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Changes in free and glycosidically bound volatile compounds of nectarine fruit during low-temperature storage

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Abstract

Free and glycosidically bound volatiles are two essential aroma compounds contributing to the flavour of nectarine fruits. To explore the relationship between free and bound volatiles in nectarine fruits during postharvest storage, they were first harvested and then subjected to the temperatures of 1, 5, and 8°C for 35 d, and the changes in volatile compounds, *β*-glucosidase (*β*-Glu) activity, and the expression of UGT (UDP-glucosyltransferase) involved in the accumulation of bound linalool were determined. Results showed that nectarine fruits stored at 5°C had the lowest contents of free volatile compounds due to damage from chilling injury, and the contents of esters and lactones decreased at 1 and 5°C. The contents of bound volatiles increased during the early storage period, and decreased afterwards due to an increase in *β*-Glu activity. Corresponding to the higher contents of bound volatiles at 1°C, the *β*-Glu activity in nectarine fruits stored at 1°C was significantly lower than that in nectarine fruits stored at the other two temperatures.

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Introduction

Nectarine (*Prunus persica* L. Batsch) fruits are cultivated extensively worldwide, and favoured by consumers for their pleasant aroma and sweet taste. Nectarine fruits are rich in vitamins and various phenols and flavonoids which contribute to their antioxidant activities (Chen *et al*., 2016; Xi *et al*., 2017). The shelf life of nectarine fruits is short (approximately 4 - 8 days) due to rapid softening, despite preservation efforts (Xi *et al*., 2017; Aubert *et al*., 2019). Low-temperature storage is an effective method of delaying the decay of nectarine fruits after harvest. However, during prolonged low-temperature storage, nectarine fruits exhibit chilling injury (CI) symptoms, a physiological disorder characterised by internal browning, lack of juiciness, and flavour loss. When severe CI occurs in fruits, the overall taste is greatly affected, and sucrose contents decrease, thus resulting in a decrease in fruit quality (Wang *et al*., 2020a). It has been reported that physiological disorder develops faster when fruits are stored at temperatures between 2.2 and 7.6°C, then when they are stored at 0°C (Lurie and Crisosto, 2005).

Aroma is one of the most critical parameters responsible for fruit quality and consumer acceptance. Nectarine fruits' volatiles have been intensively investigated, and more than 100 compounds have been identified, including alcohols, aldehydes, terpenoids, esters, and lactones (Aubert and Milhet, 2007; Ceccarelli *et al*., 2020). The characteristic nectarine fruits' aroma contains C6 compounds such as hexanal, 2-hexenal, hexanol, and lactones, mainly γ- and δ-decalactones. C6 aldehydes and alcohols are the major components in immature nectarine fruits, but the concentrations of C6 compounds decrease, and the concentrations of lactones increase significantly during maturation (Visai and Vanoli, 1997; Aubert *et al*., 2003a). Lactones and esters have been reported to play important roles in determining the aromatic features of nectarine fruits, and fruity note volatile compounds contribute to the overall nectarine fruits' aroma (Xi *et al*., 2014).

In addition to free volatiles, flavour compounds can also accumulate as non-volatiles in fruits and plant tissues, and they are known as flavourless glycoconjugates. The free volatiles are conjugated with sugar to form glycoside bonds, and

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stored in vacuoles. During processing and postharvest storage, aroma precursors are catalysed by acid hydrolysis or glycoside hydrolase, and further decompose into free forms (Chen *et al*., 2020). As the fruit ripens, the cell wall softens, and the internal tissue disappears, the related hydrolases come into contact with the previously inaccessible substrate, and participate in the degradation reaction. Releasing these potential glycosidically bound volatile compounds during storage or processing can improve the overall aroma of fruits. The free terpene linalool, characterised by sweet and alcoholic flavours, is a major contributor to the aroma of peach fruit (Tian *et al*., 2017). The conversion of free and bound terpene linalool is closely related to the *Pp*UGT gene, which could catalyse the glycosylation of free linalool (Wu *et al*., 2019).

To date, there is only one report on the bound volatiles of nectarine fruits (Aubert *et al*., 2003b), and most studies of bound aromas are still concentrated on fruits during growth. No studies have been conducted on the changes in bound volatile compounds in nectarine fruits during postharvest storage. In the present work, nectarine fruits were stored at three different low temperatures for 35 days. Both free and bound volatile compounds in nectarine fruits were extracted and analysed to explore their changes in composition and contents during cold storage. In addition, the activity of *β*-Glu, which hydrolyses the bound volatile compounds into free forms, and the gene expression of UGT, which is involved in the accumulation of glycosylated linalool, were analysed to further explore the mechanism of the changes in bound volatiles.

Materials and methods

Plant materials

Yellow-fleshed nectarine fruits (*P. persica* L. Batsch var. *nectarine* cv. Ruiguang 1) were hand-harvested at a mature commercial stage (approximately 110 days after full bloom, firmness: 25 ± 2 N, SSC: 10.5 ± 0.5%) on July 4, 2019 from an orchard in Nanjing, Jiangsu Province, China (east longitude 118°, north latitude 32°). Harvested nectarine fruits were transported to the laboratory within 2 h after harvesting, and immediately selected with the criteria of uniform in size and without visible defects or decay. A total of 540 nectarine fruits were selected and randomly divided into three groups. The first group was stored at 1°C to delay chilling injury, the second group was stored at 5°C to induce the occurrence of chilling injury, and the third group was stored at 8°C to prevent chilling injury. The nectarine fruits were stored at three temperatures for 7, 14, 21, 28, and 35 days without packing, and the relative humidity was maintained between 90 and 95%. The flesh was cut and combined at each sampling time, frozen in liquid nitrogen, and stored at -80°C for further analyses.

Quality analysis

At each sampling point, nectarine 15 fruits were randomly selected to form three replicates, with five fruits in each replicate. A texture analyser (TMS-Pro, FTC, USA) fitted with a 6 mm diameter head was used to determine firmness, and the measurements were made on three opposite sides at the equatorial region of each nectarine fruit. The penetration rate was 1 mm/s, and the penetration depth was 5 mm. Unit of firmness was expressed in newtons (N). The soluble solids contents (SSC) were measured by a digital refractometer (PAL-1, Atago, Tokyo, Japan), and the titratable acidity (TA) was measured by an acidity metre (PAL-Easy ACID F5, Atago, Tokyo, Japan). Both SSC and TA were expressed as percentage (%). The internal browning (IB) index was assessed on the degree of browning area according to Zhou *et al*. (2019a): 0 = no browning, 1 = browning area is 0 - 25%, 2 = browning area is 25 - 50%, 3 = browning area is 50 - 75%, and 4 = the browning area is more than 75%. The IB index was calculated by ∑ (IB level × number of fruit at the IB level)/(4 × total number of fruits). Ascorbic acid was measured according to Li *et al*. (2019), and expressed as μg/g. The total carotenoid contents were measured according to Peng *et al*. (2017) with some modifications. Briefly, a total of 2 g frozen powder was mixed with 20 mL extraction solvent OF acetone-ethanol-hexane (1:1:2) containing 0.1% BHT, and stirred for 20 min. Next, 6 mL distilled water was added to the mixture, stirred for another 10 min, and then centrifuged for 5 min at 1,500 rpm to obtain the organic layer. The absorbance of the organic layer was measured at 450 nm, and the carotenoid contents were expressed as μg/g.

Extraction of free volatile compounds

Free volatile compounds were extracted according to Zhou *et al*. (2019b) with some modifications. Briefly, a total of 5 g frozen powder supplemented with 1 g NaCl was put into a 20 mL

vial, and 10 μL 3-octanol (83 mg/L) was added into the vial as an internal standard. The vial was immediately sealed with a PTFE-silicone septum, and placed in a 45°C water bath for 5 min. The volatiles were extracted by a fibre coated with polydimethylsiloxane and divinylbenzene (PDMS/DVB, 65 μm; Supelco, USA) at 45°C for 45 min. Each sample was measured in triplicate.

Extraction of bound volatile compounds

Bound volatile compounds were extracted according to Gao *et al*. (2018) with some modifications. A total of 20 g frozen powder was transferred into a beaker containing 100 mL distilled water, homogenised and centrifuged for 20 min at 4°C to obtain the supernatant. Glycosides were extracted by adsorption onto an SPE LC-18 column (CNW, Düsseldorf, Germany) that was preconditioned with 10 mL methanol and 10 mL distilled water. Next, 25 mL clear supernatant sample was poured onto the column and then rinsed with 30 mL distilled water to elute soluble sugars, acids, and other low molecular weight polar compounds. Free volatiles were removed with 35 mL methylene chloride, and bound volatiles were eluted with 35 mL methanol. The methanol eluate was concentrated on a rotary evaporator (RE-2000A, Yarong, Shanghai, China) to 1 - 2 mL (water bath temperature, 35°C), followed by blowing to dryness with a nitrogen blower (YY-N100-1; Yunyan, Shanghai, China). The residue was dissolved in 1.8 mL citric acid-phosphate buffer (pH 5, 0.2 M). The buffered mixture was washed five times with 9 mL methylene chloride to remove the possible remaining free volatile compounds.

Enzymatic hydrolysis

A total of 15 mg *β*-Glu (15 U/mg; Yuanye, Shanghai, China) was added to the glycosidic precursors dissolved in the buffer solution. The vial containing the mixture was immediately sealed with a PTFE-silicone septum, and placed in a 37°C water bath for 48 h for hydrolysis, and 10 μL 3-octanol was added as an internal standard. The extraction of the released volatiles was the same as the extraction of free volatiles.

GC-MS analysis

The aromatic volatiles were analysed on an Agilent 7890A gas chromatography apparatus (7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a 5975A mass spectrometer (5975A, Agilent Technology, Santa Clara, CA, USA). The fibre was inserted into the injection port of GC for 5 min. For separation, an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA) was employed. The operating conditions for GC-MS analysis were as follows: injector temperature 250°C, oven temperature held at 40°C for 2 min, then increased to 150°C at 3°C/min, and maintained for 2 min, finally increased to 250°C at 10°C/min, and maintained for 2 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. Analysis was conducted in splitless mode. The MS detector was set to electronic impact (EI) mode at 70, and mass spectra were scanned in the range *m/z* 30 - 450. The ion source and quadrupole temperatures were 250 and 150°C, respectively. The compounds detected by GC-MS were identified by comparing their mass spectra with the NIST library. All of the compounds were quantified as 3-octanol equivalents, and expressed as μg/kg.

Determination of β-glucosidase activity

β-Glu assays were performed according to Garcia *et al*. (2013) with some modifications. Samples of 2 g frozen powder were soaked in 10 mL citrate/phosphate buffer (pH 5.5, 0.1 M) containing 0.3 g PVP for 30 min at 4°C, followed by centrifugation at 10,000 rpm at 4°C for 20 min. The supernatant was the crude *β*-Glu solution.

For the enzymatic assay, a total of 1.2 mL crude extract mixed with 0.2 mL *p*-nitrophenyl-*β*-D-glucopyranoside (p-NPG, 10 mM) solution and 0.6 mL precooled citrate/phosphate buffer (pH 5.5, 0.1 M) was incubated at 37°C for 90 min. The reaction was stopped by adding 2 mL Na_2CO_3 solution (1 M), the absorbance of the above incubated mixture was measured at 402 nm, and inactivated enzyme solution (crude extract in boiling water for 5 min to ensure that the enzyme was inactivated) was treated in the same way as a blank. The *β*-Glu activity was expressed as U/g of fresh weight. One U was defined as the amount of enzyme that released 1 nmol *p*-nitrophenol at 37°C per 1 min.

Real-time quantitative PCR analysis of gene expression

Total RNA was isolated from approximately 5 g frozen tissues using RNAplant Plus Reagent (Tiangen, Beijing, China), and cDNA synthesis was performed using a HisScriptTM Q RT SuperMix kit

(Vazyme, Nanjing, China). Quantitative real-time PCR gene expression analysis was performed on QuantStudio 6 Flex system (Applied Biosystems, Foster City, CA, USA) using AceQ® qPCR SYBR Green Master Mix (Vazyme, Nanjing, China). The q-PCRs were performed in a total volume of 20 μL, including 10 μL SYBR® Premix Ex Taq™, 2 μL diluted cDNA, 0.4 μL forward and reverse primers, 0.4 μL ROX reference dye, and ddH_2O to a final volume of 20 μL. The PCR conditions were as follows: a preliminary step of 5 min at 95°C, followed by 40 cycles of 95°C for 10 s, and 60°C for 30 s. The TEF2 gene was used as an internal control gene to normalise the subtle differences in template amounts. The relative expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Each determination contained three biological replications with three additional parallel experiments in each replication. Data were tabulated with Microsoft Excel 2019, and Duncan's test of One-way analysis of variance (ANOVA) was used to test the significance level of multiple groups with SAS Software (Version 9.2; SAS Institute, Cary, USA) at 5% confidence level.

Results

Changes in qualities of nectarine fruits during cold storage

Changes in the postharvest quality of nectarine fruits stored at different temperatures are shown in Table 1. Nectarine fruits softened after harvest, and the firmness decreased sharply after 7 d of storage at 8°C. Nectarine fruits stored at 5°C showed a softening pattern, and the firmness was almost maintained in nectarine fruits stored at 1°C. During postharvest storage, chilling injury symptoms were observed on the flesh of nectarine fruits stored at 5°C after 14 d storage, and 1°C after 21 d storage. The SSCs in all three groups showed fluctuating contents during storage. Nectarine fruits subjected to 5 and 8°C exhibited significantly higher contents of SSC than those subjected to 1°C storage, except for 14 d. The contents of TA increased first, and then decreased thereafter, and nectarine fruits stored at 5°C had higher contents of TA than those stored at 1 and 8°C. Ascorbic acid contents decreased progressively during cold storage, with no significant difference observed among the three temperatures. Carotenoid contents constantly increased at 1 and 8°C, while there was a decrease at 28 d at 5°C.

Table 1. Changes in firmness, IB index, SSC, TA, ascorbic acid, and carotenoids in nectarine fruits during cold storage at 1, 5, and 8°C.

	T (°C)	Firmness (N)	IB index	Soluble solid content (%)	Titratable acid (%)	Ascorbic acid (μg/g)	Carotenoid (μg/g)
0 d		24.58 ± 0.08	0.00 ± 0.00	10.28 ± 0.44	0.30 ± 0.01	79.85 ± 3.86	54.17 ± 1.26
7 d	1	22.86 ± 0.31^{a}	0.00 ± 0.00^{a}	11.12 ± 0.39^{a}	0.31 ± 0.00^{a}	71.07 ± 3.13^{a}	53.06 ± 1.96^{b}
	5	24.98 ± 1.16^{a}	0.00 ± 0.00^{a}	11.16 ± 0.67^{a}	0.29 ± 0.01^{a}	69.77 ± 1.89^{a}	54.90 ± 1.68^{b}
	8	14.85 ± 1.82^{b}	0.00 ± 0.00^{a}	11.58 ± 0.22^{a}	0.30 ± 0.02^{a}	69.52 ± 2.03^{a}	57.92 ± 2.44^{a}
14 d	1	24.68 ± 1.56^{a}	0.00 ± 0.00^{b}	12.20 ± 0.58^{a}	0.31 ± 0.01^{b}	61.68 ± 2.21^{a}	53.42 ± 1.61^{c}
	5	18.30 ± 1.66^{b}	0.21 ± 0.04^{a}	11.38 ± 0.50^{b}	0.35 ± 0.02^{a}	55.86 ± 2.91^{b}	60.68 ± 2.11^{b}
	8	4.86 ± 0.43^{c}	0.00 ± 0.00^{b}	11.77 ± 0.09^{b}	0.30 ± 0.01^{b}	61.05 ± 2.69^{a}	63.89 ± 0.89^{a}
21 d	1	25.92 ± 0.96^{a}	0.27 ± 0.02^{b}	11.91 ± 0.16^{a}	0.27 ± 0.01^{c}	50.34 ± 1.34^{a}	55.01 ± 2.30^{b}
	5	13.89 ± 0.36^{b}	0.33 ± 0.02^{a}	11.68 ± 0.63^{a}	0.35 ± 0.00^{a}	52.91 ± 2.72^{a}	64.88 ± 0.74^{a}
	8	3.00 ± 0.17^{c}	0.00 ± 0.00^{c}	12.13 ± 0.40^{a}	0.31 ± 0.03^{b}	49.17 ± 1.69^{a}	64.70 ± 2.12^{a}
28 d	1	23.69 ± 0.13^{a}	0.35 ± 0.03^{b}	11.74 ± 0.08^{b}	0.26 ± 0.00^{b}	41.30 ± 1.63^{b}	58.60 ± 0.21^{c}
	5	7.19 ± 0.39^{b}	0.53 ± 0.03^{a}	12.52 ± 0.11^{a}	0.31 ± 0.03^{a}	45.77 ± 3.41^{a}	62.23 ± 1.15^{b}
	8	2.56 ± 0.24^{c}	0.00 ± 0.00^{c}	12.59 ± 0.90^{a}	0.32 ± 0.05^{a}	41.25 ± 1.18^{b}	68.72 ± 1.54^{a}
35 d	1	22.22 ± 1.44^{a}	0.48 ± 0.02^{b}	11.34 ± 0.25^{b}	0.28 ± 0.01^{a}	35.75 ± 3.40^{a}	69.97 ± 0.44^{b}
	5	4.35 ± 0.12^{b}	0.61 ± 0.03^{a}	11.73 ± 0.71^{b}	0.30 ± 0.02^{a}	38.02 ± 0.57^{a}	62.35 ± 0.89^{c}
	8	2.48 ± 0.09^{c}	0.00 ± 0.00^{c}	13.98 ± 0.33^{a}	0.28 ± 0.02^{a}	35.57 ± 0.74^{a}	78.42 ± 0.78^{a}

Values are mean ± standard deviation of three replicates (n = 3). Different lowercase superscripts in the same row at the same day indicate significant difference at $p < 0.05$.

Changes in free volatile compounds in nectarine fruits during cold storage

As indicated in Table 2, 31 compounds, including four terpenes, three alcohols, nine aldehydes, six lactones, four esters, and five other components were identified in nectarine fruits, with aldehydes accounting for a large part of the total aroma compounds.

Total terpenes generally showed a decreasing trend at the three temperatures. Nectarine fruits stored at 8°C exhibited significantly higher contents of total terpenes than those stored at 1 and 5°C, except for 28 d. The contents of linalool decreased sharply at 7 d, and then decreased slowly afterwards regardless of temperature. The contents of 6-Pentyl-2H-pyran-2-one was significantly influenced by temperature, and the contents in nectarine fruits stored at 8°C was much higher than those in nectarine fruits stored at 5°C, but not identified at 1°C.

(E)-2-Hexene-1-ol, hexanol, and 2-ethylhexanol were three alcohols identified in nectarine fruits. For (E)-2-hexene-1-ol, notable decreases were found in nectarine fruits stored at 1 and 5°C, while this compound exhibited an increase before 14 d, and decreased afterward in nectarine fruits stored at 8°C. Hexanol accounted for most of the alcohols, and exhibited fluctuating contents at 1°C, and decreased at 5°C, whereas it was not detected at 8°C.

Total aldehydes decreased progressively at the three temperatures, except for an increase at 8°C at 7 d. Chilling injury-inducing temperature (5°C) suppressed the production of total aldehydes after 14 d storage. (E)-2-hexenal and hexenal were the most abundant aldehydes in nectarine fruits. The contents of hexanal and (E)-2-hexenal decreased progressively with the storage time processing at 1 and 5°C, while increased at 7 d and then decreased afterward at 8°C. Nectarine fruits stored at 8°C had the lowest contents of hexenal after 14 d storage.

Total lactones showed fluctuating contents at 1°C, while increased slightly before 14 d, and then decreased over the remaining cold storage at 5°C. Total lactones showed an upward trend under 8°C storage, and were significantly higher than those stored at 1 and 5°C. γ-decalactone and γ-caprolactone were the major compounds accounting for approximately 40 - 75% of total lactones. Nectarine fruits stored at 8°C had the highest contents of γ-decalactone and γ-caprolactone, while those stored at 1°C had the lowest.

Total esters decreased progressively during storage regardless of temperatures; the production of esters was significantly inhibited by low temperature. In addition, total esters in nectarine fruits stored at 8°C were significantly higher than those stored at 1 and 5°C. Significant lower contents of hexyl acetate were observed in 5°C-stored nectarine fruits during whole storage. The (3Z)-3-hexen-1-yl acetate and hex-2-enyl acetate contents both decreased at the three temperatures, and nectarine fruits stored at 8°C exhibited significant higher contents of the two compounds during the entire storage.

Changes in bound volatile compounds in nectarine fruits during cold storage

Table 3 shows the changes in bound volatile compounds during postharvest storage. A total of 34 bound volatiles compounds were identified, and could be grouped into five classes including 11 terpenes, four alcohols, seven aldehydes, seven phenols, and five other compounds. Aldehydes and terpenes were two predominant compounds in nectarine fruits.

Total terpenes generally showed a decreasing trend at both 1 and 5°C, while increased sharply at 7 d, and decreased thereafter at 8°C. Among them, linalool was one of the major compounds in nectarine fruits. The contents of glycosylated linalool increased before 7 d (5 and 8°C) or 14 d (1°C), and then decreased thereafter. The contents of *α*-terpineol remained constant before 21 d, and increased slightly until the end of storage, regardless of temperatures. Four isomers of lilac aldehyde were detected including lilac aldehydes A, B, C, and D, and they showed a general downward trend during cold storage. The isomers neral and geranial were identified at low contents during the entire storage. In 1°C-stored nectarine fruits, neral and geranial increased during the storage, while the two compounds increased before 14 d, and remained constant for the remaining days for 5°C-stored nectarine fruits.

A total of four bound alcohol volatiles were identified. Among them, benzyl and phenethyl alcohol were the major constituents accounting for 57 - 90% of the total bound alcohols identified. The benzyl alcohol contents fluctuated during storage at

Table 2. Changes in free volatile compounds in nectarine fruits during postharvest storage at 1, 5, and 8°C.

Category	Compound	0 d	7 d			14 d			21 d			28 d			35 d		
			1°C	5°C	8°C	1°C	5°C	8°C	1°C	5°C	8°C	1°C	5°C	8°C	1°C	5°C	8°C
Monoterpene	Linalool	68.28	2.44^{b}	2.16^{b}	3.76^{a}	1.28^{b}	1.29^{b}	2.16^{a}	1.03^{b}	0.97^{b}	2.22^{a}	1.03^{a}	0.69^{b}	1.17^{a}	0.80^{a}	0.73^{a}	1.15^{a}
	Geranylacetone	0.47	0.24^{a}	nd	nd	0.23^{a}	nd	nd	0.42^{a}	nd	nd	3.32^{a}	nd	nd	nd	nd	nd
	β-ionone	0.40	nd	nd	0.38^{a}	nd	nd	nd	nd	nd	nd	nd	nd	0.39^{a}	nd	nd	0.63^{a}
	6-Pentyl-2H-pyran-2-one	0.31	nd	1.35^{a}	1.00^{a}	nd	0.60^{b}	31.69^{a}	nd	0.65^{b}	25.95^{a}	nd	nd	nd	nd	nd	nd
	Total	69.46	2.68^{b}	3.51^{ab}	5.14^{a}	1.51^{b}	1.89^{b}	33.85^{a}	1.45^{b}	1.62^{b}	28.17^{a}	4.35^{a}	0.69^{b}	1.56^{b}	0.80^{b}	0.73^{b}	1.78^{a}
Alcohol	Hexanol	17.20	20.05^{a}	21.62^{a}	nd	nd	13.80^{a}	nd	28.14^{a}	7.70^{b}	nd	nd	nd	nd	16.15^{a}	nd	nd
	(E)-2-Hexene-1-ol	7.68	7.07^{b}	7.71^{ab}	9.80^{a}	8.07^{b}	6.00^{b}	15.90^{a}	6.78^{b}	6.00^{b}	14.07^{a}	5.28^{b}	5.06^{b}	14.90^{a}	nd	4.96^{b}	11.80^{a}
	2-Ethylhexanol	0.80	nd	0.79^{a}	nd	nd	0.51^{a}	nd	nd	0.63^{a}	nd	nd	0.55^{a}	nd	nd	0.88^{a}	nd
	Total	25.68	27.12^{a}	30.12^{a}	9.80^{b}	8.07^{b}	20.31^{a}	15.90^{a}	34.92^{a}	14.33^{b}	14.07^{b}	5.28^{b}	5.61^{b}	14.90^{a}	16.15^{a}	5.84^{b}	11.80^{a}
Aldehyde	*n*-Hexanal	239.59	175.58^{b}	220.28^{b}	365.24^{a}	170.52^{a}	142.24^{a}	144.99^{a}	157.33^{a}	90.66^{b}	54.10^{b}	144.57^{a}	75.27^{b}	35.44^{c}	129.57^{a}	69.06^{b}	nd
	(E)-2-Hexenal	391.99	358.68^{b}	383.15^{b}	533.92^{a}	328.60^{b}	297.55^{b}	433.85^{a}	312.87^{b}	270.83^{c}	459.36^{a}	250.23^{b}	231.22^{b}	362.40^{a}	220.42^{a}	266.87^{a}	298.91^{a}
	Benzaldehyde	22.86	32.07^{a}	29.08^{a}	22.62^{a}	41.43^{a}	27.07^{a}	22.58^{a}	74.46^{b}	39.87^{c}	184.79^{a}	86.94^{b}	59.51^{c}	305.55^{a}	72.35^{b}	59.18^{c}	187.01^{a}
	2,4-Hexadienal	1.30	10.55^{a}	0.57^{b}	7.00^{a}	7.07^{a}	0.12^{b}	4.95^{a}	5.95^{a}	1.48^{b}	6.29^{a}	6.99^{a}	2.34^{b}	8.11^{a}	5.46^{a}	2.30^{b}	5.92^{a}
	Decanal	1.20	1.05^{a}	0.82^{a}	1.34^{a}	0.96^{a}	0.60^{b}	0.72^{b}	1.48^{a}	nd	1.41^{a}	1.83^{a}	nd	1.65^{a}	1.37^{a}	nd	0.72^{b}
	Nonanal	5.07	3.99^{a}	nd	3.43^{a}	3.45^{a}	nd	1.64^{b}	4.17^{a}	1.99^{b}	4.46^{a}	3.10^{a}	nd	5.45^{a}	2.02^{b}	nd	28.60^{a}
	Phenylacetaldehyde	0.21	0.48^{b}	1.39^{a}	nd	0.60^{a}	0.82^{a}	nd	2.11^{a}	0.75^{a}	nd	2.07^{a}	0.49^{b}	nd	2.46^{a}	0.75^{a}	nd
	(2E)-2-Octenal	1.56	1.10^{a}	nd	1.85^{a}	1.27^{a}	nd	nd	2.04^{a}	nd	nd	2.03^{a}	nd	nd	1.25^{a}	nd	nd
	(E,E)-2,6-Nonadienal	0.68	0.85^{a}	nd	nd	0.82^{a}	nd	nd	1.20^{a}	nd	nd	1.93^{a}	nd	nd	nd	nd	nd
	Total	664.46	584.35^{b}	635.29^{b}	935.40^{a}	554.72^{b}	478.40^{b}	608.73^{a}	561.61^{b}	405.58^{c}	710.41^{a}	499.69^{b}	361.27^{c}	718.60^{a}	434.90^{b}	348.16^{c}	521.16^{a}
Lactone	δ-Decalactone	1.34	0.57^{c}	4.69^{b}	9.02^{a}	nd	3.06^{b}	15.07^{a}	1.80^{b}	3.03^{a}	2.89^{ab}	0.49^{b}	0.77^{b}	1.38^{a}	nd	0.44^{b}	1.26^{a}
	γ-Caprolactone	1.64	2.41^{a}	2.04^{a}	3.09^{a}	1.80^{c}	3.58^{a}	2.68^{b}	2.42^{c}	3.50^{b}	6.61^{a}	3.24^{b}	3.33^{b}	10.91^{a}	2.46^{b}	2.82^{b}	41.43^{a}

	γ-Decalactone	1.77	nd	7.27^{a}	9.88^{a}	nd	9.10^{a}	8.81^{a}	nd	6.50^{b}	9.27^{a}	nd	3.76^{b}	10.03^{a}	nd	2.72^{b}	30.33^{a}
	γ-Octalactone	nd	nd	0.78^{a}	0.58^{a}	nd	0.60^{a}	0.37^{a}	nd	0.55^{a}	0.96^{a}	nd	0.40^{b}	1.05^{a}	nd	0.50^{b}	4.24^{a}
	1,5-Octanolactone	nd	nd	nd	nd	nd	nd	2.37^{a}	nd	nd	15.16^{a}	nd	nd	2.16^{a}	nd	nd	1.55^{a}
	γ-Undecalactone	1.05	nd	nd	2.29^{a}	nd	nd	nd	nd	nd	1.78^{a}	nd	nd	2.25^{a}	nd	nd	9.55^{a}
	Total	5.80	2.98^{c}	14.78^{b}	24.86^{a}	1.80^{c}	16.34^{b}	29.30^{a}	4.22^{c}	13.58^{b}	36.67^{a}	3.73^{b}	8.26^{b}	27.78^{a}	2.46^{c}	6.48^{b}	88.36^{a}
Ester	Hexyl acetate	18.28	7.56^{c}	15.48^{b}	26.04^{a}	11.35^{a}	2.53^{b}	12.23^{a}	13.61^{a}	2.14^{c}	6.64^{b}	2.80^{a}	0.95^{b}	2.86^{a}	1.96^{a}	0.45^{b}	1.40^{a}
	(3Z)-3-Hexen-1-ylacetate	29.68	24.85^{b}	31.43^{ab}	48.91^{a}	nd	8.13^{b}	30.72^{a}	nd	5.96^{b}	24.97^{a}	nd	4.57^{b}	18.34^{a}	nd	1.96^{b}	16.70^{a}
	Methyl benzoate	0.51	0.80^{a}	nd	0.62^{a}	0.50^{a}	0.73^{a}	0.79^{a}	2.32^{b}	0.68^{c}	16.57^{a}	2.09^{b}	0.36^{c}	16.56^{a}	2.34^{b}	0.21^{c}	14.07^{a}
	Hex-2-enyl acetate	25.05	nd	22.17^{a}	30.42^{a}	nd	4.23^{b}	19.76^{a}	nd	4.24^{b}	18.01^{a}	nd	2.54^{b}	7.33^{a}	nd	0.89^{b}	12.21^{a}
	Total	73.52	33.21^{c}	69.08^{b}	105.99^{a}	11.85^{b}	15.62^{b}	63.50^{a}	15.93^{b}	13.02^{b}	66.19^{a}	4.89^{b}	8.42^{b}	45.09^{a}	4.30^{b}	3.51^{b}	44.38^{a}
Other	Carveol	0.56	nd	1.45^{a}	0.87^{a}	nd	0.68^{a}	0.78^{a}	nd	nd	0.56^{a}	nd	nd	nd	nd	nd	nd
	Thymol	0.42	nd	1.37^{a}	nd	nd	nd	nd	nd	0.47^{a}	nd	nd	0.26^{a}	nd	nd	0.60^{a}	nd
	3-Octanone	5.97	7.82^{a}	5.68^{a}	8.57^{a}	7.54^{a}	5.35^{b}	4.50^{b}	9.47^{a}	4.63^{c}	7.27^{b}	7.58^{a}	4.47^{b}	4.80^{b}	2.16^{b}	4.64^{a}	4.71^{a}
	Camphor	1.48	1.19^{b}	2.29^{a}	2.64^{a}	1.09^{c}	2.24^{b}	3.49^{a}	1.65^{b}	1.90^{b}	5.36^{a}	1.77^{b}	2.31^{b}	7.65^{a}	1.25^{c}	4.68^{b}	7.67^{a}
	(Z)-7-decen-5-olide	nd	nd	0.44^{a}	nd	nd	0.46^{a}	nd	nd	nd	nd	nd	1.09^{a}	nd	nd	0.62^{a}	nd
	Total	847.35	659.35^{b}	764.01^{b}	1093.27^{a}	586.58^{b}	541.29^{b}	760.05^{a}	629.25^{b}	455.13^{c}	868.70^{a}	527.29^{b}	392.38^{c}	820.38^{a}	462.02^{b}	375.26^{c}	679.86^{a}

Values are mean ± standard deviation of three replicates (n = 3). Different lowercase superscripts in the same row at the same day indicate significant difference at $p < 0.05$. nd = not detected. All compounds were quantified as 3-octanol equivalents, and expressed in μg/kg.

Table 3. Changes in bound volatile compounds in nectarine fruits during postharvest storage at 1, 5, and 8°C.

Category	Compounds	0 d	7 d			14 d			21 d			28 d			35 d		
			1°C	5°C	8°C	1°C	5°C	8°C	1°C	5°C	8°C	1°C	5°C	8°C	1°C	5°C	8°C
Monoterpene	Linalool	14.02	15.97^{b}	17.56^{b}	25.62^{a}	24.30^{a}	14.85^{b}	13.72^{b}	16.65^{a}	14.51^{a}	11.70^{b}	13.30^{a}	13.71^{a}	11.18^{a}	11.44^{a}	11.79^{a}	12.68^{a}
	(E)-linalool oxide	8.68	4.75^{b}	7.66^{ab}	9.97^{a}	5.56^{a}	6.55^{a}	4.82^{a}	4.65^{a}	5.88^{a}	4.44^{a}	2.36^{c}	4.31^{a}	3.45^{b}	2.61^{b}	6.99^{a}	3.35^{ab}
	(Z)-linalool oxide	nd	nd	nd	9.84^{a}	12.79^{a}	10.43^{a}	5.79^{b}	nd	6.13^{a}	5.86^{a}	5.69^{a}	nd	6.32^{a}	0.80^{b}	2.29^{b}	5.82^{a}
	α-Terpineol	0.53	nd	1.25^{a}	1.71^{a}	nd	1.48^{a}	1.68^{a}	2.31^{a}	1.11^{b}	1.10^{b}	nd	1.72^{a}	3.77^{a}	3.80^{a}	3.75^{a}	4.32^{a}
	Lilac aldehyde A	98.36	49.18^{a}	57.85^{a}	117.01^{a}	36.54^{a}	51.32^{a}	47.48^{a}	62.80^{a}	56.15^{a}	53.10^{a}	47.30^{a}	48.18^{a}	41.11^{a}	35.80^{a}	nd	30.47^{a}
	Lilac aldehyde B	57.84	66.27^{b}	nd	121.01^{a}	41.26^{a}	30.21^{a}	35.20^{a}	34.79^{a}	38.46^{a}	39.76^{a}	28.68^{a}	39.19^{a}	36.94^{a}	32.61^{a}	35.31^{a}	39.97^{a}
	Lilac aldehyde C	74.15	95.44^{a}	72.23^{b}	76.44^{ab}	49.96^{a}	68.14^{a}	64.53^{a}	35.09^{b}	42.42^{b}	73.92^{a}	nd	46.29^{a}	14.18^{b}	nd	48.30^{a}	15.24^{b}
	Lilac aldehyde D	64.25	25.66^{b}	27.87^{b}	62.29^{a}	nd	30.27^{a}	nd	nd	20.12^{a}	16.78^{a}	15.01^{a}	14.41^{a}	16.06^{a}	15.77^{a}	13.84^{a}	13.36^{a}
	Neral	1.52	1.88^{b}	1.86^{b}	3.39^{a}	4.20^{a}	3.20^{a}	2.81^{a}	3.45^{a}	3.54^{a}	1.48^{b}	2.96^{a}	3.06^{a}	3.12^{a}	1.09^{b}	3.66^{a}	3.15^{a}
	Geranial	1.61	3.71^{b}	3.06^{b}	7.02^{a}	5.03^{a}	3.34^{b}	4.03^{ab}	5.47^{a}	3.65^{b}	2.65^{b}	5.01^{a}	3.66^{ab}	2.81^{b}	2.84^{a}	3.91^{a}	2.94^{a}
	(+)-4-Carene	nd	2.68^{a}	nd	nd	2.02^{a}	nd	nd	3.03^{a}	nd	nd	1.64^{a}	nd	nd	2.01^{a}	nd	nd
	Total	320.96	265.54^{b}	189.34^{c}	434.3^{a}	181.66^{a}	219.79^{a}	180.06^{a}	168.24^{a}	191.97^{a}	210.79^{a}	121.95^{b}	174.53^{a}	138.94^{b}	108.77^{a}	129.84^{a}	131.30^{a}
Alcohol	Benzyl alcohol	79.71	nd	45.99^{b}	56.53^{a}	6.23^{c}	53.72^{a}	26.17^{b}	20.07^{a}	5.54^{b}	31.18^{a}	41.93^{a}	nd	30.47^{a}	5.28^{b}	5.67^{b}	19.64^{a}
	Phenethyl alcohol	15.57	16.10^{a}	22.67^{a}	16.99^{a}	21.80^{a}	26.65^{a}	25.60^{a}	24.18^{a}	24.05^{a}	19.91^{a}	31.53^{a}	23.03^{a}	20.88^{a}	19.44^{a}	18.65^{a}	12.83^{a}
	2-Ethylhexanol	5.38	nd	10.71^{a}	nd	nd	10.56^{a}	5.53^{b}	10.20^{b}	14.93^{a}	1.45^{c}	16.36^{a}	20.39^{a}	1.41^{b}	3.43^{b}	8.90^{a}	1.11^{b}
	Isoamylol	1.87	3.00^{b}	8.76^{a}	1.49^{b}	4.40^{b}	12.38^{a}	1.21^{c}	nd	6.39^{a}	1.88^{b}	12.61^{a}	13.65^{a}	4.14^{b}	4.76^{b}	9.12^{a}	3.75^{b}
	Total	102.53	19.10^{b}	88.13^{a}	75.01^{a}	32.43^{b}	103.31^{a}	58.51^{b}	54.45^{a}	50.91^{a}	54.42^{a}	102.43^{a}	57.07^{b}	56.90^{b}	32.91^{a}	42.34^{a}	37.33^{a}
Aldehyde	Benzaldehyde	1085.60	2002.44^{b}	1767.32^{b}	2914.05^{a}	2584.90^{a}	2398.92^{a}	2095.90^{a}	2095.60^{a}	1980.67^{a}	1797.68^{a}	2007.62^{a}	1834.65^{a}	1704.59^{a}	1600.66^{a}	1682.36^{a}	1671.61^{a}
	Phenylacetaldehyde	5.13	5.15^{b}	12.97^{a}	11.45^{a}	12.08^{b}	20.37^{a}	6.88^{b}	10.48^{ab}	16.18^{a}	6.90^{b}	9.19^{b}	14.17^{a}	8.82^{b}	8.90^{a}	13.66^{a}	8.32^{a}
	Nonanal	4.18	8.41^{a}	18.57^{a}	8.66^{a}	14.87^{a}	14.56^{a}	8.89^{b}	20.37^{a}	8.80^{b}	3.15^{b}	10.29^{a}	3.95^{b}	1.94^{c}	6.99^{a}	3.27^{b}	1.17^{c}
	Decanal	8.68	7.76^{a}	2.08^{b}	5.98^{a}	7.20^{a}	1.84^{b}	2.88^{ab}	4.09^{a}	1.79^{a}	2.14^{a}	1.59^{b}	1.75^{b}	4.29^{a}	2.10^{a}	3.37^{a}	2.07^{a}

	Salicylaldehyde	3.77	nd	nd	nd	3.12^{a}	nd	nd	2.53^{a}	nd	nd	3.47^{a}	nd	nd	nd	nd	nd
	2, 4-dimethyl benzaldehyde	nd	5.27^{a}	nd	nd	12.91^{a}	nd	nd	8.73^{a}	nd	nd	10.46^{a}	nd	nd	1.56^{a}	nd	nd
	Heptaldehyde	nd	0.33^{a}	nd	nd	nd	nd	nd	4.55^{a}	nd	nd	4.66^{a}	nd	nd	1.51^{a}	nd	nd
	Total	1107.36	2029.36^{b}	1800.94^{b}	2940.14^{a}	2635.08^{a}	2435.69^{a}	2114.55^{a}	2146.35^{a}	2007.44^{a}	1809.87^{a}	2047.28^{a}	1854.52^{a}	1719.64^{a}	1621.72^{a}	1702.66^{a}	1683.17^{a}
Phenol	Chavicol	1.33	2.15^{b}	2.64^{b}	10.45^{a}	4.48^{a}	2.80^{a}	2.81^{a}	4.90^{a}	2.11^{a}	nd	5.81^{a}	2.33^{b}	2.62^{b}	2.62^{b}	3.34^{b}	5.04^{a}
	Isoeugenol	1.25	0.90^{b}	1.51^{ab}	2.25^{a}	2.21^{a}	1.18^{b}	3.25^{a}	3.53^{a}	1.74^{b}	nd	2.27^{a}	1.55^{a}	0.77^{a}	2.76^{b}	2.82^{b}	3.76^{a}
	M-eugenol	55.98	35.85^{a}	40.42^{a}	36.40^{a}	72.54^{a}	43.38^{a}	58.37^{a}	92.69^{a}	38.27^{b}	35.53^{b}	101.75^{a}	38.68^{b}	nd	104.13^{a}	20.87^{b}	nd
	Trans-isoeugenol	4.08	2.92^{b}	2.68^{b}	9.40^{a}	4.87^{a}	1.97^{b}	2.13^{b}	3.71^{a}	1.87^{a}	nd	3.63^{a}	2.69^{a}	1.49^{b}	2.93^{a}	4.40^{a}	3.39^{a}
	Eugenol	1.33	nd	0.48^{a}	nd	0.80^{a}	0.51^{b}	nd	nd	nd	nd	0.27^{a}	nd	nd	0.33^{a}	nd	nd
	Carvacrol	nd	nd	nd	25.56^{a}	nd	nd	nd	nd	nd	12.61^{a}	nd	nd	2.34^{a}	nd	nd	1.44^{a}
	3-allylguaiacol	nd	nd	nd	228.45^{a}	nd	nd	64.71^{a}	nd	nd	33.84^{a}	nd	nd	56.32^{a}	nd	nd	32.87^{a}
	Total	63.97	41.82^{b}	47.73^{b}	312.51^{a}	84.90^{b}	49.84^{c}	131.27^{a}	104.83^{a}	43.99^{b}	81.98^{a}	113.73^{a}	45.25^{b}	63.54^{b}	112.77^{a}	31.43^{b}	46.50^{b}
Other	Methyl benzoate	0.39	1.32^{a}	0.45^{a}	nd	2.69^{a}	1.39^{a}	0.97^{a}	nd	1.80^{a}	0.88^{b}	0.99^{b}	1.79^{a}	1.36^{ab}	2.28^{a}	1.52^{a}	1.68^{a}
	Acetophenone	nd	0.26^{a}	nd	nd	1.15^{a}	nd	nd	nd	nd	nd	1.24^{a}	nd	nd	nd	nd	nd
	3-Octanone	7.64	4.97^{a}	nd	nd	5.44^{a}	nd	nd	nd	nd	nd	3.00^{a}	nd	nd	nd	nd	nd
	Isovaleric acid	2.23	14.18^{a}	19.95^{a}	nd	16.22^{a}	25.21^{a}	nd	42.06^{a}	22.88^{b}	nd	47.44^{a}	23.03^{b}	nd	50.62^{a}	31.01^{b}	nd
	2-Methylbutyric acid	nd	nd	nd	nd	nd	nd	nd	3.91^{a}	nd	nd	4.83^{a}	nd	nd	4.39^{a}	nd	nd
	Total	1605.08	2376.55^{b}	2146.54^{b}	3761.95^{a}	2959.57^{a}	2835.23^{a}	2485.36^{a}	2519.84^{a}	2318.99^{a}	2157.94^{a}	2442.89^{a}	2156.19^{ab}	1980.38^{b}	1933.46^{a}	1938.80^{a}	1899.98^{a}

Values are mean ± standard deviation of three replicates (n = 3). Different lowercase superscripts in the same row at the same day indicate significant difference at $p < 0.05$. nd = not detected. All compounds were quantified as 3-octanol equivalents, and expressed in μg/kg.

1°C, while this compound showed a decreasing trend at 5 and 8°C during the entire storage. The contents of phenethyl alcohol increased before 28 d (1°C) or 21 d (5 and 8°C), and then decreased thereafter; no significant differences were observed among different groups.

Total aldehydes showed a tendency of increasing at first and then decreased thereafter. Aldehydes were the most abundant bound volatile compounds detected in the nectarine fruits. Benzaldehyde appeared to be one of the major aldehydes in this cultivar, accounting for approximately 90% of the total bound aldehydes; its concentration peaked at 14 d (1 and 5°C) or 7 d (8°C), and then decreased progressively thereafter.

Total phenols decreased at first 7 d, and then remained constant thereafter at 1 and 5°C. However, an increase at 7 d and a gradual decrease afterwards were observed at 8°C. Among them, m-eugenol was the most abundant.

Methyl benzoate was the only ester identified in bound form in nectarine fruits. The contents at 1 and 5°C were similar with the free form at 1 and 5°C, while the contents of free form at 8°C were much higher than the bound form after 14 d storage.

Changes in β-Glu activity and gene expression of PpUGT in nectarine fruits during storage

The changes in *β*-Glu activity during storage are shown in Figure 1A. The *β*-Glu activities first increased at the three temperatures, and then decreased at later storage times. The *β*-Glu activities at 1°C were significantly lower than those at 5 and 8°C after 21 d of storage. During cold storage, *Pp*UGT transcript levels in nectarine fruits peaked at 7 d (8°C) or 14 d (1°C), and then decreased thereafter (Figure 1B). The relative expression of *Pp*UGT fluctuated before 21 d of storage, and decreased afterwards at 5°C, and the expression of *Pp*UGT at 1°C was lower than that at 5 and 8°C after 14 d of storage.

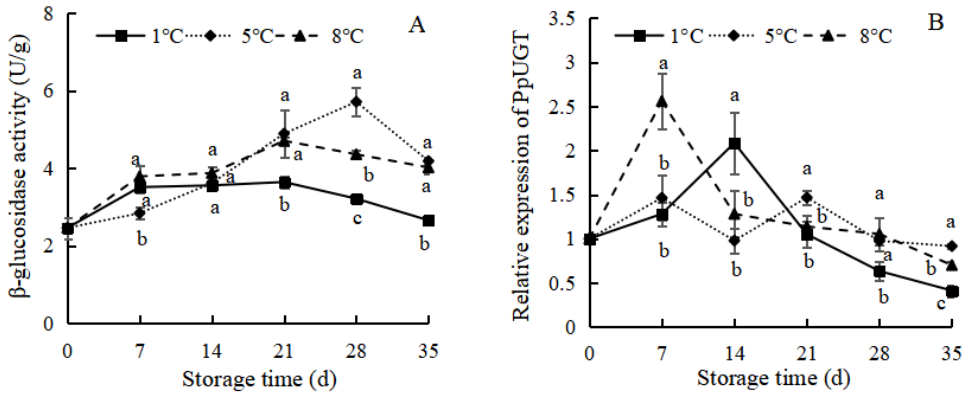

Figure 1. (A) The activities of β-glucosidase in nectarine fruits during cold storage, and **(B)** the relative expression of *Pp*UGT in nectarine fruits during cold storage. Error bars indicate ± SD.

Discussion

Marketing research has shown that consumers purchase fruits based on their appearance, and that they take the fruit quality and aroma into consideration (Wang *et al*., 2020b). Fruit quality is generally measured in terms of texture, total soluble solids, and titratable acidity (Zhang *et al*., 2011). Nectarine fruits are sensitive to low-temperature storage which can induce physiological disorders. In the present work, internal browning, the most apparent symptom, was observed on the flesh of nectarine fruits during storage at 5 and 1°C. Similar results were also found in peach fruit; similar to many fruits that are sensitive to low-temperature storage, chilling injuries occur after cold storage (Wang *et al*., 2020c). During storage, ascorbic acid contents decreased progressively which was consistent with a previous study in nectarine fruit (Aubert *et al*., 2014). The carotenoid contents significantly increased during storage at 1 and 8°C, and with the development of chilling injury, the carotenoid contents decreased after 21 d of storage at 5°C.

As aroma is one of the most appreciated components influencing fruit flavour, and volatile compounds play a crucial role in consumer acceptance. As nectarine fruit softens quickly during storage at room temperature, low temperature is an effective and common way to prolong the storage time. However, effects from low-temperature stress

may cause the loss of aroma. The present work demonstrated that different storage temperatures had different impacts on nectarine fruits' volatiles during postharvest storage. Generally, the contents of total free volatiles tended to decrease gradually during low-temperature storage, which is consistent with a previous study in nectarine fruit (Aubert *et al*., 2014). During postharvest storage, the contents of these so-called 'green aromas' with a 'grassy' flavour, especially hexanol and hexanal, decreased gradually regardless of temperature. High contents of C6 aldehydes were observed throughout the storage time, which was consistent with a previous study in peach fruit (Zhang *et al*., 2011). It is worth noting that (E)-2-hexanal and hexenal decreased faster at 8°C than at 1 and 5°C at the end of storage (21 - 35 d), and that the hexenal contents at 8°C were significantly lower than that at 1 and 5°C. In the present work, the ability of nectarine fruits to accumulate lactones and esters was maintained at 8°C. After 14 d of storage, chilling injury developed at 5°C, and lower contents of lactones and esters were observed. With storage time processing, chilling injury also developed at 1°C, and the same case was observed, which was consistent with the results previously reported by Aubert *et al*. (2014). As earlier mentioned, a sharp decline in C6 aldehydes and lower contents of hexanol and hexanal were observed in nectarine fruits stored at 8°C, and higher contents of esters were detected in 8°C-stored nectarine fruits. This indicated that 8°C promoted the conversion of aldehydes and alcohol into esters through the lipoxygenase pathway. Linalool is one of the major compounds in mature nectarine fruits, and the typical terpene is abundant in nectarine fruits and some juicy honey peaches (Li *et al*., 2015). In the present work, the contents of linalool significantly decreased during storage regardless of temperature, and nectarine fruits stored at 8°C had higher contents of linalool than those stored at 1 and 5°C, which was consistent with the results previously reported by Aubert *et al*. (2014).

Fruit aroma is determined mainly by the aroma compounds in the free and glycosidically bound forms. Aroma compounds can be liberated from glycosides by acid or enzymatic hydrolysis during maturation and postharvest storage. The present work showed that glycosidically bound volatiles increased at early storage, and decreased progressively thereafter at the three tested temperatures. Results showed that terpenes, alcohols, aldehydes, and phenols were present in nectarine fruits in bound form, but only eight of them were found to exist in both free and bound forms, namely linalool, benzaldehyde, phenylacetaldehyde, decanal, nonanal, methyl benzoate, 2-ethylhexanol, and 3-octanone. Most of them are powerful potential sources of aroma as they could occur at higher concentrations than their odour thresholds such as linalool (6 μg//kg), benzaldehyde (350 μg//kg), phenylacetaldehyde (4 μg//kg), methyl benzoate (0.52 μg//kg), and decanal and nonanal (1 and 2 μg//kg, respectively) (Garcia *et al*., 2012; Wen *et al*., 2014; Yang *et al*., 2019). Except for nonanal, methyl benzoate, and 3-octanone, the concentrations of the other five volatiles in the bound forms were higher than those in the free forms. Great differences in the variety of free and bound volatile compounds were observed in many other studies, and linalool oxide was the only compound in both free and bound forms detected in citrus fruit (Ren *et al*., 2015a). In agreement with a previous study in other nectarine fruit (Aubert *et al*., 2003b), glycosidically bound linalool, (E)-linalool oxide, (Z)-linalool oxide, *α*-terpineol, benzaldehyde, methyl benzoate, benzyl alcohol, eugenol, chavicol, and isoeugenol were also detected in the present work. Among them, eugenol and isoeugenol are not expected to have a major contribution to the overall aroma of nectarine fruit, as their concentrations were lower than their odour thresholds (Wen *et al*., 2014). Furthermore, the present work also identified some glycosidically bound volatiles that were not previously detected in nectarine fruits. In the present work, terpenes were a major part of the bound volatile extracts. The rose-like smelling terpene and lilac aldehydes exhibited the highest concentrations in nectarine fruits after harvest. Glycosylated linalool, one of the most important volatiles with low odour thresholds, plays a significant role in contributing to flavour quality and consumer perception (Wu *et al*., 2019). In the present work, its contents were second only to lilac aldehydes. It is worth noting that no lactone was found in the enzymatic hydrolysate of nectarine fruits in the present work. In a previous study, δ-decalactone was the only lactone found in the enzymatic hydrolysates of yellow-fleshed nectarine glycosides, and the contents were much lower than those of the free form (Aubert *et al*., 2003b). This compound has also been detected as a bound compound in pineapple (Wu *et al*., 1991).

Regarding the bound esters, methyl benzoate in bound form was detected in the present work, which was consistent with the results reported by Aubert *et*

al. (2003b). Here, we found that most lactones and esters in nectarine fruits existed in free form. Indeed, lactones are characteristic volatile compounds in nectarine and peach fruits, and while they do not have the chemical function of being glycosylated, their glycosylated precursor may be a glycoconjugate of the corresponding hydroxy acid (Aubert *et al*., 2003b). In a previous study on kiwifruit (Garcia *et al.*, 2013), there were no lactones or esters found in the enzymatic hydrolysate, but esters and aldehydes in free form were major contributors to the aroma of kiwifruit. Studies illustrated that they do not occur as glycosides but are synthesised from precursors.

Considering the interaction between free and bound aromas, the activity of *β*-Glu and the relative expression of UGT-glycosyltransferase were assayed. In the present work, *β*-Glu activities first increased at the three tested temperatures, while total bound volatiles increased at early storage. It is possible that the expression of other genes involved in bound volatiles also increased. As *β*-Glu activities increased, a substantial decrease in bound volatiles was also observed, but the activities decreased at the end of storage, accompanied by a slight decrease in the contents of bound volatiles (Figure 1A). It is possible that the cell walls and membranes were degraded with storage time, which stimulated the release of *β*-Glu to contact bound volatiles, and further catalysed the deglycosylation of bound volatiles into free form (Ren *et al*., 2015b).

Regarding the changes in linalool in free and bound forms, *β*-Glu and UGT-glycosyltransferase both played important roles in the conversion of bound and free forms. In the present work, most of the linalool existed in bound form during cold storage, except for the nectarine fruits on harvest day. Interestingly, the contents of free linalool were high, but the contents of bound linalool were low in nectarine fruits stored at 8°C, especially in the middle storage (14 - 21 d).

Conclusion

In summary, chilling injury developed on ‘Ruiguang 1’ nectarine fruits stored at 5°C for approximately 14 d, and at 1°C for approximately 21 d, and was accompanied by significant decreases in free aroma volatiles. For the changes in free aromas, nectarine fruits stored at 8°C exhibited the highest contents of terpenes, lactones, and esters when compared with those stored at 1 and 5°C. However, chilling injury-inducing temperature (5°C) significantly suppressed the production of terpenes, lactones, and esters. Regarding the changes in bound aromas, most bound volatiles in nectarine fruits stored at 1 and 5°C were higher than those stored at 8°C. Together with the higher *β*-Glu activity found in nectarine fruits stored at 8°C, this indicated that 8°C might promote the conversion of bound aromas into free forms by inducing *β*-Glu activity. Notably, the contents of glycosylated linalool were higher than those of free linalool. Nectarine ruits stored at 8°C exhibited lower contents of glycosylated linalool during middle storage (14 - 21 d) than those stored at other temperatures, accompanied by higher contents of free linalool. The bound volatile compounds detected in nectarine fruits observed in the present work were quite different from the characteristic free volatiles contributing to the aroma of nectarine fruits. This suggested that bound volatile compounds are not the most important contributor to nectarine fruits’ aromas but could be a potential source of nectarine aromas.

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