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Research Article

The Effect of Short-Term Temperature Pretreatments on Sugars, Organic Acids, and Amino Acids Metabolism in Valencia Orange Fruit

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Temperature pretreatment is one of the most important factors which significantly affects the postharvest quality of citrus fruit. In this study, late-ripening Valencia orange (*citrus sinensis*) fruits were used to investigate the effect of short-term treatment at low (6°C), room (20°C), and high (40°C) temperatures on fruit quality. Our results revealed that both low and room-temperature treatments maintained the content of sugars and organic acids, whereas high-temperature treatments elevated the accumulation of sugars but decreased the content of citric acid. In fruit peel (flavedo and albedo), the accumulation of sugars and organic acids responding to temperatures was diverse and mostly different from that in the pulp. Meanwhile, GABA and several amino acids were upregulated under short-term high-temperature treatment but downregulated in response to low-temperature treatment in both peel and pulp. Furthermore, PCA and correlation analysis revealed that the short-term temperature treatments changed the metabolic flow, and GABA was positively correlated with sugars and organic acids. Our study analyzed the metabolic changes of fruit peel and pulp in response to short-term temperature treatments and revealed that GABA may act as a signaling molecular involved in temperature-controlled quality changes.

1. Introduction

Citrus is one of the most popular and important fruits in the world because of its high nutritional and commercial value. Citrus fruits are rich in organic acids (particularly citric acid and malic acid), solute sugars (such as sucrose and fructose), and multiple amino acids that give them excellent flavor [1]. With the development of the citrus industry, the improvement and maintenance of fruit quality have been attracting more and more attention. Postharvest treatments are considered important strategies to maintain the fruit quality and extend the shelf life of citrus fruit [2].

To date, various preservation methods, including physical preservation and chemical preservation, have been widely used in the industry [3–5]. Physical preservation

methods, such as temperature treatments, show excellent effects on the maintenance of nutrition and natural flavors and are more environmentally friendly and safe approaches compared with chemical preservation methods [6].

Temperature treatments have been widely used as a preharvest and postharvest strategy in fruit preservation. Low-temperature (LT) treatment can effectively repress quality deterioration and delay fruit senescence, thereby extending the shelf life of fresh fruit [7–9]. Short-term LT, or precooling treatment, is a typical method to remove the respiratory heat and regulate fruit metabolism and has a good effect on maintaining fruit quality during postharvest [10, 11]. Recently, transcriptomic and metabolomic analyses revealed that appropriate LT or cold storage improved the inner and external quality via modulating multiple metabolic pathways [12, 13].

On the other hand, short-term high temperature (HT) treatment, such as hot water, hot steam, and hot air treatment, is another important physical preservation method and has become a necessary and effective pretreatment method before the long-term storage of fresh fruit. It has been reported to play an important role in maintaining postharvest quality and extending the shelf life of fresh fruit [14, 15]. HT treatment can not only repress postharvest respiration and water loss but also maintain the content of flavor metabolites such as sugar and organic acid [16–21].

Delaying the quality deterioration of citrus fruit is the primary goal throughout the storage. As is known, the late-ripening citrus fruit will reach commercial maturity in the spring or summer seasons of rising temperatures. The metabolic activity of citrus fruit is very high, which causes difficulty in maintaining the postharvest quality of fruit [22]. Meanwhile, preharvest treatments (such as heat treatment) have been determined to effectively maintain or improve fruit quality during long storage [20, 23]. However, the information about the quality changes and direct effects of short-term temperature treatments on late-ripening citrus fruit is limited. Therefore, this study aimed to evaluate the effects of different temperature treatments on the fruit quality of late-ripening citrus and provide more information on the improvement of physical preservation.

2. Materials and Methods

2.1. Plant Materials. Valencia orange (*citrus sinensis*) fruits were harvested at the commercially mature stage (April) from the orchard of Zigui Country in Yichang City, Hubei Province, China. After the harvest, the fruits with obvious damage were removed.

2.2. Experimental Design and Treatments. Valencia orange fruits of uniform size and homogeneous color were selected for experiments. For cold or heat shock treatments, Valencia orange fruits were stored in storage chambers (LRH-70F, Yiheng, China) with different parameters (relative humidity, 85%–90%; temperature, 6°C (low temperature, LT), 20°C (room temperature, CK), and 40°C (high temperature, HT)). The storage chambers with low and high temperatures were used to simulate the cold and heat shock treatments, respectively. The samples treated with different temperatures were conducted at 6 and 24 hours after treatment (HAT), respectively. 80 fruits of uniform size and color and free of visible injury or blemishes were used in each treatment. Three replications, each containing 10 fruits, were analyzed and measurements were performed. The flavedo (outer colored part of the peel), albedo (inside colorless part of the peel), and pulp were, respectively, sampled, frozen, and homogenized in liquid nitrogen, and kept at −80°C for later analysis.

2.3. Extraction of the Primary Metabolite. The primary metabolites of different tissues in citrus fruit were detected with the approach of GC-MS analysis according to the method as described previously with minor modifications [24]. Fruit tissues were grounded in liquid nitrogen, and then 0.3 g of different samples were used for the following metabolite extraction. The primary metabolites were extracted with 2700 μL precooled methanol and 300 μL ribitol (2 mg·mL^{-1}, as an internal standard).

2.4. Derivatization of the Primary Metabolite. The extracts (100 μL) were vacuum-concentrated at 30°C for 180 min. The concentrated extracts were incubated with 80 μL methoxamine hydrochloride (20 mg mL^{-1} in pyridine) at 37°C for 90 min, followed by 80 μL MSTFA (N-methyl-N-(trimethylsilyl) trifluoroacetamide) at 37°C for 30 min.

2.5. GC-MS Analysis of Primary Metabolite. After derivatization, each sample was analyzed by GC-MS with the programs described previously [23]. Briefly, each sample was injected into the gas chromatograph onto a fused-silica capillary column (30 m × 0.25 mm i.d., 0.1 μm, Agilent Technologies) with a split ratio of 20 : 1. The injector temperature was 230°C, and the carrier gas was at a flow rate of 1.2 mL/min. The column temperature was held at 100°C for 1 min, increased to 184°C with a temperature gradient of 3°C/min, increased to 190°C at 0.5°C/min for 1 min, and increased to 280°C with a temperature gradient of 15°C/min. The column temperature was held at 280°C for 5 min. The flow rate of carrier helium (99.999%) gas was 1 mL/min. Total ion current spectra were recorded over a mass range of *m*/*z* 45–600 in a scan mode. The final concentration of the metabolite was qualified using the internal standard (mg/g).

2.6. Statistical Analysis. All data are shown as the (mean ±SD) of one representative experiment. Significant differences between treatments were determined using an ANOVA followed by a Tukey's test. The partial least squares discriminant analysis (PLS-DA) was performed using the mixOmics package. The heatmaps and hierarchical clustering were performed using the pheatmap package. The correlation analysis was performed using *R* studio software. Figures were drawn using a GraphPad Prism (GraphPad Software, CA, USA).

3. Results

3.1. Effect of Short-Term Temperature Treatments on the Total Sugar and Organic Acid in Fruit Pulp. The late-ripening orange fruits, namely Valencia orange fruits, were treated at different temperatures. After 24 hours of treatment, no significant differences were observed in fruit appearance between different treatments (Figure 1(a)). As shown in Figure 1(b), after 6 hours of treatment, the content of total sugar in the pulp of all treated fruits was similar (35 mg/g to 42 mg/g). After 24 hours, the sugar content in the pulp treated at relatively high temperatures (CK, 44 mg/g; high, 53 mg/g) was higher than that of low (34 mg/g). Meanwhile, the content of total organic acid in the pulp treated at relatively high temperatures (CK and high) was much higher than that treated at low temperatures after 6 and 24 hours. The content of total organic acid was observed to be lower after 24 hours of
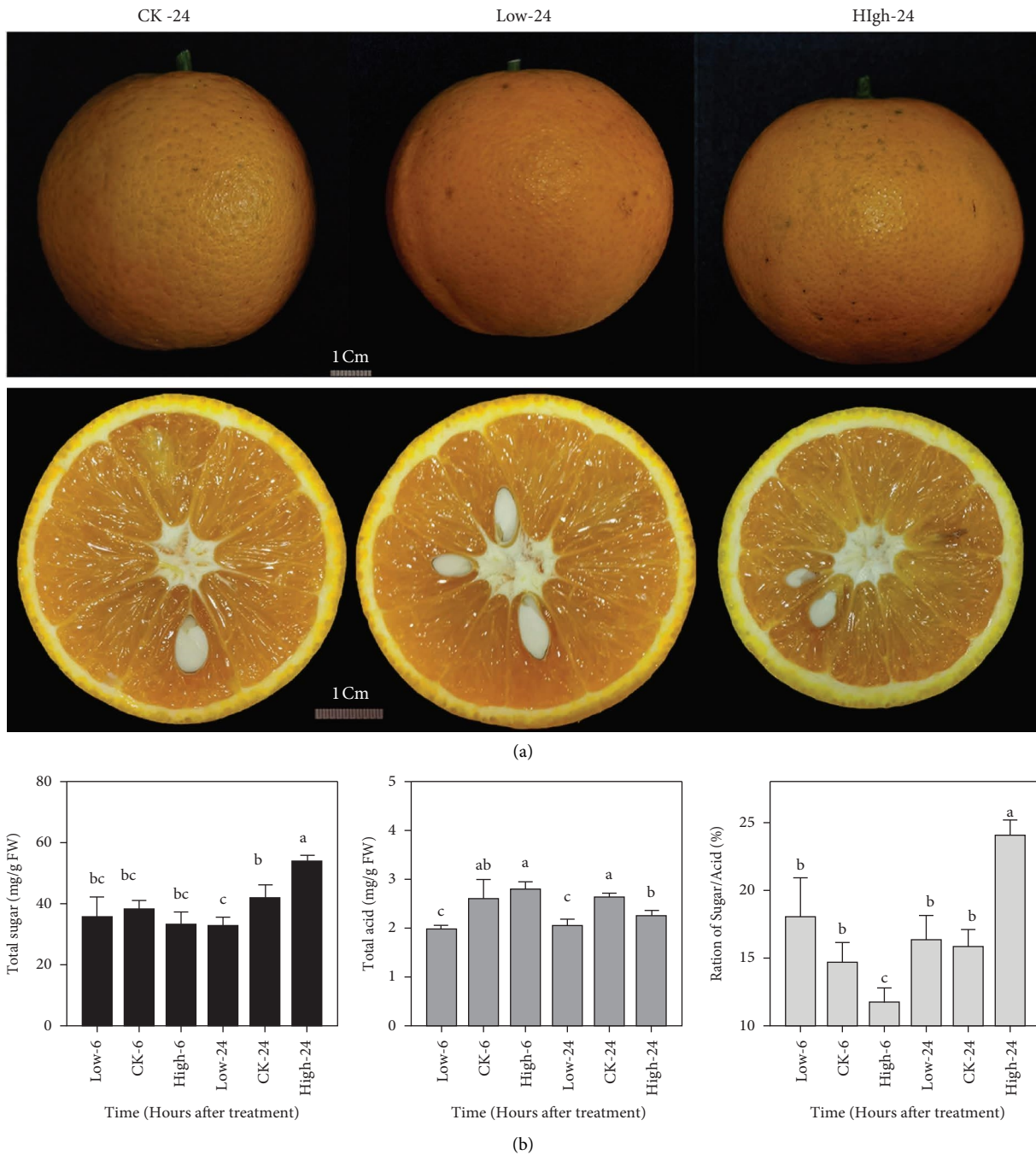
(a)

(b)

Figure 1: Content of total sugar and organic acid in the pulp of Valencia orange fruit. The vertical bars represent the standard errors of the mean. (a) The appearance of fruits with different treatments. (b) Content of total sugar, total organic acid, and the ratio of sugar to acid under different treatments. The vertical bars represent the standard errors of the mean. Values with different letters within the same figure are significantly different according to the ANOVA test at $p < 0.05$.

treatment with high temperature (HT) compared with that after 6 hours. Besides, the ratio of sugar to acid in fruit treated with HT was much higher at 24 hours but lower at 6 hours compared with other treatments (Figure 1(b)).

3.2. Effect of Short-Term Temperature Treatments on Sugar Content in Fruit Tissues.
The three main forms of soluble sugar in fruits, namely fructose, glucose, and sucrose, were

further analyzed. As shown in Figure 2, the content of these sugars in the pulp shared similar trends with the total sugar, and the content of those treated with HT (high temperature) after 24 hours was significantly higher than other treatments. In the flavedo, fructose and glucose showed the highest content in CK after 6 hours of treatment and the lowest content in CK after 24 hours of treatment. Besides, the content of fructose and glucose in LT treatment was observed to increase over time, while that of them in CK
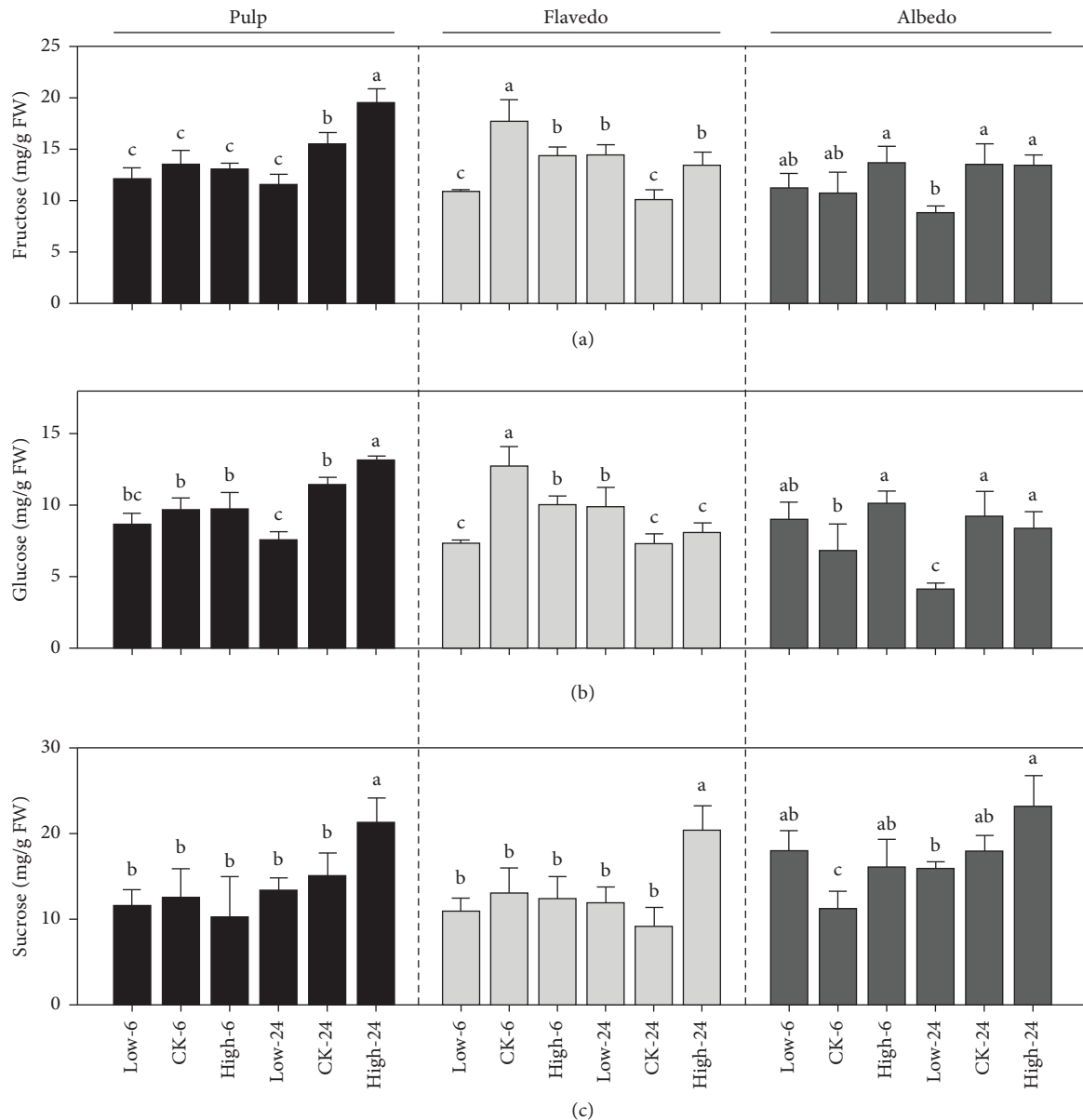
FIGURE 2: Effects of short-term temperature treatments on sugar contents ((a) fructose; (b) glucose; and (c) sucrose) in the pulp, flavedo, and albedo of Valencia orange fruits. The vertical bars represent the standard errors of the mean. Values with different letters within the same figure are significantly different according to the ANOVA test at $p < 0.05$.

decreased. In albedo, the content of fructose and glucose was lower after 24 hours of LT treatment than that after CK and HT treatments (Figures 2(a) and 2(b)). Notably, the content of sucrose in different fruit tissues shared similar trends, and it was highest after 24 hours of HT treatment (Figure 2(c)). These results indicated that the effect of different temperatures on the three main sugars in the pulp was similar, as well as the sucrose in the peel (flavedo and albedo).

3.3. Effect of Short-Term Temperature Treatments on the Organic Acid Content in Fruit Tissues. As the most important organic acid in fruit pulp, citric acid was observed to significantly increase under the relatively high temperature (CK

and high) and showed an unchanged content under LT treatment (Figure 3(a)). Meanwhile, the malic acid showed similar trends with a total organic acid content, but its content in CK after 6 hours of treatment was significantly lower than in the other treatments (Figure 3(b)). The content of quininic acid was observed to be elevated by HT in the pulp of the fruit (Figure 3(c)). In flavedo, citric acid had a lower accumulation (0.01 mg/g to 0.02 mg/g), compared with that in the pulp (more than 2 mg/g to 3 mg/g) (Figure 3(a)). As the main organic acid in flavedo, malic acid showed decreased content over time under different temperature treatments and had the highest content under LT treatment after 6 hours (Figure 3(b)). The content of quininic acid was much higher in flavedo (0.1 mg/g to 0.2 mg/g)
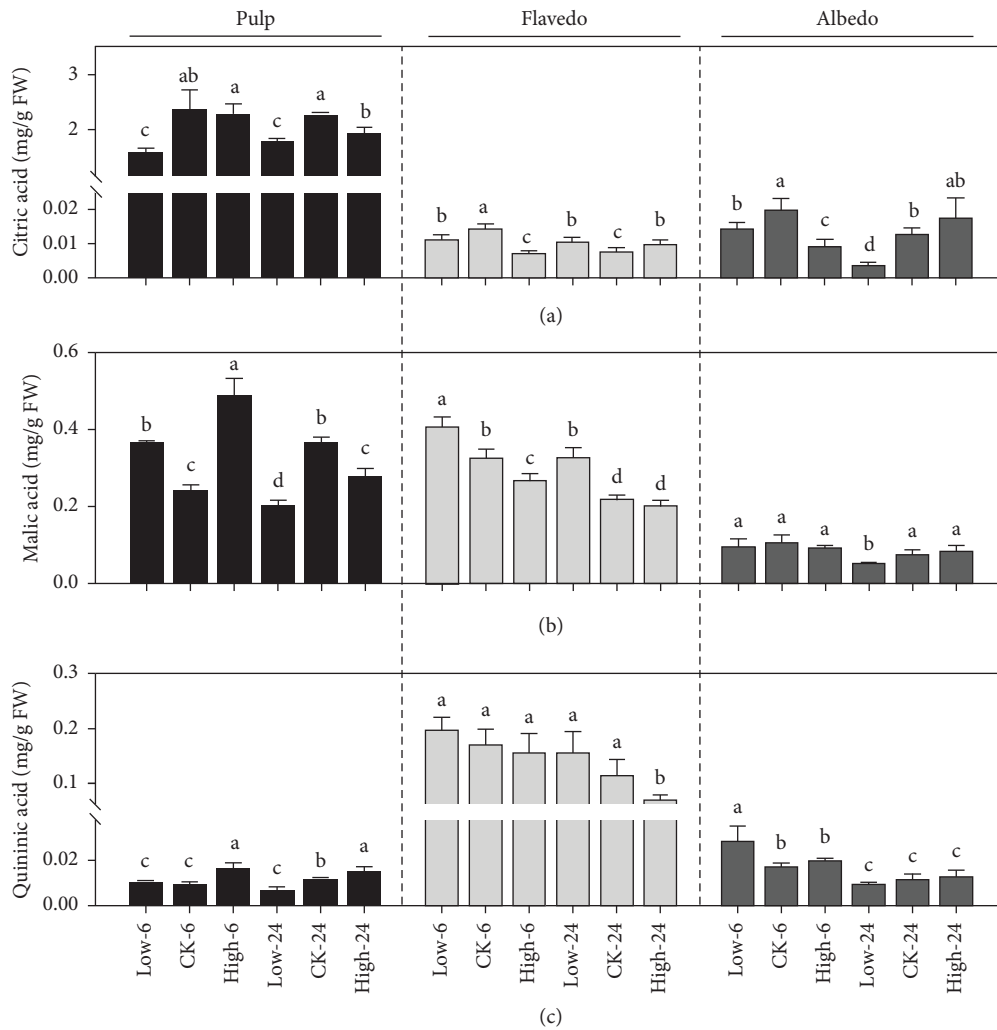
FIGURE 3: Effects of short-term temperature treatments on the organic acid contents ((a) citric acid; (b) malic acid; and (c) quininic acid) in the pulp, flavedo, and albedo of Valencia orange fruits. The vertical bars represent the standard errors of the mean. Values with different letters within the same figure are significantly different according to the ANOVA test at $p < 0.05$.

than that in other tissues (less than 0.03 mg/g). In albedo, all these organic acids showed low accumulation (less than 0.1 mg/g). Citric acid and malic acid were observed to be decreased under LT treatment after 24 hours. Besides, the content of quininic acid decreased significantly over time in the albedo of fruit (Figure 3(c)). In summary, the changes in the content of citric acid almost accounted for the changes in total organic acid in the pulp. Besides, HT treatment induced the decline of major organic acids in different tissues.

3.4. Effect of Short-Term Temperature Treatments on Amino Acid Content in Fruit Tissues.

In total, 11 amino acids were detected in this assay, as well as 3 other metabolites. As shown in Figure 4(a), most of these metabolites (particularly glycine, GABA, and Myo-inositol) were significantly reduced in the pulp under LT treatment after 24 hours. In the pulp under CK, the contents of aspartic acid, oxoproline, palmitic acid, and stearic acid were observed to accumulate after 24 hours, while GABA and asparagine were decreased.

After HT treatment, valine, alanine, glycine, and Myo-inositol showed higher accumulation than those under other treatments. Meanwhile, the content of GABA, proline, and threonine was increased after 24 hours of treatment (Figure 4(a)). In flavedo, oxoproline, palmitic acid, and stearic acid were significantly accumulated 24 hours under relative higher temperature treatments (CK and high) after whereas, aspartic acid, asparagine, and glutamic acid had high content after 6 hours of treatment followed by obviously decreasing after 24 hours' treatment under LT and CK treatments (Figure 4(b)). Additionally, valine, GABA, and proline showed higher content after 24 hours of HT treatment (Figure 4(b)). In albedo, the of content oxoproline, Myo-inositol, palmitic acid, and stearic acid highly accumulated after 6 hours of HT treatment; however, aspartic acid, asparagine, and glutamic acid shared similar content trends with that in flavedo (Figure 4(c)). The content of several amino acids (such as serine, glycine, and threonine) was much higher than that in other treatments. Notably, GABA was found to significantly accumulate after 24 hours
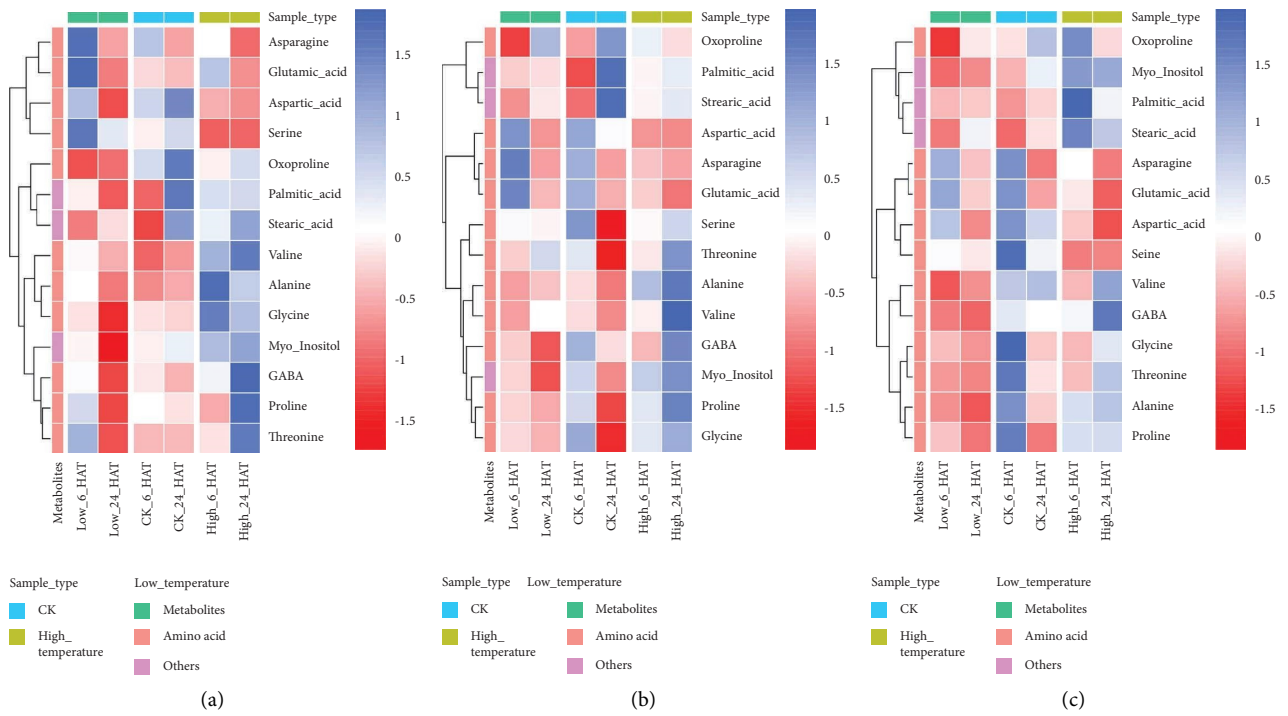
(a)

(b)

(c)

Figure 4: Effects of different temperature treatments on amino acid content in the pulp (a), flavedo (b), and albedo (c) of Valencia orange fruits. The content of each metabolite was scaled by row with the Z-score method. In the color key, the blue color indicates a high expression level, and the red color indicates a low expression level.
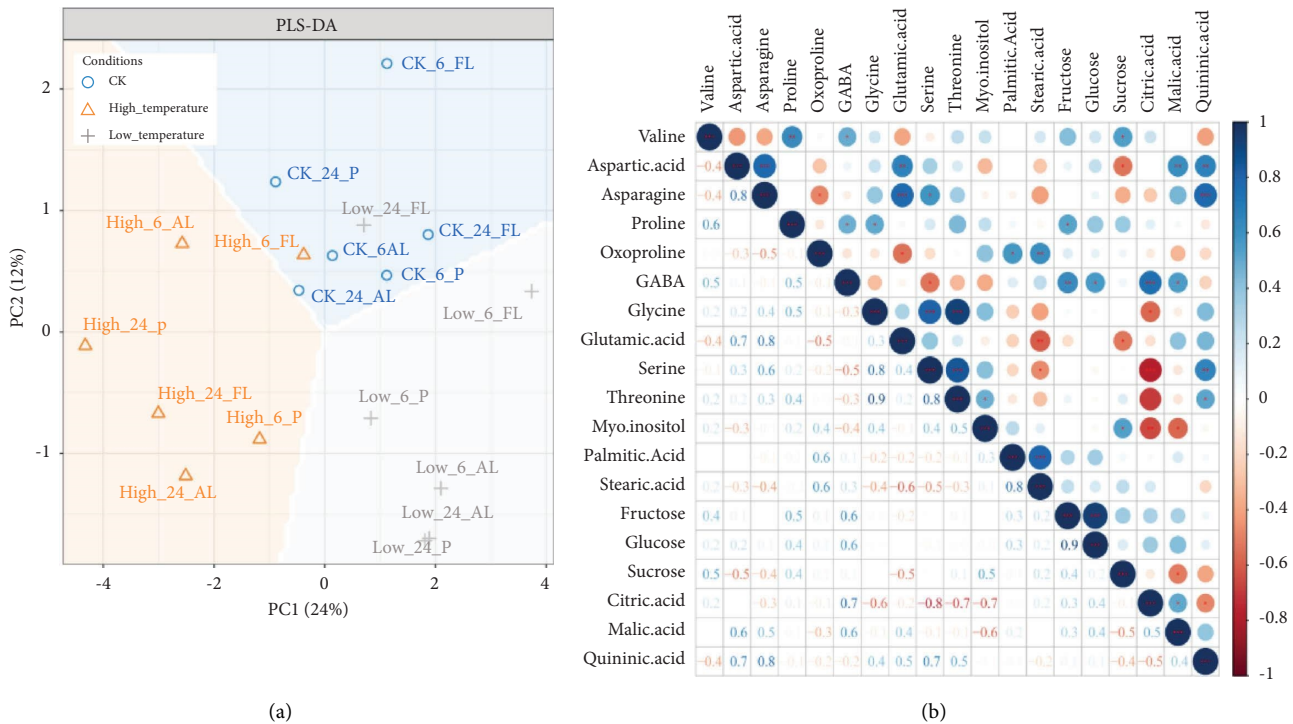

(a)

(b)

Figure 5: Multivariate analysis of metabolites in Valencia orange fruits with different temperature treatments. (a): PLS-DA score plot. The blue circle represents the control group (CK); the orange triangle represents high-temperature treatment; the gray cross represents low-temperature treatment. (b): Correlation analysis between metabolites. The color depth and the dot size indicate the correlation strength; red indicates a negative correlation, and blue indicates a positive correlation. ***indicates $p < 0.001$, **indicates $p < 0.01$, and *indicates $p < 0.05$.

of HT treatment, while decreasing after 24 hours of LT treatment (Figure 4(c)). Together, the LT treatment caused the decline of most amino acids, while the HT treatment contributed to the accumulation of them (particularly GABA and proline) in different tissues.

3.5. PLS-DA and Correlation Analysis. To better understand the effect of short-term temperature treatments on fruit metabolites, partial least-squares discrimination analysis (PLS-DA) was used to investigate the differences in metabolites among different treatments. As shown in Figure 5(a), PC1 and PC2 accounted for 24% and 12% of the total variance, respectively. Most samples under the same treatment were clustered together, while the flavedo after 6 hours of HT treatment (High_6_FL) and the flavedo after 24 hours of LT treatment (Low_24_FL) were found to be clustered with tissues under CK treatment. Besides, there was an obvious time-specificity under the same temperature treatment. Furthermore, Pearson correlation analysis was constructed to analyze the relationships between different metabolites. As shown in Figure 5(b), fructose was positively related to glucose ($R^2 = 0.9$), and sucrose was closely related to several amino acids. Besides, GABA was observed to be positively related to citric acid and malic acid, which were negatively related to Myo-inositol (Figure 5(b)). These results indicate that temperature treatments have a significant effect on the metabolic flow of citrus fruit.

4. Discussion

The preharvest or postharvest treatments have become crucial parts of the citrus industry and contribute to the annual supply of citrus fruit. To date, various strategies have been developed and demonstrated to effectively maintain the fruit quality and extend the shelf life [25–28]. It is worth noting that temperature acts as an essential environmental factor and modulates the respiration and metabolic activities in various metabolism pathways [2, 29, 30].

As it is known, the temperature has been indicated to be a fundamental factor influencing the quality of citrus fruit. Maintaining storage temperature or removing field heat from fresh fruit will decrease the deterioration and senescence processes, which obviously preserves fruit quality [29, 31]. Previously, the sweating treatment, which was used to remove the field heat of fruit, was also found to contribute to maintaining fruit quality and increasing disease resistance [23]. In the present study, short-term LT treatment was also found to maintain the content of main quality components, including solute sugars and organic acids, in the pulp of Valencia orange fruit (Figures 2 and 3). The slight changes in the content of sugar and organic acid may be mainly due to the low activity of primary metabolism caused by low temperatures [32, 33]. Whereas, multiple amino acids (particularly GABA and proline) were observed to show decreased content after short-term LT treatment compared with that after HT treatment (Figure 4). The degradation of amino acids modulated by LT may account for the changes in amino acid metabolic enzymes and transporters [34, 35].

Similar to short-term LT treatment, HT treatment is usually applied as a pretreatment method to treat fresh fruits (such as citrus and apples) before long-term storage [14, 36, 37]. Recent studies have revealed that HT treatment can modulate metabolic flow by regulating lots of genes and enzyme activity [15, 18, 38]. Herein, HT treatment was found to elevate the accumulation of three main sugars and several amino acids (such as GABA) in fruit pulp (Figure 2). Whereas, the content of citric acid and malic acid significantly decreased after 24 hours of HT treatment (Figure 3). Similarly, the content of soluble solids in mandarin fruit and persimmon fruit treated with HT also increased, but citric acid showed decreased content [39, 40]. As reported previously, the HT treatment stimulated sugar metabolism and induced the accumulation of sugars; meanwhile, it activated the transcription of many genes related to citric degradation [23, 38]. Besides, our results showed that HT treatment resulted in a higher sugar-to-acid ratio after 24 hours of treatment and a lower ratio after 6 hours (Figure 1(b)). As reported previously, the different effects of HT treatment on fruit quality may account for the treatment duration, the temperature, handling, or even the fruit species [14, 41, 42]. Hence, to investigate the treatment duration or number of handlings on citrus fruits will maximize the effect of HT treatment on quality improvement and maintenance.

Our study found that several amino acids accumulated in the peel (flavedo and albedo) after HT treatment but decreased with LT or CK treatment. Notably, the nonprotein amino, namely GABA, showed the highest content after 24 hours of HT treatment but the lowest content after LT treatment (Figure 4). In mandarin and peach fruit, GABA was also found to be upregulated after sweating or hot water treatment [23, 43]. However, GABA in *longan* fruit showed increased accumulation after precooling treatments, which was different from our results [44]. Studies have shown that GABA is mainly metabolized via the GABA shunt, which is one of the pathways involved in citric acid degradation [45, 46]. Hence, the up or downregulated GABA may be due to the specialized primary metabolic activity response to temperature treatments. Notably, GABA has been investigated to be an important signal molecule involved in the regulation of fruit preservation, disease resistance, and plant development [47, 48]. Exogenous GABA treatments were also found to improve fruit quality and delay postharvest senescence [24, 49, 50]. Therefore, it is worth investigating the regulation mechanism of GABA in response to temperature and its potential functions in fruit quality control.

5. Conclusions

Temperature treatments have a significant effect on the citrus fruit quality. In this study, short-term LT treatment contributes to the maintenance of sugars (including fructose, glucose, and sucrose) and citric acid, but decreases the content of several amino acids in the pulp of citrus fruit. HT treatment significantly promotes the accumulation of sugars and lots of amino acids but decreases the content of organic acids. Meanwhile, the signaling molecular GABA was upregulated after HT treatment but downregulated after LT

treatment. Our results indicated that the short-term temperature treatments affected the metabolic activity in different tissues of citrus fruit, and GABA may play a part in the regulation of fruit quality. The content of metabolites, metabolic activity, and activation of signal pathways are important factors affecting the fruit's storage quality before long-term storage. Our results provide new insights for the study of temperature-regulated fruit quality.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Mingfei Zhang oversaw conceptualization, validation, formal analysis, investigation, resources, writing-original draft, and visualization. Resources, investigation, and project administration were done by Rangwei Xu, Guochao Sun, and Yunjiang Cheng. Zhihui Wang was responsible for supervision, project administration, and funding acquisition.

Acknowledgments

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