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Comparison of physicochemical characteristics of *Acer truncatum* seed oil based on three extraction methods

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ABSTRACT: Studies have revealed that *Acer truncatum* seed oil has a high nutritional value. However, the constituents of seed oil may vary depending on the extraction and separation techniques employed. Here, we aimed to explore the effect of three extraction methods on *A. truncatum* seed oil. After comparing the extraction rate, acid value (AV), moisture content (MC), fatty acid composition, Fourier transform infrared spectroscopy characteristic, and anti-oxidation activities of three *A. truncatum* seed oil samples extracted by refluxed with petroleum ether (*S1*), petroleum ether extraction combining ultrasonic power (*S2*) and solvent lixiviation at room temperature (*S3*). The results showed that *S3* had a higher extraction rate than the others, but lower unsaturated fatty acid content and DPPH scavenging rate. The DPPH scavenging rate and nervonic acid (NA) concentration of *S2* sample were higher than the others, but with larger MC and lower extraction rate. As for *S1*, the unsaturated fatty acid content was higher than the others; but the extraction rate was lower, and the acid value was higher. Therefore, the results demonstrated that extraction methods should be selected according to the actual application target, as different methods presented different advantages and disadvantages. However, further studies on some other properties (such as antioxidant activity) of the extracted *A. truncatum* seed oil are required.

KEYWORDS: *Acer truncatum*, seed oil, extraction methods, essential fatty acids

INTRODUCTION

Acer truncatum, a member of the *Aceraceae* family, is a native arbor tree species with a well-developed root system and strong environmental adaptability. The tree also has other traits, such as resistance to barrenness, drought, and low temperature, and the ability to grow normally in arid, windy, and low-temperature environments [1]. In recent years, *A. truncatum* has become an economically significant and city-friendly tree.

Numerous studies have been conducted on *A. truncatum* seed oil (ATSO). The studies have analyzed the variations in seed oil content, fatty acids, and other *A. truncatum* substances based on its morphological characteristics and growth environment. Specifically, it has extensively been reported that ATSO contains more unsaturated fatty acids than many other plants [2–5]. One study found that *A. truncatum* seeds contained more than 42.0% oil, of which more than 92% was unsaturated fatty acids, including 25.8% oleic acid, 37.3% linoleic acid, and 5.5% nervonic acid (NA) [6]. NA, one of the fatty acids in rapeseed oil, has been identified as a substance with exceptional clinical value [7]. The National Health Commission of the People's Republic of China approved the use of ATSO as one of 57 new raw materials in 2021 based on the potential nutritional value (No. 9 Announcement, issued in 2011) [8]. A previous study isolated 247 chemical components from the flowers, seeds, and seed oils of *A. truncatum*, which brought the total of isolated and identified compounds to 288, including polyphenols, acids or esters, biogenic volatile compounds, and other types of compounds [9]. Numerous experiments have been performed based on these substances to explore the pharmacological properties of ATSO. For instance, antitumor or antibacterial activities have been identified based on inhibitory activity against fatty acid synthase [10]. In addition, Li et al [11] demonstrated that ATSO is a potential therapeutic food supplement for delaying aging in mice through multiple mechanisms.

Isolating and identifying the chemical components of ATSO has become a research hotspot in recent years. Common ATSO extraction and separation techniques include mechanical pressing, organic solvent, water enzymatic, and supercritical extraction techniques. Utilizing simplicity, low cost, convenience, and simple recycling in solvent extraction may be the top priority in substance extraction experiments, particularly the refluxed method with a higher-temperature solvent. Even in instances where the raw materials are identical, researchers believe that different separation methods will alter the properties of the extracted seed oil. To date, few studies have compared the results of the different ATSO extraction methods based on *A. truncatum* seeds from the same source. Therefore, the primary objective of this paper was to evaluate the properties of ATSO based on various extraction

methods, which were refluxed with petroleum ether in a water bath (*S1*), petroleum ether extraction combining ultrasonic power (*S2*) and solvent lixiviation at room temperature (*S3*). Specifically, we explored the extraction rate, acid value (AV), moisture content (MC), fatty acid composition, Fourier transform infrared spectroscopy (FT-IR) characteristics, and NA content of seed oil to determine the characteristics of ATSO extracted using the various techniques. We also evaluated the antioxidant activity and the free radical scavenging ability of the extracted seed oil.

MATERIALS AND METHODS

Materials and reagents

A. truncatum seeds were supplied by Taicang NovoSana Co., Ltd. (Jiangsu, China). Standard mixtures of fatty acid methyl esters (FAME) (Supelco 37 component FAME mix) were purchased from Sigma Chemical Co., Ltd. (Shanghai, China). All solvents were of analytical grade. Chromatographic grade acetic acid, tetrahydrofuran, methanol, and acetonitrile were purchased from Shanghai Aladdin Biochemical Technology Co. Ltd. (Shanghai, China). In addition, petroleum ether, absolute ethanol, diethyl ether, and methanol were obtained from Tianjin Fuyu Chemical Co., Ltd. (Tianjin, China).

Methods

ATSO extraction methods

A. truncatum seeds were first dried for 24 h at 60 °C, followed by crushing and extraction of oil from 10 g of raw material powder by solutions using the following different methods with a material-to-liquid ratio of 1:5.

First, 10 g sample was refluxed with 100 ml petroleum ether in a water bath at 50 °C for 60 min with continuous stirring. This procedure was repeated three times, after which the extraction solution was gathered. Next, ATSO was obtained after the petroleum ether was recovered using a rotary evaporator. Notably, this oil sample was given the code *S1*. The *S2* oil sample was obtained by combining both petroleum ether extraction and ultrasonic power by mixing 10 g sample with 100 ml petroleum ether and then ultrasound extraction with 100 W for 30 min. The procedure was repeated three times and the remaining steps following the *S1* extraction method. The third extraction method was solvent lixiviation of 10 g sample mixed with 100 ml petroleum ether at room temperature for 24 h. Similarly, the procedure was repeated three times with the remaining steps following the *S1* extraction method, and the obtained sample was designated *S3*. The following Eq. (1) was utilized to calculate the extraction rate of the individual methods:

$$\text{Yield} = \frac{\text{mass of oil (g)}}{\text{mass of materials (g)}} \times 100\%. \quad (1)$$

AV and MC of the extracted seed oil

It is crucial to determine the acid value (AV) of oil samples because seed oil can be used as an edible oil. The AV test was conducted following the National Standards of the People's Republic of China (GB/T 5009.37 and GB/T 5530). Firstly, 0.5 ml phenolphthalein and 50 ml ethanol solution were mixed and heated to boiling. When the temperature was higher than 70 °C, the solution was titrated with 0.1 mol/l potassium hydroxide solution to the end point to prepare a neutral ethanol solution. Secondly, 20.0 g of sample oil was weighed and fully mixed with neutralized ethanol solution. After boiling, the solution was titrated with potassium hydroxide standard solution to the end point, and the volume of potassium hydroxide consumed was recorded. The AV was calculated by Eq. (2).

$$\text{AV} = \frac{56.1 \times V \times c}{m}, \quad (2)$$

where V represents the consumed volume of potassium hydroxide standard solution (ml); c represents the concentration of potassium hydroxide solution (mol/l); m represents the amount of oil sample (g); 56.1 is the molar mass of potassium hydroxide (g/mol).

10.0 g of sample was placed in a petri dish previously dried to a constant weight (m_0) and weighed (m_1), then incubated in an oven at 103 °C for 60 min. Next, the sample was weighed and cooled in a desiccator for at least 30 min (m_2). The MC of the sample was calculated according to Eq. (3).

$$\text{MC}\,(\%) = \frac{m_1 - m_2}{m_1 - m_0} \times 100\%, \quad (3)$$

where m_0 represents the weight of an empty petri dish, m_1 represents the weight of the petri dish and sample before drying, and m_2 represents the weight of the petri dish and sample after drying.

Analysis of fatty acids

A 50 mg sample was placed in a centrifuge tube and dissolved in 4.0 ml of 25% isooctane. Next, 0.2 ml of 2 mol/l potassium hydroxide methanol solution was added, and the solution was shaken until it was clear. We then added one gram of sodium bisulfate to the solution. After 30 min, the organic phase was analyzed using gas chromatography (GC). The following conditions were applied for GC detection: CD-2560 column (100 m×0.25 mm, 0.20 µm); the stationary phase of the column HP-5 ((5% phenyl) - dimethylpolysiloxane); carrier gas N_2, flow rate 1.50 ml/min; the temperature of injection and detection with FID as a detector and the ports was 250 °C and 280 °C, respectively; injection volume: 1 µl, split ratio 25:1; temperature rise procedure: maintained at 120 °C for 5 min and then increased to 240 °C at 2 °C/min for

10 min. Notably, 37 fatty acid methyl ester standards were employed for qualitative and quantitative analyses using the peak area normalization technique.

It was previously demonstrated that NA had a high nutritional value and could potentially enhance human memory and cognitive function [12]. Herein, high-performance liquid chromatography (HPLC) was applied to determine the concentration of NA in the oil samples. The LC-2030 plus HPLC (Shimadzu Corporation, Japan) was equipped with an ODS-C18 reverse-phase chromatography column (4.6 mm×250 mm, 5 µm) and an SPD-16 UV/Vis detector with a wavelength of 205 nm. The following NA mobile phase analysis was used: acetonitrile; methanol; 0.4% acetic acid; tetrahydrofuran = 80:12:5:3. The flow rate was set to 1.0 ml/min, the volume was 20 µl (filtered through a 0.22 µm membrane before the testing), and the temperature of the column was set to 26 °C. To obtain a 0.1 mg/ml standard solution, the NA standard solution was diluted with acetonitrile to 100 ml.

Fourier transform infrared spectroscopy analysis

The dried samples were mixed with KBr, formed into discs, and analyzed with an FT-IR spectrometer (IRAffinity-1S, Shimadzu, Japan) between 4000 cm^{-1} and 500 cm^{-1}.

Antioxidant capacity assay

Currently, the DPPH method is the standard *in vitro* investigation technique for free radical scavengers [13]. A 0.4 mg/ml DPPH reagent was prepared by dissolving 40 mg of DPPH in 100 ml absolute ethanol [14]. To obtain a 100 mg/ml sample solution, 1.0 g of oil sample was dissolved in 10 ml of absolute ethanol. Next, 3 ml of DPPH reagent was added to sample solutions, then the absorbance (A) of these samples was assessed using an ultraviolet spectrophotometer at 517 nm. Clearance rate (CR), which is the speed of free radical scavenging and represents the anti-oxidation ability of a sample, was determined using Eq. (4). In addition, we investigated the relationship between reaction time and the CR of the three oil samples at a certain concentration by detecting the A value at different times. Each sample was examined three times, and the average value was recorded.

$$\mathrm{CR}\,(\%) = \frac{A_1 - A_0}{A_1} \times 100, \tag{4}$$

where A_1 and A_0 represent the absorbance of the DPPH solution and the tested sample, respectively.

Statistical analyses

Statistical analyses of the data were performed with the SPSS Statistics package. Significant differences between samples were determined by applying the Tukey test. Multivariate analysis of variance (ANOVA) was used to assess significant differences in fatty acid composition and yield of the three sample groups ($p < 0.05$). All samples were run in triplicate.

RESULTS AND DISCUSSION

Extract yields of the various extraction methods

Fig. 1 shows the yields and appearances of oil samples extracted using various techniques. The yields of the *S1* sample (petroleum ether extraction), the *S2* sample (combined petroleum ether extraction and ultrasonic power), and the *S3* sample (petroleum ether lixiviation) were 27.71%, 25.67%, and 31.02%, respectively (Fig. 1a). Hence, *S3* had the highest extraction rate. Theoretically, *S1* should have the highest yield due to its strong molecular movement ability at higher temperatures; however, the results demonstrated that *S1* had the lowest extraction rate. Moreover, *S1* exhibited less turbidity than the other samples, suggesting that more substances were extracted from seed powder with a lower yield during solvent-internal reflux at a specific temperature. It is worth noting that the post-treatment procedure for *S1* oil samples is complex, with the choice of either purifying the seed oil or separating the target components. Interestingly, the extraction rate of *S3* was higher at lower temperatures. Altogether, these results suggest that temperature may not significantly influence the ATSO extraction rate. The obvious difference between the three techniques was the extraction time, where the *S3* underwent a 24-h procedure. Therefore, the extraction time may be a significant influencing factor. The solvent lixiviation method may be the preferred method for extracting oil from *A. truncatum* seed due to its simplicity, portability, and recyclability.

Acidity and moisture content of seed oil

Acid value (AV, mg/g), a measure of fatty acid oxidation and fat decomposition, indicates the amount of potassium hydroxide required to neutralize free fatty acids in a 1 g sample of oil. Typically, the lower the AV, the higher the degree of refinement and freshness of the oil. However, seed oil may be oxidized or rancid under various conditions, such as higher extraction temperature or storage process, resulting in the production of free fatty acids, which may then be converted into aldehyde, ketone, acid, and other complex substances. These changes not only affect the appearance, flavor, and physicochemical properties of the oil but also pose a significant threat to human health and even cause death. Therefore, AV is a significant indicator of oil rancidity and one of the most frequently employed physical and chemical indicators for determining the quality of edible oils. Fig. 2(a) shows the AV of the extracted oil samples. Although the AV data of the three samples were different, *S3*

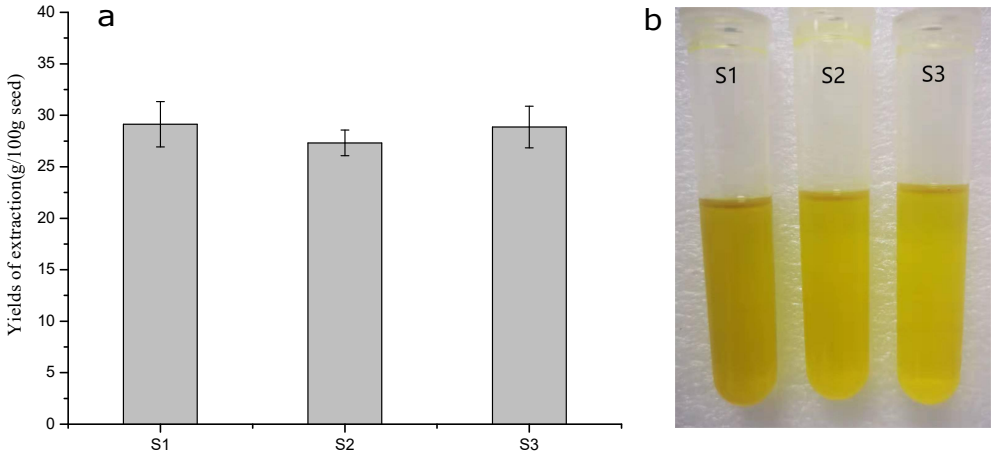

Fig. 1 Extraction yields (a) and appearances (b) of the three samples.

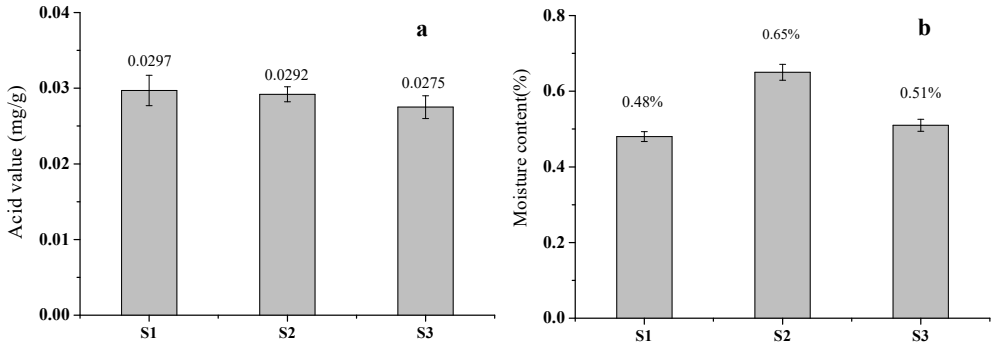

Fig. 2 The acid value (a) and moisture content (b) of the three samples.

had the lowest AV. This could be attributed to the fact that *S3* was stored at a lower temperature than *S1* and *S2*. In addition, *S3* may contain more antioxidants or reducing substances than *S1* and *S2*, which can inhibit or reduce the oxidation or rancidity process.

In general, the lower the amount of water in oil, the greater its stability, the greater the preservation of active components (such as sterols, vitamin E, polyphenols, and unsaturated fatty acids), and the lower production of saturated fatty acids [15]. The moisture content (MC) in the oil can cause accelerated rancidity and microbial growth, even at lower concentrations and temperatures, which can significantly impact the nutritional quality and economic value of edible oil. In addition, the MC can change the appearance of oil. Fig. 2(b) shows the MC of the three samples. It was evident that *S2* had the highest MC, and *S1* had the lowest MC slightly lower than *S3*. The results could be due to the mechanical action of ultrasound that promoted the emulsification of liquids and the liquefaction and dispersion of solids. Notably, numerous tiny bubbles can form when an ultrasonic wave interacts with a liquid. In addition, an ultrasonic wave can produce a significant thermal effect due to its high frequency and high energy. Therefore, the MC value of *S2* was greater than that of *S1* and *S3* because water molecules could be emulsified, liquefied, or dispersed within the oil sample due to the ultrasound function. Considering the higher temperature of the S1 procedure, the MC of *S1* should be higher than that of *S3*. However, we found that *S1* had a slightly lower MC than *S3*, suggesting that extraction temperature might not be a crucial factor in the extraction method. This was consistent with the results of the extraction yields. It is possible that the substances in *S3* might have a higher affinity for water molecules, and thus they absorbed the water molecules.

Fatty acids analysis

Fatty acids are the main constituent of lipids, and can be classified as saturated or unsaturated according to the existence of double bonds in the molecule. Saturated fat can provide energy for human body, but excessive intake of the fat will increase the risk of coronary heart disease and obesity [16]. Unsaturated fatty acid is categorized into mono- and polyunsaturated fatty acids based upon the number of double bonds [17]. The fatty acids in the three samples were analyzed based on 37 types of standard fatty acid methyl esters. The results were displayed in Table 1.

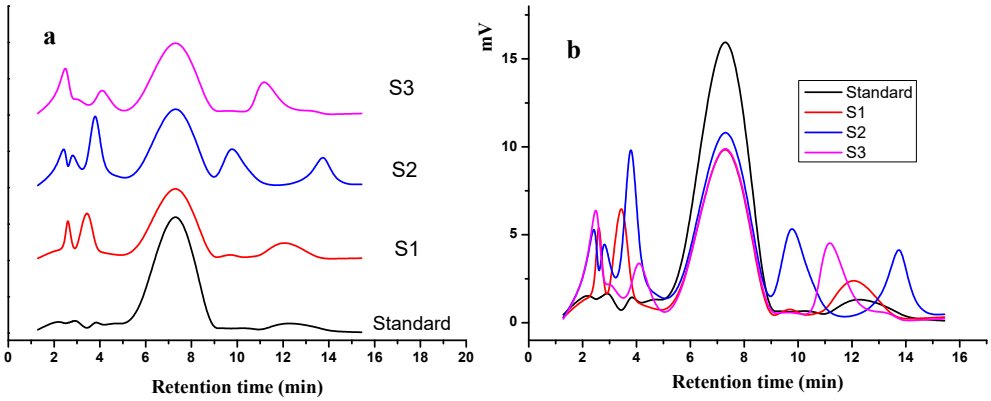

Fig. 3 HPLC results of the three samples. (a), comparison of characteristic peaks; (b) , comparison of NA peaks.

Table 1 Types and concentrations of fatty acids in the three samples.

Type of fatty acids	Content (g/100 g oil)		
	S1	*S2*	*S3*
Carbonic acid 16 (C16:0)	4.03	4.00	4.15
Cis-9-hexadecenoic acid (C16:1)	0.109	0.106	0.113
Heptadecanoic acid (C17:0)	0.066	0.066	0.070
Cis-10-heptadecaenoic acid (C17:1)	0.039	0.037	0.038
Octadecanoic acid (C18:0)	2.36	2.35	2.35
Cis-9-octadecaenoic acid (C18:1n9c)	22.6	22.4	22.6
Linoleic acid (C18:2n6c)	34.0	33.6	34.2
Eicosanoic acid (C20:0)	0.274	0.273	0.266
γ-Linolenic acid (C18:3n6)	0.516	0.515	0.524
α- linolenic acid (C18:3n3)	1.88	1.88	1.92
Cis-11-eicosenoic acid (C20:1)	8.35	8.40	8.32
Cis, cis-11,14-eicosadienoic acid (C20:2)	0.314	0.314	0.310
Docosaic acid (C22:0)	0.941	0.953	0.914
Cis-13-docosamonoenoic acid (C22:1n9)	17.8	18.1	17.5
Tricosanoic acid (C23:0)	0.038	0.039	0.038
Cis-13,16-docosadienoic acid (C22:2)	–	0.022	–
Tetracosanoic acid (C24:0)	0.411	0.419	0.399
Cis-15- Tetracosenic acid (C24:1) (NA)	6.28	6.47	6.19
saturated fatty acids	8.08	8.10	8.19
monounsaturated fatty acids	55.2	55.5	54.8
polyunsaturated fatty acids	36.7	36.3	37.0
unsaturated fatty acids	91.9	91.8	91.8

–, not detected; the deviations in the values are in the range of 0.5 to 0.9% statistical error.

It was found that the *S1*, *S2* and *S3* contained 17, 18, and 17 different types of fatty acids, respectively. The contents of saturated fatty acids in the three samples were 8.08, 8.10, and 8.19 g/100 g of oil, with slight variations. *S3* had a slightly higher total content of saturated fatty acids than the others. The respective percentages of unsaturated fatty acids detected were 91.9, 91.8, and 91.8 g/100 g of oil. The concentrations of three unsaturated fatty acids, namely oleic acid, linoleic acid and linolenic acid, were 58.990, 58.395, and 59.244 g/100 g of oil, respectively. In addition, linoleic acid concentrations in the three samples were 34.0, 33.6, and 34.2 g/100 g of oil, respectively. Fatty acids, mainly unsaturated fatty acids, are essential nutrients in oil. Numerous studies have demonstrated that unsaturated fatty acids can lower blood lipids, reduce arteriosclerosis, and prevent hypertension and cardiovascular disease [18–20]. Moreover, essential fatty acids, such as linoleic acid and linolenic acid, are essential for human growth and must be consumed from the diet because the body cannot synthesize them. Linoleic acid and linolenic acid have also been shown to have antitumor properties. Therefore, our results suggested that ATSO had essential nutritional functions for human health and might have clinical application potential. Polyunsaturated fatty acids, such as α-linolenic acids, and linoleic acid are the source of ω-3 and ω-6 fatty acids which are crucial fatty acids because it cannot be produced in the body and required through diets [21].

Erucic acid is a type of unsaturated long-chain fatty acid. Animal experiments demonstrated that a high dose of erucic acid could result in myocardial fibrosis and cardiomyopathy [22]. In this study, the erucic acid concentrations in the three seed oil samples were 17.8%, 18.1%, and 17.5%, respectively. This finding suggested that the effect of erucic acid must be considered if ATSO is prepared as an edible oil [23].

NA was initially identified in mammalian nerve tissue as a major component of sphingolipids in myelin membranes [24]. It has been reported that NA is an essential component of brain nerve cells and tissues, which is advantageous for the biosynthesis and maintenance of nerve cell myelin. NA is an important “advanced nutrient" for the growth, redevelopment, and maintenance of nerve cells, especially brain cells, optic nerve cells, and peripheral nerves [25].

Although NA has a nutritional effect on human brain, it is difficult to synthesize in the human body. Therefore, *in vitro* intake of NA is particularly crucial. A previous study demonstrated that *A. truncatum* seeds contained more than 5.5% of NA [24]. In the present study, we analyzed the concentration of NA in the three

samples using HPLC, and the obtained results were shown in Fig. 3.

Importantly, the concentrations of NA in the three samples were higher than in other studies [6, 25] with the respective NA concentrations of 5.04% (*S1*), 5.35% (*S2*), and 4.97% (*S3*) (Fig. 3(a)). Fig. 3(b) shows that the concentration of NA in *S2* was slightly higher than in the other samples, whereas the concentrations in *S1* and *S3* were nearly identical. However, we expected the difference of NA concentration in *S2* to be higher under large-scale production conditions than under our laboratory conditions. Therefore, given that the NA is one of the quality evaluation staqndards for an oil sample, *S2* could be the most appropriate extraction method, particularly for large-scale processes, compared with the other two. Overall, the high concentration of NA in ATSO suggests that it has a potential clinical application for both senile degenerative diseases and anti-aging effects. It should be noted that the results of NA analysis by HPLC differed from those of GC, suggesting that further experiments should be conducted to obtain more accurate NA concentration data.

Only the *S2* sample contained Cis-13,16 docosadienoic acid, an agonist of free fatty acid receptor 4 (FFAR4 or GPR120, the LCFA receptor), which is believed to have an anti-dependence effect. Theoretically, ultrasonic technology can promote or accelerate the movement of molecules, contribute to molecule migration, increase the dissolution rate of substances, and ultimately increase the extraction rate. Nonetheless, this study has demonstrated that ultrasound might also enhance the molecular activity of certain substances, such as NA and C22:2, or be selective for specific substances.

FT-IR analysis

Fig. 4 shows the FT-IR spectra of the three samples. The results indicated that the infrared spectral transmission of the three samples was comparable not only in peak shape but also in the position of the characteristic spectral peaks (Fig. 4(a)), indicating that the three samples shared a nearly identical composition. The absorption peak at approximately 3008 cm^{-1} could be attributed to the C—H stretching vibration of an unsaturated carbon chain. The absorption peaks at 2924 cm^{-1} and 2854 cm^{-1} in the samples were caused by the C—H stretching vibration of saturated carbon and the C=O stretching vibration peak, respectively, which appeared at around 1745 cm^{-1}. Moreover, the absorption peaks at 1436 cm^{-1} and 1162 cm^{-1} corresponded to the methyl and C—O stretching vibrations of triglycerides, respectively. Although the absorption peak positions of the three samples were comparable, the FT-IR spectra exhibited varying characteristics, especially the absorption intensity of *S3*, which was distinct from that of the other samples (Fig. 4(b)).

When molecules vibrate, the peak intensity of FT-IR spectra is generally proportional to the moment of dipole and probability of energy level transition. The higher the intensity of the spectral peak, the more significant the change in the dipole moment. Nevertheless, the change in dipole moment is associated with the polarity of molecules or groups. The stronger the polarity, the greater the dipole moment, and the stronger the peak in the absorption spectrum corresponding to that polarity. Similarly, the greater the probability of an energy transition, the stronger the absorption spectrum peak. The reason *S3* was slightly stronger than the others may be attributed to higher concentration of polarity substances in the seed. Nonetheless, the precise mechanism requires further study.

Antioxidation activity assay

Some physiological functions are associated with the production of free radicals *in vivo*, including activation of the immune system and signal transduction processes. However, radical body reactions must be in dynamic equilibrium. Several diseases, such as heart disease, Alzheimer's disease, Parkinson's disease, and tumors, are caused by oxidative damage induced by excessive free radicals. Therefore, studying free radical scavenging would be crucial for combating anti-aging and cardiovascular diseases.

In an absolute ethanol solution, DPPH, also known as 1,1-diphenyl-2-trinitrophenylhydrazine, has maximum absorption at 517 nm. In addition, the absorbance value of a solution is linearly proportional to its DPPH concentration. However, free radical scavengers can combine with or replace DPPH. Based on this theory, when free radicals combine with or replace DPPH, radical scavengers will reduce the absorbance of the solution. The ability of free radical scavengers and the antioxidant capacity of the sample can be determined by measuring the absorbance at a wavelength of 517 nm [26].

Fig. 5 shows the CR of DPPH in the three samples. It was evident that all samples possessed antioxidant properties (Fig. 5(a) and Fig. 5(b)). Results showed that the CR increased as sample concentration increased, and all CR exceeded 65% within 30 min (Fig. 5(a)). *S3* displayed a slightly higher CR than the other samples at low concentration (1.0 mg/ml), indicating having a higher antioxidant capacity. However, *S1* displayed a higher CR at the highest concentration (25 mg/ml). In addition, we measured the DPPH CR of the three samples with the same concentration at different times, and the results were shown inFig. 5(b). It was found that the CR of samples was raised as the reaction time increased. Notably, *S2* had a higher CR than the others when the reaction time exceeded 8 h. Theoretically, DPPH can absorb the hydrogen atom from an antioxidant and convert it to the corre-

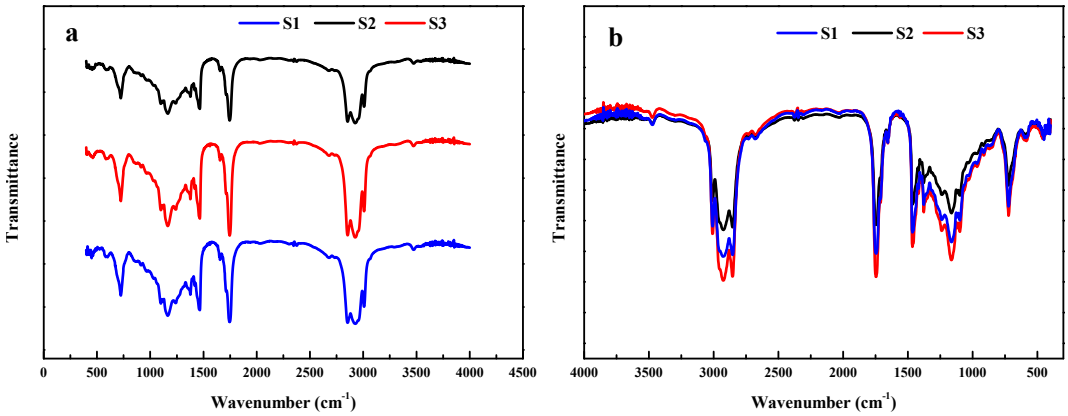

Fig. 4 FT-IR spectra of the three samples. (a), FT-IR characteristic peaks; (b), intensity comparison of infrared characteristic peaks.

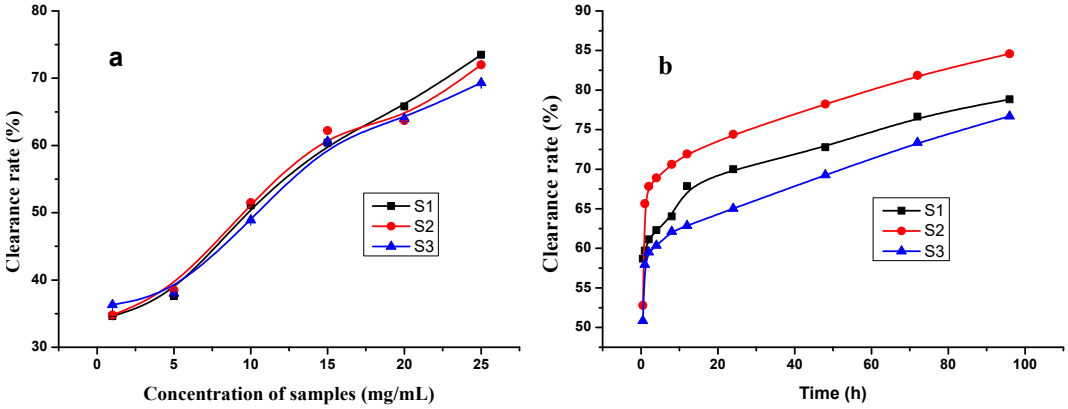

Fig. 5 Clearance rate of DPPH in the three samples. (a), within 30 min of different concentrations; (b), same concentration at different times.

sponding hydrazine. After reduction by antioxidants, DPPH radicals become stable molecules and cause a color change from deep violet to pale yellow in the solution. Generally, the CR of DPPH increases as the concentration of antioxidants in a solution increases. Therefore, our results suggested that ATSO had an obvious antioxidant effect that might help delay the aging process and the onset of cardiovascular diseases in humans.

The oil samples had an obvious antioxidant activity, which might be due to the unsaturated fatty acids and other antioxidant substances. In addition, the CR of the three samples varied, indicating the varied composition or concentration of antioxidant compounds in the samples. From Fig. 5(b), the results demonstrated that, with the same concentration, the antioxidant capacity of *S2* was stronger than *S1* and *S3* over a longer period of time, suggesting that *S2* contained more antioxidants than *S1* and *S3*. However, the contents of unsaturated fatty acids in the three samples were 91.9, 91.8, and 91.8 g/100 g of oil, respectively. These results demonstrated that the samples might contain other antioxidant substances such as vitamin E. Therefore, further studies are needed to evaluate the precise chemical composition of the three oil samples and to elucidate the antioxidation mechanism of the *S2* resulting in a more significant effect. Furthermore, although the CR of DPPH has not been applied as a kinetic parameter in numerous sources, including this article, the secondary rate constant of DPPH might be a valuable parameter for predicting the antioxidant capacity. DPPH kinetics, and mechanism studies might also be useful parameters [27].

CONCLUSION

The *S1* extraction method is the most commonly used for extracting active ingredients in natural plants. This study showed that the oil sample extracted using the *S1* method had lower values of extraction rate, fatty acid content, and antioxidant capacity, as well as high MC and turbid appearance, indicating that *S1* would require additional steps if applied for food or medicine. Furthermore, results showed that the *S1* method had a higher thermal energy consumption than *S2* and

S3. With regard to the *S2* method, the ultrasonic extraction aided by petroleum ether resulted in higher concentrations of NA and erucic acid and a higher DPPH CR over time. In addition, we found that solvent extraction with ultrasonic power might not be more efficient than solvent extraction alone, and ultrasound increases energy consumption. The total content of the three unsaturated fatty acids (oleic acid, linoleic acid, and linolenic acid) was greater in the *S3* sample than in the other two, suggesting that the *S3* sample could have a high nutritional value. Moreover, the *S3* procedure was performed at room temperature and still had a high extraction rate, providing simplicity and low consumption benefits. Regarding the DPPH clearance, *S3* demonstrated a marginally higher value within 30 min. Therefore, when considering the nutritional value, solvent lixiviation at room temperature has more potential benefits than ATSO extraction.

By comparing the physiochemical characteristics of the three extraction methods in this study, we found that the properties of seed oil extracted by the different methods vary. Despite the difficulty of accurately analyzing the chemical composition of oil samples, the best extraction technique should be chosen based on the actual objective of the application. In addition, certain properties of the three oil samples, such as infrared characteristics and DPPH differences, require further investigation. In general, the lower the MC of the oil, the greater its stability. The current findings also suggested that erucic acid might contribute unexpected effects on the human body. Given that the MC and erucic acid concentrations of the three oil samples were greater than edible rapeseed oil, these characteristics of seed oil should be considered in future practical applications or explored using the corresponding solvent methods.

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