Extraction technology of isoflavone from Dabie Mountain's *Pueraria lobata* and determination of its reducing power

Huang Renshu[1, 2]*, He Huili[2], Fang Jiaojiao[2] and Ren Yuanjun[2]
[1]Plant Cell Engineering of Anhui Province Engineering Technology Research Center, Lu'an, China
[2]Faculty of Biological and Pharmaceutical Engineering, West Anhui University, Lu'an, China

Abstract: In order to determine the optimum extracting process of the isoflavone from Dabie Mountain's *Pueraria lobata* and its reducing power, a 5-level, 4-factor Box-Behnken center-united experiment was conducted on the basis of single factor experiment. The Box-Behnken experiment analyzed and optimized the processing conditions by response surface methodology, and determined the reducing power of the Pueraria Isoflavone's extract under the optimum conditions. The results showed that the extraction rate of the Pueraria Isoflavone was 24.87%, of which the relative error was only 0.4% compared with the predicted value of 24.77%, under the optimum conditions of ethanol concentration 7.5%, liquid-to-solid ratio17.50mL/g, treatment temperature 94.30□, treatment time 47.36min. The experiment also concluded that response surface methodology was suitable for the regression analysis of isoflavone's extraction rate and parameter optimization; the reducing power of Pueraria Isoflavone's EC50 value was 0.098mg/mL.

Keywords: Pueraria lobata; isoflavone; response surface; extraction technology; reducing power.

INTRODUCTION

Pueraria lobata (P. Lobata (Willd.) Ohwi) is commonly used as Traditional Chinese Medicine's drug of dispelling wind and detoxification, of which the main active ingredients are isoflavone compounds, such as puerarin, daidzein and its derivatives (Makambila-Koubemba M C *et al*., 2011). A large number of studies has shown that the isoflavone compounds act effectively protecting the heart, reducing blood sugar, improving cerebral circulation, delaying arteriosclerosis, anti-aging, anti-oxiding, anti-tumor, enhancing immunity, and liver-protection from the damage of alcohol. Domestically it is mainly used in the clinical treatment of angina, coronary heart disease, hypertension and arrhythmia (Wang Yang, 2011 J Gupta *et al*., 2008). At present, it is a leading edge of the chemical research to make puerarin and daidzein compounds from Pueraria, a renewable biological resource, instead of from the traditional petrochemical raw materials ((Zhou Li, 2012). In the relative studies, Zhuo Lv *et al*'s extraction rate of isoflavone was 22.9% with 50%-90% alcohol as solvent under the optimum conditions (Zhou lv *et al*., 2008). Chen Yaya *et al* compared the effect of distilled water on the extraction rate of Pueraria Isoflavone with the effect of 40%-95% ethanol, showing little difference between 60% ethanol and distilled water (Chen Ya-wei, *et al*., 2012). But the studies haven't examined the effect of low-density ethanol on the extraction rate of Pueraria Isoflavone. Therefore this study took Dabie Mountain's *Pueraria lobata* as the object, and systematically studied the effect of 0-80% ethanol on the extraction process of Puerariae isoflavone through response surface methodology. The research has practical significance to promote economic development in Dabie Mountain's forest area and the industria production of Pueraria Isoflavone. In addition, in view that the present researches of Pueraria Isoflavone's inoxidizability mainly focus on hydroxyl radical, superoxide anion free radicals, DDPH free radicals and lipid per oxidation clearance rate (Zhang Jiahe *et al.,* 2010; Li Zhirui *et al.,* 2011; Wang Aiping *et al.,* 2003; Zhang Fengqing, 2010; Pei Lingpeng *et al.*, 2003), but no research on the determination of reducing power, which is, however, a commonly used method to evaluate antioxidant's activity, this study focuses on the determination of the extract's reducing power under optimum conditions, with the purpose of perfecting the evaluation system of Pueraria Isoflavone' s inoxidizability.

MATERIALS AND METHODOLOGY

Apparatus and materials

Q/TPYCI UV visible spectrophotometer (Shanghai Spectral Element Instrument Co. Ltd.), DK-8D three-hole, three-temperature water bath (Jintan Jierui'er Electric Appliance Co., Ltd.), JA2003B electronic balance (Henan Brother Equipment Co. Ltd.), CX-500A high speed multi-function mill (Haissenai Machinery Co., Ltd.), Q/IMVP1-2006 intelligent eltectrothermal constant-temperature drying box (Shanghai Langxuan Experimental Equipment Co., Ltd.), SHB-IIIS type multi-use of recycled water vacuum pump (Zhengzhou the Great Wall Branch Trade and Industry Co. Ltd.), RE-2000A rotary evaporator (Shanghai Yarong Biochemical Instrument Factory). Anhydrous ethanol (mass fraction 99.7%, Shanghai Yihai Chemical Reagent Co., Ltd.), phosphate buffer solution (Xilong Chemical Industry Inc. Ltd, 99%), three

*Corresponding author: e-mail: ahhrs@126.com

chloroacetic acid (Shanghai Ling Feng No.1 Chemical Reagent Co. Ltd., 99%), potassium ferricyanide (Chinese Shanghai Reagent factory, 99.5%), ascorbic acid (Tianjin Bodi Chemical Co., Ltd., 99.7%) ferric chloride (Guangdong, Taishan Chemical Plant, 99%), puerarin (purity ≥ 98%, Shanghai Yuanye Biotechnology Co. Ltd.). *Pueraria lobata* was collected from the wild area of Dabie Mountain.

Determination of pueraria isoflavone

Configuring the standard liquid: Given that the Isoflavone can be determined by means of the UV spectrophotometry (Zhao Ying *et al.*, 2012), 5.0mg puerarin of standard variety was accurately weighed into 50mL volumetric flask, adding 95% ethanol as constant volume, shaking dissolved as the standard liquid.

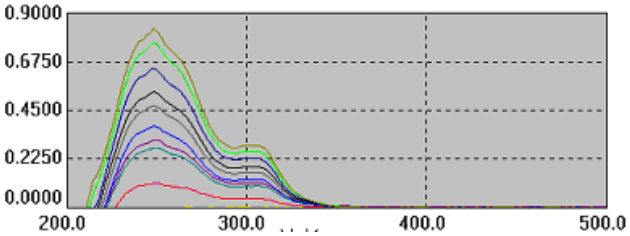

Fig. 1: The full spectrum scan graph of puerarin

Determining the maximum absorption wavelength: Gradient solution of the standard solution was scanned by ultraviolet spectrophotometer at 200-500nm wavelength range, obtaining puerarin's full wavelength scannogram to determine the wavelength λ.

Drawing standard curve: by determining the absorbance A at the point of wavelength λ, the linear relationship between absorbance and Pueraria Isoflavone concentration C (mg/mL) was established.

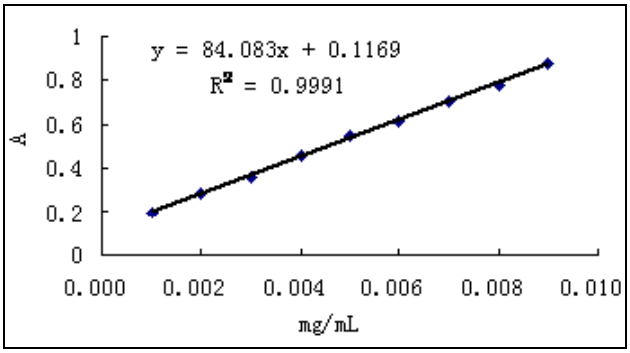

Fig. 2: The Standard curve of puerarin

Extraction of pueraria isoflavone with single factor experiment

Screening test of ethanol concentration: accurately weighing dry Pueraria powder 5.0g, 15 portions in all, divided into 5 groups, each was soaked in 60°C Water Bath 30min, with a liquid-to-solid ratio of 20ml/g in 0% (distilled water), 20%, 40%, 60%, 80% ethanol respectively. Then filtered and removed the 10μL extraction to a 10mL volumetric flask with a pipette, using 95% ethanol as constant volume, measuring its absorbance to determine the concentration of ethanol.

Screening test of the liquid-to-solid ratio: Based on the previous results, extracting with 10, 20, 30, 40, 50mL/g ethanol respectively, to determine the ratio of liquid to solid.

Screening test of treatment temperature: as above, bathing in 20, 40, 60, 80, 100□ water respectively, to determine the treatment temperature

Screening test of treatment time: As above, modifying the bathing time periods to 10, 30, 50, 70, 90 min respectively, to determine the treatment time.

Optimizing with the response surface methodology

In contrast to the fact that traditional optimal experiments are difficult to analyze the interaction among existing factors, response surface methodology (RSM) can be used effectively to evaluate the effects of multiple factors and their effects on the interactions of one or more response values, by which we can not only reduce the number of experiments by reasonable design, but also obtain optimum conditions among the examining points through the establishment of mathematical model (Zhu Aishi, 2013). Therefore, this test, based on the determination of single factor range, used Design-Expert. 8.05b software to conduct Box-Behnken experiment, a 5-level, 4-factor experiment, with the concentration of ethanol (X_1), the ratio of liquid to solid (X_2), treatment temperature (X_3), treatment time (X_4) as dependent variables, and the extraction rate of isoflavone (Y) as response value.

Test of determining the reducing power

Prepare Pueraria Isoflavone under the condition of optimum RSM, filter the extraction liquid, rotary-evaporate and concentrate the filtrate, soak the concentrated sample with 95% alcohol for 12h. then repeat the rotary evaporation, concentration, centrifugation and filtration, thus 25.380mg/mL mother liquor of Pueraria Isoflavone obtained. Take Phosphate buffer solution of 2mL 0.2mol/L & pH6.6, then add 1% potassium ferricyanide solution 2mL, sample solution 2mL in sequence, mix them and react for 20min at 50□. Cool it and add 10% three-chloroacetic acid 2mL to it, centrifuging with a speed of 3000r/min for 10min after blending. Then take 2mL of supernatant, 2mL of distilled water, 0.4mL of 0.1% three-ferric chloride solution and blend, let react for 10min at room temperature, measuring the absorbance at 700nm A. The more the absorbance, the bigger the reducing power. Sample liquors were distilled water, Vc solution 0.25mg/mL, and 10^1, 10^2, 10^3, 10^4, 10^5, 10^6 times of the mother liquor of Pueraria Isoflavone. The experiment repeated for 3 times.

Table 1: Comparison of the extracting effects of different ethanol concentrations

Ethanol concentration of (%)	Average extraction rate Y_i (%)	Y_i-11.32	Y_i-14.35	Y_i-14.77	Y_i-17.10
0	20.04	8.72**	5.69**	5.27**	2.94**
20	17.10	5.78**	2.75**	2.33**	
60	14.77	3.45**	0.42		
40	14.35	3.03**			
80	11.32				

Note: $df_A = 4$, $df_e = 10$, SS_A=127.5506 , SS_e=4.9136, $S_x = 0.4047$

Table 2: Comparison of the extracting effects of different liquid-to-solid rations

Liquid-to-solid ratio (mL·g^{-1})	Average extraction rate Y_i (%)	Y_i-15.70	Y_i-16.89	Y_i-17.14	Y_i-17.71
20	19.39	3.69**	2.50**	2.25**	1.68**
30	17.71	2.01**	0.82*	0.57*	
10	17.14	1.44**	0.25		
50	16.89	1.19**			
40	15.70				

Note: $df_A = 4$, $df_e = 10$, SS_A=21.7394, SS_e=0.9157, $S_x = 0.1747$

Table 3: Comparison of the extracting effects of different treatment temperatures

Treatment tmp (□)	Average extraction rate Y_i (%)	Y_i-17.92	Y_i-18.03	Y_i-18.85	Y_i-21.72
100	24.65	6.73**	6.62**	5.80**	2.93*
80	21.72	3.80*	3.69*	2.87*	
20	18.85	0.93	0.82		
60	18.03	0.11			
40	17.92				

Note: df_A=4, df_e=10, SS_A=101.5360, SS_e=18.4002, $S_x = 0.7832$

Table 4: Comparison of the extracting effects of different treatment time

Treatment time (min)	Average extraction rate Y_i (%)	Y_i - 22.76	Y_i - 23.75	Y_i - 24.61	Y_i - 25.85
50	26.51	3.75**	2.76**	1.90*	0.66
70	25.85	3.09**	2.10*	1.24	
30	24.61	1.85*	0.86		
90	23.75	0.99			
10	22.76				

Note: $df_A = 4$, $df_e = 10$, SS_A=27.8279 , SS_e=6.7743, $S_x = 0.4752$

RESULTS

Methods of determining pueraria isoflavone

By The full spectrum scan graph of puerarin, we can see a stable characteristic absorption peak at the ultraviolet wavelength 250nm, so puerarin's wavelength $\lambda = 250$nm; the concentration of standard solution as abscissa, the corresponding absorbance as ordinate, the standard curve of Pueraria Isoflavone is established (fig. 2), standard curve at 250nm wavelength is A=84.083C+0.1169, R=0.9995. Refer to the provisions of *Quality Control in Foodstuff Physical and Chemical Lab*: for screening methods, the correlation coefficient of linear regression equation should not be less than 0.98, for the confirmation methods, correlation coefficient should be no less than 0.99 (GB/T 27404-2008, 2008). Therefore, we conclude that the standard curve is reasonable.

Single factor experiment

Screening test of ethanol concentration

By F test, F=64.90>F0.01 $_{(4,10)}$ =5.99, showing the effects of different concentrations of ethanol on the extraction of Puerariae isoflavone has extremely significant difference (P<0.01). Further SSR test (table 1) showing that extraction ratio of Pueraria Isoflavone increased significantly with concentrations of ethanol decreasing (P<0.01). When extracted with ethanol of 0% concentration, i.e. distilled water, extraction ratio of Pueraria Isoflavone reached 20.04%. Therefore, it is concluded that suitable extract ant is distilled water;

Table 5: Design of the response surface for extracting the Pueraia Isoflavone and its results

Serial number	Ethanol concentration X_1/%	Liquid-to-solid ratio X_2/mL·g^{-1}	Processing tmp X_3/°C	Processing time X_4/min	Average extraction rate Y/%
1	2.5	17.5	92.5	45	21.04
2	7.5	17.5	92.5	45	24.06
3	2.5	22.5	92.5	45	20.94
4	7.5	22.5	92.5	45	21.22
5	2.5	17.5	97.5	45	20.74
6	7.5	17.5	97.5	45	25.31
7	2.5	22.5	97.5	45	21.74
8	7.5	22.5	97.5	45	19.39
9	2.5	17.5	92.5	55	20.65
10	7.5	17.5	92.5	55	23.66
11	2.5	22.5	92.5	55	21.49
12	7.5	22.5	92.5	55	21.76
13	2.5	17.5	97.5	55	20.24
14	7.5	17.5	97.5	55	19.74
15	2.5	22.5	97.5	55	23.95
16	7.5	22.5	97.5	55	22.27
17	0	20	95	50	21.12
18	10	20	95	50	23.53
19	5	15	95	50	19.59
20	5	25	95	50	22.39
21	5	20	90	50	18.34
22	5	20	100	50	21.77
23	5	20	95	40	19.07
24	5	20	95	60	20.14
25	5	20	95	50	24.35
26	5	20	95	50	25.77
27	5	20	95	50	23.92
28	5	20	95	50	23.8
29	5	20	95	50	24.43
30	5	20	95	50	22.37

response surface methodology can narrow the screening range of ethanol concentrations, at intervals of 0-10%.

Screening test of liquid-to-solid ratio

By F test, F=59.35 **>** $F_{0.01(4,10)}$=5.99 ， , showing the effects of different ratios of liquid-to-solid on the extraction of Puerariae isoflavone has extremely significant difference (P<0.01). Further SSR test (table 2) showing that extraction ratio of Pueraria Isoflavone reached 19.39%, the highest value at liquid-to-solid ratio of 20mL/g, which has extremely significant difference with other groups (P<0.01). Therefore, it is concluded that suitable liquid-to solid ratio is 20mL/g; response surface methodology can narrow the screening range of liquid-to-solid ratio, at intervals of 15-25mL/g.

Screening test of treatment temperature

By F test, F=13.80 **>** $F_{0.01(4,10)}$=5.99, showing the effects of different treatment temperatures on the extraction of Puerariae isoflavone has extremely significant difference (P<0.01). Further SSR test (table 3) showing that the effect of temperature on the extraction of Puerariae isoflavone was not significant at 20-60□ (P **>** 0.01), but that effect increased significantly at 80-100□ (P<0.05). extraction ratio reached 24.65% at 100□, which has extremely significant difference with the temperatures below 60□ (P<0.01). Therefore, it is concluded that suitable treatment temperature is 100□; response surface methodology can narrow the screening range of treatment temperature, at intervals of 90-100□.

Screening test of treatment time

By F test, F=10.27>$F_{0.01(4,10)}$=5.99, showing the effects of different treatment time on the extraction of Puerariae Isoflavone has extremely significant difference (P<0.01). Further SSR test (table 3) showing that Further SSR test (table 2) showing that extraction ratio of Pueraria Isoflavone reached 26.51%, at 50mmin, which has no significant difference with the treatment time of 70min (P>0.01), but has extremely significant difference with

other treatment time (P<0.01) or (P<0.05). Therefore, it is concluded that suitable treatment time 50min; response surface methodology can narrow the screening range of treatment time, at intervals of 40 -60 min.

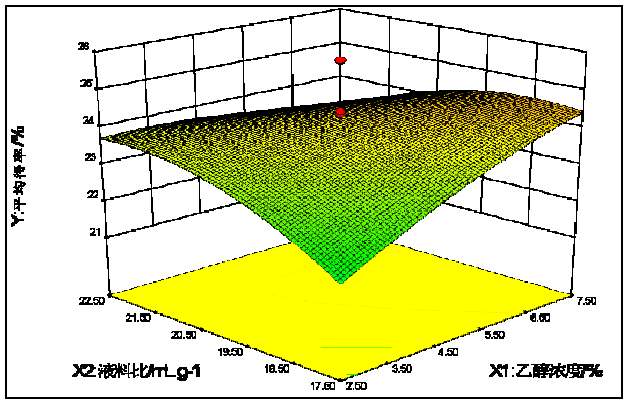

Fig. 3.1: Response surface of ethanol solution and liquid-to-solid ratio.

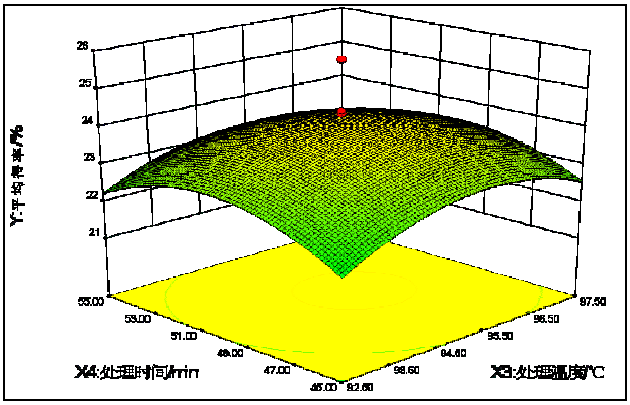

Fig. 3.2: Response surface of treatment temperatures and time.

Optimal test of RSM

Establishment of regression equation and statistical test

The design of the response surface for extracting Puerariae Isoflavone and the testing results are shown in table 5. By square regression analysis through Design-Expert.8.05b software, the regression equation is as following: Y=-1254.64428+10.67209X_1-3.67834X_2 +25.30892X_3+3.24516X_4-0.13586X_1X_2-0.065456X_1X_3-0.022114X_1X_4+0.053117X_2X_3+0.065181X_2X_4-6.32559E$^{-0.03}X_3X_4$-0.044071X_1^2-0.097471X_2^2-0.13493X_3^2-0.038253X_4^2.

From the result of the digital model of variance analysis (to see table 6), we find that F=2.6018 and (Prob>F) < 0.05, the probability that the model's F value has a relatively large error duo to noises is only 3.82%, which shows that the model is significant, with statistical significance; the model's lack of fit (Prob>F) >0.05, i.e., the lack of fit was not significant, indicating that the fitting degree of the experiment and the model is high, so it's unnecessary to introduce the items with more high frequency. Therefore, the results of extraction process of Pueraria Isoflavone can be predicted by this model. From the testing results of the significance of the regression model's coefficient, we find that the partial regression coefficients of X_1X_2, X_2X_4 in this model of is significant (P<0.05), showing that the interaction items of the ethanol concentration with the ratio of liquid-to-solid, and the ratio of liquid-to-solid with the treatment time have a significant effect on the extraction rate of Pueraria Isoflavone; the significance of the quadratic term X_2^{2}' s partial regression coefficient (with P<0.05) and the extreme significance of X_3^2, X_4^2 partial regression coefficient (P<0.01) indicate that the quadratic terms of the square of the liquid-to-solid ratio, the square of the treatment temperature and the square of the treatment time have significant effect on the extraction rate of Pueraria Isoflavone.

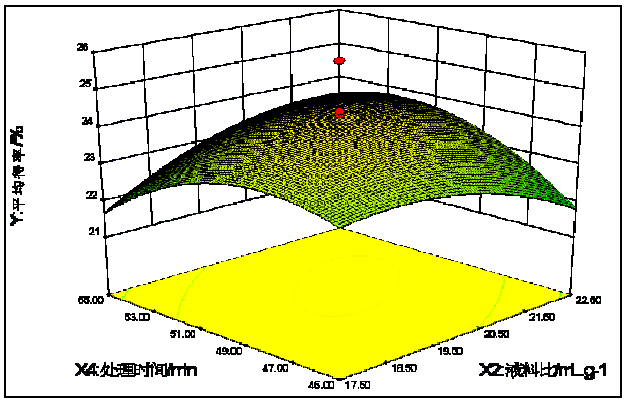

Fig. 3.3: Response surface of liquid-solid ratio and treatment time.

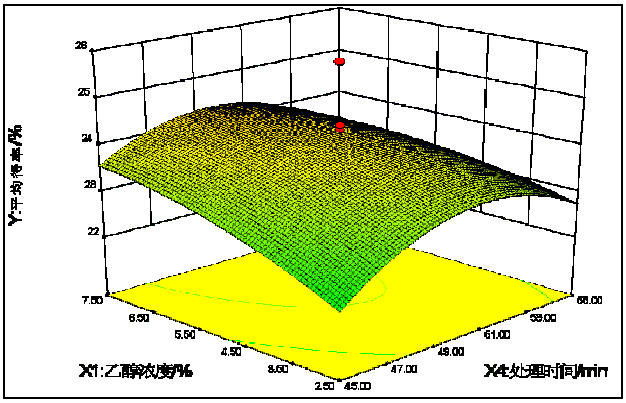

Fig. 3.4: Response surface of ethanol solution and treatment time.

Response surface analysis of pueraia isoflavone' extraction

From fig. 3-1, we can find the obvious interaction between the ethanol concentration and the liquid-to-solid ratio. In the low ethanol concentration, extraction rate of Pueraria Isoflavone increased with the raise of the liquid-to-solid ratio; when the ethanol concentration was raised, extraction rate of Pueraria Isoflavone dropped after increased at first with the raise of liquid-to-solid ratio. fig.

Table 6: Variance analysis for optimum mathematical model of the Pueraia Isoflavone's extraction process

Source of variation	Sum of squares	Degree of freedom	Mean square	*F* value	*Prob>F*
Model	78.2797	14	5.5914	2.6018	0.0382
Ethanol concentration X_1	5.4215	1	5.4215	2.5228	0.1331
Liquid-to-solid ratio X_2	0.3559	1	0.3559	0.1656	0.6898
Processing tmpX_3	1.2235	1	1.2235	0.5693	0.4622
Treatment time X_4	0.0865	1	0.0865	0.0402	0.8437
X_1X_2	11.5367	1	11.5367	5.3682	0.0351
X_1X_3	2.6778	1	2.6778	1.2460	0.2819
X_1X_4	1.2225	1	1.2225	0.5689	0.4624
X_2X_3	1.7634	1	1.7634	0.8205	0.3793
X_2X_4	10.6214	1	10.6214	4.9424	0.0420
X_3X_4	0.1000	1	0.1000	0.0465	0.8321
X_1^2	2.0810	1	2.0810	0.9683	0.3407
X_2^2	10.1791	1	10.1791	4.7366	0.0459
X_3^2	19.5076	1	19.5076	9.0773	0.0087
X_4^2	25.0847	1	25.0847	11.6724	0.0038
residual	32.2359	15	2.1491		
Lack of fit	26.1571	10	2.6157	2.1515	0.2058
Pure error	6.0788	5	1.2158		
Total	110.5156	29			

Table 7: The comparison of reducing power between Pueraia Isoflavone and Vc solution

Group	Absorbance (A_I±SD)	A_i - 0.191	A_i - 0.206	A_i - 0.206	A_i - 0.216	A_i - 0.402	A_i - 0.666	A_i - 2.389
Vc group	2.431±0.0036	2.240**	2.225**	2.225**	2.215**	2.029**	1.765**	0.042
Diluted by 10^1 times	2.389±0.0005	2.198**	2.183**	2.183**	2.173**	1.987**	1.723**	
Diluted by 10^2 times	0.666±0.0001	0.475**	0.460**	0.460**	0.450**	0.264**		
Diluted by 10^3 times	0.402±0.0003	0.211**	0.196**	0.196**	0.186*			
Diluted by 10^4 times	0.216±0.000001	0.025	0.010	0.010				
Diluted by 10^5 times	0.206±0.00001	0.015	0					
Diluted by 10^6 times	0.206±0.00001	0.015						
Distilled water	0.191±0.00001							

Note: $df_A = 7$, $df_e = 16$, SS_A=20.3042 , SS_e=0.0089, $S_x = 0.0136$

3-2 shows that extraction rate of Pueraria Isoflavone increased at first and then decreased with the treatment time or processing temperature increasing. From fig. 3-3, we can find the obvious interaction between the liquid-to-solid ratio and the treatment time. In the low treatment time, extraction rate of Pueraria Isoflavone decreased as the liquid-to-solid ratio increased; when the treatment time was added, extraction rate of Pueraria Isoflavone appeared to increase with the treatment time increasing. fig. 3-4 shows that extraction rate of Pueraria Isoflavone increased with the raise of the ethanol concentration but it increased at first and dropped later along with the raise of the treatment time.

Experimental verification

Data optimized by the software Design-Expert.8.05b, we obtained the maximum extraction rate of 24.77% under the following conditions: The concentration of ethanol 7.5%, ratio of liquid to solid 17.50mL/g, temperature 94.30□, time 47.36min. To verify the optimization results, under the optimum conditions, the extraction rate of Pueraria Isoflavone is 24.87%, with an only 0.4% relative error compared with the predicted value.

Test of determining the reducing power

Reducing power of different concentrations of Pueraria Isoflavone are shown in table 7. By F test, F=5195.87, $F_{0.01(7,16)}$ =4.03, showing the effect of different concentrations on the reducing power of Puerariae Isoflavone has extremely significant difference (P<0.01). Further SSR test showing that when the concentrated solution of the Puerariae Isoflavone extract stayed in the dilution range of 10^1~10^3 times, the reducing power showed extreme significance (P<0.01); and the reducing power of the Puerariae Isoflavone of 2.538 mg/ml is equal to that of Vc solution of 0.25mg/mL (P>0.05). In the effective concentration range the linear relationship was established between absorbance and Pueraria Isoflavone concentra-

tion C: A=0.7760C+0.4237, R^2=0.9984: by this linear relationship we got the EC50 value of Pueraria Isoflavone (the sample concentration when absorbance value is 0.5 at 700nm in reducing power test) is 0.098mg/mL.

DISCUSSIONS

The experiment shows that the optimal solvent for extracting Pueraria Isoflavone is very-low density ethanol (7.5%). β ring in the master Nuclear structure of Pueraria Isoflavone is stericly hindered by the pyran ring carbonyl, and a planar molecule can not be formed, so it is not tightly arranged, and intermolecular force is small, which is conducive to water molecules' entry, therefore it has certain hydrophilic (Cheng Gang *et al.*, 2012). Meanwhile, sugar chain of glycosides compounds in Pueraria Isoflavone is rather short, and the solubility of flavonoids is reduced in high concentration ethanol. In addition, the dissolved quantity of the elements like ethanol-soluble impurities of pigments increases, and the elements compete with flavonoids to combine with ethanol-water molecules, resulting into the decrease of flavonoids' extraction rate (Zhou Xianjiao *et al*., 2012). While the study finds that the extraction rate of isoflavone in very-low density ethanol is higher than that in water or high density ethanol, the limitation lies in that no comparisons of the efficacy among the extracted Pueraria Isoflavone in different solvents has been made.

CONCLUSION

This research took Dabie Mountain's Pueraria as the raw material, the extraction rate of Puerariae Isoflavone as the index, and established mathematical regression model of optimum conditions of extracting Puerariae Isoflavone through RSM on the basis of the single factor test. By the optimization of the quadratic regression equation we obtained the optimum processing conditions for extracting Puerariae Isoflavone: The concentration of ethanol 7.5%, ratio of liquid to solid 17.50mL/g, temperature 94.30□, time 47.36min; and under this condition, the extraction rate of Pueraria Isoflavone is 24.87%, with an only 0.4% relative error compared with the predicted value, showing the model fits well with the real situation, and RSM is suitable for the regression analysis of extraction rate of Puerariae Isoflavone and parameter optimization. Determination of Puerariae Isoflavone' reducing power under this condition indicates that reducing power increases along with the raise of the extracting solution's concentration, the value of EC50 is 0.098mg/mL.

ACKNOWLEDGMENT

This work is financially supported by Key provincial nature science foundation of Anhui higher schools (KJ2012A280); National Undergraduates' Innovation and Entrepreneurship Training Program (201310376002); 2014 Funding Program for Anhui Provincial Outstanding Young Talents in Colleges.

REFERENCES

Chen Gang, Su Tongfu and Zhao Shiju (2012). Study on effects of polar from solvents on extraction rate of pueraria flavonoids. *Henan Sci.*, **30**(11): 1585-1588.

Chen Ya-wei, Zhang Yan-ping and Zhou Dong-ju *et al* (2012). Response surface methodology for optimization of ultrasound-assisted extraction of flavonoids from radix puerariae. *Food Sci.*, **33**(14): 41-44.

GB/T 27404 (2008). The summary on quality control in foodstuff-Physical and Chemical Lab.

J Gupta, YH Siddique and T Beg *et al* (2008). A review on the beneficial effects of tea polyphenols on human health. *Int. J Pharm.*, **4**(6): 314.

Li Zhirui, Han Shuqin and Zhuang Xiaomei (2011). Purification and antioxidant activity of pueraria flavonoids. *Acad. Period. Farm Prod. Process.*, **7**(07): 33-35.

Makambila-Koubemba MC, Mbatchi B and Ardid D *et al* (2011). Pharmacological studies of ten medicinal plants used for Analgesic purposes in Congo Brazzaville. *Int. J. Pharm.*, **7**(5): 608-615.

Pei Ling-peng, Li Wen-sa and Tang Fen-fang (2003). Study on extraction of total flavone from *Pueraria lobata* ohwi by ultrasonic wave. *J. Beijing Union Univ. (Nat. Sci.)*, **17**(03): 25-27.

Wang Aiping, Zhou Liming and Lin Guowei (2003). Study on water extraction technology of antioxidant substances in pueraria residues. *Jiangsu Agr. Sci*, **41**(02): 249-250.

Wang Yang (2011). Separation and purification of puerarin from radix puerariae. Wuhan: Wuhan Inst. Technol., p.3.

Zhang Fengqing (2010). Study on extract of radix pueraria flavones and its antioxidant. *Food Ind.*, **32**(01): 68-70.

Zhang Jiahe and Han Liqin (2010). Effects of different extraction process on anti-oxidation of total Flavonoids in puerariae. *J. Anhui Agr. Sci.*, **38**(24): 1302-1303.

Zhao Ying, Wang Ran (2012). Determination of puerarin and total Flavonoids. *Bever. Ind.*, **27**(1): 37-40.

Zhou Li (2012). Studies on extraction, purification and hydrolysis of radix puerariae isoflavone. Harbin: Northeast Agr. Univ., p. 1.

ZHOU Lv, ZHANG Bin and ZHOU Wu *et al* (2008). Study on extracting total Flavone from *Pueraria Lobata* and its property of antioxidation. *J. Anhui. Agr. Sci*, **36**(17): 7402-7404.

Zhou Xianjiao, Yang Linhua and Pan Jinuan (2012). Study on the extraction process of Flavonoids from radix pueraria. *J. Zhanjiang Norm.Univ.*, **33**(6): 88-93.

Zhu Aishi (2013). Optimum extracting technology of Flavonoids from radix pueraria. *J. Zhejiang Univ. Sci. Technol.*, **25**(5): 366-372.