南京中医药大学学报 2017 年 9 月第 33 卷第 5 期
J Nanjing Univ Tradit Chin Med Vol.33 No.4 Sep. 2017

·实验研究·

板蓝根蛋白的表征及响应曲面法优化其超声辅助提取工艺研究

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摘要：目的 表征板蓝根蛋白的分子量组成，优化板蓝根蛋白超声提取工艺。**方法** 运用响应曲面法，以蛋白得率为评价指标，对提取时间、液料比及 pH 值 3 个影响因素进行考察，优化板蓝根蛋白的提取工艺，另以 Design Expert 软件对数据进行综合统计分析；采用聚丙烯酰胺凝胶电泳的方法表征板蓝根蛋白的分子量分布；应用扫描电镜技术观察超声提取前后板蓝根药材粉末的微观结构。**结果** 最优工艺条件是以 pH 值为 7.8 的 50 mmol/L Tris-HCl 为溶剂，液料比为 80∶1，超声提取 2 次，每次提取 65 min，按该工艺进行试验所得板蓝根蛋白得率为 0.705%；板蓝根蛋白 5 个分子量段的蛋白分别为 19.2、21.5、24.8、34.0～43.0 kDa 和＞170 kDa。**结论** 运用响应曲面法优选的板蓝根蛋白提取工艺稳定可行，可用于板蓝根蛋白的提取。

关键词：板蓝根蛋白；超声辅助提取；响应曲面法；蛋白表征

中图号：R284.2　　**文献标志码：**A　　**文章编号：**1672－0482(2017)05－0504－05

DOI：10.14148/j.issn.1672-0482.2017.0504

Characterization of Radix Isatidis Protein and its Ultrasound-assisted Extraction Process Optimization with Response Surface Methodology

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ABSTRACT: OBJECTIVE To characterize the molecular weight of Radix Isatidis protein (RIP) and optimize the extraction process of protein from Radix Isatidis. **METHODS** Response surface methodology (RSM) was applied to optimize the extraction of protein from Radix Isatidis. Extraction time, liquid-to-solid ratio and pH value were set as the investigated factors with respect to the protein yield. In addition, Design Expert software was used for data analysis. The RIP was characterized for composition using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Scanning electron microscopy (SEM) analysis was performed to observe microstructure of the Radix Isatidis powder before and after ultrasound-assisted extraction (UAE). **RESULTS** Based on the RSM analysis, optimum conditions were determined as follows: twice ultrasonic extraction in 50 mmol/L Tris-HCl buffer solution, pH at 7.8, liquid-to-solid ratio at 80∶1 and extraction for 65 min each time. Under the optimized conditions, the experimental values were 0.705%, which is in close agreement with values predicted by the model. The characterization of the RIP demonstrated that it contained five major groups of protein bands, namely bands of 19.2 kDa, 21.5 kDa, 24.8 kDa, 34～43 kDa and ＞170 kDa respectively. **CONCLUSION** RSM can be applied for the optimization of extraction process of RIP, which is effective, stable and feasible.

KEY WORDS: Radix Isatidis protein; Ultrasound-assisted extraction; Response surface methodology; Protein characterization

收稿日期：2017-07-02；**修稿日期：**2017-08-28

基金项目：国家自然科学基金(81703642)；江苏省中药资源产业化过程协同创新中心重点课题项目(ZDXM-3-19)；江苏省中药优势学科Ⅱ期建设工程项目(ysxy-2014)；江苏高校品牌专业建设工程资助项目(TAPP-PPZY2015A070)；南京中医药大学 2017 年大学生创新训练计划省级重点项目

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Radix Isatidis, a commonly used medicinal herb in clinic is initially documented in*The Divine Husbandman's Herbal Foundation Canon*[1]. It has been widely used in the treatment of the diseases caused by viruses or infection, especially in terms of its antiviral efficacy. Previously studies indicated that Radix Isatidis has various of pharmacological bioactivities, including anti-virus, anti-endotoxic, anti-bacterial, anti-inflammatory, anti-tumor and immune regulatory effects, etc[2-5]. In addition to supply essential nutrients, it is now well accepted that some plant proteins can offer additional health benefits beyond nutrition[6]. Radix Isatidis protein (RIP) was found to have anti-fungal and anti-viral properties[7]. Our previous research showed that RIP had strong antioxidant activity *in vitro*[8]. RIP has variety of pharmacological activities, but extraction methods of the protein from Radix Isatidis have not been reported. Thus, it is very important to develop an optimized novel extraction technique to obtain high extraction yield of RIP.

Response surface methodology (RSM) refers to the design of experiments (DOE) and analysis of data which can simulate surface relationship. It conducts the function estimation based on the multiple quadratic regression method[9]. Compared with signal factor and orthogonal experiments, RSM gets the relevant data through designing a series of experiments using methods such as full factorial design, fractional factorial designs, Plackett-Burman design, central composite design, and so on. Based on these data, a response surface, which can simulate the real limit state surface by utilizing regression estimate, analysis of variance, steepest ascent, canonical analysis and so forth, will be fit[10]. Moreover, the response surface can be further optimized through reliability analysis. Besides, it is also an efficient tool for the identification of unremarkable main effect, interaction effect and secondary effect. In conclusion, RSM is a convenient and important method in the design of experiments.

Ultrasound-assisted extraction (UAE) has many advantages such as high extraction efficiency, short extraction time and low temperatures. It is very useful for extraction of thermal instability compounds. Protein is a kind of material that is sensitive to heat. Many studies have reported that different industrial applications of ultrasound in the intensification of extraction of proteins[11-13]. Most research has focused on the effects of UAE parameters on yields of protein. However, there is still a need to evaluate and understand the effects of ultrasound treatment on the molecular properties of protein as well as on the extraction process. Therefore, the present study was carried out to examine the effects of UAE on the yield, protein compositions, and microstructure of the Radix Isatidis powder.

In the present study, we investigated how the conditions of ultrasound treatment affects the yield and compositions of RIP. Effect of different extraction parameters (ultrasound time, liquid-solid ratio and pH) on the yield of RIP were investigated and analyzed using RSM. The RIP was characterized for composition using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Scanning electron microscopy (SEM) analysis was performed to observe microstructure of the Radix Isatidis powder before and after UAE. This study could provide a basis for the comprehensive utilization of RIP in food and pharmaceutical industry.

1　Materials and Methods

1.1　Materials and chemicals

Radix Isatidis was purchased from a medical market in Tongling, Anhui province (China). The botanical origins were identified by Professor Jianwei Chen, Nanjing University of Chinese Medicine. The voucher specimens were deposited at the Herbarium in Nanjing University of Chinese Medicine (Nanjing, China). 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) was purchased from Shanghai Shanpu chemical instrument co., Ltd. Bovine serum albumin (BSA) and coomassie brilliant blue G-250 were obtained from Solarbio (Shanghai, China). All chemicals and reagents used were of analytical grade, and deionized water was prepared using a MilliQ-Plus system (Millipore, Bedford, MA).

1.2　Extraction methods

1.2.1　Ultrasound-assisted extraction (UAE)　Ultrasound-assisted extraction was performed in a temperature controlled ultrasonic cleaner (KQ-500DE type, 40.0 kHz, 500 W; Kunshan Ultrasonic Instrument Co. Ltd., Jiangsu Province, China). Radix Isatidis powder was put in a conical flask mixed with 50 mmol/L Tris-HCl at a liquid-solid ratio varying from 5∶1 to 80∶1 (v/w) and pH ranging from 7.0 to 8.5 at 4 ℃ for 10～120 min. The heat generated during sonication was offset by the ice bath. The extraction mixture was centrifuged at 3 000 r/min for 5 min and the supernatants were collected for the determination of protein content.

1.2.2　Magnetic stirring extraction (MSE)　Magnetic stirring extraction was carried out on a magnetic stirring apparatus (Jintan Ronghua Instrument Manufacturing Co., LTD, Jiangsu). For comparison, the extraction conditions of MSE were consistent with the optimal extracting conditions of UAE. The protein extract was centrifuged at 3 000 r/min for 5 min at 4 ℃ and the supernatants were collected for subse-

quent analysis.

1.3　Protein determination

The protein concentration was determined by Bradford method[14]. The extraction yield (%) was calculated with the formula below:

Yield (%) = 100%× C ×V/W_0

where C (g/mL) is the concentration of protein in extract, V (mL) is the volume of the extract solvent, and W_0 represented dried Radix Isatidis power weight (g).

1.4　Experimental design of RSM

RSM was employed for experimental design, data analysis and model building with software Design Expert (Design-Expert v. 8. 0. 5b) (Stat-Ease Inc., Minneapolis, USA). According to the results of single-factor experiments, a five-level Box-Behnken design (BBD) with three factors was applied to determine the optimal levels for ultrasound time (X_1), liquid-solid ratio (X_2) and pH (X_3). The experimental range of the selected process parameters was given in Table 1. The whole design consisted of 17 experimental points carried out in random order (Table 2). Three replicates at the center of the design were used to allow for estimation of a pure error sum of squares. The response value in each trial was average of duplicates.

Table 1　Level and code of factors chosen for experiment design

Factors	Symbol	Code and level				
		−1.732	−1	0	1	1.732
Time/min	X_1	10	33	65	97	120
Liquid-solid ratio	X_2	5	21	42.5	64	80
pH	X_3	7.0	7.3	7.8	8.2	8.5

Experimental data were fitted to the following second order polynomial model (Eq. (1)). Regression coefficients were obtained to describe relationship between the responses and the independent variables.

$$Y_i = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} x_i x_j + \sum_{i=1}^{n} \beta_{ii} x_i^2 \quad (1)$$

Where Y is the predicted response, β_0 is the constant coefficient, β_i, β_{ii} and β_{ij} are the coefficients of the linear, quadratic and interactive terms, respectively. X_i and X_j represent the independent variables[15].

1.5　Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was done according to the reported method with slight modifications[16]. Protein samples (100 μL) were mixed with the same volume of the samples buffer (pH 6.8; 1% SDS; 12.5% glycerol; 0.005% bromophenol blue; 2.5% 2-mercaptoethanol). The mixture was then heated in water bath (95 ℃) for 5 min. The protein samples were loaded onto a criterion bis-tris precast 12% gel and electrophoresis was done in a Mini Protean Ⅱ Dual Slab Cell system (BIO-RAD). The running buffer consisted of 25 mmol/L Tris, 192 mmol/L glycine and 0.1% SDS and was run at 15 mA. The gel was finally stained with Coomassie Brilliant Blue.

1.6　Scanning electron micrographs (SEM)

A scanning electron microscopy spectrometer (Sirion-200, Philips-FEI Company, Amsterdam, Netherlands) was used to observe the surface appearance of Radix Isatidis powder when processed by either MSE, UAE or without any extraction. Vacuum was set at 15 Pa and operating voltage was 20 kV. Samples were deposited on a silicon wafer and coated with a thin conductive material (gold) to ensure sufficient electron refraction[17]. High resolution topographic images were digitally recorded with short dwell times to prevent beam induced damage[18].

1.7　Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS 13.0 (SPSS, Chicago, IL, USA). $P<0.05$ was considered statistically significant. The fitness of the polynomial model equation was expressed as the coefficient of determination R^2. The significances of the regression coefficients were tested by F-test.

2　Results and Discussion

2.1　Fitting the models

In order to determine the best combination of variables for the yield of RIP, an experimental design was adopted on the basis of coded levels from three independent variables (Table 1). According to BBD, a number of 17 experimental runs were carried out in random to investigate the extraction process. The experimental and model-predicted values are listed in Table 2. Results showed that the yield of RIP ranged from 0.108% to 0.706%. Multiple regression analysis was applied on the experimental data, the response variable and the test variables are related by the following second order polynomial equation:

$Y = -0.001\,469X_1^2 - 0.034\,471X_2^2 - 0.021\,137X_3^2 + 0.021\,500X_1X_2 - 0.007\,750X_1X_3 + 0.019\,250X_2X_3 + 0.009\,157X_1 + 0.145\,925X_2 + 0.017\,936X_3 + 0.471\,003$

where Y represents the predicted yield of RIP; X_1, X_2 and X_3 are the coded values of the test variables for extraction time, liquid-solid ratio and pH, respectively.

The statistical significance ofthe equation above was checked by F-test, and the results of analysis of variance (ANOVA) are shown in Table 3. The quadratic regression model had a high F-value ($F = 48.73$) and a very low P-value ($P<0.01$), indicating that the model was highly significant. In this experiment, the linear coefficients (X_2) and cross product coefficients (X_1X_2, X_1X_3) were significant at the level of $P<0.05$ or $P<0.01$. The results indicated that liquid-solid ratio had the largest effect on the extraction yield of RIP. The result of ANOVA demonstrated that the experimental values for the yield of RIP were well-matched with the predicted values, producing a high coefficient of determination ($R^2=0.9843$) and an insignificant lack of fit ($P>0.05$), meaning that our regression model is applicable. Furthermore, the satisfactory level of C.V. value (0.8790%) indicated that the model is reproducible. Taken together, we conclud

that the developed model has outstanding reliability and high precision, and can be applied to the extraction of RIP.

Table 2　Box-Behnken design matrix and the yield of RIP

No.	Time	Liquid-solid ratio	pH	Protein yield	
				Experimental	Predieted
1	−1.732	0	0	0.463	0.451
2	−1	−1	−1	0.302	0.273
3	−1	−1	1	0.286	0.286
4	−1	1	−1	0.483	0.484
5	−1	1	1	0.569	0.574
6	0	−1.732	0	0.108	0.114
7	0	0	−1.732	0.364	0.368
8	0	0	0	0.476	0.471
9	0	0	0	0.467	0.471
10	0	0	0	0.480	0.471
11	0	0	1.732	0.428	0.431
12	0	1.732	0	0.706	0.707
13	1	−1	−1	0.261	0.264
14	1	−1	1	0.254	0.246
15	1	1	−1	0.503	0.502
16	1	1	1	0.613	0.619
17	1.732	0	0	0.502	0.483

Table 3　The extraction technology of quadratic polynomial analysis of variance

Model term	Sum of square	Degree of free	Mean square	F-value	P-value
X_1	0.001 174	1	0.001 174	0.188 66	0.677 106
X_2	0.298 108	1	0.298 108	47.916 35	0.000 227
X_3	0.004 504	1	0.004 504	0.723 91	0.423 010
X_1^2	0.000 026	1	0.000 026	0.004 23	0.949 969
X_2^2	0.014 488	1	0.014 488	2.328 68	0.170 842
X_3^2	0.005 447	1	0.005 447	0.875 55	0.380 572
X_1X_2	0.003 698	1	0.003 698	0.594 40	0.046 594
X_1X_3	0.000 480	1	0.000 480	0.077 23	0.019 112
X_2X_3	0.002 964	1	0.002 964	0.476 50	0.512 243

2.2　Response surface analysis

The relationship between different extraction parameters and yields was investigated by response surface plots. Fig. 1 shows the effect of extraction time, liquid-solid ratio and pH and their mutual interaction on the yield of RIP. The highest yield of RIP was observed at longer extraction time and higher liquid-solid ratio (Fig. 1A). Initially, the effect of liquid-solid ratio and extraction time shown in Fig. 1A demonstrated that the yield of RIP increased sharply with increasing liquid-solid ratio and time but subsequently increased smoothly, which is probably due to saturation of the solvent. Fig. 1B described interactive effects of pH and extraction time on the extraction yield. The interaction effects of pH and extraction time were insignificant for the extraction yield ($P>0.05$). As shown in Fig. 1C, it was clear that the interactions between pH and extraction time were significant. Expected yield value increases with increasing liquid-solid ratio and pH.

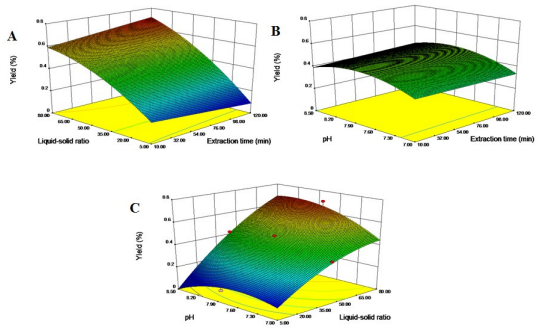


Fig. 1　Response surface plots showing the combined effects of the extraction parameters on the yield of Radix Isatidis protein (RIP)

2.3　Verification of predictive models

The results were shown in Table 2, no significant difference ($P>0.05$) was found between the experimental and predicted yield of RIP. By prediction with software Design Expert, the optimal conditions to obtain the highest yield of RIP were determined as follows: the extraction time of 65 min, liquid-solid ratio of 78.42 and pH of 7.8. Because of the limit of instruments and convenience, the optimal conditions were chosen at the extraction time of 65 min, liquid-solid ratio of 80, the pH of 7.8. Under these conditions, the average yield of RIP was determined to be 0.692% (*RSD*, 3.18%), similar to the predicted value 0.707%.

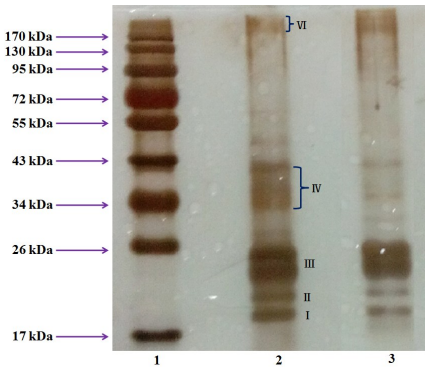
2.4　Comparison of different extraction methods

2.4.1　The yield of RIP extracted by different methods　Significant differences in yield of RIP were recorded when Radix Isatidis was extracted by UAE versus MSE ($P<0.01$). The extraction yield of RIP was (0.705 ± 0.022)% by UAE, which was markedly higher than that obtained by MSE (0.483 ± 0.034)%.

2.4.2　SDS-PAGE analysis　The SDS-PAGE profiles of RIP extracted by different methods (UAE and MSE) are shown in Fig. 2. The molecular weight of RIP was calculated by comparison with standard protein markers (Fig. 2, lane 1). The proteins extracted by UAE showed five major groups of protein bands, namely bands 19.2 kDa (Ⅰ), 21.5 kDa (Ⅱ), 24.8 kDa (Ⅲ), 34.0～43.0 kDa (Ⅴ) and >170 kDa (Ⅵ) (Fig. 2, lane 2). Based on intensity, the band of 24.8 kDa (Ⅲ) was the most abundant protein in RIP. The RIP extracted by MSE (lane 3) also showed these major groups of protein bands except the band that appeared ranging from 34.0 to 43.0 kDa. This results indicated that the protein extracted by UAE was more sufficiently than MSE.

2.4.3　Scanning electron microscopy (SEM)　In order to investigate the potential mechanism for UAE of protein from Radix Isatidis, scanning electron microscopy (SEM) analysis was performed. The morphology of Radix Isatidis powder was studied for untreated powder as well as for that extracted by MSE or UAE. The microstructures of different samples are presented in Fig. 3. The SEM analysis clearly showed visible structural changes of the plant tissues after treatment with different extraction methods. The images showed that the cells were damaged and broken much more noticeably by UAE. There were many interspaces and holes on the surface

of samples. UAE could reduce the mechanical strength of the cell wall and improve the permeability of extraction solvent into cells. In contrast, the surfaces of samples untreated or treated by MSE were relatively smooth and intact (Fig. 3B). These results demonstrated that UAE was an effective extraction method for RIP. It could disrupt plant tissues and cell walls, enhancing the access of solvent to the protein molecules and their release into the extraction solvent.



Lane 1: standard protein markers; lane 2: RIP extracted by UAE; lane 3: RIP extracted by MSE

Fig. 2 SDS-PAGE profiles of Radix Isatidis protein (RIP)

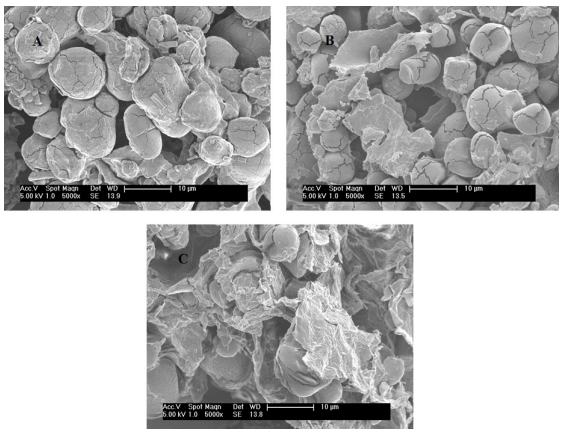


Fig. 3 Scanning electron micrographs of Radix Isatidis powder from treatments of (A) non-extraction, (B) magnetic stirring extraction (MSE), and (C) ultrasound-assisted extraction (UAE)

3 Conclusions

RSM was successfully applied to optimise the extraction conditions of RIP by UAE. Optimized conditions for maximum extraction RIP were determined including ultrasound time, liquid-solid ratio and pH. The characterization of the RIP extracted by UAE or MSE methods demonstrated that it contained five major groups of protein bands, namely bands of 19.2 kDa (Ⅰ), 21.5 kDa (Ⅱ), 24.8 kDa (Ⅲ), 34～43 kDa (Ⅴ) and ＞170 kDa (Ⅵ). Based on the results of protein yield, SDS-PAGE and SEM analysis, we concluded that UAE could provide higher extraction yield and take a much shorter extraction time when compared to MSE, without changing the protein profile. In this research, UAE was firstly performed for the protein extraction from Radix Isatidis. Furthermore, the application of UAE in enhancing protein extraction from Radix Isatidis will provide basis for developing other protein functional foods for health purposes.

References:

[1] XIAO P, YE WY, Chen JW, et al. Antiviral activities against influenza virus (FM1) of bioactive fractions and representative compounds extracted from Banlangen (Radix Isatidis)[J]. J Tradit Chin Med, 2016, 36(3):369-376.

[2] FANG JG, LIU YH, WANG WQ, et al. The anti-endotoxic effect of o-aminobenzoic acid from Radix Isatidis[J]. Acta Pharmacol Sin, 2005, 26(5):593-597.

[3] XIAO P, HUANG HZ, Chen JW, et al. In vitro antioxidant and anti-inflammatory activities of Radix Isatidis extract and bioaccessibility of six bioactive compounds after simulated gastro-intestinal digestion[J]. J Ethnopharmacol, 2014, 157:55-61.

[4] KONG W, ZHAO Y, SHAN L, et al. Thermochemical studies on the quantity - Antibacterial effect relationship of four organic acids from Radix Isatidis on Escherichia coli growth[J]. Biol Pharm Bull, 2008, 31(7):1301-1305.

[5] YE W, LI X, ChENG J. Screening of eleven chemical constituents from Radix isatidis for antiviral activity[J]. Afr J Pharm Pharmacol, 2011, 5(16):1932-1936.

[6] MALAGUTI M, DINELLI G, LEONCINI E, et al. Bioactive Peptides in Cereals and Legumes: Agronomical, Biochemical and Clinical Aspects[J]. Int J Mol Sci, 2014, 15(11):21120-21135.

[7] YANG X, WANG Q, WANG Y, et al. Analysis on Isatidis Radix Protein in Different Areas by SDS-PAGE[J]. Chin J Inform Tradit Chin Med, 2015, 22(7):86-88.

[8] XIAO P, HUANG HZ, Chen JW, et al. Nutritional evaluation, characterization and antioxidant activity of Radix Isatidis protein hydrolysates under simulated gastrointestinal digestion[J]. J Food Nutr Res, 2014, 11(2):831-838.

[9] ORDONEZ-SANTOS LE, PINZON-ZARATE LX, GONZALEZ-SALCEDO LO. Optimization of ultrasonic-assisted extraction of total carotenoids from peach palm fruit (Bactris gasipaes) by-products with sunflower oil using response surface methodology[J]. Ultrason Sonochem, 2015, 27:560-566.

[10] CELLI GB, GHANEM A, BROOKS MS. Optimization of ultrasound-assisted extraction of anthocyanins from haskap berries (Lonicera caerulea L.) using Response Surface Methodology[J]. Ultrason Sonochem, 2015, 27: 449-455.

[11] KARKI B, LAMSAL BP, GREWELL D, et al. Functional properties of soy protein isolates produced from ultrasonicated defatted soy flakes[J]. J Am Oil Chem Soc, 2009, 86(10):1021-1028.

[12] ZHU J, FU Q. Optimization of ultrasound-assisted extraction process of perilla seed meal proteins[J]. Food Sci Biotechnol, 2012, 21(6):1701-1706.

[13] ZHU K, SUN X, ZHOU H. Optimization of ultrasound-assisted extraction of defatted wheat germ proteins by reverse micelles[J]. J Cereal Sci, 2009, 50(2):266-271.

[14] BRADFORD MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding[J]. Anal Biochem, 1976, 72:248-254.

[15] TOMSIK A, PAVLIC B, VLADIC J, et al. Optimization of ultrasound-assisted extraction of bioactive compounds from wild garlic (Allium ursinum L.)[J]. Ultrason Sonochem, 2016, 29: 502-511.

[16] PRANDI B, BENCIVENNI M, FACCINI A, et al. Composition of peptide mixtures derived from simulated gastrointestinal digestion of prolamins from different wheat varieties[J]. J Cereal Sci, 2012, 56(2):223-231.

[17] ZHANG Q, FAN X, ZHANG Z, et al. Optimization of SC-CO_2 extraction of oil from almond pretreated with autoclaving[J]. LWT-Food Sci Technol, 2009, 42(9):1530-1537.

[18] VILKHU K, MAWSON R, SIMONS L et al. Applications and opportunities for ultrasound assisted extraction in the food industry - A review[J]. Innov Food Sci Emerg, 2008, 9(2): 161-169.

(编辑:董宇)