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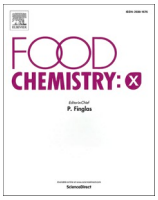

Metabolomics analyses provide insights into the nutritional quality profiling in 95 avocado germplasms grown in China

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ABSTRACT

Avocado is widely grown in tropical and subtropical regions of China. Diverse germplasms have been generated through natural hybridization and selective breeding. Here, to screen high-quality avocado germplasms, we characterized the nutritional quality of 95 avocado germplasms grown in China based on metabolomics. The oil content of the 95 avocado germplasms was 2.18 %–16.60 % and followed a normal distribution. We further profiled nine fatty acids, 16 hydrolyzed amino acids, and eight mineral elements in avocado fruit, which varied widely among different germplasms. Correlation analysis revealed significant positive correlations between fatty acid components, as well as between amino acid components and between mineral elements. Clustering analysis and evaluation of the 95 avocado germplasms identified 14 germplasms with high nutritional quality. These findings provide a basis for evaluation of avocado fruit quality and utilization of high-quality avocado germplasms, as well as important implications for the breeding of avocado.

1. Introduction

Avocado (*Persea americana* Mill.) is an evergreen tree of Lauraceae native to the tropical and subtropical regions of Mexico and Central America. Its fruit is pear-shaped or spherical with a large single seed in the center (Ayala-Silva & Ledesma, 2014). Avocado fruit is rich in unsaturated fatty acids, proteins, minerals, and dietary fibers (Dreher & Davenport, 2013). Long-term consumption of the fruit can lower the cholesterol level as well as improve weight management and cardiovascular and intestinal health (Ford et al., 2023). As a natural "superfood" with great health benefits, the nutritional quality of avocado has always been the focus of breeders and consumers (Bhuyan et al., 2019).

The main nutritional quality indicators of avocado include the contents and composition of oil, fatty acids, proteins, and mineral elements. Due to differences in genotype and genetic background, the nutritional quality of avocado varies greatly among different germplasms. Among various avocado varieties, 'Hass' avocado is the most representative variety with high nutritional quality (Dreher & Davenport, 2013). Hence, it is often used as a reference for screening high-quality avocados in nutritional quality evaluation of avocado germplasms. Acosta-Díaz et al. (2019) investigated the oil content and fatty acids of 36 avocado germplasms from Nuevo León, Mexico, and screened out some high-

Abbreviations: C14:0, Myristic acid; C16:0, Palmitic acid; C16:1(n-7), Palmitoleic acid; C18:0, Stearic acid; C18:1(n-9), Oleic acid; C18:1(n-7), 11-octadecenoic acid; C18:2(n-6), Linoleic acid; C18:3(n-3), Linolenic acid; C20:0, Arachidic acid; Asp, Aspartic acid; Thr, Threonine; Ser, Serine; Glu, Glutamic acid; Gly, Glycine; Ala, Alanine; Val, Valine; Met, Methionine; Ile, Isoleucine; Leu, Leucine; Tyr, Tyrosine; Phe, Phenylalanine; His, Histidine; Lys, Lysine; Arg, Arginine; Pro, Proline; K, Potassium; P, Phosphorus; Mg, Magnesium; Ca, Calcium; Fe, Ferrum; Zn, Zinc; Mn, Manganese; Cu, Cuprum.

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quality germplasms ('Platano temprano', 'Pato', and 'Especial') with higher oil content than 'Hass'. Additionally, Gonçalves et al. (2024) evaluated five avocado varieties from Madeira, Portugal, and found that the 'RCF' variety had the highest oil content. It has been reported that avocado fruit has a protein content ranging from 1 % to 3 %, which is higher than that of many other fruits (Araújo et al., 2018; Blakey et al., 2012). Rozan et al. (2021) further investigated the amino acid composition of proteins in six avocado varieties, and found that Glu has the highest contents, while Cys has the lowest content. Hardisson et al. (2001) determined the mineral elements of four avocado varieties ('Arona', 'Fuerte', 'Hass', and 'Orotava'), and found that the contents of mineral elements vary significantly among different varieties. Collectively, resource evaluation can screen out avocado germplasms with higher nutritional quality, providing a basis for future avocado breeding programs.

Avocado was first introduced to China in 1918, and hundreds of avocado varieties were subsequently spread to China from the United States, Mexico, South Africa, and some Central American countries, which are grown in Hainan, Yunnan, Guangxi, Guangdong, Fujian and other provinces in China (Ge et al., 2019). As Chinese consumers gradually recognize the nutritional and health benefits of avocado, the consumption of avocado in China has been continuously increasing over the past decade, and the avocado import in China had reached 65,600 tons in 2023. The avocado industry is of broad market prospects in China, while there is a lack of independently bred high-quality avocado varieties. Although China is not the region of origin for avocado, a large number of diverse avocado germplasms have been generated in China through natural hybridization during the long cultivation (Liu et al., 2020). There has been a lack of comprehensive evaluation on the nutritional quality of these diverse germplasms. In this study, we investigated the dry matter content, oil content, fatty acid composition, hydrolyzed amino acid content, and mineral elements of 95 avocado germplasms grown in China. Based on metabolomics, we analyzed the nutritional quality of different avocado germplasms, and screened some high-quality avocado germplasms. This study provides basic data for studying the nutritional quality of avocado and the breeding of high-quality avocado varieties.

2. Materials and methods

2.1. Materials

The 95 avocado germplasms used in this study included 54 local germplasms in China, 26 seedling germplasms from our resource nursery and 15 commercial avocado varieties (Table S1). These germplasms were collected from Hainan Province, Guangdong Province, Guangxi Province and some other regions, covering the main avocado growing areas in China (Table S1). The fruits of these germplasms were highly diverse in shape, size, color, weight, glossiness, and surface morphology, suggesting their different genetic backgrounds and genotypes. Therefore, we performed a comparative analysis of the nutritional quality of these germplasms.

The above avocado germplasms were uniformly grown in the Hainan Provincial Avocado Germplasm Resource Nursery in Danzhou City, Hainan Province, China under the same soil, irrigation, and nutrition conditions (Table S1). Since the dry matter content of fruits of different germplasms varies widely, dry matter content cannot be used as a criterion to determine the avocado maturity and harvest time. To make the nutritional quality of fruit of all germplasms comparable, we used the following criteria to determine the harvest time of different avocado germplasms, including the appearance of a gap between seed and flesh on some germplasms, fruit size similar to previous years, the appearance of small rust-brown specks on some germplasms, the change of seed coat from ivory to dark brown on some germplasms, the fruit becoming somewhat duller in appearance on some germplasms, days after full bloom for commercial varieties such as 'Hass', and the harvest time in previous years. The fruit of 95 avocado germplasms were harvested from July to September of 2022. At least 20 healthy fruit were harvested from each avocado germplasm and transported back to the laboratory within a few hours after harvesting. The sample fruit were stored at 25 °C and 60 % relative humidity to ripen. The avocado is ripe and edible when the fruit feels slightly soft (but not mushy) when gently squeezing it. The postharvest ripened fruit were used for subsequent experiments.

2.2. Determination of dry matter content and oil content of fruit

At least 20 postharvest ripened fruit were equally divided into four biological replicates for determination of dry matter content. For each biological replicate, the fruit with the removal of seed and peel was quickly blended into homogenate and mixed thoroughly. Subsequently, about 50 g of avocado sample was immediately weighed (W1) and quickly frozen in liquid nitrogen. Next, the frozen avocado sample was placed in a freeze dryer (SCIENTZ-18 N, China) for 72 h, and the weight of the freeze-dried sample (W2) was recorded. The dry matter content of the sample could be calculated based on the values of W1 and W2. The freeze-dried sample was ground into powder and then stored at −80 °C for further analysis.

The oil content of the fruit was determined according to the method of Ge et al. (2021) through Soxhlet extraction (Soxtec 8000, Foss, Denmark) with petroleum ether in a boiling range of 30–60 °C. The extraction procedure was boiling for 40 min, elution for 30 min, and recovery for 30 min. The oil extracted by Soxhlet extraction was weighed to calculate the oil content and then stored at −80 °C for subsequent fatty acid analysis.

2.3. Fatty acid identification and quantification in avocado fruit

Analysis of fatty acids in avocado fruit was performed by referring to the previous method with modifications (Meyer & Terry, 2008). The reagents used in the experiment were purchased from Aladdin (Shanghai, China). Briefly, about 40 μg recovered oil extracted by Soxhlet extraction was pipetted into a glass bottle, and the weight of the oil was accurately weighed with a one ten-thousandth balance and recorded. The glass bottle with oil was added with 5 mL NaOH-MeOH solution (0.2 M) and then saponified at 80 °C for 30 min. After cooling, 200 μL internal standard (10 mg/mL of pentadecanoic acid) and 2.5 mL 14 % BF3-MeOH were added, and methyl esterification was conducted at 80 °C for 30 min. After cooling again, the sample was added with 2 mL saturated NaCl and 4 mL n-hexane and mixed thoroughly. After stratification, the supernatant was taken into a clean glass tube, and anhydrous sodium sulfate was added, followed by shaking and 1 h of standing. Finally, the sample was filtered through a 0.22 μm filter membrane and transferred into a brown injection bottle, and the fatty acid methyl esterification was completed. Methyl esterified samples were analyzed by Agilent 7890b gas chromatography with a flame ionization detector (FID) and a DB-FastFAME capillary column (60 m × 0.25 mm i.d., 0.25-mm film thickness) using N_2 as the carrier gas in constant pressure mode. The column temperature was programmed at 80 °C for 0.5 min, and then raised to 165 °C at 40 °C min^{-1} and held for 1 min, increased to 206 °C at 4 °C min^{-1} and held for 0.5 min, raised to 210 °C at 1 °C min^{-1} and held for 0.5 min, and increased to 230 °C at 4 °C min^{-1} and held for 8.5 min. The FID detector temperature was 260 °C; the injection port temperature was 250 °C, the split ratio was 10:1. Nitrogen was used as the carrier gas; the constant pressure mode was selected at 28 psi; and the injection volume was 1 μL. The fatty acids were identified by comparing the retention time of gas chromatographic peaks with those of free fatty acid methyl ester standards. The quantification of fatty acids was based on internal standard method.

2.4. Analysis of hydrolyzed amino acids in fruit

Fruit hydrolyzed amino acids were determined according to the

national standards of People's Republic of China (GB 5009.124–2016). Briefly, 0.2 g of freeze-dried avocado powder and 10 mL of 6 mol/L HCl were added to a hydrolysis tube. Then, the hydrolysis tube was filled with N_2 and sealed, and the sample was hydrolyzed at 110 °C for 24 h and then cooled to room temperature. The hydrolyzed sample was filtered and diluted to 50 mL. Then, 1 mL of the sample was blown dry with nitrogen, and re-dissolved with 2 mL of sodium citrate (pH 2.2). Finally, the sample was filtered through a 0.22 μm filter membrane into a sample injection bottle for testing. The hydrolyzed amino acids were analyzed using A300 automatic amino acid analyzer (membraPure, Germany).

2.5. Determination of mineral elements in fruit

The eight mineral elements in avocado fruit, including K, P, Mg, Ca, Fe, Zn, Mn and Cu, were determined by referring to the national standards of People's Republic of China (GB 5009.268–2016). Briefly, 0.2 g freeze-dried avocado powder was added with nitric acid and then microwave digested. The digested sample was then determined by ICP-MS (PerkinElmer NexION 300×, USA). Mineral elements were qualitatively analyzed by specific mass number (m/z), and quantitatively analyzed by external standard method.

2.6. Statistical analysis

All results were obtained from four independent biological replicates. Statistical analysis and graphics were performed using R version 4.1.2, including frequency distribution histograms, box plots, heatmaps, pie charts, Pearson correlations, ANOVA, Tukey's multiple comparison test and normality test.

3. Results

3.1. Fruit dry matter content and oil content in 95 avocado germplasms

To explore the distribution and correlation of fruit dry matter content and oil content in diverse avocado germplasms, we measured the fruit dry matter and oil contents in 95 avocado germplasms. The results showed that the dry matter content ranged from 9.75 % ('HY-24-13') to 28.98 % ('GD-3-6'), with an average of 18.16 % and a CV (coefficient of variation) of 23.36 % (Table S2 and S3). Statistical analysis of oil content revealed that the average oil content of 95 avocado germplasms was 8.55 %, with a CV of 42.55 %. 'GD-4-6' had the lowest oil content (2.18 %), while 'GD-3-6' exhibited the highest oil content (16.60 %). Among the 95 avocado germplasm resources, 'CM-51-8', 'HY-23-13', and 'GD-3-6'all had oil contents exceeding 16 %, which were close to that of the most valuable commercial variety 'Hass' (16.54 %) (Table S3). We further analyzed the distribution of dry matter content and oil content in 95 avocado germplasms by frequency distribution histogram and boxplot. The results revealed that the dry matter and oil contents showed continuous variations (Fig. 1A-D), and Kolmogorov-Smirnov test showed that both of them followed a normal distribution ($P > 0.05$) (Table S4). We also tested the correlation between dry matter content and oil content. The scatter plot and fitting curve showed that the oil content was significantly positively correlated with the dry matter content with a correlation coefficient of 0.904 (Fig. 1E).

3.2. Determination of fruit fatty acid composition among 95 avocado germplasms

Fatty acid composition is an important indicator for evaluating avocado quality. Therefore, we further investigated the content and proportion of each fatty acid in 95 avocado germplasms. The results showed that all avocado germplasms had nine fatty acids, including C14:0,

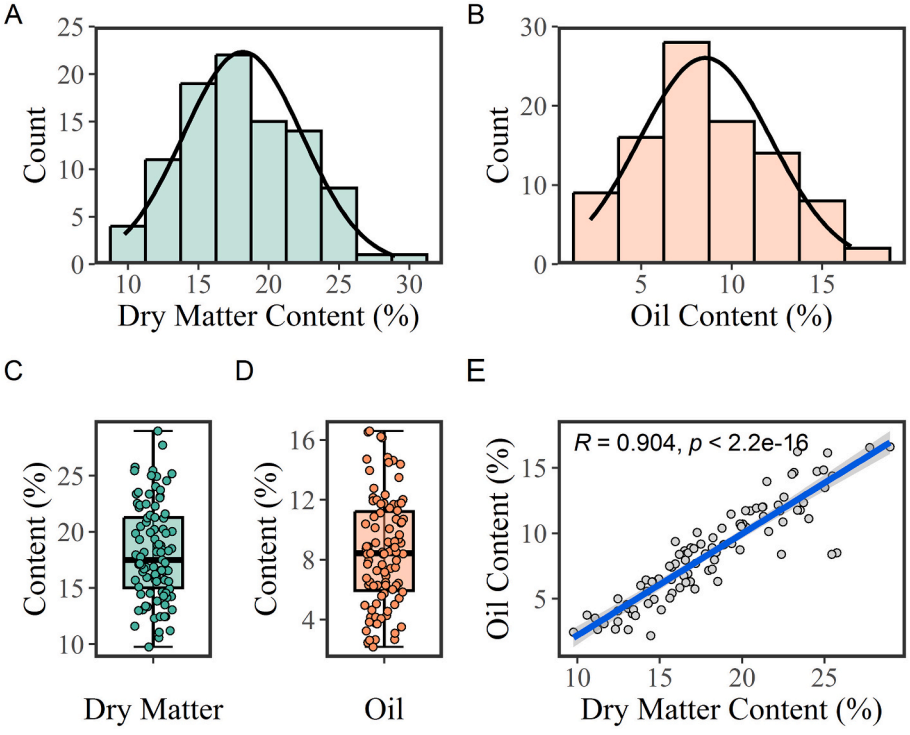

Fig. 1. Variations of fruit dry matter content and oil content among 95 avocado germplasms. A and B show the frequency distribution histograms of fruit dry matter content and oil content of 95 avocado germplasms, respectively. C and D show the boxplots of fruit dry matter content and oil content of 95 avocado germplasms, respectively. E, Scatter plot of dry matter content and oil content in 95 avocado germplasms. The '*R*' in Fig. E represents the Pearson correlation coefficient between dry matter content and oil content.

C16:0, C16:1(n-7), C18:0, C18:1(n-9), C18:1(n-7), C18:2(n-6), C18:3(n-3), and C20:0 (Fig. 2), while the content and proportion of each fatty acid varied among different germplasms (Table S3). The contents of the four SFAs (saturated fatty acids), including C14:0, C16:0, C18:0 and C20:0, were 0.032–0.228, 3.550–47.743, 0.112–1.570, and 0.001–0.189 mg/g (Table S2), and the contents of the five UFAs (unsaturated fatty acids), including C16:1(n-7), C18:1(n-9), C18:1(n-7), C18:2(n-6), and C18:3(n-3), were 0.680–17.044, 3.961–59.829, 0.441–7.732, 2.931–21.312, and 0.330–2.064 mg/g, respectively (Table S2). The frequency distribution histogram further indicated that the content of each fatty acid in the 95 avocado germplasms was continuously variable and followed a normal distribution (Fig. S1 and Table S4). The same results were obtained for the proportion of each fatty acid except for C14:0 and C18:3(n-3) (Fig. S2 and Table S4). UFAs are important nutrients with great beneficial effects to health. Among the 95 avocado germplasms, the total UFA content of seven germplasms ('ZPC-12-1', 'CM-43–6', 'HY-6-8', 'HY-19-13', 'HY-23-13', 'CM-51-8' and 'HY-4-10') exceeded 78 mg/g, which was higher than that of the recognized high-nutrition commercial variety 'Hass' (76.345 mg/g) (Table S3).

We further performed clustering and heatmap analysis of different avocado germplasms. As shown in Fig. 2A, the 95 avocado germplasms were divided into three clusters (FA-I, FA-II, and FA-III) based on their fruit fatty acid composition. In cluster FA-I, C16:0 was the dominant fatty acid, followed by C18:1(n-9). On the contrary, in cluster FA-III, C18:1(n-9) was the dominant fatty acid, followed by C16:0. However, in cluster FA-II, the proportion of C16:0 was similar to that of C18:1(n-9), both of which were significantly higher than that of other fatty acids (Fig. 2B). Among all avocado germplasms, more than 68 % of them belonged to cluster FA-III, while the commercial variety 'Hass' belonged to cluster FA-II (Fig. 2A). Despite variations in fatty acid composition among FA-I, FA-II and FA-III, the proportion of UFAs was always significantly higher than that of SFAs in all three clusters (Fig. 2B).

3.3. *Content and composition of hydrolyzed amino acids in the fruit of 95 avocado germplasms*

To explore the protein content and composition of avocado fruit, we hydrolyzed the protein with hydrochloric acid to determine the amino acid composition. As a result, a total of 16 hydrolyzed amino acids were detected, including seven EAA (essential amino acids; Thr, Val, Met, Ile, Leu, Phe, and Lys) and nine nonessential amino acids (Asp, Ser, Glu, Gly, Ala, Tyr, His, Arg, and Pro) (Table 1 and Fig. 3A). The total hydrolyzed amino acid content of 95 avocado germplasms ranged from 0.188 g/100

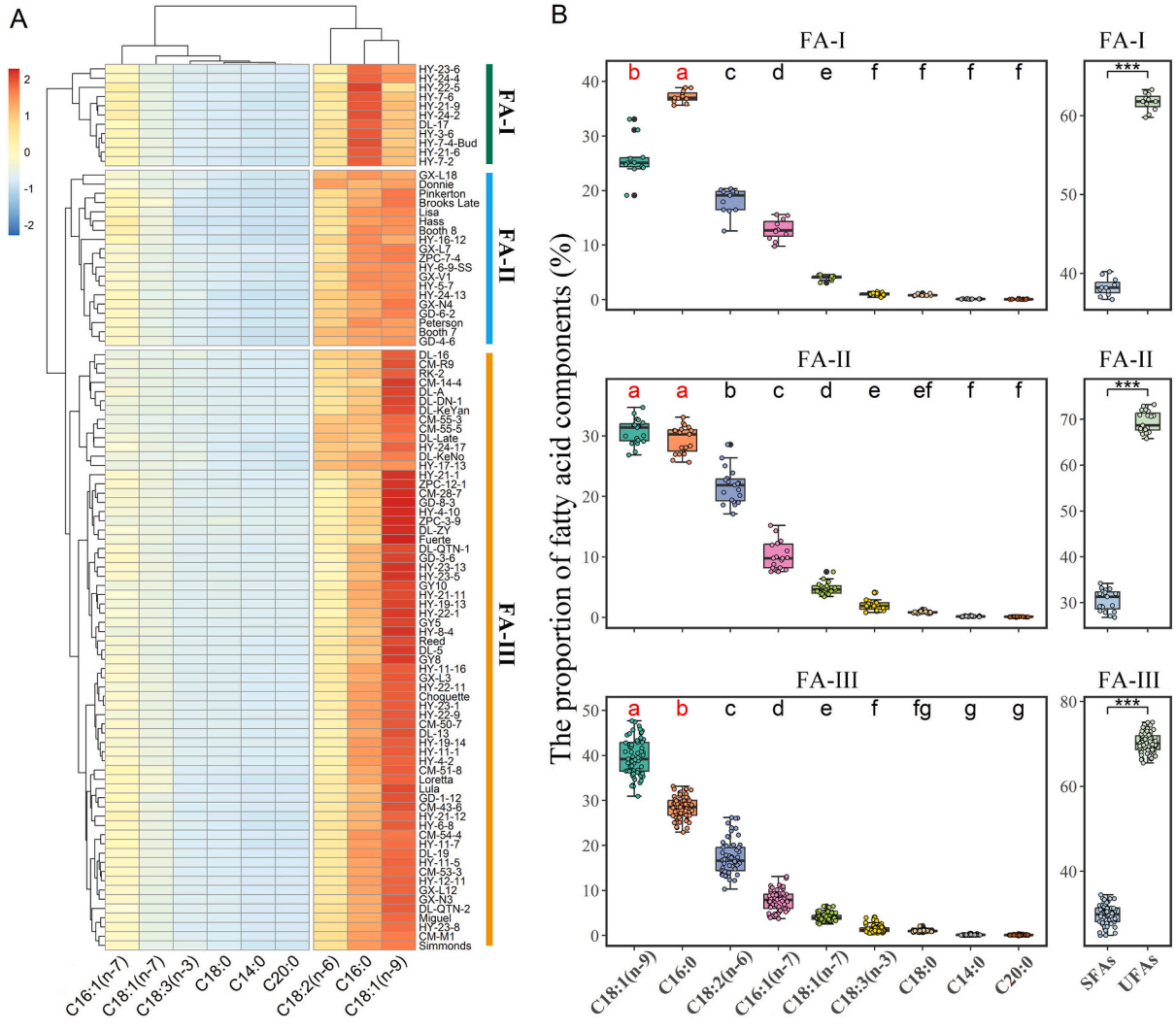

Fig. 2. Fruit fatty acid composition of the 95 avocado germplasms. A, cluster heatmap of 95 avocado germplasms based on fruit fatty acids. 'FA' is the abbreviation for fatty acid. Cluster FA-I, cluster FA-II, and cluster FA-III were three avocado germplasm clusters with different fatty acid compositions. B, multiple comparisons of the proportion of fatty acid components in FA-I, FA-II, and FA-III. SFAs, saturated fatty acids; UFAs, unsaturated fatty acids. '***' in Fig. 2B represents *P* value <0.001.

g ('DL-19') to 2.873 g/100 g ('Booth 7'), with an average value of 1.118 g/100 g and a CV of 44.315 % (Table 1). The EAA content in different avocado germplasms ranged from 0.103 g/100 g ('DL-19') to 0.922 g/100 g ('Hass') (Table 1; Table S3). Furthermore, the frequency distribution histogram indicated that the total hydrolyzed amino acid content, the EAA content and content of each amino acid in different avocado germplasms were continuously variable (Fig. S3), and followed a normal distribution except for Arg and Met (Table S4). In this work, the total hydrolyzed amino acid content and the EAA content of the reference group (the commercial variety 'Hass') reached 2.236 g/100 g and 0.922 g/100 g, respectively (Table S3). Among the 95 germplasms, six germplasms ('ZPC-7-4', 'GX-N4', 'GX-L12', 'GD-1-12', 'DL-A' and 'Booth 7′) had total hydrolyzed amino acid content and EAA content close to 'Hass' avocado (Table S3), which were potential high-quality germplasms with high protein composition.

As shown in the heatmap of Fig. 3A, Asp, Glu, Phe, Leu, Val, Lys, and Arg were seven amino acids with high proportions among the 16 amino acids and great variations among different germplasms. Based on the composition of hydrolyzed amino acids, the 95 avocado germplasms could be divided into four clusters (AA-I, AA-II, AA-III, and AA-IV) (Fig. 3A). Fig. 3B shows the proportions of different amino acid components in each cluster. In cluster AA-I, Phe accounted for the highest proportion of 18.83 %. In cluster AA-II, Asp and Glu were the top two amino acids, accounting for similar proportions of 12.44 % and 12.94 %, respectively. In cluster AA-III, Arg, Asp, and Glu were the top three amino acids, which together accounted for up to 44.44 % of the total. In cluster AA-IV, Asp accounted for the highest proportion (11.00 %), followed by Leu (10.85 %) and Glu (10.13 %) (Fig. 3B).

3.4. Fruit mineral elements in 95 avocado germplasms

In this study, we investigated four macroelements (K, P, Mg, and Ca) and four microelements (Fe, Zn, Mn, and Cu) in different avocado germplasms. The frequency distribution histogram revealed that the contents of four macroelements and four microelements were continuously variable (Fig. 4A-H). Kolmogorov-Smirnov test revealed that the contents of P, Mg, and Zn followed a normal distribution ($P > 0.05$); however, those of the remaining mineral elements showed a non-normal distribution ($P < 0.05$) (Table S4).

Furthermore, we performed a comparative analysis of the contents of four macroelements and four microelements. The results showed that K was the dominant mineral element in avocado fruit (Fig. 4I and Fig. 4J). The K content of 95 avocado germplasms ranged from 158.44 mg/100 g ('GX-V1') to 1287.19 mg/100 g ('HY-23-13'), with an average of 419.53 mg/100 g (Table S2 and Table S3). The contents of other three macroelements, namely P, Mg, and Ca, were 17.19–106.05, 8.68–49.75, and 3.44–30.88 mg/100 g, respectively (Table S2). Multiple comparative analysis results showed that the content of P was significantly higher than that of Ca, and the Mg content was not significantly different from the P and Ca content (Fig. 4I). K is one of the essential nutrients for the human body and is also the mineral element with the highest content in avocado. Through resource evaluation, four germplasms ('GX-N4', 'Booth 7', 'DL-Late' and 'HY-23-13') with higher K contents than the reference group (the commercial variety 'Hass') were found (Table S3). In terms of the four microelements, Fe, Zn, Mn, and Cu showed the contents of 0.23–2.11, 0.16–1.31, 0.07–1.55, and 0.05–0.51 mg/100 g, respectively (Table S2). Multiple comparative analysis showed that Fe and Cu were the microelements with the highest and lowest content, respectively, and the Zn content was not significantly different from the Mn content (Fig. 4J).

Table 1
Statistics of hydrolyzed amino acids in the fruit of 95 avocado germplasms.

Traits	Average (g/100 g FW)	SD (g/100 g FW)	Min (g/100 g FW)	Max (g/100 g FW)	CV (%)
Total hydrolyzed amino acid	1.118	0.496	0.188	2.873	44.315
EAA	0.452	0.178	0.103	0.922	39.512
Asp	0.140	0.090	0.019	0.714	64.724
Thr	0.054	0.023	0.006	0.123	42.599
Ser	0.057	0.024	0.007	0.130	42.240
Glu	0.142	0.089	0.021	0.523	62.398
Gly	0.062	0.028	0.007	0.148	45.189
Ala	0.061	0.029	0.005	0.148	47.837
Val	0.082	0.035	0.012	0.194	42.456
Met	0.006	0.003	0.000	0.021	56.429
Ile	0.051	0.020	0.007	0.114	39.618
Leu	0.102	0.041	0.010	0.241	40.398
Tyr	0.045	0.018	0.002	0.105	40.644
Phe	0.079	0.050	0.005	0.275	63.675
His	0.034	0.013	0.006	0.063	37.033
Lys	0.077	0.033	0.008	0.183	42.197
Arg	0.059	0.037	0.005	0.238	62.346
Pro	0.066	0.041	0.005	0.205	62.957

Note: FW, fresh weight; EAA, essential amino acid; SD, standard deviation; CV, coefficient of variation.

3.5. Correlation analysis among nutritional quality traits of avocado fruit

To investigate the relationships among various avocado nutritional quality traits, a correlation analysis was performed among oil content, fatty acid, hydrolyzed amino acid, and mineral element (Fig. 5). The results showed that the oil content was significantly positively correlated with all fatty acid components, with correlation coefficients ranging from 0.34 to 0.92. The oil content had no correlation with amino acid content, but was significantly positively correlated with nearly all mineral elements except for Zn. In addition, there were strong correlations among different fatty acid components, while most fatty acid components had no correlation with amino acid components. However, C18:3(n-3) had significant positive correlations with all amino acids except for Phe; C18:2(n-6) exhibited weak positive correlations with six amino acids (Ser, Gly, Met, Ile, Tyr, and His), with correlation coefficients between 0.21 and 0.29; and C18:1(n-7) also showed a weak positive correlation with His. Moreover, the fatty acid components were significantly correlated with macroelements, but not or weakly correlated with microelements. There were significant correlations among the 16 amino acid components. In addition, most amino acid components were positively correlated with K, P, Mg, Fe, Zn, and Cu, but had no correlation with Ca and Mn. Most mineral elements had significant positive correlations with each other, except for Mn, which had no correlation with P, Zn, and Cu, and weak correlations with K, Mg, Ca and Fe, with correlation coefficients of 0.27, 0.22, 0.23, and 0.21, respectively.

3.6. Comprehensive evaluation of 95 avocado germplasms

To screen avocado germplasms with high quality, we performed an evaluation of the 95 avocado germplasms. Based on the oil content, nine fatty acids, 16 amino acids, and eight mineral elements, these avocado germplasms were divided into six groups, namely Group-A, Group-B, Group-C, Group-D, Group-E, and Group F (Fig. 6A). We further performed multiple comparisons among the six groups in terms of oil content, total fatty acid content, total hydrolyzed amino acid content, and total mineral element content (Fig. 6B). The results revealed that Group-A, B, E, and F had significantly higher oil content and total fatty acid content than Group-C and Group-D. Moreover, Group-A had the highest total hydrolyzed amino acid content, followed by Group-D, Group-B, and Group-F. Group-C and Group-E had the lowest total amino acid content with no significant difference between the two groups. Furthermore, Group-B had the highest total mineral element content, followed by Group-A and Group-F. Group-C, Group-D, and Group-E had the lowest total mineral element content with no significant difference between each other. Overall, the avocado germplasms in

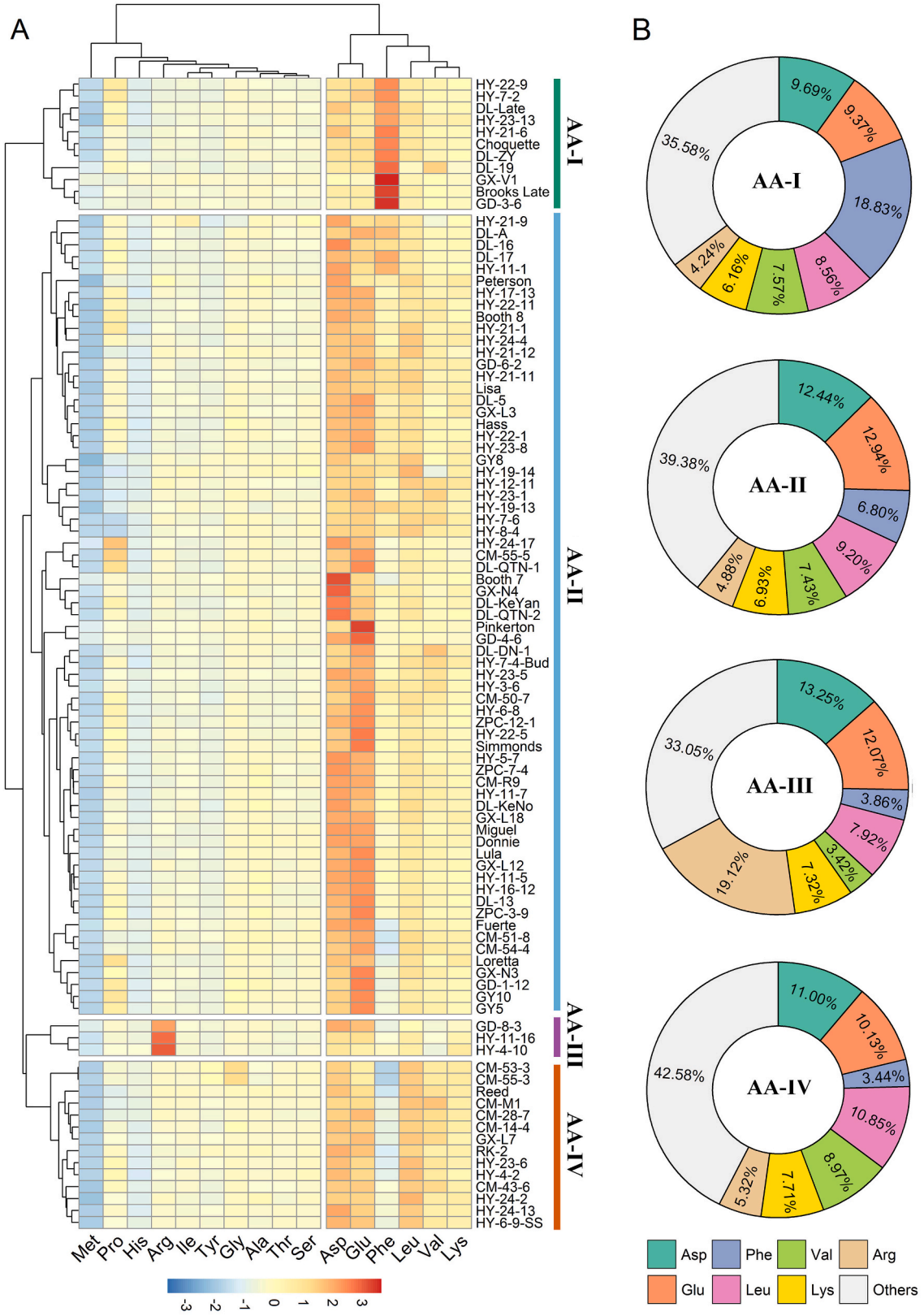

Fig. 3. Comparative analysis of hydrolyzed amino acids in the fruit of 95 avocado germplasms. A, cluster heatmap of 95 avocado germplasms based on fruit hydrolyzed amino acids. 'AA' is the abbreviation for amino acid. Cluster AA-I, cluster AA-II, cluster AA-III, and cluster AA-IV were four avocado germplasm clusters with different amino acid compositions. B, Donut charts of amino acid composition of four clusters of avocado germplasms (AA-I, AA-II, AA-III, and AA-IV). The "Others" in Fig. 3B represents the proportion of the sum of Met, Pro, His, Ile, Tyr, Gly, Ala, Thr and Ser in the total amino acid content.

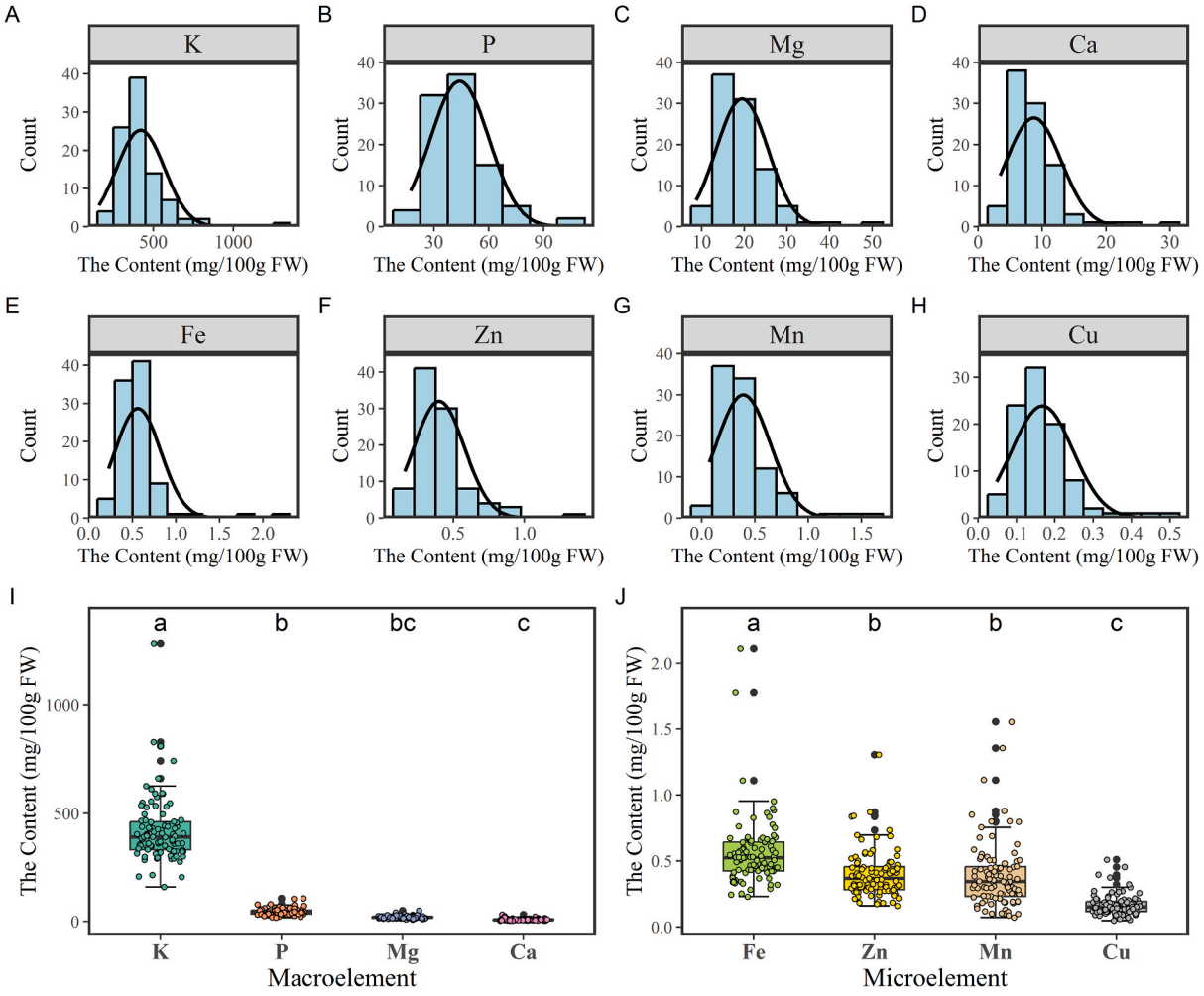

Fig. 4. Distribution of fruit mineral element contents among 95 avocado germplasms. A, B, C and D show the frequency distribution histograms of macroelements (K, P, Mg and Ca). E, F, G and H display the frequency distribution histograms of microelements (Fe, Zn, Mn and Cu). I and J show multiple comparisons of macroelements (K, P, Mg, and Ca) and microelements (Fe, Zn, Mn, and Cu) among 95 avocado germplasms, respectively. FW, fresh weight.

Group-A had higher contents of oil, fatty acids, hydrolyzed amino acids, and mineral elements. Moreover, as the reference for screening high-nutritional avocados, the commercial variety 'Hass' also belonged to group A. Therefore, the 14 avocados in group A were considered as germplasms with higher nutritional value. These 14 avocado germplasms included 11 germplasms from China ('HY-6-8', 'GD-1-12', 'GX-N4', 'CM-55-5', 'ZPC-3-9', 'DL-A', 'HY-21-6′, 'HY-7-2′, 'ZPC-12-1′, 'HY-11-7′, and 'GX-L12') and three commercial avocado varieties ('Hass', 'Booth 7′, and 'Pinkerton'). Moreover, two germplasms in Group-B, 'DL-late' and 'HY-23-13′, had significantly higher mineral element contents than other germplasms, and are therefore excellent germplasms for the breeding of varieties with high mineral elements.

4. Discussion

Fruit is an important source of nutrition for humans. Compared with the nutritional composition of citrus (Guo et al., 2023), apple (Musacchi & Serra, 2018), pear (Chen et al., 2007), banana (Ranjha et al., 2022), grape (Aubert & Chalot, 2018), peach (Petruccelli et al., 2023) and most other fruits, avocado has a lower sugar content, but can accumulate a large amount of oils mainly composed of UFAs in its flesh, making it a good source of UFAs for humans (Meyer & Terry, 2010). As the core competitive advantages over other fruits, the oil content and fatty acid composition have been the focus of research on avocado, and many varieties and germplasms with different oil contents have been screened through resource evaluation (Salazar-López et al., 2020). For example, Gómez-López (1998, 2002) characterized the oil content in 49 local avocado germplasms in Venezuela and found 12 germplasms with very low oil contents such as 'Nelan' and 'Simmonds', whose oil contents were only 3 %–6.70 %, and four germplasms with high oil contents such as 'Ryan' and 'Duke', whose oil contents were over 15 %. In this study, we measured the oil content in 95 avocado germplasms from China and found some high-oil content germplasms such as 'CM-53-3', 'HY-4-10', 'CM-51-8', 'HY-23-13', and 'GD-3-6'. These germplasms had oil contents close to those of the commercial variety 'Hass' and are suitable for the breeding of high-oil varieties. It has been reported that the fatty acid composition of avocado is mainly composed of C14:0, C16:0, C16:1(n-7), C18:0, C18:1(n-9), C18:2(n-6), C18:3(n-3) and C20:0, and UFAs such as C18:1(n-9) and C18:2(n-6) are dominant fatty acids (Ford et al., 2023; Yanty et al., 2011). The 95 avocado germplasms in this study not only contained the above fatty acids, but also C18:1(n-7), an UFA similar to C18:1(n-9). However, except for the report of Plaza et al. (2009), C18:1(n-7) has been rarely mentioned or detected in previous reports on avocado fatty acid composition (Ford et al., 2023; Opiyo et al., 2023). Similarly, Gonçalves et al. (2024) also detected arachidonic acid/C20:4 (n-6), which has been rarely detected in higher plants, in regional avocado varieties in Portugal. Nevertheless, we did not detect arachidonic acid/C20:4(n-6) in the 95 avocado germplasms in this work. The fatty acid composition of avocado is affected by variety, cultivation conditions, and determination methods and techniques (Opiyo et al., 2023),

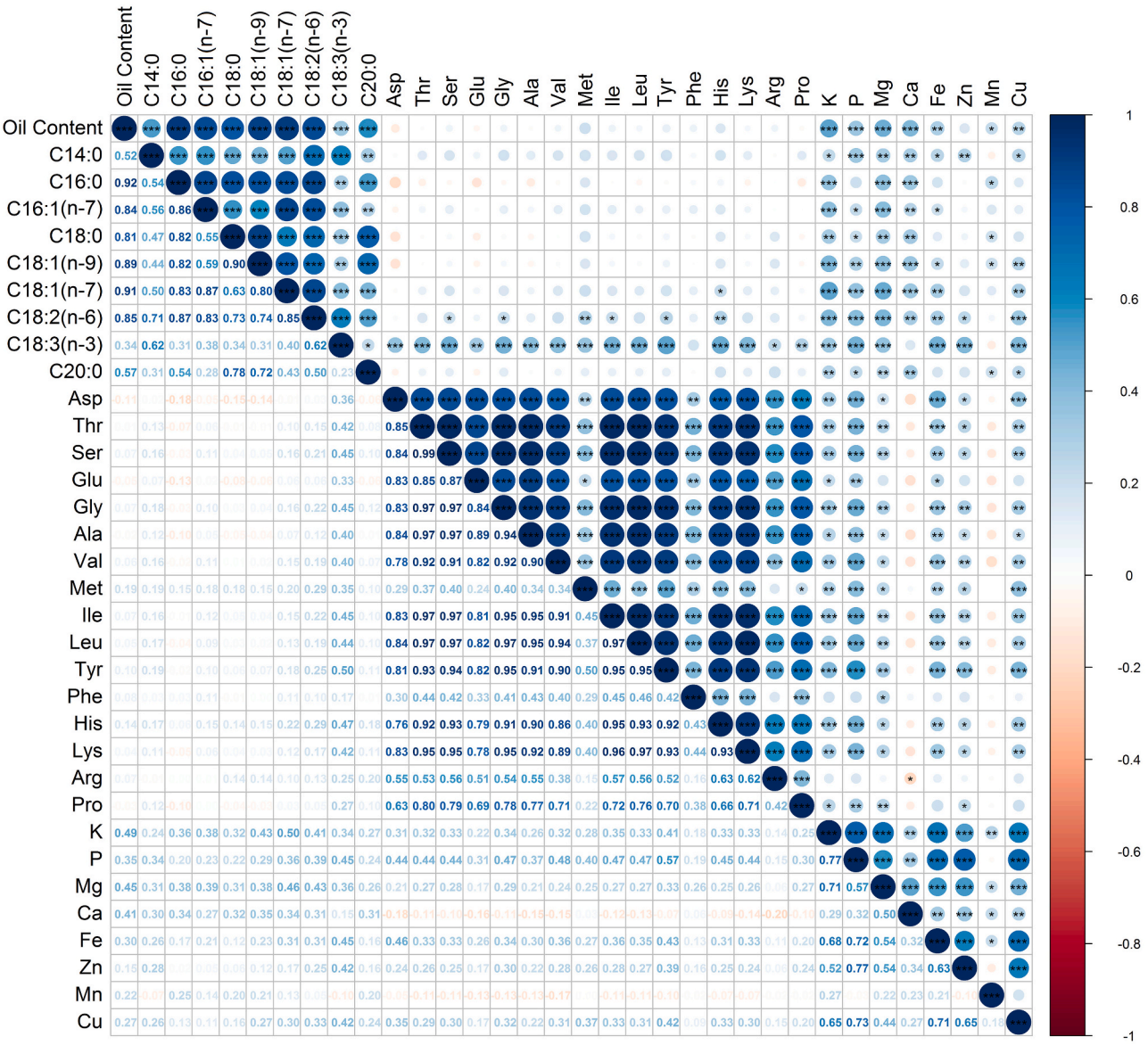

Fig. 5. Correlation heatmap among oil content, fatty acid, hydrolyzed amino acid, and mineral element in avocado fruit. The numbers in the lower half of the figure represent the pearson correlation coefficient. '*', '**', and '***' in the upper half of the figure represent *P* value <0.05, 0.01, and 0.001, respectively. Red indicates negative correlation, and blue indicates positive correlation. The depth of the color indicates the strength of the correlation coefficient. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

which may be the reason for the differences in fatty acid components reported by different studies.

In this work, although the 95 avocado germplasms had differences in the proportion of each fatty acid, the proportion of UFAs mainly composed of C18:1(n-9) and C18:2(n-6) was higher than that of SFAs mainly composed of C16:0 in all avocado germplasms, which is consistent with the results of previous studies (Ford et al., 2023). It has been reported that the stearoyl-ACP desaturase (SAD) is responsible for converting C18:0 into C18:1(n-9), and the fatty acid desaturase 6 (FAD6) can convert C18:1(n-9) into C18:2(n-6) (Yang et al., 2024). They were both key genes for the biosynthesis of UFAs in avocado fruit (Yang et al., 2024). SAD and FAD6 might be highly expressed in all avocado germplasms, resulting in the dominance of UFAs. In addition, 95 avocado germplasms could be divided into three clusters (FA-I, FA-II, and FA-III) based on different proportions of C16:0 and C18:1(n-9). It has been demonstrated that two key enzymes KAS II and SAD are required for the conversion of C16:0 to C18:1(n-9), which are responsible for the conversion of C16:0 to C18:0 and C18:0 to C18:1(n-9), respectively (Pedreschi et al., 2019). The different expression levels of KAS II and SAD genes in clusters FA-I, II, and III might account for the different proportions of C16:0 and C18:1(n-9) among different clusters. Fatty acid composition is an important indicator for evaluating the nutrition of avocado. In the future, it is necessary to further clarify the biosynthesis and regulatory pathways of unsaturated fatty acids in avocado fruit through transcriptome, genome, molecular biology and other means for the genetic improvement of oil content and unsaturated fatty acids in avocado.

Protein and mineral elements are also important components of avocado quality. In this study, we quantified the 16 amino acids that make up protein by hydrochloric acid hydrolysis. We found that Asp and Glu are the main components in most avocado fruit proteins, and Met is the least abundant, which is consistent with previous studies (Rozan et al., 2021). It has been reported that Trp is completely destroyed during acid hydrolysis and Cys cannot be directly determined by acid hydrolysis method (Fountoulakis & Lahm, 1998). According to the data of USDA Food Data Central, the contents of both Trp and Cys in avocado are very

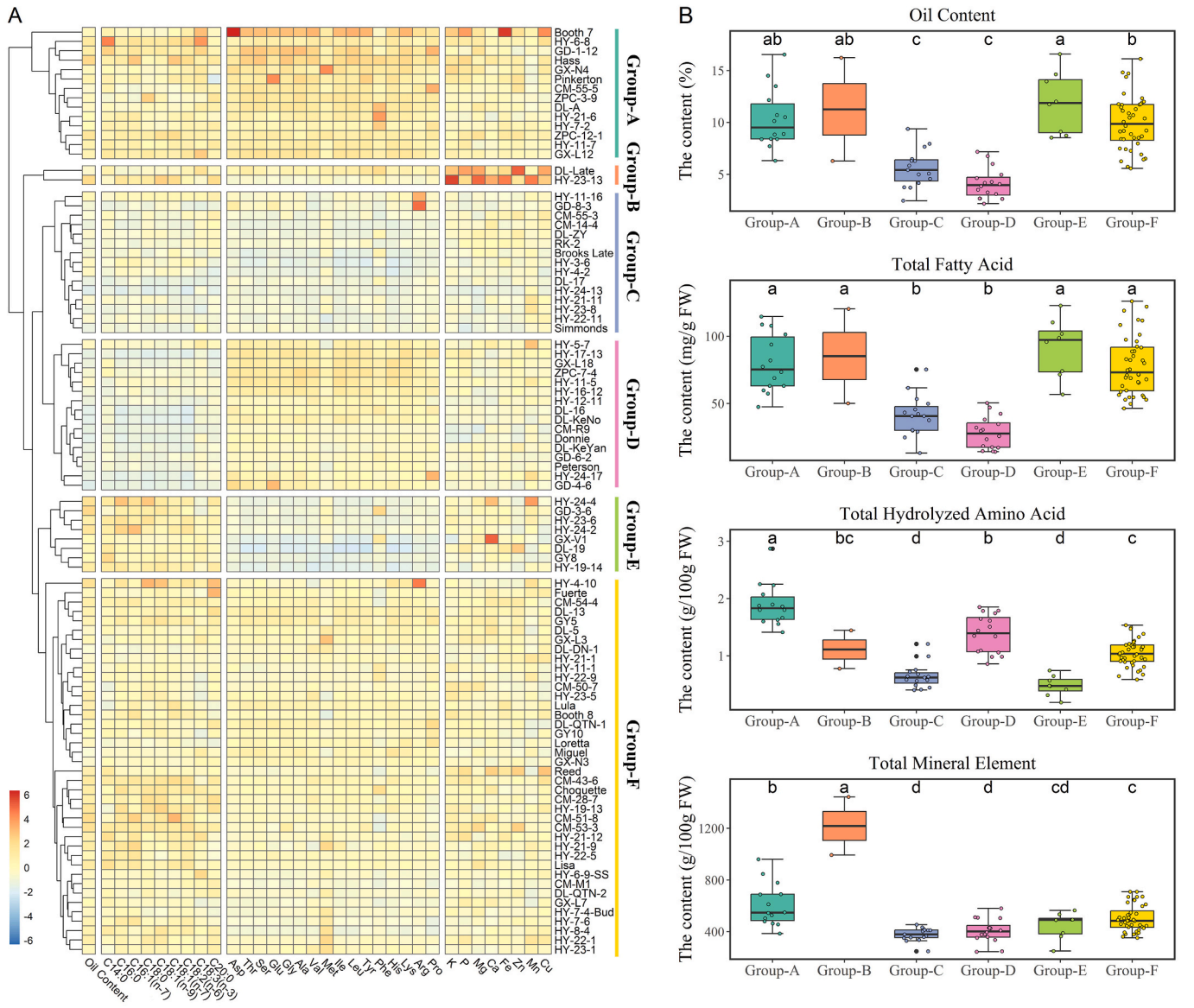

Fig. 6. Clustering and intergroup comparison of 95 avocado germplasms. A, clustering of 95 avocado germplasms based on fruit oil, fatty acid, hydrolyzed amino acid, and mineral element contents. The 95 avocado germplasms were divided into six groups, namely Group-A, Group-B, Group-C, Group-D, Group-E and Group-F. B, multiple comparisons of fruit oil, total fatty acid, total hydrolyzed amino acid, and total mineral element contents among groups. FW, fresh weight.

low, and even lower than that of Met. Hence, this work did not quantify these two amino acids. In addition, since Asn and Gln can be converted into corresponding acidic amino acids under acid hydrolysis conditions (Fountoulakis & Lahm, 1998), these two amino acids cannot be detected by acid hydrolysis. The contents of Asn and Gln in this study were actually included the contents of Asp and Glu, respectively, which is consistent with the results of Rozan et al. (2021) and USDA Food Data Central. In this work, the total content of hydrolyzed amino acids in 95 avocado germplasms (0.19 %–2.87 %) was close to the protein content in avocado (1 %–3 %) reported by previous studies (Araújo et al., 2018). Therefore, the 16 amino acids detected in this study could approximately represent the protein content in avocado fruit. Essential amino acids are also nutrients that are beneficial to human health. Among the 95 germplasms, although some germplasms with high essential amino acid contents were screened, the content did not exceed that of the commercial variety 'Hass'. The content and proportion of essential amino acids affect the nutritional value and commercial value of avocado fruit, and genetic improvement of essential amino acids is an important part of the avocado breeding program. In this study, we found that the average K content of 95 avocado germplasms reached 419.53 mg/100 g, which is close to the data from the USDA Food Data Central and the values reported by Hardisson et al. (2001). Banana has always been considered as a good source of K, whose K content reaches up to 358 mg/100 g (Ranjha et al., 2022; Sidhu & Zafar, 2018). In this work, over 60 % of the avocado germplasms were found to have a higher K content than banana. Therefore, avocado can also serve as a functional food to supplement K.

In this study, the dry matter content of avocado was significantly positively correlated with the oil content, and the fatty acid components were also significantly positively correlated with each other, which is in agreement with the results reported by previous studies (Carvalho et al., 2014; Vergara-Pulgar et al., 2019). Olive is another fruit that can accumulate oil in its flesh, and its dry matter content is also significantly positively correlated with its oil content (Fahadi Hoveizeh et al., 2023). However, in olive, C18:1(n-9) was negatively correlated with C18:2(n-6) and C16:0, and C16:0 was positively correlated with C16:1(n-7) (Dabbou et al., 2012). There are differences in the accumulation mechanism of fatty acids in flesh between avocado and olive. There were

significant positive correlations among the hydrolyzed amino acids in avocado, as well as among mineral elements such as K, P, Ca and Fe. The same pattern was also found in the study of hydrolyzed amino acids and mineral elements in jujube (Zhao et al., 2023), pear (Liu et al., 2023), and hazelnut (Yaman et al., 2023), suggesting that the accumulation mechanism of hydrolyzed amino acids and mineral elements may be similar between different species. In addition, we also found significant positive correlations between fatty acids and macronutrients, and between some mineral elements (K, P, Fe, and Cu) and amino acid components. In order to breed avocado varieties with high nutritional value, it is worth of further exploring the reasons for the positive correlations between different types of nutrients from the molecular and metabolic perspectives.

The methods for breeding new varieties of fruit trees mainly include cross breeding, mutation selection, seedling selection, radiation breeding, and molecular marker-assisted breeding. In avocado, artificial cross breeding is limited by the extremely small flowers and severe flower and fruit drop, and seedling selection of open-pollinated and controlled-pollinated offspring is the most widely used breeding technique in avocado (Schaffer et al., 2013). During the long cultivation in China, a large number of avocado germplasms have been generated through natural hybridization due to protogynous diurnally synchronous dichogamy in avocado. Thus, we also carried out seedling selection to screen high-quality avocado germplasms in this study. Through evaluation of the nutritional quality of 95 avocado germplasms grown in China, 11 high-quality local avocado germplasms were screened, including 'HY-6-8', 'GD-1-12', 'GX-N4', 'CM-55-5', 'ZPC-3-9', 'DL-A', 'HY-21-6′, 'HY-7-2′, 'ZPC-12-1′, 'HY-11-7′, and 'GX-L12'. These germplasms are close to or exceed 'Hass', the most representative commercial variety with high nutritional quality, in terms of dry matter, oil, fatty acids, hydrolyzed amino acids, and mineral elements. In future work, we will further evaluate the stability of the fruit nutritional quality of the 11 selected local avocado germplasms under different cultivation time and conditions, assess the yield, disease resistance, and abiotic stress tolerance of the above germplasms, and breed high-yield and high-quality avocado varieties suitable for growth in China.

5. Conclusion

Overall, we characterized the nutritional quality of 95 avocado germplasms grown in China by metabolomics. The results showed that the oil content ranged from 2.18 % to 16.60 %, and varied widely among different avocado germplasms. The 95 avocado germplasms could be divided into three clusters based on the proportions of nine fatty acids, where the proportion of UFAs mainly composed of C18:1(n-9) and C18:2 (n-6) was significantly higher than that of SFAs mainly composed of C16:0 in all germplasms. Furthermore, 16 hydrolyzed amino acids were detected in avocado and the content and proportion of each amino acid were different in different germplasms. We also investigated the distribution of eight mineral elements, including K, P, Mg, Ca, Fe, Zn, Mn and Cu, and found that K had the highest content while Cu had the lowest content. Finally, through evaluation of nutritional quality of different avocado germplasms, the 95 germplasms were divided into five groups and the 14 germplasms in Group-A showed higher nutritional value and were considered as high-quality germplasms. Collectively, the nutritional quality evaluation of avocado at the germplasm level contributes to the screening of germplasms with higher nutritional value and accelerates the breeding of high-quality avocado.

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CRediT authorship contribution statement

Hongbin Yang: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Funding acquisition, Formal analysis, Data curation. **Fuqiang Wang:** Methodology, Investigation. **Yingqin Li:** Methodology, Investigation. **Yake Guo:** Methodology, Investigation. **Xiuhua Tang:** Resources, Investigation. **Shuailei Gu:** Resources, Investigation. **Haihong Chen:** Resources, Investigation. **Chaohai Pang:** Methodology, Data curation. **Yanxia Li:** Data curation. **Jiali Zhang:** Data curation. **Weihong Ma:** Resources, Methodology, Investigation, Data curation. **Jiashui Wang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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