Journal of Applied Botany and Food Quality 88, 152 - 163 (2015), DOI:10.5073/JABFQ.2015.088.022

[1]College of Quartermaster Technology, Jilin University, Changchun, China
[2]Laboratory of Nutrition and Functional Food, Jilin University, Changchun, China

Optimization of ultrasound-assisted extraction of polyphenols from *maize filaments* by response surface methodology and its identification

Lv Lingzhu[1], Wei Lu[1], Chen Dongyan[1], Liu Jingbo[2], Lin Songyi[2], Ye Haiqing[1], Yuan Yuan[1*]

(Received February 16, 2015)

Summary

Maize filaments (MF) are the outer thread-like part of corn, which are widely used in traditional and official medicine. Besides, MF polyphenols are important secondary metabolites in MF which have their significant biological activities. However the existing MF is underutilized. This study aimed to raise the comprehensive utilization of MF and to increase the economic value and benefit of MF. In the current study, central composite design (CCD) was first used to investigate the effect of process variables on polyphenols contents from MF by ultrasound-assisted extraction (UAE). Results showed that the obtained optimal UAE conditions were as follows: extraction power of 520.01 W, ethanol concentration of 61.08 %, and a solvent-to-material ratio of 26.83 mL/g for polyphenols extraction. These experimental values under optimal conditions were consistent with the predicted values with polyphenols content of 7.1±0.015 mg/g. Sixteen phenolic compounds, including gallic acid, catechin, epicatechin, hyperoside were identified in MF polyphenols extractions by HPLC-MS/MS method. Antioxidant activity assays demonstrated that MF phenols had excellent scavenging ability for ABTS radicals, •OH, DPPH radicals and $•O_2$-, and 42.56 ± 1.24 % of lipid oxidation inhibition.

Introduction

Maize filaments (MF), as an indispensable part of corn, have high pharmaceutical values, as they are mild diuretic, urinary demulcent, which passes stones and gravel from the kidneys and the urinary bladder; these filaments function against benign prostatic hyperplasia, cystitis, gout, chronic nephritis, and similar ailments (British Herbal Pharmacopoeia, 1996; Czygan and Maydis, 1997; Maksimović et al., 2005; Stagos et al., 2012). In previous studies, a series of phenolic compounds such as rutin, quercetin, epicatechin, vanillic acid, benzoic acid, syringic acid, and gallic acid have been identified and isolated from MF (Chen et al., 2010; Pedreschi and Cisneros-Zevallos, 2007).

Phenols (Hydroxybenzenes) and polyphenols (containing two or more phenol groups) are ubiquitous in plant foods. Phenols have been traditionally considered as "anti-nutrients" because these substances could reduce the digestibility of proteins and subsequently increase fecal nitrogen excretion (Alzueta et al., 1992; Longstaff and Mcnabb, 1991). Pharmacological investigations have shown that phenolic compounds have an important function in human health (Vijaya Kumar Reddy et al., 2010), including anti-platelet and antithrombotic actions (Lu et al., 1999), anti-cancer activity (Sun et al., 2011; Stagos et al., 2012), inhibition of HeLa cell multiplication (Sun et al., 2010), and antioxidant activity (Gladine et al., 2007; Lee et al., 2010; Wang et al., 2012).

Conventional extraction methods such as maceration, boiling, refluxing, heating, and Soxhlet extraction are used for extraction of biologically active polyphenols from various plants. However, these conventional extraction methods are generally time-consuming and low efficient of the yield of extract (Li et al., 2005; Ma et al., 2008). In the recent years, novel extraction techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE) have been developed to increase efficiency of the extraction process with respect to time and solvent consumption as well as the extraction yield and active compound concentration (Yang and Zhai, 2010; Liu et al., 2011; Skalicka-Woźniak et al., 2011; Pan et al., 2012). Among these methods, UAE method is advantageous for polyphenols extraction due to the process simplicity, low working temperatures (from 40 to 60 °C, high recovery rate, low oxidation loss, high energy-saving efficiency, and high extraction yield (Pan et al., 2012)). The mechanism of UAE involves mechanical and cavitation efficacies which can result in disrupting the cell wall, reducing the particle sizes, and enhancing the mass to transfer across the cell membrane (Hossain et al., 2012).

Phenols and polyphenols very soluble in a variety of solvents. The most common solvents are aqueous mixtures with methanol, ethanol and acetone. Methanol and acetone have toxic effects on the human body, besides, the production cost is high, so ethanol is always used as a extraction solvent.

Response surface methodology (RSM) is a modelling tool used to optimize the extraction conditions by evaluation of multiple parameters and their interaction effects based on quantitative data. Therefore RSM can not only effectively optimize complex extraction procedures statistically, but can also reduce the number of experimental trials (Brachet et al., 1999). RSM has been often used to optimize UAE of phenolic compounds from different plant sources (Liu et al., 2011; Skalicka-Woźniak et al., 2011; Hossain et al., 2012). Li et al. (2015) have evaluated an ionic-liquid-based MAE and UAE method for the extraction of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one and 6-methoxy-benzoxazolin-2-one from etiolated maize seedlings. However, there are no published reports on the UAE of polyphenols from MF as well as on use of RSM for its optimization of UAE.

The aim of the present work was to use RSM to establish the optimum parameters of UAE of MF polyphenols and their identification. Three factors with three level central composite designs were used to optimize and investigate the effects of ultrasound, ethanol concentration, and solid-to-liquid ratio of UAE on the maximum yield of polyphenols from MF. HPLC-MS/MS method was used to identify the main phenol compounds in MF extracts obtained by UAE.

Materials and methods

Plant materials

The plant materials with Poaceae female flowers used in this experiment were mature corn silk of *Zea mays* L. exhibiting a regular growth pattern, which were gathered from corn fields in October 2012. Collected corn silks were dried in a shaded and well-ventilated

* Corresponding author

place, homogenized by a blender (DS-1, Shanghai Precision Instruments Co., Ltd, Shanghai, China), and sieved through a 100 meshes sieve. The powder was stored in sealed polyethylene bags at 4 ºC.

Chemicals and reagents

Absolute ethanol and sodium bicarbonate were purchased from Beijing Chemical Works (Beijing, China). Pyrogallic acid was purchased from the Tianjin Guangfu Fine Chemical Research Institute. Folin-Ciocalteu (FC reagent) was purchased from Shanghai Sunny Chemical Industry Tech Co., Ltd. Deionized water was purified by Milli-Q system (Millipore Corp., Bedford, MA, USA). All of the organic solvents used in the study were of analytical grade.

UAE of polyphenols from MF

MF powder (1.000 g) was placed in a capped tube and mixed with ethanol. Extraction was performed using an ultrasonic cell crushing apparatus [JY92-2D, 20 kHz to 25 kHz, 1800 W (MAX), Ningbo New Cheese Biological Technology Co., Ltd., Zhejiang, China]. After extracting, the sample was centrifuged at 4000 rpm/min for 15 min to collect the supernatant. The MF powder was extracted twice with the same method, and the supernatant of the three extracts were collected together into a 50 mL dark volumetric flask. The sample solution was evaporated using the RE-52A-type rotary evaporator apparatus enrichment (RE-52AA, Shanghai Yarong-Biochemical Instruments Factory, Shanghai, China), and then dried with the vacuum freeze-drying system (ALPHA 1-2, Martin Christ GmbH Germany).

RSM experimental design for optimal extraction conditions

Prior to RSM design, the single factors influencing the extraction yields have been conducted and suitable ranges of each independent variable have been obtained. The results showed that three main factors affect the extraction efficiencies, including the power of ultrasound (W, X_1), ethanol concentration (%, X_2), and solvent-to-material ratio (mL/g, X_3). In the RSM design, these factors were used as independent variables for optimizing extraction conditions. According to the single factors experiments, we determined the variables and their ranges were as following, extraction power of ultrasound (400 W to 600 W), ethanol extraction concentration (50% to 70%), and solvent-to-material ratio (20:1 mL/g to 40:1 mL/g). Temperature was not considered in this study since the sample was processed at 35 ºC to avoid the degradation of temperature-sensitive polyphenols compounds. In this study, the experiments were performed based on a central composite design (CCD).

The coded values of the experimental factors and their levels in CCD were shown in Tab. 1. The complete design was performed randomly and consisted of twenty combinations, including six replicates at the central point (Tab. 2). The data from CCD were analyzed by multiple regressions to fit the following quadratic polynomial model (Prakash et al., 2013):

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_{ij} + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum \sum_{j=2}^{k} \beta_{ij} X_i X_j + \ell_i \quad (1)$$

Tab. 1: Levels of variables for the experimental design.

Symbols	Independent variables	-1	0	+1
X_1	Ultrasound power (w)	400	500	600
X_2	Ethanol concentration (%)	50	60	70
X_3	Solvent-to-material ratio (ml/g)	20:1	30:1	40:1

where Y is the response, X_i and X_j are variables (i and j range from 1 to k), β_0 is the model intercept coefficient, β_j, β_{jj}, and β_{ij}, are the interaction coefficients of linear, quadratic, and second-order terms, respectively, k is the number of independent parameters (k=4 in this study), and e_i is the error (Prakash et al., 2013).

Determination of polyphenols

Polyphenols were determined by Folin-Ciocalteu (FC) procedure according to the method of Hagerman et al. (2000) with slight modifications. In brief, 1.0 mL of polyphenol extract was mixed with 2.5 mL of FC reagent. Then 2.5 mL of sodium bicarbonate (10%, w/v) was added to the mixture. The volume was increased to 25 mL by adding distilled water. The solution was allowed to stand for 1 h at room temperature and the absorbance was measured by spectrophotometry at 785 nm. The results were expressed as μg gallic acid equivalent per g sample. Polyphenols content can be calculated using the following equation:

$$\text{Polyphenolyield (mg/g)} = C \times N \times 25 \times 10^{-3}/m \quad (2)$$

where C is the sample fluid concentration of the polyphenols calculated based on gallic acid standard curve, μg/mL ; N is the diluted amount; and m is the weight of MF powder, g.

Antioxidant activity analysis of MF polyphenols extractions

The antioxidant activity of MF polyphenols extractions was evaluated by scavenging ABTS radical, hydroxyl radical (•OH), DPPH radical, and superoxideanion ($\bullet O_2^-$), which were followed by the method of Yuan et al. (2013). The lipid oxidation inhibition assay of MF polyphenols extraction was measured using the similar method of Muñiz-Márquez et al. (2013).

Identification of the main polyphenols compounds by HPLC-MS/method

This analysis was conducted according to method described by Wang et al. (2008). A Waters e2695-TQD system (Waters Corporation, USA), consisting of Waters e2695 HPLC system (including vacuum degasser, binary pump, autosampler, column oven), Waters TQ detector (TQD) and Waters 2489 ultraviolet/visible light detector were used for separation and identification of polyphenols compounds.

Chromatographic separations were carried out using a Waters Symmetry C_{18} column (4.6×150 mm, 5 μm). The mobile phase was constituted of 0.1% glacial acetic acid in water (solvent A) and methanol (solvent B). The gradient conditions used was run as described hereafter: from 0 to 10.0 min, a linear gradient from 30% solvent B to 45% solvent B; from 10.0 to 12.0 min, a linear gradient from solvent B at 45% to 70%; from 12.0 to13.0 min, solvent B at 70% to 0%; and from 13.0 to15.0 min, a linear gradient of solvent B from 0% to 30%. The column was reconditioned at 30% solvent B from 15.0 to 20.0 min. The column temperature was set at 40 ºC with a solvent flow rate of 0.3 mL/min. A total of 5 μL of sample was injected into the column for HPLC-MS/MS analysis. The detection was carried out on a Waters TQD mass spectrometer from Waters (Waters Corporation, USA) equipped with an electrospray ionization source in positive mode. The ionization parameters were:

nitrogen sheath gas flow, 10 / min; spray voltage, 30 psi; capillary temperature, 300 ºC; capillary voltage, 4000 V; and tube lens voltage, 80 V. The full scan mass spectrum in the range of m/z 30 to 3000 amu was measured.

Tab. 2: Central composite design (CCD) plan in coded value and results of experiment

Treatment	Factors			Yield of polyphenols (mg/g)	
	X_1 Power(w)	X_2 Ethanol concentration (%,V/V)	X_3 Solid-liquid ratio (mL/g)	Experimental	Predicted
1	-1	1	1	5.7768	5.8460
2	-1	-1	-1	5.8448	6.1089
3	0	1.6818	0	5.7072	5.9020
4	0	0	-1.6818	7.3070	7.2700
5	1	-1	-1	6.2346	6.4154
6	1	1	1	6.4925	6.4784
7	-1	1	-1	5.8459	5.7979
8	0	0	0	7.9686	7.9974
9	0	0	0	7.9789	7.9974
10	0	0	0	7.9812	7.9974
11	1.6818	0	0	6.4789	6.4016
12	-1.6818	0	0	5.8884	5.6121
13	0	0	0	7.9777	7.9974
14	0	0	0	7.9914	7.9974
15	0	-1.6818	0	5.7080	5.1596
16	1	1	-1	6.9063	6.7742
17	0	0	1.6818	6.6761	6.3595
18	-1	-1	1	4.9399	5.3220
19	1	-1	1	4.9866	5.2846
20	0	0	0	8.0257	7.9974

Statistical analysis

The experimental results of the response surface design were analyzed by Design-Expert 8.0.6 software (Trial version, State-Ease Inc., Minneapolis, MN, USA). The modeling was started with a quadratic model including linear, squared, and interaction terms. Significant terms in the model for each response were found by analysis of variance (ANOVA) and the significance was judged by the F-statistic calculated from the data. The experimental data was evaluated with various descriptive statistical analyses such as *P* value, *F* value, degrees of freedom (DF), sum of squares (SS), and mean sum of squares (MSS) to reflect the statistical significance of the developed quadratic mathematical model. After fitting the data to the models, the generated data were used for plotting response surfaces and contour plots. *P*-values ≤ 0.05 were considered as statistically significant. All of the experiments were conducted in triplicate unless specified otherwise.

Results

Fitting the response surface models

UAE was conducted in 20 runs to study the effect of different variables on the amount of polyphenols extracted from MF. The polyphenols responses of the experimental design were presented in Tab. 2. The decoded values of independent variable for the experiment were also presented. Polyphenols content of MF extract varied from 4.3183 to 7.0206 mg per g dried mass based on different extraction conditions.

Multiple regression analysis and Pareto analysis of variance (ANOVA) were used to check the adequacy and fitness of the developed models and results of ANOVA were given in Tab. 3. Considering the single factor test results, the response surfaces analysis results, and the experimental results, we summarized the test data result of linear regression and binomial fitting analysis in Tab. 3.

The results showed that the response of worth rate (Y) model was highly significant. An item ultrasound power (X_1) and ethanol concentration (X_2) was significant and solvent-to-material ratio (X_3) was extremely significant. The second order items were reached extremely significant level.

To calculate the polyphenols content in MF (Y) encoded by independent variables ultrasound power (X_1), ethanol concentration (X_2), and solvent-to-material ratio (X_3), we used the quadratic multinomial regression equation as follows:

$$Y = 34.11660 + 0.057147X_1 + 0.80761X_2 + 0.12396X_3 + 0.000146625X_1X_2 + 0.001828X_2X_3 - 0.0000616296X_1^2 - 0.00763692X_2^2 - 0.00366157X_3^2 \quad (3)$$

The ANOVA results and regression coefficients were presented in Tab. 3 and Tab. 4, indicating that the contribution of the quadratic model was significant ($p < 0.05$). The ''fitness'' of the model was investigated using the lack-of-fit test ($p > 0.05$), indicating the suitability of models to predict variations accurately (Prasad et al., 2011).

The F value of the model was 25.89 and F statistical analysis shows that the model of "Prob > F" return value was < 0.05, indicating that the model was significant. The proposed item is not significant. In these test results, X_1, X_2, X_3, X_1^2, X_2^2, and X_3^2 were significant.

Tab. 3: Results of analysis of variance of the primarily established quadric regression mode.

Source	Squares	df	Square	Value	Prob > F	Remark
Model	15.85	9	1.76	25.89	<0.0001	**
X_1	0.58	1	0.58	8.48	0.0155	*
X_2	0.51	1	0.51	7.50	0.0209	*
X_3	0.77	1	0.77	11.28	0.0073	**
X_1X_2	0.17	1	0.17	2.53	0.1429	
X_1X_3	0.05	1	0.05	0.67	0.4332	
X_2X_3	0.27	1	0.27	3.93	0.0756	
X_1^2	5.47	1	5.47	80.46	<0.0001	**
X_2^2	8.41	1	8.41	123.54	<0.0001	**
**						
X_3^2	1.93	1	1.93	28.40	0.0003	
Residual	0.68	10	0.07			
Lack of Fit	0.68	5	0.14	3.63	0.16	
Pure Error	0.001565	5	0.00031			
Cor Total	16.53	19				

df means degrees of freedom; * Showed significant difference, $p< 0.05$; * * showed very significant difference, $p< 0.01$;

Although X_3, X_1^2, X_2^2, and X_3^2 were very significant, the interactive items were not significant. This result shows that the effect of the three factors on the polyphenols extraction yield was summarized as follows: ultrasonic power > solid-to-liquid ratio > ethanol concentration.

Effect of extraction parameters on polyphenols content

The response surface and contour plots of the effects of extraction parameters on the polyphenol content were presented in Fig. 1 and Fig. 2.

As shown in Fig. 1A, the efficiency of ultrasound power on the polyphenols was evaluated and the results showed that the yield increased with the increase of ultrasound power. It can be concluded that maximum polyphenols extraction could be achieved when the ethanol concentration and ultrasonic power were 61.08% and 520.01 W, respectively. The polyphenols yield increased with an increase of ethanol concentration from 43% to 61.08%, while decreased when the ethanol concentration was above 61.08%. As for the effect of ultrasound power on the polyphenols yield, the yield increased with prolonged the extraction power from 400 to 520.01 W, and decreased after 520.01 W.

The interaction effect of extraction power and solvent-to-material ratio on the polyphenols was presented in Fig. 1B. The increase extraction yield of polyphenols was observed with an increase solvent-to-material ratio from 13 to 26.83. Further increase of solvent-to-material ratio resulted in decrease of the polyphenol content.

The interaction effect of ethanol concentration and the solvent-to-material ratio on the polyphenols was also presented in Fig. 1C. It was found that maximum polyphenols yield is achieved when the ethanol concentration was 61.08% and the solvent-to-material ratio was 26.83 mL/g. Ethanol volume fraction and the material liquid have significant interaction effect, the phenolic yield was first increased with the increase of the ethanol volume fraction and solid-liquid ratio, and then decreased.

Verification of predictive models

An optimum UAE conditions for obtaining maximum MF polyphenols content in the extract were determined. All the factors and responses with the respective high-limit and low-limit experimental region have to satisfy the criteria defined for the optimum operations were stated in Tab. 1. As shown in Tab. 2, the predictive values matched well with experimental values obtained using optimum extraction parameters, which were confirmed with a good regression coefficient ($R^2 = 0.9588$). Applying the methodology of desired function, the optimum level of various parameters was obtained and it indicated that extraction power of 520.01 W, ethanol concentration of 61.08% and solvent-to-material ratio of 26.83 mL/g given a phenol content of 7.1±0.015 mg/g. These optimize conditions could be considered as optimum as well as feasible conditions.

Antioxidant activity of MF polyphenols extractions

The antioxidant activity of MF polyphenols extractions was evaluated using five assays and the results were shown in Tab. 5. The ABTS radical scavenging ability, •OH-scavenging ability, DPPH radical scavenging ability, $\bullet O_2^-$ scavenging ability and lipid oxidation inhibition increased with increased MF polyphenols extractions concentration. The IC_{50} values of various antioxidant assays were used to evaluate the antioxidant activity of MF polyphenols extractions concentration. The IC_{50} values of MF polyphenols extractions concentration for the ABTS radical, •OH, DPPH radical and $\bullet O_2^-$ were 0.7228, 0.301, 12.79, and 0.010 mg/mL, respectively. Lower IC_{50} values indicated that MF polyphenols extractions had excellent antioxidant properties. Among the assays, the best inhibiting capacity of MF polyphenols was observed in $\bullet O_2^-$ (0.010 mg/mL). In lipid oxidation inhibition assay, the general ability of extracts to prevent lipid oxidant was tested, and results showed 42.56 ± 1.24% of lipid oxidation inhibition. The results showed that the polyphenols compounds extracted from the MF also have ability to inhibit lipid oxidation.

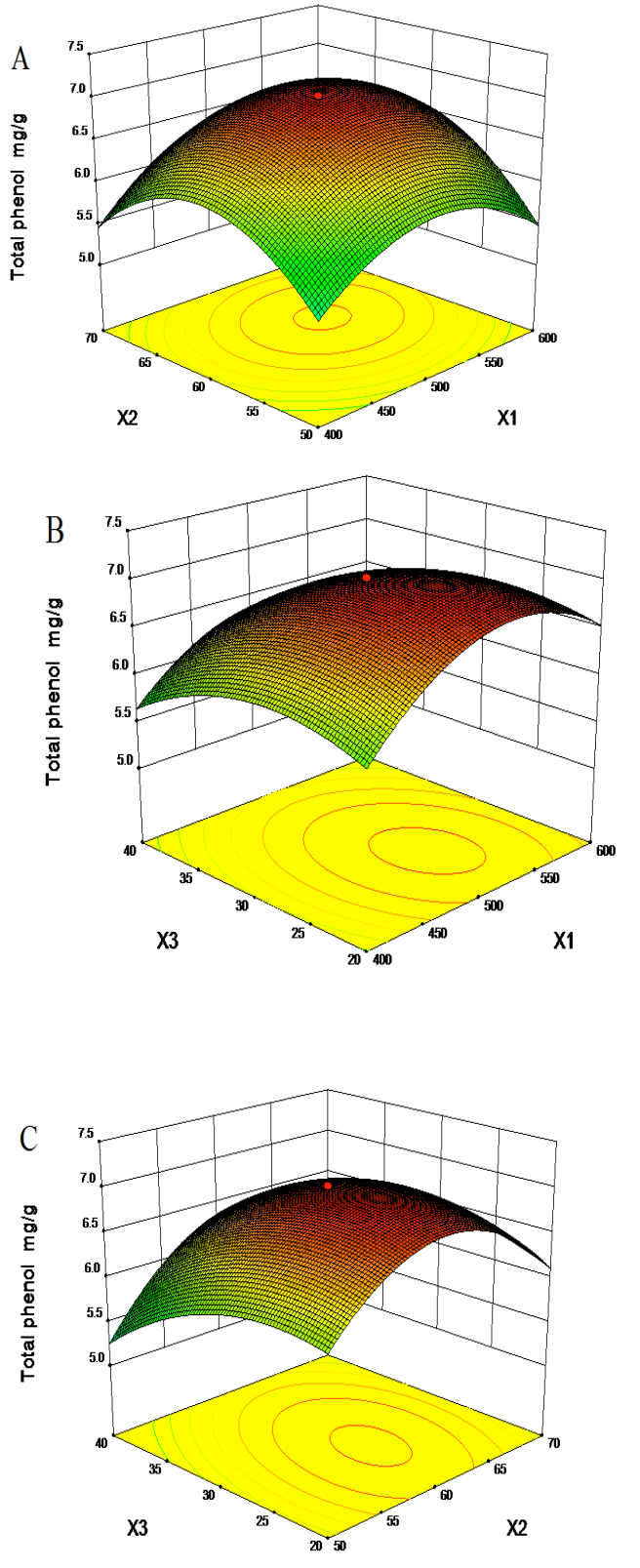


Fig. 1: Response surface for the effect of independent variables on polyphenols extraction yield.
(A) Response surface graph showing interaction between ultrasonic power (X_1/W) and ethanol concentration (X_2/%); (B) response surface graph showing interaction between ultrasonic power (X_1/W) and solvent-to-material ratio (X_3/mL/g); (C) response surface graph showing interaction between ethanol concentration (X_2/%) and solvent-to-material ratio (X_3/mL/g).

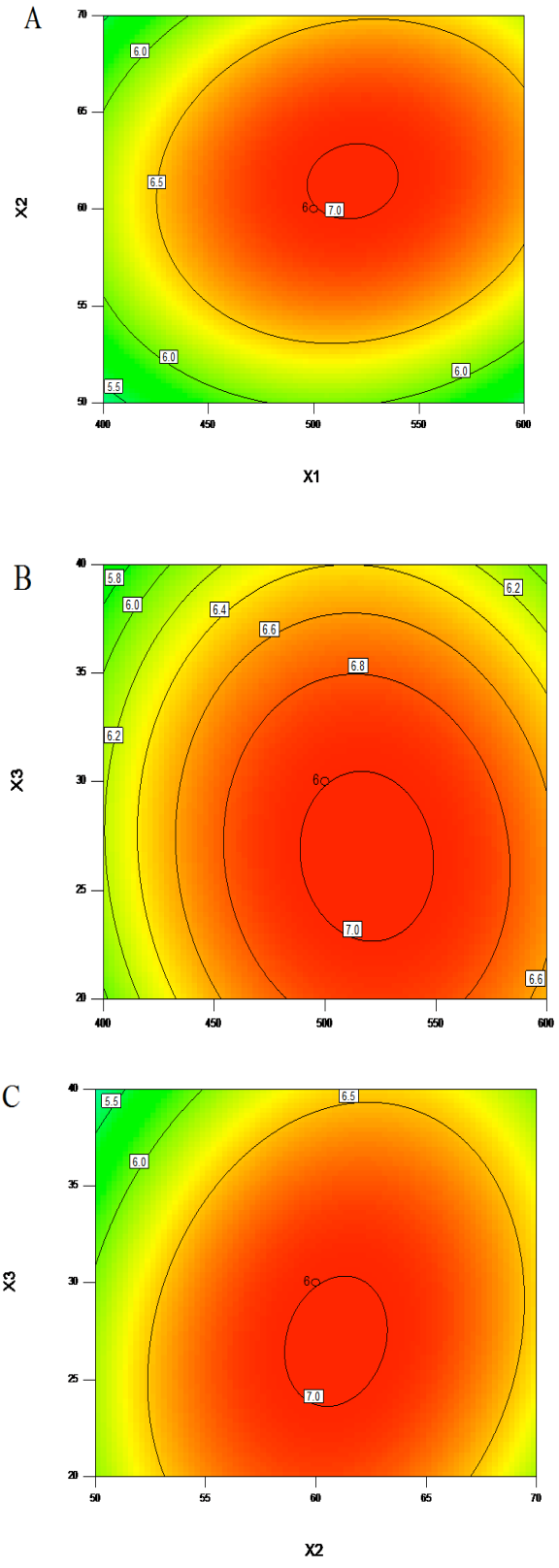


Fig. 2: Contour plots for the effect of independent variables on polyphenols extraction yield.
(A) Contour plot graph showing interaction between ultrasonic power (X_1/W) and ethanol concentration (X_2/%); (B) Contour plot graph showing interaction between ultrasonic power (X_1/W) and solvent-to-material ratio (X_3/mL/g); (C) Contour plot graph showing interaction between ethanol concentration (X_2/%) and solvent-to-material ratio (X_3/m/g).

Tab. 4: Significance test table of regression coefficient.

Std. Dev.	0.2608	R-Squared	0.9588
Mean	5.8034	Adj R-Squared	0.9218
C.V. %	4.4945	Pred R-Squared	0.6887
PRESS	5.1468	Adeq Precision	13.4740

Tab. 5: Antioxidant activity of MF phenol extractions by scavenging free radicals.

Free radical	Concentration of MF phenol extractions (mg/mL)	Scavenging Rate	IC_{50} (mg/mL)
DPPH	0.5333	44%	0.72
	0.7999	52%	
	1.066	62%	
	1.33	76%	
ABTS	0.083	7%	0.30
	0.16	18%	
	0.25	38%	
	0.333	59%	
•OH	5	13%	12.79
	10	23%	
	15	87%	
	20	94%	
$•O_2^-$	0.05	36%	0.01
	0.01	54%	
	0.02	72%	
	0.03	80%	

HPLC-MS/MS analysis and identify the main polyphenols in MF extractions

The HPLC-MS/MS chromatograms of MF extraction were given in Fig. 3. Sixteen phenolic compounds, including gallic acid, catechin, epicatechin, hyperoside were identified according to the method of HPLC-MS/MS. The chemical structures of sixteen phenolic compounds were provided in Tab. 6.

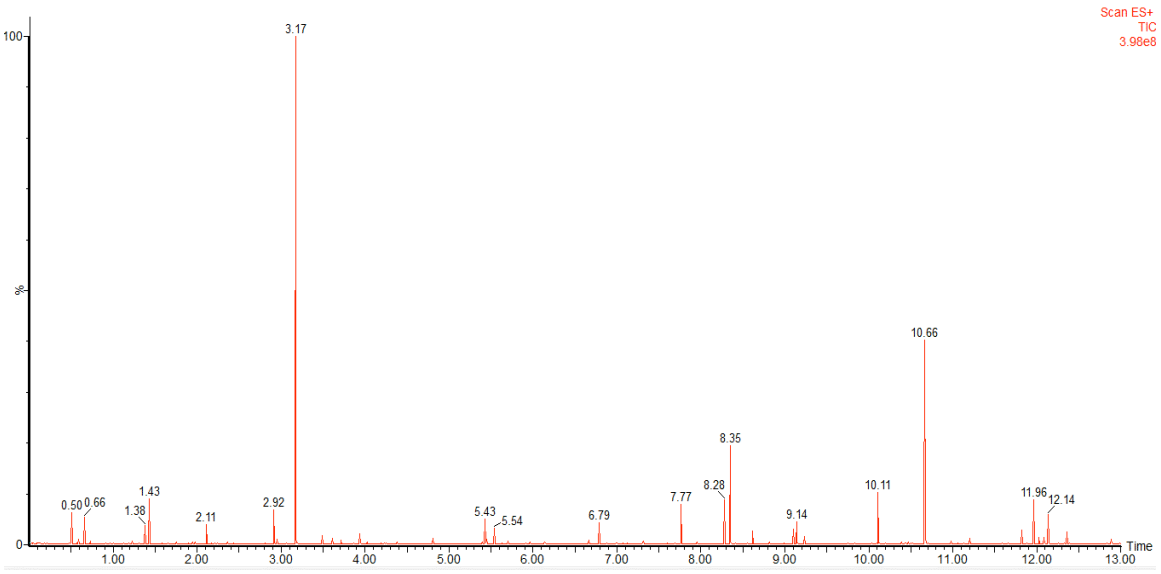
Discussion

In the current study, ultrasonic extraction and RSM design were successfully used to optimize the extraction conditions of polyphenols from MF, and optimal extraction conditions were also established. The best extraction conditions were extraction power of 520.01 W, ethanol concentration of 61.08%, and solvent-to-material ratio of 26.83 mL/g. From the statistical analysis, we learned that ultrasonic power was the most significant factor influencing the extraction yield. The yield increased to 3.56% with the increase of extraction power from 400 to 520.01 W. The results might due to the collision of molecules when the ultrasound power increased, thus increased the release of phenolic compounds. Besides, the increases of the ultrasound power may lead to the increase of temperature together with the degradation of the phenols during oxidation at high temperature (Spigno and De Favari, 2009).

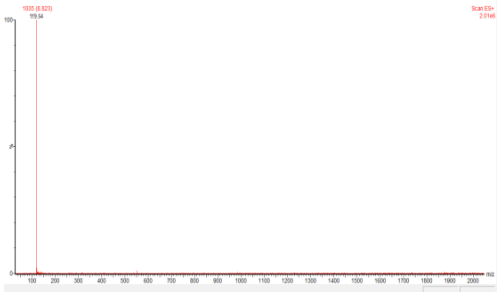
The ethanol concentration was another important factor. The polyphenols content increased to 18.07% when the ethanol concentration increased from 43% to 61.08%. The findings obtained from our study were in good agreement with Bucić-Kojić et al. (2011), where the total phenolics yield from lyophilized fig fruits increased to 54.17% when ethanol concentration increased from 50 to 80%. Although pure water is the most polar solvent of all solvents, it did not yield the best results with respect to extract and polyphenols content. This phenomenon might be attributed to the higher viscosity of water than that of the other solvents, which is a matter of mass transfer. As the polyphenols yield depended largely on the polarity of solvents and compounds, a single solvent might not be effective for the extraction of a bioactive compound. Hence, a combination of ethanol with water was more effective in extracting phenolic compounds than ethanol alone (Markom et al., 2007). Sixty percent of ethanol showed the greatest yield of polyphenols, as a result of lower viscosity and altering the plant structure by swelling the matrix, which enabled the solvent to more completely penetrate the leaves. Accordingly, water is acting as the plant swelling agent, while ethanol is believed to disrupt the bonding between the solutes and plant matrices. Therefore, the mixture of water and ethanol as solvent agent exhibited the best performance to extract phenolic among all the extractants used. Another explanation might be the high dielectric constant of water, which leads to increase polarity indices of ethanol in aqueous solution (Sahin and Saml, 2013). In the present study, the increase extraction yield of polyphenols was observed with the increase solvent-to-material ratio from 13 to 26.83. This was probably due to the fact that more solvent could enter plant cells while more phenolic compounds could permeate into the solvent under the higher solvent-to-material ratio conditions (Prasad et al., 2009).

Many plants have been examined have strong antioxidant ability (Vijaya, et al., 2010; Wang et al., 2012). In the present study, MF total phenolic extractions exhibited strong *in vitro* antioxidant ability and could be considered as a good source of natural antioxidant compounds, which was in agreement with the study of Mohammad et al. (2008), they showed that MF have potent antioxidant ability and might exert a protective effect against oxidative damage. Besides, the antioxidant activity of MF polyphenols extractions increased with the increase of its concentration.

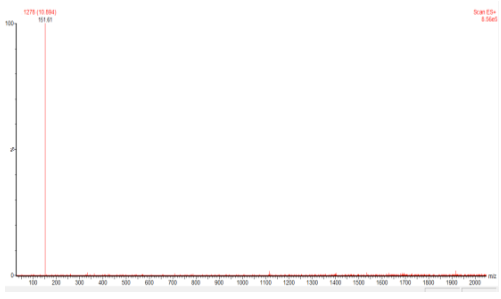
Sixteen phenolic compounds, including gallic acid, catechin, epicatechin, hyperoside were identified in MF polyphenols extractions by HPLC-MS/MS method, which was the first time UAE polyphenols from MF were identified. It can be concluded that the chemical properties of the phenolic compounds vary significantly because these compounds have different hydroxyl groups and glycoside.The number of identified phenolic compounds is higher that previously reported by Chen et al. (2010), they used HPLC technology to identify eight phenolic compounds in sweet corn silk, including gentisidc acid, rutin, quercetin, epitatechin, syringic, catechin, gallic acid and caffeic acid. Pedreschi et al. (2007) analysed phenolic compounds of Andean purple corn and found some major phenolic compounds similar to our study, such as protocatechuic acid. However, there was some dissimilarity in the composition of phenolic acids, including vanillic acid and p-coumaric acid, which might be due to the different maize varieties and experimental methods. Therefore, it is concluded that UAE is an efficient and credible method for the extraction of polyphenols from MF. The isolated polyphenols possess strong antioxidant activity. Sixteen phenolic compounds were firstly identified in MF extracts by HPLC-MS/MS method. All results can provide scientific reference of analysis and utilization of natural plant resources.


Scan ES+
TIC
3.98e8
100
%
0
3.17
10.66
8.35
0.50
0.66
1.43
1.38
2.11
2.92
5.43
5.54
6.79
7.77
8.28
9.14
10.11
11.96
12.14
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
Time

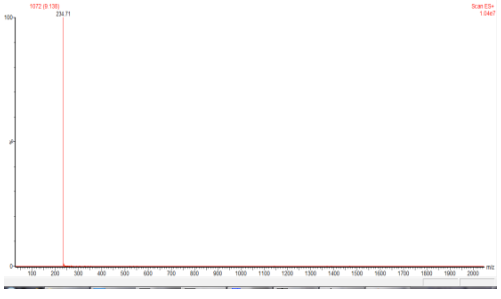
(1)



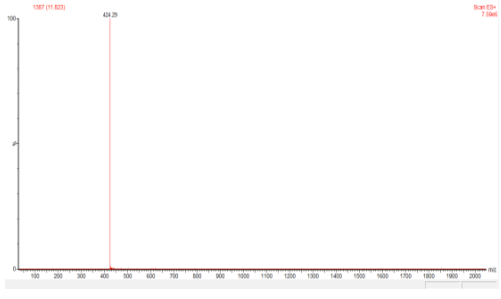
(2)



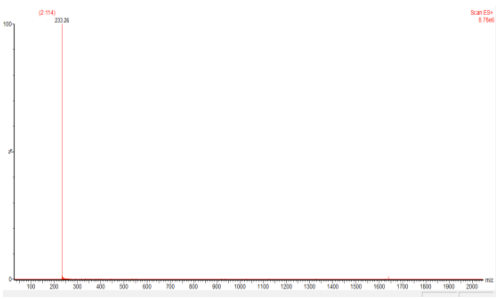
(3)



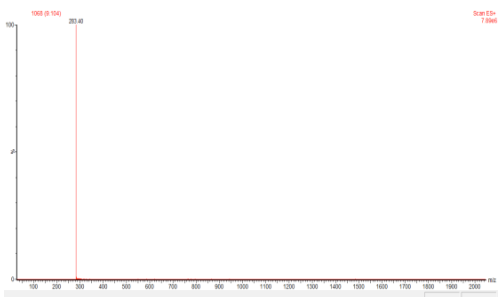
(4)



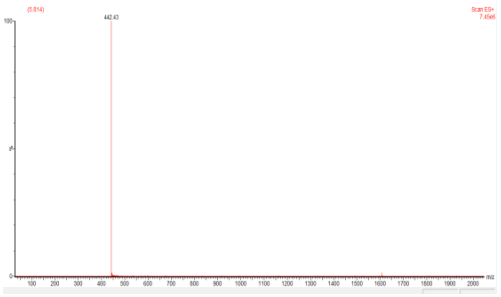
(5)



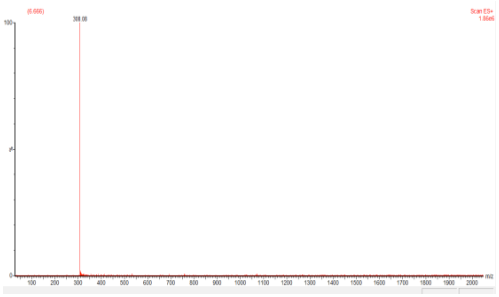
(6)



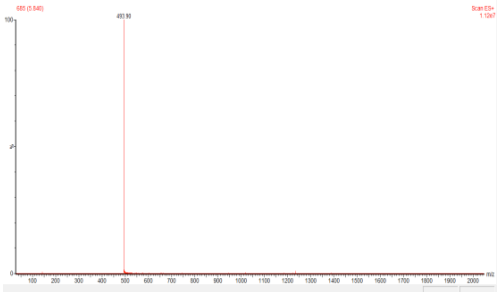
(7)



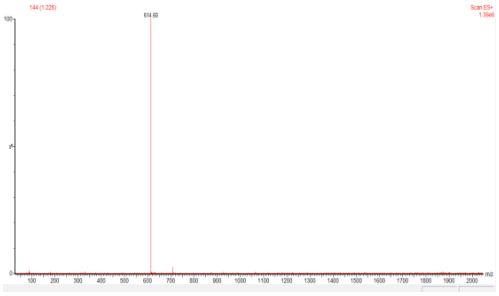
(8)



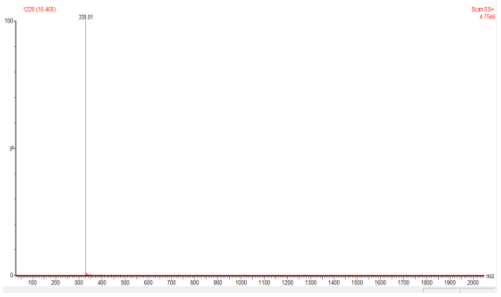
(9)



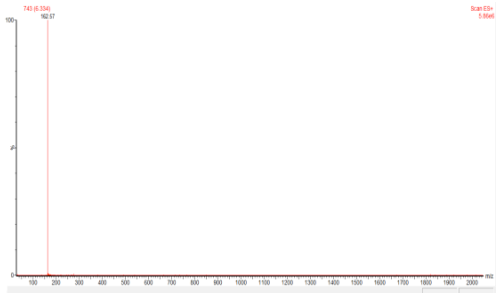
(10)



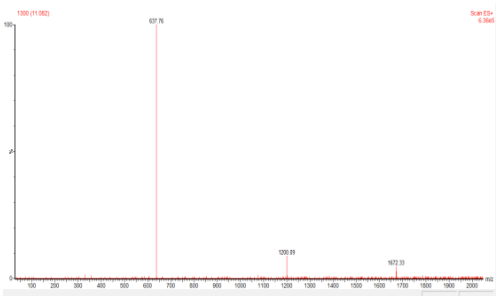
（11）



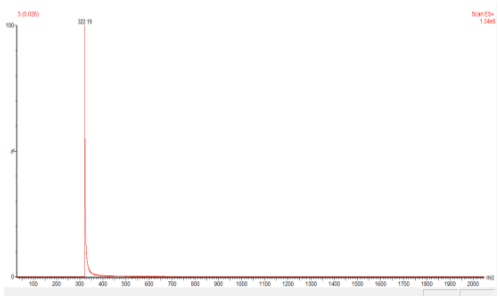
（12）



（13）



（14）



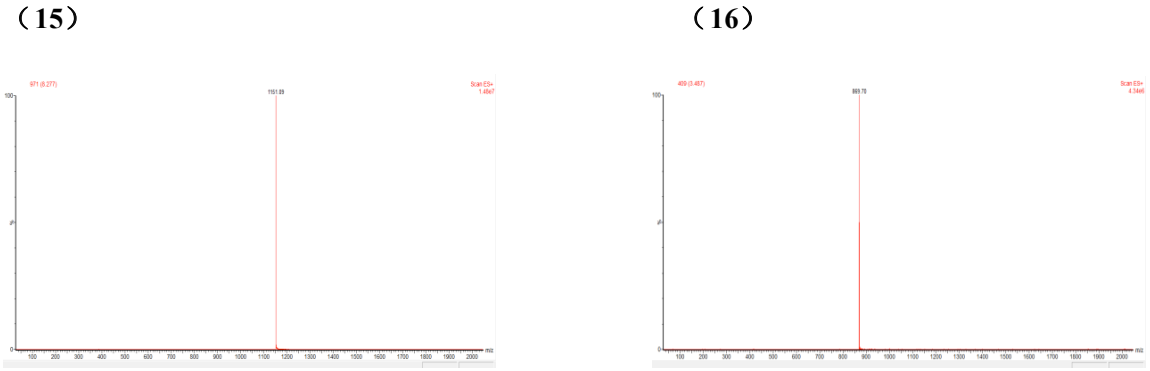


Fig. 3: HPLC-MS/MS chromatograms of MF polyphenols extract and analysis mass spectrum of the sixteen phenolic compounds. MS: (1) Benzoic acid; (2) Gentisic acid; (3) Epicatechin; (4) B2 Benzoic acid; (5) syringic acid; (6) Catechin; (7) Catechin gallate; (8) Quercetin; (9) Quercetin-3-O-rhamnoside; (10) Rutin;(11) chlorogenic acid; (12) Gallic acid; (13) Gallic acid-6 hydroxy diphenyl-pyran glucose; (14) Ellagic acid; (15) Epicatechin trimer; (16) Epicatechin tetramer.

Acknowledgements

This work was supported by Jilin province science and technology development plan project (20130206098SF) and the youth scientific innovation leading talent and team building project of Jilin province (20140519014JH). Accordingly, the authors gratefully acknowledge the funds support.

References

Alzueta, C., Trevino, J., Ortiz, L., 1992: Effect of tannin from fava beans on protein utilisation in rats. J. Sci. Food Agric. 59, 551-553.

British Herbal Pharmacopoeia, 1996: Corn Silk, British Herbal Medicine Association.

Brachet, A., Mateus, L., Cherkaoui, S., Christen, P., Gauvrit, J.Y., Lanteri, P., Veuthey, J.L., 1999: Application of central composite designs in the supercritical fluid extraction of tropane alkaloids in plant extracts. Analusis 27, 772-778.

Bucić-Kojić, A., Planinić, M., Tomas, S., Jokić, S., Mulić, I., Bilić, M., Velić, D., 2011: Effect of extraction conditions on the extractability of phenolic compounds from lyophilised fig fruits (*Ficus carica* L.). Pol. J. Food Nutr. Sci. 61, 195-199.

Czygan, F.C., *Maydis stigma,* 1997: Teedrogen und Phytopharmaka, Wissenschaftliche Verlagsgesellshaft mbH, Stuttgart, In: M. Wichtl (ed.), 362.

Chen, Z.Y., Xu Y.J., Yin,Y., Liu, X.M., Li, X.Q., 2010: Analysis of polyphenol composition of different parts of ear of sweet corn. Food Sci. 31, 235-238.

Gladine, C., Morand, C., Rock, E., Bauchart, D., Durand, D., 2007: Plant extracts rich in polyphenols (PERP) are efficient antioxidants to prevent lipoperoxidation in plasma lipids from animals fed n-3 PUFA supplemented diets. Anim. Feed Sci. Tech. 136, 281-296.

Hagerman, A., Harvey-Mueller, I., Makkar, H.P.S., 2000: Quantification of tannins in tree foliage-a laboratory manual, 4-7. FAO/IAEA, Vienna.

Hemwimol, S., Pavasant, P., Shotipruk, A., 2006: Ultrasound-assisted extraction of anthroquinones from roots of Morinda citrifolia. Ultrason. Sonochem. 13, 543-548.

Hossain, M.B., Brunton, N.P., Patras, A., Tiwari, B., O'Donell, C.P., 2012: Optimization of ultrasound assisted extraction of antioxidant compounds from marjoram (*Origanum majorana* L.) using response surface methodology. Ultrason. Sonochem. 19, 582-590.

Longstaff, M., McNabb, J.M., 1991: The inhibitory effects of hull polysaccarides amd tannins of field beans (*Vicia faba* L.) on the digestion of amino acids, starch and lipid, and on digestive activities in young chicks. Brit. J. Nutr. 65, 199-216.

Lu, T.L., Ye, D.J., Mao, C.Q., Liu, X.S., 1999: Studies on antiplatelet aggregation and antithrombosis action of total flavonoids of Common Burreed (*Sparganium stoloniferum*). Chin. Tradit. Herbal Drugs. 30, 439-441.

Lang, Q., Wai, C.M., 2001: Supercritical fluid extraction in herbal and natural product studies-a practical review. Talanta. 53, 771-782.

Li, H., Chen, B., Yao, S.Z., 2005: Application of ultrasonic technique for extracting chlorogenic acid from Eucommia ulmodies Oliv. (E. ulmodies). Ultrason. Sonochem. 12, 295-300.

Lee, S.Y., Choi, S.U., Lee, J.H., Lee, D.U., Lee, K.R., 2010: A new phenypropane glycoside from the rhizome of Sparganium stoloniferum. Arch. Pharm. Res. 33, 515-521.

Liu, J., Lin, S.Y., Wang, Z.Z., Wang, C.N., Wang, E.L., Zhang, Y., Liu, J.B., 2011: Supercritical fluid extraction of flavonoids from Maydis stigma and its nitrite-scavenging ability. Food Bioprod. Process. 89, 333-339.

Li, C.Y., Lu, Z.C., Zhao, C.J., Yang, L., Fu, Y.J., Shi, K., He, X., Li, Z., Zu, Y.G., 2015: Ionic-liquid-based ultrasound/microwave-assisted extraction of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one and 6-methoxy-benzoxazolin-2-one from maize (*Zea mays* L.) seedlings. J. Sep. Sci. 38, 291-300.

Maksimović, Z., Malencić, D., Kovacević, N., 2005: Polyphenol contents and antioxidant activity of Maydis stigma extracts. Biores. Technol. 96, 873-877.

Markom, M., Hasan, M., Ramli, W., Daud, W., Singh, S., Jahim, J.M., 2007: Extraction of hydrosable tannins from Phyllanthus niruri Linn. Effect of solvents and extraction methods. Sep. Purif. Technol. 52, 487-496.

Ma, Y.Q., Chen, J.C., Liu, D.H., Ye, X.Q., 2008: Effect of ultrasonic treatment on the total phenolic and antioxidant activity of extracts from citrus peel. J. Food Sci. 73, 115-120.

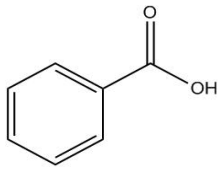
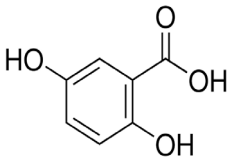
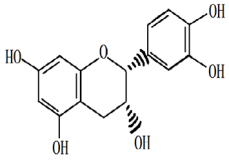
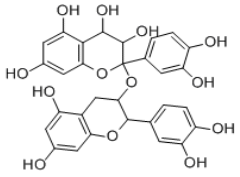
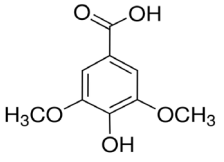
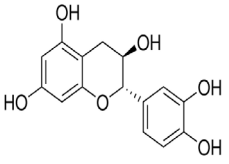
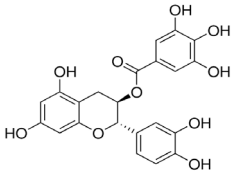
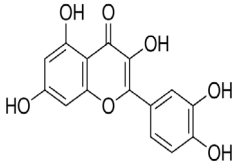
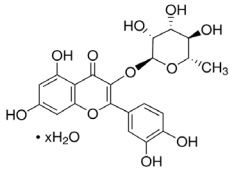
Mohammad, A.E., Fereshteh P., Samira H., 2008: Antioxidant activities of iranian corn silk. Turk. J. Biol. 32, 43-49.

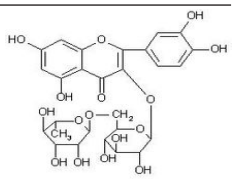
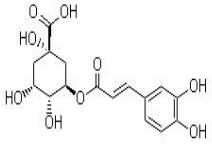
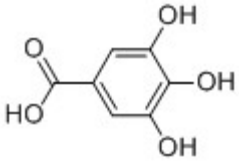
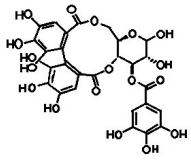
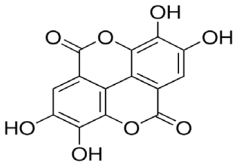
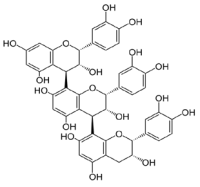
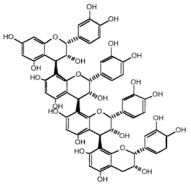
Muñiz-Márquez, D.B., Martínez-Ávila, G.C., Wong-Paz, J.E., Belmares-Cerda, R., Rodríguez-Herrera, R., Aguilar, C.N., 2013: Ultrasound-assisted extraction of phenolic compounds from *Laurus nobilis* L. and their antioxidant activity, Ultrason. Sonochem. 20, 1149-1154.

Maran, J.P., Manikandan, S., Nivetha, V., Dinesh, R., 2013: Ultrasound assisted extraction of bioactive compounds from *Nephelium lappaceum* L. fruit peel using central composite face centered response surface design. Arab. J. Chem. DOI: 10.1016/j.arabjc.2013.02.007

Maran, J.M., Mekala, V., Manikandan, S., 2013: Modeling and optimiza-

Tab. 6: Analysis of polyphenols in maize filaments by HPLC-MS/MS method.

Peak no.	Rentention time (min)	[M-H]-/fragments	[M+H]+/fragments	Molecular mass	Proposed compounds	Chemical structure
1	8.823	117.54	119.54	122.12	Benzoic acid	
2	10.894	149.61	151.61	154.10	Gentisic acid	
3	9.128	232.71	234.71	290.27	Epicatechin	
4	11.823	422.29	424.29	577.00	B2 Benzoic acid	
5	2.114	231.26	197.12	198.18	Syringic acid	
6	9.104	281.4	283.4	308.28	Catechin	
7	5.814	440.43	442.43	443.00	Catechin gallate	
8	6.6666	306.08	300.08	302.28	Quercetin	
9	5.840	491.9	493.90	448.40	Quercetin-3-O-rhamnoside	

Peak no.	Rentention time (min)	[M-H]-/fragments	[M+H]+/fragments	Molecular mass	Proposed compounds	Chemical structure
10	1.228	612.6	614.60	610.51	Rutin	
11	10.468	328.01	330.01	354.31	Chlorogenic acid	
12	6.334	160.57	162.57	170.10	Gallic acid	
13	11.082	635.76	637.76	634.00	Gallic acid-6 hydroxy diphenyl-pyran glucose	
14	0.0026	320.19	322.19	302.00	Ellagic acid	
15	3.487	867.7	869.70	865.00	Epicatechin trimer	
16	8.277	1149.09	1151.09	1153.30	Epicatechin tetramer	

tion of ultrasound-assisted extraction of polysaccharide from *Cucurbita moschata*. Carbohydr. Polym. 92, 2018-2026.

Pedreschi, R., Cisneros-Zevallos, L., 2007: Phenolic profiles of Andean purple corn (*Zea mays* L.). Food Chem. 100, 956-963.

Prasad, K.N., Chun, Y., Yang, E., Zhao, M., Jiang, Y., 2009: Effects of high pressure on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp. Innov. Food Sci. Emerg. 10, 155-159.

Prasad, K.N., Hassan, F.A., Yang, B., Kong, K.W., Ramanan, R.N., Azlan, A., Ismail, A., 2011: Response surface optimisation for the extraction of phenolic compounds and antioxidant capacities of underutilized Mangifera pajang Kosterm, Peels. Food Chem. 128, 1121-1127.

Pan, G.Y., Yu, G.Y., Zhu, C.H., Qiao, J.L., 2012: Optimization of ultrasound-assisted extraction (UAE) of flavonoids compounds (FC) from hawthorn seed (HS). Ultrason. Sonochem. 19, 486-490.

Spigno, G., De Favari, D.M., 2009: Microwave-assisted extraction of tea phenols: A phenomenological study. J. Food Eng. 93, 210-217.

Sun, J., Wang, S., Ma, D., Li, H., Guo, B., Wei, Y.H., 2010: *Sparganium stoloniferum* flavonoids extraction and HPLC analysis of ingredients against HeLa cervical cancer. Acta Bot. Boreali-Occident. Sin. 30, 2530-

2535.

Skalicka-Woźniak, K., Szypowski, J., Głowniak, K., 2011: HPLC analysis of kaempherol and quercetin derivatives isolated by different extraction techniques from plant matrix. J. Aoac Int. 94, 17-21.

Sun, J., Wang, S., Guo, B., Wei, Y.H., 2011: Flavonoids of Rhizoma Sparaganii induce S/G2stage arrest in A549 and MCF-7 cells. Nat. Prod. Res. Dev. 23, 224-227.

Stagos, D., Amoutzias, G.D., Matakos, A., Spyrou, A., Tsatsakis, A.M., Kouretas, D., 2012:Chemoprevention of liver cancer by plant polyphenols. Food Chem.Toxicol. 50, 2155-2170.

Sahin, S., Saml, R., 2013: Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrason. Sonochem. 20, 595-602.

Toma, M., Vinatoru, M., Paniwnyk, L., Mason, T.J., 2001: Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrason. Sonochem. 8, 137-142.

Vijaya Kumar Reddy, C., Sreeramulu, D., Raghunath, M., 2010: Antioxidant activity of fresh and dry fruits commonly consumed in India. Food Res. Int. 43, 285-288.

Wang, L.J., Weller, C.L., 2006: Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Tech. 17, 300-312.

Wang, D.M., Lu, J.L., Miao, A.Q., Xie, Z.Y., Yang, D.P., 2008: HPLC-DAD-ESI-MS/MS analysis of polyphenols and purine alkaloids in leaves of 22 tea cultivars in China. J. Food Compos. Anal. 21, 361-369.

Wang, X., Wu, Q., Wu, Y., Chen, G., Yue, W., Liang, Q., 2012: Response surface optimized ultrasonic-assisted extraction of flavonoids from Sparganii rhizome and evaluation of their in vitro antioxidant activities. Molecules, 17, 6769-6783.

Yang, Z.D., Zhai, W.W., 2010: Optimization of microwave-assisted extraction of anthocyanins from purple corn (*Zea mays* L.) cob identification with HPLC–MS. Innov. Food Sci. Emerg. 11, 470-476.

Yuan, Y., Wu, S.J., Liu, X., Zhang, L.L., 2013: Antioxidant effect of salidroside and its protective effect against furan-induced hepatocyte damage in mice. Food Funct. 4, 763-769.

Address of the corresponding author:
College of Quartermaster Technology, Jilin University, Changchun, China, 130062.
E-Mail: yuanyuan1024@gmail.com