Microwave-low-pressure process (MWLP): An effective technology applied in extraction of total polyphenols

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Abstract: The microwave-low-pressure process (MWLP) of total polyphenols from *Chaenomeles speciosa* fruit was studied, and the advantages of MWLP were further evaluated by comparing with ultra high pressure (UHP) and microwave-assisted extraction (MAE). The influences of liquid/solid ratio, extraction time, pressure, and ethanol concentration on the performance of MWLP were investigated. Thereafter, the interactive variables were further optimized by the stepwise multiple quadratic regression model on the basis of the previous univariate analysis. The results showed that temperature as an intermediate variable in MWLP significantly affected the yields of polyphenols and 3-o-caffeoyl-quinic acid, which was determined by pressure and ethanol concentration. The optimized parameters were proved to be valid because the results predicted by the stepwise multiple quadratic regression model equations fit well with the experimental results. Compared with UHP, the predominance of MWLP was that the extraction time was shortened and the cost of extraction equipment was lowered. MWLP is an effective technology since MWLP was superior to MAE based on extraction yield, solvent loss and reproducibility.

Keywords: Microwave-low-pressure process (MWLP), total polyphenols, *Chaenomeles speciosa* fruit, model optimization, performance evaluation

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1 Introduction

The plant phenolics are the most important natural antioxidants because of their diversity and extensive distribution. They have attracted special attention due to their health-promoting characteristics. For example, they may protect the human body from oxidative stress which can cause many diseases such as cancer, aging, and cardiovascular diseases[1-6]. Chlorogenic acids are major antioxidants among phenolic compounds, 3-o-caffeoyl-quinic acid (3-O-CQA) as one isomer of chlorogenic acids showed similar antioxidation with chlorogenic acids[7]. It was found that the IC50 values (antioxidant activity) of phenolic fractions of quince pulp, peel, and jam extracts were correlated with the total content of caffeoylquinic acids[8]. Therefore, it is important for pharmaceutical industry to find effective process technology to extract herb plant phenolics containing high content of 3-O-CQA.

Chaenomeles speciosa as shown in Figure 1 is a genus in the family Rosaceae, which is called "Zou Pi Mu Guan" in China. It is native to China, Korea and Japan, its fruit contributes to analgesic, anti-inflammatory, antispasmodic, astringent and digestant[9-11]. The

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alcohol decoction of the fruit is internally used for the treatment of nausea, joint pains, cholera and associated cramps[12]. Moreover, its yield is very large, e.g. the total yield had over 113 000 tons in 2012 just in Tongbo County, Henan Province, China. Hence it is significant to study its processing.

For decades, the increasing hydrostatic pressure progressively became a research hotspot which can enhance mass transport phenomena[13]. However, Most of previous literatures[14-19] nearly focused on high pressure (HP, 10 MPa $\leqslant$ p < 100 MPa) and ultra-high pressure extraction (UHP, p $\geqslant$ 100 MPa)[20]. Few studies involved in low pressure (LP, 0.1 MPa $\leqslant$ p < 1.6 MPa)[20] extraction technique have been reported. It is well known that too high pressure process entails extremely high equipment costs and elevated risks in operation. In some occasions, the effect of UHP on the target compound may be limited or similar with LP. Therefore, the investigation of LP and UHP for the process of polyphenols from *Chaenomeles speciosa* fruit would be valuable.

Figure 1 *Chaenomeles speciosa* fruit

Microwave-assisted extraction (MAE) was another hotspot that had been used to extract interesting components from a wide variety of sample matrices for a number of applications[21-24]. Compared with conventional methods, MAE could considerably reduce both extraction time and solvent consumption[25-27]. As a concomitant result of MAE, highly localized temperature is easy to cause the loss of solvents with the increasing time, which resulted in poor reproducibility owing to lack of homogeneity[28]. Liu et al.[29] modified ordinary household microwave oven by adding a cooling water system to have solved the problem to a certain extent. However, it will be difficult to make the temperature to be uniformly distributed in large volumes if it is applied in the industrial equipments. On the other hand, the added cooling system is used to decrease extraction temperature whereas extraction rates are generally temperature-dependent. Therefore, the loss of solvent and inhomogeneity should be taken into account when MAE is employed.

The aim of the present study was to investigate a new technology named MWLP that microwave(MW) assisted extraction combined with low pressure (LP) process, whose destination is not only decrease the evaporation loss of solvent but also LP could improve temperature homogeneity of solvent in MAE. In addition, the transfer of target compounds might simultaneously be enhanced by MW and LP. The technology was applied in the extraction of polyphenols including 3-O-CQA from *Chaenomeles speciosa*. Its performance was evaluated through comparison with two popular methods UHP and MAE.

2 Materials and methods

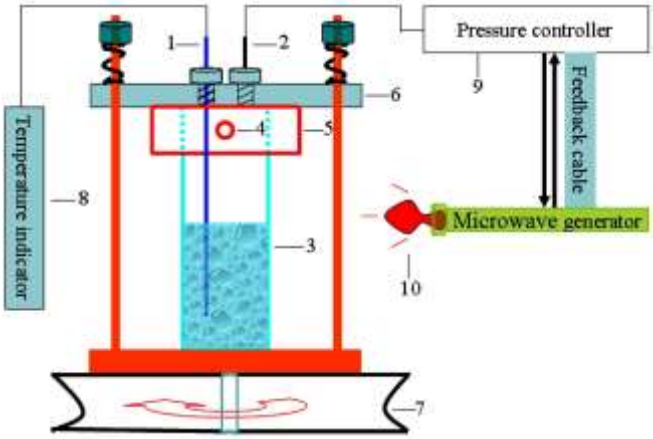
2.1 Materials and chemicals

Ripe fruit of *Chaenomeles speciosa* were purchased from Xi'an pharmaceutic market (Xi'an, Shaanxi Province, China) in October, 2011. They were sliced, sun dried for three days and grounded into powder using a pulverizer (Tianjin Taisite Instrument Co., Ltd, Tianjin, China), and then sieved powder with particle sizes between 60 meshes and 80 meshes. The reference standard 3-o-caffeoyl-quinic acid and chlorogenic acid with the purity no less than 98% were obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade acetonitrile (Fisher Scientific, USA) and phosphoric acid were used as-received. All other chemicals with analytical grade were obtained from Beijing Reagent Company (Beijing, China). All solvents and non-concentrated sample solutions were filtered through 0.45 μm nylon membrane filters before determination using high-performance liquid chromatography. All aqueous solutions were prepared

by doubly de-ionized distilled water (dd-water) produced by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2 MWLP instrumentation and procedure

A pressurized microwave extraction device, equipped with 70 mL dimethyl sulfoxde vessels, was obtained from Sineo Microwave Chemistry Technology Co. Ltd. (Shanghai, China). The multimode microwave generator has a twin magnetron (2×800 W, (2 450±50) MHz) with a maximum delivered power of 1000 W in 200 W increments. A rotating turnplate ensures homogeneous microwave distribution throughout the extraction vessels. The temperature was indicated by a shielded thermocouple (ATC-300) inserted directly into solvent in the corresponding vessels. The preselected pressure was monitored by a HQ-3051 pressure sensor (Huai An, China) and controlled by feedback to the pulse microwave regulator. The MWLP apparatus is described in Figure 2.



1. Temperature probe 2. Pressure probe 3. Extraction cup 4. Pressure balance hole 5. Sealed lid 6. Bracket 7. Turnplate 8. Temperature indicator 9. Pressure indicator and controller 10. Microwave generator

Figure 2 Schematic of a MWLP instrumentation

In a typical MWLP procedure, 1.00 g fruit powder was accurately weighed, transferred into the extraction vessel, mixed well with a given volume of extraction solvent (liquid/solid ratios 10:1-20:1-30:1-40:1-50:1-60:1) and rigorously soaked for 15 min. The extraction vessel with a sealed lid was fixed into the bracket shown in Figure 2. Then the bracket with the extraction vessel was put on the turn-plate circumrotated at 60 r/min so that it was symmetrically exposed to MW field in the chamber. At the beginning of the procedure, the actual pressure in the extraction vessel began to rapidly increase with some liquid solvent molecules vaporized into vapor phase with the irradiation of MW. As soon as the practical pressure reached the preset pressure (0.5-0.7-0.8-0.9-1.1-1.2-1.3-1.5 MPa), the pressure was maintained by the pulse microwave regulator through feedback regulation of controller (Shanghai, China), the temperature of solvent was simultaneously monitored by a temperature indicator. The procedure was carried out continuously at the preset pressure until the extraction time (1-2-3-4-5-6 min) was over. Subsequently the extraction vessel was cooled spontaneously to room temperature. Naturally, the pressure of the vessel went down to normal atmosphere pressure with the condensation of vaporous solvent in closed vessel. The extract obtained was centrifuged for five minutes at 4 250 × g and transferred into a 50 mL volumetric flask. The sediment was rinsed three times with extraction solvent. The rinsed solvent was coalesced into the flask and the total volume was added to the mark. The mixture in the flask was filtered by a 0.45 μm filter membrane prior to the determination of polyphenols using spectrophotometric method and the analysis of 3-O-CQA using HPLC. The different aqueous ethanol solutions (60%, 70%, 80%, 90%, v/v) were used as extraction solvent.

2.3 UHP

A parallel ultrahigh pressure (UHP) procedure was performed. 1.00 g of fruit powder was mixed well with 40 mL 70% aqueous ethanol and rigorously soaked for 15 min, and then placed into an aluminum foil bag. Subsequently, the bag was sealed after eliminating air and subjected to UHP treatment in the machine UAPF-750MPa (Baotou KeFa New Type Hi-tech Food Machine Co., Ltd. Baotou, China). An optimized UHP condition was obtained by two factors orthogonal design for extraction time (1, 2, 3, 4, 5, 6 min) and extraction pressure (100, 200, 300, 400, 500 MPa). When the UHP extraction was finished, the treatment of the extract was the same as MWLP procedure above mentioned prior to the determination of polyphenols using pectrophotometric method and the analysis of 3-O-CQA using HPLC.

2.4 MAE

A parallel MAE control was carried out in an open

system, i.e., under normal atmosphere pressure. The other operations were the same with MWLP.

2.5 Determination and analysis of extract

2.5.1 Determination and analysis of total polyphenols

The contents of total polyphenols in any extracts obtained were determined by Folin-Ciocalteu (FC) method based on procedures described by several researchers[30,31] with some modifications. Briefly, 1 mL of extract was mixed well with 9 mL of dd-water and one milliliter of FC reagent in turn in a 25-mL volumetric flask. After five minutes, 10 mL of 7% sodium carbonate was added into the flask, the total volume of reaction mixture was diluted to mark (25 mL) with dd-water and mixed well. Promptly, it was kept in the dark at room temperature (23℃) and incubated for 90 min. The absorbance of the resulting reaction mixture was measured at 733 nm with UV-2550 UV-visible Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The standard stock solutions (20, 40, 60, 80, and 100 mg/L) of chlorogenic acid were used for constructing the standard curve, the results of total polyphenols were expressed as g of chlorogenic acid equivalents/per 100 g dry weight (DW±SD) (standard deviation), which represented the average of three independent analysis.

2.5.2 Determination and analysis of 3-O-CQA

Quantification of 3-O-CQA was carried out by Shimadzu LC 2010 HPLC system equipped with a quaternary pump, an on-line solvent vacuum degasser, a variable wavelength detector and an auto sampler with a 10 μL injection loop. Analyses were conducted at 30℃ using a Dikma reverse phase C18 column (250 mm×4.6 mm, 5 μm) protected by a Dikma SecurityGuard™ guard cartridge (4×3.0 mm, Dikma Technologies, Beijing, China). The mobile phase was acetonitrile at 0.1% phosphoric acid-water solution (15:85, v/v) at a flow rate of 1 mL/min, and the effluent was monitored at 327 nm by UV detector. Standard stock solution of 3-O-CQA was prepared at 200 mg/L as the external standard. The data were acquired and processed by Shimadzu software. The method was validated to achieve the satisfactory precision and recovery. To evaluate the precision of the method, six replicate analyses of a standard solution on different days were performed. The precision expressed as relative standard deviation always remained <2% for all. In order to determine accuracy of the HPLC procedure, the known amount (two levels of 20, 40 mg) of 3-O-CQA dissolved in 100 mL extraction solvent (70% ethanol solution) was mixed with 20 g of the same batch of samples. The recovery of 3-O-CQA is between 96.6% and 98.4%. Quantification of 3-O-CQA in extracts was achieved by the peak-area ratio method compared with the external standard reference. The extraction yields of 3-o-caffeoyl-quinic acid (abbreviation: YCQA %) were calculated according to the following equation (1).

$$YCGA(\%) = [C \times V \times N \times 10^{-6} / M] \times 100\% \quad (1)$$

where, C is the concentration of 3-O-CQA in μg/mL; V is the total volume of extraction solution in mL; N is the diluted times; and M is the dry mass of *Chaenomeles speciosa* fruit in g.

Qualitative analysis of 3-O-CQA was performed by a Shimadzu LC-MS-IT-TOF liquid chromatograph mass spectrometer (Kyoto, Japan). The sample solutions were injected directly into the system. The MS/MS parameters were as follows: positive mode; ion spray voltage, 5 500; the first collision energy, 53.0000 volts; scan range m/z, 50-370 Da. The LC-MS Solution software (version 1.5.1) (Shimadzu, Tokyo, Japan) was used for data collection and analysis.

2.6 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 DPS (Version 6.55). The confidence level for statistical significance was set at a probability value of 0.05.

3 Results and discussion

3.1 3-O-CQA as a main polyphenols component in Chaenomeles speciosa

Qualitative analysis results of the extract and 3-O-CQA standard solution by using LC-MS were showed in Figure 3. Panel A and Panel B were the mass spectrum of 3-O-CQA standard product and the extract, respectively. The m/z fragments of standard product

were 73.1, 91.0, 251.1 in turn, those of the extract were 73.1, 91.3, 251.2 in turn. The data were automatically analyzed by Analyst software (version 1.5.1), the results obtained indicated the identical degree matched each other was 96.4%, the molecular weight was 354.20 Da. The liquid chromatograms of 200 mg/L 3-O-CQA standard product and the extract of 20 g dry fruit powder plus 200 mg/L 3-O-CQA were illustrated in Panel C, respectively. It can be seen that the main peak in the extract kept the same retention time with 3-O-CQA standard product. All evidences indicated that 3-O-CQA was a main component in aqueous ethanol extract from *Chaenomeles speciosa* fruit.

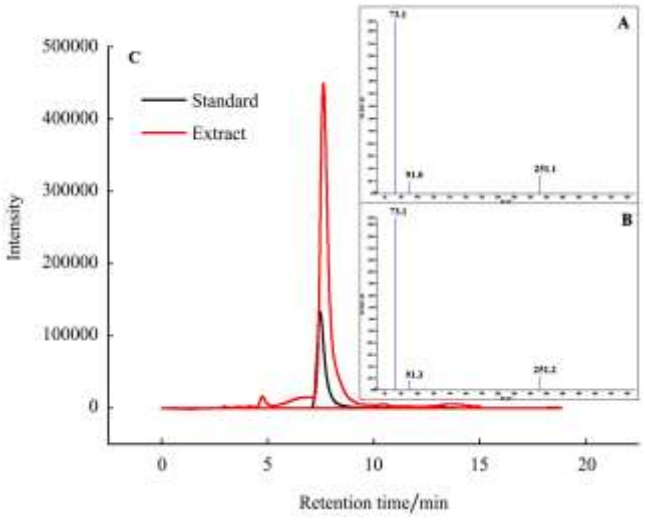


Figure 3 Mass spectrum and liquid chromatograms of 3-O-CQA standard product and the extract from *Chaenomeles speciosa* fruit. "Standard": 200 mg/L 3-O-CQA standard product; "Extract": the extract of 20 g dry fruit powder plus 200 mg/L 3-O-CQA.

3.2 Influence factors and the stepwise multiple quadratic regressions of MWLP

According to the previous reports[19,27,32], liquid/solid ratio, extraction time, ethanol concentration, pressure, MW power and temperature were the influence factors on the extraction of polyphenols. However, for the present MWLP procedure, MW time in boosted pressure stage was not considered as the extraction time, and the extraction time was only pressure holding time. On the other hand, MW was pulsed in feedback regulation style in maintained pressure stage. There are three levels (600, 800, 1 000 W) to be available. In the practice, MW power feedback regulation was performed at 800 W. Another particularity of MWLP was that the extraction temperature was a passive factor, which was decided by pressure and ethanol concentration in sealed vessel. Hence, the influences of liquid/solid ratio, extraction time, ethanol concentration and pressure were investigated. Trials were performed using different liquid/solid ratios (10:1-20:1-30:1-40:1-50:1-60:1) and different aqueous ethanol concentrations (60%-70%-80%-90%, v/v) at different pressures (0.6-0.7-0.8-0.9-1.1-1.2-1.3-1.4 MPa) in the selected time period (1-2-3-4-5-6 min).

3.2.1 Effects of liquid/solid ratio on yields of polyphenols and 3-O-CQA by MWLP

As Figure 4a showed, for the trials with 1 g constant dry fruit powder, there was an apparent increase in polyphenols and 3-O-CQA yields for liquid/solid ratios increasing from 10:1 to 40:1 (mL/g) when other isocratic conditions were fixed, viz., extraction at 1.5 MPa for one minute using 80% aqueous ethanol as solvent, the yields of total polyphenols increased from (5.88±0.26)% to (8.69±0.30)%, meanwhile, the yields of 3-O-CQA was lifted from (1.96±0.43)% to (2.81±0.36)%. It was due to the fact that increasing liquid/solid ratio could improve internal and external solute concentration differential. It is inferred that the diffusion is driven by potential physical gradients, together with gradients due to local dissolved reactions, such as the different temperature and concentration profiles developed in the boundary layer, which consequently prompted diffusion rate of solute particles and made more polyphenols molecules enter solvent at the beginning of the process. However, for liquid/solid ratios higher than 40:1, the yields of polyphenols and 3-O-CQA yield both decreased slightly. It may be developed a dynamic dissolution balance or limit. Simultaneously, the changes of 3-O-CQA yields showed the same trends with those of polyphenols. For an efficient extraction, the amount of solvent should be enough to dissolve the target compounds. On the other hand, it simultaneously should not be too much to dilute the concentration of polyphenols or increase the cost of solvent recovery. For the present extract, the efficient extraction had better set up at liquid/solid ratio 40:1 according to above-mentioned analysis.

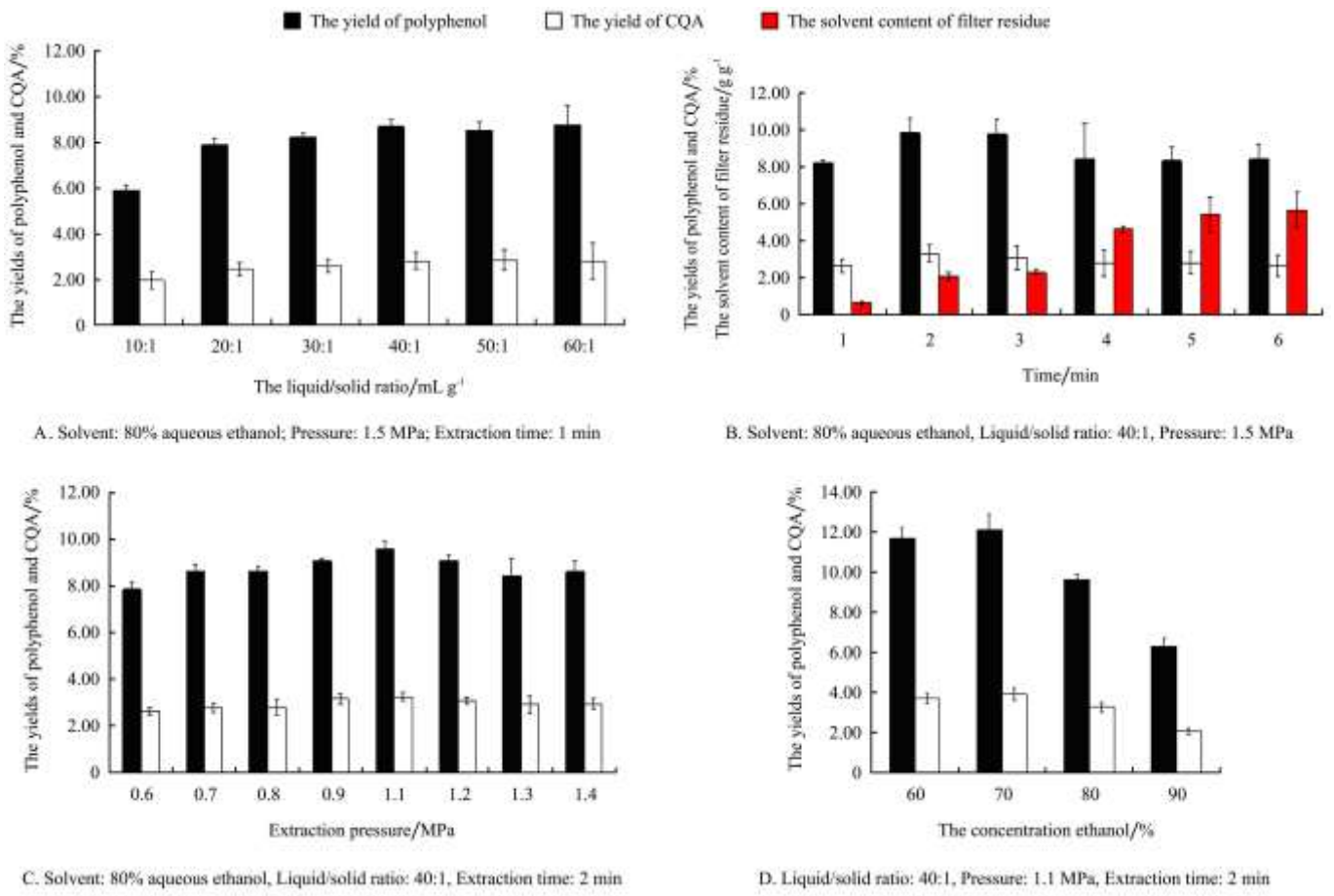
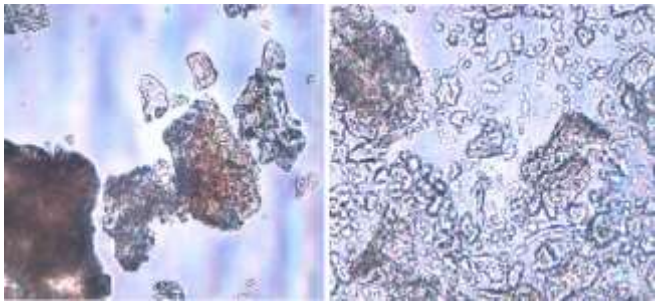


Figure 4 Effects of liquid/solid ratio, extraction time, extraction pressure and ethanol concentration on yields of polyphenols and 3-O-CQA

3.2.2 Effects of extraction time on yields of polyphenols and 3-O-CQA in MWLP

All preselected pressure values could be obtained in 20 s under 800 W microwave radiation in all cases, the pressure-elevated time was not credited to the extraction time. Hence, the extraction time only represented pressure holding time. After a preset pressure to be built up, 1-6 min was taken into account in order to find when the dissolution equilibrium of polyphenols between intra and extra particle solutions formed. As Panel B of Figure 4 showed, when other isocratic conditions were fixed at 1.5 MPa using 80% aqueous ethanol and optimized liquid/solid ratio 40:1 under 800W microwave feedback control, extraction time was from one minute to two minutes, yields of polyphenols and 3-O-CQA increased from (8.26±0.14)% to (9.86±0.80)%, (2.66±0.35)% to (3.29±0.46)%, respectively. However, yields were slightly declined when extraction time was from three minutes to five minutes. From five minutes to six minutes, yields of polyphenols and 3-O-CQA maintained a dynamic equilibrium with the further increase of extraction time, which were relatively stable at (8.4±0.83)%, (2.78±0.61)%, respectively. The cause may be explained by the solvent content per gram filter residue (g/g). As Panel B of Figure 4B illustrated, the solvent content per gram filter residue (g/g) continuously increased with increasing extraction time form one minute to six minutes, which was 0.64 g/g, 2.07 g/g, 2.29 g/g, 4.61 g/g, 5.43 g/g, 5.67 g/g in turn. The data meant the penetrable rate of extraction solvent increased with the increasing extraction time. However, it was interesting that yields of polyphenols and 3-O-CQA did not isochronously increase with the increase of the solvent content per gram filter residue. It may be due to the excessive infiltration and swelling developed when the solvent content per gram filter residue increased to a certain extent, which inversely led to the loss of extraction solvent so that simultaneous loss of the dissolved polyphenols happened. In order to confirm the inference, extracts at 1 min and 2 min were sampled

for electron microscopy analysis. The equiponderant residues were equally resuspended well in 1 mL water, respectively. Three microlitre of each resuspended extract was sampled and fixed on slides with a cover slip. The two slides were examined using a DMBA400 electron microscopy (Motic China Group Co., LTD., China) with a digital imaging system. It showed the residue particles at the end of one minute were kept relatively bigger sizes than those at the end of two minutes presented in Panel B of Figure 5. In the case of two minutes, puny damage and smaller particles were observed, indicating cell walls of the fruit tissues were broken, which was helpful to the release of target components. But with extending of extraction time, capillarity and surface adsorption may become prominent, which caused the rise of the solvent content per gram filter residue and the loss of target components. In addition, as polyphenols were unstable, they could not be heated for a long time by MW. Thus the maximum yields of polyphenols and 3-O-CQA were obtained at two minutes, but not between three minutes and six minutes. Another reason may be that, the pyrolysis of some hemicellulose or lignin may develop a granular structure to increase surface area when extraction time is longer than two minutes, which results in the adsorption of polyphenols and causes the loss of polyphenols and 3-O-CQA. The results indicated that the maximum loss limit of solvent happened within five minutes to six minutes; hence the yield of polyphenols appears to be stable at that time. The analysis above mentioned suggested that two minutes was the best extraction time.



A B

Figure 5 Microscopic pictures of residues treated by MWLP in 1 min and 2 min. Panel A: 1 minute (1 000 times); panel B: two minutes (1 000 times), other isocratic conditions were as the same with Panel B in Figure 4

3.2.3 Effects of extraction pressure on yields of polyphenols and 3-O-CQA in MWLP

Because LP ranges from 0.1 MPa $\leqslant$ p < 1.6 MPa, the univariate analysis of pressure was performed in the range when the other isocratic conditions were fixed at liquid/solid ratio 40:1, 80% ethanol as solvent and extraction time for two minutes under 800 W microwave feedback control, the effect of extraction pressure on yields of polyphenols and 3-O-CQA was investigated. As illustrated in Panel C of Figure 4, the yields of polyphenols and 3-O-CQA increased from 7.84% to 9.61%, 2.61% to 3.25%, respectively, with increasing extraction pressure from 0.6 MPa to 1.1 MPa. However, for pressure higher than 1.1 MPa, the yields of polyphenols and 3-O-CQA yield both decreased slightly and finally stabilized at 1.3-1.4 MPa. The maximum yields of them were achieved at 1.1 MPa. It indicated that the effects of pressure on yields of them were limited. It was reported that there was no significant improvement for the yields of polyphenolic compounds even at high pressure ranging from 300 to 689 bar[33]. In the case the extraction curve show the three distinct regions usually found in the extraction of fruit power using MWLP: constant extraction rate period, falling rate period (transition) and diffusion-controlled rate period. As pressure increases at first, all easily accessible polyphenols around the surface of the particles is dissolved rapidly in the solvent when it was in constant extraction rate period and falling rate period (transition), namely, at this time LP accelerates mass transfer. However, the function of LP may become limited when it was in diffusion-controlled rate period.

3.2.4 Effects of ethanol concentration on yields of polyphenols and 3-O-CQA in MWLP

The effects of ethanol concentration on yields of polyphenols and 3-O-CQA from by univariate analysis were shown in Panel D of Figure 4. When other isocratic conditions were using liquid/solid ratio 40:1 at 1.1 MPa for two minutes under 800 W microwave feedback control, ethanol concentration increased from 60% to 70% (v/v), the yields of polyphenols and 3-O-CQA increased from 11.68% to 12.12%, from 3.71% to 3.90%, respectively. As seen in Panel D of Figure 4,

the highest yield of total polyphenols was obtained with 70% ethanol as solvent. For ethanol concentration higher than 70%, the extraction yield of total polyphenols decreased quickly. It was could be explained that water content has a remarkable effect on partition coefficient. This might be due to the optimal solubility power of polyphenols compounds in 70% ethanol. It indicated that ethanol concentration was one of the critical factors to be taken into consideration in MWLP. Therefore, the optimized ethanol concentration appears to be 70% according to univariate analysis.

3.2.5 Interactive effects of the extraction pressure and aqueous ethanol concentration on yields of polyphenols and 3-O-CQA from Chaenomeles speciosa fruit in MWLP

In the practical MWLP procedure, the extraction temperature was found to be a dependent variable which depended on ethanol concentration and the extraction pressure in MWLP. According to engineering principle, when the pressure rose to the preset value, the temperature of solvent at a given composition should be one-to-one correspondence with the pressure and the given composition. Therefore, the temperature of MWLP was bound by ethanol concentration and pressure. In order to investigate the interactive effects of the extraction pressure and aqueous ethanol concentration on temperature, a series of combinations of extraction pressure and aqueous ethanol concentration was carried out according to the results of the univariate analysis above-mentioned, which was investigated in a range from 60% to 80% for ethanol concentration, from 0.5 to 1.3 MPa for pressure, respectively. Meanwhile, the other conditions were constant (extraction time two minutes and liquid/solid ratio 40:1 according to univariate analysis above-mentioned). The resulting temperatures related to different pressures and ethanol concentrations in MWLP procedure were enumerated in Table 1. Assuming aqueous ethanol concentration to be constant, it can be seen the temperature of solvent would increase with increasing pressure. On the other hand, assuming pressure to be constant, the temperature of solvent decreased with increasing ethanol concentration.

The corresponding yields of polyphenols and 3-O-CQA for the resulting temperatures were also displayed in Table 1. As shown in Table 1, the higher yields were basically obtained at higher temperatures. It can be seen that increasing temperature is beneficial to the extraction of polyphenols. The extraction temperature had great influence on extraction kinetics, solvents viscosities, extraction efficiencies and overall recoveries. Higher temperatures could allow better penetration of solvent in matrix particle, 3-O-CQA was greater solubility in hot water, so heat was favorable for its extraction to a certain extent. Hence the extraction yield of polyphenols increased with increasing temperature at a certain range. However, the chemical change of some compounds may occur at too high temperature that could easily make some molecules contained unsaturated bonds formed isomers, which may reduce the yield of polyphenols. Therefore, there was an optimized temperature to exist in the extraction yield of polyphenols.

Table 1 Results of orthogonal design about the interactive effects of extraction pressure and ethanol concentration on temperature, yields of polyphenols and 3-O-CQA

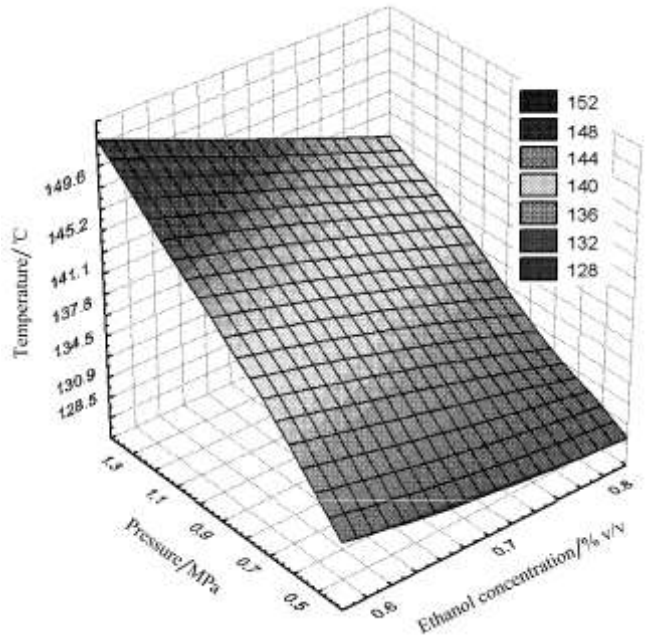
Ethanol concentration /%-v/v	Pressure /MPa	Temperature / ℃	The yield of polyphenols /%	The yield of 3-O-CQA /%
0.6	0.5	132.6±2.6	7.47±0.58	2.37±0.29
0.6	0.7	137.8±3.2	8.38±0.62	2.71±0.35
0.6	0.9	141.8±3.5	9.27±0.48	3.07±0.33
0.6	1.1	145.4±1.8	11.68±0.51	3.67±0.28
0.6	1.3	149.6±3.7	11.5±0.65	3.71±0.47
0.7	0.5	130.1±3.3	8.32±0.38	2.73±0.40
0.7	0.7	134.7±1.4	9.21±0.43	3.07±0.38
0.7	0.9	138.5±2.9	11.6±0.49	3.91±0.57
0.7	1.1	142±1.5	12.12±0.77	3.9±0.33
0.7	1.3	145.2±2.0	12.15±0.32	4.08±0.41
0.8	0.5	128.5±3.1	6.96±0.55	2.21±0.24
0.8	0.7	130.9±2.7	8.65±0.31	2.77±0.22
0.8	0.9	134.5±1.9	9.09±0.76	2.93±0.56
0.8	1.1	138.3±3.6	9.61±0.36	3.25±0.19
0.8	1.3	141.1±3.9	8.46±0.45	2.82±0.27

To fully understand the interactive effects of the extraction pressure and aqueous ethanol concentration on temperature, yields of polyphenols and 3-O-CQA in MWLP. The related mathematical models were developed to describe the preparation of polyphenols and 3-O-CQA from *Chaenomeles speciosa* fruit by MWLP. Quadratic regression had been used with considerable success in industrial applications for many years[34,35].

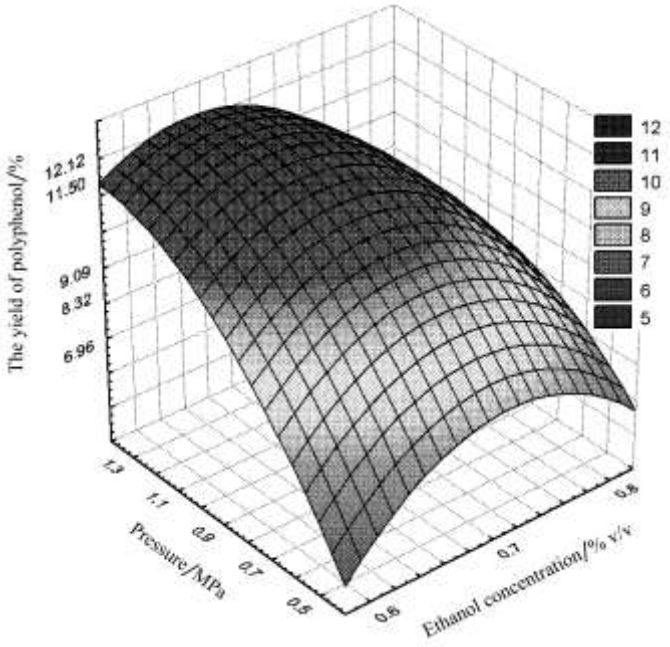
In addition, quadratic regression results always could reflect the interaction of two factors. A stepwise multiple quadratic regression was carried out using the data in Table 1 to establish the relationship among temperature, extraction pressure and ethanol concentration. Equation (2) obtained showed a favorable regression for the temperature (R^2=0.996). In order to illustrate their function relationship, the corresponding curve of Equation (2) was simulated in Panel A of Figure 6. It can be seen the extraction temperature was up-regulated by the pressure and down-regulated by ethanol concentration as Panel A of Figure 6 showed.

$$Y=126.11+34.45X_2-9.66X_1\times X_1-22.62X_1\times X_2 \quad (2)$$

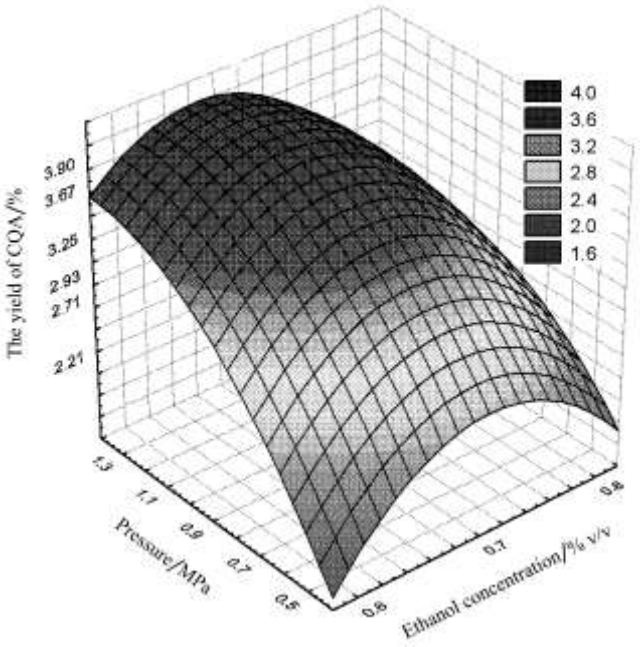
where, Y is temperature (℃); X_1 is aqueous ethanol concentration (v/v, %); X_2 is Extraction pressure (MPa).



A. Simulation curve about the interactive effects of extraction pressure and ethanol concentration on extraction temperature



B. Simulation curve about the interactive effects of extraction pressure and ethanol concentration on polyphenols yield



C. Simulation curve about the interactive effects of extraction pressure and ethanol concentration on 3-O-CQA yield

Figure 6 Simulation curves about the interactive effects of extraction pressure and ethanol concentration on extraction temperature, total polyphenols and 3-O-CQA. Other isocratic conditions were using liquid/solid ratio 40:1 for 2 min under 800W MW feedback control

Similarly, the co-effect of extraction pressure and ethanol concentration on the yield of polyphenols was fitted into a function as Equation (3). The corresponding model plot profile was illustrated as Panel A of Figure 6.

$$Y = -81.80+229.66X_1+27.93X_2-156.1X_1\times X_1-5.93X_2\times X_2-18.50X_1\times X_2 \quad (3)$$

where, Y is yield of total polyphenols (%); X_1 is aqueous ethanol concentration (v/v, %); X_2 is extraction pressure (MPa). 0.0001 of p-value indicated the regression equation was significant, the correlation coefficient 0.96 showed the model equation met well with the relationship between response variable and independent variables. As seen in Equation (3), the first order items of X_1 and X_2 revealed a positive partial correlation, but all second order items were negative partial correlation. The optimum combination for extraction pressure and ethanol concentration was predicted by the model equation, i.e. when ethanol concentration was 65.9% (approximate to 70%), extraction pressure was 1.3 MPa, the maximum yield of polyphenols predicted by Equation (3) was 12.18%. At that time, the temperature predicted by Equation (2) was 147.32 ℃.

In similar condition, the co-effect of extraction pressure and ethanol concentration on the yield of 3-O-CQA was fitted into a function as Equation (4). The regression relationship was similar with that of polyphenols above-mentioned. Its model plot profile was displayed as Panel C of Figure 6

$$Y = -29.98+85.0X_1+8.46X_2-58.7X_1\times X_1-1.99X_2\times X_2-4.85X_1\times X_2 \quad (4)$$

where, Y is extraction yield of 3-O-CQA (%); X_1 is aqueous ethanol concentration (v/v, %); X_2 is extraction pressure (MPa). The correlation coefficient was 0.96, which showed both responses and regressors were functional. The optimum extraction pressure and ethanol concentration predicted by the model Equation (4) was 1.3 MPa and 67% (approximate to 70%), respectively. Under the optimized combination condition, the temperature predicted by Equation (2) was 146.86 ℃, the predicted maximum yield of 3-O-CQA was 4.02%.

Actually as shown in Table 1, when ethanol concentration was 70%, extraction pressure was 1.3 MPa, the result showed the practical temperature was (145.2± 2.0) ℃, the practical yields of polyphenols and 3-O-CQA were 12.15% and 4.08%, respectively, which were very close to the maximum values predicted by the stepwise multiple quadratic regressions. The theoretical results predicted by Equations (2), (3) and (4) very well approximated the practical values, but not the results (70% ethanol and 1.1 MPa extraction pressure) by univariate analysis. Therefore, these optimized results are valid and reliable.

3.3 Comparison of MWLP with UHP and Conventional MAE

To further evaluate the advantages of MWLP method, parallel experiments were carried out with UHP extraction methods. The comparison of the extraction yields of total polyphenols and 3-O-CQA obtained by UHP method with MWLP was shown in Table 3. The yield of total polyphenols by MWLP was only less 0.65% than that by UHP. P-value analyzed by the mean difference test (P<0.05, Student's t-test) was 0.52 revealed that there was no significant difference for the yields of total polyphenols obtained by the two methods. The yield of 3-O-CQA was also similar with that of polyphenols. Ultra high pressure is mainly due to the mechanical effects of the rapid pressure changes, which enhances both solvent penetration into the plant material and the intracellular product release by disrupting the cell walls[14]. The mechanical effects may reach a limit with increasing pressure to a certain extent[33]. In addition, UHP was a non-thermal processing method[19], it is performed around room temperature, hence UHP took longer time to enhance the mass transfer than MWLP because the operation temperature at 25 ℃ of UHP was too lower than that of MWLP, it needed time to make up, because temperature can significantly improve the solvent penetration rate except the role of high pressure. Furthermore, the cost of UHP was obviously higher than that of MWLP because of higher requirements for equipment and higher energy consumption.

Likewise, MAE parallel experiments compared with MWLP were also carried out. As Table 4 showed, p-values were 0.002 and 0.01 for yields of total

polyphenols and 3-O-CQA, respectively. The results indicated the remarkable difference between MWLP and common MAE was so most significant that the yields of total polyphenols and 3-O-CQA of the former were 2.32 folds and 2.47 folds of the later, respectively.

The biggest difference between conventional MAE and MWLP was pressure factor, which directly resulted in the difference of temperature in vessel. The temperature of MAE was (76±2)℃ when ethanol concentration varied from 60% to 90%, which was much lower than those of MWLP. That may be the main reason to cause the differences of the two methods. Another reason may be that MAE confined within a localized area[29]. But for MWLP, LP forced the solvent into the matrix pores and hence should facilitate extraction of analyte, which enabled advantageous dissolution and rapid extractions happened, and which changed the localized situations happened in MAE. In parallel MAE experiment, it was found there always were different solvent losses (in open system), which affected reproducibility. For MWLP, the solvent losses could be ignored.

Table 3 Comparison of MWLP with UHP under respective optimized conditions

Extraction method	MWLP	UHP	Analysis of the mean difference test for the two methods
Optimized conditions	40:1, 70% ethanol, 1.3 MPa, 2 min, 800 W, (2450 MHz)	40:1, 70% ethanol 300 MPa, 25 ℃, 5 min	
Yields of polyphenols	12.15±0.32	12.80±1.56	p = 0.52
Yields of 3-O-CQA	4.08±0.41	4.14±0.76	p = 0.91

Table 4 Comparison of MWLP with conventional MAE

Extraction method	MWLP	MAE	The analysis of the mean difference test for the two methods
Optimized Extraction conditions	40:1, 70% ethanol, 1.3 MPa, 2 min, 800 W, (2450 MHz)	40:1, 70% ethanol, atmosphere pressure, 2 min, 800 W, (2450 MHz)	
Yields of polyphenols	12.15±0.32	5.23±0.88	p = 0.002
Yields of 3-O-CQA	4.08±0.41	1.65±0.79	p = 0.01

4 Conclusions

Compared with UHP, the predominance of MWLP was its shorter extraction time and lower cost of extraction equipment. Compared with conventional MAE, whatever for extraction yield, solvent loss and reproducibility, MWLP was superior to MAE. Therefore, MWLP is a promising extraction technique for fast extraction of solute from plant matrices.

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[References]

[1] Huang M T, Ferraro T. Phenolic compounds in food and cancer prevention. Washington: ACS Publications. 1992. pp. 8-34.

[2] Islam M S, Yoshimoto M, Yahara S, Okuno S, Ishiguro K, Yamakawa O. Identification and characterization of foliar polyphenolic composition in sweetpotato (*Ipomoea batatas L.*) genotypes. Journal of Agricultural and Food Chemistry, 2002; 50(13): 3718-3722.

[3] Kaul A, Khanduja L. Polyphenols inhibit promotional phase of tumorigenesis: relevance of superoxide radicals. Nutrition and Cancer, 1998; 32(2): 81-85.

[4] Robards K, Prenzler P D, Tucker G, Swatsitang P, Glover W. Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry, 1999; 66(4): 401-436.

[5] Ronald L P, Cao G H, Martin A, Martin A, Sofic E, McEwen J, et al. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. Journal of Agricultural and Food Chemistry, 1998; 46(7): 2686-2693.

[6] Tung Y T, Wu J H, Huang C Y, Kuo Y H, Chang S T. Antioxidant activities and phytochemical characteristics of extracts from *Acacia confusa* bark. Bioresource

Technology, 2009; 100(1): 509-514.

[7] Nakatani N, Kayano S, Kikuzaki H, Sumino K, Katagiri K, Mitani T. Identification, quantitative determination, and antioxidative activities of chlorogenic acid isomers in Prune (*Prunus domestica L.*). Journal of Agricultural and Food Chemistry, 2000; 48(11): 5512-5516.

[8] Silva B M, Andrade P B, Valentão P, Ferreres F, Seabra R M, Ferreira M A. Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and jam: antioxidant activity. Journal of Agricultural and Food Chemistry, 2004; 52(15): 4705-4712.

[9] A Barefoot Doctor's Manual. The American translation of the official Chinese paramedical manual. Philadelphia: Running Press. 1977. 640p.

[10] Brown D. Encyclopaedia of Herbs and their Uses. London: DK ADULT. 1995. pp. 69-422.

[11] Duke J A, Ayensu E S. Medicinal plants of China. Michigan: Reference Publications Algonac. 1985. 896p

[12] Liu W, Yang X, Qian J. Analgesic and antiinflammatory activity of ethanol extract of the fruit of *Chaenomeles speciosa*. Journal of Sichuan of Traditional Chinese Medicine. 2004; 22(8): 7-8. (in Chinese).

[13] Welti-Chanes J, Velez-Ruiz J F, Barbosa-Canovas G V. Transport phenomena in food processing. New York: CRC Press. 2010. 568p.

[14] Chen R, Meng F, Zhang S, Liu Z. Effects of ultrahigh pressure extraction conditions on yields and antioxidant activity of ginsenoside from ginseng. Separation and Purification Technology, 2009; 66(2): 340-346.

[15] Zhang S, Chen R, Wang C. Experiment study on ultrahigh pressure extraction of Ginsenosides. Journal of Food Engineering, 2007; 79(1): 1-5.

[16] Wang B, Li D, Wang L, Chiu Y L, Chen X D, Mao Z. Effect of high-pressure homogenization on the structure and thermal properties of maize starch. Journal of Food Engineering, 2008; 87(3): 436-444.

[17] Corrales M, García A F, Butz P, Tauscher B. Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure. Journal of Food Engineering, 2009; 90(4): 415-421.

[18] Bi H, Zhang S, Liu C, Wang C. High hydrostatic pressure extraction of salidroside from Rhodiola Sachalinensis. Journal of Food Process Engineering, 2009; 32(1): 53-63.

[19] Jun X. Caffeine extraction from green tea leaves assisted by high pressure processing. Journal of Food Engineering, 2009; 94(1): 105-109.

[20] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. Supervision regulation on safety technology for stationary pressure vessel. Beijing: Xinhua publishing house, 2010. 76p.

[21] Gao M, Song B Z, Liu C Z. Dynamic microwave-assisted extraction of flavonoids from *Saussurea medusa* Maxim cultured cells. Biochemical Engineering Journal, 2006; 32(2): 79-83.

[22] Rostagno M A, Palma M, Barroso C G. Microwave assisted extraction of soy isoflavones. Analytica Chimica Acta, 2007; 588(2): 274-282.

[23] Zhang W, Xu S. Microwave-assisted extraction of secoisolariciresinol diglucoside from flaxseed hull. Journal of the Science of Food and Agriculture, 2007; 87(8): 1455-1462.

[24] Zhou H Y, Liu C Z. Rapid determination of solanesol in tobacco by high-performance liquid chromatography with evaporative light scattering detection following microwave-assisted extraction. Journal of Chromatography B, 2006; 835(1): 119-122.

[25] Bartolomé L, Cortazar E, Raposo J C, Usobiaga A, Zuloaga O, Etxebarria N, et al. Simultaneous microwave-assisted extraction of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters and nonylphenols in sediments. Journal of Chromatography A, 2005; 1068(2): 229-236.

[26] Fulzele D P, Satdive R K. Comparison of techniques for the extraction of the anti-cancer drug camptothecin from Nothapodytes foetida. Journal of Chromatography A, 2005; 1063(1): 9-13.

[27] Zhang B, Yang R, Liu C Z. Microwave-assisted extraction of chlorogenic acid from flower buds of *Lonicera japonica* Thunb. Separation and Purification Technology, 2008; 62(2): 480-483.

[28] Kaufmann B, Christen P. Recent extraction techniques for natural products: Microwave-assisted extraction and pressurised solvent extraction. Phytochemical Analysis, 2002; 13(2): 105-113.

[29] Liu Z, Han W, Li X. Extraction of chlorogenic acid from *Flos Lonicerae* by icrowave-assisted extraction. Journal of East China University of Science and Technology (Natural Science Edition), 2008; 34(4): 543-546.

[30] Kyoung Chun O, Kim D O. Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums. Food Research International, 2004; 37(4): 337-342.

[31] Singleton V L, Rossi Jr J A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture, 1965; 16(3): 144-158.

[32] Spigno G, De Faveri D. Microwave-assisted extraction of tea phenols: A phenomenological study. Journal of Food Engineering, 2009; 93(2): 210-217.

[33] Hamburger M, Baumann D, Adler S. Supercritical carbon dioxide extraction of selected medicinal plants-effects of high pressure and added ethanol on yield of extracted substances. Phytochemical Analysis, 2004; 15(1): 46-54.

[34] Andres T. Sampling methods and sensitivity analysis for large parameter sets. Journal of Statistical Computation and Simulation, 1997; 57(1-4): 77-110.

[35] Meyer R, Montgomery D. Response surface methodology: Process and product optimization using designed experiment. Hoboken, New Jersey: John Wiley & Sons Inc. 1995. 704p.