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UPLC-Q-TOF/MS-based metabolomic analysis reveals the effects of asomate on the citrus fruit

Guangyun He [a,b], Xi Chen [c], Xue Hou [a,b,*], Xi Yu [d], Mei Han [a,b], Shiting Qiu [a,b], Ying Li [a,b], Shudi Qin [a,b], Fengyi Wang [a,b]

[a] *Institute of Quality Standard and Testing Technology for Agro-Products, Sichuan Academy of Agricultural Sciences, Chengdu, 610066, China*
[b] *Laboratory of Quality and Safety Risk Assessment for Agro-products (Chengdu), Ministry of Agriculture, Chengdu, 610066, China*
[c] *SCIEX Analytical Instrument Trading Co., Shanghai, 200335, China*
[d] *Faculty of Medicine, Macau University of Science and Technology, Macau, 999078, China*

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ABSTRACT

The regulation of the sugar-acid ratio is of great significance to the improvement of citrus fruit quality. The citric acid level in fruit is influenced by many factors. Among them, cultivar selection and production practices are the most important strategies under the grower's control. In recent years, an arsenic-containing preparation called "Tianmisu", with the main ingredient of asomate, has occasionally been reported to be used in citrus cultivation to improve the sweetness of fruits. In order to reveal the effects of the pesticide on citrus fruits, 'Harumi' tangor was treated with "Tianmisu", and the impact of this pesticide on fruit quality and metabolites was investigated through UPLC-Q-TOF/MS-based metabolomic analysis. Compared with the control, the concentration of titratable acidity, in particular citric acid, in the pulp of 'Harumi' tangor treated with the pesticide, was significantly reduced by 60.5%. The differences in metabolites between the pesticide-treated samples and the control were illustrated by Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). The PLS-DA analysis demonstrated a clear discrimination, with R^2Y and Q^2 values of 0.982 and 0.933 in the positive mode and 0.984 and 0.900 in the negative mode, respectively. A total of 155 compounds were identified, and 63 characteristic components were screened out from the pesticide-treated samples compared to the control. Aside from the upregulation observed for a few metabolites, the majority of the compounds, including citric acid and various lipids, were down-regulated in the treated citrus fruits compared to the control. This study can serve as a basis for understanding the regulatory mechanism of organic acids in citrus and will be helpful in developing different strategies to improve citrus quality.

1. Introduction

Citrus is one of the world's leading fruit crops, with an estimated production of more than 124 million tons per year (N. Liu et al., 2021). Citrus fruits are consumed worldwide due to their nutritional, sensory, and health-promoting attributes associated with a variety of metabolites, such as sugars, organic acids, vitamins, flavonoids, limonoids, carotenoids, etc. (Kim et al., 2021; Saini et al., 2022; Gao et al., 2022). In general, the quality of citrus fruits includes external and internal qualities as well as nutritional and nutraceutical properties, such as peel coloration, juice percentage, and soluble solids/acidity ratio. However, the relative importance of these parameters varies and depends on species, varieties, growing regions, and market demands (Goldenberget al., 2018). For example, in some cities of the USA, consumer preferences for fresh citrus fruits indicated that freshness, flavor, appearance, and juiciness were the most important attributes (Baldwin et al., 2014).

The taste of citrus fruit is primarily determined by the levels of sugars and acids in the juice sacs, as well as the relative ratio of the two. The sugar-acid ratio is one of the key indicators of fruit flavor and is mainly affected by organic acid contents (Lado et al., 2014; Lado et al., 2018). As the main components of total soluble solids (TSS), the three important carbohydrates, namely, fructose, glucose, and sucrose, comprise the major sugars in citrus, and the major acid is citric acid, which accounts

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[*] Corresponding author. Institute of Quality Standard and Testing Technology for Agro-Products, Sichuan Academy of Agricultural Sciences, Chengdu, 610066, China.

E-mail addresses: 527489328@qq.com (G. He), houxue127@163.com (X. Hou).

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for 90% of the total organic acid content (Sadka et al., 2000).

Organic acid accumulation is a complex process that is influenced by genetic factors, rootstock, environmental factors, and agronomic and cultural practices (Hussain et al., 2017). Fruit development is typically accompanied by sugar accumulation and organic acid degradation, and can be divided into three stages: cell division (stage I), cell expansion (stage II), and fruit maturation (stage III). Organic acids, mostly citric acid, typically accumulate during the early stage of fruit development and decrease during fruit ripening and storage (Sadka et al., 2000). In recent years, the accumulation and modification of fruit acidity have drawn considerable attention from researchers. Environmental factors, cultural practices, and the use of fertilizers or pesticides have been reported to be correlated with the accumulation of organic acids (Sadka et al., 2000; Chen et al., 2012; Jiang et al., 2014; Asai et al., 2017; Zhou et al., 2018; Liao et al., 2019; X. C. Liu et al., 2021). Most recently, research has discovered that asomate has the ability to enhance the sweetness and flavor of citrus fruit, while also accelerating the ripening process (Qiu et al., 2022). However, the physiological and molecular mechanisms underlying these correlations are not well understood and require further research. In recent years, an arsenic-containing preparation called “Tianmisu”, with the main ingredient of asomate, has occasionally been reported to be used in citrus cultivation to improve the sweetness and quality of fruits. However, little information is available concerning the effects of this pesticide on fruit quality, metabolites, and the underlying regulatory mechanisms.

Metabolomics based on high-throughput technologies can provide comprehensive information regarding the similarities and differences in metabolite composition among samples, and are powerful tools for analyzing complex regulatory processes in terms of metabolic pathways or regulatory networks (Lin et al., 2015; Feng et al., 2018). Metabolite profiling studies, especially metabolomics based on high-resolution mass spectrometry with high selectivity, have been widely applied in citrus research (Zhang et al., 2020; Yan et al., 2021; Huang et al., 2021; Dadwal et al., 2022; Wang et al., 2022).

To reveal the effects of pesticide on the citrus fruit, ‘Harumi’ tangor [*Citrus reticulate* × (*C.reticulata* × *C.sinenesis*)] were treated with the pesticide “Tianmisu”, and the influence of this pesticide on the citrus fruit quality and metabolites were investigated via the untargeted metabolomics based on UPLC-Q-TOF/MS. This research is expected to be helpful in understanding the mechanism of organic acid accumulation in citrus and developing different strategies to improve citrus quality.

2. Materials and methods

2.1. Chemicals and reagents

The methanol and acetonitrile (HPLC grade) were obtained from Tedia (Fairfield, OH, USA). The formic acid (HA, HPLC grade) was purchased from ANPEL Laboratory Technologies (Shanghai, China). The pesticide “Tianmisu” was obtained during a survey on the usage of this pesticide in the cultivation of citrus crops.

2.2. Plant materials

A field study was conducted from June 1 to December 31, 2021, in an experimental orchard located in Qionglai County, Sichuan Province, China. The 5 years old ‘Harumi’ tangor grafted on ‘tangerine’ (*Citrus reticulata* Blanco, cv. Hongjv) was used to conduct this experiment. The ‘Harumi’ tangor trees were divided into two groups: a pesticide-treated group (Sample) and an untreated group (Control). The ‘Harumi’ tangor trees in the Sample group were sprayed with aqueous solutions of “Tianmisu” (1.25 g L^{-1}) to fully wet them, while the trees in the Control group located in the same orchard, were treated with the same quantity of water. There were two spray schedules in June with a 15 days interval.

2.3. Fruit sampling

The fruits of ‘Harumi’ tangor (20 batches, each comprised of 3 kg of fruits) were harvested at maturity stage. The fruits were washed with distilled water and the peels were removed. The flesh was homogenized and stored at −18 °C for further extraction.

2.4. Sample preparation

The samples were extracted according to the previously reported method with some modifications (Guo et al., 2021; Feng et al., 2018). The samples (2.0 g) were weighed into 50-mL centrifuge tubes. A volume of 15.0 mL methanol 0.1% formic acid was added into the tube and shaken for 30 s. The mixtures were extracted in an ultrasonic bath at 25 °C for 15 min. The extract was then centrifuged at 8000 rpm for 5 min at −10 °C. The supernatant was transferred to a 50-mL tube, and this step was repeated for two times. The supernatants were collected, then an aliquot of them was filtered through a 0.2 μm polytetrafluoroethylene (PTFE) filter before UPLC-QTOF/MS analysis. To ensure the stability and reproducibility of the method, a quality control (QC) sample was prepared by combining 100 μL of each individual sample.

2.5. Analysis of sugar and acid concentrations, and the total soluble solids

The water soluble sugars (WSS), the titratable acidity (TA), and the total soluble solids (TSS) were measured according to literature methods (Wu et al., 2021a,b; Moreno et al., 2018).

2.6. UPLC-Q-TOF/MS analysis

The chromatographic separation of the samples was carried out by means of a Nexera X2 UPLC system (Shimadzu, Tokyo, Japan), on a Waters HSS T3 C_{18} column (100 × 3 mm, 1.7 μm) at 40 °C. The flow rate was 0.3 mL·min^{-1} and the injection volume was 1 μL. The mobile phases consisted of (A) 0.05% FA aqueous solution and (B) methanol/acetonitrile ($v/v = 1/1$) containing 0.1% FA. The linear gradient programs were listed as follows: 0–1.0 min.5% (B); 1.0–7.0 min. 5%→50% (B); 7.0–13.0 min. 50%→70% (B); 13.0–17.0 min.70%→98% (B); 17.0–22.0 min. 98% (B); 22.0 → 25.0 min. 5% (B).

Mass spectrometry analysis was performed on a SCIEX ZenoTOF™ 7600 (AB SCIEX, USA) which was calibrated every five samples in highly sensitive mode as recommended. ESI source was operated in positive and negative mode, and the spectra was obtained in TOF/MS-IDA-TOF MS/MS mode. The Zeno trap functionality was turned on for all experiments. Declustering potential (DP) was set at 80 V/-60 V. IDA MS/MS was conducted at the collision energy (CE) of 40 V ± 20 V/-40 V ± 20 V (intensity≥100 cps, ion tolerance≤10 ppm). SCIEX OS 2.2 (Framingham, MA, USA) and PeakView 2.2 were used for data acquisition and processing.

2.7. Data processing and multivariate statistical analysis

Raw data files acquired from UPLC-Q-TOF/MS analysis were collected by SCIEX OS 2.2 (Framingham, MA, USA) and then imported into R (Framingham, MA, USA) for peak extraction and peak alignment to obtain a peak table, including information on retention time (RT), m/z and MS intensity of the metabolites in the sample. The signals with the RT of 0.5–22 min and the m/z of 50–1200 were processed. The intensity threshold for peak detection was set at 100. The retention time tolerance and mass tolerance for the peak alignment was set at 0.2 min and 0.01 Da, respectively. Principal component analysis (PCA), Partial least-squares discriminant analysis (PLS-DA) and Heat Map Analysis (HMA) were performed with Metaboanalyst. The SCIEX Natural Products Library was used to perform library searching.

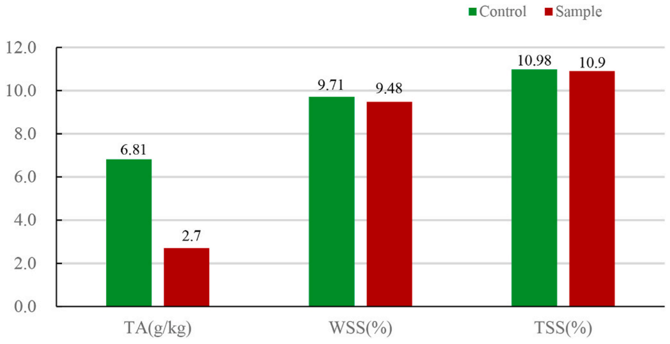


Fig. 1. The effects of pesticide on the citrus fruit quality.

3. Results and discussion

3.1. Effects of pesticide on the sugar and organic acid levels in citrus fruits

As organic acids and soluble sugars significantly contribute to the flavor and quality of citrus fruits, the impact of pesticide on the concentrations of these compounds were investigated. The results were presented in Fig. 1. In comparison to the control, the concentration of TA in the pesticide-treated fruits pulp was significantly reduced by 60.5%, from 6.81 g/kg to 2.70 g/kg. The WSS of the treated group and control were 9.71% and 9.48%, respectively. Additionally, the TSS values of 10.98% and 10.90% for the sample and the control, respectively, showed a similar pattern. This is consistent with the literature report (Qiu et al., 2022). The findings suggested that pesticide treatment has a considerable effect on the TA concentrations. However, no meaningful difference was identified in the WSS and TSS between the two groups (Zhang et al., 2012). Consequently, the WSS/TA and TSS/TA ratios were

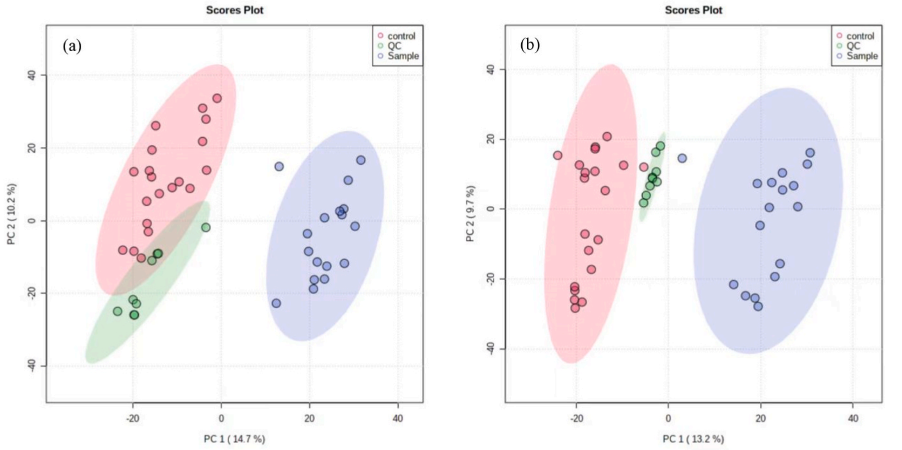
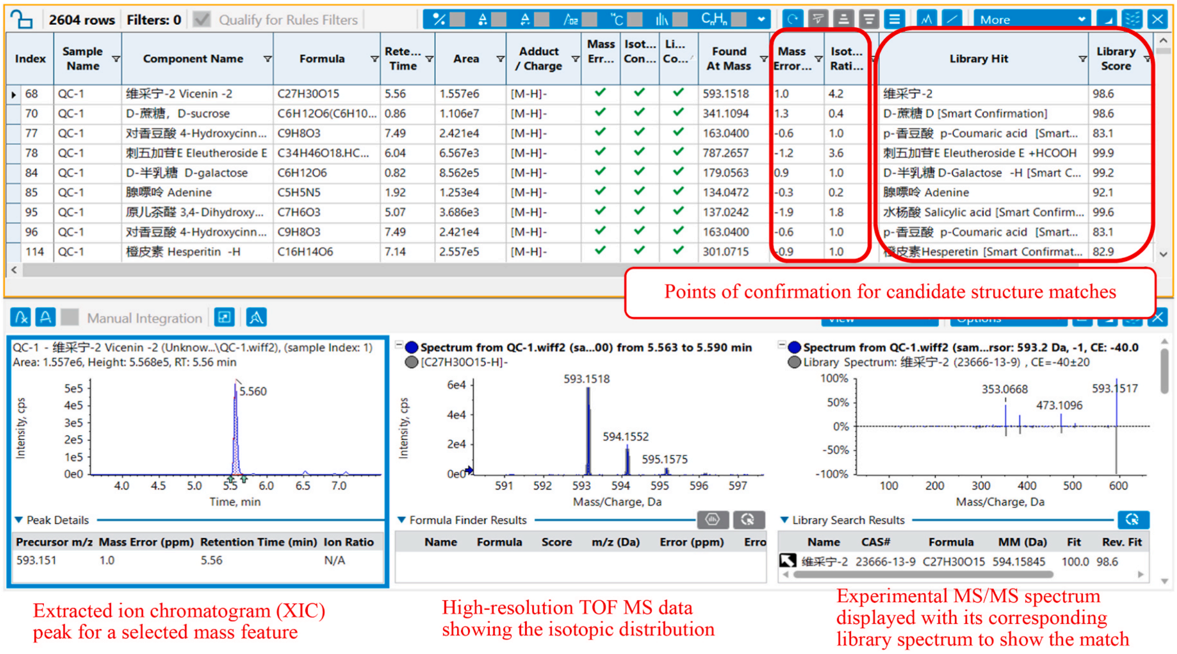


Fig. 2. PCA plots of the pesticide treated samples, control and QC samples in positive (a) and negative (b) ionization modes.



Index	Sample Name	Component Name	Formula	Rete... Time	Area	Adduct / Charge	Mass Err...	Isot... Con...	Li... Co...	Found At Mass	Mass Error...	Isot... Rati...	Library Hit	Library Score
68	QC-1	维采宁-2 Vicenin -2	C27H30O15	5.56	1.557e6	[M-H]-	✓	✓	✓	593.1518	1.0	4.2	维采宁-2	98.6
70	QC-1	D-蔗糖，D-sucrose	C6H12O6(C6H10...	0.86	1.106e7	[M-H]-	✓	✓	✓	341.1094	1.3	0.4	D-蔗糖 D [Smart Confirmation]	98.6
77	QC-1	对香豆酸 4-Hydroxycinn...	C9H8O3	7.49	2.421e4	[M-H]-	✓	✓	✓	163.0400	-0.6	1.0	p-香豆酸 p-Coumaric acid [Smart...	83.1
78	QC-1	刺五加苷E Eleutheroside E	C34H46O18.HC...	6.04	6.567e3	[M-H]-	✓	✓	✓	787.2657	-1.2	3.6	刺五加苷E Eleutheroside E +HCOOH	99.9
84	QC-1	D-半乳糖 D-galactose	C6H12O6	0.82	8.562e5	[M-H]-	✓	✓	✓	179.0563	0.9	1.0	D-半乳糖 D-Galactose -H [Smart C...	99.2
85	QC-1	腺嘌呤 Adenine	C5H5N5	1.92	1.253e4	[M-H]-	✓	✓	✓	134.0472	-0.3	0.2	腺嘌呤 Adenine	92.1
95	QC-1	原儿茶醛 3,4-Dihydroxy...	C7H6O3	5.07	3.686e3	[M-H]-	✓	✓	✓	137.0242	-1.9	1.8	水杨酸 Salicylic acid [Smart Confirm...	99.6
96	QC-1	对香豆酸 4-Hydroxycinn...	C9H8O3	7.49	2.421e4	[M-H]-	✓	✓	✓	163.0400	-0.6	1.0	p-香豆酸 p-Coumaric acid [Smart...	83.1
114	QC-1	橙皮素 Hesperitin -H	C16H14O6	7.14	2.557e5	[M-H]-	✓	✓	✓	301.0715	-0.9	1.0	橙皮素Hesperetin [Smart Confirmat...	82.9

Points of confirmation for candidate structure matches

QC-1 - 维采宁-2 Vicenin -2 (Unknow...\QC-1.wiff2), (sample Index: 1)
Area: 1.557e6, Height: 5.568e5, RT: 5.56 min

Peak Details

Precursor m/z	Mass Error (ppm)	Retention Time (min)	Ion Ratio
593.151	1.0	5.56	N/A

● Spectrum from QC-1.wiff2 (sa...00) from 5.563 to 5.590 min
● [C27H30O15-H]-

Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Erro

● Spectrum from QC-1.wiff2 (sam...rsor: 593.2 Da, -1, CE: -40.0
● Library Spectrum: 维采宁-2 (23666-13-9) , CE=-40±20

Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit
维采宁-2	23666-13-9	C27H30O15	594.15845	100.0	98.6

Extracted ion chromatogram (XIC) peak for a selected mass feature

High-resolution TOF MS data showing the isotopic distribution

Experimental MS/MS spectrum displayed with its corresponding library spectrum to show the match

Fig. 3. Typical results display of the screening data in SCIEX OS software. The points of confirmation highlighted in the red boxes are, from left to right: TOF MS1 mass error within 2 ppm of the candidate molecular formula; isotope ratio matching to theoretical within 2%; and the library hits with >70% library scores in the match between the experimental and reference spectra. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

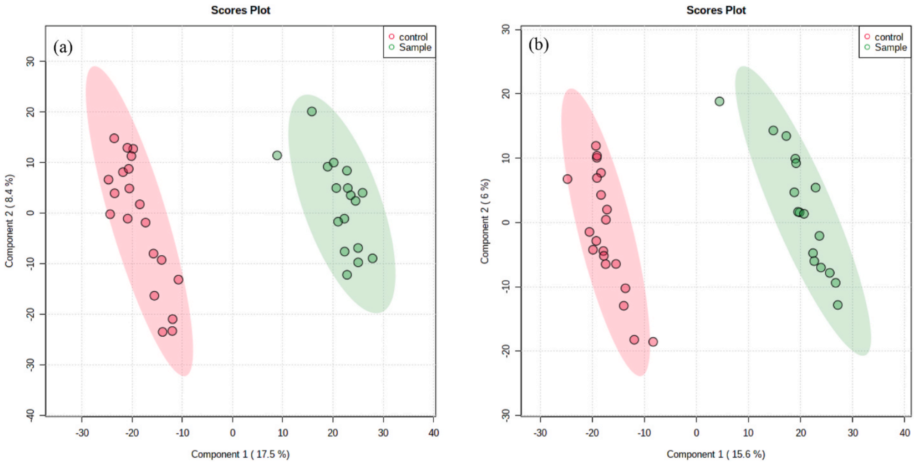


Fig. 4. PLS-DA plots constructed with the complete data set of pesticide-treated samples and control in positive (a) and negative (b) ionization modes.

considerably increased by 146.2% and 150.4%, respectively. Since the TSS/TA ratio is a crucial factor that impacts consumer acceptance, the taste of the fruit was significantly enhanced. However, information regarding the safety of the pesticide and its regulatory mechanism on the acidity levels of citrus or other plants is currently very limited and unclear (Pereira et al., 2014; Sánchez-Bravo et al., 2022). The findings from this study offer a foundation for comprehending the organic acid regulatory mechanisms in citrus and may aid in discovering safe and effective alternatives to enhance the quality of citrus fruits.

3.2. The metabolic profile of citrus fruits

After background drift correction, peak extraction, and peak alignment of raw data files, a peak table containing information on the metabolites in the samples, including their retention time (RT), m/z, and MS intensity, was obtained. After filtering, 13293 ions and 10584 ions were produced in positive mode and negative mode, respectively. To gain an overview of the differences in metabolite levels between the pesticide-treated citrus fruits and the control, an unsupervised PCA was performed as the first step in data analysis (Reisdorph et al., 2021). The 2D scatter plots depicting the scores in positive and negative mode are shown in Fig. 2. The results show that the pesticide-treated samples and the control group were well segregated and could be easily distinguished into two distinct clusters. The QC samples were interspersed between samples and clustered into a tight group, demonstrating good reproducibility. This indicates that the precision and repeatability of the data in this metabolomics-based study can be ensured (Xin et al., 2018). The first and second principal components account for 24.9% and 22.9% of the total variance in positive and negative mode, respectively. The results indicate that it is possible to distinguish the pesticide treated and control citrus fruits completely, based on their metabolic profiles. Additionally, the pesticide treatment was found to have a significant impact on the composition of the citrus pulp.

3.3. Compound identification based on non-targeted metabolic analysis

The analysis of the citrus metabolome is challenging due to the multiplicity of metabolites, some of which are present in small quantities, making them difficult to detect in complex matrices like citrus pulp (Reisdorph et al., 2021). The ZenoTOF 7600 system offers an approximately 10-fold enhancement in MS/MS sensitivity, which contributes to the availability of superior quality MS/MS spectra used for matching against published databases. This significant improvement enables identification of compounds at previously undetectable levels. Screening for suspect or non-targeted compounds often yields a substantial list of candidate compounds that is both time consuming and labor intensive to review. SCIEX OS software allows the user to define confidence thresholds on key confirmation criteria like mass error, isotopic ratio matches, and MS/MS library matches, which are collectively assessed to identify a compound. A fast and straightforward screening workflow identified a total of 155 natural components (Table S1) present in citrus pulp. These components comprised sugars, organic acids, lipids, flavonoids and their derivatives, amino acids and their derivatives, nucleosides and nucleotides, phenols, terpenoids, coumarin and its derivatives, as well as vitamins (Chhikara et al., 2018), typical results display of the screening data were showed in Fig. 3.

3.4. Characteristic metabolites of citrus fruits

To confirm the differentiating metabolites induced by pesticide treatment, the peak table was imported into Metaboanalyst for conducting a supervised PLS-DA (Chaji et al., 2021; Shi et al., 2022). As shown in Fig. 4, the 2D score scatter plots created with the entire dataset of pesticide-treated and control samples in positive and negative ionization modes demonstrated a more distinct separation between two groups, with most samples falling within the 95% confidence intervals (Hotelling's T-squared ellipse). The results indicated that PLS-DA achieved remarkable predictive power, displaying R^2Y and Q^2 of 0.982 and 0.933 in positive mode and 0.984 and 0.900 in negative mode, respectively (He et al., 2022).

To explore the characteristic metabolites induced by the pesticide, *t*-test in conjunction with variable importance in projection (VIP) from the PLS-DA model were employed for significance testing. The metabolites with a VIP value greater than 1.0 are usually considered as potential markers to the model being studied. Meanwhile, the *t*-test is commonly performed to estimate significant differences of metabolite contents among different groups of samples (Lee et al., 2022). In this study, a total of 63 distinctive components (Table 1) including various lipids, organic acids, hesperitin, hesperidin, and other compounds, were screened out between the pesticide-treated and control samples using analysis criteria of p-value <0.01 and VIP value > 1.0. The VIP score plot of the characteristic compounds are presented in Fig. S1.

PLS-DA plot generated with the screened target components was showed in Fig. 5. It was observed that using differentially expressed metabolites, PLS-DA enabled the discrimination of the effects of pesticide treatment by grouping most samples together within their respective groups, except for two distinct points. The first and second principal components cumulatively explained 96.6% of the total variance, with 88.6% accounted for by the first component and 8.0% by the second. The R^2Y and Q^2 values obtained through cross-validation in PLS-DA score plot were 0.953 and 0.948, respectively, indicating the excellent fitness and reliability of the model.

Table 1
Sixty-three characteristic components between the pesticide-treated citrus and control.

No.	Component	VIP values	*P*-value
1	LPE 18:0	1.61	8.87E-24
2	PE 36:5	1.60	1.39E-21
3	LPC 16:0	1.59	1.10E-17
4	PC 36:5	1.59	2.52E-21
5	PC 36:4	1.59	5.31E-22
6	PE 36:6	1.59	3.33E-18
7	LPE 20:1	1.59	8.39E-21
8	LPC 18:1	1.58	4.16E-20
9	LPC 16:1	1.58	7.14E-20
10	LPC 15:0/0:0	1.58	2.39E-20
11	LPC 18:3	1.57	3.60E-19
12	LPE 20:0	1.57	8.39E-21
13	LPE 18:3	1.56	1.26E-19
14	2,5-Furandicarboxylic acid	1.56	2.11E-20
15	PC 36:6	1.56	3.33E-18
16	2-Ketoglutaric acid	1.55	4.40E-20
17	PE 34:4	1.55	2.15E-17
18	LPC 18:2	1.55	2.14E-18
19	2-Furoic acid	1.55	1.55E-20
20	Citric acid	1.55	5.75E-21
21	cis-Aconitic acid	1.54	8.07E-20
22	PC 34:3	1.54	1.82E-17
23	LPE 16:0	1.54	6.05E-23
24	Homoisocitrate	1.53	2.46E-17
25	C18:3-glycer-Glu	1.51	2.99E-15
26	LPE 18:2	1.51	2.12E-16
27	LPE 18:1	1.49	1.42E-14
28	LPE 16:1	1.45	2.48E-13
29	L-leucyl-L-proline	1.44	2.86E-10
30	PE 34:3	1.44	3.56E-12
31	PE 36:4	1.42	1.04E-11
32	Folinic acid	1.41	1.60E-11
33	C18:3-glycer-2Glu	1.40	1.87E-12
34	Caffeic acid	1.38	8.37E-09
35	Gamma-Aminobutyric acid	1.37	3.21E-09
36	LPC 18:0	1.35	2.29E-10
37	Glutamic acid	1.33	1.93E-11
38	synephrine	1.30	6.38E-09
39	Ferulic acid	1.26	4.57E-08
40	Syringa vulgaris	1.26	4.82E-08
41	Alpha-Lactose	1.25	5.38E-08
42	Hesperidin	1.23	1.25E-07
43	D-raffinose	1.22	1.57E-07
44	Malic acid	1.21	2.29E-07
45	Neoeriocitrin	1.19	4.23E-07
46	Eriodictyol	1.18	6.20E-07
47	D-(+)-Glucose	1.17	7.88E-07
48	Beta-Leucine	1.14	1.96E-06
49	Hesperitin	1.14	2.48E-06
50	Hesperetin 7-glucoside	1.13	3.13E-06
51	Coumarin	1.13	3.31E-06
52	Stachydrine	1.12	3.85E-06
53	FFA 18:2-O	1.11	5.49E-06
54	Anthranilic acid	1.11	5.72E-06
55	Guanosine	1.10	6.79E-06
56	4-Hydroxy-6-methyl-2-pyron	1.10	7.02E-06
57	C16:0-glycer-2Glu	1.10	7.40E-06
58	beta-Citryl-L-glutamate	1.08	1.24E-05
59	D-sucrose	1.08	1.26E-05
60	Rhamnocitrin 3-rutinoside	1.06	2.02E-05
61	Vitamin B2	1.05	2.54E-05
62	Pipecolic acid	1.05	2.56E-05
63	Neodiosmin	1.03	3.80E-05

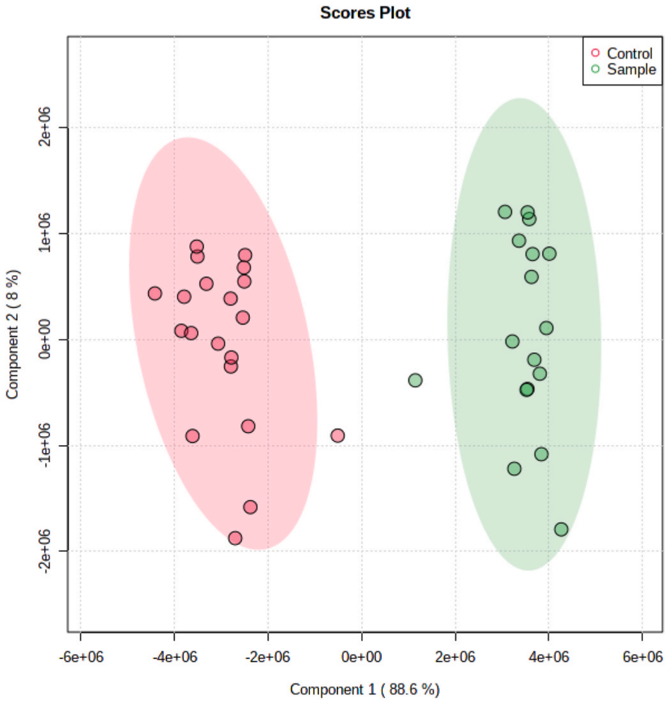


Fig. 5. PLS-DA plots constructed with characteristic components between the pesticide treated samples and control.

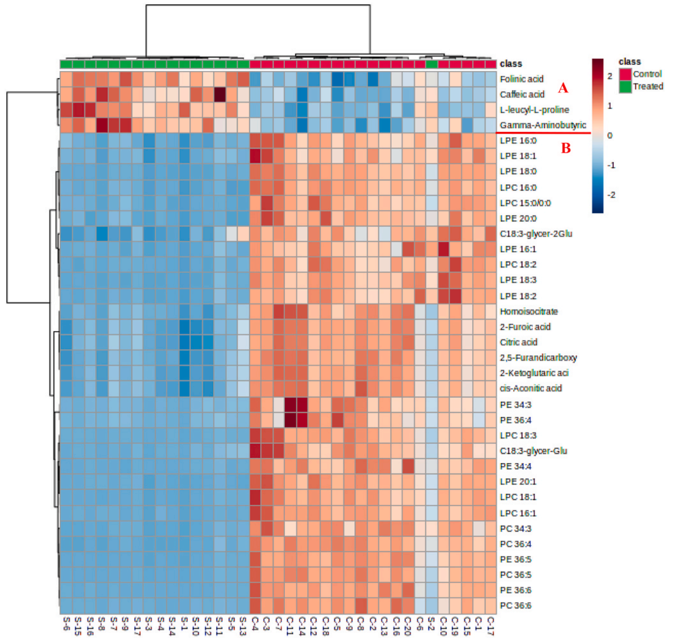


Fig. 6. Heatmap of the top 35 compounds with the greatest differences between the pesticide treated samples and control.

3.5. *Accumulation patterns of characteristic metabolites in citrus fruits*

In order to visualize the relationship of differential metabolites in the pesticide-treated samples and control, HMA was utilized (Wang et al., 2016). The comparative quantitative changes in the top 35 characteristic compounds with the most significant differences were illustrated in the Heatmap (Fig. 6). As it was shown that the compounds could be categorized into two groups. Group A comprised a small number of metabolites, such as L-leucyl-L-proline, folinic acid, caffeic acid, and gamma-aminobutyric acid, which were found to be upregulated in the pesticide-treated citrus fruits in comparison to the control. In contrast, most of the compounds belonging to Group B, which primarily comprised citric acid, glutamic acid, 2-furoic acid, 2-ketoglutaric acid, cis-aconitic acid, and various lipids, were present at lower levels in the pesticide-treated group than in the control group. The VIP score plot (Fig. S1) of the characteristic compounds also proved that accumulation patterns of these metabolites. The box and whisker plots of typical metabolites were showed in Fig. S2, which also demonstrate the statistical significance between the pesticide-treated samples and the control samples.

The sweet and sour tastes of citrus fruits are primarily determined by the ratio of sugar and organic acid content present in them. Citric acid is one of the most prevalent organic acids in citrus fruits, whereas fructose, glucose, and sucrose are the primary sugars found in them. As it was shown in Fig. S1, The quantity of citric acid in the treated pulp samples was considerably lower than that of the control, whereas no noteworthy difference in sugar content was observed between the two groups. In the process of fruit development, citric acid is synthesized from soluble sugars through the tricarboxylic acid cycle (Hussain et al., 2017). According to the previous literature, the enzyme activity related to citrate biosynthesis was not inhibited, and the content of the related metabolites was also not reduced in As-treated fruits (Qiu et al., 2022). However, the expression of proton pump genes *CitPH5* and *CitPH1*, which are responsible for controlling the vacuolar citric acid accumulation, was affected, and their transcription factor genes *CitTT8* and *CitMYB5* were downregulated. This suggests that As treatment may indirectly impact citrate biosynthesis by modulating the expression of genes involved in regulating the proton pumps. Further research is needed to gain a thorough understanding of the impact of pesticide on citrate biosynthesis.

4. Conclusions

Since the sugar content in fruits only slightly varies, while the acid content varies significantly, the regulation of organic acid metabolism is crucial to enhancing the quality of citrus fruits. The level of citric acid in fruits is affected by numerous factors, but the most crucial factor under a grower's control is cultivar selection and production methods. The present study successfully illustrated the impact of the pesticide "Tianmisu", an arsenic-containing preparation with the main component of asomate, on the concentrations of sugar, organic acid, and the metabolic profile of citrus fruits. Compared with the control, the titratable acidity concentrations mainly the citric acid in the pesticide-treated 'Harumi' tangor pulp was notably reduced by 60.5%. However, the pesticide did not have significant impact on the sugar content of the citrus fruit. Additionally, the pesticide treatment had a notable effect on the metabolic profile of the fruit pulp. A total of 155 compounds were identified, and 63 characteristic components were screened out between the treated sample and the control. Apart from the upregulation of a few metabolites, the majority of the compounds, such as citric acid and various lipids, were found to be downregulated in the treated citrus fruits as compared to the control. These results will lay the groundwork for comprehending the mechanism of organic acid regulation in citrus and serve as a useful reference for developing different strategies to improve citrus quality.

CRediT authorship contribution statement

Guangyun He: Conceptualization, Methodology, Writing – original draft, preparation. **Xi Chen:** Technical consultation. **Xue Hou:** Supervision, instruction. **Xi Yu:** Consultation. **Mei Han:** Reviewing. **Shiting Qiu:** Reviewing. **Ying Li:** Sampling. **Shudi Qin:** Literature research. **Fengyi Wang:** Literature research.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100523.

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