Extraction and separation of unsaturated fatty acids from sunflower oil

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Abstract. In this paper, natural sunflower seed oil was used as the raw material to extract mixed fatty acids with potassium hydroxide-methyl alcohol solution, and then benzene and methanol were added for methylation reaction, and finally the saturated and unsaturated fatty acid methyl ester were separated by urea encapsulation method. The optimal reaction temperature of the mixed fatty acid extraction process, the optimal dosage ratio of urea to mixed fatty acid methyl ester and the optimal freezing temperature in the urea encapsulation process were explored. Fourier transform infrared spectrometer (FT-IR) and GC-MS were used to analyze. The results show that after methyl esterification of the extracted fatty acids, the separation efficiency of the saturated fatty acid methyl esters by the urea encapsulation method was extremely high, and the content of the unsaturated fatty acid methyl ester in the filtrate was up to 99.67%, which made a solid foundation for further demethylation to prepare unsaturated fatty acids.

1. Introduction

With the improvement of living standards, people pay more and more attention to health. Unsaturated fatty acids are indispensable to the human body, which play a positive role in regulating blood lipids, cleaning blood clots, immune regulation and so on[1-6]. The main components in vegetable oil are various fatty acids, which are divided into saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. The essential unsaturated fatty acids needed by the human body are mainly derived from vegetable oils. Therefore, the extraction and analysis of the components in vegetable oils, especially the extraction of unsaturated fatty acids, have received extensive attention and importance[7,8]. As for sunflower seed oil, its output accounts for a large proportion of the total vegetable oil. Therefore, the separation and extraction of fatty acids from sunflower seed oil has gradually become a focus in the fatty acid production industry. At present, the common methods for separation and purification of unsaturated fatty acids include low-temperature crystallization method, adsorption separation method, supercritical fluid extraction method, molecular distillation method, urea encapsulation method, etc[9-12]. However, the traditional urea encapsulation method is directly applied to the fatty acid extracted from vegetable oil. Due to the complex composition of fatty acids, it brings to defects such as low urea encapsulation rate and poor separation rate of saturated and unsaturated fatty acids[13-15].

This paper uses natural sunflower seed oil as raw material. Firstly, the mixed fatty acids extracted by the optimized acidification method was subjected to the methyl esterification reaction. Then, saturated
and unsaturated fatty acid methyl esters were extracted by urea encapsulation method to achieve the purpose of high encapsulation rate and high separation rate. Specifically, this paper explored the optimal reaction conditions: the optimal extraction temperature of mixed fatty acids, the optimal mass ratio of urea to mixed fatty acid methyl esters, and the optimal freezing time for urea encapsulation. Fourier transform infrared spectrometer (FT-IR) and GC-MS were used to analyze the mixed fatty acid methyl ester, fatty acid methyl ester in the urea inclusion compound, and fatty acid methyl ester in the filtrate, respectively. This paper laid solid foundation for further demethylation to prepare unsaturated fatty acids necessary for the human body.

2. Experimental

2.1. Materials and equipment
Sunflower seed oil, absolute ethanol, petroleum ether, methanol, urea, benzene, sodium bisulfate (NaHSO₄), anhydrous sodium sulfate (Na₂SO₄); Fourier Transform Infrared Spectrometer, Nicolet-6700, Shanghai Sile Instrument Company; GC-MS QP2010, Japan Shimadzu Company.

2.2. Sample characterization and test conditions
FT-IR: Nicolet-6700 FT-IR equipment is used for testing, potassium bromide is used as the carrier, and the test scanning range is 400-4000cm⁻¹;
GC-MS: gasification chamber temperature: 300°C; detector temperature: 320°C; Column temperature with gradient heating: keep the initial temperature at 100°C for 2min, and then increase the temperature to 245°C at a rate of 10°C/min lasting for 5min. Ion source temperature: 250°C; transmission line temperature: 250°C; sample volume: 0.5ml.

2.3. Experimental processes

2.3.1. Extraction of mixed fatty acids. A 500ml round-bottomed flask was added 20g of sunflower oil and 0.4mol/L KOH-CH₃OH solution. Stirring and refluxing for about 1h at 65°C in a thermostatic magnetic stirrer until the solution was transparent and clear. The solution was transferred to a beaker for being cooled to room temperature, NaHSO₄ solution was added dropwise to adjust the pH to about 3 and then the upper oil phase was separated with a separatory funnel.

2.3.2. Methyl esterification of mixed fatty acids. The above upper oil phase was transferred into a three-necked flask, 4g of NaHSO₄ dissolved with an appropriate amount of water was added into a three-necked flask, and then 4ml benzene and 40ml methanol were added. The three-neck flask equipped with a thermometer, a water separator and a ground stopper was heated and stirred at 85°C for about 4h until the height of the liquid level in the water separator kept constant. The evaporation tube of the water separator was wrapped with cotton. The product was taken out for being cooled to room temperature. It was transferred to a separatory funnel and washed with deionized water for 4 times. Petroleum ether was used as the extractant to extract the upper oil phase and then dry it with Na₂SO₄ powder. Furthermore, it was filtered and evaporated with a rotary evaporator (40°C) to obtain the mixed fatty acid methyl esters.

2.3.3. Separation of mixed fatty acid methyl esters by urea encapsulation method. A three-necked flask was added 8g of urea and 160ml of ethanol (preparing 95% ethanol with absolute ethanol). 2g of mixed fatty acid methyl esters was then added. The flask was placed on a magnetic stirrer for stirring, heating and refluxing at 65°C for about 40 min until the solution was light yellow and clear. It was transferred to a beaker for being cooled to room temperature. Then, it was placed in a refrigerator and frozened at -20°C. After 10-16 hours, it was taken out and quickly filtered to obtain filter residue (urea crystals) and filtrate, respectively.
2.3.4. Extraction of fatty acid methyl esters in the filter residue. The urea crystals were soaked with a proper amount of petroleum ether and then filtered. This process was repeated for 3 times. Furthermore, it was soaked in petroleum ether for 10 h in a sealed state. Both the above filtrate and the soaked solution were merged into the filtrate of step of 1.3.3; The treated urea encapsulation compound was dissolved with deionized water and extracted with petroleum ether. The upper oil substance was dried with Na₂SO₄ powder. Then it was filtered and finally evaporated with a rotary evaporator (40°C) to obtain the mixed fatty acid methyl esters in the urea encapsulation compound.

2.3.5. Extraction of fatty acid methyl esters in the filtrate. Transfer the filtrate of step of 1.3.3 to a separatory funnel, wash 2 to 3 times, extract with petroleum ether as an extractant, separate the upper oil substance from the separatory funnel, and repeat the process of 2 to 3 times; The obtained oil was dried with anhydrous Na₂SO₄ powder. Then it was filtered and finally evaporated with a rotary evaporator (40°C) to obtain the mixed fatty acid methyl esters in the filtrate, which was sealed for storage.

3. Results and discussion

3.1. Exploration of the optimal reactive conditions
Figure 1(a) is to explore the optimal reaction temperature for the extraction of mixed fatty acids. It can be seen that the extraction rate rises significantly as the temperature rises before 65°C. After reaching at 65°C, the extraction rate decreases slightly with the increase of temperature. Therefore, the optimal reaction temperature for the extraction of mixed fatty acids is 65°C, and the optimal extraction rate is up to 95%. Figure 1(b) demonstrates the effect of different mass ratios of urea and mixed fatty acid methyl esters on the encapsulation rate during urea crystallization. It should be aware that the overall encapsulation rate is above 75%. Compared with previous researchers who used urea encapsulation method to directly separate mixed fatty acids, the encapsulation rate has been greatly improved due to the introduction of methyl esterification process. When the mass ratio of urea to mixed fatty acid methyl ester is 4:1, the encapsulation rate is largest and is up to 83%. With continuous increase of the amount of urea, the encapsulation capacity does not change significantly. Figure 1(c) shows the impact of freezing time on the encapsulation rate. We can conclude that the encapsulation rate reaches the maximum amount at 12 h. Continuing to extend the freezing time, the content of fatty acid methyl esters encapsulated by urea keeps unchanged.

In general, it can be concluded that the optimal temperature for extraction of mixed fatty acids is 65°C, the optimal mass ratio of urea to mixed fatty acid methyl ester is 4:1, and the optimal freezing time for urea encapsulation is at 12 h.

3.2. Mixed fatty acid methyl esters
Figure 2 shows the FT-IR spectrum and GC-MS results of the mixed fatty acid methyl esters obtained after methyl esterification of the mixed fatty acids in sunflower seed oil. It can be seen from Figure 2(a)
that there are obvious C=C double bonds absorption peaks at 1610 cm\(^{-1}\) and 3010 cm\(^{-1}\), and there is a very distinct ester group peak at 1740 cm\(^{-1}\), indicating that the mixed fatty acids extracted from natural sunflower seed oil has been successfully converted into mixed fatty acid methyl esters. In addition, it can be seen from Figure 2(b) that the mixed fatty acid methyl esters contain saturated fatty acid methyl esters and unsaturated fatty acid methyl esters. There are mainly six different kind of products, which are labeled 1-6. The relative content of each product can be seen from the table of Figure 2(b).

![Figure 2 FT-IR spectrum and GC-MS analyzed results of mixed fatty acid methyl esters.](image)

**3.3. Mixed fatty acid methyl esters in the filter residue**

Figure 3(a) and Figure 4(a) are the infrared spectra of the mixed fatty acid methyl esters in the filter residue and in the filtrate, respectively. By comparing the peaks of two graphs at the wavenumber of 3010 cm\(^{-1}\), it can be seen that after the urea encapsulation process, the content of unsaturated fatty acid methyl esters in the filter residue is significantly reduced while the content of unsaturated fatty acid methyl esters in the filtrate increased significantly. It can be concluded that the urea encapsulation method was used in the urea crystallization process to encapsulate saturated fatty acid methyl esters as much as possible, while left a large amount of unsaturated fatty acid methyl esters in the filtrate. In this way, a good separation effect can be achieved. However, it can also be seen from the FT-IR spectra that not only saturated fatty acid methyl esters were completely encapsulated, but also a small amount of unsaturated fatty acid methyl esters were contained\(^{16-18}\).

![Figure 3 FT-IR spectrum and GC-MS results of mixed fatty acid methyl esters in the filter residue.](image)

The GC-MS chromatography was used to further analyze the components in the filter residue, as shown in Figure 3(b). It can be aware that after urea encapsulation, the saturated fatty acid methyl esters in the mixed fatty acid methyl esters increased from 14.44% to 26.04%. The methyl oleate
increased from 31.63% to 47.57%; methyl linoleate was reduced from 22.35% to 10.15%; methyl linolenate was reduced from 25.06% to 10.74%. Therefore, in the encapsulation process, the encapsulation ability of saturated fatty acid methyl ester and methyl oleate is very strong, but the encapsulation ability of polyunsaturated fatty acid methyl ester is very weak.

3.4. Mixed fatty acid methyl esters in the filtrate

Figure 4(b) is a gas chromatogram of mixed fatty acid methyl esters in the filtrate. Compared with Figure 2(b), it can be seen that peaks at 1, 3 and 4 are obviously decreased, peaks at 5 and 6 are obviously increased and peak at 2 has little change. It shows that in the crystallization process of urea, the encapsulation ability of saturated fatty acid methyl esters and monounsaturated fatty acid methyl esters is very strong and the inclusion ability of polyunsaturated fatty acid methyl esters such as methyl linoleate and methyl linolenate is very weak. Therefore, the relative content of methyl oleate in the urea encapsulation was significantly increased, while the relative content of methyl linoleate and methyl linolenate was significantly reduced. It can be judged that the urea encapsulation method has a significant separation effect on unsaturated fatty acid methyl esters.

Figure 4 FT-IR spectrum and GC-MS results of mixed fatty acid methyl esters in the filtrate.

Furthermore, it can be seen from the table that after encapsulation with urea, the saturated fatty acid methyl esters in the filtrate was reduced from 14.44% to 0.33%. Methyl oleate was reduced from 31.63% to 5.37%; methyl linoleate increased from 22.35% to 38.66%; methyl linolenate increased from 25.06% to 48.39%. Thus, it can be concluded that in the urea crystallization process, saturated fatty acid methyl esters are basically removed and the main components of the filtrate is the methyl esterification product of unsaturated fatty acids required by the human body.

3.5. Separation effect of urea encapsulation method

Figure 5 Comparison of fatty acid methyl ester content before and after encapsulation with urea.
Figure 5 is a comparison of fatty acid methyl ester content before and after encapsulation with urea. It can be seen that in the filter residue the content of saturated fatty acid methyl esters has increased significantly and the content of unsaturated fatty acid methyl esters has decreased significantly. In the filtrate, the content of saturated fatty acid methyl esters dropped sharply and approached zero, while the content of unsaturated fatty acid methyl esters rose sharply. The results show that after the mixed fatty acid methyl esters are encapsulated by the urea encapsulation method, the separation rate of unsaturated fatty acid methyl esters up to 99.67% can be achieved.

4. Conclusions
In this paper, KOH-CH₃OH solution was used to extract the mixed fatty acids from natural sunflower seed oil, then the mixed fatty acids were methylated and the unsaturated fatty acid methyl esters were successfully separated by urea encapsulation method. The optimal reaction temperature for the extraction of mixed fatty acids is 65°C, the optimal ratio of urea to mixed fatty acid methyl esters during the urea encapsulation process is 4:1 and the optimal freezing time for urea encapsulation is 12h. The results show that saturated fatty acid methyl ester and methyl oleate are mainly contained in the filter residue; unsaturated fatty acid methyl esters are mainly contained in the filtrate and the content is as high as 99.67%. The efficient separation of unsaturated fatty acid methyl esters provides technical basis and theoretical guidance for further demethylation to prepare high-purity unsaturated fatty acids needed by human body.

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References
[1] Li, F., Y. G. Zhang, Li. M, et al. Journal of Bingtuan Education Institute, 2002, 12 (3): 35-38.
[2] M. Y. Cai, B. Li, X. H. Yuan. Science and Technology Knowledge, 2003, 11 (2): 37-39.
[3] J. K. Liu, X. H. Li, H. Yuan, H. Wu, Z. H. Xiao, C. Z. Li. Food Engineering, 2015, (02): 4-5+22.
[4] X. G. Jiang. Liaoning Normal University, 2008.
[5] Y. Q. Song. Food Industry Science and Technology, 1990 (6): 53-54+49.
[6] H. Li. Shanxi University, 2014.
[7] B. Li. Shandong Agricultural University, 2014.
[8] L. H. Sheng, Z. Huang, J. Wang. Chemical Analysis and Metrology, 2010 (2): 35-38.
[9] Y. Wu. Hunan Normal University, 2014.
[10] B. B. Huang. Huaqiao University, 2015.
[11] Y. L. Sun, W. Lu, X. M. Xiao, et al. Progress in Chemical Industry, 2014, 33 (12): 3319-3343.
[12] X. H. Hu, C. J. Pan, C. Wang. Western Cereals and Oils Technology, 2001, 26 (2): 16-18.
[13] G. M. Zhang, M. Y. He, S. K. Jiang. Tropical Agriculture Science and Technology, 2008, 31 (4): 48-53.
[14] M. Y. Wu. Tianjin University, 2007.
[15] W. Chao, Y. D. Lu, J. F. Zhao. Grain and Oil Processing, 2010 (12): 11-12.
[16] M. Li, L. F. Zhang, J. X. Li, H. O. Wang, H. Li. Journal of the Chinese Cereals and Oils Association, 2005, (05): 109-111.
[17] G. H. Lu, W. Liu. Grain and Oil, 2017, (06): 45-49.
[18] M. Ling. Northwest A&F University, 2011.