American Journal of Chemical and Biochemical Engineering
2017; 1(1): 7-16
http://www.sciencepublishinggroup.com/j/ajcbe
doi: 10.11648/j.ajcbe.20170101.12

Study on Extract Methodology of Total Flavonoids from Ginger and Hydroxyl Radicals Scavenging Effect

Li Huiduan*, Yu Jianzhong

Department of Chemistry and Life Science, Chuxiong Normal University, Chuxiong, P. R. China

Email address:

lhd08@cxtc.edu.cn (Li Huiduan)

*Corresponding author

To cite this article:

Li Huiduan, Yu Jianzhong. Study on Extract Methodology of Total Flavonoids from Ginger and Hydroxyl Radicals Scavenging Effect. *American Journal of Chemical and Biochemical Engineering*. Vol. 1, No. 1, 2017, pp. 7-16. doi: 10.11648/j.ajcbe.20170101.12

Received: September 8, 2016; **Accepted**: December 7, 2016; **Published**: January 7, 2017

Abstract: Extract methodology of total flavonoids from ginger and hydroxyl radicals scavenging effect, were researched in this paper. Methanol concentration, solid-liquid ratio, extraction temperature and time were determined as four single-factor in the experiment. The central points of Box-Benhnken design were selected according to the experimental results of single-factor experiment, the extract process was further optimized by RSM and BBD. The optimum extract conditions were methanol concentration of 60%, solid-liquid ratio of 3:30 (g:mL), extraction temperature of 60°C and time of 3 h, The maximum experimental extraction ratio was 0.497% by RSM. The experimental extraction ratio matched well with the theoretical value of 0.538% by solving the multiple regression equation. RSM has been proved to be an effective technique for optimization of extraction process and the fitted quadratic model has a predictive effect on target extracts. The scavenging effect of *ginger* extracts, BHT and L-ascorbic acid on·OH with the same concentration were sorted by L-ascorbic acid > *ginger* extract > BHT, and all the three antioxidant regents displayed a significant dose-effect relationship.

Keywords: Extract Methodology, Total Flavonoids, *Ginger*, Extraction Ratio, Response Surface Methodology, Hydroxyl Radicals Scavenging Effect

1. Introduction

Flavonoids are natural polyphenolic antioxidants with significant effects on anti-peroxidation and free radical scavenging. Flavonoids have significant antioxidant, anti-cancer, anti-inflammatory, bactericidal, anti-virus and regulating body immunity, and etc [1-4]. The Extraction technology of flavonoids reported in the literature includes organic solvent extraction, ultrasonic extraction, microwave extraction, supercritical fluid extraction and Enzyme-assisted extraction [5-6].

There were related reports on extraction of total flavones from ginger in the literature [7-9]. Z. Wang from Central south University reported ethanol extraction of total flavonids from ginger leaf by Response Surface Methodology and antioxidant activity in vitro, the maximum extraction ratio was 15.42±0.09mg/g under the experimental of ethanol concentration of 70%, extraction temperature of 76°C and time of 95 min [7]. Mo kai-ju from Hubei Institure for Nationalities reported study on extraction technology, purification and structural appraisal of flavonoid from ginger, the reported maximum experimental extraction ratio was 1.25% under the experimental conditions of methanol concentration of 75%, solid liquid ratio of 1:40(g:mL), extraction temperature of 60-65°C and time of 2 h. A preliminary judgment that the double hydrogen flavonoids were the main composition of total flavonoid from ginger was obtained by UV spot monitoring Ultraviolet spectrum analysis of color reaction, etc [8]. Gao shu-yun from Xuzhou Institute of Technology reported soxhlet extraction of flavonoids from ginger, the optimal experimental conditions were ethanol concentration of 66.4%, solid-liquid ratio of 1:39.9 (g:mL), extraction temperature of 96.95% and time of 4.02 h by response surface methodology, with corresponding maximum extraction ratio of 1.611 mg/g [9].

Response Surface Methodology (RSM) is a statistical method in order to solve problems containing multiple variables. Multiple quadratic function was obtained by reasonable experimental design to fit the factors and response value, and the optimal process parameters were determined by analyzing regression equation. RSM has advantages of shorten experimental cycle, high accuracy of the regression equation, Interactions between multiple factors, etc [10]. RSM was demonstrated an effective statistic technique for optimizing complex processes, which has been successfully used to optimize the extraction of total flavonoids from many medicine plants [11-12]. The total flavonoids extraction ratio was greatly influenced by extraction conditions, Box-Behnken Design was performed to predict the optimal extraction conditions [13].

Here, methanol extract methodology of flavonoids from *ginger* was optimized by RSM. flavonoids content of *ginger* extracts was further determined by spectrophotometry. Additionally, hydroxyl radicals scavenging effect of *ginger* extracts was also studied. This research was helpful in providing valuable flavonoids content data for development and utilization of *ginger.*

2 Materials and Methods

2.1. Materials

Ginge, Rutin, Methanol, Macroporous Resin, $NaNO_2$, $Al(NO_3)_3$, NaOH, $AlCl_3$, $FeCl_3$, NH_3(aq).

Ginger (purchased in chuxiong's agricultural market) → dry → crush → Spare.

2.2. Experimental Methods

Methanol extract methodology of total flavonoids from *ginger* was initially determined by single-factor experiment, Methanol concentration, solid-liquid ratio, extraction temperature and time were determined as four single-factor in the experiment. The central points of Box-Benhnken Design were selected according to the experimental results of single-factor experiment, the extract process was further optimized by RSM and BBD. Finally, Research on hydroxyl radicals scavenging effect of *ginger* extracts was studied. The experimental illustration of optimization of flavonoids extraction by RSM and study on radical scavenging effects were illustrated in Figure 1. Methanol extraction from *ginger*, Chromogenic reaction of *ginger* extracts, and obtains of linear equations from Rutin standard curve were performed according to the literature [14-15].

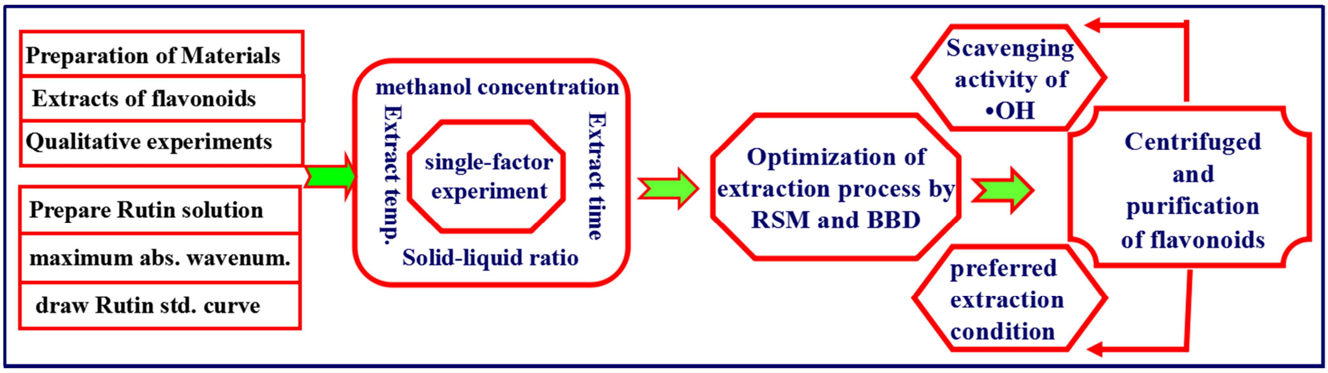

Figure 1. *The experimental illustration of RSM optimization of ginger flavonoids extraction and radical scavenging effects.*

2.3. Optimization of the Extraction Process by RSM

Optimization of flavonoids extraction by RSM was operated as references [16]. Box-Benhnken Design combining with quadratic response model of four factors and three levels were performed to optimize the extract methodology. First, the independent variables of four single-factors were determined, the level of variables were coded by -1, 0, 1 based on the results of single-factor experiment (as shown in Table 1). A total of 25 points were designed, including points 16 factorial, 8 star points and 1 central points to ensure the precision of experiment.

Table 1. *Factors and levels of Response Surface Methodology.*

Code levels of independent variables	Independent variables			
	A: Methanol concentration (%)	B: Solid-liquid ratio ($mL \cdot g^{-1}$)	C: Extraction temperature (°C)	D: Extraction time (h)
-1	60	3:30	50	2.0
0	65	4:30	60	3.0
+1	70	5:30	70	4.0

2.4. Study on Hydroxyl Radical Inhibition Activity

Total flavonoids were extracted from *ginger* under the preferred conditions by RSM. The extracts was centrifuged, purification by macroporous resin, methanol elution (methanol volume fraction 60%), solvent evaporation, freeze-dried to obtain the total flavonoids powder. *ginger* flavonoids solutions with different concentrations were prepared. Hydroxyl radical scavenging effect was operated as reference [16-18], *ginger* total flavonoids, Rutin and BHT solutions with different concentrations were added, The absorbance was measured under maximum absorption wavelength of 495nm, The scavenging ratio was calculated as (1):

$$\text{The scavenging ratio of hydroxyl radical (\%)} = [A_0 - (A_x - A_{x0})] / A_0 \times 100 \quad (1)$$

A_0 is the absorbance of control solution, A_x was the absorbance of *Broccoli* extract; A_{x0} was background absorbance of the extract without H_2O_2.

3. Results and Discussion

Table 2 shows the Chromogenic reaction of *ginger* extracts and Rutin solution, the phenomenons of Chromogenic reaction were consistent with each other. It confirms that *ginger* contained total flavonoids. The absorption spectra of *ginger* extracts and Rutin determined the maximum absorption wavelength of 495nm, As shown in Figure 2. The linear regression equation was formulated as A=3.283C-0.009, R^2=0.999 by the standard curve.

Table 2. *Chromogenic reaction of flavonoids extract from ginger and rutin solution.*

Reagents	**4%NaOH(aq)**	**$NH_3 \cdot H_2O$(aq)**	**$AlCl_3$(aq)**	**Fe Cl_3(aq)**
Ginger extracts	Bright -yellow	yellow	Light-yellow	Dark-green
Rutin	Bright -yellow	yellow	Light-yellow	Dark-green

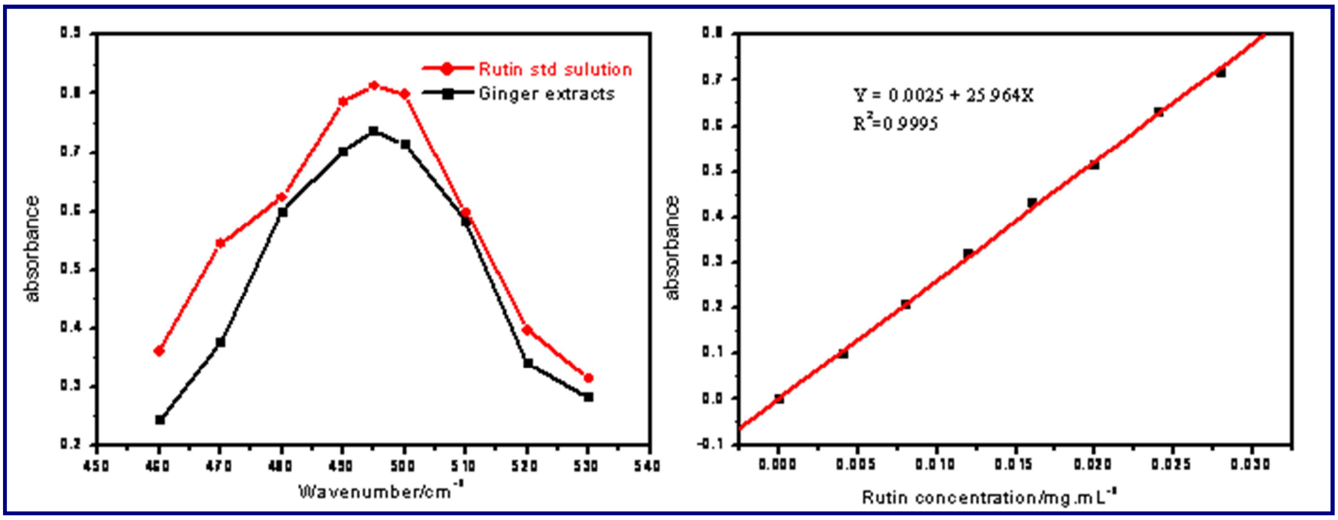

Figure 2. *the absorption spectrum of diagram of ginger extracts and Rutin solution; the standard curve of Rutin solution.*

3.1. Result of Single-Factor Experiment

Influences of each single factor on extraction ratio were shown in Figure 3. The maximum extraction ratio of 0.338% was achieved as the methanol concentration of 65% for ginger, which was attributed to that flavonoid glycosides with moderately polar was rich-contained in ginger. With increasing methanol concentration the polarity of the dissolution system was reduced and increased the dissolution of fat-soluble impurities. The presences of impurities were not conductive to post-separation and purification of total flavonoids. The optimum methanol concentration and the central points of Box-Benhnken Design was determined as 65%. Extraction ratio was significantly increased with the increasing of solid-liquid ratio, when the solid-liquid ratio was higher than 4:30 (g:mL), extraction ratio began to decrease. The maximum extraction ratio of 0.303 was obtained under the solid-liquid ratio of 4:30 (g:mL). Lower than 4:30 (g:mL), the concentration gradient of solid-liquid phase was two small, which was not conducive to the dissolution of total flavonoids. Higher than 4:30 (g:mL), resulted in a waste of materials; The preferred solid-liquid ratio was 4:30 (g:mL), which was selected as the central points of Box-Benhnken design. The optimum extraction temperature was 60°C with corresponding extraction ratio of 0.468%, The higher temperature would lead to methanol evaporation and oxidation degeneration of flavonoids. The lower temperature decreases the dissolution rate of flavonoids. The optimum extraction time was 3.0 h with the responding extraction ratio of 0.346% from *ginger*. Lower than 2.0h, the dissolution balances were not achieved, higher than 2.0h, the dissolution of other fat-soluble impurities complicated the post-separation and purification of total flavonoids.

According to the results of single factor experiments, methanol concentration of 60~70%; solid-liquid ratio of 3.0:30~5.0:30 (g:mL), extraction temperature of 50~70°C and extraction time of 2.0 ~ 4.0 h were determined as the factors and scope for response surface analysis.

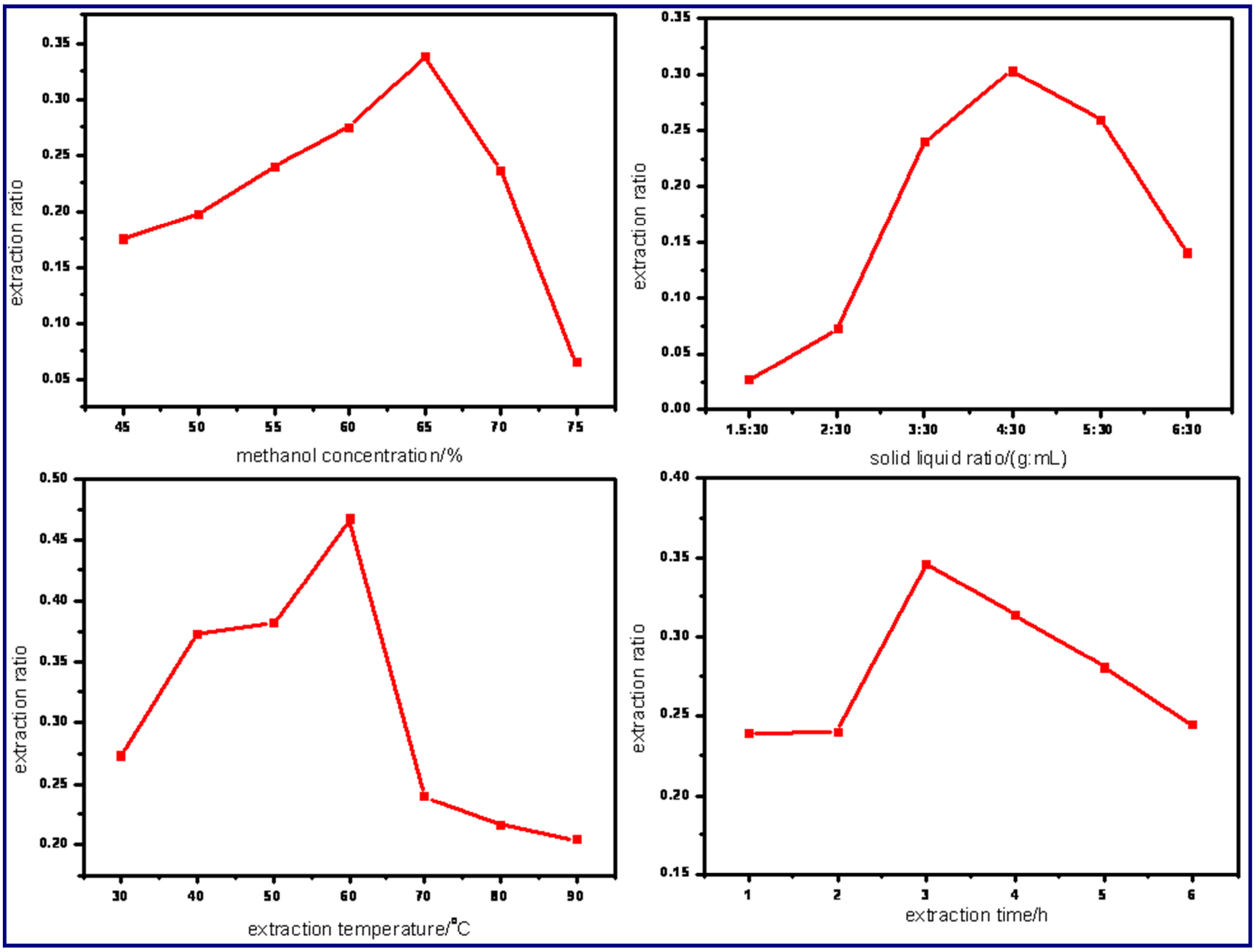

Figure 3. *Effect of methanol concentration, solid-liquid ratio, temperature and time on the extraction ratio of total flavonoids.*

3.2. Response Surface Optimization of Extraction Process

3.2.1. Multiple Regression Model and Analysis of Variance (ANOVA)

Extract methodology of total flavonoids from *ginger* was further optimized by RSM. According to the single-factor experimental results of figure 3, methanol concentration of 60~70%; solid-liquid ratio of 3.0:30~5.0:30 (g:mL), extraction temperature of 50~70°C and time of 2.0 ~ 4.0 h were selected as the actual levels of factors to maximize extraction ratio by Box-Benhnken Design, as listed in table 1. A total of 25 experiments were designed, including 16 factorial experiments, 8 star experiments and 1 central experiments to estimate the absolute error.

Table 3. *Experimental design and results for extraction ratio by using Box-Behnken design and RSM.*

Std	Run	Independent variables				Response 1 extraction ratio (%)
		A: methanol concentration (%)	B: Extraction time (h)	C: Extraction temperature (°C)	D: Solid-liquid ratio (g:mL)	
5	1	65	3	50	3:30	0.310
3	2	60	4	60	4:30	0.459
19	3	60	3	70	4:30	0.412
18	4	70	3	50	4:30	0.412
4	5	70	4	60	4:30	0.445
8	6	65	3	70	5:30	0.238
13	7	65	2	50	4:30	0.428
24	8	65	4	60	5:30	0.220
11	9	60	3	60	5:30	0.226
7	10	65	3	50	5:30	0.471
22	11	65	4	60	3:30	0.452
6	12	65	3	70	3:30	0.462

Std	Run	Independent variables				Response 1 extraction ratio (%)
		A: methanol concentration (%)	B: Extraction time (h)	C: Extraction temperature (°C)	D: Solid-liquid ratio (g:mL)	
17	13	60	3	50	4:30	0.405
15	14	65	2	70	4:30	0.379
12	15	70	3	60	5:30	0.270
21	16	65	2	60	3:30	0.348
14	17	65	4	50	4:30	0.356
10	18	70	3	60	3:30	0.342
9	19	60	3	60	3:30	0.497
16	20	65	4	70	4:30	0.353
20	21	70	3	70	4:30	0.324
23	22	65	2	60	5:30	0.432
1	23	60	2	60	4:30	0.264
25	24	65	3	60	4:30	0.309
2	25	70	2	60	4:30	0.324

The Box-Behnken design and results of extraction ratio for *ginger* was shown in Table 3. The experimental extraction ratio ranged from 0.220% to 0.497%. the optimal experimental conditions were methanol concentration of 60%; solid-liquid ratio of 3:30 (g:mL), extraction temperature of 60°C and extraction time of 3.0h with corresponding maximum extraction ratio of 0.497%. The experimental data was analyzed by using Design-Expert 8.0, the response variabl and the four independent variables were related by the following multiple regression equation of (2) and (3):

$$\text{Extract ratio} = +0.31-0.012*A+9.167E-003*B-0.018*C-0.046*D-0.018*AB-0.024*AC+0.050*AD+0.011*BC-0.079*BD-0.096*CD+0.025*A^{\wedge 2}+0.035*B^{\wedge 2}+0.046*C^{\wedge 2}+0.011*D^{\wedge 2} \quad (2)$$

Extract ratio = +4.10308-0.13328* methanol concentration+0.28592* extraction time+8.49167E-003* extraction temperature+0.032583* solid liquid ratio-3.70000E-003*methanol concentration* extraction time-4.75000E-004 * methanol concentration * extraction temperature+9.95000E-003 * methanol concentration * solid liquid ratio+1.15000E-003 * extraction time * extraction temperature-0.079000 * extraction time * solid liquid ratio-9.62500E-003 * extraction temperature * solid liquid ratio+1.00500E-003 * methanol concentration$^{\wedge 2}$+0.035125 * extraction time$^{\wedge 2}$+4.63750E-004 * extraction temperature$^{\wedge 2}$+0.011125 * solid liquid ratio$^{\wedge 2}$ (3)

The analysis of variance (ANOVA) for the above multiple regression equation was listed in table 4. the analysis result indicated that the response variable value of extraction ratio and the four test factor were not a simple linear relationship. The "Model F-value" of 1.86 implies the model is not significant relative to the noise. There is a 16.28% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, the interaction terms of extraction time and solid-liquid ratio, extraction temperature and solid-liquid ratio, the linear term of solid-liquid ratio are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The analysis result of ANVOA indicated the response variable and the four independent variables were not a simply linear relationship. The value of R^2 (0.7229) and R_{Adj}^2 (0.3349) for the multiple regression equation was not approaching, indicated that the model need to be further optimized. The adequate precision value of 5.491 was higher than the desirable value of 4.00, which indicated an adequate "signal (response) to noise (deviation)" and the model could be used to navigate the design space. The P value of 0.1628 for the model were higher than 0.05, indicated that the model was not significant. The value of coefficient of the variance (C. V. =18.30%) also indicated that the model have a predictable effect for the extraction process of total flavonoids.

The result of analysis of variance (ANOVA) showed that significant levels of the four factors were sorted by solid-liquid ratio > extraction temperature > methanol concentration > extraction time. The linear term of solid liquid ratio, the interaction terms of extraction time and solid-liquid ratio, extraction temperature and solid-liquid ratio, were significant for the response variables.

Table 4. *ANOVA for Response Surface Quadratic Model for ginger.*

Source of deviation	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	significant
Model	0.116965	14	0.008355	1.863	0.1628	
A	0.001776	1	0.001776	0.396	0.5432	
B	0.001008	1	0.001008	0.225	0.6455	s
C	0.003816	1	0.003816	0.851	0.3780	
D	0.025576	1	0.025576	5.704	0.0381	

Source of deviation	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	significant
AB	0.001369	1	0.001369	0.305	0.5927	
AC	0.002256	1	0.002256	0.503	0.4943	
AD	0.0099	1	0.0099	2.208	0.1681	
BC	0.000529	1	0.000529	0.118	0.7384	s
BD	0.024964	1	0.024964	5.567	0.0400	
CD	0.037056	1	0.037056	8.264	0.0165	
A^2	0.001782	1	0.001782	0.397	0.5425	
B^2	0.003484	1	0.003484	0.777	0.3988	
C^2	0.006072	1	0.006072	1.3547	0.2716	
D^2	0.000349	1	0.000349	0.078	0.7858	
Residual	0.044841	10	0.004484			
Cor Total	0.161806	24				
Pure Error		0				
Lack Of Fit		10				
R-Squared	0.7229		Adj R-Squared	0.3349		
Adeq Precision	5.491		C.V.%	18.32		

3.2.2. RSM Analysis and Research on the Optimum Extract Process

The multiple regression models could be vividly reflected by the 3D response surface and Contour lines plots, as listed in figure 4-9. The 3D response surface plots reflected the effects of multiple independent variables on the response value, the sensitivity of response value to different factors could also be analyzed. The corresponding surfaces were more steeper, indicated its extremely significant impact on response value. Contour lines plots with oval indicated strong interaction between independent variables, the closer the curve to the center, the greater significant impact of the variable on the response value.

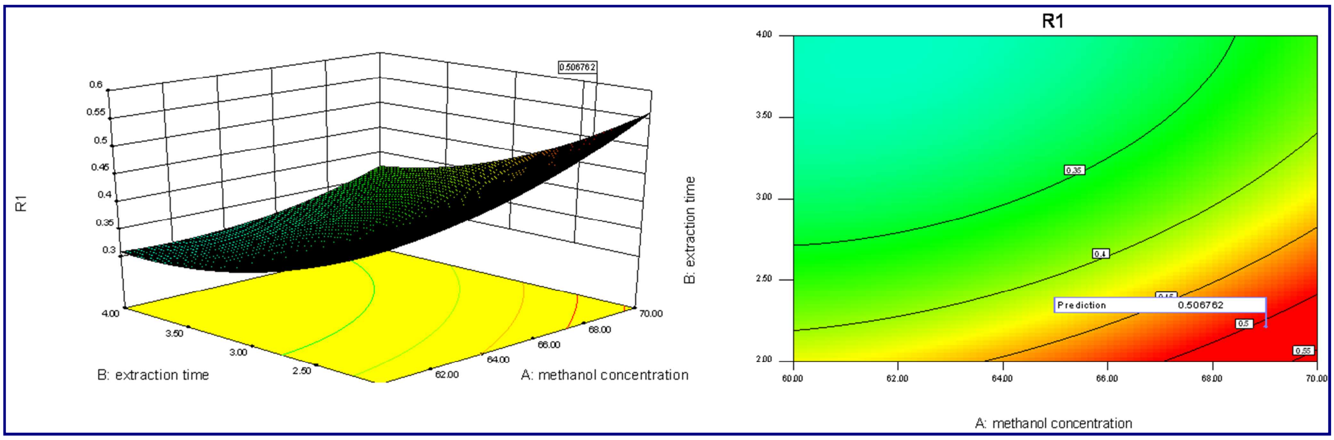

Figure 4. *RSM analyses for interactive effects of methanol concentration and extraction time.*

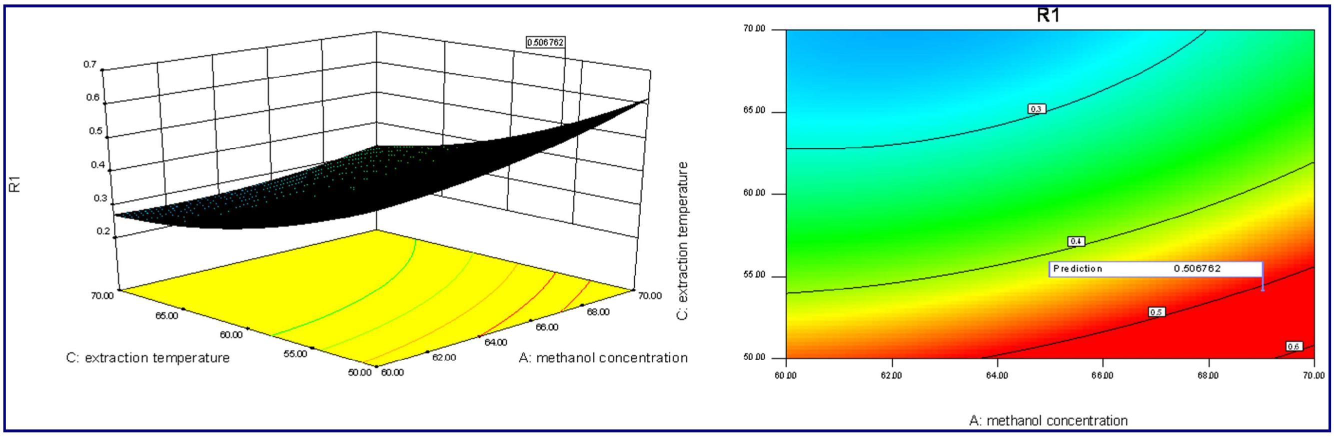

Figure 5. *RSM analyses for interactive effects of methanol concentration and extraction temperature.*

The interaction effect of methanol concentration and extraction time on the extraction ratio was illustrated in the 3D response surface plots of figure 4, the corresponding surface of methanol concentration was more steeper than that of extraction time, indicated its more significant impact on responding value. Circle-like Contour lines plot indicated the weak interaction effect of the two terms on responding values with the p value of 0.5927. the maximum extraction ratios of 0.538% was achieved as methanol concentration of 60.46% and extraction time of 2.57h. The interaction terms of methanol concentration and extraction temperature on the responding values were displayed in the 3D response surface plots of figure 5, the impact of the latter term on extraction ratio was more significant than that of the former. Contour lines plots was approaching to circle-like, indicated weak impact of above two interaction terms on the responding value with P =0.4943. The maximum extraction ratios of 0.538% was obtained as methanol concentration of 60.46% and extraction temperature of 69.91°C.

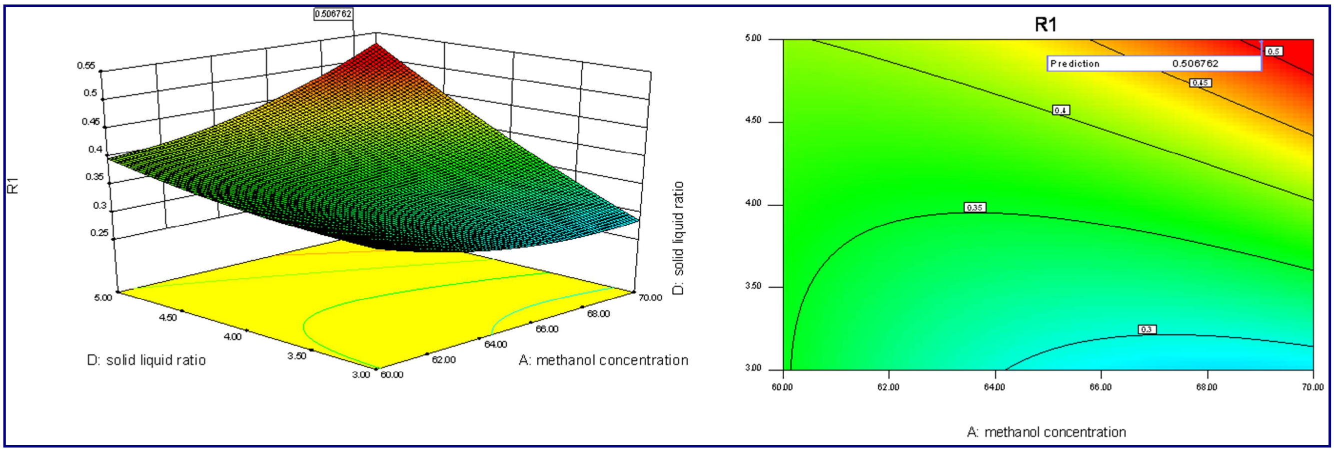

Figure 6. *RSM analyses for interactive effects of methanol concentration and solid liquid ratio.*

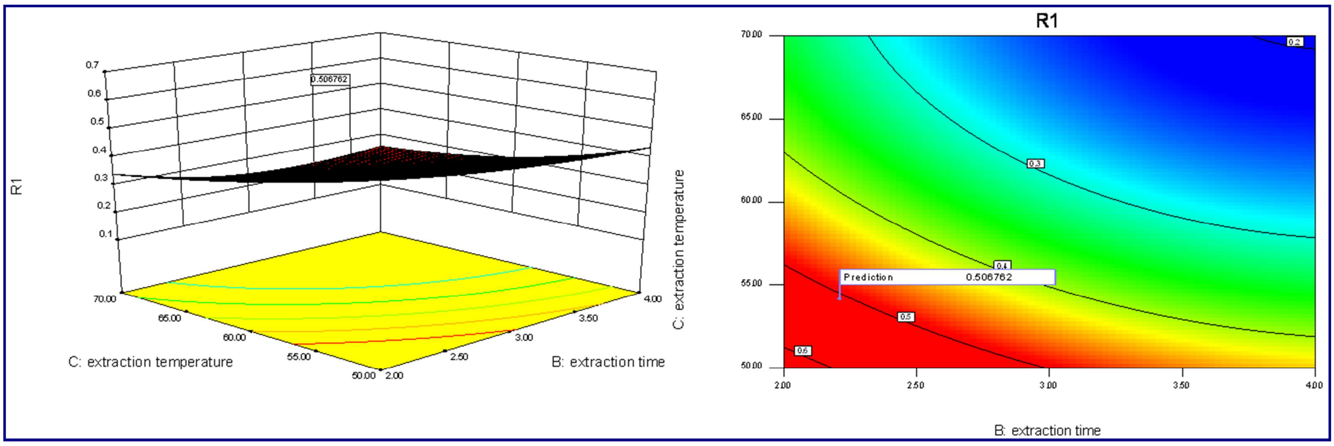

Figure 7. *RSM analyses for interactive effects of extraction temperature and time.*

The impact of solid-liquid ratio on the responding value was more significance than that of methanol, as shown in figure 6. Contour lines plots also indicated the weak interaction effects of the above two terms on the response values P = 0.1681. The maximum extraction ratios of 0.538% was obtained as methanol concentration of 60.46% and solid-liquid ratio 3.03:30 (g:mL). Figure 7 showed that impact of the interaction terms of extraction time and temperature on the responding value was not significant with the responding value with p=0.7384. Circle-like Contour lines plots also indicated weak interaction effect of above two terms on the response values. the maximum extraction ratios of 0.538% was achieved as extraction time of 2.57 h and temperature of 69.91°C.

The significant impact of interaction terms of extraction time and solid-liquid ratio on extraction ratio was illustrated in the 3D response surface and Contour lines plots of figure 8. the linear of solid-liquid ratio has a more significance effect on the responding value from the more steeper surface. Contour lines plots was approaching to oval, also indicated strong impact of above two interaction terms on the responding value with P = 0.04. Both the linear and interaction effects of extraction temperature and solid-liquid ratio on the extraction ratio were significance with p value of 0.0165, as listed in the 3D response surface and Contour lines plots of figure 9. The maximum extraction ratio of 0.538% was achieved under extraction temperature 69.91°C and solid-liquid ratio of 3.03:30 (g:mL).

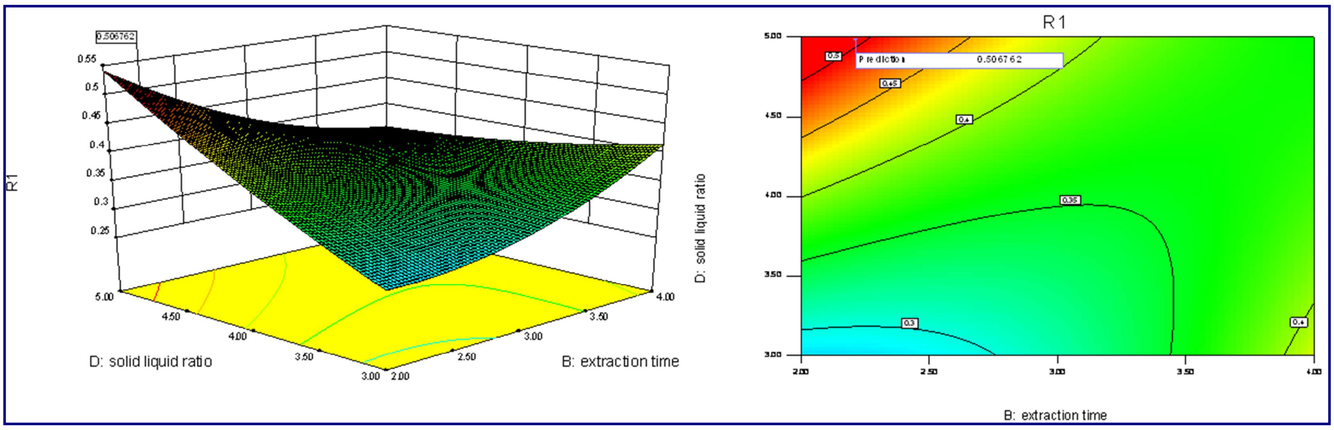

Figure 8. *RSM analyses for interactive effects of solid-liquid ratio and extraction time.*

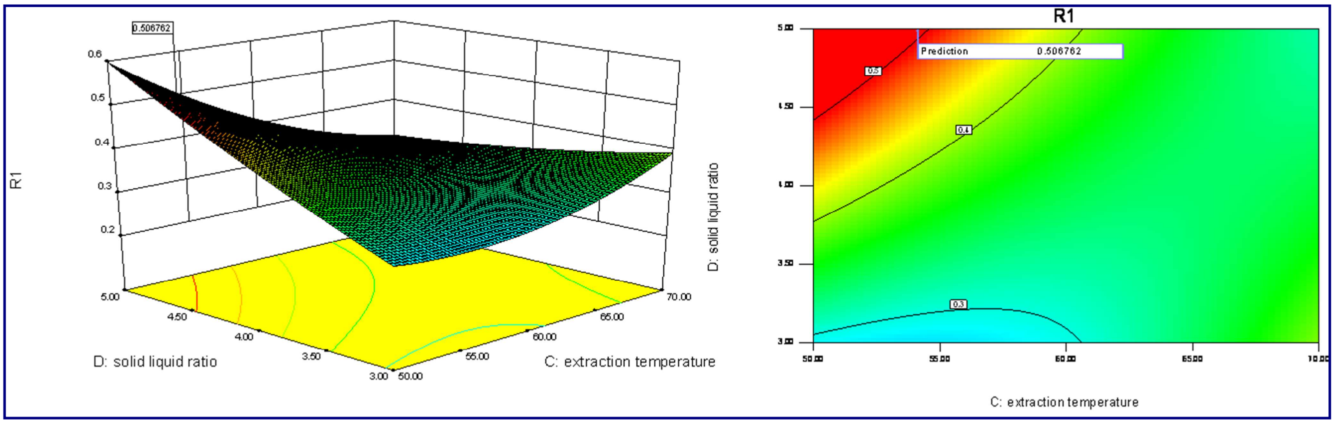

Figure 9. *RSM analyses for interactive effects of solid liquid ratio and extraction temperature.*

The optimum values of the selected variables were obtained by solving the multiple regression equation. The values obtained were A=60.46%, B=2.57 h., C=69.91°C and D=3.03:30 (g:mL), with the corresponding extraction ratio of 0.538% for *ginger*, calculated by Design-Expert 8.0 software.

Table 5. *The obtained experimental data of extraction ratio under preffer experimental conditions.*

Run	Optimal extraction condition determined by BBD and RSM	absorbance	extraction ratio (%)	Average value
1	A=60	0.779	0.498	
2	B=3 C=60	0.775	0.496	0.499
3	D=3:30	0.786	0.503	

In the experiment, three triplicate experiments were performed under preferred extract conditions of methanol concentration of 60%, extraction time of 3h, extraction temperature of 80°C and solid liquid ratio of 3:30 (g:mL), the average maximum value of extraction ratio was 0.499% for *ginger*. The obtained experimental data were listed in table 5, the experimental extraction ratio and calculated values of response variable were not well consistent with each other, which indicated that the model have a predictable effect for the extraction methodology of flavonoid from *ginger*, the experimental extraction value and the calculated values by the Design-Expert 8.0 software were not approaching, indicated that the model need to be further optimized.

3.3. Study on Hydroxyl Radical Scavenging Activity

The flavonoids compounds had a scavenging effect on hydroxyl radical, superoxide radicals and DPPH radicals as the *o*-dihydroxy from the structural benzene ring[14-16]. Hydroxyl radical scavenging effects of *ginger* extracts, BHT and *L*-ascorbic acid with different concentrations were measured and listed in Figure 10. With the concentration increasing, the scavenging ratio for hydroxyl radicals increased, which showed a significant degree of dose-effect relationship. Hydroxyl radical scavenging effects of the three antioxidant with the same concentration were sorted by *L*-ascorbic acid >*ginger* extract> BHT. The experimental result of antioxidant was consistent with that reported in the literature [7]. The lower antioxidant activity of ginger extracts contrast to *L*-ascorbic acid could be attributed to the following two reasons: firstly, *o*-dihydroxy from benzene rings were partly methylated, leading to the reduction of scavenging activity on hydroxyl radical [16-18]. Secondly, the lack of necessary separation and identification for extracts, and the presence of impurities also affected their scavenging effects.

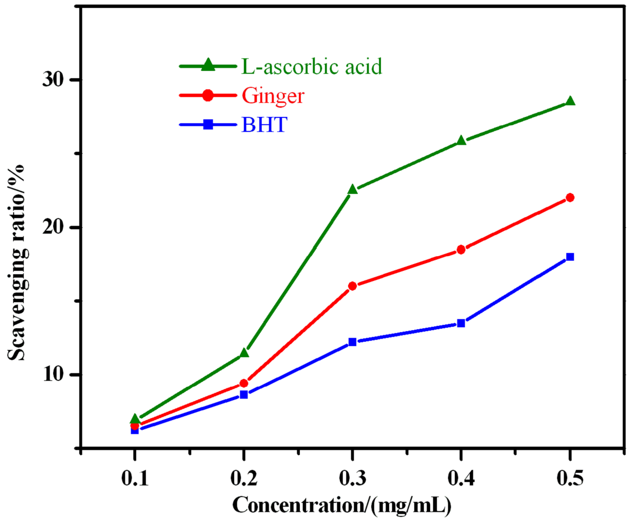

Figure 10. *scavenging activity comparison of ginger extracts, BHT and L-ascorbic acid.*

4. Conclusions

Extract methodology of total flavonoids from ginger and hydroxyl radicals scavenging effect, were researched in this paper. Methanol concentration, solid-liquid ratio, extraction temperature and time were determined as four single-factor in the experiment. The central points of Box-Benhnken Design were selected according to the experimental results of single-factor experiment, the extract process was further optimized by RSM and BBD. The optimum extract conditions were methanol concentration of 60%, solid-liquid ratio of 3:30 (g∶mL), extraction temperature of 60°C and time of 3 h with maximum experimental extraction ratio of 0.497% by RSM. the theoretical value was 0.538% by solving the multiple regression equation, which indicated that the fitted quadratic model has a predictive effect on target extracts. The above experimetal and theoretical value were not approching, also indicated that the model need to be further optimized.

The scavenging effect of *ginger* extracts, BHT and *L*-ascorbic acid on ·OH with the same concentration were sorted by *L*-ascorbic acid >*ginger* extract> BHT, and all the three antioxidant regents displayed a significant dose-effect relationship. The isolation, purification and structure identification of ginger extracts, relationship between antioxidant activity and structure of flavonoids, and related research works are underway.

Acknowledgements

This work was financially supported by China Scholarship Council and the Natural Science Foundation of China (Grant no. 21643006). The authors also acknowledge the financial support of National College students' innovative entrepreneurial training program (Grant no. 16449) and College students' innovative entrepreneurial training program of yunnan Education Department.

References

[1] Yang L., Cao Y.-L., Jiang J. G., etal. Response surface optimization of ultrasound-assisted flavonoids extraction from the flower of *Citrus aurantium* L. var. *amara* Engl [J]. Journal of Separation Science, 2010, 33(9), 1349-1355.

[2] Daffodil E. D., Mohan V. R. Total phenolics, flavonoids and in vitro antioxidant activity of *Nymphaea Pubescens* wild rhizome [J]. World Journal of Pharmacy and Pharmaceutical Sciences, 2013, 2(5), 3710-3722.

[3] Huang W., Xue A., Niu H., etal. Optimized ultrasonic-assisted extraction of flavonoids from Folium eucommiae and evaluation of antioxidant activity in multi-test systems in vitro [J]. 2009, 114(3): 765-1172.

[4] Li Y. H., Jiang B., Zhang T., etal. Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate [J]. Food Chemistry, 2008, 106(2): 444–450.

[5] Zhang Y., Cao G.. J., Zhang Y., etal. Research on the extraction and identification of flavonoids [J]. Food Research and Development, 2008, 29(1), 154-157.

[6] Wang L., Weller C. L. Recent advances in extraction of nutraceuticals from plants [J]. Trends in Food Science and Technology, 2006, 17: 300–312.

[7] Wang ZC, Jiang YR, Liu XW, et al. Response Surface Methodology Optimization of total flavonids from ginger leaf and study on antioxidation effect[J]. Natural product research and developmen, 2015, 27(9): 1582-1588.

[8] Mo KJ, Cheng C. Huang P. et al. Study on extract technology, purification and structural appraisal of flavonoid from ginger [J]. Food science, 2005, 26(9): 229-233.

[9] Gao SY, Ge ZZ. Optimization of extraction technique of flavonoids from ginger by response surface methodology [J]. China condiment, 2012, 37(12): 32-35.

[10] Mu YD. Response surface metnodology and its application in food industry [J]. Journal of Zhengzhou Institute of technololy, 2001, 22(3): 91-94.

[11] Amado I. R., Franco D., Sάnchez M., etal. Optimization of antioxidant extraction from *Solanum tuberosum* potato peel waste by surface response methodology [J]. Food Chemistry. 2014, 165, 290-299.

[12] Ranic M., Nikolic M., Pavlovic M., etal. Optimization of microwave-assisted extraction of natural antioxidants from spent espresso coffee grounds by response surface methodology [J]. Journal of Cleaner Production, 2014, 80: 69-79.

[13] Liu W., Yu Y., Yang R., etal. Optimization of Total Flavonoid Compound Extraction from *Gynura medica* Leaf Using Response Surface Methodology and Chemical Composition Analysis [J]. Int. J. Mol. Sci. 2010, 11, 4750-4763.

[14] Li H D. Enzyme-assisted extraction of total flavonoids from Wisteria and study on radicals scavenging effect [J]. Journal of Henan normal university (Natural Science Edition), 2014, 42(03): 79-84.

[15] Li Huiduan. Response surface optimization of flavonoids extraction fromtwo kinds of chinese tea andresearch on antioxidant effect, NPAIJ, 2015, 11(1): 001-011.

[16] Zhang L. M., Li R. C., Hao L. M., etal. Response surface methodology for optimization of extracting total flavonoids from maca leaves and antioxidant evaluation [J]. Modern Food Science and Technology, 2014, 30(4): 233-239.

[17] Smirnoff N., Cumbes Q. J. Hydroxyl radical scavenging activity of compatible solutes [J]. Photochemistry, 1989, 28(4): 1057-1060.

[18] Heim K. E., Taglicferro A. R., Bobilya D. J. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships [J]. Journal of Nutritional Biochemistry, 2002, 13(10):572-584.