Investigation on Walnut Kernel Oil Extraction Using Different Methods

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Extraction of oil from walnut kernel as raw material was explored using the method of pressing. Soxhlet and ultrasonic-assisted extractions of walnut oil, its evaluation index, the extraction process optimization by single factor experiment, the physical and chemical properties of oil, fatty acid composition, element composition, and the main functional group analysis were studied. The results showed that walnut kernel could be extracted effectively with petroleum ether. The optimal extraction conditions of walnut oil by ultrasonic assisted extraction were as follows: solid/liquid ratio of 1:11 (g/mL), ultrasonic power 180 W, extraction time 60 min, and the extraction efficiency of walnut oil was 59.13%. The optimal extraction conditions of walnut oil by Soxhlet were solid to liquid ratio of 1:7, temperature of 76 °C, time of 4 h, and the extraction efficiency of walnut oil was 58.3%. The extraction efficiency of pressing was 43.2%. Walnut oil mainly contains phosphorus, zinc, magnesium, potassium, calcium, and other elements. The fatty acids of walnut oil mainly consist of palmitic acid, palmitoleic acid, *cis*-linoleic acid, *cis,cis,cis*-9,12,15-octadecanotrienoic acid and other fatty acids. The *cis* linoleic acid accounted for the highest proportion in GC content, and the content of both was more than 90%.

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INTRODUCTION

Due to the non-renewable nature of fossil fuels, it will face the problem of energy exhaustion in the future. Therefore, nowadays, the world is paying more attention to renewable clean energy, and countries are beginning to develop alternate renewable and sustainable energy sources that can replace fossil fuels. Biodiesel has the advantages of extensive raw material resources, renewable nature, high calorific value, biodegradability, harmless and non-toxic nature, safe transportation process, and wide use of glycerin as byproduct (Li *et al*. 2004).

At present, one of the factors restricting the development of biodiesel is the high cost of oil crops, which accounts for about 75% of the total cost of biodiesel production (Zhu and Zhang 2004; Khan *et al*. 2016). Walnut, a forest species abundant in the northeast of Inner Mongolia, was studied and has been developed as an energy resource. Different extraction technologies for walnut seeds oil, the oil physical and chemical properties, and fatty acid composition have been explored for the development and utilization of walnut

oil biodiesel. In order to alleviate the energy crisis and ensure the sustainable use of renewable energy, it is of great significance to increase the use of domestic oil tree species and reduce the dependence on foreign energy (Jiang *et al*. 2006; Xu *et al*. 2006; Zhu *et al*. 2006; Chen and Lin 2010).

In this study, walnut oil was extracted from walnut kernel by a pressing method, Soxhlet extraction, and ultrasonic-assisted extractions, respectively. The physical and chemical properties (moisture and volatile, relative density, saponification value, acid value, molecular weight, *etc*.) of extracted walnut oil by these three methods were determined. The effects of different extraction methods and conditions on the oil yield of walnut oil were compared.

EXPERIMENTAL

Materials

The walnut kernel was purchased from Gongyi, Henan, China. The seeds were peeled, cleaned, dried, and broken into several pieces for storage.

Reagents and Instruments

Petroleum ether (boiling range 60 to 90 °C), zinc chloride, potassium hydroxide, potassium iodide, and methanol used were all analytically pure and purchased from Tianjin Xin Bote Chemical Reagent Technology Co., Ltd.

A digital display constant temperature water bath pot was obtained from Changzhou Jiangnan Experimental Instrument Factory (HH-1, Jiangsu, China). An ultrasonic disperser from Shanghai Shengxi Ultrasonic Co., Ltd. (Shanghai Shengxi Ultrasonic Co., Ltd., China) and an oil press from Zhengzhou Qixin Machine Co. Ltd. (BOZY-01G, Henan, China) were used. A 7890B GC system and an inductively coupled plasma emission spectrometer from Agilent Technologies (Santa Clara, CA, USA) were used for analysis. Fourier transform infrared spectrometry was performed using a Bruker (Tensor, Billerica, MA, USA) and scanning electron microscopy was done using Thermo Fischer Scientific (Phenom ProX, Netherlands) instruments.

Methods

Pressing method

The walnut powder of certain quality was taken in a drying box and dried for 10 to 50 min, and then placed it into a household oil press to extract walnut oil (Güneşer and Yilmaz 2019; Ashirov *et al*. 2020). In this experiment, walnut oil was obtained using the cold pressing method. The oil press contained material used for solid-liquid separation. When the oil press runs, the processed oil enters the chamber from the hopper. The spiral press continuously pushes the embryo forward with pressure.

Soxhlet method

The walnut powder was taken in a single flask, and the flask was kept in a water bath pot. A Soxhlet extractor and a condenser were connected with the flask. The extraction was done at a certain reaction temperature (60 to 90 ℃) and solid/liquid ratio (1:3 to 1:11) using petroleum ether. After a certain extraction time (2 to 6 h), the extracted solution was filtered, and the extract was recovered by rotary evaporation (Wang *et al*. 2019).

Ultrasonic-assisted extraction

A desired amount of walnut powder was mixed with petroleum ether (60 to 90 °C) in a beaker according to a certain material to liquid ratio (1:5 to 1:13). The ultrasonic disperser was purchased from Shanghai Shengxi Ultrasonic Co., Ltd., with a size of 400 * 250 * 240 mm and a motor power of 0.60 kW, which is suitable for processing liquid materials such as oil and water.

In this experiment, the beaker was placed in the ultrasonic disperser, and the ultrasonic probe was immersed in a third of the mixed liquid, and the extraction temperature was set at 50 °C (Li *et al*. 2004; Luque-Garcia and Luque de Castro 2004; Zhang *et al.* 2009; Li *et al.* 2015). Walnut oil was extracted under a certain range of extraction time (20 to 100 min) and ultrasonic power (90 to 210 W). The extracted solution was filtered, and the solvent was recovered by rotary evaporation (Cravotto *et al.* 2008; Dong *et al.* 2010; Lou *et al.* 2010; Ning *et al.* 2020).

The walnut kernel oil was extracted by three methods, pressing, ultrasonic-assisted extraction, and Soxhlet extraction. The oil yields of the different methods were calculated according to Eq. 1 (Zhang 2010),

$$Y_{oil}(\%) = \frac{M_{oil}}{M_{ds}} \times 100, \quad (1)$$

where Y_{oil} is the oil yield (%), M_{oil} is the mass of the extracted oil (g), and M_{ds} is the mass of the dried walnut kernel seeds (g).

Analysis of physical and chemical properties

According to the national standards GB 5009.236 (2016), GB 5009.2 (2016), GB/T 5534 (2008), GB 5009.239 (2016), walnut oil moisture and volatiles, relative density, saponification value, acid value, molecular weight, *etc*. were determined. The composition, and contents of fatty acids, and elements were determined according to GB 5009.168 (2016) and GB 5009.268 (2016) national standards. The PHENOM scanning electron microscope was used to observe the microstructure of the residue before and after walnut oil extraction, and the acceleration voltage used was 5kV. Walnut oil obtained by different methods were analyzed by Fourier transform infrared spectroscopy (Tensor 27) with a wavelength of 4000 to 400 cm^{-1} and a resolution of 4 cm^{-1}.The obtained data were processed by Excel software (Microsoft, Redmond, WA, USA), and the images were processed using Origin software (Northampton, MA, USA).

RESULTS AND DISCUSSION

Pressing Method

Drying time was the most influential factor in the pressing method (Fig. 1). It can be seen from Fig. 1 that extraction firstly increased and then decreased with the increase of drying time. This may be because the walnut powder contains certain volatile substances (moisture, *etc*.) during 10 to 20 min. As the volatile substances volatilized out, the extraction efficiency rose. When the drying time continued to increase, walnut powder oil due to the influence of time and temperature led to partial decomposition, such that the extraction efficiency then decreased (Güneşer and Yilmaz 2019; Ashirov *et al*. 2020).

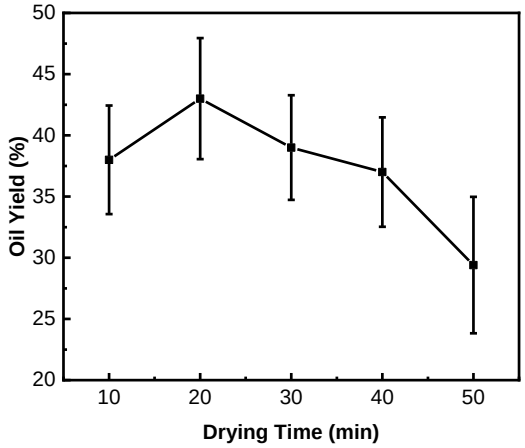

Fig. 1. The influence of drying time to walnut kernel oil yield (pressing)

Soxhlet Method

Extraction temperature

Under the condition of solid-liquid ratio of 1:7 and extraction time of 2 h, the influence of extraction temperature on the extraction efficiency of walnut oil was explored, and the results are shown in Fig. 2. The extraction efficiency first increased with the increase of temperature and reached the peak value at 76 ℃. Then it tended to be stable and slightly decreased. This may be because with the increase of temperature, the boiling of the extract was accelerated, and the refluxing increased at the same time. Thus, the extraction efficiency was improved, and the extraction efficiency increased. However, with the increase of temperature, most of the extract was present in the reaction in a gaseous state, and some walnut oil was decomposed, which ultimately led to the decrease of the extraction efficiency (Zhang 2010).

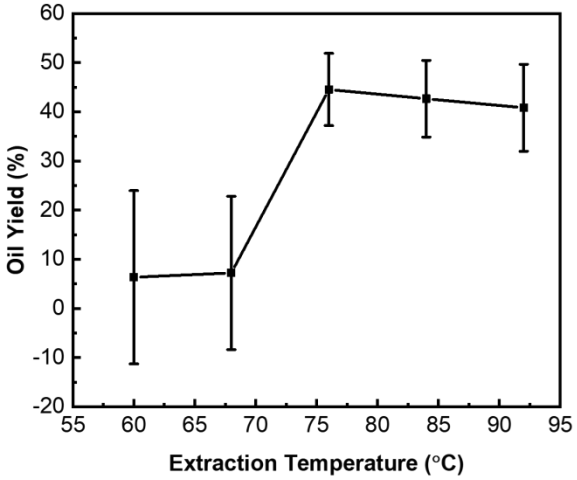

Fig. 2. The influence of temperature to walnut kernel oil yield (Soxhlet)

Extraction time

With solid/liquid ratio of 1:7 and extraction temperature of 76 ℃, the influence of extraction time on the extraction efficiency of walnut oil was explored, and the results are shown in Fig. 3. It is apparent that the extraction efficiency increased with time at the beginning and then decreased slowly after reaching the peak value after 4 h. This could

be attributed to the increase in refluxing with the increase of time, as most of the oil was dissolved, and the extraction efficiency increased (Zhang 2010). However, with the increase of time, the partial decomposition of oil under the influence of time and temperature made the extraction efficiency decrease.

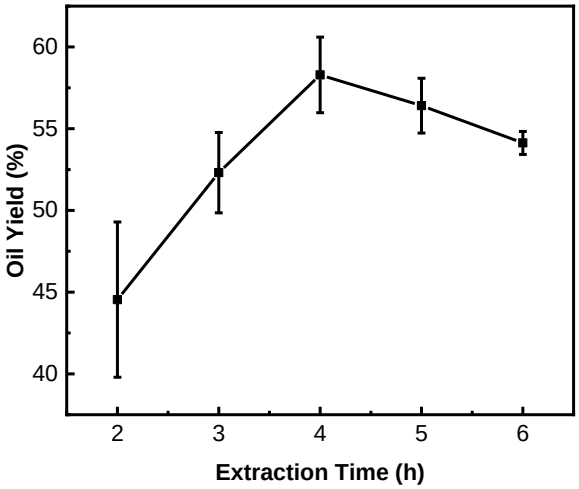

Fig. 3. The influence of time to walnut kernel oil yield (Soxhlet)

Solid-to-liquid ratio

With an extraction temperature of 76 ℃ and time of 4 h, the influence of solid/liquid ratio on the extraction efficiency of walnut oil was analyzed, as shown in Fig. 4. As can be seen, the extraction efficiency firstly increased with the increase of solid/liquid ratio, and then it tended to be stable and slightly decreased, reaching the peak at the solid/liquid ratio of 1:7. When the solid/liquid ratio was low, the dosage of the extraction agent was too small for refluxing to begin. The extraction agent was only soaking walnut powder at this stage, and the extraction efficiency stabilized near the peak value when the solid/liquid ratio increased to the reflux condition (Zhang 2010). However, the excessive extraction could also cause the volatilization of the extraction agent, which further reduced the extraction efficiency.

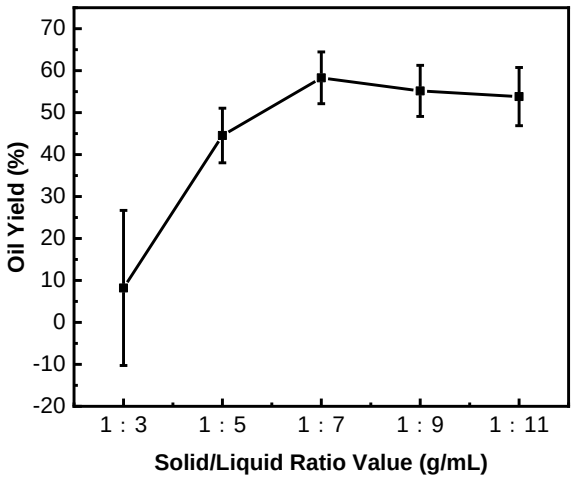

Fig. 4. The influence of solid/liquid ratio to walnut kernel oil yield (Soxhlet)

Ultrasonic-assisted Extraction

Extraction time

With solid/liquid ratio of 1:7 and ultrasonic power of 50 W, the effect of extraction time on the extraction efficiency of walnut oil was investigated, and the results are shown in Fig. 5. As can be seen, with the extension of extraction time, extraction initially increased and then began to decline slowly. The peak value was reached at 60 min. This was because the cell wall of walnut kernel began to break under the action of ultrasonic oscillation in less than 60 min. The cell wall damage increased with time and reached the maximum value when it was 60 min. However, as time continued to increase, insoluble impurities in cells gushed out and mixed in the extract, affecting the permeability of the extract to cells. The efficiency of the extraction agent was reduced and the extraction efficiency was reduced (Dong *et al*. 2010).

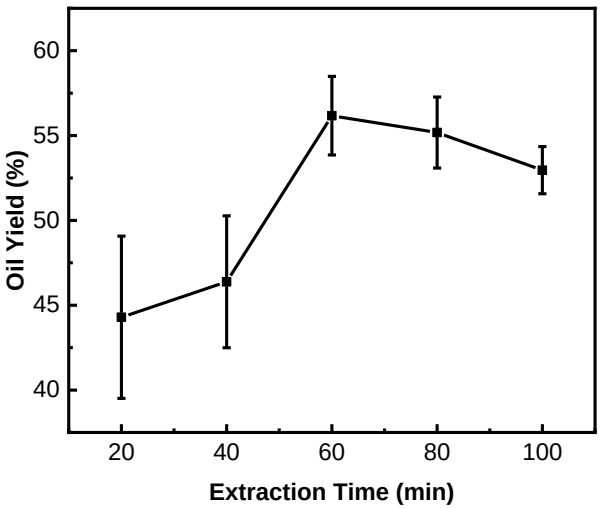

Fig. 5. The influence of time to walnut kernel oil yield (ultrasonic)

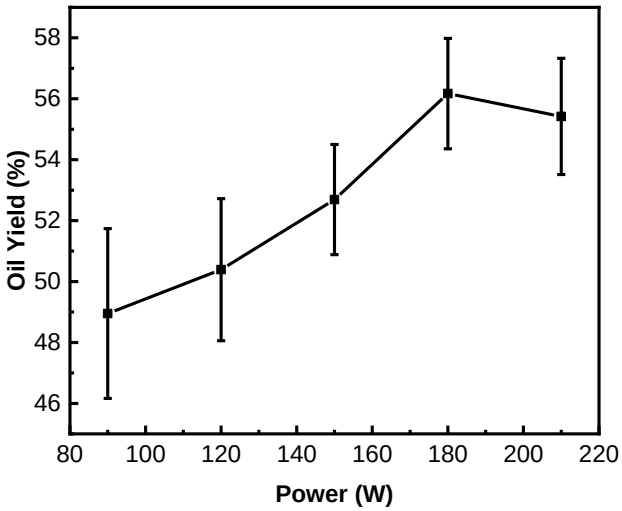

Fig. 6. The influence of ultrasonic power to walnut kernel oil yield (ultrasonic)

Ultrasonic power

The effect of ultrasonic power on the extraction rate of walnut oil was studied when the extraction time was 60 min and the solid/liquid ratio was 1:7. As can be seen from Fig. 6, the extraction efficiency gradually increased with the increase of ultrasonic power and peaked at 180 W and began to show a downward trend. This may be due to the increased mechanical effect caused by the increase in ultrasonic power, which accelerated the efficiency of the extraction agent and also sped up the mass transfer efficiency. But when the power was too large to accelerate the molecular thermal movement, the walnut oil underwent decomposition, and the extraction agent was volatilized (Wang *et al*. 2019).

Solid-to-liquid ratio

With extraction time of 60 min and ultrasonic power of 180 W, the influence of ultrasonic power on the extraction efficiency of walnut oil was examined. As shown by Fig. 7, the extraction efficiency gradually increased with the increase of solid/liquid ratio, and it reached the highest value at 1:11. Then it began to show a downward trend. This result was consistent with the mass transfer principle: the increase of extraction agent increased the concentration gradient between the solid phase and the liquid phase, and the driving force in the mass transfer process increased, so a large amount of oil dissolved

during extraction. When the extraction agent was excessive, most of the oil in walnut kernel was extracted. This effect would gradually weaken or even restrict the extraction process, resulting in the decline of extraction efficiency (Zhang *et al*. 2009; Lou *et al*. 2010).

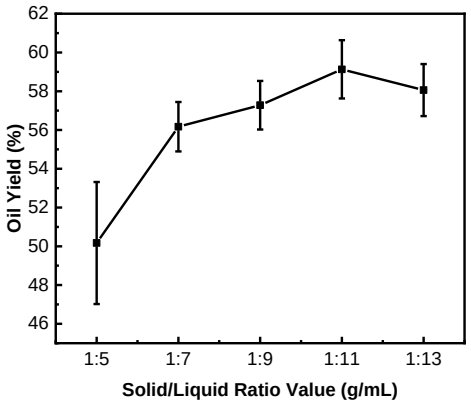

Fig. 7. The influence of solid/liquid ratio to walnut kernel oil yield (ultrasonic)

Comparison of the Three Methods of Walnut Oil Extraction

Table 1 shows the results of walnut oil extraction efficiency using various extraction methods. As can be seen from the table, the extraction efficiency was 43.2% after 10 min of pressing (Zheng *et al*. 2013). The optimum conditions for Soxhlet extraction were a solid/liquid ratio of 1:7, an extraction time of 4 h, an extraction temperature of 76 °C, and the extraction efficiency was 58.3%. The optimal conditions of ultrasonic assisted method involved a power of 180 W, solid/liquid ratio of 1:11, 60 min, and the extraction efficiency was 59.1%. Although the extraction time of pressing method was lower than that of Soxhlet extraction and ultrasonic-assisted extraction methods, the extraction efficiency was too low, the utilization efficiency of raw materials was low, and the energy consumption was large. Although the extraction efficiency of Soxhlet extraction was similar to that of ultrasonic-assisted method, the extraction time of Soxhlet method was much higher than that of ultrasonic assisted extraction. Therefore, the ultrasonic-assisted extraction method was more suitable for the extraction of walnut oil.

Table 1. The Optimal Parameters of Walnut Oil Extraction

Methods	Power (W)	Solid/Liquid Ratio ($g.mL^{-1}$)	Temperature (°C)	Time (min)	Efficiency (%)
Pressing	—	—	—	10	43.23
Soxhlet	—	1:7	76	240	58.29
Ultrasonic	180	1:11	—	60	59.13

Analysis and Characterization of Walnut Oil

The physical and chemical property of walnut oil and elemental analysis

As shown in Tables 2 and 3, the ultrasonic-assisted extraction and the pressing method revealed slightly higher acid value of walnut oil than that of Soxhlet extraction. It may be that the ultrasonic-assisted method and pressing will lead to increased hydrolysis of triglycerides in the walnut oil, thereby causing a rise of acid value, and Soxhlet extraction does not lead to the hydrolysis of triglycerides.

Table 2. Comparison of the Physical and Chemical Properties of Extracted Oil by Different Methods

Method	Acid Value ($mg.g^{-1}$)	Saponification Value ($mg.g^{-1}$)	Refractive Index	Relative Density ($g.mL^{-1}$)	Water and Volatile Matter	Surface Tension ($N.m^2$)
Pressing	1.48	193.01	1.46	0.92	0.14%	30.72
Soxhlet	1.30	176.36	1.46	0.92	0.32%	30.10
Ultrasonic	1.38	190.21	1.46	0.93	0.31%	32.24

Table 3. Elemental Composition and Contents of Walnut Oil

Element	Content ($mg\ kg^{-1}$)
Calcium (Ca)	11.3
Chromium (Cr)	1.3
Iron (Fe)	2.3
Potassium (K)	14.8
Magnesium (Mg)	15.9
Manganese (Mn)	0.4
Sodium (Na)	0.4
Phosphorus (P)	45.5
Selenium (Se)	0.1
Zinc (Zn)	32.7

The saponification values of Soxhlet and ultrasonic-assisted extraction of walnut oil were 176 $mg.g^{-1}$ and 190 $mg.g^{-1}$, respectively, indicating that the relative molecular weight of walnut oil was medium, but the walnut oil with low saponification value had better fluidity (Wei *et al*. 2009, 2011; Geng *et al*. 2018; Jedidi *et al.* 2020; Maestri *et al.* 2020). There was little difference in the relative densities of the three fatty acids, which indicated that the fatty acid composition of the three was almost the same. Walnut oil extracted by pressing and ultrasonic-assisted extraction had the same refractive index, indicating that the number of fatty acid double bonds was the same and possessed the same unsaturation level, while the lower refractive index of Soxhlet extraction revealed lower number of fatty acid double bonds, indicating lower unsaturation. According to GB5009.268-2016 national food safety standard, the multi-elements in food were determined. The highest content elements in walnut oil were phosphorus, zinc, magnesium, potassium, and calcium, with contents of 45.50, 32.70, 15.90, 14.80, and 11.30 mg/kg, respectively. The content of elements in walnut oil is extremely rich. Long-term consumption is conducive to physical and mental health.

GC-MS analysis

The influence of various extraction methods on the fatty acid compositions of walnut oil was analyzed, as shown in Fig. 8. The main fatty acid composition and GC contents of walnut oil extracted by different methods are shown in Table 4. It can be seen from Fig. 8 and Table 4 that the fatty acid composition of walnut oil obtained by the three extraction methods was the same, and the GC contents of main fatty acids such as palmitic acid, *cis*-oleic acid, and *cis*-linoleic acid were also similar, indicating that the fatty acid composition of the oil was not damaged by any of these extraction methods (Nde *et al*. 2015). Walnut oil mainly contains fatty acids such as palmitic acid, palmitoleic acid, *cis*-linoleic acid, *cis*,*cis*-9, and 12,15-octadecanotrienoic acid.

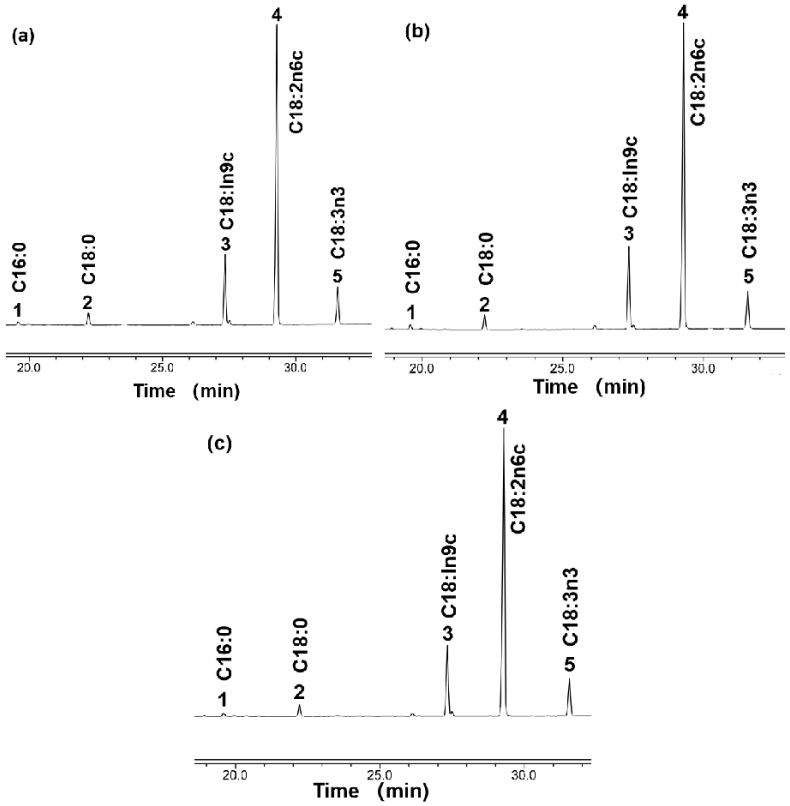

Fig. 8. Analysis of walnut oil fatty acids from different methods: (a) Soxhlet; (b) ultrasonic; and (c) pressing. 1. C16:0—Hexadecanoic acid (palmitic acid); 2. C18:0—Octadecarbonic acid (stearic acid); 3. C18:ln9c—*cis*-9-Octadecarbonic acid (*cis*-oleic acid); 4. C18:2n6c—*cis*-9,12-Octadecadienoic acid (*cis*-linoleic acid); and 5. C18:3n3—*cis*-9, *cis*-9,12,15-Octadecadienoic acid

Table 4. Main Fatty Acid Compositions and GC Contents of Walnut Oil

Number	Name	Fatty Acid	Relative Hazard Peak Area (%)		
			Pressing	Soxhlet	Ultrasonic
1	Palmitic Acid	C16:0	2.48	2.51	3.01
2	Stearic Acid	C18:0	0.71	0.74	0.86
3	*cis*-Oleic Acid	C18:ln9c	15.60	15.60	17.10
4	*cis*-Linoleic Acid	C18:2n6c	71.50	72.00	69.90
5	*cis*-9, *cis*-9,12,15, Octadecadienoic Acid	C18:3n3	9.31	9.04	8.61

The contents of *cis*-linoleic and cis-9,12,15-octadecanotrienoic acids in GC accounted for the highest proportion. The content of both was more than 90%, which indicated that walnut oil has high practical value.

FT-IR analysis of walnut oil

The FT-IR analysis revealed that the structural components of walnut oil extracted using three methods were consistent, as shown in Fig. 9. This was same as that of previous fatty acid analysis. The peaks due to the stretching vibrations of C-H bond in walnut oil appeared at 3008 cm^{-1}, and that of saturated hydrocarbon group appeared at 2930 cm^{-1} and 2857 cm^{-1}. The stretching vibration peak of triglyceride ester C-O-C was at 1160 cm^{-1} and the peak at 718 cm^{-1} denoted the stretching vibration peak of carbonyl group (Zheng *et al*. 2013). According to FT-IR results, the fatty acid structures of walnut oil obtained by various methods were similar.

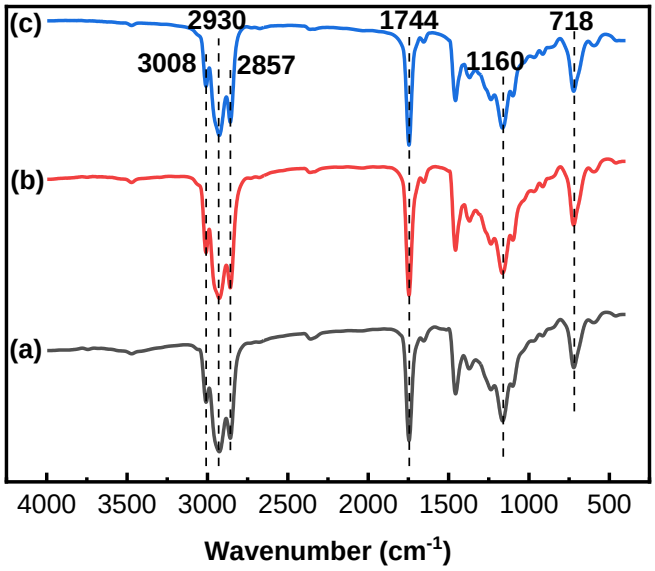

Fig. 9. FT-IR analysis of walnut oil from different methods pressing; (b) soxhlet; and (c) ultrasonic

SEM analysis of walnut oil before and after extraction

As shown in Fig.10, the walnut cell wall is dark (Fig. 10a), and the white luminous area is the floating oil (Guo *et al*. 2016; Komartin *et al*. 2021; Shi *et al*. 2021). However, the walnut powder can be seen in Fig. 10b. After Soxhlet extraction, most of the luminous area had disappeared obviously, indicating that the extractant broke the cell wall in large quantities and the oil was extracted. This phenomenon can also be seen in Fig. 10d. It can be seen from Fig. 10c that the degree of cell wall breakage was not as obvious as in Figs.10b and 10d, and only a few cell wall damages are observed, indicating that among the three methods, ultrasonic-assisted and Soxhlet extraction methods have high utilization efficiency of raw materials and are good extraction methods.

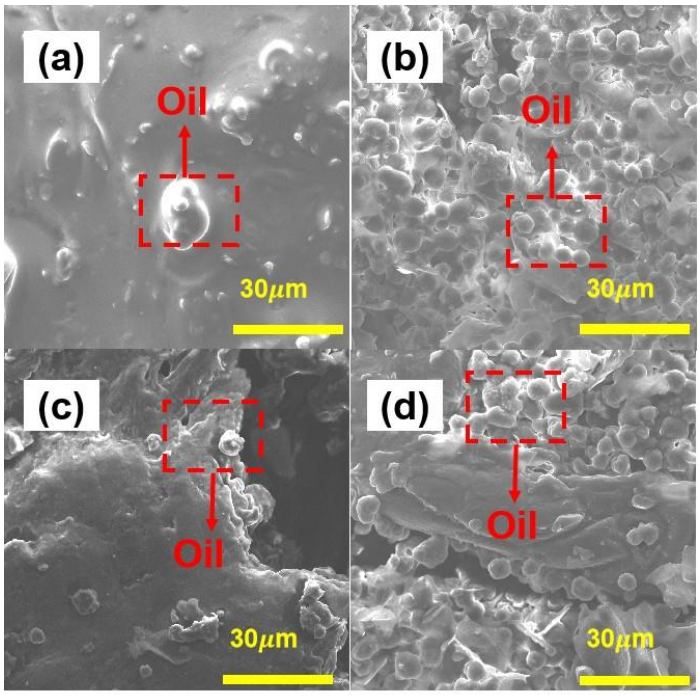

Fig. 10. SEM images of walnut before and after extraction: (a) Walnut; (b) After Soxhlet; (c) After pressing; and (d) After ultrasonic

Nuclear magnetic analysis of walnut oil

The NMR data in Figs. 11 and 12 show that the solvent peak of triglyceride were at 4.1 to 4.3 ppm in the ^{1}H NMR of walnut oil (Yang *et al*. 2015; Merchak *et al*. 2017; Fadhil *et al*. 2019; Xiao *et al*. 2020). By NMR analysis of walnut oil, the presence of triglycerides and fatty acid methyl esters were confirmed (Pretsch *et al*. 2002).

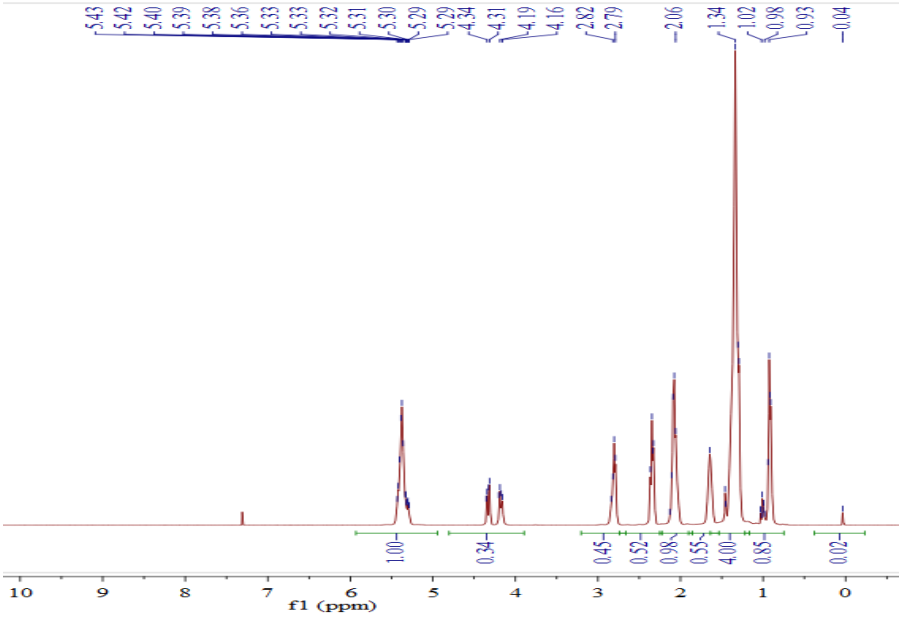

Fig. 11. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of walnut oil. Data: δ 7.94 to 3.67 (m, 101H), 2.35 (ddd, J = 107.5, 98.0, 6.1 Hz, 131H), 2.27 to 1.90 (m, 73H), 1.71 to 0.53 (m, 384H), and 0.04 (s, 1H)

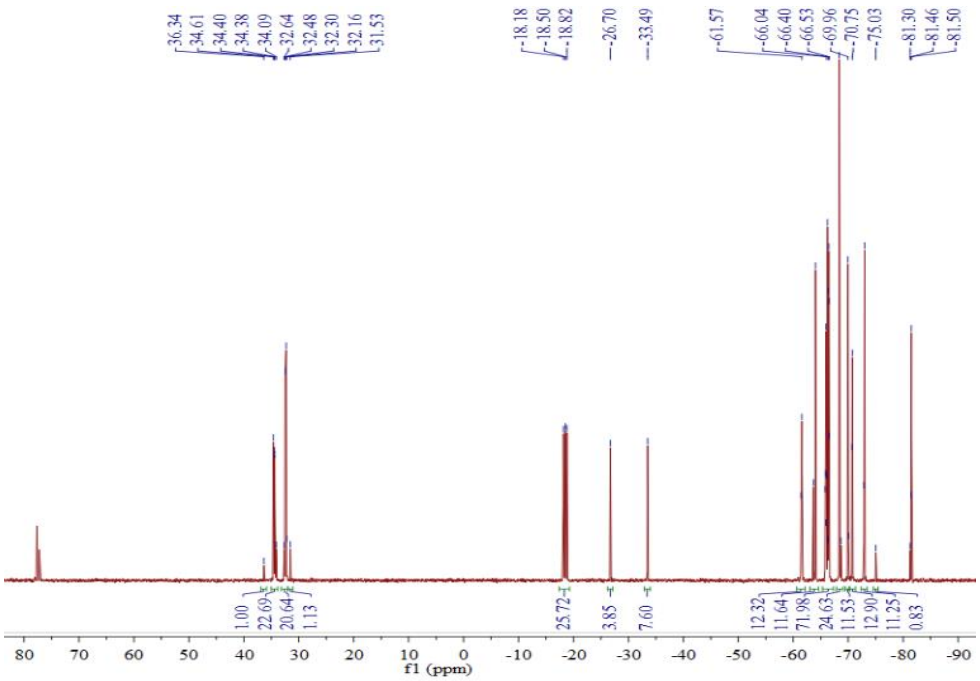

Fig.12. ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of walnut oil. Data: δ 173.16 (s, 1H), 140.18 to 123.42 (m, 4H), 91.40 to 61.08 (m, 3H), 34.02 (d, J = 16.6 Hz, 1H), 32.48 to 20.26 (m, 12H), and 14.02 (d, J = 3.8 Hz, 1H)

CONCLUSIONS

1. Through single factor experiments, the optimum conditions for each extraction process were obtained. For the pressing method, the maximum extraction efficiency was 43.2% when drying time was 20 min. The optimal extraction conditions were as follows: extraction temperature of 50 ℃, extraction time of 60 min, ultrasonic power of 180 W, solid/liquid ratio of 1:11 g.mL^{-1}, and the yield of walnut oil was 59.1%. The optimal extraction conditions for the Soxhlet method were as follows: time 4 h, solid/liquid ratio 1:7, temperature 76 ℃, for which the extraction efficiency of walnut oil was 58.29%.
2. The physical and chemical properties, Fourier transform infrared (FT-IR) spectra, scanning electron microscopy (SEM) images, and the composition and contents of fatty acids and elements of walnut oil extracted by Soxhlet, and ultrasonic assisted methods were similar. As the Soxhlet extraction method of extraction time is too long, the ultrasonic assisted method was selected to be practical.
3. Through FT-IR and nuclear magnetic resonance (NMR) results of walnut and oil revealed that the walnut kernel can be successfully used to prepare biodiesel. The carbon chain length of walnut oil was mainly concentrated in C16:0-C18:3n3, unsaturated acid content accounted for more than 80%, and primarily *cis*-oleic and *cis*-linoleic acid contents were high.

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