



# A comprehensive foodomics analysis of rambutan seed oils: Focusing on the physicochemical parameters, lipid concomitants and lipid profiles

Jingtao Cui, Siqi Zhao, Yanchi Zhou, Tian Li, Weimin Zhang\*

School of Food Science and Engineering, Hainan University, Haikou 570228, China

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## ABSTRACT

A foodomics approach was employed to systematically characterize and compare the quality parameters, antioxidant activity, minor-components, fatty acid composition, and lipid profiles of the seed oils from the three most popular rambutan varieties in China. The total lipid content ranged from 23.40 to 25.77 g/100 g. The fatty acids 9cC18:1 (39.84%–40.92%) and C20:0 (28.45%–30.23%) were identified as the dominant ones, which are uncommon among higher plants. All oil samples exhibited low AI and TI values. BR-7 exhibited the highest levels of squalene (21.48 mg/kg), cholesterol (144.43 mg/kg), and tocopherol (17.42 mg/kg), and the lowest levels of polyphenols (24.21 mg GAE/kg). Additionally, a total of 807 lipid species were identified, with TAG, DGTS, and PE being the predominant ones. Multivariate statistical analyses revealed significant variations in lipid profiles among the varieties, particularly in glycerophospholipids and sphingolipids. Fifty-seven distinct lipids were identified as potential markers for distinguishing between rambutan varieties. Furthermore, a hypothetical scenario was developed by linking relevant lipid metabolism pathways. These findings establish a theoretical framework for comprehending rambutan seed oil in depth and unlocking its high-value potential.

## 1. Introduction

The rambutan (*Nephelium lappaceum* L.), a significant tropical fruit, belongs to the Sapindaceae family and the genus *Nephelium*, predominantly found in Southeast Asia. Thailand, Malaysia, Indonesia, and the Philippines are the primary producers and exporters of rambutan (Hernández-Hernández et al., 2019). Additionally, small-scale cultivation occurs in Hainan Province, China, and Hawaii, USA. Due to its exotic appearance, refreshing taste, and superior nutritional value, rambutan enjoys significant popularity among consumers. In addition to being consumed fresh, rambutan fruit is frequently processed into juices, wines, canned goods, and jams to prolong its shelf life and consumption opportunities. As the consumption primarily targets the pulp, which accounts for approximately 40% of the total fresh fruit weight, substantial agro-industrial byproducts are produced during processing. Among these by-products, seeds constitute the second major component, aside from the peel, representing approximately 6% of the total fruit weight (Hernández-Hernández et al., 2019). Statistical data indicate that in 2017, Thailand alone disposed of an average of 1900 tons of rambutan seeds annually as waste (Mahisanunt et al., 2017). Similar practices occur in other rambutan-growing countries, posing significant

challenges related to resource wastage and environmental pollution. Therefore, it is crucial to utilize rambutan seeds efficiently.

Given the global emergence of green and sustainable concepts in recent years, numerous researchers have directed their focus towards rambutan seeds (Jahurul et al., 2020). Lipids represent a crucial nutrient component in rambutan seeds, constituting approximately 14.7%–41.3% of the dry weight basis (Jahurul et al., 2020; Solís-Fuentes et al., 2010). Apart from providing energy, dietary lipids supply essential fatty acids, aromatic substances, and various functional components, playing a vital role in the metabolism of living organisms. Utilizing CiteSpace software, we visualized and analyzed the research conducted on rambutan seed lipids over the past five years (Fig. 1). Presently, research on rambutan seed lipids has primarily centered on their physicochemical properties and fatty acid composition. However, there is limited information available on their comprehensive lipid profile, notably glycerolipids (GL), glycerophospholipids (GP), and trace lipids such as saccharolipids (SL) and sphingolipids (SP), which also serve crucial regulatory functions in physiological metabolism. Furthermore, the levels of lipid concomitants in rambutan seed oil, including sterols and tocopherols, are equally significant factors influencing its nutritional value (Jahurul et al., 2020). One previous study by Soeng et al. (2015)

\* Corresponding author.

E-mail address: [zhwm1979@163.com](mailto:zhwm1979@163.com) (W. Zhang).

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demonstrated the inhibitory effects of rambutan seed extract on alpha-glucosidase activity and triacylglycerols (TAG) levels, indicating its potential for preventing and treating Type 2 diabetes. Hence, it is imperative to conduct a more comprehensive characterization of the lipid concomitant profiles and lipid profiles of rambutan seed oils. This will contribute to a deeper understanding of the nutritional value of rambutan seed oils.

With the advancements in high-capacity chromatography and high-resolution mass spectrometry, lipidomics methodology has emerged. In the past two decades, lipidomics has emerged as the foremost technique for examining the lipid profiles of food matrices, offering a comprehensive and detailed analysis at the molecular level (Sun et al., 2020). Previous studies have confirmed the reliability and potential of lipidomics in characterizing lipid composition in food matrices and monitoring lipid molecule dynamics during food processing and storage (Sun et al., 2020; Tietel et al., 2023; Wu et al., 2021). To our knowledge, there are no relevant literature reports on the application of lipidomics techniques to investigate lipid profiles and lipid concomitants in rambutan seed oil.

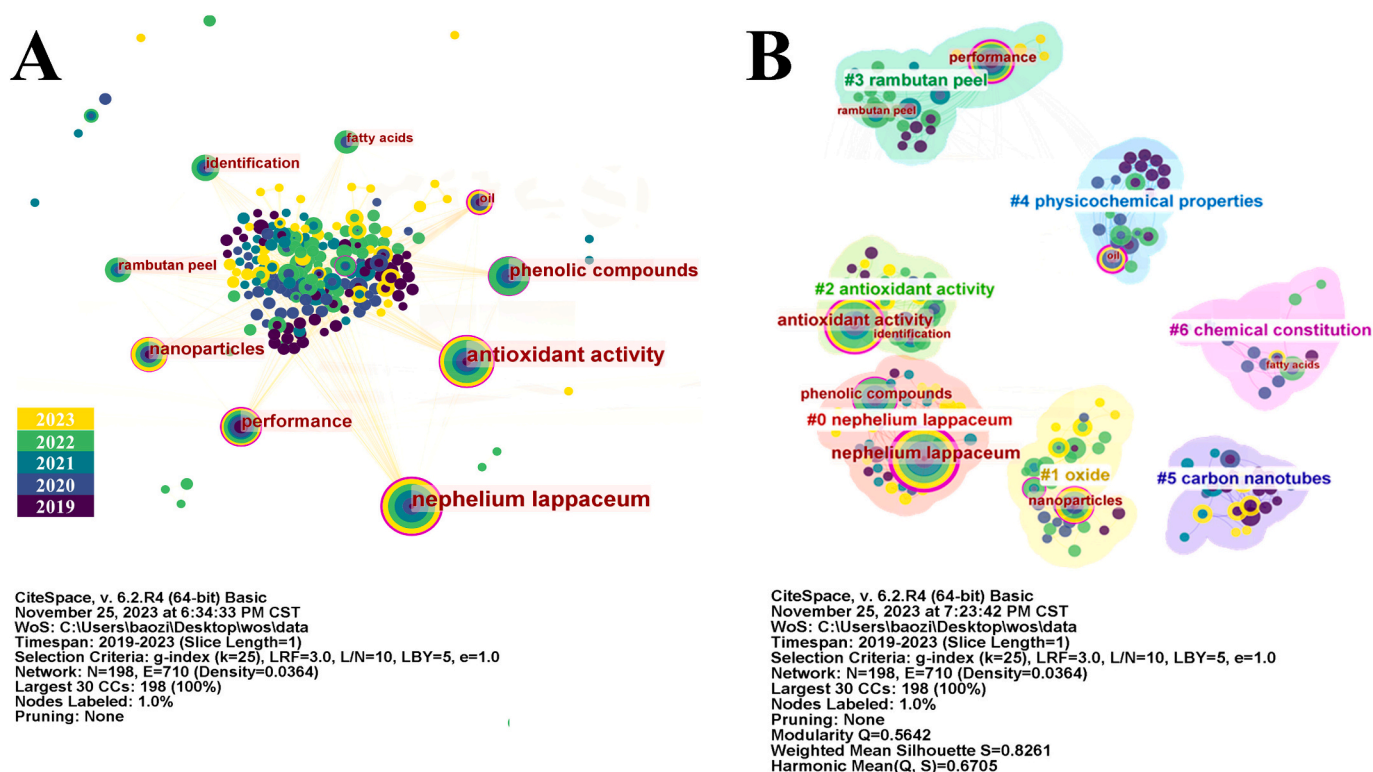
Due to its climate similarity to Malaysia and Thailand, Baoting Li and Miao Autonomous County has become China's primary region for rambutan cultivation. BR-4, BR-5, and BR-7 are favored rambutan varieties in the Chinese market, thus selected as experimental ingredients. The study aimed to preliminarily explore the physicochemical properties, lipid concomitant compositions, and lipid profiles of the seed oils from three rambutan varieties using foodomics techniques. Additionally, chemometric approaches were employed to analyze and compare seed oils from three rambutan varieties, identifying differential lipid molecules and potential lipid markers for discrimination. These findings will enhance understanding of rambutan seed lipid profiles, supplement plant seed lipid databases, and offer theoretical guidance for selecting rambutan seed oil as a food ingredient.

## 2. Materials and methods

### 2.1. Materials and reagents

Three rambutan varieties, BR-4 ( $41.88 \pm 1.54$  g), BR-5 ( $42.04 \pm 1.79$  g), and BR-7 ( $43.12 \pm 2.11$  g), were sourced from the Baoting Rambutan Germplasm Resource Nursery in Hainan Province (latitude:  $18^{\circ}23'18''$  N, longitude:  $109^{\circ}21'109''$  E). All rambutan varieties in the nursery are cultivated and managed to uniform standards. All of the rambutan fruit trees are 11 years old. The harvesting of rambutan was completed on the same day in July 2023. Following harvest, rambutans were promptly transported to the laboratory. The entire fruit was then dissected into pulp, skin, and seeds. The seed portions were dried in a DHG-9070 constant temperature blast drying oven (Shanghai Yilin Scientific Instrument Co., Ltd., Shanghai, China) at  $45^{\circ}\text{C}$  for 24 h. Subsequently, the samples were ground using a DE-100 g grinder (Zhejiang Hongjingtan Industry & Trade Co., Ltd., Zhejiang, China), sieved through a 60-mesh screen, vacuum-sealed, and stored in a  $-20^{\circ}\text{C}$  refrigerator until further analysis.

The fatty acid methyl ester (FAME, GLC-463) standard mixture was procured from NuChek-Prep (Elysian, MN, USA). Methyl tert-butyl ether (MTBE), methanol, acetonitrile, ammonium formate, dichloromethane, isopropanol, and n-hexane were procured from ANPEL Laboratory Technologies (Shanghai) Inc. (Shanghai, China) for lipid extraction and chromatographic analysis. Ethanol, phenolphthalein, potassium hydroxide, potassium iodide, sodium thiosulphate, starch, cyclohexane acetic, Wijs solution, and potassium hydroxide for determining the physicochemical parameters of oils were all obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). For antioxidant analyses, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), trolox, 2,4,6-Triphenyltriazine (TPTZ),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , HCl, sodium acetate buffer solution, potassium persulphate, and 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS), were all obtained from Xilong Scientific Co., Ltd. (Shantou, China). Folin-ciocalteu reagent, gallic acid,  $\text{Na}_2\text{CO}_3$ , acetone,



**Fig. 1.** Current research status on rambutan seed oil. Retrieving the rambutan and seed and lipid at Web of Science. Overlay visualization of the title and abstract in selected 209 references over the past five years (2019–2023). Keyword co-occurrence map (A). Key words Cluster map (B).

cholesterol, 1-methylimidazole, and *N*-methyl-*N*-trimethylsilylheptafluorobutyramide for lipid concomitant assays were all obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Standard substances, including squalene, cholesterol, brassicasterol, stigmasterol,  $\Delta^5$ -avenasterol,  $\beta$ -sitosterol, campesterol, 24-methylene-cycloartanol,  $\alpha$ -tocopherol,  $\delta$ -tocopherol,  $\beta$ -tocopherol, and  $\gamma$ -tocopherol, were procured from Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China).

## 2.2. Total lipid extraction

The MTBE-methanol solvent system was chosen for lipid extraction due to its lower density and toxicity, simplified collection process, and reduced risk of contamination compared to the traditional chloroform-methanol system (Matyash et al., 2008). Precisely 1 g of samples were weighed into centrifuge tubes. Then, 8 mL of distilled water and 19.20 mL of MTBE-methanol solution (5:1, v/v) were added. The samples were vortexed for 3 min, followed by sonication in an ice-water bath for 10 min. This vortex-ultrasound cycle was repeated three times. The mixture was then incubated at  $-40^\circ\text{C}$  for 60 min and subsequently centrifuged at 5000 rpm for 15 min. Finally, the supernatant was collected, and the solvent was removed via rotary evaporation to obtain the total lipids. The recovered lipids were weighed to determine the total lipid yield, sealed, and stored at  $-20^\circ\text{C}$  until further analysis.

## 2.3. Determination of physicochemical parameters of rambutan seed oils

### 2.3.1. Acid value

Determination of acid value was done using the AOAC (2000) method. Briefly, 10 g of the oil sample was mixed with 50 mL of ethanol in a conical flask. The mixture was boiled for 5 min, then 1 mL of phenolphthalein reagent was added. The mixture was subsequently titrated with 0.1 mol/L potassium hydroxide until a slight red coloration indicated the endpoint. The acid value was calculated using the following formula:

$$\text{Acid value (mg KOH/g oil)} = \frac{56.1 \times V \times C}{M}$$

where *V* represents the titration volume of 0.1 mol/L potassium hydroxide, *C* represents the concentration of potassium hydroxide (0.1 mol/L), and *M* represents the weight of the oil sample (g).

### 2.3.2. Peroxide value

The peroxide value was determined according to the official method recommended by AOAC (2000). Briefly, 5 g of the oil sample was mixed with 30 mL of acetic acid-trichloromethane (3:2, v/v) solution in a 250 mL conical flask and shaken vigorously. Then, 1.00 mL of saturated potassium iodide solution was added, and the mixture was shaken and placed in the dark for 3 min, followed by the addition of 100 mL of distilled water. After thorough shaking, the mixture was titrated with 0.01 mol/L sodium thiosulfate solution. When the yellow color disappeared, 1 mL of starch solution was added as an indicator, and titration continued until the blue color disappeared. Blank experiments were conducted without oil samples using the same steps. The peroxide value was calculated using the following formula:

$$\text{Peroxide value (mEq O}_2\text{/kg oil)} = \frac{1000 \times (V - V_0) \times C}{M}$$

where *V* means the titration volume of sodium thiosulphate used for the actual sample (mL), *V*<sub>0</sub> means the titration volume of sodium thiosulphate in the blank experiment (mL), *C* means the concentration of sodium thiosulphate (0.01 mol/L), and *M* means the weight of the oil sample (g).

### 2.3.3. Iodine value

The iodine value was determined according to the official method recommended by AOAC (2000). Briefly, 1 g of oil sample, 15 mL of cyclohexane-acetic acid solution (1:1, v/v), and 25 mL of Wijs reagent were added to a 250 mL conical flask, shaken to homogeneity, and placed in the dark for 1 h. After standing for 10 min, 20 mL of 15% potassium iodide solution and 150 mL of distilled water were added. The shaken solution was then titrated with 0.1 mol/L sodium thiosulfate to a pale-yellow color. Finally, 1 mL of starch solution was added as an indicator, and titration was continued until the blue color disappeared. Blank experiments were conducted without oil samples using the same steps. The iodine value was calculated using the following formula:

$$\text{Iodine value (g I}_2\text{/100g oil)} = \frac{12.6 \times C \times (V_1 - V_2)}{M}$$

where *V*<sub>1</sub> is the titration volume of sodium thiosulphate in the blank experiment (mL), *V*<sub>2</sub> is the titration volume of sodium thiosulphate consumed by the actual sample (mL), *C* is the concentration of sodium thiosulphate (0.1 mol/L), and *M* is the weight of the oil sample (g).

### 2.3.4. Saponification value

The saponification value was determined according to the official method recommended by AOAC (2000). Briefly, 2 g of oil sample and 25 mL of 0.1 mol/L potassium hydroxide-ethanol solution were mixed in a 250 mL conical flask. The mixture was boiled under reflux until a clear solution was observed. It was then titrated with 0.5 mol/L hydrochloric acid using phenolphthalein as an indicator until the pink color disappeared. Blank experiments were conducted without oil samples using the same steps. The saponification value was calculated using the following formula:

$$\text{Saponification value (mg KOH/g oil)} = \frac{56.1 \times (V_3 - V_4) \times C}{M}$$

where *V*<sub>3</sub> represents the titrated volume of hydrochloric acid in the blank solution (mL), *V*<sub>4</sub> represents the titrated volume of hydrochloric acid in the actual sample (mL), *C* represents the hydrochloric acid concentration (0.5 mol/L), and *M* represents the weight of the oil sample (g).

## 2.4. Determination of antioxidant capacity of rambutan seed oils

### 2.4.1. Oxidative-stability index

The oxidative stability index (OSI) of the sample oils was determined using an oil and fat oxidative stability apparatus (892 Professional Rancimat; Metrohm, Herisau, Switzerland). Briefly, 3 g of rambutan seed oil samples were weighed into test tubes equipped with the instrument and analyzed for oxidative induction at a temperature of  $110^\circ\text{C}$  and an air flow rate of 20 L/h. Results were expressed as the induction period and reported in hours.

### 2.4.2. DPPH assay

The DPPH radical scavenging activity of the oil samples was conducted following the method outlined by Gao et al. (2019b). Briefly, 100 mg of the oil sample was mixed with 1.5 mL of methanol in a centrifuge tube and vigorously shaken. The mixture was centrifuged at 5000 rpm for 10 min, and the methanol phase was subsequently collected. A 0.5 mL aliquot of the methanol extract was combined with 0.1 mmol/L DPPH methanol solution (0.25 mL), vortexed, and allowed to stand for 10 min before measuring the absorbance at 515 nm. The results were expressed as  $\mu\text{g Trolox/g oil}$  using a Trolox calibration curve.

### 2.4.3. FRAP assay

The ferric ion reducing antioxidant power (FRAP) assay was performed according to the steps of the Lee et al. (2019). The methanol

extract of rambutan seed oil underwent the same procedure as described in Method 2.4.2. The FRAP reagent was prepared in advance by mixing 20 mmol/L  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 10 mmol/L TPTZ solution in 40 mmol/L HCl, and 0.1 mol/L sodium acetate buffer (pH 3.6) in a 1:1:10 ratio by volume. Combine 0.3 mL of the methanol extract with 2 mL of FRAP reagent, and then adjust the volume to 10 mL with distilled water. Following incubation at 37 °C for 10 min in a water bath, the absorbance was measured at 593 nm. The results were shown as  $\mu\text{g Trolox/g oil}$ .

#### 2.4.4. ABTS assay

The ABTS test was performed following the previously reported methodology (Uoonlue & Muangrat, 2019). The methanol extract of rambutan seed oil was subjected to the procedure outlined in Method 2.4.2. The stock solution of ABTS was prepared by combining 25 mL of 7 mmol/L aqueous ABTS solution with 0.44 mL of 2.45 mmol/L potassium persulfate solution and allowing it to stand for 12–16 h at room temperature. The ABTS working solution was prepared by diluting the ABTS stock solution with methanol. One hundred microliters of the methanol extract were mixed with 1000  $\mu\text{L}$  of the ABTS working solution and allowed to stand for 5 min, followed by recording the absorbance at 734 nm. The results were presented as  $\mu\text{g Trolox/g oil}$ .

#### 2.5. Fatty acid composition analysis of rambutan seed oils

Fatty acid methyl esterification was conducted following a previously established method in our laboratory (Cui et al., 2023). Briefly, 2 mg of the sample was dissolved in 1.5 mL of n-hexane, followed by the addition of 100  $\mu\text{L}$  of sodium methoxide-methanol solution and 40  $\mu\text{L}$  of methyl acetate. After reacting at room temperature for 20 min, the mixture was chilled at  $-20$  °C for 10 min. Next, 60 mL of oxalic acid solution was added, and the mixture was centrifuged at 4200 rpm for 10 min. The supernatant was passed through an anhydrous sodium sulfate column to eliminate water and dried under nitrogen to obtain fatty acid methyl esters. The methyl esterified sample was then dissolved in 1 mL of n-hexane in the injection vial.

Fatty acid separation was conducted using a Mode 7890 A gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a flame ionization detector and a CP-Sil 88 capillary column (100 m  $\times$  0.25 mm, 0.20  $\mu\text{m}$ , Agilent, USA). The heating protocol was as follows: the initial temperature was 45 °C for 4 min, then raised to 175 °C at a rate of 13 °C/min and maintained for 27 min, followed by an increase to 215 °C at a rate of 4 °C/min and held for 35 min. The injector temperature was set at 250 °C, and the injection volume was 1  $\mu\text{L}$ . Qualification involved comparing the retention times of the sample peaks with those of standards, and quantification of fatty acids (expressed as % of total fatty acids) was accomplished using peak area normalization.

The nutritional value of fatty acids was further evaluated by calculating the atherosclerotic index (AI) and thrombotic index (TI) using the calculation method described by Chen and Liu (2020).

$$\text{AI} = (\text{C12} : 0 + (4 \times \text{C14} : 0) + \text{C16} : 0) / (\text{monounsaturated fatty acids (MUFA)} + \text{polyunsaturated fatty acids (PUFA)})$$

$$\text{TI} = (\text{C14} : 0 + \text{C16} : 0 + \text{C18} : 0) / ((0.5 \times \text{MUFA}) + (0.5 \times n - 6 \text{ PUFA}) + (3 \times n - 3 \text{ PUFA}) + (n - 3 \text{ PUFA}/n - 6 \text{ PUFA}))$$

#### 2.6. Polyphenol content determination of rambutan seed oils

The total polyphenol content was determined according to the method of Xu et al. (2022). Briefly, 2.0 g of the oil sample was dissolved in 5 mL methanol-water solution (7,3, v/v) and centrifuged at 5000 rpm for 25 min. This extraction process was repeated three times. The supernatant was collected and combined, stored in a brown vial, and was

volume-filled to 10 mL with methanol to obtain the test solution. Mix 3 mL of the test solution with 0.5 mL of folin-ciocalteu reagent and 1 mL of 10% sodium carbonate solution in a 10 mL brown volumetric flask, and then bring the volume to 10 mL using distilled water. After incubating the working solution in the dark for 2 h, the absorbance was measured at 765 nm. Gallic acid served as the standard, and the polyphenol content was expressed as milligrams of gallic acid equivalent per 100 g of oil.

#### 2.7. Squalene and phytosterol determination in rambutan seed oils

Squalene and phytosterol content were determined following the method outlined by Yang et al. (2019). Simply, 50 mg of oil sample, 200  $\mu\text{L}$  of cholesterol solution (100  $\mu\text{g/mL}$ ) as an internal standard, and 5 mL of potassium hydroxide-ethanol solution (2 mol/L) were mixed and vortexed. The mixture was then saponified by incubating at 75 °C for 30 min. After cooling, 2 mL of distilled water and 5 mL of n-hexane were added to the centrifuge tubes. The mixture was centrifuged at 4000 rpm for 10 min to collect the upper n-hexane layer containing the target substance. The residue was then extracted three times with n-hexane, and the supernatants were combined and dried under nitrogen. The dried extract was then dissolved in the derivatization solution (1-methylimidazole/N-methyl-N-trimethylsilylheptafluorobutyramide, 95:5, v/v), vortexed, shaken, and incubated at 75 °C for 30 min. After cooling, the volumes were adjusted to 1 mL using n-hexane. Squalene and phytosterol standards were diluted with acetone to prepare standard solutions and curves, respectively.

A TQ-8040 gas chromatograph-mass spectrometer (Shimadzu Corp., Kyoto, Japan) equipped with a DB-5 MS capillary column (0.25 mm i.d.  $\times$  30 m length, 0.25  $\mu\text{m}$  film, Agilent, USA) was utilized for analysis. Helium was used as the carrier gas with a flow rate of 1.2 mL/min. The injection volume was 1  $\mu\text{L}$  with a split ratio of 20:1. The column oven was programmed with a starting temperature of 100 °C held for 1 min, then ramped up to 290 °C at 40 °C/min and held for 15 min. Mass spectrometry parameters included selected ion monitoring, an ion source temperature of 250 °C, a transmission line temperature of 290 °C, and an ionization voltage of 70 eV. Components were characterized by comparing the mass spectral retention times and characteristic peaks with corresponding standards. Squalene and phytosterol contents were quantified using standard curves obtained by the external standard method.

#### 2.8. Tocopherol determination in rambutan seed oils

Tocopherol composition was analyzed using the approach of Chen et al. (2023). Briefly, 2 g of the oil sample was weighed into a 15 mL centrifuge tube, and 5 mL of n-hexane was added. The mixture was vortexed for 1 min and then sonicated at 40 °C for 30 min. Subsequently, the mixture was vortexed for 30 s and centrifuged at 4500 rpm for 5 min. Finally, 1 mL of the supernatant was filtered through a 0.22  $\mu\text{m}$  organic phase filtration membrane and transferred to a clean injection vial for liquid chromatography (LC) analysis. Additionally, four standards ( $\alpha$ -tocopherol,  $\delta$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol) were diluted with isopropanol to establish the standard curve.

Analysis of the four tocopherols was conducted using an Agilent 1200 liquid chromatography system equipped with a VWD detector and a Porsohell 120 SB-C18 column (2.1 mm i.d.  $\times$  150 mm length, 2.7  $\mu\text{m}$  particle size). The mobile phase comprised 90% methanol and 10% water. Parameters were set as follows: column temperature at 40 °C, flow rate of 0.90 mL/min, absorption drag time of 30 min, UV wavelength at 294 nm, and injection volume of 10  $\mu\text{L}$ . Tocopherols were characterized by comparing the retention times of samples with standards. Tocopherols were quantified based on the standard curve obtained by the external standard approach.

## 2.9. Lipidomics analysis

### 2.9.1. Sample preparation

Lipid extraction was conducted following the procedures outlined in Method 2.2, except that MTBE-methanol solution containing the internal standard was used as the extraction solvent. The recovered lipids were dissolved in a 500  $\mu$ L dichloromethane/methanol solution (1:1, v/v), vortexed for 30 s, and sonicated for 10 min in an ice-water bath. Afterwards, the mixture was centrifuged at 12000 rpm, at 4 °C for 15 min. Lastly, the supernatant (100  $\mu$ L) was filtered through a 0.22  $\mu$ m organic filtration membrane and transferred to a clean injection vial for LC-MS analysis.

### 2.9.2. LC-MS/MS analysis

Lipid molecules were separated using an ultra-high performance liquid chromatograph (UHPLC, Vanquish, Thermo Fisher Scientific) equipped with a Phenomen Kinetex C18 column (2.1 mm  $\times$  100 mm, 1.7  $\mu$ m). The mobile phase consisted of two components: phase A (40% water, 60% acetonitrile, containing 10 mmol/L ammonium formate) and phase B (10% acetonitrile, 90% isopropanol, with 50 mL of a 10 mmol/L aqueous ammonium formate solution added per 1000 mL). The flow rate of the mobile phase was 0.3 mL/min. The mobile phase gradient elution proceeded as follows: 0–1.0 min, 40% B; 1.0–12.0 min, 40%–100% B; 12.0–13.5 min, 100% B; 13.5–13.7 min, 100%–40% B; 13.7–18.0 min, 40% B. The column temperature was set to 55 °C, while the sample tray temperature was 4 °C. An injection volume of 2  $\mu$ L was used for both positive and negative ionization modes.

The liquid chromatographic effluent was directed into a Q Exactive Orbitrap mass spectrometer (MS, Thermo Fisher Scientific, USA) for subsequent analysis and annotation. The mass spectrometer was controlled by Xcalibur version 4.0.27 (Thermo) acquisition software, operating in Data Dependent Acquisition (DDA) mode, which continuously assessed full-scan MS spectra for primary and secondary mass spectral information. The electrospray ionization (ESI) source conditions included a sheath gas flow rate of 30 Arb, Aux gas flow rate of 10 Arb, capillary temperatures of 320 °C (positive mode) and 300 °C (negative mode), full MS resolution of 70,000, MS/MS resolution of 17,500, collision energies of 15/30/45 in the NCE mode, and spray voltages of 5 kV (positive mode) and – 4.5 kV (negative mode).

### 2.9.3. Data processing

The original data files were converted to mzXML format using the “mconvert” program in ProteoWizard. Subsequently, peak detection, extraction, alignment, and integration were carried out using the CentWave algorithm controlled by XCMS (R package, version: 3.6.2). Parameters minfrac and cutoff were set to 0.5 and 0.3, respectively, for compound annotation. Lipid molecules were qualified through spectral matching with the LipidBlast database, with the intensity of component peaks used to detect differences in mass spectra among various rambutan seed oils.

## 2.10. Statistical analysis

Literature visualization was performed using CiteSpace software version 6.2.R4 (School of Information Science and Technology, Redsell University, USA). Experiments were replicated three times, and the results are presented as mean  $\pm$  standard deviation. Statistical analysis was conducted using SPSS statistical software (Version 26.0, SPSS Inc., Chicago, IL, USA) and Metaboanalyst 5.0 (<https://www.metaboanalyst.ca/>). Tukey’s test and analysis of variance (ANOVA) were used to assess parameters, with statistical significance set at the 5% level ( $P < 0.05$ ). Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed using SIMCA 14.1 software (Umetrics, Umea, Sweden). Heat maps, volcano maps, and dynamic distribution maps of differential molecules were generated using TTools-II version 2.042 software and the Metware Cloud online

platform (<https://www.metware.cn/>).

## 3. Results and discussion

### 3.1. Total lipid content

The lipid content of the three rambutan seeds was presented in Table 1, showing that BR-5 had the highest lipid content (25.77 g/100 g dry basis) and BR-4 had the lowest (23.40 g/100 g). Although the total lipid content varied among the analyzed varieties, there were no statistically significant differences ( $P > 0.05$ ). Likewise, Chai et al. (2018) observed no significant difference in lipid content among the seeds of rambutan varieties R7, R10, and R153. Interestingly, Ghobakhlou et al. (2019)’s study revealed significant differences in lipid content among the seeds of three rambutan varieties (R4, R7, and Serjan) from Malaysia. Furthermore, prior research has shown that the lipid content of rambutan seeds ranges from 14.70% to 41.30% on a dry weight basis (Chai et al., 2018; Jahurul et al., 2020), consistent with our findings. It is important to note that variations in reported lipid yields of rambutan seeds across studies may also stem from differences in pretreatment conditions, species, extraction methods, origin, and maturation time (Jahurul et al., 2020; Sirisompong et al., 2011). In summary, the substantial lipid content of rambutan seeds makes them a promising and distinct resource for the oil and fat industry.

### 3.2. Physicochemical parameters of rambutan seed oils

The acid value is a crucial indicator of oil quality, with higher values suggesting poorer quality and increased rancidity. The acid values of the three rambutan seed oils, presented in Table 1, varied significantly ( $P < 0.05$ ), ranging from 2.88 to 3.10 mg/g. BR-7 exhibited the highest value, whereas BR-4 had the lowest. These values are below the maximum acid value limit ( $\leq 4$  mg/g) specified for crude vegetable oils in China’s national food safety standards. The Thai Rongrien variety exhibited an even higher acid value (4.35 mg/g) compared to our findings (Lourith et al., 2016). This variation can be attributed primarily to differences in species and extraction solvent; n-hexane was used in their study (Lourith et al., 2016). Furthermore, the acid value of the three rambutan seed oils is comparable to that of coconut, avocado, and pomegranate oils, which are commercially manufactured as food and cosmetic ingredients (Lourith et al., 2016).

The peroxide value assesses the extent of primary oxidation in the oil, thereby indicating its quality to some degree. The peroxide values for BR-4, BR-5, and BR-7 were 7.46, 7.43, and 7.49 mEq O<sub>2</sub>/kg oil, respectively, showing no significant differences (Table 1). These values fall below the maximum peroxide value limits set by the Codex Alimentarius Commission, which permits a maximum of 10 mEq O<sub>2</sub>/kg oil for vegetable oils (Ghobakhlou et al., 2019). Ghobakhlou et al. (2019) observed lower peroxide values (ranging from 1.74 to 2.00 mEq O<sub>2</sub>/kg) in three rambutan seed oils from Malaysia.

The iodine value reflects the relative degree of unsaturation in oil components, serving as an estimate for quality factors such as melting point and oxidative stability. Higher iodine values indicate greater unsaturation and heightened susceptibility to oxidation. Table 1 illustrates significant variations in iodine values among different rambutan seed oils ( $P < 0.05$ ). BR-5 (50.42 g I<sub>2</sub>/100 g oil) and BR-7 (50.21 g I<sub>2</sub>/100g oil) displayed markedly higher iodine values compared to BR-4 (46.47 g I<sub>2</sub>/100g oil). These values fall within the range of iodine values previously documented for rambutan seed oils (32.31–58.13 g I<sub>2</sub>/100g oil) (Jahurul et al., 2020). Compared to other common vegetable oils, rambutan seed oils exhibited lower iodine values than peanut oil (86–107 g I<sub>2</sub>/100g oil) and corn oil (107–135 g I<sub>2</sub>/100g oil), but higher values than coconut oil (6.0–11.0 g I<sub>2</sub>/100g oil) and palm kernel oil (14.0–21.0 g I<sub>2</sub>/100g oil) (Bockisch, 2015).

The saponification value relies on the average molecular weight of the fatty acid mixture constituting the oil, serving as an indicator of its

**Table 1**

Total lipid content, physicochemical parameters, oxidative-stability index, antioxidant activity, fatty acid composition, and minor-components content of rambutan seed oils.

	BR-4	BR-5	BR-7
Total lipid content (g/100 g dry matrix)	23.40 ± 0.77 <sup>a</sup>	25.77 ± 1.49 <sup>a</sup>	24.99 ± 2.34 <sup>a</sup>
Acid value (mg KOH/g oil)	2.88 ± 0.02 <sup>c</sup>	2.99 ± 0.07 <sup>b</sup>	3.10 ± 0.03 <sup>a</sup>
Peroxide value (mEq O <sub>2</sub> / kg oil)	7.46 ± 0.07 <sup>a</sup>	7.43 ± 0.09 <sup>a</sup>	7.49 ± 0.05 <sup>a</sup>
Iodine value (g I <sub>2</sub> /100g oil)	46.47 ± 0.57 <sup>b</sup>	50.42 ± 0.76 <sup>a</sup>	50.21 ± 0.38 <sup>a</sup>
Saponification value (mg KOH/g oil)	203.64 ± 0.57 <sup>b</sup>	205.96 ± 0.88 <sup>a</sup>	203.35 ± 0.45 <sup>b</sup>
Oxidative-stability index (h)	3.51 ± 0.06 <sup>b</sup>	3.41 ± 0.06 <sup>ab</sup>	3.37 ± 0.05 <sup>a</sup>
<i>Antioxidant activity (μg Trolox equivalent/g oil)</i>			
DPPH	28.43 ± 0.86 <sup>a</sup>	30.25 ± 0.65 <sup>a</sup>	17.91 ± 2.60 <sup>b</sup>
FRAP	5.96 ± 0.70 <sup>a</sup>	2.95 ± 0.12 <sup>b</sup>	1.34 ± 0.02 <sup>c</sup>
ABTS	11.99 ± 1.02 <sup>a</sup>	9.83 ± 1.40 <sup>b</sup>	11.00 ± 1.63 <sup>a</sup>
<i>Fatty acid (% total fatty acids)</i>			
C12:0	0.09 ± 0.02 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>a</sup>
C14:0	0.09 ± 0.01 <sup>b</sup>	0.13 ± 0.00 <sup>a</sup>	0.08 ± 0.02 <sup>a</sup>
C14:1	0.19 ± 0.05 <sup>a</sup>	0.19 ± 0.04 <sup>a</sup>	0.16 ± 0.01 <sup>a</sup>
C16:0	7.89 ± 0.50 <sup>a</sup>	7.53 ± 0.18 <sup>a</sup>	7.26 ± 0.35 <sup>a</sup>
C16:1	1.43 ± 0.10 <sup>b</sup>	2.09 ± 0.07 <sup>a</sup>	1.73 ± 0.28 <sup>b</sup>
C18:0	5.24 ± 0.26 <sup>a</sup>	5.20 ± 0.26 <sup>a</sup>	5.29 ± 0.46 <sup>a</sup>
9cC18:1	40.56 ± 0.85 <sup>a</sup>	40.92 ± 0.15 <sup>a</sup>	39.84 ± 0.38 <sup>a</sup>
9c12c18:2 n-6	2.28 ± 0.08 <sup>b</sup>	3.27 ± 0.21 <sup>a</sup>	3.18 ± 0.23 <sup>a</sup>
C20:0	30.23 ± 1.20 <sup>a</sup>	28.45 ± 0.53 <sup>a</sup>	29.58 ± 1.28 <sup>a</sup>
9c12c15c18:3 n-3	7.93 ± 0.31 <sup>b</sup>	8.36 ± 0.20 <sup>b</sup>	8.69 ± 0.50 <sup>ab</sup>
C22:0	2.77 ± 0.16 <sup>a</sup>	2.40 ± 0.09 <sup>b</sup>	2.66 ± 0.11 <sup>a</sup>
C20:4 n-6	0.88 ± 0.03 <sup>b</sup>	1.13 ± 0.08 <sup>a</sup>	1.12 ± 0.14 <sup>a</sup>
C22:3 n-6	0.40 ± 0.04 <sup>a</sup>	0.23 ± 0.03 <sup>b</sup>	0.35 ± 0.03 <sup>a</sup>
Total SFA	46.32 ± 1.11 <sup>a</sup>	43.81 ± 0.65 <sup>a</sup>	44.94 ± 1.50 <sup>a</sup>
Total MUFA	42.18 ± 0.97 <sup>ab</sup>	43.20 ± 0.23 <sup>a</sup>	41.73 ± 0.65 <sup>b</sup>
Total PUFA	11.50 ± 0.26 <sup>b</sup>	12.99 ± 0.45 <sup>a</sup>	13.33 ± 0.85 <sup>a</sup>
EFA	11.10 ± 0.23 <sup>b</sup>	12.77 ± 0.43 <sup>a</sup>	12.98 ± 0.82 <sup>a</sup>
n-3 PUFA	7.93 ± 0.31 <sup>a</sup>	8.36 ± 0.20 <sup>a</sup>	8.69 ± 0.50 <sup>a</sup>
n-6 PUFA	3.57 ± 0.06 <sup>b</sup>	4.63 ± 0.29 <sup>a</sup>	4.65 ± 0.40 <sup>a</sup>
SFA/UFA	0.86	0.78	0.82
n-3/n-6	2.22	1.81	1.87
AI	0.16	0.14	0.14
TI	0.27	0.25	0.25
Polyphenols (mg GAE/kg oil)	40.71 ± 1.26 <sup>a</sup>	42.04 ± 1.54 <sup>a</sup>	24.21 ± 1.55 <sup>b</sup>
Squalene (mg/kg oil)	10.35 ± 1.00 <sup>c</sup>	13.73 ± 1.20 <sup>b</sup>	21.48 ± 0.61 <sup>a</sup>
<i>Phytosterol (mg/kg oil)</i>			
Cholesterol	1.08 ± 0.09 <sup>a</sup>	1.11 ± 0.12 <sup>a</sup>	1.03 ± 0.08 <sup>a</sup>
Brassicasterol	6.71 ± 0.63 <sup>a</sup>	7.10 ± 0.62 <sup>a</sup>	6.79 ± 0.61 <sup>a</sup>

**Table 1 (continued)**

	BR-4	BR-5	BR-7
Campesterol	16.10 ± 1.53 <sup>a</sup>	17.11 ± 1.42 <sup>a</sup>	16.22 ± 1.44 <sup>a</sup>
Stigmasterol	30.36 ± 1.64 <sup>c</sup>	34.69 ± 0.67 <sup>b</sup>	38.01 ± 0.15 <sup>a</sup>
β-Sitosterol	20.18 ± 1.94 <sup>a</sup>	20.98 ± 1.99 <sup>a</sup>	20.45 ± 1.76 <sup>a</sup>
Δ5-Avenasterol	7.24 ± 0.68 <sup>a</sup>	7.66 ± 0.69 <sup>a</sup>	7.17 ± 0.64 <sup>a</sup>
Cycloartenol	2.26 ± 0.24 <sup>b</sup>	2.42 ± 0.13 <sup>b</sup>	2.97 ± 0.14 <sup>a</sup>
24-MethyleneCycloartanol	48.53 ± 8.50 <sup>a</sup>	46.40 ± 4.15 <sup>a</sup>	51.80 ± 15.03 <sup>a</sup>
Total	132.46 ± 11.23 <sup>a</sup>	137.47 ± 8.73 <sup>a</sup>	144.43 ± 19.81 <sup>a</sup>
<i>Tocopherol (mg/kg oil)</i>			
α-Tocopherol	6.80 ± 0.57 <sup>b</sup>	3.69 ± 0.76 <sup>c</sup>	8.37 ± 0.29 <sup>a</sup>
β-Tocopherol	8.59 ± 1.02 <sup>ab</sup>	10.38 ± 1.03 <sup>a</sup>	5.30 ± 0.10 <sup>b</sup>
γ-Tocopherol	ND	ND	3.75 ± 0.38
Total	15.39 ± 1.47 <sup>a</sup>	14.07 ± 1.54 <sup>a</sup>	17.42 ± 0.48 <sup>a</sup>

All data represents the mean of three replications (Mean ± SD). The superscript letters indicate the statistical difference in rows in significant level at 5%. ND, not detected. AI, atherosclerotic index; EFA, essential fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TI, thrombotic index; UFA, unsaturated fatty acids.

quality. BR-5 exhibited a significantly higher saponification value (205.96 mg KOH/g oil) compared to BR-4 (203.64 mg KOH/g oil) and BR-7 (203.35 mg KOH/g oil) ( $P < 0.05$ ). No statistical difference was observed between BR-4 and BR-7 (Table 1). These values align with the saponification values of rambutan seed oil from the Rongrien variety (199.38 mg KOH/g oil) (Sonwai & Ponprachanuvut, 2012) but exceed those of Anak sekolah (157.07 mg KOH/g oil) (Manaf et al., 2013) and Clone R99 (182.10 mg KOH/g oil) (Harahap et al., 2012). Interestingly, these values closely resemble the saponification value of cocoa butter (190.74 mg KOH/g oil), indicating potential cocoa butter-like properties in rambutan seed oil (Kaur et al., 2022). Collectively, this information offers valuable insights into the utilization of rambutan seed oil as a food ingredient.

### 3.3. Antioxidant capacity of rambutan seed oils

The oxidative stability index, often denoted as the oxidation induction period (h), reflects oils' capacity to resist auto-oxidation, thereby indicating their storage resistance. Table 1 presents the OSI values of the three rambutan seed oils, ranging from 3.37 h to 3.51 h. The BR-4 group exhibited a longer oxidation induction time compared to BR-7 and BR-5. This suggests that secondary oxidation products are produced at a slightly slower rate in BR-4 seed oil than in the other two oils during storage. Similarly, walnut oils have been reported to have OSI values ranging from 3.37 to 5.55 h (Gao et al., 2019b).

Three antioxidant assays (DPPH, ABTS, and FRAP) were conducted in this study to assess the antioxidant capacity of rambutan seed oils, and the results are detailed in Table 1. BR-5 exhibited the highest scavenging capacity for DPPH radicals (30.25 μg Trolox equivalent/g oil), followed by BR-4 (28.43 μg Trolox equivalent/g oil) and BR-7 (17.91 μg Trolox equivalent/g oil). There was no statistically significant difference in the DPPH radical scavenging ability between BR-5 and BR-4. In the FRAP assay, significant differences ( $P < 0.05$ ) were observed among the three rambutan seed oils, with antioxidant capacities ranging from 1.34 (BR-7) to 5.96 (BR-4). The ABTS assay was employed to assess the antioxidant activity of hydrophilic and lipophilic antioxidants. The results ranged from 9.83 to 11.99, with BR-5 exhibiting the highest activity, followed by BR-4 and then BR-7. Furthermore, there was no significant difference in ABTS free radical scavenging capacity between BR-4 and

BR-7. Generally, free radical scavenging capacity can to some extent reflect the antioxidant capacity of rambutan seed oil. Despite variations in absolute values among different antioxidant capacity assays, the trend remained consistent, with BR-4 demonstrating stronger free radical scavenging capacity than BR-5 and BR-7. Nevertheless, due to the lack of antioxidant testing data for rambutan seed oil, we only compared the data with that of other seed oils. Unfortunately, rambutan seed oil exhibits lower antioxidant capacity compared to Assam tea seed oil (Uoonlue & Muangrat, 2019), walnut oil (Gao et al., 2019b), and milk thistle seed oil (Fathi-Achachlouei et al., 2019), *Bischofia polycarpa* oil (Wang, Su, et al., 2023; Wang, Wang, et al., 2023), and cactus fruit seed oil (Li et al., 2023). Nevertheless, it is important to acknowledge that discrepancies in the data may arise from variations in sample pre-treatment and oil extraction methods (Wang, Su, et al., 2023; Wang, Wang, et al., 2023).

### 3.4. Fatty acid composition of rambutan seed oils

Table 1 presents the fatty acid composition of the three rambutan seed oils. Thirteen fatty acids ranging from C12:0 to C22:3 n-6 were identified and compared across different cultivars. Regarding fatty acid families, the samples exhibited the following order of content: saturated fatty acids (SFA, ranging from 43.81% in BR-5 to 46.32% in BR-4) > MUFA (ranging from 41.73% in BR-7 to 43.20% in BR-5) > PUFA (ranging from 11.50% in BR-4 to 13.33% in BR-7). This trend aligns with findings from Solís-Fuentes et al. (2010), indicating that SFA constituted the predominant fatty acid family in Mexican rambutan seed oil (comprising 50.7% of total fatty acids). Nevertheless, in rambutan seed oil (Clome 99) extracted from Selangor, Malaysia, the percentage content of MUFA was slightly higher than that of SFA (Manaf et al., 2013). These variations may arise from differences in varieties, growing regions, and environmental conditions (Manaf et al., 2013). No significant differences in SFA levels were observed among the three varieties of rambutan seed oils analyzed in this study. Furthermore, the MUFA content in BR-4 and BR-5 was significantly higher than that in BR-7, whereas the PUFA content in BR-4 was significantly lower compared to the other varieties ( $P < 0.05$ ).

Concerning individual fatty acid molecules, their content varies in different varieties of rambutan seed oils. Oleic acid (9cC18:1) is the predominant fatty acid, constituting 39.84% in BR-7 to 40.92% in BR-5. This is followed by C20:0 (28.45%–30.23%), 9c12c15cC18:3 n-3 (7.93%–8.69%), C16:0 (7.26%–7.89%), and C18:0 (5.20%–5.29%). Notably, there were no statistically significant differences in the content of these five major fatty acids among the three varieties. These five fatty acids collectively account for over 90% of the total fatty acids in rambutan seed oils. Chai et al. (2018) examined the fatty acid composition of 11 Malaysian rambutan seed oils, and their findings differed from ours in terms of both the actual percentage and ranking of fatty acids, as well as the fatty acid families. In their study, oleic acid was the most abundant, ranging from 33.35% to 45.14%, followed by C20:0 (26.03% to 33.27%), C20:1 (5.75% to 10.55%), C18:0 (5.22% to 8.97%), 9c12c15cC18:3 n-3 (0.14% to 9.90%), and C16:0 (2.40% to 3.39%). Another study conducted by Sirisompong et al. (2011) examined the fatty acid profile of Thai rambutan seed oil, revealing that oleic acid (36.79%) was the predominant fatty acid, followed by C20:0 (34.32%), 9c12c15cC18:3 n-3 (6.48%), C18:0 (7.03%), and C16:0 (4.69%). Both studies noted that 9cC18:1 and C20:0 were the predominant fatty acid molecules in rambutan seed oil, comprising over 65% of the total fatty acids, a trend that closely aligns with our findings.

Oleic acid is crucial in the metabolic processes of living organisms. However, the body's endogenous synthesis of oleic acid is often insufficient to meet demands. Therefore, consuming cooking oil rich in oleic acid is beneficial for health. In comparison to common edible oils, the oleic acid content in rambutan seed oil closely resembles that of rice bran oil (39.52%) (Liu et al., 2019) and is lower than that of peanut oil (47.69%–50.73%) (Akhtar et al., 2014; Wang et al., 2019). This

indicates that rambutan seed oil serves as a promising source of oleic acid. Another beneficial fatty acid, C20:0, finds application in the production of photographic materials, detergents, and lubricants owing to its distinctive physicochemical properties. Prior research indicates that this fatty acid is commonly present in plant and animal lipids, albeit typically in low concentrations (< 1%). Remarkably, the C20:0 content in rambutan seed oil, as reported in this study, ranged from 26.03% to 33.27%, significantly surpassing that of peanut oil (< 5%) and flaxseed oil (< 5%), which were traditionally considered natural sources of C20:0. These findings collectively suggest that rambutan seed oil stands as a superior natural source of C20:0. Additionally, five trace fatty acid molecules (< 1%), comprising C12:0, C14:0, C14:1, C20:4 n-6, and C22:3 n-6, were detected in rambutan seed oil. Significantly divergent contents of these trace fatty acid molecules were observed among the measured samples ( $P < 0.05$ ). To the best of our knowledge, this is the initial documentation of these five fatty acids in rambutan seed oil.

To comprehensively evaluate the fatty acid nutritional value of rambutan seed oil, we computed several evaluation indices based on the formulas provided by Chen and Liu (2020). Essential fatty acids (EFA) are vital for maintaining bodily functions but cannot be synthesized internally, necessitating their provision through diet (Cunnane, 2003). Among the three rambutan seed oils analyzed, the essential fatty acid content ranged from 11.10% in BR-4 to 12.98% in BR-7 ( $P < 0.05$ ). The SFA/unsaturated fatty acids (UFA) ratio is a widely used indicator for assessing the cardiovascular health impact of diet. It posits that all UFA in the diet decrease low-density lipoprotein cholesterol and serum cholesterol levels, while all SFA increase serum cholesterol levels (Chen & Liu, 2020). Consequently, a lower ratio signifies a more positive effect. Table 1 demonstrates that all three rambutan seed oils exhibited low SFA/UFA values ranging from 0.78 to 0.86. Similarly, the n-3/n-6 PUFA ratio serves as a crucial indicator of the nutritional value of edible oils. A high dietary intake of n-3/n-6 PUFA is advantageous in reducing the incidence of vascular disease and cancer, with a FAO/WHO recommendation exceeding 0.25 (Dohrmann et al., 2019). The n-3/n-6 values in the rambutan seed oil analyzed in this study ranged from 1.81 to 2.22, significantly surpassing the official recommended value of 0.25. Additionally, the AI and TI are frequently employed to characterize the atherosclerotic and thrombotic potentials of fatty acids, respectively (Chen & Liu, 2020). Both indicators aid in comprehending the nutritional value of cooking oils, with Weng et al. (2020) reporting that a low dietary intake of AI and TI may decrease the risk of atherosclerosis and thrombosis. In our study, both AI (0.14–0.16) and TI (0.25–0.27) values of rambutan seed oil were found to be low. In conclusion, the peculiar fatty acid composition of rambutan seed oil confers upon it significant nutritional value and utilization potential.

### 3.5. Polyphenol content

Research has demonstrated that rambutan seeds contain abundant phenolic compounds, averaging 58.6 mg/g (Thitilertdecha et al., 2008). These compounds can be simultaneously extracted during the oil extraction process, significantly enhancing the nutritional value and applicability of rambutan seed oil. Table 1 shows the polyphenol content of the three rambutan seed oils. To our knowledge, this is the inaugural study reporting the polyphenol content of Chinese rambutan seed oil. The polyphenol content in rambutan seed oil samples ranged from 24.21 to 42.04 mg GAE/kg oil, as depicted in Table 1. BR-5 exhibited the highest polyphenol content, followed by BR-4 and BR-7. Notably, the polyphenol content of the seed oils extracted from BR-5 and BR-4 exceeded that of BR-7 by >1.5 times. These values are comparable to the polyphenol content found in walnut oil (28.0 to 45.40 mg GAE/kg) (Gao et al., 2021) and macadamia oil (31.62 to 39.74 mg GAE/kg) (Shuai et al., 2022). Walnut oil, in particular, has gained widespread acceptance and consumption globally. These findings suggest a significant potential for the development and utilization of rambutan seed oil. Nonetheless, there is a scarcity of studies on rambutan seed oil,

necessitating further investigation into its phenolic composition.

### 3.6. Squalene content

Squalene, a 30-carbon straight-chain hydrocarbon steroid precursor classified as a terpene, finds extensive application in food and cosmetics owing to its hypoxia-resistant properties and excellent emulsification ability. According to Table 1, the squalene content in the three rambutan seed oils ranged from 10.35 to 21.48 mg/kg oil, with BR-7 exhibiting the highest content, followed by BR-5 and BR-4. The squalene content in the rambutan seed oil analyzed in this study surpassed that in walnut oil (9.4 mg/kg) and was comparable to that in macadamia nut oil (22.9 mg/kg) and coconut oil (20.37 mg/kg) (Cicero et al., 2018). With increasing concerns about marine life, exploration of new natural sources of squalene is underway, as the traditional source from shark liver oil raises ethical and environmental issues, highlighting rambutan seed oil as a potentially valuable alternative source of this compound.

### 3.7. Phytosterol composition

Phytosterols are crucial lipid components found in vegetable oils. Due to their significant biological activity, phytosterols serve as essential benchmarks for assessing the nutritional value of oils. Table 1 reveals the characterization of eight phytosterols in rambutan seed oils, namely cholesterol, brassicasterol, campesterol, stigmasterol,  $\beta$ -sitosterol,  $\Delta$ 5-avenasterol, cycloartenol, and 24-methylenecycloartanol. The total phytosterol content ranged from 132.46 mg/kg oil in BR-4 to 144.43 mg/kg oil in BR-7, with no statistically significant differences observed among the samples ( $P > 0.05$ ). The 24-methylenecycloartanol, stigmasterol,  $\beta$ -sitosterol and campesterol were the major components, accounting for >85% of the total phytosterols. Previous research also identified  $\beta$ -sitosterol (0.32 mg/g) and stigmasterol (0.61 mg/g) in rambutan seed oil from Thailand; however, 24-methylenecycloartanol and campesterol were not detected (Sirisompong et al., 2011). These variations may be influenced by factors such as rambutan variety, origin, processing method, and analytical technique. Apparently, the contents of stigmasterol (38.01 mg/kg oil) and cycloartenol (2.97 mg/kg oil) in BR-7 were significantly higher than those in BR-5 (34.69 and 2.42 mg/kg oil, respectively) and BR-4 (34.69 and 2.42 mg/kg oil, respectively) ( $P < 0.05$ ). Interestingly, except for stigmasterol and cycloartenol, the levels of the remaining six phytosterols showed no statistically significant differences between samples. To our knowledge, this is the first report of cholesterol, brassicasterol, campesterol,  $\Delta$ 5-avenasterol, cycloartenol, and 24-methylenecycloartanol in rambutan seed oil. Due to the limited current data on the phytochemical composition of rambutan seed oil, further comprehensive studies are warranted. Moreover, consideration of diverse cultivars, larger sample sizes, and broader sampling ranges is imperative.

### 3.8. Tocopherol composition

Tocopherol, a natural oily antioxidant, is a crucial bioactive component of vegetable oils, offering numerous benefits including antiproliferative, antioxidant, and neuroprotective properties (Lee et al., 2022). Table 1 illustrates the identification of three tocopherol isomers ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherol) in rambutan seed oil, with the total tocopherol content ranging from 14.07 mg/kg oil in BR-5 to 17.42 mg/kg oil in BR-7. The  $\alpha$ -Tocopherol (8.37 mg/kg oil) emerged as the predominant tocopherol in BR-7, consistent with findings by Sirisompong et al. (2011), who exclusively detected  $\alpha$ -tocopherol in rambutan seed oil at 1.03 mg/kg oil. In contrast,  $\beta$ -tocopherol predominates in BR-4 and BR-5. Additionally,  $\alpha$ -tocopherol content in BR-7 significantly exceeded that in BR-4 and BR-5, whereas  $\beta$ -tocopherol content in BR-5 significantly surpassed that in other samples ( $P < 0.05$ ). Notably,  $\gamma$ -tocopherol was exclusively detected in BR-7, with a concentration of 3.75 mg/kg oil. Overall, among the three cultivars examined, BR-7 exhibited the highest

suitability for tocopherol provision, owing to its diverse tocopherol fractions and elevated tocopherol content.

### 3.9. Correlation between antioxidant capacity and minor-compounds

The antioxidant properties of vegetable oils are tightly correlated with their active phytochemicals. Pearson correlation analysis was conducted to investigate the potential relationship between minor-components and the antioxidant capacity of rambutan seed oils (Fig. S1). Pearson's correlation coefficient ( $r$ ) was calculated between the variables, with  $r > 0.5$  indicating a positive correlation and  $r < -0.5$  implying a negative correlation. OSI is a crucial parameter for assessing the sensory and nutritional characteristics of fats and oils, and their susceptibility to oxidative deterioration can be affected by trace components. OSI exhibited a positive correlation with  $\gamma$ -Tocopherol ( $r = 0.752$ ), stigmasterol ( $r = 0.830$ ), squalene ( $r = 0.826$ ), and cycloartenol ( $r = 0.895$ ), implying a potentially significant dose-response relationship between these bioactive compounds and antioxidant activity. In contrast, OSI displayed a negative correlation with  $\beta$ -tocopherol ( $r = -0.76$ ). Similar findings have been reported in previous studies, wherein OSI exhibited a notable negative correlation with  $\beta$ -tocopherol in walnut oil (Gao et al., 2019a, 2019b). Regarding free radical scavenging capacity, polyphenols showed a positive correlation with both DPPH ( $r = 0.980$ ) and FRAP ( $r = 0.610$ ), indicating that the presence of polyphenols enhances the antioxidant activity of rambutan seed oil. These compounds primarily function as chain breakers by donating hydrogen to the alkyl peroxy radical (Ballus et al., 2015). Similar correlations have been found in other edible vegetable oils (Gao et al., 2019a; Wang, Su, et al., 2023; Wang, Wang, et al., 2023). A positive correlation was also noted between DPPH and  $\beta$ -tocopherol. Conversely, both FRAP and DPPH exhibited negative correlations with  $\gamma$ -Tocopherol ( $r = -0.676$  and  $r = -0.964$ ), stigmasterol ( $r = -0.966$  and  $r = -0.686$ ), squalene ( $r = -0.842$  and  $r = -0.855$ ), and cycloartenol ( $r = -0.758$  and  $r = -0.777$ ), respectively. Wang, Su, et al. (2023) and Wang, Wang, et al. (2023) also reported a negative correlation between stigmasterol and squalene and the free radical scavenging capacity (FRAP and DPPH) in *Bischofia polycarpa* seed oil. Additionally, positive correlations ( $r$  values ranging from 0.549 to 0.995) were observed among the phytosterol fractions. Together, these findings provide additional insights into the relationship between the antioxidant capacity and the minor compounds present in rambutan seed oil.

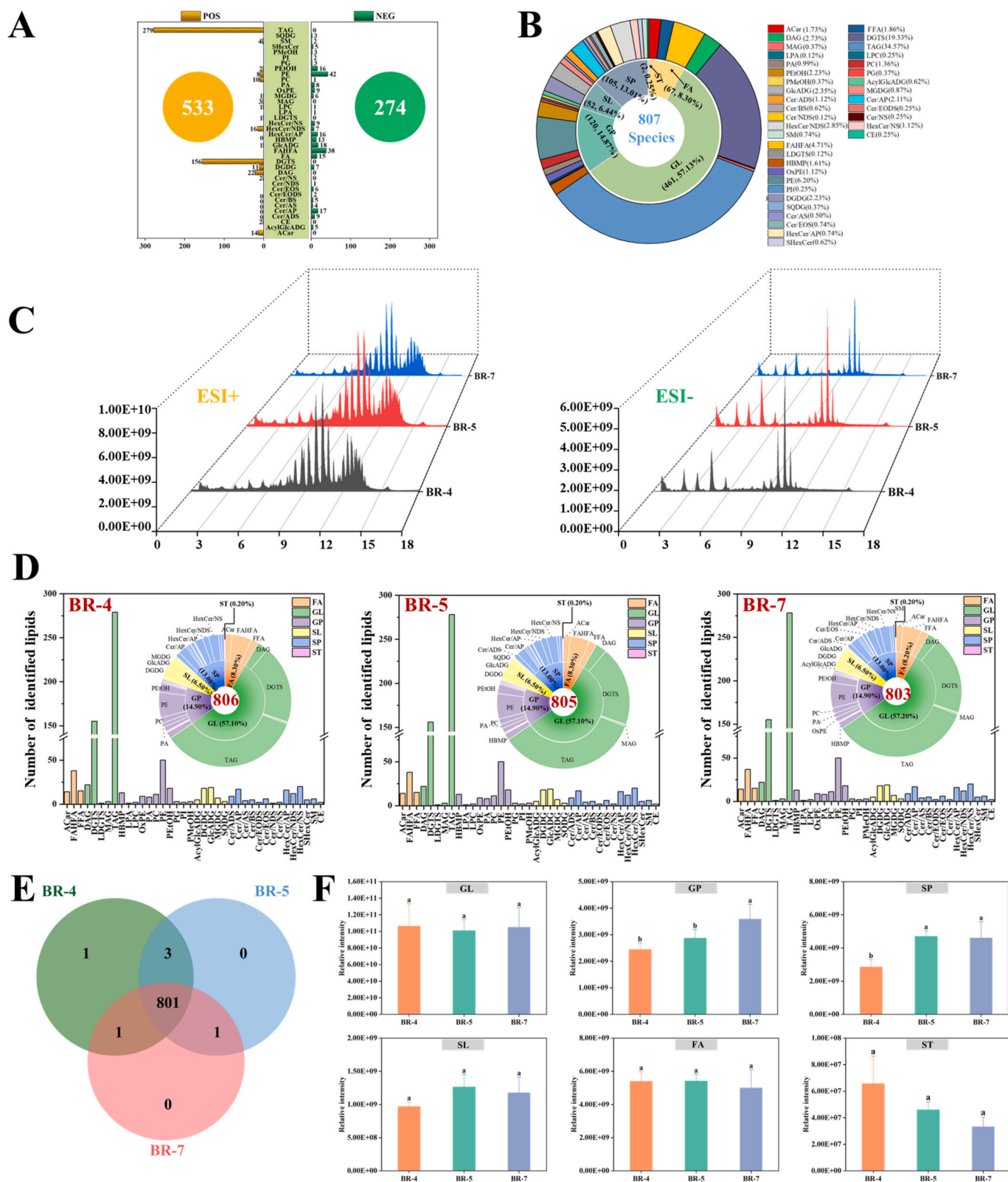
### 3.10. Lipidomics analysis of rambutan seed oils

#### 3.10.1. Overview of lipids in rambutan seed oils

Lipids are crucial nutrients necessary for the human body, serving as a primary energy source and providing essential fatty acids, and they play an indispensable role in human physiological metabolism. Advances in analytical and detection technologies have facilitated the widespread adoption of lipidomics in food chemistry, utilizing high-throughput and high-resolution mass spectrometry capabilities. This approach enables a comprehensive understanding of the lipid profile of vegetable oils at the molecular level and also tracks the dynamics of lipid molecules throughout the processing of vegetable oils. Hence, lipidomic analysis of rambutan seed oil is essential to further our understanding of its composition and properties.

The lipid profiles of seed oils extracted from three rambutan cultivars were analyzed using an untargeted lipidomics approach with UHPLC-Q Exactive Orbitrap-MS. In total, 807 lipid molecules from six lipid classes, including GL, GP, SP, SL, fatty acyls (FA), and steroids (ST), were identified in rambutan seed oils (Fig. 2A). Furthermore, these lipids were classified into 38 lipid subclasses according to the classification rules proposed by the Lipid Metabolites and Pathways Strategy (LIPID MAPS®). In the positive ion mode, 533 lipid molecules from six classes and 17 subclasses were identified, including 14 acylcarnitines (ACar), 2 cholesteryl esters (CE), 2 ceramide non-hydroxyfatty acid-sphingosines





**Fig. 2.** Overview of lipids in rambutan seed oils. Number of lipids in rambutan seed oils (A). Percentage of lipids in rambutan seed oils (B). Total ion chromatograms of actual specimens in positive (POS) and negative (NEG) ionization modes (C). Numbers and percentages of subclasses and categories in BR-4, BR-5, and BR-7 (D). Venn plot of lipids in three rambutan seed oils (E). Intensity of all lipid classes (including GL, GP, SP, SL, FA, and ST) in rambutan seed oils (F). The different letters in the bar chart were shown as a significant difference between samples ( $P < 0.05$ ).

(Cer/NS), 22 diacylglycerols (DAG), 11 digalactosyldiacylglycerols (DGDG), 156 diacylglyceryl trimethylhomoserines (DGTS), 1 glucuronosyldiacylglycerol (GlcADG), 16 hexosylceramide non-hydroxyfatty acid-dihydrospingosines (HexCer/NDS), 1 lysodiacylglyceryl trimethylhomoserine (LDGTS), 1 lysophosphatidylcholine (LPC), 3 mono-unsaturated fatty acids (MAG), 1 monogalactosyldiacylglycerol (MGDG), 10 phosphatidylcholines (PC), 8 phosphatidylethanolamines (PE), 2 phosphatidylethanolols (PEtOH), 4 sphingomyelin (SM), and 279 TAG. These subclasses were predominantly detected as [M]<sup>+</sup>, [M + NH<sub>4</sub>]<sup>+</sup>, [M + H]<sup>+</sup>, and [M + Na]<sup>+</sup> ions (Table S1). Simultaneously, 274 lipid molecules from 4 classes and 30 subclasses, primarily including 42 PE, 38 fatty acid ester of hydroxyl fatty acids (FAHFA), 18 GlcADG, 16 PEtOH, and 16 hexosylceramide alpha-hydroxy fatty acid-phytospingosines (HexCer/AP), etc., were identified in the negative ion mode, predominantly as [M-H]<sup>-</sup> ions (Table S1). Similar results were reported by Wang et al. (2022), wherein GL (including TAG and DAG) were exclusively detected in the positive ion mode, fatty acyls (except for the ACar subclass) were solely identified in the negative ion mode, while PC and PE were detected in both modes. Fig. 2B depicts the relative proportions of lipids in rambutan seed oil. Generally, GL (57.13%) constituted the predominant lipids in rambutan seed oil, followed by GP (14.87%) and SP (13.01%). Similar findings were noted in *Camellia oleifera* oil (Zeng et al., 2024) and *hazelnut oil* (Sun et al., 2022). Additionally, TAG and DGTS emerged as the top two GL subclasses, comprising 34.57% and 19.33%, respectively, while PE (6.20%), PEtOH (2.23%), and PC (1.36%) constituted the primary GP subclasses. This finding aligns with the research by (Bakhytkyzy et al., 2022), where TAG was identified as the predominant lipid subclass in flaxseed oil, followed by PC and PE.

### 3.10.2. Differences in lipid content of three rambutan seed oils

The total ion chromatograms (Fig. 2C) reveal that the lipid compositions of the three rambutan seed oils are comparable, albeit with variations in abundance. In Fig. 2D, 806, 805, and 803 lipid molecules were identified in BR-4, BR-5, and BR-7, respectively. The Venn diagram (Fig. 2E) illustrates the overlap of lipid molecules among samples, with a total of 801 lipid molecules shared by all three rambutan seed oils. Cer/NDS (d14:0/26:2) was exclusively detected in BR-4, while DGTS (16:2/20:5), FAHFA (18:1/18:1), and TAG (12:2/12:2/17:2) were found in BR-4 and BR-5. Moreover, TAG (20:2/20:2/20:2) and DGTS (16,4/18:5) were absent in BR-5 and BR-4, respectively. These findings suggest that the lipid molecule composition of rambutan seed oil is influenced by the variety. Further analysis of lipid composition variation in different rambutan seed oils involved categorizing each lipid molecule and summing the peak intensities of all lipid molecules from the same subclass to represent their relative contents, as shown in Fig. 2F and Fig. S2. High levels of GL, approaching  $1.0 \times 10^{11}$ , are observed in terms of the summed abundance of the different lipids (Fig. 2F). GP are major components of biological membranes and serve as precursors of signaling molecules involved in numerous biological processes, making them of particular interest (Chen et al., 2023). The relative abundance of GP varied significantly among the three rambutan seed oils, with BR-7 exhibiting significantly higher content than BR-5 and BR-4 ( $P < 0.05$ ). Moreover, BR-5 displayed the highest SP abundance, while the lowest was observed in BR-4 ( $P < 0.05$ ). No statistical differences were observed for SL, FA, and ST, despite fluctuations in their relative abundances among samples. These findings suggest that there may have been more significant changes in phospholipid metabolism and sphingolipid metabolism among the three examined rambutan species.

The GL detected in this study were primarily in the form of TAG and DGTS (Fig. S2). TAG serves as a predominant energy reserve substance in most eukaryotes (Coleman & Lee, 2004). Among the three rambutan seed oils analyzed, BR-4 exhibited the highest relative TAG content, while BR-7 had the lowest. Notably, there were no statistically significant differences in TAG levels among the samples. DGTS, a lipid subclass with ether bonds similar to PC in structure and function, has

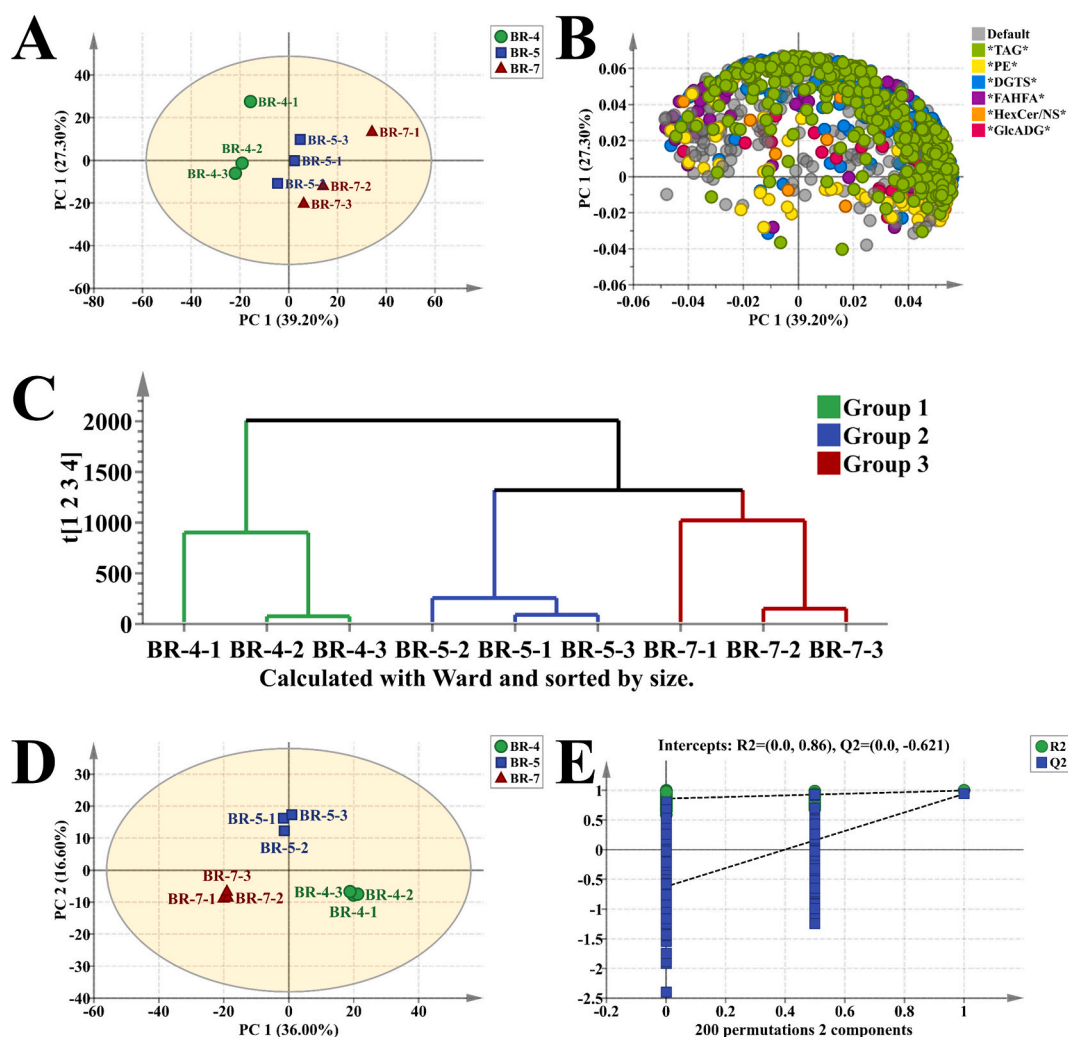
demonstrated improved high-density lipoprotein function (Khattib et al., 2020). Interestingly, the relative content of DGTS was higher in BR-7 compared to the other samples, although the difference was not statistically significant. Similarly, the relative abundances of other GL subclasses, such as DAG, MAG, and LDGTS, did not exhibit statistically significant differences among the analyzed rambutan seed oils. These findings reaffirmed that the variations in GL among the analyzed rambutan seed oils were not statistically significant.

In this study, GP were primarily detected as PE, PEtOH, and PC, with PE being the most abundant. These lipid subclasses play crucial roles in maintaining the integrity and functionality of biological membranes. The relative abundance of PE was significantly higher in BR-7 compared to BR-4 and BR-5 ( $P < 0.05$ ). The PEtOH content was significantly higher in BR-7 and BR-5 compared to BR-4 ( $P < 0.05$ ). However, there were no significant differences in the relative abundance of PC among all samples. Moreover, the relative contents of hemi-bismonoacylglycerophosphates (HBMP) and lysophosphatidic acids (LPA) were significantly higher in BR-7 compared to the other two groups. BR-4 exhibited the highest levels of relative phosphatidylglycerols (PG) and oxidized phosphatidylethanolamines (OxPE) ( $P < 0.05$ ). The relative contents of PA, PMeOH, phosphatidylinositols (PI), and LPC in the three rambutan seed oils exhibited similar trends to those of PC. Basically, significant differences were observed in both the number and content of major GP molecules among the three rambutan seed oils.

Despite being a smaller proportion of total lipids compared to GP and GL, SP containing a sphingomyelin backbone play crucial roles in cell membrane formation and signal transduction processes (Wang et al., 2022). The SP identified in the analyzed rambutan seed oil primarily consisted of HexCer/NDS, Cer/AP, and HexCer/AP. BR-7 exhibited the highest levels of HexCer/NDS, while BR-5 had the highest levels of Cer/AP ( $P < 0.05$ ) (Fig. S2). In contrast, the relative amount of HexCer/AP was significantly lower in BR-4 compared to the other two groups. Interestingly, trace lipids with biological activity, specifically SL and FAHFA, were also identified in the analyzed rambutan seed oils. The former exhibits a wide range of biological activities such as antioxidant, antibacterial and anti-inflammatory (Ng et al., 2023). GlcADG and DGDG were the primary SL subclasses identified in the analyzed rambutan seed oils, with BR-5 exhibiting the highest relative amounts of both lipid subclasses. FAHFA constitute a recently identified class of bioactive lipids known to alleviate inflammation linked to obesity and mitigate type II diabetes (Brejchova et al., 2020; Zhu et al., 2018). Although the relative content of FAHFA was slightly higher in BR-5 compared to BR-4 and BR-7, no statistically significant difference was observed ( $P > 0.05$ ) (Fig. S2). In summary, the lipid profiles of the analyzed rambutan seed oils exhibited some variation.

### 3.10.3. Multivariate statistical analysis of lipid molecules in rambutan seed oils

To compare the lipidomic profiles of various rambutan seed oils and discern differences between species, we conducted multivariate statistical analyses on the samples. The unsupervised PCA model analyzes samples solely based on data characteristics (Chen et al., 2023). Thus, we employed this model initially to comprehend overall differences among the three rambutan seed oils. The PCA score plot (Fig. 3A) illustrates that all samples were categorized into three distinct clusters, suggesting significant differences in lipid profiles among samples. The first two principal components of the PCA model, explaining 39.20% and 24.30% of the variance, respectively, were extracted. The loading diagram (Fig. 3B) elucidates this trend, with colors denoting various lipid subclasses, as illustrated in Fig. 3B, where lipids tend to aggregate into three clusters. Notably, TAG molecules were evenly distributed across quadrants I, II, and IV, while PE predominated in quadrants III and IV, indicating the role of lipid molecules in sample differentiation to some extent. Furthermore, we employed another unsupervised analytical model, Hierarchical Clustering Analysis (HCA), to evaluate the similarity among the three rambutan seed oils. Fig. 3C clearly



**Fig. 3.** Multivariate statistical analysis of lipid profiles of rambutan seed oils. Score plot of PCA model (A). Loading plot (B). Hierarchical clustering analysis plot (C). Score plot of OPLS-DA model (D). 200 times permutation test plot (E).

demonstrates the clustering effect among the different varieties BR-5 and BR-7 were clustered into one group, while BR-4 was in a separate group, implying a certain degree of similarity in the lipid profiles of BR-5 and BR-7. These findings tentatively confirm substantial alterations in the lipid profiles of the three rambutan seed oils. Additionally, to mitigate confounding factors (e.g., within-group variances), magnify between-group disparities, and reflect the discriminative and predictive capacities of the model, we constructed the OPLS-DA model using supervised learning. Comparable classification trends to the PCA model were observed, as depicted in the OPLS-DA score plot (Fig. 3D), wherein the three distinct clusters were entirely segregated. This further validated the PCA results, demonstrating the diversity of lipid extracts in BR-4, BR-5, and BR-7. The 200 permutation tests were conducted to further validate the model. As illustrated in Fig. 3E, the parameters  $R^2$  and  $Q^2$  were 0.86 and  $-0.621$ , respectively, demonstrating the robust fitting and predictive capability of the OPLS-DA model.

To elucidate differences among samples and identify potential differential lipid molecules, we employed pairwise comparative analysis to develop an OPLS-DA model for the three varieties (Wang et al., 2022). Pairing included BR-4 vs BR-5, BR-4 vs BR-7, and BR-5 vs BR-7. Fig. S3A, D, and G illustrate clear separation among all paired groups, indicating distinct differences in lipids between any two groups. Table S2 summarizes classification parameters for the three paired OPLS-DA models, including  $R^2X_{cum}$ ,  $R^2Y_{cum}$ , and  $Q^2_{cum}$ . These values exceed 0.5, confirming the validity of all models (Wang, Su, et al., 2023;

Wang, Wang, et al., 2023). S-plots of the OPLS-DA model offer a graphical projection of lipid molecules. The vertical axis denotes the correlation coefficient, while the horizontal axis represents the covariance between the principal component and the lipid molecule. Red diamonds indicate lipid molecules with variable influence on projection (VIP)  $> 1$ , while green dots denote those with  $VIP \leq 1$ . In total, 138 (BR-4 vs BR-5), 133 (BR-4 vs BR-7), and 117 (BR-5 vs BR-7) lipid molecules had VIP values  $> 1$  (Fig. S3B, E, and H). These lipids are distributed in both positive and negative directions away from the origin, with greater distance indicating a stronger contribution to sample differentiation. The top 10 differential lipid molecules with VIP values for each paired group were depicted in Fig. S3C, F, and I. For instance, TAG (18:0/18:1/20:0) in BR-4 vs BR-5, DAG (18:1/18:1) in BR-4 vs BR-5, and TAG (16:0/18:0/18:1) in BR-5 vs BR-7. Therefore, potential differential lipids in the seed oils of various rambutan varieties were preliminarily filtered.

The fold change (FC) value is a common statistical threshold used to filter differential markers. To illustrate the differences in lipid molecule content between samples, we calculated FC values for the pairwise comparison groups, using a screening criterion of  $FC > 2$  or  $< 0.5$ . Subsequently, dynamic distribution maps of lipid content differences were generated by arranging the lipid molecules based on FC values, ranging from smallest to largest (Fig. S4A, B, and C). Additionally, to refine the screening criteria for identifying differential lipid molecules,  $P$ -values obtained from  $t$ -tests were integrated to establish screening thresholds. Volcano plots comparing the 3 sets of samples in pairs are

depicted in Fig. S4D, E, and F. Based on the screening criteria of  $FC > 2$  or  $< 0.5$  and  $P < 0.05$ , a total of 139 and 214 differential lipid molecules were identified in BR-4 vs BR-5 and BR-4 vs BR-7, respectively. In BR-4 vs BR-5, out of the 139 differential lipid molecules identified, 125 (e.g., HBMP (18:0/18:0/18:2) and TAG (12:0/12:0/12:0/12:0)) were significantly up-regulated, while 14 (e.g., FAHFA (18:1/16:0) and DGTS (16:4/18:5)) were significantly down-regulated (Fig. S4D). In BR-4 vs BR-5, among the 214 differential lipid molecules identified, 169 (e.g.,

FAHFA (16:0/18:2) and PC (18:1/18:1)) were significantly up-regulated, while 45 (e.g., SM (d14:0/24:1) and HBMP (12:0/16:1/20:0)) were significantly down-regulated (Fig. S4E). In the comparison between BR-5 and BR-7, 92 differential lipid molecules were identified ( $FC > 2$  or  $< 0.5$  and  $P < 0.05$ ), with 46 being up-regulated and 46 being down-regulated (Fig. S4F).

Furthermore, we integrated the screening criteria discussed above, namely  $VIP > 1$ ,  $FC > 2$  or  $< 0.5$ , and  $P < 0.05$  (Table S3, S4, and S5).

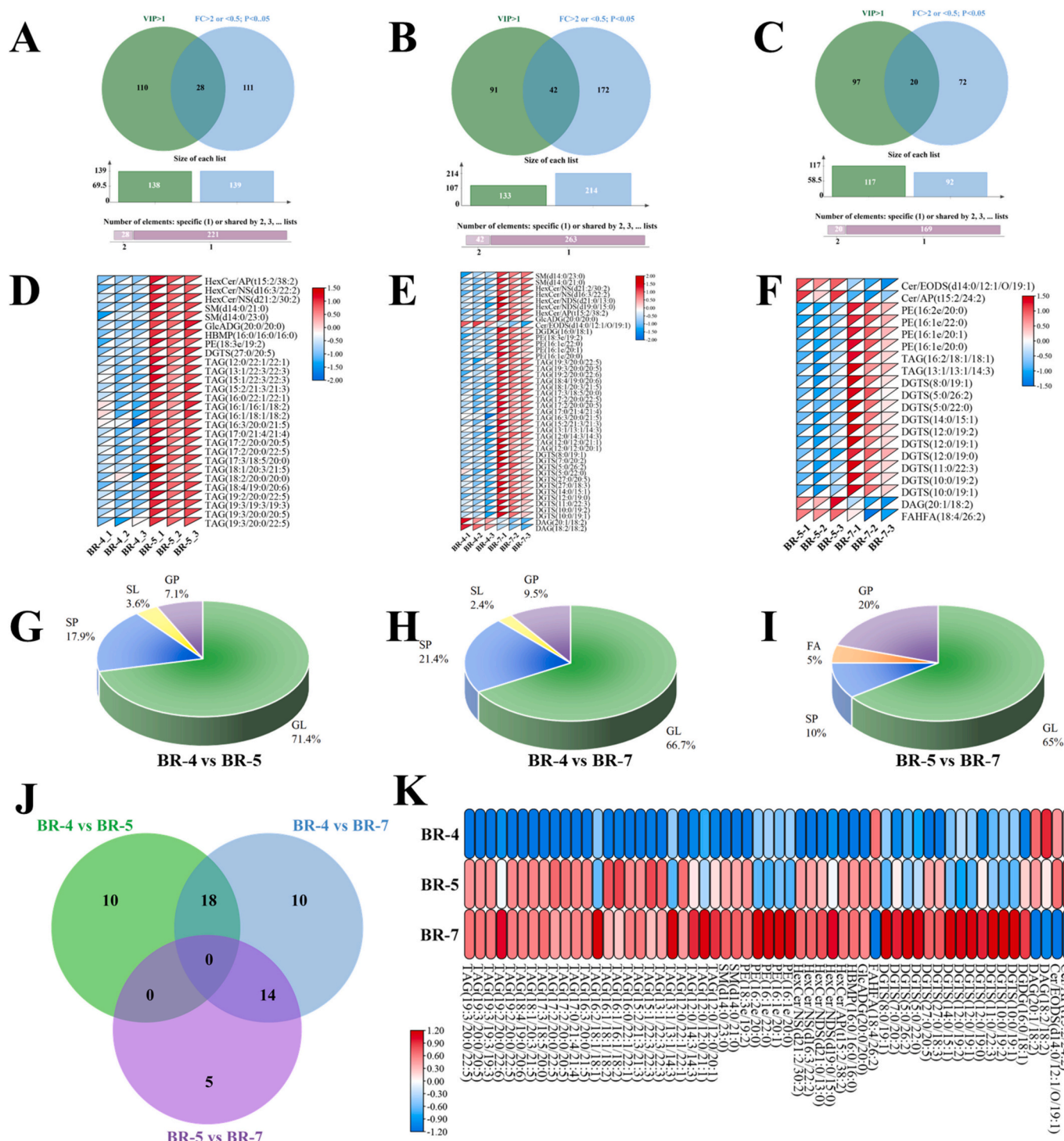


Fig. 4. Screening and comparison of differential lipid molecules. Venn diagram of variable influence of projection (VIP) and fold change (FC), lipids meeting the conditions were differential lipids between BR-4 and BR-5 (A), BR-4 vs BR-7 (B), BR-5 and BR-7 (C). Heat map analysis and percentage of the categories of 28 differential lipids in BR-4 and BR-5 (D, G), 42 differential lipids in BR-4 vs BR-7 (E, H), and 20 differential lipids in BR-5 vs BR-7 (F, I). Venn diagram of differential lipids in BR-4 and BR-5, BR-4 vs BR-7, and BR-5 vs BR-7 (J). Heat map analysis of 57 key differential lipids in rambutan seed oils (K).

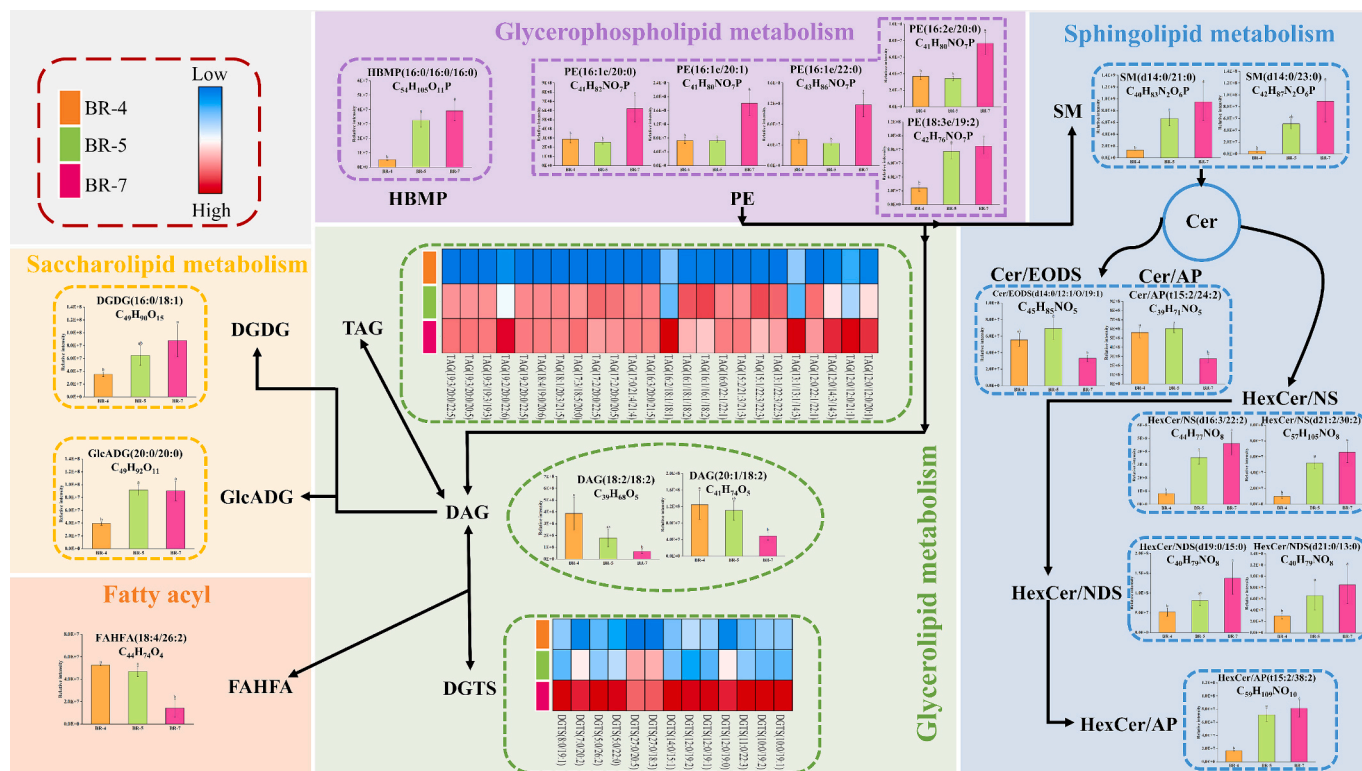


Fig. 5. Potential relationship between key differential lipid molecules based on pathway network from KEGG (<https://www.kegg.jp>) and LIPID MAPS (<https://www.lipidmaps.org>).

Fig. 4A, D, and G show the filtration of 28 key differential lipid molecules in BR-4 vs BR-5, with lower content observed in BR-4 compared to BR-5. These include 19 TAG, 2 SM, 2 hexosylceramide non-hydroxyfatty acid-sphingosines (HexCer/NS), 1 PE, 1 HexCer/AP, 1 HBMP, 1 GlcADG, and 1 DGTS. There were 42 key lipid molecules screened in BR-4 vs BR-7, with DAG (20:1/18:2) and DAG (18:2/18:2) being more abundant in BR-4 than in BR-7, as shown in Fig. 4B, E, and H. The remaining 39 lipids were less abundant in BR-4 than in BR-7, including 15 TAG, 11 DGTS, 4 PE, 2 SM, 2 DAG, 2 HexCer/NS, 2 HexCer/NDS, 1 HexCer/AP, 1 GlcADG, 1 DGDG, and 1 ceramide esterified omega-hydroxy fatty acid-dihydroxyphingosine (Cer/EODS). Fig. 4C, F, and I depict significant differences between BR-5 and BR-7, with 20 lipids showing variation. Among these, 16 lipids exhibited lower levels in BR-5 compared to BR-7, comprising 10 DGTS, 4 PE, and 2 TAG. The levels of the remaining four lipid molecules (i.e., FAHFA (18:4/26:2), DAG (20:1/18:2), and Cer/AP (15:2/24:2)) were notably higher in BR-5 than in BR-7. Pairwise comparisons integrated to identify 57 lipid molecules as potential biologically significant markers, comprising 24 TAG, 2 SM, 5 PE, 2 HexCer/NS, 2 HexCer/NDS, 1 HexCer/AP, 1 HBMP, 1 GlcADG, 1 FAHFA, 13 DGTS, 1 DGDG, 2 DAG, 1 Cer/EODS, and 1 Cer/AP. GL predominated numerically, followed by SP (Fig. 4G, H, and I). In Fig. 4J, 10, 10, and 5 lipid markers were exclusive to BR-4 vs BR-5, BR-4 vs BR-7, and BR-5 vs BR-7, respectively. Additionally, 18 markers were shared between BR-4 vs BR-5 and BR-4 vs BR-7. Fourteen markers overlapped between BR-4 vs BR-7 and BR-5 vs BR-7. Heat maps depicts the variation in the 57 differential lipids across the three rambutan seed oils (Fig. 4K). BR-7 exhibited significantly higher abundance of most differential lipids compared to BR-4, except for Cer/EODS (d14:0/12:1/O/19:1), DAG (18:2/18:2), DAG (20:1/18:2), FAHFA (18:4/26:2), and Cer/AP (15:2/24:2), which were higher in BR-4. Majority of GL lipid molecules showed no significant difference between BR-5 and BR-7. Except for PE (18:3e/19:2), other PE lipid molecules (PE (16:1e/20:0), PE (16:1e/20:1), PE (16:1e/22:0), and PE (16:2e/20:0)) were less abundant in BR-5 than BR-7 ( $P < 0.05$ ). These findings demonstrate significant differences in lipid

composition among the three rambutan seed oils, suggesting the potential utility of these 57 lipid molecules as markers for distinguishing between the oils extracted from different rambutan varieties.

#### 3.10.4. Correlation analysis and potential lipid metabolic networks

Pearson's correlation analysis (Fig. S5) was employed to investigate lipid relationships, with a correlation coefficient threshold set at  $|r| > 0.5$ . The solid yellow line indicates a positive correlation, while the dashed blue line signifies a negative correlation. Correlations between metabolites can depict the synthesis and transformation dynamics. For instance, positive correlations suggest metabolites stemming from the same anabolic pathway, while negative correlations indicate potential substrate-product relationships within catabolic pathways. Most lipid subclasses within the same class exhibited positive correlations, barring a few exceptions (Fig. S5A). TAG demonstrated positive correlations with PC ( $r = 0.848$ ), FAHFA ( $r = 0.780$ ), Cer/NS ( $r = 0.866$ ), Cer/AS ( $r = 0.678$ ), Cer/AP ( $r = 0.708$ ), and ACar ( $r = 0.825$ ). DAG, a pivotal nexus for integrating glycerophospholipid, glycerolipid, and sphingolipid metabolism, exhibited significant positive correlations with PI ( $r = 0.606$ ), PC ( $r = 0.747$ ), LPA ( $r = 0.910$ ), FAHFA ( $r = 0.868$ ), Cer/NS ( $r = 0.918$ ), Cer/NDS ( $r = 0.562$ ), and Cer/EODS ( $r = 0.692$ ), while displaying a negative correlation with OxPE ( $r = -0.541$ ). Furthermore, PC exhibited significant positive correlations with fatty acyls (FAHFA and ACar), saccharolipids (MGDG, GlcADG, and DGDG), and sphingolipids (Cer/NS, and Cer/AP). PE exhibited negative correlations with Cer/EODS ( $r = -0.777$ ), and CE ( $r = -0.562$ ), while showing positive correlations with SM ( $r = 0.780$ ), MGDG ( $r = 0.573$ ), HexCer/NS ( $r = 0.763$ ), HexCer/NDS ( $r = 0.678$ ), HexCer/AP ( $r = 0.652$ ), and DGDG ( $r = 0.514$ ). Cer/NDS and PG exhibited negative correlations with the majority of lipid subclasses.

Additionally, we examined the correlations among the differential lipid molecules and represented them in a Pearson correlation network diagram (Fig. S5B). TAG (16:1/18:1/18:2) exhibited significant correlations with 34 other differential lipid molecules, comprising 19 TAG, 4

DGTS, 2 SM, 2 HexCer/NS, 2 HexCer/NDS, 1 HexCer/AP, 1 HBMP, 1 GlcADG, and 1 DGDG, all showing positive correlations. Notably, besides showing positive correlations with DAG (20:1/18:2), Cer/EODS (d14:0/12:1/O/19:1), and FAHFA (18:4/26:2), DAG (18:2/18:2) exhibited significant negative correlations ( $|r| > 0.5$ ) with nearly all the differentiated lipid molecules (47 species, including 21 TAG, 2 SM, 5 PE, 2 HexCer/NS, 2 HexCer/NDS, 1 HexCer/AP, 1 HBMP, 1 GlcADG, 11 DGTS, 1 DGDG), underscoring the pivotal role of DAG molecules in integrating diverse lipid metabolism pathways. These analyses suggest that the variations in the lipid profiles of the three rambutan seed oils primarily arise from the collective influence of these 57 differential lipid molecules.

Different lipids in living organisms' function coordinately to perform various biological roles (Sun et al., 2020; Tietel et al., 2023). Studying and annotating lipid metabolic pathways can elucidate the mechanisms contributing to variations in lipid profiles. We correlated lipid metabolic pathways associated with differential lipids using the KEGG library and the LIPID MAP database, then integrated and visualized them into a correlation network (Fig. 5). Hypothetical integration scenarios encompass key metabolic pathways such as glycerolipid metabolism, glycerophospholipid metabolism, sphingolipid metabolism, and saccharolipid metabolism. In summary, these findings contribute to elucidating the discrepancies in lipid profiles of seed oils from three rambutan cultivars.

#### 4. Conclusion

The present study aimed to investigate and compare the quality of seed oils from three rambutan cultivars using a foodomics methodology. No significant differences were found in the content of total lipids, phytosterols, and tocopherols. The acid value, peroxide value, iodine value, and saponification value of the three rambutan seed oils complied with internationally recommended standards for crude vegetable oils. BR-4 showed relatively high antioxidant capacity. 9cC18:1 and C20:0 were the predominant fatty acid molecules. BR-4 contained the lowest UFA and EFA content. Rambutan seed oils had low AI and TI values. BR-7 exhibited the lowest polyphenol content and the highest squalene content. Additionally, a total of 807 lipid species belonging to 38 lipid subclasses were identified, with TAG, DGTS, and PE being the major lipid subclasses. BR-7 showed dominance in GP and SP. Significant differences were observed in the lipid molecular profiles of the three rambutan seed oils, and 57 potentially differential lipid molecules were identified through chemometric modeling.

Overall, our findings contribute to a comprehensive understanding of the nutritional value and potential applications of rambutan seed oil, thus fostering advancements in rambutan processing within the industry. Nevertheless, despite these promising findings demonstrating the potential of rambutan seed oil, further in-depth research is warranted. Given that this study represents a pioneering investigation into these specific varieties and considering Hainan Province's adaptation to rambutan cultivation, there are still numerous aspects awaiting exploration in this domain.

#### CRediT authorship contribution statement

**Jingtao Cui:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Siqi Zhao:** Data curation, Formal analysis. **Yanchi Zhou:** Investigation, Software. **Tian Li:** Investigation, Methodology, Writing – review & editing. **Weimin Zhang:** Funding acquisition, Project administration, Supervision, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work

reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2024.101699>.

#### References

- Akhtar, S., Khalid, N., Ahmed, I., Shahzad, A., & Suleria, H. A. R. (2014). Physicochemical characteristics, functional properties, and nutritional benefits of peanut oil: A review. *Critical Reviews in Food Science and Nutrition*, 54(12), 1562–1575. <https://doi.org/10.1080/10408398.2011.644353>
- AOAC. (2000). *Official methods of analysis of the AOAC international* (19th ed.). Washington DC, USA: Association of Official Analytical Chemists.
- Bakhytkyzy, I., Hewelt-Belka, W., & Kot-Wasik, A. (2022). A method for a comprehensive lipidomic analysis of flaxseed (*Linum usitatissimum*) with the use of LC-Q-TOF-MS and dispersive micro-solid-phase ( $\mu$ DSPE) extraction. *Food Chemistry*, 381, Article 132290. <https://doi.org/10.1016/j.foodchem.2022.132290>
- Ballus, C. A., Meinhart, A. D., de Souza Campos, F. A., & Godoy, H. T. (2015). Total phenolics of virgin olive oils highly correlate with the hydrogen atom transfer mechanism of antioxidant capacity. *Journal of the American Oil Chemists' Society*, 92(6), 843–851. <https://doi.org/10.1007/s11746-015-2629-0>
- Bockisch, M. (2015). *Fats and oils handbook*. Nahrungsfette und Öle: Elsevier.
- Brejchova, K., Balas, L., Paluchova, V., Brezinova, M., Durand, T., & Kuda, O. (2020). Understanding FAHFA: From structure to metabolic regulation. *Progress in Lipid Research*, 79, Article 101053. <https://doi.org/10.1016/j.plipres.2020.101053>
- Chai, K. F., Mohd Adzahan, N., Karim, R., Rukayadi, Y., & Ghazali, H. M. (2018). Characteristics of fat, and saponin and tannin contents of 11 varieties of rambutan (*Nephelium lappaceum* L.) seed. *International Journal of Food Properties*, 21(1), 1091–1106. <https://doi.org/10.1080/10942912.2018.1479857>
- Chen, J., & Liu, H. (2020). Nutritional indices for assessing fatty acids: A mini-review. *International Journal of Molecular Sciences*, 21(16), 5695. <https://doi.org/10.3390/ijms21165695>
- Chen, Z., Fu, J., Dou, X., Deng, Z., Wang, X., Ma, F., Yu, L., Yun, Y.-H., Li, P., & Zhang, L. (2023). Comprehensive adulteration detection of sesame oil based on characteristic markers. *Food Chemistry: X*, 18, Article 100745. <https://doi.org/10.1016/j.fochx.2023.100745>
- Cicero, N., Albergamo, A., Salvo, A., Bua, G. D., Bartolomeo, G., Mangano, V., ... Dugo, G. (2018). Chemical characterization of a variety of cold-pressed gourmet oils available on the Brazilian market. *Food Research International*, 109, 517–525. <https://doi.org/10.1016/j.foodres.2018.04.064>
- Coleman, R. A., & Lee, D. P. (2004). Enzymes of triacylglycerol synthesis and their regulation. *Progress in Lipid Research*, 43(2), 134–176. [https://doi.org/10.1016/S0163-7827\(03\)00051-1](https://doi.org/10.1016/S0163-7827(03)00051-1)
- Cui, J., Yang, Z., Xu, Y., Tan, C.-P., & Zhang, W. (2023). Lipidomics insight on differences in lipid profiles and phytosterol compositions of coconut oils extracted by classical and green solvents. *Food Research International*, 174, Article 113653. <https://doi.org/10.1016/j.foodres.2023.113653>
- Cunnane, S. C. (2003). Problems with essential fatty acids: Time for a new paradigm? *Progress in Lipid Research*, 42(6), 544–568. [https://doi.org/10.1016/S0163-7827\(03\)00038-9](https://doi.org/10.1016/S0163-7827(03)00038-9)
- Dohrmann, D. D., Putnik, P., Bursac Kovačević, D., Simal-Gandara, J., Lorenzo, J. M., & Barba, F. J. (2019). Japanese, Mediterranean and Argentinean diets and their potential roles in neurodegenerative diseases. *Food Research International*, 120, 464–477. <https://doi.org/10.1016/j.foodres.2018.10.090>
- Fathi-Achachlouei, B., Azadmard-Damirchi, S., Zahedi, Y., & Shaddel, R. (2019). Microwave pretreatment as a promising strategy for increment of nutraceutical content and extraction yield of oil from milk thistle seed. *Industrial Crops and Products*, 128, 527–533. <https://doi.org/10.1016/j.indcrop.2018.11.034>
- Gao, P., Liu, R., Jin, Q., & Wang, X. (2019a). Comparative study of chemical compositions and antioxidant capacities of oils obtained from two species of walnut: *Juglans regia* and *Juglans sigillata*. *Food Chemistry*, 279, 279–287. <https://doi.org/10.1016/j.foodchem.2018.12.016>
- Gao, P., Liu, R., Jin, Q., & Wang, X. (2019b). Comparison of solvents for extraction of walnut oils: Lipid yield, lipid compositions, minor-component content, and antioxidant capacity. *LWT*, 110, 346–352. <https://doi.org/10.1016/j.lwt.2019.04.100>

- Gao, P., Liu, R., Jin, Q., & Wang, X. (2021). Effects of processing methods on the chemical composition and antioxidant capacity of walnut (*Juglans regia* L.) oil. *LWT*, 135, Article 109958. <https://doi.org/10.1016/j.lwt.2020.109958>
- Ghobakhlou, F., Ghazali, H. M., Karim, R., & Mohammed, A. S. (2019). Characterization of three varieties of Malaysian rambutan seed oil. *Revista Española de Nutrición Humana y Dietética*, 23(3), 183–193. <https://doi.org/10.14306/renhyd.23.3.756>
- Harahap, S. N., Ramli, N., Vafaei, N., & Said, M. (2012). Physicochemical and nutritional composition of rambutan anak sekolah (*Nephelium lappaceum* L.) seed and seed oil. *Pakistan Journal of Nutrition*, 11(11), 1073.
- Hernández-Hernández, C., Aguilar, C. N., Rodríguez-Herrera, R., Flores-Gallegos, A. C., Morlett-Chávez, J., Govea-Salas, M., & Ascacio-Valdés, J. A. (2019). Rambutan (*Nephelium lappaceum* L.): Nutritional and functional properties. *Trends in Food Science & Technology*, 85, 201–210. <https://doi.org/10.1016/j.tifs.2019.01.018>
- Jahurul, M. H. A., Azzatul, F. S., Sharifudin, M. S., Norliza, M. J., Hasmadi, M., Lee, J. S., ... Zaidul, I. S. M. (2020). Functional and nutritional properties of rambutan (*Nephelium lappaceum* L.) seed and its industrial application: A review. *Trends in Food Science & Technology*, 99, 367–374. <https://doi.org/10.1016/j.tifs.2020.03.016>
- Kaur, G., Kaur, D., Kansal, S. K., Garg, M., & Krishania, M. (2022). Potential cocoa butter substitute derived from mango seed kernel. *Food Chemistry*, 372, Article 131244. <https://doi.org/10.1016/j.foodchem.2021.131244>
- Khattib, A., Atrahimovich, D., Dahli, L., Vaya, J., & Khatib, S. (2020). Lyso-diacylglyceryltrimethylhomoserine (lyso-DGTS) isolated from *Nannochloropsis* microalgae improves high-density lipoprotein (HDL) functions. *Biofactors*, 46(1), 146–157. <https://doi.org/10.1002/biof.1580>
- Lee, A. R., Tariq, A., Lau, G., Tok, N. W., Tam, W. W., & Ho, C. S. (2022). Vitamin E, alpha-tocopherol, and its effects on depression and anxiety: A systematic review and meta-analysis. *Nutrients*, 14(3), 656. <https://doi.org/10.3390/nu14030656>
- Lee, J., Kang, Y.-R., Kim, Y. J., & Chang, Y. H. (2019). Effect of high pressure and treatment time on nutraceuticals and antioxidant properties of *Lonicera japonica* Thunb. *Innovative Food Science & Emerging Technologies*, 54, 243–251. <https://doi.org/10.1016/j.ifset.2019.05.005>
- Li, X., Qi, B., Zhang, S., & Li, Y. (2023). Foodomics revealed the effects of ultrasonic extraction on the composition and nutrition of cactus fruit (*Opuntia ficus-indica*) seed oil. *Ultrasonics Sonochemistry*, 97, Article 106459. <https://doi.org/10.1016/j.ultsonch.2023.106459>
- Liu, R., Liu, R., Shi, L., Zhang, Z., Zhang, T., Lu, M., Chang, M., Jin, Q., & Wang, X. (2019). Effect of refining process on physicochemical parameters, chemical compositions and in vitro antioxidant activities of rice bran oil. *LWT*, 109, 26–32. <https://doi.org/10.1016/j.lwt.2019.03.096>
- Lourith, N., Kanlayavattanakul, M., Mongkonpaibool, K., Butsaratrakool, T., & Chinmuang, T. (2016). Rambutan seed as a new promising unconventional source of specialty fat for cosmetics. *Industrial Crops and Products*, 83, 149–154. <https://doi.org/10.1016/j.indcrop.2015.12.045>
- Mahisanunt, B., Na Jom, K., Matsukawa, S., & Klinkesorn, U. (2017). Solvent fractionation of rambutan (*Nephelium lappaceum* L.) kernel fat for production of non-hydrogenated solid fat: Influence of time and solvent type. *Journal of King Saud University, Science*, 29(1), 32–46. <https://doi.org/10.1016/j.jksus.2016.08.004>
- Manaf, Y. N. A., Marikkar, J. M. N., Long, K., & Ghazali, H. M. (2013). Physico-chemical characterisation of the fat from red-skin rambutan (*Nephelium lappaceum* L.) seed. *Journal of Oleo Science*, 62(6), 335–343.
- Matyash, V., Liebisch, G., Kurzchalia, T. V., Shevchenko, A., & Schwudke, D. (2008). Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics\* s. *Journal of Lipid Research*, 49(5), 1137–1146.
- Ng, Y. J., Chan, S. S., Khoo, K. S., Munawaroh, H. S. H., Lim, H. R., Chew, K. W., ... Show, P. L. (2023). Recent advances and discoveries of microbial-based glycolipids: Prospective alternative for remediation activities. *Biotechnology Advances*, 68, Article 108198. <https://doi.org/10.1016/j.biotechadv.2023.108198>
- Shuai, X., Dai, T., Chen, M., Liang, R., Du, L., Chen, J., & Liu, C. (2022). Comparative study on the extraction of macadamia (*macadamia integrifolia*) oil using different processing methods. *LWT*, 154, Article 112614. <https://doi.org/10.1016/j.lwt.2021.112614>
- Sirisompong, W., Jirapakkul, W., & Klinkesorn, U. (2011). Response surface optimization and characteristics of rambutan (*Nephelium lappaceum* L.) kernel fat by hexane extraction. *LWT - Food Science and Technology*, 44(9), 1946–1951. <https://doi.org/10.1016/j.lwt.2011.04.011>
- Soeng, S., Evacuasiany, E., Widowati, W., Fauziah, N., Manik, V. T., & Maesaroh, M. (2015). Inhibitory potential of rambutan seeds extract and fractions on adipogenesis in 3T3-L1 cell line. *Journal of Experimental and Integrative Medicine*, 5(1), 55–60.
- Solís-Fuentes, J. A., Camey-Ortiz, G., Hernández-Medel, M. d. R., Pérez-Mendoza, F., & Durán-de-Bazúa, C. (2010). Composition, phase behavior and thermal stability of natural edible fat from rambutan (*Nephelium lappaceum* L.) seed. *Bioresource Technology*, 101(2), 799–803. <https://doi.org/10.1016/j.biortech.2009.08.031>
- Sonwai, S., & Ponprachanuvut, P. (2012). Characterization of physicochemical and thermal properties and crystallization behavior of krabok (*Irvingia Malayana*) and rambutan seed fats. *Journal of Oleo Science*, 61(12), 671–679. <https://doi.org/10.5650/jos.61.671>
- Sun, J., Hu, P., Lyu, C., Tian, J., Meng, X., Tan, H., & Dong, W. (2022). Comprehensive lipidomics analysis of the lipids in hazelnut oil during storage. *Food Chemistry*, 378, Article 132050. <https://doi.org/10.1016/j.foodchem.2022.132050>
- Sun, T., Wang, X., Cong, P., Xu, J., & Xue, C. (2020). Mass spectrometry-based lipidomics in food science and nutritional health: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 19(5), 2530–2558.
- Thitilertdecha, N., Teerawutgulrag, A., & Rakariyatham, N. (2008). Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. *LWT - Food Science and Technology*, 41(10), 2029–2035. <https://doi.org/10.1016/j.lwt.2008.01.017>
- Tietel, Z., Hammann, S., Meckelmann, S. W., Ziv, C., Pauling, J. K., Wölk, M., ... Domingues, M. R. (2023). An overview of food lipids toward food lipidomics. *Comprehensive Reviews in Food Science and Food Safety*, 22(6), 4302–4354.
- Uoonlue, N., & Muangrat, R. (2019). Effect of different solvents on subcritical solvent extraction of oil from Assam tea seeds (*Camellia sinensis* var. *assamica*): Optimization of oil extraction and physicochemical analysis. *Journal of Food Process Engineering*, 42(2), Article e12960.
- Wang, H., Wu, Y., Xiang, H., Sun-Waterhouse, D., Zhao, Y., Chen, S., Li, L., & Wang, Y. (2022). UHPLC-Q-Exactive Orbitrap MS/MS-based untargeted lipidomics reveals molecular mechanisms and metabolic pathways of lipid changes during golden pomfret (*Trachinotus ovatus*) fermentation. *Food Chemistry*, 396, Article 133676. <https://doi.org/10.1016/j.foodchem.2022.133676>
- Wang, J., Wang, D., Huang, M., Sun, B., Ren, F., Wu, J., Meng, N., & Zhang, J. (2023). Identification of nonvolatile chemical constituents in Chinese Huangjiu using widely targeted metabolomics. *Food Research International*, 172, Article 113226. <https://doi.org/10.1016/j.foodres.2023.113226>
- Wang, W., Yang, X., Ye, Z., Li, Y., Liu, Y., & Cao, P. (2019). Extraction technology can impose influences on peanut oil functional quality: A study to investigate the lipid metabolism by Sprague-dawley rat model. *Journal of Food Science*, 84(4), 911–919. <https://doi.org/10.1111/1750-3841.14457>
- Wang, Y., Su, Y., Shehzad, Q., Yu, L., Tian, A., Wang, S., Ma, L., Zheng, L., & Xu, L. (2023). Comparative study on quality characteristics of Bischofia polycarpa seed oil by different solvents: Lipid composition, phytochemicals, and antioxidant activity. *Food Chemistry: X*, 17, Article 100588. <https://doi.org/10.1016/j.fochx.2023.100588>
- Weng, C., Deng, Z.-Y., Zhang, N., Zou, Q., Fan, Y.-W., Liu, R., Zhen, L.-F., & Li, J. (2020). Lipid profiles of Chinese soft-shell turtle eggs (*Pelodiscus sinensis*). *Journal of Food Composition and Analysis*, 94, Article 103627. <https://doi.org/10.1016/j.jfca.2020.103627>
- Wu, B., Wei, F., Xu, S., Xie, Y., Lv, X., Chen, H., & Huang, F. (2021). Mass spectrometry-based lipidomics as a powerful platform in foodomics research. *Trends in Food Science & Technology*, 107, 358–376. <https://doi.org/10.1016/j.tifs.2020.10.045>
- Xu, L., Zhu, C., Liu, T., Karrar, E., Ouyang, Y., & Li, D. (2022). Effect of microwave heating on lipid composition, chemical properties and antioxidant activity of oils from *Trichosanthes kirilowii* seed. *Food Research International*, 159, Article 111643. <https://doi.org/10.1016/j.foodres.2022.111643>
- Yang, R., Xue, L., Zhang, L., Wang, X., Qi, X., Jiang, J., Yu, L., Wang, X., Zhang, W., Zhang, Q., & Li, P. (2019). Phytosterol contents of edible oils and their contributions to estimated phytosterol intake in the Chinese diet. *Foods*, 8(8), 334. <https://doi.org/10.3390/foods8080334>
- Zeng, W., Liu, X., Chao, Y., Wu, Y., Qiu, S., Lin, B., Liu, R., Tang, R., Wu, S., Xiao, Z., & Li, C. (2024). The effect of extraction methods on the components and quality of *Camellia oleifera* oil: Focusing on the flavor and lipidomics. *Food Chemistry*, 447, Article 139046. <https://doi.org/10.1016/j.foodchem.2024.139046>
- Zhu, Q.-F., Yan, J.-W., Zhang, T.-Y., Xiao, H.-M., & Feng, Y.-Q. (2018). Comprehensive screening and identification of fatty acid esters of hydroxy fatty acids in plant tissues by chemical isotope labeling-assisted liquid chromatography–mass spectrometry. *Analytical Chemistry*, 90(16), 10056–10063. <https://doi.org/10.1021/acs.analchem.8b02839>