Optimization of Microwave-Assisted Extraction of Antihepatotoxic Triterpenoid from *Actinidia deliciosa* Root and Its Comparison with Conventional Extraction Methods

Xinpeng Bai[1,2], Aiyong Qiu[1]* and Junjun Guan[3]

[1]The Key Laboratory of Food Science and Safety, Ministry of Education, Southern Yangtze University, Wuxi 214036, PR China

[2]College of Science and Engineering, South China University of Tropical Agriculture, Hainan 571737, PR China

[3]College of Bioengineering, Henan University of Technology, Zhengzhou 450052, PR China

Summary

A simple and rapid microwave-assisted extraction (MAE) procedure has been developed and optimized for extracting triterpenoids (TTP) from the *Actinidia deliciosa* root. Several variables that could potentially affect the extraction efficiency, namely extraction time (min), ethanol fraction (%), liquid:solid ratio (volume per mass) and microwave power (W) have been optimized by means of response surface methodology (RSM). The results suggest that ethanol concentration and microwave power were statistically most significant factors. The optimal conditions were determined and three-dimensional response surfaces were plotted from the mathematical models. The optimum conditions were ethanol fraction 72.67 %, microwave power 362.12 W, liquid:solid ratio (volume per mass) 15:1 and extraction time 30 min. Under the optimum operating conditions, the percentage of extracted TTP was 84.96 %, and MAE showed significantly higher recoveries than those obtained by the conventional extraction methods (ultrasonic and reflux extraction). In addition, a drastic reduction of the extraction time (30 min *vs.* 8 h) and solvent consumption (15 *vs.* 30) was achieved, comparable with that provided by the reflux extraction as a reference method.

Key words: microwave-assisted extraction (MAE), *Actinidia deliciosa* root, triterpenoid, response surface methodology

Introduction

Actinidia deliciosa (A. Chev.) C. F. Liang *et* A. R. Ferguson is a subfamily of the genus *Actinidia*, which is known as Chinese gooseberry, kiwifruit, yangtao, *etc.* in China, and consists of 55–60 species. The genus *Actinidia* is a dioecious plant and widely distributed on the Asian continent. It is also native to China and most species are cultured in the southwest of China. Out of all species, only *A. deliciosa* is intensely cultivated all over the world.

In addition, the fruit of *A. deliciosa* has been acclaimed for its native and medicinal values.

The root of *A. deliciosa* has been used as a traditional drug in China for a long time and has recently acquired interest due to its attractive potential application in indigenous drugs. It has been reported that folk remedy for adult diseases, such as potent antihepatotoxic, anti--pyrrheal and gingival inflammation, was observed in the root of *A. deliciosa* (*1*). Furthermore, the ethanol ex-

*Corresponding author; Phone/Fax: ++86 510 85 864 893; E-mail: aiyongqiu2001@yahoo.com.cn

tracts of *A. deliciosa* root have been proven to possess anticancer properties *in vitro* (*2*) and *in vivo* (*3,4*). However, few studies have been reported on the effect of ethanol extracts of *A. deliciosa* roots on liver damage caused by hepatotoxicants, and the investigations of antihepatotoxic chemical constituents of *A. deliciosa* root have not been undertaken yet in a systematic way. In our previous studies, we proved that ethanol extracts from roots of *A. deliciosa* had antihepatotoxic effects (*5*), and the main antihepatotoxic chemical constituents were investigated by column chromatography techniques, physico-chemical constants and spectroscopic analysis. Their chemical structures were confirmed by EI-MS, ^{1}H NMR and ^{1}C NMR, and two triterpenoids (TTP), ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) and oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid), were determined (Fig. 1).
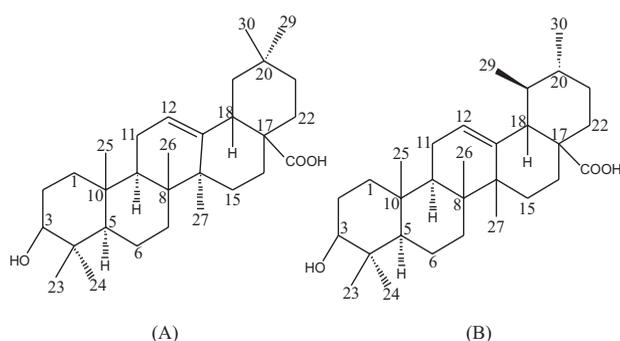

Fig. 1. Structure of oleanolic acid (A) and ursolic acid (B)

The increasing interest in plant bioactive components is accompanied by a need to expand and modify the arsenal of plant-extraction protocols. Conventional methods for the extraction of natural products from plant material, *e.g.* Soxhlet, liquid–liquid, and solid–liquid extractions are characterized by the consumption of large volumes of solvent and energy, lengthy extraction procedures, and the potentially deleterious degradation of labile compounds. In recent years, new extraction techniques have been developed to reduce the volume of solvent needed for extraction, to reduce extraction and extract clean-up time, and to improve the reproducibility of compound recovery. These recent extraction techniques include accelerated solvent extraction (ASE), super-critical-fluid extraction (SFE), extraction with supercritical or subcritical water, and microwave-assisted extraction (MAE) (*6–10*). Most of these methods have similar pros and cons with regard to solvent volume, extraction time and extraction efficiency. The use of SFE or ASE, however, requires greater financial investment, and the presence of water in samples can cause blockage in both techniques.

MAE is a process of using microwave energy to heat solvents and specific molecules in contact with a sample to enhance the extraction of soluble components contained therein. Its main advantages are reduced solvent volume and time consumption, and increased sample throughput (*11,12*). It was reported that MAE was used for the extraction of saponins from ginseng (*6,13*), and glycyrrhizic acid from licorice root (*14*). Moreover, optimization of microwave-assisted extraction for gin-

seng components by response surface methodology was reported by Kwon *et al.* (*15–17*), but the optimized conditions of MAE used in TTP extraction from *A. deliciosa* root have not been reported yet.

When many factors and interactions affect the desired response, response surface methodology (RSM) is an effective tool for optimizing the process, which was originally described by Box and Wilson (*18*). The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process. Response surface methodology has been successfully used to model and optimize biochemical processes (*19,20*), including extraction processes, to obtain effective substances from the stem of *Opuntia ficus-indica* (*21*), anthocyanins from black currants (*22*), or phenolic compounds from wheat (*23*).

As part of our research on antihepatotoxic bioactive constituents of *A. deliciosa* root, the objective of this study was to optimize the MAE conditions for the extraction of TTP from *A. deliciosa* root. RSM was designed to study the effects of extraction time, ethanol concentration, liquid:solid ratio and the microwave power on the yield of total TTP from *A. deliciosa* root. The obtained recoveries were compared with those corresponding to both extracts under reflux and sonication methods.

Materials and Methods

Materials

The roots of *A. deliciosa* were collected from the plants grown at School of Medicine campus, Jishou University (Hunan Province, China) in November, 2004. The plant material was identified by Prof. Liu Zhonghua, Department of Botany, Jishou University. The roots were separated, washed, cut into small pieces, air-dried, ground by a mill (A11 basic, IKA®-Werke GmbH, Germany), sieved, and the particles of $(0.5–1.5) \cdot 10^{-2}$ mm diameter were selected. The standard ursolic acid was obtained from Sigma Chemical Co. (St Louis, MO, USA). All other solvents and chemicals used were of analytical grade and purchased from Shanghai Chemicals Co. (Shanghai, China). The process of MAE was performed in a laboratory scale MW oven (model NJL08-3, Jiequan Microwave Equipment Co., Ltd., Nanjing, China), which was equipped with an inboard thermometer, power control switch, time control switch, stirrer and water condenser.

Experimental design

To optimize the MAE conditions of TTP, a three-step design was used that included fractional factorial design (FFD), steepest ascent design, and central composite design (CCD). FFD was used for screening the most important factor of the MAE conditions of TTP, steepest ascent design was used to determine the direction toward predicted higher responses, and CCD was used to optimize the important factor and maximize the total yield of TTP.

Consequently, a full factorial design (FFD) of 2^4 (*24*) involving 16 experiments was undertaken at random; it was used to obtain a first approximation to the response

surface of the microwave extraction process. On the basis of preliminary experiments for extract yield, we considered four variables that could potentially affect the extraction efficiency, and the following parameters were chosen for screening design: X_1 (ethanol fraction, %), X_2 (extraction time, min), X_3 (liquid:solid ratio, volume per mass) and X_4 (microwave power, W). The values corresponding to the upper (coded as +1) and lower (coded as –1) levels taken by each variable in this design are listed in Table 1. Other factors implicated in the extraction were kept constant: the mass of ground roots (10 g) and final volume of the extract (200 mL).

Table 1. Factors and levels of FFD applied in MAE of TTP

Independent variable	Symbol		Lower	Upper
	Uncoded	Coded	–1	+1
Ethanol fraction/%	X_1	x_1	30	90
Extraction time/min	X_2	x_2	10	50
Liquid:solid ratio (volume per mass)	X_3	x_3	5	25
Microwave power/W	X_4	x_4	200	600

X_1 is the actual value of ethanol fraction, %; X_2 is the actual value of extraction time, min; X_3 is the actual value of liquid::solid ratio, volume per mass; X_4 is the actual value of microwave power, W; x_1 is the coded value of ethanol fraction; x_2 is the coded value of extraction time; x_3 is the coded value of liquid:solid ratio; x_4 is the coded value of power

The correspondence between the coded and uncoded values can be obtained by using the following formula:

$$x_i = \frac{X_i - X_i^0}{\Delta X_i} \qquad /1/$$

where x_i is the coded value, X_i is the corresponding actual value, X_i^0 is the actual value in the centre of the domain, and ΔX_i is the increment of X_i corresponding to 1 unit of x_i. Eqs. 2–5 give x_1 (coded value of ethanol fraction), x_2 (coded value of extraction time), x_3 (coded value of liquid:solid ratio), and x_4 (coded value of power):

$$x_1 = \frac{X_1 - 60}{30} \qquad /2/$$

$$x_2 = \frac{X_2 - 30}{20} \qquad /3/$$

$$x_3 = \frac{X_3 - 15}{10} \qquad /4/$$

$$x_4 = \frac{X_4 - 400}{200} \qquad /5/$$

These factorial designs do not allow exhaustive exploration of a wide range of the experimental space, but they do indicate with minimum experimental effort the possible direction that future experiments should take.

Once the significant factors have been shown, the curvature of the response surface and the accurate position of the optimum extraction of TTP are evaluated by means of second experimental design 3^2 (*24*) with five

central points. The values for the three levels (upper, central and lower) taken by the variables in this new design are presented in Table 2.

Table 2. Factors and levels of CCD applied in MAE of TTP (γ=1.414)

Factors	Levels				
	$-\gamma$	–1	0	+1	$+\gamma$
x_1 fraction/%	55.86	60	70	80	84.14
x_4 power/W	279.30	300	350	400	420.70

x_1 is the coded value of ethanol fraction, x_4 is the coded value of power

Extraction of TTP from *A. deliciosa* root by MAE

In this study, ground roots (10 g) were transferred to the extraction vessels. According to a predesigned experimental trial, the respective volumes of the extracting solvents were added to the vessels. Extractions were carried out under different MAE conditions. The microwave power was adjusted according to the experimental trial. After extraction time was completed, the vessels were allowed to cool to room temperature, the extract was filtered, then diluted to 200 mL with an extractant and the mixture was centrifuged at 4000 rpm for 10 min to separate the fine particulates. A volume of 20 mL of supernatant was taken from the original 200 mL of solution, then transferred to a round-bottomed flask and evaporated to dryness in a rotary evaporator. The flask was cooled in a desiccator and weighted to the constant mass.

Extraction under reflux

Ground roots (10 g) were extracted under reflux with 300 mL of ethanol-water (70 %, by volume, 8 h). The final extracts were filtered, and the following steps were performed as above.

Ultrasonic extraction

Ground roots (10 g) were extracted in an ultrasonic bath (Fritsch, Germany), with 150 mL of solvent (70 % by volume, ethanol in water), for 90 min at room temperature. Following sonication, the final extracts were filtered, and the following steps were performed as above.

Quantification of the total TTP by colorimetric method

In this work, the measurement of total extracted TTP was based on a colour reaction of TTP (*i.e.* sapogenins) with vanillin (*6,25,26*). Ursolic acid, a pentacyclic TTP, was used as the standard. After optimizing all experiment parameters, the content of the total TTP was determined by the colorimetric method (*6*) with slight modifications: the extract obtained as described above was dissolved in 20 mL of methanol; 0.02 mL of methanol solution were applied to TLC plate (silica gel G) for refining. The mobile phase was chloroform-methanol-water at a ratio of 70:30:10. A TTP spot was located with ursolic acid standard, then scratched off, mixed with 0.3 mL of 5 % vanillin/glacial acetic acid (mass per volume) and 1.0 mL of perchloric acid to react for 15 min at 60

°C, and then cooled to ambient temperature using ice bath. The absorbance of the sample was measured at 520 nm after 5 mL of glacial acetic acid were added to the sample. The amount of total TTP (equivalent to ursolic acid, $C_{ursolic\ acid}$ (µg)) in the reaction sample was detected against a calibration curve established with the ursolic acid standard.

Under the optimal colorimetric conditions used in this study, calibration graphs were constructed in the range of 0–16.5 µg. The overall intra- and inter-day variations were less than 4.21 % for the total TTP. The recovery assay of the total TTP was carried out by adding the standard to the treated materials, and the recovery was in the range of 93–115 %. The regression equation and correlation coefficient (R^2) were as follows:

$$C_{ursolic\ acid} = 25.56 \times A_{520} - 0.078,\ R^2 = 0.9937 \quad /6/$$

The yield of total TTP can be estimated by

$$C = \frac{C_{ursolic\ acid} \times V_0}{V_1 \times W} \times 100\ \% \qquad /7/$$

where C is the yield of total TTP (mg/100 mg, dry mass), $C_{ursolic\ acid}$ is the TTP amount from Eq. 6, V_0 is the volume of total TTP solution (mL), V_1 is the test volume of the methanol solution (0.02 mL), W is the test sample mass (weight).

In the present work, the percentage of extracted TTP (Y) was the ratio of TTP in total yield (%):

$$Y = (C_1\ /\ C_0) \times 100\ \% \qquad /8/$$

where Y is the ratio of TTP in total yield (%), C_1 is the yield of TTP from each step of the extraction, C_0 is the total yield of TTP from all steps. C_0 was detected to be 7.65 mg/100 mg.

Data analysis

In all cases, SAS procedures for experimental design (SAS ver. 8.10, SAS Institute Inc., USA) were used to design and analyze the experimental data. Experimental data were fitted to a second-order polynomial model and regression coefficients obtained. The generalized second-order polynomial model used in the response surface analysis was as follows:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum \sum_{i=j=1}^{4} \beta_{ij} X_i X_j \qquad /9/$$

where β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j are the independent variables. The SAS was used to generate response surfaces and contour plots while holding a variable constant in the second-order polynomial model.

Results and Discussion

The screening design

In this experiment, FFD was used to screen the main factors of the extraction process. The 16 experiments of design matrix and the measured average ratio of TTP in total yield are shown in Table 3. The effects of the parameters were analyzed by multiple regression tech-

Table 3. Experimental designs and the results of FFD

Run	x_1	x_2	x_3	x_4	$Y/\%$
1	−1	−1	−1	−1	35.29
2	1	−1	−1	−1	63.65
3	−1	1	−1	−1	37.25
4	1	1	−1	−1	73.58
5	−1	−1	1	−1	38.50
6	1	−1	1	−1	71.21
7	−1	1	1	−1	41.37
8	1	1	1	−1	77.94
9	−1	−1	−1	1	37.25
10	1	−1	−1	1	62.72
11	−1	1	−1	1	36.80
12	1	1	−1	1	60.81
13	−1	−1	1	1	36.12
14	1	−1	1	1	59.24
15	−1	1	1	1	43.14
16	1	1	1	1	60.75

x_1 is the coded value of ethanol fraction, x_2 is the coded value of extraction time, x_3 is the coded value of liquid:solid ratio, x_4 is the coded value of power, Y is the ratio of TTP in total yield (%)

niques. A linear regression equation could be obtained from the regression results of fractional factorial experiment:

$$Y = 52.22625 + 14.01125\ x_1 + 1.72875\ x_2 + \\ + 1.3075\ x_3 - 2.6225\ x_4 \qquad /10/$$

where Y is the response (the ratio of TTP in total yield) and x_1, x_2, x_3, x_4 are the coded values of test variables: ethanol fraction, %; extraction time, min; liquid:solid ratio, volume per mass; and microwave power, W; respectively.

The values of the regression coefficient and a first order equation with coefficient of determination, $R^2=0.98$ were calculated. The results determined using the F-test and p-value showed that ethanol fraction (x_1), and microwave power (x_4) were more significant than other factors at 95 % confidence level (p<0.05), which were not statistically significant. ANOVA showed that the p-value of the model was 0.00049, indicating that the model was statistically significant at 95 % confidence level (p<0.05).

The analysis of these results produced the graph of main effects shown in Fig. 2. Fig. 2 shows that the extraction efficiency was directly proportional to ethanol fraction (x_1) and inversely proportional to microwave power (x_4). The results showed that the ranges previously established in order to vary ethanol fraction (x_1) and microwave power (x_4) did not include the optimum.

The steepest ascent experiment and analysis

In this situation, a directional search method was used to determine the next set of experiments. Steepest ascent experiment uses the sign of the linear effects to determine the direction of the experiment. This experiment started at the centre point of the current design and stretched beyond the current design. Since statistical
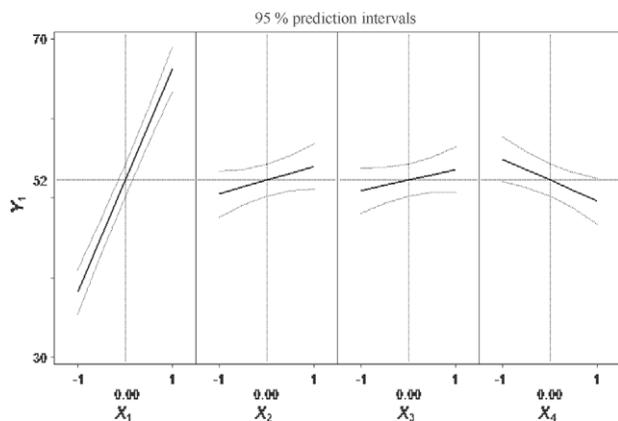
Fig. 2. Influence of the main effects on the ratio of TTP in total yield
The lines indicate the magnitude and sign (increase or decrease) of the variation of the extraction efficiency with the factor level (from low to high)

analysis showed that ethanol fraction (x_1) and microwave power (x_4) were more significant than other factors at 95 % confidence level ($p<0.05$), these two factors were selected for the steepest ascent experiment and the other factors were fixed at their mean level (30 min and 15:1 volume per mass). The results indicate that the extraction efficiency was increased when x_1 increased and x_4 decreased during the first step of the experiment, but after the second step in the experiment, the extraction efficiency decreased (Table 4). This suggested that the highest extraction efficiency was achieved during the second step of experiment.

Table 4. Experimental designs of the steepest ascent and experimental data

No.	1	2	3	4	5
Fraction X_1/%	60	70	80	90	95
Power X_4/W	400	350	300	250	200
Extraction rate Y/%	78.12± ±1.26	83.96± ±2.21	76.35± ±0.95	53.10± ±4.18	45.20± ±2.28

Y is the ratio of TTP in total yield (%)

Central composite design (CCD) and response surface analysis

Using these results, a new central composite design (CCD) was established. Due to their small influence on extraction efficiency, the extraction time (min) and liquid:solid ratio (volume per mass) were eliminated from the new experimental design and set at their mean level (30 min and 15:1 volume per mass, respectively) for the following experiments. The effects of the variables ethanol fraction (x_1) and microwave power (x_4) at five variation levels (Table 2) in the process of extraction were investigated in CCD. A set of 13 experiments was carried out. Table 5 shows the measured experimental results and the design matrix. The following regression equation was obtained after the regression results of CCD:

Table 5. Design matrix and experimental results in the CCD

No.	x_1	x_4	Y/%
1	–1	–1	69.86
2	–1	1	68.28
3	1	–1	73.21
4	1	1	75.16
5	–1.414	0	63.12
6	1.414	0	78.02
7	0	–1.414	74.21
8	0	1.414	82.87
9	0	0	83.92
10	0	0	85.01
11	0	0	84.96
12	0	0	84.34
13	0	0	84.59

Y is the ratio of TTP in total yield (%), x_1= (X_1–70)/10, x_4= (X_4–350)/50

$$Y = 84.564 + 3.913\ x_1 + 1.577\ x_4 - 7.729\ x_1^2 + \\ + 0.883\ x_1\ x_4 - 3.744\ x_4^2 \qquad /11/$$

where Y is the response (the ratio of TTP in total yield) and x_1 and x_4 are coded values of the test variables ethanol fraction and microwave power, respectively.

ANOVA was performed on the experimental results of the CCD to assess the significance of the model with the initial summary of the model statistics given in Table 6. The coefficient of determination, R^2 value, provides a measure of variability in the response values and can be explained by the experimental factors and their interactions. R^2 was calculated to be 0.9243 for the extraction efficiency. This implies that the sample variation of 92.43 % could be attributed to the independent variables and the model did not explain only 7.5 % of the total variations (Table 6). The model's F-value of 17.09 and the p-value of 0.0008 were less than 0.05, implying that the model is significant.

Table 6. Analysis of variance for CCD

Source	DF	Sum of square	Mean square	F-ratio	p-value
x_1	1	122.4762	122.4762	17.0484	0.0044
x_4	1	19.8991	19.8991	2.7699	0.1400
x_1^2	1	415.5513	415.5513	57.8437	0.0001
$x_1\ x_4$	1	3.1152	3.1152	0.4336	0.5313
x_4^2	1	97.5065	97.5065	13.5727	0.0078
Model	5	614.0069	122.8014	17.0937	0.0008
Error	7	50.2882	7.1840	7	
Total	12	664.2951			

The regression model (Eq. 11) allowed the prediction of the effects of the two parameters on the ratio of TTP in total yield. The relationship between independent and dependent variables was illustrated in three-di-

mensional representation of the response surfaces and two-dimensional contour plots generated by the model for the ratio of TTP in total yield (Fig. 3). Two variables were depicted in one three-dimensional surface plot while the two other variables were kept constant to elucidate the interaction of ethanol fraction and microwave power on the ratio of TTP in total yield and determine the level of these two factors, which are required for the optimum ratio of TTP in total yield. The plot showed that the ratio of TTP in total yield reached its maximum value (85.28 %) at a combination of coded level 0.2670 (x_1) and 0.2427 (x_4). The possible extraction conditions of ethanol fraction of 72.67 % and microwave power of 362.12 W could optimize the extraction of total TTP.

In view of this finding, the optimum MAE conditions were: ethanol fraction 72.67 %, microwave power 362.12 W, liquid:solid ratio (volume per mass) 15:1 and extraction time 30 min.

Verification experiments

The suitability of the model equation for predicting the optimum response values was tested using the selected optimal conditions. The five individual experiments were carried out, and the data showed that the ratio of TTP in total yield was 84.96 %. It was found to be in agreement with the predicted one. The above results indicate that the model was adequate under these extraction conditions and was useful to produce the ratio of TTP in total yield.

Comparison of techniques

The conventional extraction of boiling and refluxing the solvent (RE), and ultrasound-assisted extraction (UAE) were carried out in order to compare MAE with traditional extraction methods. In Table 7, optimum experimental conditions for MAE and UAE were compared to RE parameters. Significant benefits in terms of extraction time, solvent consumption, and simultaneous extraction indicated that MAE provides a very good and reliable extraction method.

Conclusion

The antihepatotoxic TTP found in *A. deliciosa* root, ursolic acid and oleanolic acid, could be efficiently extracted using optimized MAE method. From the obtained results, it could be concluded that the optimum
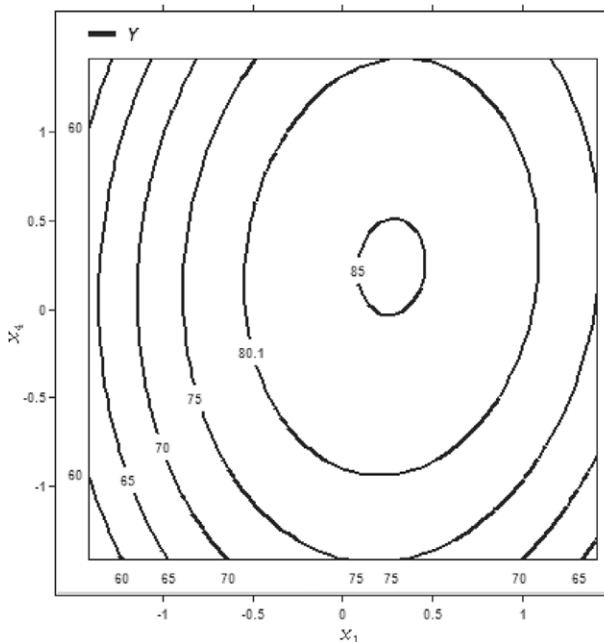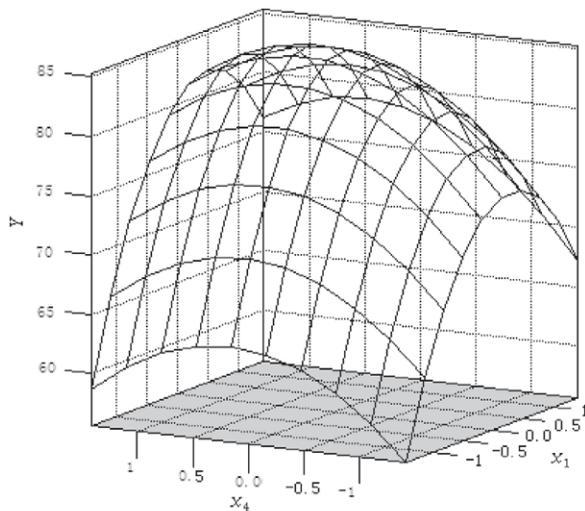

Fig. 3. Responsive surfaces and contours estimated from CCD of $Y=f(x_1, x_4)$

conditions for the ratio of TTP in total yield were ethanol fraction 72.67 %, microwave power 362.12 W, liquid:solid ratio (volume per mass) 15:1 and extraction

Table 7. Comparison of RE parameters employed and the optimum values for UAE and MAE

Parameter	Extraction mode		
	RE	UAE	MAE
Sample size/g	10	10	10
Extraction solvent	70 % ethanol	70 % ethanol	73 % ethanol
Temperature/°C	b.p. of solvent	ambient	b.p. of solvent
Extraction time/h	8	1.5	0.5 (static+dynamic)
Liquid:solid ratio (volume per mass)	30	15	15
No. of samples extracted simultaneously	2	3	5
The ratio of TTP in total yield/%	81.26±1.23	84.14±3.16	84.66±2.85

b.p. boiling point

time 30 min. The experimental yield (84.96 %) was close to the predicted yield (85.28 %). Compared with conventional extraction methods employed in this study, the main advantages of the MAE procedure are low consumption of organic solvent and, particularly, the rapid extraction, which was performed in only 30 min, achieving relatively higher yield for TTP, compared with RE method.

On the basis of these considerations, the optimized MAE method should be favoured for the routine screening analysis of *Actinidia* plants. The potential use of MAE for the efficient extraction of natural products may assist in expediting the chemical analysis and characterization of the biological activities of such compounds. With all these merits, MAE should be considered for wider application in the extraction and purification of phytochemicals from plants. It was found that RMS could be used to optimize MAE process.

References

 1. *Chinese Traditional Medicine Glossary*, Shanghai Science and Technology Publishing Co. (1977) p. 2211 (in Chinese).

 2. Z.G. Zhong, F.F. Zhang, H.S Zhen, Experimental study on the antitumor effects of extracts from roots of *Actinidia deliciosa* in carcinoma cell lines, *Chin. Arch. Trad. Chin. Med. 22* (2004) 1705–1707 (in Chinese).

 3. Y. Li, The experiment of the anticancer functions of 8 kinds of Chinese medicinal herbs, *Lishizhen Med. Mat. Med. Res. 2* (2001) 587–588 (in Chinese).

 4. Y.Y. Zhou, G.S. Tan, Z.X. Xie, Experimental study on the antitumor effects of roots of *Actinidia* in carcinoma cell lines, *Hunan Guid. J. TCMP, 5* (1999) 37–39 (in Chinese).

 5. X.P. Bai, A.Y. Qiu, The liver-protecting effect of extract from the root of *Actinidia deliciosa* in mice, *J. Chin. Inst. Food Sci. Technol. 6* (2006) 247–249 (in Chinese).

 6. W. Vongsangnak, J. Gua, S. Chauvatcharin, J.J. Zhong, Towards efficient extraction of notoginseng saponins from cultured cells of *Panax notoginseng, Biochem. Eng. J. 18* (2004) 115–120.

 7. S.B. Hawthorne, D.J. Miller, Direct comparison of Soxhlet and low- and high-temperature supercritical CO$_2$ extraction efficiencies of organics from environmental solids, *Anal. Chem. 66* (1994) 4005–4012.

 8. O.P. Heemken, N. Theobald, B.W. Wenclawiak, Comparison of ASE and SFE with Soxhlet, sonication, and methanolic saponification extractions for the determination of organic micropollutants in marine particulate matter, *Anal. Chem. 69* (1997) 2171–2180.

 9. M.P. Llompart, R.A. Lorenzo, R.F. Cela, K. Li, J.M.R. Belanger, J.R.J. Pare, Evaluation of supercritical fluid extraction, microwave-assisted extraction and sonication in the

10. determination of some phenolic compounds from various soil matrices, *J. Chromatogr. A, 774* (1997) 243–251.

10. J.R.J. Pare, J. Sigouin, M. Lapointe, Microwave-assisted natural products extraction. *US patent 5 002 784* (1990).

11. C.S. Eskilsson, E. Bjorklund, Analytical-scale microwave-assisted extraction, *J. Chromatogr. A, 902* (2000) 227–250.

12. K. Zohar, G.S. Hilla, Y. Oded, Microwave-assisted extraction of bioactive saponins from chickpea (*Cicer arietinum* L.), *J. Sci. Food Agr. 85* (2005) 406–412.

13. J.H. Kwon, J.M.R. Bélanger, J.R.J. Paré, V.A. Yaylayan, Application of the microwave-assisted process (MAP^{TM}) to the fast extraction of ginseng saponins, *Food Res. Int. 36* (2003) 491–498.

14. X. Pan, H. Liu, G. Jia, Y.Y. Shu, Microwave-assisted extraction of glycyrrhizic acid from licorice root, *Biochem. Eng. J. 5* (2000) 173–177.

15. J.H. Kwon, J.M.R. Bélanger, J.R.J. Paré, Optimization of microwave-assisted extraction (MAP) for ginseng components by response surface methodology, *J. Agric. Food Chem. 51* (2003) 1807–1810.

16. J.H. Kwon, J.M.R. Bélanger, J.R.J. Paré, Effect of ethanol concentration on the efficiency of extraction of ginseng saponins when using a microwave-assisted process (MAP^{TM}), *Int. J. Food Sci. Technol. 38* (2003) 615–622.

17. J.H. Kwon, G.D. Lee, K. Kim, J.M.R. Bélanger, J.R.J. Paré, Monitoring and optimization of microwave-assisted extraction for total solid, crude saponin, and ginsenosides from ginseng roots, *Food Sci. Biotechnol. 13* (2004) 309–314.

18. G.E.P. Box, K.G. Wilson, On the experimental attainment of optimum conditions, *J. Roy. Stat. Soc. 13* (1951) 1–45.

29. I.H. Boyacı, A new approach for determination of enzyme kinetic constants using response surface methodology, *Biochem. Eng. J. 25* (2005) 55–62.

20. S.G. Ozkal, M.E. Yener, L. Bayındırlı, Response surfaces of apricot kernel oil yield in supercritical carbon dioxide, *Lebensm. Wiss. Technol. 38* (2005) 611–616.

21. G.D. Lee, J.O. Kim, G.J. Loo, J.H. Kwon, Optimum conditions for the extraction of effective substances from the stem of *Opuntia ficus-indica, Food Sci. Biotechnol. 14* (2005) 190–195.

22. J.E. Cacace, G. Mazza, Optimization of extraction of anthocyanins from black currants with aqueous ethanol, *J. Food Sci. 68* (2003) 240–248.

23. C.L. Pathirana, F. Shahidi, Optimization of extraction of phenolic compounds from wheat using response surface methodology, *Food Chem. 93* (2005) 47–56.

24. G.E.P. Box, N.G. Hunter, J.S. Hunter: *Statistics for Experimenters. An Introduction to Design, Data Analysis and Model Building*, Wiley, New York, USA (1978).

25. S. Hiai, H. Oura, T. Nakajima, Color reaction of some sapogenins and saponins with vanillin and sulfuric acid, *Planta Med. 29* (1976) 116–122.

26. W.A. Oleszek, Chromatographic determination of plant saponins, *J. Chromatogr. A, 967* (2002) 147–162.