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Influence of ripening stage and meteorological parameters on the accumulation pattern of polyphenols in greengages (*Prunus mume* Sieb. Et Zucc) by widely targeted metabolomic

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ABSTRACT

Greengage is a Chinese traditional medicine food homology plant that contains abundant polyphenols. Greengages with different levels of maturity have different medicinal functions and applications. Therefore, this study comprehensively analysed the phenolic compounds in greengage of whole mature stage. Notably, the influence of climate conditions on greengage polyphenol synthesis was deeply explored. The polyphenols of greengages were profiled based on the widely targeted metabolomic technology using ultra-performance liquid chromatography–electrospray ionisation triple quadrupole mass spectrometry (UPLC–QTRAP–MS/MS). A total of 214 polyphenols were detected, covering 11 subclasses. During the ripening of greengages, these polyphenols first increased and then decreased, peaking during the swell stage. Multivariate statistical methods, including redundancy analysis (RDA), random forest analysis, Mantel test, and Spearman's correlation indicated that temperature, sunshine hours, humidity, and radiation were important factors driving the formation and changes in the polyphenols of greengages. In particular, flavanones and flavonols, showed a structure-dependent response to temperature and radiation.

1. Introduction

Greengage (*Prunus mume* Sieb. Et Zucc) is a plant belonging to the Rosaceae family that is small, green or yellow-green, sour and astringent, like apricot. It is native to China and now widely cultivated in other Asian countries, including Korea, and Japan, and was treated as a medicine food homology plant, which have been used in food and medicinal herb for over 2000 years in Asia. Greengages exhibit antibacterial, antioxidant, antifatigue, and anticancer properties owing to the high content of bioactive compounds such as polyphenols, terpenes, and organic acids (Gong et al., 2021; Tian et al., 2021). Controlling the maturity of the greengage as a raw material is essential for ensuring the content of bioactive substances in the processed products (Bailly, 2020). Unripe greengage is suitable for the production of traditional Chinese medicine "Wu Mei" (Xia et al., 2011), while ripe fruit is mainly used for the production of alcoholic beverages and preserved fruits (Chen et al., 2021). It is well known that plant substances, particularly polyphenols, change significantly during fruit growth and development (Kalisz et al., 2020; Cendrowski et al., 2017). However, little research has focused on the accumulation patterns of greengage phenolic compounds during the fruit ripening. It is particularly necessary for elucidating the nutritional properties of greengages and its processed products.

The release and accumulation of secondary metabolites including plant polyphenols are affected by soil, altitude, and especially climate (Akhatou et al., 2017). Moreover, the composition and content of polyphenols in fruits change in response to the variations in meteorological parameters: UV radiation significantly increases the content of flavonols and anthocyanins (Yang et al., 2019), the accumulation of proanthocyanidins can be promoted by appropriate temperatures, whereas excessive temperatures inhibit their accumulation (Cohen et al., 2012). Greengage differs from other fruits in that it blooms in winter, grows in spring, and matures in summer (Tian et al., 2022), which may greatly influence the synthesis and metabolism of greengage polyphenols. Thus, this makes it that the accumulation of greengage polyphenols is possibly related to "terroir", including climate and region.

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Widely targeted metabolomics is a method that combines the advantages of untargeted and targeted metabolomics with high throughput and sensitivity (Feng et al., 2020; Li et al., 2022), thus allowing the qualification and precise quantification of thousands of metabolites. Nowadays, this technique has been widely used in plant metabolomics (Wang et al., 2022; Hu et al., 2020). Previous studies have focused on specific categories of greengage polyphenols, such as phenolic acids (chlorogenic acid) and proanthocyanidins (Horinishi et al., 2021). Nevertheless, there have been no comprehensive research on the profile of greengage polyphenols.

Hence, in this study, widely targeted metabolomic technique was used to comprehensively identify the composition of greengage polyphenols. The dynamic variations and accumulation patterns of phenolic compounds in greengage during the ripening was investigated. Besides, we investigated the correlation between greengage polyphenol accumulation and meteorological parameters. This study provides a guide for determining the best harvesting time—to ensure that more bioactive compounds are transferred into processed products—and for controlling the quality of processed greengage products. Furthermore, exploring the correlation between polyphenol accumulation and climatic factors can help identify chemical markers of greengages with the potential to establish the geographical indication.

2. Materials and methods

2.1. Reagent and standards

Methanol and acetonitrile were of chromatographic purity and purchased from CNW Technologies (ANPEL Laboratory Technologies, Shanghai, China). Chromatographic-grade formic acid was obtained from Sigma–Aldrich (St. Louis, MO, USA). All chromatographic-grade standards, such as catechin, epicatechin, apigenin, naringenin, chlorogenic acid, astragalin, and isoquercitrin, were purchased from Sigma and J&K Scientific Ltd. (Beijing, China).

2.2. Materials

Greengages (*Prunus mume* Sieb. Et Zucc) were collected from Dayi County, Chengdu City, Sichuan Province, southwest China. Four sampling time points were selected according to the ripening stage of the greengages: the green stage (March 30, 2020, D1), swell stage (April 28, 2020, D2), veraison stage (June 5, 2020, D3), and ripening stage (June 29, 2020, D4) (Fig. 1). These four stages fruits were picked from greengages trees. The greengages without any physical injury or abnormalities were selected. The content of water was determined by water content tester (DSH-50A-1, Youke Instrument, Shanghai, China) after sampling. Then the fruits were immediately placed in an icebox and transported to the laboratory. All samples were stored at −20 °C before analysis.

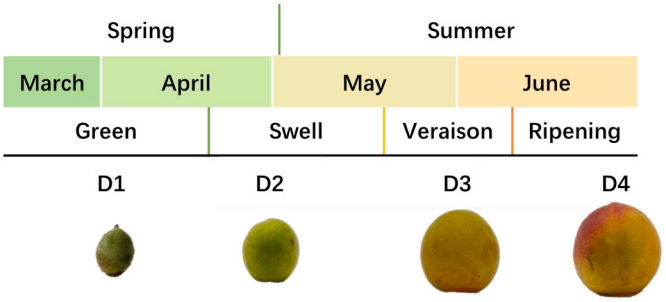

Fig. 1. Ripening stages of greengages.

2.3. Sample preparation

Greengage polyphenol was extracted using a previously reported method with modifications (Zha et al., 2018). The greengages were rapidly frozen in liquid nitrogen, and the flesh was crushed in a mixer mill for 30 s at 60 Hz. Thereafter, 50 mg of the flesh was weighed and extracted with 700 μL of methanol/water (3:1, v/v). After vortexing for 30 s, the samples were homogenised at 40 Hz for 4 min and sonicated for 5 min in an ice-water bath. Homogenisation and sonication were repeated three times, and the samples were shaken overnight at 4 °C. After centrifuging at 1,2000 rpm and 4 °C for 15 min, the supernatant was filtered using a 0.22 μm microporous membrane. The resulting supernatants were diluted 15 times with the extraction solution and vortexed for 30 s. Afterward, the samples were transferred to 2 mL glass vials and stored at −80 °C until the mass spectrometry analysis.

2.4. Widely targeted metabolomics analysis

2.4.1. UPLC parameters

Widely targeted metabolomic analysis was performed according to a previously reported method with minor modifications (Zha et al., 2018). UPLC separation was performed using an EXIONLC system (Sciex Technologies, Framingham, MA, USA). Mobile phase A was 0.1% formic acid in water, and mobile phase B was acetonitrile. The gradient program was as follows: 0 min, 2% B; 10 min, 50% B; 11 min, 95% B; 13.1 min, 2% B; 15 min, 2% B. The UPLC column was Waters ACQUITY UHPLC HSS T3 (1.8 μm, 2.1 × 100 mm) (Waters Corporation, Milford, MA, USA). The flow rate was 0.40 mL/min and the column temperature was 40 °C. The automatic injection temperature was 4 °C, and the injection volume was 2 μL.

2.4.2. ESI–QTRAP–MS/MS parameters

An Electrospray ionisation-triple quadrupole-linear ion trap mass spectrometer (ESI-Q TRAP-MS/MS, AB Sciex QTRAP 6500 System, AB SCIEX Pet. Ltd., Framingham, Massachusetts, USA) was used for the analysis. The ion source parameters were as follows: ion spray voltage set as +5500 V (positive ion mode)/−4500 V (negative ion mode); curtain gas was set to 35 psi; source temperature 400 °C; ion source gas I was set as 60 psi; ion source gas II was set as 60 psi; declustering potential: ±100 V. Triple quadrupole (QQQ) scans were acquired for multiple reaction monitoring (MRM). The MRM parameters including precursor ions (Q1), characteristic fragment ions (Q3), and collision energy (CE) were listed in Table S1.

2.4.3. Qualification and relative quantification of polyphenols

SCIEX Analyst Workstation Software (Version 1.6.3) was employed for the acquisition and processing of the MRM data. MS raw data (.wiff) files were converted to TXT format using MSconventer. To identify the substances, mass spectrometry data were analysed based on internal databases and public databases (MassBank, HMDB, and METLIN). Some metabolites were identified according to the mass spectral information and retention times of the standards. After the mass spectrometry data were obtained for different samples, all mass spectrum peaks were subjected to area integration, and the mass spectrum peaks of the same substance in different samples were subjected to integration correction. Structurally similar standards for each metabolite were selected for relative quantification, as detailed in Table S2.

2.5. Meteorological data

The meteorological data recorded at the Dayi meteorological station were extracted from the China Meteorological Data Service Centre (http://data.cma.cn/), which covers the entire ripening stages of greengages. The meteorological data of the two weeks before the sampling day were averaged, including the mean temperature, mean precipitation, mean atmospheric pressure, mean sunshine hours, mean relative

humidity, mean evaporation, and mean ultraviolet intensity (radiation). The specific data are presented in Fig. S1.

2.6. Data analysis

All results were expressed as the mean ± standard deviation (SD) of three replicates. Statistical analysis was conducted using Duncan's Multiple Range Test ($p < 0.05$) in SPSS (IBM SPSS 26.0, SPSS Inc). Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed using MetaboAnalyst 5.0 (www.metaboanalyst.ca). The variable importance projection (VIP) values of greengage polyphenols were calculated, and the polyphenols with VIP values greater than 1.0 were selected as polyphenols that significantly changed during the ripening of greengages. Greengage polyphenols were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www. kegg. jp/kegg/) to obtain metabolic pathways related to the synthesis of polyphenols at different ripening stages. To analyse the driving factors of the metabolic pathways, redundancy analysis (RDA) was conducted using Canoco (version 5.0, Microcomputer Power, New York, USA). Random forest analysis (rfPermute function in the rfPermute package in R) was performed to estimate the importance of meteorological parameters for the metabolism of greengage polyphenols. The Mantel test (the mantel function of the vegan package for R) was conducted to evaluate the influence of meteorological factors on the variations in greengage polyphenols. Spearman's rank correlations between polyphenols and meteorological parameters were determined using R (version 3.6.3).

3. Results and discussion

3.1. Composition of polyphenols in greengages

The qualification of polyphenols in greengages based on an internal database and public database, and specific information of the identified polyphenols are listed in Table S1. Totally, 214 phenolic compounds were identified from greengages at different ripening stages by widely targeted metabolomics analysis. Greengage polyphenols could be divided into 11 different classifications according to their structure: phenolic acids (61), flavones (50), flavanones (30), flavonols (23), isoflavones (20), flavanols (8), chalcones (7), anthocyanins (5), anthocyanidins (3), pterocarpans (5), and proanthocyanidins (2). Based on the type and proportion, phenolic acids were the largest class of polyphenols, which accounted for 28.50% of the total greengage phenolic compounds, followed by flavones (23.36%), flavanones (14.02%), and flavonols (9.81%), as shown in Fig. 2A.

Phenolic acids are the largest class of polyphenols in greengage fruit (Xia et al., 2010). Except for chlorogenic acid, protocatechuic acid, gallic acid, and caffeic acid, fifty-seven phenolic acids were identified for the first time in greengages. Among them, the bioactive functions of some phenolic acids have been reported in previous studies. Ferulic acid is a ubiquitous effective constituent of Chinese medicinal herbs, such as *Rhizoma Chuanxiong* and *Angelica sinensis*, which has been reported to have many bioactive functions, including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities (Ou and Kwok, 2004). Vanillin is a compound related to food flavour and is the dominant component in vanilla plants. Studies have suggested that vanillin has the potential to alleviate obesity-related gut microbiota disorders (Guo et al., 2018).

Some low-content substances can be detected by widely targeted metabolomic techniques, which led us to identify flavones from greengage fruits for the first time. Flavones are common phenolic compounds in plants, and were detected in other *Rosaceae* fruits, such as plums (Treutter et al., 2012). Narcissoside was shown to be an important bioactive flavone in lotus seed, with antioxidant and antiviral activities (Ha et al., 2022). And scolymoside were obtained from the artichoke extract and clinical trials have confirmed the potential of scolymoside for treating digestive problems (Nassar et al., 2013).

Flavanones, except naringenin and narirutin, were identified for the first time in greengages. Pinobanksin was detected in honey and has anticancer and antioxidant activities (Subramanian et al., 2016). Epicatechin class and catechin class are the main flavanols in greengage. EGCG, the main active compound in green tea, is an ester of (−)-epigallocatechin and gallic acid, which was identified in greengage fruits for the first time. The anticancer, antiobesity, and neuroprotective effects of EGCG have been confirmed in previous studies (Pervin et al., 2019).

Flavonols are a major class of polyphenols in greengage fruits, predominantly in the form of quercetin and kaempferol glycosides (Jang et al., 2018). The identified flavonols had been studied except myricetin, quercetin 3-*O*-rhamnoside-7-*O*-glucoside, and kaempferitrin. Five anthocyanins were identified from greengage, and they may contribute to the greenage peel colour (Bailly 2020). Pelargonidin-3,5-diglucoside, Pelargonidin-3-glucoside and Peonidin-3-glucoside were identified for the first time in greengage fruits. Proanthocyanidins are also important

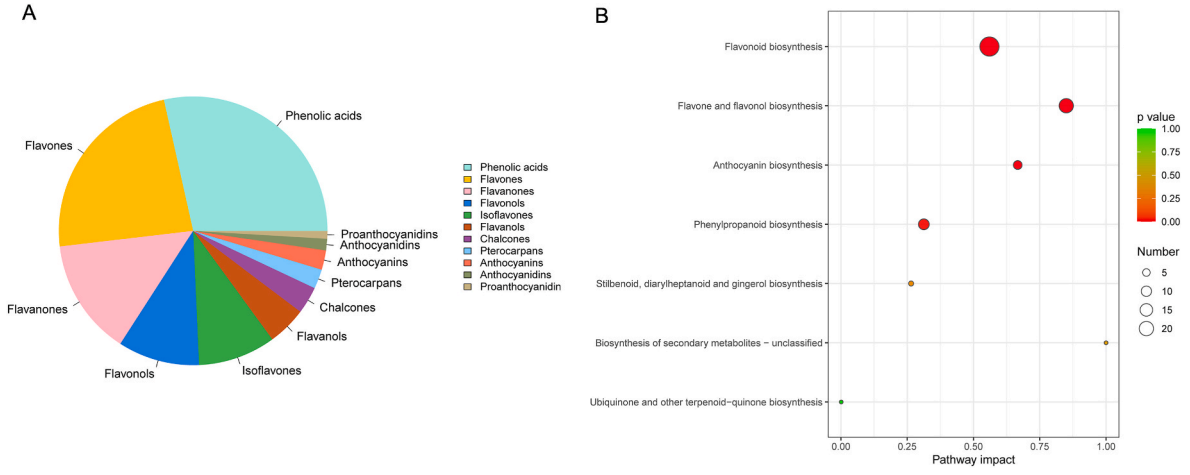

Fig. 2. Greengage polyphenols and related metabolic pathways
(A) Composition of the polyphenols of greengages; (B) Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis of greengage polyphenols. The ordinate is the pathway name, and the abscissa indicates the impact corresponding to each pathway. The colour of the bubble represents the p-value. The size of the bubble represents the number of substances enriched. The deeper the red colour, the more significant the enrichment. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

phenolic compounds in *Rosaceae* fruits, such as peaches and plums. Two proanthocyanidins were identified from greengages. Procyanidin B2 have been found in flower of greengages (Zhang et al., 2015), while procyanidin B4 was first identified from greengage fruits. This result revealed that greengages were abundant in phenolic compounds, which were helpful for further elucidation of the chemical basis of nutritional value of greengages.

Subsequently, to obtain a global understanding and analysis of the phenolic compounds in greengages, the detected greengage polyphenols were mapped to the KEGG databases. The enrichment results and detailed metabolic pathways are shown in Fig. 2B. The pathway enrichment analysis revealed that greengage polyphenols were distributed in seven metabolic pathways, including flavonoid biosynthesis pathway, flavone and flavonol biosynthesis pathway, phenylpropanoid biosynthesis pathway, anthocyanin biosynthesis pathway, stilbenoid, diarylheptanoid, gingerol biosynthesis pathways, and biosynthesis of secondary metabolites: unclassified, ubiquinone, and other terpenoid–quinone biosynthesis pathways. Based on the *p*-values of enrichment analysis, the flavonoid, flavone and flavonol, phenylpropanoid, and anthocyanin biosynthesis pathways were the most significant pathways in greengage ($p < 0.05$). Further investigation into the four metabolic pathways can help globally profile the composition of phenolic compounds in greengages and clarify the links among the polyphenols.

3.2. Polyphenol content of greengages during ripening stages

To accurately analyse the changes in the content of polyphenols in greengages, the quantification of the detected phenolic compounds was determined using standards for single-point calibration (Table S2). The total polyphenols content (TPC) of greengage ranged from 5.31 to 21.862 mg/g dry weight (DW) over the entire ripening stage. Our result was basically consistent with reported studies. You et al. (2021) and Liu et al. (2022) had been reported the to TPC in greengage 11.96 mg/g DW and 8.58–15.00 mg/g DW, respectively, using the Folin–Ciocalteu method (calculated based on gallic acid). However, the fluctuation of TPC in greengage depends on the fruit variety, maturity and detection method. The high throughput and sensitivity of widely targeted metabolomics technique, allowing us to identify more polyphenols from greengages, which would lead to determine a higher polyphenol content than that in the previous studies.

As shown in Fig. 3, the TPC showed a trend of increasing and then decreasing as the fruits ripened, reaching a maximum value at the swell stage (D2). Deytieux-Belleau et al. (2009) found that as the water content increased could increase the risk of pests and diseases in fruits. In the result of water contents in greengages at different ripening stages (shown in Fig. S2), we found the dramatic growth of water content in swell stage (D2). Therefore, the increasing polyphenol content may be related to the mechanism of self-protection. In unripe fruits, phenolic compounds are accumulated to resist pathogens and prevent plant diseases. Meanwhile, the high content of phenolic compounds in the early stages can prevent the fruit from being eaten by insects and birds, because some polyphenols are a source of bitterness and astringency (Muñoz et al., 2011). It's worth noting that unripe greengages are often used as the raw material to produce the traditional Chinese medicine "Wu Mei" or conduct pharmacological studies (Bailly, 2020), which may be related to the relatively high content of polyphenols in unripe greengage fruits.

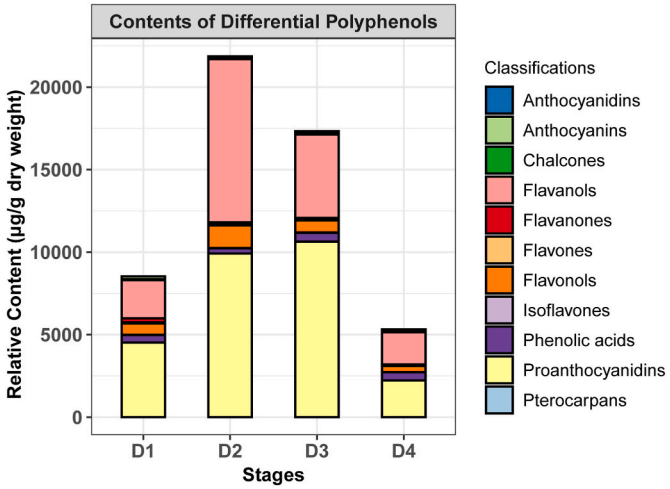

Fig. 3. Contents of polyphenols in greengages during ripening.

We observed that proanthocyanidins, flavanols, flavonols, and phenolic acids were the dominant phenolic compounds in greengages. These compounds are responsible for the astringency and bitterness of raw plant materials (Sáenz-Navajas et al., 2010; Grobelna et al., 2019), which are related to the sensory characteristics of greengages. During the mature stage, the content of proanthocyanidin reached a maximum value at the veraison stage (D3), whereas the flavanols and phenolic acids reached a maximum value at the swell stage (D2). The individual polyphenol compounds in greengages showed different patterns of variation (Table S2). Overall, the majority of phenolic compounds decreased as the fruit grew. Such as, *p*-coumaric acid, caffeoyl quinic acid, caffeoyl shikimic acid, caffeic acid and ferulic acid, which are all the precursors of polyphenol, they all presented decreasing tendency during the ripening stage. Also, flavanones— naringenin, pinobanksin, and isosakuranetin were downregulated over the entire ripening stage. However, the contents of apigenin, baimaside, vitexin 2″-*O*-glucoside, and *p*-hydroxymandelic acid increased with fruit ripening. The contents of some phenolic compounds fluctuated with fruit growth, such as vanillin, hesperidin, and rutin. Previous studies have shown that plants respond to environmental stress and climate change by modulating secondary metabolic pathways, including those related to flavonoid production (Akhatou et al., 2017). Our results explain the influence of the ripening stage on greengage polyphenols, indicating that greengages with different levels of maturity may be selected as raw materials for functional products based on their polyphenol content.

3.3. Correlations between greengage polyphenols and meteorological parameters

The life cycle of greengage is quite different from other fruit, which blossom in winter, and the fruits grow from spring until they ripen in summer. Hence, it is worthwhile to deeply study the influence of climate on greengage polyphenol synthesis. All detected greengage polyphenolic compounds belonged to four main polyphenol synthesis pathways, including flavonoid, flavone and flavonol, phenylpropanoid, and anthocyanin biosynthesis pathways (Fig. 2B). Here, we used chemometrics to reveal the correlation between the greengage polyphenol and meteorological parameters.

The phenolic compounds related to the four pathways were subjected to PCA, and the PC1 score of each sample was used to indicate the comprehensive variations in all phenolic compounds in the pathways. RDA was conducted to evaluate the differences between greengage polyphenols at different ripening stages and to clarify the relationship between meteorological parameters and the variations in polyphenols. As shown in Fig. 4A, differences were observed in various polyphenols during the ripening of greengages. The greengage polyphenols related to the flavonoid biosynthesis pathway were abundant at the swell and veraison stages (D2 and D3), while the phenolic compounds related to flavone and flavonol biosynthesis pathway, phenylpropanoid biosynthesis pathway, and anthocyanin biosynthesis pathway were the highest at the ripening stage (D4) of greengages. Variations of polyphenols related to different pathways were different, which suggest that different polyphenol metabolic pathways may be activated at different

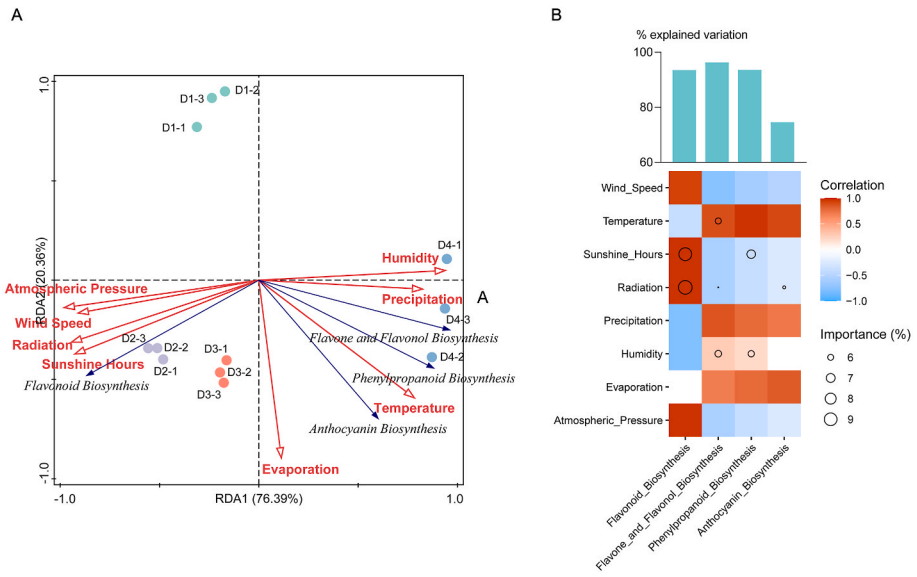

Fig. 4. Correlations between meteorological parameters and metabolic pathways
(A) RDA of meteorological parameters and the pathways related to the synthesis of polyphenols in greengages; (B) contributions of meteorological parameters to polyphenol metabolism pathways based on Spearman's correlations and random forest models; circle size represents the importance of the meteorological parameters (i.e. percentage of increase in mean square error calculated via random forest model). Colours represent Spearman's correlations. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ripening stages. The total explanation for RDA1 and RDA2 was 96.75% (Fig. 4A), suggesting a correlation between meteorological parameters and the variations in greengage polyphenols. The polyphenols related to the flavonoid biosynthesis pathway were positively correlated with radiation, sunshine hours, wind speed, and atmospheric pressure, and negatively correlated with temperature, precipitation, and humidity; the flavone and flavonol biosynthesis pathway were the opposite. Polyphenols related to the phenylpropanoid biosynthesis pathway and anthocyanin biosynthesis pathway were negatively correlated with most meteorological parameters but positively correlated with temperature, precipitation, humidity, and evaporation. The results suggest that variations in polyphenols are correlated with meteorological parameters. Additionally, the changes in meteorological parameters caused the metabolic pathways to become active at different ripening stages, indicating that meteorological factors are vital drivers of the synthesis and variations in greengage polyphenols.

To further evaluate the influence of meteorological parameters on the changes of greengage polyphenols, Mantel tests were performed. As shown in Fig. S3, Mantel tests revealed that the polyphenols enriched in the flavonoid biosynthesis pathway were significantly correlated with precipitation, wind speed, atmospheric pressure, sunshine hours, and radiation. In the flavone and flavonol biosynthesis pathway, polyphenols were significantly correlated with all meteorological parameters ($p < 0.001$), except for evaporation. Temperature, atmospheric pressure, sunshine hours, and radiation were the strongest parameters that affected the variations in phenolic compounds in the phenylpropanoid biosynthesis pathway ($p < 0.001$), whereas no significant correlation was found between meteorological factors and polyphenols in the anthocyanin biosynthesis pathway, except for temperature and evaporation ($p < 0.001$). Overall, meteorological factors are critical for driving the variations in greengage polyphenols at different ripening stages, and the pathways associated with the synthesis of phenolic compounds can be regulated by meteorological parameters.

Spearman's correlation and random forest models were applied to identify the meteorological parameters driving the variations in greengage polyphenols (Fig. 4B). The bar charts indicate that meteorological parameters are responsible for the variations in greengage polyphenols. The size of the circle in the heatmap indicates the importance of the factor, and the colour represents Spearman's correlation. Radiation is an important meteorological parameter for regulating the variations of polyphenols, including the flavonoid biosynthesis pathway, flavone and flavonol biosynthesis pathway, and anthocyanin biosynthesis pathway, during the ripening of greengages. Sunshine hours are vital for regulating the changes of polyphenols related to flavonoid biosynthesis and the phenylpropanoid biosynthesis pathway, while humidity is an important factor for regulating the flavone and flavonol biosynthesis pathway and phenylpropanoid biosynthesis pathway. These were consistent with the above results of RDA and Mantel tests. However, the temperature only contributed to the variations of polyphenols related to flavone and flavonol biosynthesis pathway. Additionally, the effects of temperature and humidity on the variations in the flavone and flavonol biosynthesis pathway of greengages were stronger than those of radiation. Sunshine hours had the strongest effect on the variation in the phenylpropanoid biosynthesis pathway, followed by humidity. It is indicated that temperature, humidity, sunshine hours, and particularly radiation contributed the most to the variations in greengage polyphenols at different ripening stages.

The variations in individual polyphenols in different polyphenol synthesis pathways during the ripening of greengage fruits vary. PLS-DA combined with the VIP, was adopted to select the phenolic compounds that significantly changed during the ripening of greengages according to the criterion that VIP >1. The results are shown in Fig. S4.

A more in-depth analysis was adopted to assess the effects of meteorological parameters on significant greengage polyphenols. Phenolic compounds with significant changes in the flavonoid biosynthesis pathway mainly included flavanones, such as homoeriodictyol, sakuranetin, garbanzol, and flavanols, such as catechin and epicatechin (Fig. 5A). They were significantly correlated with radiation and sunshine hours; however, the different classifications of polyphenols responded differently to the meteorological parameters. For example, the content of homoeriodictyol was positively correlated with radiation ($p < 0.001$), in contrast to sakuranetin and garbanzol, which may be related to the methoxy group on the B-ring of homoeriodictyol. In previous studies on peppermint, it was found that the methoxy group in flavanones responded to UV light, and flavanones that did not contain methoxy groups were unchanged or decreased after UV-B irradiation (Dolzhenko et al., 2010). Although temperature changes had a little influence on the overall flavonoid synthesis pathway, they may have a more significant effect on certain branches, such as those synthesizing flavanones (Fig. 5A). Eriodictyol, which contains two hydroxy groups on the B-ring, showed a significant negative correlation with temperature ($p < 0.001$), whereas sakuranetin and garbanzol, which contain only one hydroxy group, showed a non-significant correlation with temperature. This is because flavanones with a relatively high number of hydroxy groups on the B-ring are more sensitive to temperature changes (Julkunen-Tiitto and Sorsa, 2001). Therefore, the differences were existed in

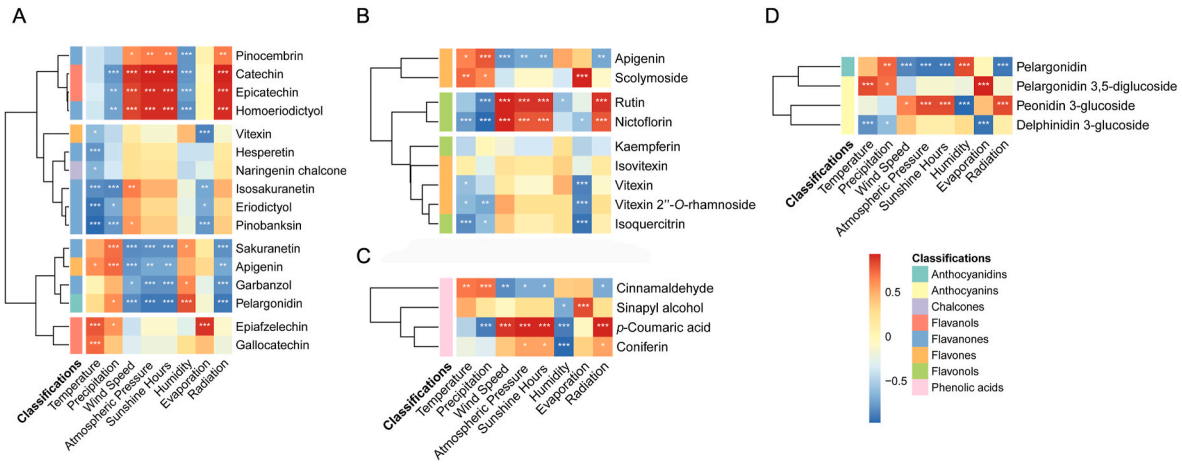

Fig. 5. Correlations between meteorological parameters and polyphenols in different metabolic pathways
(A) Flavonoid biosynthesis pathway; (B) flavone and flavonol biosynthesis pathway; (C) phenylpropanoid biosynthesis pathway; (D) anthocyanin biosynthesis pathway.

the responses of the different structures of flavanones to temperature changes.

Flavanols related to the flavonoid synthesis pathway were significantly altered during the greengage ripening. As shown in Fig. 5A, most flavanols, such as catechin and epicatechin, showed a significant positive correlation ($p < 0.001$) with radiation and sunshine hours. Plants can resist radiation through a series of morphological and physiological changes, reducing UV transmission by increasing the content of flavonoids, alleviating oxidative stress caused by UV exposure, and reducing UV damage to plants. UV radiation increases the expression of key enzyme genes in the flavonol biosynthesis pathway, which in turn increases the flavonol content. We found differences in the correlation between different flavanols and UV radiation, probably because of the selective induction of key enzyme genes on different branch pathways by UV at different ripening stages (Zhang et al., 2013).

Flavonols are the primary differential polyphenols in the flavone and flavonol biosynthesis pathways and are also major responders to climate change and abiotic stresses. For example, UV-B significantly induces the accumulation of flavonols, particularly flavonol glycosides (Fig. 5B). However, we found that rutin (quercetin-3-rutinoside) and nictoflorin (kaempferol-3-rutinoside) were more sensitive to changes in radiation than the other flavonol glycosides. This is because the flavonol 1,6-Rhamnosyltransferase synthesis gene in peach, which catalyses the formation of the flavonol rutinoside, was more sensitive to UV-B than others (Xie et al., 2022). This result demonstrated that flavonols showed a structure-dependent response to meteorological parameters.

Further, the flavones related to the flavone and flavonol biosynthesis pathway were significantly altered by meteorological parameters. Flavone glycosides, such as isovitexin, vitexin, and vitexin 2″-*O*-rhamnoside, were positively correlated with radiation, although the correlations were not statistically significant (Fig. 5B). The increased flavone glycoside content increased the UV tolerance of the plants owing to natural variation in the glycosyltransferase alleles as a result of UV exposure (Peng et al., 2017).

Four phenolic acids related to the phenylpropanoid biosynthesis pathway were differentially changed, of which *p*-Coumaric acid is a vital precursor substance for the synthesis of polyphenols (Fig. 5C). The conversion of phenylalanine to cinnamic acid catalysed by PAL enzymes is followed by the synthesis of *p*-Coumaric acid catalysed by C4H enzymes (Shen et al., 2022). PAL and C4H are the two primary enzymes in the polyphenol biosynthesis pathway and play a vital role in the response to abiotic stress in plants. Relatively strong UV irradiation can induce the expression of *PAL* and *C4H* genes in plants (Jiao et al., 2015), and we found that radiation was positively correlated with the variations in *p*-Coumaric acid content ($p < 0.001$). The increase in *p*-Coumaric acid synthesis could protect greengages from the damage caused by intense UV radiation. Although changes in precipitation were significantly negatively correlated with the variations in *p*-Coumaric acid content ($p < 0.001$), more precipitation may affect the activity of PAL and C4H (Duan et al., 2019), which is detrimental to the synthesis of polyphenol precursor substances and thereby reduces the content of polyphenols. Therefore, *p*-Coumaric acid, a precursor substance for polyphenol synthesis, significantly responded to changes in meteorological parameters, suggesting that meteorological parameters have an important influence on the synthesis of polyphenols in greengages.

Anthocyanins can be involved in abiotic stresses in plants; however, their mechanism of action is not yet clear. Intense UV radiation can increase the content of anthocyanin compounds; however, only peonidin 3-glucoside was consistent with this rule (Fig. 5D), probably because different structures of anthocyanins may respond differently to meteorological parameters (Kovinich et al., 2014). Since different anthocyanins have different sensitivities to temperature changes (Stiles et al., 2007), only the content of delphinidin 3-glucoside showed a significant negative correlation with temperature changes ($p < 0.01$). In summary, the variations in meteorological factors influence the changes in greengage phenolic compounds, while the response to meteorological parameters, including temperature and radiation, is structure-dependent, particularly in flavanones and flavonols.

4. Conclusions

Greengages are a significant source of polyphenols, and variations in meteorological factors result in the variations of greengage polyphenols during fruit ripening. The polyphenols of greengages at different ripening stages were first investigated by widely targeted metabolomic analysis based on UPLC–ESI-QTRAP–MS/MS. Phenolic acids were the most common type of greengage polyphenols, and proanthocyanidins had the highest polyphenol content. As the fruits ripened, the total number of greengage polyphenols increased and then decreased, peaking during the swell stage. Variations in meteorological parameters cause variations in different polyphenols during ripening. Additionally, greengage polyphenols, such as flavanones and flavonols, showed a structure-dependent response to meteorological parameters, including temperature and radiation. In conclusion, our findings provide new insights into greengage fruit nutrition and guidance for improving the quality and stability of greengages. These results can help guide the selection of suitable cultivars, maturity, and production regions for developing deep-processing food of greengages.

CRediT authorship contribution statement

Chang Liu: Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Minxin Liu:** Sample preparation, Investigation. **Lili Yang:** Writing – review & editing, Review. **Xiaoxu Zhang:** Project administration, Conceptualization, Supervision.

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

I have shared the link to my data at the Attach File step.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.10.013.

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