



Widely targeted metabolic profiling provides insights into variations in bioactive compounds and antioxidant activity of sesame, soybean, peanut, and perilla



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ABSTRACT

Oilseeds are important sources of diversified nutraceuticals with marked health attributes. Thus, a better understanding of metabolome differences between common oilseeds will be conducive to the food pharmacy. This study aimed to compare the metabolite profiles and antioxidant activity of sesame, soybean, peanut, and perilla seeds and reveal the variation in bioactive compounds. LC-MS-based widely targeted metabolic profiling identified a total of 975 metabolites, of which 753 were common to the four crops. Multivariate analyses unveiled a crop-specific accumulation of metabolites, with 298–388 DAMs (differentially accumulated metabolites) identified. Amino acid metabolism, phenylpropanoid biosynthesis, flavonoid biosynthesis, and lipid metabolism were the most differentially regulated pathways. Furthermore, we revealed the variation in the relative content of 48, 20, 18, 9, 18, 11, and 6 differentially accumulated bioactive flavonoids, phenolic acids, amino acids, vitamins, terpenoids, alkaloids, and coumarins, respectively. Most of the flavonoids accumulated highly in soybean, followed by perilla. Sesame exhibited a better amino acid profile than other oilseeds. DPPH and FRAP assays showed that the antioxidant activity of perilla seed extracts was the highest, followed by soybean, peanut, and sesame. Our results provide data support for the comprehensive use of sesame, perilla, soybean, and peanut seeds in food, and pharmaceutical industries.

1. Introduction

The awareness of chronic diseases' damage to humans' health has triggered research on sustainable functional foods (Nayak et al., 2021). Among the most common functional foods used worldwide, oilseeds represent not only the major source of human energy but also critical reservoirs of essential nutritional compounds and nutraceuticals (Sangwan et al., 2022; Sarwar, 2013; Tanwar & Goyal, 2021). Oilseeds rank fourth in major food commodities behind cereals, vegetables and melons, and fruits and nuts, and are grown worldwide due to their high economic value (Ahmad et al., 2021; Tanwar & Goyal, 2021). Accordingly, the production area of oilseeds has expanded by over 82 %, occupying about 213 M ha of arable land over the world (Ahmad et al., 2021; Zhang et al., 2022a). The health benefits attributes of oilseeds'

phytonutrients have earned the attention of food scientists and other researchers (Tanwar & Goyal, 2021). Oilseeds and their derived edible oils are rich in protein, fatty acids, dietary fiber, vitamins, phytosterols, phenolic compounds, and other functional components (Tanwar & Goyal, 2021; Zhang et al., 2022). Studies have demonstrated that the chemical compositions of different oilseed plants are diverse (Tanwar & Goyal, 2021; Zhou et al., 2020). Among oilseed phytochemicals, polyphenols, including flavonoids, phenolic acids, lignans, etc., are the most diversified and the essential material basis for their various biological activities, such as anti-bacterial, antioxidant, anti-inflammatory, anti-viral, anti-tumor, anti-depressant, cardioprotective, anti-diabetic, anti-obesity, and neuroprotective (Hou et al., 2022; Zhang et al., 2022a). With the implementation of the food pharmacy, it is of global interest to thoroughly access and understand the variation in bioactive compounds

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in the different oilseeds.

Diverse oilseed crops, such as rapeseed, sunflower, soybean, cotton, sesame, peanut, perilla, etc., are produced worldwide. Of them, rape-seed and sunflower oilseeds are cultivated mainly for oil extraction, while cotton is produced primarily for its fibers. Sesame (*Sesamum indicum* L.), soybean (*Glycine max*), peanut (*Arachis hypogea* L.), and perilla (*Perilla frutescens*) are well-recognized oilseed crops for their edible oil and the pharmacological properties of their derived products (Bi et al., 2022; Dossou et al., 2022a; Hou et al., 2022; Xu et al., 2022). Particularly, in China, Korea, Japan, and other Asian countries sesame, soybean, peanut, and perilla seeds are used to produce various foods, and represent critical ingredients in traditional medicine (Zhang et al., 2022b; Kim et al., 2020; Toomer, 2018). The edible oils and other important seed-derived products from sesame, peanut, soybean, and perilla are used in diverse industries, such as food, cosmetics, pharmaceuticals, biofuels, etc., due to their excellent phytochemical profiles and health benefits (Ahmed, 2019; Pathak et al., 2014; Shang et al., 2023; Tanwar & Goyal, 2021). Due to their multi-functionality, these crops have gained higher attention in multi-omics research especially, in food-omics (Ku et al., 2022; Toomer, 2018). For instance, metabolomics studies have shown that sesame (Dossou et al., 2022b; Wang et al., 2018), soybean (Li et al., 2018; Lin et al., 2014; Xu et al., 2022), perilla (Ahmed, 2019; Chen et al., 2022; Zhou et al., 2021), and peanut (Klevorn et al., 2019; Patel et al., 2022; Zhang et al., 2023) seeds contain diverse metabolites, including amino acids, sugars, and lipids, phenolic acids, flavonoids, terpenoids, organic acids, alkaloids, vitamins, stilbenes, etc., most of which are active metabolic compounds with recorded biological functions. These studies unveiled the intra-species metabolite diversity and variability. However, knowledge of the diversity and variation of metabolites in among these oilseeds is scarce, limiting the well development of their respective industries and their insertion in specific nutritional and health programs. Only a slight comparative metabolomics analysis of sesame and perilla seeds (Kim et al., 2020), and sesame, flax, and chia seeds (Brigante et al., 2022) have been performed. Therefore, a comparative analysis of the metabolic profiles of sesame, soybean, peanut, and perilla seeds is of great interest. It will provide important data support for their comprehensive use, and for food traceability, authenticity, quality assessment, and quality breeding purposes (Brigante et al., 2020; Kim et al., 2020). In vegetables (Tao et al., 2023; Baky et al., 2022), and fruits (Khakimov et al., 2016) comparative metabolomics analysis was carried out providing insights into the variation of important food components and nutraceuticals among these crop species.

With the expansion of the food omics concept, defined as a comprehensive and high-throughput approach to take full advantage of food science in the light of improving human nutrition, advanced analytical methods have been developed to link food components and the diet to human health (Balkir et al., 2021; Capozzi & Bordoni, 2013). While traditional food analytical methods only allow access to global food composition, omics approaches permit understanding changes in different food samples at the molecular level (Srinivasan, 2020). Widely targeted metabolic profiling is one of the most advanced omics tools for deciphering nutrients that compose the global metabolome of plant organs. It allows an understanding of the diversity in plants via an accurate identification and evaluation of the relative content of all metabolites and the exploration of metabolite profile changes due to stresses or perturbations (Chen et al., 2013; Chen et al., 2020; Dossou et al., 2021; Xu et al., 2022; Farag et al., 2019; Scalbert et al., 2011; Xiao et al., 2021). It has been applied to explore metabolome underlying phenotypic variations in many plant species such as tea, rice, sesame, peanut, and tomato (Chen et al., 2018; Chen et al., 2013; Dossou et al., 2022a; Sharma et al., 2020; Zhang et al., 2023). The diversity and the relative content variability of metabolites among the crops as well as genotypes could have an association with their end use and bioactivities (Kim et al., 2021). With the evolution of the oilseed markets, revealing the variation in bioactive compounds and antioxidant activities between

sesame, soybean, peanut, and perilla will contribute to refining demands and promote the development of novel varieties with improved therapeutic potentials.

In this study, we applied UHPLC_MS/MS (ultra-high performance liquid chromatography-mass spectroscopy)-based widely targeted metabolic profiling to differentiate between the metabolite profiles of sesame, soybean, peanut, and perilla seeds. Moreover, we revealed crop specific accumulation, differentially regulated pathways that could potentially influence the variation characteristics of bioactive compounds, and antioxidant activities among the four oilseeds. Our findings may allow the comprehensive use of sesame, perilla, soybean, and peanut seeds in food pharmacy.

2. Materials and methods

2.1. Description of the plant material

The experimental seeds of sesame, soybean, peanut, and perilla ($4 \times 6 = 24$) were obtained from the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (OCRI-CAAS) (Supplementary Table 1). These crops were grown and managed under a controlled environment in the experimental area of the institute from July to October in the year 2021. All necessary agronomic management practices were applied equally as per the crop demand. The seed samples from each crop genotype were collected in triplicate from ten individual plants after harvest. Then, these samples were taken and placed in the refrigerator at -80°C until the metabolomic profiling analysis. The other group of representative samples was prepared similarly and kept in the OCRI-CAAS seed storage room until the evaluation of the antioxidant activities.

2.2. Reagents and chemical standards

The chemicals, including acetonitrile, acetic acid, and methanol, were purchased from the Merck Company (Germany, Hesse, Darmstadt) for use in ultrahigh-performance liquid chromatography mass-spectrometry (UHPLC-MS) gradient-grade solvents. Standard metabolites and additional chemicals were purchased from Sigma Aldrich (St. Louis, Missouri, USA) and BioBioPha (Kunming, Yunan, China). Methanol or dimethyl sulfoxide was used to dissolve the standards to prepare standard stock solutions (which were stored at -20°C). Before mass-spectrometry analysis, the standards were dissolved with 70 % methanol to produce a gradient of various concentrations.

2.3. Experimental sample preparation and metabolite extraction

The seeds (100 mg) taken from each genotype were individually grounded with liquid nitrogen, and the homogenate mix was resuspended with 80 % prechilled methanol using the well-vortex. Subsequently, the extracts were changed to new and clean tubes and subjected to centrifugation at 15,000 g for 20 min at 4°C after being incubated for 5 min on ice. The supernatants were diluted with LC-MS grade water to a final concentration that contained 53 % of methanol. The supernatants were then added to the LC-MS/MS system analysis to complete the process (Want et al., 2013). A mixture of equal quantities of each sample extract made up the quality control (QC) samples.

2.4. UHPLC-MS/MS analysis

UHPLC-MS/MS analysis (Shim-pack UFCL SHIMADZU CBM30A) was performed using an ExionLC™ AD system (SCIEX) coupled with a QTRAP® 6500 + mass spectrometer (SCIEX) at Novogene Co., Ltd. (Beijing, China). Samples were injected onto an Xselect HSS T3 (2.1 × 150 mm, 2.5 μm) using a 20 min linear gradient at a 0.4 mL/min flow rate for the positive/negative polarity mode. The eluents A (0.1 % formic acid water) and B (0.1 % formic acid acetonitrile) were used (Luo

et al., 2015). The different solvent gradients such as 2–100 % B, 15 min; 2 % B, 2 min; 100 % B, 17 min; 100–2 % B, 17.1 min; and 2 % B, 20 min were used. The positive polarity mode QTRAP® 6500 + mass-spectrometer was operated with a curtain gas of 35psi, collision gas of medium, ion spray voltage of 5500 V, a temperature of 550 °C, ion source gas 1:60, and ion source gas 2:60. Similarly, the negative polarity mode of QTRAP® 6500 + mass-spectrometer was operated with a curtain gas of 35psi, collision gas of medium, ion spray voltage of –4500 V, temp. 550 °C, ion source gas 1:60, and ion source gas 2:60.

2.5. The identification and quantification of metabolites

The internal database of Novogene. Co., Ltd. served as the foundation for the MRM (Multiple Reaction Monitoring) detection and identification of metabolites in the experimental samples. Q3 was considered to quantify the metabolites, whereas each compound Q1/Q3 (ion-pair), retention time (RT), de-clustering potential (DP), and collision energy (CE) were considered to identify the metabolites. The data produced from UHPLC-MS/MS was subjected to SCIEX-OS version 1.4 to integrate and adjust the peaks. The three main criteria taken into account were the minimum peak height of 500, a signal-to-noise ratio of 5, and Gaussian smooth breadth of 1, and the relative content of each peak as represented by the peak area.

2.6. Determination of antioxidant activity

The sample preparation method was adopted from (Dossou et al., 2022b). Hence, 0.5 g of ground powder from each crop sample was extracted with 5 mL of 80 % ethanol in a shaking incubator for four hours at room temperature. The supernatants were filtered and carefully collected individually after centrifugation of 5000 rpm for 15 min. Then the DPPH and FRAP assays were performed as follows;

DPPH assay. The 1,1-diphenyl-1-picrylhydrazino radical (DPPH) assay was performed with a slight modification of the method reported by (Kim et al., 2014a,b). Sample extracts of each crop seed (10 µL), 1,1-diphenyl-1-picrylhydrazino radical (DPPH, 0.2 mM) (190 µL), and absolute ethanol solution in 96-well plates were incubated for 30 min at room temperature in darkness. The absorbance of the experiment was determined at 515 nm wavelength using a microplate reader. The blank consists of 10 µL of 80 % ethanol and 190 µL DPPH, whereas the control consists of absolute ethanol 190 µL and sample extracts of 10 µL. Finally, the scavenging activity result was expressed as a percentage using the following formula:

$$\text{DPPH radical scavenging effect (\%)} = [1 - (\text{AS} - \text{AC}) \div \text{AB}] \times 100.$$

Where, AS = absorbance of sample, AC = Absorbance of control, AB = absorbance of blank

FRAP assay. The ferric ion-reducing antioxidant power (FRAP) assay was performed according to the method reported by Ahmed and Tavaszi-sarosi (2019) with little modification. Sodium acetate (3.1 g), acetic acid (16 mL), and distilled water (DW) (11 mL) were mixed to prepare acetate buffer in a measuring cylinder. Using 0.03123 g of TPTZ, 10 mL distilled water, and 33.6 µL HCl, 2,4,6-tripiridil-S-triazine (TPTZ) solution was prepared. Then, iron chloride (FeCl3) 0.054 g was diluted with 10 mL of DW. Finally, the FRAP solution was prepared by mixing 50 mL acetate buffer, TPTZ solution (5 mL), and iron chloride (5 mL) solutions. To calibrate the standard curve, ascorbic acid (0.017613 mg) was dissolved in 10 mL of DW. Then, 100 µL was taken from this solution and diluted by 900 µL of DW. A serial dilution of an ascorbic acid stock solution containing 10 µL, 20 µL, 30 µL, 40 µL, 50 µL, and 100 µL was prepared as a standard reference. A blank, having 6 µL of 80 % ethanol, 180 µL FRAP solution, and 18 µL DW was prepared. Then, 6 µL of sample extract, 180 µL FRAP solution, and 18 µL DW were added to test tubes.

Then, the samples were subjected to a microplate reader Thermo Evolution 201 spectrophotometer at 593 nm absorbance. The FRAP values were calculated from the standard curve equation ($X=(Y + 0.0038)/0.005$). They revealed a good regression quotient ($R^2 = 0.999$) close to 1, where Y and X represent the absorbance and ascorbic acid concentration, respectively. The result was presented as Mg ascorbic acid equivalent (mg AAE/g DM). The measurements were performed in triplicate.

2.7. Data analysis

The two databases KEGG (<http://www.genome.jp/kegg/>) and HMDB (www.hmdb.ca), were used to annotate the identified metabolites. These databases were also used to annotate metabolites and study the metabolite function and metabolic pathways. Then, the KEGG pathway database (<http://www.kegg.jp/kegg/pathway.html>) was used to map the annotated metabolites. Significant enrichment pathways were determined at $P < 0.05$ significant level. MetaboAnalyst5.0 (<https://www.metaboanalyst.ca/MetaboAnalyst/>) was used to draw principal component analysis (PCA) and partial least discriminant analysis (PLS-DA) figures. To identify differential metabolites, metabolites with variable importance in projection (VIP) > 1 and $P < 0.05$ and fold change (FC) ≥ 2 or FC ≤ 0.5 were considered. Based on the volcano plots Log2(FC) and -Log10(P-value), differential metabolites were screened using the R-programming language ggplot2 program. The data were normalized using the intensity of differential metabolites Z-score and Pheatmap package in R-language. Simple pearson correlation analysis was performed to evaluate the association between phenolic compounds and antioxidant activities at $P < 0.05$.

3. Results

3.1. Metabolic profiles and variation in sesame, peanut, soybean, and perilla

To provide insights into the distribution and variation of metabolites in important edible oilseed crops, we subjected sesame (SS), soybean (SB), peanut (PN), and perilla (PL) seeds to widely targeted metabolic profiling (Chen et al., 2013; Dossou et al., 2021; Dossou et al., 2022b). The metabolites were detected at both the positive (+) and negative (-) electrospray ionization (ESI) as shown in the ion chromatograms (TICs) of quality control (QC) samples (Supplementary Fig. 1). In total, we structurally identified 975 metabolites (Supplementary Table 2), of which 753 were common to perilla, soybean, sesame, and peanut seeds (Fig. 1A). Four (sesamin, forsythoside-A, hydro-protopine, and O-feruloyl coumarin), one (Dl-glyceraldehyde 3-phosphate), and ten metabolites were specifically identified in SS, SB, and PL, respectively (Fig. 1A, Supplementary Table 3).

The classification of the identified metabolites showed that amino acids and derivatives (19.28 %), saccharides and alcohols (11.2 %), lipids (10.97 %), flavonoids (10.26 %), organic acid and its derivatives (9.54 %), nucleotide and its derivatives (8.10 %), phenolic acids (7.4 %), phenols and derivatives (5.44 %), terpenoids (4.2 %), and vitamins (2.15 %) were the ten major dominants metabolite classes in SS, SB, PN, and PL seeds (Fig. 1B). The sum of the intensities of all metabolites within each major class revealed significant variations ($P < 0.05$) (Fig. 1C-L). Accordingly, PN seeds contain significantly lower lipid content than sesame, soybean, and perilla seeds (Fig. 1C). Phenols and derivatives, phenolic acids and derivatives, and vitamins exhibited the highest relative content in PL seeds (Fig. 1E-G). Meanwhile, flavonoids, organic acids and derivatives, nucleotides and derivatives, and terpenoids showed the highest relative content in SB seeds (Fig. 1I-K). There was no statistically significant variation on the relative intensities of amino acids and derivatives between four crops (Fig. 1L).

To precisely examine the variability of metabolites between and within groups, we performed multivariate data analyses (Fig. 2). The PCA and HCA analyses showed that the metabolite profiles of SS, SB, PN,

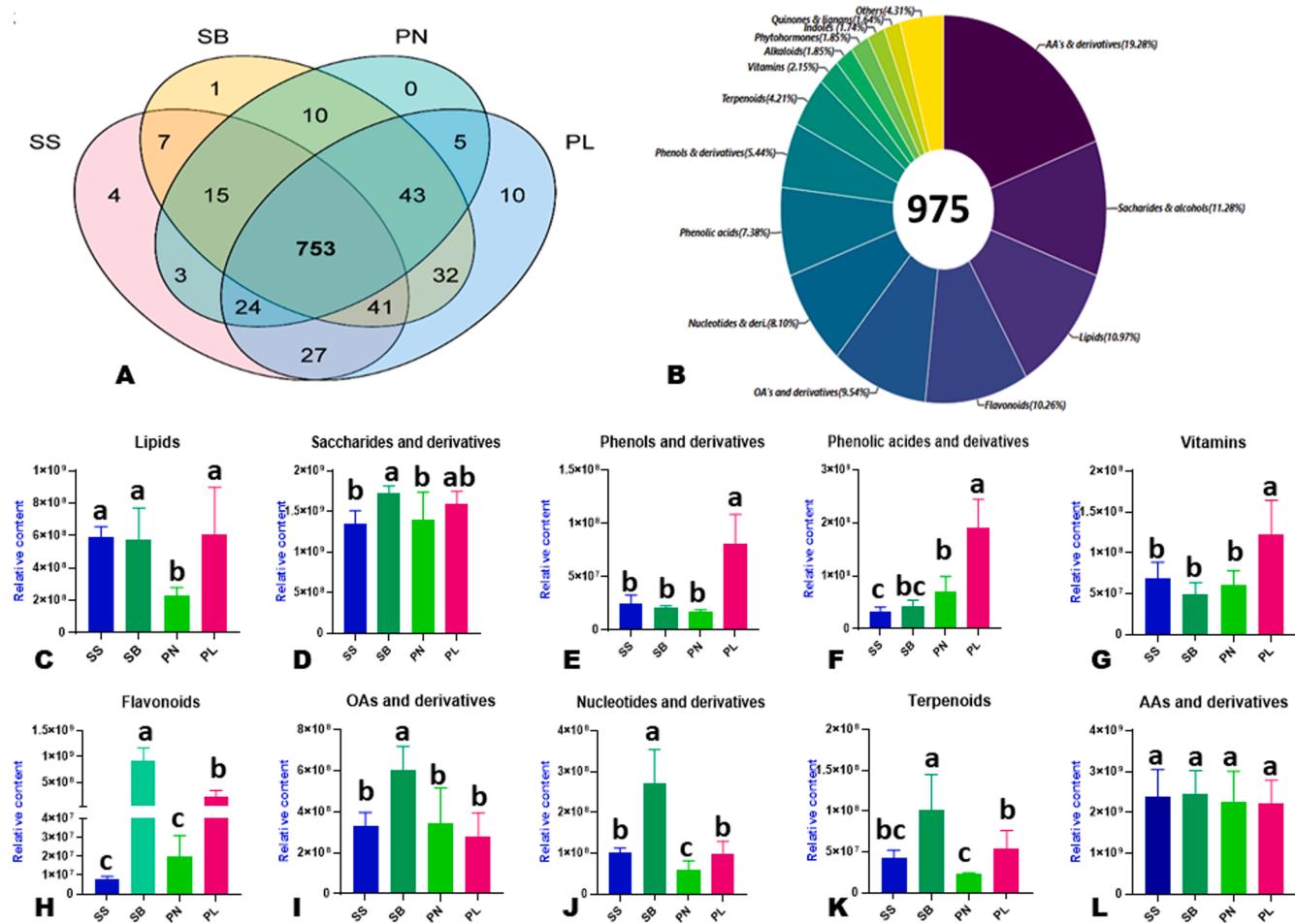


Fig. 1. (A) Venn diagram showing the numbers of common and specific metabolites accumulated in the four oilseeds. (B) Classification of the 975 identified metabolites. (C)-(L) Relative contents of major metabolite classes in the four oilseeds. SS, sesame; SB, soybean; PN, peanut; PL, perilla.

and PL seeds were very different (Fig. 2A, B). On the PCA plot, samples were clearly separated into four clusters without outliers corresponding to SS, SB, PN, and PL (Fig. 2A). Supportively to the above results, more metabolites exhibited higher relative content in SB, followed by PL, inferring their seeds might be richer than sesame and PN seeds (Fig. 2B). To confirm the observed variation in the metabolite profiles, we carried out OPLS-DA analysis (Fig. 2C-D, Supplementary Fig. 2). The R^2Y and Q^2Y values of all pairwise comparisons were close to 1, supporting the reliability of the experiments analysis (Fig. 2C-D, Supplementary Fig. 2).

3.2. Differentially accumulated metabolites (DAMs) and pathways between Sesame, Soybean, Peanut, and perilla

In order to screen out all DAMs in pairwise comparison between the four oilseed groups, we applied three thresholds of $VIP > 1.0$, $FC > 1.2$ or $FC < 0.833$, and $P\text{-value} < 0.05$. In the pairwise comparison between SB vs. PN, SS vs. SB, SB vs. PL, PN vs. PL, SS vs. PL, and SS vs. PN, there were 388 (350 up-regulated in SB), 363 (64 up-regulated in SS), 358 (232 up-regulated in SB), 355 (66 up-regulated in PN), 319 (97 up-regulated in SS), and 298 (204 up-regulated in SS) DAMs, respectively (Fig. 3A). The volcano plots of the DAMs are shown in Supplementary Fig S3. Venn diagram analysis showed that 37 overlapped DAMs in the pairwise comparison between SS against other oilseed crops (Fig. 3B, C, Supplementary Table 4). These 37 key overlapping DAMs were mainly amino acids and their derivatives (21.62 %), phenolic acids (16.2 %), flavonoids (13.5 %), saccharides and alcohols (8.11 %), and nucleotides and derivatives (8.11 %) (Supplementary Fig. 4).

The classification of DAMs between groups revealed that amino

acids, flavonoids, phenolic acids, saccharides and alcohols, lipids, and organic acids were dominant components (Fig. 3D). Further, we achieved KEEG enrichment analysis of DAMs, and the results were consistent with the classification (Supplementary Fig. 5). The main pathways differentially regulated in SS, SB, PN, and PL seeds were amino acid metabolism, phenylpropanoid biosynthesis, flavonoid biosynthesis, and lipid metabolism (Supplementary Fig. 5). To enable an easy overview of key DAMs and differentially regulated pathways between SS, SB, PN, and PL, we constructed a diagram (Fig. 4).

3.3. Variation characteristics of bioactive compounds in Sesame, Soybean, Peanut, and perilla seeds

Phytochemicals, including flavonoids, phenolic acids, alkaloids, terpenoids, essential vitamins, etc., have shown therapeutic properties against chronic diseases such as diabetes, cancers, oxidation stresses, insomnia, amnesia, angiogenesis, osteoclastogenesis, neurodegeneration, and inflammation (Ben et al., 2015; Choi et al., 2020; Gong et al., 2019; Hussain et al., 2018; Imran et al., 2019; Koirala et al., 2016; Manayi et al., 2016; Yamamoto et al., 2017). To reveal the variation characteristics of bioactive compounds in the four oilseed crops, we examined the relative content of 48, 20, 18, 9, 18, 11, and 6 differentially accumulated flavonoids, phenolic acids, amino acids, vitamins, terpenoids, alkaloids, and coumarins, respectively.

3.3.1. Flavonoids

We observed a great variation in the relative content of bioactive differentially accumulated flavonoids with most of them exhibiting the

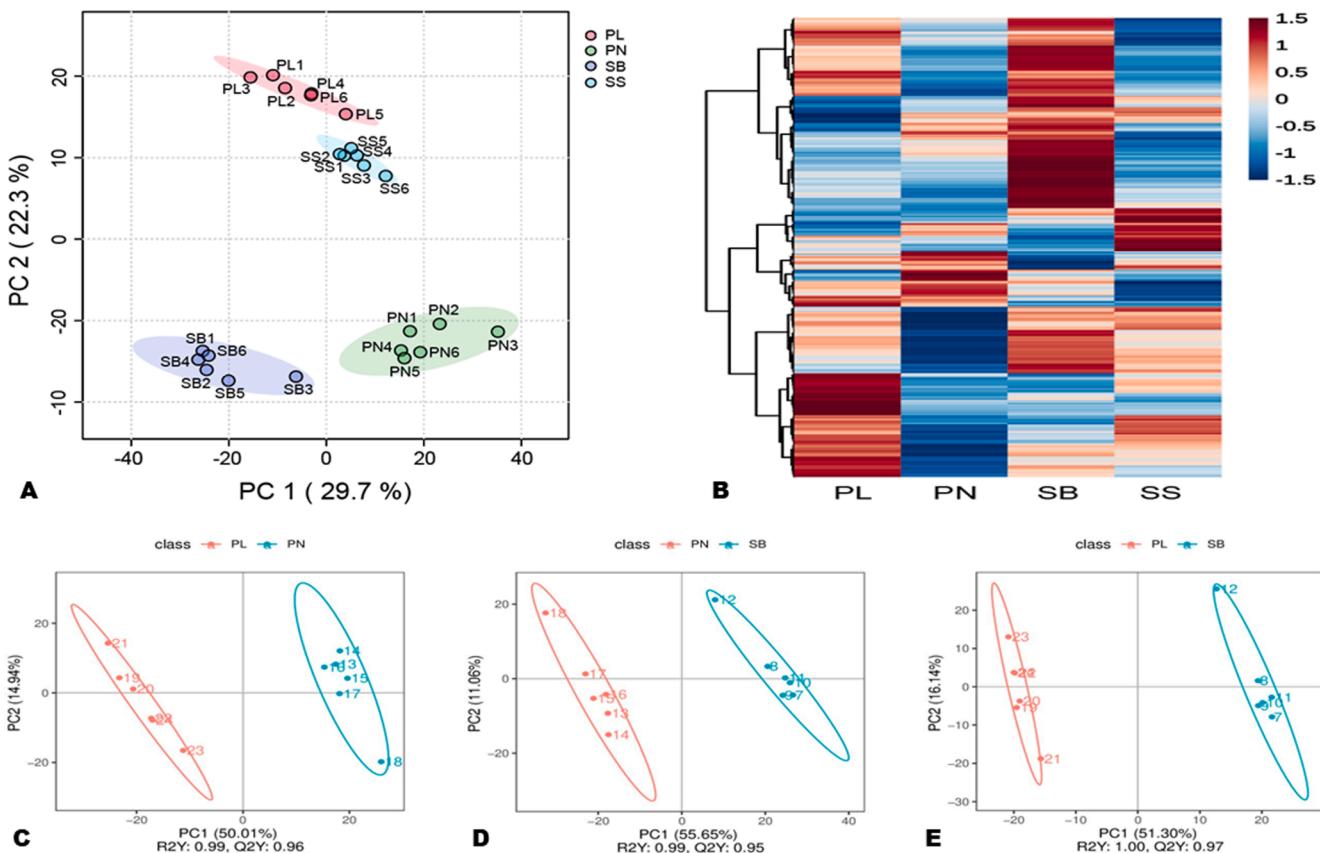


Fig. 2. Overview of the diversity and variation of metabolites in the four oilseeds. (A) principal component analysis (PCA); (B) hierarchical clustering analysis (HCA); (C)-(E) Loading plots of OPLS-DA results of pairwise comparisons between PL.vs.PN, PN.vs.SB, and PL.vs.SB, respectively. SS, sesame; SB, soybean; PN, peanut; PL, perilla.

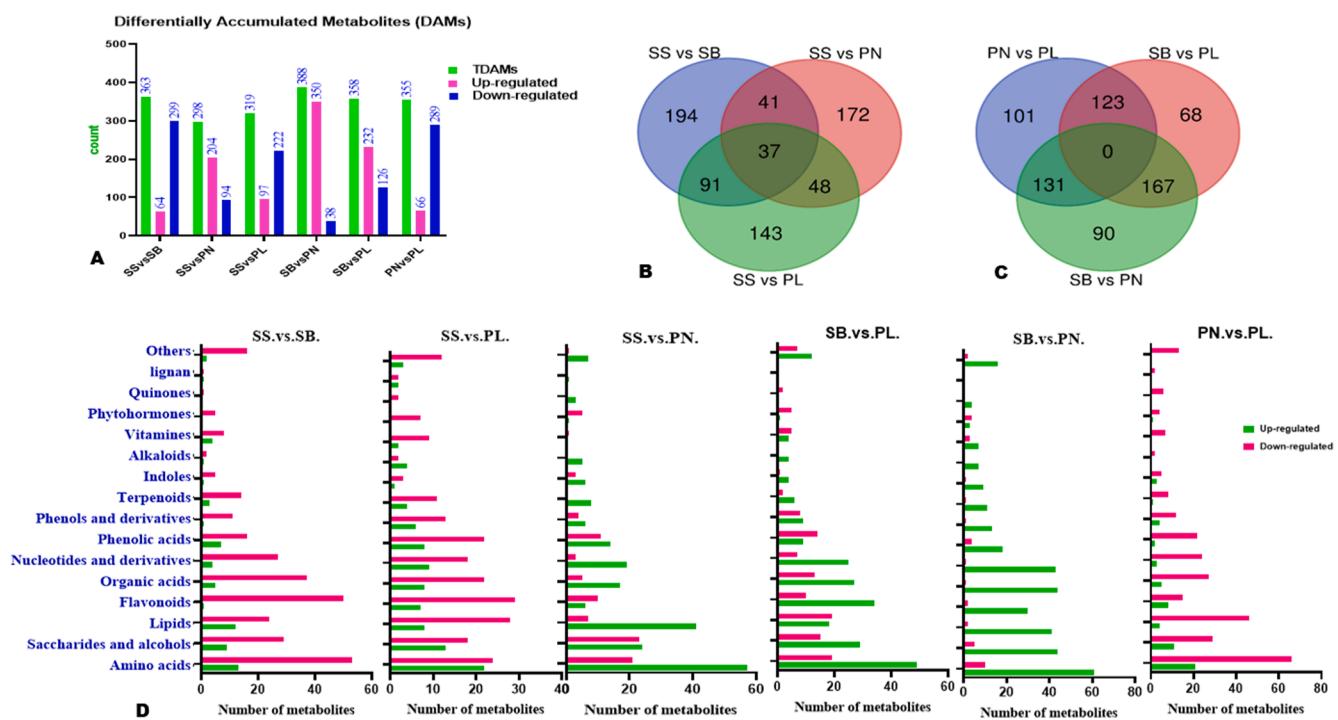


Fig. 3. Differentially accumulated metabolites (DAMs) across the four oilseeds. (A) Numbers of significant DAMs in the pairwise comparison between groups. (B) and (C) Venn diagram exhibiting the number of the key DAMs. (D) Classification of the DAMs. Up-regulation for “X.vs.Y” indicates the metabolite has higher relative content in X. TDAMs, total DAMs; SS, sesame; SB, soybean; PN, peanut; PL, perilla.

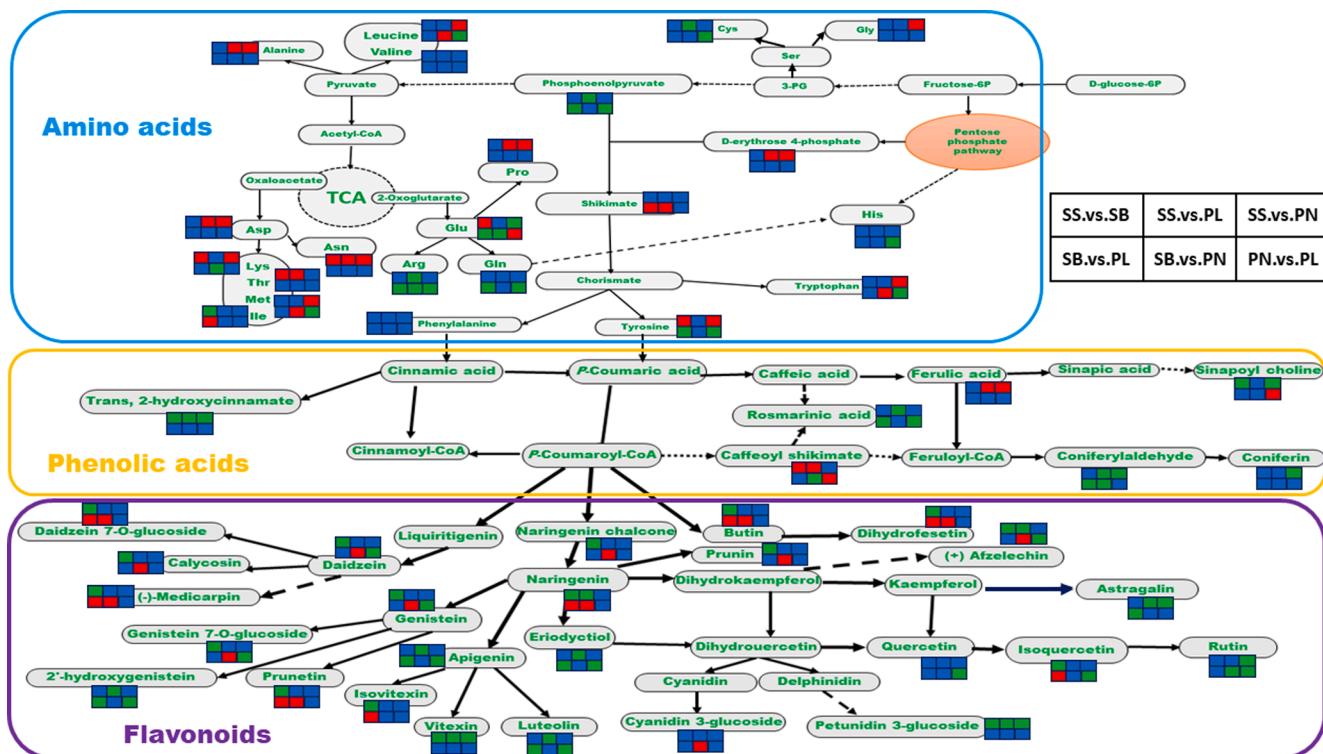


Fig. 4. Changes in some key metabolites mapped to metabolic pathways in pairwise comparisons between the four oilseed groups. The red, green, and blue color small rectangles indicate that the metabolite content is significantly up-regulated, down-regulate, and no significant difference, respectively. Up-regulation for "X-vs. Y" indicates the metabolite has higher relative content in X. SS, sesame; SB, soybean; PN, peanut; PL, perilla. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

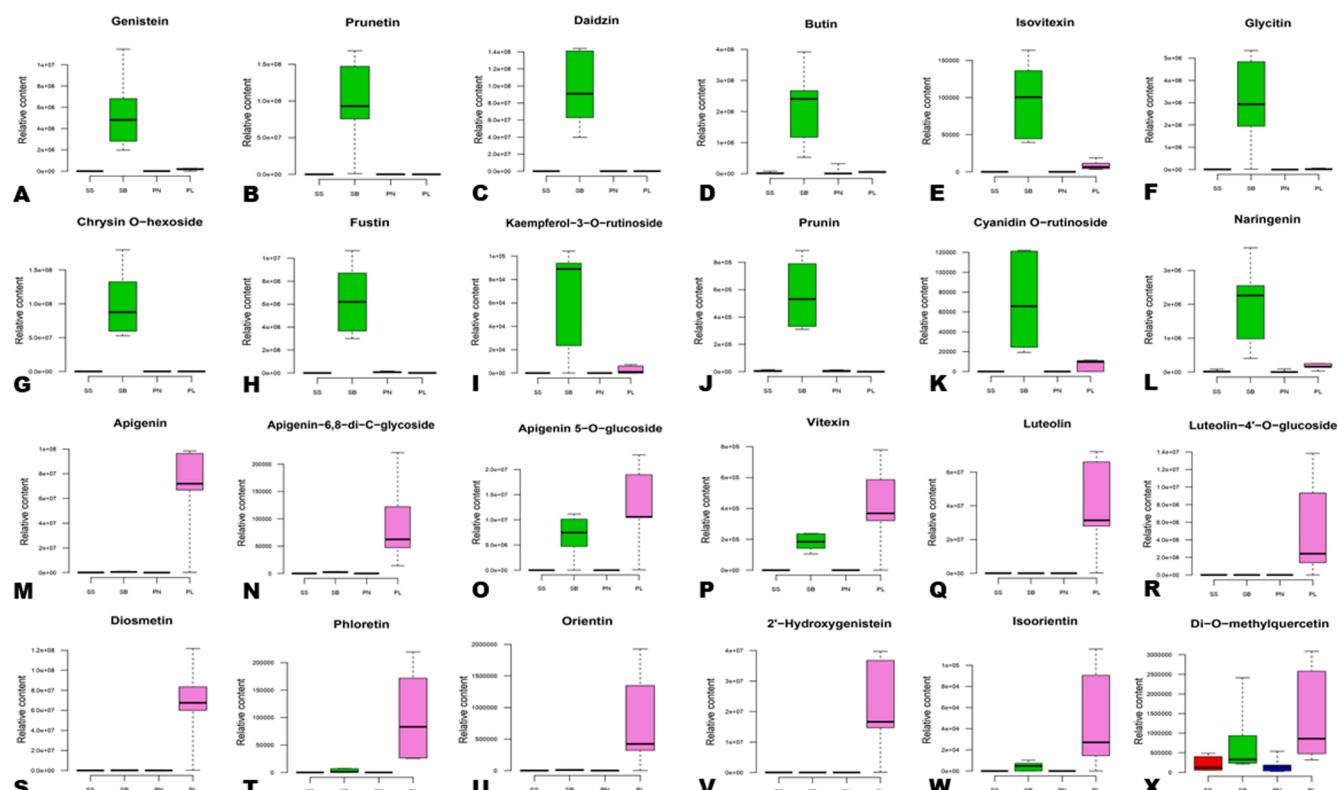


Fig. 5. Box plots showing the variation of twenty-four selected differentially accumulated bioactive flavonoids in the four oilseeds. SS, sesame; SB, soybean; PN, peanut; PL, perilla.

highest content in SB and the remaining in PL (Fig. 5 and Supplementary Fig. 6). For instance, 30 of the 48 examined flavonoids, including genistein, prunetin, daidzin, butin, isovitexin, fustin, naringenin, toringin, kaempfeol3-O-rutinoside, cyanidin O-rutinoside, etc. showed higher relative content in SB than other oilseeds (Fig. 5 and Supplementary Fig. 6). Meanwhile, 15 flavonoids, including apigenin, apigenin-5-O-glucoside, luteolin, luteolin-4'-O-glucoside, vitexin, phloretin, orientin, iso-orientin, 2'-hydroxygenistein, diosmetin, etc. exhibited the highest relative content in PL (Fig. 5 and Supplementary Fig. 6). Two (rutin and tricin 5-O-hexoside) flavonoids exhibited higher relative content in PN (Fig. 5 and Supplementary Fig. 6).

3.3.2. Phenolic acids and terpenoids

The variation in the differentially accumulated phenolic acids is presented as a box plot in Fig. 6. SS exhibited the highest relative content of isoacteoside, isovanillic acid, vanillyl alcohol, and 5-cafeoylshikimic acid (Fig. 5A-E). Sinapic acid, sattabacin, isomangiferin, 5-O-p-coumaroyl shikimic acid, and 2-O-caffeoylemalic acid showed higher relative content in SB than other oilseeds (Fig. 5F-K). Compared to other oilseeds, the relative contents of rosmarinic acid, rosmarinic acid-3'-O-glucoside, caffeoic acid O-glucoside, gallic acid O-hexoside, and N'-p-coumaroyl agmatine were higher in PL (Fig. 5L-P). Meanwhile, PN exhibited the highest relative content of p-coumaric acid, hydroxycinnamate, 2-hydroxycinnamate, p-hydroxy cinnamic acid, and N-p-coumaroyl spermidine (Fig. 5Q-U).

Of the 18 differentially accumulated terpenoids examined, 5 (asiatic acid, fraxinellone, alpha-lonone, geranylacetone, and geniposidic acid), 9 (maslinic acid, kolavic acid, valepotriate, pterosin A, capsianoside I, etc.), and 3 (oleanic acid, ursolic acid, and isomangiferolic acid) exhibited the highest relative content in SS, SB, and PL, respectively

(Supplementary Fig. 7).

3.3.3. Alkaloids and coumarins

In total, 11 and 6 differentially accumulated alkaloids and coumarins were examined, respectively (Supplementary Figs. 8 and 9). Of the alkaloids, five (vasicine, arecoline, beta-nicotyrine, D-hydroevodiamine, trigogelline, and hirsuteine), three (hydropropotopine, tabersonine, and N'-nitrosoanabasine), and two (hirsuteine and swainsomine) showed the highest relative content in SB, SS, and PL, respectively (Supplementary Fig. 8). Among the coumarins, skimmmin and psoralen showed the highest relative content in SS and SB, respectively (Supplementary Fig. 9). Ayapin and esculin exhibited the highest relative content in PL (Supplementary Fig. 9).

3.3.4. Amino acids and vitamins

The box plots showing the variations of amino acids and vitamins are presented in Figs. 7 and 8A-I. Five amino acids, including proline, threonine, asparagine, ornithine, and alanine, showed higher relative content in SS than the other oilseeds (Fig. 7A, B, D-F). Lysine, tyrosine, and glutamine exhibited higher relative content in SS and PL than in SB and PN (Fig. 7C, J, L). Histidine, arginine, and serine exhibited the highest relative content in PL, followed by SS (Fig. 7G-I). Meanwhile, tryptophan, leucine, aspartic acid, and methionine exhibited the highest relative content in SB, followed by SS and PL (Fig. 7M-P). The relative content of glutaric acid in PN was the highest, followed by SS and PL (Fig. 7Q).

Four (vitamins C, U, K, and isonicotinic acid), two (vitamins B6 and B7), and two (vitamin B3 and B1) exhibited the highest relative content in PL, SB, and SS, respectively (Fig. 8A-H). The relative content of vitamin B2 was higher in SB and PL than in SS and PN (Fig. 8I).

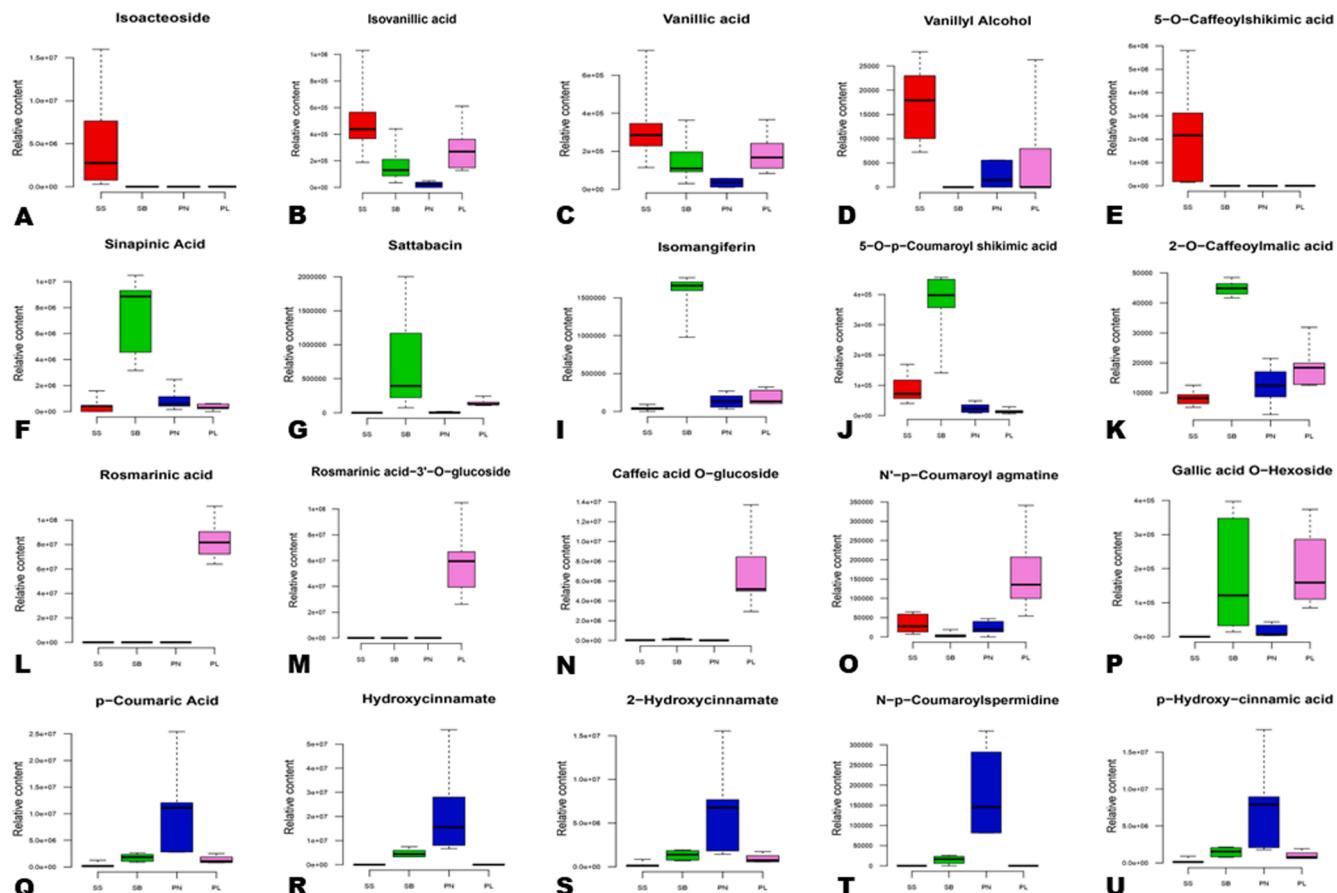


Fig. 6. Box plots showing the variation of twenty selected differentially accumulated bioactive phenolic acids in the four oilseeds. SS, sesame; SB, soybean; PN, peanut; PL, perilla.

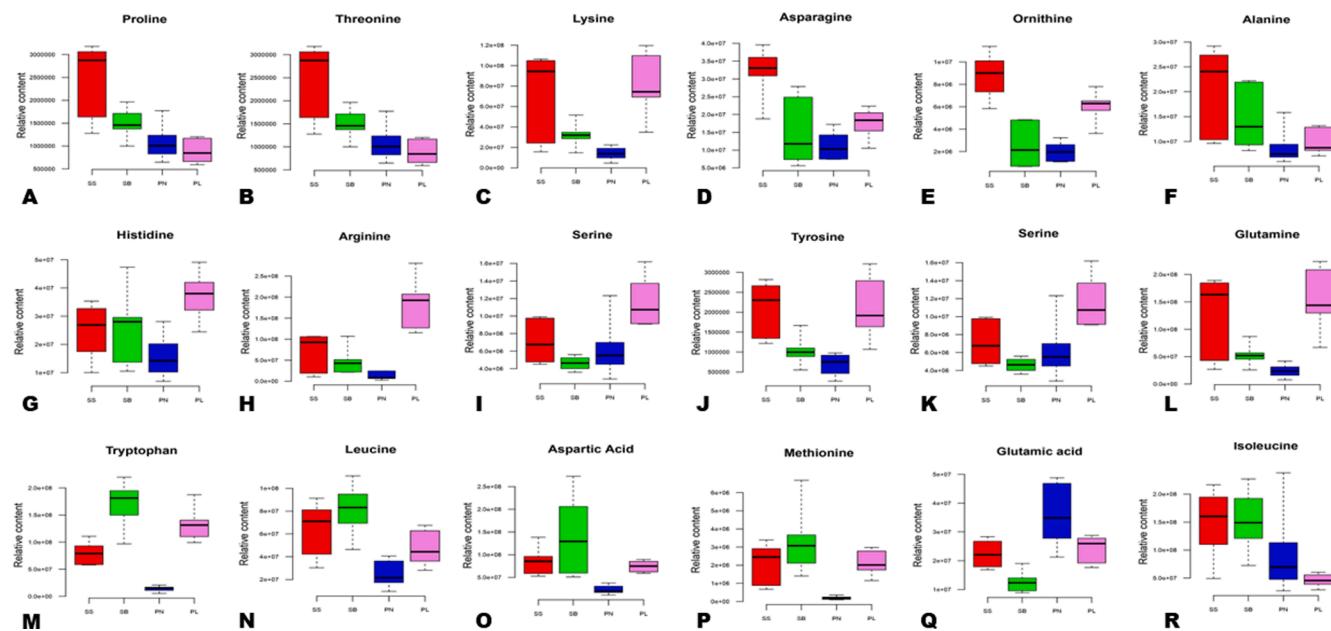


Fig. 7. Box plots showing the variation of eighteen differentially accumulated bioactive amino acids in the four oilseeds. SS, sesame; SB, soybean; PN, peanut; PL, perilla.

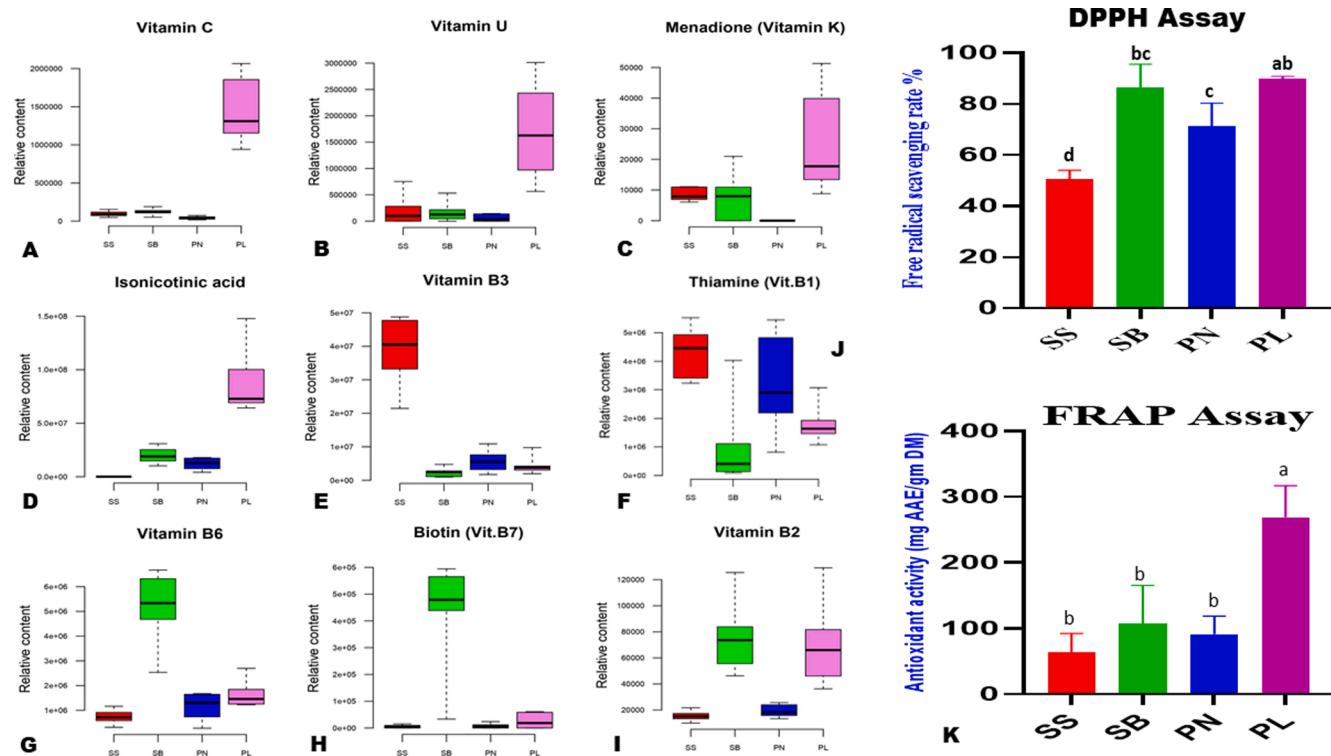


Fig. 8. Box plots showing the variation of nine differentially accumulated bioactive vitamins in the four oilseeds, (A)-(I). DPPH free radical scavenging rate of the four oilseeds, (J). FRAP, ferric reducing antioxidant potential (mg AAE/g DM), (K). Different letters above bars indicate statistically significant differences at $p < 0.05$. SS, sesame; SB, soybean; PN, peanut; PL, perilla.

3.4. Antioxidant activities of sesame, soybean, peanut, and perilla seeds

To explore the potential relationship between the metabolic profiles and bioactivity of SS, SB, PN, and PL seeds, we performed a comparative antioxidant activities analysis using the most common DPPH and FRAP assays. The DPPH assay showed that the antioxidant activities of PL and SB seeds were the highest, followed by PN and SS seeds (Fig. 8J). The

FRAP assay showed that the antioxidant activity of PL seeds was significantly higher than other oilseeds (Fig. 8K). There were no statistical differences between the antioxidant activities of SS, SB, and PN via FRAP. However, the FRAP-reducing capacity of SB seed extract was slightly higher (Fig. 8K).

4. Discussion

With the evolution of food pharmacy, it is crucial to seize the diversity and variation of bioactive metabolites within foods of the same categories. Sesame, soybean, peanut, and perilla seeds are part of the most important sources of high-quality vegetable oils and diverse products used worldwide (Tanwar & Goyal, 2021). They provide humans with energy, important nutrients, and nutraceuticals (Tanwar & Goyal, 2021; Zhang et al., 2022b). Although numerous studies have been conducted on each of the oilseeds, no comparative metabolic profiling was reported, limiting a balanced and science-based use of sesame, soybean, peanut, and perilla seed oils and products. Hence, the present study investigated changes in the metabolome profiles of sesame, soybean, peanut, and perilla seeds, with emphasis on the distribution characteristics of bioactive compounds and variation in antioxidant activities.

We identified and chemically characterized a total of 975 metabolites from sesame, soybean, peanut, and perilla seeds. Of them, four (sesamin, frysanthoside-A, hydro-protopine, and O-feruloyl coumarin), one, and ten metabolites were specific to sesame, soybean, and perilla, respectively. These specific metabolites may play various crop-specific functions and could serve as potential metabolic biomarkers for distinguishing the four oilseeds. This agrees with previous reports indicating that lignans sesamin and its derivative compounds are specific to sesame and may serve as biochemical markers for discriminating sesame products (Brigante et al., 2022; Dossou et al., 2023). Multivariate analyses (PCA, HCA, and OPLS-DA) revealed great differences among the metabolite profiles of sesame, soybean, peanut, and perilla seeds. Concurrently, we found a great variation in the relative contents of the major classes of metabolites, including lipids, phenolic acids, flavonoids, terpenoids, vitamins, etc. These results show a crop-specific accumulation of metabolites. Moreover, they indicate great differences in the regulation of metabolic processes during the four oilseeds' development.

Further analysis revealed that there were over 298 DAMs in pairwise comparisons between the four oilseed groups. The classification and KEGG analysis of DAMs unveiled that amino acid metabolism, phenylpropanoid biosynthesis, flavonoid biosynthesis, and lipid metabolism were the most differentially regulated pathways between the four oilseeds. Further comparative studies on the regulation of these pathways in the four oilseeds are needed for identifying key candidate genes or genetic markers for oilseed crops' quality improvement. Amino acids (AA) are, together with carbohydrates, the primary sources of energy and play vital roles in the central metabolism of plants (Aguirre et al., 2018; Amir et al., 2018). For instance, AAs serve as the building block of storage proteins, as well as precursors for the biosynthesis of various secondary metabolites (Amir et al., 2018). Among the amino acids, glutamate, tyrosine, tryptophan, and arginine exhibited great variations between the four oilseeds. Glutamate plays a critical role in the central metabolism of plants (Forde & Lea, 2007; Liao et al., 2022). Through its multifunctional functions, including modulation of carbon and nitrogen metabolism (precursor of glutamine, acidic amino acids, glutathione, folate, γ -amino butyric acid, salicylic acid, etc.), modulation of stomatal closure, amino donor for transamination reactions, and conjugation with IAA (indole acetic acid), glutamate represents a vital signaling molecule regulating growth, development, and defense mechanisms in plants (Forde & Lea, 2007; Liao et al., 2022; Yoshida et al., 2016). Phenolic compounds in plants are synthesized from aromatic AA, namely phenylalanine, tyrosine, and tryptophan (Maeda & Dudareva, 2012). Taken together, these findings infer that differences in glutamate and aromatic AA metabolism may be the major contributors to the great variation in metabolite profiles and antioxidant activities of the four oilseeds. The study by Gregorio et al. found that glutamate modulates enzyme inhibitory properties, phenolic profile, and antioxidant traits in lettuce (De Gregorio et al., 2023). An in-depth dissection of glutamate and aromatic AA metabolism during the four oilseeds' development is required to better understand the global metabolism in oilseed crops.

Flavonoids, phenolic acids, alkaloids, terpenoids, AA, vitamins, and coumarins have shown therapeutic properties against chronic diseases such as diabetes, cancers, oxidation stresses, insomnia, amnesia, angiogenesis, osteoclastogenesis, neurodegeneration, and inflammation (Alagawany et al., 2017; Ben Othman et al., 2015; Choi et al., 2020; Gong et al., 2019; Hattori et al., 2021; Hussain et al., 2018; Imran et al., 2019; Kalinowska et al., 2021; Koira et al., 2016; Manayi et al., 2016; Yamamoto et al., 2017). Soybean showed the highest relative content of most differentially accumulated flavonoids, terpenoids, and alkaloids, followed by perilla, peanut, and sesame. The relative content of phenolic acids was higher in perilla than in the other oilseeds. These results suggest that perilla and soybean may possess marked biological properties compared to peanut and sesame seeds. Consistently, we found that the antioxidant activity of perilla seed extract was the highest, followed by soybean, peanut, and sesame. It is reported that crops or genotypes with higher contents of polyphenols have higher antioxidant activities (Salami et al., 2023). As each bioactive compound plays specific functions, our results may promote the science-based use of sesame, soybean, peanut, and perilla seeds in various industries. Furthermore, they represent data support for using the different oilseeds in specific food pharmacy formulations.

5. Conclusions

Overall, this study differentiated between the metabolite profiles and antioxidant activities of sesame, soybean, peanut, and perilla seeds and unveiled the distribution characteristics of bioactive compounds. Our analysis revealed a crop-specific accumulation and significant variability of antioxidant, and key bioactive compounds between compared crop species. Perilla seed extracts showed the highest antioxidant activity, followed by soybean, peanut, and sesame. Soybean seeds may represent a rich source of many important bioactive flavonoids (genistein, butin, daidzin, prunatin, isovitexin, fustin, naringenin, toringin, kaempferol 3-O-rutinoside, cyanidin O-rutinoside, etc.), phenolic acids (sinapic acid, sattabacin, isomangiferin, 5-O-p-coumaroyl shikimic acid, and 2-O-caffeoylemalic acid), terpenoids (maslinic acid, kolavic acid, valepotriate, pterosin A, capsianoside I, etc.), alkaloids (vasicine, arecoline, beta-nicotyrine, D-hydroevodiamine, trigogelline, and hirsuteine), amino acids (tryptophan, leucine, aspartic acid, and methionine), and vitamins (B6 and B7). Meanwhile, perilla seeds may represent major source of key nutraceuticals, such as apigenin, apigenin-5-O-glucoside, luteolin, luteolin-4'-O-glucoside, vitexin, phloretin, orientin, isoorientin, 2'-hydroxygenistein, and diosmetin (flavonoids); rosmarinic acid, rosmarinic acid-3'-O-glucoside, caffeic acid O-glucoside, gallic acid O-hexoside, and N'-p-coumaroyl agmatine (phenolic acids); oleanic acid, ursolic acid, and isomangiferolic acid (terpenoids); hirsuteine and swainsonine 9alkaloids); and amino acids (lysine, tyrosine, glutamine, histidine, and arginine). Peanut could be recommended as major source of five phenolic acids (p-coumaric acid, hydroxycinnamate, 2-hydroxycinnamate, p-hydroxy cinnamic acid, and N-p-coumaroyl spermidine), and two flavonoids (rutin and tricin 5-O-hexoside). Sesame could be the major source of five bioactive phenolic acids (isoacteoside, isovanillic acid, vanillic acid, vanillyl alcohol, and 5-caffeylshikimic acid), five terpenoids (asiatic acid, fraxinellone, alpha-lonone, geranylacetone, and geniposidic acid), three alkaloids (hydroprotopine, tabersonine, and N'-nitrosoanabasine), skimming (coumarin), amino acids, and its specific lignans (sesamin and derivatives). Amino acid metabolism, phenylpropanoid biosynthesis, flavonoid biosynthesis, and metabolism of lipids and organic acids were the most differentially regulated pathways. Our research is the first comprehensive and comparative metabolomic analysis result between sesame, soybean, peanut, and perilla. Therefore, our findings may contribute to enhancing oilseed industries and promoting data-based comprehensive use of sesame, soybean, peanut, and perilla in food pharmacy and various industries.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

CRediT authorship contribution statement

Habtamu Kefale: Investigation, Methodology, Formal analysis, Software, Writing – original draft, Writing – review & editing, Data analysis. **Senouwa Segla Koffi Dossou:** Perform data analysis, Visualization, Formal analysis, Methodology, Writing – review & editing. **Feng Li:** Involved in experiment design. **Nanjun Jiang:** Involved in experiment design. **Rong Zhou:** Prepared materials and original data. **Lei Wang:** Prepared materials and original data. **Yanxin Zhang:** Prepared materials and original data. **Donghua Li:** Prepared materials and original data. **Jun You:** Designed the experiment, Resources, Supervision, Funding, Review, Validation. **Linhai Wang:** Designed the experiment, Resources, Supervision, Validation, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2023.113586>.

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