PAPER

OPTIMIZATION OF EXTRACTION PROCESS OF PUMPKIN SEED OIL CHARACTERIZED WITH HIGH PUFA CONTENT VIA DOUBLE RESPONSE SURFACE IN COMBINATION WITH MATLAB METHODOLOGY

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ABSTRACT

Box-behnken-double response surface optimization combined with Matlab analysis was used to optimize the extraction of Inner Mongolia pumpkin seed crude oil based on the relative content of polyunsaturated fatty acids (PUFA) (Y_1) and crude oil yield (Y_2). The influence of three single factors (solid-liquid ratio, extraction temperature, extraction time) of pumpkin seed was investigated. By the optimization of dual response surface analysis, the optimal extraction process parameter was as follows: solid-liquid ratio of 1:26.8 g/mL, extraction temperature of 30°C, extraction time of 2.5 h. On this condition, the relative content of PUFA was 43.54±0.14%, and the crude oil yield was 14.84±0.10 %, which showed no significant difference from theoretical value (p >0.05). In addition, by Matlab analysis, when the extraction time was taken as the lower level (C=2.5 h), Y_1 was able to obtain larger theoretical values (45.8485%), however, when the temperature was taken as the higher level (C=3.5 h), Y_2 can obtain larger theoretical values (17.0563%). When the extraction time was fixed as 2.5 h, and the solid-liquid ratio was within the range of 1:24 ~ 1:27 g/mL, extraction temperature was within the range of 30～32.5°C, Y_1 and Y_2 both can get the theoretical maximum value. The results of dual response surface optimization and matlab optimization are consistent. The combination of the two methods can not only accurately obtain the best extraction scheme, but also more intuitively find out the reasonable process parameter interval of Pumpkin seed oil.

Keywords: pumpkin seed oil, polyunsaturated fatty acid (PUFA), crude oil yield, double response surface, matlab methodology

1. INTRODUCTION

The pumpkin, which is of relatively high economic importance, belongs to the family Cucurbitaceae (GEMROT *et al*., 2006; DURANTE *et al*., 2014). In the naked seed, there are about 22～64% lipids (YOUNIS *et al*., 2000; JIAO *et al*., 2014), which contains abundant oleic, linoleic and linolenic acids (DURANTE *et al*., 2014). The pumpkin seed oil contains various bioactive compounds, such as phytosterols (NISHIMURA *et al*., 2014), phenolic (REZIG *et al*., 2012), antioxidants (REZIG *et al*., 2012), tocopherols (RABRENOVIĆ *et al*., 2014; WANG *et al*., 2017) and small levels of carotenoids (WANG *et al*., 2017; PROCIDA *et al*., 2013), which has been widely used not only in the food industry as an edible oil, but also in different regions of the world as the commercial oil (CAN-CAUICH *et al*., 2019). In addition, the FAO indicates that unrefined pumpkin oil is considered to be of high quality for its taste, aroma and color, the characteristics of which have defined the use of this oil for salads and cold dishes (OJEDA-AMADOR *et al*., 2018).

As is known to all, the two essential polyunsaturated fatty acids (PUFAs) namely linoleic acid (omega-6 fatty acids) and alpha-linolenic acid (omega-3 fatty acids) are considered very important for human body to survive. Studies have shown that essential fatty acids can treat certain diseases such as cancers and diabetes (ORSAVOVA *et al*., 2015). Particularly, linoleic acid is recognized as the essential nutrient for humans. Moreover, the pumpkin seed oil is a good source of linoleic acid (C18:2,cis-9,12) (ORSAVOVA *et al*., 2015; LIANG *et al*., 2018). In the literature, a number of reports can be found describing the different methods for the extraction of oil from the seeds of pumpkins, such as organic solvent extraction (REZIG *et al*., 2018), mechanical pressing (RABRENOVIĆ *et al*., 2014), and supercritical fluid (DURANTE *et al*., 2017) etc. However, almost all the research optimized the extraction process based on the oil extraction rate. No research has been carried out to determine the influence of extraction method and condition on the content of bioactive compounds of PUFA portion in pumpkin oil. Thus, the aim of this study was to investigate the optimization of extraction process based on the PUFA content of pumpkin seed oil, by the methodology of double response surface in combination with Matlab analysis, in order to prepare the superior pumpkin seed oil characterized with high PUFA proportion, as well as the higher extraction rate.

2. MATERIALS AND METHODS

2.1. Samples of pumpkin seed

The pumpkin seed (Gold No. 2) were acquired in the locality of Hetao plain, Inner Mongolia (North latitude of 40°, East longitude of 107°), China, in September 2017. The complete seeds were selected manually, removing broken or cracked seeds. The selected seeds were dried (up to 4% humidity) in a convection oven at 30±1.0°C for 48 h and subsequently stored at 4°C in polyethylene bags until use. Each sample was analyzed as the whole nut, with the shell.

2.2. Oil extraction

The extraction of oil by organic solvent, was carried out according to the method described by MOO-HUCHIN *et al*. (2013), with a few modifications. 10 g of seeds were crushed in grinder (YB-2500A, Yongkang Fastfront Industrial and Trade Co. LTD.) until the powder passed through No. 40 (about 0.43 mm) mesh. The oils from pumpkin seeds were extracted with petroleum ether (350 mL) in a Soxhlet apparatus by thermal cycles at 45°C for 4 h. In order to obtain the oil, the solvent was removed using a rotary vacuum evaporator at 40°C (Buchi, Flawil, Switzerland). The yield of extracted oil was calculated gravimetrically after collection.

2.3. The analysis of PUFAs in pumpkin seed oil

The pumpkin seed oil was methylated with boron fluoride-methanol (Sigma Aldrich) according to XUE (2016). The fatty acid methyl esters were analyzed by gas chromatograph by the method of XUE (2016). The fatty acids methyl esters were analyzed by a QP-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a split injector. One microliter of FA methyl esters was injected in split mode (5:1) onto a Rtx-Wax capillary column (Restek, Bellefonte, PA, USA; 30 m length × 0.25 mm id × 0.25 μm film thickness). The temperature of the column was programmed as follows: 1 min at 140°C, increments of 8°C/min to 180°C and held at 180°C for 2 min, increments of 3°C/min to 210°C then increments of 5°C /min to 230°C and held at 230°C for 10 min. The temperature of the injector and the detector both were 250°C. The flow rate of the carrier gas (N_2) was 1.5 mL/min. Identification of fatty acids was performed by comparison of the retention times with those of standards (Sigma-aldrich, Shanghai, China). The results were expressed as percent of the total fatty acids methyl esters present.

2.4. Single factor test

The effects of solid-liquid ratio (1:15, 1:25, 1:35, 1:45 and 1:55 g/mL), extraction temperature (25, 35, 45, 55, 65°C), and the extraction time (2, 3, 4, 5, 6 h) on the relative content of PUFA in pumpkin seed oil were investigated, respectively.

2.5. Response surface test

The box-behnken design (BBD) of double response surface methodology was employed using the Design Expert 7.0 software, based on the result of single factor test. The double response values are the relative content of PUFA (Y_1) and the crude oil yield (Y_2), in order to prepare the superior pumpkin seed oil characterized with not only higher PUFA proportion, but also the higher extraction rate. Three variables were optimized such as solid-liquid ratio, extraction temperature and extraction time. The range and level employed in the experiment are shown in Table 1.

Table 1. Experimental range and levels of independent variables in optimization study.

Level	Factors		
	A Solid-liquid ratio (mL/g)	B Extraction temperature (°C)	C Extraction time (h)
-1	1:20	30	2.5
0	1:25	35	3
1	1:30	40	3.5

2.6. Verification experiment of optimal combination

According to the results of box-behnken double response surface and matlab methodology, the optimal process parameters were obtained and verified by experiments.

2.7. Optimization experiment of matlab

The matlab methodology was based on the optimization calculation method and graphic processing function of algorithm language. By programming M (the program code), calculate the four-dimensional and three-dimensional interaction results, which can explain the effect of three factors (solid to liquid ratio (A), extraction temperature (B), extraction time (C)) on the relative content of PUFA (Y_1) and crude oil yield (Y_2) of pumpkin seed oil. The levels or range of values of the variables followed here were based on the results of the parametric study previously conducted.

2.8. Statistical analysis

All analysis was performed in duplicate and the data collected were subjected to ANOVA using Excel® 2010 software and Tukey tests (with a 95% confidence interval), to evaluate differences between the results.

3. RESULTS AND DISCUSSION

3.1. GC analysis of pumpkin seed oil

Fig. 1 shows the GC chromatogram of fatty acid methyl esters extracted from pumpkin seed oil by petroleum ether. The extracts of the seeds showed the presence of saturated fatty acids (such as palmitic acid (C16:0) and stearic acid (C18:0)), and unsaturated fatty acids (such as oleic acid (C18:1,cis-9), linoleic acid (C18:2,cis-9,12), and α-linolenic acid (C18:3,cis-9,12,15). Similar results for the fatty acid composition of pumpkin seed oil have been reported by MITRA *et al*. (2009), SALGIN and KORKMAZ (2011), DURANTE *et al*. (2014) and CUCO *et al*. (2019).

As shown in Table 2, abundant PUFA was observed (34.61%), especially the α-Linoleic acid (34.02%), which was higher than the amount extracted by pressurized CO_2 (CUCO *et al*, 2019). According to CUCO (2019), the extracts obtained using organic solvent, such as petroleum ether and *n*-hexane had higher concentrations of linoleic and stearic acids in relation to the extracts obtained with pressurized CO_2, which was consistent with the research results in this paper. Besides, the eicosanoic acid ($C_{20:0}$) portion was not detected, since the extraction of fatty acids may be related to the polarity of the solvents used (MEZZOMO *et al*., 2010). Differences observed in fatty acid composition of pumpkin seed oil may be attributed to the variation in climatic conditions, cultivation locations, agronomical practices, and genetic backgrounds of the seeds, as well as esterification and analysis methods (ÖZCAN *et al*., 2019).

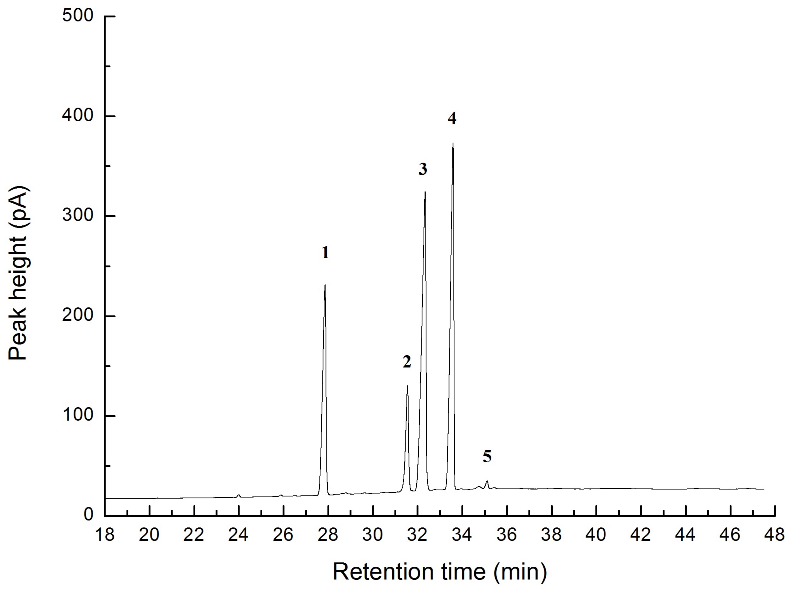

Figure 1. Gas chromatogram of fatty acid methyl ester extracted from pumpkin seed oil by petroleum ether.

Table 2. Composition of fatty acids of pumpkin seed oil.

No.	fatty acid	The relative content (%)
1	C16:0 Palmitic acid	20.75±0.23
2	C18:0 Stearic acid	8.79±0.01
3	C18:1,cis-9 Oleic acid	35.85±0.16
4	C18:2,cis-9,12 Linoleic acid	34.02±0.15
5	C18:3,cis-9,12,15 α-Linolenic acid	0.59±0.09
	Saturated fatty acid, SFA	29.54±0.22
	Monounsaturated fatty acid, MUFA	35.85±0.16
	Polyunsaturated fatty acid, PUFA	34.61±0.05
	N-3 PUFA	0.59±0.09
	N-6 PUFA	34.02±0.15

In recent years, the seed oil from vegetable, such as olive oil, sunflower seed oil, and algerian peanut landraces oil had been obtained for food use and biodiesel production (GIUFFRÈ *et al.*, 2017a; GIUFFRÈ *et al.*, 2017b; GIUFFRÈ *et al.*, 2016). The balance of fatty acids in the diet is important for health, and the fatty acids are among the most important parameters to establish the edibility of a vegetable oil (DENG, 2014). As for the monounsaturated fatty acids (MUFA), the content of oleic acid in the pumpkin seed oil (35.15%) was higher than that in the sunflower seed oil (32.47%) (GIUFFRÈ *et al.*, 2017b) and soybeans oil extracted by supercritical carbon dioxide (21.191%) (JOKIĆ *et al.*, 2013). The pumpkin seed oil was characterized with higher content of linoleic acid (34.02%), compared to that in olive oil (18.45%) (GIUFFRÈ *et al.*, 2017a), algerian peanut landraces oil (29.92%) (GIUFFRÈ *et al.*, 2016) and rapeseed (*Brassica napus ssp. oleifera* L.) oil (25.02%)

(BEYZI *et al.*, 2019). In addition, the α-linolenic acid portion in this pumpkin seed oil (0.59%) was significantly higher than that in the sunflower seed oil (0.06%) (GIUFFRÈ *et al.*, 2017b) and algerian peanut landraces oil (0.09%) (GIUFFRÈ *et al.*, 2016). Thus, pumpkin seed oil was a nutrient and biologically active vegetable oil, which had abundant PUFA playing the important beneficial role.

3.2. Single factor test results

The effect of solid-liquid ratio, extraction temperature, and extraction time on the relative content of PUFA in pumpkin seed oil was represented in Figs. 2-4, respectively. As shown in Fig. 2, with the increase of solid-liquid ratio, the relative content of PUFA in pumpkin seed crude oil first increased (p >0.05) and then decreased significantly (p <0.05). When at 1:25 g/mL, the content of PUFA reached the highest. From Fig. 3, at the range of 25～65°C, the relative PUFA content first increased from 25 to 35°C, while decreased with the rising of extraction temperature. The max content of PUFA was observed in 35°C. Similarly, when the extraction time was at 3 h, the PUFA have the maximum, and decreased significantly with extension extraction time (Fig. 4). Since heat treatment may cause lipid oxidation, and the temperature and time are key factors (BYRNEA *et al.*, 2002).

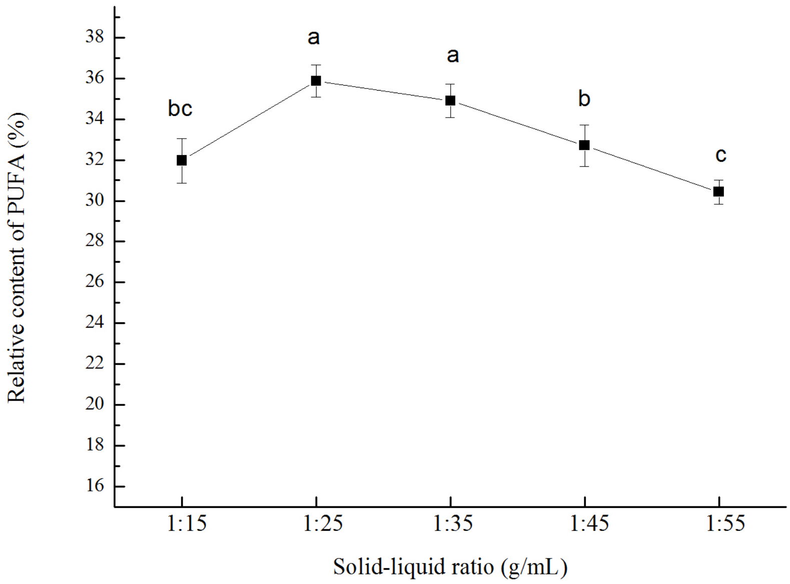

Figure 2. Effect of solid-liquid ratio on relative content of PUFA from pumpkin seed oil.

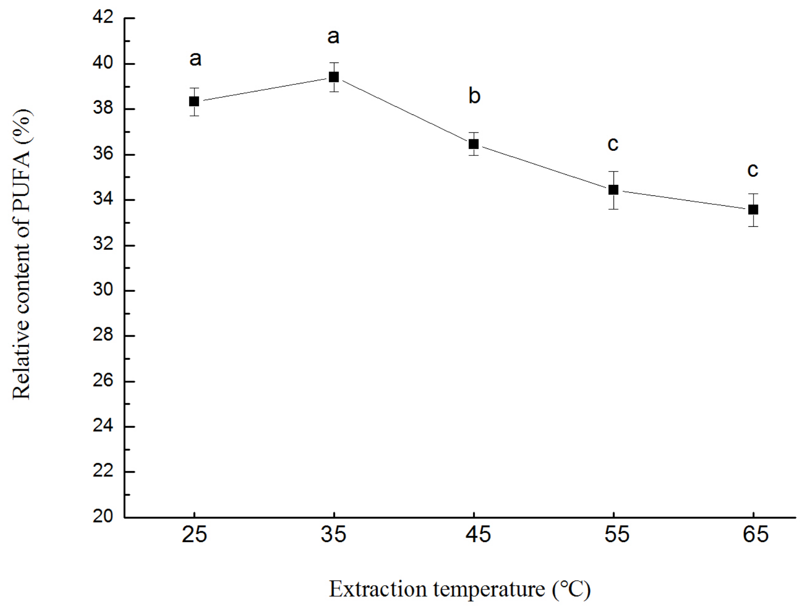

Figure 3. Effect of extraction temperature on relative content of PUFA from pumpkin seed oil.

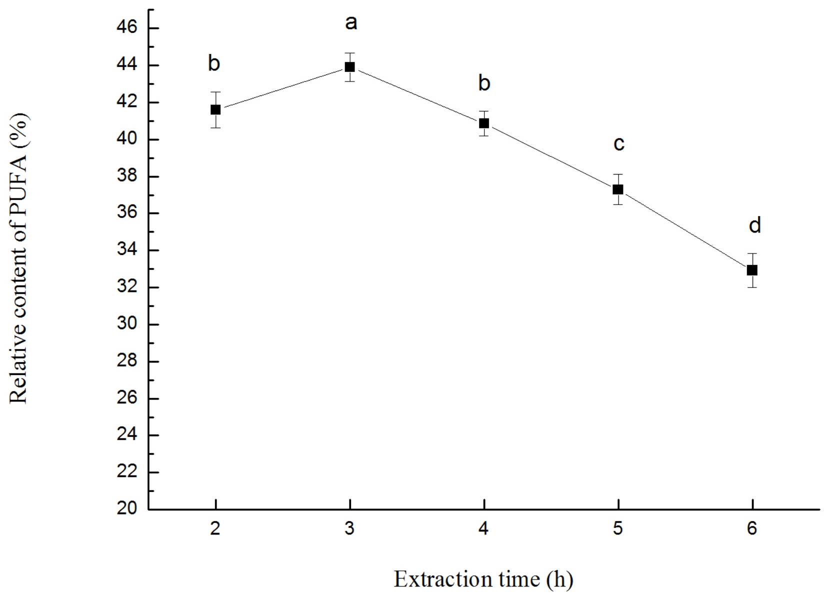

Figure 4. Effect of extraction time on relative content of PUFA from pumpkin seed oil.

3.3. Double response surface experiment results

BBD is an independent, rotatable or nearly rotatable second-order design based on three-level incomplete factorial designs. BBD is more efficient compared to other response surface designs, such as central composite designs. In addition, BBD can provide sufficient information for testing the lack of fit, and therefore is one of the best quadratic models for response surface methodology and has been widely used in analytical fields (SONG *et al*., 2009). The quadratic rotary combination design and test results can be seen in Table 3. In this study, coefficients of the models were calculated and the predicted models were as follows, respectively:

$$Y_1=38.29-1.31*A-1.74*B-2.59*C-0.84*AB-2.45*AC-0.025*BC+0.70*A^2+0.66*B^2-0.091*C^2 \quad (1)$$

$$Y_2=14.88+1.15*A+0.45*B+0.33*C+1.13*AB+0.38*AC+1.25*BC-0.38*A^2-0.18*B^2-0.26*C^2 \quad (2)$$

The results of ANOVA for the quadratic model were shown in Table 4 (Y_1) and Table 5 (Y_2), and the significance of each coefficient was determined by F-value and p-value. Generally, the larger magnitude of F-value and the smaller *p*-value had more significant effect on the corresponding coefficient. The model F-value of Y_1 and Y_2 implied that the model were significant. There was only a 0.0006 and 0.0071 chance that a model F-value of this size could occur due to statistical noise, respectively. The coefficient of determination (R^2) meant the proportion of the total variation in the response expected by the model. Higher coefficient of determination (R^2) of model implied that the response surface model was reasonable (HU *et al*., 2017).

Table 3. Experimental design and results of double response surface optimization.

Test No.	A：Solid-liquid ratio (mL/g)	B：Extraction temperature（°C)	C：Extraction time (min)	Y1: Relative content of PUFA (%)	Y2: Crude oil yield（%)
1	-1	-1	0	42.33	13.96
2	1	-1	0	40.08	13.8
3	-1	1	0	40.91	12.6
4	1	1	0	35.3	16.95
5	-1	0	-1	39.62	12.82
6	1	0	-1	43.22	14.57
7	-1	0	1	39.48	13.17
8	1	0	1	33.28	16.43
9	0	-1	-1	43.42	15.14
10	0	1	-1	39.61	13.55
11	0	-1	1	38.16	12.84
12	0	1	1	34.25	16.25
13	0	0	0	37.71	14.56
14	0	0	0	38.18	15.65
15	0	0	0	37.93	13.76
16	0	0	0	38.2	15.15
17	0	0	0	39.42	15.29

All data are mean values of duplicate measurements.

Table 4. The variance analysis of Y_1.

Source	Sum of squares	df	Mean square	F-value	*p*-value (Prob>F)	Significant
Model	122.4503	9	13.60559	17.17016	0.0006	**
A- Solid-liquid ratio	13.67645	1	13.67645	17.25959	0.0043	**
B- Extraction temperature	24.2208	1	24.2208	30.56649	0.0009	**
C- Extraction time	53.56125	1	53.56125	67.59395	<0.0001	**
AB	2.8224	1	2.8224	3.56185	0.1011	
AC	24.01	1	24.01	30.30046	0.0009	**
BC	0.0025	1	0.0025	0.003155	0.9568	
A^2	2.083841	1	2.083841	2.629794	0.1489	
B^2	1.853609	1	1.853609	2.339243	0.1700	
C^2	0.035252	1	0.035252	0.044487	0.8390	
Residual	5.54678	7	0.792397			
Lack of fit	3.7837	3	1.261233	2.861432	0.1680	
Pure error	1.76308	4	0.44077			
Cor Total	127.997	16				
Adj-R^2	0.9009					
Pred-R^2	0.5055					
C. V. (%)	2.29					

Note: *: $p<0.05$; **: $p<0.01$.

As for Y_1, the graphic plot of predicted values by the model vs. observed experimental values showed a linear distribution (R^2=0.9567) for the response, indicating that the predicted values obtained from the model had a linear relationship with the observed values.

As for Y_2, the R^2 for the predictive model was 0.9067, which also indicated that the experimental data well fitted the second-order polynomial equation. In addition, the ANOVA analysis presented in Table 4 (Y_1) and Table 5 (Y_2) indicated that the model was highly appropriate for the prediction. Overall, R^2, the fraction of the variation of the response by the model, adjusted R^2 and regression *p*-value and lack of fit values > 0.05 all indicated that model well fitted the experimental data points (BILGIC *et al*., 2012; BILGIÇ-KELEŞ *et al*., 2019).

The conditions for the maximum Y_1 and Y_2 were both generated by the optimizer function of the Design Expert 7.0 software. Based on the mathematical model established, the optimal experimental conditions were as follows: solid-liquid ratio (1:27 g/mL), extraction temperature (30°C) and extraction time (2.5 h), respectively. The Y_1 was obtained as 43.54%, and Y_2 obtained as 14.84%, which was satisfactorily close to the predicted value 43.44%, 14.78% at this point, respectively.

Table 5. The variance analysis of Y_2.

Source	Sum of squares	df	Mean square	F-value	*p*-value (Prob>F)	Significant
Model	26.08134	9	2.897927	7.556931	0.0071	**
A- Solid-liquid ratio	10.58	1	10.58	27.5895	0.0012	**
B- Extraction temperature	1.629013	1	1.629013	4.24798	0.0782	
C- Extraction time	0.851513	1	0.851513	2.220492	0.1798	
AB	5.085025	1	5.085025	13.26023	0.0083	**
AC	0.570025	1	0.570025	1.486456	0.2623	
BC	6.25	1	6.25	16.29814	0.0050	**
A^2	0.595267	1	0.595267	1.55228	0.2529	
B^2	0.134157	1	0.134157	0.349841	0.5728	
C^2	0.281357	1	0.281357	0.733695	0.4200	*
Residual	2.684355	7	0.383479			
Lack of fit	0.493675	3	0.164558	0.30047	0.8246	
Pure error	2.19068	4	0.54767			
Cor Total	28.76569	16				
Adj-R^2	0.7867					
Pred-R^2	0.6064					
C. V. (%)	4.27					

Note: *: $p < 0.05$; **: $p < 0.01$.

3.4. Analysis of the response surface

Three-dimensional response surfaces were applied to test the mutual effects of different variables, so as to better understand the independent variables' effect on relative content of PUFA, as well as crude oil yields. Contour plot and response surface plot of the interaction of two parameters can be seen in Fig. 5 (Y_1) (5a-5c) and Fig. 6 (Y_2) (6a-6c). Fig. 5a shows the mutual effects between solid- liquid ratio and extraction temperature extracted at fixed extraction time (3 h). It can be found that content of PUFA obviously decreased with growth of solid liquid ratio at a designated extraction temperature (Figure 5a), however, the growth of crude oil yield was increasingly slow when solid liquid ratio increased (Fig. 6a). The obviously reduction of PUFA content was found with growth of solid liquid ratio at a designated extraction time (Fig. 5b), whereas the growth of crude oil yield was increasingly when solid liquid ratio increased (Fig. 6b), similar with Fig. 6a. The PUFA content obviously decreased with growth of extraction temperature at a designated extraction time (Fig. 5c), while crude oil yield firstly decreased and then increased with the growth of extraction temperature, which had dual influences on crude oil yields (Fig. 6c).

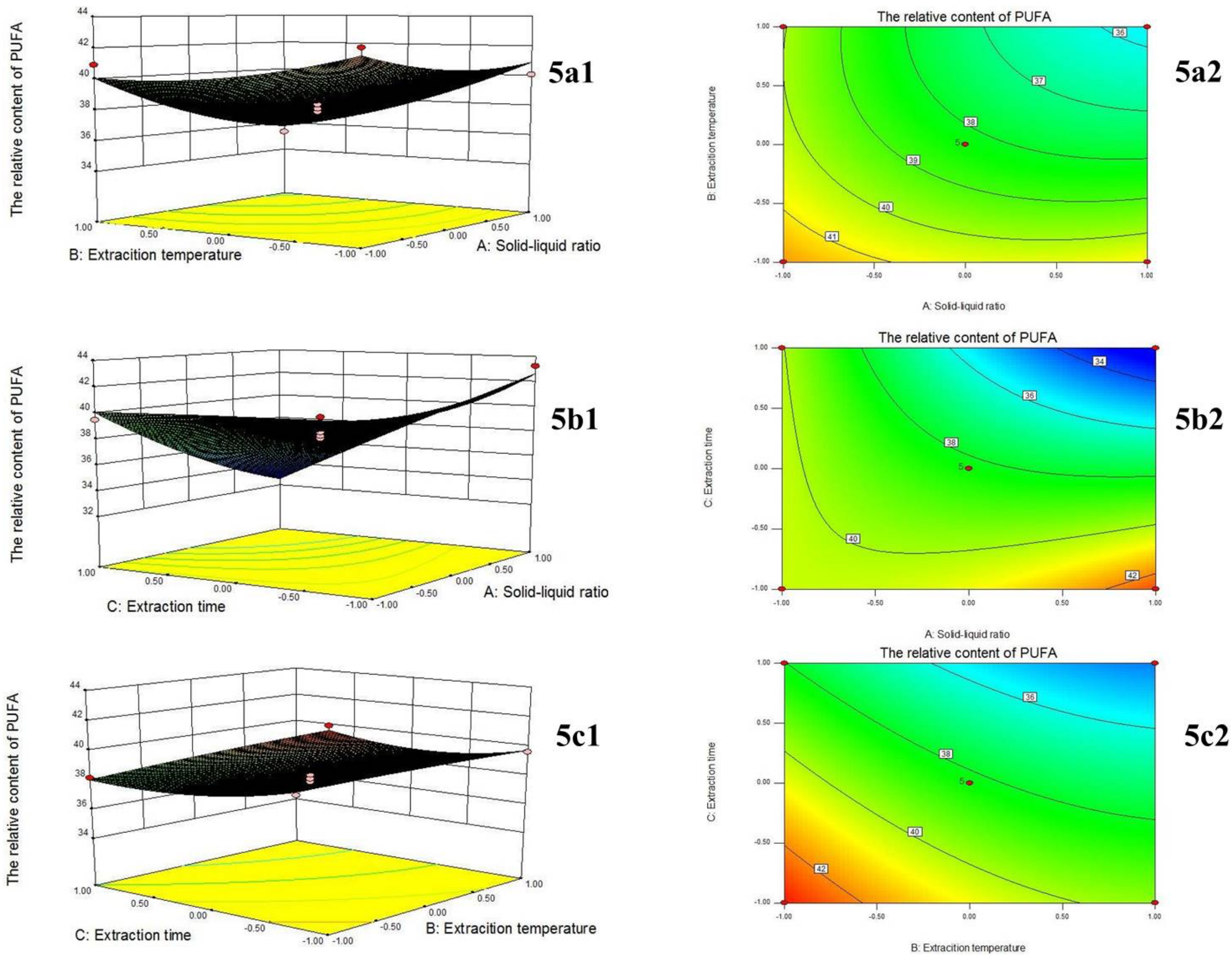

Figure 5. Contour plot and response surface plot of the influence of the interaction of various factors on the relative content of PUFA.

3.5. Matlab experimental results

Through programming, the 4-dimensional diagram of PUFA content and crude oil yield of pumpkin seed effected by solid-liquid ratio (g/mL), extraction temperature (°C) and extraction time (min) was presented (Figs. 7, 8). When the theoretical maximum value of Y_1 (45.8485%) was obtained, the conditional parameters were calculated by the matrix as bellow: the solid-liquid ratio of 1: 30 mL /g, the extraction temperature of 30°C, and the extraction time of 2.5 h. Similarly, when Y_2 reached the theoretical maximum value (18.7515%), the solid-liquid ratio was 1:30 g/mL, the extraction temperature was 40°C, and the extraction time was 3.5 h.

In order to better describe and analyze the interaction effects between data, three-dimensional response surface and contour projection diagrams of the interaction effects of Y_1 and Y_2 were drawn respectively (Fig. 9), when the extraction time was set short (2.5 h), medium (3 h) and long (3.5 h).

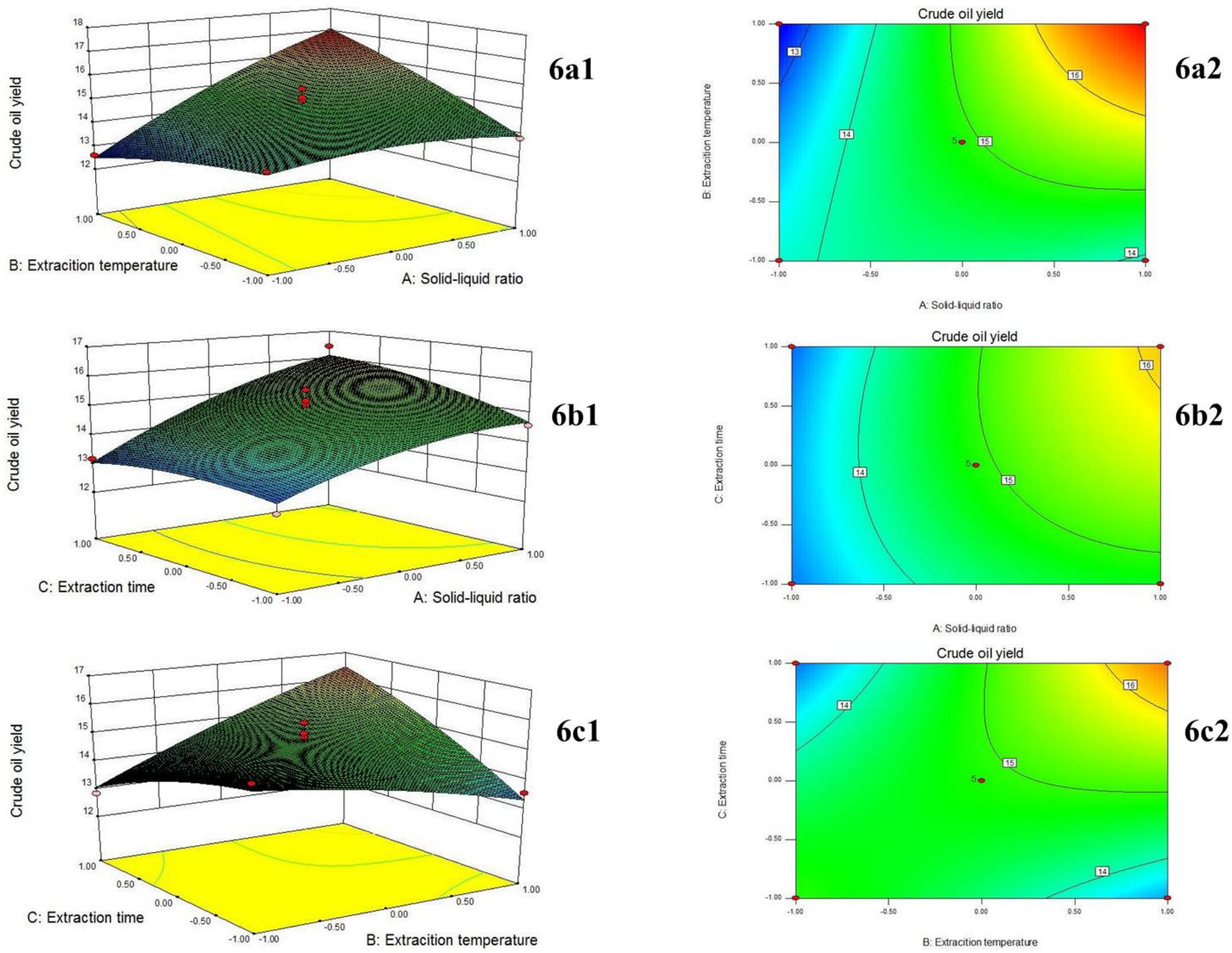

Figure 6. Contour plot and response surface plot of the influence of the interaction of various factors on the crude oil yield.

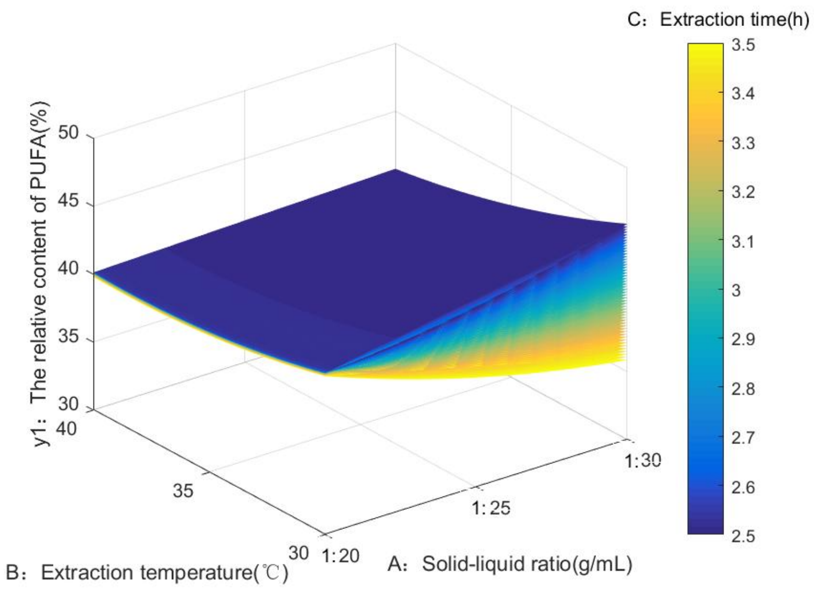

Figure 7. The 4-D interactive surface based on the optimizing of Y_1.

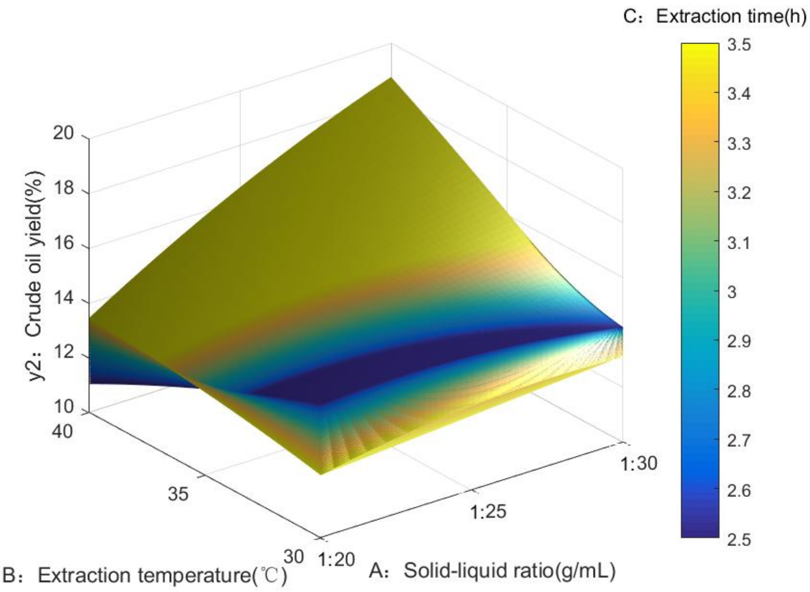

Figure 8. The 4-D interactive surface based on the optimizing of Y_2.

When the extraction time (C) set as lower level (C=2.5 h), the extraction temperature (B) was fixed, the value of Y_1 and Y_2 gradually increased with the increasing of solid-liquid ratio (A). When A value is fixed, both Y_1 and Y_2 decrease continuously with the increase of B. When the A value ranged from 1:24 to 1:27 g/mL, and the B value ranged from 30 to 32.5°C (the larger the value of A, the smaller the value of B), the Y_1 and Y_2 can approach the maximum value simultaneously. On this condition, the value range of Y_1 was 39.7~ 45.8485%, and the value range of Y_2 was 11.0440 ~ 15.0013%.

When the middle value (C) of extraction time (C=3 h) was set, and B value is fixed, with the increase of A, Y_1 continued to decrease, while Y_2 gradually increased. When A value was fixed, with the increasing of B value, Y_1 decreased and Y_2 increased. As A and B get closer to the minimum, Y_1 get closer to the maximum, and as A and B get closer to the maximum, Y_2 will get closer to the maximum. On this condition, the value range of Y_1 was 35.7675 ~ 41.8625%, and the value range of Y_2 was 12.5013 ~ 17.0563%, thus Y_1 and Y_2 cannot reach the maximum value simultaneously.

When the higher level of extraction time (C) (C=3.5h) was set, and B value was fixed, with the increasing of A, Y_1 continued to decline, while Y_2 showed a gradual increase trend. When fixed A value, as B increased, Y_1 continued to decline, while Y_2 gradually increased. The change rule was similar to what happened when C was set as the medium level (C=3 h). On this condition, the value range of Y_1 was 30.6135 ~ 41.6585%, and the value range of Y_2 was 12.2940 ~ 18.7515%. Only when A and B took a specific middle interval, can we ensure that both Y_1 and Y_2 get larger value simultaneously.

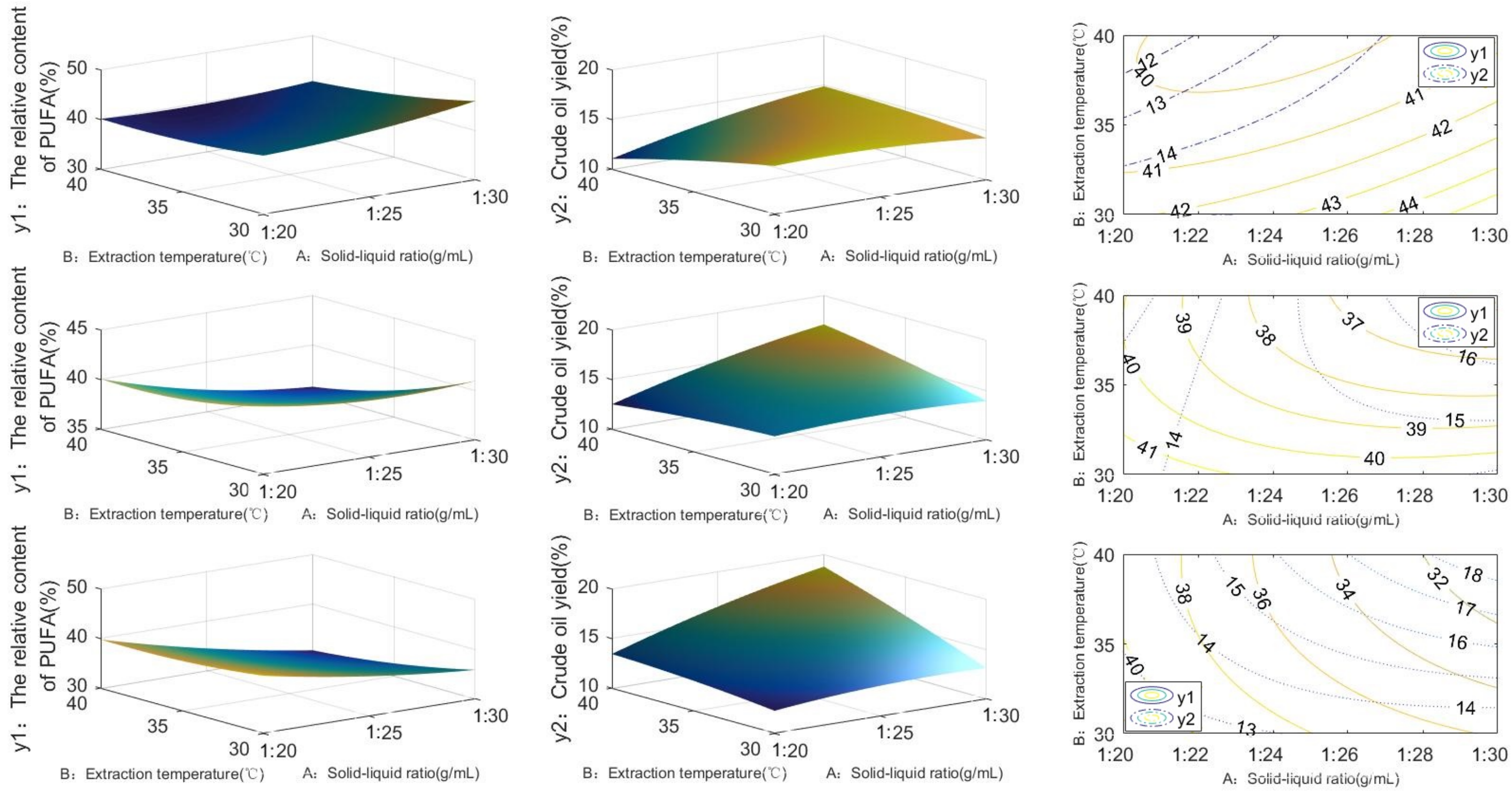

Figure 9. Contour plots and response surface plots of the effects of the interaction of various factors on relative content of PUFA and crude oil yield.

In conclusion, according to the software calculation, when the extraction time was set as the lower level (C=2.5 h), Y_1 can obtain the maximum theoretical value (45.8485%); when the extraction time was set as the higher level (C= 3.5 h), Y_2 can obtain the maximum theoretical value (17.0563%). When the value of C was 2.5 h, and the value of A was 1:24 ~ 1:27 g/mL, the value of B was 30 ~ 32.5°C, both Y_1 and Y_2 can obtain the larger theoretical value simultaneously, which is consistent with the above analysis conclusion.
Matlab, an acronym for MATrix LABoratory, is a product of the MathWorks, Inc.of Natick, MA (GILAT, 2011). The matlab is a high-performance and powerful graphics system for presenting and visualizing data. It integrates computation, visualization, and programming in an easy-to-use environment where problems and solutions are expressed in familiar mathematical notation (JALALVAND *et al*., 2019). Nowadays, the matlab is widely used in various research fields, such as optimization of oil extraction in chemical industry (HØRSHOLT *et al*., 2018), design and manufacturing of food machinery (CAO *et al*., 2014; ZHANG and DENG, 2018), and optimization of nutritional catering (TIAN and YU, 2009). At present, there is hardly any research on the optimization of pumpkin seed crude oil extraction process using matlab analysis method, which may be the development trend of food industry in the future.

4. CONCLUSIONS

The box - behnken dual response surface optimization method combined with matlab analysis method was used to optimize the extraction process of crude oil from Inner Mongolia pumpkin seed, based on the relative contents of PUFA, as well as the crude oil yield. The optimal process parameters were as bellow: solid-liquid ratio of 1:26.8 g/mL, extraction temperature 30°C, extraction time 2.5 h. At this point, the relative contents of PUFA pumpkin seeds oil was 43.54±0.14%, and the yield was 14.84±0.10%, which was not significantly different from the theoretical value (p >0.05). In addition, by the analysis of matlab, when the C value was set as the lower level (C=2.5 h), the solid-liquid ratio was within the range from 1:24 to 1:27 g/mL, the extraction temperature was within the range from 30 ~ 32.5°C, both Y_1 and Y_2 can obtain the larger theoretical value simultaneously, which was consistent with the conclusion of double response surface analysis. According to results, the optimization method combined dual response surface with matlab can not only accurately obtain the best extraction scheme, but also more intuitively find out the reasonable process parameter range, so that can provide theoretical basis and innovative basis for the research on industrial processing and utilization of pumpkin seed oil.

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