Process optimization and characterization of arachidonic acid oil degumming using ultrasound-assisted enzymatic method

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\begin{abstract}
Ultrasound assisted enzymatic method was applied to the degumming of arachidonic acid (ARA) oil produced by Mortierella alpina. The conditions of degumming process were optimized by response surface methodology with Box- Behnken design. A dephosphorization rate of 98.82\% was achieved under optimum conditions of a 500 U/kg of Phospholipase A\textsubscript{1} (PLA\textsubscript{1}) dosage, 2.8 mL/100 g of water volume, 120 min of ultrasonic time, and 135 W of ultrasonic power. The phosphorus content of ultrasonic assisted enzymatic degumming oil (UAEDO) was 4.79 mg/kg, which was significantly lower than that of enzymatic degumming oil (EDO, 17.98 mg/kg). Crude Oil (CO), EDO and UAEDO revealed the similar fatty acid compositions, and ARA was dominated (50.97 ~ 52.40\%).

The oxidation stability of UAEDO was equivalent to EDO and weaker than CO, while UAEDO presented the strongest thermal stability, followed by EDO and CO. Furthermore, aldehydes, acids and alcohols were identified as the main volatile flavor components for the three oils. The proportions of major contributing components such as hexanal, nonanal, (E)-2-nonanal, (E, E)-2,4-decadienal, (E)-2-nonenal and aldehydes in UAEDO and EDO were all lower than CO. Overall, Ultrasound assisted enzymatic degumming proved to be an efficient and superior method for degumming of ARA oil.
\end{abstract}

1. Introduction

Mortierella alpina (M. alpina) is an oil producing fungus belonging to Zygomycetes, Mucorales, Mortierellaceae and Mortierella family. In recent years, M. alpina has passed the FDA safety assessment (GRAS), and its fermentation broth has gradually become an important method for industrial production of ARA oil [1–3]. Arachidonic acid (ARA), a pertinent \(\omega-6\) polyunsaturated fatty acid, exists as a structural lipid in muscle, liver, brain and other tissues and organs, and it is also the direct precursor of prostaglandins, thromboxanes, leukotrienes and other twenty carbon derivatives [4]. These derivatives play a pivotal role in human cardiovascular system and immune system. Furthermore, ARA can affect the physical, mental and visual development of infants. ARA is an essential fatty acid in infancy [5].

Similar to other oils, ARA oil contains colloidal impurities such as phospholipids, which lead to the decline of the quality of the product oil in the oil refining process. Therefore, the gum in the oil must be removed, that is, degumming. At present, the commonly used degumming methods include traditional degumming (hydration and acidification), physical adsorption and enzymatic degumming. Traditional methods can effectively remove hydrated phospholipids, but difficulty for non-hydrated phospholipids [6]. Physical adsorption process is simple and convenient operation, but trace components (such as tocopherol, phytosterol, \(\beta\)-carotene, etc.) in oil would be lost together, thus reducing the functionality of the oil. Enzymatic degumming is based on the characteristics of phospholipase to hydrolyze non-hydrated phospholipids in crude oil into hydrophilic phospholipids, which are eliminated by hydration [7]. Enzymatic degumming has been widely used in soybean oil [8], rapeseed oil [9], sunflower seed oil [10], corn oil [11], rice bran oil [12] and other vegetable oil processing. Nonetheless, enzymatic degumming is usually time-consuming. How to shorten the time is one of the key problems for its successful industrial application.

Ultrasonic wave is an elastic mechanical wave with the frequency range of \(10^{4} \sim 10^{12}\) Hz. When the ultrasonic wave propagates in the medium, it produces pressure wave, which forms high pressure region (compression region) and low pressure region (rarefied region) in the medium. In the region of high pressure and low pressure, the medium molecules contract and expand respectively. In the process of expansion,
molecules are pulled apart to produce tiny cavities or microbubbles. When the critical radius is reached, a very high local energy density is released due to implosion, which is called cavitation effect. At the moment of collapse of ultrasonic cavitation bubble, local high temperature, high pressure and accompanying strong shock wave are generated around it, and high energy intermediates are also generated [13–16]. The mechanical effects, such as shock wave, shear and vibration, enhance the movement of macromolecules, particles and suspended cells in the medium, and significantly increase the mass transfer rate, which is very important for chemical reactions in heterogeneous systems. Jiang et al. [17] found that compared with mechanical stirring system, ultrasonic treatment could accelerate the enzyme reaction rate in the first 2 h, and the residual phosphorus content in rapeseed oil could be rapidly reduced to less than 10 mg/kg. In mechanical stirring system, the reaction rate firstly increased and then decreased, and it took 4 h to reduce the residual phosphorus to below 10 mg/kg. Moreover, More and Gate [18] observed that an increase in ultrasonic power from 20 W to 40 W resulted in an increased in extent of degumming (EOD) from 85% to 89%. Further increase from 60 W to 100 W, resulted in EOD from 96.71% to 98.38%. Currently, the ultrasonic assisted enzymatic degumming is relatively less studied in microbial oil. In this experiment, Box-Behnken response surface method was used to optimize the ultrasonic assisted enzymatic degumming process of ARA oil. Additionally, the quality characteristics of ultrasonic assisted enzymatic degumming oil, enzymatic degumming oil and curd oil were compared respect to fatty acid composition, volatile components, thermal stability and oxidation stability, so as to provide technical support for the processing of high-quality ARA oil.

2. Materials and methods

2.1. Materials

Crude ARA oil was supplied by CABIO Biotech (Wuhan) Co, Ltd (Wuhan, China) with an original phosphorus content of 405.48 mg/kg. Phospholipase A$_1$ (PLA$_1$, Lecitase ultra) was procured from Novozymes ( Bagsvaerd, Denmark). The PLA$_1$ activity was analyzed and found to be 4954 U/mL. Other reagents were of analytical grade and were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China)

2.2. PLA$_1$ activity assay thermal stability analysis

PLA$_1$ activity was determined according to Yu’s method [19] with slight modifications. The enzyme activity was expressed as the amount of enzyme that released 1 μmol of titratable free fatty acid per minute under the assay conditions. 4 g of defatted soybean lecithin and 100 mL of disodium hydrogen phosphate - citric acid buffer (0.01 mol/L, pH 5.0) was emulsified at 50°C. Subsequently, 4 mL of 100 times dilution enzyme solution as commercial PLA$_1$ was added to lecithin emulsion and incubated for 15 min. The reaction was terminated by 60 mL of alcohol (95%, v/v). The released free fatty acids were titrated with 0.05 mol/L sodium hydroxide solution.

2.3. Ultrasonic assisted enzymatic degumming process

The ultrasonic assisted enzymatic degumming process of ARA oil was established using a JY92-IIDN ultrasonic homogenizer (Xinzhi Biotechnology Co., Ltd, Ningbo, China) equipped with a 6 mm of horn. The fixed frequency of 20 kHz has been used in the study. ARA oil (25 g) was heated to 70°C in a magnetic stirrer under mechanical stirring (500 rpm). 0.2 mL of citric acid solution (45%, w/w) was added under high shear rate (10000 rpm) for 1 min and then the mixture was continued to stir for 20 min. Subsequently, the oil was cooled to 45°C, and NaOH (4%, w/w) solution was used to adjust the pH of mixture to 4.8. A required quantity of diluted PLA$_1$ and distilled water were added into the oil with high shear rate (10000 rpm) for 1 min. Ultrasonic horn was inserted into the mixture, the ratio of action / interval was set to 30 s/30 s. Under a certain ultrasonic power, the enzymatic degumming reaction was completed by stirring in combination with ultrasonic. The mixture was heated to 95°C for 10 min, and quickly centrifuged at 4863 × g for 10 min. The upper oil was collected for rotary evaporation dehydration (~0.01 MPa, 60°C). The residual phosphorus content was analyzed and the dephosphorization rate was calculated as follow:

\[
Y = \frac{m_0 - m_1}{m_0} \times 100
\]

where Y was the dephosphorization rate (%), the $m_0$ and $m_1$ was phosphorus amount in raw oil (mg) and degumming oil (mg), respectively.

2.4. Phosphorus content assay

Phosphorus content in oil was determined by modifying the method of Han et al. [20]. 10 g of oil and 0.5 g of zinc hydroxide were accurately weighed in a crucible. The samples was carbonized completely and then send to a 575°C muffle furnace for ashing for 2 h. Afterword, the ash was dissolved with 10 mL hydrochloric acid solution (1:1, v/v) and heat to micro boiling for 5 min. The mixture was filtered and diluted to 100 mL. 10 mL of sample solution was neutralized with potassium hydroxide solution (50%, w/v) and hydrochloric acid solution (1:1, v/v) was slowly added to absolutely dissolve the precipitation. 8 mL of hydrazine sulfate solution (0.015%, w/v) and 2 mL of sodium molybdate dilute sulfuric acid solution (2.5%, w/v) were added. After mixing, it was heat in boiling water bath for 10 min and cool to room temperature. The solution was dilute to 50 mL and measured using a UV/Visible Spectrophotometer (UV-1900, Shimadzu, Tokyo, Japan) at 650 nm.

2.5. Fatty acid composition analysis

Samples were methylated with 0.5 mL of sodium methoxide (20 mg/mL) as the method described by Guo et al. [21] and analyzed using Agilent 7890A gas chromatography system (Agilent, Santa Clara, CA, USA) on a DB-FFAP column (30 m × 0.25 mm, 0.25 μm) with minor modifications and determined by solid phase micro extraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS). Oil (5 g) was accurately weighted in the headspace bottle and sealed. Sample was equilibration at 50°C for 20 min, and the volatile components were extracted with 1 cm 50/30 μm DVB/CAR/PDMS fiber (Stableflex 24 Ga, Gray) for 30 min. It was inserted into the gas chromatography injection port and desorbed at 250°C for 5 min. The gas chromatograph analysis was carried out by an Agilent 7890A gas chromatograph on a DB-WAX (0.32 mm × 30 mm, 0.25 μm). The initial temperature of oven was 40°C, holding for 2 min, followed by programming to 180°C at the rate of 5°C/min and lasting for 2 min, then to 240°C at the rate of 8°C/min. The injection temperature was 250°C. The carrier gas was helium (99.99%) at a flow rate of 1.5 mL/min. The injection volume was 1 μL and the split ratio was 10:1. The mass spectrometry analysis was performed by a 5975C mass spectrometer (Agilent Technologies, USA). MS conditions were electron bombardment ion source (EI) with electron beam energy of 70 eV. The temperature of transmission line, ion source and quadrupole were 280°C, 230°C and 150°C, respectively. The m/s ranged from 35 to 500.
2.7. Thermal stability analysis

Thermogravimetric analysis (TGA) was applied to evaluate the thermal stability of ARA oils according to the method of Mohammadpour et al. [23] with some modifications. It was carried out using a thermogravimetric analyzer (TGA/DSC 1/1100, Mettler Toledo, Zurich, Switzerland) under a nitrogen atmosphere (30 mL/min). Approximately 15 mg of samples were weighed into aluminum crucibles. The analysis was conducted at 30 ~ 800 °C with a constant heating rate of 10 °C/min.

2.8. Oxidative stability analysis

The oxidative stability of ARA oils were assessed by the oxidation induction time (OIT) using a Rancimat apparatus (743, Metrohm KEBO Lab AB, Herisau, Switzerland) and according to Cong et al. [24]. 3.0 g of sample was heated to 110 °C in the air atmosphere with the flow rate of 20 L/min. Small molecules produced from oil oxidation were brought into the conductive chamber, and the polar small molecules were dissolved in deionized water, which caused the changes of conductivity. The OIT was recorded by calculating the change and reported in minutes (min).

2.9. Statistical analysis

All experiments were executed in triplicate, and the results were expressed as mean ± standard deviation. Significance of difference between the means were evaluated by one-way analysis of variance and Tukey test (p < 0.05) using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Data of ultrasonic assisted enzymatic degumming were processed by Design-Expert 8.0.6 (Stat-Ease, Inc., Minneapolis, MN, USA).

3. Result and discussion

3.1. Response surface optimization of UAED

Ultrasonic-assisted enzymatic degumming parameters of ARA oil were optimized via response surface methodology with Box-Behnken design (BBD). A total of 29 experiments (including 24 discrete factorial points and 5 repeated central points) were carried out and the pure error of whole experiment was analyzed by the central point of repeated design. The codes and levels of four independent variables are shown in Table 1, and the trial results are shown in Table 2.

Regression equation of dephosphorization rate of UAED was obtained by quadratic polynomial stepwise regression fitting of the experimental results (Table 2):

\[ Y = 96.73 + 0.67 A + 4.30B + 1.28C + 0.31 D - 0.063 AB + 0.26 AC + 0.12 AD + 0.13 BC + 0.27 BD + 0.015CD - 0.96 A^2 - 4.45 B^2 - 0.68 C^2 - 0.43 D^2 \]

where Y was dephosphorization rate (%), A was PLA1 dosage (U/kg), B was water volume (mL/100 g), C was ultrasonic time (min) and D was ultrasonic power (W).

Variance analysis of regression model is revealed in Table 3, the model F value of 378.47 and p value of 0.0001 demonstrated the fit of the regression model was significant. Meanwhile, F value and p value of the lack of fit were 3.69 and 0.1098 (>0.05), respectively, thereby the model was feasible to fit the relationship between variables and dephosphorization rate by equation. The correlation coefficient (R²) and adjusted determination coefficient (R²adj) were 0.99992 and 0.9959, respectively, which indicated that the predicted values presented a good correlation with actual values. The model was applicable to predict the dephosphorization rate of degumming process. Furthermore, results showed that A, B, C and D had extremely significant effect on dephosphorization rate (p < 0.01). The order of influence of each factor on dephosphorization rate was as follows: water volume (B), ultrasonic time (C), PLA1 dosage (A), ultrasonic power (D). Interaction terms AC and BD had significant effect (p < 0.05), but AB, AD, BC and CD had no significant effect (p > 0.05). Quadratic terms A², B², C² and D² also had

| Table 1 Factors and levels of Box-Behnken design (BBD). |
|-------------------|-------------------|-------------------|
| Factor            | Code              | Level             |
| PLA1 dosage (U/kg)| A                 | -1 0 1            |
| water volume (mL/100 g)| B | 550 450 350 |
| ultrasonic time (min)| C | 3.0 2.5 2.0 |
| ultrasonic power (W)| D | 120 90 60 |

| Table 2 Experimental design and results of Box-Behnken. |
|-------------------|-------------------|-------------------|
| Run | PLA1 dosage (U/kg) | water volume (mL/100 g) | ultrasonic time (min) | ultrasonic power (W) | dephosphorization rate (%) |
| 1  | 350 | 2.0 | 90 | 126 | 86.22 |
| 2  | 550 | 2.0 | 90 | 126 | 97.74 |
| 3  | 350 | 3.0 | 90 | 126 | 94.95 |
| 4  | 550 | 3.0 | 90 | 126 | 96.22 |
| 5  | 450 | 2.5 | 60 | 108 | 93.93 |
| 6  | 450 | 2.5 | 120 | 108 | 96.67 |
| 7  | 450 | 2.5 | 60 | 144 | 94.48 |
| 8  | 450 | 2.5 | 120 | 144 | 97.28 |
| 9  | 350 | 2.5 | 90 | 108 | 94.50 |
| 10 | 550 | 2.5 | 90 | 108 | 95.41 |
| 11 | 350 | 2.5 | 90 | 144 | 94.90 |
| 12 | 550 | 2.5 | 90 | 144 | 96.29 |
| 13 | 450 | 2.0 | 60 | 126 | 86.19 |
| 14 | 450 | 3.0 | 60 | 126 | 94.42 |
| 15 | 450 | 2.0 | 120 | 126 | 88.42 |
| 16 | 450 | 3.0 | 120 | 126 | 97.15 |
| 17 | 350 | 2.5 | 60 | 126 | 93.50 |
| 18 | 550 | 2.5 | 60 | 126 | 94.44 |
| 19 | 350 | 2.5 | 120 | 126 | 95.43 |
| 20 | 550 | 2.5 | 120 | 126 | 97.40 |
| 21 | 450 | 2.0 | 90 | 108 | 87.56 |
| 22 | 450 | 3.0 | 90 | 108 | 95.73 |
| 23 | 450 | 2.0 | 90 | 144 | 87.64 |
| 24 | 450 | 3.0 | 90 | 144 | 96.88 |
| 25 | 450 | 2.5 | 90 | 126 | 96.60 |
| 26 | 450 | 2.5 | 90 | 126 | 96.81 |
| 27 | 450 | 2.5 | 90 | 126 | 96.70 |
| 28 | 450 | 2.5 | 90 | 126 | 96.79 |
| 29 | 450 | 2.5 | 90 | 126 | 96.76 |

| Table 3 Test of significance for regression coefficient. |
|-------------------|-------------------|-------------------|
| Variable | Sum of Squares | df | Mean square | F-value | P-value |
| Model | 378.47 | 14 | 27.03 | 1289.34 | <0.0001 |
| A | 5.33 | 1 | 5.33 | 254.37 | <0.0001 |
| B | 221.71 | 1 | 221.71 | 10574.13 | <0.0001 |
| C | 19.74 | 1 | 19.74 | 941.37 | <0.0001 |
| D | 1.12 | 1 | 1.12 | 53.53 | <0.0001 |
| AB | 0.016 | 1 | 0.016 | 0.075 | 0.4025 |
| AC | 0.27 | 1 | 0.27 | 12.65 | 0.0032 |
| AD | 0.508 | 1 | 0.508 | 2.75 | 0.1197 |
| BC | 0.063 | 1 | 0.063 | 2.98 | 0.1062 |
| BD | 0.29 | 1 | 0.29 | 13.65 | 0.0024 |
| CD | 9.000E-004 | 1 | 9.000E-004 | 0.043 | 0.8389 |
| A² | 6.03 | 1 | 6.03 | 287.44 | <0.0001 |
| B² | 128.39 | 1 | 128.39 | 6123.24 | <0.0001 |
| C² | 2.96 | 1 | 2.96 | 141.02 | <0.0001 |
| D² | 1.20 | 1 | 1.20 | 57.25 | <0.0001 |
| Residual | 0.29 | 14 | 0.021 | | |
| Lack of Fit | 0.26 | 10 | 0.026 | 3.69 | 0.1098 |
| Pure Error | 0.029 | 4 | 7.170E-003 | | |
| Cor Total | 378.76 | 28 | | | |
significant effect \((p < 0.01)\).

The three-dimensional curved surface and contour plots can be used to intuitively express the effects of any independent variable on dephosphorization rate and significant degree of interaction between every two variables [25]. The three-dimensional curved surface and contour plots about PLA\(_1\) dosage and water volume are shown in Fig. 1 (a), while ultrasonic time and ultrasonic power were at the central level, there was no interaction between PLA\(_1\) dosage and water volume. With increasing PLA\(_1\) dosage, dephosphorization rate increased. It can be attributed to the cavitation effect produced by ultrasound [26]. Wang et al. [27] found that the molecular structure of the free enzyme changed after ultrasonic treatment, which made the enzyme easier to attack the substrate in the hydrolysis process. Under the optimal ultrasonic treatment conditions, enzyme activity increased by 18.17% compared with the control. Differently, with the increase of water volume, dephosphorization rate increased and then gradually decreased. The peak appeared at 2.8 mL/100 g. Phospholipid absorbs more water in oil, so that colloid can be fully expanded, condensed and precipitated to be removed, which made dephosphorization rate increased firstly. However, too much water may exceed the maximum absorption capacity of phospholipids. Phospholipids with strong amphiphilic properties were easy to produce emulsification, which led to difficulties in separating phospholipids from oil [28]. On the other hand, excessive water caused the pH of reaction system deviated from the optimum range of PLA\(_1\), which reduced its activity. Fig. 1(b) showed the three-dimensional curved surface and contour plots based on changed PLA\(_1\) dosage and ultrasonic time at constant water volume and ultrasonic power (2.5 mL/100 g and 126 W, respectively). When PLA\(_1\) dosage was fixed, dephosphorization rate first increased and then tended to be flat with the extension of ultrasonic time. It is generally known that non-hydrated phospholipids are dissolved in the oil phase, while PLA\(_1\) is dissolved in the water phase. Consequently, the degumming reaction between these two took place at the water/oil interface. Ultrasound treatment can promote two-phase emulsification and increase the specific surface area. Moreover, the cavitation effect of ultrasound facilitated the conversion of non-hydrated phospholipids to hydrophilic phospholipids, making the removal of them more easily. With the extension of time, the dissolution of phospholipids gradually reached
transition stage and slow stage, and the reaction reached dynamic equilibrium. More and Gate [18] investigated the effect of enzymatic time on EOD using the ultrasonic horn at 34 °C of temperature, 3.0 mL/L enzyme loading and 6.75 of pH, and reported that enzymatic time from 10 to 80 min resulted in an increase in EOD, and it kept about 93.45% at 80 ~ 120 min. Furthermore, the comparative studies of EOD using conventional mechanical stirring based approach (82.04%) revealed better yield for the approach of combination of enzyme with ultrasound. Borah et al. [29] concluded that using ultrasonic instead of mechanical stirring can enhance kinetics of enzymatic hydrolysis by ~ 10-fold. There was an interaction between the dosage of PLA dosage and ultrasonic time, which was coincident with the results in Table 2. Similarly, interaction also acted between water volume and ultrasonic power (Fig. 1(c)). At the same water volume, depolarization rate increased with the increase of ultrasonic power. It might be attributed to a finer dispersion of water and enzyme in the oil phase with higher ultrasonic power. [30,31]. Fig. 1(c) exhibited that setting water volume and ultrasonic time at the central level, PLA dosage and ultrasonic power had no interaction. Similar results also could be observed in Fig. 1(d) and Fig. 1(e), there was no direct interaction between water volume and ultrasonic power or ultrasonic time.

The optimal parameters of UAEDO obtained by Design Expert software was as follows: PLA dosage was 500 U/kg, water volume was 2.8 mL/100 g, ultrasonic time was 120 min, and ultrasonic power was 136.9 W. In order to verify the optimal conditions, it was slightly modified to PLA dosage of 500 U/kg, water volume of 2.8 mL/100 g, ultrasonic time of 120 min, and ultrasonic power of 135 W. The depolarization rate revealed 98.82%, which was close to predicted value (98.80%), indicating that the model could predict the actual depolarization rate of degumming process. The phosphorus content of degumming oil was 4.79 mg/kg, which was significantly lower than that of non-ultrasonic assisted enzymatic degumming oil (17.98 mg/kg).

3.2. Fatty acid composition

The fatty acid composition (FAC) of ARA oils are shown in Table 4. In this study, eleven different fatty acids were identified. The FAC of crude oil (CO) was practically similar to enzymatic degumming oil (EDO) or ultrasonic assisted enzymatic degumming oil (UAEDO), which indicated that degumming process had little effect on FAC. Li et al. [32] also confirmed that the FAC in the refined oil was basically unchanged. In all samples, arachidonic acid (C20:4) was the most abundant fatty acid (50.97% ~ 52.40%), which was consistent with the report of Wang et al. [33]. Eicosapentaenoic acid (EPA, C20:5) was the second main fatty acid found higher values from 8.23% to 8.94%. EPA is a ω-3 class polyunsaturated fatty acid [34] that can protect the nervous system by regulating the differentiation of neural stem cells [35], promoting synaptogenesis [36] and regulating the balance of inflammation [37]. Although linolenic acid can be converted into EPA in human body, the reaction speed is very slow and the amount of conversion is very small, which is far from meeting the needs of EPA, so it must be directly supplemented from food [38]. In addition, samples were rich in oleic acid (C18:1, 5.53% ~ 5.88%), linoleic acid (C18:2, 5.81% ~ 5.96%) and eicosatrienoic acid (C20:3, 5.40% ~ 5.64%).

Unsaturated fatty acids (UFAs) dominated (83.80% ~ 84.00%) in all ARA oils. Monounsaturated fatty acids (MUFAs) constituted 6.03% ~ 6.32%, mainly oleic acid (C18:1), which accounted for ~ 5.88%. The content of docosaoenoic acid (C22:1) was less than 0.51%; polyunsaturated fatty acids (PUFAs) were ARA, EPA, linoleic acid, eicosatrienoic acid and linolenic acid (C18:3), the content was 77.48% ~ 77.83%. The saturated fatty acids (SFAs) such as palmitic (C16:0), stearic acid (C18:0) and behenic acid (C22:0) contributed 7.67% ~ 8.00%, 7.27% ~ 7.55% and 0.78% ~ 0.90%. The lipid biosynthesis in Mortierella alpine with high content of UFAs, especially ARA and EPA, may be used as functional food.

3.3. Volatile flavor compounds

As shown in Table 5, volatile flavor compounds were detected in ARA crude oil and degummed oils, including aldehydes, alcohols, acids, ketones, hydrocarbons and a small amount of heterocyclic compounds. 46 volatile compounds were detected in ARA crude oil, of which aldehydes, acids and alcohols were the major components and the relative proportion were 31.57%, 22.51% and 20.36%, respectively. Most aldehydes were the decomposition products of peroxides formed after oxidation of lipid oxidation [39], and had a significant effect on the flavor of oils. There were 14 kinds of aldehydes in crude oil, among which hexanal (14.51%), (E,E)-2-octenal (4.08%), (E)-2-heptanal (3.07%), benzeneacetaldhyde (2.06%), benzaldehyde (1.59%), nonanal (1.47%), (E)-2,4-decadienal (1.36%) and (E)-2-nonenal (0.83%) were relatively high in oil. Acids were primarily formed from lipase hydrolysis, alcohol dehydrogenation and aldehyde dehydrogenation. From high to low, the content of volatile acids in CO was acetic acid, hexanoic acid, propionic acid, isobutyric acid and valeric acid. Moreover, there are many kinds of alcohols in CO, among which unsaturated alcohol 1-pentanol (2.11%) and saturated alcohol 1-pentanol (2.11%). One of the main formation pathways was the peroxide reaction of n-3 and n-6 unsaturated fatty acids [40]. Comparatively, 45 volatile components of the following chemical classes were identified from EDO: aldehydes (fourteen), acids (four), ketones (three), hydrocarbons (eight) and others (six). 43 kinds were detected in UAEDO, including 10 aldehydes, 10 acids, 6 alcohols, 1 ketone, 10 hydrocarbons and 6 others. Similar to crude oil, aldehydes, acids and alcohols were the main flavor components. After degumming, the proportions of hexanal, nonanal, (E)-2-nonenal, (E,E)-2,4-decadienal and (E)-2-nonenal were decreased, and the total aldehydes were decreased by 4.25% (EDO) and 7.32% (UAEDO), respectively. On the contrary, acids and alcohols increased by 1.43% and 0.93% and 6.63% and 5.81% respectively.

More than content, threshold of volatile components contributed to flavor. The threshold of aldehydes was comparatively lower than that of ketones, alcohols and other compounds [41]. The saturated like aldehydes, hexanal and nonanal presented unpleasant grass and pungent flavor [42,43]; (E,E)-2,4-octadienal presented cucumber flavor [44]; (E), (E)-2,4-heptadienal, (E,E)-2,4-decadienal and (E)-nonanal presented fishy odor [44,46]. Aromatic aldehydes such as benzaldehyde had bitter almond flavor. The above may be the reason why ARA oil was
appearance with fishy and irritating smell. Acids with low threshold can have certain effect on the flavor of oil, conversely, that of saturated acids was higher, and made little contribution [47]. In the same vein, the contribution of ketones and hydrocarbons were negligible.

3.4. Thermal and oxidative stability

Table 6 shows the results of thermogravimetric analysis of ARA oils, in which six critical temperature values for thermal stability evaluation can be obtained: initial decomposition temperature (To), maximum weight loss rate temperature (Tmax) and temperature at 5%, 10%, 50% and 90% weight loss (T90%) (expressed by T95%, T10%, T50%, T90%, respectively). UAEDO revealed the highest initial decomposition temperature of 287.83 °C, which was significantly higher than EDO (281.33 °C) and CO (273.50 °C). The 90% mass loss of UAEDO occurred at 452.50 °C, followed by EDO at 451.67 °C and CO 453.50 °C. Similarly, it was presented other critical temperatures of UAEDO were all higher than CO and EDO. Overall, UAEDO had the strongest thermal stability, followed by EDO, and CO was the worst, which suggested enzymatic degumming, especially ultrasonic-assisted enzymatic degumming, can effectively improve the thermal stability of oil. The thermal decomposition of oil was attributed to volatilization of polyunsaturated fatty acids, followed by monounsaturated and saturated fatty acids [48].

The oxidative stability of the oils was evaluated by measuring their oxidation induction time with the Rancimat test (Fig. 2). The oxidation induction time values for CO, UAEDO and EDO were 27.6, 10.8 and 10.8 min, respectively, which were approximate with DHA algal oil (0.25 h) [49] and significantly shorter than those of rapeseed oil (11.8 ~ 21.1 h) [24], yellow horn seed oil (7.63 ~ 11.36 h) [50] and tiger nut oil (6.64 ~ 12.37 h) [21]. Obviously, the ARA oils presented poor oxidative stability. Therefore, it is necessary to add antioxidants or microencapsulate the oil to improve their stability in the process of processing, storage or preservation. Moreover, the oxidative stability of the crude oil was significantly higher than the other two degummed oils. It was probably own to the high content of phospholipids in crude oil. On the one hand, phospholipid can chelate metal ions to inhibit lipid oxidation due to the negative charge of phospholipid group [51]. On the other hand, phospholipids contain free amino groups, which can react with lipid oxidation products by Maillard reaction to produce antioxidants such as dihydroxyproline phospholipids [52].

4. Conclusion

In this experiment, ultrasound assisted enzymatic degumming process of ARA oil was established with response surface methodology. Results showed the optimal conditions were as follows: PLA dosage of 500 U/kg, water volume of 2.8 mL/100 g, ultrasonic time of 120 min, and ultrasonic power of 135 W. The dephosphorization rate was found to be significantly higher than that of ED. Moreover, ED or UAEDO showed negligible effect on fatty acid compositions, but had significant effect on the decrease of major volatile flavor components (including aldehydes, acids and alcohols). Evaluation of sample stability presented that EDO and UAEDO had the similar oxidative stability. UAEDO had the uppermost thermal stability as compared with EDO and CO. Thus, ultrasound assisted enzymatic degumming was an efficient method for the degumming of ARA oil with higher dephosphorization rate, and it appeared great potential for application in oil refining process.

| Component | CO | UAEDO | EDO |
|-----------|----|-------|-----|
| Aldehydes |    |       |     |
| hexanal   | 31.57 | 24.25 | 27.32 |
| octanal   | 14.51 | 13.40 | 14.13 |
| nonanal   | 0.38  | 0.21  | 0.21 |
| butanal   | 1.47  | 0.31  | 0.50 |
| 1,1-eicosane | 0.53 | 0.00  | 0.26 |
| 5-methyl-2-furanaldehyde | 0.00 | 3.05  | 1.57 |
| 2-burnyl-2-octenal | 0.83 | 1.01  | 0.85 |
| 3-(3,4-dicarboxyphenyl)propanal | 0.16 | 0.21  | 0.20 |
| 2-pyridinecarboxaldehyde | 0.00 | 1.16  | 1.06 |
| 2-heptenal | 3.07  | 0.00  | 1.81 |
| 2-octenal  | 4.08  | 0.20  | 2.03 |
| benzeneacetaldehyde | 0.83 | 0.00  | 0.00 |
| Ketones   |    |       |     |
| 1-pentanol | 20.36 | 26.17 | 26.99 |
| 1,1-eicosane | 0.21 | 2.12  | 2.00 |
| 1-octen-3-ol | 12.16 | 11.11 | 11.53 |
| 2-ethyl-1-hexanol | 0.12 | 0.09  | 0.00 |
| 2-furanmethanol | 0.00 | 3.64  | 2.60 |
| 5-methyl-2-furanaldehyde | 0.08 | 0.23  | 0.28 |
| Picolon   | 1.76  | 0.14  | 0.18 |
| benzaldehyde | 1.45  | 1.53  | 1.45 |
| Phenylethyl alcohol | 0.58 | 0.78  | 0.72 |
| 1-hexanol  | 0.00  | 0.00  | 1.27 |
| Dimethylethanol | 0.61 | 0.00  | 0.00 |
| 3,5-dioxan-2-ol | 0.00 | 1.37  | 1.62 |
| Acids     |    |       |     |
| Acetic acid | 22.51 | 23.44 | 23.94 |
| Propanoic acid | 1.80 | 2.95  | 3.42 |
| Butanoic acid | 0.00 | 0.79  | 0.00 |
| Hexanoic acid | 31.57 | 24.25 | 27.32 |
| Ketones   |    |       |     |
| 2-undecanone | 0.12 | 0.14  | 0.15 |
| Aceton     | 0.00  | 0.00  | 0.00 |
| 2,2-dihydroxy-3,3,5-trimethyl-3-cyclohexene-1-one | 0.00 | 0.00  | 0.00 |
| 3-octen-2-one | 0.00 | 3.61  | 2.60 |
| 3-methyl-1,2-cyclopentanediene | 0.36 | 0.00  | 0.00 |
| Hydrocarbons |    |       |     |
| Benzocyclooctene | 17.50 | 20.50 | 13.03 |
| Tetracosen | 8.83  | 8.41  | 7.89 |
| Octamethylcyclotrisiloxane | 1.19 | 0.00  | 0.00 |
| Nonadecane | 2.64  | 1.96  | 1.14 |
| Eicosane | 2.64  | 0.34  | 0.12 |
| 1,1′-[3-(2,3-dihydroxypropyl)phenyl]bis-cyclopentane | 0.52 | 2.43  | 1.46 |
| 1,2,3-trimethylbenzene | 0.12 | 2.27  | 0.00 |
| Mesitylene | 0.00  | 0.43  | 0.00 |
| 1,3-dimethylbenzene | 0.78 | 1.19  | 0.00 |
| 2-ethyl-2-methyl-1,3-hexadiene | 0.54 | 0.44  | 0.00 |
| 1-undecene | 0.00  | 0.92  | 1.00 |
| 1-dodecene | 0.00  | 0.08  | 0.54 |
| 1-tridecene | 0.00 | 0.00  | 0.59 |
| 2,6-di-tert-butylphenol | 6.29 | 5.51  | 6.84 |
| MTalol | 0.05  | 0.08  | 0.17 |
| 2-ethyl-3,5-dimethylpyrazine | 0.05 | 0.08  | 0.17 |
| 2-furanmethanol | 0.18 | 0.00  | 0.00 |
| 1-(3-hydroxy-2-furyl)ethanone | 0.24 | 0.32  | 0.30 |
| 2,4-diacetylpyridine | 0.57 | 2.49  | 2.66 |
| Butylatedhydroxytoluene | 0.10 | 0.17  | 0.18 |
| 2,4-di-tert-butylphenol | 0.00 | 2.45  | 2.84 |
| Malol | 0.56  | 0.00  | 0.69 |

Table 6 Results of thermogravimetric analysis of ARA oils.

| Component | CO | UAEDO | EDO |
|-----------|----|-------|-----|
| T95%/°C  | 324.00 | 343.67 | 398.50 |
| T10%/°C  | 423.50 | 273.50 | 386.67 |
| T50%/°C  | 451.67 | 281.33 | 414.17 |
| T90%/°C  | 453.50 | 281.33 | 414.17 |
| Tmax/°C  | 386.67 | 414.17 | 415.50 |
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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[16] T. Guo et al. Fig. 2. The oxidation induction time of ARA oils.

CRediT authorship contribution statement

Tingting Guo: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft. Chuyun Wan: Resources, Methodology, Funding acquisition, Writing - review & editing, Supervision. Fenghong Huang: Project administration, Funding acquisition. Chunlei Wei: Investigation. Xia Xiang: Investigation.
