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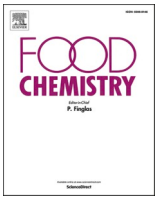

Untargeted foodomics for authenticating the organic farming of water spinach (*Ipomoea aquatica*)

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ABSTRACT

This study aimed to conduct a comprehensive analysis of the primary and secondary metabolites of water spinach (*Ipomoea aquatica*) using hydrophilic interaction liquid chromatography coupled with Orbitrap high-resolution mass spectrometry (HILIC-Orbitrap-HRMS). Certified samples from two cultivars, Green stem water spinach (G) and White stem water spinach (W) cultivated using organic and conventional farming methods, were collected from the Hong Kong market. Multivariate analysis was used to differentiate water spinach of different cultivars and farming methods. We identified 12 metabolites to distinguish between G and W, 26 metabolites to identify G from organic farming and 8 metabolites to identify W from organic farming. Then, two metabolites, isorhamnetin and jasmonic acid, have been proposed to serve as biomarkers for organic farming (in both G and W). Our foodomics findings provide useful tools for improving the crop performance of water spinach under abiotic/biotic stressesand authentication of organic produce.

1. Introduction

Organic farming is a more sustainable food production approach, abstaining synthetic pesticides and fertilizers by other means, such as crop rotations, with more consideration on soil fertility and closed nutrient cycles (Knapp & van der Heijden, 2018). Despite the sizeable global organic food market, low consumer trust impedes its long-term sustainable development (Vega-Zamora, Torres-Ruiz, & Parras-Rosa, 2019). Consumer trust is more fragile as publicly accessible scientific information on their nutrition, food safety and sensory quality is lacking (Popa, Mitelut, Popa, Stan, & Popa, 2019). Furthermore, organic foods are particularly susceptible to food fraud due to the absence of effective authentication method that can differentiate organic from conventional foods (Ferreira, Tucker, Rakola, & Skorbiansky, 2021). Currently, documentary-based certification system is the only way for consumers to identify food products that are organically produced. Organic foods are classic examples of credence goods in which the authenticity/quality of the product (farming practices used in organic food) cannot be ascertained even after purchase. In general, consumers pay a premium price for organic foods, but it varies across the globe. For example, American consumers are willing to pay 31% to 41% higher prices for organic food than conventional food (de Morais et al., 2023). As a result, organic food is prone to fraud and opportunistic behaviour in the supply chain, leading to difficulties in building consumer trust (Lee & Hwang, 2016). For the long-term sustainable development of the global organic food market, it is crucial to foster high consumer trust in the organic labelling (Inácio, Chalk, & Magalhães, 2015). Therefore, there is an urgent need to develop a rapid authentication method specifically for certified organic products to support the existing organic certification system and promote more sustainable farming practices.

There may be a wide range of interpretations of what organic refers, particularly in the perceptions of different stackholders. For most of the general public or consumers, organic farming is generally considered as “chemical-free” farming without the use of synethetic nutrient inputs

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and chemical pesticides as they are thinking for healthier argricultural products. In a more comprehensive view, organic farming is conceived as a holistic farming sytem aimed to maintain sustainable production by careful balance of soil health and environment, biological diversities of the farm lands. This view is embodied in the standards developed by International organizations which attempt to harmonize the meaning of organic farming. The most influential standards would be the standards developed by the Codex Alimentarius (set up by the Food and Agriculture Organization and the World Health Organization) and The International Federation of Organic Agriculture Movement (Seufert, Ramankutty, & Mayerhofer, 2017). In Hong Kong, where the samples were collected for this study, the organic farming practices are based on the guidelines and standards of these two major international organizations. The Agriculture, Fisheries and Conservation Department of HKSAR provides Organic Farming Support Service (previously known as Organic Farming Conversion Scheme) to help farmers to switch to organic farming since 2000 (AFCD, 2023). With funding support from local government, the Hong Kong Organic Resource Center (HKORC) was established to provide organic certification services (HKORC-Cert, 2024).

Various analytical platforms have been proposed and utilized in the literature to develop methods of identifying crops with different farming practices. The stable isotope ratio and elemental profile of crops, with the help of chemometrics, have been demonstrated to be effective in identifying organic vegetables. Most studies suggested using nitrogen isotope value (δ15N), which tends to be zero in synthetic fertilizers, to discriminate between organic and conventional vegetables, such as tomatoes, carrots and leafy vegetables (Barroso, 2010; de Lima & Barbosa, 2019). Although stable isotope and elemental profile successfully authenticate the farming methods of crops in the field studies, the application on the markets might be limited because of the significant influence of the synthetic fertilizer residues commonly found in organic farms (Capuano, Boerrigter-Eenling, van der Veer, & van Ruth, 2013), and types of fertilizer used (Inácio et al., 2020). For example, Inácio et al. reported inconsistency in δ15N for differentiating between organic and conventional farming in tomatoes and lettuces (Inácio et al., 2020).

Foodomics, and specifically metabolomics, is another approach to authenticate crops grown using different practices. The changes induced by agronomic techniques, on the composition of fruit and vegetables, were studied using a foodomics approach, which allowed the identification of a pool of compounds, capable of discriminating between grapes produced with biodynamic or organic practices, including GABA (Picone et al., 2016). This molecule brings health benefits (stress relief, improved immune system). Therefore, through foodomics, useful information was provided to evaluate whether changes influence nutritional values and factors with implications on the health and, therefore, on the quality of vegetables.

Metabolomics has emerged as a powerful analytical tool to assist the development of next-generation crops. Numerous reviews showed the potential of metabolomics in studying the environmental influence on plants (Villate et al., 2021), helping the farming method modification (Alawiye & Babalola, 2021) and assisting the plant breeding selection (Enfissi et al., 2021).

Two analytical platforms commonly used in metabolomics are nuclear magnetic resonance (NMR) and mass spectrometry (MS). The complementary advantages of both platforms are well known, with abundant literature highlighting higher sensitivity in favor of MS, while NMR is considered more robust in producing reproducible data (Picone, Mengucci, & Capozzi, 2022). With the rapid development of mass spectrometers, MS-based metabolomics has been the most commonly applied approach to examine plant-related issues, including the authentication of organic crops. Previous MS-based metabolomics studies on authenticating the organic origin of crops and plant-based products mainly focused on secondary metabolites due to their value for human health (Mihailova et al., 2021). For example, nine secondary metabolites, pelargonidin-glucoside, kaempferol glucuronide, cyanidin-glucoside, quercetin-rutinoside, kaempferol-coumaroyl-glucoside, quercetin-glucuronide, proanthocyanidin B2, quercetin-glucoside and pelargonidin-rutinoside, were proposed as markers for differentiating organic from conventional strawberries (D'Urso, d'Aquino, Pizza, & Montoro, 2015). In rice, nine secondary metabolites were identified for signifying the organic origin, including histidinol, malvin, pinoresinol, lagochiline, methylumbelliferyl glucuronide, coumarin, benzoyl-l-arginine, and hydrocinchonine (Xiao, Ma, Zhang, & Qian, 2018). Compared with secondary metabolites, there is still much room to investigate how organic farming influences the primary metabolites and the regulation of the conversion between primary and secondary metabolites.

Water spinach (*Ipomoea aquatica* Forsk., IA), also called kangkong and swamp cabbage, is a protein-rich leafy vegetable available in Asia during summer. Two major cultivars of IA with distinctive morphology are commonly consumed in Asia. Green stem water spinach (G), "Ching Quat" variety, has a green and succulent stem with smaller hollow and narrower leaves. White stem water spinach (W), "Pak Quat" variety, has a white and thin-walled stem with larger hollow arrow-shaped leaves. The traditional farming methods applied to the two cultivars are totally different. G is planted in moist soils (dry-land method), while W is grown in flooded paddy fields (wet-land method) (Edie & Ho, 1969; Gangopadhyay, Das, Bandyopadhyay, & Das, 2021). Despite the high annual consumption of IA and the increasing yield of the organic IA, there is a lack of knowledge on the difference in nutritional values of the Asian IA cultivars and the impact of organic farming practices on these cultivars (Umar, Hassan, Dangoggo, & Ladan, 2007).

In this study, a mass spectrometry-based metabolomics method for the identification of organic water spinach is reported. The present study applied an untargeted metabolomics approach with ultra-high-performance liquid chromatography-Orbitrap-mass spectrometry (UPLC-Orbitrap-MS) analytical platform to reveal the difference between two cultivars and the influence of organic farming on water spinach. The hydrophilic interaction liquid chromatography (HILIC) column was used to increase the polar metabolites' differentiation power. This approach provides insight into the full complexity of farming practices based on a more comprehensive range of polar to semi-polar metabolites, including primary and secondary metabolites. The overall objective of this study is to uncover a common set of metabolites associated with cultivation from organic farming and understand the impact of farming practices on the nutritional values of the water spinach produced.

2. Materials and methods

2.1. Reagents

High-performance liquid chromatography (HPLC)-grade solvents, acetonitrile, chloroform and methanol were purchased from Duksan Pure Chemicals (Gyeonggi-do, South Korea). Optima LC/MS grade formic acid was purchased from Fisher Chemical (Radnor, PA, USA). Double-deionized water was freshly prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Deuterated cholic acid (2,2,4,4-d_4) and L-tryptophan (indole-d5) was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Ammonium formate, 2-hydroxyIsocaproic acid, aspartic acid, caftaric acid, coumaroyltyramine, daphnetin, gentisic acid, glutamic acid, glutamine, isoleucine, jasmonic acid, leucine, mesaconic acid, methionine, nicotinamide, N-trans-p-coumaroyltyramine, phenylalanine, threonine, tyrosine and uric acid were commercially obtained from Sigma-Aldrich (St. Louis, MO, USA). 3-methyladenine, 2-aminoadipic acid, maltose, salicylic acid, shikimic acid, tryptamine and xylitol were acquired from Acros Organics (Morris Plains, NJ, USA). Alpha-ketoglutarate, fumaric acid and glutaric acid were purchased from Honeywell International (Phoenix, AZ, USA). Quinic acid and 7-hydroxycoumarin were commercially obtained from LGC Labor GmbH (Augsburg, Germany)

and Chengdu Herbpurify (Chengdu, China), respectively. 4-aminobutyric acid, adenosine, catechin, epicatechin, hypoxanthine, mannose and protocatechuic acid were acquired from FUJIFILM Wako Pure Chemicals (Osaka, Japan). Isorhamnetin and kaempferol were purchased from International Laboratory USA (South San Francisco, CA, USA). Isoquercitrin was acquired from Shanghai Tauto Biotech (Shanghai, China).

2.2. Sample collection

Hong Kong has limited agricultural land, with only 7 km^2, which is only sufficient to supply about 1.6% of fresh vegetables consumed in the territory (AFCD, 2024). Yet, the HKSAR Government has Accredited Farms Scheme and Organic certification in collaboration with the Agriculture, Fisheries and Conservation Department and the Vegetable Marketing Organization (VMO) to help the local farmers to establish good farming practices and to better market their vegetables in order to maintain some food security. A total of twenty-eight green-stem IA samples farmed in dry-land (G) and twenty white- stem IA samples farmed in wet-land (W), were collected from the local farms in Hong Kong, China. In detail, the organic G (GO, $n = 14$) and organic W (WO, $n = 10$), were cultivated in the local farms with Hong Kong Organic Resource Centre (HKORC) Certification and provided by the VMO, while the conventional G (GF, n = 14) and conventional W (WF, n = 10) were planted in Accredited Farms and purchased from VMO regulated markets. The mentioned certification and accreditation ensure the authenticity and reliability of the samples used in the study. It is also noted that Hong Kong's small territory and localized farm lands contribute to minimizing variations in factors such as rainfall and soil characteristics, making the observed differences in the study more relevant to the cultivation practices. The summary of the collection information was shown in the supplementary material (Table S1).

2.3. Metabolomics analysis

2.3.1. Quality control (QC) sample preparation

The quality control (QC) samples were prepared for monitoring the extraction efficiency and the instrumental stability by mixing two grams of each freeze-dried IA samples. The QC sample extraction and redissolution procedures were the same as those followed for the entire sample batch, as described in Section 2.3.2. Before analysing the sample batch, a QC sample was injected five times to confirm the repeatability of the UPLC-MS. During the sample batch analysis, a QC sample was analyzed for every five sample runs to monitor the stability of the UPLC-MS throughout the data acquisition process.

2.3.2. Sample preparation

After sample collection, 100 g of samples were immediately cleaned, homogenized and freeze-dried. A modified extraction method (Gevi, Fanelli, Zolla, & Rinalducci, 2019) was applied to extract the metabolites and inactivate the enzymes. The freeze-dried sample powder was precisely weighed at 100 ± 5 mg into 2 mL microcentrifuge tubes and subsequently vortex-mixed with 1400 μL methanol. Then, the samples were centrifuged at 14000 rpm for 10 min at 4 °C after incubation at 70 °C for 10 min. Liquid-liquid extraction was used to remove the lipid component in the samples, which cannot be well separated in the aqueous-based HILIC column condition. The 750 μL water and 375 μL chloroform were vortex-mixed with 350 μL supernatant in the glass test tube. The upper aqueous layers were collected in microcentrifuge tubes after centrifugation at 14000 rpm for 10 min at 4 °C. Then, the aqueous layer was dried under a nitrogen stream and then re-dissolved in 150 μL of pre-chilled HILIC solvent (85% *v*/v acetonitrile: 15% v/v water with 10 mM ammonium acetate) with a 1 ppm internal standard (cholic acid-d_4 and tryptophan-d_5). The supernatant was transferred into an HPLC vial for HILIC-UPLC-Orbitrap-MS analysis after centrifugation at 14,000 rpm at 4 °C for 15 min.

2.3.3. UPLC-Orbitrap-MS data acquisition

The metabolite was separated by Thermo UltiMate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Merck SeQuant Z.I.C. cHILIC column (100 mm × 2.1 mm, 3 μm) with Merck SeQuant Z.I.C. cHILIC guard column (2.1 mm × 20 mm) (Merck, Darmstadt, Germany) at 60 °C column temperature. The mobile phase consisted of 10 mM ammonium acetate (pH 3) in water (A) and 85% *v*/v acetonitrile (B). The metabolites in 3 μL injection sample were eluted from the column at a flow rate of 0.3 mL/min and a sample chamber temperature of 4 °C using a gradient mode as the following: 0–0.5 min, 98% B; 0.5–7.5 min, 98–70% B; 7.5–8 min, 70–50% B; 8–9.5 min, 50% B; 9.5–11 min, 50–98% B; 11–15 min, 98% B.

The untargeted metabolomics platform used was the Thermo UltiMate 3000 UHPLC system coupled Thermo Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) using heated electrospray ionization (H-ESI). The MS parameters were listed as follows: spray voltage, 3500 V for (+)ESI and 2300 V for (−)ESI; nebulizer auxiliary gas, 10 arbitrary units; sheath gas, 35 arbitrary units; sweep gas, 0 arbitrary units; ion transfer tube temperature of 350 °C and vaporizer temperature of 300 °C. The mass range was set at 70–1200 *m/z* with 120,000 mass resolutions. The maximum injection time was set as 100 ms with 2.0×10^5 automatic gain control target.

2.3.4. UPLC-Orbitrap-MS data pretreatment and analysis

The UPLC-Orbitrap-MS data were processed with Progenesis QI (version 2.0; Nonlinear Dynamics) to pick, align and deconvolute the extracted ion chromatographic peaks. To remove the systematic variations in different sample injections, for each extracted feature, an in-house Python program was developed to fit peak areas of QC samples and injection orders using a cubic spline and divide all peak area values of the feature by the cubic spline calculated values. The cubic spline has the advantage of flexibility in fitting any shapes of variations so that systematic variations, either derived from the instrument or samples, could be effectively removed. The peak filtering criteria was set as the coefficient of variation (CV) below 20% in all QC samples and the highest peak mean in the QC or biological samples. Extended Statistical tool (EZinfo v2.0 software, Waters Corp.) was used to perform the multivariate statistical analysis. The scaling mode applied in the multivariate statistical analysis was unit variance. The potential marker selection was based on the variable importance in the projection (VIP, threshold ≥1.0) and jackknife confidence. After multivariate statistical analysis, potential markers were identified by MS/MS with the METLIN tandem mass spectrometry database (https://metlin.scripps.edu/), RefMetaPlant database (https://www.biosino.org/RefMetaDB/) and commercial metabolite standards.

2.4. Statistics analysis

Statistically significant differences in potential markers among the sample groups were analyzed using a two-tailed, unpaired Student *t*-test with *p*-values using GraphPad Prism 5.02 (San Diego, CA, USA). *p* values ≤0.05 were considered statistically significant. Principal component analysis (PCA) was performed on data matrix which was standardized with mean zero and variance one in advance. The orthogonal projections to latent structures discriminant analysis (OPLS-DA) was performed with leave-one-out cross-validation and pareto scaling conducted using the Python package *pypls* (https://github.com/Omicometrics/pypls). A permutation test with 10,000 permutations was then employed to estimate the significance of the OPLS-DA model, using Q2 as the metric.

3. Results and discussion

3.1. Metabolomic profiles of water spinach

A wide variety of water-soluble metabolites, ranging from primary to secondary metabolites, was detected in the aqueous extracts of IA

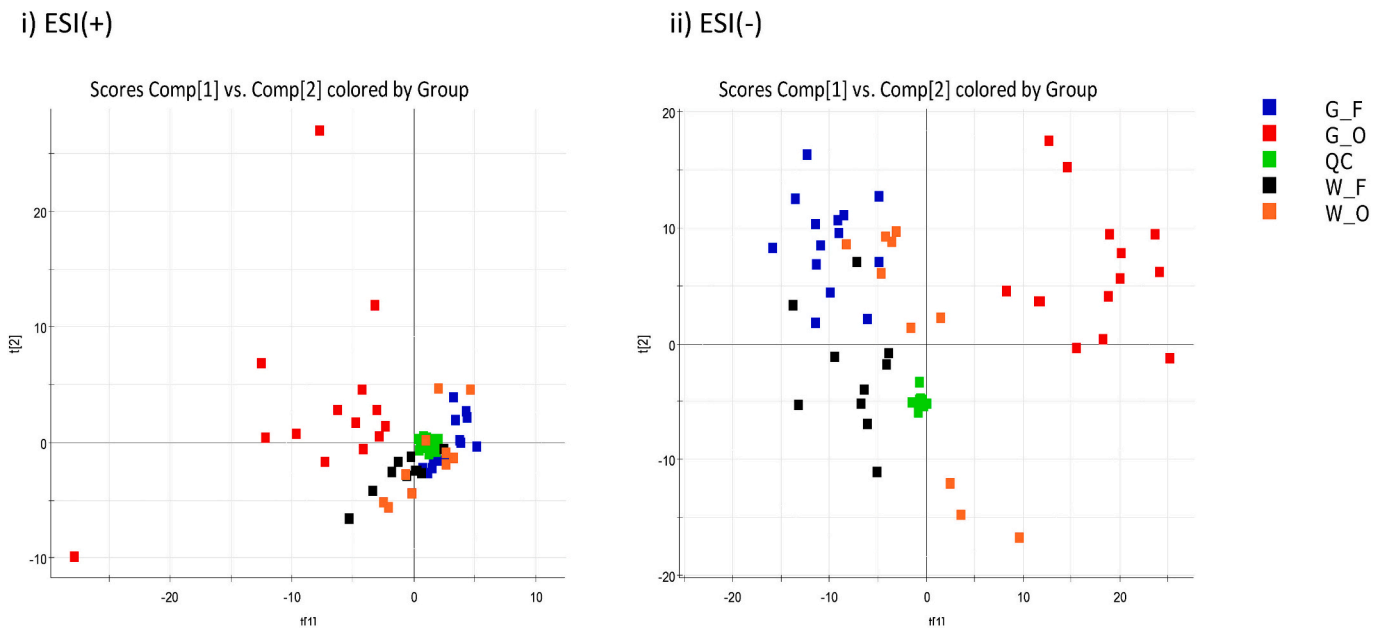

Fig. 1. PCA score plots of all water spinach samples obtained from i) ESI(+)-MS and ii) ESI(−)-MS.

samples to investigate the impact of environmental conditions (Fig. S1). Endogenous plant metabolites are categorized into primary and secondary metabolites (Adetunji et al., 2021). Primary metabolites are the essential components formed in the metabolism that are directly involved in plant growth, development and reproduction (Rojas, Senthil-Kumar, Tzin, & Mysore, 2014). Numerous primary metabolites detected in IA included amino acids, purine and its derivatives, organic acids, tricarboxylic acids, and simple sugars such as monosaccharides, disaccharides, and sugar alcohols. Secondary metabolites are specific chemicals, modified from primary metabolites, that plants produce in response to the environmental stress (Erb & Kliebenstein, 2020; Teoh, 2015). The secondary metabolites found included vitamins, flavonoids and phenylpropanoids. These metabolites, found in the aqueous extracts of IA, were subjected to multivariate analysis and further validated using commercially available standards. Thus, the potential markers annotated in our study have been achieved by the highest level of identification according to the Metabolomics Standards Initiative (MSI), referred to as level 1 compound identification (Sumner et al., 2007).

3.2. *Reliability of the metabolomics models and markers selection*

The metabolomics profiles acquired from positive and negative electrospray ionization (ESI) mode of HILIC-UPLC-Orbitrap-MS (HILIC-MS) were plotted using principal components analysis (PCA) (Fig. 1). The PCA score plots revealed that the QCs samples exhibited high levels of aggregation, demonstrating excellent repeatability and stability of the untargeted metabolomics platforms throughout the experiment. The robust metabolomics models fully support the study's findings, pointing out that the observed difference between groups is primarily driven by biological variations, such as cultivars and farming methods.

Orthogonal partial least squares discriminant (OPLS-DA) analysis with leave-one-out cross-validation (Fig. S2 to S4) was applied, which included comparisons between IA cultivars (G vs W) as well as between organic and conventional farming methods within each cultivar (GO vs GF and WO vs WF). The results were summarized in Table 1, and all metabolomics models showed over 80% correct assignment of the samples in cross-validated score plots. The performance of (−)ESI mode was generally better than (+)ESI mode. In (−)ESI mode, >97% correct sample assignment was achieved in all metabolomics models. When comparing the cultivar in (+)ESI mode, 95% and 86% correct sample assignment were obtained in W and G, respectively. GF and WF in (+)ESI mode also achieved 100% correct assignment in comparison with their organic farming counterparts, while the GO and WO attained 95% and 80% correct samples assignment, respectively. The permutation plots of permutation tests, each of which derived from 10,000 random permutations, consistently displayed negative Q2 intercepts, indicating that the observed segregation was not a result of overfitting.

The MS peak features with high covariance and correlation were screened out based on variable importance in the projection (VIP) scores and jackknife confidence analysis. By comparing two cultivars in the OPLS-DA plot, 239 and 182 MS peak features from the (+)ESI and (−)ESI modes had VIP scores >1, respectively. The Jackknife resampling analysis revealed that both the features of 126 and 54 MS peaks could withstand the analysis. Subsequently, the 54 MS peak features were provisionally annotated, resulting in the identification of 12 MS peak features at MSI level 1. These findings are summarized in Table 2. When comparing GO and GF, the results showed that 295 MS peak features in the (+)ESI mode and 236 MS peak features in the (−)ESI mode had VIP scores >1 in the OPLS-DA plot. A total of 102 and 177 MS peak features remained after undergoing Jackknife resampling analysis; 81 MS peak features were then tentatively annotated, and 26 MS peak features could be confirmed with MSI level 1 identification and summarized in Table 3. Finally, the comparison between WO and WF in the OPLS-DA plot indicated that 268 and 114 MS peak features in (+)ESI and (−)ESI

Table 1
Results of the differentiation powers of the metabolomics models for cultivars and farming practices authentication.

Platform		IA Cultivars		Farming practices in G		Farming practices in W	
		G	W	GO	GF	WO	WF
Metabolomics	HILIC-ESI(+)-MS	86%	95%	95%	100%	80%	100%
	HILIC-ESI(−)-MS	97%	100%	100%	100%	100%	100%

Table 2
A summary of the diffential metabolites between green stem water spinach (G) and white stem water spinach (W).

Class	R.T. (min)	Adduct form	Theoretical m/z	Observed m/z	Mass difference (ppm)	Level of annotation	Higher concentration detected	p value	Fold change (to W)
2-Hydroxy-3-(beta-D-xylopyranosyloxy)benzoic acid	4.04	[M + H]-	285.0610	285.0618	−2.66	2	G	< 0.0001	0.32
3-beta-D-Galactosyl-sn-glycerol	4.76	[M + H]-	253.0923	253.0930	−2.99	2	G	0.0020	0.31
3-O-(alpha-L-rhamnopyranosyl)-D-ribitol	4.73	[M + H]-	297.1186	297.1194	−2.84	2	G	< 0.0001	0.35
7-hydroxycoumarin	1.05	[M + H]-	161.0245	161.0244	−0.62	1	G	< 0.0001	0.48
Adenosine	2.24	[M + H]-	266.0895	266.0900	1.88	1	G	< 0.0001	0.55
alpha-ketoglutarate	5.26	[M + H]-	145.0142	145.0141	−0.69	1	W	0.0006	2.66
Aminoadipic acid	6.48	[M + H]-	160.061	160.0614	−2.50	1	G	0.0117	0.39
Catechin/epicatechin	4.76	[M + H]-	289.0712	289.0698	4.84	1	G	< 0.0001	0.30
Glutaric acid	1.64	[M + H]-	131.0351	131.0350	−0.76	1	G	0.0011	0.69
Isorhamnetin	2.13	[M + H]+	317.0661	317.0649	3.78	1	G	0.0063	0.79
Jasmonic acid	1.02	[M + H]-	209.1183	209.1184	−0.39	1	G	0.0001	0.35
Nicotinamide	1.26	[M + H]+	123.0553	123.0552	−0.81	1	G	0.0229	0.74
Salicylic acid	1.33	[M + H]-	137.0245	137.0243	−1.46	1	G	0.0156	0.80
Shikimic acid	3.53	[M + H]-	173.0456	173.0456	0.00	1	W	< 0.0001	1.36
Xylitol	3.64	[M + H]-	151.0613	151.0611	−1.32	1	W	0.0006	1.49

modes had VIP scores >1, respectively. After implementing the Jackknife resampling analysis, a total of 54 and 95 MS peak features were preserved, and 45 MS peak features were putatively annotated. 11 MS peak features were successfully recognized at MSI level 1 identification and summarized in Table 4.

3.3. Differences in metabolome of two IA cultivars

The metabolite profiles of the two cultivars did not exhibit a considerable difference in PCA score plots, except for GO. In both the (+)ESI and (−)ESI modes, GO showed a clear separation from the other groups along PC1 (Fig. 1). Additionally, in the PCA score plots of (−)ESI mode, the organic IA, WO, and GO noticeably distinguished from their conventional counterparts, WF and GF, along PC1. This revealed that fertilization had a significant impact on the metabolite profile of IA compared to genetic differences and other abiotic stresses, such as water stress and hypoxia. With the help of leave-one-out cross-validation in OPLS-DA analysis (shown in Fig. S2), 12 metabolites, out of 54 putatively annotated MS peak features, were identified with significant differences between two cultivars, G and W. Most of these markers are highly associated with environmental stress, suggesting that the wetland and dry-land cultivated water spinach withstand different abiotic and biotic stresses.

Secondary metabolites are a specific class of molecules produced by plants to combat biotic and abiotic stress due to the absence of the circulation of immune cells. >50,000 secondary metabolites with pharmacological activities have been discovered in plants (Teoh, 2015). In our study, four secondary metabolites derived from shikimic acid; salicylic acid, 7-hydroxycoumarin, catechin and isorhamnetin, were more abundant in G, while shikimic acid, the precursor of the secondary metabolites and a connecting metabolite between primary and secondary metabolic pathway in plants, showed lower abundance in G. The shikimic acid pathway is the common route for aromatic amino acid biosynthesis in bacteria, fungi, yeasts, plants and algae and is involved in protein synthesis, vitamins, and electron-carrier compounds such as cofactors, and ubiquinone. However, there was no significant difference in aromatic amino acids between W and G. The accumulation of shikimic acid in G may be attributed to its conversion to other secondary metabolites like salicylic acid and isorhamnetin. This could explain why G has a stronger flavour and bitter taste than W. Previous studies have pointed out that flavonoids and isoflavonoids are the sources of the bitter taste in various plants by activating the bitter taste receptors, including salicylic acid (Jones, 2011), 7-hydroxycoumarin (Rimal, Sang, Dhakal, & Lee, 2020), catechin (Narukawa et al., 2011) and isorhamentin (Yang et al., 2022).

The results of a higher concentration of salicylic acid in G could reveal a slight increase in the growth rate of W when compared with that of G in the cultivation field (Edie & Ho, 1969). Salicylic acid is a phytohormone influencing the plant growth and development (de Torres Zabala, Bennett, Truman, & Grant, 2009). Recent studies have shown that the concentration of salicylic acid is inversely proportional to the growth rate of plants. In a study on *Arabidopsis* plant, the growth rate of roots and aerial parts was reduced by upregulating the salicylic acid-inducible DOF (DNA binding with one finger) transcription factor OBP3 (Kang & Singh, 2000). In contrast, Arabidopsis NahG transgenic plants' growth rate increased by reducing the salicylic acid content (Abreu & Munné-Bosch, 2009). In addition, shikimic acid was also found in higher concentration in W. Regarding its application as a growth promoter in cultivation, shikimic acid has also been used to increase the growth rate and phase change of tomato (Al-Amri, 2013) and Chinese jujube (Meng et al., 2023).

Adenosine is the precursor of adenosine triphosphate (ATP), and its accumulation may suggest energy metabolism dysfunction in the presence of abiotic stresses. Based on the results obtained, adenosine was found in higher concentration in G. In contrast, alpha-ketoglutarate was found in higher concentrations in W. Our trend aligns with a previous

Table 3
A summary of the diffential metabolites between organic farming of green stem water spinach (GO) and conventional farming green stem water spinach (GF).

Class	R.T. (min)	Adduct form	Theoretical m/z	Observed m/z	Mass difference (ppm)	Level of annotation	Higher concentration detected	p value	Fold change (to F)
2-HydroxyIsocaproic acid	2.04	[M + H]-	131.0708	131.0712	−3.05	1	GF	0.0007	0.76
2-Hydroxy-3-methylbutyric acid	2.68	[M + H]-	117.0552	117.0556	−3.68	2	GO	0.0003	0.49
4-aminobutyric acid	6.97	[M + H]-	102.0561	102.0559	1.96	1	GF	0.0025	0.51
5'-S-Methylthioadenosine	1.40	[M + H]+	298.0974	298.0972	0.67	1	GF	< 0.0001	0.68
Aminoadipic acid	6.48	[M + H]-	160.0610	160.0614	−2.50	1	GF	< 0.0001	0.22
Aspartic acid	7.55	[M + H]-	132.0302	132.0301	−0.76	1	GF	< 0.0001	0.39
Coumaroyltyramine	1.10	[M + H]+	284.1287	284.1276	3.87	1	GF	0.0203	0.37
Dihydroxybenzoic acid	2.21	[M + H]-	153.0188	153.0193	−3.37	2	GF	< 0.0001	0.96
Glutamic acid	6.97	[M + H]-	146.0459	146.0458	−0.68	1	GF	0.0022	0.29
Glutamine	6.09	[M + H]+	147.0763	147.0763	0.00	1	GO	0.0348	1.70
Glutaric acid	1.64	[M + H]-	131.0351	131.0350	−0.76	1	GF	< 0.0001	0.57
Hypoxanthine	2.39	[M + H]+	137.0458	137.0457	−0.73	1	GO	0.0001	1.42
Isoquercitrin	3.59	[M + H]-	463.0883	463.0887	0.86	1	GO	0.0275	1.69
Isorhamnetin	2.13	[M + H]+	317.0661	317.0649	3.78	1	GF	0.0119	0.55
Jasmonic acid	1.02	[M + H]-	209.1183	209.1184	−0.39	1	GO	0.0001	3.04
Leucine	3.48	[M + H]-	130.0874	130.0872	−1.54	1	GO	0.0021	4.27
Mesaconic acid	3.51	[M + H]-	129.0188	129.0192	−3.10	1	GF	0.0041	0.47
Methionine	4.02	[M + H]+	150.0582	150.0581	−0.67	1	GO	0.0140	3.65
N4-Acetylsulfamethazine	3.09	[M + H]-	319.0865	319.0861	1.21	2	GF	0.0201	19.43
Oxaloglutarate	7.98	[M + H]-	203.0192	203.0199	−3.56	2	GF	0.0258	0.23
Phenylalanine	3.33	[M + H]+	166.0862	166.0861	−0.60	1	GO	0.0375	0.73
Protocatechuic acid	1.42	[M + H]-	153.0193	153.0193	0.00	1	GF	0.0002	0.35
Quinic acid	3.59	[M + H]-	191.0556	191.0562	−3.14	1	GF	< 0.0001	0.38
Salicylic acid	1.33	[M + H]-	137.0245	137.0243	−1.46	1	GF	0.0140	0.66
Shikimic acid	3.53	[M + H]-	173.0456	173.0456	0.00	1	GF	0.0002	0.69
Threonine	5.71	[M + H]+	120.0654	120.0654	0.00	1	GO	0.0308	2.19
Tryptophan	3.76	[M + H]+	205.0971	205.0969	−0.98	1	GO	0.0456	0.97
Tyrosine	4.99	[M + H]+	182.0811	182.081	−0.55	1	GO	0.0409	2.67
Uric acid	4.52	[M + H]-	167.0211	167.021	−0.60	1	GO	0.0396	1.35
Xylitol	3.64	[M + H]-	151.0613	151.0611	−1.32	1	GF	0.0039	0.76

Table 4
A summary of the diffential metabolites between organic farming of white stem water spinach (WO) and convention farming white stem water spinach (WF).

Class	R.T. (min)	Adduct form	Theoretical m/z	Observed m/z	Mass difference (ppm)	Level of annotation	Higher concentration detected	p value	Fold change (to F)
3-methyl adenine	3.41	[M + H]+	150.0774	150.0772	−1.33	1	WO	0.0057	3.84
Caftaric acid	7.25	[M + H]-	311.0403	311.0413	−3.22	1	WO	< 0.0001	3.63
Daphnetin	1.38	[M + H]-	177.0188	177.0194	−3.39	1	WO	0.0029	1.33
Fumaric acid	5.89	[M + H]-	115.0038	115.0035	−2.61	1	WO	0.0153	1.30
Gentisic acid	2.21	[M + H]-	153.0188	153.0193	−3.35	2	WO	0.0015	1.95
Homoisocitrate	5.11	[M + H]-	205.0348	205.0355	−3.26	2	WO	0.0221	2.41
Isoleucine	3.65	[M + H]+	132.1018	132.1018	0.00	1	WO	0.0299	2.51
Isorhamnetin	2.13	[M + H]+	317.0661	317.0649	3.78	1	WF	0.0002	0.30
Jasmonic acid	1.02	[M + H]-	209.1183	209.1184	−0.39	1	WF	0.0051	0.50
Kaempferol	1.17	[M + H]-	285.0405	285.0408	1.05	1	WF	0.0002	0.50
Maltose	6.00	[M + Na]+	365.1054	365.1046	−2.19	1	WO	0.0039	4.19
Mannose	4.02	[M + H]-	179.0562	179.0561	−0.56	1	WO	0.0101	2.03
Tryptamine	2.47	[M + H]+	161.1073	161.1072	−0.62	1	WF	0.0435	0.42

study on metabolic changes associated with flooding stress in soybean (Komatsu et al., 2011), in which a set of 81 mitochondria-associated metabolites suggested a boost in concentrations of metabolites involved in respiration and glycolysis coupled with the depletion of free ATP.

Salicylic acid, also called 2-hydroxybenzoic acid, is a well-known pain killer used several thousand years ago by our ancestors, and its importance in plant growth has been proven in the past few decades. It is an essential endogenous phytohormone with a broad range of biological functions, such as acting as a signaling molecule (Peng, Yang, Li, & Zhang, 2021) and inducing the response to pathogens (Koo, Heo, & Choi, 2020; Vlot, Dempsey, & Klessig, 2009). Numerous plant studies have shown a positive correlation between endogenous salicylic acid content and resistance responses against bacteria and virus infection. Klessig (Klessig, Choi, & Dempsey, 2018) found that the endogenous salicylic acid content increased 20-fold in the *Tobacco mosaic* virus-infected resistant tobacco plants. Salicylic acid treatment also triggered resistance responses and defense activities in various crops against disease-caused pathogens, for example, *Fusarium oxysporum* (Daw, Zhang, & Wang, 2008; Li et al., 2022), *Magnaporthe grisea* (Mandal, Mallick, & Mitra, 2009) and *Xanthomonas spp* (Song et al., 2022; Srinivasa et al., 2022; Sun et al., 2022).

Xylitol, a pentose sugar alcohol, followed the same trend as the secondary metabolites in IA. Lee (Lee et al., 2016) reported that xylitol content in family *Rosaceae* plants was influenced by the fungal pathogen *Gymnosporangium asiaticum*. The tendency of the change depended on the pathogen species. The infected Rosaceae plant *Pyrus pyrifolia* increased the xylitol content, while the *Chaenomeles sinensis* and *Crataegus pinnatifida* infection reduced the xylitol content in Rosaceae plants. Therefore, a higher content of xylitol in G reveals that biotic stress in dry-land cultivation was generically different from that in wet-land cultivation.

Nicotinamide, known as vitamin B3, is an essential human nutrient. Long-term inadequate intake of vitamin B3 is related to the risk of pellagra (Kohlmeier, 2015). Green leafy vegetables are one of the dietary sources of nicotinamide (Marcus & Coulston, 1990), especially for vegetarians. A significant difference in nicotinamide content was found between two cultivars of IA. This may induce a higher functional value of G than W. Meanwhile, nicotinamide is a stress-related metabolite in the plants. The main source of endogenous nicotinamide in plants is the decomposition of nicotinamide adenine dinucleotide (NAD) by poly (ADP-ribose) polymerases, which is activated by DNA-strand breakage under oxidation stress (Matsui, Yin, Yamanaka, Iwasaki, & Ashihara, 2007). For example, the content of nicotinamide in plant tissue was raised after UV-B exposure, which caused DNA damage (Berglund, Kalbin, Strid, Rydström, & Ohlsson, 1996). Exogenous nicotinamide protected plants against the oxidation stress from 2,2′-azobis(2-amidinopropane) dihydrochloride (Berglund, Wallström, Nguyen, Laurell, & Ohlsson, 2017). This suggests that G withstands higher oxidative stress in dry-land cultivation.

3.4. Discrimination between organic and conventional green stem water spinach

A distinctive distribution in OPLS-DA score plots was observed between organic and conventional green stem water spinach (Fig. S3). We identified 26 metabolites with significant differences between GO and GF, summarized in Table 2. The multivariate analysis revealed that farming practices greatly influenced the formation of amino acids in G. Amino acids accounted for 9 out of 26 metabolites that could differentiate the farming practices of G. Previous metabolomics studies on wheat (Bonte et al., 2014), pepper (Novotná et al., 2012), and potato (Shepherd et al., 2014) reported significant differences in amino acids between organic and conventional farming. In the present study, five essential amino acids (EAAs), including leucine, phenylalanine, tryptophan, threonine and methionine, were upregulated in GO. It indicates

that organic G has a higher nutritional value than conventional since the EAAs cannot be synthesized in the human body and are obtained only through our diet. Genetic engineering is the most effective technology applied to enhance the EAAs production in plants (Beauregard & Hefford, 2006; Ufaz & Galili, 2008). Our results suggest that organic farming is an alternative way to improve the abundance of EAAs in crops without using genetic engineering.

Glutamic acid and glutamine are key intermediates in nitrogen assimilation that converts the inorganic nitrogen compounds absorbed from the environment to amino acids in plant (Liu, Hu, & Chu, 2022). GO exhibited a decrease in glutamic acid and its downstream metabolite gamma-aminobutyric acid (GABA), while glutamine significantly accumulated, indicatind downregulation of glutamate synthase (GOGAT) in GO. Several studies have consistently reported similar regulatory effects under ammonium exposure in plants (Setién et al., 2013; Song, Yang, & Jeong, 2022; Sun et al., 2020) and microalgae (Wang et al., 2019). These findings suggest that ammonium accumulation occurred in G, potentially due to the presence of ammoniacal nitrogen species in organic fertilizers (Frerichs, Daum, & Pacholski, 2020). The soil ammonium accumulation inhibits plant growth by reducing the nitrogen fixation (Song, Yang, & Jeong, 2022). Our results suggest that using non-ammoniacal nitrogen species bearing organic fertilizer that avoids ammonium accumulation in the soil could potentially improve the growth of G in organic farming.

Salinity is one of the harshest environmental factors reducing crop production over the long term (Shrivastava & Kumar, 2015). Organic farming is one of the proposed agricultural methods to minimizing soil salinity (Wichern, Islam, Hemkemeyer, Watson, & Joergensen, 2020). In G, the opposite regulation of two sulphur-bearing metabolites, methionine and its downstream metabolite 5'-*S*-methylthioadenosine (MTA), in the methionine salvage pathway indicated lower soil salinity in organic farming. Several studies on salt stress also found a reduction of methionine content under increased soil salinity conditions (Farhangi-Abriz & Ghassemi-Golezani, 2016; Xie et al., 2020). Besides, The accumulation of MTA, a by-product in the polyamine biosynthesis (Watanabe, Chiba, & Hirai, 2021), reflects high salinity and the elevated polyamine levels, high salt pressure indicator (Chen, Shao, Yin, Younis, & Zheng, 2019; Mutlu & Bozcuk, 2005), in conventional farming.

Under conventional farming, G accumulated shikimic acid while experiencing a reduction in its downstream aromatic amino acids, including tyrosine, tryptophan, and phenylalanine. This trend may relate to glyphosate use or contamination in conventional farming. Glyphosate, high effective and non-selective in weed control, has gained widespread use as the most extensively employed non-selective herbicide globally (Duke, 2018), including its usage in Hong Kong (Tsui, 1999). Intensive studies reported that glyphosate promotes shikimic acid accumulation and inhibits aromatic amino acid biosynthesis in many plants and bacteria (Malalgoda, Ohm, Howatt, Green, & Simsek, 2020; Pline, Wilcut, Duke, Edmisten, & Wells, 2002; Yokoyama, de Oliveira, Kleven, & Maeda, 2021). This suggests that organic G produced in Hong Kong might be relatively safer than conventional G in terms of herbicide contamination.

The secondary metabolites contents has been the major focus area in previous studies on the discrimination of organic farming due to their unique phytoactivities and bioactivities (Mihailova et al., 2021). Previous studies on Danish wheat (Weesepoel et al., 2016) and apple (Juozas et al., 2018), also exhibited the significantly lower content of protocatechuic acid (Weesepoel et al., 2016) and higher isoquercitrin content (Juozas et al., 2018) in organic farming. Our study aligns with th literaturethat protocatechuic acid showed significant lower in GO, while isoquercitrin was upwnregulated.

3.5. Discrimination between organic and conventional white stem water spinach

A distinctive distribution in OPLS-DA score plots was observed among organic and conventional white stem water spinach (Fig. S4). We identified and summarized 12 metabolites with significant differences between GO and GF in Table 4.

Soil salinity, a leading cause of osmotic stress (Hui & Jian-Guo, 2010), degrades soil quality in farming and reduces crop yield, particularly in wetland cultivation (Lim et al., 2020). Numerous researchers reported that organic amendments in high salt soil protect nutrient cycle-related microorganisms (Evelin, Devi, Gupta, & Kapoor, 2019; Naveed et al., 2021; Wichern et al., 2020) and enhance crop growth (Park et al., 2022). Three osmoprotective metabolites, including maltose, mannose and fumaric acid, were elevated in WO. Maltose, the primary soluble sugar from starch degradation in chloroplasts, adjusts the cytosol osmosis in roots and leaves (Thalmann & Santelia, 2017). Mannose also regulates water balance to tolerate osmotic stress in white

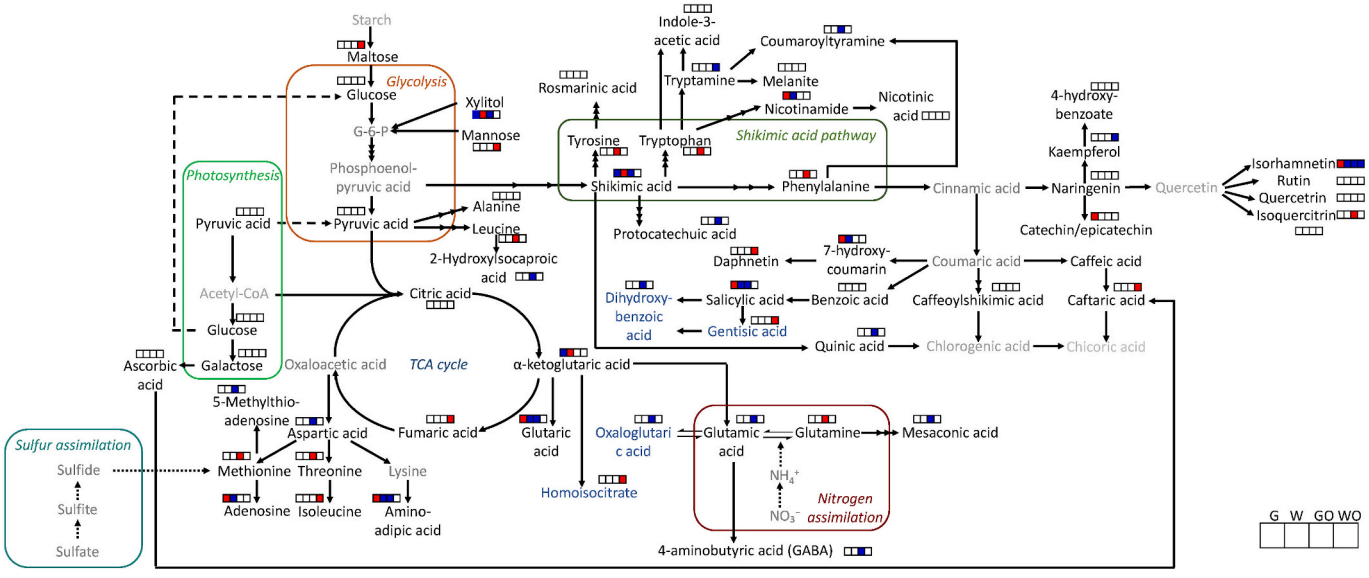

Fig. 2. The major metabolic alterations of water spinach in response to the cultivars and farming method. The black and blue colored metabolites were confirmed at MSI levels 1 and 2, respectively. The increase and decrease in metabolites are highlighted in red and blue, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

clover (Zhao, Zeng, Li, & Peng, 2020). Fumaric acid, an organic acid, acts as an osmolyte in plant vacuoles to alleviate the osmatic stress damage (Sheng, Tang, Zhang, & Huang, 2011). The accumulation of these soluble organic metabolites suggests that IA has high salt tolerance with the aid of microorganisms under organic agriculture.

Tryptamine, a tryptophan-derived indole alkaloid, has broad biological and pharmaceutical significance for humans (Kousar, Anjuma, Jaleel, Khan, & Naseema, 2017). In the plant, tryptamine bridges primary and secondary metabolites (Negri, Commisso, Avesani, & Guzzo, 2021). It is synthesized through the tryptophan decarboxylation within the shikimic acid pathway and rapidly converts to melanite and serotonin. In WF, tryptamine was upregulated, whereas changes in upstream and downstream metabolites, including shikimic acid, tryptophan, indole-3-acetic acid, and melanite, were absent. Similar regulation also occurs in tobacco with overexpressing tryptophan decarboxylase. Surprisingly, this regulation induces anti-pest activities in *Nicotiana tabacum* and *Camptotheca acuminate* (Gill & Ellis, 2006; Poulsen, Goddijn, Hoge, & Verpoorte, 2005). This contradicts our hypothesis that the biotic stress in organic farming is higher than conventional farming due to the higher efficiency of synthetic pesticides. One possible reason is the biotic stress from pesticide-resistant pests. Under the overuse of pesticides (Hawkins, Bass, Dixon, & Neve, 2019) and climate change (Ma et al., 2021), over 580 species have been reported with insecticide resistance (Sparks & Nauen, 2015). Many common insect pests in crops have developed biocide resistance, including diamondback moth (Troczka et al., 2012), common fruit fly (Catania et al., 2004), beet armyworm (Wang et al., 2018) and two-spotted spider mite (Xu et al., 2018).

Kaempferol, a well-known plant functional flavonoid, is an antioxidant, antibacterial, insect repellent and abiotic stress mitigator (Shah & Smith, 2020). Kaempferol accumulation is triggered in response to numerous abiotic stress, including nutrient deficiency (Mosa, Ali, Ramamoorthy, & Ismail, 2022), water-logging (Khan, Ulrichs, & Mewis, 2011), high salinity (Jan et al., 2021), high daily photosynthetically active radiations (Trejo-Téllez et al., 2019) and heat stress (Jan et al., 2021).

In a previous study, kaempferol content in peppers showed a significant farming system × genotype interaction (Ribes-Moya et al., 2020). Most pepper cultivars had slightly higher kaempferol content in the conventional system. However, the trend was opposite or absent in other agricultural products, such as herbs (Hallmann & Sabała, 2020), tomatoes (Mitchell et al., 2007) and some leafy vegetables (Young et al., 2005). This suggests that kaempferol could be a potential marker for differentiating organic and conventional systems in some vegetables, although the direction of regulation depends on the species and cultivar.

3.6. Impact of organic farming on water spinach

Consumer attitudes and behaviours worldwide towards organic food have changed over the last decade, especially regarding its characteristics and consumption patterns. Due to the unavailability of simple chemical testing for organic food, documentary-based certification systems have emerged to inform consumers and protect food producers against fraud. Our study provides a starting point for understanding and systematically evaluating different cultivars and farming methods of water spinach. Fig. 2 summarizes the major metabolic alterations of water spinach in response to the cultivars and farming method. Both W and G possess an identical set of metabolites with varying abundances. Nonetheless, as shown by the results in the PCA plots, the impact of organic farming on the two water spinach cultivars varied, and the metabolite profile of G changed more compared to W. Consequently, distinct groups of organic practices-related metabolites were identified for both cultivars. G showed a unique change in amino acid profiles, while sugars and secondary metabolites were the major metabolite classes related to organic farming found in W.

In the comparison of biomarkers for cultivar distinction and organic farming authentication, it was found that seven metabolites exhibited certain differentiation power in both authentication purposes. These metabolites include aminoadipic acid, glutaric acid, isorhamnetin, jasmonic acid, salicylic acid, shikimic acid, and xylitol. These metabolites can serve as potential markers for distinguishing cultivars and authenticating organic origin in G, while only two of them have the ability to authenticate organic origin in W.

Isorhamnetin and jasmonic acid are the only metabolites found in two water spinach cultivars associated with the organic farming. WO and GO displayed low isorhamnetin content, while jasmonic acid accumulated in WF and GO. The environmental stress-induced isorhamnetin regulation in plants was seldom observed in literature. Isorhamnetin, a methylated quercetin, was documented to correlate with the precipitations of the wettest month negatively (Fang et al., 2022). Additionally, it has been found to possess inhibitory effects on lipid peroxidation (Singh, Arif, Bajguz, & Hayat, 2021). Although the reasons behind the downregulation of isorhamnetin in organic farming remain inadequately elucidated, this unique regulatory pattern suggests that isorhamnetin could serve as a valuable biomarker, reflecting the organic farming characteristics of water spinach.

Jasmonic acid (JA) is a well-known phytohormone in plant defense system (Li et al., 2022; Schuman & Baldwin, 2016) and plays a crucial role in responses to abiotic stress (Wang, Mostafa, Zeng, & Jin, 2021). The water stress is one of abiotic reason causing the accumulation of endogenous JA (Pedranzani, Sierra-de-Grado, Vigliocco, Miersch, & Abdala, 2007; Shan & Liang, 2010), however, the opposite trend was observed in our study that G from dry-land showed higher JA content compared to W from wet-land. This finding suggests that water stress may not be the primary factor contributing to JA accumulation in these IA cultivars. A water-stress study on *Pinus pinaster* from two Spainsh provenances proved that the JA regulation correlated to geographical origin and might correlated to diverse ecological conditions (Pedranzani et al., 2007). Instead of abiotic stress, plant JA level is highly sensitive to the biotic stress. Al-Zahrani proved the *Spodoptera exigua* attack in tomato and maize foliage indicated the JA accumulation (Al-Zahrani, Bafeel, & El-Zohri, 2020). A soil amendment study revealed the endogenous JA level in rice was affected by three key factors, including organic matter in the soil, pest infestation, and cultivars (Waqas et al., 2018). Two rice cultivars planted in the biochar-amended semi-hydroponic soil with white-backed plant hopper (WBPH) infestation exhibited higher JA levels, while the biochar-treated rice without WBPH infestation showed an opposite JA trend. In *Arabidopsis*, the JA upregulation also inducing the protection against *Botrytis cinerea* infection and cabbage looper (*Trichoplusia ni*) infestation (Chehab, Yao, Henderson, Kim, & Braam, 2012). This explaines the opposite JA regulation in water spinach cultivars may be related to pest infestation and genetic variation.

4. Conclusion

This study demonstrates that the untargeted metabolomic UPLC-MS analytical platform is valuable for authenticating the organic origins of IA. With the help of the integrated approach, we have revealed the influence of organic farming practices on a wide range of primary and secondary metabolites. A set of metabolites has been discovered as potential biomarkers in IA associated with organic farming. Isorhamnetin and jasmonic acid stand out as the distinctive marker that accurately reflects the organic farming practices employed for IA. The metabolite regulation trends of IA revealed that pest-resistance induced by synthetic pesticides is a possible future source of distinction between organic and conventional faming. Metadata about diseases and infections in field are necessary in future studies. Our results suggest that this integrated approach could potentially be applied to develop a routine analytical-based food traceability system for differentiating organic and conventional farming.

CRediT authorship contribution statement

Ka-Yi Man: Writing – original draft, Investigation, Formal analysis. **Chi-On Chan:** Writing – review & editing, Methodology, Formal analysis. **Siu-Wai Wan:** Formal analysis. **Kevin Wing Hin Kwok:** Methodology, Funding acquisition, Conceptualization. **Francesco Capozzi:** Writing – review & editing, Methodology. **Nai-ping Dong:** Validation, Supervision, Software, Data curation. **Ka-Hing Wong:** Writing – review & editing, Supervision, Resources, Conceptualization. **Daniel Kam-Wah Mok:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT 4.0 to improve readability and language. After using this service, the authors reviewed and edited the content and take full responsibility for the content of the publication.

Declaration of competing interest

The authors have no conflict of interests to declare.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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