperature, respectively. After depressurization, the mixture was filtered through a filter paper. The extracts were centrifuged at 4000 ×*g* for 10 min, and the supernatants were pooled. The supernatants obtained were combined and concentrated in a rotary evaporator under reduced pressure at 40 °C and then the supernatant was lyophilized (Xi, 2009; Xi et al., 2009). In this manner, the green tea extracts (GTE) by UPE were prepared.

The freeze-dried extracts were weighed and the extract yields were calculated as the weight (g) from 10 g raw material, and the yields were expressed in percentage on a dry basis.

2.3. Conventional extraction

Conventional extraction was performed as described by Jin et al. (Jin, Jin, & Row, 2006) with some modifications. Sample was weighed exactly (10 g) in a three-neck flask, and 200 ml 50% ethanol extraction solvent was added. A stirring apparatus and a reflux condenser were fixed. Extraction was carried out at boiling state for 4 h. Then the extracts were prepared as UPE procedure.

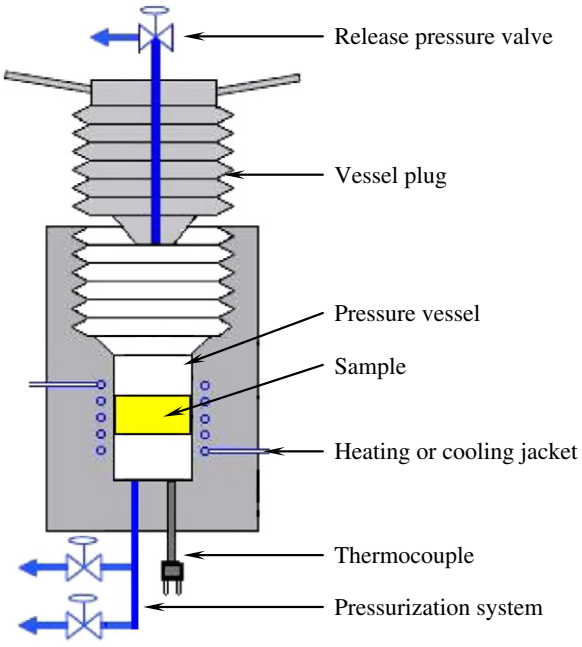

Fig. 1. Schematic diagram of ultrahigh pressure extraction system.

2.4. Determination of total polyphenol content of green tea extracts

The amount of polyphenol was reference measured by a photometric Folin–Ciocalteu assay according to a proposed international standard method (Chen, Zhao, Liu, Cai, & Liu, 2008; Turkmen, Sari, & Velioglu, 2006). The method is based on the reduction of phosphotungstic acid ($H_3P[W_3O_{10}]_4$) in alkaline solution to phosphotungstic blue. The absorbance of formed phosphotungstic blue is proportional to the number of aromatic phenolic groups and is used for their quantification with gallic acid as the standard. Briefly, a calibration curve of gallic acid (ranging from 0.005 to 0.05 mg/ml) was prepared and the results, determined by regression equation of the calibration curve ($y = 60.56x - 0.72, R^2 = 0.9996$), were expressed as mg gallic acid equivalents per gram of the sample. In this method, 1 ml of tea extract diluted 10–75 times with de-ionized water (to obtain absorbance in the range of the prepared calibration curve) was mixed with 1 ml of 3-fold-diluted Folin–Ciocalteu phenol reagent. Two milliliters of 35% sodium carbonate solution is added to the mixture, which was then shaken thoroughly and diluted to 6 ml by adding 2 ml of water. The mixture is allowed to stand for 30 min and blue color formed is measured at 700 nm using a spectrophotometer. The total phenolic contents were determined using the standard gallic acid calibration curve.

2.5. Scavenging activity on DPPH radical

The DPPH radical scavenging activities of α-tocopherol, GTE by difference UPE and CE were analyzed by the method of Sheng et al. (Sheng, Zhou, Wang, Xu, & Hu, 2007) with some modifications. Initially, 2 ml aliquot of each solution (50 μg/ml, 100 μg/ml) was added to 2 ml of 2×10^{-4} mol/l ethanolic DPPH solution in a cuvette. The mixture was shaken vigorously. The reaction mixture was incubated at 28 °C in a dark room for 30 min. The control contained all reagents except the extract sample while ethanol was used as blank. The scavenging activity against DPPH radicals was determined by measuring the absorbance at 517 nm with a spectrophotometer. The inhibition of DPPH radicals by the test samples was calculated as scavenging activity (%) = (1 − absorbance of sample/absorbance of control) × 100. The DPPH radical scavenging activities of CE and α-tocopherol samples were also assayed for comparison.

2.6. Determination of total antioxidant capacity

The total antioxidant capacity of α-tocopherol, GTE by difference UPE and CE were determined according to the method of Prieto et al. (Prieto, Pineda, & Aguilar, 1999) with some modifications. 0.1 ml sample solutions at 50 μg/ml and 100 μg/ml were mixed with 0.3 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was then incubated for 90 min at 95 °C. After the mixture had cooled to room temperature (25 °C), the absorbance of the mixture was measured at 695 nm against a blank. The blank contained the reagent solution and solvent excepting the test sample. The total antioxidant activity was expressed as the absorbance of the sample. The antioxidant activities of α-tocopherol and CE samples were also assayed for a comparative analysis.

2.7. Statistical analysis

All computations were performed by SAS (version 8.0). The data were presented as means ± standard deviations of three replicates. Means for significant differences were compared using the Student's *t*-test. Multiple comparisons of means were done by LSD (least significant difference) test. A probability value of $P < 0.05$ was considered significant.

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