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Physiochemical changes in *Citrus reticulata* cv. Shatangju fruit during vesicle collapse

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ABSTRACT

'Shatangju' (*Citrus reticulate*) is a popular mandarin that easily develop vesicle drying, which is one of the most common internal physiological disorders of citrus fruit. The vesicle drying occurred firstly in the peel-side of the segment of 'Shatangju' fruit, and then the juice sac shrank gradually with the development of the syndrome, which finally resulted in the collapse and hollowing of the segments. The incidence of vesicle collapse increased with the storage temperature and the seed number. After storage at 10 °C and 20 °C for 90 d, over 80 % of the fruit developed vesicle collapse syndromes. The physiochemical characteristics of healthy segments and the vesicle collapsed segments were subsequently compared in detail. The results showed that, the physiochemical changes of 'Shatangju' fruit during vesicle collapse were characterized by the increase of H_2O_2, MDA, flavonoids and cell wall compounds, and by the decrease of carotenoids, ethanol, ethyl acetate, D-limonene and β-myrcene, as well as by the modest decrease of sugars and acids. In addition, the contents of amino acids in the vesicle collapsed segment changed slightly. Taken together, the results indicated that the vesicle collapse of 'Shatangju' fruit involved in the changes of primary metabolism, secondary metabolism as well as the peroxidation of the tissue. Present study provided detailed information about the physiochemical alterations occurring in the vesicle collapsed segment of 'Shatangju' mandarin.

1. Introduction

Citrus are globally consumed fruit with great popularity, but easily suffer from various physiological disorders, such as vesicle drying. The pulp of citrus fruit infected by vesicle drying usually tastes off, while the appearance of fruit, especially the peel, seems to be normal. Thus, it is difficult to distinguish the quality of citrus fruits through sensory evaluation.

The vesicle drying is also called segment drying, granulation, crystallization, scorification and dryness. The symptoms of drying vesicle vary among different citrus species. In the pomelo, orange, grapefruit and some mandarin fruit, the drying vesicle becomes enlarged, hardened and light gray in color, which is most commonly termed as "granulation" (Kong et al., 2010). In some mandarin fruit, the drying vesicle becomes soft and shrunken instead of hardened and enlarged, which is termed as "vesicle collapse". Some mandarin fruit develop both granulation and vesicle collapse (Kong et al., 2010).

So far, the characteristics of granulation have been reported in several citrus species, including pomelo, orange, grapefruit and some mandarin fruit. The granulated fruit showed a decrease of TSS, acidity, juice content, and phenolics content (Sharma et al., 2016, 2006), while an increase of respiration rate (Hwang et al., 1990), hydrogen peroxide (H_2O_2), malonaldehyde (MDA), and cell wall components (She et al., 2009). The sugar and acid metabolism pathways are reported to be involved in the granulation (Wang et al., 2014; Yao et al., 2018a). The hardening of juice sac is due to the change of cell wall components (She et al., 2008). The development of granulation can be affected by several factors, including cultivar (Sharma et al., 2006), imbalance of trace elements level in the tissue (Goto, 1989; Manchanda et al., 1972; Wang et al., 2014; Zhang et al., 2019), tree-age (She et al., 2008), root-stock (Sharma and Saxena, 2004), and postharvest temperature (Hu et al., 2015a). However, the causes and the underlying mechanisms for granulation remain obscure.

Compared with the granulation, vesicle collapse received much less attention. There is cultivar variation in this two vesicle drying types, in that granulation is commonly seen in the pomelo, orange and

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grapefruit, while vesicle collapse is more commonly seen in the mandarin. However, in some mandarin cultivars, both types are existing (Kong et al., 2010). The symptoms of vesicle collapse are different from granulation, in that the dried vesicle of the former is soft, and shrunken. Puffiness always accompanies with the vesicle collapse, with the peel separated from the pulp. Some researchers have compared the difference between granulation and vesicle collapse, and found that the vesicle collapsed fruit possessed a higher respiratory rate than the granulated fruit, which can be effectively reduced by the respiratory inhibitor Na_3PO_4 (Wang, 2005, 1997). Wang et al. (2005) found that the low humidity during the storage led to the vesicle collapse of the mandarin.

'Shatangju' is one of a variety of mandarins grown natively in the southern part of China, which is popular for its delicious taste and attractive appearance. In a pilot study, we found that 'Shatangju' fruit have a high incidence of vesicle collapse, especially after long-term storage. As a result, it can be regarded as a suitable material for investigation of vesicle collapse. In present study, we systematically evaluated the physiochemical changes of 'Shatangju' fruit during the vesicle collapse, aiming to determine out the characteristic physiochemical indexes that are closely correlated with the vesicle collapse, and thus setting foundation for the further study of vesicle collapse in mandarin.

2. Materials and methods

2.1. Materials

'Shatangju' mandarin fruit (*Citrus reticulate*) was harvested in December 2018 from the orchard of Guangzhou city, China. The fruit were stored in 0 °C, 6 °C, 8 °C, 10 °C and 20 °C (RH 90 %), respectively, with 200 fruit in each temperature. After storage for 90 d, the incidence of vesicle collapse under different temperatures was determined. The fruit stored in the 8 °C was used for the physiochemical analysis. The fruit with vesicle collapse symptoms in all the segments were regarded as the completely vesicle collapsed fruit (CVC), those with vesicle collapse symptoms in some of the segments was regarded as the partial vesicle collapsed fruit (PVC), and those without vesicle collapse symptoms were regarded as the healthy fruit (N). For the PVC fruit, the vesicle collapsed segments and healthy segments were collected separately and named as PVC' and PN' respectively. Three repetitions were conducted for each class, with the mixture of tissue from ten fruits as one repetition.

2.2. Distribution of lignin

The Weisner reagent test was employed to provide an overview about the distribution of lignin on the equatorial plane of segment. The schedule was described as follows. 'Shatangju' fruit were directly cut-through at the equatorial plane into halves with a hand knife. A few drops of 1 % phloroglucinol ethanol solution was poured on the equatorial plane of segments, following by adding drops of concentrated HCl to start the Wiesner reaction. Images of the lignin-stained segments were acquired immediately. Cryosection was conducted to obtain the microsections of juice sac in the native fresh state (Zhu et al., 2018). The juice sac was impregnated directly with the optimum cutting temperature (OCT) freeze medium, snap frozen in liquid nitrogen, and then were mounted onto the 'specimen disc' and sectioned to a thickness of about 40 μm by a microtome (Leica, Wetzlar, Germany). The temperature of the microtome chamber was kept at −20 °C. The prepared section was carefully put on a glass slide and observed under the optical microscope.

2.3. Qualification of alcohol insoluble solids (AIS), lignin, pectin and cellulose

The contents of AIS and lignin were determined according to Barnes and Anderson (2017) with slight modification. The grounded lyophilized pulp of 0.1 g was extracted subsequently with 1.5 mL of 95 % ethanol, 1.5 mL of chloroform / methanol mixture (v/v, 1/1) and 1.5 mL of acetone. The supernatant of each extraction was removed by centrifugation at 8000 *g* for 10 min. The final residue was obtained and dried in the oven at 65 °C for 12 h to obtain AIS and then weighted. Three repetitions were done for each sample. The AIS was washed twice with 1 mL of 90 % DMSO by oscillating for 12 h to remove the starch. The residue was obtained by centrifugation and then washed with 1 mL of 70 % ethanol for six times, followed by washing with 1.5 mL of acetone. The residue was dried in the oven at 65 °C for 12 h and used for the lignin qualification by the acetyl bromide soluble lignin (ABSL) assay. Three repetitions were done for each sample.

The extraction of pectin and cellulose was carried out according to Dietz and Rouse (1953) and Rose et al. (1998) with slight modification. The grounded lyophilized pulp of 1 g was extracted with 25 mL of 95 % ethanol at 100 °C water bath for three times, 30 min each time. The supernatant was removed by centrifugation at 8000 *g* for 15 min. The residue was extracted with 20 mL H_2O at 50 °C water bath for 30 min, the supernatant was collected by centrifugation at 8000 *g* for 5 min. Repeated the extraction one more time, and combined the supernatant to obtain water soluble pectin. The residue was extracted with 25 mL of 0.5 mol L^{-1} H_2SO_4 for 10 min at 85 °C water bath, followed by centrifugation at 8000 *g*, the supernatant was collected to obtain protopectin. The residue was washed with H_2O for three times, and dried in oven to obtain cellulose and weighted. The content of water-soluble pectin and protopectin was determined using hydroxybiphenyl method as described by Blumenkrantz and Asboe-Hansen (1973). Three repetitions were conducted for each sample.

2.4. H_2O_2 and MDA content detection

The H_2O_2 content in the tissue was detected according to the instruction of the H_2O_2 qualification kit (Comin Biotechnology Co. Ltd., Suzhou, China). In brief, the grounded fresh sample of 0.1 g was ultrasonically extracted at 4 °C with 1 mL of pre-cooled acetone for 15 min. The supernatant was collected after centrifugation at 8000 *g* for 10 min under 4 °C. Three repetitions were conducted for each sample. For H_2O_2 detection, 200 μL of the supernatant was obtained, and added subsequently with 20 μL of solution II and 40 μL of solution III, then centrifugated at 8000 *g* for 10 min at 25 °C. The precipitate was dissolved in 200 μL of solution IV. The absorbance of the solution was detected at 415 nm. The content of H_2O_2 was calculated according to the standard curve.

The MDA content in the tissue was detected according to the instruction of the MDA qualification kit (Comin Biotechnology Co. Ltd., Suzhou, China). Three repetitions were conducted for each sample. The content of MDA was calculated according to the standard curve.

2.5. Soluble sugars, organic acids and amino acids composition

The contents of soluble sugars, organic acids and amino acids were evaluated using GC–MS method as previously described by Lin et al. (2015) with modification. For the sample extraction, 0.1 g of the grounded lyophilized sample was extracted with 1.4 mL of methanol by vortexed at 70 °C for 15 min. The supernatant was collected by centrifugation at 8000 *g* for 10 min. Then, 1.5 mL of H_2O and 750 μL of chloroform were added into the supernatant and vortexed. The mixture was centrifuged at 8000 *g* for 10 min, and 1 mL of upper layer liquid was collected and stored in -40 °C for later use. Three repetitions were conducted for each sample. For the derivation, aliquots of 100 μL upper phase were obtained, added with 10 μL ribitol (0.2 g L^{-1}) as internal

standard, and dried in centrifugal vacuum evaporator at 30 °C. The residue was dissolved in 40 μL of 20 g L^{-1} pyridine methoxyamine hydrochloride, and incubated for 1.5 h at 37 °C. The sample was then mixed with 40 μL of BSTFA reagent (containing 1 % trimethylchlorosilane (TCMS)) and incubated at 37 °C for 30 min.

The GC–MS analysis was carried out in an Agilent 7980B gas chromatograph instrument coupled with an Agilent 7000C Network Mass Selective Detector (Agilent J & W, Folsom, CA, USA). Compounds were separated using a HP-5 ms column (5 % phenyl methyl siloxane, 30 m × 0.25 mm i.d., 0.25 μm, Agilent J & W, Folsom, CA, USA), with helium as carrier gas at a flow rate of 1.0 mL min^{-1}. One microliter of each sample was injected at a 10:1 split ratio. The injector temperature was 250 °C. The temperature program for the compounds separation was as following: 100 °C for 1 min, ramped to 184 °C at 3 °C min^{-1}, then ramped to 190 °C at 0.5 °C min^{-1}, held for 1 min, then ramped to 250 °C at 10 °C min^{-1}, held for 1 min, then increased to 280 °C at 5 °C min^{-1} and held for 3 min. The soluble sugars, organic acids and amino acids contents were calculated according to the standard curves of authorized compounds.

2.6. Carotenoids composition

The carotenoids were detected using HPLC methods established by Xu et al. (2006). In brief, 0.1 g of grounded lyophilized pulp was added subsequently with 350 μL of methanol, 700 μL of chloroform and 350 μL of H_2O. The lower phase containing carotenoids was collected by centrifugation at 8000 *g* for 10 min. The upper phase was extracted with chloroform for two more times. The chloroform phase of three extractions was combined and the solvent was removed by vacuum evaporation. The residue reacted with 350 μL of methanol containing 6 % KOH in the dark at 60 °C for 30 min, followed by extracting with the mixture of 700 μL chloroform and 350 μL H_2O. The chloroform phase was collected by centrifugation at 8000 *g* for 10 min, and then extracted repeatedly with 700 μL ddH_2O until the aqueous phase become neutral. The solvent in the chloroform phase was removed by vacuum centrifuge evaporation. The residue was dissolved with 100 μL ethyl acetate. Three repetitions were conducted for each sample. HPLC analysis was carried out in a Waters Alliance system (2695 pump, 2996 diode array detector; Waters, Milford, MA, USA) coupled with a C30 column (250 mm × 4.6 mm i.d., 5 μm), with column temperature of 25 °C, flow rate of 1 mL min^{-1}, and injection volume of 20 μL. The elution was carried out using methanol (A), 80 % methanol containing 0.2 % ammonium acetate (B) and methyl tertiary butyl ether (C) as mobile phase, with gradient program as follows: 0 − 6 min, 95 % of A and 5 % of B; 6 − 7 min, ramping to 80 % A, 5% B and 15 % C; 7 − 12 min, 80 % A, 5 % B and 15 % C; 12 − 32 min, ramping to 30 % A, 5 % B and 65 % C; 32 − 48 min, 30 % A, 5 % B and 65 % C; 48 − 50 min, ramping to 95 % A and 5 % B; 50 − 60 min, 95 % A and 5 % B. The ζ-carotene was detected at 400 nm, and the other compounds were detected at 450 nm. The compounds were calculated according to the standard curves of authorized compounds.

2.7. Flavonoids composition

The grounded lyophilized sample of 1 g was extracted with 20 mL of methanol for three times. The supernatants of three extractions were combined for the flavonoid composition analysis. Three repetitions were conducted for each sample. The flavonoid compounds were analyzed using UPLC-DAD assay (2695 pump, 2996 diode array detector; Waters, Milford, MA, USA) coupled with Acquity UPLC ® HSS R3 Column (100 mm × 2.1 mm i.d., 1.8 μm, Waters), by wavelength scanning from 200 nm to 400 nm. The elution was carried out using water (A) and acetonitrile (B) as mobile phase, with a flow rate of 0.4 mL min^{-1} and column temperature of 25 °C. The gradient program was as follows: 0–0.5 min, 2–20 % of B; 0.5–6.5 min, 20–28 % of B; 6.5 − 9 min, 28–50 % of B; 9 − 14 min, 50–98 % of B; 14 − 15 min, 98-2 % of B; 15 − 16 min, 2 % of B. The flavonoid contents were calculated according to the relevant standard curves of authorized compounds under 280 nm.

2.8. Volatile compounds

The volatile compounds were detected using static headspace extraction and GC–MS. The grounded fresh sample of 3 g was mixed with 4 mL of saturated NaCl solution. The mixture of 3 mL was put into the 10 mL headspace extraction bottles, added with 10 μL of 1-hexanol (0.1 %, v/v) as internal control, and heated at 85 °C for 30 min in an Agilent 7697A automatic headspace sampler (Agilent J & W, Folsom, CA, USA). Three repetitions were conducted for each sample. One milliliter of the gas was extracted from the head space of the bottle for the GC–MS analysis. Volatile contents were determined with an Agilent 7980B gas chromatograph instrument (Agilent J & W, Folsom, CA, USA) coupled to an Agilent 5977C Network Mass Selective Detector (Agilent J & W, Folsom, CA, USA). Compounds were separated using a HP-5 ms column (5 % phenyl methyl siloxane, 30 m × 0.25 mm i.d., 0.25 μm, Agilent J & W, Folsom, CA, USA), with helium as carrier gas at a flow rate of 1.0 mL min^{-1}. The oven temperature was programmed to start at 35 °C for 3 min, and then ramped to 40 °C at a rate of 1 °C min^{-1}, held for 2 min, followed by a second ramp to 100 °C at a rate of 5 °C min^{-1}, and a third ramp to 200 °C at a rate of 10 °C min^{-1}. MS scan range was 20–350 mass units. Volatiles were identified based on the database of the NIST/EPA/NIH Mass Spectral Library (http://chemdata.nist.gov/). The identities of the volatiles were then confirmed by comparison with authentic standards. The internal standards were used for compensating for differences between samples, and the abundance of each volatile was calculated as its peak area.

2.9. Statistics

The data were statistically assessed using SPSS version 20.0. Statistical significance of differences was calculated using one-way ANOVA. Comparisons between the groups were performed using Student's *t*-test or Tukey method. Principal component analysis (PCA) was carried out online (https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml), with the data normalized by scaling (mean-centered and divided by the standard deviation of each variable).

3. Results

3.1. Effect of the storage temperature and seed number on the vesicle collapse

The vesicle collapse happened initially in part of the segments, and the symptoms occurred firstly in the peel-side of the segment (Fig. 1A). At the early stage, the transparency of the juice vesicle decreased, and then the juice sac shrank gradually with the development of the syndrome, which finally led to the shrinking and hollowing of the segments (Fig. 1B, 1C, 1D). The process and symptoms were different from the granulation that has been reported. In pomelo, the granulation is most frequently observed initially in the middle and stylar end sections of the fruit segment (Lacerna et al., 2018), where the juice vesicle becomes enlarged, hard and non-transparent (Wu et al., 2014). In the ponkan fruit, the granulation starts from the stem end and then spreads to the stylar end section of the fruit segment, in which the segment becomes shriveled and grayish in color, and the disordered juice vesicles are granulated and become hard, dry and cloudy white in color (Yao et al., 2018a). Thus, the vesicle drying process in 'Shatangju' fruit is different from pomelo or ponkan fruit, for the reason that it starts from the peel-side of the segment, with obvious shrinkage of juice sac. The similarity was that, the occurrence of the vesicle collapse sacs is not visible from the outside. Therefore, it is difficult to separate the disordered fruit

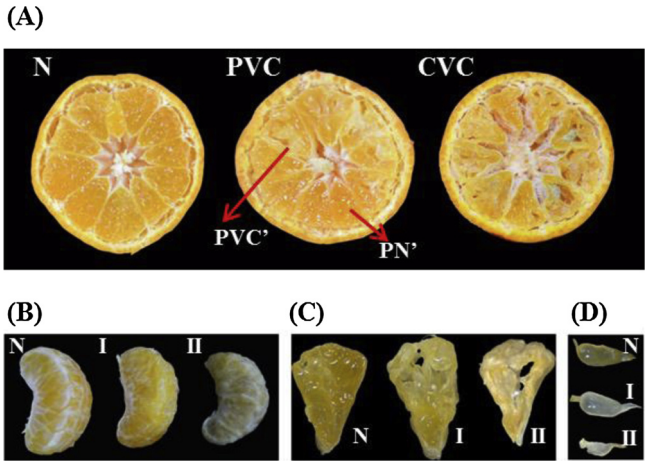

Fig. 1. . 'Shatangju' fruit: (A) Transection of the fruit with different degrees of vesicle collapse (N, PVC and CVC); (B) Segments of the fruit with different degrees of vesicle collapse (N, I and II); (C) Transection of the segments with different degrees of vesicle collapse (N, I and II); (D) Juice vesicle with different degrees of vesicle collapse (N, I and II). N, healthy fruit; PVC, partial vesicle collapsed fruit; PN', healthy segments in the partial vesicle collapsed fruit; PVC', vesicle collapsed segments in the partially vesicle collapsed fruit; CVC, completely vesicle collapsed fruit; The vesicle drying was more severe in II than in I.

from the healthy ones until the peel is removed.

We compared the incidence of vesicle collapse in 'Shatangju' fruit under different storage temperatures. The results showed that, the higher storage temperature led to higher incidence of vesicle collapse. After storage at the temperature above 10 °C for 90 d, the incidence of vesicle collapse was more than 80 % compared to the incidence at 6 °C/8 °C (lower than 40 %) and 0 °C (5.7 %), respectively (Fig. 2A). Yao et al. (2018a) found that a proportion of ponkan fruit developed the granulation after four months of storage at 10 °C. Hu et al. (2015) found that 10 °C could effectively alleviate the granulation and extend the storage life of Shatianyou pomelo than that of 20 °C in the long-term storage. Thus, low storage temperature had been proved to be effective in preventing citrus granulation. Our results indicated that storage temperature was also an important factor for vesicle collapse of 'Shatangju' fruit. The storage temperature higher than 10 °C led to higher vesicle collapse incidence, and storage temperatures of 6 °C and 8 °C were much superior in alleviating vesicle collapse.

We also compared the vesicle collapse incidence between the segments containing seeds and those without seeds. The vesicle collapse incidence was 78 % in the segments containing seeds, while the incidence was only 49 % in the seedless segments (Fig. 2B).

3.2. Physiochemical changes of 'Shatangju' fruit during the vesicle collapse

The pulps from fruits of different vesicle collapse stages (N, PN', PVC', PVC) were collected respectively. The physiochemical characteristics of different tissues were listed in Table 1.

3.2.1. Sugars, acids and amino acids

The sugars, acids and amino acids are the primary metabolites that are necessary for maintaining the basic functions of fruit, and also are important contributors for the taste. Generally, the contents of sugars and acids tend to decrease in the vesicle collapse segments, though in a mild extent. Four sugars (sucrose, fructose, glucose and sorbitol) were identified in the pulp. Among the four sugars, only glucose showed steep decrease in the collapsed-vesicle (19.41 % decrease in PVC compared to N, and 21.51 % decrease in PVC' compared to PN'). However, the contents of sucrose, sorbitol and fructose in the collapsed-vesicle were similar to those in the healthy tissue. Two organic acids were identified, with the citric acid being the major organic acid. The organic acids showed a trend of decrease in the collapsed-vesicle, with the decrease of citric acid (13.61 % decrease in CVC compared to N, 12.83 % decrease in PVC' compared to PN') more significant than that of the malic acid. Ten amino acids were identified. Generally, the amino acids contents showed small change in the vesicle collapse segments (Table 1). In previous studies, the decrease of sugars and acids was one of the characteristic symptoms of the segment drying (Sharma et al., 2006). One study reported the nutrient translocation from the pulp to the peel (Chen et al., 1996), and it is hypothesized that the sugars and acids were consumed to provide substance and energy for the synthesis of cell wall compounds (Yao et al., 2018a). In addition, the vesicle collapsed or granulated fruit were found to have higher respiration rate. Thus, a respiration-based consumption might also be the reason for the decrease of sugars and acids (Wang, 2005, 1997).

3.2.2. Carotenoids

The carotenoids are important secondary metabolites giving the appealing yellow color of 'Shatangju' fruit. A total of 14 carotenoids compounds were detected, in which 7 compounds were identified while the other 7 compounds were not (Fig. S1). The 9-cis-violaxanthin was the main carotenoid in the pulp of 'Shatangju' fruit, accounting for 22–32 % of the total carotenoids. Most of the individual carotenoids components were decreased in the collapsed segments (Table 1), resulting in the paler looks of the vesicle collapsed segments compared to healthy tissue. The carotenoids are the chromoplast contents. Therefore, the decrease of carotenoids might suggest the cellular organelle disintegration of collapsed vesicle.

3.2.3. Volatiles

Like other citrus fruit, 'Shatangju' fruit had its characteristic pleasant smell, which was contributed by the volatiles. By the headspace

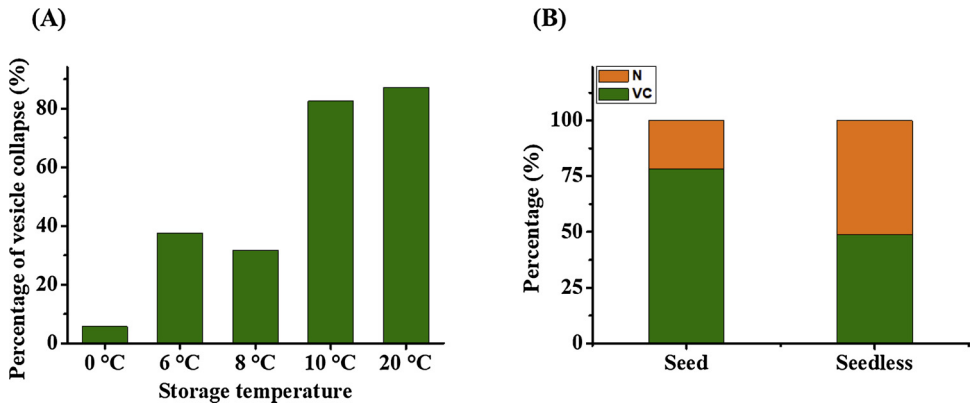

Fig. 2. Effect of the storage temperature (A) and seed number (B) on the vesicle collapse. N, healthy segment; VC, vesicle collapsed segment.

Table 1
The contents of nutrients and flavor compounds in the healthy and vesicle collapsed segment.

	N	CVC	PVN'	PVC'
Sugars (g kg^{-1})				
Fructose	47.47 ± 3.46 a	49.11 ± 9.05 a	44.52 ± 1.76 a	39.36 ± 2.73 a
Glucose	32.81 ± 4.32 a	26.44 ± 6.52 a	30.31 ± 2.08 a	23.79 ± 3.40 a
Sucrose	179.35 ± 9.43 a	154.74 ± 9.54 b	181.79 ± 3.98 a	181.51 ± 2.71 a
Sorbitol	0.19 ± 0.16 a	0.28 ± 0.06 a	0.17 ± 0.08 a	0.13 ± 0.04 a
Acids (g kg^{-1})				
Malic acid	0.95 ± 0.08 a	0.94 ± 0.06 a	0.97 ± 0.05 a	0.86 ± 0.06 a
Citric acid	6.44 ± 0.16 a	5.56 ± 0.14 a	6.55 ± 0.70 a	5.71 ± 0.28 a
Amino acids (g kg^{-1})				
Alanine	0.28 ± 0.02 a	0.28 ± 0.01 a	0.28 ± 0.02 a	0.25 ± 0.03 a
Valine	0.23 ± 0.02 a	0.24 ± 0.03 a	0.24 ± 0.02 a	0.26 ± 0.03 a
Leucine	0.20 ± 0.01 a	0.19 ± 0.00 a	0.20 ± 0.01 a	0.21 ± 0.01 a
Proline	0.91 ± 0.08 a	1.01 ± 0.17 a	0.83 ± 0.30 a	0.84 ± 0.27 a
Isoleucine	0.21 ± 0.01 b	0.21 ± 0.00 ab	0.21 ± 0.01 ab	0.23 ± 0.02 a
Glycine	0.23 ± 0.01 ab	0.21 ± 0.00 b	0.22 ± 0.01 ab	0.24 ± 0.01 a
Serine	0.30 ± 0.02 a	0.26 ± 0.01 a	0.30 ± 0.04 a	0.31 ± 0.04 a
Threonine	0.19 ± 0.00 a	0.19 ± 0.00 a	0.19 ± 0.01 a	0.19 ± 0.01 a
Aspartic acid	0.23 ± 0.01 a	0.25 ± 0.01 a	0.23 ± 0.02 a	0.26 ± 0.02 a
Glutamic acid	0.89 ± 0.01 a	0.90 ± 0.06 a	0.88 ± 0.05 a	0.86 ± 0.06 a
Carotenoids (mg kg^{-1})				
Violaxanthin	9.87 ± 1.98 a	8.48 ± 1.17 a	9.17 ± 2.74 a	6.39 ± 1.32 a
Luteoxanthin	12.03 ± 3.12 a	11.84 ± 1.25 a	11.91 ± 2.07 a	8.24 ± 1.54 a
9-cis-Violaxanthin	46.13 ± 8.25 a	44.44 ± 2.94 a	42.13 ± 10.20 ab	30.63 ± 3.88 b
Lutein	7.14 ± 2.69 a	5.84 ± 0.92 a	6.24 ± 0.61 a	2.49 ± 0.21 b
Zeaxanthin	9.37 ± 4.39 a	6.21 ± 3.28 a	8.50 ± 1.73 a	4.61 ± 1.36 a
β-cryptoxanthin	9.11 ± 2.86 a	10.05 ± 0.80 a	9.20 ± 3.00 a	5.79 ± 1.92 a
ζ-carotene	5.45 ± 3.02 a	4.87 ± 0.24 a	3.19 ± 1.30 a	3.19 ± 1.28 a
UK	71.78 ± 18.62 a	66.53 ± 8.36 ab	67.35 ± 11.74 ab	43.16 ± 8.31 b
Volatiles (mg kg^{-1})				
Ethanol	0.28 ± 0.04 a	0.18 ± 0.01 bc	0.24 ± 0.01 ab	0.18 ± 0.00 c
Ethyl acetate	9.53 ± 3.41 a	3.22 ± 0.69 b	5.01 ± 0.63 ab	2.41 ± 0.54 b
Hexanal	1.55 ± 0.40 b	3.17 ± 0.32 a	1.63 ± 0.26 b	1.74 ± 0.32 b
Pentanal	0.00 ± 0.00 b	0.34 ± 0.02 a	0.00 ± 0.00 b	0.35 ± 0.07 a
D-Limonene	124.24 ± 37.41 a	58.01 ± 12.42 b	63.01 ± 11.36 b	31.97 ± 13.17 b
β-Myrcene	2.38 ± 0.71 a	1.29 ± 0.33 ab	1.14 ± 0.65 ab	0.37 ± 0.07 b
α-Phellandrene	1.02 ± 0.28 ab	1.67 ± 0.50 a	0.50 ± 0.20 b	0.56 ± 0.13 b
γ-Terpinene	ND	0.27 ± 0.14 a	0.18 ± 0.10 a	0.20 ± 0.06 a
Flavonoids (mmol kg^{-1})				
Eriocitrin	0.24 ± 0.01 b	0.30 ± 0.04 b	0.24 ± 0.00 b	0.26 ± 0.02 b
Narirutin	1.23 ± 0.11 ab	1.51 ± 0.19 a	1.07 ± 0.12 b	1.32 ± 0.10 ab
Naringin	6.67 ± 0.28 a	8.52 ± 2.03 a	6.11 ± 0.71 a	7.29 ± 0.13 a
Didymin	0.93 ± 0.03 ab	1.10 ± 0.14 a	0.86 ± 0.03 b	1.00 ± 0.01 ab
Poncirin	0.39 ± 0.01 ab	0.43 ± 0.02 a	0.38 ± 0.02 b	0.40 ± 0.01 ab

extraction combining with GC–MS, a total of eight volatile compounds were identified in the pulp, including 4 monoterpenes. D-limonene was the most abundant volatile in the pulp, following by the β-myrcene and α-phellandrene and γ-terpinene. D-limonene and β-myrcene were decreased dramatically in the vesicle collapsed segments. The α-phellandrene was slightly increased in the vesicle collapsed segments, while γ-terpinene was detected only in the vesicle collapsed fruit (PN', PVC', CVC). Interestingly, ethanol and ethyl-acetate, which had been considered as the off-flavor compounds, were significantly decreased in the PVC' and CVC. However, the PN' had higher ethanol and ethyl-acetate content than N (Table 1). As ethanol and ethyl-acetate are the products of anaerobic respiration and lipid degradation (Tietel et al., 2011), it can be assumed that these bioproducts were elevated in the initiation of vesicle collapsing process and down-regulated at the later stage due to the gradual cell death. Further study is needed to understand the mechanism.

3.2.4. Flavonoids

Flavonoids are important secondary metabolites, which had been reported to be beneficial to human health (Yi et al., 2017), as well as involved in responses to environmental factors (Liu et al., 2019). By UPLC analysis, a total number of 5 major flavonoids were identified in the pulp of the 'Shatangju' fruit, and the sum of their peak areas accounted for more than 80 % of the total phenolics peak areas. Naringin was the most abundant flavonoid compound found in the pulp of Shatangju fruit, which was more than 65 % of the total phenolics (Fig. S2). The other 4 main flavonoid compounds were eriocitrin, narirutin, didymin and poncirin, respectively. All the flavonoids tended to increase in the vesicle collapsed segment, indicating that the secondary metabolism was elevated in the vesicle collapsed tissues (Table 1). To the best of our knowledge, this was the first report on the elevation of flavonoids contents in the dried segment.

3.2.5. AIS, lignin, pectin and cellulose content

The AIS in citrus included pectin, lignin, cellulose and hemi-cellulose, which are the cell wall components. According to our results, the vesicle collapsed segment had a higher AIS content than the healthy tissue. In addition, the contents of water-soluble pectin, protopectin, cellulose and lignin were also increased significantly in the vesicle collapsed segment (Fig. 3A-E). The in-situs staining using Weisner reagent showed the distribution of lignin in the segments. The results showed that the lignin was mainly distributed in the vesicles collapsed sections, which was located in the peel-side of the segment (Fig. 3F). The cryosection of dried juice sac showed light-purple staining in the

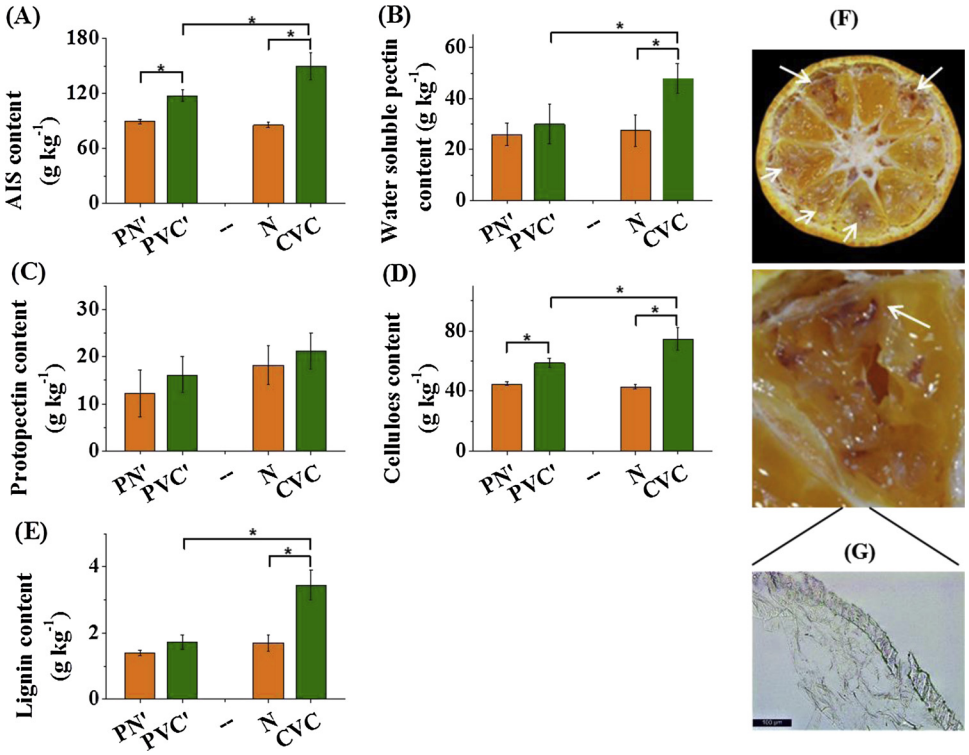

Fig. 3. Alcohol insoluble substance (AIS) and lignin contents in the healthy and vesicle collapsed segment: (A) AIS; (B) water soluble pectin; (C) protopectin; (D) cellulose; (E) lignin; (F) distribution of lignin in the segments; (G) distribution of lignin in the juice sac (purple stained area). Arrows showed the stained tissue. The contents were expressed as g kg^{-1} on a dry weight basis. Data were represented as mean ± standard deviation of three replicates (n = 3). * indicated significant difference at $p < 0.05$ level by Student's t test (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

cell wall area, indicating lignin accumulation (Fig. 3G). Lignin is the polymerized macromolecules by phenolics compounds, which is an important component of secondary cell wall. Previous study deduced that the sugars and acids were consumed to support the synthesis of the cell wall compounds (Yao et al., 2018a). Thus, the increment of cell wall compounds echoed the decrease of sugars and acids as well as the elevation of secondary metabolism.

3.2.6. H_2O_2 and MDA

The H_2O_2 is a signal molecule in the plant cell that could be induced under stress environment (Saxena et al., 2016). MDA is a product of lipid peroxidation. In the present study, the H_2O_2 and MDA levels were increased in the collapsed vesicle (Fig. 4), indicating that the peroxidation happened in the process of vesicle collapse. This phenomenon is similar to that of granulation in the pomelo (She et al., 2009; Xiong et al., 2017). The accumulation of H_2O_2 was also involved in the lignin synthesis (She et al., 2009). Thus, the increase of H_2O_2 echoes the increase of lignin content in the vesicle collapsed segments.

UK, unidentified; ND, not detected; the contents of sugars, acids, amino acids, flavonoids and carotenoids were expressed on a dry weight basis; the contents of volatiles were expressed on a fresh weight basis. Data were represented as mean ± standard deviation of three replicates (n = 3). Values with lowercase letters in the same row represented significantly difference at $p < 0.05$ level by Tukey testing.

3.3. Comprehensive evaluation on the physiochemical changes of collapsed vesicle and the characteristic compounds determination

Taking all the components into a comprehensive evaluation, the down-regulated compounds in the collapsed segment included the sugars, acids, carotenoids and most of the volatile compounds. The up-regulated compounds included the flavonoids, cell wall constituents, and the products of peroxidation reaction. Among the differential metabolites, great variation on the contents of lutein, ethyl acetate, D-limonene, β-myrcene and H_2O_2 were found between healthy and collapsed vesicle, with more than two-fold changes. In comparison, the changes of the primary compounds were relatively modest (Fig. 5).

PCA was applied to discriminate the tissues of different vesicle collapse level by compounds. The distribution of compounds affected by vesicle collapse can be visualized in a PCA plot. Fig. 6 showed the

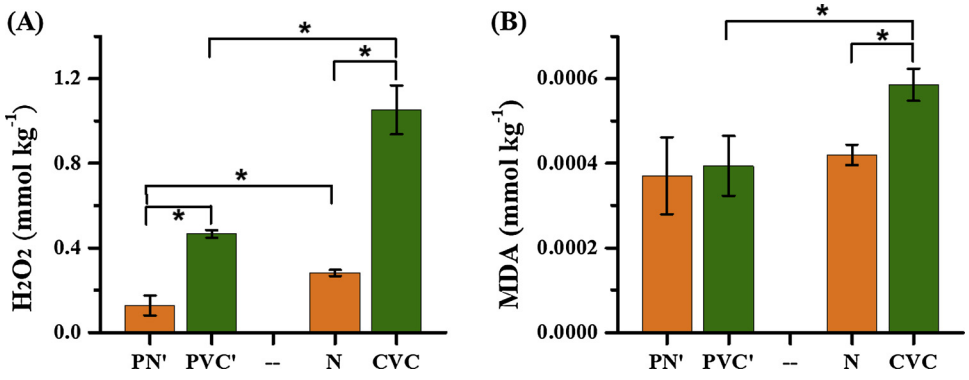

Fig. 4. Contents of H_2O_2 (A) and MDA (B) in the healthy and vesicle collapsed segment. The contents were expressed as mmol kg^{-1} on a fresh weight basis. Data were represented as mean ± standard deviation of three replicates (n = 3). * indicated significant difference at $p < 0.05$ level by Student's *t*-test.

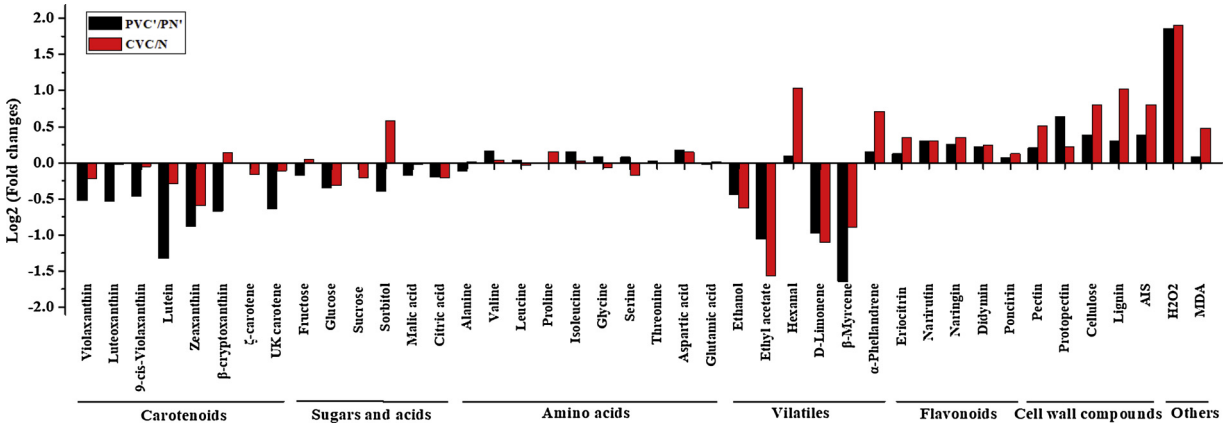

Fig. 5. The general pattern on the changes of compounds.

score bi-plot and loading of the first two principle components in 'Shatangju' pulp. The results confirmed that the compounds were separated between the collapsed and healthy tissue. The vesicle collapsed samples (PVC' and CVC) were located in the negative side of the PC1 (Fig. 6A), indicating the higher amounts of cell wall compounds, H_2O_2, MDA, flavonoids, lignin, and hexanol, which also had very high negative loading of PC1 (Fig. 6B, Table S1). The healthy samples were located in the positive side of the PC1 (Fig. 6A), indicating high in ethanol, ethyl acetate, limonene, carotenoids, and citric acid, which were also located in the positive side of the PC1 (Fig. 6B, Table S1). The healthy tissues from the normal fruit and partial vesicle collapsed fruit cannot be separated (Fig. 6A), indicating the similarity in the physiochemical characteristics between these two samples.

4. Discussion

Vesicle collapse is characterized by the decrease of taste, flavor and color substances (sugars, acids, monoterpenes and carotenoids), and the increase of cell wall compounds, flavonoids and peroxide metabolites in the pulp. The results indicated that both primary and secondary metabolisms were elevated in the collapsed vesicle. The quality changes were similar to that of the segment drying reported in the Ponkan fruit (Yao et al., 2018a, b), as well as similar to that of the granulation that had been reported in other citrus fruit (Hu et al., 2015b; Pan et al., 2013; She et al., 2008; Wu et al., 2014).

There are several theories on the segment drying, especially on the granulation of citrus fruits. Some studies concluded that the segment drying is due to the reallocation of nutrients, for example, ^{14}C-glucose in the pulp was transferred into the peel in the granulated fruit (Chen et al., 1996). While other studies reported that the segment drying was due to the excessive consumption of nutrients, with the evidence from the formation of cavity in the juice sec (Liu et al., 1988) and the increased respiration rate in the disordered fruit (Burns, 1990).

Plant hormones seem to be involved in the segment drying. In satsuma mandarin, the IAA level in the peel was higher and increased faster than that in the pulp, while the ABA level in the peel was lower than that in the pulp, indicating that the senescence of the peel is lagging behind that of the pulp (Chen et al., 1997). Plant growth regulator, such as exogenous spermidine, alleviates fruit granulation in a citrus cultivar 'Huangguogan' through the antioxidant pathway (Xiong et al., 2017). Competitive inhibitor of ethylene, 1-MCP does not influence the granulation of pomelo (Lacerna et al., 2018). The minerals such as calcium and boron are reported to be important factors affecting the segment drying (Zhang et al., 2019). The preservative containing Ca^{2+} significantly reduces the incidence of segment drying (Chen et al., 2005). However, the exact mechanisms are unknown.

One of the difficulties in studying the mechanism of segment drying of citrus fruit is that, it is difficult to track the course of disorder as the early symptoms cannot be observed without peeling the fruit (Fumuro et al., 2014; Theanjumpol et al., 2019). In fact, all the physiochemical changes found in present study as well as in most of the other studies are the end results of the physiological disorders (Sharma et al., 2016, 2006; She et al., 2009). There might be complex physiological changes that happened in the early stage of the segment drying process, which is

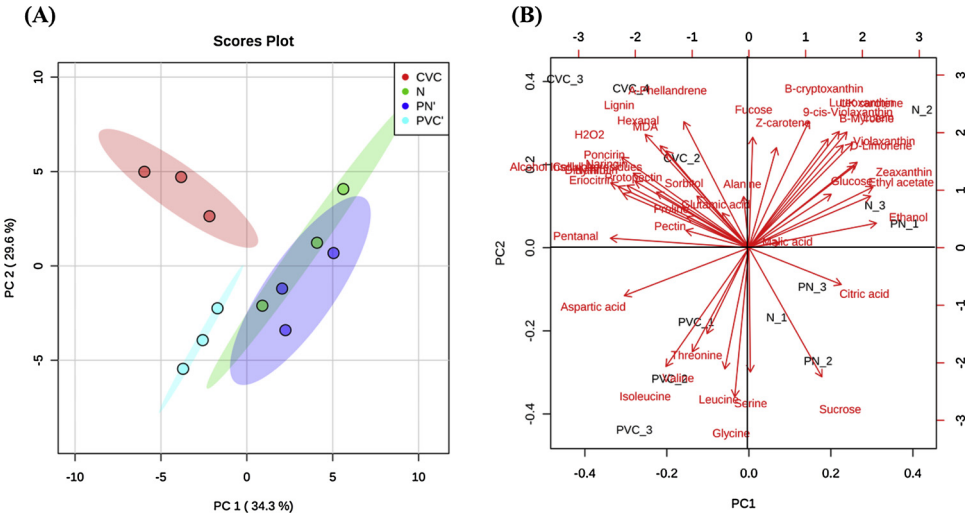

Fig. 6. PCA analyses of physiochemical indexes: (A) Scores plot; (B) Biplot.

important to understand the phenomenon. Thus, it is important to develop a model of vesicle collapse by finding appropriate materials, or by exogenous inductions. In addition, it is also important to establish a sensitive non-destructive inspection method to track the development process of vesicle collapse.

It is really interesting to find the relationship between the storage temperature and the incidence of vesicle collapse in Shatangju fruit. In the former studies, we also stored the satsuma mandarin and ponkan fruit at a relatively higher temperature (> 10 °C), however, the vesicle collapse was not necessarily occurred (data not shown). Thus, 'Shatangju' fruit was more susceptible to the segment drying compared to other citrus fruit, and is useful to study vesicle collapse. By storing the 'Shatangju' fruit at a relatively higher temperature, it might be possible for us to track the dynamic changes of vesicle collapse processes.

5. Conclusion

'Shatangju' fruit was sensitive to vesicle drying, with the incidence of vesicle collapse being higher than 80 % after storage at 10 °C and 20 °C for 90 d. The vesicle drying in 'Shatangju' fruit occurred firstly in the peel-side of the segment, then the juice sac shrank gradually with the development of the syndrome, which finally led to the collapse and hollowing of the segments. The vesicle collapse of 'Shatangju' fruit was characterized by the decrease of carotenoids, flavor compounds, sugars and acids contents, and the increase of flavonoids, lignin, pectin, and cellulose contents, as well as the accumulation of H_2O_2 and MDA contents. These changes indicated the degradation of the primary metabolites, and the synthesis of the secondary metabolites, as well as the peroxidation happening in the vesicle collapsed tissue. In conclusion, this study provides important perspectives of the physiochemical characteristics occurring in the vesicle collapsed segment of 'Shatangju' mandarin, and illustrates that this fruit was a suitable material for studying the dynamic changes of vesicle collapse.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2020.111180.

References

Barnes, W.J., Anderson, C.T., 2017. Acetyl bromide soluble lignin (ABSL) assay for total lignin quantification from plant biomass. BioProtocol 7.

Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54, 484–489.

Burns, J.K., 1990. Respiratory rates and glycosidase activities of juice vesicles associated with section-drying in citrus. Hortscience 25, 544–546.

Chen, K.S., Zhang, S.L., Ye, Q.F., Li, F., Bei, Z.M., Ye, X.Y., 1996. Studies on the mechanism of postharvest granulation and its control of' Huyou' fruit. Journal of Basic Science and Engineering 4 (1), 17–21.

Chen, K.S., Chen, Q.J., Zhang, S.L., 1997. The Relationship between changes of endogenous ABA and IAA levels and granulation in 'Bendizao' (*Citrus reticulata*) citrus fruit during storage. Acta Horticulturae Sinica 24 (3), 291–292.

Chen, K.S., Xu, C.J., Li, F., Zhang, S.L., 2005. Postharvest granulation of' Huyou' (*Citrus Changshanensis*) fruit in response to calcium. Isr. J. Plant Sci. 53, 35–40.

Dietz, J.H., Rouse, A.H., 1953. A rapid method for estimating pectic substances in citrus juices. J. Food Sci. 18, 169–177.

Fumuro, M., Horikawa, Y., Sakurai, N., 2014. Application of acoustic vibration method for discrimination of fruit with freezing and granulation of juice sacs in blood orange (*Citrus sinensis*) and Hassaku (*Citrus hassaku*). Hortic. Res. 13, 365–370.

Goto, A., 1989. Relationship between pectic substances and calcium in healthy, gelated, and granulated juice sacs of sanbokan (*Citrus sulcata* hort. Ex Takahashi) fruit. Plant Cell Physiol. 30, 801–806.

Hu, W.R., Kong, W.N., Pang, X.Q., Liu, S.Z., Zhang, Z.Q., 2015a. Effects of two storage temperatures on storage quality of Shatianyu pomelo. Food Sci. Technol. 40, 334–339.

Hu, W., Liu, S., Jiang, Y., Lin, Y., Wang, Y., Li, B., 2015b. Characteristics of granulated juice sacs of red-fleshed sweet pomelo. Food Science 36, 233–238.

Hwang, Y.S., Huber, D.J., Albrigo, L.G., 1990. Comparison of cell-wall components in normal and disordered juice vesicles of grapefruit. J. Am. Soc. Hortic. Sci. 115, 281–287.

Kong, W., Liu, S., Li, X., Zhang, Z., Hu, W., 2010. Advances in mechanism and control of granulation of citrus fruit. Journal of Tropical and Subtropical Botany 18, 453–458.

Lacerna, M.M., Bayogan, E.V., Secretaria, L.B., 2018. Rind color change and granulation in pummelo [*Citrus maxima* (Burm. Ex Rumph.) Merr.] fruit as influenced by 1-methylcyclopropene. Int. Food Res. J. 25, 1483–1488.

Lin, Q., Wang, C.Y., Dong, W.C., Jiang, Q., Wang, D.L., Li, S.J., Chen, M., Liu, C.R., Sun, C.D., Chen, K.S., 2015. Transcriptome and metabolome analyses of sugar and organic acid metabolism in Ponkan (*Citrus reticulata*) fruit during fruit maturation. Gene 554, 64–74.

Liu, S.X., Chen, M.D., Li, Y.B., 1988. Morphological structure and microcellular changes of mandarin fruit with postharvest section-drying. China Citrus 17, 2.

Liu, S.Y., Hu, L.P., Jiang, D., Xi, W.P., 2019. Effect of post-harvest LED and UV light irradiation on the accumulation of flavonoids and limonoids in the segments of newhall navel oranges (*Citrus sinensis* Osbeck). Molecules 24 (9), 1755.

Manchanda, H.R., Shukla, U.C., Randhawa, N.S., 1972. Effect of soil and foliar applications of Zn and foliar applications of Cu, Mn, Fe alone and in combination with Zn on the incidence of chlorosis, fruit fall, granulation and fruit quality in sweet orange *Citrus sinensis* L. Osbeck variety blood red. Indian J. Hortic. 29, 19–29.

Pan, T., Zhu, X., Pan, D., Guo, Z., She, W., Chen, G., 2013. Relationship between granulation and lignin metabolism in Guanximiyou pummelo fruit during storage. J. Fruit Sci. 30, 294–298.

Rose, J.K., Hadfield, K.A., Labavitch, J.M., Bennett, A.B., 1998. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol. 117, 345–361.

Saxena, I., Srikanth, S., Chen, Z., 2016. Cross Talk between H_2O_2 and interacting signal molecules under plant stress response. Front. Plant Sci. 7, 570.

Sharma, R.R., Saxena, S.K., 2004. Rootstocks influence granulation in Kinnow mandarin (*Citrus nobilis* × *C. deliciosa*). Sci. Hortic. 101, 235–242.

Sharma, R.R., Singh, R., Saxena, S.K., 2006. Characteristics of citrus fruits in relation to granulation. Sci. Hortic. 111, 91–96.

Sharma, R.R., Awasthi, O.P., Kumar, K., 2016. Pattern of phenolic content, antioxidant activity and senescence-related enzymes in granulated vs non-granulated juice-sacs of' Kinnow' mandarin (*Citrus nobilis* × *C. deliciosa*). Journal of Food Science and Technology-Mysore 53, 1525–1530.

She, W.Q., Zhao, X.L., Pan, D.M., Lin, H.T., 2008. Relationship between cell wall metabolism and fruit juicy sac granulation during fruit mature stage of pummelo *Citrus grandis* (L.) Osbeck 'Guanxi-miyou'. Journal of Tropical and Subtropical Botany 16, 545–550.

She, W.Q., Pan, D.M., Lin, H.T., 2009. Relationship between granulation and active oxygen metabolism of juice sac in pummelo fruit during maturation. Scientia Agricultura Sinica 42 (5), 1737–1743.

Theanjumpol, P., Wongzeewasakun, K., Muenmanee, N., Wongsaipun, S., Krongchai, C., Changrue, V., Boonyakiat, D., Kittiwachana, S., 2019. Non-destructive identification and estimation of granulation in' Sai Num Pung' tangerine fruit using near infrared spectroscopy and chemometrics. Postharvest Biol. Technol. 153, 13–20.

Tietel, Z., Feldmesser, E., Lewinsohn, E., Fallik, E., Porat, R., 2011. Changes in the transcriptome of' Mor' mandarin flesh during storage: emphasis on molecular regulation of fruit flavor deterioration. J. Agric. Food Chem. 59, 3819–3827.

Wang, X.Y., 1997. Effects of respiratory inhibitors on granulation of ponkan (*Citrus reticulata* var. poonensis) fruits. Science and Technology of Food Industry 21–23.

Wang, X., 2005. Studies on the difference of some physiological indexes of vesicle collapse and granulation of Ponkan mandarin fruit. Journal of Fruit Science 22, 216–219.

Wang, X.Y., Wang, P., Qi, Y.P., Zhou, C.P., Yang, L.T., Liao, X.Y., Wang, L.Q., Zhu, D.H., Chen, L.S., 2014. Effects of granulation on organic acid metabolism and its relation to mineral elements in *Citrus grandis* juice sacs. Food Chem. 145, 984–990.

Wu, J.L., Pan, T.F., Guo, Z.X., Pan, D.M., 2014. Specific lignin accumulation in granulated juice sacs of *Citrus maxima*. J. Agric. Food Chem. 62, 12082–12089.

Xiong, B., Ye, S., Qiu, X., Liao, L., Sun, G., Luo, J., Dai, L., Rong, Y., Wang, Z., 2017. Exogenous spermidine alleviates fruit granulation in a citrus cultivar (Huangguogan) through the antioxidant pathway. Acta Physiol. Plant. 39, 98.

Xu, C.J., Fraser, P.D., Wang, W.J., Bramley, P.M., 2006. Differences in the carotenoid content of ordinary citrus and lycopene-accumulating mutants. J. Agric. Food Chem. 54, 5474–5481.

Yao, S., Cao, Q., Xie, J., Deng, L., Zeng, K., 2018a. Alteration of sugar and organic acid metabolism in postharvest granulation of Ponkan fruit revealed by transcriptome profiling. Postharvest Biol. Technol. 139, 2–11.

Yao, S., Cao, Q., Xie, J., Deng, L., Zeng, K., 2018b. Changes in volatile compounds and expression pattern of genes involved in terpenoid biosynthesis in pulp of Ponkan fruit

during section-drying. Food and Machinery 34, 23–27.
Yi, L.Z., Ma, S.S., Ren, D.B., 2017. Phytochemistry and bioactivity of Citrus flavonoids: a focus on antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities. Phytochem. Rev. 16, 479–511.
Zhang, S., Cheng, C., Lin, W., Li, X., Zhu, D., Chen, L., Guo, J., Li, Y., 2019. Analysis of boron nutrition status in soils and trees and its relationship with fruit granulation in Guanximiyou pomelo. J. Fruit Sci. 36, 468–475.
Zhu, N., Wu, D., Chen, K.S., 2018. Label-free visualization of fruit lignification: raman molecular imaging of loquat lignified cells. Plant Methods 14, 58.