

Supporting information

Enzymatic Degumming of Arachidonic Acid Oil Using Immobilized Phospholipase A1 on Hollow Double-Layer Mesoporous Silica Nanoparticles

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1. Supplementary methods:

1.1. Determination of the Enzyme Immobilization Amount

According to the method reported previously (Li, et al., 2023). The immobilization amount of HdlMS was determined using a BCA kit. The immobilization amount was calculated by measuring the protein concentrations in the enzyme solution and the supernatant after immobilization. The specific operations are as follows: In a 96-well plate, add PBS buffer (10 μ L, 50 mmol/L, pH = 7.0) and the enzyme solution to be tested (10 μ L). Then add the BCA working solution (200 μ L), incubate at 37 °C for 30 minutes, and finally measure the protein content at 256 nm. The immobilization amount is calculated using the following formulas.

$$\text{Immobilization amount} = \frac{(C_0 - C)V}{m}$$

Where:

C_0 is the initial protein concentration of the lipase solution (mg/mL),

C is the protein concentration of the supernatant obtained by centrifugation after the immobilization of the lipase (mg/mL),

V represents the volume of the protease solution added (mL),

m is the mass of the immobilized carrier (g)

1.2. Determination of Enzyme Activity

To determine the activity of the immobilized phospholipase, referring to the reference method (Wang, et al., 2024). Egg yolk lecithin (10 g) was dispersed in a citric acid buffer solution (100 mL, 0.01 mol/L, pH 6.0). Then, it was mixed with a 0.5% polyvinyl alcohol solution at a volume ratio of 1:4 for emulsification. The emulsion was combined with the citric acid buffer solution at a ratio of 1:1 to form the substrate solution. 15 mL of the substrate solution was added to each of four 50 mL conical flasks and pre-heated in a water bath at a predetermined temperature for 5 minutes. 50 mg of the immobilized enzyme was added to two of the flasks as samples, while the other two served as controls without the enzyme. The reaction was carried out in a constant-temperature water bath for 10 minutes. Subsequently, 15 mL of 95% ethanol was added to terminate the reaction. Phenolphthalein was added to the flasks, and the reaction mixture was titrated with a standard sodium hydroxide solution (0.05 mol/L). The average consumption of the alkali was calculated to represent the enzyme activity. The enzyme activity was expressed as the number of micromoles of sodium hydroxide consumed per minute, which was equivalent to the release of 1 millimole per liter of fatty acid per minute under the assay conditions.

$$\text{Calculation formula: } X = \frac{50(V - V_0)cn}{(0.05t)}$$

Where:

X : the enzyme activity (U),

V : the volume of the NaOH standard solution consumed during the titration of the sample (mL),

V_0 : the volume of the NaOH standard solution consumed during the titration of the blank (mL),

C : the concentration of the NaOH standard solution (mol/L),

N : the dilution factor of the enzyme solution sample;

T : the reaction time for measuring the enzyme activity (min).

1.3. Determination of Phosphorus Content

According to the method described in the literature (Eddehech, et al., 2023), the phosphorus content of the oil and fat was determined: Accurately weigh 3 g of oil and 0.5 g of

zinc oxide and place them in a crucible. First, completely carbonize them on an electric furnace. Subsequently, put the crucible into a muffle furnace and burn it at 580 °C for 3 hours until it is completely ashed. Take out the crucible and, after it cools down to room temperature, dissolve the ash with 10 mL of hydrochloric acid solution and heat it to a slightly boiling state. Stop heating after 5 minutes. When the solution cools down to room temperature, carry out the filtration operation, and rinse the crucible and the filter paper three times with hot water at the same time. Transfer the filtrate to a 100 mL volumetric flask, add potassium hydroxide solution to it for neutralization until the solution becomes turbid. Then, slowly add hydrochloric acid dropwise until the zinc oxide precipitate is completely dissolved, and add 2 more drops of hydrochloric acid. Finally, after the solution cools down to room temperature, make up the volume to the mark line and shake it well. Use a pipette to accurately suck 10 mL of the solution to be tested and add it to a colorimetric tube. Add 8 mL of hydrazine sulfate solution and 2 mL of sodium molybdate solution in sequence, and shake well. Put the colorimetric tube into a boiling water bath and heat it for 10 minutes. Take it out and cool it down to room temperature, then dilute it to the mark line with water and let it stand for 10 minutes. Transfer the solution to a dry and clean colorimetric cuvette, and measure its absorbance at a wavelength of 650 nm using an ultraviolet spectrophotometer. Each group of experiments is repeated for measurement three times.

1.4. Determination of fatty acid composition of ARA oil

The method for determining the fatty acids of ARA oil is as follows: Dissolve 10 µL of the oil sample in 2 mL of n-hexane. Subsequently, add 0.5 mL of a 0.5 mol/L potassium hydroxide-methanol solution as the methyl esterification reagent. Vortex-mix for 1 minute to ensure thorough mixing. Conduct the methylation at room temperature for 10 minutes. Carefully aspirate the upper-layer n-hexane solution using a pipetting tool and transfer it into a sample vial containing a small amount of anhydrous sodium sulfate to absorb the excess water in the solution. Filter the obtained supernatant through a 0.22-µm organic membrane filter, and then analyze the fatty acid composition by a gas chromatography analyzer. The detection scheme for fatty acid methyl esters is as follows: Use an Agilent 7890A system, which is a gas chromatograph (GC, Shimadzu, Japan) equipped with a flame ionization detector (FID). Determine the fatty acid composition according to the retention time of the fatty acid methyl ester standards, and calculate the content of each fatty acid by the peak area normalization method.

2. Supplementary Figure

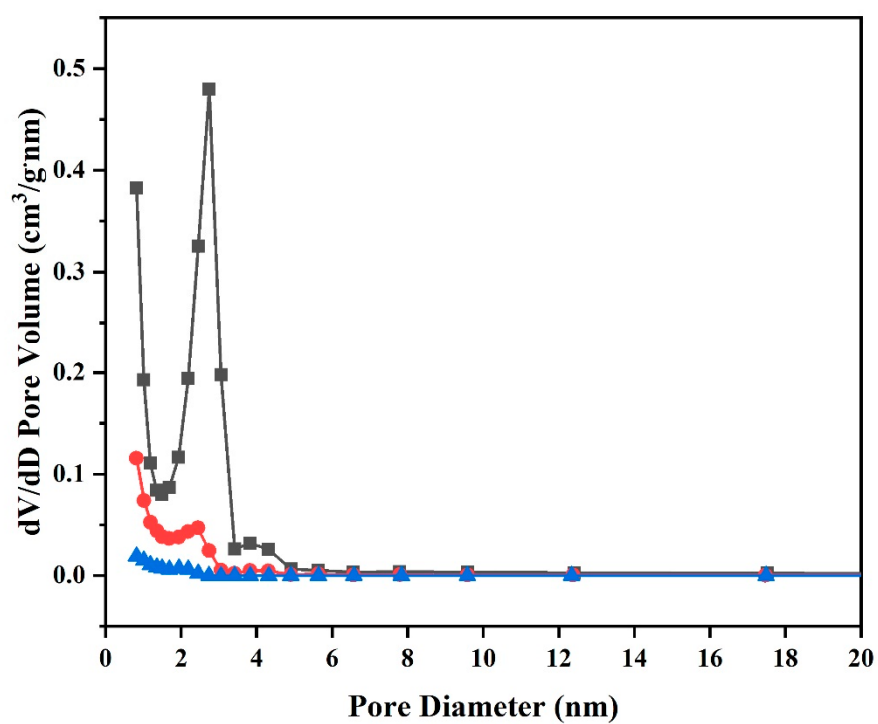


Figure S1. Pore size distribution of HdlMS, $\text{NH}_2/\text{C}_8\text{-HMSS}$, and $\text{PLA1@NH}_2/\text{C}_8\text{-HdlMS}$.

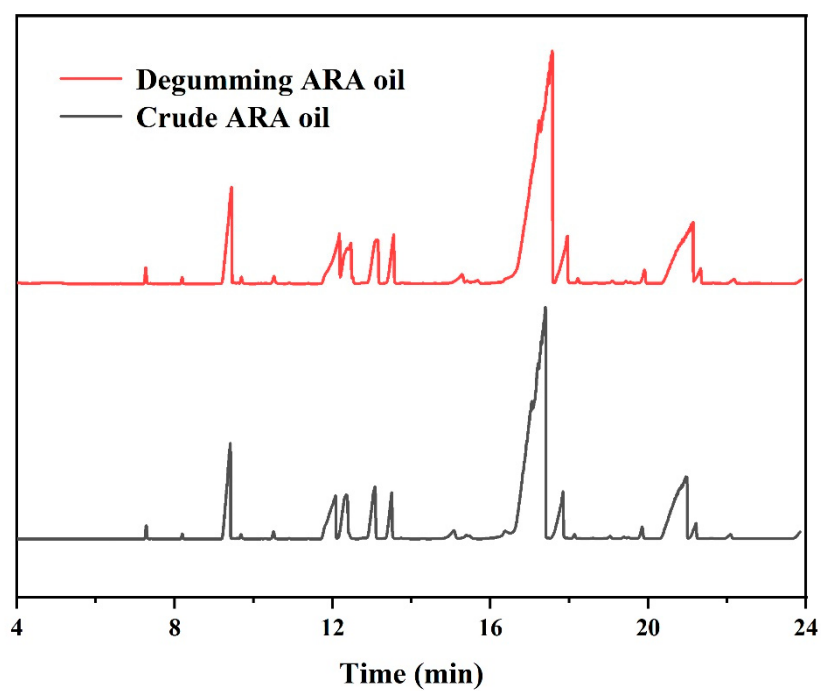


Figure S2. Gas chromatograms of fatty acid methyl esters of ARA oil before and after degumming

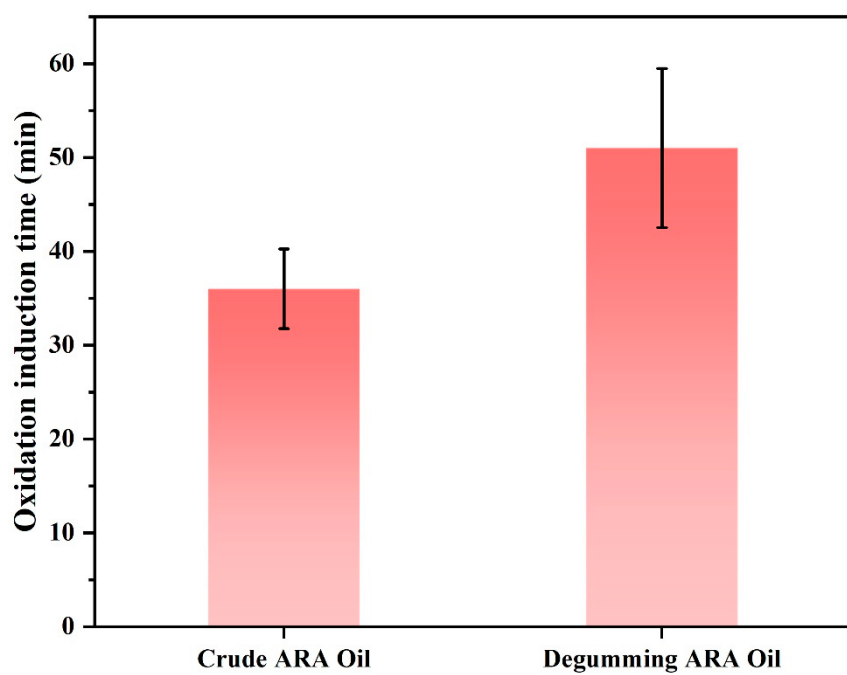


Figure S3. Changes in the oxidation induction time before and after degumming

3. Supplementary Tables

Table S1. The specific surface area, pore size distribution, and pore volume of HdIMS, NH₂/C₈-HdIMS, and PLA1@NH₂/C₈-HdIMS.

Specimen	Surface Area (m ² /g)	Pore Diameter (nm)	Pore Volume (cm ³ /g·nm)
HdIMS	1158.3	2.74	0.808
NH ₂ /C ₈ -HdIMS	129.7	2.44	0.147
PLA1@NH ₂ /C ₈ - HdIMS	1.8	-	0.004

Table S2. The elemental composition and content of HdIMS, NH₂/C₈-HdIMS and PLA1@NH₂/C₈-HdIMS.

Element (Atomic %)	C	N	O	Si
HdIMS	16.37	0.45	57.86	25.33
NH ₂ /C ₈ -HdIMS	33.86	2.13	44.34	19.67
PLA1@NH ₂ /C ₈ - HdIMS	52.65	10.23	30.84	6.28

Reference

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- Li, Z.; Zhou, H.; Liu, X.; Wang, W.; Lan, D.; Wang, Y. A novel thermo-responsive phospholipase a1 with high selectivity and efficiency in enzymatic oil degumming. *Food Chem.* **2024**, 456, 139624.