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Comparison of physiochemical characteristics of *Citrus reticulata* cv. Shatangju fruit with different fruit sizes after storage

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ABSTRACT

'Shatangju' (*Citrus reticulata*) is a loose-skin mandarin cultivar with delicious taste quality. However, its fruit size varied greatly which might affect the storage results. This study investigated the physiochemical characteristics of 'Shatangju' fruit with different sizes after a long-term storage, and found that small fruit tended to have a longer shelf life. According to the results, the fruit with a higher transverse diameter tended to contain more seeds and had lower edible rate. The storability of 'Shatangju' fruit decreased with the increase of fruit size, in that the big fruit suffered more severe vesicle collapse after storage. Correspondingly, the physiochemical characteristics of the flesh varied among stored fruit of different sizes. The stored larger fruit tended to have higher content of cellulose, hemicellulose and lignin, as well as a lower content of soluble sugars, organic acids, and amino acids in the flesh, which led to the bad texture and tasteless flavor. We also found that the vesicle collapse of 'Shatangju' fruit was a very complicated syndrome involving a series of physiochemical changes rather than simple water loss. The results of the present study provided guidance for the classification and storage of 'Shatangju' fruit after harvest.

1. Introduction

'Shatangju' (*Citrus reticulate* cv. Shatangju) is a popular citrus variety extensively grown in the southern part of China, which belongs to the loose-skin mandarin category (Li et al., 2012). 'Shatangju' is characterized by its excellent features, such as easy-peeling, sweet and juicy flesh and attractive appearance, and thus has become one of the most competitive citrus varieties in the market (Li et al., 2016, 2019).

The fruit size is one of the most important quality parameters of citrus fruit, which affects the acceptability by customers (Combrink, Labuschagne, & Bijzet, 2013). As is reported in previous studies, the fruit size was determined by several factors, including the genetic factors (Zhang, Liu, & Hu, 2016; Zhang, Wang, et al., 2016), root stock (Liu, Li, Huang, & Chen, 2015), plant hormones (Turgut, Salih, & Berken, 2017) and cultivation techniques (i.e., fertilization, irrigation, fruit thinning and girdling) (Davis, Stover, & Wirth, 2004). As for 'Shatangju' mandarin fruit, the percentage of fruit with different sizes in a cropping period was uncertain and variable. 'Shatangju' fruit with different sizes existed in one tree, and even in the same branch, which seems unrelated to the genetic difference. Liu et al. (2015) found that rootstocks of 'Shatangju' fruit affected fruit size by modulating the transcription of several auxin response factor genes. Besides, the number of seed per fruit, crop load and pruning were also reported to be correlated with fruit size of 'Shatangju' fruit (Ketsa, 2010).

For some citrus species, the fruit size has been reported to be correlated with fruit quality and the storability. For the 'Shatian' pomelo, the medium-size fruit possessed a higher soluble solids content compared to the large-size and small-size fruit (Peng et al., 2020). For the navel orange, the medium- and small-size fruit also had higher soluble solids after a period of storage (Diao, Zeng, Zhu, & Zhong, 2015). In addition, the fruit size had been found to be correlated with the storage results. For instance, the decay rate of medium-size 'Shatian' pomelo

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was lower than that of the large-size and small-size fruit (Peng et al., 2020); the weight loss and granulation index of stored satsuma mandarin tended to increase with the fruit size (Wen, Shi, Wang, & Wu, 2013); the granulated 'Ougan' fruit after storage tend to have a large size than the healthy fruit (Jin, Gao, Chen, & Wu, 2015).

Considering that fruit size variation is commonly seen in the 'Shatangju' fruit, it is important to get a better understanding of the relationship between its fruit size and the fruit qualities during the postharvest period. However, to the best of our knowledge, there had been few studies on the relationship between fruit size and the physiochemical characteristics of stored 'Shatangju' fruit. Thus, the present study investigated the external and internal quality of stored 'Shatangju' fruit with different sizes, aiming to elucidate the relationship between fruit size and fruit quality of 'Shatangju' fruit in the later storage period.

In order to reduce losses and maximize economic benefits after harvest, many effective postharvest handling methods such as cold storage, edible coating and heat shock have been put forward to extend the shelf life of citrus fruit (Corato, Salimbeni, Pretis, Avella, & Patruno, 2018). However, we found that the shelf life of 'Shatangju' fruit was also affected by fruit size. In the later storage period, it is hard to maintain the quality of large fruit even under modified storage conditions. Thus, fruit classification is quite an effective measure to extend the shelf life of 'Shatangju' fruit. The results of this study provide a theoretical basis for the production and marketing of 'Shatangju' fruit.

2. Materials and methods

2.1. Plant material and storage condition

'Shatangju' mandarin fruit were harvested on December 13, 2019 from a local orchard in Guangzhou city, China. A total of 300 fruit were divided into five classes according to their transverse diameters: 2S (< 40 mm), S (40–45 mm), M (45–50 mm), L (50–55 mm) and 2L (55–65 mm), with more than 50 fruit in each class. For storage, the fruit were put into the porous plastic baskets and laid on the shelf, as the common packing manner in the industry. The fruit were stored at $10 \pm 0.5\,^{\circ}C$ (RH 90%) for 150 d, and then 50 fruit without decay in each group were used for the physiochemical characteristics determination.

2.2. General fruit quality analysis

The transverse diameter and longitudinal diameter of 'Shatangju' fruit were measured using a digital caliper (SATA, China). The fruit shape index was calculated according to the following formula:

$$\text{Fruit shape index} = \frac{\text{Longitudinal diameter(mm)}}{\text{Transverse diameter(mm)}}$$

The weight of the whole fruit and the peel were measured using an electronic balance (Sartorius, Germany). The edible rate was calculated according to the following formula:

$$\text{Edible rate (\%)} = \frac{\text{Total fruit weight (g)} - \text{Peel weight (g)}}{\text{Total fruit weight (g)}} \times 100$$

The seed number of each fruit was counted separately, and average seed number of each group was also calculated.

The rind color was measured using a colorimeter (Hunterlab, USA). Four evenly distributed equatorial points of each fruit were selected for the measurement. The CIE 1976 $L^*a^*b^*$ color scale was used to evaluate the rind color of 'Shatangju' fruit. Citrus color index (CCI) of each fruit was calculated using the equator below according to Cao et al. (2019).

$$\text{CCI} = \frac{a^* \times 1000}{b^* \times L^*}$$

2.3. Evaluation of vesicle collapse level

Vesicle collapse index and vesicle collapse rate was adopted to evaluate the severity of the vesicle collapse of 'Shatangju' fruit. The vesicle collapse index was evaluated according to the method of Chen, Zhang, Li, Chen, and Liu (1995) and Nie, Huang, Chen, Wan, and Chen (2020) with slight modification. Fruit were cut at the equatorial plane and the vesicle collapse level was determined according to the portion of vesicle collapsed area on each transection: No vesicle collapse, level 0; vesicle collapsed area $\leq$ 10%, level 1; 10% $\leq$ vesicle collapsed area $\leq$ 25%, level 2; 25% $\leq$ vesicle collapsed area $\leq$ 50%, level 3; vesicle collapsed area $\geq$ 50%, level 4. The vesicle collapse index was calculated according to the following formula:

$$\text{Vesicle collapse index} = \frac{\sum \text{Vesicle collapse level} \times \text{Number of fruit in this level}}{\text{The highest vesicle collapse level(level4)} \times \text{Total fruit number}}$$

The vesicle collapse rate was used to reflect the percentage of vesicle collapse fruit in each group, which was calculated according to the following formula.

$$\text{Vesicle collapse rate(\%)} = \frac{\text{Number of vesicle collapsed fruit}}{\text{Total fruit number of eachgroup}} \times 100$$

2.4. Measurement of water content and dry matter content

Water content was employed to evaluate the edible quality of 'Shatangju' fruit, which was represented by the moisture content in the flesh. The flesh was collected, weighed as fresh and recorded as W_0. Then the fresh segments were frozen in liquid nitrogen and subjected to lyophilization in a vacuum freeze dryer (Virtis, USA) to completely remove moisture. The lyophilized samples were weighed again and recorded as W_1. Three replicates were conducted for each group, with 15 fruit in each replicate. The water content and dry matter content were calculated according to the following formulas.

$$\text{Water content (\%)} = \frac{W_0 - W_1}{W_0} \times 100$$

$$\text{Dry matter content (\%)} = \frac{W_1}{W_0} \times 100$$

2.5. Qualification of alcohol insoluble solids (AIS), pectin, protopectin, cellulose, hemicellulose and lignin content

The flesh was lyophilized and ground into fine powder. The AIS was prepared according to the method of Dietz and Rouse (1953). Briefly, 1 g of lyophilized flesh was homogenized in 25 mL of 95% ethanol, and then incubated in 90 °C water bath for 30 min. After cooling to room temperature, the supernatant was removed by centrifugation at $3000 \times g$ for

5 min. The extraction above was repeated 3 times. The final residue was then dried at 60 °C for 12 h and weighed. Three replicates were conducted for each group, with 15 fruit in each replicate. The AIS content was calculated by the following equator.

$$\text{AIS content}\ (\%) = \frac{\text{Dried residue weight}\ (\text{g})}{\text{Sample weight}\ (\text{g})} \times 100$$

The qualification of water-soluble pectin and protopectin was performed based on the method of Dong, Xia, Wang, Xiao, and Liu (2008). Approximately 200 mg of AIS powder from each group was extracted with 20 mL distilled water, and then incubated in 50 °C water bath for 30 min. The supernatant was obtained by centrifugation at 3000 × *g* for 5 min. This procedure was repeated an additional two times. The supernatants of the three extractions were combined as water-soluble pectin extracts. The residue was homogenized in 25 mL of 0.5 mol/L H_2SO_4 and incubated in a water bath at 85 °C for 10 min. After centrifugation at 3000 × *g* for 5 min, the supernatant was collected to get the protopectin extracts. For the determination of water-soluble pectin and protopectin, 0.6 mL of H_2SO_4 (containing 0.48% sodium tetraborate) was added to 0.1 mL water-soluble pectin and protopectin extracts separately and stored at room temperature for 12 h. After that, 10 μL of 0.15% M-hydroxy biphenyl (dissolved in 0.5% NaOH) was added to the solution. Three replicates were conducted for each group, with 15 fruit in each replicate. The absorbance of the solution was measured at 530 nm using a microplate reader (BIO-RAD, USA). The concentration of water-soluble pectin and protopectin was calculated using a standard curve of galacturonic acid and expressed as mg/g dry weight (DW).

The extraction of cellulose and hemicellulose was performed according to the method of Zhou, Li, Yan, and Xie (2011) with some modifications. Briefly, the residue from the protopectin extraction was collected and homogenized in 10 mL of 4 mmol/L NaOH containing 100 mmol/L $NaBH_4$. After ultrasonic-assisted extraction for 6 h, the supernatant was obtained by centrifugation at 3000 × *g* for 5 min to get the hemicellulose. The remaining residue was washed with distilled water twice in order to obtain the cellulose. For the hydrolyzation of hemicellulose, 12 mL of 2 mol/L H_2SO_4 was added to the 10 mL of hemicellulose extracts and then incubated in a water bath at 100 °C for 5 h. For the hydrolyzation of cellulose, 3 mL of 80% H_2SO_4 was added to the cellulose extracts. After hydrolyzation for 2 h, 6 mL ddH_2O was added to the mixture. Then the mixtures were incubated in a water bath at 100 °C for 5 h. The content of cellulose and hemicellulose was determined using the anthrone method described by Chen et al. (2017). Briefly, 3 mL H_2SO_4 (containing 0.25% anthrone) was added to the cellulose and hemicellulose extracts separately and then incubated in a water bath at 90 °C for 15 min. Three replicates were conducted for each group, with 15 fruit in each replicate. The absorbance of the solution was detected at 620 nm. The concentration of cellulose and hemicellulose was calculated using a standard curve for glucose and expressed as mg/g DW.

The lignin content determination was performed using the method published by Chang, Chandra, Berleth, and Beatson (2008) with some modifications. Briefly, 15 mg of AIS powder was homogenized in 1.5 mL of 90% DMSO and oscillated overnight in order to remove the starch. The supernatant was removed by centrifugation at 10,000 × *g* for 5 min. The remaining residue was washed with 1 mL of 70% ethanol six times, and then washed with 1.5 mL of acetone. After that, the residue was dried in a vacuum concentrator (Eppendorf, Germany) for 12 h to obtain de-starched AIS. For the determination of lignin, 1 mL of 25% acetyl bromide was added in de-starched AIS and then incubated in a 70 °C water bath for 1 h, followed by adding 5 mL of acetic acid. Then, 300 μL of the supernatant was obtained, and mixed with 400 μL of 1.5 mol/L NaOH and 300 μL of 0.5 mol/L freshly made hydroxylamine hydrochloride, and the absorbance of the mixture was detected at 280 nm. Three replicates were conducted for each group, with 15 fruit in each replicate. The lignin content was determined by the acetyl bromide soluble lignin (ABSL) assay and expressed as ABSL%.

2.6. Determination of soluble sugars, organic acids and amino acids contents

The gas chromatography-mass spectrometer (GC-MS) method was applied to determine the contents of soluble sugars, organic acids and amino acids. Sample preparation was carried out according to the method of Lin et al. (2015). Briefly, 0.1 g of lyophilized sample was homogenized in 1.4 mL of methanol and then vortexed at 950 rpm for 15 min at 70 °C, followed by centrifugation at 10,000 × *g* for 10 min. The supernatant was homogenized with 1.5 mL of distilled water and 0.75 mL of chloroform. After centrifugation at 4000 × *g* for 10 min, 100 μL of the supernatant was collected and 10 μL of ribitol (0.2 mg/mL) added as an internal standard, then dried in a vacuum concentrator (Eppendorf, Germany) at room temperature for 3 h. The residue was resuspended in 60 μL of pyridine methoxyamine hydrochloride (20 mg/mL) and vortexed at 37 °C for 90 min. Finally, the sample was mixed with 40 μL of BSTFA (containing 1% trimethylchlorosilane (TMCS)) and vortexed at 37 °C for 30 min. Three replicates were conducted for each group, with 15 fruit in each replicate.

A GC-MS analysis was performed according to the protocol described by Cao et al. (2020). Agilent 7980B GC and Agilent 7000C MSD (Agilent Technology, USA) were used for analysis. A sample of 1 μL was injected into a HP-5ms column (30 m × 0.25 mm i.d., 0.25 μm; Agilent Technology, USA), with a split ratio of 10:1. The temperature of the injector was 250 °C, with helium as carrier gas at a flow rate of 1 mL/min. The oven temperature conditions were set as follows: held at 100 °C for 1 min and then increased to 184 °C at a rate of 3 °C /min; ramped up to 190 °C at 0.5 °C /min and held for 1 min; ramped up to 250 °C at 10 °C /min and held for 1 min; ramped up to 280 °C at 5 °C /min and finally held at 280 °C for 3 min. The ionization voltage was set as − 70 eV, with an ion source temperature of 230 °C. Mass spectra were obtained with a mass range of 30–550 *m/z* (Detailed information about GC-MS analysis can be found at Fig. S3 and Table S1 in Supplementary data). The contents of soluble sugars, organic acids and amino acids were calculated according to the standard curves of the corresponding authorized standards.

2.7. Statistical analysis

Data were statistically analyzed using SPSS (Version 20.0, USA) and represented as mean ± standard deviation (SD) in the figures. Comparisons among groups were carried out using Tukey multiple range test at $P < 0.05$ level. A principal component analysis (PCA) was performed online (https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml). Figures were drawn using GraphPad Prism (Version 8.0.2, USA).

3. Results and discussion

3.1. The relationship between the general fruit quality and the fruit size

Fruit size is an important quality parameter of citrus fruit. The fruit size of 'Shatangju' varied considerably, with the transverse diameters ranging from 33.7 mm to 63.91 mm (Fig. 1A). The fruit shape index ranged from 0.74 to 0.77, and the large fruit (L and 2L) and small fruit (2S) tended to be more oblate than the middle size fruit (S and M) (Fig. 1B). The weight proportion of the peel ranged from 15.77 to 32.72 (Fig. 1C), correspondingly, the edible rate decreased with the increase of fruit size, with a correlation coefficient of 0.5082 ($p < 0.05$) (Fig. 1F).

Rind color is an important external trait of citrus fruit, which affects the acceptability by customers (Farag, Abib, Ayad, & Khattab, 2020). There was no significant difference on the CCI value among different groups (Fig. 1D), thus, the correlation between CCI value and transverse diameter was very low ($r^2 = 0.0159$) (Fig. 1G), which indicated that the fruit transverse diameter will not affect the rind color of 'Shatangju' fruit. Interestingly, we observed that there was no significant difference in the rind color of 'Shatangju' fruit with different sizes since the initial

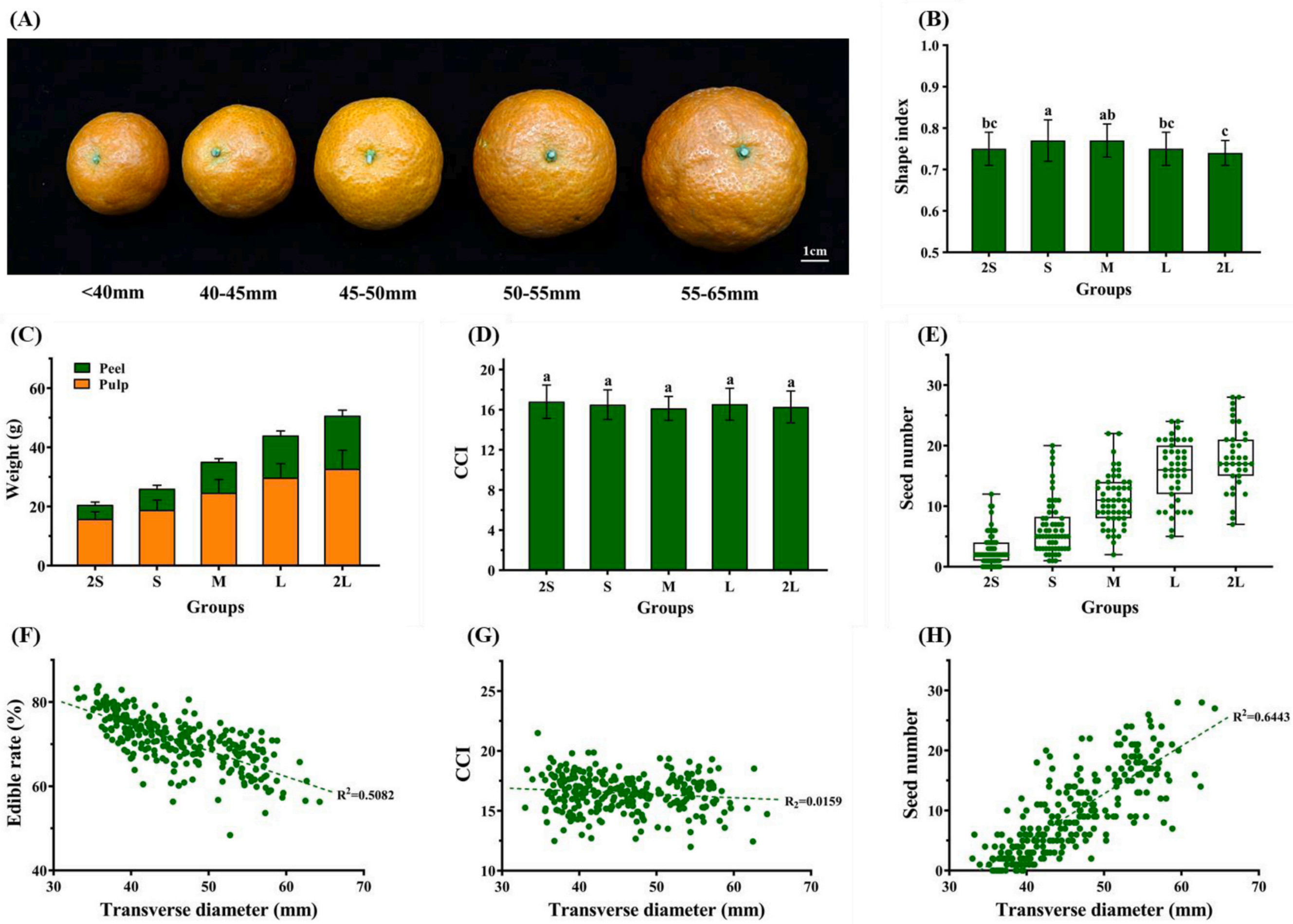

Fig. 1. The general quality of 'Shatangju' fruit with different fruit sizes. (A) Appearance of fruit with different sizes; (B) Fruit shape index; (C) Citrus color index (CCI); (D) The weight proportion of peel and pulp; (E) Seed number; (F) Correlation analysis between citrus color index (CCI) and transverse diameter; (G) Correlation analysis between seed number and transverse diameter; (H) Correlation analysis between edible rate and transverse diameter. Fruit were classified according to their transverse diameter: 2S (< 40 mm), S (40–45 mm), M (45–50 mm), L (50–55 mm) and 2L (55–65 mm). Data were represented as mean ± standard deviation of 50 replicates (n = 50). Different lowercase letters in the column represented a significant difference at $p < 0.05$ level by Tukey testing.

storage period (Fig. S1A–E).

Seed number is one of the essential internal properties of mandarin. The seed number of 'Shatangju' varied considerably, ranging from 0 to 27 (Fig. 1E). It is interesting to find that there was a link between seed number and fruit size. A considerable portion of the 2S fruit (17%) were seedless, while the fruit of other size contained certain number of seeds. Generally, the seed number increased with the fruit size, with the median of seed number being 3, 7, 11, 16, and 18 in the 2S, S, M, L and 2L fruit respectively (Fig. 1E). A correlation analysis showed that the seed number of 'Shatangju' fruit was positively correlated with the transverse diameter, with a correlation coefficient of 0.6443 ($p < 0.05$) (Fig. 1H).

Fruit size affects the consumer acceptability and marketability (Combrink et al., 2013). Previous studies indicated that, in some citrus species, the fruit size was correlated with certain general quality indexes. For instance, Peng et al. (2020) found that in 'Shatian' pomelo, the fruit > 1200 g had a significantly thicker peel and a lower edible rate compared to the small sized fruit; Chao (2005) found a positive correlation between seed number per fruit and fruit diameter in mandarin. These results coincided with our findings in 'Shatangju' fruit.

3.2. The relationship between the interior fruit quality with the fruit size

Although no significant difference was seen on the fruit surface appearance, the appearance of the pulp varied among fruit with different sizes after long-term storage. Although the interior pulp quality of 'Shatangju' fruit was insignificant among fruit with different sizes at 0 d (Fig. S2A and B), the syndromes of vesicle drying, shrinking, peel and pulp separation appeared gradually with the elongation of storage time and the incidence increase with the fruit size, which were the typical syndromes of puffing and vesicle collapse (Fig. 2A). The vesicles of 2S fruit maintained a good juicy appearance, and the peel combined tightly with the pulp. In contrast, the vesicle of 2L fruit was shrunken, grayish in color and had almost no extractable juice, with the peel and pulp combined loosely. The vesicle collapse syndrome of the fruit was quantitatively analyzed using a vesicle collapse index and vesicle collapse rate. The result indicated that vesicle collapse index and vesicle collapse rate both increased with fruit transverse diameter. The vesicle collapse index of 2S fruit was only 0.07, which was significantly lower than the other groups (Fig. 2B). In the 2L fruit, almost all the fruit developed the vesicle collapse syndromes (vesicle collapse rate was 97.37%), which was more than 3 folds that of the 2S fruit (26.39%) (Fig. 2C). These results indicated a correlation between the fruit size and vesicle collapse in the postharvest storage of 'Shatangju' fruit.

Vesicle drying is a serious physiological disorder in citrus fruit, which diminishes the nutritional and taste quality of fruit (Wu, Pan, Guo, & Pan, 2014). In 'Shatangju' fruit, vesicle-drying juice sacs become dry, colorless, hardened and have little extractable juice, which is also termed as "vesicle collapse" (Zhang, Liu, et al., 2016; Zhang, Wang,

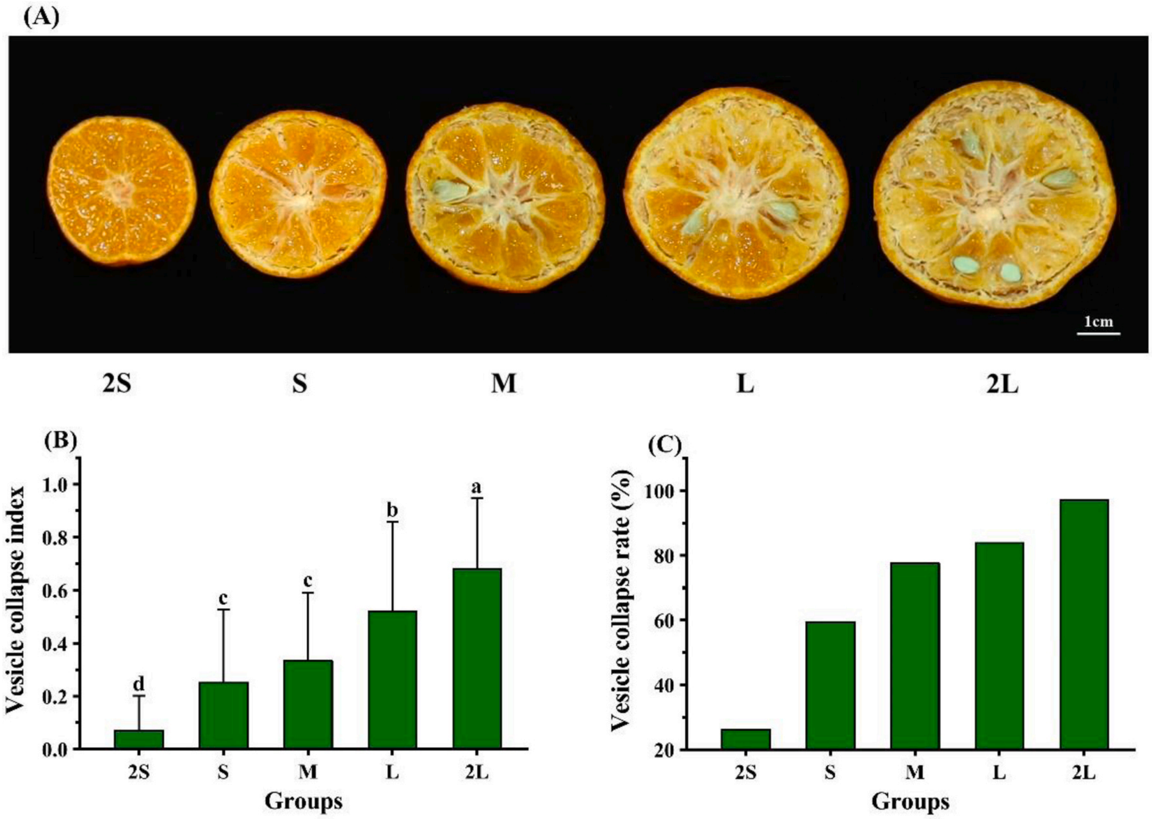

Fig. 2. The interior quality of 'Shatangju' fruit with different fruit sizes. (A) Inner appearance of fruit with different sizes; (B) Vesicle collapse index; (C) Vesicle collapse rate. Fruit were classified according to their transverse diameter: 2S (< 40 mm), S (40–45 mm), M (45–50 mm), L (50–55 mm) and 2L (55–65 mm). Data were represented as mean ± standard deviation of 50 replicates (n = 50). Different lowercase letters in the column represented a significant difference at $p < 0.05$ level by Tukey testing.

et al., 2016). Jin et al. (2015) observed that the vesicle drying was more common in the 'Ougan' fruit with a larger size. Wen et al. (2013) reported that the vesicle drying index increased during the storage of Satsuma mandarin, and the level was higher in the large fruit. These findings were in good consistence with our results.

3.3. The flesh components of fruit with different size

3.3.1. Water content and dry matter content

As shown in Figs. 3, 4A, the water content of 'Shatangju' fruit ranged from 77.57% to 80.73%. It is interesting that there was no significant difference on the proportion of water and dry matter among different groups ($p > 0.05$). The 2L fruit, which had the highest vesicle collapse index and vesicle collapse rate, had even slightly higher water content than that of other groups ($p > 0.05$). The highest proportion of dry matter was recorded in 2S fruit (22.43%) and the lowest in 2L fruit (19.27%). These results indicate that the vesicle collapse was not due to the water loss of the juice sacs.

Therefore, we could assume that the vesicle collapsed segments contained a lower water content. In previous studies, the juice yield of the vesicle dried tissue had a lower juice yield than the normal tissue, in almost all types of citrus fruit (Sharma, Singh, & Saxena, 2006; Yao, Cao, Xie, Deng, & Zeng, 2018; Yao et al., 2020). The water content in the juice sac also decreased with the development of granulation (Wu et al., 2020). However, Tan et al. (1985) found that the vesicle-drying juice sacs had a higher water content compared to the normal juice sacs in 'Ponkan' fruit, which coincided with our result. This indicated that vesicle collapse is a very complicated syndrome involving a series of physiochemical changes rather than simple water loss.

3.3.2. Soluble sugars, organic acids and amino acids content

Soluble sugars, organic acids and amino acids are important primary metabolites in citrus fruit, and also contributed to the taste quality (Cao et al., 2020). Glucose, fructose and sucrose are the main soluble sugars in 'Shatangju' fruit. Generally, the contents of glucose, fructose and sucrose in the 2S fruit were all higher than that in the 2L fruit, though in a mild extent ($p < 0.05$) (Fig. 3A). Citric acid and malic acid are the main organic acids in 'Shatangju' fruit. In this study, citric acid was only detected in the 2S fruit, with the content of 2.47 g/kg. Malic acid existed in the fruit and no significant difference was seen among different groups (Fig. 3B). The decline of organic acid had been considered as an important postharvest phenomenon affecting the taste quality and the senescence of citrus fruit, which was mainly due to the decline of citric acid (Hussain et al., 2017; Sun et al., 2013; Yun et al., 2010). Our previous study found that, the citric acid content in the 'Shatangju' fruit decreased rapidly while the malic acid content remained stable with the elongation of storage time. Among all the storage temperatures, 10 °C led to the highest decrease of citric acid (data unpublished). In addition, we previously found that the vesicle collapsed segments of 'Shatangju' fruit contained lower citric acid than the normal segments (Cao et al., 2020). In the present study, we found that the large fruit have higher vesicle collapse index than the small fruit, which was echoed to the significantly lower citric content in large fruit. The above information supported our results that the citric acid might not be detectable in the large size fruit after a long-term storage under 10 °C, and the 2S fruit maintained the best taste quality by maintaining considerable amount of citric acid.

Amino acids are important contributors for the taste of citrus fruit. A total of six amino acids were identified in the pulp of 'Shatangju' fruit. As shown in Fig. 3C, the amino acids in the 2S fruit were richer in species

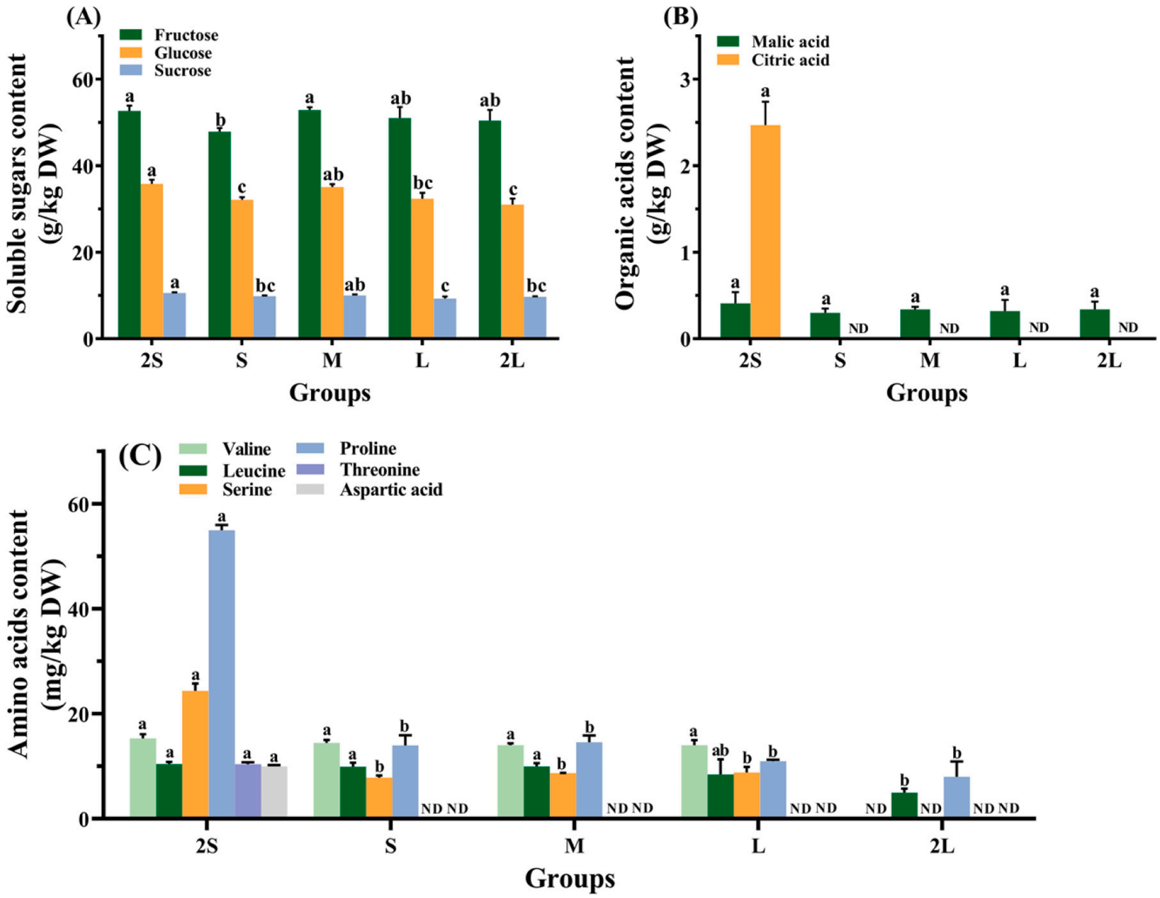

Fig. 3. Composition of soluble sugars (A), organic acids (B) and amino acids (C) in the segments from fruit of different sizes. Fruit were classified according to their transverse diameter: 2S (< 40 mm), S (40–45 mm), M (45–50 mm), L (50–55 mm) and 2L (55–65 mm). The contents of soluble sugars and organic acids were expressed as g/kg on a dry weight basis; The contents of amino acids were expressed as mg/kg on a dry weight basis. ND, not detected. Data were represented as mean ± standard deviation of three replicates ($n = 3$), with 15 fruit in each replicate. Different lowercase letters in the column of the same component represented a significant difference between different groups at $p < 0.05$ level by Tukey testing.

and higher in contents compared to the other groups. In the 2L fruit, only the leucine and proline were detected in the pulp. These results indicated that the large-size fruit contained less flavor components, especially organic acids and amino acids, than the small-size fruit after long-term storage, which led to the tasteless flavor.

There had been several studies on the relationship between fruit size and the sugars and acids contents in citrus fruit. Peng et al. (2020) used 'Shatian' pomelo as experimental material and found that small-size fruit had a significantly higher soluble solids content. Ketsa (1988) found that the content of total soluble solids had a negative correlation with fruit size in tangerine. In our present study, we found that the fruit with a large size tended to develop severe syndromes of vesicle drying after storage. According to the previous studies, the vesicle drying led to the decrease of soluble sugars and organic acids (Cao et al., 2020; Yao et al., 2018, 2020). Thus, it can be deduced that, the decrease of soluble sugars and organic acids might be at least partially due to the development of vesicle drying.

3.3.3. AIS, pectin, cellulose and lignin content

The insoluble components are important contributors to the texture quality of the fruit. The alcohol insoluble solids (AIS) in 'Shatangju' fruit mainly consisted of cell wall components, including water-soluble pectin, protopectin, cellulose, hemicellulose and lignin (Cao et al., 2020). In general, the AIS content in the dry matter of the pulp increased with the transverse diameter of 'Shatangju' fruit. The AIS content in 2S fruit (15.61%) was significantly lower than that of 2L (19.24%) ($p < 0.05$) (Fig. 4B). A similar pattern was found in cellulose and lignin content of 'Shatangju' fruit (Fig. 4E and G). However, there was no obvious difference in water-soluble pectin and protopectin content among different groups (Fig. 4C–D).

The accumulation of cellulose, hemicellulose and lignin plays a critical role in maintaining mechanical strength of plant cells, but is undesirable for the quality of citrus fruit (Shi et al., 2020). Previous studies have demonstrated that the content of cellulose and lignin increased with the vesicle drying level in citrus fruit (Wu et al., 2020; Zhang, Liu, et al., 2016; Zhang, Wang, et al., 2016). Thus, the lower cellulose and lignin content echoed the lower vesicle collapse level in small-size 'Shatangju' fruit.

3.4. PCA of fruit quality indexes

To discriminate the quality of 'Shatangju' fruit with different fruit size, a PCA was carried out based on the physiochemical indexes. The distribution of physiochemical indexes correlated to the fruit size can be visualized in a PCA plot. As shown in Fig. 5A, 'Shatangju' fruit with similar transverse diameters were clustered together. The scores plot showed the variance contribution rate of 47.7% for the first principal component (PC1) and 29.7% for the second principal component (PC2) (Fig. 5A). The results indicated that the general physiochemical characteristics varied among 'Shatangju' fruit depending on their transverse diameters. 'Shatangju' fruit with a larger transverse diameter (2L and L) were located on the negative side of the PC1 (Fig. 5A), indicating the

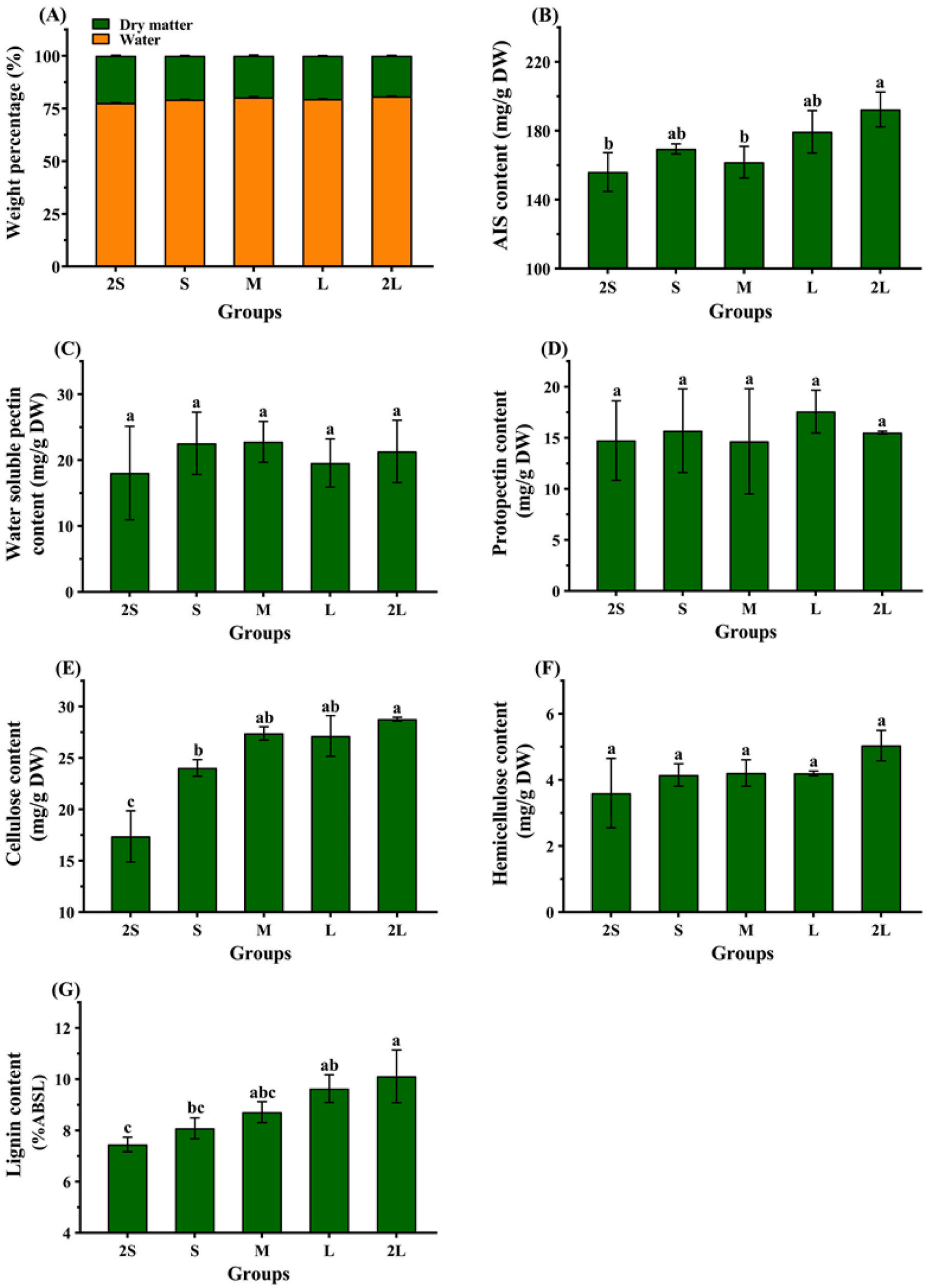

Fig. 4. Contents of dry matter and cell wall components in the segments from fruit of different sizes. (A) Weight percentage of water and dry matter; (B) Alcohol insoluble solids (AIS); (C) Water soluble pectin; (D) Protopectin; (E) Cellulose; (F) Hemicellulose; (G) Lignin. Fruit were classified according to their transverse diameter: 2S (< 40 mm), S (40–45 mm), M (45–50 mm), L (50–55 mm) and 2L (55–65 mm). The contents of AIS, water soluble pectin, protopectin, cellulose and hemicellulose were expressed as mg/g on a dry weight basis; The content of lignin was expressed as %ABSL on a dry weight basis. Data were represented as mean ± standard deviation of three replicates (n = 3), with 15 fruit in each replicate. Different lowercase letters in the column represented a significant difference at $p < 0.05$ level by Tukey testing.

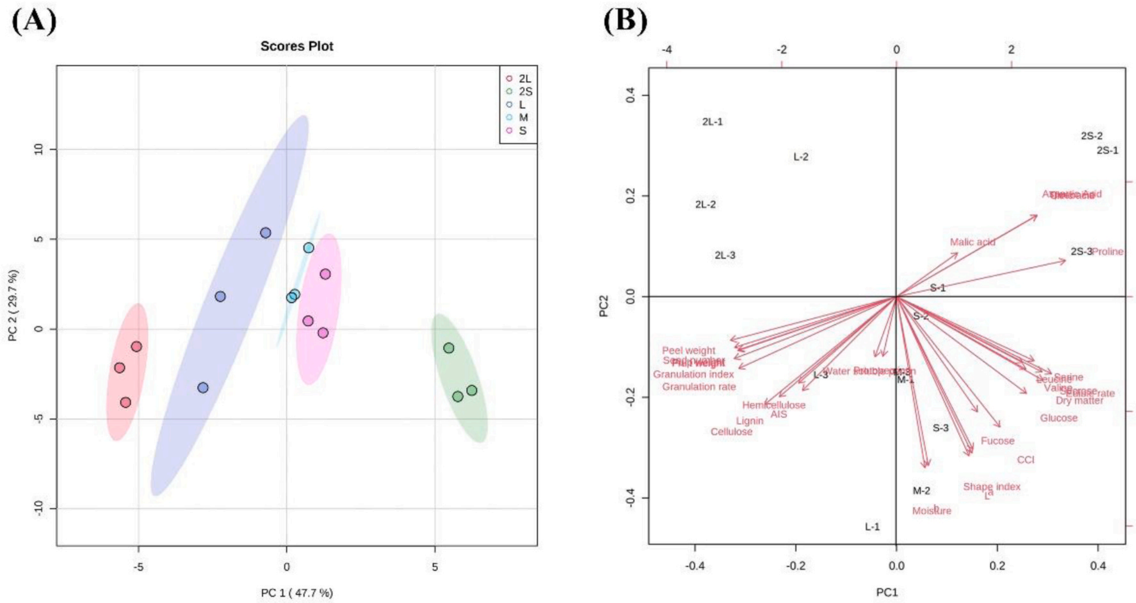

Fig. 5. Principle component analysis of fruit quality indexes (A) Scores plot; (B) Biplot. Fruit were classified according to their transverse diameter: 2S (< 40 mm), S (40–45 mm), M (45–50 mm), L (50–55 mm) and 2L (55–65 mm).

higher contents of cellulose and lignin, higher vesicle collapse level, more seeds and a higher fruit weight but lower edible rate, which also had very high negative loads on PC1 (Fig. 5B, Table S2). In contrast, 'Shatangju' fruit with a smaller transverse diameter (2S, S and M) were located on the positive side of the PC1 (Fig. 5A), indicating the higher content of proline, organic acids, sucrose, dry matter and higher edible rate, which were also located on the positive side of the PC1. They also had less seeds, lower contents of cellulose and lignin, and lower vesicle collapse level, which were located on the negative side of the PC1 (Fig. 5B, Table S2).

From the above results, we get a general understanding on the relationship between the fruit size and the physiochemical characteristics of 'Shatangju' fruit after the long-term storage. Most notably were the relationships among the fruit size, the seed numbers, and proportion of the peel and the vesicle collapse incidence, the underlying mechanisms of which are worthy to be studied further. Furthermore, during the growth of 'Shatangju' fruit, reasonable management of fertilization, irrigation, fruit thinning and girdling also should be put forward in order to decrease the proportion of large fruit.

In addition, this study indicated that the fruit quality and shelf life of 'Shatangju' fruit varied depending on the fruit size, which were largely affected by the vesicle collapse incidence. According to our results, large-size fruit became almost inedible after 150 d storage, possibly due to the high level of vesicle collapse rate. In contrast, small-size fruit had a significantly lower vesicle collapse rate, which resulted in a better overall quality in the later storage period, indicating a longer shelf life in the postharvest storage. Hence, selecting appropriate fruit is an effective strategy for extending the shelf life of 'Shatangju' fruit. In order to reduce losses and maximize economic benefits in the production of 'Shatangju', the large fruit which is unsuitable to be stored is suggested to be sold at early period, while small-size fruit can be selected for long-term storage. Thus, classification of 'Shatangju' fruit basing on the fruit size is very important for the postharvest quality maintenance and marketing of this fruit.

4. Conclusion

'Shatangju' fruit varied greatly in size, which affected its fruit quality and shelf life. The fruit with smaller transverse diameter tends to have better fruit quality and longer shelf life, in that the 'Shatangju' fruit with small transverse diameter had a lower vesicle collapse incidence. In the large size fruit, along with the event of vesicle collapse, the contents of cellulose, hemicellulose and lignin increased, and the contents of soluble sugars, organic acids and amino acids content decreased, and thus led to the bad texture and tasteless flavor. The study indicated that the storability and shelf life of 'Shatangju' fruit varied among different fruit sizes and the fruit size classification was very important for the post-harvest quality maintenance and marketing of this fruit. We also found that the 'Shatangju' fruit size was correlated with the seed numbers, the peel proportion and the vesicle collapse incidence, and the underlying mechanisms on the relationship among these features are worthy to be studied further.

CRediT authorship contribution statement

Chen Kang: Data curation, Visualization, Writing – original draft. **Jinping Cao:** Methodology, Writing – review & editing. **Jun Sun:** Resources, Formal analysis. **Guixia Zheng:** Resources. **Yue Wang:** Conceptualization, Investigation. **Kunsong Chen:** Validation, Project administration. **Chongde Sun:** Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2021.100774.

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