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Dynamic Changes in Polyphenols in Fruit Development of Red Flesh Apple 'Hongxun 2'

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Abstract: In this study, fruits of the red flesh *Malus* plant 'Hongxun 2' (*Malus neidzwetzkyana* (Dieck) Langenf.) and green flesh *Malus* plant 'Xinye 13-11' (*Malus sieversii* (Led.) Roem.) were used as experimental materials. Both of them came from Xinjiang, China, and *Malus neidzwetzkyana* (Dieck) Langenf. is believed to be a variant of *Malus sieversii* (Led.) Roem. The components and contents of polyphenols in the peel and pulp of the two kinds of fruit during the development period were detected, and the dynamic changes and differences in the polyphenols between the two kinds of fruit were discussed. The results showed that the total polyphenol content of 'Xinye 13-11' was higher in the peel and pulp than that of 'Hongxun 2', and the content of peel was higher than that of pulp in the two kinds of fruit. An analysis of five types of polyphenols showed that anthocyanins were only contained in the peel and pulp of 'Hongxun-2', and the peel had a higher content than the pulp. Cyanidin 3-O-galactoside was the main anthocyanin component. Four other types of substances, except hydroxycinnamics, were higher in 'Hongxun-2' than 'Xinye 13-11', while the contents of other substances in 'Xinye 13-11' were higher than those of 'Hongxun 2'. The accumulation of major polyphenol components in the peel and flesh of 'Hongxun 2' and 'Xinye 13-11' apples was significant in the period before and after 65 days after flowering, and the contents of procyanidin B1 and procyanidin C1 were the highest in this period. In addition to the difference in anthocyanin content between 'Hongxun 2' and 'Xinye 13-11', the chlorogenic acid content in the peel and pulp of 'Hongxun 2' was significantly higher than that of 'Xinye 13-11', and the contents of other components were lower than those of 'Xinye 13-11'. Moreover, based on the components and contents of polyphenol components, this paper supports the viewpoint that *Malus neidzwetzkyana* (Dieck) Langenf is a separate species to *Malus sieversii* (Led.) Roem.

Keywords: apple; red flesh; polyphenol; dynamic changes

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1. Introduction

Polyphenols are an indispensable component of the human diet and are widely found in fruits, vegetables, nuts, and other crops. Apples are the third-largest dietary source of polyphenols after tea and onions [1,2]. Polyphenols are closely related to their potential health value in apples, and substances such as procyanidins and catechins have a strong antioxidant capacity and can resist low-density lipoprotein oxidation [3]. Chlorogenic

acid has the ability to scavenge alkyl peroxide free radicals and plays an important role in anti-tumor capabilities [4,5]. Flavonols can inhibit platelet aggregation, calcium activation, and tyrosine protein phosphorylation and effectively resist the occurrence of cardiovascular diseases [6]. Anthocyanins belong to a class of polyphenols stored in plant vacuoles with strong antioxidant, anti-cancer, and free radical scavenging capabilities, in addition to enhancing resistance, restoring vision, and preventing cardiovascular diseases [7].

Malus neidzwetzkyana (Dieck) Langenf. has become a research hotspot due to its high anthocyanin content and strong anti-oxidation, anti-cancer, and free radical scavenging potential. In addition, the flowers, leaves, fruits, and branches of *Malus neidzwetzkyana* (Dieck) Langenf. show different degrees of red, and it is considered to be an ornamental tree. The attractive color is popular with consumers [7–9]. In recent years, Shandong Agricultural University has selected and bred a series of new red flesh varieties with high flavonoids as its main line [10]. Qingdao Agricultural University bred a red flesh variety 'Daihong' [11]. The Research Institute of Pomology of Chinese Academy of Agricultural Sciences bred a red flesh variety 'Hongyun' [12]. Red flesh apple varieties such as 'JPP35', 'Weirouge', 'Baya Marise', and 'Redlove' have also been bred abroad [13,14].

The breeding parents of domestic red flesh varieties mostly come from *Malus neidzwetzkyana* (Dieck) Langenf. Two red flesh superior strains, "Hongxun 1" and "Hongxun 2", with excellent traits, were obtained through the local selection of red flesh apples. In the fruit ripening stage, red flesh apples mainly contain anthocyanins and flavanols, while non-red flesh apples mainly contain flavonols and flavanols. The contents of polyphenolic substances and the antioxidant capacity of red flesh apples are higher than those of non-red flesh apples. In the process of fruit development, except anthocyanin, the polyphenolic substances of red flesh and non-red flesh apples showed a downward trend in the early stage of development, and the change trends of different red flesh types were different. The anthocyanin content of 'Hongxun 1' was low in the early stage, then continued to increase, and significantly decreased before maturity. The initial content of "Xiahongrou" (*Malus neidzwetzkyana* (Dieck) Langenf.) was very high, then it continued to decline, and increased again at maturity [15]. It was found that there were differences in the anthocyanin accumulation patterns among different red flesh types. 'Maypole' always had a large amount of anthocyanin accumulation in the flesh during the whole development period of the fruit, while 'JPP35' only had anthocyanin accumulation at the mature stage [16]. A physical and chemical analysis of "Hongxun 1" cider found that the "Hongxun 1" produced in Tacheng had a rich flavor and red color after aging, the color gradually deepened during the aging process, and had better fermentation and aging habits [17].

There have been many reports on the polyphenol content and dynamic changes in red flesh apples, and many data have shown that *Malus neidzwetzkyana* (Dieck) Langenf. is a variant of the *Malus sieversii* (Led.) Roem. However, there are few studies on the comparison of the polyphenol contents and dynamics between red flesh apples and *Malus sieversii* (Led.) Roem. Polyphenols are a basis for apple classification, and whether red flesh apples are varieties of *Malus sieversii* (Led.) Roem can also be verified by polyphenol studies. In this study, the components and contents of polyphenols in the peel and pulp of 'Hongxun 2' (*Malus neidzwetzkyana* (Dieck) Langenf.) and 'Xinye 13-11' (*Malus sieversii* (Led.) Roem.) during the fruit growth period were determined and analyzed. The differences in the polyphenol components and contents between them were analyzed. The periods of 'Hongxun 2′ suitable for extracting polyphenol components for processing were determined. The relationship between *Malus neidzwetzkyana* (Dieck) Langenf. and *Malus sieversii* (Led.) Roem. was studied.

2. Materials and Methods

2.1. Experimental Material

'Hongxun 2' and 'Xinye 13-11' with good growth were selected as experimental materials. The first samples were collected about 50 days after flowering (21 June 2023), and then samples were collected at 65, 80, 95, and 110 days after flowering. A total of

five samples were collected from the outer crown of the tree with a uniform size. There were 30–50 ripe fruits free of pests, diseases, and mechanical damage in each collection, 10–15 per replicate, and 3 biological replicates were set. The samples were transported to the laboratory at room temperature, then the fruit cores were removed, the peel was sliced, and the flesh was cut into pieces. The peel and flesh were frozen in liquid nitrogen and stored in the refrigerator at −80 °C to be tested.

2.2. Methods

The extraction method for polyphenols referred to the method of Nie et al. and Wang et al. [18,19], which was slightly modified. The apple peel and pulp were frozen and ground into a powder, weighed at 5.0 g (fresh weight, FW), placed in a 50 mL centrifuge tube (BD Falcon®, Corning, New York, NY, USA) with 25 mL of 80% ethanol, shaken well, and placed away from light for 12 h, in an ultrasonic apparatus (SB 25-12 DTD, Ningbo, China). After 20 min of vibration, they were centrifuged for 5 min at 10,000 r/min (CF 16 RX, Hitachi, Japan), the supernatant was absorbed by a pipette carefully, and then discarded. The residue was mixed with 20 mL of 80% ethanol for repeated extraction, the supernatant was combined twice, and the volume of 80% ethanol was fixed to 50 mL. The ethanol was removed by the evaporation of 10 mL of the extract on a 40 °C rotary evaporator (R-215, Buchi, Switzerland). An OasisHLB solid-phase extraction column (Oasis®HLB, Waters, MA, USA) was activated with 10 mL of methanol and 10 mL of purified water. The solid-phase extraction column was washed with 5 mL of deionized water twice and the waste liquid was discarded. With 5 mL of methanol, the solid-phase extraction column was washed twice, and the filtrate was collected. The filtrate collected by the rotary evaporator was evaporated at 40 °C to nearly dry, the methanol volume was fixed to 5 mL, and the filtrate was filtered through a 0.22 μm nylon (Nylon66) organic-phase filter membrane (Jinteng, Tianjin, China) into a brown vial to be measured.

Polyphenols were detected using a UPLC-XeVo/TQ ultra-high-performance liquid chromatograph (UPLC-XEVO/TQ) with a PDA eλ detector (Waters, MA, USA), and the LC-10 ATvp high-performance liquid chromatograph (Shimadzu, Shimane, Japan). The columns were ACQUIITY UPLC® HSS T3 1.8 μm and XSelect® HSS T3 5 μm from the Waters Corporation.

The conditions for the UPLC-Xevo/TQ ultra-high-performance liquid chromatograph and ultra-performance liquid chromatography (UPLC) were as follows: the flow rate was 0.3 mL/min, the sample size was 2.0 μL, the column temperature was 40 °C, the wavelength scanning range was 200–600 nm, the quantitative detection wavelengths were 280 nm (flavanol), 320 nm (hydroxycinnamic acid), 360 nm (flavonol), the mobile phase A was 0.5% formic acid solution, and the mobile phase B was acetonitrile. Gradient dewashing, liquid B, 0% (0 min) → 10% (1 min) → 20% (10 min) → 25% (16 min) → 40% (18 min) → 100% (19 min), 20 min back to the initial state, balance for 5 min.

Dihydrochalcone polyphenols should be detected separately under UPLC conditions. The UPLC conditions were as follows: a flow rate of 0.3 mL·min^{-1}, a sample size of 2.0 μL, a column temperature of 40 °C, a quantitative detection wavelength of 280 nm, mobile phase A as 0.5% formic acid solution, and mobile phase B as acetonitrile. Gradient dewashing, B solution, 0% (0 min) → 8% (2 min) → 15% (10 min) → 23% (20 min) → 100% (20.5 min) → 100% (21.5 min), 22 min return to the initial state, balance for 5 min.

The conditions for liquid chromatography–mass spectrometry (LC-MS) were as follows: in the electrospray ionization (ESI) and multiple reaction monitoring (MRM) modes, the ion source temperature was 150 °C, the desolvent temperature was 450 °C, and the desolvent gas flow rate was 650 L/h. The flow rate of cone hole gas was 50 L/h, and the flow rate of collision gas (high purity argon) was 0.14 mL/min.

The LC-10 ATvp High-Performance Liquid Chromatography (High-Performance Liquid Chromatography, HPLC) conditions were as follows: the flow rate was 0.7 mL·min^{-1}, the sample size was 1 μL, the column temperature was 40 °C, and the wavelength was quantitatively detected at 520 nm (anthocyanins). The mobile phase A was 5% formic acid,

and B was a 1:1 mixture of formic acid and acetonitrile. Gradient dewashing, solution B: 5% (0 min) →10% (10 min) →20% (30 min) →30% (40 min) →90% (40.5 min) →90% (44.5 min) →5% (50 min), return to the initial state, balance for 20 min.

2.3. Quantitative and Qualitative Measurements of Polyphenol Components

The polyphenolic compositions without standards were identified using LC-MS and quantified using UPLC. Polyphenol standards of catechin, chlorogenic acid, epicatechin, rutin, quercetin 3-O-xyloside, cyanidin 3-O-galactoside, cyanidin 3-O-glucoside, cyanidin 3-O-arabinoside, peonidin 3-O-galactoside, cyanidin 3-O-xyloside, and phlorizin were purchased from Sigma-Aldrich (St. Louis, MO, USA); procyanidin B1, procyanidin B2, procyanidin C1, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-arabinoside, and quercetin3-O-rhamnoside were purchased from ChromaDex (Irvine, CA, USA). 4-*p*-coumarylquinic acid and 5-*p*-coumarylquinic acid were quantified by the chlorogenic acid standard. 3-hydroxyphloretin-xylglucoside, 3-hydroxyphloretin-glucoside, phloridin-hexose-hexose, and phloretin xyloglucoside were quantified by phloridzin.

2.4. Statistical Analysis

Microsoft Office Excel 2021 was used for data analysis, and SPSS 27 was used for single-factor ANOVA tests to calculate significance, mean values, and standard deviations. Origin 2021 was used to plot the dynamic changes in the total polyphenol and polyphenol component contents. The total polyphenol content was calculated as the sum of the contents of each polyphenol component, and the contents of five types of polyphenols were calculated as the sum of the contents of each type of polyphenol component.

3. Results

3.1. Dynamic Changes in Total Polyphenol During Fruit Development

The total polyphenol contents of 'Hongxun No. 2' and 'Xinye 13-11' were different during development. The content of total polyphenols in 'Xinye 13-11' was higher than that in 'Hongxun 2' in all periods, in both the peel and pulp. From 50 days to 110 days after flowering, with the gradual development and ripening of fruits, the overall content showed a decreasing trend, with the reduction becoming getting smaller and smaller. In all periods, the total polyphenol contents of 'Hongxun 2' and 'Xinye 13-11' were higher in the peel than in the pulp.

During the development stage from 50 to 110 days after flowering, the total polyphenol content decreased by 66.20% and 63.15% in the peel and pulp of 'Hongxun 2', while the total polyphenol content decreased by 47.74% and 59.78% in the peel and pulp of 'Xinye 13-11' (Figure 1).

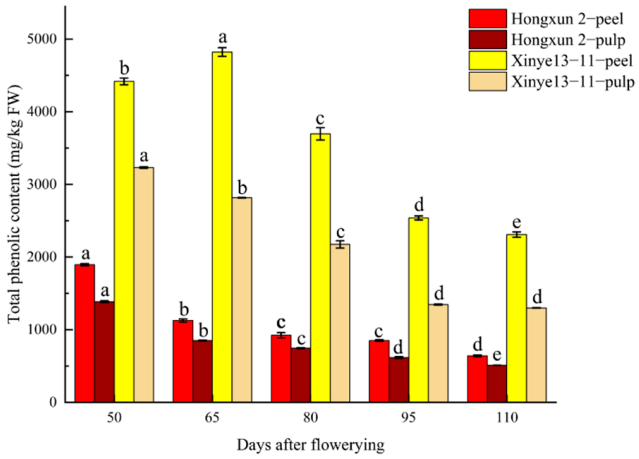

Figure 1. Total polyphenolic contents during fruit development of 'Hongxun 2' and 'Xinye 13-11'. Note: Different letters indicate differences at $p < 0.05$ levels.

3.2. Dynamic Changes in Five Types of Polyphenols During Fruit Development

The flavanol contents of 'Xinye 13-11' were significantly higher than those of 'Hongxun 2' in the peel and pulp, and the flavanol content reached 3203.45 mg/kg in the peel of 'Xinye 13-11' at 65 days after flowering, and then gradually decreased. The flavanol contents were 2762.99 mg/kg and 394.00 mg/kg in the pulp of 'Xinye 13-11' and the peel of 'Hongxun 2' at 50 days after flowering, respectively, and gradually decreased after flowering. The flavanol content of the pulp was 62.80 mg/kg in 'Hongxun 2' at 50 days after flowering, which decreased from 50 days to 65 days after flowering and then began to rise again, reaching 61.31 mg/kg at 95 days after flowering, and then decreased again (Figure 2A).

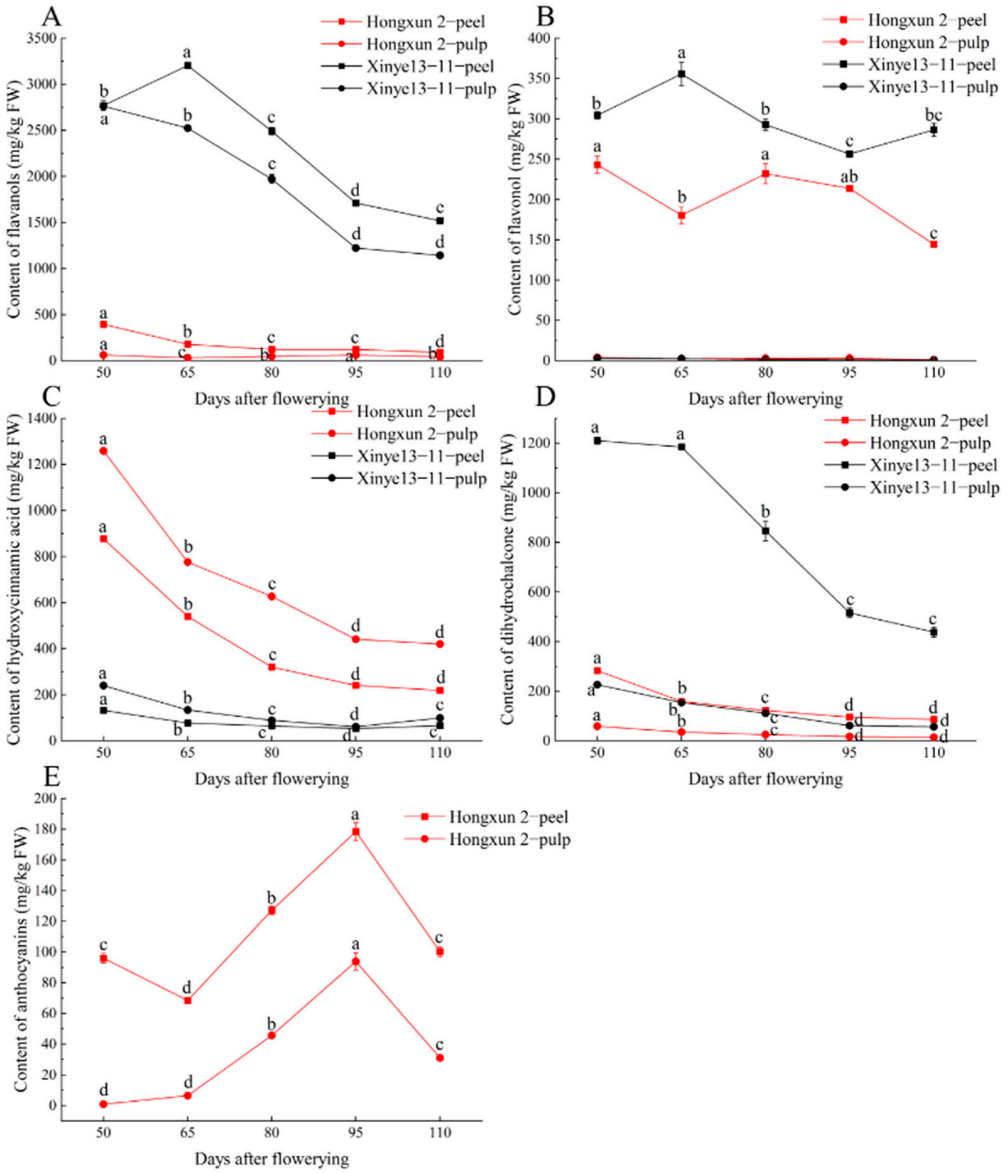

Figure 2. Changes in the contents of five types of polyphenolic substances at different developmental stages in 'Hongxun 2' and 'Xinye 13-11'. (**A**) Changes in the contents of flavanol at different developmental stages in 'Hongxun 2' and 'Xinye 13-11'. (**B**) Changes in the contents of flavonol at different developmental stages in 'Hongxun 2' and 'Xinye 13-11'. (**C**) Changes in the contents of hydroxycinnamic acid at different developmental stages in 'Hongxun 2' and 'Xinye 13-11'. (**D**) Changes in the contents of dihydrochalcone at different developmental stages in 'Hongxun 2' and 'Xinye 13-11'. (**E**) Changes in the contents of anthocyanins at different developmental stages in 'Hongxun 2' and 'Xinye 13-11'. Note: Different letters indicate differences at $p < 0.05$ levels.

The flavonol content was lower in the flesh of 'Hongxun 2' and 'Xinye 13-11', but higher in the peel, and its variation trends were different. The flavonol content was 243.24 mg/kg in the peel of 'Hongxun 2' at day 50 after flowering, decreased at day 65, then increased, and decreased again at 80 days after flowering. The flavonol content was 355.82 mg/kg in the peel of 'Xinye 13-11' at 75 days after flowering, the content increased from 50 days to 65 days, then gradually decreased, and gradually increased at 95 days after flowering (Figure 2B).

The content of hydroxycinnamic acid in 'Hongxun 2' was significantly higher than that of 'Xinye 13-11' in the peel and pulp. The contents of hydroxycinnamic acid in 'Hongxun-2' and 'Xinye 13-11' were 877.36 mg/kg, 1258.80 mg/kg, 132.55 mg/kg, and 239.96 mg/kg in the peel and pulp, respectively, 50 days after flowering. Except for in the pulp of 'Xinye 13-11', the content of 'Xinye 13-11' decreased gradually with development, and the content of 'Xinye 13-11' increased significantly from 95 days to 110 days after flowering (Figure 2C).

The dihydrochalcone content of 'Hongxun 2' was higher than that of 'Xinye 13-11' in the pulp, and it showed a downward trend, tending to be stable after 95 days of flowering. The contents of dihydrochalcone were 283.13 mg/kg, 58.74 mg/kg, 1210.43 mg/kg, and 226.13 mg/kg in the peel and pulp of 'Hongxun 2' and 'Xinye 13-11' at 50 days after flowering, respectively (Figure 2D).

The dynamic change trend of the anthocyanin content of 'Hongxun 2' was similar in the peel and pulp, and the highest contents were 178.51 mg/kg and 93.93 mg/kg at 95 days after flowering for the peel and pulp, respectively. However, the anthocyanin content decreased, to a certain extent, in the peel of 'Hongxun 2' at 65 days after flowering, then gradually increased, and reached its highest at 95 days after flowering (Figure 2E).

3.3. The Difference in Polyphenol Components and Contents During Fruit Development

There were significant differences in the contents of polyphenol components in 'Hongxun 2' in the peel and pulp during the development of fruits (Tables 1 and 2). In the peel, the content of polyphenols decreased significantly from 50 to 65 days after flowering. The contents of 29 polyphenol components, including procyanidin B1, catechin, procyanidin B2, epicatechin, etc., were significantly decreased, while the contents of kaempferol 3-O-galactoside, and 5-*p*-coumaryl quinic acid showed no significant difference, and paeoniflorin 3-o-galactoside was not detected during this period. The contents of flavonols such as 3-hydroxyphloretin-xyloglucose, rutin, and quercetin 3-O-galactoside and anthocyanins such as cyanidin 3-O-galactoside and cyanidin 7-O-arabinoside increased significantly at this stage from 65 to 80 days after flowering. The other components were significantly reduced. From 80 to 95 days after flowering, the contents of most components tended to be stable without significant differences. The contents of rutin, quercetin 3-O-glucoside, and anthocyanin components, except cyanidin 7-O-arabinoside, increased significantly at this stage. The contents of quercetin 3-O-xyloside, quercetin 3-O-rhamnoside, chlorogenic acid, 3-hydroxyphloretin-xyloglucose, and phloretin-xyloglucose were significantly reduced at this stage. Kaempferol 3-O-rhamnoside and 4-*p*-coumarylquinic acid were not detected from 95 to 110 days after flowering. The contents of catechin, procyanidin B2, procyanidin C1, kaempferol 3-O-galactoside, 3-hydroxyphloretin-xyloglucose, phloridin-hexose-hexose, 3-hydroxyphloretin-glucose, phloretin-xyloglucose, and phloretin showed no significant difference in the maturation stage, while the contents of other components were significantly reduced (Table 1).

Table 1. Polyphenol components and content in the peel of 'Hongxun 2' during fruit development.

Polyphenol Components	Contents of Polyphenol Components (mg·kg^{-1} FW)				
	50 d	**65 d**	**80 d**	**95 d**	**110 d**
PROB1	17.93 ± 0.39 a	7 ± 0.51 b	4.66 ± 0.07 c	4.97 ± 0.14 c	3.18 ± 0.3 d
CATE	15.97 ± 0.51 a	3.63 ± 0.08 b	2.01 ± 0.1 c	2.02 ± 0.03 c	1.35 ± 0.07 c
PROB2	121.98 ± 4.34 a	65.47 ± 3.58 b	49.81 ± 3.31 c	52.72 ± 0.6 c	41.25 ± 0.83 c
EPI	153.08 ± 2.6 a	60.11 ± 1.93 b	35.03 ± 1.91 c	33.42 ± 0.44 c	23.42 ± 0.79 d
PROC1	85.03 ± 8.16 a	42.21 ± 6.17 b	30.8 ± 1.28 bc	28.67 ± 0.58 bc	20.86 ± 0.46 c
RUTIN	0.35 ± 0.01 bc	0.2 ± 0.02 c	0.43 ± 0.03 b	0.46 ± 0.04 a	0.32 ± 0.01 bc
QUEGA	79.46 ± 5.29 ab	64.7 ± 3.75 c	100.33 ± 7.19 a	98.97 ± 3.04 a	67.92 ± 2.82 b
QUEGL	7.05 ± 0.23 c	5.6 ± 0.2 d	9.47 ± 0.4 b	12.31 ± 0.17 a	8.71 ± 0.13 b
QUEXY	34.41 ± 1.26 a	24.43 ± 0.42 c	29.12 ± 1.58 b	25.2 ± 0.07 c	15.98 ± 0.18 d
QUEPY	3.27 ± 0.13 ab	2.56 ± 0.13 c	3.6 ± 0.19 a	3.78 ± 0.05 a	2.94 ± 0.1 bc
QUEFU	84.06 ± 3.17 a	59.64 ± 6.39 b	59.77 ± 2.41 b	49.21 ± 0.62 b	30.03 ± 0.37 c
QUERH	33.25 ± 0.56 a	22.55 ± 0.16 c	28.32 ± 1.11 b	23.24 ± 0.44 c	18.08 ± 0.24 d
KAEGA	0.26 ± 0.02 a	0.19 ± 0.03 a	0.29 ± 0.03 a	0.28 ± 0.02 a	0.2 ± 0.03 a
KAEGL	nd	nd	nd	nd	nd
KAEFU	0.9 ± 0.01 a	0.43 ± 0.01 c	0.5 ± 0.02 b	0.29 ± 0.01 d	0.19 ± 0.02 e
KAERH	0.23 ± 0.01 a	0.13 ± 0.01 b	0.17 ± 0.03 ab	0.13 ± 0.02 b	nd
CHLAC	850.86 ± 11.61 a	525.93 ± 8.02 b	312.65 ± 12.34 c	235.76 ± 1.96 d	215.14 ± 4.88 d
4COUAC	19.87 ± 0.49 a	6.81 ± 0.4 b	3.06 ± 0.17 c	2.24 ± 0.28 c	nd
5COUAC	6.63 ± 0.21 a	7.09 ± 0.11 a	4.52 ± 0.28 b	2.82 ± 0.16 b	4.02 ± 0.17 c
HYDXY	5.61 ± 0.3 a	4.55 ± 0.08 b	5.3 ± 0.25 ab	3.48 ± 0.07 c	3.49 ± 0.37 c
PHLHE	1.07 ± 0.06 a	0.77 ± 0.01 b	0.59 ± 0.06 bc	0.44 ± 0.06 c	0.5 ± 0.03 c
HYDGL	12.45 ± 0.32 a	6.19 ± 0.38 b	5.21 ± 0.47 bc	4.12 ± 0.18 cd	3.01 ± 0.21 d
PHLXY	110.21 ± 2.09 a	74.68 ± 3.27 b	65.48 ± 0.33 c	52.29 ± 1.61 d	52.87 ± 2.41 d
PHLPE	2.33 ± 0.19 a	1.77 ± 0.17 b	1.47 ± 0.14 b	1.2 ± 0.07 b	1.3 ± 0.07 b
PHLPE1	5.81 ± 0.41 a	4.21 ± 0.31 b	3.4 ± 0.51 b	2.99 ± 0.26 b	2.88 ± 0.18 b
PHLZI	145.66 ± 6.44 a	66.64 ± 1.57 b	40.63 ± 4.22 c	31.18 ± 1.71 cd	22.12 ± 2.37 d
CYAGA	72.45 ± 2.24 d	53.89 ± 1.65 e	106.6 ± 2.36 b	155.08 ± 4.83 a	87.23 ± 2.72 c
CYAGL	0.38 ± 0.02 c	0.28 ± 0.02 c	0.75 ± 0.05 b	1.11 ± 0.1 a	0.61 ± 0.01 b
CYA3AR	1.41 ± 0.05 c	0.93 ± 0.03 d	1.99 ± 0.01 b	3.2 ± 0.12 a	1.88 ± 0.11 b
PEOGA	nd	nd	0.17 ± 0.01 c	0.37 ± 0.02 a	0.29 ± 0.03 b
CYA7AR	10.51 ± 0.32 a	5.9 ± 0.16 b	6.72 ± 0.18 b	6.02 ± 0.21 b	3.73 ± 0.19 c
CYAXY	11.34 ± 0.4 b	7.46 ± 0.2 c	10.97 ± 0.24 b	12.73 ± 0.52 a	6.57 ± 0.18 c

Note: PROB1: procyanidin B1; CATE: catechin; PROB2: procyanidin B2; EPI, epicatechin; PROC1: procyanidin C1; RUTIN: rutin; QUEGA: quercetin 3-O-galactoside; QUEGL: quercetin 3-O-glucoside; QUEXY: quercetin 3-O-xyloside; QUEPY: quercetin 3-O-arabinopyranoside; QUEFU: quercetin 3-O-arabinofuranoside; QUERH: Quercetin 3-O-rhamnoside; KAEGA: kaempferol 3-O-galactoside; KAEGL: kaempferol 3-O-glucoside; KAEFU: kaempferol 3-O-arabinofuranoside; KAERH: kaempferol 3-O-rhamnoside; CHLAC: chlorogenic acid; 4COUAC: 4-*p*-coumaryl quinic acid; 5COUAC: 5-*p*-coumaryl quinic acid; HYDGL: 3-hydroxyphloridin-glucose; HYDXY: 3-hydroxyphloridin-xyloglucose; PHLHE: phloridin-hexose-hexose; PHLXY: phloridin-xyloglucose; PHLPE: phloridin-pentose-hexose; PHLPE1: phloridin-pentose-hexose 1; PHLZI, phloridin; CYAGA: cyanidin 3-O-galactoside; CYAGL: cyanidin 3-O-glucoside; CYA3AR: cyanidin 3-O-arabinoside; PEOGA: Peonidin 3-O-galactoside; CYA7AR: cyanidin 7-O-arabinoside; CYAXY: cyanidin 3-O-xyloside; and nd: No detected. Different letters in the same line indicate differences at the $p < 0.05$ level, as shown in Tables 2–4.

Table 2. Polyphenol components and content in the pulp of 'Hongxun 2' during fruit development.

Polyphenol Components	Contents of Polyphenol Components (mg·kg^{-1} FW)				
	50 d	**65 d**	**80 d**	**95 d**	**110 d**
PROB1	5.71 ± 0.15 a	3.05 ± 0.14 b	2.39 ± 0.2 b	2.86 ± 0.15 b	2.63 ± 0.16 b
CATE	1.66 ± 0.04 a	1.13 ± 0.06 b	1.17 ± 0.01 b	1.26 ± 0.08 b	1.14 ± 0.04 b
PROB2	25.13 ± 1.24 a	10.73 ± 0.36 c	15.95 ± 1.27 b	25.42 ± 0.33 a	19.65 ± 1.28 b
EPI	14.16 ± 0.48 b	7.96 ± 0.24 c	9.58 ± 0.23 c	16.45 ± 0.66 a	9.25 ± 0.42 c
PROC1	16.14 ± 1.29 a	7.57 ± 0.09 b	16.62 ± 1.16 a	15.31 ± 0.45 a	10.41 ± 0.66 b
RUTIN	nd	nd	nd	nd	nd
QUEGA	1.44 ± 0.1 a	0.67 ± 0.12 c	1.01 ± 0.08 b	1.37 ± 0 a	nd
QUEGL	nd	nd	nd	nd	nd

Table 2. *Cont.*

Polyphenol Components	Contents of Polyphenol Components (mg·kg^{-1} FW)				
	50 d	**65 d**	**80 d**	**95 d**	**110 d**
QUEXY	nd	nd	nd	nd	nd
QUEPY	nd	nd	nd	nd	nd
QUEFU	nd	nd	nd	nd	nd
QUERH	2.44 ± 0.36 a	1.68 ± 0.17 ab	1.89 ± 0.32 ab	1.76 ± 0.16 ab	1.1 ± 0.12 b
KAEGA	nd	nd	nd	nd	nd
KAEGL	nd	nd	nd	nd	nd
KAEFU	nd	nd	nd	nd	nd
KAERH	nd	nd	nd	nd	nd
CHLAC	1222.6 ± 11.37 a	758.14 ± 6.47 b	609.74 ± 4.96 c	431.51 ± 7.41 d	412.55 ± 0.61 d
4COUAC	22 ± 0.37 a	8.41 ± 0.46 b	5.88 ± 0.05 c	3.89 ± 0.12 d	2.12 ± 0.14 e
5COUAC	14.19 ± 0.45 a	9.91 ± 0.43 c	11.19 ± 0.32 b	5.6 ± 0.07 d	5.78 ± 0.15 d
HYDXY	2.44 ± 0.2 a	2.03 ± 0.17 a	1.38 ± 0.08 b	1.2 ± 0.06 b	1.21 ± 0.06 b
PHLHE	1.24 ± 0.09 a	1.15 ± 0.11 a	1.26 ± 0.05 a	1.14 ± 0.06 a	1.2 ± 0.08 a
HYDGL	2.44 ± 0.1 b	3.62 ± 0.13 a	2.03 ± 0.2 b	1.1 ± 0.09 c	0.95 ± 0.02 c
PHLXY	19.35 ± 0.59 a	10.97 ± 0.32 b	7.82 ± 0.21 c	5.67 ± 0.22 d	5.43 ± 0.44 d
PHLPE	2.51 ± 0.03 a	1.69 ± 0.13 b	1.66 ± 0.06 b	nd	nd
PHLPE1	3.69 ± 0.51 a	2.21 ± 0.03 b	2.17 ± 0.21 b	1.86 ± 0.2 b	nd
PHLZI	27.07 ± 0.93 a	13.79 ± 0.77 b	8.48 ± 1.05 c	6.56 ± 0.27 c	5.88 ± 0.38 c
CYAGA	0.59 ± 0.05 d	4.76 ± 0.32 d	38.07 ± 1.38 b	81.12 ± 4.87 a	26.65 ± 1.06 c
CYAGL	nd	nd	0.31 ± 0.03 b	0.62 ± 0.03 a	0.21 ± 0.01 c
CYA3AR	nd	nd	0.62 ± 0.02 b	1.48 ± 0.11 a	0.34 ± 0.02 c
PEOGA	nd	nd	nd	nd	nd
CYA7AR	0.16 ± 0.02 d	0.66 ± 0.06 cd	2.13 ± 0.13 b	2.83 ± 0.23 a	1.07 ± 0.11 c
CYAXY	0.18 ± 0.02 d	1.07 ± 0.14 d	4.62 ± 0.13 b	7.87 ± 0.45 a	2.79 ± 0.16 c

nd: No detected. Different letters in the same line indicate differences at the $p < 0.05$ level.

Table 3. Polyphenol components and contents in the peel of 'Xinye 13-11' during fruit development.

Polyphenol Components	Contents of Polyphenol Components (mg·kg^{-1} FW)				
	50 d	**65 d**	**80 d**	**95 d**	**110 d**
PROB1	116.3 ± 6.62 c	214.64 ± 1 a	187.94 ± 7.03 b	131.94 ± 6.25 c	118.99 ± 4.12 c
CATE	386.16 ± 7.5 a	321.57 ± 1.4 b	187.47 ± 3.59 c	81.97 ± 2.05 d	61.03 ± 1.52 e
PROB2	556.65 ± 18.8 c	811.89 ± 3.62 a	729.51 ± 9.97 b	600.97 ± 21.9 c	544.93 ± 6.87 c
EPI	928.6 ± 7.37 a	879.27 ± 7.84 b	692.33 ± 15.22 c	452.82 ± 14.47 d	382.88 ± 7.05 e
PROC1	782.53 ± 27.12 b	976.09 ± 34.77 a	695.34 ± 6.67 c	444.54 ± 6.93 d	410.21 ± 13.94 d
RUTIN	5.25 ± 0.31 a	4.37 ± 0.56 a	4.08 ± 0.15 a	4.4 ± 0.3 a	4.97 ± 0.48 a
QUEGA	73.62 ± 1.28 c	90.49 ± 2.85 b	82.49 ± 3.3 bc	84.92 ± 0.6 b	113.7 ± 2.31 a
QUEGL	37.88 ± 1.01 c	49.5 ± 1.91 b	44.5 ± 1.69 b	48.3 ± 0.07 b	56.07 ± 1.11 a
QUEXY	26.08 ± 0.99 a	28.91 ± 1.73 a	21.97 ± 0.07 b	16.97 ± 0.17 c	16.28 ± 0.65 c
QUEPY	2.22 ± 0.06 ab	2.64 ± 0.22 ab	2.32 ± 0.06 ab	1.97 ± 0.07 c	2.88 ± 0.27 a
QUEFU	91.91 ± 1.84 a	100.66 ± 3.18 a	75.63 ± 2.44 b	54.14 ± 0.2 c	52.2 ± 2.94 c
QUERH	64.49 ± 1.56 b	76.78 ± 4.07 a	59.84 ± 0.22 b	44.33 ± 1.04 c	38.78 ± 1.22 c
KAEGA	0.25 ± 0.03 ab	0.24 ± 0.01 ab	0.22 ± 0.01 ab	0.19 ± 0.01 b	0.28 ± 0.02 a
KAEGL	0.82 ± 0.02 a	0.64 ± 0.03 b	0.55 ± 0.01 c	0.5 ± 0.02 c	0.53 ± 0.03 c
KAEFU	1.17 ± 0.02 a	0.95 ± 0.03 b	0.66 ± 0.01 c	0.4 ± 0.02 d	0.33 ± 0.02 d
KAERH	0.49 ± 0.02 a	0.49 ± 0.02 a	0.34 ± 0.01 b	0.24 ± 0.02 c	0.21 ± 0.02 c
CHLAC	121.16 ± 0.88 a	73.93 ± 0.66 b	62.03 ± 0.44 d	51.92 ± 1.44 e	65.92 ± 1.21 c
4COUAC	11.38 ± 0.48 a	3.76 ± 0.09 b	2.21 ± 0.21 c	1.52 ± 0.2 c	nd
5COUAC	nd	nd	nd	nd	nd
HYDXY	9.58 ± 0.57 a	9.02 ± 0.57 a	9.72 ± 1.08 a	7.89 ± 0.08 a	4.94 ± 0.39 b
PHLHE	2.85 ± 0.12 a	2.93 ± 0.14 a	2.51 ± 0.12 a	1.78 ± 0.1 b	1.63 ± 0.09 b
HYDGL	43.54 ± 1.34 a	45.7 ± 0.78 a	36.69 ± 2.06 b	28.61 ± 1.03 c	18.47 ± 0.75 d
PHLXY	252.11 ± 4.1 b	304.63 ± 10.25 a	228.52 ± 7.76 b	162.25 ± 4.33 c	146.17 ± 6.67 c
PHLPE	6.54 ± 0.95 a	6.97 ± 0.12 a	4.56 ± 0.4 b	3.25 ± 0.2 b	3.29 ± 0.36 b
PHLPE1	11.36 ± 0.95 ab	12.52 ± 0.58 a	9.97 ± 0.6 b	5.64 ± 0.31 c	5.53 ± 0.26 c

Table 3. *Cont.*

Polyphenol Components	Contents of Polyphenol Components (mg·kg^{-1} FW)				
	50 d	**65 d**	**80 d**	**95 d**	**110 d**
PHLZI	884.45 ± 11.54 a	803.03 ± 9.03 b	554.24 ± 28.07 c	306.62 ± 15.66 d	258.35 ± 12.73 d
CYAGA	nd	nd	nd	nd	nd
CYAGL	nd	nd	nd	nd	nd
CYA3AR	nd	nd	nd	nd	nd
PEOGA	nd	nd	nd	nd	nd
CYA7AR	nd	nd	nd	nd	nd
CYAXY	nd	nd	nd	nd	nd

nd: No detected. Different letters in the same line indicate differences at the $p < 0.05$ level.

Table 4. Polyphenol components and contents in the pulp of 'Xinye 13-11' during fruit development.

Polyphenol Components	Contents of Polyphenol Components (mg·kg^{-1} FW)				
	50 d	**65 d**	**80 d**	**95 d**	**110 d**
PROB1	99.72 ± 2.14 c	142.66 ± 6.05 a	122.93 ± 5.74 b	76.69 ± 2.16 d	74.72 ± 0.22 d
CATE	241.76 ± 12.1 a	181.07 ± 4.63 b	103.81 ± 4.66 c	45.79 ± 1.02 d	38.18 ± 0.21 d
PROB2	792.75 ± 9.71 a	736.77 ± 10.11 b	659.1 ± 23.05 c	452.5 ± 9.8 d	447.7 ± 2.28 d
EPI	870.56 ± 6.71 a	778.33 ± 7.6 b	580.98 ± 11.43 c	357.51 ± 1.46 d	316.03 ± 1.36 e
PROC1	758.21 ± 2.05 a	686.98 ± 4.77 b	505.35 ± 11.95 c	289.76 ± 0.44 d	267.28 ± 1.12 d
RUTIN	nd	nd	nd	nd	nd
QUEGA	nd	nd	nd	nd	nd
QUEGL	nd	nd	nd	nd	nd
QUEXY	nd	nd	nd	nd	nd
QUEPY	nd	nd	nd	nd	nd
QUEFU	1.37 ± 0.12 a	0.98 ± 0.13 b	nd	nd	nd
QUERH	1.64 ± 0.2 a	1.66 ± 0.28 a	1.41 ± 0.17 ab	0.81 ± 0.1 b	0.79 ± 0.06 b
KAEGA	nd	nd	nd	nd	nd
KAEGL	nd	nd	nd	nd	nd
KAEFU	nd	nd	nd	nd	nd
KAERH	nd	nd	nd	nd	nd
CHLAC	223.7 ± 0.51 a	129.53 ± 1.77 b	87.16 ± 2.24 d	59.32 ± 0.66 e	98.11 ± 1.63 c
4COUAC	16.26 ± 0.1 a	3.99 ± 0.1 b	2.16 ± 0.12 c	1.96 ± 0.06 c	0.8 ± 0.19 d
5COUAC	nd	nd	nd	nd	nd
HYDXY	nd	nd	6.15 ± 0.21 a	5.81 ± 0.27 a	4.29 ± 0.14 b
PHLHE	8.88 ± 0.28 a	7.5 ± 0.21 b	4.81 ± 0.25 c	3.1 ± 0.06 d	2.9 ± 0.09 d
HYDGL	4.86 ± 0.11 b	6.73 ± 0.08 a	4.67 ± 0.12 b	2.38 ± 0.08 c	1.94 ± 0.24 c
PHLXY	49.86 ± 0.41 a	42.96 ± 0.33 b	31.86 ± 0.91 c	18.64 ± 0.29 d	16.71 ± 0.53 d
PHLPE	2.36 ± 0.52 a	2.51 ± 0.04 a	2.32 ± 0.33 a	nd	nd
PHLPE1	16.15 ± 0.94 a	12.79 ± 0.43 b	9.13 ± 0.65 c	4.65 ± 0.39 d	4.42 ± 0.26 d
PHLZI	144.03 ± 3.38 a	81.95 ± 1.35 b	52.34 ± 1.44 c	26.6 ± 0.73 d	26.16 ± 0.2 d
CYAGA	nd	nd	nd	nd	nd
CYAGL	nd	nd	nd	nd	nd
CYA3AR	nd	nd	nd	nd	nd
PEOGA	nd	nd	nd	nd	nd
CYA7AR	nd	nd	nd	nd	nd
CYAXY	nd	nd	nd	nd	nd

nd: No detected. Different letters in the same line indicate differences at the $p < 0.05$ level.

In the pulp of 'Hongxun 2', rutin, quercetin 3-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-arabinopyranoside, quercetin 3-O-arabinofuranoside, kaempferol 3-O-galactoside, kaempferol 3-O-glucoside, kaempferol 3-O-arabinoside, kaempferol 3- O-glucoside, kaempferol 3-O-arabinoside, kaempferol 3-O-rharnoside, and paeoniflorin 3-O-galactoside were not detected (Table 2). From 50 days to 65 days after flowering, the contents of 3-hydroxyphloretin-glucose were significantly increased, while the contents of 3-hydroxyphloretin-xyloglucose, phloridin-hexose-hexose, quercetin 3-O-rhamnoside, and anthocyanin were not significantly different, and the contents of other polyphenol components were significantly

decreased. Cyanidin 3-O-xyloside and entaurin 7-O-arabinoside were not detected at this stage. From 65 to 80 days after flowering, the contents of procyanidin B2, procyanidin C1, quercetin 3-O-galactoside, 5-*p*-coumaryl quinic acid, and anthocyanin polyphenols were significantly increased. The contents of chlorogenic acid, 4-*p*-coumaryl quinic acid, 3-hydroxyphloretin-xyloglucose, 3-hydroxyphloretin-glucose, phloretin-xyloglucose, and phloretin were significantly decreased, while the contents of other polyphenol components saw no significant difference. From 80 to 95 days after flowering, the contents of 3-hydroxyphloretin-glucose, phloretin-xyloglucose, and hydroxy-cinnamic acid polyphenols decreased significantly, while the contents of procyanidin B2, epicatechin, quercetin 3-O-galactoside, and anthocyanin increased significantly, and the contents of other polyphenols saw no significant difference. Phloretin-pentose-hexose was not detected at 95 days after flowering. The contents of procyanidin B2, epicatechin, procyanidin C1, 4-*p*-coumaryl quinic acid, phloretin-pentose hexose 1, and anthocyanin polyphenols were significantly decreased from 95 days to 110 days after flowering, while the contents of other polyphenol components were not significantly different. Quercetin 3-O-galactoside was not detected at 110 days after flowering (Table 2).

'Xinye 13-11' is a green pulp apple, so anthocyanins were not detected at any time, and 5-*p*-coumaryl quinic acid was not detected either (Tables 3 and 4). In the peel of 'Xinye 13-11', the contents of procyanidin B1, procyanidin B2, procyanidin C1, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-rhamnoside, and phloretin-xyloglucose were significantly increased from 50 days to 65 days after flowering. The contents of catechin, epicatechin, kaempferol 3-O-glucoside, kaempferol 3-o-arabinofuranoside, chlorogenic acid, 4-*p*-coumaryl quinic acid, and phloridzin were significantly decreased, while the other polyphenol components saw no significant difference. From 65 to 80 days after flowering, the contents of quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-arabopyranoside, kaempferol 3-O-galactoside, 3-hydroxyphloretin-xyloglucose, and phloretin-hexosaccharide saw no significant difference, while the contents of other polyphenol components were significantly decreased. From 80 to 95 days after flowering, the contents of quercetin 3-O-galactoside, quercetin 3-O-glucoside, kaempferol 3-O-galactoside, kaempferol 3-O-glucoside, 4-*p*-coumaryl quinic acid, 3-hydroxyphloretin-xyloglucose, and phloretin-pentose hexose saw no significant difference, while the contents of other polyphenol components were significantly decreased. From 95 days to 110 days after flowering, the quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-arabopyranoside, kaempferol 3-O-galactoside, and chlorogenic acid contents increased significantly, while the catechin, epicatechin, 3-hydroxyphloretin-xyloglucose, and phloretin-xyloglucose contents decreased significantly. There were no significant differences in other polyphenol components, and 4-*p*-coumaryl quinic acid was not detected at 110 days after flowering (Table 3).

In the pulp of 'Xinye 13-11', rutin, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-xyloside, quercetin 3-O-arabopyranoside, kaempferol 3-O-galactoside, kaempferol 3-O-glucoside, kaempferol 3-o-arabinoside, kaempferol 3-O-rhamnoside, and 5-*p*-coumarylquinic acid were not detected (Table 4). From 50 days to 65 days after flowering, the contents of procyanidin B1 and 3-hydroxyphloretin-glucose were significantly increased, while the contents of quercetin 3-O-rhamnoside and phloretin-pentose hexose were not significantly different. The contents of other polyphenol components were significantly decreased, and 3-hydroxyphloretin-xyloglucose was not detected. There were no significant differences in the contents of quercetin 3-O-rhamnoside and phloretin-pentose hexose from 65 days to 80 days after flowering, 3-hydroxyphloretin-xyloglucose was detected at 80 days after flowering, and the contents of other polyphenol components were significantly reduced. From 80 to 95 days after flowering, the quercetin 3-O-rhamnoside, 4-*p*-coumaryl quinic acid, and 3-hydroxyphloretin-xyloglucose contents were not significantly different, phloretin-pentose hexose was not detected at 95 days after flowering, and the contents of other polyphenol components were significantly decreased. From 95 days to 110 days after flowering, the contents of all polyphenol components tended

to be stable, only the chlorogenic acid content increased significantly, and the contents of epicatechin, 4-*p*-coumaryl quinic acid, and 3-hydroxyphloretin-xyloglucose decreased significantly, while the contents of other polyphenol components showed no significant differences (Table 4).

3.4. Dynamic Changes in Main Polyphenol Components During Fruit Development

From 50 days to 110 days after flowering, the fruits developed and matured gradually, and the main polyphenol components and contents of the peel and pulp saw significant changes (Figure 3). The content of procyanidin B2 reached 121.98 mg/kg in the peel of 'Hongxun 2' at 50 days after flowering, then gradually decreased, and slightly increased at 95 days after flowering, but this difference was not significant. In peel, the content of procyanidin B2 decreased gradually at first, reached its highest value of 25.42 mg/kg at 95 days after flowering, and then decreased again. The content of procyanidin B2 in the peel of 'Xinye 13-11' was the highest at 65 days after flowering, reaching 811.89 mg/kg, and then gradually decreased, but in the pulp, its content was 792.75 mg/kg at 50 days after flowering, and then gradually decreased (Figure 3A).

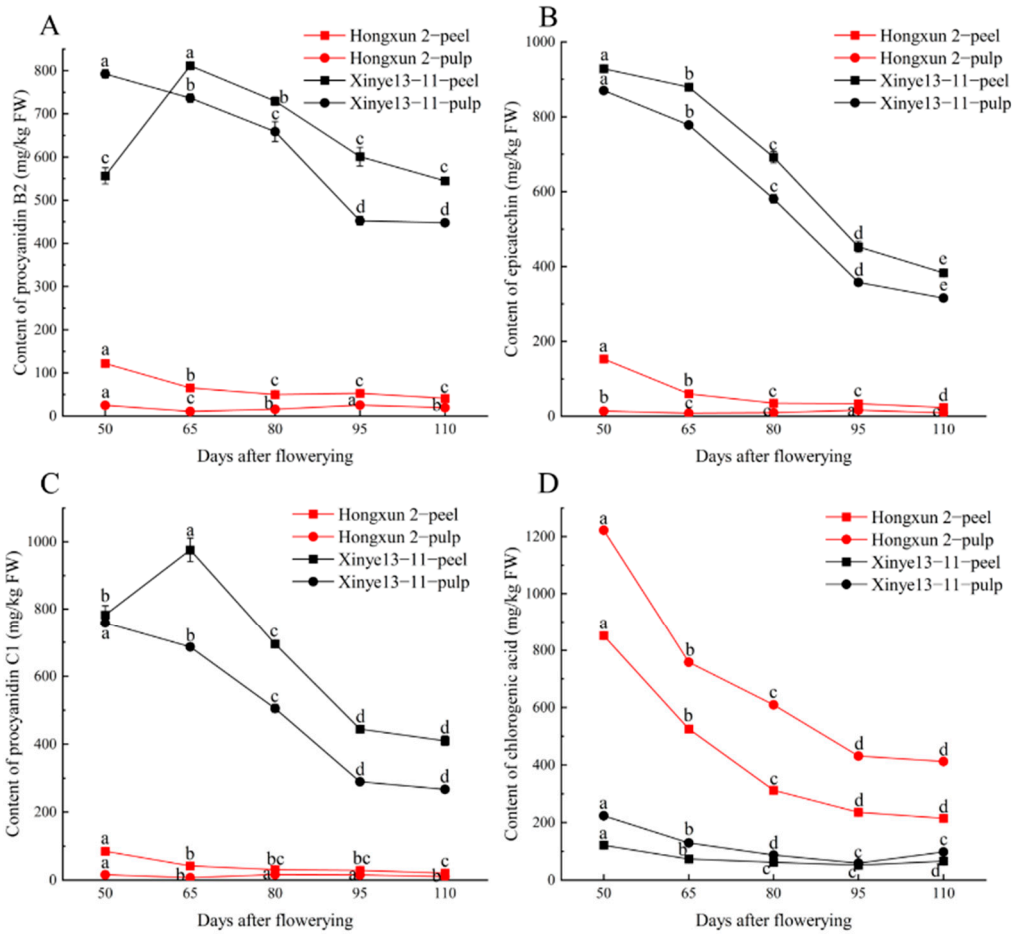

Figure 3. Dynamic changes in the contents of major polyphenol components during fruit development. (**A**) Dynamic changes in the contents of procyanidin B2 during fruit development. (**B**) Dynamic changes in the contents of epicatechin during fruit development. (**C**) Dynamic changes in the contents of procyanidin C1 during fruit development. (**D**) Dynamic changes in the contents of chlorogenic acid during fruit development. Note: Different letters indicate differences at $p < 0.05$ levels.

The dynamic changes in epicatechin content in the peel and pulp of 'Hongxun2' and 'Xinye 13-11' were similar to those of catechin, with values of 153.08 mg/kg, 928.60 mg/kg, and 870.56 mg/kg at 50 days after flowering, respectively, when it then gradually decreased.

The content in the pulp of 'Hongxun 2' was the highest at 95 days after flowering at 16.45 mg/kg (Figure 3B).

The contents of procyanidin C1 in the peel of 'Hongxun 2' and the pulp of 'Xinye 13-11' were 85.03 mg/kg and 758.21 mg/kg, respectively, and then gradually decreased at 50 days after flowering. The highest procyanidin C1 content was 16.62 mg/kg in the pulp of 'Hongxun 2' at 95 days after flowering, while the highest content was 976.09 mg/kg in the peel of 'Xinye 13-11' at 65 days after flowering (Figure 3C).

The contents of chlorogenic acid in the peel and pulp of 'Hongxun 2' and 'Xinye 13-11' were 850.86 mg/kg, 1222.60 mg/kg, 121.16 mg/kg, and 223.76 mg/kg at 50 days after flowering, respectively. Among them, the chlorogenic acid content in the peel and pulp of 'Hongxun 2' and 'Xinye 13-11' gradually decreased and then tended to be stable, but the content of chlorogenic acid in the pulp of 'Xinye 13-11' saw a great increase at 110 days after flowering (Figure 3D).

4. Discussion

4.1. Dynamic Changes in Total Phenols and Five Types of Polyphenols in Different Developmental Stages of Fruit

These results show that the contents of polyphenols in the peel and pulp of apple gradually decreased with the ripening of the fruit, and the contents of flavanols, flavonols, and chlorogenic acid showed a downward trend during the growth and development of the apple fruits. The contents of flavanols, flavonols, and chlorogenic acid decreased rapidly in the early stage of apple development, then this decline rate gradually slowed down, and finally tended to be stable or slightly decreased [20,21]. In the young fruit stage, the proportions of flavanols and flavonols were small, and chlorogenic acid was the main polyphenol substance in the pulp. During fruit development and expansion, the proportion of chlorogenic acid gradually decreased, while the proportions of flavanols and flavonols gradually increased. The contents of flavanols and procyanidins in the ripe apple fruits were more than that of chlorogenic acid, becoming the main polyphenols [22]. In this study, the total polyphenol contents of 'Hongxun 2' and 'Xinye 13-11' gradually decreased with fruit development, and the magnitude of this decline diminished and tended to be stable from 95 to 110 days after flowering, which is consistent with previous results [23]. However, the total polyphenol contents of the 'Xinye 13-11' pericardium increased at 65 days after flowering and then gradually decreased, showing some differences from previous studies. Kevers et al. also conducted a related study on the effect of harvesting time on the total polyphenol content of apples. In total, 14 kinds of ripe apple fruits were collected three times, each with an interval of two weeks, and the total polyphenol contents of the apples were detected. The results showed that there were no significant differences in the total polyphenol contents of the three harvests [24].

The five types of polyphenol components in the apples gradually decreased with the development of fruits. Renard et al. studied two wine apple varieties and found that the total phenol contents of the fruits decreased from 35 to 100 days after flowering, and the contents of flavanols, dihydrochalcone, and flavonols all decreased rapidly, while that of hydroxyl cinnamic acid decreased slowly [25]. In this study, the flavonol content fluctuated with the development period, and its overall trend decreased, while the other four polyphenol contents gradually decreased. In general, flavanol contents decreased rapidly during the rapid fruit expansion phase [26]. However, in this study, the flavanol content in the peel of 'Xinye 13-11' increased significantly from 50 to 65 days after flowering, which was an important reason for the increase in the total polyphenol content. Studies have shown that flavanols are synthesized in large quantities in the early stage of fruit development and then increase slowly during the development process, with the expansion of fruit development leading to a reduction in flavanol content. Increases in the total polyphenol content may be due to the fact that the fresh weight proportion of fruit expansion is less than that of flavanol content [27].

Previous studies on the dynamic changes in anthocyanin content in red flesh apples were of two types, the single-peak type and the double-peak type [28]. However, Sun et al. found that red flesh apples had two content peaks in early fruit development (30 d after flowering) and near maturity (105 d to 120 d after flowering) [11]. Wang et al. also found that the contents of red flesh apples gradually increased during fruit development and reached a peak value 150 days after flowering [29]. In this study, the peel of 'Hongxun 2' showed a decreasing trend from 50 to 65 days after flowering, indicating that there was a peak content in the peel before 50 days after flowering, which was similar to the results of Wang et al. [30]. The contents of anthocyanins in the peel and pulp were the highest at 95 days after flowering, which was consistent with research results on the change trend of polyphenol contents in 'Hongmantang' during development [31]. Studies have shown that red flesh apples have a higher antioxidant activity than non-red flesh apples, and anthocyanins play a major role in this [9]. Wang et al. studied four red flesh apple varieties and two white flesh varieties and found that the red flesh apples had a higher antioxidant activity. The key period for anthocyanin extraction and utilization was 95 days after flowering [32].

4.2. Dynamic Changes and Differences in Polyphenol Components in Fruits

Catechins, epicatechin, chlorogenic acid, and phloridzin have strong antibacterial abilities and can effectively inhibit food spoilage [33,34]. In this study, the contents of catechin, epicatechin, chlorogenic acid, and phlophorin in 'Hongxun 2' and 'Xinye 13-11' gradually decreased with fruit development from 50 days after flowering. Similar results were obtained in a study of "*Malus rockii* Schneid." and "Tsugaru" [35]. The extraction and utilization of these components should be carried out in the early stage of fruit development. The contents of procyanidin B1, procyanidin B2, and procyanidin C1 all decreased after 65 days of flowering, but the contents of procyanidin B1 and procyanidin C1 decreased significantly more than that of procyanidin B2. Flavonols gradually accumulate during fruit development, and other flavanols are transformed into each other, mainly into procyanidin B2. The content of each component decreases with an increase in single fruit weight, and the content of procyanidin B2 decreases the least and is the highest in mature fruits [25]. It can be inferred that procyanidin B2 can be obtained from mature fruits when a specific flavanol component is required for production and processing.

The main polyphenols of flavanols are procyanidin C1 and procyanidin B2, and the main polyphenol of hydroxycinnamic acid is chlorogenic acid [34,36]. The differences in polyphenol contents between 'Hongxun 2' and 'Xinye 13-11' were mainly reflected in these three components. The contents of chlorogenic acid in 'Hongxun 2' and flavonols in 'Xinye 13-11' were higher. Flavonols and chlorogenic acid are the main substances contributing to the astringency of fruit [37,38]. However, other studies have shown that the contents of flavonols and chlorogenic acid are positively correlated with the degree of fruit browning, and the contributions of flavonols and chlorogenic acid to the degree of browning are affected by the level of polyphenol content and variety due to differences in polyphenol oxidase substrates [27,39,40]. Chlorogenic acid is the main cause of enzymatic browning. Although procyanidins are positively correlated with fruit browning, they have a high antioxidant activity. Fresh-cut apple preservation was prolonged by applying the procyanidins from *Aronia melanocarpa* (Michx.) Elliott [41]. Therefore, in the breeding process of processed varieties, resources with a low chlorogenic acid content and a high procyanidin content can be selected as parents to reduce the degree of fruit browning and achieve a high antioxidant activity level. However, in the breeding process of fresh food varieties, the edible property of fruits should be considered, so the flavonol content should not be too high, which will lead to severe fruit astringency and affect the fruit flavor.

4.3. Dynamic Variation in Flavonol Components in Fruits

The overall flavonol content in fruit development was decreased [34]. In this study, quercetin 3-O-galactoside, quercetin 3-O-arabinofuranoside, and quercetin 3-O-rhanoside

in the peel of 'Hongxun 2' and 'Xinye 13-11' were the main flavonol components. Their contents fluctuated during fruit ripening and showed a downward trend as a whole, which was consistent with previous studies [42,43]. The content of flavanols in the peel of 'Honghun 2' decreased first from 50 to 80 days after flowering and then increased gradually from 80 to 110 days after flowering, while the content of 'Xinye 13-11' increased first from 50 to 95 days and then decreased from 95 to 110 days after flowering, showing an opposite trend to that of 'Honghun 2'. The contents of flavonols and anthocyanins in quercetin glycosides are both affected by light. Light can affect the substrate synthesis and use of quercetin glycosides and anthocyanins by controlling enzymes. Generally, fruits with sufficient light have higher contents of quercetin glycosides and anthocyanins. Meanwhile, cyanidin 3-O-galactoside, the main substance of quercetin glycosides and anthocyanins, has the same biosynthetic pathway as other quercetin substances [44–48]. From 60 to 75 days after flowering is the key period for the rapid expansion of fruit and light absorption, and usually, the quercetin glycoside and anthocyanin contents increase [27,43]. However, the flavonol content of Honghun 2 decreased during this period. It is speculated that 'Hongxun 2' is rich in anthocyanins, which begin to accumulate gradually 65 days after flowering, while the enzymes used to synthesize quercetin glycoside simultaneously synthesize anthocyanins, resulting in a decrease in quercetin glycoside synthesis and a decrease in flavanol content. The dynamic changes between the components of flavonols of 'Hongxun 2' and 'Xinye 13-11' during the development process were different, and whether they are applicable to other red flesh apples and non-red flesh apples needs to be further studied and verified.

4.4. Relationship Between Malus neidzwetzkyana (Dieck) Langenf and Malus sieversii (Led.) Roem

Based on fruit morphological diversity, early studies generally believed that *Malus neidzwetzkyana* (Dieck) Langenf was a variant of *Malus sieversii* (Led.) Roem. This view was adopted in books such as "*Malolog*", "*Annals of Chinese Fruit Trees (Apple Scroll)*", and "*Research on Germplasm Resources of Apple Genus*" [49–51]. Through many years of field investigation, field records, and molecular biological technology research, Yan et al. concluded that the genetic distance between *Malus sieversii* (Led.) Roem. and *Malus neidzwetzkyana* (Dieck) Langenf was far, and red flesh apples should be regarded as an independent species [52]. The composition and contents of polyphenols such as flavonoids can be used as the basis for the classification of apple plants [53]. In this study, it was found that, during the fruit development of 'Hongxun 2' and 'Xinye 13-11', the change trends of the five polyphenols were different, except for the same trend observed for hydroxylcinnamic acid. Therefore, this paper supports the viewpoint that *Malus neidzwetzkyana* (Dieck) Langenf is a separate species to *Malus sieversii* (Led.) Roem.

5. Conclusions

There were significant differences in the components and contents of polyphenols between red flesh apples and green flesh apples, and the total polyphenol contents in the peel and pulp of 'Xinye 13-11' were significantly higher than those of 'Hongxun 2'. Anthocyanins were the main substances displaying differences in the components of polyphenols in the peel and pulp of red flesh apples and green flesh apples, and they are also the reason for the red color of red flesh apples. In addition, the hydroxycinnamic acid of 'Hongxun 2' was higher than that of 'Xinye 13-11' during fruit development, especially chlorogenic acid. Red flesh apples can be used as raw materials for chlorogenic acid extraction, and the extraction period was better at 50 days after flowering. Based on the classification of polyphenols, the differences in the components and contents of polyphenols support the existence of *Malus neidzwetzkyana* (Dieck) Langenf as an independent species of *Malus*.

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