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Analysis on quality differences associated with metabolomics of rambutan during different temperature storage

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ABSTRACT

This study aimed to understand how temperatures differentially impact the crucial quality indices and metabolites in rambutan during storage. Rambutan browned quickly at room temperature from 0 d (control). After ten days at 5 ℃, browning index and lightness were 4.2% and 147.5%, compared with rambutan stored at 1 ℃, which was the best quality achieved. An UPLC-MS/MS was performed to uncover the metabolism underlying those quality differences, followed by the analysis of KEGG pathways. Results showed that 276 differentially expressed metabolites (DEMs) screened were enriched in 18 KEGG pathways. The pathways related to carbohydrates, aliphatic metabolites, and organic acids were highly active in rambutan stored at room temperature, whereas the pathways related to amino acids biosynthesis and nucleotides were highly active in rambutan stored at 1 ℃, 5 ℃. These findings indicated that increased scopoline was associated with serious browning at room temperature. L-leucine and L-isoleucine both increased in response to low temperature and reduced browning. Glutathione and ascorbate decreased to 4.89% and 4.36%, compared with 0 d (CK) in rambutan with severe browning stored at 1 ℃ for ten days. However, no significant changes in those two metabolites were observed in rambutan stored at optimal 5 °C for ten days. Thus glutathione and ascorbate could be used as potential indicators of browning degree. Our study provided a metabolic insight into the role of temperature on rambutan quality and browning.

1. Introduction

Rambutan (*Nephelium lappaceum* L.) belongs to Sapindaceae spp., the same family as the sub-tropical fruits lychee and longan (Wall, 2006). This fruit is an important commercial crop in Asia, where it is consumed fresh, canned, and processed. Products are appreciated for their refreshing flavor and appearance (Ong, Acree, & Lavin, 1998). Rambutan fruits are a good source of Vitamin C, Cu, Mn and can provide 2–6% of the DRI for five minerals (P, K, Mg, Fe, Zn) (Wall, 2006). Because of high content of nutritional and bioactive compounds, rambutan is more and more popular recently. However, rambutan pericarp turns brown easily under ambient conditions after harvest (O'Hare, 1995), which greatly shortens shelf life and economic value.

Many studies have investigated the causes and mechanisms of pericarp browning in postharvest rambutan fruit. Low-temperature stress (O'Hare, Prasad, & Cooke, 1994), water loss (Landrigan, Morris, Eamus, & McGlasson, 1996), polyphenoloxidase (Sun et al., 2010) have been recognized as the main factors inducing pericarp browning. Among them, low-temperature stress is significantly crucial because rambutan is a temperature-sensitive sub-tropical fruit. Small molecular metabolites such as soluble solids, organic acids, malondialdehyde will change at low temperature. However, the limited number of physiological indicators cannot accurately and comprehensively reflect the changing rules and internal relations of specific metabolites in response to low temperature.

Metabolomics deals with detecting, identifying, quantitating, and cataloging the history of time-related metabolic changes in an integrated biological system rather than the individual cell (Nicholson, Lindon, & Holmes, 2008). Such multidimensional metabolic trajectories are then related to biological events like the deterioration of fruit. Metabolomics

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has been successfully applied to study the deterioration and browning mechanism of various agricultural products, including litchi (Yun et al., 2016), apple (Zhao et al., 2020), volvariella volvacea (Zhao et al., 2020) caused by low-temperature stress. Despite these reports, there is no information, at least to the best of our knowledge, about the browning of rambutan pericarps analyzed by metabolomics under low-temperature stress.

In this paper, the optimal and chilling injury temperatures for rambutan storage were obtained by comparing key quality indexes. With the help of metabolomics, we compared the changes of different metabolites after browning at room-temperature, optimal storage temperature, and chilling injury temperature. Finally, we discovered the maps of KEGG pathways, which could provide a metabolic insight into the deterioration and browning mechanism of rambutan during different temperature storage.

2. Materials and methods

2.1. Chemicals and reagents

Packaging materials: Rambutan was packed in 0.01 mm polyethylene (PE) bags, sealed in polypropylene (PP) preservation box, and then stored at different temperatures. AR-grade NaOH was purchased from Guangzhou chemical reagent factory (Guangzhou, China). HPLC-grade methanol and ethanol acetonitrile were purchased from Merck (Wuhan, China). HPLC-grade dimethyl sulfoxide was purchased from Sigma-Aldrich (Wuhan, China).

2.2. Materials, treatments, and sampling

"Baoyan-7", one of the main varieties of rambutan in China, was selected and bred by Baoting Tropical Crops Institute. 100 kg commercial mature "Baoyan-7" rambutan were collected from the orchard of Hainan Baoting Hengtong Co., Ltd., and their pericarps were all red, which were sent to the laboratory immediately after harvest. 40 kg harvested rambutan of the same size were collected, with no mechanical damage and no disease. 3 kg rambutan fruits were weighed into PE bags and sealed in the fresh-keeping box. The fruits were stored at 1 ℃ (L1), 5 ℃ (L5) and room temperature (RT), respectively. Six physicochemical parameters related to fruit quality and browning were measured at 0, 3, 7, 10 days, including browning index, lightness, respiratory intensity, relative conductivity, soluble solid and titratable acid.

2.3. Measurement of browning index

Browning index was determined with the method proposed by Zhang et al. (2015), with some modifications. Rambutan pericarp browning was visually assessed by evaluating the browned area's extent on each fruit surface using the following scale: Class 0 = no browning; Class 1 = 1%-25% browning; Class2 = 26%-50% browning; Class 3 = 51%-75% browning; Class 4 = 76%-100% browning. The browning index was calculated as $\sum$ (browning scale × number of corresponding fruit within each class) / (4 × total number of fruit). Each treatment contained three replicates, each of which contained 30 fruits.

2.4. Measurement of pericarp lightness

Pericarp color was measured using a portable spectrophotometer (NS800, Shenzhen Threenh Technology Co., Ltd. China), providing the L*, a*, b* values according to the CIE system. L* value represents lightness. The smaller lightness value, the more serious the browning. The soft thorns of the fruit were all cut off before measuring. Three measurements were conducted at equidistant points on the equatorial axis. Five replicates for each treatment were performed, with 15 fruits for each replicate.

2.5. Quality analysis

1 kg rambutan were placed in a closed container for one hour, and the top gas was analyzed by gas analyzer (Check Point II, AMETEK MOCON Co., Ltd., Denmark). The mass of CO_2 produced per kilogram of rambutan in one hour was calculated in terms of respiratory intensity. Relative conductivity was measured with a conductivity meter (COM-100, HM Digital, Inc. USA) using the method of Fan et al. (2016).

The rambutan pulps were separated, juiced, and used to determine the total soluble solid (TSS) content by a brix refractometer (PAL-1, ATAGO Co., Ltd., Japan). The content of titratable acid (TA) was measured according to GB 5009.239–2016 (National Standard for Determination of Food Acidity). The analysis was repeated three times at each time point.

2.6. UPLC-MS/MS analysis

The pericarp of rambutan was freeze-dried in a vacuum and crushed with zirconia beads at 30 Hz for 1.5 min. Then 0.1 g crushed powder was extracted with 1.0 mL 70% methanol solution at 4 ℃ overnight. After centrifugation at a speed of 10,000 r/min for 10 min, the extract was purified by CNWBOND Carbon-GCB SPE Cartridge (250 mg, 3 mL) and filtered with SCAA104 (0.22 μm) membrane. Finally, UPLC-MS/MS analysis was performed.

The analyses were performed using a UPLC (Shim-pack UFLC SHIMADZUCBM30A system, Shimadzu) equipped with a column (ACQUITY UPLC HSS T3 C18, 2.1 mm × 100 mm, 1.80 am, Waters) and coupled to an Applied Biosystems 6500 QTRAP tandem mass spectrometer. 2.0 μL sample was loaded into the column and maintained at 40 °C with 0.4 mL/min flow rate. The mobile phase was composed of buffer A (ultrapure water with 0.04% acetic acid) and buffer B (acetonitrile with 0.04% acetic acid). The mobile phase gradient was programmed as follows: 95% A was linearly reduced to 5% within 11 min and kept at 5% for 1 min and then increased to 95% within 0.1 min, with a 3 min re-equilibration period employed. A specific set of MRM (multiple reaction monitoring) transitions was monitored for each period.

2.7. Statistical analysis

Based on multiple orthogonal partial least squares discriminant analysis (OPLS-DA), variable importance in project (VIP) and fold change, the metabolites with fold change ≥ 2 or fold change ≤ 0.5 and VIP ≥ 1 were considered to be differentially expressed metabolites (DEMs) in the rambutan pericarp with different temperature treats.

SigmaPlot software (version 12.3, Systat Software Inc.) and Adobe Illustrator software (CS4, Adobe Systems Inc.) were used for image processing. SPSS software (version 22.0, SPSS Inc.) was used to analyze the least significant difference (LSD) at the 5% level.

3. Results and discussion

3.1. Changes in the appearance and quality of rambutan during storage

The marketability of rambutan is often limited due to rapid skin desiccation and browning during storage (O'Hare, 1995). The spinterns (hair-like protuberances) can facilitate water loss from the pericarp, which makes the fruit highly perishable (Yingsanga, Srilaong, Kanlayanarat, Noichinda, & McGlasson, 2008). At 0 day (CK) after harvest, the pericarp and spinterns of rambutan were all red. In contrast, after five days of storage at room temperature (RT), the spinterns of rambutan shrunk, and the pericarps were dark brown, which lost commercial value. The appropriate low temperature could reduce this browning, and rambutan had the best appearance quality at 5 ℃ (L5) for ten days. The whole pericarp of rambutan became browning after stored at 1 ℃ (L1) for ten days, and the top of spinterns were severely browning (Fig. 1).

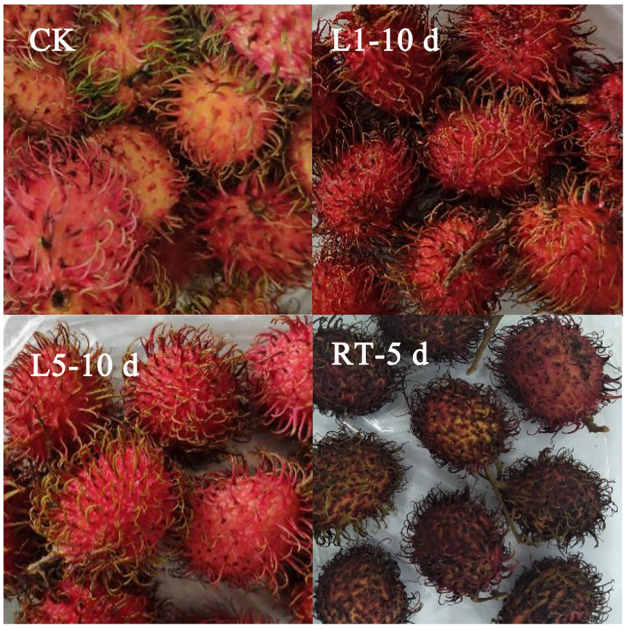

Fig. 1. Browning of rambutan stored at 0 day (CK), 1 ℃ for 10 days (L1-10 d), 5 ℃ for 10 days (L5-10 d) and 5 days at room temperature (RT-5 d).

There was a big difference between the indexes related to browning of rambutan under different temperature conditions. As showed in Fig. 2A, the browning index of fruits increased continuously during storage. After three days of storage, the browning index of RT rambutan was 0.45, which was significantly higher than that of other temperature treatments at the same period ($p < 0.05$). As shown in Fig. 2B, after ten days stored at 1 ℃, the chilling injury symptoms were evident, the red color of L1 pericarp became dark and the lightness was 28, which was significantly lower than that of L5 ($p < 0.05$). On the 10th day, the browning index and lightness of L5 rambutan pericarps were 4.2% and 147.5% of L1, indicating that browning was the least.

Low temperature could inhibit the respiration of rambutan pericarp, and there was no significant difference between L1 and L5 (Fig. 2C). However, from the 3rd day, the relative conductivity of L5 rambutan pericarp was always significantly lower than that of L1 ($p < 0.05$). As the indicator of membrane integrity, a greater electrical conductivity rate usually represents the lower integrity of the cell membrane (Liu et al., 2019). The relative conductivity of L5 rambutan pericarp was the lowest, indicating that its cell membrane integrity was the best, which played an important role in inhibiting browning caused by low-temperature stress (Fig. 2D).

Total soluble solid (TSS), titratable acidity (TA), and the ratio of TSS/TA are important factors for evaluating fruit quality (Kafkas, Koşar, Paydaş, Kafkas, & Başer, 2007). From the 7th day, the TSS of L5 rambutan was significantly higher than that of L1, but TA was lower than L1 (Fig. 2E/F). It was shown that the rambutan pericarp stored at 5 ℃ exhibited higher TSS and lower TA, so as to ensure a high TSS/TA ratio and maintain the high quality of rambutan.

Previous studies have shown that the sweetness, acidity and flavor of fruits are mainly influenced by the types and levels of carbohydrates, organic acids, and amino acids (Keutgen & Pawelzik, 2008; Malundo, Shewfelt, & Scott, 1995). The above data showed that rambutan browned severely within five days at room temperature. 5 ℃ was the best temperature to store rambutan, with the least browning and the best quality. When stored at 1 ℃, the chilling injury would increase the

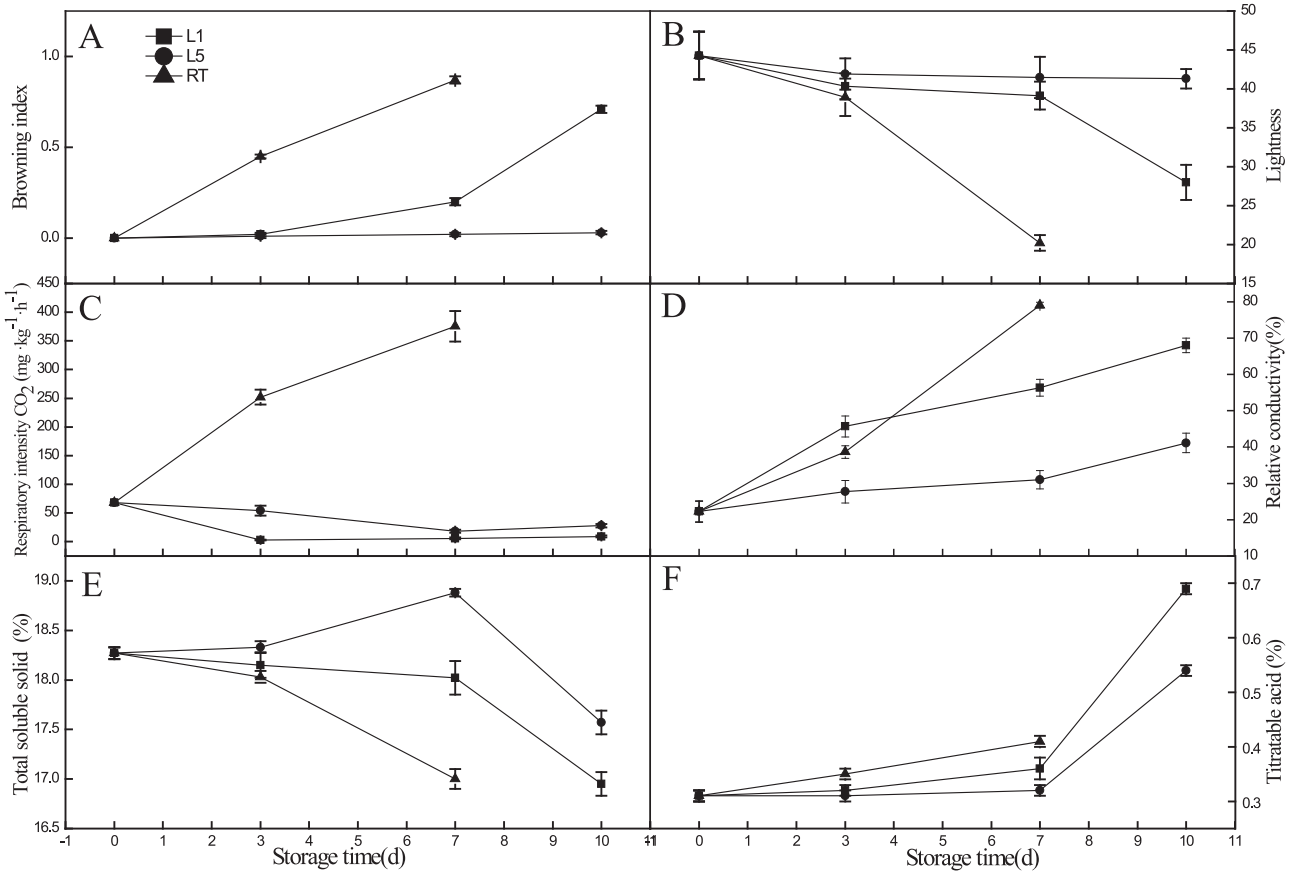

Fig. 2. Browning index (A), lightness (B), respiratory intensity (C), relative conductivity (D), soluble solid (E), titratable acid (F) in rambutan stored at 1 ℃ (L1), 5 ℃ (L5) and room temperature (RT).

pericarps browning, and the six quality indexes were significantly different from those at 5 ℃ on the 10th day during storage. However, the data mentioned above were not enough to explain all the metabolites' variation and connection during pericarp browning, so a metabolomics analysis was thus performed to interpret the changes.

3.2. Common DEMs in L1-10 d, L5-10 d and RT-5 d compared with CK

To analyze the fundamental metabolic processes in browning rambutan pericarp related to temperature, the metabolites of rambutan pericarp at 0 d (CK), RT-5 d, L1-10 d, and L5-10 d were analyzed by UPLC-MS/MS. The results showed that compared with CK, 276 differentially expressed metabolites (DEMs) were screened in L1-10 d, L5-10 d, and RT-5 d, 47 of which were common DEMs, which may be involved in the browning of rambutan pericarp. According to Table 1, there were 12 amino acids and derivatives, 11 lipids and derivatives, eight organic acids and derivatives, three carbohydrates and derivatives, three phenylpropanoid and derivatives, and ten other metabolites. Interestingly, the browning of rambutan pericarp was the most serious at room temperature for five days (RT-5 d), and there were 218 DEMs, 91 of which were unique. In contrast, the browning of rambutan pericarp was the lightest at 5 ℃ for ten days (L5-10 d), and there were only 94 DEMs, 19 of which were unique (Fig. 3A). The appearance of a large number of metabolites indicated that the pericarp's physiological balance was broken, which was harmful to the quality. Those findings also confirmed that 5 ℃ is an optimal storage temperature for rambutan compared with 1 ℃ and room temperature, showing the best appearance and quality.

Carbohydrates are important respiratory substrates and osmotic regulators (Tarczynski, Jensen, & Bohnert, 1993). Compared with CK, all the carbohydrates and derivatives were decreased in L1-10 d, L5-10 d, and RT-5 d (Table 1). The more serious the browning, the more significant the decline. These findings are consistent with the measured TSS content during storage (Fig. 2E).

The abundances of all lipids and derivatives except for acetyl tryptophan were increased in L1-10 d, L5-10 d, and RT-5 d. The fold changes of lipids and derivatives in L1-10 d compared with CK were greater than those of L5-10 d compared with CK. In particular, MAG (18:3) isomer3, MAG (18:3) isomer 2 and 13-HPODE of L1-10 d increased mostly compared with L5-10 d. These changes might be related to respiratory metabolism and low-temperature stress response. Rambutan pericarp under low temperature will induce accumulation of reactive oxygen species and results in oxidative damage to cellular components such as membrane lipids. Membrane damage or deterioration of plants will lead to a substantial increase in electrolyte leakage (Yabuta et al., 2002). These findings are consistent with the measured relative conductivity during storage (Fig. 2D).

Amino acids are closely related to the ability of plant to resist stress (Yang et al., 2019). Except for L-citrulline, N-acetyl-L-tyrosine, DL-homocysteine, the levels of amino acids and derivatives in RT-5 d rambutan pericarps were all decreased. We conjectured that these amino acids might be transformed into ketone and melanin finally, which aggravated the browning of rambutan pericarps at room temperature. Glutamine and glutamic acid are important precursors for the synthesis of amino acids in plants, and their content directly affects the synthesis of amino acids (Lea & Miflin, 1974; Yang et al., 2019). L-glutamic acid in L1-10 d, L5-10 d, and RT-5 d was all decreased, resulting in degradation of other amino acids, with aspartic acid decreased mostly. L-leucine and L-isoleucine in rambutan pericarp decreased at room temperature but increased significantly during low-temperature storage. Previous studies have shown that increased concentrations of branched-chain amino acids, like isoleucine, leucine, and valine during low-temperature stress were detected (Tian, Lam, & Shui, 2016). The abundances of organic acids and derivatives were all accumulated in L1-10 d, L5-10 d, and RT-5 d, with ethyl gallate increased

Table 1
Fold changes of common differentially expressed metabolites of rambutan under different temperatures.

Metabolites	Log_2 fold change			Metabolites	Log_2 fold change		
	L1-10 days vs.CK	L5-10 days vs.CK	RT-5 days vs.CK		L1-10 days vs.CK	L5-10 days vs.CK	RT-5 days vs.CK
Carbohydrates and derivatives							
D-Glucose 6-phosphate	-1.05	-1.05	-2.44	Glucose-1-phosphate	-1.11	-1.05	-2.45
D-Fructose 6-phosphate-disodium salt	-1.12	-1.04	-2.52				
Lipids and derivatives							
Lys PE 16:0	2.9	1.22	3.92	LysoPE 18:0 (2n isomer)	3.27	1.19	2.52
LysoPE 18:0	2.48	1.26	3.8	Punicic acid	3.62	1.09	4.72
MAG (18:3) isomer3	4.8	1.29	5.7	LysoPC 19:0	2.57	1.23	3.16
MAG (1 8:3) isomer2	4.26	1	5.28	LysoPC 17:0	3.6	1.17	4.85
13-HPODE	4.11	1.04	5.35	LysoPC 18:0	2.91	1.12	4.61
Acetyl tryptophan	-1.29	-1.35	-1.24				
Amino acids and derivatives							
L-Glutamic acid O-glucoside	-1.86	-1.01	-1.75	L-Citrulline	1.97	1.77	1.54
L-Aspartic acid	-3.84	-2.05	-1.48	L-Glutamic acid	-1.89	-1.29	-1.23
L-Leucine	1.78	1.31	-1.52	N6-Acetyl-L-lysine	1.68	2.21	-1.29
L-Isoleucine	1.34	1.05	-1.29	N-Acetyl-L-tyrosine	2.09	2.27	2.38
H-HomoArg-OH	1.12	1.79	-1.08	DL-Homocysteine	2.52	2.7	2.37
Aspartic acid	-3.76	-1.87	-1.47	Glutamic acid	-1.85	-1.38	-1.23
Organic acids and derivatives							
2-Isopropylmalate	3.31	1.85	1.54	3-Hydroxybutyrate	3.03	1.28	1.23
Azelaic acid	4.57	1.72	2.84	Ethyl gallate	14.32	8.77	12.27
2,5-Dihydroxybenzoic acid (Gentisic acid)	2.36	1.49	3.9	3-Hydroxyanthranilic acid	1.96	1.22	3.56
2,3-Dihydroxybenzoic acid	2.32	1.49	3.93	2,4-Dihydroxybenzoic acid	2.31	1.48	3.93
Phenylpropanoids and derivatives							
3,4,5-Trimethoxycinnamic acid	16.19	14.06	15.97	p-Coumaric acid	2.85	1.63	2.68
Scopolin	2.01	1.23	4.68				
Others							
Nordihydrocapsaicin	3.97	1.18	4.71	Acid orange 20	3.82	1.13	3.79
"N',N"",N""-p-coumaroyl-cinnamoyl-caffeoyl spermidine"	4.01	1.66	1.79	Di-O-methylquercetin	4.09	1.07	5.46
Pinocembrin	2.33	2.4	2.71	Protocatechuic acid	2.41	1.57	4.02
6-Gingerol	3.58	1.27	5.27	Vitamin A	-1.74	-1.35	-2.3
2,6-Dimethyl-7-octene-2,3,6-triol	4.59	1.75	2.86	3-Indoleacetonitrile	1.77	1.72	1.57

The colur shades represents the value of Log_2 fold change.

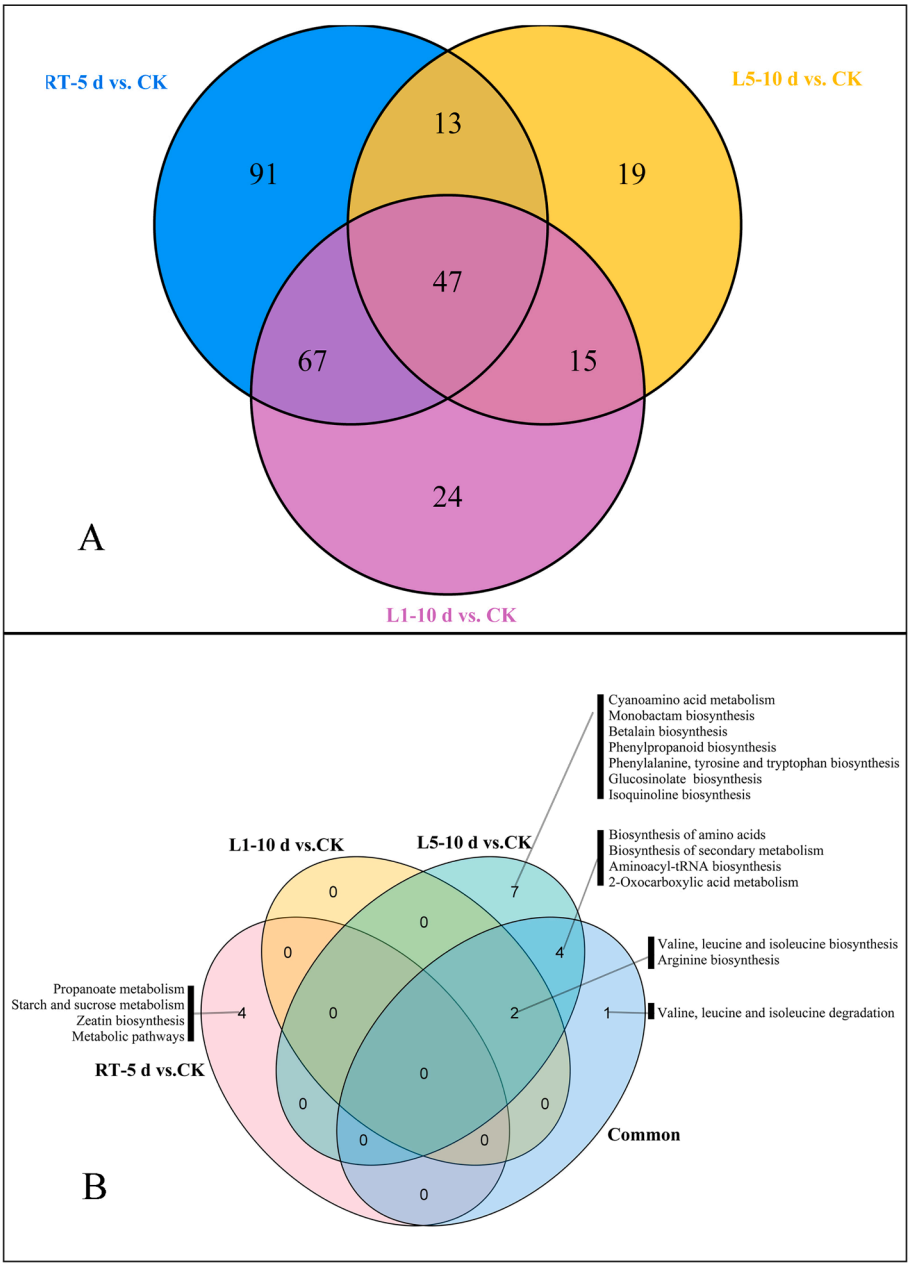

Fig. 3. Venn diagram of differentially expressed metabolites in each group (A), KEGG pathways enrichment relationship in each group (B).

mostly. The organic acid levels in L1-10 d were always higher than those in L5-10 d, which is consistent with the measured TA content during storage (Fig. 2F).

Phenylpropanoids have been considered to play pivotal roles in tolerance to biotic and abiotic stress (Vogt, 2010; Wang et al., 2020). It was reported that the enhancement of chilling tolerance in lemon fruit possibly resulted from increased production of total phenolics and the activation of enzymes involved in the phenylpropanoid pathway (Siboza, Bertling, & Odindo, 2014). Three phenylpropanoids and derivatives were found to have increased in L1-10 d, L5-10 d, and RT-5 d, in which 3,4,5-trimethoxycinnamic acid increased mostly in L1-10 d. These increased phenylpropanoids could affect the browning of rambutan pericarp under low-temperature stress by regulating the activities of phenylalanine ammonia-lyase (PAL), cinnamic acid-4-hydroxylase (C4H), 4-coumaric acid: CoA-ligase (4CL) (Wang et al., 2020).

3.3. The most variable DEMs in L1-10 d, L5-10 d and RT-5 d compared with CK

In addition to 47 common DEMs, the metabolites with the largest variation in L1-10 d, L5-10 d, and RT-5 d compared with CK may also be related to rambutan pericarp browning. As shown in Table 2, delphinidin 3-O-glucoside in RT-5 d decreased mostly compared with CK, followed by 5-hydroxy-L-tryptophan, C-rhamnosyl-apigenin O-feruloylhexoside, MAG (18:4) isomer1. Abundant anthocyanins content has been thought to contribute to the bright red color of fruit pericarp, and enzymatic or non-enzymatic degradation of anthocyanin is one of the main factors contributing to postharvest browning (Jiang, Duan, Joyce, Zhang, & Li, 2004; Zhang et al., 2015). During storage at room temperature, the phenolics and other anthocyanidins generated from the enzymatic degradation of delphinidin 3-O-glucoside may serve as substrates to be oxidized by peroxidase (POD) and polyphenol oxidase (PPO) to other compounds, contributing to pericarp browning. The up-

Table 2
Fold changes of most variable differentially expressed metabolites of rambutan under different temperatures.

Metabolites	Log_2 fold change			Metabolites	Log_2 fold change		
	L1-10 days vs.CK	L5-10 days vs.CK	RT-5 days vs.CK		L1-10 days vs.CK	L5-10 days vs.CK	RT-5 days vs.CK
Delphinidin 3-O-glucoside	–	–	−21.32	L-Glutamic acid	−1.89	−1.29	–
5-Hydroxy-L-tryptophan	–	–	−16.4	Eudesmoyl quinic acid	−1.88	–	–
C-rhamnosyl-apigenin O-feruloylhexoside	–	–	−14.56	L-Glutamic acid O-glucoside	−1.86	–	–
MAG (18:4) isomer1	–	–	−13.79	MAG (18:3) isomer3	4.8	–	–
2-Aminoethanesulfinic acid	–	–	−13.54	p-Coumaryl alcohol	5.56	–	–
Reduced form glutathione	−4.35	–	−8.49	13-HpOTrE(r)	10.94	–	–
N', N''-disinapoylspermidine	–	–	−8.42	2-Hydroxyisocaproic acid	13.13	–	–
CYS-GLY	−3.89	–	−7.45	3-Hydroxypropanoic acid	13.94	–	–
L-Ascorbate	−3.83	–	−5.17	(S)-(-)-2-Hydroxyisocaproic acid	16.36	14.7	–
Serotonin	–	–	−3.78	Sesamolin	19.79	–	–
LysoPC 16:0 (2n isomer)	–	–	6.5	O-Phosphocholine	–	−2.16	–
LysoPC 18:3	–	–	6.73	O-Phosphorylethanolamine	–	−1.53	–
LysoPC 12:1	–	–	7.09	Scopoletin	–	−1.5	–
trans-Cinnamate	–	–	11.98	Glutamic acid	–	−1.38	–
Ethyl gallate	14.32	8.77	12.27	Vitamin A	–	−1.35	–
Ethylmalonate	–	–	12.49	Acetyl tryptophan	–	−1.35	–
13-HpOTrE(r)	–	–	13.69	Pinoresinol	–	−1.35	–
LysoPC 10:0	–	–	14.61	N6-Acetyl-L-lysine	–	2.21	–
3,4,5-Trimethoxycinnamic acid	16.19	14.06	15.97	N-Acetyl-L-tyrosine	–	2.27	–
Nandrolone	14.58	–	16.02	Pinocembrin	–	2.4	–
L-Aspartic acid	−3.84	−2.05	–	DL-Homocysteine	–	2.7	–
Aspartic acid	−3.76	−1.87	–	2-Hydroxyisocaproic acid	–	11.54	–
2-Hydroxy-6-aminopurine	−2.57	–	–	Gentiopicroside	–	13.44	–
Cholesterol	−2	–	–	3-Hydroxypropanoic acid	–	13.5	–

regulation order of metabolites in RT-5 d was as follows: nandrolone, 3,4,5-trimethoxycinnamic acid, lysoPC (10:0); The level of reduced form glutathione in L1-10 d was decreased mostly compared with CK, followed by CYS-GLY, L-aspartic acid, L-ascorbate. The up-regulation order of metabolites in L1-10 d was as follows: sesamolin, (S)-(−)-2-hydroxyisocaproic acid, 3,4,5-trimethoxycinnamic acid; The level of O-phosphocholine in L5-10 d decreased mostly compared with CK, followed by L-aspartic acid, aspartic acid, which reduced significantly in L1-10 d as well. The up-regulation order of metabolites in L5-10 d was as follows: (S)-(−)-2-hydroxyisocaproic acid, 3,4,5-trimethoxycinnamic acid, which was promoted significantly in L1-10 d, L5-10 d, and RT-5 d compared with CK.

According to the above results described, glutathione and ascorbate decreased to 4.89% and 4.36%, compared with CK in rambutan stored at 1 ℃ for ten days. However, no significant changes in those two metabolites were observed in rambutan stored at optimal 5 °C for ten days. Glutathione is essential for ascorbic acid, which functions in metabolism, catalysis, transport, and cell protection (Meister, 1992). We speculated that glutathione and ascorbic acid were involved in the scavenging of free radicals during the browning process. Therefore, a serious decrease of them was found in the pericarps of RT-5 d and L1-10 d with severe browning. In contrast, nandrolone was up-regulated in RT-5 d and L1-10 d pericarps with severe browning, but no significant change was found in L5-10 d.

3.4. Pathways associated with the DEMs in L1-10 d, L5-10 d and RT-5 d compared with CK

The DEMs in the L1-10 d, L5-10 d and RT-5 d pericarps compared with the pericarps at 0 d were analyzed using the KEGG database by Fisher's exact test, and the similarities and differences in the main metabolic pathways were compared (Kanehisa, Goto, Sato, Furumichi, & Tanabe, 2011). Among 192 metabolic pathways, 18 of which had significant changes ($p < 0.05$) and may be related to browning. The main metabolic pathways were different in the L1-10 d, L5-10 d, and RT-5 d pericarps compared with CK. As showed in Fig. 3B, the metabolic pathways found only in RT-5 d pericarp with most serious browning were "propanoate metabolism", "starch and sucrose metabolism", "zeatin biosynthesis", "metabolic pathways". The main metabolic pathways of L1-10 d pericarps with serious browning were "valine, leucine and isoleucine degradation", "arginine biosynthesis", which were also significantly changed in L5-10 d pericarps. L5-10 d pericarps with slight browning had seven unique metabolic pathways, including "cyanoamino acid metabolism", "monobactam biosynthesis", "betalain biosynthesis", "phenylpropanoid biosynthesis", "phenylalanine, tyrosine and tryptophan biosynthesis", "glucosinolate biosynthesis", "isoquinoline biosynthesis" and six pathways also found in common DEMs. 47 common DEMs compared with CK were significantly enriched in seven metabolic pathways. The first four of them, including "biosynthesis of amino acids", "biosynthesis of secondary metabolism", "aminoacyl-tRNA biosynthesis", "2-oxocarboxylic acid metabolism" were also significantly enriched in L5-10 d pericarps with slight browning. The metabolic pathway of "valine, leucine and isoleucine degradation" was only found in 47 common DEMs.

These findings demonstrated that the respiratory metabolic pathways of rambutan pericarps were notably changed during storage at either room or low temperatures, resulting in losses in fruit quality and browning. During this process, the metabolic pathways related to carbohydrates, aliphatic metabolites, and organic acids might be highly active in RT-5 d rambutan pericarps, whereas the metabolic pathways related to amino acids biosynthesis and nucleotides might be highly active in L1-10 d and L5-10 d rambutan pericarps.

Respiratory metabolism played crucial roles in browning and senescence in harvested crops (Lin et al., 2018). The map of metabolic pathways (Fig. 4) showed that all the key DEMs participating in glycolysis and TCA, including sucrose, maltotetraose, glucose-6-P, fructose-6-P, phosphoenolpyruvate, and *trans*-citridic acid exhibited notable changes in RT-5 d rambutan pericarps. The large consumption of carbohydrates had two main effects on the browning process of rambutan. Firstly, the reduced fructose-6-P and phosphoenolpyruvate entered the phenylpropanoid biosynthesis pathway and produced a lot of ferulic acids, scopolin, scopoletin, and other substances in RT-5d rambutan pericarps. Oxidation of scopoletin and scopolin by hydrogen peroxidase yields a blue-black color (Bayoumi, Rowan, Beeching, & Blagbrough, 2008; Liu, Zainuddin, Vanderschuren, Doughty, & Beeching, 2017). Therefore, the browning of RT-5 d rambutan pericarps was the most serious. Secondly, pyruvic acid entered the valine, leucine and isoleucine biosynthesis pathway, resulting in an apparent decrease of

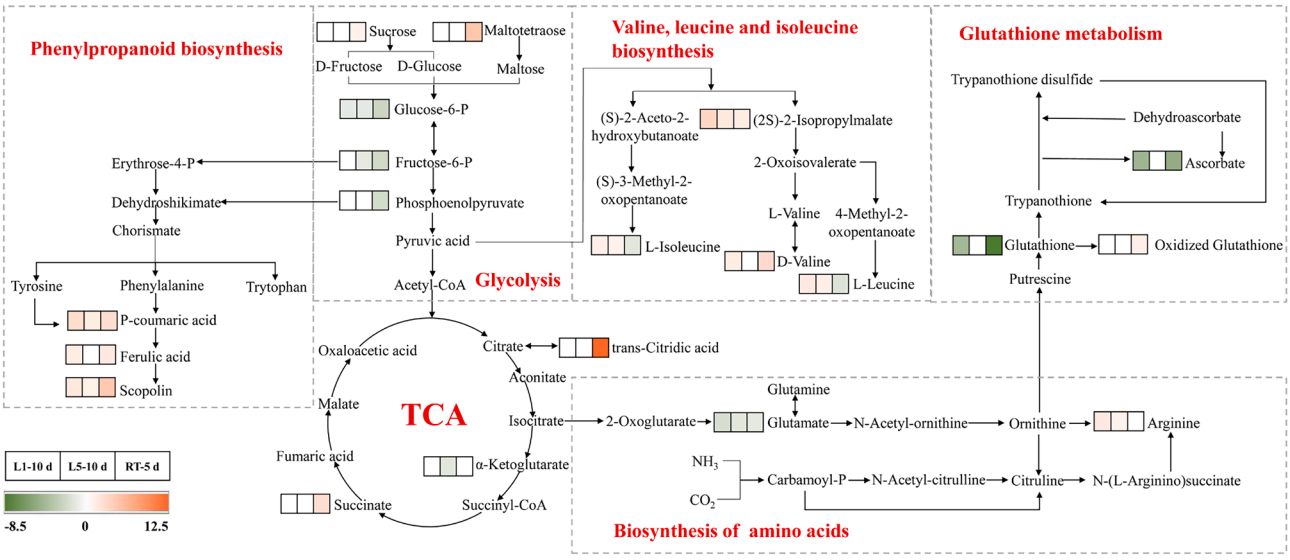

Fig. 4. Screening for maps of metabolic pathways involved in key differentially expressed metabolites.

leucine and isoleucine in RT-5 d. However, those two amino acids increased in L1-10 d and L5-10 d rambutan pericarps. We conjectured that leucine and isoleucine in RT-5d rambutan pericarps might convert to acetyl-CoA, then entered the TCA cycle again, participated in active respiration metabolism and accelerated the browning process.

Isocitrate from the TCA cycle entered the biosynthesis amino acids pathway, causing a significant decline of glutamate. Glutamate is a central molecule in amino acid metabolism in higher plants. The α-amino group of glutamate is directly involved in both the assimilation and dissimilation of ammonia and is transferred to ornithine and other amino acids (Forde & Lea, 2007). Ornithine entered the glutathione metabolism pathway, causing significant changes in glutathione and ascorbate. Interestingly, glutathione and ascorbate decreased significantly in L1-10 d and RT-5 d pericarps with severe browning, while the above two metabolites were not notably changed in L5-10 d pericarps with minimal browning. We speculate that glutathione and ascorbate might be used as enhancers to remove reactive oxygen species caused by browning.

4. Conclusion

The differences in the browning index, lightness, respiratory intensity, relative conductivity, total soluble solid, titratable acid demonstrated that room and low temperatures had notably different effects on the quality of "Baoyan-7" rambutan during storage. The browning of rambutan pericarp was the most serious under room temperature and lost its commercial value within five days. After ten days stored at 5 ℃, the browning index and lightness of rambutan pericarps were 4.2% and 147.5% of that at 1 ℃, indicating the least browning and best quality. An UPLC-MS/MS analysis was performed to uncover the metabolism underlying those effects, followed by the analysis of KEGG pathways of key DEMs. Our results demonstrated that the majority of the carbohydrates and derivatives were consumed, while lipids, organic acids, phenylpropanoids, and their derivatives were accumulated during low and room-temperature storage of rambutan. Moreover, the decreased fructose-6-P and phosphoenolpyruvate from glycolysis pathway entered the phenylpropanoid biosynthesis pathway. They converted into ferulic acids, scopolin, and blue-black coloring matter, which was one of the main reasons for the browning of rambutan pericarp during storage at room temperature. L-leucine and L-isoleucine in the L1-10 d and L5-10 d pericarps both increased to cope with cold stress. The consumption of glutathione and ascorbate could be used as potential indicators of browning degree, and there were no significant changes observed in rambutan stored at optimal 5 °C for ten days. These results provided a metabolic fingerprint that reveals how the room and low temperatures differentially influenced the quality of rambutan during storage.

CRediT authorship contribution statement

Hao Deng: Conceptualization, Methodology, Investigation, Writing original draft. **Qingchun Yin:** Conceptualization, Supervision, Writing original draft. **Yuqin Lin:** Methodology, Investigation, Review & Editing. **Jiancheng Feng:** Investigation, Review & Editing. **Zhe Chen:** Review & Editing. **Ronghu Zhang:** Conceptualization, Supervision, Review & Editing.

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.

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References

Bayoumi, S. A. L., Rowan, M. G., Beeching, J. R., & Blagbrough, I. S. (2008). Investigation of biosynthetic pathways to hydroxycoumarins during post-harvest physiological deterioration in Cassava roots by using stable isotope labelling. *Chembiochem, 9*(18), 3013–3022. https://doi.org/10.1002/cbic.v9:1810.1002/cbic.200800515

Fan, L., Wang, Q., Lv, J., Gao, L., Zuo, J., & Shi, J. (2016). Amelioration of postharvest chilling injury in cowpea (Vigna sinensis) by methyl jasmonate (MeJA) treatments. *Scientia Horticulturae, 203*, 95–101. https://doi.org/10.1016/j.scienta.2016.03.010

Forde, B. G., & Lea, P. J. (2007). Glutamate in plants: Metabolism, regulation, and signalling. *Journal of Experimental Botany, 58*(9), 2339–2358. https://doi.org/10.1093/jxb/erm121

Jiang, Y., Duan, X., Joyce, D., Zhang, Z., & Li, J. (2004). Advances in understanding of enzymatic browning in harvested litchi fruit. *Food Chemistry, 88*(3), 443–446. https://doi.org/10.1016/j.foodchem.2004.02.004

Kafkas, E., Koşar, M., Paydaş, S., Kafkas, S., & Başer, K. H. C. (2007). Quality characteristics of strawberry genotypes at different maturation stages. *Food Chemistry, 100*(3), 1229–1236. https://doi.org/10.1016/j.foodchem.2005.12.005

Kanehisa, M., Goto, S., Sato, Y., Furumichi, M., & Tanabe, M. (2011). KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Research, 40*(D1), D109–D114. https://doi.org/10.1093/nar/gkr988

Keutgen, A. J., & Pawelzik, E. (2008). Contribution of amino acids to strawberry fruit quality and their relevance as stress indicators under NaCl salinity. *Food Chemistry, 111*(3), 642–647. https://doi.org/10.1016/j.foodchem.2008.04.032

Landrigan, M., Morris, S. C., Eamus, D., & McGlasson, W. B. (1996). Postharvest water relationships and tissue browning of rambutan fruit. *Scientia Horticulturae, 66*(3), 201–208. https://doi.org/10.1016/S0304-4238(96)00915-6

Lea, P. J., & Miflin, B. J. (1974). Alternative route for nitrogen assimilation in higher plants. *Nature, 251*(5476), 614–616. https://doi.org/10.1038/251614a0

Lin, Y., Lin, Y., Lin, H., Chen, Y., Wang, H., & Shi, J. (2018). Application of propyl gallate alleviates pericarp browning in harvested longan fruit by modulating metabolisms of respiration and energy. *Food Chemistry, 240*, 863–869. https://doi.org/10.1016/j.foodchem.2017.07.118

Liu, S., Zainuddin, I. M., Vanderschuren, H., Doughty, J., & Beeching, J. R. (2017). RNAi inhibition of feruloyl CoA 6'-hydroxylase reduces scopoletin biosynthesis and post-harvest physiological deterioration in cassava (Manihot esculenta Crantz) storage roots. *Plant Molecular Biology, 94*(1–2), 185–195. https://doi.org/10.1007/s11103-017-0602-z

Liu, X., Yang, Q., Lu, Y., Li, Y., Li, T., Zhou, B., & Qiao, L. (2019). Effect of purslane (Portulaca oleracea L.) extract on anti-browning of fresh-cut potato slices during storage. *Food Chemistry, 283*, 445–453. https://doi.org/10.1016/j.foodchem.2019.01.058

Malundo, T. M. M., Shewfelt, R. L., & Scott, J. W. (1995). Flavor quality of fresh tomato (Lycopersicon esculentum Mill.) as affected by sugar and acid levels. *Postharvest Biology and Technology, 6*(1), 103–110. https://doi.org/10.1016/0925-5214(94)00052-T

Meister, A. (1992). On the antioxidant effects of ascorbic acid and glutathione. *Biochemical Pharmacology, 44*(10), 1905–1915. https://doi.org/10.1016/0006-2952(92)90091-V

Nicholson, J. K., Lindon, J. C., & Holmes, E. (2008). 'Metabonomics': Understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica, 29*(11), 1181–1189. https://doi.org/10.1080/004982599238047

O'Hare, T. J., Prasad, A., & Cooke, A. W. (1994). Low temperature and controlled atmosphere storage of rambutan. Postharvest Biology and Technology, 4(1-2) 147-157. https://doi.org/ 10.1016/0925-5214(94)90016-7.

O'Hare, T. J. (1995). Postharvest physiology and storage of rambutan. *Postharvest Biology and Technology, 6*(3), 189–199. https://doi.org/10.1016/0925-5214(95)00022-X

Ong, P. K. C., Acree, T. E., & Lavin, E. H. (1998). Characterization of volatiles in rambutan fruit (Nephelium lappaceum L.). *Journal of Agricultural and Food Chemistry, 46*(2), 611–615. https://doi.org/10.1021/jf970665t

Siboza, X. I., Bertling, I., & Odindo, A. O. (2014). Salicylic acid and methyl jasmonate improve chilling tolerance in cold-stored lemon fruit (Citrus limon). *Journal of Plant Physiology, 171*(18), 1722–1731. https://doi.org/10.1016/j.jplph.2014.05.012

Sun, J., Su, W., Peng, H., Zhu, J., Xu, L., & Bruñá, N. M. (2010). Two endogenous substrates for polyphenoloxidase in pericarp tissues of postharvest rambutan fruit. *Journal of Food Science, 75*(6), C473–C477. https://doi.org/10.1111/j.1750-3841.2010.01660.x

Tarczynski, M. C., Jensen, R. G., & Bohnert, H. J. (1993). Stress protection of transgenic tobacco by production of the osmolyte mannitol. *Science, 259*(5094), 508–510. https://doi.org/10.1126/science.259.5094.508

Tian, H., Lam, S., & Shui, G. (2016). Metabolomics, a powerful tool for agricultural research. *International Journal of Molecular Sciences, 17*(11), 1871. https://doi.org/10.3390/ijms17111871

Vogt, T. (2010). Phenylpropanoid biosynthesis. *Molecular Plant, 3*(1), 2–20. https://doi.org/10.1093/mp/ssp106

Wall, M. M. (2006). Ascorbic acid and mineral composition of longan (Dimocarpus longan), lychee (Litchi chinensis) and rambutan (Nephelium lappaceum) cultivars grown in Hawaii. *Journal of Food Composition and Analysis, 19*(6–7), 655–663. https://doi.org/10.1016/j.jfca.2005.12.001

Wang, J., Lv, M., He, H., Jiang, Y., Yang, J., & Ji, S. (2020). Glycine betaine alleviated peel browning in cold-stored 'Nanguo' pears during shelf life by regulating phenylpropanoid and soluble sugar metabolisms. *Scientia Horticulturae, 262*, 109100. https://doi.org/10.1016/j.scienta.2019.109100

Yabuta, Y., Motoki, T., Yoshimura, K., Takeda, T., Ishikawa, T., & Shigeoka, S. (2002). Thylakoid membrane-bound ascorbate peroxidase is a limiting factor of antioxidative systems under photo-oxidative stress. *The Plant Journal: for Cell and Molecular Biology, 32*(6), 915–925. https://doi.org/10.1046/j.1365-313x.2002.01476.x

Yang, M., Yang, J., Su, L., Sun, K., Li, D., Liu, Y., … Guo, T. (2019). Metabolic profile analysis and identification of key metabolites during rice seed germination under low-temperature stress. *Plant Science, 289*, 110282. https://doi.org/10.1016/j.plantsci.2019.110282

Yingsanga, P., Srilaong, V., Kanlayanarat, S., Noichinda, S., & McGlasson, W. B. (2008). Relationship between browning and related enzymes (PAL, PPO and POD) in rambutan fruit (Nephelium lappaceum Linn.) cvs. Rongrien and See-Chompoo. *Postharvest Biology and Technology, 50*(2–3), 164–168. https://doi.org/10.1016/j.postharvbio.2008.05.004

Yun, Z., Qu, H. X., Wang, H., Zhu, F., Zhang, Z. K., Duan, X. W., … Jiang, Y. M. (2016). Comparative transcriptome and metabolome provides new insights into the regulatory mechanisms of accelerated senescence in litchi fruit after cold storage. *Scientific Reports, 6*(1). https://doi.org/10.1038/srep19356

Zhang, Z., Huber, D. J., Qu, H., Yun, Z., Wang, H., Huang, Z., & Jiang, Y. (2015). Enzymatic browning and antioxidant activities in harvested litchi fruit as influenced by apple polyphenols. *Food Chemistry, 171*, 191–199. https://doi.org/10.1016/j.foodchem.2014.09.001

Zhao, J., Quan, P. K., Liu, H. K., Li, L., Qi, S. Y., Zhang, M. S., & Xing, L. B. (2020). Transcriptomic and metabolic analyses provide new insights into the apple fruit quality decline during long-term cold storage. *Journal of Agricultural and Food Chemistry, 68*(16), 4699–4716. https://doi.org/10.1021/acs.jafc.9b07107

Zhao, X., Chen, M., Li, Z., Zhao, Y., Yang, H., Zha, L., … Song, X. (2020). The response of volvariella volvacea to low-temperature stress based on metabonomics. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.01787