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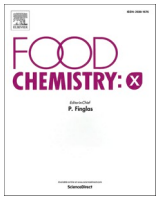

Comparison of the characteristics of phenolic compounds in Se-enriched kiwifruit and conventional kiwifruit

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ABSTRACT

While selenium (Se) fortification significantly influences plant secondary metabolism, there has been limited research on its effects on the phenolic profile of kiwifruit. This study aimed to characterize and compare the phenolic profile of Se-enriched and conventional kiwifruit. A total of 463 phenolic compounds were identified. Among these, nine common differential metabolites, including six flavonoids, two phenolic acids, and one lignan (massoniresinol), were identified between Se-enriched and conventional kiwifruit of the same variety. Further comparative analysis of diverse varieties indicated that massoniresinol was the sole differential metabolite. This metabolite was found to be significantly up-regulated in Se-enriched kiwifruit compared to conventional kiwifruit. In addition, correlation analysis showed that the total Se, phenol, and flavonoids content were highly significantly positively correlated with antioxidant activities in kiwifruit. These findings enhance our understanding of the phenolic composition in Se-enriched and conventional kiwifruit and provide a potential biomarker for distinguishing Se-enriched from conventional kiwifruit.

1. Introduction

Kiwifruit (*Actinidia deliciosa*), a nutrient-dense green fruit, is particularly rich in antioxidants including vitamin C, polyphenols, and flavonoids. These bioactive compounds have been shown to possess significant health-promoting properties, including anti-inflammatory, anti-diabetic, and immunomodulatory effects (Cheng, Guo, Du, & Zhou, 2022). Selenium (Se), an essential trace element for humans and animals, exerts its biological functions primarily through the synthesis of Se-containing proteins. These proteins play critical roles in antioxidant defense, anti-aging mechanisms, and immune regulation (Chen, Zhao, & Zhang, 2021). Se-enriched kiwifruit is a type of functional food rich in vitamin C, polyphenols, and Se. Consequently, it is considered a promising dietary source of Se.

Selenium fortification affects the secondary metabolism of plants and their products. Previous studies have demonstrated that Se exerts a considerable regulatory influence on the levels of sulfur compounds, nitrogen compounds, terpenoids, phenols, and flavonoids in plants (Gui et al., 2022). For instance, the application of a spray containing 50 mg/L sodium selenite increased vitamin C and flavonoid content in winter jujube by 20.94 % and 43.48 %, respectively (Jing et al., 2017). Additionally, the supplementation with 10–100 mM sodium selenate enhanced anthocyanins, flavonoids, and polyphenols in strawberry fruits (Mimmo et al., 2017). The application of Se nanoparticles led to an enhancement in the levels of carbohydrates, amino acids, and volatile organic compounds in grains, concurrently regulating the phenylpropanoid pathway to improve their nutritional quality. (Zhou et al., 2022). Furthermore, the capsaicinoid pathways in peppers were activated by Se nanoparticles, which enhanced the capsaicin synthesis, thereby promoting the accumulation of secondary metabolites and antioxidants (Li et al., 2020). Secondary metabolites have been shown to endow plants with physiological functions such as antioxidant and disease resistance. Treatment with 150 mg/L of sodium selenite in fertilizer could improve antioxidant enzyme activity to enhance plant stress resistance and citrus quality (Wen, Wang, Gao, Wu, & Huang, 2021). Furthermore, Se nanoparticles activated phenylalanine biosynthesis and branched-chain fatty acid pathways, thereby promoting the accumulation of jasmonic acid and cucurbitacin in cucumber plants, thus enhancing their resistance to *Botrytis cinerea* (Jia et al., 2023). Consequently, Se not only elevates the levels of secondary metabolites but also

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synergistically improves fruit quality through dual mechanisms of antioxidant enhancement and pathogen resistance.

Research on Se fortification of kiwifruit has primarily concentrated on its effects on kiwifruit quality and function. There is a scarcity of studies investigating the function of Se-enriched kiwifruit. Notably, supplementation with Se-enriched kiwifruit has been demonstrated to reduce blood lipids and protect hepatic function in hyperlipidaemic mice (Zhang et al., 2021), as well as increase the abundance of potentially beneficial gut microbiota such as *Bacteroides*, *Bifidobacterium*, and *Clostridium sporogenes* (He et al., 2023). The majority of the research has focused on the effects of Se fortification on kiwifruit quality. The organic Se in the Se-enriched kiwifruit consisted mainly of Se-protein and Se-polysaccharide, which accounted for 62.32 % of the total Se content (Xu et al., 2018). The organic Se content in kiwifruit treated with 50 mg/L sodium selenite increased by 9.04 times compared to untreated controls. The antioxidant activity of kiwifruit showed a highly significant positive correlation with the organic Se concentration (Lu, Mu, Wang, & Huang, 2024). In addition, sodium selenite supplementation increased soluble solids and vitamin C content while reducing titratable acid content in kiwifruit (Deng et al., 2018). At a concentration of 15 mg/L, Se nanoparticles significantly enhanced the expression levels and enzymatic activities of antioxidant enzymes in kiwifruit, while concomitantly up-regulating genes associated with secondary metabolism (Liu et al., 2024). Moreover, spraying at a concentration of 2 mg/L Se led to a significant delay in the senescence of kiwifruit, accompanied by a promotion of antioxidant activity, as evidenced by an increase in phenylalanine deaminase activity and a decrease in polyphenol oxidase activity (Ghafouri, Razavi, Arghavani, & Gheshlaghi, 2022). Nevertheless, there remains limited research comparing the characteristics of phenolic compounds in Se-enriched kiwifruit and conventional kiwifruit from a metabolomics perspective. The evaluation and identification of Se-enriched kiwifruit are the prerequisites for effective utilization of Se-enriched kiwifruit resources.

Therefore, we aimed to characterize the phenolic compounds present in Se-enriched kiwifruit and compare them with those found in conventional kiwifruit. In our study, two types of Se-enriched kiwifruit and three types of conventional kiwifruit were selected as experimental materials. The content of total Se, total phenolic, total flavonoids, antioxidant activities, and phenolic profiles of Se-enriched kiwifruit and conventional kiwifruit were analyzed. Multivariate statistics were then employed to identify differential metabolites between Se-enriched and conventional kiwifruit samples. This study enhances a comprehensive understanding of the phenolic profile in Se-enriched versus conventional kiwifruit and provides a potential marker to identify and differentiate Se-enriched kiwifruit varieties from their conventional counterparts.

2. Materials and methods

2.1. Kiwifruit samples

Kiwifruit variety “Yanhuang Jinguo” Se-enriched kiwifruit (YH-Se) and conventional kiwifruit (YH), “Jinlei No.1” Se-enriched kiwifruit (JL-Se) and conventional kiwifruit (JL), and conventional kiwifruit “Jianxiang” (JX), harvested in Suizhou (Hubei Province, China), Qianjiang (Chongqing Province, China), and Enshi (Hubei Province, China), respectively, were obtained at commercial maturity in October 2023. The kiwifruit were then transported to the laboratory, where they were washed, sliced, and freeze-dried. The lyophilized samples were then ground and stored at −80 °C for subsequent testing and analysis.

2.2. Total selenium content analysis

The total selenium content (TSC) was determined by atomic fluorescence spectrometry with hydride generation according to GB 5009.93–2017. Freeze-dried kiwifruit (0.2 g) was digested with HNO_3 (7 mL) in a closed microwave system. The conditions applied were as follows: the temperature was maintained at 120 °C for 5 min, raised to 150 °C for 3 min, raised to 200 °C for 10 min, and finally cooled to 55 °C for 20 min. After digestion, the acid was evaporated to 1 mL at 240 °C. Then, 50 % hydrochloric acid (5 mL) was added, and the solution was evaporated to reduce its volume to 1 mL. The solution was then diluted with an appropriate amount of solvent to attain a final volume of 10 mL. The analysis of TSC was then conducted using a liquid chromatography-atomic fluorescence spectrometer (LC-AFS 8530) (Beijing Haiguang Instrument Co., Ltd., Beijing, China.) following microwave digestion.

2.3. Total phenol and flavonoids content analysis

0.2 g of kiwifruit freeze-dried powder was mixed with 5 mL 70 % methanol that had been preheated at 70 °C in a water bath. The mixture was then extracted at 70 °C for 10 min, followed by centrifugation at 4000 rpm for 10 min. The supernatant was transferred to a 10 mL volumetric flask, and the residue underwent two sequential re-extractions with 5 mL of 70 % methanol. The combined extracts were then brought to a final volume of 10 mL with fresh 70 % methanol.

2.3.1. Total phenolic content

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu assay (GB/T8313–2018). The extract solution (2 mL), deionized water (4 mL), and freshly prepared 10 % Folin reagent (1 mL) were added sequentially, shaken evenly, and 7.5 % sodium carbonate solution (3 mL) was added immediately. Absorbance was measured at 765 nm after 2 h under conditions that protected the samples from light. The TPC in the samples was calculated and expressed as gallic acid equivalents (GAE mg/g).

2.3.2. Total flavonoids content

The total flavonoids content (TFC) was determined according to the aluminum chloride colorimetric method (GB/T20574–2006). The extract solution (5 mL), deionized water (20 mL), 5 % (*w/v*) sodium nitrite solution (1.5 mL), and 10 % (w/v) aluminum nitrate solution (1.5 mL) were added sequentially. Following a 10-min incubation period, the absorbance was measured at 510 nm. The TFC in the samples was calculated and expressed rutin equivalents (RE mg/g).

2.4. Preparation and extraction procedures for metabolomic analysis

The extraction, identification, and quantification of the metabolites of kiwifruit were conducted by Met Ware Biotechnology Ltd. (Wuhan, China). The powder of each sample (50 mg) was immersed in 70 % aqueous methanolic solution pre-cooled to −20 °C (1200 μL) to extract the metabolites. The mixture was vortexed for 30 s at 30-min intervals for a total of six times and then filtered the supernatant after centrifugation (12,000 *g*, 3 min). The resulting liquid is used for subsequent metabolic analysis. The detailed method for quantitative analysis of metabolites was described by Wang et al. (2023).

2.5. Determination of antioxidant activities

2.5.1. Ferric reducing antioxidant power (FRAP)

For both the control and test samples, 180 μL of the FRAP reagent was combined with 5 μL of distilled water or kiwifruit extract (as described in section 2.3). The mixture was then subjected to incubation at 37 °C for 3–5 min prior to the measurement of the optical density (OD) value at 593 nm.

2.5.2. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

0.9 mL of a freshly prepared DPPH methanol solution (0.1 mM) was mixed with 0.1 mL of kiwifruit extract (as described in section 2.3). The same volume of methanol was used to control. Subsequent to this, the optical density (OD) was measured at a wavelength of 515 nm following a 30-min incubation period in the dark for 30 min at room temperature.

2.5.3. Radical scavenging activity of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)

The $ABTS^+$ working solution is prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) and storing it for 16 h in a dark place at room temperature. The $ABTS^+$ solution was then diluted to an absorbance of 0.750 ± 0.025 at 734 nm using 0.1 M sodium phosphate buffer (pH 7.4). Subsequently, 2 mL of the $ABTS^+$ solution and 2 mL of kiwifruit extract (as described in 2.3) were added in succession. Absorbance was measured at 734 nm after incubation for 6 min in the dark at room temperature.

2.6. Data analysis

Results are presented as mean ± standard deviation from three replicates. The analysis of differences among group means was conducted using a one-way analysis of variance (ANOVA) followed by Duncan's test ($p < 0.05$) with SPSS 24.0 software (SPSS Inc., Chicago, IL, USA). The data was visualized using Origin Pro 8.6 (Origin Lab Corporation, Northampton, MA, USA) and GraphPad Prism v6.01 (GraphPad Software, San Diego, CA, USA). The integration of the fold change (FC) values from the orthogonal partial least squares-discriminant analysis (OPLS-DA) model with the variable importance in projection (VIP) values was utilized for the identification of differentially abundant metabolites for each comparison group. The screening criteria employed were as follows: $|\log_2 FC| \geq 1$ and $VIP \geq 1$.

3. Results and discussion

3.1. Total selenium, phenolic, and flavonoids content in Se-enriched and conventional kiwifruit

From the results shown in Fig. 1A, the TSC of Se-enriched kiwifruit was found to be significantly higher than that of conventional kiwifruit ($p < 0.05$). The TSC of JL-Se and YH-Se were found to be 0.14 μg/g and 0.17 μg/g, respectively. This represents an increase of almost 161.29 % and 169.96 % over JL and YH, respectively. It is hypothesized that the Se content of kiwifruit may be influenced by soil composition, variety, fertilizers, climate, and management practices (Li & Ji, 2011). The TPC of YH-Se, JL-Se, YH, JL, and JX kiwifruit was 10.49 mg/g, 12.65 mg/g, 10.47 mg/g, 11.02 mg/g, 11.33 mg/g, respectively (Fig. 1B). The TFC was 8.36 mg/g, 9.24 mg/g, 6.78 mg/g, 5.89 mg/g, and 5.27 mg/g, respectively (Fig. 1C). The TPC and TFC of JL-Se samples were significantly higher than those of conventional JL samples ($p < 0.05$). The TPC and TFC of YH-Se were higher than those of conventional YH kiwifruit, though not significant. A previous study found that phenolic compounds can be enhanced at suitable Se concentrations (Dall'Acqua, Ertani, Pilon-Smits, Fabrega-Prats, & Schiavon, 2019). The TFC of Se-enriched kiwifruit was higher than that of conventional kiwifruit ($p < 0.05$). The TSC of JL-Se and YH-Se were higher than JL and YH, which might help to synthesize flavonoids. It has been demonstrated that Se increases the level of flavonoids accumulation in plants by regulating the expression of key genes in the flavonoids biosynthetic pathway (such as chalcone synthase and dihydroflavonol-4-reductase) (Lammi & Qu, 2018). The antioxidant properties of Se have been demonstrated to play an important role in protecting total phenolic and flavonoids antioxidants, which have been shown to mitigate damage to plant cells caused by free radicals (Gao et al., 2017). In addition, Se plays a regulatory role in the activity of crucial enzymes associated with the metabolic pathways of these compounds, including phenylalanine decarboxylase and tyrosine hydroxylase (Ledley, 1986), thereby enhancing the plant's antioxidant system, which in turn promotes the accumulation of flavonoids and other beneficial compounds (Ueno, Sekine-Suzuki, Shimokawa, & Nakanishi, 2018). The TSC of YH-Se was higher than that of JL-Se ($p < 0.05$), but the TPC and TFC of YH-Se were lower than that of JL-Se ($p < 0.05$). It indicated not only Se fortification but also the variety, growing region, and environmental conditions that affect the TPC and TFC in kiwifruit (Li et al., 2022).

3.2. Metabolites identified in Se-enriched and conventional kiwifruit

To further investigate the differences in the specific polyphenols and flavonoids between the Se-enriched and conventional kiwifruit, the phenolic profiles were determined using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and the self-built database MWDB (Met Ware Biological Science and Technology Co., Ltd., Wuhan, China) (Zhang, Qiu, Tan, Xiao, & Mei, 2020). A total of 463 metabolites were detected in our kiwifruit samples (Supplementary Table S1). These included 189 flavonoids (40.82 %), 175 phenolic acids (37.80 %), 71 lignin and coumarin (15.33 %), 23 tannins (4.97 %) and five other substances (1.08 %) (Fig. 2A). Of the 189 flavonoids identified, 58 were flavonols, 42 were flavones, 27 were flavanols, 21 were flavanones, 14 were chalcones, nine were flavanonols, six were anthocyanidins and 12 were other flavonoids. Flavanols and other flavonoids were found to be the most relatively abundant flavonoids. The six most abundant flavonoids were identified as 3,5,3′,4′,5′-penta-hydroxyflavan-7-gallate, epicatechin, epicatechin gallate, epigallocatechin-3-O-gallate, gallocatechin-3-O-gallate and 7-methoxy-3-[1-(3-pyridyl)methylidene]-4-chromanone. Among them, the relative abundance of epigallocatechin-3-O-gallate and gallocatechin-3-O-gallate were the highest. Among the 175 phenolic acids examined, the top four in terms of content were grevilloside F, diisobutyl phthalate, dibutyl phthalate, and butylisobutyl phthalate. Notably, grevilloside F, often designated as a glycosidic form of ferulic acid, exhibited the highest content among these compounds. According to the findings of preceding studies on kiwifruit, the presence of significant polyphenolic compounds, including catechin, epigallocatechin, and ferulic acid, has been documented (Hettihewa, Hemar, & Rupasinghe, 2018).

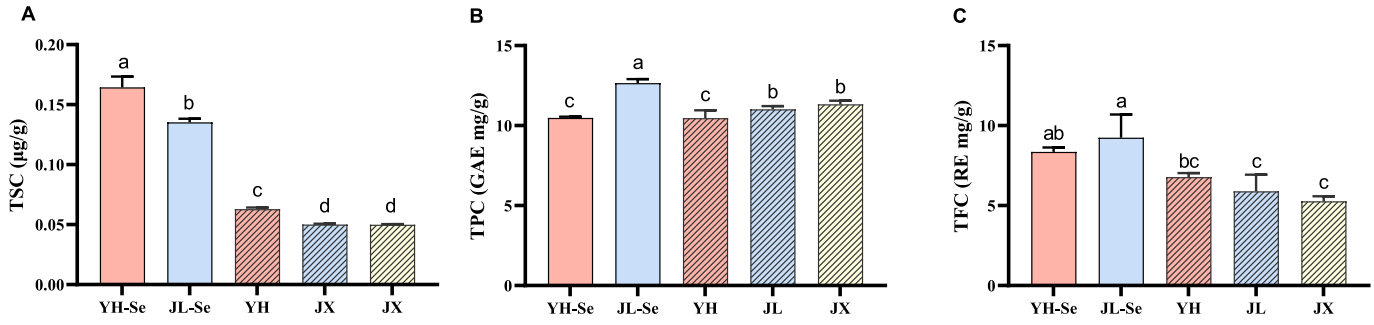

Fig. 1. Content of total (A) selenium, (B) phenol, and (C) flavonoids in kiwifruit. Note: YH-Se and YH: Yanhuang Jinguo Se-enriched kiwifruit and conventional kiwifruit, YH-Se and YH: Jin lei No.1 Se-enriched kiwifruit and conventional kiwifruit, JX: Jianxiang conventional kiwifruit. Values with different letters are significantly different ($p < 0.05$) from each other.

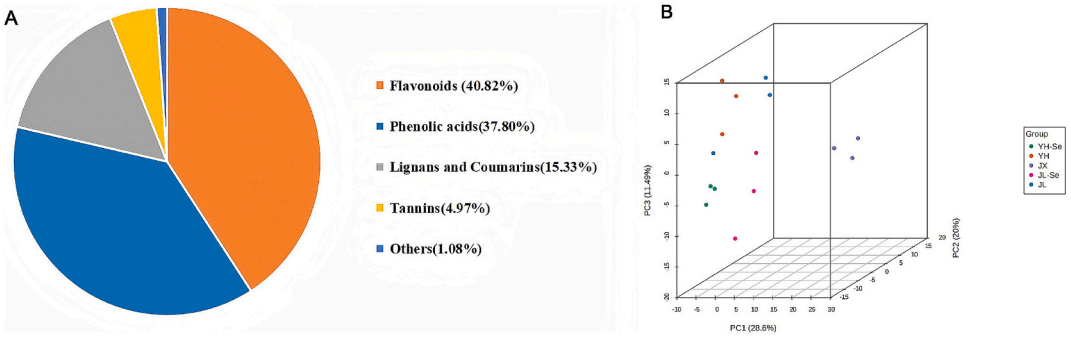

Fig. 2. (A) Classification of 463 metabolites in kiwifruit based on the self-built database MWDB. (B) Principal component analysis (PCA) score 3D plot of kiwifruit samples based on the relative variation of the metabolites. Note: YH-Se and YH: Yanhuang Jinguo Se-enriched kiwifruit and conventional kiwifruit, YH-Se and YH: Jin lei No.1 Se-enriched kiwifruit and conventional kiwifruit, JX: Jianxiang conventional kiwifruit.

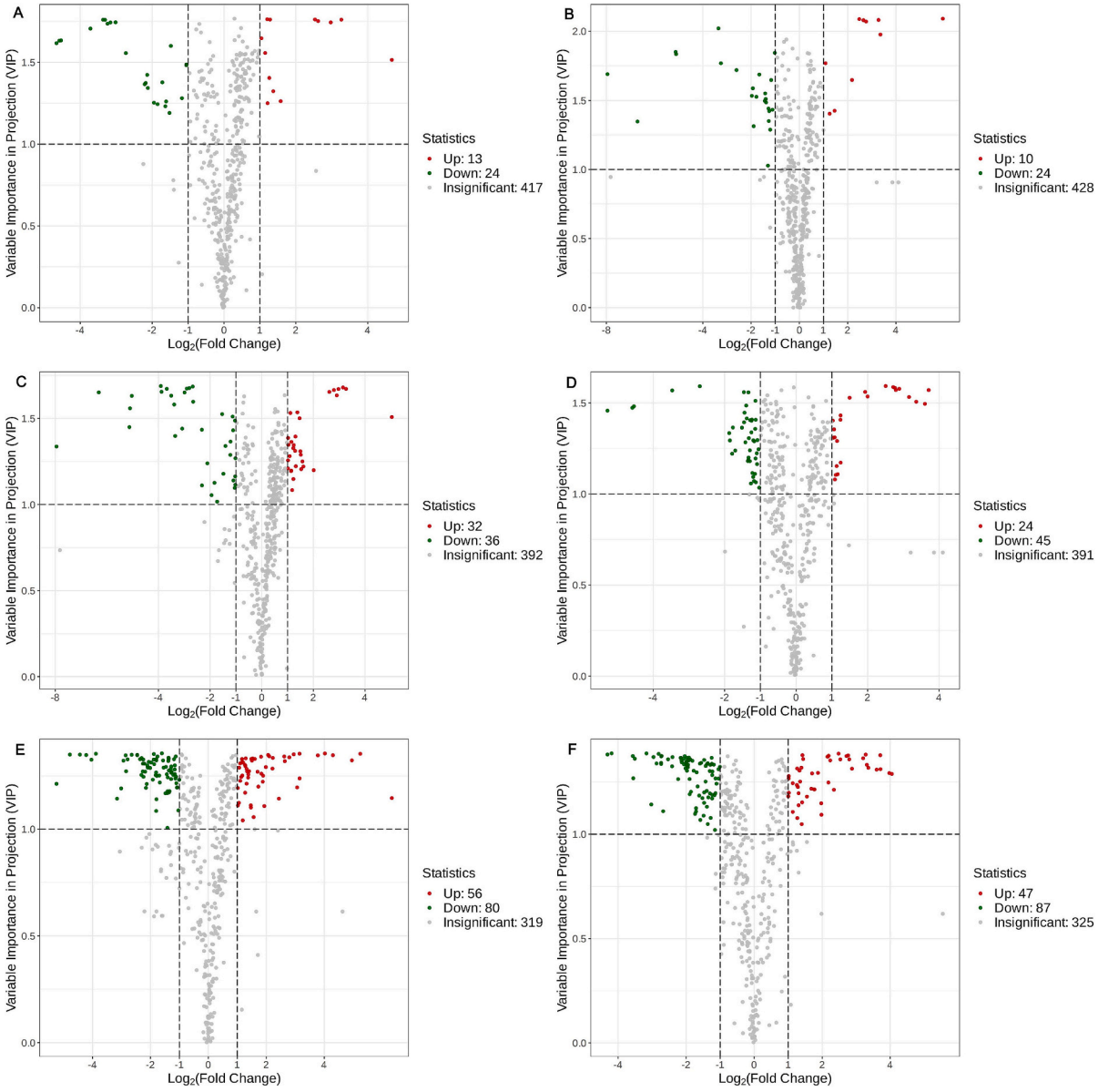

Fig. 3. Volcano plots of the differential metabolites between different kiwifruit samples. (A) YH-Se VS YH, (B) JL-Se VS JL, (C) YH-Se vs JL, (D) JL-se VS YH, (E) YH-Se vs JX, (F) JL-Se vs JX. Red and green dots indicate upregulated and down-regulated metabolites, respectively; gray dots represent the metabolites showing no significant difference. Note: YH-Se and YH: Yanhuang Jinguo Se-enriched kiwifruit and conventional kiwifruit, YH-Se and YH: Jin lei No.1 Se-enriched kiwifruit and conventional kiwifruit, JX: Jianxiang conventional kiwifruit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Differences in metabolite composition between Se-enriched and conventional kiwifruit

Principal component analysis (PCA) was performed to better understand the variation in metabolites between the Se-enriched kiwifruit and conventional kiwifruit. Principal components(PC) 1, PC2, and PC3 explained 28.6 %, 20 %, and 11.49 % of the total variance across all samples, respectively (Fig. 2B). In the PCA score plot, points that are closer together indicate more similar metabolic profiles. The PCA score plot demonstrated that a clear distinction could be made between all samples. This indicates the presence of substantial disparities in the metabolic profiles of Se-enriched and conventional kiwifruit. The samples were divided into two distinct regions. The first region consists of YH-Se, YH, JL-Se, and JL, indicating that they exhibit metabolic similarities. Among them, Se-enriched kiwifruits (YH-Se, JL-Se) separate from their conventional counterparts (YH, JL) along the horizontal axis (PC1), which accounts for the greatest variation (28.6 %). This finding suggests that Se treatment has a substantial impact on the metabolic profile of kiwifruit. The second region is a sample of JX. All samples from the JX region cluster tightly in the upper right quadrant, forming a distinct group, suggesting that the JX kiwifruit is characterized by its unique phenolic metabolism.

The orthogonal partial least squares-discriminant analysis (OPLS-DA) was performed to further investigate the compositional differences in flavonoids and phenolic acids composition between Se-enriched and conventional kiwifruit. The identification of differential metabolites was based on predefined thresholds of $|\log_2 FC| \geq 1$ and Variable Importance in Projection (VIP) ≥ 1 as detailed in Supplementary Table S2. The results of screening for differential metabolites between groups are shown in Fig. 3(A-F). The horizontal axis is the fold change in the difference in relative content ($\log_2$ FC) and the vertical axis is the significance level (VIP). Red and green dots indicate up-regulated and down-regulated metabolites, respectively, while gray dots represent the metabolites that do not show a significant difference. The analysis identified 37 differential metabolites (13 up-regulated, 24 down-regulated) between YH-Se and YH (Fig. 3A), 34 differential metabolites (10 up-regulated, 24 down-regulated) between JL-Se and JL (Fig. 3B), 68 differential metabolites (32 up-regulated, 36 down-regulated) between YH-Se and JL (Fig. 3C), 69 differential metabolites (24 up-regulated, 45 down-regulated) between JL-Se and YH (Fig. 3D), 136 differential metabolites (56 up-regulated, 80 down-regulated) between YH-Se and JX (Fig. 3E), and 134 differential metabolites (47 up-regulated, 87 down-regulated) between JL-Se and JX (Fig. 3F).

A comparative analysis was conducted of the flavonoids and phenolic acids present in Se-enriched kiwifruit and conventional kiwifruit of the same variety. The identification of nine compounds was achieved as the common differential metabolites between YH-Se and YH (Fig. 3A) and JL-Se and JL (Fig. 3B), including six flavonoids (6-geranylnaringenin, epigallocatechin, gossypetin-3-O-rutinoside, leucocyanidin, myricetin-3-O-β-D-glucoside, naringenin-7-O-rutinoside-4'-O-glucoside), two phenolic acids (3-O-digalloyl quinic acid, methyl gallate) and one lignan (massoniresinol). Among them, six flavonoids were down-regulated, while massoniresinol was up-regulated in Se-enriched kiwifruit compared to the same variety of conventional kiwifruit. 6-Geranylnaringenin, a kind of flavonoids, has been shown to possess a variety of biological effects, including antioxidant and anti-inflammatory properties (Venturelli et al., 2016). Epigallocatechin has been shown to possess significant antioxidant activity, as well as the capacity to treat allergic reactions and possess anti-cancer properties (Gąsowska-Bajger & Wojtasek., 2024). Leucocyanidin, a naturally occurring compound, has been identified as having multiple bioactive properties, including antiviral and antifungal effects as well as anti-inflammatory and antioxidant activities (de Souza Farias, da Costa, & Martins, 2023). In addition, the two phenolic acids were up-regulated in the YH-Se group in comparison to the YH group and down-regulated in the JL-Se group in comparison to the JL group. The analysis of differential metabolites in Se-enriched kiwifruit and conventional kiwifruit of the same variety suggests that Se enrichment has a significant impact on the secondary metabolism of kiwifruit. This may result in the production or disappearance of certain characteristic metabolites (Ahmad et al., 2024).

To clarify whether there are also common differential metabolites among different varieties and to explore whether such differences are related to varieties, further screening of the nine common differential metabolites was conducted with the differential metabolites of YH-Se and JL (Fig. 3C). This process led to the identification of six common differential metabolites (6-geranylnaringenin, epigallocatechin, leucocyanidin, myricetin-3-O-β-D-glucoside, naringenin-7-O-rutinoside-4'-O-glucoside, massoniresinol). Further comparison and screening between JL-Se and YH (Fig. 3D) identified five common differential metabolites: 6-geranylnaringenin, epigallocatechin, myricetin-3-O-β-D-glucoside, naringenin-7-O-rutinoside-4'-O-glucoside, and massoniresinol. Of these, massoniresinol was found to be up-regulated in different varieties of conventional kiwifruit, while the other substances were downregulated. This result indicates that specific metabolic pathways may be influenced by Se, and this effect is consistent across diverse kiwifruit varieties.

To further extend the scope of the study, a comparison was made between the metabolites of Se-enriched kiwifruit and those of the JX variety of conventional kiwifruit. The results demonstrated that a greater number of differential metabolites were identified in the comparisons between YH-Se and JX (Fig. 3E) and JL-Se and JX (Fig. 3F), which reflected the inherent metabolic differences among the different varieties and corresponded to the results in the PCA plot (Fig. 2B). By comparing and screening YH-Se and JX (Fig. 3E), two common differential metabolites (myricetin-3-O-β-D-glucoside*; massoniresinol) were identified. Furthermore, a comparison of JL-Se vs JX (Fig. 3F) yielded the identification of a single common differential metabolite: massoniresinol.

A comprehensive comparison of Se-enriched and conventional kiwifruit was undertaken, which revealed massoniresinol as the common differential metabolite. In comparison to all varieties of conventional kiwifruit, massoniresinol was found to be up-regulated in Se-enriched kiwifruit. The pharmacological potential of massoniresinol was further substantiated by Schumacher et al., who isolated this metabolite from valerian (Schumacher et al., 2002). This finding suggests that massoniresinol may serve as a potential marker to differentiate between Se-enriched and conventional kiwifruit, independent of variety.

3.4. Antioxidant activities of Se-enriched and conventional kiwifruit

A significant disparity in metabolites was identified between Se-enriched and conventional kiwifruit, with these phenolic compounds potentially being closely associated with the antioxidant activities of kiwifruit. Consequently, the antioxidant activities of Se-enriched and conventional kiwifruit were measured. As shown in Fig. 4 (A-C), the antioxidant activities (FARP, ABTS, DPPH) of the Se-enriched JL kiwifruit (JL-Se) were significantly higher than that of the conventional kiwifruit. A similar trend was observed for the ABTS antioxidant activities of the Se-enriched YH kiwifruit (YH-Se), which also exhibited significantly higher levels compared to the conventional kiwifruit. This finding indicates that Se-enriched kiwifruit exhibits superior antioxidant activity in comparison to conventional kiwifruit. This phenomenon may be attributed to the elevated level of Se, total phenol, and flavonoids present in Se-enriched kiwifruit. In addition, the unique compound was only found in Se-enriched kiwifruit, massoniresinol, which has been reported to have some antioxidant activities (Li, Feng, Zhang, & Cui, 2015). However, no significant difference was observed between the DPPH and FARP antioxidant activity of Se-enriched YH kiwifruit (YH-Se and conventional YH) kiwifruit, which is consistent with the content of total phenol and flavonoids in kiwifruit. Variations in antioxidant activities might be related to the different mechanisms for antioxidant

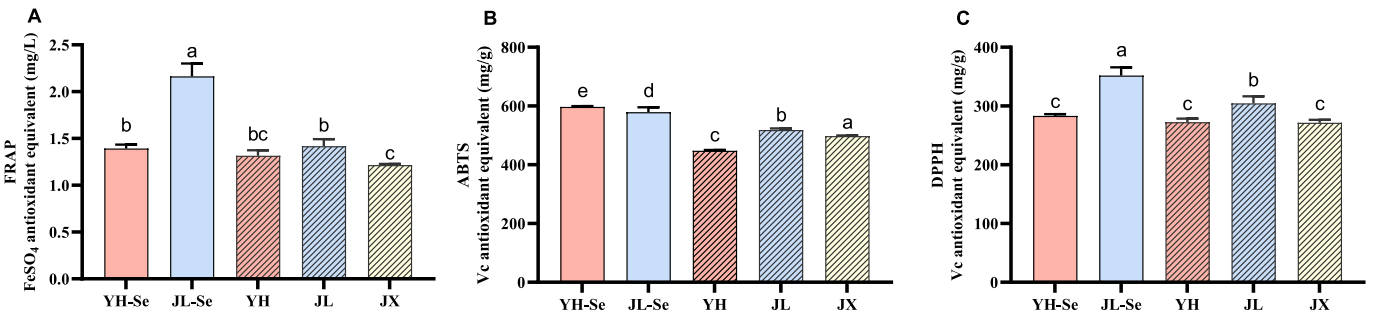

Fig. 4. The antioxidant activities of kiwifruit. (A) FRAP, (B) ABTS,(C) DPPH. Note: YH-Se and YH: Yanhuang Jinguo Se-enriched kiwifruit and conventional kiwifruit, YH-Se and YH: Jin lei No.1 Se-enriched kiwifruit and conventional kiwifruit, JX: Jianxiang conventional kiwifruit. Values with different letters are significantly different ($p < 0.05$) from each other.

activities (Narváez-Ortiz et al., 2018). Se has been observed to bind to proteins, forming Se-proteins that have been shown to possess potent antioxidant properties within the context of plant metabolism. Specifically, Se-proteins have been shown to enhance the clearance of enzymatic (superoxide dismutase, catalase, and ascorbate peroxidase) and non-enzymatic (ascorbic acid, flavonoids, and tocopherols) compounds against reactive oxygen species (ROS) to protect plant tissues from oxidative stress (Wang et al., 2022). In addition, research has demonstrated that Se can enhance the antioxidant activities of fruit by reducing phenolase activity and reducing lipid peroxidation (Wang et al., 2023). These findings further confirm the importance of Se in plant antioxidant systems.

3.5. Correlation analysis of antioxidant activity and antioxidant compounds

The Pearson correlations were established to comprehend the impact of the TSC, TPC, and TFC on the antioxidant activities of kiwifruit.

As shown in Fig. 5, the TSC exhibited remarkably strong positive correlations with TFC ($r^2 = 0.89$) and ABTS ($r^2 = 0.86$); the TPC showed very strong positive correlations with DPPH ($r^2 = 0.86$) and FRAP ($r^2 = 0.85$); the TFC showed very strong positive correlations with FRAP ($r^2 = 0.84$), ABTS ($r^2 = 0.81$) and DPPH ($r^2 = 0.75$). These results indicate a robust correlation between TSC, TPC, and TFC with antioxidant activities. Additionally, a notable association is observed between TSC and TFC. Se, phenolic compounds, and flavonoids have been identified as significant important antioxidant substances in fruits with strong antioxidant activity (Wilczyńska & Żak., 2024; Pinto et al., 2023). Previous studies have identified a significant correlation between Se and flavonoids with antioxidant activity (Niu et al., 2022). In addition, Se has been demonstrated to promote the synthesis of flavonoids, thereby increasing the content of flavonoids in plants (Deng et al., 2022). Consequently, it can be hypothesized that Se may influence the content of phenolic compounds, which in turn affects the antioxidant activities of the kiwifruit.

4. Conclusion

In our study, the levels of TSC, TPC, TFC, antioxidant activities, and phenolic profiles were determined in various Se-enriched kiwifruit and conventional kiwifruit samples. The TSC, TPC, and TFC of the Se-enriched kiwifruit were found to be higher than those of the conventional kiwifruit. The results demonstrated that Se enrichment significantly influenced the secondary metabolism of kiwifruit. The presence

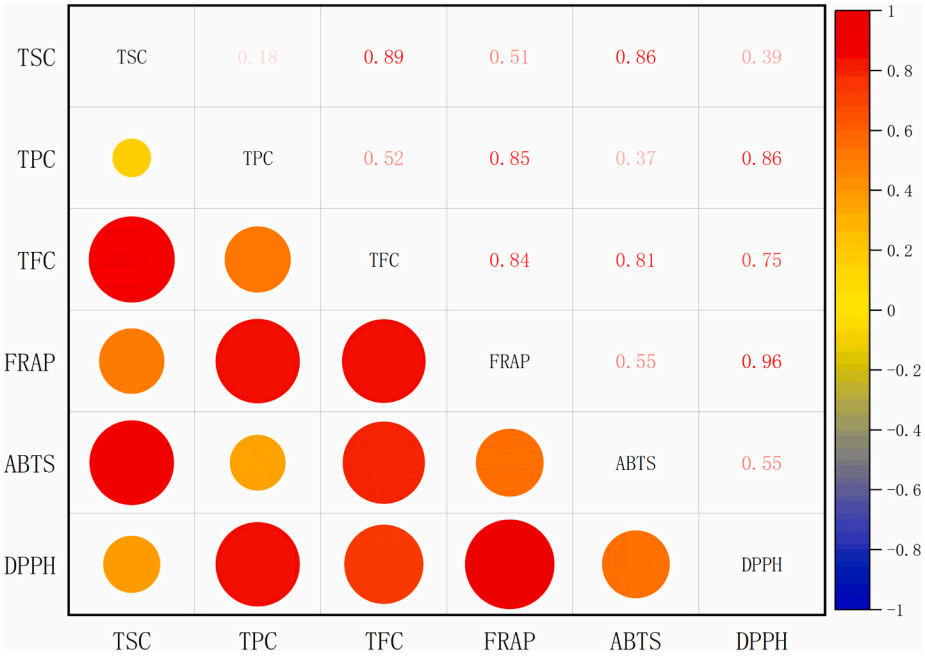

Fig. 5. Correlation heatmap between TSC, TPC, TFC, and antioxidant activity in Se-enriched kiwifruit. Note: Red in the heatmap indicates a positive correlation and blue indicates a negative correlation. Darker colors indicate stronger correlations. Note: TSC: Total selenium content, TPC: Total phenolic content, TFC: Total flavonoids content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of massoniresinol, a metabolite identified as a common differential metabolite between Se-enriched and conventional kiwifruit of different varieties, was identified as a potential marker to distinguish between the two. However, the kiwifruit varieties utilized in our study are limited. Consequently, further data from a substantial number of diverse kiwifruit varieties is required for the validation of massoniresinol as a potential marker to differentiate Se-enriched kiwifruit from conventional kiwifruit. Additionally, the antioxidant activity of Se-enriched kiwifruit is significantly higher than that of conventional kiwifruit. Correlation analysis demonstrated a close relationship between the antioxidant activity of kiwifruit and the levels of TSC, TPC, and TFC. It was suggested that the enhancement of antioxidant activity in Se-enriched kiwifruit is the result of a collaborative regulation by Se-enrichment and the secondary metabolism, particularly the polyphenol and flavonoid metabolic processes. This study provides a comprehensive understanding of the difference in polyphenolic composition between Se-enriched and conventional kiwifruit, and also a potential approach for differentiating Se-enriched from conventional kiwifruit in terms of phenolic profile, which is helpful for the effective utilization of Se-enriched kiwifruit resources.

CRediT authorship contribution statement

Ye Zheng: Writing – original draft, Methodology, Investigation, Data curation. **Fang Luo:** Writing – original draft, Methodology, Data curation. **Li Wang:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Shuyi Li:** Validation, Supervision, Resources, Investigation. **Xuehua Wang:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Zhenzhou Zhu:** Validation, Supervision, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102453.

Data availability

Data will be made available on request.

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