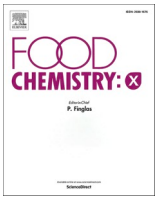
Food Chemistry: X 23 (2024) 101629

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



UPLC-MS/MS reveals the differences in lipids composition of *Camellia oleifera* from northern margin distribution area

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ARTICLE INFO

Keywords:
Camellia oleifera
Northern margin distribution area
Lipidomics
Functions
Metabolic pathways

ABSTRACT

The lipids accumulation characteristics in 23*Camellia oleifera* lines from northern margin distribution area were investigated through quantitative lipidomics. Combined lipids content-function analysis indicated that NQ1, HT1, HT2, ZA2, ZB1, ZB2, and SN2 lines had potential to develop functional foods due to abundant glycerolipids (GLs), glycerophospholipids (GPs), fatty acids (FAs), and prenol lipids (PRs). 673 lipids components were detected, and 293 differential components were identified in NQ1, ZA2, HB1, and HT1. 4 kinds free fatty acids (FFAs) were higher in NQ1, 5 triglycerides (TGs) were higher in HT1, and 2 phosphatidyl serines (PSs) and 1 phosphatidyl glycerol (PG) were higher in ZA2. GLs, GPs, and FFAs had strong relation at intra- and inter-category level. Glycerolipid metabolism, glycerophospholipid metabolism, and fatty acid biosynthesis were the significantly differential lipids pathways. Our study elucidated lipids differences of 23 *C. oleifera* lines, and offered valuable references for lipids biosynthesis, directional breeding, and lipids utilization.

1. Introduction

Camellia oleifera is a peculiar woody oil-bearing tree in China, which has been cultivated and utilized for >2300 years (Zhang, Guo, et al., 2022). Compared with *Elaeis guineensis*, *Olea europaea* and *Cocos nucifera*, it is honored as the world's four main woody edible oil plants, praised as the oriental olive oil (Yang et al., 2016; Zeng, Huang, et al., 2023). Camellia oil is richer in unsaturated fatty acids, among which oleic acid and linoleic acid are the highest, and it also contains a variety of fat-soluble components (Gao et al., 2022; Gong et al., 2020). Camellia oil has antioxidant and pharmacological effects on the nervous system, improving sleep, anti-depression and improving memory, which has been recognized as a safe natural and healthy edible vegetable oil (Lee & Yen, 2006; Ye, 2022).

Lipids, the main components of vegetable oil, were composed of neutral lipids (TGs: triglyceride; DGs: diglyceride, FFAs: free fatty acids) and polar lipids (GPs: glycerophospholipids; SPs: sphingolipids) (Hu et al., 2022; Shi et al., 2020). At present, the researches on the lipids composition of Camellia oil have been concentrating on fatty acids composition determination through GC–MS method following methyl esterification, and to compare the effects of variety, origin and processing technology on fatty acid composition (Li et al., 2023). The functional properties of Camellia oil are also mostly attributed to the higher unsaturated fatty acid content, which displayed the preventive effects of lowering blood lipid and cardiovascular disease on human health (Yang et al., 2018). Studies on other vegetable oils have been confirmed that other lipid components had important health effects (Shen et al., 2013). For example, phospholipids richer in olive oil have been found to play an important role in inflammatory processes and signaling pathways (Alves et al., 2021). Therefore, determining lipids composition and abundance was of great significance to evaluate the biological function and nutritional health quality of Camellia oil.

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https://doi.org/10.1016/j.fochx.2024.101629
Received 21 May 2024; Received in revised form 5 July 2024; Accepted 5 July 2024
Available online 6 July 2024

However, the lack of research on lipids components and function have become the main limiting factors for the promotion of excellent traits of *C. oleifera* and the development and utilization of high-end products of Camellia oil.

South Shaanxi (Hanzhong, Ankang and Shangluo cities), the northernmost edge of the suitable distribution area of *C. oleifera* in China, is planned as the core development area in the Fourteenth Five-Year Plan for *C. oleifera* (Zeng, Xu, et al., 2023). Ancient trees and germplasm Resources were distributed in south Shaanxi, establishing a better foundation for elite tree breeding and directed breeding of *C. oleifera* (Xu et al., 2021). In previous studies, the phenotypic characters, oil content, and fatty acid composition in Camellia oil has always been the focus, which were affected by different clones or cultivars (Ye, 2022; Zeng, Huang, et al., 2023). Oil contents and FFAs composition in camellia seeds from ten 'Changlin' *C. oleifera* lines were significantly affected by geographical and climatic heterogeneity (Yang et al., 2016). However, to our knowledge, there is no literature on the comparison of the lipid contents and profiles in *C. oleifera* from the northern margin distribution area (southern Shaanxi). There, it is of great significance to characterize the lipids profile in *C. oleifera* lines from southern Shaanxi.

Lipidomics, a branch of metabolomics, has rapidly become an emerging omics technology to carry out lipid profile analysis in foods (Cao et al., 2024; Zeng et al., 2024). Lipidomics has been widely utilized in food lipid composition characterization, quality identification and evaluation, geographical origin traceability (Chen et al., 2024; Yang et al., 2024). Lipidomics based on UPLC-MS/MS (ultra performance liquid chromatography tandem mass spectrometry) have been successfully detected the lipids profile analysis of olive oil (Shen et al., 2013), peanut oil (Zhang, Zhu, et al., 2022), flaxseed oil (Zhang et al., 2021) and hazelnut oil (Sun et al., 2022). Therefore, it has become feasible to elucidate the variations and functional characteristics of lipids profile in *C. oleifera* lines from southern Shaanxi. However, the researches on difference and functional characteristics of *C. oleifera* lines from southern Shaanxi were still little.

In this study, lipidomics based on UPLC-MS/MS method was employed to investigate the lipids profiles in 23*C. oleifera* lines from northern margin distribution area (South Shaanxi), aiming to characterize the lipid composition. The functional characteristics of different lipids components were revealed to provide a scientific theoretical basis for the development and utilization of Camellia oil. The representative varieties of *C. oleifera* were selected for comparative analysis of lipids profile, which provided a theoretical basis for further study of the molecular mechanism of lipids metabolism and molecular directed breeding.

2. Materials and methods

2.1. Plant materials

From October to November 2023, the mature *C. oleifera* fruits were collected from northernmost edge of suitable area (South Shaanxi), including 23*C. oleifera* lines distributed in Hanzhong city, Ankang city and Shangluo city, Shaanxi Province. The detailed information of the 23*C. oleifera* lines were demonstrated in **Fig. S1** and **Table S1**. The fruits were randomly selected from the middle and upper parts of the canopy in individuals with healthy-looking. The *C. oleifera* fruits without obvious mechanical damage, pest and disease were taken back to the experimental laboratory and the seed kernels were dried at room temperature.

2.2. Sample preparation and extraction

20 mg post-thaw *C. oleifera* fruits were transferred into 2 mL centrifuge tube with a steel bead (internal diameter about 4 mm). 1.00 mL lysis solution (0.75 mL Methyl tertiary-butyl ether+0.25 mL methanol, including the internal standard mixed solution) was added and homogenized for 30 min, and the internal standards were summarized in **Table S2**. 0.30 mL high-purity water was added, homogenized, incubated for 10 min at 4 °C, and centrifuged at 12,000 r/min for 3 min at 4 °C. 0.40 mL supernatant was transferred into 1.50 mL tube, concentrated to near dryness, dissolved with reconstituted solution (Isopropanol: acetonitrile = 1:1), homogenized for 3 min, and centrifuged at 12,000 rpm for 10 min at 4 °C. A total of 120 μL reconstituted solution was collected for UPLC-MS/MS analysis (Yang et al., 2024).

2.3. Determination of the lipid components

Lipids components in 23*C. oleifera* lines were determined by UPLC-MS/MS system (Yang et al., 2024). 2.0 μL solution above was injected into the Thermo Accucore™C30 column (2.6 μm, 2.1 mm × 100 mm i.d.) equipped with solvent system A (60% Acetonitrile +40% Water +0.10% formic acid +10 mmol/L ammonium formate) and solvent system B (10% Acetonitrile +90% Isopropanol +0.10% formic acid +10 mmol/L ammonium formate), through a 20 min linear gradient with the flow rate of 0.35 mL/min. The move phase gradient was set as follows: 80.0% A, 0 min; 70.0% A, 2 min; 40.0% A, 4 min; 15.0% A, 9.0 min; 10.0% A, 14.0 min; 5.0% A, 15.0 min; 5.0% A, 17.3 min; 80.0% A, 17.4 min; 80.0% A, 20.0 min.

LIT and triple quadrupole scans were acquired on a triple quadrupole-linear ion trap mass spec-trometer (QTRAP), QTRAP® 6500+ LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (SCIEX). Temperature of positive and negative ion mode of the ESI was 500 °C, ion spray voltage of positive ion mode was 5500 V, ion spray voltage of negative ion mode was −4500 V, ion source gas 1 was 45 psi, gas 2 was 55 psi, curtain gas was 35 psi. In the triple quadrupole, each ion pair was scanned and detected according to the optimized decluster voltage and collision energy (Yang et al., 2024).

2.4. MS data analysis

The MWDB database was constructed based on standard substances to qualitatively analyze the lipids MS data. The lipids quantitative analysis was performed through the MRM mode of triple quadrupole mass spectrometry. After obtaining the lipids MS data of 23*C. oleifera* lines, peak area integration was performed on the chromatographic peaks of each lipid component, and each lipids content was quantitatively analyzed and computed by internal standard method (**Table S2**).

2.5. Data statistical analysis

The differences among the lipid contents of 23*C. oleifera* lines were conducted by Duncan's test, and generated on SPSS 26.0 software for Windows. The grouped columns plots, scatter matrix plots, heatmap plots and bar plots were achieved and completed by Origin Pro 2024b software. The differential lipids were selected through multivariate statistical analyses (principal component analysis, PCA; orthogonal partial least squares discrimination analysis, OPLS-DA; unweighted correlation network analysis, UCNA), among which the PCA, OPLS-DA, and UCNA were successfully completed by using R (obtained on 20 May 2024).

3. Results

3.1. Lipids content of the 23C. oleifera lines

The remarkable differences of lipids contents of the 23*C. oleifera* lines were observed ($p < 0.05$), and described in Fig. 1A. The average lipids content was 51,690.17 ± 1527.21 nmol/g. The highest lipids content was achieved from NQ1 samples (69,603.31 ± 1978.56 nmol/g), followed by HT1 (59,528.26 ± 1261.77 nmol/g) and HT2 samples (58,356.61 ± 703.77 nmol/g), ZA2 (55,271.23 ± 1686.31 nmol/g),

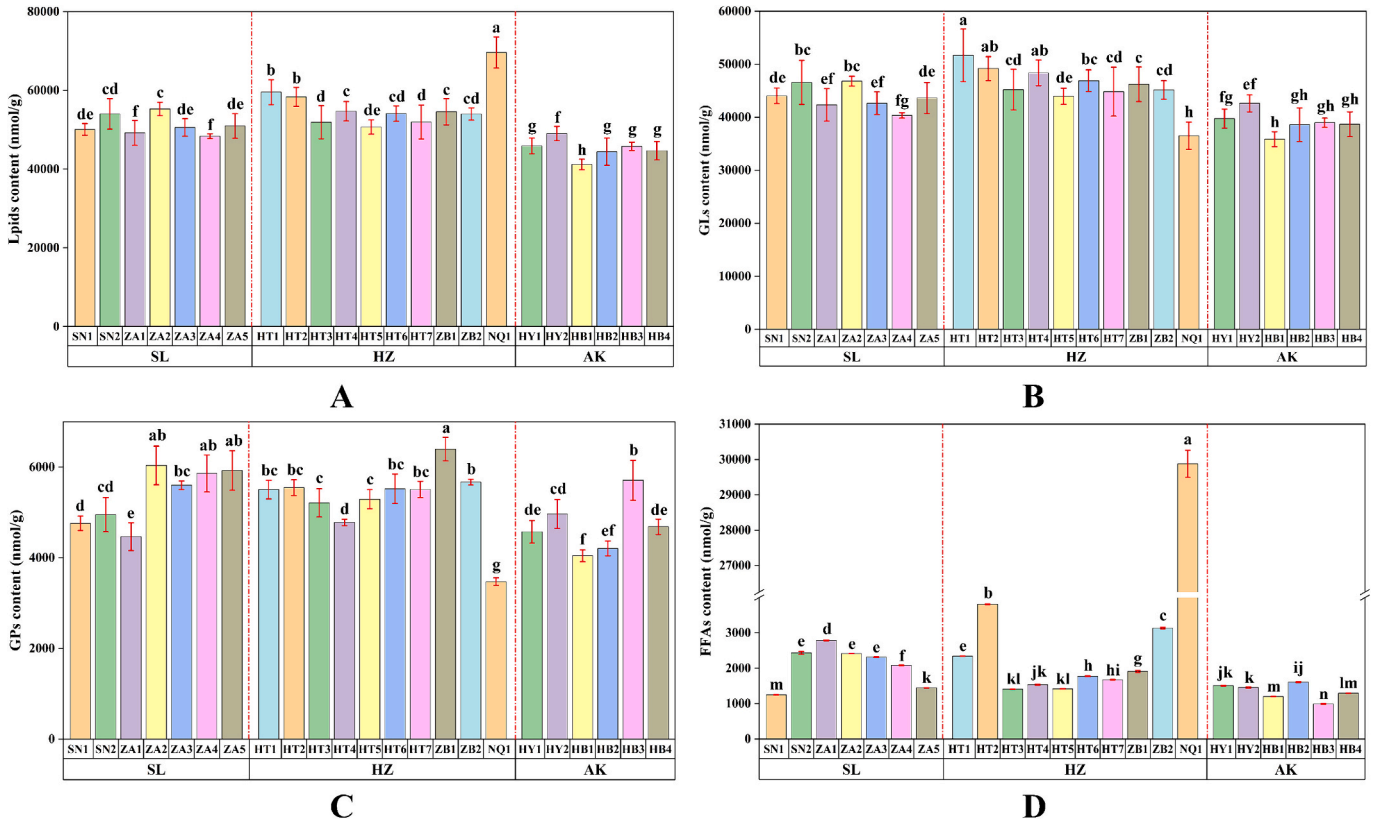

Fig. 1. Grouped columns plots of lipids contents (**A**), Glycerolipids (GLs) content (**B**), GPs content (**C**), and FFAs content (**D**) of 23*C. oleifera* lines. Units: nmol/g. Different lowercase letters in the figure indicated the significant difference at 0.05 level.

while the lower lipid content was found in the samples from HB, such as HB3 (45,778.97 ± 1072.02 nmol/g), HB4 (44,691.36 ± 1393.53 nmol/g), HB2 (44,413.30 ± 1949.22 nmol/g), and HB1(41,169.06 ± 1348.25 nmol/g). The results implied that the NQ1, HT1, HT2, ZA2, SN2, and ZB1 were identified as the high lipids content lines.

3.2. Lipids profiles and function analysis of 23*C. oleifera* lines

673 lipids components were detected and identified in 23*C. oleifera* lines through UPLC-MS/MS method, which were divided into 25 subclasses belonging to 5 categories. All the lipid components were composed of 30 FAs, 413 GLs, 196 GPs, 3 PRs, and 31 SPs (**Table S3**). Among them, the 413 GLs were subdivided into 298 TGs, 59 DGs, 17 DGTSs, 10 DGDGs, 9 SQDGs, 7 MGDGs, 5 ADGGAs, 4 MGs, 3 LDGTSs, and 1 DGGAs. The 196 GPs were classified into 49 PEs, 35 PCs, 25 PGs, 21 PIs, 12 PSs, 11 PAs, 15 LPCs, 7 LPEs, 7 LPAs, 4 LPIs, 4 LPGs, and 6 PMeOHs. The 31 SPs were composed of 17 Certs, 8 HexCers, 5 Cers, and 1 SPH. The GLs occupied a dominate place in lipids among those 5 categories, accounting for 61.37%, ranked by GPs (29.12%), SPs (4.61%), FAs (4.46%), and PRs (0.45%). From the perspective of lipid categories, GLs had the highest content in all *C. oleifera* lines, indicating that GLs was the main lipids composition. And, GLs, FFAs and GPs were the most important lipid components. The higher GLs content was found in HZ city, especially in HT1 (51,691.11 ± 4952.80 nmol/g), ranked by HT2 (49,196.55 ± 2249.57 nmol/g) (Fig. 1B). The highest GPs content was observed in ZB1 (6396.11 ± 259.85 nmol/g), ranked by ZA2 (6037.62 ± 727.06 nmol/g) (Fig. 1C). The highest FFAs content was achieved in NQ1 (29,879.92 ± 382.46 nmol/g), ranked by HT2 (3809.65 ± 13.01 nmol/g) (Fig. 1D).

The chain length and unsaturation degree of lipids could affect the structure and functional properties of their complexes with other chemicals, which in turn affected digestion performance (Morstein & Trauner, 2020). Our results illustrated that the carbon chain length of lipids in Camellia oil was concentrated between 34 and 66, belonging to medium and long chain lipids, with an average content of 48,219.53 nmol/g. Lipids, especially unsaturated lipids, are essential nutrients for human body, which exhibit an important role in the control of blood lipid, anti-thrombosis and prevention of atherosclerosis (Liu et al., 2015). The lipid composition of Camellia oil was mainly unsaturated lipids, and the content was ranged from 38,971.99 nmol/g to 71,723.27 nmol/g. The content of lipid categories in different Camellia oils was also obviously different, which in turn influenced the functional properties of edible oils. To systematically understand the lipids composition and functional characteristics, 5 major lipid subclass functions were summarized based on the known functional characteristics. These differential accumulated lipids with higher content exhibited various functions, such as cardiac protection and inhibition of cardiovascular disease (FAs, GLs, PRs), reduction of cholesterol and improvement of cognitive and brain function (GPs), antioxidant, anti-inflammatory (GPs, PRs, FAs) and energy supply (GLs). PRs (CoQ8, CoQ9, CoQ10), the lipids subclasses seldom mentioned in other edible vegetable oil, were detected in our study, especially CoQ10 exhibiting excellent antioxidant, anti-inflammatory and anti-cancer functions.

Thus, the lines of NQ1, HT1, HT2, ZA2, ZB1, ZB2, and SN2 could be extended from a single cooking oil to various functional products, based on the contents and functions of GLs, GPs, FAs, and PRs.

3.3. Lipids profiles of NQ1, ZA2, HB1, and HT1 lines

Interestingly, the FFAs, GLs, GPs, and SPs contents in NQ1, ZA2, and HT1 were obviously different (Fig. 2), among which NQ1 was richer in SPs and FFAs, ZA2 was richer in GPs, and HT1 was richer in GLs. The contents of the 4 lipids categories were calculated with the lipids detected in the same category (Fig. 2). HT1 samples had the highest GLs

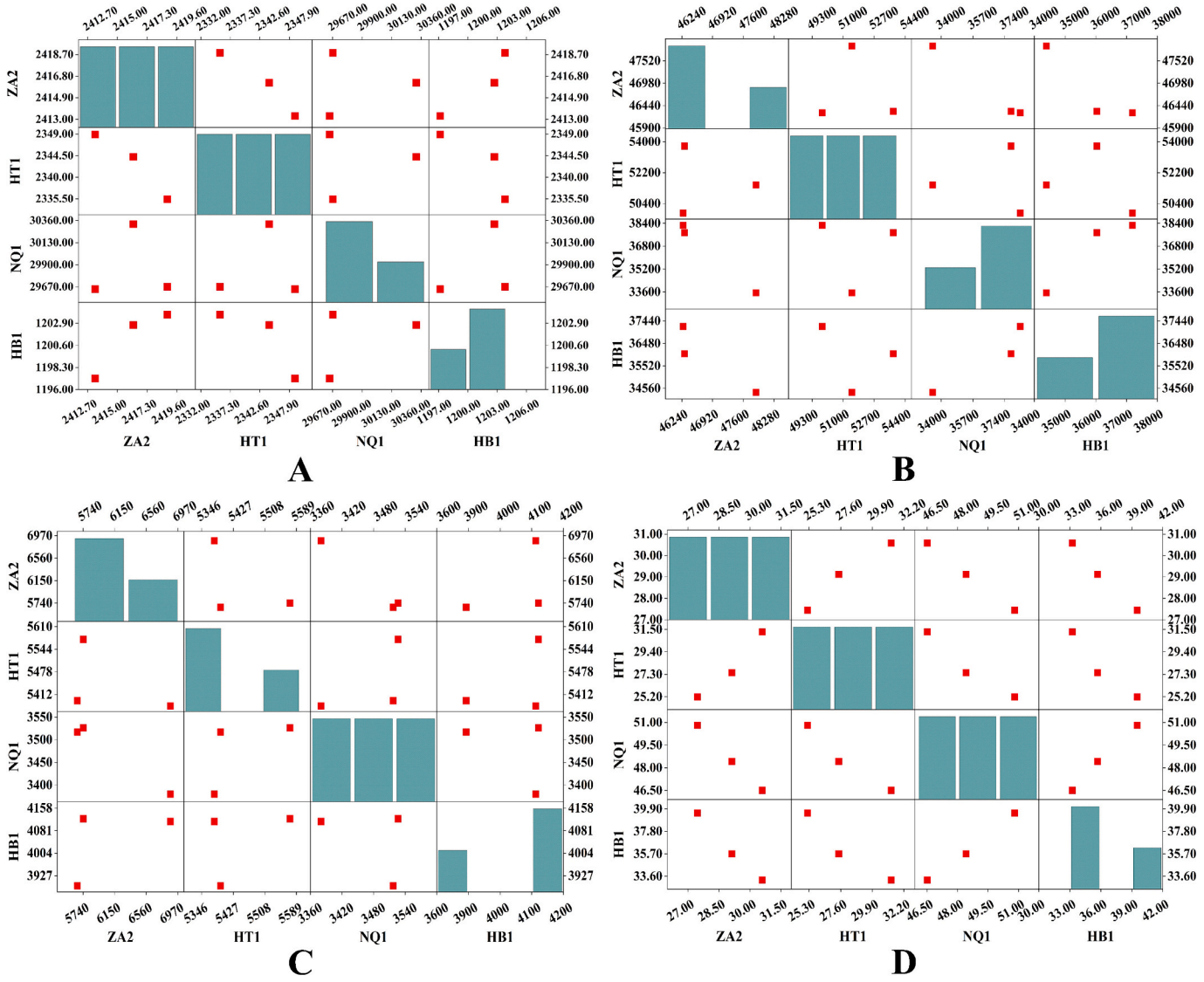

Fig. 2. Scatter matrix plots of the main lipid subgroups in NQ1, ZA2, HB1, and HT1. **A**: FFAs content, **B**: GLs content, **C**: GPs content, **D**: SPs content. Units: nmol/g.

content (51,691.11 ± 2952.80 nmol/g), ranked by ZA2 (46,815.53 ± 919.12 nmol/g) ($p < 0.05$). ZA2 samples exhibited the highest GPs content (6037.62 ± 727.06 nmol/g). The highest contents of FFAs (29,879.92 ± 382.46 nmol/g) and SPs (48.58 ± 5.50 nmol/g) were observed in NQ1 samples. The lowest FFAs and GLs contents were found in HB1 samples. Compared to HT1 samples, the GLs and GPs were decreased markedly in NQ1 samples, whereas the FAAs and SPs were increased remarkably.

From the perspective of lipid monomers, 69 lipid monomers content in ZA2 were >100 nmol/g, 66 lipid monomers in HT1, 64 lipid monomers in NQ1, and 63 lipid monomers in HB1, among which most lipid monomers belonged to TGs, and FFAs. Next, the quantitative lipids method was employed to comprehensively analyze the lipids of NQ1, ZA2, HB1, HT1 with different lipids contents, and to elucidate the differential lipids and lipids metabolic pathway of Camellia oil.

3.4. Lipid pattern recognition analysis NQ1, ZA2, HB1, and HT1 lines with different lipids content

The differences among NQ1, ZA2, HB1, and HT1 were generated and achieved through the PCA and OPLS-DA analysis, with regard to the 673 lipids components. In the 2D-PCA plot (**Fig. S2A**), 2 principal components were constructed with the interpretation of 50.04% (PC1) and 24.96% (PC2) of the variability, and the cumulative proportion rate was 75.00%. A distinct separation was observed from the 2D-PCA plot that NQ1, ZA2, HB1, and HT1 differed significantly. It could be seen from **Fig. S2B** that NQ1, ZA2, HB1, and HT1 samples were also gathered into 4 groups. The permutation test was applied to validate the OPLS-DA model, and the results exhibited that R^2Y (0.998) and Q^2 (0.954) were closer to 1 (**Fig. S2C**), and the model was without overfitting phenomenon (**Fig. S2D**). The quantitative assessment of lipids differences between NQ1, ZA2, HB1, and HT1 with different lipids content was achieved by the utilization of PCA and OPLS-DA results, which offered a detailed understanding for further analysis of differential lipids components and differential lipids metabolic pathways in *C. oleifera*.

3.5. Identification of differential lipids between NQ1, ZA2, HB1, and HT1 lines

293 differential lipids (VIP > 1, $p < 0.05$, Fold change>2 or Fold change<0.5) were identified among NQ1, ZA2, HB1, and HT1 samples (**Table S4**). Those differential lipids were statistically analyzed in Fig. 3, including TGs (Fig. 3A), FFAs (Fig. 3B), PGs and PEs (Fig. 3C), LPCs and LPAs (Fig. 3D).

Compared to HT1 samples, the NQ1 samples exhibited 100 up-regulated lipids and 104 down-regulated lipids (**Table S5**), the ZA2

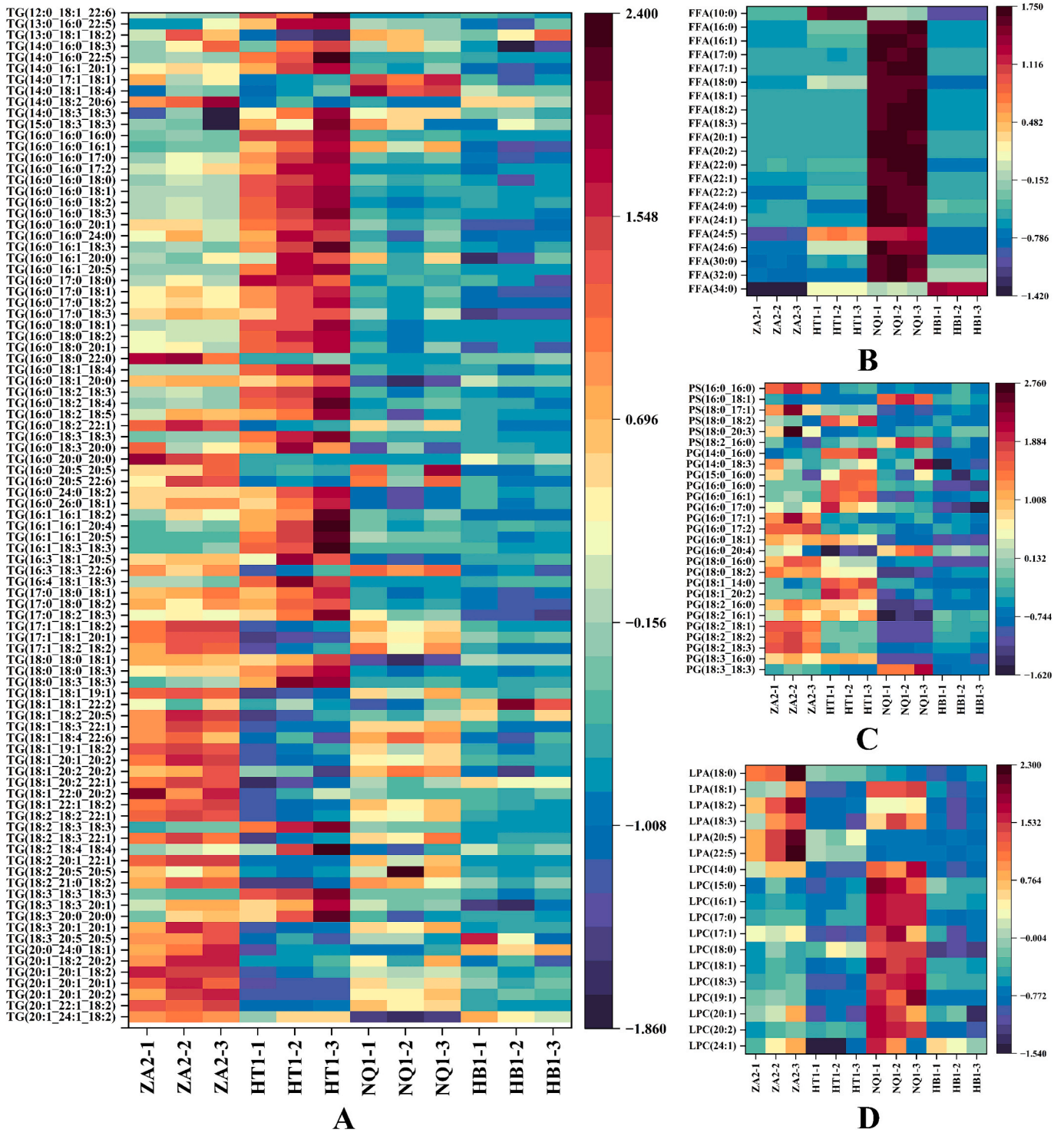

Fig. 3. Heat map analysis of differential lipids with higher content in NQ1, ZA2, HB1, and HT1 samples. **A**: Differential lipids belonging to TGs, **B**: Differential lipids belonging to FFAs, **C**: Differential lipids belonging to PGs and PSs, **D**: Differential lipids belonging to LPCs and LPAs. The blue color indicated a lower accumulation level of each lipid component and the red color indicated a higher accumulation level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

samples displayed 71 up-regulated lipids and 43 down-regulated lipids (**Table S6**), whereas the HB1 samples showed 27 up-regulated lipids and 127 down-regulated lipids (**Table S7**). In the comparison of NQ1_vs_HT1, the differential lipids with higher content were FFAs and TGs, among which most of the TGs were down-regulated and all FFAs were up-regulated (Fig. 4). TG(16:0_18:0_18:1), TG(16:0_16:0_18:1), and TG(16:0_16:0_18:2) were the differential lipids with higher content in HT1, and their contents were 2721.72 ± 179.95 nmol/g, 2165.45 ± 7.31 nmol/g, and 1493.29 ± 120.53 nmol/g. TG(16:0_18:0_18:1), TG (16:0_16:0_18:1), TG(16:0_16:0_18:2), TG(18:0_18:0_18:3), and TG (18:0_18:0_18:1) in NQ1 were 0.38-, 0.32-, 0.32-, 0.49-, and 0.45-fold of that in HT1. Among the FFAs, the FFA(18:1) was observed with the highest content in NQ1, followed by FFA(18:2), FFA(16:0), FFA(18:0), and FFA(20:1), and their contents were 22,643.71 ± 272.21 nmol/g,

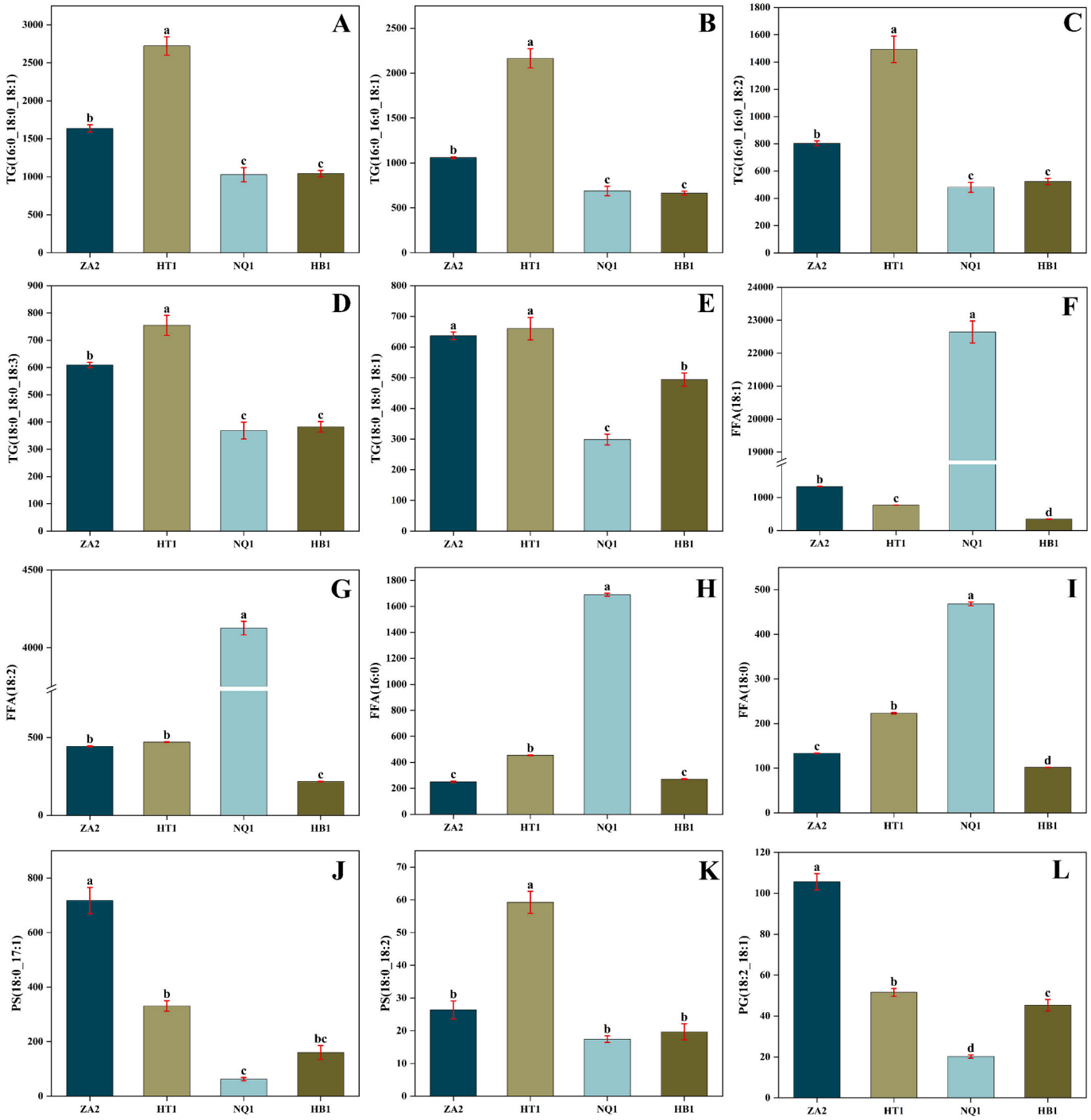

Fig. 4. Comparison of the differential lipids with higher content among NQ1, ZA2, HB1, and HT1. Different lowercase letters in the bar charts represented the significant difference ($p < 0.05$).

4125.63 ± 35.92 nmol/g, 1689.69 ± 99.90 nmol/g, 468.22 ± 7.31 nmol/g and 293.59 ± 7.47 nmol/g, respectively. The FFA(18:1) and FFA (20:1) in NQ1 exhibited the most obvious differences compared to HT1, and the fold change values of FFA(18:1) and FFA(20:1) were 29.43 and 17.99. The FFA(18:2), FFA(16:0), and FFA(18:0) in NQ1 also displayed a remarkable increase, which were 8.76-, 3.72-, and 2.10- fold of those in HT1. The highest PG(18:2_18:1) and PS(18:0_17:1) were found in ZA2, and their content were 717.27 ± 96.39 nmol/g and 105.64 ± 3.23 nmol/g, which were 2.17- and 2.05-fold than those in HT1, respectively. The TG(16:0_16:0_18:1) in ZA2 (1059.63 ± 7.06 nmol/g) displayed a notable down-regulation compared to HT1 (2165.45 ± 7.31 nmol/g), while TG(18:1_18:1_19:1), TG(17:1_18:1_18:2), and TG(18:1_19:1_18:2) in ZA2 were present with obvious up-regulation. In the comparison of HB1_vs_HT1, the TG(16:0_18:0_18:1), TG(16:0_16:0_18:1), TG (16:0_16:0_18:2), TG(16:0_18:0_18:2), and TG(16:0_18:2_18:3) in HB1 were present with a down-regulation. The FFA(18:1) and FFA(18:2) in HB1 were exhibited the lowest content, with the content of 343.34 ± 4.12 nmol/g and 217.21 ± 6.41 nmol/g. Besides the above differential lipids, functional lipids were also identified in NQ1, ZA2, HB1, and HT1, among which the content of LPCs was the highest in NQ1, and the content of LPAs was higher in ZA2.

3.6. Correlation analysis of differential lipids in NQ1, ZA2, HB1, and HT1 lines

Owing to various lipids were possessed with similar physiological, molecular characteristics and same trends at ripening stage, the hypothesized that they were correlated/co-regulated was valid (Yang et al., 2024). Unweighted correlation network analysis was performed on the significantly differential lipids with higher content in NQ1_vs_HT1, ZA2_vs_HT1, and HB1_vs_HT1 to reveal the interaction between these differentially expressed lipids. As shown in Fig. 5, most lipids exhibited a highly distinct correlation between each other ($p < 0.05$). 54 differential lipids with strong correlation were screened out in NQ1_vs_HT1 ($p < 0.05$), 59 differential lipids in ZA2_vs_HT1 ($p < 0.05$), and 66 differential lipids in HB1_vs_HT1 ($p < 0.05$). The differential lipids with strong correlations were GLs and FFAs in NQ1_vs_HT1 (Fig. 5A), GLs and GPs in ZA2_vs_HT1 (Fig. 5B), and GLs and GPs in HB1_vs_HT1 (Fig. 5C). Among those differential lipids, intra-category and inter-category correlation of GLs, GPs, and FFAs were relatively strong in NQ1_vs_HT1, ZA2_vs_HT1, and HB1_vs_HT1. Moreover, our results also indicated that the GLs exhibited the stronger correlation with other lipids at intra-category and inter-category level.

3.7. Metabolic pathways of differential lipids in NQ1, ZA2, HB1, and HT1 lines

To further explore the differential lipids metabolism among NQ1, ZA2, HB1, and HT1, the 293 differential lipids identified were utilized to map the differential pathways. The top 50 differential lipids were main GLs, FFAs, and GPs, among which the FFAs were the leading differential lipids in NQ1_vs_HT1 (Fig. 6A), GLs and GPs were the main differential lipids in ZA2_vs_HT1 (Fig. 6B), and GLs, GPs and FFAs were the predominant differential lipids in HB1_vs_HT1(Fig. 6C), respectively. Among these differential lipids, the correlation of GLs and GPs were relatively stronger among NQ1, ZA2, HB1, and HT1, indicating that the lipids difference was caused by GLs and GPs.

The pathways most obviously relevant to lipids metabolism were glycerolipid metabolism, glycerophospholipid metabolism, sphingolipid metabolism, and fatty acid biosynthesis (Fig. 6D). Hence, FFA(18:1), FFA(18:2), FFA(16:0), FFA(18:0), and FFA(20:1) were the important differential lipids involved in fatty acid biosynthesis. TG (16:0_18:0_18:1), TG(16:0_16:0_18:1), TG(16:0_16:0_18:2), TG (18:1_18:1_19:1), TG(17:1_18:1_18:2), and TG(18:1_19:1_18:2) were the major differential lipids related to glycerolipid metabolism. PS (18:0_17:1), PS(18:0_18:2), PG(18:2_18:1), PE(20:1_18:2), PG (16:0_16:0), and PS(18:0_20:3) were the leading differential lipids linked to glycerophospholipid metabolism. In addition, linoleic acid metabolism, inositol phosphate metabolism, arachidonic acid metabolism, and alpha linolenic acid metabolism were also included in the differential lipid metabolic pathway, which had little effect on lipids metabolism. Among those, glycerolipid metabolism and glycerophospholipid metabolism pathway was the most significantly relevant lipids metabolic pathway.

4. Discussion

Vegetable edible oils, rich sources of dietary fats and various phytochemicals, are objects related to health and commercial interest (Sun et al., 2020). Lipids have been considered as nutrients in vegetable oil, and the in-depth investigation of lipids nutrition is of great significance to food quality and safety (Banas et al., 2023). 94 lipids components were identified in olive oil through lipidomics based on UPLC-MS/MS (Alves et al., 2022), which provided an important basis for nutritional evaluation of olive oil. 103 lipid components were identified in flaxseed oils by un-roasting and roasting (Sun et al., 2022), and 238 lipid components were detected in hazelnut oil during storage (Zhang et al., 2021), which provided a reference for the processing and storage of edible oil.

Southern Shaanxi is the northernmost edge of the suitable distribution area of *C. oleifera* in China, but most of the *C. oleifera* growing in southern Shaanxi was still in a semi-wild state, and its development and utilization were still in primary stage and immature. In this study, based on UPLC-MS/MS, the lipids content of 23*C. oleifera* in southern Shaanxi was determined. A total of 673 lipid components in 25 subclasses of 5 categories were obtained, which further clarified the lipid composition of Camellia oil. Further analysis of the carbon chain length and saturation of lipids in Camellia oil revealed that the medium and long chain lipids with the length of 34 to 66 accounted for about 82.46%, and unsaturated lipids (lipid saturation > 0) accounted for about 86.03%. Studies have illustrated that medium and long chain lipids have the effects on reducing body weight and body fat content, promoting energy consumption, which could be developed into health foods with special functions (Hopiavuori et al., 2019; Wang et al., 2023). Unsaturated lipids also have the effects on regulating blood lipids, antithrombotic and improving visual fatigue (Nieto-Ruiz et al., 2022; Stupin et al., 2019). Therefore, the length of the carbon chain and the degree of unsaturation of the lipids in Camellia oil were contributed to its various

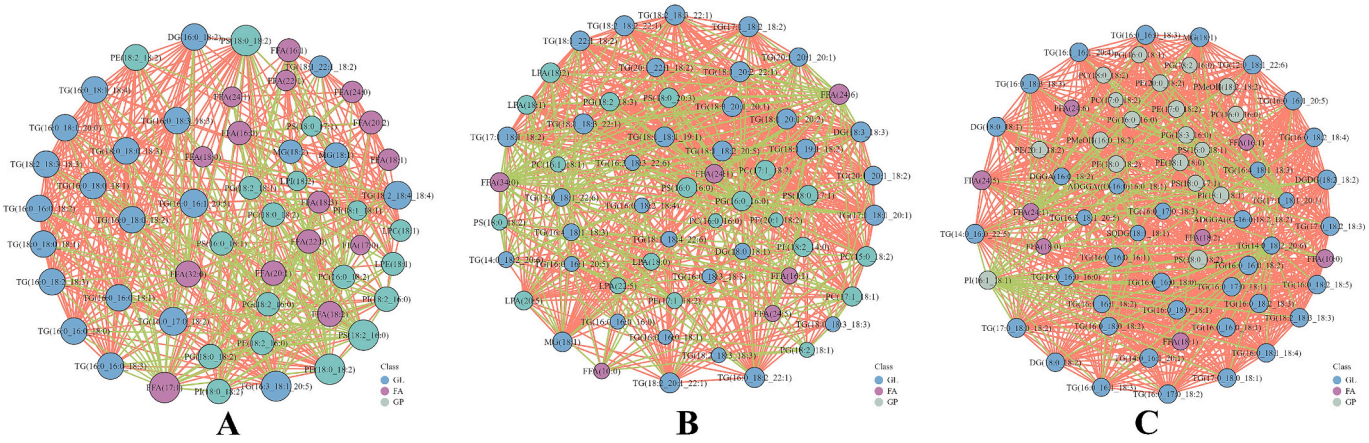

Fig. 5. Unweighted correlation network analysis ($p < 0.05$) of 54 differential lipids with higher content in NQ1_vs_HT1 (**A**), 59 differential lipids with higher content in ZA2_vs_HT1 (**B**), and 66 differential lipids with higher content in HB1_vs_HT1 (**C**). The node sizes indicated the relationship level, and the larger the node, the more differential lipids associated. The green solid lines represented a negative correlation, and the red line represented a positive correlation. The width of edges represented the credibility of relevance (*p*-value), the wider the edge (the smaller the *p*-value), the higher the credibility of the correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

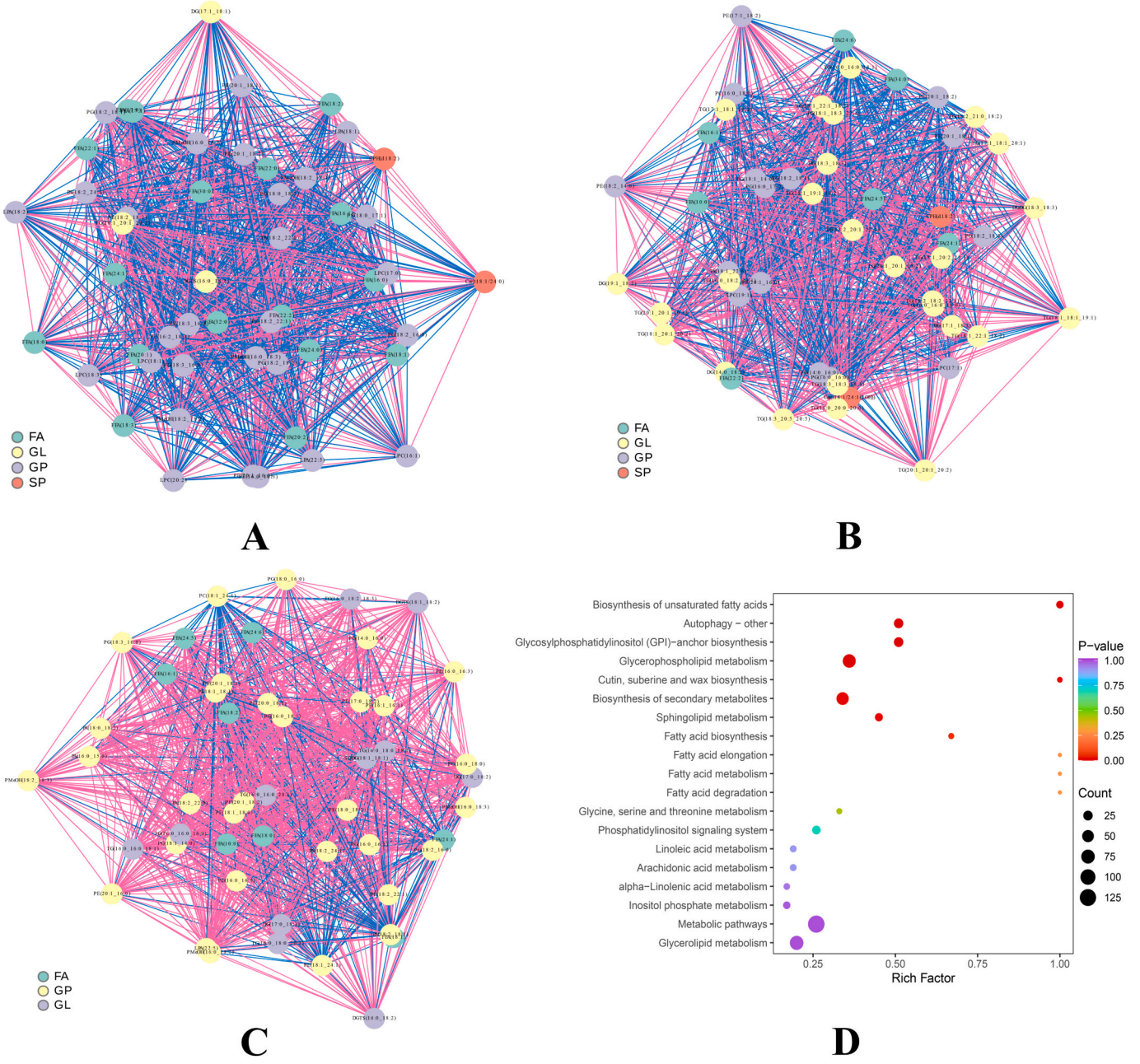

Fig. 6. The correlation network of differential lipids and metabolic pathways. **A**: Spearman's correlation network ($p < 0.05$) of differential lipids in the comparison of NQ1_vs_HT1. **B**: Spearman's correlation network ($p < 0.05$) of differential lipids in the comparison of ZA2_vs_HT1. **C**: Spearman's correlation network ($p < 0.05$) of differential lipids in the comparison of HB1_vs_HT1. Different colored circles represented different lipids categories. The connection line of nodes indicated the relationship level, and the number of lines and more, the more differential lipids associated. **D**: Significant metabolic pathways of differential lipids among NQ1, ZA2, HB1, and HT1.

biological activities, such as lowering blood lipids, anti-depression, anti-oxidation, and anti-tumor.

The lipids compositions of vegetable oils from different plant sources were obviously different, which were contributed to various biological activity (Dou et al., 2024; Koopman, 2018). Camellia oil is a natural edible vegetable oil with a variety of biological activities (He et al., 2022; Xiao et al., 2023). Therefore, based on previous studies, the differences in lipids compositions between Camellia oil and other plant-derived oils were achieved in our study. The results demonstrated that the proportion of GLs in Camellia oil was 84.53%, which was different from proportion of GLs (95.00%) in other vegetable oils. 413 GLs subclasses were identified in 23*C. oleifera* lines, which was the woody vegetable oil with the largest number of GLs reported at present, compared to olive oil (44 GLs subclasses), hazelnut oil (46 GLs subclasses), peanut oil (25 GLs subclasses) and jujube kernel oil (292 GLs subclasses). It was also found that the ratio of TGs to DGs in Camellia oil (40.61) was much lower than that in hazelnut oil (68.67), peanut oil (51.27) and palm oil (53.26). The DGs content in Camellia oil was higher than 1052.43 nmol/g, which could decrease the fats contents and blood lipids in human body after meals. Thus, Camellia oil has the potential to develop special formula low-calorie foods for obese patients, due to its higher DGs content. GPs, the important lipids linked to human health, exhibited vital roles in maintaining the body basic activities and improving the body immunity. 196 kinds of GPs components were identified with the average content of 5160.32 nmol/g, and it was speculated that Camellia oil had the potential to develop into functional

foods for enhancing immunity. Moreover, it was noteworthy that PRs (CoQ8, CoQ9, CoQ10) were detected in 23*C. oleifera* lines, with the content ranging from 11.07 nmol/g to 23.49 nmol/g, which had the functions of controlling blood pressure and dyslipidemia. The clinical trials had been verified that the cerebrovascular and hypertension might be elicited by the lack of PRs (Ayers et al., 2018). Particularly, the PRs (CoQ10) have been utilized as unique lipid-soluble antioxidants and nutritional supplements in the treatment of various chronic diseases, with excellent antioxidant, anti-inflammatory and anti-cancer properties (Ghaffari & Roshanravan, 2020). Consequently, Camellia oil exhibited extensively potential ability for preventing hypertension and metabolic syndrome. Therefore, Camellia oil could be developed into various health foods and household products for different requirements.

NQ1, ZA2, HB1 and HT1 with different lipids contents were applied to elucidate the differential lipids and lipids metabolic pathway of Camellia oil. 293 differential lipids were identified among NQ1, ZA2, HB1 and HT1, among which the differential lipids with higher content were TGs, FFAs, PGs, and PEs. TGs were the main storage forms in vegetable oil, which was a glycerol skeleton formed by sequential esterification of three fatty acids. The synthesis and assembly process of TGs in plants were as follows: the fatty acid was located in the endoplasmic reticulum, and then acyl-CoA was converted to the sn-3 position of DG to produce TG under the catalysis of diacylglycerol acyltransferase or transferred the sn-2 acyl group of phospholipids to DG to form TG under the catalysis of diacylglycerol acyltransferase (Bates & Browse, 2011; Lu et al., 2009). Phosphatidylglycerol phosphate (PGP) forms PGs under the action of phosphatase dephosphorylation. DGs reacted with CDP-choline and CDP-ethanolamine to form PC and PS respectively under the action of amino acetaldehyde phosphate transferase (Haslam et al., 2016). Our results displayed that NQ1 samples were richer in FFAs, ZA2 was richer in GPs, and HT1 was richer in GLs, which suggested that the expression of diacylglycerol acyltransferase and diacylglycerol acyltransferase in HT1 were higher, and the expression of PGP phosphatase and amino acetaldehyde phosphate transferase were higher in ZA2. The stronger correlation of GLs, GPs, and FFAs among NQ1, ZA2, HB1 and HT1 demonstrated that fatty acid synthesis would be converted into TGs at the mature stage of ZA2 and HB1, while the synthesis in NQ1 was opposite. Among them, TG(16:0_18:0_18:1), TG(16:0_16:0_18:1), and TG(16:0_16:0_18:2) were specific differential TGs, with the highest content in HT1, ranked by ZA2 and NQ1. Among FFAs, the FFA(18:1), FFA(18:2), FFA(16:0), FFA(18:0), and FFA(20:1) were particular differential FFAs, observed with highest content in NQ1. The PG (18:2_18:1) and PS(18:0_17:1) were found with the highest content in ZA2.

The unweighted correlation network analysis revealed that GLs, GPs, and FFAs were relatively stronger among NQ1, ZA2, HB1 and HT1 at intra-category and inter-category level, and GLs were present with strongest correlation with other lipids, which indicated that the GLs and GPs metabolism were contributed to the lipids difference. Glycerolipid metabolism and glycerophospholipid metabolism were the most influential pathways among NQ1, ZA2, HB1 and HT1 through pathway enrichment analysis. Although larger number of lipids metabolites have been found, how different lipids transformed into each other was still unknown. A mass of lipid metabolites had not been mapped to KEGG pathway, and the regulation and mechanism of enzymes in these pathways were still unclear. Therefore, it is desiderate to explore the mechanism of these enzymes in lipids synthesis in the further study.

5. Conclusion

The study presented the lipids profiles of 23*C. oleifera* lines from northern margin distribution area (South Shaanxi). 673 lipids components were detected, among which GLs, GPs, and FFAs occupied higher proportions. The lipids contents were between 69,603.31 ± 1978.56 nmol/g and 41,169.06 ± 1348.25 nmol/g of 23*C. oleifera* lines. Combined lipid content-function analysis indicated that camellia oil had advantages over other common vegetable oils in terms of medium and long chain lipids and unsaturated lipids, such as GLs, GPs, FFAs, TGs to DGs ratio, and PRs (CoQ10), and NQ1, HT1, HT2, ZA2, ZB1, ZB2, and SN2 lines could be extended to other functional products based on the unique lipid composition. Interestingly, the FFAs, GLs, and GPs contents in NQ1, ZA2, and HT1 were obviously different, among which FFAs were richer in NQ1, GPs were richer in ZA2, and GLs were richer in HT1. 293 differential lipids components were identified among NQ1, ZA2, HB1, and HT1. GLs, GPs, and FFAs were relatively stronger in NQ1, HT1, ZA2, and HB1 at intra- and inter-category level, especially GLs exhibiting strongest correlation with other lipids. The glycerolipid metabolism, glycerophospholipid metabolism, and fatty acid biosynthesis were the obviously differential lipids pathways, which established foundation for exploring the lipids biosynthesis mechanism. The above results provided the comprehensive description of lipids compositions and abundances in 23*C. oleifera* lines from northern margin distribution area (South Shaanxi), and were contributed to the utilization of functional food products and directional breeding.

Fundings

This financial support of this study was supplied by the Talent Initiation Project of Shaanxi University of Technology (SLGRCQD-2307), State Key Laboratory (Cultivation) of Qinba Mountains Biological Resources and Ecological Environment, 'co-construction of city and school' scientific research project industrialization project (SXC-2309), and The Shaanxi Province Natural Science Basic Research Plan Project (2016JM3034).

CRediT authorship contribution statement

Tao Zheng: Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Min Tian:** Investigation, Formal analysis. **Zhuang Deng:** Investigation, Formal analysis. **Qi Tang:** Investigation, Data curation. **Zhubing Hu:** Software, Methodology. **Guodong Wang:** Software, Methodology. **Haitao Zeng:** Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

All authors declared that they had no known competitive financial interests or personal relationships that could have appeared to influence the study reported in this paper.

Data availability

All the lipid composition and contents data of 23 C. oleifera lines demonstrated in this study are obtained from corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101629.

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