



Changes in sugars and organic acids in wolfberry (*Lycium barbarum* L.) fruit during development and maturation



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ABSTRACT

Wolfberry (*Lycium barbarum* L.) fruits of three cultivars ('Damaye', 'Baihua' and 'Ningqi No.1') were harvested at five different ripening stages and evaluated for sugars and organic acids. Fructose, glucose and total sugar contents increased continually through development and reached their maxima at 34 days after full bloom (DAF). Fructose and glucose were the predominant sugars at maturity, while sucrose content had reduced by maturity. *L. barbarum* polysaccharides (LBP) content was in the range of 13.03–76.86 mg g⁻¹ FW during ripening, with a maximum at 20 DAF. Citric, tartaric and quinic acids were the main organic acid components during development, and their levels followed similar trends: the highest contents were at 30, 14 and 20 DAF, respectively. The significant correlations of fructose and total sugar contents with LBP content during fruit development indicated that they played a key role in LBP accumulation.

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1. Introduction

Free sugars and organic acids of many fruits and vegetables are important components of taste, and together with aroma play important roles in maintaining fruit quality and determining nutritive value (Ashoor & Knox, 1982; Wu et al., 2012). The nature and the concentration of these constituents in fruits are of interest due to their important influence on organoleptic properties. Therefore, food analysts and plant physiologists are interested in changes in the nature and amounts of these various chemical components during ripening in the edible parts of fruit, due to their impact on market quality of the food product (Wrolstad, 1981).

Wolfberry (*Lycium barbarum* L., also known as *Fructus lycii*, family Solanaceae) is a perennial, deciduous shrub growing in north-west China and the Mediterranean region. It is fast growing, has a deep, well-developed root system, is tolerant to drought and cold and has extensive adaptability (Chang & So, 2008; Chen et al., 2004). Its fruit has been used for centuries in China as a traditional herbal medicine and as a valuable nourishing tonic (Committee of Chinese Pharmacopoeia, 2010). Recently, medical research has

indicated that these fruits have many pharmacological functions, such as improving visual acuity, nourishing the liver and kidneys, reducing blood sugar levels, reducing the risk of cancer and cell senescence and improving immunity (Amagase, Sun, & Borek, 2009; Chang & So, 2008; Xie et al., 2001; Xu et al., 2000). The main active components of wolfberry have been identified as *L. barbarum* polysaccharides (LBP) and zeaxanthin, and other small molecules, such as betaine, cerebroside, beta-sitosterol, p-coumaric acid and various vitamins (Chang & So, 2008). LBP are one of the most important of these compounds, it has been proved to have many health benefits, including anti-aging, improvement of blood function and immunity regulation (Cao et al., 1994; Qian et al., 1989; Xu et al., 2000). LBP is a glycoprotein complex, in which sugar chains (including glucose, arabinose, galactose, mannose, xylose and rhamnose) account for 70% of its total content (Tian, 2003; Tian et al., 1995). Additionally, fructose, glucose and sucrose are the three major components that contribute to the total sugar content in ripe fruit (Tian, 2003). Thus, both the biosynthesis and accumulation of LBP and total sugars can be significantly affected by the types and contents of sugars.

Today, an increasing number of consumers are not only searching for a sweet tasting wolfberry cultivar, but also health promoting compounds in the fruit, such as LBP. However, although there

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have been several studies of sugar contents of various wolfberry cultivars, there is little information on the contents at various ripening stages (Zhang et al., 2006; Zheng et al., 2008, 2010). Research papers concerning the sugar and organic acid compositions and contents of wolfberry fruit are scarce. The characteristics of sugar and organic acid compositions and contents in different cultivars are not well known. The objective of this study was to compare the changes in sugars, organic acids and LBP in three cultivars at five ripening stages, analyse the composition of sugars and organic acids in wolfberry fruit and to find correlations between LBP content and free sugars. Our findings will provide useful information for improving the flavour and especially the nutritional quality of wolfberry fruit via enhancing the content of LBP.

2. Materials and methods

2.1. Materials

Wolfberry fruit of three cultivars ('Damaye', 'Baihua' and 'Ningqi No.1') were collected from three 15 year old trees at the Wolfberry (Lycium) Germplasm Repository of Ningxia (Academy of Agriculture and Forestry Sciences, Ningxia Hui Autonomous Region, China (38°38'N, 106°09'E and altitude 1100 m)). The blooms were marked on 20th June 2012, and the fruits were collected at 9, 14, 20, 30 and 34 days after full bloom (DAF) (Fig. 1). The flowers were randomly marked with red wool as soon as the flowers blossomed, and then the fruit was sampled in the aforementioned time increments. Sampled fruit was immediately evaluated for weight, length and major diameter on 30 fruits, and the remaining fruit was immediately treated with liquid nitrogen and stored at -80°C until further analysis. All reagents, solvents and standards were of analytical reagent grade.

2.2. Extraction and determination of LBP

Extraction and determination of LBP was conducted with reference to the national standard method (GB/T 18672-2002), with slight modifications. After the fresh fruit was sampled, 2.5–3.0 g of sample was ground to a fine powder and placed in a heart-shaped bottle, 75 ml of 80% ethanol solution was added and reflux extracted for 1 h. After 80°C filtering, the residues were washed with 80% hot ethanol solution (about 25 ml) and placed in a flask and 75 ml of distilled water was added. After boiling bath extraction for 1 h, the residues were repeatedly washed with distilled water, the wash solution was combined with the filtrate and the

volume set to 50 ml of sample solution. Then, 0.3 ml of sample solution was added to 2 ml of distilled water and 1 ml of phenol solution, and 5 ml of concentrated sulphuric acid was quickly added drop-wise with mixing until remaining at room temperature for 5 min. The mixture was boil-bathed for 15 min, removed, cooled to room temperature and the absorbance measured at 490 nm. The glucose content was calculated according to the standard curve of glucose ($y = 0.1533x - 0.0269$, $R^2 = 0.9972$, where y is glucose content and x is absorbance). The LBP content was calculated according to the formula $W = \rho \times 250 \times f / (m \times V \times 10^6) \times 100$: where W is the content of LBP (%); ρ is the content of glucose, (g); f is a conversion coefficient of glucose conversion of polysaccharides, (3.19); m is sample mass (g); and V is the measured volume (0.3 ml).

2.3. Extraction and determination of sugars and organic acids

2.3.1. Extraction and determination of sugars

Of frozen fruit samples, 3 g (accurate to 0.0001 g) was weighed and rapidly powdered with liquid nitrogen in a mortar, mixed with 20 ml of ethanol solution (80%, v/v) and then transferred to a heart-shaped bottle, where 55 ml of ethanol solution was added (80%, v/v). The mixture was reflux extracted for 60 min and then filtered at 80°C and the extraction procedure repeated three more times. All filtered extracts were combined and made up to 100 ml after cooling to room temperature. Then, 4 ml of the filtrate was placed in a glass tube and pumped dry by an aspirator pump, and then 1 ml of pyridine was added and shaken until the filtrate dissolved. Then, 0.4 ml of hexamethyl disilylamine and 0.2 ml of trimethylsilyl chloride were added to the solution while in an ice water bath, kept for 30 min at 20°C , then centrifuged and the supernatant collected for gas chromatography (GC) analysis. The chromatographic column was a $30\text{ m} \times 0.25\text{ mm} \times 25\text{ }\mu\text{m}$ Rtx-5 quartz capillary column (Restek, USA): temperature program was initial temperature 180°C for 20 min, increased at $20^{\circ}\text{C min}^{-1}$ to 280°C for 10 min; FID detector temperature was 300°C ; inlet temperature was 280°C ; H_2 flow rate was 30 ml min^{-1} ; N_2 flow rate was 30 ml min^{-1} ; airflow speed was 300 ml min^{-1} ; split ratio was 20:1; and injection volume was $1\text{ }\mu\text{l}$. Sugar concentrations were determined according to the standard curve method and expressed as mg g^{-1} FW of fruit. All sugar standards were obtained from Sigma–Aldrich (USA).

2.3.2. Extraction and determination of organic acids

Three g of frozen fruit samples, (accurate to 0.0001 g) were rapidly powdered with liquid nitrogen in a mortar and then transferred to a screw-cap Eppendorf tube with 5 ml of ethanol solution (80%, v/v). The reaction mixture was placed in a water bath for 20 min at 35°C and centrifuged at $4000 \times g$ for 15 min and the extraction was repeated three times. The supernatant was made up to a constant volume of 20 ml. Then, 1 ml of the mixture was centrifuged at $10,000 \times g$ for 10 min at 4°C and the supernatant collected for high performance liquid chromatography (HPLC) analysis. The chromatographic conditions for organic acids were 50 mM $(\text{NH}_4)_2\text{PO}_4$ buffer, (adjusted to pH 2.3 with phosphoric acid) with a flow rate of 0.5 ml min^{-1} . A Waters Sunfire™ C18, $5\text{ }\mu\text{m}$, $4.6 \times 250\text{ mm}$ column (Shiseido, Japan), with diode array detector (Waters e2695) was used, with detection wavelength of 210 nm. Organic acid concentrations were calculated by a standard curve method and expressed as mg g^{-1} FW of fruit. All organic acids standards were obtained from Sigma–Aldrich (USA).

2.4. Estimation of wolfberry taste parameters

Sweetness index for the different cultivars analysed was calculated as described by Keutgen and Pawelzik (2007). Briefly, the



Fig. 1. Morphological characteristics of five stages of wolfberry fruit development in three varieties.

contribution of each major sugar found in wolfberry fruits was calculated, considering that fructose and glucose are 1.75 and 0.7 times sweeter than sucrose, respectively. Accordingly, sweetness index = 1.0 [sucrose] + 1.75 [fructose] + 0.7 [glucose].

2.5. Statistical analyses

Data analysis was performed with DPS statistical software (version 8.0) and Excel. Figures were constructed using OriginPro 8.5 G (Microcal Software Inc., Northampton, MA, USA). All data was subjected to analysis of variance (ANOVA) and using least significant difference values (LSD; $P = 0.05$). The results are expressed as mean $\pm$ standard error ($n \geq 3$).

3. Results and discussion

3.1. Changes in morphological characteristics during fruit ripening

The fresh weight (FW) of single fruits increased significantly (Fig. 2A) with fruit growth and development in three varieties, but the growth range was different in the three wolfberry varieties over the five developmental stages. The FW of single fruits did not change significantly among the three varieties at 9 and 34 DAF. The FW of single fruits of 'Baihua' and 'Ningqi No.1' was significantly greater than that of 'Damaye' during 14–30 DAF, and there was

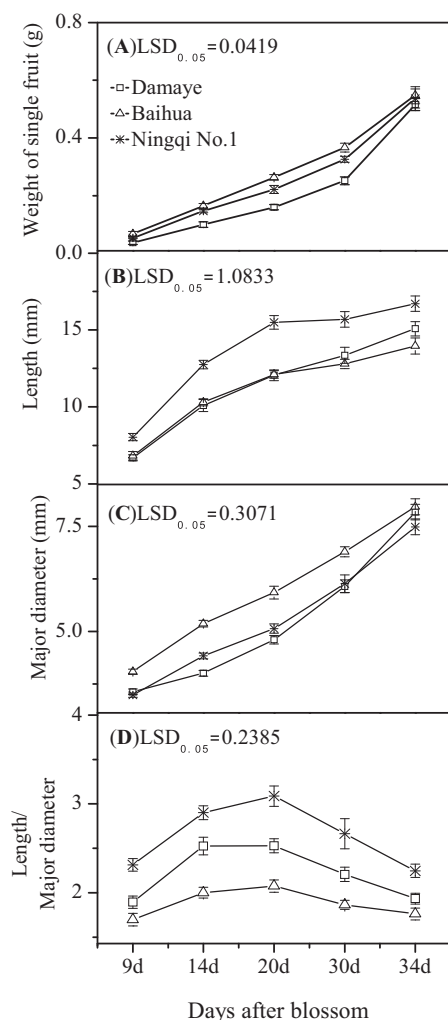


Fig. 2. Weight of single fruit (A), length (B), major diameter (C) and length/major diameter (D) in wolfberry fruit at five stages of ripening. Error bars indicate standard error from 30 replicates, LSD is least significant difference ($P < 0.05$).

no significant difference between 'Baihua' and 'Ningqi No.1'. The fastest growth rate of single fruits was for 'Baihua' (0.012 g d^{-1}) followed by 'Ningqi No.1' (0.011 g d^{-1}) and then 'Damaye' (0.008 g d^{-1}) before 30 DAF. However, during 30–34 DAF, the fastest growth rate of single fruit was for 'Damaye' (0.066 g d^{-1}) followed by 'Ningqi No.1' (0.052 g d^{-1}) and then 'Baihua' (0.045 g d^{-1}). Therefore, the development of wolfberry fruit had two general stages: slow growth during 9–30 DAF; and fast growth during 30–34 DAF. In contrast, Zheng et al. (2010) found that the development of 'Ningqi No.1' fruit could be roughly divided into three stages. The first stage spanned 3–8 DAF, followed by a slow growth stage (8–24 DAF) and after that, rapid growth (24–34 DAF).

Length and major diameter increased (Fig. 2B and C) with fruit growth and development, and length of 'Ningqi No.1' and major diameter of 'Baihua' were significantly higher than the other varieties during all development stages. Length increased rapidly during 9–20 DAF, and showed a slow growth trend during 20–30 DAF; and that of 'Ningqi No.1' did not change during this period, but increased slowly during 30–34 DAF, with the increment obviously lower than the previous rapid growth. Major diameter had a generally linear increasing trend with fruit growth, but the growth trend of different varieties at different developmental stages differed slightly – the growth rate of 'Damaye' during 9–20 DAF was distinctly less than that at 20–34 DAF, but had a lower rate of increase than the other varieties.

Length/major diameter increased in the earlier stage and decreased later (Fig. 2D). Length/major diameter reached maximum values at 20 DAF: the greatest was 'Ningqi No.1' (3.08) followed by 'Damaye' (2.53) and 'Baihua' (2.08). Length/major diameter reached a minimum at 34 DAF for the three varieties: 'Ningqi No.1' was 2.25, 'Damaye' was 1.93 and 'Baihua' was 1.76. Therefore, the growth of wolfberry fruit was mainly longitudinal elongation during 9–20 DAF, and mainly horizontal thickening during 20–34 DAF. The fruit shape of 'Ningqi No.1' and 'Damaye' was a long ellipse, and 'Baihua' fruits were wide ellipses, indicating that the differences in length/major diameter were largely due to fruit shape.

3.2. Changes in LBP contents during fruit ripening

The LBP contents in fruit showed a trend of initial increase followed by a decrease (Fig. 3), with a change trend similar to that of length/major diameter (Fig. 2D). The LBP contents in fruit reached a maximum at 20 DAF: 76.86, 42.53 and 27.6 mg g^{-1} FW for 'Damaye', 'Baihua' and 'Ningqi No.1', respectively. During the whole developmental process the average LBP content in 'Damaye' fruit was highest, followed by 'Baihua' and 'Ningqi No.1' with 57.8, 33.86 and 19.98 mg g^{-1} FW, respectively. Therefore, the LBP content in fruit showed significant differences at different development stages for the different varieties. Zheng et al. (2010) found that LBP content in 'Ningqi No.1' fruit increased and peaked at 34 days, with 48.6 mg g^{-1} dry weight, which cannot be directly

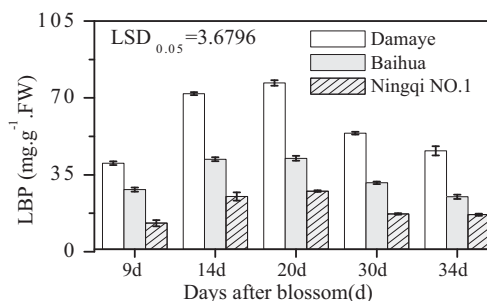


Fig. 3. LBP contents in wolfberry fruit at five stages of ripening. Error bars indicate standard error from four replicates, LSD is least significant difference ($P < 0.05$).

compared to results in the present study as values are always lower for fresh fruit samples.

3.3. Changes in sugar contents during fruit ripening

Wolfberry fruit contains abundant sugars, with 11 kinds of monosaccharides detected (Sung, Oh, & Kim, 1994) and oligosaccharides and polysaccharides. In the present study, there were high contents of fructose, glucose, sucrose, erythrose, galactose and arabinose in the fruit (Table 1) according to GC results. The main composition of sugars changed at different developmental stages with fruit growth and development: the sugars in fruit were mainly sucrose, fructose and erythrose during 9–208 DAF; and mainly fructose, sucrose and glucose during 20–30 DAF. At 34 DAF, there was mainly fructose, glucose and sucrose with contents >33, >2 and >0.38 mg g⁻¹ FW, respectively, with percentages of total sugar of >91%, >4.5% and >0.7% in the three varieties. Similarly, Zheng et al. (2010) demonstrated that fructose and glucose contents increased continuously during ripening in wolfberry fruit. However, Kafkas, Kosar, Paydas, Kafkas, and Baser (2007) found that fructose and glucose contents decreased from the pink to red stage of ripening in strawberry fruit of 'Camarosa' genotype. Wolfberry fruit showed general hexose (i.e., fructose and glucose) accumulation, confirming previously reported data (Zheng et al., 2010). Furthermore, it is in agreement with a general trend, as numerous studies have found increasing levels of fructose and glucose at advanced stages of fruit maturity: e.g., apple (Ackerman, Fischer, & Amado, 1992; Zhang et al., 2010), medlar (Glewa et al., 2003), strawberry (Basson, Groenewald, Kossmann, Cronjé, & Bauer, 2010) and grape (Wu, Liu, Guan, Fan, & Li, 2011).

The contents of fructose and glucose in wolfberry fruit increased (Table 1) in the three varieties with fruit growth and development. In particular, fructose and glucose contents in fruit increased gradually during 9–14 DAF, and were not significantly different among the three varieties; they increased significantly during 14–34 DAF, reaching maxima at 34 DAF of 49.08 and 2.34 mg g⁻¹ FW in 'Damaye', respectively, and correspondingly 33.57 and 2.06 mg g⁻¹ FW in 'Baihua', and 40.7 and 2.49 mg g⁻¹ FW in 'Ningqi No.1'. Fruit of the different varieties showed different growth trends during development; the contents of both fructose and glucose in 'Baihua' fruit were significantly higher than that of 'Damaye' and 'Ningqi No.1' at 20 DAF. At 30 DAF, the fructose content in 'Damaye' fruit was significantly higher than in 'Baihua' and 'Ningqi No.1', and there was no significant difference in glucose among the varieties. At 34 DAF, there were significant differences in fructose content among varieties, but not in glucose content.

The sucrose content in wolfberry fruit decreased with fruit development (Table 1). The sucrose content in fruit gradually declined during 9–14 DAF, with that of 'Ningqi No.1' being significantly higher than 'Damaye' and 'Baihua'. The sucrose content in fruit generally rapidly decreased during 20–34 DAF, with that of 'Damaye' increasing slightly during 20–30 DAF. At 30 DAF, the sucrose contents in wolfberry fruit reached minima of 0.38, 0.84 and 0.68 mg g⁻¹ FW in 'Damaye', 'Baihua' and 'Ningqi No.1', respectively, with percentages of total sugar of 0.72%, 2.29% and 1.58%. Zheng et al. (2010) reported that the sucrose content increased steadily at 24 DAF, but then reduced to a minimum value at 34 DAF in extracts of dry wolfberry fruit samples, this cannot be directly compared to the results reported in the present study as values are always lower in extraction of fresh fruit samples.

3.4. Changes in organic acid contents during fruit ripening

Six organic acids in wolfberry fruit, which were citric acid, tartaric acid, quinic acid, malic acid, oxalic acid and fumaric acid, were

Table 1
Sugar and organic acid contents (mg g⁻¹ FW) of wolfberry fruit at five stages of ripening. Results are expressed as the mean ± standard error of four separate extractions and determinations. Values in the same row can be compared by least significant difference (LSD).

Stage	Sugar (mg g ⁻¹ FW)						Organic acid (mg g ⁻¹ FW)					
	Fructose	Glucose	Sucrose	Erythrose	Galactose	Arabinose	Citric acid	Tartaric acid	Quinic acid	Malic acid	Oxalic acid	Fumaric acid
9 DAF	Damaye	0.72 ± 0.055	0.014 ± 0.001	5.64 ± 2.893	0.0753 ± 0.0054	0.0301 ± 0.0015	0.0099 ± 0.0008	2.878 ± 0.068	3.381 ± 0.149	0.863 ± 0.042	0.644 ± 0.029	0.0553 ± 0.0005
	Baihua	2.62 ± 0.246	0.028 ± 0.002	5.77 ± 0.117	0.3923 ± 0.0026	0.0712 ± 0.0034	0.0120 ± 0.0001	12.24 ± 0.464	2.181 ± 0.031	1.452 ± 0.023	1.078 ± 0.018	0.0568 ± 0.0000
	Ningqi No.1	2.46 ± 0.243	0.063 ± 0.003	9.81 ± 1.467	0.2916 ± 0.0094	0.1252 ± 0.0008	0.0110 ± 0.0001	13.19 ± 0.248	1.409 ± 0.063	1.444 ± 0.044	1.093 ± 0.020	0.0581 ± 0.0002
14 DAF	Damaye	1.82 ± 0.142	0.037 ± 0.003	4.38 ± 0.737	0.0881 ± 0.0012	0.0636 ± 0.0008	0.0104 ± 0.0004	14.37 ± 1.203	3.254 ± 0.259	1.251 ± 0.092	0.559 ± 0.062	0.0567 ± 0.0003
	Baihua	2.56 ± 0.202	0.073 ± 0.004	5.37 ± 0.230	0.2709 ± 0.012	0.0395 ± 0.0022	0.0154 ± 0.0002	13.54 ± 0.038	3.901 ± 0.014	1.881 ± 0.030	0.972 ± 0.016	0.0580 ± 0.0009
	Ningqi No.1	3.83 ± 0.086	0.082 ± 0.004	9.63 ± 1.551	0.3079 ± 0.0035	0.0871 ± 0.0014	0.0154 ± 0.0005	13.81 ± 0.404	3.382 ± 0.029	1.917 ± 0.082	0.670 ± 0.031	0.0579 ± 0.0004
20 DAF	Damaye	6.39 ± 0.115	0.188 ± 0.003	1.61 ± 0.288	0.0432 ± 0.0021	0.0169 ± 0.0003	0.0086 ± 0.0002	17.62 ± 0.103	3.433 ± 0.048	0.532 ± 0.022	0.502 ± 0.007	0.0563 ± 0.0005
	Baihua	18.52 ± 0.434	0.780 ± 0.005	4.59 ± 0.132	0.1257 ± 0.0006	0.0324 ± 0.0002	0.0136 ± 0.0003	14.64 ± 0.027	4.661 ± 0.882	0.808 ± 0.058	0.717 ± 0.021	0.0588 ± 0.0005
	Ningqi No.1	14.82 ± 0.879	0.521 ± 0.041	4.36 ± 0.207	0.1331 ± 0.0082	0.0420 ± 0.0041	0.0123 ± 0.0001	16.50 ± 0.881	3.576 ± 0.139	0.957 ± 0.021	0.541 ± 0.009	0.0584 ± 0.0015
30 DAF	Damaye	27.16 ± 0.782	1.011 ± 0.029	1.83 ± 0.310	0.0967 ± 0.0009	0.0351 ± 0.0007	0.0109 ± 0.0003	17.09 ± 0.135	2.857 ± 0.128	0.470 ± 0.018	0.694 ± 0.006	0.0550 ± 0.0001
	Baihua	23.50 ± 0.296	0.994 ± 0.060	2.03 ± 0.226	0.0926 ± 0.0006	0.0206 ± 0.0006	0.0132 ± 0.0004	16.57 ± 0.878	3.895 ± 0.030	0.946 ± 0.025	0.705 ± 0.011	0.0569 ± 0.0001
	Ningqi No.1	21.75 ± 1.200	0.928 ± 0.025	2.43 ± 0.095	0.1002 ± 0.0031	0.0349 ± 0.0005	0.0148 ± 0.0007	17.29 ± 0.059	3.344 ± 0.022	0.749 ± 0.016	0.755 ± 0.005	0.0571 ± 0.0005
34 DAF	Damaye	49.08 ± 1.037	2.335 ± 0.061	0.38 ± 0.074	0.0693 ± 0.0031	0.0230 ± 0.0011	0.0407 ± 0.0003	16.93 ± 0.160	3.060 ± 0.084	0.251 ± 0.006	0.803 ± 0.016	0.0547 ± 0.0002
	Baihua	33.57 ± 0.680	2.063 ± 0.011	0.84 ± 0.041	0.0788 ± 0.0017	0.0148 ± 0.0002	0.0293 ± 0.0006	14.02 ± 0.062	3.032 ± 0.090	0.638 ± 0.070	0.669 ± 0.008	0.0563 ± 0.0003
	Ningqi No.1	40.70 ± 3.760	2.494 ± 0.014	0.68 ± 0.026	0.0692 ± 0.0006	0.0245 ± 0.0007	0.0451 ± 0.0007	13.92 ± 0.316	2.383 ± 0.389	0.253 ± 0.066	0.721 ± 0.069	0.0551 ± 0.0003
LSD (P < 0.05)		2.0583	0.0631	1.8833	0.0277	0.0058	0.0069	1.1337	0.6371	0.8002	0.0974	0.0030

detected by HPLC (Table 1). The main composition of acids changed at different developmental stages with fruit growth and development. During 9–20 DAF, the contents of acids were in the order of citric acid, quinic acid, tartaric acid, malic acid, oxalic acid and fumaric acid; during 30–34 DAF the order was citric acid, tartaric acid, quinic acid, malic acid, oxalic acid and fumaric acid. During the whole development process the contents of citric acid, tartaric acid and quinic acid were in the ranges of 12.24–17.62, 1.14–4.66 and 0.80–4.07 mg g⁻¹ FW, respectively, which corresponded to >58%, >6.7% and >4.15% of the total organic acids. This study suggests that the major organic acid component was citric acid. Similarly, other researchers have shown that citric acid was the most common organic acid in ripe *Passiflora edulis* f. *flavicarpa* Degener fruit (Chan, Chang, & Chenchin, 1972), citrus (Yamaki, 1989), mango (Gil et al., 2000), medlar (Glewa et al., 2003) and strawberry (Basson et al., 2010; Ornelas-Paz et al., 2013). In contrast, malic acid was the most common in ripe apple (Ackerman, Fischer, & Amado, 1992; Krotkov, Wilson, & Street, 1951), sweet cherry (Girard & Kopp, 1998), peach (Moing et al., 1998), blackberry (Kafkas, Koşar, Türemiş, & Başer, 2006) and persimmon (Veberic, Jurhar, Mikulic-Petkovsek, Stampar, & Schmitzer, 2010).

With fruit growth and development, the contents of citric, tartaric and quinic acids in wolfberry fruit initially increased and then decreased (Table 1) in the three varieties. The content of citric acid in 'Damaye' fruit reached a maximum (17.62 mg g⁻¹ FW) at 20 DAF, while that of 'Baihua' and 'Ningqi No.1' reached maxima (16.57 and 17.29 mg g⁻¹ FW, respectively) at 30 DAF. The differences in citric acid content among the three varieties were not obvious during growth and development. The content of tartaric acid in 'Baihua' and 'Ningqi No.1' rapidly increased during 9–14 DAF, while that in 'Damaye' slowly increased. However, tartaric acid content in 'Baihua' rapidly increased during 14–20 DAF, while that in 'Damaye' and 'Ningqi No.1' slowly increased. At 20 DAF the tartaric acid contents reached maxima: 3.43, 4.66 and 3.58 mg g⁻¹ FW in 'Damaye', 'Baihua' and 'Ningqi No.1', respectively. After that, the content of tartaric acid in 'Baihua' and 'Ningqi No.1' rapidly decreased during 20–34 DAF, while that in 'Damaye' slowly decreased. During growth and development, the tartaric acid content in 'Baihua' was significantly higher than in 'Ningqi No.1'. The quinic acid content in 'Damaye', 'Baihua' and 'Ningqi No.1' rapidly increased during 9–14 DAF, reaching maxima at 20 DAF of 4.07, 2.72 and 3.82 mg g⁻¹ FW, respectively; quinic acid content rapidly decreased during 14–34 DAF, reaching corresponding minima at 34 DAF of 1.97, 0.80 and 1.87 mg g⁻¹ FW. The content of quinic acid in 'Baihua' was significantly lower than that of 'Damaye' and 'Ningqi No.1', and there were small differences between 'Damaye' and 'Ningqi No.1'. However, in mango fruit, the content of citric acid increased in earlier stages, and then decreased (Léchaudel, Joas, Caro, Génard, & Jannoyer, 2005). Glewa et al. (2003) concluded that medlar fruit exhibited a steady increase in malic acid content, reaching a maximum of 4.28 mg g⁻¹ FW at ripe maturity, while the level of citric acid showed fluctuations throughout the development period. Basson et al. (2010) found that the citric acid content decreased slightly as strawberries ripened and was 2–3 times higher than malic acid content. Thus, the composition of fruit differed greatly among the different varieties during growth and development.

3.5. Changes in total sugars, total organic acids, total sugars/total organic acids and sweetness index during fruit ripening

Total sugars, total sugars/total organic acids and sweetness index increased with fruit growth and development (Fig. 4A, C and D). These measures slowly increased in 'Damaye' and 'Ningqi No.1' during 9–14 DAF, while those in 'Baihua' showed a slight downward trend; and 'Damaye' had significantly lower total sugar,

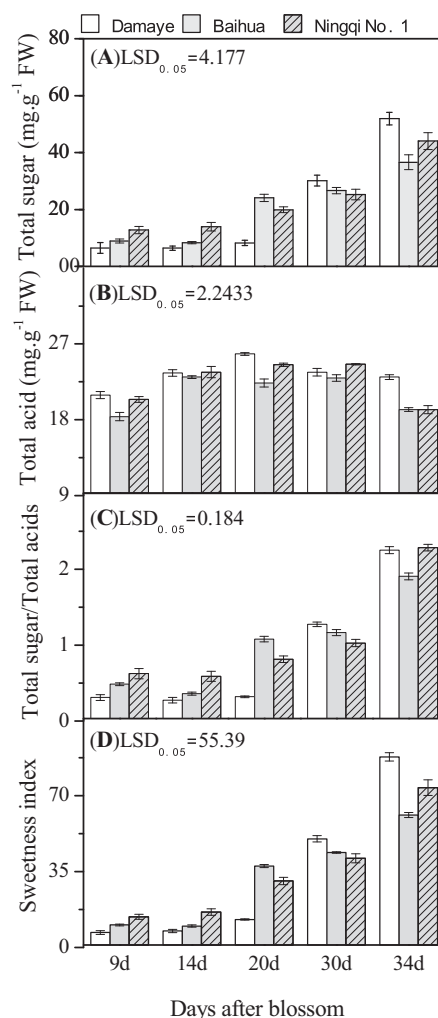


Fig. 4. Total sugar content, total organic acid content, total sugars/total organic acids and sweetness index in wolfberry fruit at five stages of ripening. Error bars indicate standard error from four replicates, LSD is least significant difference ($P < 0.05$).

total sugars/total organic acids and sweetness index compared to the other varieties during this period. Total sugars, total sugars/total organic acids and sweetness index in 'Baihua' and 'Ningqi No.1' slowly increased during 20–30 DAF, while those in 'Damaye' rapidly increased. During 30–34 DAF, total sugars, total sugars/total organic acids and sweetness index in the three varieties rapidly increased, and those of 'Damaye' were significantly higher than of 'Baihua' and 'Ningqi No.1', and then reached maxima of 51.92, 2.25 and 85.9 mg g⁻¹ FW in 'Damaye' at 34 DAF, respectively, and correspondingly 36.6, 1.9 and 61.03 mg g⁻¹ FW in 'Baihua' and 44.02, 2.30 and 73.66 mg g⁻¹ FW in 'Ningqi No.1'. With fruit growth and development, total organic acids initially increased and later decreased (Fig. 4B). Total organic acids in 'Baihua' reached a maximum of 23.07 mg g⁻¹ FW at 14 DAF, 'Damaye' reached 25.81 mg g⁻¹ FW at 20 DAF and 'Ningqi No.1' reached 24.59 mg g⁻¹ FW at 30 DAF. Then, total organic acids had a decreasing trend, with 'Baihua' and 'Damaye' significantly lower compared to 'Ningqi No.1' at 34 DAF.

The ratio of sugar/acid and the sweetness index play important roles that can characterize acceptance of fruit by consumers (Basson et al., 2010; Bordonaba & Terry, 2010; Glewa et al., 2003; Veberic et al., 2010). Our results showed that there were high correlation coefficients between sweetness index and total

Table 2

Correlation coefficients (r) between *Lycium barbarum* polysaccharides (LBP) and sugar contents of wolfberry fruit at five stages of ripening.

Compounds	LBP				
	9 DAF	14 DAF	20 DAF	30 DAF	34 DAF
Fructose	−0.71*	−0.89**	−0.81**	0.83**	0.60*
Glucose	−0.98**	−0.94**	−0.72**	0.39	−0.11
Sucrose	−0.70*	−0.80**	−0.91**	−0.47	−0.70*
Erythrose	−0.61*	−0.96**	−0.95**	−0.23	−0.19
Galactose	−0.98**	−0.34	−0.93**	0.14	0.10
Arabinose	−0.34	−0.89**	−0.84**	−0.88**	−0.03
Σ Sugar	−0.76**	−0.82**	−0.84**	0.81**	0.58*

* and ** represent significance at $P < 0.05$ and $P < 0.01$, respectively.

sugar content or total sugars/total organic acid ratios ($r = 0.96$ and $r = 0.89$, respectively). Additionally, for the whole development process, the ratio of sugar/acid maintained a rising trend, although total organic acid content increased at the immature stage, followed by a decrease at the ripe stage. Fully ripe 'Damayse' fruit had higher total sugar and sweetness index than the other tested cultivars (Fig. 2A and Fig. 3).

3.6. Correlation analysis between LBP and sugar contents during fruit ripening

The correlations among LBP and sugar contents of wolfberry fruit were statistically analysed (Table 2). The correlations between LBP and sugar contents varied between sugars ($r = -0.34$ to -0.98) at 9–20 DAF, with significant negative correlations except from galactose and arabinose; there were significant positive correlations only for fructose and total sugar during 30–34 DAF. Therefore, the LBP content accumulated largely by the decreased free sugar content before 20 DAF, but the LBP content increased significantly only with the increase in fructose and total sugar after 20 DAF. During development, the change in LBP content was affected by all kinds of free sugars: total sugars, fructose, arabinose, glucose and sucrose. Similarly, Zheng et al. (2010) established that LBP content was positively correlated with total sugar content ($r = 0.830$, $P < 0.05$) and fructose ($r = 0.830$, $P < 0.05$), but negatively correlated with sucrose content ($r = -0.812$, $P < 0.05$) in ripe fruit. This further confirmed that total sugars and fructose played a critical role during LBP accumulation.

4. Conclusions

Developmental changes of seven sugars and six organic acids in the flesh of wolfberry were determined. The results suggest that wolfberry fruit was rich in total sugars and fructose, but glucose was most abundant in fully ripe fruit. Citric acid was the major organic acid during fruit development. According to literature, fully ripe fruit has high total sugar content, a high ratio of total sugars/total organic acids and a moderate content of LBP, which results in good quality (i.e., morphological characteristics and sweetness index). The experiments showed that LBP content was closely correlated with total sugar and fructose contents. Our findings will provide useful information for improving the flavour quality and especially for nutritional quality of wolfberry fruit via enhancing content of LBP.

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