Extraction and Characterization of Flaxseed Oil Obtained with Subcritical n-Butane

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Abstract: In this study, subcritical n-butane was adopted to extract oil from flaxseed. The extraction conditions i.e. extraction temperature, extraction time, and liquid-solid ratio were investigated and optimized by response surface methodology. The flaxseed oil obtained by subcritical n-butane were characterized and compared with those prepared by n-hexane and cold pressing. Results indicated that the optimal combination of parameters was 53.93°C, 56.82 min, and 19.98:1 mL/g. Subcritical n-butane had higher yield (28.75%) than n-hexane and cold pressing. GC analysis indicated that subcritical n-butane extraction had no obvious influence on the fatty acid composition. Nevertheless, the oil obtained by subcritical n-butane with higher contents of phytosterols (2.93 mg/g) and carotenoids (46.56 mg/kg), and presented a higher oxidation stability (9.27 h). Thus, it was suggested that subcritical n-butane extraction is a promising alternative to extract high quality flaxseed oil.

Key words: flaxseed oil, subcritical n-butane, extraction, response surface optimization, oil quality

1 Introduction

Flaxseed (Linum usitatissimum L.) is a potential oilseed crop and widely planted in China, North America, Ethiopia and India. Flaxseed contains 30-40% of oil. It has been reported that daily intake of flaxseed oil can reduce the risk of colon tumors, mammary cancer, atherosclerosis, etc. The efficacy of flaxseed oil is mainly attributed to the richness in unsaturated fatty acids, especially α-linolenic acid (ALA 18:3, n-3), which accounts for 40-68%. The consumption of flaxseed oil helps to balance the proportion of n-3 and n-6 fatty acids in the human diet. Flaxseed oil has been considered a healthy oil for its nutritional and pharmaceutical value.

Mechanical pressing and organic solvent extraction are the two most common methods for vegetable oil production. However, organic solvent extraction is unacceptable on account of the inevitable residual solvent that is harmful to human health. While mechanical pressing is high energy consumption and low extraction rate. Supercritical CO₂ extraction seems to be an innovative and acceptable method because it is non-toxic, organic solvents-free, and allows more selectivity for target production. However, it is suffering from some serious problems such as high operating pressure, high cost of instrument, and limited production capacity. Compared with supercritical CO₂ extraction, subcritical fluid extraction has advantages of mild operating environment and low equipment cost. In this method short-chain hydrocarbons such as n-propane and n-butane is commonly used as solvent, which allow to enhancement of extraction efficiency due to their high diffusivity, density, and low viscosity. The mild temperature and pressure used favors the retention of bioactive components and reduction of lipid oxidation. Besides, as with those extracted by supercritical CO₂ extraction, the final products from subcritical fluid extraction are free of toxic solvent residue. Subcritical fluid extraction has been widely applied to prepare oil from various crops such as pepper seeds, sapucaia seeds, chia seeds, crambe seed, wheat germ, sunflower seeds, soybean germ, sesame, and so on. The previous investigations suggested subcritical fluid extraction is safe, efficient, and environmental compatibility.

It is noteworthy that subcritical n-propane have been applied to extract flaxseed oil in some previous studies. Zanqui et al. extracted flaxseed oil with subcritical n-propane and found the extracted oil had higher purity and greater oxidation stability than those obtained via conventional solvents. Afterward, the extraction process of subcritical n-propane for flaxseed oil was modeled by Piva et al. Compared to n-propane (critical conditions: T = 95.7°C, P = 4.4 Mpa), n-butane (critical conditions: T = 152.8°C, P = 3.6 Mpa) is much safer and profitable due to
the lower operating pressure and equipment investment. However, review of literature shows that there is no report on extraction of flaxseed oil using subcritical \textit{n}-butane. In this study, subcritical \textit{n}-butane was firstly used as extraction solvent to extract oil from flaxseed. The purposes of this study were to evaluate the effect of the extraction conditions, i.e. extraction time, temperature, liquid-solid ratio, on the oil yield and to optimize the extraction parameters by response surface methodology (RSM) and statistical techniques. Moreover, the physicochemical properties, fatty acid composition, content of phytochemicals (carotenoids and phytosterols), and oxidation stability of subcritical \textit{n}-butane extracted oil was evaluated and compared with those extracted with \textit{n}-hexane extraction and cold pressing.

2 Materials and Methods

2.1 Sample preparation and chemicals
Flaxseed was obtained from the Gansu Academy of Agricultural Sciences, China. The raw material was cleaned to remove dust, stone, other seeds, etc. The cleaned seed was dried at 50°C until reducing to the moisture of approximately 6%. The dried sample was ground in a blender and then pass through a 30-mesh sieve. The ground seed was sealed and stored in the dark at 4°C until use.
\textit{n}-Butane solvent (99.5%) was obtained from Longyu Chemical Co., Ltd. (Puyang, China). Fatty acids and phytosterols standards (Campesterol, Stigmasterol, \(\beta\)-Sitosterol, Sitostanol and 5\(\alpha\)-cholestanol) were purchased from Sigma-Aldrich Chemical Co., Ltd. (Shanghai, China). All other chemicals used in study were at least of analytical grade.

2.2 Extraction of flaxseed oil

2.2.1 Subcritical \textit{n}-butane extraction (SBE)
The SBE was carried out on a CEB-5L subcritical fluid system (Henan Subcritical Extraction Biological Technology Co., Ltd, Anyang, China). The schematic diagram of the system is shown in Fig. 1. For each extraction, 200 g of flaxseed powder was placed into the extraction vessel (\(\varphi = 15.0\) cm, \(h = 25.0\) cm). Then the oxygen in the extraction vessel and separation vessel was drawn off by a vacuum pump. The liquid \textit{n}-butane was added to the extraction vessel using a metering pump with a digital scale. The temperature was controlled by the heating jacket connecting with hot water tank, and the extraction pressure was 0.5 Mpa. The extraction temperature, time, and liquid-solid ratio were determined according to experimental design. After the procedure completed, subcritical \textit{n}-butane containing extracted oil was allowed to flow into the separation vessel. The \textit{n}-butane was gasified and separated from the dissolved oil by depressurization. Subsequently, the separated \textit{n}-butane was sent to solvent vessel after compressing and liquefying, enabling the solvent circulation. The extracted oil was collected for calculating oil yield and stored at \(-20°C\) until further analysis.

2.2.2 Cold pressing
Cold pressing of flaxseed was performed on an YKY-6YL-550 hydraulic equipment (Zhengzhou Bafang Machinery Equipment Co. LTD, Zhengzhou, China). Five hundred grams of flaxseed powder were packed using absorbent gauze and pressed at 45 Mpa for 1 h. The oil was collected and stored as above.

2.2.3 \textit{n}-Hexane extraction
\textit{n}-Hexane extraction was carried out with a Soxhlet apparatus\(^9\). Briefly, 100 g of ground flaxseed was eluted with 500 mL of \textit{n}-hexane under reflux in a Soxhlet extractor. The extraction was carried out at 70°C for 6 h. Then the \(n\)-
hexane was removed in a rotatory evaporator at 45°C. The recovered oil was weighted and stored at low temperature.

2.3 Determination of oil yield

The formula for calculating the oil yield was as follows:

\[
\text{Oil yield} (\%) = \frac{\text{Mass of extracted oil}}{\text{Mass of flaxseed powder}} \times 100
\]  

(1)

2.4 Single-factor experiments

The effect of extraction temperature (25, 35, 45, 55, and 65°C) on the oil yield was determined under a fixed extraction time (40 min) and liquid-solid ratio (10:1 mL/g). Moreover, the effect of extraction time (10, 20, 30, 40, and 50 min) on the oil yield was analyzed under extraction temperature 45°C and liquid-solid ratio 10:1 mL/g. Finally, the effect of liquid-solid ratio (5:1, 10:1, 15:1, 20:1, and 25:1 mL/g) on the oil yield was evaluated at extraction temperature 45°C and time 40 min.

2.5 Optimization of extraction conditions with RSM

Based on the results of single-factor experiment, the preliminary proper ranges of extraction temperature \(X_1\), extraction time \(X_2\), and liquid-solid ratio \(X_3\) was chosen to be 45-65°C, 20-60 min and 10:1-20:1 mL/g, respectively. To determine the optimal combination of variables, a three-factor, three-level Box-Behnken design (BBD) was performed. The design consisted of 17 experimental runs, in which 5 replicates at the center point to estimate the pure error of experiment and the repeatability of the method. The experiment was carried out in random order. The BBD matrix and response values are shown in Table 1.

The yield of flaxseed oil \(Y\) was estimated using the following quadratic model equation:

\[
Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{2} \sum_{j=1}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j
\]

(2)

Where \(Y\) is theoretical yield, \(X_i\) and \(X_j\) are independent parameter \((i \neq j)\), \(\beta_0\) is a constant, and \(\beta_i\), \(\beta_{ij}\) and \(\beta_{ij}\) are coefficients of linear, quadratic and interactive, respectively. The software of Design Expert (Version 8.0.6.1, Stat-Ease, Inc., Minneapolis, U.S.) was used to collect data and conduct analysis of variance (ANOVA), regression analysis, mathematical modeling, and optimization of variables.

2.6 Determination of physicochemical properties

The refractive index and specific gravity were measured using an abbe refractometer and a specific gravity bottle, respectively\(^{19}\). The standards of American Oil Chemists’ Society (AOCs) Cd 8b-90, Cd 3d-63, Cc 13e-92, Cd 3-25, Cd 1-25 were applied to determine the peroxide value, acid value, color, saponification value, and iodine value of flaxseed oil, respectively.

Table 1  Response surface experiment design and results.

| Run | Coded (Actual) | Coded (Actual) | Coded (Actual) | Experimental Yield (%) | Predicted Yield (%) |
|-----|---------------|---------------|---------------|-----------------------|-------------------|
| 1   | 1 (65)        | -1 (20)       | 0 (15:1)      | 26.13                 | 26.39             |
| 2   | -1 (45)       | 0 (40)        | -1 (10:1)     | 22.90                 | 23.30             |
| 3   | -1 (45)       | 1 (60)        | 0 (15:1)      | 27.33                 | 27.14             |
| 4   | 1 (65)        | 0 (40)        | 1 (20:1)      | 28.66                 | 28.26             |
| 5   | 0 (55)        | -1 (20)       | -1 (10:1)     | 23.73                 | 23.21             |
| 6   | 0 (55)        | 0 (40)        | 0 (15:1)      | 26.53                 | 27.14             |
| 7   | 0 (55)        | 0 (40)        | 0 (15:1)      | 27.27                 | 27.14             |
| 8   | 1 (65)        | 0 (40)        | -1 (10:1)     | 23.83                 | 24.09             |
| 9   | 0 (55)        | 1 (60)        | -1 (10:1)     | 25.60                 | 25.46             |
| 10  | 0 (55)        | 0 (40)        | 0 (15:1)      | 27.27                 | 27.14             |
| 11  | 1 (65)        | 1 (60)        | 0 (15:1)      | 26.43                 | 26.31             |
| 12  | 0 (55)        | 1 (60)        | 1 (20:1)      | 28.33                 | 28.85             |
| 13  | 0 (55)        | -1 (20)       | 1 (20:1)      | 27.80                 | 27.94             |
| 14  | 0 (55)        | 0 (40)        | 0 (15:1)      | 27.33                 | 27.14             |
| 15  | 0 (55)        | 0 (40)        | 0 (15:1)      | 27.30                 | 27.14             |
| 16  | -1 (45)       | 0 (40)        | 1 (20:1)      | 27.53                 | 27.27             |
| 17  | -1 (45)       | -1 (20)       | 0 (15:1)      | 23.73                 | 23.85             |
2.7 Determination of fatty acid composition

The fatty acid methyl esters (FAMEs) were prepared according to the procedure reported previously. The analysis of fatty acid composition was carried out by a gas chromatography system (7890 B, Agilent Co., Santa Clara, CA, USA) with a flame ionization detector (FID). The separation of FAMEs was executed on a HP-88 capillary column (100 m × 0.25 mm × 0.20 μm, Agilent Co., Santa Clara, CA, USA). The column temperature was programmed from 140°C to 240°C at a rate of 4°C/min and maintained at initial and final temperature for 5 and 10 min, respectively. The injection volume was 1 μL in a split mode (split ratio 50:1). Carrier gas was nitrogen with a flow rate of 1 mL/min. The injector and detector temperature were 250°C and 280°C, respectively. The retention times were compared to standard methyl esters to identify the unknown fatty acids. The area normalization method was used for quantitative analysis of fatty acids.

2.8 Determination of carotenoid

Carotenoid content of flaxseed oil was determined according to the method as reported by Suri et al. Briefly, 7.5 g of flaxseed oil was dissolved and diluted with cyclohexane in a 25 mL of volumetric flask. The absorbance of the solution at 470 nm was measured by a spectrophotometer (UV-6000 PC, Shanghai Metash Instruments Co., Ltd, Shanghai, China). The concentration of carotenoid was calculated using the equation (3) provided by Suri et al.:

\[
\text{Carotenoid content (mg/kg)} = \frac{(\text{Abs}_{470} \times 10^3)}{(2000 \times 100 \times \text{density})}
\]

2.9 Determination of phytosterols

The phytosterols of flaxseed oil was determined based on the procedure reported by Zhang et al. The phytosterols composition was analyzed on an Agilent 7890 A gas chromatograph (Agilent Co., Santa Clara, CA, USA) equipped with a FID detector. An HP-5 column (30 m × 320 μm × 0.25 μm, Agilent Co., Santa Clara, CA, USA) was used for the separation of phytosterols. The carrier gas was nitrogen with a flow rate of 1 mL/min, split ratio was 20:1, and the injection volume was 1 μL. The temperature of the column oven was 285°C kept for 30 min, then increased to 300°C at 10°C/min and maintained for 5 min. Injector temperature was 300°C; detector temperature was 360°C. The qualitative analysis of phytosterols was performed by comparing the retention times with standards. The quantitative analysis was carried out using 5α-cholestanol as an internal standard.

2.10 Determination of oxidative stability

The oxidation stability of flaxseed oils was evaluated using a Metrohm Rancimat model 743 (Herisau, Switzerland). Oil samples (3.0 g) were weighed exactly and oxidized at 110°C with an airflow rate of 20 L/min. The induction time of the samples was recorded automatically, and the result was expressed in hours.

2.11 Statistical analyses

All data are reported as mean value ± standard deviation of triplicate analyses. The analysis of variance (ANOVA) and Duncan test of data were performed on the IBM SPSS statistical software (version 21.0, IBM Corporation, NY, USA). The significance of difference values with \( p < 0.05 \) were regarded as statistically significant.

3 Results and Discussion

3.1 Effect of extraction temperature on oil yield

As it can be seen from Fig. 2A, the yield of flaxseed oil increased significantly \( (p < 0.05) \) with the increase of extraction temperature, ultimately reaching a maximum (25.63%) at 55°C. Subsequently, the oil yield decreased slightly with further increasing of temperature. The phenomenon can be explained by the fact that the rise of temperature increased the vapor pressure of extraction solvent and reduced its viscosity. As a result, the mass transfer resistance was diminished, which facilitates the oil extraction. However, excessive temperature accelerated the gasification of \( n \)-butane, and subsequently reduced the contents of liquid \( n \)-butane in the extractor, resulting in a decrease of oil solubility. Similar tendency was observed in the process of SBE for fenugreek seed oil and red pepper seed oil. The high temperature would cause an increase of production cost and a loss of temperature-sensitive components. Therefore, the optimal temperature was selected as 55°C in the RSM experiment.

3.2 Effect of extraction time on oil yield

Extraction time had a dominant effect on the yield of flaxseed oil. As shown in Fig. 2B, the migration speed of flaxseed oil was fast in the initial stage of extraction. The oil yield reached to 24.40% at 40 min. However, the trend stabilized when extraction time was above 40 min. The increment of oil yield was only 0.1% (from 24.4 to 24.5%) during the extraction time from 40 to 50 min. This phenomenon can be attributed to that the solvent dissolved the lipid in the superficial layer of the matrix at a faster rate at the initial stage of extraction. However, the system reached saturation with enough extraction time. What’s more, when the solutes on the surface layer were extracted, the residual oil in the deep layer of material cannot be obtained easily, which resulted in the reduction of extraction speed. The oil yield did not increase obviously with increasing extraction time beyond 40 min. Therefore, 40 min was chosen as the central point for further experiments.
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3.3 Effects of liquid-solid ratio on oil yield

As can be seen in Fig. 2C, the increase in liquid-solid ratio from 5:1 to 15:1 (mL/g) resulted in a significant increase of oil yield from 20.37 to 26.30%. While further increase in liquid-solid ratio from 15:1 to 25:1 (mL/g) led to a negligible increase of oil yield by 1.07%. It may be that when there was a small liquid to solid ratio, the surface oil soon dissolved in the solvent and thus the solution would become too sticky to diffuse within the solid matrix. The increase in liquid-solid ratio increased the contact area between materials and extraction solvent, facilitating the elution of bound oil from the material. However, excessive increase in liquid-solid ratio would not change substantially mass transfer driving forces as it depends on the concentration difference between solvent and matrix. Consequently, the ratio of 15:1 mL/g was selected as the center of the RSM experiment.

3.4 Optimization of SBE

3.4.1 Model fitting

To obtain the highest oil yield (Y), RSM with BBD was performed to explore the optimal extraction conditions including extraction temperature ($X_1$), extraction time ($X_2$), and liquid-solid ratio ($X_3$). Detailed experimental parameters were shown in Table 1.

A second order regression model obtained in terms of coded values was given by the following:

$$Y = 27.14 + 0.45X_1 + 0.79X_2 + 2.03X_3 - 0.83X_1X_2 + 0.05X_1X_3 - 0.34X_2X_3 - 0.94X_1^2 - 0.30X_2^2 - 0.47X_3^2$$  \(4\)

The fitting of the regression model and actual results was judged by $F$-test and $p$-value. The model $F$-value (22.43) and $p$-value (0.0002) demonstrated the regression model was significant and fitted well with the actual situation. The $F$-value (3.44) and $p$-value (0.1321) of the lack of fit indicated that the lack of fit of the model was not significant. The results revealed that the abnormal fitting was not dominant, and the model adequately represented the experimental results. It is reported that the fitting of theoretical values to actual results was successful well when $R^2$ was more than 0.75. In this study, the experimental values obtained by quadratic regression analysis are good consistency with the second-order polynomial mathematical model ($R^2 = 0.9665$), indicating that 96.65% of results can be explained using this model. The adequate precision value of 15.04 was greater than 4, indicating an adequate signal in this study.

The significance of each coefficients was determined by the ANOVA result and $p$-value. It is revealed that linear terms $X_1$, $X_2$ and $X_3$, interaction term $X_1X_2$ and quadratic terms $X_1^2$, $X_2^2$ and $X_3^2$ were significant. The $p$-value were less than 0.05, indicating the corresponding coefficients were significant.
term $X_1^2$ had significant influence ($p<0.05$) on the oil yield of flaxseed oil.

3.4.2 Response surface analysis

To evaluate the interaction between temperature and time, a three-dimensional response surface graph and a two-dimensional contour graph were generated and shown in Fig. 3. As observed in Fig. 3, the oil yield increased with the extension of extraction time at low levels of extraction temperature. However, at high temperature levels, the oil yield decreased with the prolongation of extraction time. Our studies suggested that extraction temperature had a dual influence on the flaxseed oil yield during the SBE process. More specifically, on the one hand, the extension of extraction time increased the rate of mass transfer and elevated the solubility of the solute, causing the improvement of extraction efficiency. On the other hand, the increase of temperatures reduced the density of extraction solvent and thus a reduction of dissolving capacity. Additionally, although a fixed liquid-solid ratio was maintained when analyzing the interaction of temperature and time, too long extraction time would result in a loss of liquid $n$-butane and thus a reduction in actual liquid-solid ratio, leading to a low oil yield.

3.4.3 Optimization of extraction conditions

To obtain the highest flaxseed oil yield, the extraction conditions of SBE were optimized using Design Expert software. The optimal parameter combination was as follow: extraction temperature of 53.93°C, extraction time of 56.82 min, liquid-solid ratio of 19.98:1 mL/g, and the predicted maximum oil yield was 28.87%. For convenience operation, the theoretical parameters were slightly modified to carry out verification experiment. The modified conditions were extraction temperature of 54°C, extraction time of 57 min, and the liquid-solid ratio of 20:1. Under this condition, the practical oil yield was 28.75 ± 0.19% performed by triplicate experiments. The predicted value was much closed to the actual value. It indicated that the regression model was adequate to reflect the expected optimization.

### 3.5 Yield and physicochemical properties

Table 2 demonstrated the extraction efficiency of flaxseed oil prepared by three methods. The highest amount of flaxseed oil (28.75%) was obtained by SBE, following by $n$-hexane Soxhlet extraction (27.53%) and cold pressing (19.56%). The results indicated that SBE was more efficient than conventional methods. Similar phenomenon was also observed by Sun et al., who found that the lipid recovery using SBE was 81.2%, while $n$-hexane was 70.0%. This could be attributed to the high density, diffusivity and low viscosity of $n$-butane, which makes it have excellent penetrability and solubility under high pressure condi-

![Fig. 3](image)

Fig. 3  Response surface and contour plot for interaction between extraction temperature and time.

| Table 2 | Yield and physicochemical characteristics of flaxseed oil. |
|---------|----------------------------------------------------------|
|         | Subcritical $n$-butane | $n$-Hexane extraction | Cold pressing |
| Yield (%) | 28.75 ± 0.08 a | 27.53 ± 0.06 b | 19.56 ± 0.27 c |
| Color (red and yellow) | R 4.67 ± 0.06 a | R 3.73 ± 0.06 b | R 3.23 ± 0.06 c |
| Refractive index (20°C) | Y 70.00 ± 0.00 a | Y 70.00 ± 0.00 a | Y 70.00 ± 0.00 b |
| Density (g/mL 20°C) | 1.4789 ± 0.00 a | 1.4725 ± 0.00 b | 1.4825 ± 0.00 c |
| Acid value (mg KOH/g) | 0.9299 ± 0.01 a | 0.9280 ± 0.00 b | 0.9333 ± 0.01 b |
| Peroxide value (mmol/kg) | 1.15 ± 0.03 a | 1.17 ± 0.00 b | 1.86 ± 0.11 b |
| Iodine value (g I₂/100 g) | 168.69 ± 0.58 a | 170.86 ± 0.85 b | 173.11 ± 0.67 c |
| Saponification value (mg KOH/g) | 189.79 ± 0.33 a | 186.27 ± 1.58 b | 192.47 ± 0.37 c |

Note: different letters in the same line indicate statistically significant differences ($p < 0.05$).
The physicochemical properties of vegetable oils are mainly related with their chemical composition and are the important indicators of their quality. The physical and chemical properties of flaxseed oils obtained are shown in Table 2. Color is one of the most obvious product characteristics evaluated by any consumer of edible oil \(^{31}\). Table 2 shows that the SBE oil was redder than the oils extracted with the other two methods. It may be due to a higher content of colored substances such as carotenoids in the SBE oil. In this study, the refractive index and density of flaxseed oil was within the range from 1.4725 to 1.4825 and 0.9280 to 0.9333, respectively, which were consistent with the earlier study of Piva et al. \(^{32}\).

Acid value and peroxide value are the two most common chemical tests for quality determination of vegetable oil. The acid value reflects the amount of free fatty acids, while the peroxide value determines the amount of primary oxidation product in the oil. Compared with the oils obtained by \(n\)-hexane extraction and cold pressing, the oil extracted with subcritical \(n\)-butane had a lower number of acid value (1.68 mg KOH/g) and peroxide value (1.15 mmol/kg). The results suggested that the application of SBE was favored for improving the quality of crude oil. This was due to the mild temperature and shorter extraction time used in the SBE, which reduced the decomposition and oxidation of oils, and subsequently caused a reduction in the content of free fatty acids, peroxides and hydroperoxides \(^{30}\).

The saponification value of SBE oil was 189.79 mg KOH/g which is slightly larger than that of \(n\)-hexane-extracted oil (186.27 mg KOH/g), but lower than cold pressing oil (192.47 mg KOH/g). Saponification value represents the average molecular weight and chain length of fatty acids in the oils. The larger value of saponification implies a high content of low molecular weight triacylglycerols, which are very useful for the production of liquid soap and shampoos. Iodine value is related to the quantity and degree of unsaturated fatty acids and can be used as an indicator of the susceptibility to oxidative degradation \(^{10}\). The iodine value of SBE oil (168.69 g I\(_2/100\) g) was significantly lower than the oils prepared by \(n\)-hexane extraction (170.86 g I\(_2/100\) g) and cold pressing (173.11 g I\(_2/100\) g). The result showed that the SBE oil might be good at stability and shelf-life due to with more saturated bonds.

### 3.6 Fatty acid composition

Fatty acid composition is a major determinant on the nutrition, stability and use of vegetable oils. The ideal edible oils generally contain a high percentage of unsaturated fatty acids. Table 3 shows that the oils obtained from different methods were of similar fatty acid profile. Seven major fatty acid had been identified in the oil samples, including palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), and linolenic acid (C18:3). The most prevalent fatty acids were linolenic acid (43.13-43.66\%), followed by oleic acid (32.15-32.59\%) and linoleic acid (12.93-13.08\%), which accounted for around 88% of total fatty acids. It was noteworthy that the fatty acid composition in our study was obviously different from those found in previous studies. Pradhan et al. reported flaxseed oils contained 50-55 \% of linolenic acid, in which the oils were obtained by supercritical \(\mathrm{CO}_2\) extraction, screw press, and solvent extraction. Piva et al. extracted flaxseed oil with subcritical propane and reported the linolenic acid content in the obtained oil was 54.76-56.00\% \(^{10}\). The

### Table 3 Fatty acid composition of flaxseed oil.

| Fatty acids (%) | Subcritical \(n\)-butane | \(n\)-Hexane extraction | Cold pressing |
|----------------|--------------------------|------------------------|---------------|
| C 16:0         | 6.05 ± 0.04 \(a\)        | 5.96 ± 0.01 \(b\)      | 5.60 ± 0.01 \(a\) |
| C 16:1         | 0.11 ± 0.04 \(a\)        | 0.09 ± 0.01 \(a\)      | 0.10 ± 0.04 \(a\) |
| C 18:0         | 5.00 ± 0.03 \(b\)        | 4.93 ± 0.00 \(c\)      | 5.30 ± 0.00 \(a\) |
| C 18:1         | 32.15 ± 0.04 \(b\)       | 32.22 ± 0.03 \(b\)     | 32.59 ± 0.05 \(a\) |
| C 18:2         | 13.02 ± 0.02 \(b\)       | 12.93 ± 0.01 \(c\)     | 13.08 ± 0.01 \(a\) |
| C 20:0         | 0.19 ± 0.03 \(a\)        | 0.20 ± 0.00 \(a\)      | 0.20 ± 0.01 \(a\) |
| C 18:3         | 43.48 ± 0.01 \(a\)       | 43.66 ± 0.02 \(a\)     | 43.13 ± 0.08 \(b\) |
| \(\Sigma\) MUFU | 32.26 ± 0.00 \(b\)       | 32.31 ± 0.02 \(b\)     | 32.69 ± 0.10 \(a\) |
| \(\Sigma\) PUFA | 56.50 ± 0.03 \(a\)       | 56.59 ± 0.03 \(a\)     | 56.21 ± 0.14 \(b\) |
| \(\Sigma\) SFA  | 11.24 ± 0.03 \(a\)       | 11.09 ± 0.00 \(b\)     | 11.10 ± 0.02 \(b\) |
| UFA/SFA        | 7.90 ± 0.02 \(b\)        | 8.02 ± 0.05 \(a\)      | 8.01 ± 0.01 \(a\) |

Note: MUFU: monounsaturated fatty acid, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, UFA: total unsaturated fatty acids.

Different letters in the same line indicate statistically significant differences \((p < 0.05)\).
reason for this phenomenon might be different varieties of flaxseeds. The low content of α-linolenic acid in flaxseed oil may be desirable in terms of shelf life and resistance against oxidation.

Statistical analysis indicated that there was a significant difference \((p<0.05)\) in the content of certain fatty acids for three oil samples. However, extraction methods had little influence on the fatty acid composition. Similar phenomenon was also observed by Gu et al., who investigated the fatty acid composition of *Xanthoceras sorbifolia* Bunge seed oils extracted with different methods including subcritical \(n\)-butane\(^{[30]}\). It can be inferred that fatty acids were non-selectively extracted during the process of SBE. Therefore, Subcritical \(n\)-butane can be used as an alternative to \(n\)-hexane for the industrial production of vegetable oil.

### 3.7 Content of carotenoid and phytosterols

Phytosterols are the principal constituent of unsaponifiable matter, which can reduce the level of plasma cholesterol and the incidence of heart disease and cancer\(^{[31]}\). Table 4 shows that four types of phytosterols were identified from the flaxseed oils extracted with three different methods. The dominating phytosterols were sitosterol comprising about 50\% of total sterol, followed by campessterol and sitostanol \((\sim 10\%)\), and stigmasterol \((\sim 10\%)\). Our result was in line with the phytosterols composition of flaxseed oil obtained with subcritical propane\(^{[17]}\). Statistical analysis revealed that extraction method had a significant influence \((p<0.05)\) on content of individual and total sterol. More sitosterol and campessterol was contained in SBE oil, resulting in a higher content of total phytosterols in SBE oil \((2.93 \pm 0.02 \text{ mg/g})\) comparing to hexane-extracted oil \((2.51 \pm 0.06 \text{ mg/g})\) and cold pressing oil \((2.64 \pm 0.01 \text{ mg/g})\). Similar results were also found in the extraction of wheat germ oil with subcritical butane\(^{[10]}\). This phenomenon could be interpreted that subcritical \(n\)-butane had a stronger selective extraction power than that of \(n\)-hexane for phytosterols, since the subcritical butane has a higher diffusion coefficient and lower viscosity in oil\(^{[10]}\).

| Phytosterols, carotenoids content and induction time of flaxseed oil. |
|---------------------------------|----------------|----------------|----------------|
| **Subcritical \(n\)-butane**    | **\(n\)-Hexane extraction** | **Cold pressing** |
| Campesterol (mg/g)             | 0.82 ± 0.01 \(^a\) | 0.75 ± 0.04 \(^b\) | 0.80 ± 0.02 \(^ab\) |
| Stigmasterol (mg/g)            | 0.28 ± 0.01 \(^a\) | 0.26 ± 0.01 \(^b\) | 0.26 ± 0.01 \(^b\) |
| Sitosterol (mg/g)              | 1.53 ± 0.01 \(^a\) | 1.26 ± 0.04 \(^c\) | 1.35 ± 0.03 \(^b\) |
| Sitostanol (mg/g)              | 0.3 ± 0.01 \(^a\) | 0.29 ± 0.01 \(^a\) | 0.25 ± 0.05 \(^b\) |
| \(\Sigma\) sterols (mg/g)      | 2.93 ± 0.02 \(^a\) | 2.51 ± 0.06 \(^c\) | 2.64 ± 0.01 \(^b\) |
| Carotenoids (mg/kg)            | 46.56 ± 0.59 \(^a\) | 34.98 ± 0.20 \(^b\) | 26.20 ± 1.14 \(^c\) |
| Induction time (h)             | 9.27 ± 0.12 \(^a\) | 6.45 ± 0.09 \(^b\) | 0.49 ± 0.06 \(^c\) |

Note: different letters in the same line indicate statistically significant differences \((p < 0.05)\). Carotenoids are a class of lipophilic pigment and are known as antioxidants. As shown in Table 4, the extraction methods had a significant influence \((p<0.05)\) on the carotenoids content of flaxseed oils. The highest carotenoids content \((46.56 \text{ mg/kg})\) was obtained in SBE oil, followed by hexane-extracted oil \((34.98 \text{ mg/kg})\) and cold pressing oil \((26.20 \text{ mg/kg})\). The higher content of carotenoids in SBE oil partly explained the highly intense red color of SBE oil. More carotenoids contained in SBE oil can be attributed to that carotenoids are naturally lipophilic and they have higher affinity to subcritical \(n\)-butane\(^{[30]}\). Moreover, carotenoids are sensitive to light, heat and oxygen, the mild extraction condition of SBE reduced their loss during the extraction process. Carotenoids can quench reactive oxygen and are beneficial to human health in preventing cardiovascular diseases\(^{[32]}\). Hence, carotenoids-enriched SBE oil could be developed as healthy oil to meet the demand of certain populations.

### 3.8 Oxidation stability

Table 4 shows that the induction time of subcritical extracted, hexane-extracted, and cold-pressed oils was 9.27, 6.45, and 0.49 h, respectively. There was a significant difference \((p<0.05)\) among the induction time of oils extracted by three methods. In general, the longer the induction time is, the higher the oxidation stability will be. A longer induction time of SBE oil implied the oil was more stable to oxidation than those extracted with hexane and cold pressing. This phenomenon could be explained by the lower degree of oxidation and the presence of more antioxidants such as carotenoids and phytosterols in the oil obtained with subcritical \(n\)-butane. Similar phenomenon was observed in the extraction of rice bran oil by Liu et al., who found the induction time of subcritical \(n\)-butane extracted oil was 5.02 h, while \(n\)-hexane extracted oil was 0.52 h\(^{[30]}\).

### 4 Conclusions

In this study, subcritical \(n\)-butane was firstly introduced...
to the preparation of flaxseed oil. The factorial design showed that the temperature, time and liquid-solid ratio were significant for the yield of flaxseed oil. The oil obtained applying different methods revealed no significant differences in relation to the fatty acid composition. Moreover, the oil extracted with n-butane was lower in acid value and peroxide value, higher phytosterol and carotenoids content, which resulted in a higher oxidative stability. The results suggested that SBE did not negatively on oil composition, and the increased of yield and bioactive components may make the extraction of flaxseed oil with subcritical n-butane more economically attractive.

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