

Melatonin improves the storage quality of rabbiteye blueberry (*Vaccinium ashei*) by affecting cuticular wax profile

Jia Li^{a,1}, Yaru Cao^{a,b,1}, Shicun Bian^a, Seung-Beom Hong^c, Kai Xu^a, Yunxiang Zang^{a,*}, Weiwei Zheng^{a,*}

^a Key Laboratory of Quality and Safety Control for Subtropical Fruit and Vegetable, Ministry of Agriculture and Rural Affairs, Collaborative Innovation Center for Efficient and Green Production of Agriculture in Mountainous Areas of Zhejiang Province, College of Horticulture Science, Zhejiang A&F University, Hangzhou 311300, Zhejiang, China

^b Jiaxing Vocational and Technical College, Jiaxing 314001, Zhejiang, China

^c Department of Biotechnology, University of Houston Clear Lake, Houston, TX 77058-1098, USA

ARTICLE INFO

Keywords:

Cuticular wax
Rabbiteye blueberry
Melatonin
Water loss

ABSTRACT

Cuticular wax is the first line of structural defense for plants against external stresses. This study investigated the effects of melatonin (MT) on chemical composition and accumulation profile of wax, as well as fruit quality of rabbiteye blueberry during storage. The results indicated a significant reduction in the overall wax content during storage. Nevertheless, MT effectively delayed the decline, with a higher amount of 9.8% and 15.17% in the treated 'Baldwin' and 'Garden Blue' compared to their respective controls at 21st day of storage. The wax composition significantly varied depending on storage time, MT treatment, and cultivars. Additionally, MT markedly improved the fruit quality of rabbiteye blueberries. Correlation analysis revealed water loss and decay rates were negatively correlated with triterpenoids and fatty acids. Taken together, this study highlights the positive effects of post-harvest MT application on shelf life and fruit quality of blueberry by modifying the wax profile during storage.

1. Introduction

Rabbiteye blueberry (*Vaccinium ashei*) is a widely consumed berry around the world, prized for its health-promoting bioactive compounds such as phenolic acids, flavonoids, and procyanidins (Reque et al., 2016). Global production of blueberries has risen from 439,000 tons in 2010 to over 1 million tons in 2020, with the cultivation area reaching 126,146.6 ha in 2021, reflecting its significant economic value (<http://www.internationalblueberry.org/2022-report/>). However, economic losses due to the water evaporation and other physiological disorders during storage are commonly observed in blueberries (Chu, Gao, Chen, Fang, & Zheng, 2018a; Chu, Gao, Chen, Wu, & Fang, 2018b). Recent studies have shown that the quality of postharvest fruit is closely linked to the composition and structure of cuticular wax (Lara, Belge, & Goulao, 2015; Li, Min, Song, Shao, & Zhang, 2017), which has capacity to reduce the infestation of microbial pathogens, postharvest softening, and decay rate of the fruit (Chu et al., 2018a; Dimopoulos et al., 2020; Xiao, 2017).

Cuticular wax is a crucial factor in maintaining the quality of blueberry fruit because it covers the epidermal cells, forming a water-repellent surface that reduces non-stomatal water loss and prevents the formation of a water film on which microbial pathogens can be deposited to germinate or multiply. Previous studies have demonstrated that cuticular wax is a complex mixture of very long chain fatty acids (VLCFAs) and their derivatives, including diketones, alkanes, aldehydes, esters, ketones, primary and secondary alcohols, as well as secondary metabolites such as triterpenoids (Chu et al., 2017). Triterpenoids and diketones were found to be the predominant wax components in *Vaccinium ashei* (Cao et al., 2022). The composition of cuticular wax varies among different cultivars and organs, as well as across developmental stages of the same organ (Cao et al., 2022; Zhang et al., 2021). Biosynthesis of cuticular wax usually begins with the *de novo* synthesis of C16–C18 fatty acids by β -ketoacyl-ACP synthase (KAS) in plastids. They are then elongated into VLCFAs ranging from C20 to C34 by β -ketoacyl-CoA-synthase (KCS) in endoplasmic reticulum (ER). Finally, VLCFAs are modified to produce primary and secondary alcohols via the acyl

* Corresponding authors.

E-mail addresses: yxzang@zafu.edu.cn (Y. Zang), zhengww@zafu.edu.cn (W. Zheng).

¹ These authors contributed equally to this work.

reduction pathway, and other aliphatic compounds such as alkanes, aldehydes, and ketones through decarbonylation pathway. The first step in the biosynthesis of triterpenoids involves cyclization of the 30-carbon precursor 2,3-oxidosqualene by members of the oxidosqualene cyclase (OSC) family to produce lupeol, α -amyrin and β -amyrin. Subsequently, C-28 oxidation is catalyzed by cytochrome P450 (CYP) to generate ursolic acid (UA), oleanolic acid (OA), and betulinic acid (BA) (Zhang, You, Li, & Hao, 2020b).

Melatonin (MT; *N*-acetyl-5-methoxytryptamine) is a ubiquitous indoleamine compound that can reduce the rate of respiration and ethylene release from horticultural products, scavenge reactive oxygen radicals, and delay fruit senescence, thereby extending the storage and shelf life (Gao et al., 2016; Tang et al., 2020). MT has been reported to enhance the quality and antioxidant activities of fruit, as well as increase the level of cuticular wax in plants (Ding, Wang, Wang, & Zhang, 2018; Shang et al., 2021). Exogenous MT application has also been linked to thicker exocarp tissues, caused by a dense epidermal wax layer in mangoes, pears, sweet cherry and apples (Dong et al., 2021; Fekry, Rashad, Alaraidh, & Mehany, 2021; Michailidis et al., 2021; Onik et al., 2021). Our previous study showed that exogenous MT application increased the content of total cuticular wax and certain wax constituents during fruit development in rabbiteye blueberries (Cao et al., 2022; Li et al., 2016). However, it remains unclear whether post-harvest MT application affects the composition and content of cuticular wax in blueberry fruit during storage. To address this gap, we investigated the effect of post-harvest MT application on cuticular wax accumulation in two rabbiteye blueberry cultivars during storage and examined the impact of MT treatment on fruit quality characteristics. Our results will provide valuable insights into enhancing the post-harvest quality of blueberries for commercial fruit development and utilization.

2. Materials and methods

2.1. Plant material

This study was conducted in 2020 using 10-year old rabbiteye blueberry cv. 'Baldwin' and 'Garden Blue' in Zhejiang A&F University, China. Six kilogram of fruit with uniform size and color without physical damages were harvested at S4 phenological stage of maturity and soaked in 100 μ M MT (Beijing Sola Biotechnology Co., Ltd.) or distilled water (served as the control) for 30 min. Following immersion, the fruit were dried for 30 min at room temperature and were then stored at 4 °C and 45 % relative humidity for analysis. The concentration and soaking time of MT was adopted based on the previous study (Cao et al., 2022).

2.2. Determination of wax content and wax compounds by GC–MS

Total wax load and chemical compositions were analyzed by GC–MS according to Cao et al. (Cao et al., 2022). Briefly, 20 blueberries were immersed in 40 mL chloroform for 2 min, and the extracted solution was dried and derivatized with equal amounts of pyridine and bis-*N*,*O*-(trimethylsilyl) trifluoroacetamide (BSTFA; Alfa) for 40 min at 70 °C. The mixture was dried under the stream of nitrogen, redissolved in chloroform, and filtered through a 0.2 μ m membrane. The filtered solution was run through a HP-5 MS capillary column (30 m, 0.25 mm i. d. cross-linked 100 % dimethyl polysiloxane, 0.25 μ m film thickness, Agilent Technologies) at a 1.0 mL/min flow rate of carrier gas (helium). The initial oven temperature was 70 °C, increasing to 200 °C at a rate of 10 °C/min and held for 2 min, followed by 290 °C at a rate of 3 °C/min, 310 °C at a rate of 2 °C/min, and finally kept at 310 °C for 20 min.

n-Tetracosane (C24) was added to the mixture as an internal standard. Injection, MS transfer line, and ion source temperatures were 250 °C, 250 °C and 270 °C, respectively. The split ratio of carrier gas was 10:1 (v/v). Electron impact (EI) mass spectra were obtained with a JMS-AX 505 HAD spectrometer (JEOL) at an ionization voltage of 70 eV. The mass spectrum data were collected in the full scan mode (*m/z*

50—850). Wax constituents were identified either by using the NIST.8.0 Library SW (version 2011) or by comparing their mass spectra and retention times with those of generic standards. Triterpenoids including ursolic acid, oleanolic acid, lupeol and betulinic acid were quantified using the external standard method based on calibration curves. All measures in this study were repeated 3 times.

2.3. Determination of fruit quality parameters

The fruit surface area, fruit weight, water loss, and decay rates were determined as described by Cao et al. (Cao et al., 2022). The equatorial and polar diameters of each fruit were measured using a digital vernier caliper (Guanglu Measuring Instrument, Guilin, China). Total soluble solids (TSS) content was measured using a digital refractometer (PAL-1, Atago, Tokyo, Japan). The fruit surface *L** (lightness), *a** (change of greenness to redness) and *b** (change of blueness to yellowness) were measured using a Minolta CR-400 colorimeter. Titratable acidity (TA) was determined by titration with 0.1 M NaOH. Total phenolics, flavonoids, and total anthocyanins content was measured according to the method of Shang et al. (Shang et al., 2021). Malondialdehyde content and relative conductivity were measured as previously described by Xu et al. (Xu, Chen, & Kang, 2019). All measures in this study were repeated 3 times with 10 blueberry fruits per replicate.

2.4. Statistical analysis

Statistical analyses of the data were performed using SPSS software V20.0 (IBM Corp, Armonk, USA). The data were analyzed using Student's *t*-tests, and the differences were considered significant at *p* < 0.05. All data in this study were shown as the means \pm standard error calculated from three biological replicates.

2.5. The kinetic model

In general, the deterioration of foods could be expressed in zero- or first order as follows. Zero-order $A_t = A_0 + k_a \cdot t$. First order $A_t = \exp(A_0 + k_a \cdot t)$, where A_t and A_0 are the quality indices at time *t* and zero, respectively. k_a represents the kinetic rate constant, and *t* is the storage time (day). The order of the reaction rate was determined by performing regression with the ideal coefficient of determination (R^2) at each treatment and by comparing total sums of determination coefficients among different reactions results (Liu, Qin & Lou, 2016).

3. Results and discussion

3.1. Effect of MT on wax content during storage

According to Fig. 1A, the total wax content varied between the 'Baldwin' and 'Garden Blue' in the absence of MT treatment during the 35 d storage period. This finding is consistent with a previous study comparing 'Baldwin' and 'Brightwell' (Cao et al., 2022). Regardless of MT application, the total wax content of 'Garden Blue' cultivar gradually decreased during storage at 4 °C, whereas that of 'Baldwin' cultivar sharply declined during the first 7 d of storage and then gradually decreased over the next 28 d. A similar result was previously reported for 'Brightwell' cultivar during storage at 4 °C (Chu et al., 2018b). Overall, the 'Baldwin' cultivar showed a more rapid decline in total wax content (57.14 %) than the 'Garden Blue' cultivar, which experienced a 36.54 % decline at 35th d of storage compared to 0 d. The increase in wax content induced by MT treatment began at 14th d of storage, peaked at 21st d of storage, and then slowly decreased over time. This led to a delayed decline in the total wax content in both cultivars after 14 d of storage.

The detection of various individual wax compounds, including triterpenoids, diketones, fatty acids, alkanes, esters, and primary alcohols were shown in Fig. 1B-G. Prior to storage, triterpenoids were the most

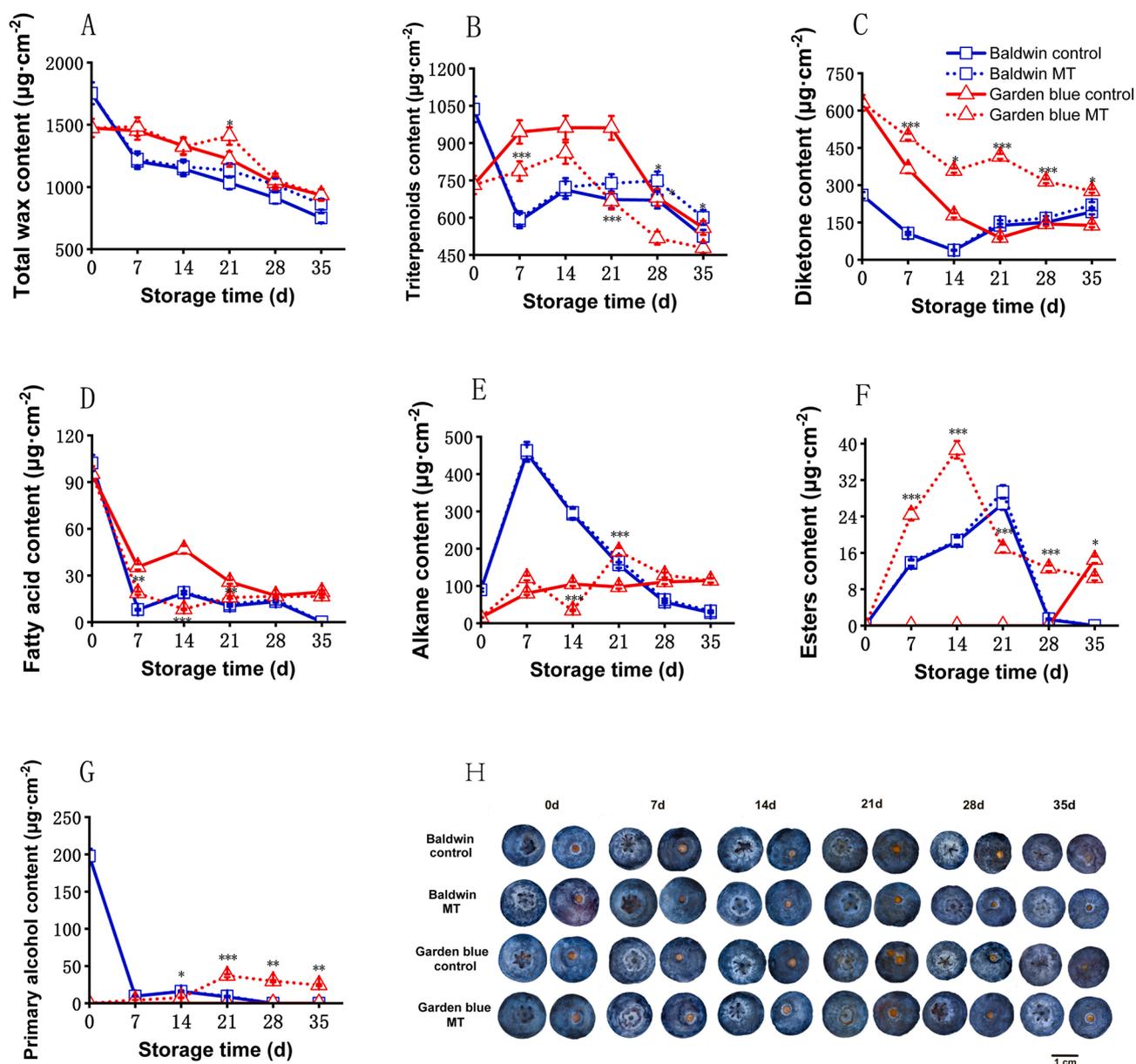


Fig. 1. MT affects the total wax contents and wax components of rabbiteye blueberry fruit during storage. Total wax content (A); Triterpenoids content (B); Diketone content (C); Fatty acid content (D); Alkane content (E); Esters content (F); Primary alcohol content (G); Photos of fruits at different storage time (H). Abbreviations: MT, melatonin. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

plentiful wax components accounting for 59.08 % and 49.65 % of the total wax content of 'Baldwin' and 'Garden Blue', respectively, followed by diketones, which represented 14.80 % and 42.83 % of the total wax content of 'Baldwin' and 'Garden Blue', respectively. Similar observations were previously noted in other cultivars (Cao et al., 2022; Chu et al., 2019; Chu et al., 2018b). The contents of terpenoids in plum fruit (Huang et al., 2022) and 'Powderblue' blueberry have been reported to exhibit an increasing-decreasing pattern (Jiang et al., 2022). Our study also observed a similar trend, with the highest content of triterpenoids occurring at day 14, accounting for 62.18 % and 72.18 % of the total wax content in the controls of 'Baldwin' and 'Garden Blue', respectively. However, the effect of MT treatment on triterpenoids load in the fruit of the two cultivars differed significantly in that MT treatment increased the content of triterpenoids in 'Baldwin' by $76.49 \mu\text{g}/\text{cm}^2$ but decreased it in 'Garden Blue' by $294.66 \mu\text{g}/\text{cm}^2$ at day 21 of storage (Fig. 1B). Interestingly, the ratio of triterpenoids to total wax increased to 80.99 % and 76.38 % in 'Baldwin' and 'Garden Blue', respectively, at day 35. This

suggests that triterpenoids may become a more component of the wax in blueberry during later stages of storage. A similar outcome was noted in the case of plum (Zhu et al., 2023), where triterpenes exhibited their highest content at 20d of storage.

In 'Garden Blue' control, the accumulation pattern of diketones showed a decreasing trend, leading to 78.17 % reduction after 35 d of storage (Fig. 1C). MT treatment significantly increased the diketones content of 'Garden Blue' throughout the storage period; however, no significant difference in diketones content was observed between the MT-treated and untreated 'Baldwin'. In contrast to diketones, the content of fatty acids in both cultivars sharply decreased during the 7 d of storage, resulting in a 92.43 % and 62.76 % reduction in 'Baldwin' and 'Garden Blue', respectively (Fig. 1D). This accumulation pattern of fatty acids is different from that of apple fruit, which exhibited an increase in fatty acids content during storage (Chai et al., 2020; Lin et al., 2022). This discrepancy could be attributed to be the genetic difference between the fruit (Sater, Bizzio, Tieman, & Munoz, 2020). Upon MT

treatment, fatty acids content of 'Baldwin' fruit remained unchanged throughout the storage period, whereas that of 'Garden Blue' fruit decreased by 82.09 % at day 28. These results suggest that the two cultivars may differently respond to chemical treatment during storage, which could have implications for food preservation and shelf life. A significant decline of fatty acids was also found in MT-treated plum fruit during cold storage (Lin et al., 2022).

Notably, the contents of primary alcohols and alkanes differed between the two cultivars before storage, with 'Baldwin' containing 197.67 $\mu\text{g}/\text{cm}^2$ of primary alcohols and 89.16 $\mu\text{g}/\text{cm}^2$ of alkanes, whereas 'Garden Blue' had 0 $\mu\text{g}/\text{cm}^2$ of primary alcohols and 15.55 $\mu\text{g}/\text{cm}^2$ of alkanes. Accumulation patterns of alkanes, primary alcohols and esters greatly differed between the two cultivars depending on storage time and MT treatment (Fig. 1E, F and G). The deposition pattern of alkanes in 'Baldwin' fruit showed an increasing trend followed by a decreasing trend, reaching the highest level of 37.85 % of the total wax content at day 7, regardless of MT treatment. In contrast, 'Garden Blue' slowly increased the content of alkanes, reaching 12.25 % of the total wax after 35 d of storage. The effect of MT treatment on the alkanes content of both cultivars appeared to be minimal. Likewise, a minimal impact on alkanes was observed in MT-treated plums on day 20, but subsequently, the inhibitory effect became more pronounced, extending throughout the duration of cold storage (Lin et al., 2022). In 'Garden Blue' control, esters were detected only at day 35. However, their synthesis was induced 7 days after MT treatment, resulting in a peak deposition of 38.66 $\mu\text{g}/\text{cm}^2$ at day 14, followed by a decline to 10.48 $\mu\text{g}/\text{cm}^2$ at day 35. Meanwhile, there was no effect of MT on the accumulation pattern of esters in 'Baldwin', which displayed a trend of increment peaking at day 21 followed by a rapid decrement. Like esters, primary alcohols were undetectable in 'Garden Blue' control, but their synthesis was induced upon MT treatment. In 'Baldwin', there was no effect of MT on the accumulation pattern of primary alcohols, which rapidly decreased to 95.11 % within 7 d of storage. Taken the results together, there appears to be cultivar-specific effects of MT on the contents of individual wax constituents.

3.2. Effect of MT on the contents of triterpenoids fractions during storage

Triterpenoids are important plant secondary metabolites derived

Table 1
MT affects the contents ($\mu\text{g}/\text{cm}^2$) of key cuticular wax in 'Baldwin' during storage.

Compound	Retention time	0d			7d		14d		21d		28d		35d	
		Control	Control	MT										
Alkanes														
Hexadecane	17.34	8.4	3.18	3.23	21.47	21.82	17.45	19.17	3.8	4.24	1.54	1.77		
Heptadecane	17.8	/	2.31	2.34	11.27	11.45	2.01	2.21	0.5	0.56	1.76	2.01		
Octadecane	18.56	5.05	7.24	7.36	38.7	39.33	2.07	2.27	0.69	0.77	/	/		
Eicosane	20.32	2.95	15.7	15.95	14.92	15.16	9.99	10.97	3.03	3.38	1.36	1.56		
Heneicosane	21.8	/	146.48	148.83	13.97	14.2	31.48	34.57	12.02	13.42	1.64	1.88		
Tetracosane	22.61	/	6.15	6.25	10.93	11.11	9.93	10.91	1.87	2.09	1.46	1.68		
Heptacosane	25.94	/	62.95	63.96	5.81	5.9	13.01	14.29	4.86	5.42	1.68	1.92		
Octacosane	27.69	10.42	51.04	51.86	10.76	10.93	21.21	23.29	2.45	2.73	5.01	5.74		
Hentriacontane	32.67	7.25	127.63	129.67	4.4	4.47	8.15	8.95	6.44	7.18	3.8	4.35		
Tetracontane	34.32	4.52	5.52	5.61	10.02	10.18	9.32	10.24	7.47	8.33	3.47	3.97		
Diketones														
3-Azabicyclo[3.2.1]octane-2,4-dione	53.24	/	105.34	107.03	22.65	23.01	68.27	74.98	87	97.09	143.93	164.92		
Fatty acids														
Hexadecanoic acid	20.11	46.97	2.78	2.82	13.36	13.58	8.94	9.81	4.54	5.07	0.13	0.15		
Octadecanoic acid	22.66	/	5.17	5.25	5.42	5.5	1.5	1.65	1.32	1.47	/	/		
Triterpenoids														
.beta.-Amyrin	53.98	621.51	289.62	294.26	347.72	353.32	382.93	420.58	361.75	403.71	293.04	335.78		
.alpha.-Amyrin	55.96	129.76	64.23	65.26	72.14	73.3	72.95	80.13	90.38	100.87	86.36	98.95		
Ursolic acid	55.67	43.9	206.52	209.83	258.56	262.72	183.28	201.3	172.6	192.62	115.02	131.79		
Oleanolic acid	56.28	60.95	4.77	4.85	7.47	7.59	6.81	7.48	14.27	15.92	7.19	8.24		
Lupeol	56.56	19.87	16.6	16.87	20.14	20.46	20.69	22.72	25.29	28.23	18.22	20.87		
Betulinic acid	58.48	160.01	5.17	5.25	5.53	5.62	5.68	6.24	6.44	7.19	4.67	5.36		

Note: Each value represents the mean of three replicates (-, undetectable). Abbreviations: MT, melatonin

from squalene, a C30 triterpene hydrocarbon (Noushahi et al., 2022), and they have various pharmacological activities such as anti-cancer, anti-virus, anti-inflammatory, and cholesterol-lowering (Perveen, 2018). In our study, six triterpenoids, namely β -amyrin, α -amyrin, ursolic acid, oleanolic acid, lupeol, and betulinic acid, were detected from 'Baldwin' and 'Garden Blue' by comparing the retention times and mass spectra of known substances and standards in the database (Table 1 and 2). The most abundant triterpenoid in both cultivars was β -amyrin, which accounted for 35.44 % and 23.03 % of the total wax, and 59.99 % and 46.39 % of the total triterpenoids of 'Baldwin' and 'Garden Blue' before storage, respectively. Interestingly, this finding contradicts a previous report that ursolic or oleanolic acid was the most abundant triterpenoids in the fruit of nine other blueberry cultivars (Chu et al., 2017). The discrepancy could be ascribed to the cultivar specificity and/or differing degree of fruit maturity. The second most abundant triterpenoids were betulinic acid in 'Baldwin' and ursolic acid in 'Garden Blue', comprising 15.44 % and 32.74 % of the total triterpenoids, respectively. The content of β -amyrin in 'Garden Blue' gradually decreased during storage, while it increased and then decreased in 'Baldwin'. Similar patterns were noted for α -amyrin in 'Garden Blue' and ursolic acid in 'Baldwin'. On the other hand, the levels of oleanolic acid, lupeol, and betulinic acid in both cultivars remained relatively low during storage. It is notable that unlike other wax constituents, all components of triterpenoids were present in both cultivars throughout the storage time, regardless of MT treatment.

MT was previously shown to significantly increase the levels of α -amyrin, oleanolic acid, lupeol, and betulinic acid during the development of 'Baldwin' fruit (Cao et al., 2022). Similarly, in this study, MT treatment induced the accumulation of all constituents of triterpenoids in 'Baldwin' during storage. However, in 'Garden Blue', MT only led to significant increases in the levels of α -amyrin and oleanolic acid, which were 1.77 and 7.48 times higher than the control, respectively, at day 35. Surprisingly, MT treatment inhibited the accumulation of β -amyrin, ursolic acid, luperol, and betulinic acid in 'Garden Blue', with a particularly significant decrease of 41.30 % in β -amyrin content at day 35 as compared to the control.

Table 2
MT affects the contents ($\mu\text{g}/\text{cm}^2$) of key cuticular wax in 'Garden Blue' during storage.

Compound	Retention time	0d Control	7d Control	MT	14d Control	MT	21d Control	MT	28d Control	MT	35d Control	MT
Alkanes												
Hexadecane	17.34	0.08	9.94	1.47	4.54	1.33	1.98	4.06	7	1.92	17.6	1.7
Heptadecane	17.8	0.02	4.83	18.3	4.23	1.62	0.79	5.66	5.56	5.68	10.92	5.04
Octadecane	18.56	0.21	3.01	9.02	4.7	1.21	4.53	2.56	2.5	2.56	4.71	2.27
Nonadecane	19.52	0.03	5.46	3.9	3.57	5.05	3.21	7.16	2.87	2.94	8.83	2.61
Eicosane	20.32	1.87	4.36	4.14	5.37	1.37	6.49	5.7	9.03	9.24	13.74	8.2
Heneicosane	21.8	0.12	4.78	4.6	0.58	1.13	2.26	14.99	1.95	2	2.74	1.77
Docosane	22.43	1.19	6.42	1.35	6.24	2.26	2.9	9.46	2.5	2.56	4.48	2.27
Heptacosane	25.94	1.72	1.98	7.48	25.43	1.49	3.22	3.87	3.8	5.27	3.33	4.68
Octacosane	27.69	0.73	2.16	7.45	2.27	1.48	7.93	7.04	10.77	11.02	5.07	9.78
Nonacosane	28.74	6.27	4.67	4.29	9.31	0.73	10.67	7.79	10.79	11.04	/	9.8
Triacosane	30.62	0.03	3.57	6.46	8.77	3.9	22.34	28.01	22.84	23.37	23.21	20.73
Hentriacontane	32.67	1.36	7.92	7.61	16.31	1.46	16.57	11.96	15.63	15.99	4.21	14.18
Dotriacontane	33.52	1.11	2.92	5.1	2.67	2.13	5.59	10.2	6.9	7.06	8.16	6.27
Tetracontane	34.32	0.04	7.46	7.17	5.98	1.22	7.28	11.7	8.26	8.45	7.5	7.5
Diketones												
1-(2-Methoxyphenyl)-2,5-dihydro-1H-pyrrole-2,5-dione	52.04	234.48	192.53	137.17	157.44	17.15	72.06	35.24	84.38	30.76	116.61	23.88
6-Phenylamino-1H-pyrimidine-2,4-dione	53.31	396.66	173.45	357.63	20.96	341.55	16.49	380.22	59.36	284.7	21.16	252.57
Fatty acids												
Hexadecanoic acid	20.11	66.73	13.85	5.88	9.56	6.35	7.3	11.35	7.72	9.55	10.47	9.41
Octadecanoic acid	22.66	26.33	4.6	12.7	22.72	2	17.05	4.54	9.35	7.04	8.92	7.15
Triterpenoids												
.beta.-Amyrin	53.98	339.38	459.62	368.34	442.77	333.06	390.12	208.15	267.02	155.86	233.55	138.27
Compound												
.alpha.-Amyrin	55.96	42.2	99.82	77.4	115.8	96.2	80.62	77.67	70.38	70.04	46.98	83.17
Ursolic acid	55.67	239.53	297.39	311.66	317.7	315.94	375.95	261.93	274.9	196.13	224.21	174
Oleanolic acid	56.28	34.54	33.48	9.83	30.25	87.85	84.74	91.45	29.49	68.47	8.12	60.74
Lupeol	56.56	57.16	35.73	8.09	29.59	14.58	16.88	16.08	16.45	12.04	23.29	10.68
Betulinic acid	58.48	18.74	18.62	11.91	25.88	12.28	12.97	11.34	22.96	15.2	22.59	11.27

Note: Each value represents the mean of three replicates (-, undetectable). Abbreviations: MT, melatonin.

3.3. Effect of MT on the contents of VLC aliphatic compounds during storage

3.3.1. Diketone

The quantities of five VLC aliphatic compounds, including diketones, primary alcohols, alkanes, fatty acids, and esters, were found to be highly variable between the two cultivars. As previously reported by Chu et al. (2018), diketones were identified as the most abundant compounds among VLC aliphatic compounds in blueberries. The present study also yielded similar findings, reaffirming the prevalence of diketones in this context. In 'Garden Blue', the two diketone compounds, 6-Phenylamino-1H-pyrimidine-2,4-dione and 1-(2-Methoxyphenyl)-2,5-dihydro-1H-pyrrole-2,5-dione, were detected in the highest amounts before storage. However, after 35 d of storage, the quantities of these compounds decreased by 49.73 % and 5.33 %, respectively (Table 2). It is worth noting that 6-Phenylamino-1H-pyrimidine-2,4-dione was detected only at 0 d and 35 d, while 1-(2-Methoxyphenyl)-2,5-dihydro-1H-pyrrole-2,5-dione was detected at 14 d-35 d of storage in 'Baldwin' (Table S1). In 'Garden Blue', the quantity of 6-Phenylamino-1H-pyrimidine-2,4-dione increased by 11.94 times, accounting for 33.54 % of total diketones, while the quantity of 1-(2-Methoxyphenyl)-2,5-dihydro-1H-pyrrole-2,5-dione decreased by 5.33 times, accounting for 83.71 % of the total diketones 35 d after MT treatment.

3.3.2. Alkanes

Ten types of alkanes in 'Baldwin' and 17 types of alkanes in 'Garden Blue' were detected before storage, and their accumulation and distribution patterns greatly varied depending on the storage time and MT treatment. Tetracontane (C44H90) accumulated at the highest level ($34.75 \mu\text{g}/\text{cm}^2$) at 0 d, and its content sharply declined to $2.45 \mu\text{g}/\text{cm}^2$ at 7 d in 'Baldwin' (Table S1). By contrast, it was not detected at 0 d but present at 7 d in 'Garden Blue' (Table S2). Nonacosane (C29H60)

accumulated at the highest level ($6.27 \mu\text{g}/\text{cm}^2$) at 0 d and decreased at 7th d, after which it increased in 'Garden Blue', whereas it was absent at 0 d and present only at 7th d in 'Baldwin'. Interestingly, MT treatment induced the synthesis of dodecane, tridecane, pentadecane, tetracosane, pentacosane, and hexacosane at varying levels throughout the period of storage in 'Garden Blue', but not in 'Baldwin' (Table S1, S2). In addition, tricontane was absent irrespective of MT treatment during storage in 'Baldwin', while its content increased until 21 d upon MT treatment in 'Garden Blue'. While tetradecane and nonacosane disappeared after 21 d regardless of MT treatment in 'Baldwin', they appeared after 28 and 35 d of storage, respectively, in 'Garden Blue' upon MT treatment. Research has substantiated the importance of alkanes in preserving fruit moisture and weight during apple storage, with a particular emphasis on tetradecane within the alkane composition. Significantly, tetradecane was found to be closely correlated with the water loss observed in apples during the storage period (Chai et al., 2020). Thus, MT treatment can induce the synthesis of specific alkanes in 'Garden Blue' but not in 'Baldwin'.

3.3.3. Fatty acids

Among the six fatty acids detected in 'Baldwin', only hexadecanoic acid was present throughout storage and before storage, corresponding to 45.94 % of the total fatty acids at 0 d (Table 1). Both hexadecanoic and octadecanoic acids were present in 'Baldwin' throughout the storage, during which their accumulation trends were similar, irrespective of MT treatment. However, their contents were higher in MT-treated 'Baldwin' fruit during storage.

In 'Garden Blue', four saturated fatty acids were detected (Table S2), and both hexadecanoic and octadecanoic acids were present throughout the storage, making up 69.99 % and 27.62 % of the total fatty acids at 0 d, respectively (Table 2). Their contents decreased by 56.82 % and 43.18 % at 35th d, respectively. MT application either enhanced or

reduced accumulation of hexadecanoic and octadecanoic acids depending on the storage time. For example, octadecanoic acid increased to 2.6-fold at 7th d and decreased by 91.2 % at 14th d. It was documented that MT treatment could preserve the levels of most long-chain saturated fatty acids contributing to cuticular wax and slow down their metabolic breakdown, and this effect was particularly notable in the increase of octadecanoic acid in blueberry cv. 'Powderblue' (Liu et al., 2023). Regardless of MT application, both cultivars lacked unsaturated fatty acids, consistent with the previous results from 'Brightwell' cultivar (Cao et al., 2022). In contrast, they were detected in peach fruit (Gao et al., 2016). This suggests that unsaturated fatty acids may not be conducive to the improvement of postharvest quality of blueberry fruit, although their contents were reduced after MT treatment in Liu's study (Liu et al., 2023).

3.3.4. Primary alcohols

Three types of primary alcohols, hopenol, 2-tridecanol, and 1-hexadecanol, were detected in 'Baldwin' (Table 1). Hopenol, which accounted for 95.49 % of primary alcohols, was detected only at 0 d, suggesting that its chemical stability is highly susceptible to cold storage at 4 °C. On the other hand, 2-tridecanol disappeared after 21 d, and 1-hexadecanol was detected only at 0 d and 21 d of storage. MT did not appear to significantly affect accumulation of primary alcohols during storage in 'Baldwin.' There were no primary alcohols detected in 'Garden Blue' at 0 d. However, the induced synthesis of 1-hexacosanol was observed in MT-treated 'Garden Blue' during storage.

3.3.5. Ester

Two esters named 4-ethylbenzoic acid, 3-chloroprop-2-enyl ester, and 1,2,4-benzenetricarboxylic acid, 4-dodecyl dimethyl ester were detected in 'Baldwin' at 7 d of storage. Tridecanoic acid, 2-ethyl-2-methyl-, ethyl ester was only detected at 21d of storage, and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester was present from 14 d – 28 d showing a decreasing trend (Table 1, S1). For 'Garden Blue', three substances of benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester; fumaric acid, 2-decyl tridecyl ester; and hexadecanoic acid, 2,3-bis[(trimethylsilyloxy)propyl] ester were produced by MT treatment at 7 d of storage, while octadecanoic acid, 2,3-bis[(trimethylsilyloxy)propyl] ester was observed at 14 d in MT-treated fruits (Table 2, S2).

Regarding other miscellaneous compounds, both 9-Octadecenamide, (Z)- and naphthalene, 1,2,3,4-tetrahydro-6-methoxy-2-(4-pentylcyclohexyl)-, trans- were detected in both cultivars, which exhibited a similar accumulating trend, reaching peaks at different times of storage (Table S1, S2). MT did not substantially affect their contents of 'Baldwin', but in 'Garden Blue', and their contents were either increased or decreased depending on storage time.

Overall, MT did not change the total number of wax constituents in 'Baldwin' but increased it in 'Garden Blue' at each day of storage. This result is in line with the fact that 'Garden Blue' accumulated higher levels of total wax content throughout the storage time (Fig. 1A). As shown in Table 1, 2, S1, and S2, there are some interesting differences between the two cultivars: (i) both primary alcohols and esters were undetectable during 28 d of storage in 'Garden Blue' control, whereas they were detectable at day 7 to 21 in 'Baldwin' control; (ii) their contents were markedly increased in 'Garden Blue' but remained unchanged in 'Baldwin' upon MT treatment; (iii) 'Baldwin' sample had much higher level of primary alcohols at the beginning of the experiment, while 'Garden Blue' sample contained primary alcohols at relatively low levels only after MT treatment. Taken together, the accumulation and distribution profiles of individual cuticular wax components varied greatly depending on cultivar type, storage time, and MT treatment.

3.4. Effect of MT on fruit quality of rabbiteye blueberry during storage

During storage, the fruit weight, diameter, and surface area of rabbiteye blueberry decreased, but MT treatment delayed the reduction of fruit weight and diameter in both cultivars, especially in 'Garden Blue' (Fig. 2). The L* and a* values gradually decreased over time, while the b* value continued to increase, reaching a peak at 35 d of storage ($p < 0.05$). MT application appeared to attenuate the variations of L*, a*, and b* values, which were all slightly higher than the control group at the end of storage. Thus, we believe that MT delayed the fruit darkness during storage. Certain fruits, such as papaya, strawberry, date palm also showed similar performance during storage after MT treatment (Fekry et al., 2021; Liu, Zheng, Sheng, Liu, & Zheng, 2018; Wang et al., 2022).

Total soluble solids (TSS) are a key factor in determining fruit quality and acceptability, particularly for soft fruit. Although TSS content was higher in 'Garden Blue' than 'Baldwin', both cultivars showed a gradual increase, especially after 14 d of storage, when compared with the untreated fruit ($p < 0.05$). It's worth noting that this differs slightly from the findings of Cai's study (Cai, 2023), which reported a trend of TSS initially increasing and then decreasing in 'Brightwell' during storage. This disparity may be attributed to variations in storage conditions between the two studies. MT application led to an increase in the accumulation of TSS and a decrease in TA in both cultivars. Consequently, this resulted in a higher TSS/TA ratio, signifying a fruit quality that is less acidic and sweeter. Furthermore, MT markedly inhibited titratable acid content at 14th d of storage in both cultivars, consistent with the previous findings in strawberry fruit during storage at 4°C (Liu et al., 2018).

Phenolic compounds are known to contribute to the flavor, sensory and nutritional quality of blueberry fruit and are responsible for their health-promoting properties, antibacterial and antioxidant activities that affect the strength of fruit during storage (Rossi, Woods, & Leisner, 2022). Since MT application is known to positively affect the contents of phenolics, flavonoids, and anthocyanins in pear, *Zizyphus jujuba* and sweet cherry fruit (Aghdam et al., 2020; Belge et al., 2014a; Sharafi, Jannatizadeh, Fard, & Aghdam, 2021; Sun et al., 2021; Wang et al., 2021), we examined the effects of post-harvest MT application on their accumulation profiles during storage. Overall, the contents of total phenolics, flavonoids, and total anthocyanins slightly increased during storage of the untreated fruit; however, their accumulation patterns upon MT application appeared to be irregular depending on cultivar and storage time. For example, the content of total phenolics in MT-treated fruit was highest in 'Baldwin' and lowest in 'Garden Blue' at 21st d, leading to the convergent and divergent accumulation profile between the two cultivars. MT treatment reduced flavonoids content in 'Garden Blue' but either increased or decreased slightly during storage in 'Baldwin'. The contents of total anthocyanins in both cultivars converged at 14th d and thereafter diverged. MT did not significantly affect anthocyanins content in both cultivars at 14th d but triggered a 34.29 % increase and a 32.89 % decrease of anthocyanins at 35th d in 'Garden Blue' and 'Baldwin', respectively, as compared to the control ($p < 0.05$). In contrast to our results, MT treatment resulted in an increased production of polyphenols, flavonoids, and anthocyanins in highbush blueberries when stored at 5°C for 3 weeks (Magri & Petriccione, 2022). This indicates that there is a genotype-specific variation in the accumulation of secondary metabolites in response to MT application.

The untreated blueberry fruit continued to experience a water loss and an increase in decay rate during storage, which occurred concomitantly with a gradual increase in MDA content and relative conductivity (Fig. 3). However, MT mitigated the adverse effects of these factors on fruit quality in both cultivars, with a more pronounced negative observed in 'Garden Blue' than 'Baldwin'. For instance, MT application reduced water loss by 35.61 % in 'Baldwin' and 62.90 % in 'Garden Blue' at day 35. The effectiveness of MT may be attributed to its highly efficient direct and indirect antioxidant properties (Gao et al., 2016;

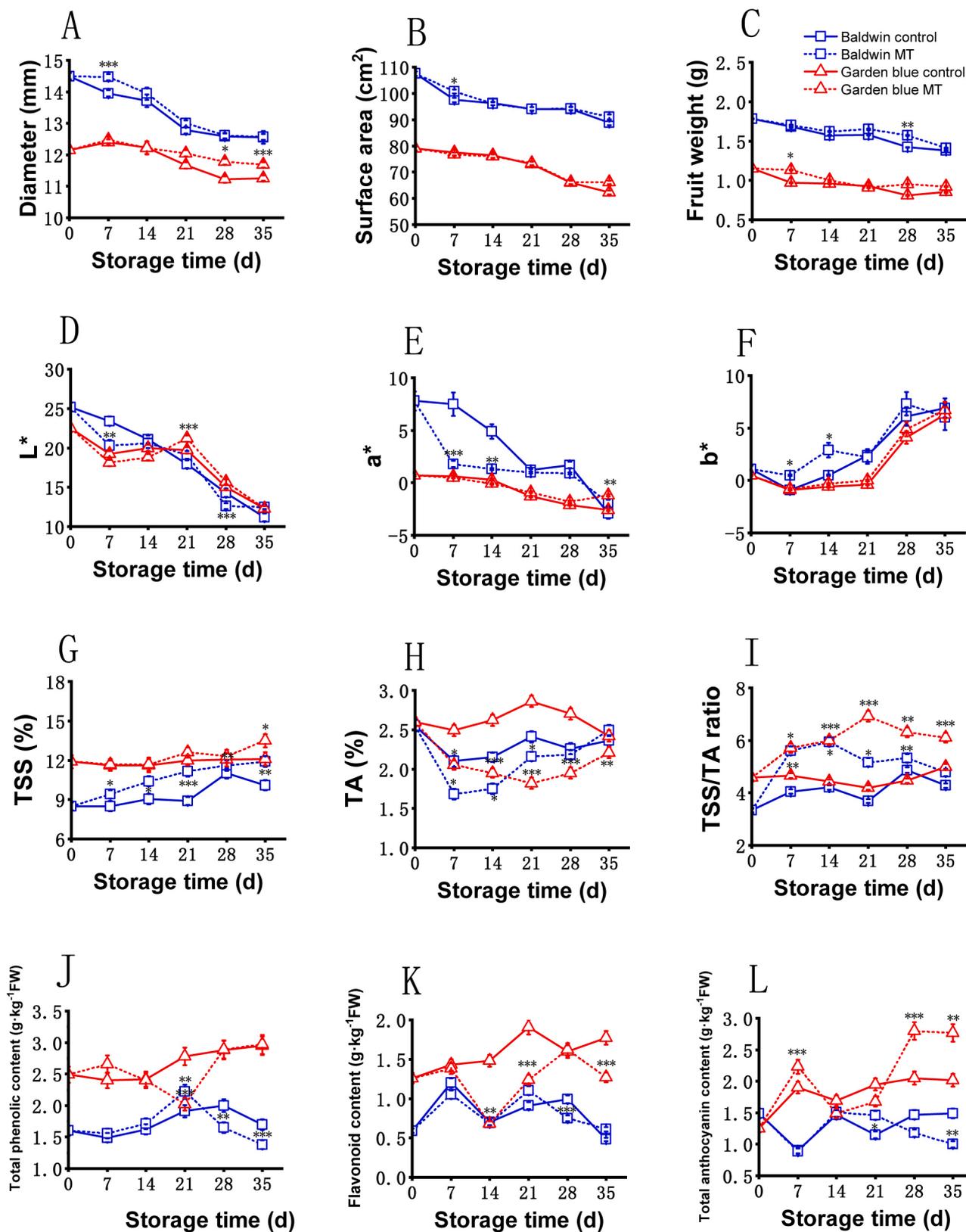


Fig. 2. MT affects fruit quality of rabbiteye blueberry during storage. Diameter (A); Surface area (B); Fruit weight (C); L* value (D); a* value (E); b* value (F); TSS (G); TA (H); TSS/TA (I); Total phenolic content (J); Flavonoid content (K); Total anthocyanin content (L). Abbreviations: MT, melatonin; TSS, total soluble solids, TA, titratable acidity; TSS/TA, total soluble solids/titratable acidity. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

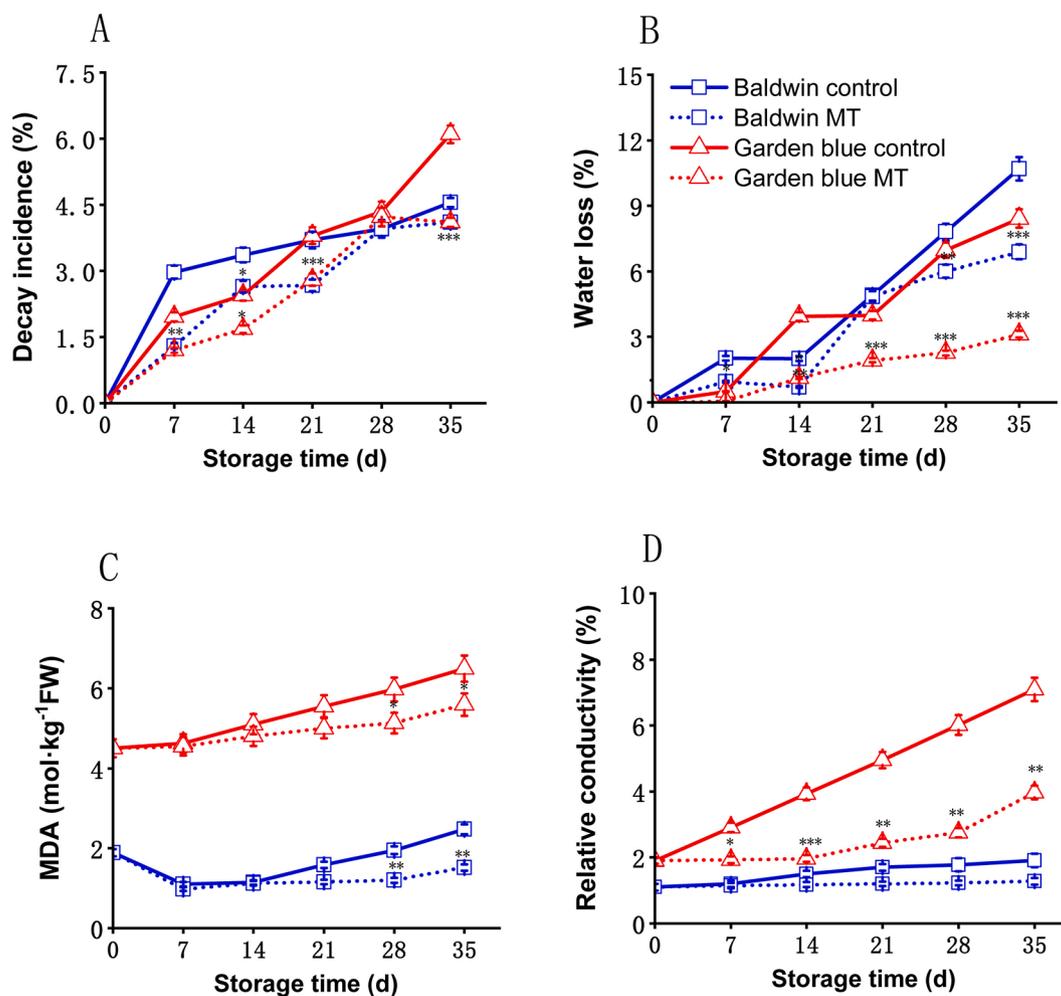


Fig. 3. MT affects on fruit decay rate, water loss, MDA content and relative conductivity of rabbiteye blueberry fruit during storage. Decay incidence (A); Water loss (B); MDA (C); Relative conductivity (D). Abbreviations: MT, melatonin; MDA, malondialdehyde. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Rastegar, Hassanzadeh Khankahdani, & Rahimzadeh, 2020).

Overall, 'Baldwin' fruit are more prone to dehydration than 'Garden Blue' fruit. The decay rate appears to positively correlate with water loss, MDA content, and relative conductivity, irrespective of MT treatment, since changes in those postharvest quality parameters were all synchronized and increased linearly with storage time. This phenomenon is in accord with the fact that levels of MDA and relative conductivity reflect the degree that expedites cell membrane damage caused by ROS followed by changes in cell membrane permeability induced by cell damage (Gao et al., 2016; Sachdev, Ansari, Ansari, Fujita, & Hasanuzzaman, 2021). The efficacy of MT in reducing decay incidence was similarly observed in wax apple, achieved by the elimination of excessive ROS through the collaborative actions of antioxidant enzyme (Chen et al., 2020). Taken together, the total wax content appears to be inversely related to b^* , TSS, decay rate, water loss, MDA content, and relative conductivity during storage, regardless of MT treatment. This suggests the important role of cuticular wax in maintaining the postharvest fruit quality. This notion is supported by previous findings that removal of wax on the apple fruit accelerates the postharvest water loss and decay, reduces the sensory and nutritional qualities, decreases the contents of antioxidants, expedites ROS accumulation and lipid peroxidation, and facilitates fruit softening (Shao, 2009).

3.5. Correlation analysis

To better understand the relationship between the postharvest fruit

quality parameters and wax constituents of MT and control, a correlation matrix graph was constructed in both cultivars (Fig. 4). In both cultivars, the fruit growth indices, including weight, surface area and equatorial diameter, were found to be inversely related to the water loss and decay rate, regardless of MT treatment. With this inverse correlation, MT treatment resulted in the reduction of the decay and water loss rates (Fig. 3). This suggests that fruit physical properties may have an impact on the postharvest water loss and decay rate, and that MT has a mitigating effect on the decay and water loss. Notably, the inverse relationship between weight loss and water loss was observed in both cultivars regardless of MT treatment. In other words, as the fruit weight increased, the water loss rate decreased. Heavier fruits may have thicker epicarps, which can act as a barrier to water loss and help retain moisture within the fruit. Conversely, lighter fruits tend to have thinner skins and may lose moisture more rapidly, leading to a higher water loss rate. This aligns with a previous report that there is an inverse relationship between the weight loss and water loss characterized by shriveling and drying in blueberry fruits during storage (Nunes & Emond, 2007). To further examine the correlation between the water loss and wax constituents during storage, we applied the kinetic model proposed by Liu et al. (2016) to determine the rate constant (k) and coefficient of determination (R^2) values for the blueberry storage process. According to R^2 (Table S3), it can be assumed that water loss is more compatible with the zero-order reaction, and that the wax components are more suitable for the first-order reaction. The k values of most quality indices in both cultivars under MT treatment were lower than those in the

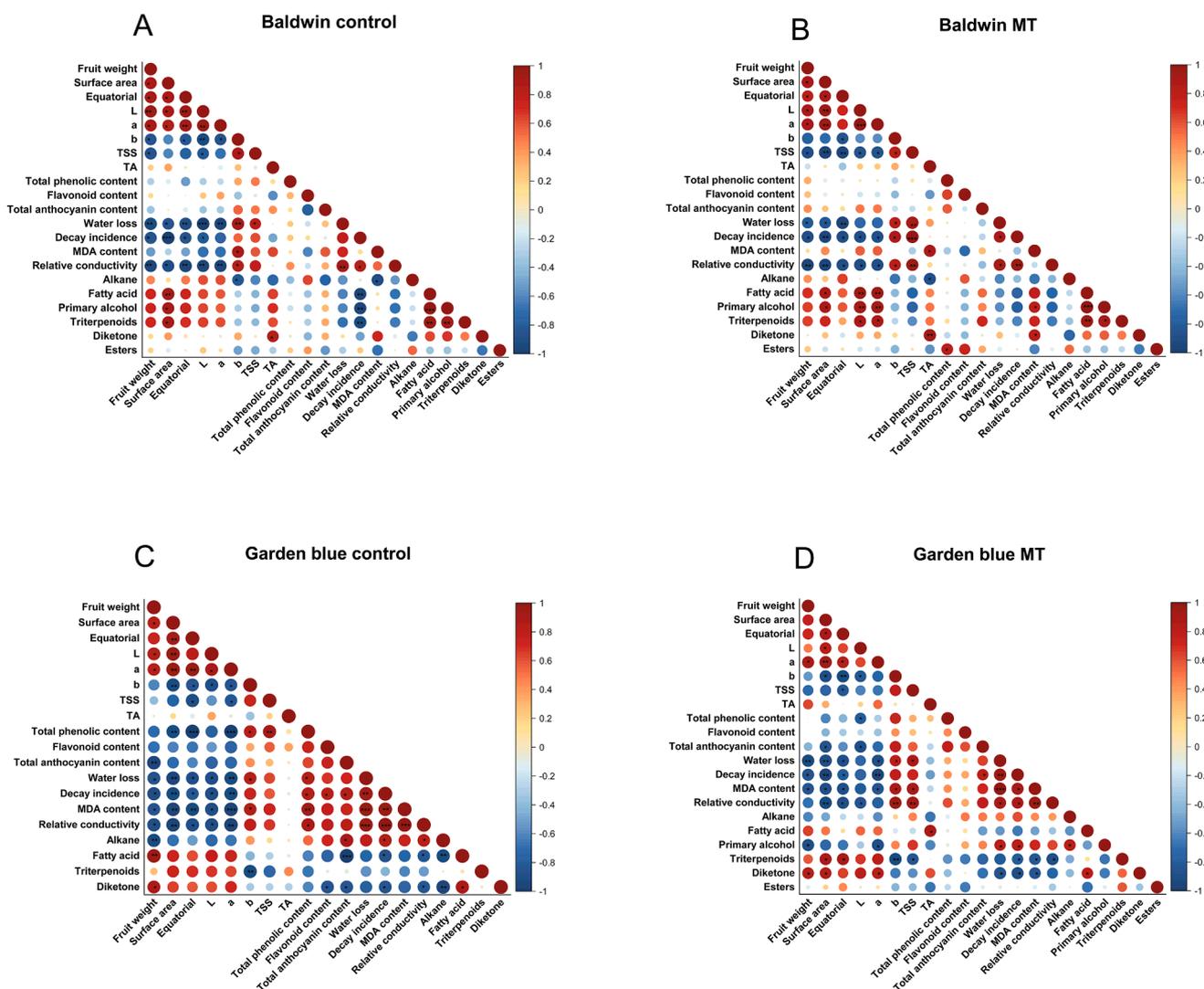


Fig. 4. Correlogram of wax composition and fruit quality attributes of two blueberry cultivars. ‘Baldwin’ control (A), ‘Baldwin’ MT (B), ‘Garden Blue’ control (C), ‘Garden Blue’ MT (D). Person correlation analysis was performed using origin 2022 software. Positive correlations are displayed in red and negative correlations in blue color. Color intensity and the size of the circle are proportional to the correlation coefficients. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

control. This suggests that MT treatment can effectively mitigate the deterioration of the blueberries during storage and extend their shelf life.

Additionally, the growth indices, L^* and a^* were positively correlated with fatty acids and triterpenoids, both of which together comprised the largest proportion of the wax content in the control and MT treatment samples of both cultivars, implicating that among the wax constituents, triterpenoids and fatty acids have a major contribution to the fruit quality during storage. This notion is substantiated by the fact that the relative conductivity as well as the rates of water loss and decay were negatively correlated with triterpenoids and fatty acids in the control and MT treatment samples of both cultivars. Meanwhile, primary alcohols were positively and negatively correlated with growth indices, L^* and a^* in the MT-treated samples of ‘Baldwin’ and ‘Garden Blue’, respectively. Diketones appeared to have positive correlations with those attributes of ‘Garden Blue’ but no significant correlation with those of ‘Baldwin’, regardless of MT application. Similar findings regarding L^* , a^* , triterpenoids, and diketones were previously reported in a study involving 12 blueberry genotypes (Yan, Dossett, & Castellarin, 2023). Alkanes appeared to have no significant correlation with fruit weight in ‘Baldwin’ but negative correlation with fruit weight in ‘Garden

Blue’, regardless of MT application. It was reported that the fruit weights of ten apple cultivars during storage were significantly correlated with total alkanes and primary C13 alcohol (Chai et al., 2020). Thus, the correlation between the wax composition and postharvest fruit quality appears to vary widely depending on plant species and cultivars.

The water loss and cell membrane integrity are reflected in the changes in relative conductivity and oxidative stress, which leads to the changes in decay rate and MDA content. The rates of water loss and decay, MDA content, and relative conductivity are important stress indicators of fruit quality and shelf life (Huang et al., 2022). All these four measures were negatively correlated with alkanes in ‘Baldwin’, but the positive correlation was observed in ‘Garden Blue’ regardless of MT treatment. It suggests that higher levels of alkanes may be associated with lower quality of ‘Baldwin’ fruits and higher quality of ‘Garden Blue’ fruits, respectively. It has been reported that the alkane content has a positive correlation with weight loss rates in stored plums (Liu et al., 2022), while exhibiting a negative correlation with weight loss in various blueberry cultivars (Cai, 2023). On the other hand, the above four parameters were negatively correlated with diketones in ‘Garden Blue’, while all but MDA content have no significant relationship with diketones in ‘Baldwin’, regardless of MT treatment. This divergence

could be due to the genetic differences in the mechanisms that regulate water loss, decay, and oxidative stress between the two cultivars. The negative correlation between alkanes and all these stress measures in the 'Baldwin' cultivar suggests that alkanes may have a protective effect against these stresses in this cultivar during cold storage. Along this line, alkanes are thought to have an important role in controlling permeability of the cuticle and have a protective effect against environmental stresses in plants, including drought, temperature, and oxidative stress, although the protective effects of alkanes may vary depending on the plant species, cultivar, and the specific stress conditions (Yang et al., 2022). The positive correlation between alkanes and all the stress measures in the 'Garden Blue' cultivar suggests that alkanes may not have a protective effect against water loss, decay, and oxidative stress in this cultivar. It is possible that the mechanisms that regulate these stresses in the 'Garden Blue' cultivar are different from those in the 'Baldwin' cultivar, and that the presence of alkanes in the 'Garden Blue' may exacerbate these stresses rather than protect against them during cold storage.

Relative conductivity had a strongly positive correlation with water loss and decay rates in the fruit of both 'Baldwin' and 'Garden Blue' cultivars, regardless of MT treatment. This implies that (i) as the extent of cellular damage or membrane permeability increases, the water loss and decay rates in the fruit also increase, and (ii) the protective effects of MT on fruit may not be effective in decreasing water loss and decay rates, at least not solely by reducing cellular damage and membrane permeability. In 'Garden Blue' fruit, there was a significant positive correlation between relative conductivity and MDA levels, which was not affected by MT treatment. In contrast, 'Baldwin' fruit showed an uncorrelated relationship between relative conductivity and MDA levels, which was also not significantly affected by MT treatment (Fig. 3C and D). This implies that the response of different fruit cultivars to MT application in terms of oxidative stress and cellular damage can vary. It has been well documented that MT functions as an effective antioxidant using both direct and indirect mechanisms (Khan et al., 2020). Thus, MT may have a protective effect against oxidative stress in 'Garden Blue' fruit, which can be reflected in a stronger correlation between relative conductivity and MDA levels. However, the protective effect of MT in 'Baldwin' fruit may not be significant, resulting in an uncorrelated relationship between these two parameters. At the molecular level, this difference could be attributed to factors such as dissimilar expression patterns of the key genes involved in wax biosynthesis and transport causing distinct cuticular wax content and composition, differences in cell membrane degradative enzyme activities, and their effect on membrane integrity. It's important to note that correlation does not necessarily imply causation, and further research would be needed to determine the exact mechanisms involved in the relationship between relative conductivity, MDA, water loss, and decay rates in fruit during storage. Nonetheless, the results highlight the importance of cellular damage and membrane permeability in understanding the mechanisms behind fruit water loss and decay.

Transpirational water loss has been assumed to be a major cause of firmness change during postharvest storage of blueberry (Paniagua, East, Hindmarsh, & Heyes, 2013). Thus, a correlogram between the wax composition and water loss was constructed to assess which constituents are significant determinants of water permeability (Fig. S1, S2, S3, S4). Regardless of MT treatment, most alkanes and primary alcohols components appeared to be negatively and positively correlated with water loss in 'Baldwin' and 'Garden Blue' fruit, respectively. Alkanes are known to form a hydrophobic layer on the surface of plant tissues, which can reduce water loss through evaporation (Huang, Wang, Qiu, & Lu, 2022). MT promoted long-chain alkanes that were negatively correlated with water loss (Lin et al., 2022). On the other hand, primary alcohols have been shown to enhance water permeability in plant tissues by disrupting the hydrophobic barriers formed by waxes and cuticles (Zhang et al., 2020b). By balancing the hydrophobic and hydrophilic components of the cuticle, fruit would be able to regulate water

permeability and maintain healthy water balance. Along this line, cytochrome P450 family member CYP96B5 has been demonstrated to catalyze the hydroxylation of alkanes into primary alcohols and plays a crucial role in the synthesis of cuticular wax in rice leaves, thereby impacting the plant's sensitivity to drought (Zhang et al., 2020a). The negative correlation in 'Baldwin' and positive correlation in 'Garden Blue' suggest that the balance between hydrophobic alkanes and hydrophilic primary alcohols molecules in the cuticle of 'Garden Blue' fruit is different from that in 'Baldwin' fruit. In fact, the untreated 'Baldwin' had higher content of primary alcohols than the untreated 'Garden Blue' (Fig. 1G; Table 1 and 2), paralleling with higher water loss in 'Baldwin' at 35 d of storage (Fig. 3B). Despite the very low content of primary alcohols, 'Garden Blue' fruits tend to increase water loss during storage. The weight loss rate was found to significantly correlate with alkanes in apples during storage (Chai et al., 2020). The results indicate that alkanes and primary alcohols, both of which are minor compound classes of cuticle in both cultivars, are not the sole determinants of water permeability in the blueberry fruit surface, and that MT did not have a significant impact on the relationship between these chemical components and water loss in the blueberry fruit.

Previous studies have suggested that triterpenoids play an important role in regulating water loss by forming a protective barrier in the cuticle (Belge, Llovera, Comabella, Graell, & Lara, 2014b). Cuticular triterpenoids were shown to accumulate exclusively in the intracuticular wax of different plant species. The intracuticular wax layer, primarily composed of aliphatic constituents, with some modification from triterpenoids, was proposed to serve as the primary component of the transpiration barrier, whereas epicuticular aliphatics play a minor role (Vogg et al., 2004). Our results showed that the most abundant component of triterpenoids in both cultivars was β -amyrin, and the second most abundant component was betulinic acid in 'Baldwin' and ursolic acid in 'Garden Blue' (Tables 1 and 2). Regardless of MT treatment, 'Baldwin' fruit showed a negative relationship of β -amyrin and betulinic acid with water loss, while 'Garden Blue' fruit displayed a negative relationship of β -amyrin and ursolic acid with water loss (Fig. S1, S2, S3, S4). This is consistent with the decreases in the contents of β -amyrin, betulinic acid and ursolic acid observed over 35 d (Tables 1 and 2) during which water loss rate increased (Fig. 3B). Regardless of MT treatment, all the fatty acid components in both cultivars seemed to have a negative relationship with water loss. Thus, β -amyrin, betulinic acid, ursolic acid and fatty acids appear to make a significant contribution to transpiration barrier.

4. Conclusions

Our findings demonstrate that MT treatment had distinct effects depending on the cultivar, affecting the accumulation and distribution of cuticular wax components during cold storage. Additionally, MT treatment improved the post-harvest quality of blueberries by minimizing water loss, reducing decay rate, and increasing TSS content. Furthermore, our study highlights the important role of cuticular wax in maintaining post-harvest fruit quality and suggests that triterpenoids and fatty acids play significant roles in determining the fruit quality of 'Baldwin' and 'Garden Blue' cultivars during storage. The study also emphasizes the importance of understanding the mechanisms underlying fruit water loss, weight loss, and decay during storage. Future research endeavors could focus on unraveling the molecular mechanisms that underlie cultivar-specific effects of MT treatment at the transcriptomic and proteomic levels and exploring the potential of cuticular wax constituents as indicators of post-harvest fruit quality.

CRedit authorship contribution statement

Jia Li: Investigation, Visualization, Writing – original draft. **Yaru Cao:** Data curation, Formal analysis. **Shicun Bian:** Formal analysis, Visualization. **Seung-Beom Hong:** Writing – review & editing. **Kai Xu:**

Resources. **Yunxiang Zang:** Investigation, Methodology. **Weiwei Zheng:** Conceptualization, Project administration, Software, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by fund from Science and Technology Bureau of Ningbo city (2019B10024). We also gratefully acknowledge the financial support provided by Zhejiang A&F University (2010FR089).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2023.101106>.

References

- Aghdam, M. S., Luo, Z., Li, L., Jannatizadeh, A., Fard, J. R., & Pirzad, F. (2020). Melatonin treatment maintains nutraceutical properties of pomegranate fruits during cold storage. *Food Chemistry*, 303, Article 125385. <https://doi.org/10.1016/j.foodchem.2019.125385>
- Belge, B., Llovera, M., Comabella, E., Gatius, F., Guillen, P., Graell, J., & Lara, I. (2014a). Characterization of cuticle composition after cold storage of "Celeste" and "Somerset" sweet cherry fruit. *Journal of Agricultural and Food Chemistry*, 62(34), 8722–8729. <https://doi.org/10.1021/jf502650t>
- Belge, B., Llovera, M., Comabella, E., Graell, J., & Lara, I. (2014b). Fruit cuticle composition of a melting and a nonmelting peach cultivar. *Journal of Agricultural and Food Chemistry*, 62(15), 3488–3495. <https://doi.org/10.1021/jf5003528>
- Cai, L. K. (2023). *The cloning and analysis of wax metabolism related gene during storage of blueberry*. Zhejiang University of Science and Technology.
- Cao, Y., Zang, Y., Wu, S., Li, T., Li, J., Xu, K., Hong, S. B., Wu, B., Zhang, W., & Zheng, W. (2022). Melatonin affects cuticular wax profile in rabbiteye blueberry (*Vaccinium ashei*) during fruit development. *Food Chemistry*, 384, Article 132381. <https://doi.org/10.1016/j.foodchem.2022.132381>
- Chai, Y., Li, A., Chit Wai, S., Song, C., Zhao, Y., Duan, Y., Zhang, B., & Lin, Q. (2020). Cuticular wax composition changes of 10 apple cultivars during postharvest storage. *Food Chemistry*, 324, Article 126903. <https://doi.org/10.1016/j.foodchem.2020.126903>
- Chen, Y., Zhang, Y., Nawaz, G., Zhao, C., Li, Y., Dong, T., Zhu, M., Du, X., Zhang, L., Li, Z., & Xu, T. (2020). Exogenous melatonin attenuates post-harvest decay by increasing antioxidant activity in wax apple (*Syzygium samarangense*). *Frontiers in Plant Science*, 11, Article 569779. <https://doi.org/10.3389/fpls.2020.569779>
- Chu, W., Gao, H., Cao, S., Fang, X., Chen, H., & Xiao, S. (2017). Composition and morphology of cuticular wax in blueberry (*Vaccinium* spp.) fruits. *Food Chemistry*, 219, 436–442. <https://doi.org/10.1016/j.foodchem.2016.09.186>
- Chu, W., Gao, H., Chen, H., Fang, X., & Zheng, Y. (2018). Effects of cuticular wax on the postharvest quality of blueberry fruit. *Food Chemistry*, 239, 68–74. <https://doi.org/10.1016/j.foodchem.2017.06.024>
- Chu, W., Gao, H., Chen, H., Wu, W., & Fang, X. (2018). Changes in cuticular wax composition of two blueberry cultivars during fruit ripening and postharvest cold storage. *Journal of Agricultural Food Chemistry*, 66(11), 2870–2876. <https://doi.org/10.1021/acs.jafc.7b05020>
- Dimopoulos, N., Tindjau, R., Wong, D. C. J., Matzat, T., Haslam, T., Song, C., Gambetta, G. A., Kunst, L., & Castellari, S. D. (2020). Drought stress modulates cuticular wax composition of the grape berry. *Journal of Experimental Botany*, 71(10), 3126–3141. <https://doi.org/10.1093/jxb/era046>
- Ding, F., Wang, G., Wang, M., & Zhang, S. (2018). Exogenous melatonin improves tolerance to water deficit by promoting cuticle formation in tomato plants. *Molecules*, 23(7), 1605. <https://doi.org/10.3390/molecules23071605>
- Dong, J., Kebbeh, M., Yan, R., Huan, C., Jiang, T., & Zheng, X. (2021). Melatonin treatment delays ripening in mangoes associated with maintaining the membrane integrity of fruit exocarp during postharvest. *Plant Physiology and Biochemistry*, 169, 22–28. <https://doi.org/10.1016/j.plaphy.2021.10.038>
- Fekry, W. M. E., Rashad, Y. M., Alaraidh, I. A., & Mehany, T. (2021). Exogenous application of melatonin and methyl jasmonate as a pre-harvest treatment enhances growth of barhi date palm trees, prolongs storability, and maintains quality of their fruits under storage conditions. *Plants (Basel)*, 11(1), 96. <https://doi.org/10.3390/plants11010096>
- Gao, H., Zhang, Z. K., Chai, H. K., Cheng, N., Yang, Y., Wang, D. N., Yang, T., & Cao, W. (2016). Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. *Postharvest Biology and Technology*, 118, 103–110. <https://doi.org/10.1016/j.postharvbio.2016.03.006>
- Huang, H., Wang, L., Qiu, D., & Lu, Y. (2022). Chemical composition of cuticle and barrier properties to transpiration in the fruit of *Clausena lansium* (Lour.) skeels. *Frontiers in Plant Science*, 13, Article 840061. <https://doi.org/10.3389/fpls.2022.840061>
- Huang, Q., Huang, L., Chen, J., Zhang, Y., Kai, W., & Chen, C. (2022). Maintenance of postharvest storability and overall quality of 'Jinshayou' pummelo fruit by salicylic acid treatment. *Frontiers in Plant Science*, 13, 1086375. <https://doi.org/10.3389/fpls.2022.1086375>
- Jiang, B., Liu, R., Fang, X., Tong, C., Chen, H., & Gao, H. (2022). Effects of salicylic acid treatment on fruit quality and wax composition of blueberry (*Vaccinium virgatum* Ait). *Food Chemistry*, 368, Article 130757. <https://doi.org/10.1016/j.foodchem.2021.130757>
- Khan, A., Numan, M., Khan, A. L., Lee, I. J., Imran, M., Asaf, S., & Al-Harrasi, A. (2020). Melatonin: Awakening the defense mechanisms during plant oxidative stress. *Plants (Basel)*, 9(4), 407. <https://doi.org/10.3390/plants9040407>
- Lara, I., Belge, B., & Goulao, L. F. (2015). A focus on the biosynthesis and composition of cuticle in fruits. *Journal of Agricultural and Food Chemistry*, 63(16), 4005–4019. <https://doi.org/10.1021/acs.jafc.5b00013>
- Li, C., Liang, B., Chang, C., Wei, Z., Zhou, S., & Ma, F. (2016). Exogenous melatonin improved potassium content in *Malus* under different stress conditions. *Journal of Pineal Research*, 61(2), 218–229. <https://doi.org/10.1111/jpi.12342>
- Li, F., Min, D., Song, B., Shao, S., & Zhang, X. (2017). Ethylene effects on apple fruit cuticular wax composition and content during cold storage. *Postharvest Biology and Technology*, 134, 98–105. <https://doi.org/10.1016/j.postharvbio.2017.08.011>
- Lin, X., Huang, S., Huber, D. J., Zhang, Q., Wan, X., Peng, J., Luo, D., Dong, X., & Zhu, S. (2022). Melatonin treatment affects wax composition and maintains storage quality in 'Kongxin' plum (*Prunus salicina* L. cv) during postharvest. *Foods*, 11(24), 3972. <https://doi.org/10.3390/foods11243972>
- Liu, C., Zheng, H., Sheng, K., Liu, W., & Zheng, L. (2018). Effects of melatonin treatment on the postharvest quality of strawberry fruit. *Postharvest Biology and Technology*, 139, 47–55. <https://doi.org/10.1016/j.postharvbio.2018.01.016>
- Liu, R., Shang, F., Niu, B., Wu, W., Han, Y., Chen, H., & Gao, H. (2023). Melatonin treatment delays the softening of blueberry fruit by modulating cuticular wax metabolism and reducing cell wall degradation. *Food Research International*, 173, Article 113357. <https://doi.org/10.1016/j.foodres.2023.113357>
- Liu, X. C., Qin, N., & Luo, Y. K. (2016). Application of a combination model based on an error-correcting technique to predict quality changes of vacuum-packed bighead carp (*Aristichthys nobilis*) filets. *LWT-Food Science and Technology*, 74, 514–520. <https://doi.org/10.1016/j.lwt.2016.08.010>
- Magri, A., & Petriccione, M. (2022). Melatonin treatment reduces qualitative decay and improves antioxidant system in highbush blueberry fruit during cold storage. *Journal of the Science of Food and Agriculture*, 102(10), 4229–4237. <https://doi.org/10.1002/jsfa.11774>
- Michailidis, M., Tanou, G., Sarrou, E., Karagiannis, E., Ganopoulos, I., Martens, S., & Molassiotis, A. (2021). Pre- and post-harvest melatonin application boosted phenolic compounds accumulation and altered respiratory characters in sweet cherry fruit. *Frontiers in Nutrition*, 8, Article 695061. <https://doi.org/10.3389/fnut.2021.695061>
- Noushahi, H. A., Khan, A. H., Noushahi, U. F., Hussain, M., Javed, T., Zafar, M., Batool, M., Ahmed, U., Liu, K., Harrison, M. T., Saud, S., Fahad, S., & Shu, S. (2022). Biosynthetic pathways of triterpenoids and strategies to improve their biosynthetic efficiency. *Plant Growth Regulation*, 97(3), 439–454. <https://doi.org/10.1007/s10725-022-00818-9>
- Nunes, M. C. N., & Emond, J. P. (2007). Relationship between weight loss and visual quality of fruits and vegetables. *Proceedings of the Florida State Horticultural Society*, 120, 235–245. <https://www.researchgate.net/publication/276848674>
- Onik, J. C., Wai, S. C., Li, A., Lin, Q., Sun, Q., Wang, Z., & Duan, Y. (2021). Melatonin treatment reduces ethylene production and maintains fruit quality in apple during postharvest storage. *Food Chemistry*, 337, Article 127753. <https://doi.org/10.1016/j.foodchem.2020.127753>
- Paniagua, A. C., East, A. R., Hindmarsh, J. P., & Heyes, J. A. (2013). Moisture loss is the major cause of firmness change during postharvest storage of blueberry. *Postharvest Biology and Technology*, 79, 13–19. <https://doi.org/10.1016/j.postharvbio.2012.12.016>
- Perveen, S. (2018). Introductory Chapter: Terpenes and Terpenoids. In *Terpenes and Terpenoids*.
- Rastegar, S., Hassanzadeh Khankahdani, H., & Rahimzadeh, M. (2020). Effects of melatonin treatment on the biochemical changes and antioxidant enzyme activity of mango fruit during storage. *Scientia Horticulturae*, 259, Article 108835. <https://doi.org/10.1016/j.scienta.2019.108835>
- Reque, P. M., Steckert, E. V., dos Santos, F. T., Danelli, D., Jablonski, A., Flôres, S. H., Rech, R., de, O., Rios, A., & de Jong, E. V. (2016). Heat processing of blueberries and its effect on their physicochemical and bioactive properties. *Journal of Food Process Engineering*, 39(6), 564–572. <https://doi.org/10.1111/jfpe.12249>
- Rossi, G., Woods, F. M., & Leisner, C. P. (2022). Quantification of total phenolic, anthocyanin, and flavonoid content in a diverse panel of blueberry cultivars and ecotypes. *HortScience*, 57(8), 901–909. <https://doi.org/10.21273/HORTSCI16647-22>
- Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., & Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants (Basel)*, 10(277), 1–37. <https://doi.org/10.3390/antiox10020277>

- Sater, H. M., Bizzio, L. N., Tieman, D. M., & Munoz, P. D. (2020). A review of the fruit volatiles found in blueberry and other vaccinium species. *Journal of Agricultural Food Chemistry*, 68(21), 5777–5786. <https://doi.org/10.1021/acs.jafc.0c01445>
- Shang, F., Liu, R., Wu, W., Han, Y., Fang, X., Chen, H., & Gao, H. (2021). Effects of melatonin on the components, quality and antioxidant activities of blueberry fruits. *LWT-Food Science and Technology*, 147, Article 111582. <https://doi.org/10.1016/j.lwt.2021.111582>
- Shao, J. P. (2009). *Study on phenolics and antioxidant activity in epicuticular wax of cold-storage apples*. Shandong Agricultural University.
- Sharafi, Y., Jannatizadeh, A., Fard, J. R., & Aghdam, M. S. (2021). Melatonin treatment delays senescence and improves antioxidant potential of sweet cherry fruits during cold storage. *Scientia Horticulturae*, 288, Article 110304. <https://doi.org/10.1016/j.scienta.2021.110304>
- Sun, H., Wang, X., Shang, Y., Wang, X., Du, G., & Lü, D. (2021). Preharvest application of melatonin induces anthocyanin accumulation and related gene upregulation in red pear (*Pyrus ussuriensis*). *Journal of Integrative Agriculture*, 20(8), 2126–2137. [https://doi.org/10.1016/S2095-3119\(20\)63312-3](https://doi.org/10.1016/S2095-3119(20)63312-3)
- Tang, Q., Li, C., Ge, Y., Li, X., Cheng, Y., Hou, J., & Li, J. (2020). Exogenous application of melatonin maintains storage quality of jujubes by enhancing anti-oxidative ability and suppressing the activity of cell wall-degrading enzymes. *LWT-Food Science and Technology*, 127, Article 109431. <https://doi.org/10.1016/j.lwt.2020.109431>
- Vogg, G., Fischer, S., Leide, J., Emmanuel, E., Jetter, R., Levy, A. A., & Riederer, M. (2004). Tomato fruit cuticular waxes and their effects on transpiration barrier properties: Functional characterization of a mutant deficient in a very-long-chain fatty acid beta-ketoacyl-CoA synthase. *Journal of Experimental Botany*, 55(401), 1401–1410. <https://doi.org/10.1093/jxb/erh149>
- Wang, D., Randhawa, M. S., Azam, M., Liu, H., Ejaz, S., Ilahy, R., Qadri, R., Khan, M. I., Umer, M. A., Khan, M. A., & Wang, K. (2022). Exogenous melatonin treatment reduces postharvest senescence and maintains the quality of papaya fruit during cold storage. *Frontiers in Plant Science*, 13, 1039373. Doi: 10.3389/fpls.2022.1039373.
- Wang, L., Luo, Z., Ban, Z., Jiang, N., Yang, M., & Li, L. (2021). Role of exogenous melatonin involved in phenolic metabolism of *Zizyphus jujuba* fruit. *Food Chemistry*, 341, Article 128268. <https://doi.org/10.1016/j.foodchem.2020.128268>
- Xiao, S. Y. (2017). Preliminary study on the relationship between outer epidermal wax of blueberry and postharvest diseases and disease resistance of fruits. Nanjing Agricultural University.
- Xu, T., Chen, Y., & Kang, H. (2019). Melatonin is a potential target for improving post-harvest preservation of fruits and vegetables. *Frontiers in Plant Science*, 10, 1388. <https://doi.org/10.3389/fpls.2019.01388>
- Yan, Y., Dossett, M., & Castellarin, S. D. (2023). Cuticular waxes affect fruit surface color in blueberries. *Plants People Planet*, 5(5), 736–751. <https://doi.org/10.1016/10.1002/ppp3.10368>
- Yang, H., Zou, Y., Li, X., Zhang, M., Zhu, Z., Xu, R., Xu, J., Deng, X., & Cheng, Y. (2022). QTL analysis reveals the effect of CER1-1 and CER1-3 to reduce fruit water loss by increasing cuticular wax alkanes in citrus fruit. *Postharvest Biology and Technology*, 185, Article 111771. <https://doi.org/10.1016/j.postharvbio.2021.111771>
- Zhang, D., Yang, H., Wang, X., Qiu, Y., Tian, L., Qi, X., & Qu, L. Q. (2020). Cytochrome P450 family member CYP96B5 hydroxylates alkanes to primary alcohols and is involved in rice leaf cuticular wax synthesis. *New Phytologist*, 225(5), 2094–2107. <https://doi.org/10.1111/nph.16267>
- Zhang, M., Zhang, P., Lu, S., Ou Yang, Q., Zhu Ge, Y., Tian, R., Jia, H., & Fang, J. (2021). Comparative analysis of cuticular wax in various grape cultivars during berry development and after storage. *Frontiers in Nutrition*, 8, Article 817796. <https://doi.org/10.3389/fnut.2021.817796>
- Zhang, Y. L., You, C. X., Li, Y. Y., & Hao, Y. J. (2020). Advances in biosynthesis, regulation, and function of apple cuticular wax. *Frontiers in Plant Science*, 11, 1165. <https://doi.org/10.3389/fpls.2020.01165>
- Zhu, S., Huang, S., Lin, X., Wan, X., Zhang, Q., Peng, J., Luo, D., Zhang, Y., & Dong, X. (2023). The relationships between waxes and storage quality indexes of fruits of three plum cultivars. *Foods*, 12(8), 1717. <https://doi.org/10.3390/foods12081717>