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Research Article

Optimization for Ultrasonic- and Microwave-assisted Extraction of Flavonoids from Burdock (*Arctium lappa* L.) Root by Response Surface Methodology

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Abstract: An efficient Ultrasonic and Microwave Assisted Extraction (UMAE) was developed to extract total flavonoids from the *Arctium lappa* L. boot. Response Surface Methodology (RSM) combined Box-Behnken Design (BBD) was employed to optimize extraction condition based on the yield of total flavonoids. The optimal condition was identified: ethanol concentration, 59% (v/v); liquid/ratio ratio, 50 mL/g; ultrasound time, 3000 sec and microwave power 480 W. The predicted total flavonoids extraction yield of 0.473 g RUE/100 g DW was obtained under optimized UMAE conditions.

Keywords: Burdock root, extraction, flavonoids, response surface methodology

INTRODUCTION

Arctium lappa Linn. (burdock), called Niubang in Chinese, is an edible perennial herb of Asteraceae family. The plant has been used as a traditional medicine and a dietary vegetable for a period of long time by the Chinese civilization (Morita *et al*., 1993). It is also popular in North America, Europe and Asia for over thousands of years due to its therapeutic qualities. Although burdock leaves, fruit and seeds all can be used, the dried burdock root is the main part used for different therapeutic intentions, such as throat pain, tonsillitis, rashes, arthritis, blood purifier and various skin diseases (Chan *et al*., 2011b). These pharmacological effects are positively believed to be related to the fact that burdock root is rich in biologically active substances (Han *et al*., 2013; Tousch *et al*., 2014). Of all the compounds presented in burdock root, the flavonoids components were considered to be critical to their pharmacological activities (Miyamoto *et al*., 1993; Tamayo *et al*., 2000) and have received most attention by food manufacturers. The extraction efficiency of flavonoid components is influenced by many factors including the extraction technology (Liu *et al*., 2010; Mao *et al*., 2008). However, there is little information concerning the optimization of extraction of the flavonoids in burdock boot by modern extraction techniques.

Microwave-Assisted Extraction (MAE) is the process by which microwave energy is used to heat solvents in contact with solid samples and to partition compounds of interest from the sample into the solvent (Spigno and De Faveri, 2009). In recent years, MAE has received a great attention as a potential prospective technique to replace conventional extraction methods, mainly due to considerable savings in processing time, solvent consumption and energy (Camel, 2000; Chan *et al*., 2011a). Ultrasonic and has been widely used as one of the most industrially technique because of its potential in improving the extraction effects (Matsumoto *et al*., 2014). Recently, simultaneous Ultrasonic/Microwave Assisted Extraction (UMAE) presented many benefits by combining the vantage of ultrasonic and microwave (Lou *et al*., 2012), However, to the best of our knowledge, there are no studies related to simultaneous UMAE of TF from burdock boot.

Therefore, in the current study the total flavonoids were extracted from burdock boot. The purpose of this study was to optimize the operational conditions, including the liquid/solid ratio, the ethanol concentration, the ultrasound time and the microwave power by applying RSM. The response variable was examined based on the total flavonoids yield under different extraction conditions.

MATERIALS AND METHODS

Materials: Burdock root was purchased in Hongqi agricultural market (Tianjin, China). The samples were washed and dried. A relatively powder was obtained by ground with a blade-mill and sieved through the 60-mesh sifter. The powder was kept in 4°C until use.

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Rutin were purchased from Sigma Chemical Company. The other chemicals were of reagent grade.

UMAE extraction procedure: The process of UMAE of burdock was carried out in a microwave extraction apparatus (CW-2000, Shanghai XTrust Instruments Company, China) equipped with a 250 mL quartz vessel and a cool water circulation system. The dried sample was placed in a round bottom flask with different volume of extraction solvent according to the BBD. After the extraction procedure, the leaves extracts were filtered through Whatman filter paper. The filtrate was transferred into a volumetric flask and diluted to 100 mL for quantitative analysis.

Determination of total flavonoids: The determination of TF in the extracts was performed according to Shao *et al*. (2012). Briefly, 6.0 mL extracts solution was added separately with 1 mL 5 wt% $NaNO_2$. One mL 10 wt% $Al_2(NO_3)_3$, 10 mL 5 wt% KOH. After mixing, the absorbance of the sample was measured at 500 nm by an ultraviolet spectrophotometer (UV-5200, Shanghai Metash instruments Company, China). Rutin standards was employed to prepare the calibration curve ($y = 9.7868x + 0.0263$, $R^2 = 0.9908$), where y is the absorbance at 500 nm and x is the concentration of flavonoids (mg/mL). TF yield of the samples was calculated using the calibration standard and expressed as rutin equivalents per 100 g dry weight (g RUE/100 g DW).

Box-Behnken design: According to a BBD with 4-variable at 3-level on the TF yield, optimization of the UMAE process was investigated. Based on previous single-factor experiments, four experimental parameters, including Microwave Power (MP), Solid/Liquid Ratio (SLR), Ethanol Concentration (EC) and Ultrasound Time (UT), were chosen as independent variables with ranges of 480-600 W, 30-50 mL/g, 50-70% and 1800-3000 sec, respectively. Multiple regression was used to fit the quadratic model by analyzing data from the BBD. The second-order model equation for the response variable was as follows:

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

where, Y is the response, β_0 is a constant and β_i, β_{ii} and β_{ij} represent the linear, quadratic and interactive regression coefficients, respectively. X_i and X_j were the levels of the independent coded variables. Table 1 shows the coded levels of the independent variables and the parallel parameter values.

Statistical analysis: Multiple regression analysis (R^2), Analysis of Variance (ANOVA), the numerical optimization and the Three Dimensional (3D) response surface plots were analyzed by The Design-Expert software (trial version 8.0.5, Stat Ease Inc., Minneapolis, USA).

Table 1: Box-Behnken design factors and levels of encoded values

	Levels		
Variables	-1	0	1
Microwave power (X_1, W)	480	540	600
Liquid/solid ratio (X_2, g/mL)	30	40	50
Ultrasound time (X_3, sec)	1800	2400	3000
Ethanol concentration (X_4, %)	50	60	70

Table 2: Box-Behnken design and experimental results

Number	X_1 (%)	X_2 (mL/g)	X_3 (sec)	X_4 (w)	TF yield (g RUE/100 g DW)
1	60	30	2400	480	0.403
2	50	40	2400	480	0.418
3	60	40	2400	540	0.434
4	60	40	2400	540	0.433
5	50	30	2400	540	0.344
6	60	40	2400	540	0.435
7	60	50	2400	480	0.458
8	60	50	1800	540	0.410
9	70	30	2400	540	0.380
10	60	50	2400	600	0.437
11	60	40	2400	540	0.434
12	60	30	2400	600	0.381
13	70	40	1800	540	0.378
14	60	40	1800	600	0.446
15	50	40	3000	540	0.408
16	70	50	2400	540	0.441
17	50	40	2400	600	0.376
18	50	50	2400	540	0.403
19	60	40	3000	600	0.404
20	50	40	1800	540	0.403
21	60	30	1800	540	0.408
22	60	50	3000	540	0.460
23	60	40	2400	540	0.435
24	60	30	3000	540	0.423
25	70	40	2400	480	0.403
26	70	40	2400	600	0.394
27	60	40	1800	480	0.431
28	60	40	3000	480	0.432
29	70	40	3000	540	0.384

Verification of the model: The optimum conditions of TF extraction of MP, UT, EC and MLR were obtained from the generated model. For verification of the model, the practical yield of TF under the optimal conditions was determined.

RESULTS AND DISCUSSION

Fitting the model: In this study, four effectual variables (MP, EC, LSR, UT) that affect UMAE of burdock boot on yield of TF were optimized using BBD. All data received from 29 experimental runs (Table 2). The highest yield of TF was obtained using treatments 22 with the values of 0.460 g RUE/100 g DW and the lowest yield of TF were obtained using treatments 5 with the values of 0.344 g RUE/100 g DW. After the regression analysis of the data shown in Table 3, the second-order polynomial equation developed for TF in terms of coded units was as follows:

$$Y = 0.43 + 0.002358X_1 + 0.023X_2 + 0.003142X_3 - 0.008975X_4 + 0.0004X_1X_2 + 0.000225X_1X_3 + 0.0082X_1X_4 + 0.000525X_2X_4 + 0.00875X_2X_3 - 0.011X_3X_4 - 0.035X_1^2 - 0.008019X_2^2 - 0.003119X_3^2 - 0.003419x_4^2$$

Table 3: Analysis of Variance (ANOVA) for response surface quadratic model for the extracted protein

Source	S.S.	DF	M.S.	F-value	Prob.>F	
Model	0.016	14	1.170E-003	3.52	0.0124	Significant
X1	6.674E-005	1	6.674E-005	0.20	0.6608	
X2	6.089E-003	1	6.089E-003	18.33	0.0008	
X3	1.184E-004	1	1.184E-004	0.36	0.5599	
X4	9.666E-004	1	9.666E-004	2.91	0.1101	
X1X2	6.400E-007	1	6.400E-007	1.927E-003	0.9656	
X1X3	2.025E-007	1	2.025E-007	6.098E-004	0.9806	
X1X4	2.690E-004	1	2.690E-004	0.81	0.3834	
X2X3	3.062E-004	1	3.062E-004	0.92	0.3532	
X2X4	1.102E-006	1	1.102E-006	3.320E-003	0.9549	
X3X4	4.622E-004	1	4.622E-004	1.39	0.2577	
X12	7.932E-003	1	7.932E-003	23.88	0.0002	
X22	4.171E-004	1	4.171E-004	1.26	0.2813	
X32	6.311E-005	1	6.311E-005	0.19	0.6695	
X42	7.583E-005	1	7.583E-005	0.23	0.6401	
Residual	4.649E-003	14	3.321E-004			
Lack of fit	4.646E-003	10	4.646E-004	648.03	<0.0001	Not significant
Pure error	2.868E-006	4	7.170E-007			

S.S.: Sum of square; M.S.: Mean square

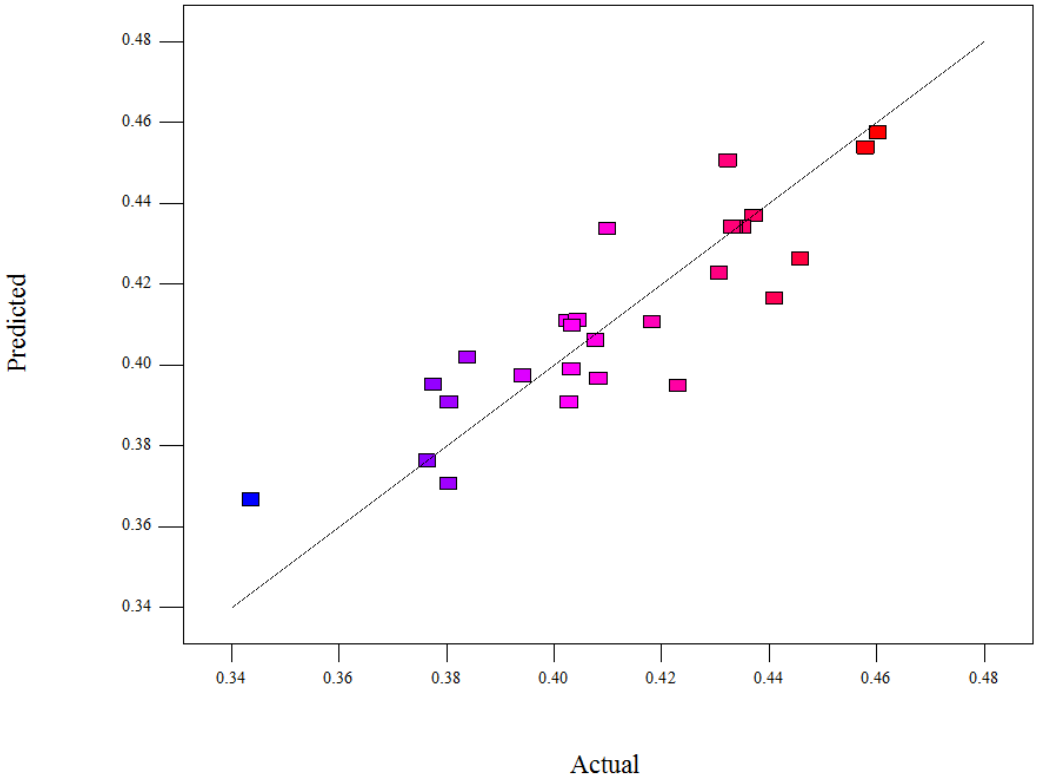
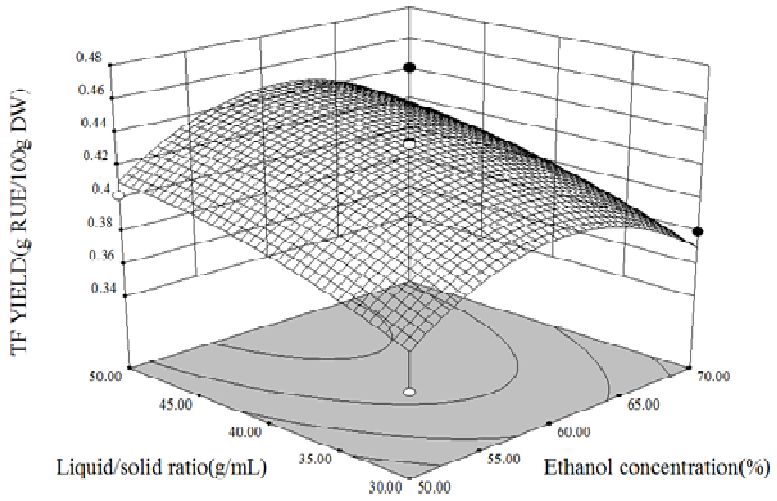
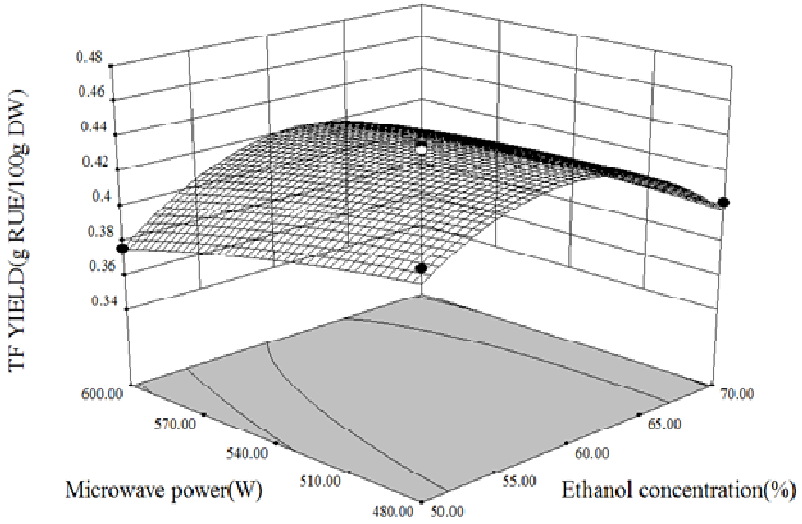


Fig. 1: Comparison between predicted and actual values of TF extraction yield

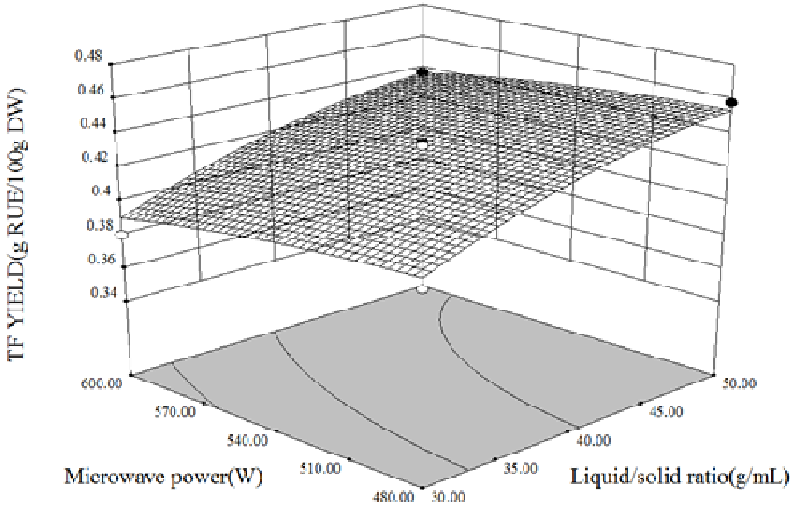
ANOVA offers the availability of the quadratic model and could evaluate the goodness of fit. It is said that a regression model would be well fitted to the experimental data if the model has a significant regression. In Table 3, the model of the TF extraction yield were statistically significant (F-value 20.13, $p<0.0001$). In addition, the model with $R^2>0.75$ was considered appropriate (Yang *et al*., 2010) and the small value of CV indicated that the variation of the mean value is low and can preferably develop an adequate response model (Liyana-Pathirana and Shahidi, 2005), in this study the correlation coefficient ($R^2 = 0.7789$) and the coefficient of variation (4.41%) demonstrated that the response predicted model was suitable for the actual situation. It was also observed that the linear term of LSR and quadratic term of EC have large significant effect on the yield of TF because of the high F-value of 18.33 and 23.88, respectively. The results indicated that the influence variables did not have a simple linear relationship. According to the


TF YIELD(g RUE/100g DW)
0.48
0.46
0.44
0.42
0.4
0.38
0.36
0.34
50.00
45.00
40.00
35.00
30.00
Liquid/solid ratio(g/mL)
50.00
55.00
60.00
65.00
70.00
Ethanol concentration(%)

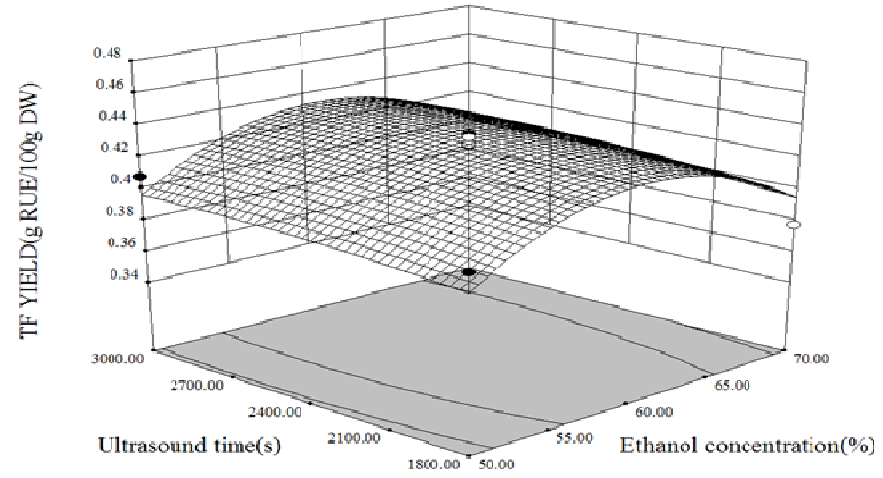
(a)


TF YIELD(g RUE/100g DW)
0.48
0.46
0.44
0.42
0.4
0.38
0.36
0.34
600.00
570.00
540.00
510.00
480.00
Microwave power(W)
50.00
55.00
60.00
65.00
70.00
Ethanol concentration(%)

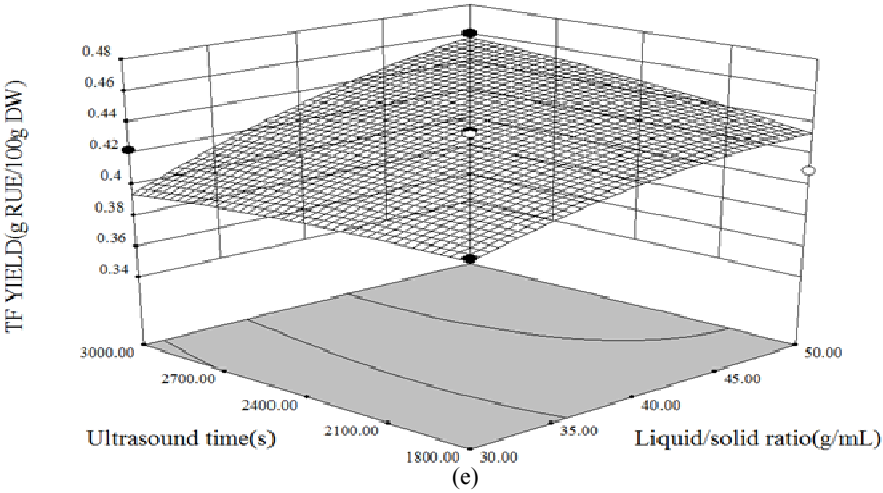
(b)


TF YIELD(g RUE/100g DW)
0.48
0.46
0.44
0.42
0.4
0.38
0.36
0.34
600.00
570.00
540.00
510.00
480.00
Microwave power(W)
30.00
35.00
40.00
45.00
50.00
Liquid/solid ratio(g/mL)

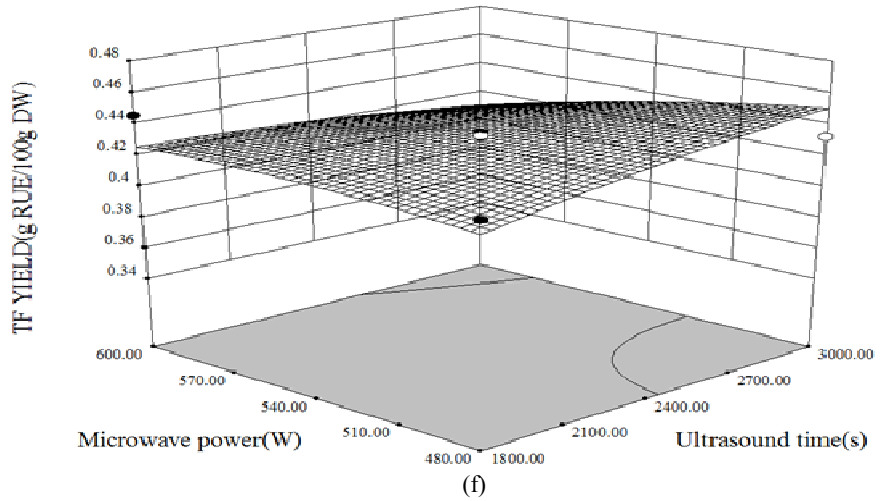
(c)



(d)



(e)



(f)

Fig. 2: Surface plots for total flavonoids extraction of *Arctium lappa L.* boot; (a): Figure plot to show the combination of liquid/solid ratio (mL/g) and ethanol concentration (%, v/v); (b): Figure plot to show the combination of microwave power (W) and ethanol concentration (%, v/v); (c): Figure plot to show microwave power (W) and liquid/solid ratio (mL/g); (d): Figure plot to show ultrasound time (sec) and ethanol concentration (%, v/v); (e): Figure plot to show ultrasound time (sec) and liquid/solid ratio (mL/g); (f): Figure plot to show microwave power (W) and ultrasound time (sec)

analysis, the studied variables influence the response variable in the following order: liquid/solid ratio > microwave power > ultrasound time > ethanol concentration.

Figure 1 depicted the plot of actual values versus predicted values for the estimated model, the relationship between the actual and predicted values shows that the actual points cluster around the diagonal line, which reveals the experimental values was in good agreement with the regression model.

Analysis of response surface: The mutual interaction of the independent variables on the extraction yield of TF can be seen on 3D response surface plots shown in Fig. 2a to f. The curves were generated by plotting the values of response variable while keeping the other two independent variables at their zero level. The steeper plots illustrated the sensitivity of the response towards the change in the extraction conditions. Otherwise, the observed effects were slight (Bezerra *et al*., 2008).

Figure 1 shows that the increase in LSR from 30 to 50 mL/g with EC from 50 to 70% increased the yield of TF. While with increase of EC over 60%, there was a gradual decline in the response. These results suggest that the LSR and EC had a quadratic effect on the response, but the mutual interactions between the two variables were not significant. Figure 2b depicts that increase of MP from 450 to 600 W at EC of 60% induce the slight decline of the yield. The yield increased when the EC changed from 50 to 60%, but decreased thereafter. Figure 2c shows that the yield of TF increased rapidly with the increase of LSR at a fixed microwave power, while the influence of the MP on the yield was less significant than the LSR. Figure 2d reveals that no obvious effect was obtained on TF yield with the increase of UT from 1800 to 3000 (s). By contrast, when the EC was lower than 60%, the TF yield increased with the enhancement of the EC, but the yield decreased when the EC exceeded 60%. The effects of the UT and LSR are shown in Fig. 2e. As LSR rose from 30 to 50 mL/g, the yield was increased. The influences of UT with range from 1800 to 3000 sec were not obvious. The highest of TF yield was obtained at 3000 sec UT with 50 mL/g LSR. Figure 2f shows that the TF yield was definitely correlated to the increase of UT, whereas no obvious change in yield was observed when MP increased from 480 to 600 W. The lowest yield of TF could be observed when extraction was performed at 600W MP with 3000 sec UT.

Validation of the model: (0.465 g) RUE/100 g DW of TF yield was obtained under the optimized operating condition (EC of 59%, LSR of 50 mL/g, UT of 3000 sec, MP of 480 W). The experimental yield of TF was in agreement with the predicted value (0.473 g RUE/100 g DW).

CONCLUSION

In this study, RSM and BBD was successfully used to optimize the UMAE process. The second order polynomial model can be applied to optimize the parameters of burdock boot extraction to obtain an extract with high TF yield (0.473 g RUE/100 g DW). The yield was significantly increased under the optimized conditions. Through this study, we managed to obtain more TF form burdock boot, dramatically increased extraction efficiency, making it possible to guide the industrial production of TF from burdock boot.

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