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Research Article

A Novel Process for the Aqueous Extraction of Linseed Oil Based on Nitrogen Protection

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Abstract: In order to prevent the oxidation of linseed oil and emulsification during extraction process, Nitrogen-protected and Salt-Assisted Aqueous Extraction (NSAE) of linseed oil was investigated in this study. Nitrogen-protected and salt-assisted were found to be the most effectively in weakening oil oxidation and improving the oil yield, respectively. The highest oil recovery of 87.55% was achieved under optimal conditions of sodium carbonate solution concentration (2 mol/L), solution-to-flour ratio (10 mL/g) and temperature (60°C). Moreover, there were no significant variations in physicochemical properties of Nitrogen-protected and Salt-assisted Aqueous Extracted Oil (NSAEO) and Salt-Assisted Aqueous Extracted Oil (SAEO), but NSAEO showed better oxidation stability. Additionally, NSAEO had a higher content of linoleic acid (18.97±0.05%), α-linolenic acid (56.48±0.12%). Therefore, NSAEO is a promising and environmental-friendly technique for oil extraction in the food industry.

Keywords: Aqueous extraction, linseed oil, nitrogen protection, response surface methodology

INTRODUCTION

Linseed (also known as flaxseed) is an important oil crop cultivated worldwide for oil and fiber. It has been cultivated in more than 50 countries, Canada is the major linseed producer, followed by China, United states and India (Coskuner and Karababa, 2007; Dixit *et al.*, 2012; Singh *et al.*, 2011). Linseed contains approximately 200~250 g/kg crude protein and 400~430 g/kg oil, consisting a potential source of protein and energy to be used in animal feeding (Collins *et al.*, 2011; Boylston *et al.*, 1996; Doreau *et al.*, 2009). It is also an excellent source of omega-3 fatty acid, which makes about 55~60% of total fatty acid (Rodriguez *et al.*, 2010; Leyva *et al.*, 2011; Yang and Luo, 2013; Mueller *et al.*, 2010; Choo *et al.*, 2007; Nykter *et al.*, 2006; Herchi *et al.*, 2012). However, this high content of omega-3 fatty acid makes linseed oil highly sensitive to heat, oxygen and light during oil extraction process (Juita *et al.*, 2013; Herchi *et al.*, 2011; Khattab and Zeitoun, 2013; Long *et al.*, 2011). Therefore, oil extraction is the crucial step which influences the quality of linseed oil.

Prepress solvent extraction and expeller press extraction is the conventional and efficient process used to extract the oilseeds. However, it comprises the use of flammable menstruums that present safety attentions and leads to volatilization of organic solvents, high running costs and undesirable influences on the quality of the oil (Kasote *et al.*, 2013; Ali and Watson, 2014; Pradhan *et al.*, 2010; Zhang *et al.*, 2008; Jiao *et al.*, 2014; Latif and Anwar, 2011). It is generally know that these extraction restrictions can be conquer by aqueous extraction method. Researchers have been done on aqueous oil extraction from diverse seeds such as olive oil, rice bran oil, soybean oil, peanut oil, coconut oil (Zhang *et al.*, 2012; Aliakbarian *et al.*, 2008; Yu *et al.*, 2013; Rostami *et al.*, 2014; Rajković *et al.*, 2013; Campbell and Glatz, 2009; Amarasinghe *et al.*, 2009). It is worth noticed that aqueous extraction method present lower efficiency and labor consuming. Therefore, we modified the aqueous oil extraction process, using a sodium carbonate solution instead of water as the extraction medium. This important step prevented emulsion formation in the aqueous system, which resulted in a very high oil yield. Meanwhile, the whole extraction process was full of the nitrogen gas, play a role of protector. Prevented the oxidation of linseed oil, contribute to produce the maximum quantity and good quality of oil.

To the best of our knowledge, an aqueous method based on nitrogen-protection and salt-assisted extraction of the linseed oil is not well documented so far. Therefore, the purpose of this study is to explore the optimal condition of oil extraction process on quantity and quality of linseed oil, with the help of the response surface methodology.

METERIALS AND METHODS

Materials: Linseed which was obtained from the Huining, Gansu province, the initial oil content of dry basis was 30.2%, the meal was produced by cleaning

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flaxseed and was milled to pass through a 20-40 mesh sieves and made the materials evenly.

Reagents: Nitrogen, sodium carbonate, sodium hydroxide, sodium thiosulfate, *et al*, all purchased from the Xi'an chemical reagent company. All reagents used for experiments were of analytical grade (>99.9%).

Instruments and equipment: High speed tabletop centrifuge (H-1650), Changsha Hunan, China Homoiothermy oscillator (SHA-C), Changzhou Jiangsu, China.

Extraction process: The linseeds were cleaned manually to remove all foreign materials, such as dust, handstone, dirt and broken and immature seeds.

Then, grinded the linseed and obtained mills pass through a 20 mesh sieves, which were then measured 10 g meals into stopper flask and commixed with distilled water at a designed ratio (8:1, v/w), prepared sodium carbonate solution (2 mol/L) were added and the nitrogen were inlet the mixtures with the speed of 0.2 m^3/h, followed the above steps, the samples were kept in a homoiothermy oscillator water bath (the temperature were set for 70°C and the vibration were at 160 r/m for 2 h), next, the emulsion phase was centrifuged at 4000 rpm for 15 min to get free oil. Finally, all free oils were collected together and weighted use to count the oil yield, the experiment was carried out in triplicate.

The relationship between the extractability and materials can be expressed by the following equation:

$$\text{Extractability} = \frac{\text{supernatant quality}}{\text{meal quality} \times \text{powder oil content}} \times 100\%$$

Analysis of linseed oil properties: The Peroxide value and Acid value of all oils was determined in accordance with the American Oil Chemist's Society (AOCS) (2003a, b) official methods (AOCS cd 8b-90; AOCS cd 3a-63).

Experiment design and statistical analysis: Based on the single-factor test, a series of experiments by RSM were designed to optimize the extraction conditions. A Box-Behnken design was used to survey the effects of three independent variables (sodium carbonate solution concentration, solution-to-flour ratio and temperature.) at three levels on the dependent variables and the test design. All trials were performed in triplicate. Design-Experts (Version 8.05, USA.) software package was used for the experimental design variance and regression analysis of the experimental data.

RESULTS

Effect of sodium carbonate solution concentration on oil extractability: The effect of sodium carbonate solution concentration on oil extractability was present in Fig. 1.

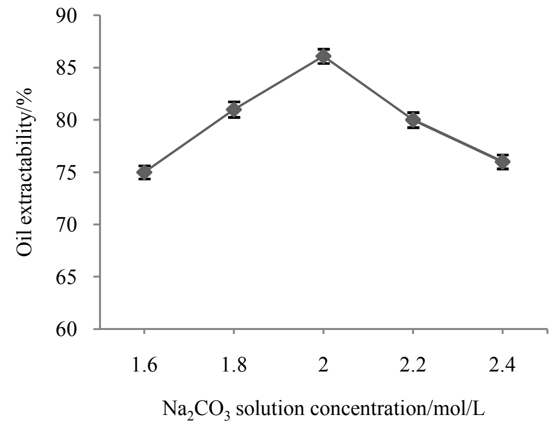


Fig. 1: Effect of Na_2CO_3 solution concentration on oil extractability

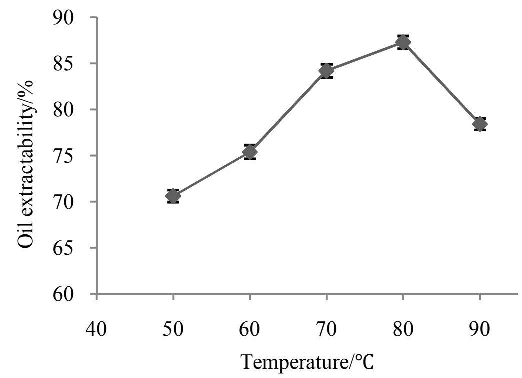


Fig. 2: Effect of extraction temperature on oil extractability

Oil extractability increased significantly with the increase of concentration of Na_2CO_3, suggesting that linseed oil can be extracted more effectively under salt effect conditions by Na_2CO_3. The highest extraction yield was achieved at 2.0 mol/ L, at which approximately 85% of the oil was extracted avoiding emulsification in the aqueous system. After that, the yield of oil was decreased as the concentration increased. These phenomena might be derived from various concentration of Na_2CO_3 had different influences on oil extraction system. Appropriated amount of Na_2CO_3 made contribution to avoid the emulsification of the oil extraction systems, accelerated the release of oil droplets, increasing the oil yield. While, over added Na_2CO_3 generated severely denaturation of protein, released a lot of no-lipid compositions, covered the oilseed, rendered the decrease of oil yield. So in this study, Na_2CO_3 solution of 1.8~2.2 mol/L was adopted for further investigation.

Effect of extraction temperature on oil extractability: The effect of extraction temperature on oil extractability was demonstrated in Fig. 2.

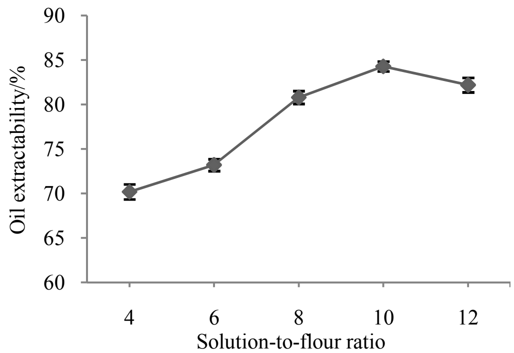


Fig. 3: Effect of solution-to-flour ratio on oil extractability

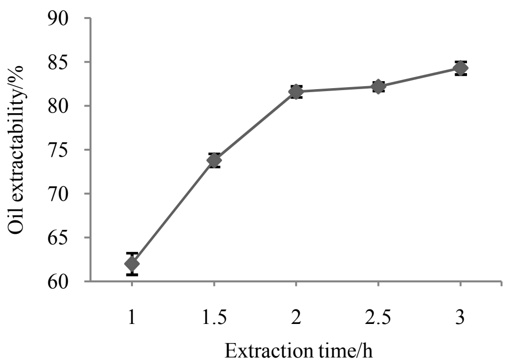


Fig. 4: Effect of extraction time on oil extractability

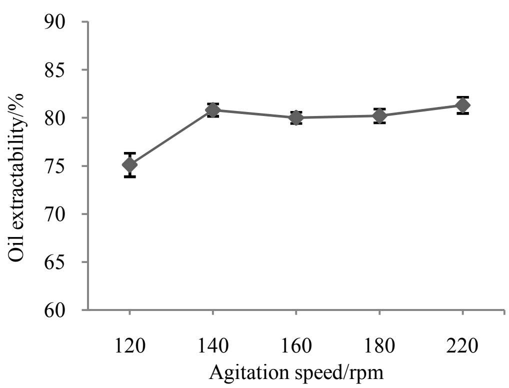


Fig. 5: Effect of agitation (vibration) speed on oil extractability

It is obviously that with the increase of temperature the oil yield increased during 50~80°C. Meanwhile, it should be observed that the increase become more and more slow. Principally due to the fasten movement of oil molecular was in favor of bigger oil droplets collision which made it easy to obtain oil and perhaps in part to the denatured protein made the emulsion system imbalance, released the oil, improved the oil yield. When the temperature is over 80°C, the oil yield reduced notably with the enhance of temperature, this could be due to the similar theory of heavy concentration of sodium carbonate. Therefore, a temperature of 60~80°C was deemed satisfactory for further research of oil extraction.

Effect of solution-to-flour ratio on oil extractability: The effect of solution-to-flour ratio on oil extractability was expressed in Fig. 3.

Oil extractability was significantly affected by solution-to-flour ratio (Fig. 3). The oil yield increased as the ratio increased until the ratio up to 10. The insufficient immersed sample and serious solution viscosity might be responsible for this phenomenon. At the ratio of 10, it is clearly that the oil yield up to the maximum, the possible reason is under these conditions the proportion of sample molecule size and water quantity were suitable, in favor of the oil release and extract. When the ratio over 10, the oil yield decreased slightly, of all possible reasons the most likely one might be the inappropriate proportion of samples and solution, excessive amounts of water which increased the difficulty of the extraction. From these experiments, it is clearly that the ratio of 8~12 is adequate for the deeper study of optimal process.

Effect of extraction time on oil extractability: The effect of extraction time on oil extractability was represented in Fig. 4.

There was a powerful evidence indicated that oil extractability increased significantly when extraction time increased. With the increase of time the oil yield was changed rarely. Considering the production efficiency and yield efficiency, an extraction time of 1.5~2.5 h was therefore for the process.

Effect of agitation (vibration) speed on oil extractability: The effect of agitation speed on oil extractability was investigated and reported in Fig. 5.

It was found that the speed variation had little influence on oil extraction. When the experiment was carried out at the lowest speed of 140 rpm, solid were observed to settle slowly, the speed of 160 rpm was therefore for the process, increased occasionally to ensure homogeneity. The result suggested that high agitation speed were not needed.

So in this research, we chose the variables depend on the level of impact on oil extractability. Finally, we employed sodium carbonate solution concentration 1.8 ~2.2 mol/L, solution-to-flour ratio 8~12 and extraction temperature 60~80°C for RSM experiments.

Optimization of aqueous extraction with the help of salt by RSM:

Fitting the mathematical model: The conditions of salt-assisted aqueous extraction of oil with nitrogen existence from linseed were optimized by RSM. According to the experimental results of BBD (Table 1) and regression analysis, a second-order polynomial equation was established to estimate the relationship between the oil extraction yield and variables. As for equation was impressed as follows:

Table 1: Experimental design and corresponding results for response surface analysis

Run	Coded variable(Actual variable) Sodium carbonate concentration -x_1/mol/L	Solution-to-flour Ratio-x_2	Extraction temperature -x_3/°C	Oil extractability -y/%
1	-1 (1.8)	-1 (8)	0 (70)	75.34
2	1(2.2)	-1	0	72.76
3	-1	1 (12)	0	78.02
4	1	1	0	75.87
5	-1	0 (10)	-1(60)	83.53
6	1	0	-1	86.91
7	-1	0	1(80)	83.29
8	1	0	1	78.17
9	0 (2.0)	-1	-1	85.03
10	0	1	-1	78.43
11	0	-1	1	69.82
12	0	1	1	76.83
13	0	0	0	84.87
14	0	0	0	86.73
15	0	0	0	86.15
16	0	0	0	82.76
17	0	0	0	85.15

Table 2: Results of ANOVA

Source	SS	df	M.S.	F-value	p-value (Prob>F)	Significant
Model	424.68	7	60.67	25.11	<0.0001	**
x_1	5.23	1	5.23	2.17	0.1752	
x_2	4.81	1	4.81	1.99	0.1921	
x_3	83.14	1	83.14	34.41	0.0002	**
x_1x_3	18.06	1	18.06	7.47	0.0231	*
x_2x_3	46.31	1	46.31	19.16	0.0018	**
x_1^2	18.56	1	18.56	7.68	0.0217	*
x_2^2	240.32	1	240.32	99.45	< 0.0001	**
Residual	21.75	9	2.42			
Lack of Fit	12.46	5	2.49	1.07	0.4861	
Pure Error	9.29	4	2.32			
Cor Total	446.42	16				

*: p = 0.05; **: p = 0.01; SS.: Sum of square; M.S.: Mean of square

$$y = 85.11-0.81x_1+0.78x_2-3.22\,x_3-2.13x_1x_3+3.40x_2x_3-2.10\,x_1^2-7.54x_2^2$$

where, x_1 x_2 and x_3 correspond to the coded values of the three independent variables(sodium carbonate solution concentration, solution-to-flour ratio and extraction temperature).

Table 2 presents the results of ANOVA, which was used to screen the important operational variables affecting the extraction oil yield.

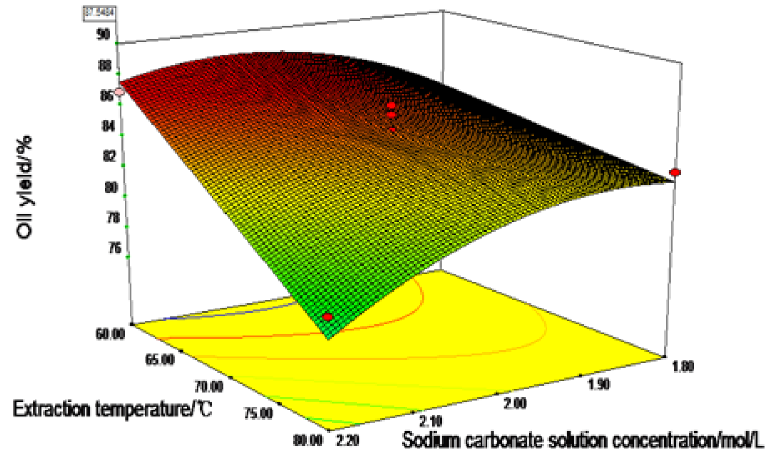
The statistical significance of the model, the three independent variables and their interactions were evaluated from their F- and p-values. At the confidence interval of 95%, the fitted model was statistically significant as implied by model F- and p-values of 25.11 and <0.0001, respectively. In addition, the coefficient of multiple determinations (R^2) of the response of oil extraction was 0.9513.

According to the results of ANOVA, it can be observed that the model was significant and the lack of fit was not significant, demonstrating that it is an excellent model. Thereby, the extraction temperature and it combined effects with solution-to-flour ratio presented significantly on the extraction of oil ($p<0.01$), respectively.

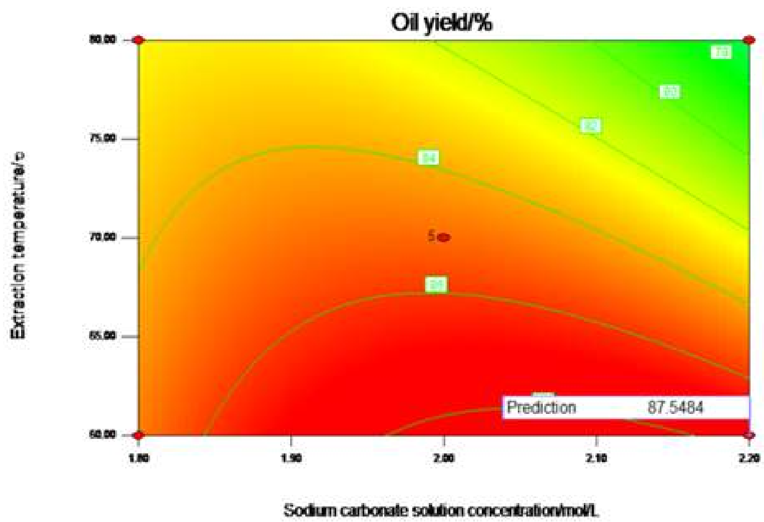
Based on the above factors, it could be deduced that the model was appropriate to predict the responses. From the equation and Table 2, it was also seen that the factors with the largest effect on the oil yield were the extraction temperature, the interactions between temperature and solution-to-flour ratio and the quadratic term of solution-to-flour ratio ($p<0.01$). The interactions between sodium carbonate solution concentration and temperature and the quadratic term of sodium carbonate solution concentration, they have significant influence on extraction yield of oil ($p<0.05$). The linear term of sodium carbonate concentration and solution-to-flour ratio had negative effects on oil yield.

The maximal value of oil extraction yield predicted by RSM was 87.55%, under the following extraction conditions: sodium carbonate concentration 2 mol/L, solution/flour ratio 10 mL/g and temperature 60°C.

The interaction between the independent variables: The best way to evaluate the effect of the independent variables on the dependent one was to draw three-dimensional (3D) response surface curves of the model. Figure 6a and b, we can know that were the 3D and 2D plots of the response surface for the interaction effects on the oil yield. It indicated that moderate temperature resulted in high extraction yield of oil. With increasing temperature, the extraction yield decreased continuously. It may ascribed to the high temperature



(a)



(b)

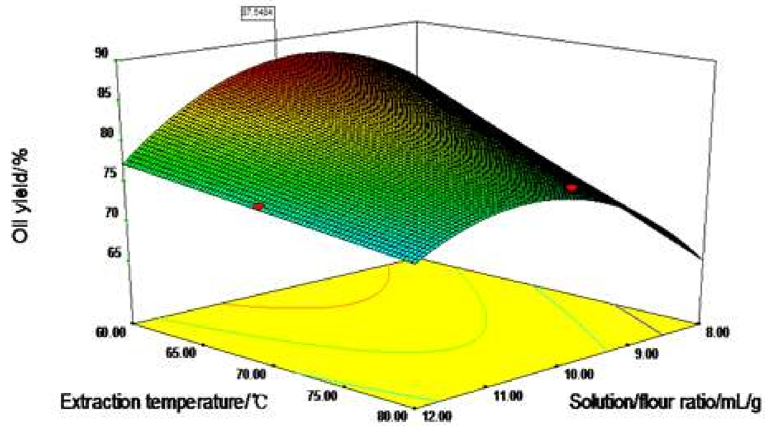
Fig. 6: Response contours of sodium carbonate concentration and temperature

generated strong emulsification that made the oil yield reduced. As to sodium carbonate solution concentration, in influence of this independent variable was very large, it would be easily seen that extraction yield increased with increased in concentration. This phenomenon might be due to the sodium carbonate solution prevented the system emulsion.

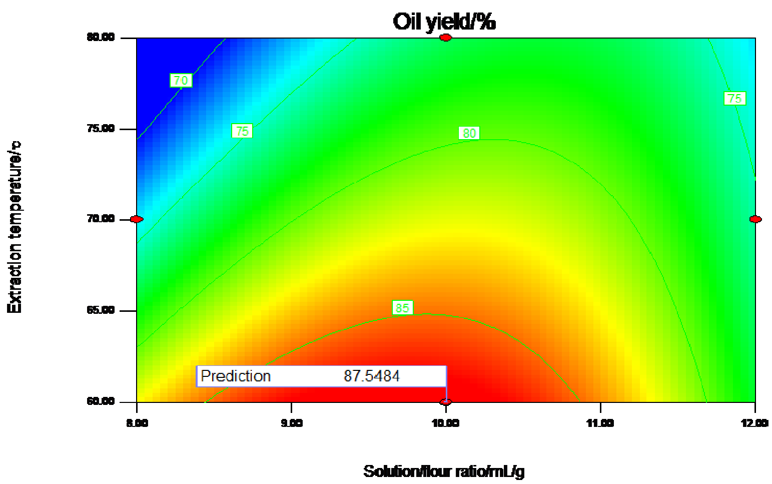
Figure 7a and b showed the 3D and 2D plots of the response surface for the extraction yield of oil as relevant to temperature and solution-to-flour ratio. It demonstrated that regulating temperature made a significant impact on extraction yield of oil. With increasing temperature, the extraction yield rose at first, but once the solution-to-flour ratio reached high levels, the extraction yield slightly decreased.

Extraction yield and properties: From Table 3, we can found the composition of fatty acid under nitrogen protection and no-nitrogen protection. The resultant of composition of linseed oil indicated that the content of palmitic, oleic, linolenic, was increased significantly with application of nitrogen protection, the level of increase was as follows, linolenic, α-linolenic, palmitic, oleic. However, there was slight decreased in the content of stearic. While, there was no substantial changes occur in the content of eicosanois acid. These rasults proved that under the application of nitrogen protected the fatty acid composition of linseed oil changed during oil extraction.

The findings by analysis is that the quality and average oil yield of linseed was 86.4%, the SD was 0.2646, the acid value was 0.4 mg/g, the SD was 0.1, the peroxide value was 1.2 mmol/kg, the SD was 0.0577, conformed to the international edible oil standards totally. Additionally, the obtained linseed oil was transparent and clear in appearance with it inherent



(a)



(b)

Fig. 7: Response contours of solution-to-flour ratio and temperature

Table 3: Comparison of fatty acid composition of linseed oil for nitrogen protection and no-nitrogen protection

Fatty acid	No-nitrogen protection Content (%)	Nitrogen protection Content (%)
Palmitic acid	5.64±0.32	7.78±0.24
Stearic acid	3.53±0.17	3.08±0.13
Oleic acid	20.57±0.66	22.46±0.60
Linoleic acid	16.33±0.12	18.97±0.05
α-linolenic acid	54.12±0.28	56.48±0.12
Eicosanoic acid	<0.5	<0.5

odor and taste, no foreign smell and it is a kind of directly health food. It is indicated that the extracted process of linseed oil was suitable to the requirements of environmental and ecological and be of great importance to the development of oil extraction industry.

DISCUSSION AND CONCLUSION

Aqueous extraction under the condition of nitrogen protection and salt-assisted, which was an efficient and environmentally friendly extraction processes. It could be used to improve the extraction yield of linseed oil. Firstly, we acquired the optimal extraction condition (sodium carbonate solution concentration, solution-to-flour ratio and extraction temperature) by orthogonal test design. Then to further optimize oil extraction, sodium carbonate solution concentration, solution-to-flour ratio and extraction temperature were explored by RSM. Finally, we measured the oil yield and investigated the properties of linseed oil. Under the optimal conditions, the experiment extraction yield of linseed oil was 87.55%, the quality of oil was conformed to the international edible oil standards totally.

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