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A comprehensive study on the fruit quality of a late-ripening mutant variety of plum

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ABSTRACT

The fruit ripening period is an important factor affecting fruit quality and commercial value.To investigate why WSQCL ripens about 15 days later than QCL and why WSQCL has a more astringent flavor. This study analyzed the dynamics of fruit development and quality of three plum varieties, including "Late-maturing Qiangcuili" (WSQCL), 'Qiangcuili' (QCL), and 'Cuihongli' (CHL), to explore the differences in plums quality formation and the ripening period between the late-maturing variant WSQCL and its parental cultivars. The results indicated that the order of the fruit growth rate was QCL > WSQCL > CHL. During fruit development of the three plum varieties, the changes in soluble sugar (SS), titratable acid (TA), starch, chlorophyll, carotenoids, and phenolic contents were consistent, while the accumulation of total phenols, SS, and TA differed significantly between WSQCL and QCL. WSQCL had higher expression of genes related to phenolic compound synthesis than QCL, and phenolic compound synthesis was closely associated with the expression of *PAL3, 4CL*, *HCT1,* and *CHS*. Principal component analysis revealed differences between WSQCL and the other two varieties during the middle and late stages of fruit development. This study provides a reference for quality formation and development of the potential value of WSQCL.

1. Introduction

Prunus salicina Lindl belong to the Rosaceae family and are popular for their flavor. This tree is originates from China and is commonly grown for its edible fruit and ornamental value. China has the greatest plum cultivation area and the highest plum production worldwide (Al-Khayri et al., 2018). Southwest China is one of China's leading plum-producing regions, and many local varieties have been bred due to the excellent climatic conditions. The main cultivars in Sichuan are the crisp plum series, such as 'Qiangculi', 'Cuihongli', and 'BashanCuili', which are popular because of their crispy texture, juicy, sweet and sour flavor, and thin-skinned characteristics (Lin et al., 2023).

Plum fruits contain bioactive substances, such as vitamin C, carotenoids, polyphenols, and dietary fiber, that can be used in functional foods (Chen et al., 2021). Previous studies have shown that consuming plums prevents diabetes, hypertension, cancer, thrombosis, and inflammation (Wang et al., 2020). The quality of plum fruit develops gradually during maturation, hence there is increasing interest in studying the changes in the bioactive substances during fruit maturation.

The content and ratio of sugars and organic acids are important components that affect the flavor of mature fruits, and their changes in content are essential signs of fruit maturity (Liu et al., 2016). Phenolics are a class of metabolites that increase the antioxidant capacity of fruits. Plums have a greater antioxidant capacity than bananas and grapes because of their great total phenolic content (TPC) (Kayano et al., 2002). In addition, the phenolic compound composition and content of plums significantly impact the flavor and color of the fruit (Yu et al., 2020). As the fruit ripens, the content of phenolic compounds decreases and the antioxidant properties decline (Aladag et al., 2020). In the past few years, researchers have focused on gene expression in synthesizing phenolic compounds. Phenolic substances are biosynthesized by a variety of enzymes in the phenylpropanoid pathway, of which phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), hydroxycinnamic acid transferase (HCT), and 4-count rate CoA ligase (4CL) are the key enzymes (Shen et al., 2022). An investigation of the connection between

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phenolic substances and antioxidant capacity in four Asian plums and fruit ripening reported that the *HCT* gene is strongly associated with the synthesis of phenolic acids and is less abundant at the ripening stage than at the half-ripening stage (Hwang et al., 2020).

Sichuan crisp plums have naturally mutated into many excellent new varieties during long-term cultivation. The selection and breeding of mature and good-quality plum varieties are essential for reducing homogenization and enriching the structure of the variety. Late-maturing Qiangcuili (WSQCL) is a late-ripening bud sport of Qiangcuili (QCL), while Cuihongli (CHL) and WSQCL are late-maturing varieties with the same ripening period. In this study, WSQCL, QCL, and CHL were used to compare and analyze the fruit quality formation patterns of the different varieties. A comprehensive fruit quality evaluation was conducted to provide a basis for developing and utilizing WSQCL.

2. Materials and methods

2.1. Plants materials

The QCL, WSQCL, and CHL fruits were harvested in 2020 from the same orchard in Maoling Village, Wenchuan County, Abaqiang-Tibetan Autonomous Prefecture, Sichuan Province (31°28 N, 103°33′ E), at an altitude of 1966 m, under the same cultivation and management conditions. We sampled the three varieties of plums at 14-day intervals beginning 45 days after flowering, recorded as S1, S2, S3, S4, S5, S6, and S7, respectively, with QCL sampled six times since 16 June, WSQCL sampled seven times since 1 July, and CHL was sampled 6 times since 15 July. Plum fruits of uniform shape and free of mechanical damage, pests or pathogens were selected and transported to the laboratory in an ice box. Each biological replicate consisted of over thirty plums collected from more than three plum tree. Peels and pulp were separated on ice, frozen in liquid nitrogen (Ritez Corp., Chengdu, China), and stored at −80 °C for later analysis.

2.2. Determination of single fruit weight and longitudinal and transverse diameters

Thirty plums without separating the peel from the pulp were used to measure per-fruit weight (g). Digital Vernier calipers (Huatongtianxia Corp., Chengdu, China) were used to measure the fruits' longitudinal and transverse diameters (mm) and calculate the fruit form index.

2.3. Determination of SS, TA, and starch content

The SS content was measured using anthrone colorimetry. TA content was measured using sodium hydroxide titration, and starch content was determined by perchloric acid hydrolysis.

2.4. Determination of color differences and pigmentation

The color indicators L *, a *, and b * of the equatorial part and both sides of the peel and flesh of the fruits were determined using a CR-400 colorimeter (Konica minolta Corp., Shanghai, China). We determined chlorophyll and carotenoid contents using the acetone (Hoboyo Corp., Chengdu, China) extraction method (acetone: ethanol = 2:1/100 mL of extract). Light was avoided throughout the process. The samples were ground at 0 °C. After grinding, 0.5 g of material was collected and added to 20 mL of extraction solution, and incubated for 24 h. Absorbance levels were calculated at 663, 646 and 470 nm. Determination of total anthocyanin content with reference to hydrochloric acid-methanol (Hoboyo Corp., Chengdu, China) solution extraction method, in which 0.5 g of material was combined with 5 mL of 1 % hydrochloric acid-methanol solution, and absorbance was determined at 530, 620, and 650 nm.

2.5. Extraction and determination of total phenolic content (TPC), total flavonoid content (TFC) and total flavanol content (TFAC)

2.5.1. Extraction of TPC, TFC and TFAC

The TPC, TFC and TFAC was extracted as previously described with slight modifications (Zhang et al., 2024). The peel and pulp were ground in liquid nitrogen, and 0.5 g of the powder was then homogenized in 6 mL of extraction solvent (methanol:formic acid:anhydrous ethanol = 70:2:28, *v*/v/v). This mixture was ultrasonicated for 30 min at 4 °C and shaken at 30 °C for 2 h at 250 r/min. Subsequently, the mixture was centrifuged at 8000 rpm (radius = 10 cm) for 10 min. The supernatant was filtered through a 0.45 μm syringe filter membrane and used for the measurement of TPC, TFC, and TFAC. All operations were conducted in the dark to avoid exposure to light.

2.5.2. Determination of TPC

A 200 μL material extract was added to 3 mL of ultrapure water and 0.2 mL of Folin-Ciocalteu reagent (Hoboyo Corp., Chengdu, China) in a tube sequentially. The mixture was mixed and reacted for one minute, and 3 mL of 20 % Na_2CO_3 solution (Hoboyo Corp., Chengdu, China) was added and mixed and reacted for 2 h away from light. A spectrophotometer was used to measure absorbance at 765 nm, with the extraction solvent serving as a control. Gallic acid was used as the standard (50–1000 mg/L), and results were shown in GAE.

2.5.3. Determination of TFC

In a centrifuge tube, 400 μL of fruit extract, 2.6 mL of methanol, 200 μL of $NaNO_2$ (0.5 M) (Hoboyo Corp., Chengdu, China), and 200 μL of $AlCl_3$ (0.3 M) (Hoboyo Corp., Chengdu, China) were mixed sequentially. Mix well and add 1 mL of NaOH (1 M) (Hoboyo Corp., Chengdu, China) after 5 min. Absorbance was determined at 510 nm. The standard was rutin (20–100 mg/L), and the values were given in terms of rutin equivalents.

2.5.4. Determination of TFAC

Add 200 μL of material extract to 3 mL of purified water and 2 mL of 1 % p-DMACA solution (Hoboyo Corp., Chengdu, China), then mix in a centrifuge tube. After ten minutes, absorbance was determined at 640 nm. The standard utilized was catechin (6.25–200 mg/L). The outcomes were presented in terms of catechin equivalents.

2.6. Determination of antioxidant capacity

2.6.1. Determination of DPPH

The DPPH determination referenced to previous methods (Brand-Williams et al., 1995). A 200 μL of materials extract was combined with 4 mL of DPPH methanol solution (6.25×10^{-5} M) (Hoboyo Corp., Chengdu, China) and reacted for 20 min away from light. Absorbance was determined at 517 nm. The results were represented as μmol/L Trolox equal antioxidant capacity, with Trolox as the benchmark.

2.6.2. Determination of FRAP

The FRAP determination referenced to previous methods (Benzie & Strain, 1999). Add 200 μL of materials extract to 2 mL of purified water and 3.6 mL of triphenyl tetrazolium chloride solution (Hoboyo Corp., Chengdu, China). Mix well and react at 37 °C for ten minutes. Absorbance values were determined at 593 nm. The results were represented as μmol/L Trolox equal antioxidant capacity.

2.7. Expression analysis of phenolic compound genes in WSQCL and QCL

RNA was extracted using CTAB method (Yi et al., 2018). The Goldenstar™ RT 6 cDNA Synthesis Ver. 2 (Tsingke Corp., Beijing, China) reverse transcription kit was used. We searched the sequence information of genes related to phenolic compounds in plums on the NCBI (National Center of Biotechnology Information), extracted the

associated sequences, compared them to the *Prunus domestica* genomic database through blast, and screened and obtained the related genes, *PAL1, PAL3, C4H, 4CL1, 4CL2, CHS, HCT1,* and *HCT2.* The sequences of the associated plum genes were detected using TBtools (South China Agricultural University, Guangzhou, China). We used Beacon Designer 7.0 (PREMIER Biosoft Corp., San Francisco, USA) software to design the primers for gene sequencing (Table S1). Using the SYBR Premix Ex TaqTM II kit (Tsingke Corp., Beijing, China), reactions were run on a CFX 96 PCR machine(Bio-Rad Laboratories, California, USA) with 40 cycles of pre-denaturation at 95 °C for 30 s, denaturation at 95 °C for 5 s, and annealing at 58 °C for 30 s. We use the $2^{-\Delta\Delta Ct}$ approach was used to calculate the relative expression.

2.8. Data analysis

The data are presented as mean ± standard deviation, with each value determined in three replicates. IBM SPSS 26.0 (IBM Corp., Armonk, N.Y., USA) analyzed the data using one-way ANOVA. PCA was carried out using SIMCA 14.1 (Umetrics Corp., Umeå, Sweden)

3. Results and discussion

3.1. The dynamics of plum fruit growth

Bud sport selection is an essential way to select new varieties of fruit trees (Xiux et al., 2019), and a phenological period investigation distinguishes the differences in biological traits between bud mutations and their parents (Wadhwa & Sihag, 2019). In this study, the phenological period of WSQCL and its parent QCL were similar, with the flowering period about 5 days later. Nevertheless, the fruit development period was about 15 days longer, and the differences were most significant in the sclerotizing, expansion, and ripening phases (Table S2). The maturation of WSQCL was similar to that of CHL.

The fruit developmental stage, color, and fruit shape index are essential indicators of a comprehensive fruit quality evaluation (Yang et al., 2019). In this study, the pericarp and pulp of WSQCL and QCL shifted from green to yellowish-green as the fruit matured (Fig. 1A, C). The CHL fruit peel changed from green to red, and the pulp changed from green to yellow (Fig. 1B). The trends in per fruit weight and longitudinal and transverse diameters of the three plum varieties were consistent (Fig. 1D, E, F), and the growth rates of the three varieties were in the order QCL > WSQCL > CHL. The WSQCL growth cycle was significantly longer than that of CHL. The fruit shape indices of the three plum varieties ranged from 0.82 to 1.16 (Fig. 1G), and the shapes of the mature fruits were nearly spherical. The main difference between WSQCL and its parents was the longer growing period. The weight of the WSQCL fruit was lower than that of QCL but larger than that of CHL.

3.2. Changes in fruit color and pigment substances

Fruit color is a crucial indicator of both commercial and nutritional value (Wang et al., 2024). In this research, the highest peel L* values in CHL occurred during the S1-S4 periods and QCL had the highest peel L* values as the fruit ripened. The WSQCL and CHL pulp L* values varied consistently with the peel, while that of CHL was substantially higher compared to the other two varieties (Fig. 2A-1, 2). The three varieties of plum peel and pulp a* values showed an overall increasing trend, and the CHL peel and pulp a* values were obviously greater than those of the other two varieties (Fig. 2B-1, 2). The b* values of the WSQCL and QCL fruits showed the same changes, as both increased in the late phases of fruit development. WSQCL had the highest fruit ripening b* value, followed by QCL and CHL (Fig. 2C-1, 2).

Fruit color is a vital indicator of fruit development and ripening, and it is primarily regulated by pigment type and content. Chlorophylls, carotenoids, and anthocyanins impact the color of plum fruits (Ranganath, 2022). Chlorophyll degradation is an essential sign of fruit ripening (Infante et al., 2011). Fruit chlorophyll content decreased in all three plum varieties, which was confirmed with the findings of previous studies (Alina et al., 2018). Chlorophyll content in the peel was in the following order: QCL > WSQCL > CHL (Fig. 2D-1). The chlorophyll content in the pulp was less than that in the peel, and the chlorophyll content of the three varieties was in the order: WSQCL > QCL > CHL (Fig. 2D-2). Fruit ripening is usually accompanied by changes in carotenoids (Kapoor et al., 2022). The carotenoid content in the WSQCL peel increased and then decreased and was higher at the young fruit stage, reaching a maximum value (75.08 μg/g) at S2. In contrast, the carotenoids in the QCL and CHL peels decreased and then increased, with the highest contents of 61.33 μg/g and 55.9 μg/g, respectively, at the fruit ripening stage. The WSQCL carotenoids were markedly lower than those of the other two cultivars at the later phases of fruit growth (S4-S6). The

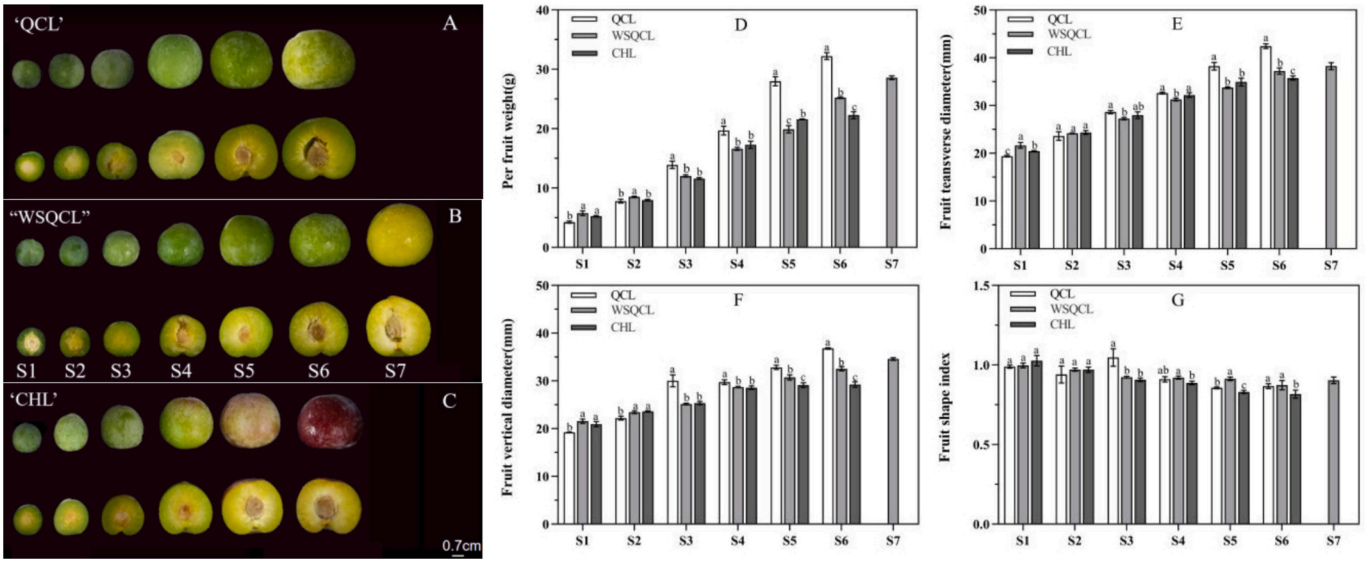

Fig. 1. Changes in the appearance and quality of three varieties of plum fruits during fruit development. Note:'Qiangcuili'(QCL), 'Late-maturing Qiangcuili'(WSQCL), 'Cuihongli'(CHL), same below; QCL as a whole and in profile (**A**), WSQCL as a whole and in profile (**B**), CHL as a whole and in profile (**C**), per fruit weight (**D**), transverse diameter (**E**), longitudinal diameter (**F**), fruit shape index (**G**). According to Duncan's test ($p < 0.05$), significant differences are indicated by a-g, where each value represents the mean and standard error for $n = 3$, the same as below.

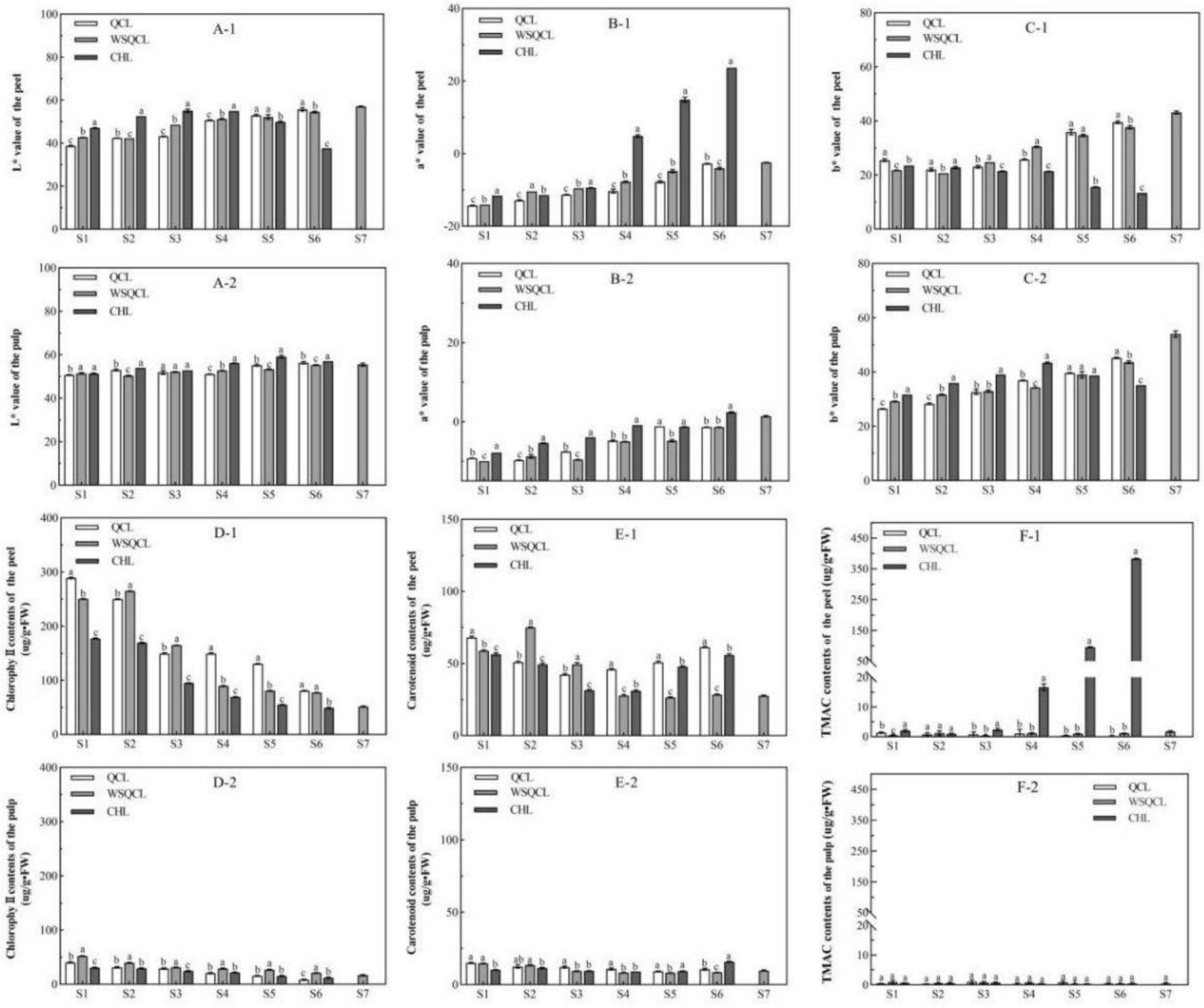

Fig. 2. Changes in color and pigment content during the development of three plum varieties. L* value of peel (**A-1**), L* value of flesh (**A-2**), a* value of peel (**B-1**), a* value of flesh (**B-2**), b* value of peel (**C-1**), b* value of flesh (**C-2**), chlorophyll content of peel (**D-1**), chlorophyll content of flesh (**D-2**), carotenoid content of peel (**E-1**), carotenoid content of flesh (**E-2**), total anthocyanin content (TMAC), TMAC of peel (**F-1**), TMAC of pulp (**F-2**).

carotenoid content in the pulp of the different plum varieties decreased and then increased, which was consistent with the trend in the pulp b* value, and the carotenoid content in the pulp accumulated during the later stages of fruit development. In line with the findings of previous author (Gil et al., 2002) on plums, the flesh had lesser carotenoid levels than the peel in all three plum varieties (Fig. 2E-1, 2). The yellowish-green coloration of WSQCL and CHL resulted from the decomposition of chlorophyll and accumulation of carotenoids. At the same time, the red color of the CHL fruits at the late stage was related to the accumulation of anthocyanins. Current studies on plum fruit coloring have concluded that the color presentation of red and purple-black plum varieties at fruit ripening is mainly determined by the anthocyanin content, with the darker the color, the higher the content (Gu et al., 2015). The yellow-green fruits of WSQCL and QCL had low total anthocyanin levels detected in the peel and flesh. Consistent with changes in the a* values, the TFAC in the CHL peel increased significantly from the coloring stage (S4). It reached the greatest at the ripening stage, and TFAC was highly significantly greater in the peel (383.07 mg/g·FW) than in the flesh (0.47 mg/g·FW) (Fig. 2F-1, 2).

3.3. Changes in SS, TA, and starch content

Fruit sugars and organic acids are essential index of fruit tastes (Forcada et al., 2019). Similar to research on 'Fengtang' plums (Nie et al., 2023), all three plum cultivars showed modest increases in the SS content during fruit maturity. At the fruit ripening stage, the SS contents of WSQCL, QCL, and CHL were 10.20 %, 11.93 %, and 12.06 % in the peels and 10.64 %, 13.06 %, and 13.89 % in the flesh. The SS content of WSQCL was lower than that of QCL and CHL (Fig. 3A-1 2). Changes in TA content are an indicator of fruit maturity. Consistent with studies on the 'Crisp red plum' (CHL), and its mutant (Liao et al., 2023), the TA content of the peel and flash of the three plum varieties declined progressively as the fruit grew and developed. The TA content of WSQCL was higher during the S6 period than that of the other plums, but there was little difference in TA content during the fruit ripening period. The TA contents of the peel were 0.71 %, 0.68 %, and 0.75 %, and those of the flesh were 0.34 %, 0.35 %, and 0.34 %, respectively (Fig. 3B-1, 2). The lower TA content of plum fruits could be attributed to increased basal metabolism and sugar or secondary chemical synthesis during ripening (Jiang et al., 2018). In non-starch-accumulating fruits, starch

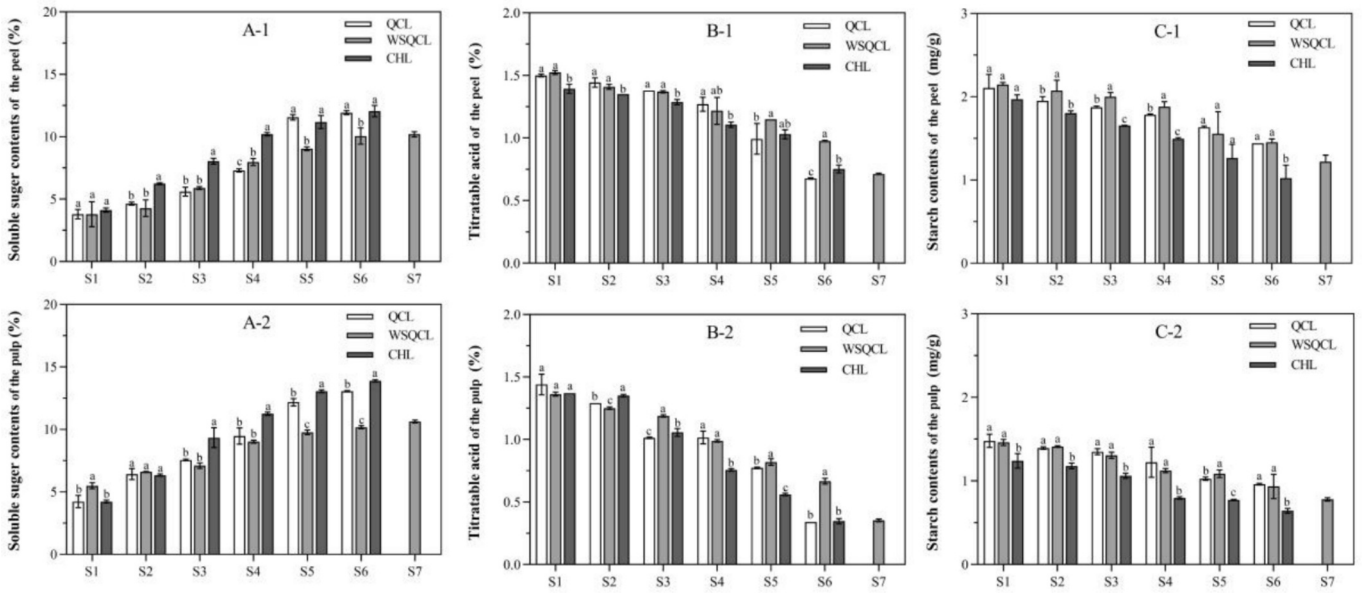

Fig. 3. Changes in SS, TA, and starch content during fruit development of three plum varieties. SS of peel (**A-1**), SS of pulp (**A-2**), TA of peel (**B-1**), TA of pulp (**B-2**), starch of peel (**C-1**), starch of pulp (**C-2**).

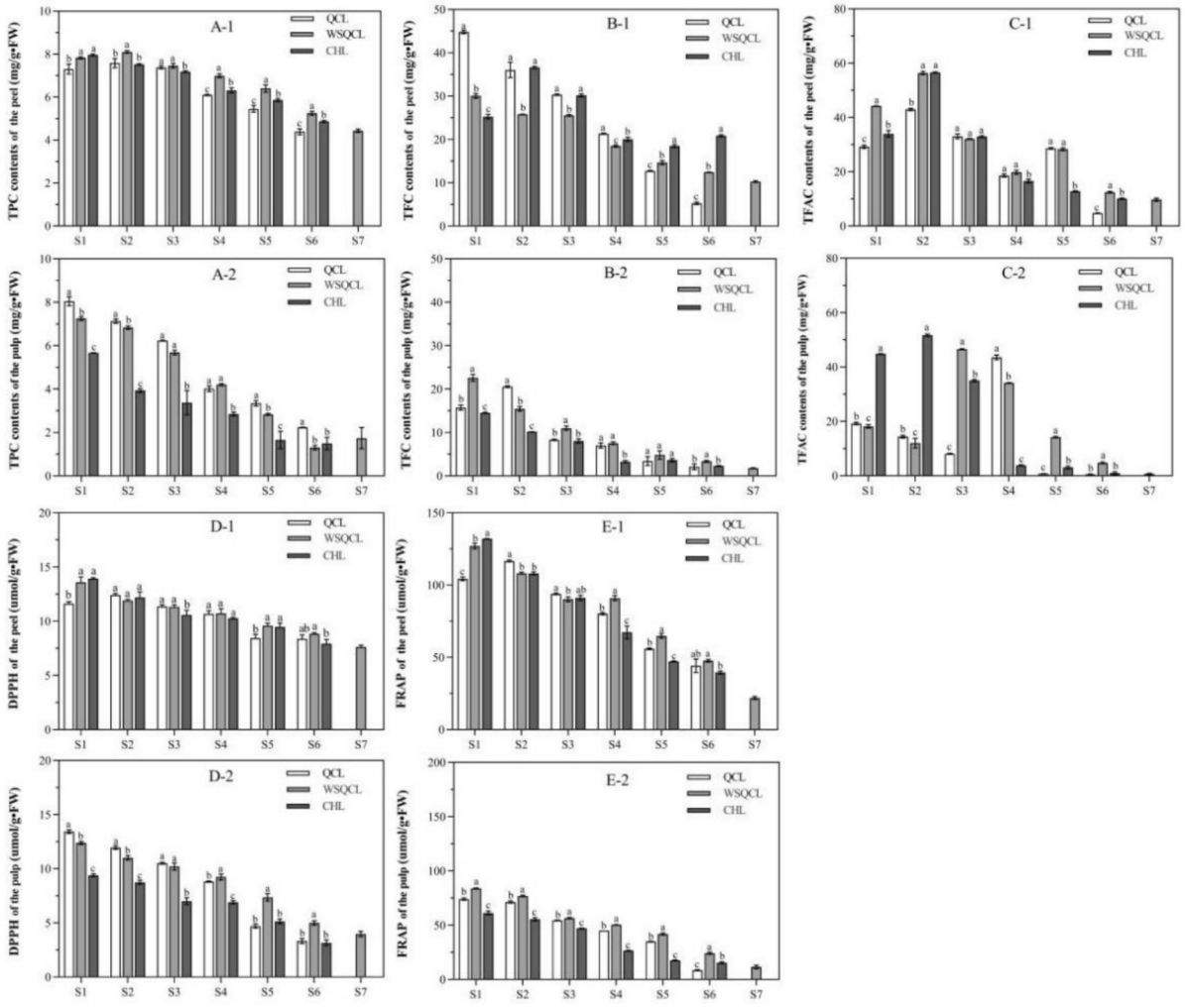

Fig. 4. Changes in phenolic compound content and antioxidant capacity of three plum varieties during fruit development. TPC of peel (**A-1**), TPC of pulp (**A-2**), TFC of peel (**B-1**), TFC of pulp (**B-2**), TFAC of peel (**C-1**), TFAC of pulp (**C-2**), DPPH of peel (**D-1**), DPPH of pulp (**D-2**), FRAP of peel (**E-1**), FRAP of pulp (**E-2**).

content decreases with fruit development (Ren et al., 2023). The overall starch content in the fruits of the three plum varieties decreased with fruit development. During fruit ripening, the starch content was in the order of QCL > WSQCL > CHL and the starch content of the flesh of the three varieties of plum was lower than that of the peel (Fig. 3C-1, 2).

3.4. Changes in phenolic content and antioxidant capacity

Plum fruits include natural phenolic compounds, flavonoids, anthocyanins, and other bioactive components with significant nutritional value. (Kim et al., 2003). Similar to a previous study (Redondo et al., 2016), TPC was greater in immature fruits and gradually decreased during fruit ripening. TPC of WSQCL was higher than that of the QCL and CHL peel, whereas QCL flesh had the highest content (Fig. 4A-1, 2). The total flavanol content (TFC) of the three plum varieties decreased with fruit development. The CHL peel contained the highest TFC content, while the TFC in the peel of WSQCL was lower than that of QCL during the S1-S4 periods and higher than that of QCL during the late phase of fruit development (S5-S7). The TFC in WSQCL pulp was higher than that in QCL and CHL, except during the S2 period (Fig. 4B-1, 2). The total flavanol content (TFAC) in the peels of the three plum varieties followed the same trend, reaching their highest levels during the S2 period, and were higher in WSQCL than in the other varieties at the ripening stage (12.45 mg/g·FW). The TFAC of fruit flesh from QCL, WSQCL, and CHL reached the highest level during the S4, S3, and S2 periods, respectively, while TFAC was low in all three varieties at the ripening stage (Fig. 4C-1, 2). The changes in TFAC observed in the earlier research are similar to those of Zhang et al. in CHL and QCL plum fruits (Zhang et al., 2022).

Fruits are an excellent source of antioxidant substances (Zhu et al., 2023). The changes in antioxidant capacity in the fruits of the three plum types corresponded to the TPC trend, which gradually decreased during fruit growth and development and were significantly lower at the maturity period than at the young fruit stage (Fig. 4D-1, 2 and Fig. 4E-1, 2). The antioxidant capacity of the fruit pulp of the three plum varieties was lower than that of the peel during the same growth period, and WSQCL demonstrated more potent antioxidant activity than QCL and CHL. Research indicates that TPC has a direct influences on the antioxidant activity of fruits (Vlaic et al., 2017). DPPH and FRAP in the WSQCL and CHL peels show a remarkable correlation with TPC, TFC, TFAC, and TMAC, with the strongest correlation with TPC. DPPH and FRAP in the QCL were significantly associated with TPC, TFAC, and total anthocyanins (Table S3). In the pulp, DPPH and FRAP were significantly correlated with TPC, TFAC, and total flavanols in WSQCL and QCL, whereas CHL fruit antioxidant properties were significantly associated with total anthocyanins. Consistent with previous studies on the peel and flesh of European plums, TPC, total flavonoids (TFC), and anthocyanins correlated well with their antioxidant capacity (DPPH and FRAP) (Trendafilova et al., 2022).

3.5. Expression analysis of genes related to synthesis of phenolic compounds

Phenolic chemicals are generated via the shikimic acid pathway, and the gene expression and enzyme activities of *PAL, C4H, 4CL, HCT*, and *CHS* in the pathway are closely related to TPC (Guodong et al., 2019). To further investigate the reasons why the phenolic and antioxidant activities of WSQCL were higher than those of QCL, we analyzed the expression changes in eight genes from the phenolic synthesis pathway (Fig. 5). Consistent with the trend in TPC, the expression of *4CL1, HCT1*, and *CHS* in WSQCL peel was downregulated during fruit development (Fig. 5A), and the expression of the *PAL3, 4CL1, 4CL2, HCT1,* and *CHS* genes in the pulp decreased (Fig. 5B). The correlation analysis revealed that the phenolic content in the fruits of WSQCL and QCL was strongly positively correlated with the expression of *PAL3*, *4CL1*, *HCT1*, and *CHS* (Tables S4 and S5). *PAL* is the primary enzyme regulating the metabolism of phenolic compounds. *4CL1* is primarily involved in pathways related to the metabolism of flavonoids, CHS is a critical rate-limiting enzyme in flavonoid synthesis and *HCT* is a crucial gene for synthesizing the phenolic substance chlorogenic acid (Goto-Yamamoto et al., 2002). The expression levels of *PAL3, 4CL1, HCT1,* and *CHS* in the WSQCL peel were higher during fruit development than that of QCL, which was consistent with the difference in phenolic content in the peel. We speculated that the elevated phenolic content in the WSQCL peel was attributable to increased expression of these genes.

3.6. Principal component analysis

The principal component analysis (PCA) is an efficient method for evaluating sample differences. (Di et al., 2022). This study used PCA to analyze 16 quality indicators of plum fruits, aiming to distinguish the differences in the developmental processes among the three plum varieties (Fig. 6). As results, two principal components explained 84.7 % of the total variance in the fruit peel of the three plum varieties, with the first principal component (PC1) and the second principal component (PC2) accounting for 67.4 % and 17.3 % of the total variance, respectively. The WSQCL and QCL quality indices were close to each other during the early and late phases of development, and the difference in peel development occurred during the WSQCL S4-S5 period. In addition, the CHL S5 and S6 periods were more discrete from the other periods (Fig. 6A), and in combination with the fruit quality PCA analysis (Fig. 6B), the more discrete CHL S5 and S6 periods were influenced by the total anthocyanin and a* values. PCA of the pulp of the three plum materials revealed that PC1 and PC2 explained 65.8 % and 12.5 % of the variance, respectively. Similar to the results in the peel, the quality of WSQCL and QCL was close to each other during the early stage (S1-S2) and fruit ripening (S6–S7), but the differences were more significant during the S3–S5 period, with the differences in the flesh showing earlier than those in the peel (Fig. 6C).

According to the loading plot (Fig. 6B and Fig. 6D), it was seen that

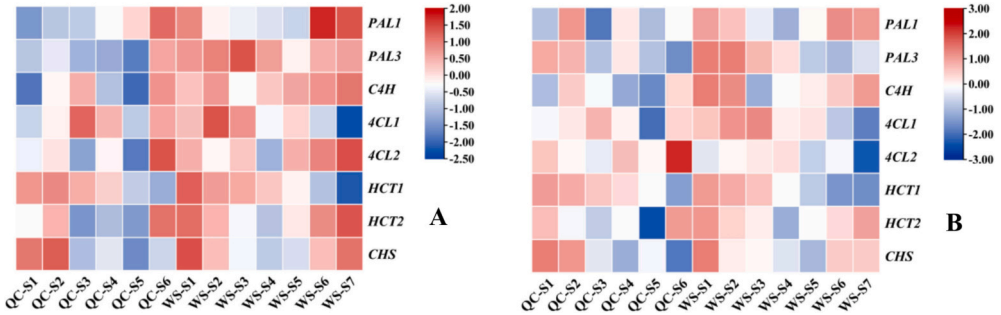

Fig. 5. Changes in gene expression related to phenolic compound synthesis during WSQCL and QCL fruit development. QC-'Qiangcuili', WS-'Late-maturing Qiangcuili', peel (**A**), flesh (**B**).

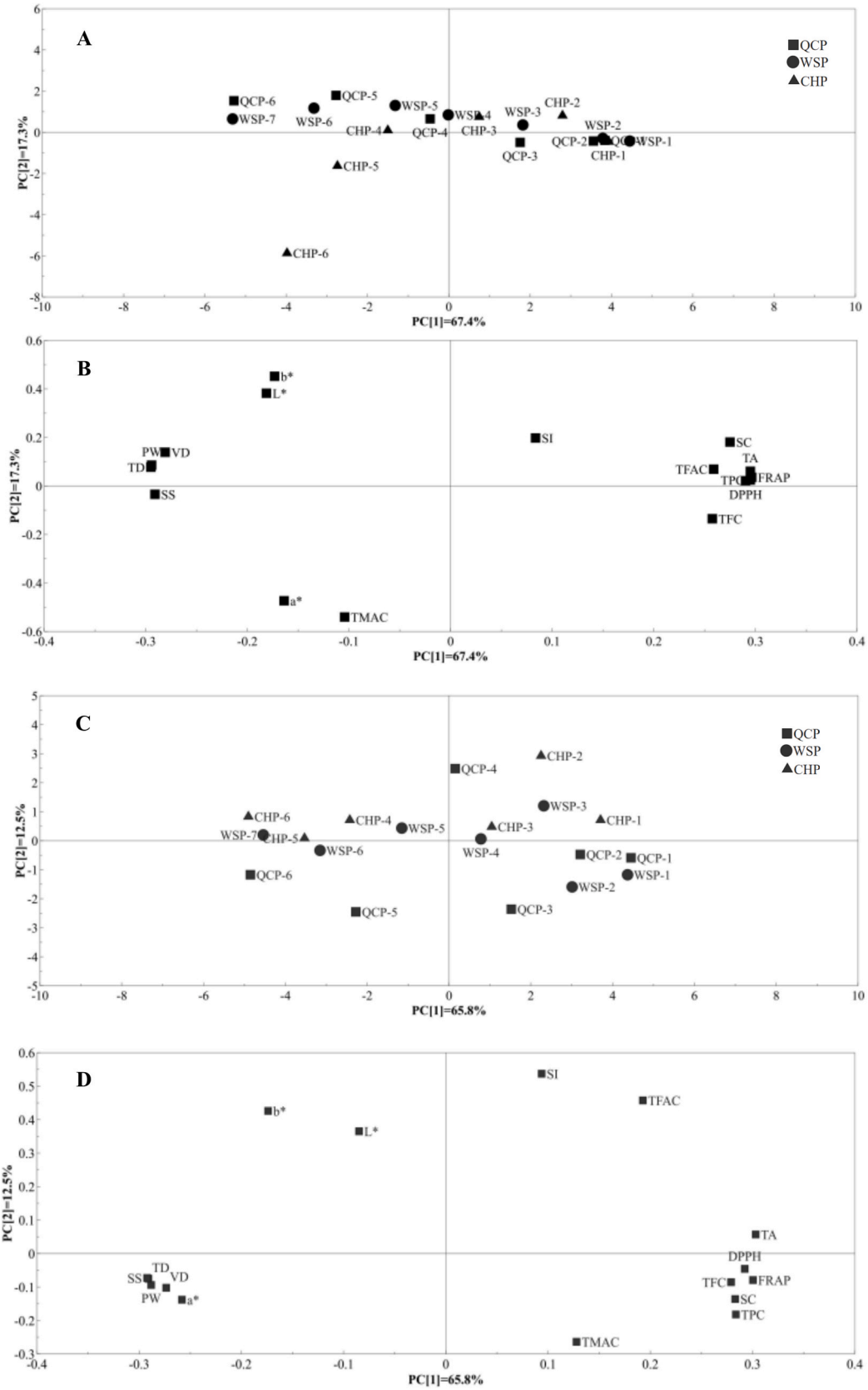

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Fig. 6. Principal component analysis of the appearance and intrinsic indices during fruit growth of three plum cultivar. Score scatter plot of peel(**A**), Loading scatter plot of peel(**B**), Score scatter plot of pulp (**C**), Loading scatter plot of pulp (**D**), the first to sixth period of 'Qiangcuili' peels (QCP1–6), the first to seventh period of 'Late-maturing Qiangcuili' peels (WSP1–7), the first to seventh period of 'Cuihongli' peels (CHP1–7), the first to sixth period of 'Qiangcuili' flesh (QCR1–6), the first to seventh period of 'Late-maturing Qiangcuili' flesh (WSR1–7), the first to seventh period of 'Cuihongli' flesh (CHR1–7), single fruit weight (PW), transverse diameter (TD), longitudinal diameter (VD), fruit shape index (SI), L* value (L*), a* value (a*), b* value (b*), total anthocyanin content (TMAC), soluble sugar (SS), titratable acid (TA), starch (SC), total phenolic content (TPC), total flavonoid content (TFC), total flavanol content (TFAC), DPPH radical scavenging capacity (DPPH), FRAP ferric reducing antioxidant capacity (FRAP).

hardness, TFAC, TPC, FARP, DPPH, and TA in the peel and pulp were clustered at the young fruit stage and were the main quality contributors during the pre-developmental stage of the fruit, whereas SS, per fruit weight, and longitudinal and transversal diameters clustered together in one group and were correlated with fruit maturity. WSQCL differed significantly from QCL and CHL mainly during the middle and late stages of fruit development.

4. Conclusion

The WSQCL growth and development cycle was significantly longer than that of its QCL parent, but the changes in the WSQCL, QCL, and CHL fruit quality indices were similar during fruit development. The carotenoid, anthocyanin, TPC, and antioxidant activity of the peel were higher than those of the pulp in the plum varieties. The TPC and antioxidant activities in the WSQCL fruit were higher than those of QCL, which may have been related to the higher expression of *PAL3*, *4CL1*, *HCT1*, and *CHS*. The PCA of 16 fruit quality indicators revealed that the differences between WSQCL and QCL occurred mainly during the middle and late stages of fruit development.

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CRediT authorship contribution statement

Huifen Zhang: Writing – review & editing, Formal analysis. **Ying Du:** Writing – original draft, Formal analysis. **Yinyin Meng:** Investigation. **Xiaofu Tang:** Formal analysis. **Jie Niu:** Writing – original draft. **Hongxia Wang:** Validation. **Yihua Ren:** Validation. **Penghan Yi:** Validation. **Xian Luo:** Resources. **Qunxian Deng:** Resources, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2025.100251.

Data availability

The data that has been used is confidential.

References

Aladag, M. O., Doğu, S., Uslu, N., Özcan, M. M., Gezgin, S., & Dursun, N. (2020). Effect of drying on antioxidant activity, phenolic compounds and mineral contents of hawthorn and wild pear fruits. *Erwerbs-Obstbau, 62*, 473–479. https://doi.org/10.1007/s10341-020-00526-6

Alina, V. R., Vlad, M., Elena, M. A., Carmen, M. C., Adriana, P., Viorel, M., Simona, C. M., & Muste, S. (2018). The changes of polyphenols, lavonoids, anthocyanins and chlorophyll content in plum peels during growth phases: From fructification to ripening. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46*, 148–155. https://doi.org/10.15835/nbha46111017

Al-Khayri, J. M., Jain, S. M., & Johnson, D. V. (2018). *Advances in plant breeding strategies: Fruits. 3*. Springer, 10.1007/978-3-319-91944-7.

Benzie, I., & Strain, J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology, 299*, 15–27. https://doi.org/10.1016/s0076-6879(99)99005-5

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT Food Science and Technology, 28*, 28–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Chen, H., Xu, Q. H., Shen, S. L., Dong, J. X., & Zheng, X. L. (2021). Effect of benzothiadiazole treatment on quality and anthocyanin biosynthesis in plum fruit during storage at ambient temperature. *Journal of the Science of Food and Agriculture, 101*, 3176–3185. https://doi.org/10.1002/jsfa.10946

Di, H., Cui, C., Fang, P., Ma, J., He, M., Li, M., … Zheng, Y. (2022). Variation in the main health-promoting compounds and antioxidant activity of different organs of wasabi (*Eutrema japonicum*) from two producing areas. *Frontiers in Plant Science, 13*, 1043378. https://doi.org/10.3389/fpls.2022.1043378

Forcada, C., Reig, G., Gimenez, R., Mignard, P., Mestre, L., & Moreno, M. (2019). Sugars and organic acids profile and antioxidant compounds of nectarine fruits influenced by different rootstocks. *Scientia Horticulturae, 248*, 145–153. https://doi.org/10.1016/j.scienta.2018.12.010

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., & Kader, A. A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry, 50*, 4976–4982.

Goto-Yamamoto, N., Wan, G. H., Masaki, K., & Kobayashi, S. (2002). Structure and transcription of three chalcone synthase genes of grapevine (*Vitis vinifera*). *Plant Science, 162*, 867–872, 10.1016/j.phytochem.2014.03.024.doi:10.1016/S0168-9452(02)00042-0.

Gu, C., Liao, L., Zhou, H., Wang, L., Deng, X., & Han, Y. (2015). Constitutive activation of an anthocyanin regulatory gene PcMYB10.6 is related to red coloration in purple-foliage plum[J]. *PLoS One, 10*, Article e0135159. https://doi.org/10.1371/journal.pone.0135159

Guodong, R., Jianguo, Z., Xiaoxia, L., & Ying, L. (2019). Identification of putative genes for polyphenol biosynthesis in olive fruits and leaves using full-length transcriptome sequencing. *Food Chemistry, 300*, Article 125246, 10.1016/j.foodchem.2019.125246. doi:10.1021/jf020136b.https://journals.tubitak.gov.tr/botany/vol22/iss1/3.

Hwang, H., Myracle, A., Moran, R., Fort, R., & Cole, B. (2020). Impact of fruit maturity and growing season on phenolic phytochemicals and antioxidant activity among four asian plum cultivars. *Journal American Pomological Society, 74*, 14–19. https://www.researchgate.net/publication/344127033.

Infante, R., Rubio, P., Contador, L., Noferini, M., & Costa, G. (2011). Determination of harvest maturity of D'Agen plums using the chlorophyll absorbance index. *Ciencia e investigación agraria, 38*, 199–203. https://doi.org/10.4067/S0718-16202011000200004

Jiang, C., Fang, Z., Zhou, D., Pan, S., & Ye, X. (2018). Changes in secondary metabolites, organic acids and soluble sugars during the development of plum fruit cv. 'Furongli' (*Prunus salicina* Lindl.). *Journal of the Science of Food and Agriculture, 99*, 1010–1019. https://doi.org/10.1002/jsfa.9265

Kapoor, L., Simkin, A. J., George Priya Doss, C., & Siva, R. (2022). Fruit ripening: Dynamics and integrated analysis of carotenoids and anthocyanins. *BMC Plant Biology, 22*, 27. https://doi.org/10.1186/s12870-021-03411-w

Kayano, S. I., Kikuzaki, H., Fukutsuka, N., Mitani, T., & Nakatani, N. (2002). Antioxidant activity of prune (Prunus domestica L.) constituents and a new synergist. *Journal of Agricultural and Food Chemistry, 50*, 3708–3712. https://doi.org/10.1021/jf0200164

Kim, D., Chun, O., Kim, Y., Moon, H., & Lee, C. (2003). Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry, 51*, 6509–6515. https://doi.org/10.1021/jf0343074

Liao, L., Li, Y., Lan, X., Yang, Y., Wei, W., Ai, J., Feng, X., Chen, H., Tang, Y., Xi, L., & Wang, Z. (2023). Integrative analysis of fruit quality and anthocyanin accumulation of plum cv. 'cuihongli' (*Prunus salicina* Lindl.) and its bud mutation. *Plants, 12*, 1357. https://doi.org/10.3390/plants12061357

Lin, Z., Li, B., Liao, M., Liu, J., Zhou, Y., Liang, Y., … Li, H. (2023). The physicochemical attributes, volatile compounds, and antioxidant activities of five plum cultivars in Sichuan. *Foods, 12*, 3801. https://doi.org/10.3390/foods12203801

Liu, S., Liu, Y. C., Liu, N., Zhang, Y. P., Zhang, Q. P., Xu, M., … Liu, W. S. (2016). Sugar and organic acid components in fruits of plum cultivar resources of genus Prunus. *Scientia Agricultura Sinica, 49*, 3188–3198. https://doi.org/10.3864/j.issn.0578-1752.2016.16.012

Nie, X., Hong, C., Wang, Q., Lu, M., & An, H. (2023). Sugar composition and transcriptome analysis in developing "Fengtang" plum (*Prunus salicina* Lindl.) reveal candidate genes regulating sugar accumulation. *Plant Physiology and Biochemistry, 202*, Article 107955. https://doi.org/10.1016/j.plaphy.2023.107955

Ranganath, K. G. (2022). Pigments that colour our fruits: An overview. *Erwerbs-Obstbau, 64*, 535–547. https://doi.org/10.1007/s10341-022-00698-3

Redondo, D., Arias, E., Oria, R., & Venturini, M. E. (2016). Thinned stone fruits are a source 413 of polyphenols and antioxidant compounds. *Journal of the Science of Food and Agriculture, 97*, 902–910. https://doi.org/10.1002/jsfa.7813

Ren, Y., Liao, S., & Xu, Y. (2023). An update on sugar allocation and accumulation in fruits. *Plant Physiology, 193*, 888–899. https://doi.org/10.1093/plphys/kiad294

Shen, N., Wang, T., Gan, Q., Liu, S., Wang, L., & Jin, B. (2022). Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chemistry, 383*, Article 132531. https://doi.org/10.1016/j.foodchem.2022.132531

Trendafilova, A., Ivanova, V., Trusheva, B., Kamenova-Nacheva, M., Tabakov, S., & Simova, S. (2022). Chemical composition and antioxidant capacity of the fruits of European plum cultivar "Čačanska Lepotica" influenced by different rootstocks. *Foods, 11*, 2844. https://doi.org/10.3390/foods11182844

Vlaic, R. A., Muresan, V., Muresan, A. E., Muresan, C. C., Paucean, A., Mitre, V., … Muste, S. (2017). The changes of polyphenols, flavonoids, anthocyanins and chlorophyll content in plum peels during growth phases: From fructification to ripening. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46*, 148–155. https://doi.org/10.15835/nbha46111017

Wadhwa, N., & Sihag, R. C. (2019). Flowering phenology of european plum (Prunus domestica L.) in the semi-arid environment of north-West India. *Asian Journal of Biological Sciences, 12*, 17–23. https://scialert.net/abstract/?doi=ajbs.2019.17.23.

Wang, L., Sang, W., Xu, R., & Cao, J. (2020). Alteration of flesh color and enhancement of bioactive substances via the stimulation of anthocyanin biosynthesis in 'friar' plum fruit by low temperature and the removal. *Food Chemistry, 310*, Article 125862. https://doi.org/10.1016/j.foodchem.2019.125862

Wang, N., Liu, W., Mei, Z., Zhang, S., Zou, Q., Yu, L., Jiang, S., Fang, H., Zhang, Z., Chen, Z., Wu, S., Cheng, L., & Chen, X. (2024). A functional indel in the WRKY10 promoter controls the degree of flesh red pigmentation in apple. *Advanced science (Weinheim), 11*, Article e2400998. https://doi.org/10.1002/advs.202400998

Xiux, D., Lir, W., Shaoh, L., Shaol, Z., Zhih, Z., Peih, C., Ganj, Y., Xues, C., Houb, C., & Caih, Z. (2019). Retrospection and prospect of fruit breeding for last four decades in China. *Journal of Fruit Science, 36*, 514–520. https://doi.org/10.13925/j.cnki.gsxb.20190094

Yang, T., Ma, H., Zhang, J., Wu, T., Song, T., Tian, J., & Yao, Y. (2019). Systematic identification of long noncoding RNAs expressed during light-induced anthocyanin accumulation in apple fruit. *The Plant Journal, 100*, 572–590. https://doi.org/10.1111/tpj.14470

Yi, S., Jin, W. T., Yuan, Y. N., & Fang, Y. H. (2018). An optimized CTAB method for genomic DNA extraction from freshly-picked pinnae of fern, *adiantum capillus-veneris L. Bio-protocol, 8*, Article e2906. https://doi.org/10.21769/BioProtoc.2906

Yu, X., Rizwan, H., Li, P., Luo, S., Sherameti, I., Wu, W., Lin, J., Zheng, S., Oelmüller, R., & Chen, F. (2020). Comparative studies on the physiochemical properties, phenolic compounds and antioxidant activities in 13 Japanese plum cultivars grown in the subtropical region of China. *Applied Ecology and Environmental Research, 18*, 3147–3159. https://doi.org/10.15666/aeer/1802_31473159

Zhang, H., Pu, J., Tang, Y., Wang, M., Tian, K., Wang, Y., Luo, X., & Deng, Q. (2022). Changes in phenolic compounds and antioxidant activity during development of 'Qiangcuili' and 'Cuihongli' fruit. *Foods, 11*, 3198. https://doi.org/10.3390/foods11203198

Zhang, K., Ma, Q., Wang, Y., Yuan, Z., Yang, Z., Luo, X., & Deng, Q. (2024). Transcriptome and biochemical analyses reveal phenolic compounds-mediated flavor differences in loquat (*Eriobotrya japonica* Lindl.) cultivars Chunhua no.1 and Dawuxing. *Food Chemistry: X*, 21. https://doi.org/10.1016/j.fochx.2024.101145

Zhu, C. W., Lü, H., Du, L. L., Li, J., Chen, H., Zhao, H. F., … Li, W. L. (2023). Five blueberry anthocyanins and their antioxidant, hypoglycemic, and hypolipidemic effects in vitro. *Frontiers in Nutrition, 10*, 1172982. https://doi.org/10.3389/fnut.2023.1172982