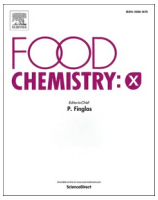
Food Chemistry: X 23 (2024) 101766

Contents lists available at ScienceDirect

Food Chemistry: X

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



Characterization of volatile aroma compounds in pak choi

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ARTICLE INFO

Keywords:
Pak choi
Volatiles
Aroma
Untargeted metabolome profiling

ABSTRACT

Aroma is a key sensory factor in the flavor evaluation of pak choi (*Brassica rapa* L. ssp. *chinensis* var. Makino). The pak choi varieties Xiangqingcai (XQC) and Xiuhuajin (XHJ) have unique aroma characteristics, but the chemical profiles of these aromas are unknown. Here, the aroma profiles of three varieties of pak choi including XQC, XHJ, and Suzhouqing (CK, non-aromatic) were determined using gas chromatography–mass spectrometry (GC–MS) and relative odor activity values (rOAV). A total of 15 categories of 716 volatile metabolites were detected in the three pak choi varieties, with terpenoid metabolites identified as the major components, although in each sample the identity of the major terpenoid metabolite varied. There were 53 aroma components in XQC and 54 aroma components in XHJ with rOAV >1, which contribute to rice aroma and fishy odor of these varieties, respectively.

1. Introduction

Pak choi (*Brassica rapa* L. ssp. *chinensis* var. Makino) belongs to the *Brassica* family of vegetables in the cruciferous family (Dong et al., 2022) and is one of the most important leafy vegetables grown in China. There are many varieties of pak choi, but the fragrances released by different varieties are highly variable. For example, variety Xiangqingcai (XQC) releases a strong fragrance of rice, while Xiuhuajin (XHJ) has a strong fishy odor. While aroma is considered one of the most important attributes affecting the sensory experience of consumers, the chemical cause of the differing aroma characteristics among pak choi varieties is unclear.

Volatile components are either primary or secondary metabolites of pak choi, and can significantly affect the taste and flavor. Wei et al. (2021) analyzed the volatile metabolites of green and purple cabbage, discovering that they volatile metabolites included 75 of hydrocarbon, ester, nitrile, alcohol, aldehyde, thiocyanate, ether, and thiazole. Breme et al. (2009) showed that the volatile extracts of two wild cabbage species gave a strong boiled potato flavor with green, spicy, herbaceous, nutty, and sulfurous notes. Baky et al. (2022) identified 190 metabolites from the leaves of six *Brassica* vegetables, namely cauliflower, turnip, broccoli, watercress, radish and cabbage, that included aldehydes, alcohols, esters, sulfur compounds, nitrogen compounds, acids, and terpenoids. Notably, aldehydes and ketones comprised up to 27 % of the relative volatile metabolite content. Seven classes of volatile metabolites were detected in fresh kale, including aldehydes, alcohols, sulfur compounds, terpenoids, esters, furans, and ketones (Rajkumar et al., 2017), with another study in 2021 identifying 25 substances, where sulfur-containing compounds were most abundant (Oh & Cho In, 2021). In 2019, Xu revealed that aldehydes, alcohols, and esters were the main volatile flavor components of non-aromatic pak choi, and Lv (2019) studied the aroma composition of XJH, finding that the primary aromatic substances were 2,6,6-trimethyl-1- cyclohexene-1-acetaldehyde, 2-bromomethyl-1-isopropenyl-3-methylcyclopentane, glutaric acid, and 4-methoxyphenyl butyl ester. Although several volatile components of the *Brassica* family of vegetables have been characterized, it has been shown that only a small proportion of these compounds contribute to the overall aroma profile and the aroma characteristics of pak choi have not been fully elucidated.

Metabolomics is a method that can analyze the chemical characteristics of samples in an undifferentiated and comprehensive way. It has been widely applied in the aroma analysis of vegetables and fruits because of its rapidity and high efficiency (Farag et al., 2021; Khalil et al., 2017). HS-SPME (headspace solid–phase microextraction) is a technique characterized by simple operation, a low monitoring threshold, good reproducibility, good sensitivity, and it is solvent-free (Abdulra'uf & Tan, 2015). In addition, when combined with gas chromatography–mass spectrometry (GC–MS) detection, it can be used to

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https://doi.org/10.1016/j.fochx.2024.101766
Received 13 May 2024; Received in revised form 7 August 2024; Accepted 22 August 2024

analyze a variety of volatile metabolites concurrently, leading to more accurate data (Jelen et al., 2012). Using HS-SPME combined with GC–MS, Yang et al. (2021) detected a total of 70 volatile components in the pulp of 85 apple varieties, including esters, alcohols, aldehydes, acids, ketones and other compounds, and Lv et al. (2022) detected a total of 57 aroma substances in five strains of kiwi fruit, including aldehydes, alcohols, alkanes, esters and terpenes.

Although some investigation has been undertaken into the aroma of *Brassica* vegetables, systematic studies to identify key aroma compounds in pak choi are lacking, and the aroma characteristics of different pak choi varieties remain unclear. The aim of this study was to identify the volatile aroma compounds in different varieties of pak choi, and to differentiate between their volatile chemical constituents and aromatic properties.

2. Materials and methods

2.1. Materials

Three pak choi varieties, Suzhouqing (CK), Xiangqingcai (XQC), and Xiuhuajin (XHJ), were collected from Jiangsu Province, China. The three varieties were all planted in the experimental garden at Shenyang Agricultural University, with identical cultivation and management techniques. Samples were taken from the fourth and fifth leaves as well as the petioles, which were cleaned and wiped. Six replicates were used for each variety. Samples were stored at −80 °C.

2.2. Instruments

An Agilent GC–MS/MS (8890-7000D) was equipped with a column (DB-5MS, 30 m × 0.25 mm × 0.25 μm) and extraction head. A Retsch ball mill (MM400) was used during sample preparation.

2.3. Sample treatment

A sample of powdered leaf (0.3 g) ground in liquid nitrogen was immediately transferred to a headspace vial (10 mL; Agilent, Palo Alto, CA, USA) containing saturated NaCl solution. Vials were sealed using crimp-top caps with TFE–silicone headspace septa (Agilent). At the time of SPME analysis, each vial was placed in a 60 °C oven for 5 min, then a 120-μm DVB/CWR/PDMS fiber (Agilent) was exposed to the headspace of the sample for 15 min at 100 °C.

2.4. GC–MS conditions

After sampling, desorption of the volatile organic compounds (VOCs) from the fiber coating was undertaken in the injection port of the GC apparatus (Model 8890; Agilent) by heating the fiber at 250 °C for 5 min in splitless mode. Identification and quantification of VOCs was used an Agilent Model 8890 GC and a 7000D mass spectrometer (Agilent), equipped with a 30 m × 0.25 mm × 0.25 μm DB-5MS (5 % phenylpolymethylsiloxane) capillary column. Helium was used as the carrier gas at a linear velocity of 1.2 mL/min. The injector temperature was kept at 250 °C and the detector at 280 °C. The oven temperature program started from 40 °C (3.5 min), increasing at 10 °C/min to 100 °C, at 7 °C/min to 180 °C, then at 25 °C/min to 280 °C, where the temperature was held for 5 min. Mass spectra were recorded in electron impact (EI) mode at 70 eV. The quadrupole mass detector, ion source and transfer line temperatures were set, respectively, at 150, 230 and 280 °C.

2.5. Qualitative and quantitative analysis of volatile metabolites

Retention time of the detected peaks was compared with standard references according to the MWDB (Metware Database). RI (rentention index) values were determined using *n*-alkane mixtures (C_7–C_{40}). Selected ion monitoring (SIM) mode was used for the identification and quantification of volatile metabolites. Each metabolite was cross-referenced with one quantitative ion and two or three qualitative ions. The quantitative ions were used to perform integration of the chromatographic peaks and for correction work to enhance the accuracy of quantification (Lin et al., 2024; Tao et al., 2024). The relative content of each metabolite in the three pak choi varieties was calculated by the internal standard method, using the formula: Relative content of each metabolite ωx (mg/kg) = $(A_x/A_s)(V_s{\cdot}C_s/M){\cdot}1000$ where A_x and A_s were the peak areas of the detected fractions and the internal standard, respectively; M was the amount of the sample (g); V_s was the volume of the internal standard (mL); and C_s was the concentration of the internal standard (μg/mL).

2.6. Relative odor activity value (rOAV)

The rOAV is the ratio between a specific aroma component and the threshold value of the aroma component (the odor threshold) provided in the relevant literature, It was calculated using the formula: rOAV = ωx/Tx where Tx is the threshold value of the aroma component (mg/kg) (Fang et al., 2024; Huang et al., 2022).

2.7. Analysis of differential metabolites

Significantly differential metabolites between groups were identified via the criteria VIP (variable importance in projection) ≥ 1, $|\log_2 \text{fold-change}| \geq 1$ and $p < 0.05$. VIP values were extracted from OPLS-DA (orthogonal partial least squares discriminant analysis) results using MetaboAnalystR (version 4.0). Data were preprocessed using a logarithmic transform ($\log_2$) and mean centering, before OPLS-DA. To avoid overfitting, a 200-time permutation test was performed.

2.8. Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis

KEGG pathway enrichment analysis was performed based on the results for differential metabolites, where the Rich Factor was the ratio of the number of differentially expressed metabolites in the corresponding pathway to the total number of metabolites annotated to that pathway detection; a higher value indicates a greater degree of enrichment. The *p*-value used was the *p*-value of the hypergeometric test. The formula for calculation of the hypergeometric distribution was:

$$P = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

where N represents the number of metabolites with KEGG annotation among all metabolites, n represents the number of differential metabolites in N, M represents the number of metabolites of a KEGG pathway in N, and m represents the number of differential metabolites of a KEGG pathway in M. The closer the *p*-value is to 0, the more significant the enrichment.

2.9. Statistical analysis

Unsupervised principal component analysis (PCA) was performed by using the statistics function prcomp in RStudio. The data were unit variance scaled before PCA. Excel 2016 was used for statistical analysis and charting of data. SPSS version 20.0 was employed for analyzing data using Duncan's multiple range tests of variance ($p < 0.05$) and significance test.

3. Results

3.1. Analysis of total volatile metabolite species and relative content in three pak choi varieties

Analysis of the total volatile metabolite compounds and relative content in three pak choi varieties revealed 15 classes of volatile metabolites: terpenes, heterocycles, esters, aldehydes, alcohols, ketones, hydrocarbons, aromatics, phenols, halogenated hydrocarbons, acids, nitrogen-containing metabolites, sulfur-containing metabolites, amines, and others (Fig. 1). In CK, 716 volatile compounds were detected (Supplementary Table 1), in XQC 714 volatile compounds were detected, and in XHJ 715 volatile compounds were detected. In all three cases, terpenoids, esters and heterocyclic compounds were dominant species, accounting for about 20 %, 15 %, and 14 % of the total volatile metabolites, respectively (Fig. 1).

3.2. Principal component analysis (PCA)

Multivariate data analysis was performed using PCA of the GC–MS dataset. The three pak choi varieties formed the corresponding confidence intervals, and the six biological replicates for each species clustered together (Fig. 2), indicating good experimental reproducibility and high reliability. The total contribution of principal components of CK and XQC was 59.59 % (PC1 = 38.7 %, PC2 = 20.89 %; Fig. 2A), with CK mainly distributed in quadrants one and two, and XQC mainly distributed in quadrants three and four. The only overlap was near the origin. The total contribution of principal components of CK and XHJ was 65.45 % (PC1 = 42.61 %, PC2 = 23.84 %; Fig. 2B), with CK mainly distributed in quadrants two and three, and XHJ mainly distributed in quadrants one and four, showing a significant difference between the main volatile metabolites of CK and XHJ. The total contribution of principal components of XQC and XHJ was 69.32 % (PC1 = 40.07 %, PC2 = 29.25 %; Fig. 2C), with XHJ mainly distributed in quadrants one and four, and XQC mainly distributed in quadrants two and three. This shows that there were differences in volatile metabolites between CK and XHJ, CK and XQC, and XQC and XHJ.

3.3. Analysis of differential volatile metabolites

The R^2Y and Q^2 values for each OPLS-DA model were > 0.9, which indicated that there were no over-fitted models, and the OPLS-DA models had good discriminatory ability for the samples in this study. Differential metabolites were screened via the criteria VIP ≥ 1, $|\log_2$ fold-change$| \geq 1$, and $p < 0.05$. Among the 716 total metabolites extracted from the three types of pak choi, 141 differential metabolites were identified in three groups: CK vs XQC, CK vs XHJ, and XQC vs XHJ. This included 13 compound classes and 42 terpenoids, which accounted for 30 % of the total. A total of 71 differential metabolites were identified in CK vs XQC (Supplementary Table 2), of which 62 were upregulated and nine were downregulated. Three terpenoids were in the top ten upregulated substances, namely (1H-cyclopropa[*a*]naphthalene,1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,[1aR-(1a.*α*.,7.*α*.,7a.*α*.,7b.*α*.)]-; p-mentha-1,5,8-triene and (1R,9R,E)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene). The down-regulated terpenes included humulene and bicyclo[2.2.1]heptane,2-methyl-3-methylene-2-(4-methyl-3-pentenyl)-, (1S-endo)- (Fig. 3A) ; A total of 91 differentially metabolized substances were identified in CK vs XHJ (Supplementary Table 3), of which 81 were upregulated and 10 were downregulated. Among the top 10 upregulated substances were five terpenoids, namely (1H-cyclopropa[*a*]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,[1aR-(1a.*α*.,7.*α*.,7a.*α*.,7b.*α*.)]-; (1R,9R,E)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene; L-fenchone; bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-; and 3-buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-,). Down-regulated substances included bicyclo[2.2.1]heptane, 2-chloro-1,7,7-trimethyl-, (1R-endo)- and cyclohexanol, 1-methyl-4-(1-methylethyl)-, cis- (Fig. 3B). A total of 84 differential metabolites were identified in XQC vs XHJ (Supplementary Table 4), of which 57 were upregulated and 27 were downregulated. Among the top 10 upregulated substances were four terpenoids, namely (bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-; L-fenchone; 3-buten-2 -one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- and (1R,9R,E)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene,). The top 10 downgraded terpenes included ((−)-.*β*.-bourbonene; bicyclo[2.2.1]heptane, 2-chloro-1,7,7-trimethyl-, (1R-endo)- and cyclohexanol, 1-methyl-4-(1-methylethyl)-, cis-) (Fig. 3C).

3.4. Analysis of aroma substances of three pak choi varieties

The significance of aroma depends on the quantity of volatile metabolites in a sample and the odor threshold of that metabolites. rOAVs are commonly used to assess the contribution of a volatile component to aroma. In the three pak choi varieties tested, a total of 55 characteristic volatile metabolites had rOAV>1 in at least one sample (Table 1), which therefore contributed to the overall aroma. Of these, terpenes were the most prevalent with 14 different species identified, followed by 12 esters, eight heterocyclic compounds, seven aldehydes, five alcohols, three ketones, and two each of sulfur-containing metabolites, phenols, and aromatic hydrocarbons.

There were 53 characteristic aroma substances in XQC, and nine substances with rOAV >10,000, namely: 2-methylisoborneol; butanoic acid, 3-methyl-, hexyl ester; 3-mercaptohexyl acetate; 6-nonenal, (*Z*)-; 2-thiophenemethanethiol; 2(5H)-furanone, 5-ethyl-3-hydroxy-4-methyl-; pyridine, 2-pentyl-; 3-methyl-2-butene-1-thiol; 3-cyclohexene-1-methanethiol, .α.,.α.,4-trimethyl-. Compared with CK, 15 substances in XQC showed significant differences in rOAV. 2-methylisoborneol; 1H-3a,7-methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, (3*R*,3a*S*,7*S*,8a*R*)-; 1H-3a,7- methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3*R*-(3.*α*.,3a.*β*.,7.*β*.,8a.*α*.)]-; 2-propenoic acid, 3-phenyl-, ethyl ester; 2,4-decadienal, (*E*,*E*)-; 4-heptenal, (*Z*)-; 2(5H)-furanone, 5-ethyl-3-hydroxy-4-methyl-; phenylethyl alcohol; butanoic acid, ethyl ester; *trans*-.*β*.-ionone; butanoic acid, 3-methyl-, hexyl ester; 2-furanmethanol, 5-ethenyltetrahydro-.*α*.,.*α*.,5-trimethyl-, *cis*-; 3-methyl-2-butene-1-thiol 3-cyclohexene-1-methanethiol,.*α*.,.*α*.,4-trimethyl-; phenol, 2-

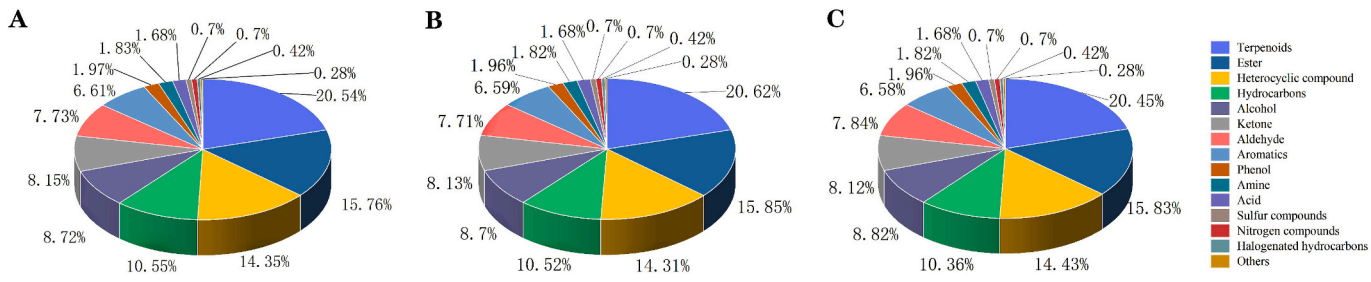

Fig. 1. Summary of volatile species in three pak choi varieties. (A) Volatile compounds in variety Suzhouqing (CK); (B) volatile compounds in variety xiangqingcai (XQC); (C) volatile compounds in variety xiuhuajin (XHJ).

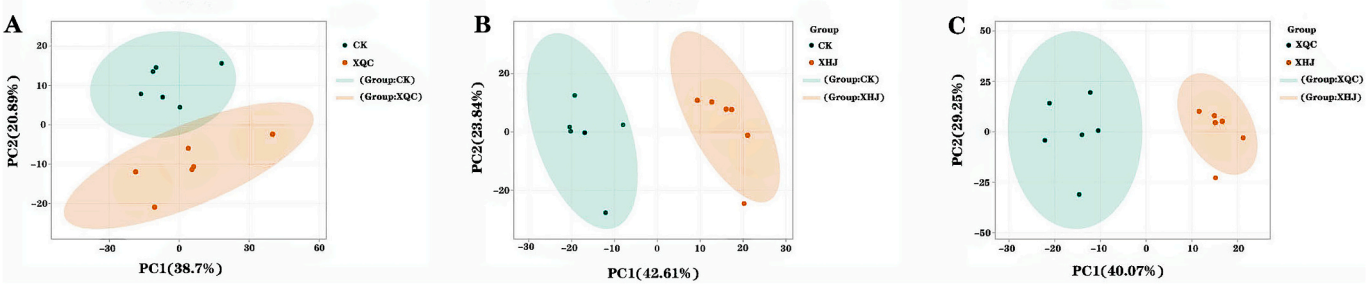

Fig. 2. Principal component analysis of three pak choi varieties. (A) CK vs XQC PCA score; (B) CK vs XHJ PCA score; (C) XQC vs XHJ PCA score.

nitro-; and 3-cyclohexene-1-methanethiol,.*α*.,.*α*.,4-trimethyl) and 15 substances showed significant differences in rOAV. Finally, in XQC, three dominant substances were identified: 5-heptenal, 2,6-dimethyl-; carvenone, and 2-hexanoylfuran, with relative contents of 15.88 mg/kg, 14.39 mg/kg, and 14.37 mg/kg, respectively (Supplementary Table 5).

There were 54 characteristic aroma substances in XHJ, among which the rOAV value of 3-mercaptohexanol was >1000; it was not detected in the other two kinds of pak choi. The rOAV for 2-propenoic acid, 3-phenyl-, ethyl ester was 11,007.64, which was significantly higher than in the other two pak choi varieties. In XHJ, there were 11 substances with rOAV >10,000, (2-methylisoborneol; *trans*-.*β*.-ionone; butanoic acid, 3-methyl-, hexyl ester; 3-mercaptohexyl acetate; 2-propenoic acid, 3-phenyl-, ethyl ester; 6-nonenal, (*Z*)-; 2- thiophenemethanethiol; 2(5H)-furanone, 5-ethyl-3-hydroxy-4-methyl-; pyridine, 2-pentyl-; 3-methyl-2-butene-1-thiol; and 3-cyclohexene-1-methanethiol, .*α*., .*α*.,4-trimethyl-). There were seven substances that differed in terms of rOAV when compared with the other two pak choi varieties: benzeneacetaldehyde, 4-methyl-; *trans*-.*β*.-ionone; 3-mercaptohexyl acetate; 2-propenoic acid, 3-phenyl-, ethyl ester; 2,4-decadienal, (*E*,*E*)-; 3-hexen-1-ol, acetate, (*Z*)-; and 3- cyclohexene-1-methanethiol, .α., .α.,4-trimethyl-). The relative content of (1*R*,9*R*,*E*)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene in XHJ was 171.40 mg/kg (Supplementary Table 6). All these aroma substances likely contribute significantly to the overall odor of XHJ.

3.5. Differential metabolite KEGG enrichment analysis

Mapping the three sets of differential metabolites to the KEGG database revealed that a total of 10 of the 71 differential metabolites in CK vs XQC arose from nine metabolic pathways, of which only one showed downregulation. A total of nine of the 91 differential metabolites in CK vs XHJ arose from eight metabolic pathways. Seven of the 84 differential metabolites in XQC vs XHJ were attributed to eight metabolic pathways, where five showed upregulation and two showed downregulation. The three comparative groups of differential metabolites were mainly from enriched metabolic pathways, such as the biosynthesis of various secondary metabolites, the biosynthesis of the secondary metabolite limonene, the pinene degradation pathway, the metabolic pathway of alpha-linolenic acid, and the biosynthesis of sesquiterpenoids and triterpenoids (Supplementary Table 7).

To determine the specific differences in metabolic pathways between the different pak choi varieties, enrichment analysis of differential metabolic pathways was performed. The differential metabolites were significantly enriched in the phenylalanine metabolic pathway when compared to CK (Fig. 4A). Significant enrichment of differential metabolites from the phenylalanine metabolic pathway was observed in bubble plots of CK vs XQC and CK vs XHJ (Fig. 4B). The metabolic pathways that were most significantly different for XQC vs XHJ were the sesquiterpene and triterpenoid biosynthesis pathways (Fig. 4C).

4. Discussion

4.1. Terpenoids are the main and main differential volatile metabolites in pak choi

Terpenoids are the main volatile metabolites in the three pak choi varieties investigated. Because consumer acceptance of fresh pak choi is, besides textural attributes, highly related to its volatile attributes, it is important to include pak choi aroma when studying its quality (Janssens et al., 2022). Previous studies identified that the main volatile compounds in the leaves of cabbage, broccoli, cauliflower, turnip, radish, and watercress were aldehydes, alcohols, esters, ketones, sulfur compounds, terpenes, furans, and miscellaneous compounds (Baky et al., 2022; Lončarić et al., 2020; Rajkumar et al., 2017), but the main volatiles in kale were sulfur compounds (Samec et al., 2018). The main volatile substances of Chinese cabbage, flowering Chinese cabbage, and cauliflower were found to be alcohols esters and sulfur compounds (Valette et al., 2003; Wu et al., 2009; Yuan et al., 2019). But in the present study, terpenoids accounted for 20.5 % of the total volatile components, which is in contrast to results from previous studies on volatile metabolites in *Brassicaceae* vegetables. Key differences in the volatile substances present in the three pak choi varieties were further probed using PCA. It was noted that the dominant family of volatile species was the same in each variety of pak choi, but the exact compound identity differed. Other substances present included esters, heterocyclic compounds and ketones. We suggest that the difference in terpene identity between the three pak choi varieties is what contributes to their differing aromas.

4.2. The aroma substances in pak choi

The aroma of pak choi is a key feature of its quality and an important trait for breeding. At present, reports on the aromatic substances present pak choi are limited. For example, Wang et al. (2022) detected 26 volatile compounds in XQC and identified β-caryophyllene, which has a clove-like fragrance, as the main aroma substance. In our study, we identified 52 aroma substances that contributed to the overall aroma of XQC, which has a strong rice flavor, with terpenes and esters being the main aromatic components. In terms of rice fragrance, 2-pentyl-furan has been shown to contribute to the key aroma of Hainan rice (Jia et al., 2022), and it is also the main aroma substances of roasted rice tea (Zhao et al., 2016). Butanoic acid, ethyl ester was present in greater quantities in XQC than in the other two kinds of pak choi studied, and it has flavors of pineapple and brandy wine. It is also a key aroma substance for brown rice and glutinous rice wine (Su, 2020), white wine (Shen, 2003) and strawberry (Fu et al., 2021). 2-Propenoic acid, 3-phenyl-, ethyl ester and butanoic acid, ethyl ester are both key aroma components in strawberries (Wang et al., 2021). *trans*-.β.-Ionone has a strong aromatic flavor and is the main aroma component in *Chaenomeles* (Meng et al., 2007). Cheng (2020) detected trace quantities of 3-cyclohexene-1-methanethiol, .α.,.α.,4-trimethyl- in mandarins. These substances are believed to constitute the rice aroma and green scent of XQC

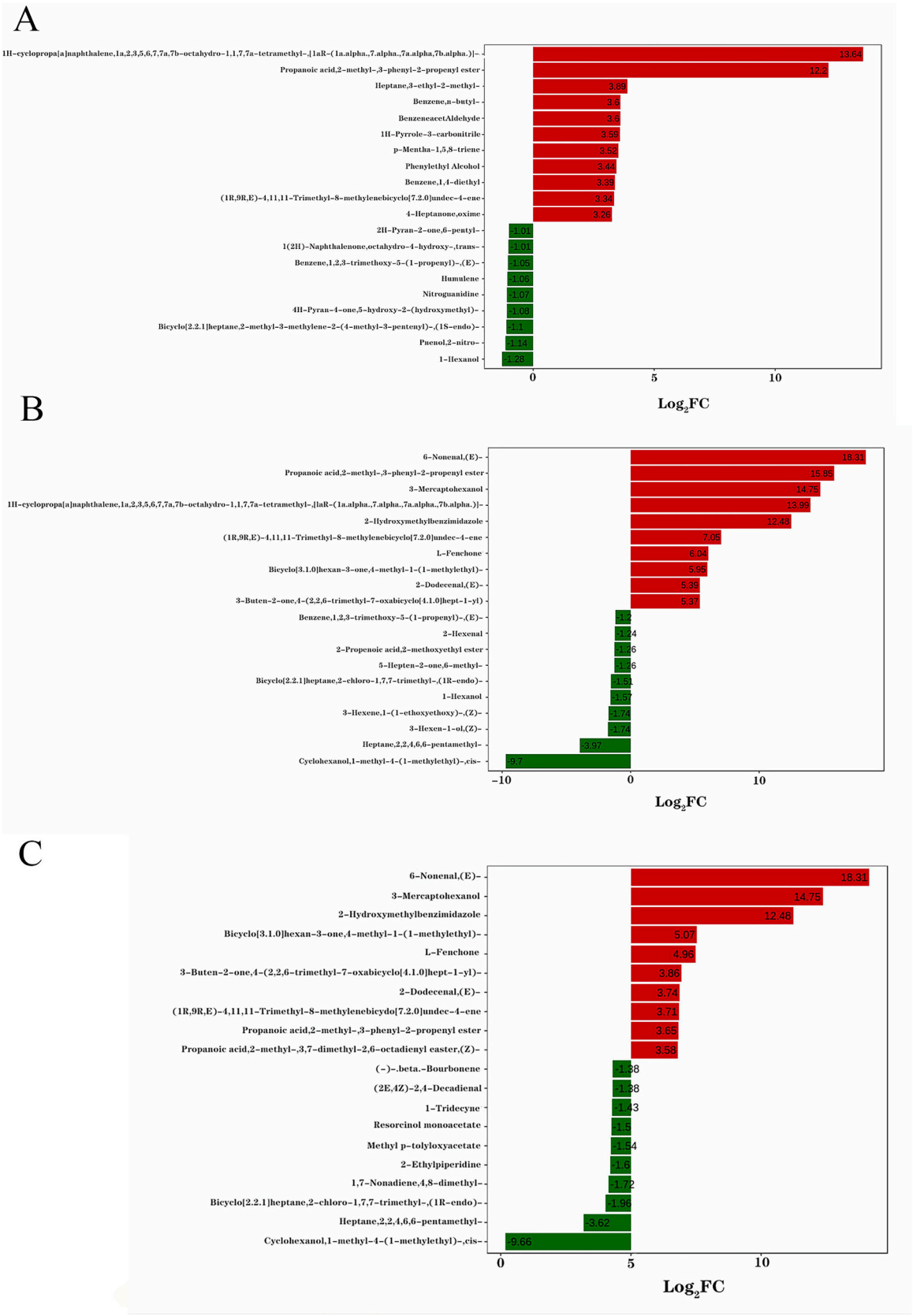

Fig. 3. Top 10 differential volatile metabolites in pak choi varieties **(A)** CK vs XQC; **(B)** CK vs XHJ; **(C)** XQC vs XHJ.

Table 1
Volatile compounds with rOAVs (>1) in three varieties of pak choi.

Group No.	Volatile compounds	Odor description	CAS	Threshold (mg/kg)	Relative odor activity values (rOAVs)		
					XQC	XHJ	CK
Terpenoids							
1	2-Methylisoborneol	earthy musty	2371-42-8	0.0000039	439,241.98	447,862.16	431,450.39
2	*trans*-.*β*.-Ionone	dry powdery floral woody orris	79–77-6	0.001	7229.13	11,562.16	8061.64
3	Linalool	citrus floral sweet bois de rose woody green blueberry	78–70-6	0.0015	1360.89	1428.87	1384.21
4	Terpinen-4-ol	pepper woody earth musty sweet	562–74-3	0.0002	531.20	543.90	474.33
5	Geraniol	sweet floral fruity rose waxy citrus	106–24-1	0.0075	185.29	199.27	187.16
6	Phenol,2-methyl-5-(1-methylethyl)-	spice woody camphor thymol	499–75-2	0.0001	129.25	143.70	140.31
7	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	woody pine balsam sweet mint medical	515–00-4	0.001	105.85	109.23	105.51
8	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3*R*-(3.*α*.,3a.*β*.,7.*β*.,8a.*α*.)]-	woody cedar sweet fresh	469–61-4	0.00003	74.70	44.82	30.04
9	2-Buten-1-one, 1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-, (*E*)-	apple rose honey tobacco sweet	23,726–93-4	0.00014	51.94	54.14	44.79
10	D-Carvone	spice mint bread caraway	2244-16-8	0.086	32.96	35.43	33.61
11	.*β*.-Myrcene	peppery terpene spicy balsam plastic	123–35-3	0.0166	4.94	6.15	4.71
12	Limonene	citrus herbal terpene camphor	138–86-3	0.01	4.15	3.21	3.93
13	.*β*.-Phellandrene	mint turpentine	555–10-2	0.036	4.05	3.88	4.09
14	Linalyl acetate	sweet green citrus bergamot lavender woody	115–95-7	0.50	3.60	4.25	3.65
Esters							
15	Butanoic acid, 3-methyl-, hexyl ester	sweet green fruity apple unripe fruit apple skin strawberry	10,032–13-0	0.00001	195,880.58	216,698.14	198,999.76
16	3-Mercaptohexyl acetate	floral fruity pear tropical passion fruit blackberry raspberry black currant bud	136,954–20-6	0.0000023	19,759.16	64,982.70	15,027.27
17	2-Propenoic acid, 3-phenyl-, ethyl ester	sweet balsam fruity spicy powdery berry plum	103–36-6	0.00006	962.43	11,007.44	292.73
18	Butanoic acid, ethyl ester	fruity juicy fruit pineapple cognac	105–54-4	0.001	277.50	217.59	253.77
19	*cis*-3-Hexenyl-.*α*.-methylbutyrate	fresh green apple sweet fruity pear	53,398–85-9	0.004	138.04	146.45	139.46
20	Benzoic acid, methyl ester	phenolic wintergreen almond floral cananga	93–58-3	0.0015	72.80	75.16	70.34
21	Propanoic acid, hexyl ester	pear green fruity musty rotting fruit	2445-76-3	0.008	22.30	34.90	21.71
22	2H-Pyran-2-one, tetrahydro-6-pentyl-	fresh sweet oily coconut fruity peach creamy dairy	705–86-2	0.001	17.33	22.83	18.54
23	Heptanoic acid, methyl ester	sweet fruit green orris waxy floral berry	106–73-0	0.004	5.52	5.36	6.15
24	Isopentyl hexanoate	fruity banana apple pineapple green	2198-61-0	0.20	5.29	5.70	5.38
25	Isobutyl acetate	sweet fruity ethereal banana tropical	110–19-0	0.30	4.79	5.01	4.76
26	3-Hexen-1-ol, acetate, (*Z*)-	fresh green sweet fruity banana apple grassy	3681–71-8	0.0121	2.90	2.67	2.66
Aldehydes							
27	6-Nonenal, (*Z*)-	green cucumber melon cantaloupe waxy	2277-19-2	0.00002	102,867.60	109,579.80	102,581.80
28	2-Nonenal	fatty green waxy cucumber melon	2463-53-8	0.0005	1453.61	2060.03	1542.48
29	2,4-Decadienal, (*E*,*E*)-	oily cucumber melon citrus pumpkin nut meat	25,152–84-5	0.00005	851.27	1055.48	596.30
30	2,4-Nonadienal, (*E*,*E*)-	fatty melon waxy green violet leaf cucumber tropical fruit chicken fat	5910-87-2	0.00006	375.35	639.50	432.62
31	Benzeneacet Aldehyde	green sweet floral hyacinth clover honey cocoa	122–78-1	0.04	279.48	287.32	22.62
32	4-Heptenal, (*Z*)-	oily fatty green dairy milky creamy	6728–31-0	0.00006	190.53	270.99	120.65
33	BenzAldehyde, 4-methoxy-	sweet powdery mimosa floral hawthorn balsam	123–11-5	0.03	7.92	8.52	8.11
34	(*E*)-2-Octenal	fresh cucumber fatty green herbal banana waxy green leaf	2548-87-0	0.02	3.99	4.00	3.95
Heterocyclic compounds							
35	2-Thiophenemethanethiol	roast coffee fishy	6258-63-5	0.000005	301,634.90	322,402.85	300,951.91
36	2(5H)-Furanone, 5-ethyl-3-hydroxy-4-methyl-	sweet fruity caramellic maple fenugreek brown sugar nutty chicory praline butterscotch	698–10-2	0.000024	190,848.15	195,911.02	187,101.60
37	Pyridine, 2-pentyl-	fatty tallow green pepper mushroom herbal	2294-76-0	0.0000012	43,321.74	47,291.67	41,408.52
38	Furaneol	sweet cotton candy caramel strawberry sugar	3658-77-3	0.012	745.94	751.14	738.98
39	Pyridine, 2-hexyl-	green fatty mutton	1129-69-7	0.00028	55.15	254.97	55.86
40	Furan, 2-pentyl-	fruity green earthy beany vegetable metallic	3777–69-3	0.0048	17.59	16.34	11.93

(*continued on next page*)

Table 1 (*continued*)

Group No.	Volatile compounds	Odor description	CAS	Threshold (mg/kg)	Relative odor activity values (rOAVs)		
					XQC	XHJ	CK
41	2-Furanmethanol, 5-ethenyltetrahydro-.α.,.α.,5-trimethyl-, *cis*-	earthy floral sweet woody	5989-33-3	0.32	0.02	0.02	0.02
Alcohols							
42	1-Nonanol	fresh clean fatty floral rose orange dusty wet oily	143–08-8	0.002	713.30	980.02	756.40
43	3-Hexen-1-ol, (*Z*)-	fresh green cut grass foliage vegetable herbal oily	928–96-1	0.20	11.92	7.42	20.44
44	3-Mercapto-3-methylbutanol	roasted spicy sweet vegetable	34,300–94-2	0.0013	8.19	6.59	7.51
45	Phenylethyl Alcohol	floral rose dried rose flower rose water	60–12-8	0.045	3.62	4.11	0.33
46	3-Mercaptohexanol	sulfurous fruity tropical	51,755–83-0	0.000022	nd	3753.14	nd
Ketones							
47	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	earthy woody mahogany orris dry amber	17,283–81-7	0.0017	52.13	77.32	39.95
48	Isophorone	cooling woody sweet green camphor fruity musty cedarwood tobacco leather	78–59-1	0.0017	13.53	44.05	10.75
49	2-Nonanone	fresh sweet green weedy earthy herbal	821–55-6	0.05	12.66	13.60	12.40
Sulfur compounds							
50	3-Methyl-2-butene-1-thiol	sulfurous smoke leek onion skunky	5287-45-6	0.00000025	309,067.64	307,904.33	325,755.30
51	3-Cyclohexene-1-methanethiol, .α.,.α.,4-trimethyl-	sulfury aromatic grapefruit naphthyl resinous woody	71,159–90-5	0.000001	26,202.85	128,890.22	27,717.12
Phenols							
52	2-Methoxy-Phenol	phenolic smoke spice vanilla woody	90–05-1	0.0055	246.58	265.36	243.69
53	Phenol, 2-nitro-	—	88–75-5	0.001	11.25	15.74	24.38
Aromatics							
54	Naphthalene, 2-methyl-	oily aromatic	91–57-6	0.004	16.97	18.95	15.19
55	Naphthalene	pungent dry tarry	91–20-3	0.05	15.66	14.01	10.32

Odor description found in the literature with database (Perflavory Information System).
All odor thresholds were obtained from: 'Odour & Flavour Detection Thresholds in Water'.
'——', no odor description information was found in the literature. 'nd', not detectable.

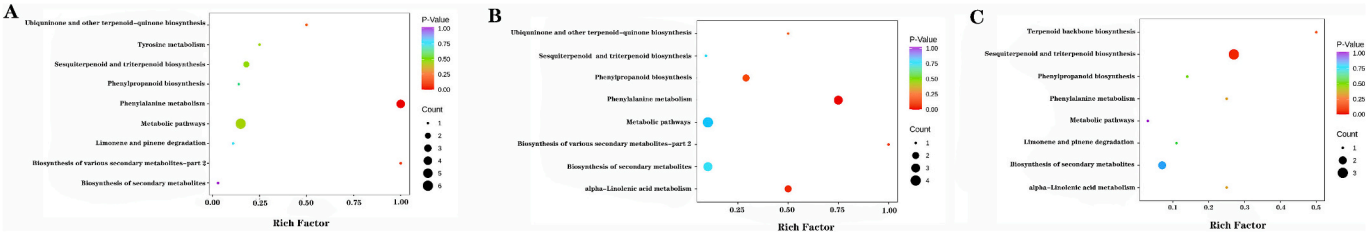

Fig. 4. Kyoto encyclopedia of genes and genomes enrichment plots of differential metabolites in three pak choi varieties: (A) CK vs XQC; (B) CK vs XHJ; (C) XQC vs XHJ. The horizontal coordinates in the graphs indicate the rich factor corresponding to each pathway, the vertical coordinates are the pathway names. The colors of the dots reflect the size of the *p*-value, with redder indicating more significant enrichment. The size of the dots represents the number of differential metabolites enriched.

and the combined effect of these aroma substances makes an important contribution to the rice flavor of XQC.

Fifty-four aroma substances were detected in XHJ, making significant contributions to its fishy flavor. The rOAV values of 3-mercaptohexanol; 2-propenoic acid, 3-phenyl-, ethyl ester; and 3-mercaptohexyl acetate differed from those in the other two pak choi varieties. Therefore it can be concluded that these three substances are required for the fishy aroma of XHJ. 3-Mercaptohexanol has been detected in mango (Xiang, 2020), but 2-propenoic acid, 3-phenyl-, ethyl ester and 3-mercaptohexyl acetate have not been reported in aroma studies. Lv (2019) showed that the aroma substances in xiuhuajin (XHJ) are 2,6,6-trimethyl-1- cyclohexene −1-acetaldehyde; 2-bromomethyl-isopropyl-3-methyl-cyclopentane and glutaric acid, 4-methoxyphenylbutyl ester, but these three substances were not detected in the present study. The main reason for this difference may be environmental differences in growth conditions and the different extraction method and temperature used, which can greatly improve extraction efficiency.

5. Conclusions

Identification of the key aroma compounds in pak choi was undertaken using HS-SPME/GC–MS, followed by calculation of rOAV The following conclusions were drawn: A total of 716 metabolites were identified in the three pak choi varieties studied, with terpenoid-based

scaffolds identified as the primary volatile species in all three cases. Through KEGG pathway enrichment analysis of differential metabolites, it was inferred that the phenylalanine metabolic pathway contributes to the formation of the special flavors of the two aromatic pak choi varieties compared with the non-aromatic CK. Sesquiterpene and triterpene biosynthesis involving terpenes may contribute to the rice aroma of XQC and the fishy odor of XHJ. The rice aroma for XQC and fishy odor for XHJ were due to 53 and 54 aromatic components with rOAV >1, respectively. This study expands our knowledge of the aroma constituents of pak choi for future commercial applications, and will be helpful for further research into the mechanisms of aroma formation in pak choi.

CRediT authorship contribution statement

Jinyan Li: Writing – original draft. **Wenfeng Zheng:** Data curation. **Weina Zhou:** Data curation. **Zhe Wang:** Data curation. **Junlong Hu:** Data curation. **Zhiyong Liu:** Writing – review & editing. **Hui Feng:** Writing – review & editing. **Yun Zhang:** Funding acquisition.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by basic scientific research project of educational department of Liaoning (JYTYB2024055).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101766.

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