



Pre-storage conditioning treatment reduces physiological disorders by mediating the targeted primary metabolites in ‘Chuhwangbae’ pears during shelf life after cold storage

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Received: 2 November 2024 / Revised: 29 January 2025 / Accepted: 1 February 2025 / Published online: 14 March 2025
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Abstract

The fruit of the late season ‘Chuhwangbae’ Asian pear cultivar is prone to the incidence of physiological disorders during cold storage and following shelf life. However, pre-storage conditioning treatments have been used to reduce the incidence and severity of numerous storage disorders in cold-stored Asian pears during cold storage and shelf life. Thus, this study aimed to evaluate the effects of a pre-storage conditioning treatment at 22 °C for seven days on fruit quality attributes, the incidence and severity of physiological disorders, and targeted primary metabolites in the fruit of late season ‘Chuhwangbae’ Asian pear cultivar for a shelf life of 21 days at 22 °C following three months of cold storage at 0.5 °C with 90% of relative humidity. The peel blackening, cortex browning, core browning, and cavity were reduced by pre-storage conditioning treatment during shelf life after short-term cold storage. Although flesh firmness responded inconsistently in earlier shelf life, titratable acidity (TA) was lower in pre-storage conditioned fruit than in untreated fruit and weight loss and the ratio of soluble solids content (SSC) to TA were higher in pre-storage conditioned fruit compared to untreated fruit as a control. The cortex hue angle values of the fruit colour variables were higher in pre-storage conditioned fruit than in the untreated fruit. The pre-storage conditioning treatment notably affected threonine and total amino acids among the targeted primary metabolites during shelf life after cold storage. The correlation coefficient network indicated that the cortex browning and cavity were positively correlated with fumaric acid, sorbitol, and sucrose in the pre-storage conditioned fruit. Therefore, the results suggested that pre-storage conditioning treatment should be highly effective in reducing the incidence and severity of physiological disorders by mediating primary metabolism during the shelf life after cold storage.

Keywords Asian pear · Cavity · Core browning · Cortex browning · Peel blackening · Targeted metabolomics

1 Introduction

As a long-term popular Asian pear cultivar in Korea, the ‘Shingo’ Asian pear cultivar, known as the ‘Niitaka’ pear in Japan, has been broadly cultivated and popular cultivar in Korea which is appreciated owing to its postharvest premium fruit quality of high storability and consistent fruit quality at harvest and during long-term cold storage (Choi et al. 1995; Lee et al. 2003). Thus, it has been reported that

more than 85% of Asian pear production is derived from one single Asian pear cultivar, ‘Shingo’, according to the results of the annual national statistics report on agricultural commodities (KOSIS 2024). Among the developed Asian pear cultivars, numerous cultivars have been actively bred, and released into the Asian pear industry; however, ‘Chuhwangbae’ as a late-season Asian pear cultivar, has garnered notable interest from Asian pear growers and is cultivated and produced extensively to meet consumer demand in the

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Communicated by Eun Jin Lee.

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Asian pear market (Hwang et al. 2001; Lee et al. 2018). There were several physiological disorders, detected in the fruit of the ‘Chuhwangbae’ Asian pears at harvest and during cold storage, or even during shelf life immediately after harvest or cold storage (Lee et al. 2018; Lwin and Lee 2021a). Chilling injury symptoms in ‘Chuhwangbae’ pears manifest as several physiological disorders, such as peel blackening, cortex and core browning, and the formation of cavities on the peel, cortex, and core tissues. The severity of the storage disorders is further aggravated as they advance in both cold storage and shelf life condition after cold storage (Latt et al. 2024a; Lwin et al. 2023b).

Among various potential technologies to control the development of physiological disorders during cold storage and the following shelf life phase, the controlled atmosphere (CA) storage technology could be involved in delaying the incidence of flesh breakdown in CA stored Asian pears (Park et al. 1999). During short-term storage, 2% CO₂ treatment delays the development of skin blackening in the fruits of late-season Asian pear cultivars (Lee et al. 2018). The incidence and severity of specific physiological disorders, including internal browning, core browning, pithiness, and water soaking, increase with an increase in storage and handling temperatures in the fruit of early-season Asian pears during shelf life followed by short-term cold storage (Lee et al. 2013b). Pre-harvest aminoethoxyvinylglycine (AVG) treatment reduces the incidence of pithiness and core browning in ‘Wonhwang’ Asian pears during the shelf life period right after harvest, with no incidence of mealiness observed (Choi et al. 2013). The application of 1-methylcyclopropene (1-MCP) postharvest treatment has been linked to a decrease in both the occurrence and intensity of flesh and core browning, as well as pithiness and mealiness, in early-season Asian pears throughout their shelf life following harvest (Lee et al. 2012c, 2014, 2017). However, pre-harvest GA₄₊₇ application enhances the severity of peel staining, cortex and core browning, water soaking, mealiness, and skin browning during shelf life immediately following harvest in early-season Asian pears (Lee et al. 2014) and during their distribution in international markets (Seo et al. 2019). In addition, the severity of physiological disorders is further aggravated by pre-harvest GA₄₊₇ treatment in the fruit of early-season Asian pears during long-term cold storage (Latt et al. 2024b). However, the pre-drying application reduces the incidence of skin blackening in the fruit of the ‘Chuhwangbae’ Asian pear cultivar during short-term cold storage (Lee et al. 2018). In addition, the implementation of pre-storage conditioning immediately following harvest has been shown to diminish both the incidence and severity of physiological disorders in Asian pear cultivars during cold storage (Seo et al. 2019).

Recently, untargeted and targeted metabolomic approaches based on highly advanced analytical technologies have been actively applied to elucidate the biochemical mechanisms underlying the incidence of physiological disorders in several horticultural commodities after and during cold storage. An untargeted metabolomics approaches using GC- and LC-MS based technologies are used to investigate the incidence of firm flesh browning in CA-stored ‘Empire’ apple (Lee et al. 2012b) and internal browning in CA-stored ‘Braeburn’ apple (Lee et al. 2012a) as well as the incidence of core breakdown in CA-stored ‘Conference’ European pear (Pedreschi et al. 2009). Furthermore, the targeted metabolomics methodology is particularly effective in elucidating the specific metabolic pathways linked to the development of physiological disorders in cold-stored fruits of Asian pear cultivars (Lwin and Lee 2022; Lwin et al. 2023c). The targeted metabolomics approach was applied to examine the biochemical mechanisms involved in physiological disorders affected by pre-storage ambient temperature conditioning in the fruit of ‘Chuhwangbae’ Asian pears during long-term cold storage (Lwin et al. 2023b). However, whether pre-storage ambient temperature conditioning after harvest affects fruit quality or the incidence of physiological disorders during the shelf life period after short-term cold storage remains unclear. Therefore, this study aimed to test the hypothesis that the pre-storage ambient temperature conditioning treatment could affect the fruit quality and reduce the incidence and severity of physiological disorders during shelf life period after short-term cold storage in the fruits of late season ‘Chuhwangbae’ Asian pear cultivar. Additionally, the targeted metabolomics approach was applied to comprehend the relationship between targeted primary metabolites and physiological disorders in pre-storage conditioned fruit of ‘Chuhwangbae’ Asian pear cultivar for up to 21 days of shelf life period after short-term cold storage.

2 Materials and methods

2.1 Fruit material, treatment and storage conditions

The fruits of the ‘Chuhwangbae’ pear (*Pyrus pyrifolia* Nakai) cultivar were collected on October 4, 2018, from the Pear Research Center, at the National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA) located in Naju, Jeollanam-do, Republic of Korea. Pears were selected according to similar size and no physiological storage disorders or mechanical damages and stored at 22 °C for 7 days as a pre-storage ambient temperature conditioning treatment. Untreated fruits were directly transferred to the cold storage facility

at 0.5 °C with 90% relative humidity (RH). Then, the fruit quality parameters and tissue sampling to evaluate the pre-storage ambient temperature conditioning treatment effects were conducted on 1, 7, 14, and 21 days during the shelf life period after three months of cold storage.

2.2 Determination of fruit quality parameters

The effects of pre-storage ambient temperature conditioning treatment were evaluated on fruit quality attributes, the incidence and severity of physiological disorders, and targeted major metabolites during the shelf life period at 22 °C after short-term cold storage. Fruit fresh weight loss was determined at harvest time and at the corresponding sampling time point during shelf life after three months of cold storage (Park et al. 2024). A chroma meter (Minolta CR 200, Konica Minolta Co., Osaka, Japan) was used to assess the colour parameters of the peel, cortex, and core tissues (Lwin et al. 2023a). Fruit flesh firmness was evaluated using a texture analyzer (TAHDI/500, TAHD, London, UK) with an 11 mm cylinder shape probe (Latt et al. 2024b). The titratable acidity content (TA, %) and soluble solids content (SSC, %) were analyzed using a Titrette bottle-top burette (BrandTech® Scientific, Inc., Wertheim, Germany) and a digital refractometer (PAL-α, Atago Co., Tokyo, Japan), respectively (Latt et al. 2024a). After cutting the equatorial portion of the fruit, the incidence and severity rate of physiological disorders were recorded according to previous studies (Latt et al. 2023; Lee et al. 2013a; Lwin et al. 2021b). The severity of each disorder was evaluated as the percentage of the area exhibiting the disorder relative to the total surface area and subjectively defined the levels as: 0, 0%; 1, 1–10%; 2, 11–25%; 3, 26–50%; 4, 51–75%; and 5, 76–100%. The incidence was determined as the proportion of the fruit exhibiting the disorder. Then, the cortex tissues from the different parts of the equatorial region of fifteen fruits were flash-frozen with liquid nitrogen and stored at -80 °C for metabolite analyses.

2.3 Determination of soluble carbohydrates and organic acids

The sample extraction protocols for the measurement of soluble carbohydrates and organic acids were adopted from previous studies (Latt et al. 2023; Lwin and Lee 2020). For analysis of soluble carbohydrates, such as sucrose, fructose, glucose, and sorbitol, 5 g of fresh sample was mixed with 30 mL of extract buffer consists of 0.1 M perchloric acid and 0.1% meta-phosphoric acid in water and a syringe filter. Measurements were performed using filtered through high-performance liquid chromatography (HPLC) with a flow

rate of 0.5 mL min⁻¹ purified water and column oven set at 70 °C.

For organic acids, 10 µL of extracted samples were injected into an Ultimate 3000 HPLC (Thermo Dionex Co., Sunnyvale, CA, USA) equipped with refractive index detector (RI detector, ERC, RefractoMAX520, Data Apex, Prague, Czech Republic) with an Aminex column (300 × 7.8 mm, Bio-Rad Co., Hercules, CA, USA) as previous studies (Latt et al. 2024b).

2.4 Determination of free amino acids

To determine the free amino acid contents, the extract was mixed with hydrochloric acid and homogenized before drying in a centrifuge. Then, the derivation was performed using a borate buffer for 15 min at a temperature of 56 °C. An Ultimate 3000 HPLC system (Thermo Dionex Co., Sunnyvale, CA, USA) was equipped with a fluorescence and UV detectors (Agilent 1260, Agilent Technologies, Santa Clara, CA, USA) was used for the analysis. The mobile phase solvents consisted of 40 mM sodium phosphate, and formulated from purified water, acetonitrile, and methanol in a ratio of 10:45:45 (v/v, %), with a flow rate was set at 0.5 mL min⁻¹ (Latt et al. 2024a).

2.5 Statistical analyses

The results were presented with mean ± standard error (SE) with three replicates. All data were analyzed using two-way ANOVA with two main factors, pre-storage ambient temperature conditioning treatment and shelf life after short-term cold storage, and their interaction effect using SAS Ver.9.3 (SAS Institute Inc., Cary, NC, USA). The mean difference between the untreated control fruits and the pre-storage ambient temperature conditioning treatments was assessed utilizing the least significant difference (LSD) test. Fruit quality parameters, physiological storage disorders, and targeted primary metabolites for the determination of pre-storage ambient temperature conditioning treatment effects were generated with a normalized heatmap system using a MetaboAnalyst Ver. 5.0 (Chong et al. 2018; Lwin et al. 2021a). The network of correlation coefficients was constructed utilizing the MetScape plugin within the Cytoscape software Ver. 3.8.2 (<https://cytoscape.org/>) (Basu et al. 2017; Lwin and Lee 2021b; Lwin et al. 2021b).

3 Results

3.1 Pre-storage ambient temperature conditioning effects on the physiological disorders

The incidence rate and severity of physiological disorders were determined during the shelf life period immediately after 3 months of cold storage at 0.5 °C with 90% RH. The physiological disorders, such as peel blackening, cortex browning, core browning, and cavity, occurred in fruits after cold storage, and the incidence and severity of physiological disorders were higher in untreated control fruit than in pre-storage conditioned fruit during shelf life after short-term cold storage. In all physiological disorders, fruits treated with pre-storage ambient temperature conditioning showed reduced incidence and severity levels compared to the control fruits during shelf life. In particular, core browning was only detected in the untreated control fruit during shelf life, with an increasing trend. Overall, an increasing trend was observed in almost all physiological disorders, whereas fruit treated with pre-storage ambient temperature conditioning treatment showed evidently reduced physiological disorders after 2 weeks of shelf life (Fig. 1).

3.2 Fruit quality attributes

The physiological fruit quality attributes were not significantly different between the untreated control fruit and pre-storage conditioned fruit, except for flesh firmness, TA, and the ratio of SSC to TA values at certain sampling points (Figs. 2 and 5). During shelf life, the fruit fresh weight loss was gradually increased in both control and treated fruits. Similarly, for fruit flesh firmness, the decreasing rate of fruit treated with pre-storage ambient temperature conditioning was lower than that of untreated control fruits during the second half of the shelf life period. However, flesh firmness was highly maintained in the fruit of the pre-storage ambient temperature conditioning treatment immediately after the removal of cold storage compared to the untreated fruit. SSC was only different on day 1 after cold storage but did not differ between treatments during the entire shelf life period. However, TA tended to be lower in the pre-storage ambient temperature conditioning treatment than in the untreated control fruit during the entire shelf life period after short-term cold storage. Thus, during the shelf life, the SSC-to-TA ratio tended to be higher in the pre-storage ambient temperature conditioning treatment than in the untreated control fruit (Fig. 2).

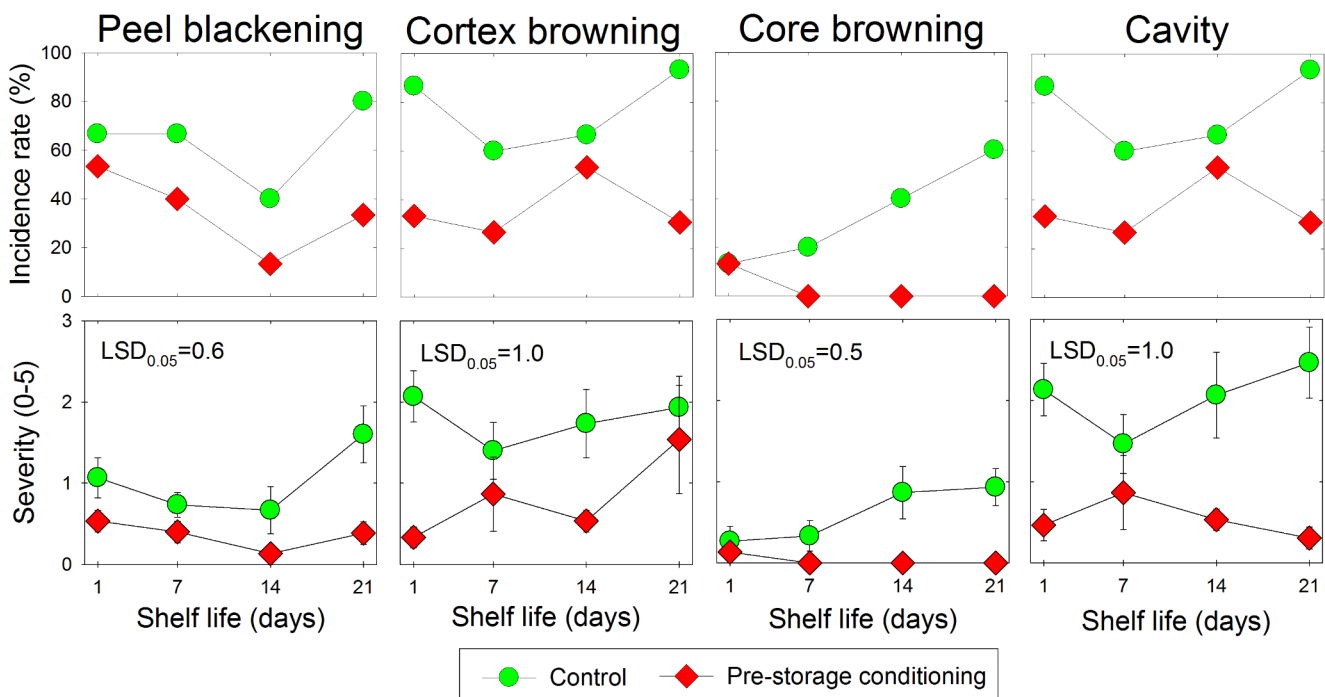


Fig. 1 The incidence rate and severity of peel blackening, cortex browning, core browning, and cavity in ‘Chuhwangbae’ pear fruit treated with pre-storage conditioning at 22 °C for 7 days after harvest and stored at 0.5 °C for 3 months followed by 21 days at 22 °C. Each datum point indicates the mean of fifteen fruit replicates ($n=15$) $\pm$ standard error, where larger than the symbols. The incidence of physiologi-

cal disorders was expressed as a percent of affected fruit ($n=15$). The severity of physiological disorders was subjectively scored as 0=0%, 1=1–10%, 2=11–25%, 3=26–50%, 4=51–75%, and 5=76–100% area coverage of the peel, cortex, and core tissues with the largest area for the corresponding symptoms. The least significant difference (LSD) test was applied to examine significance at $p=0.05$

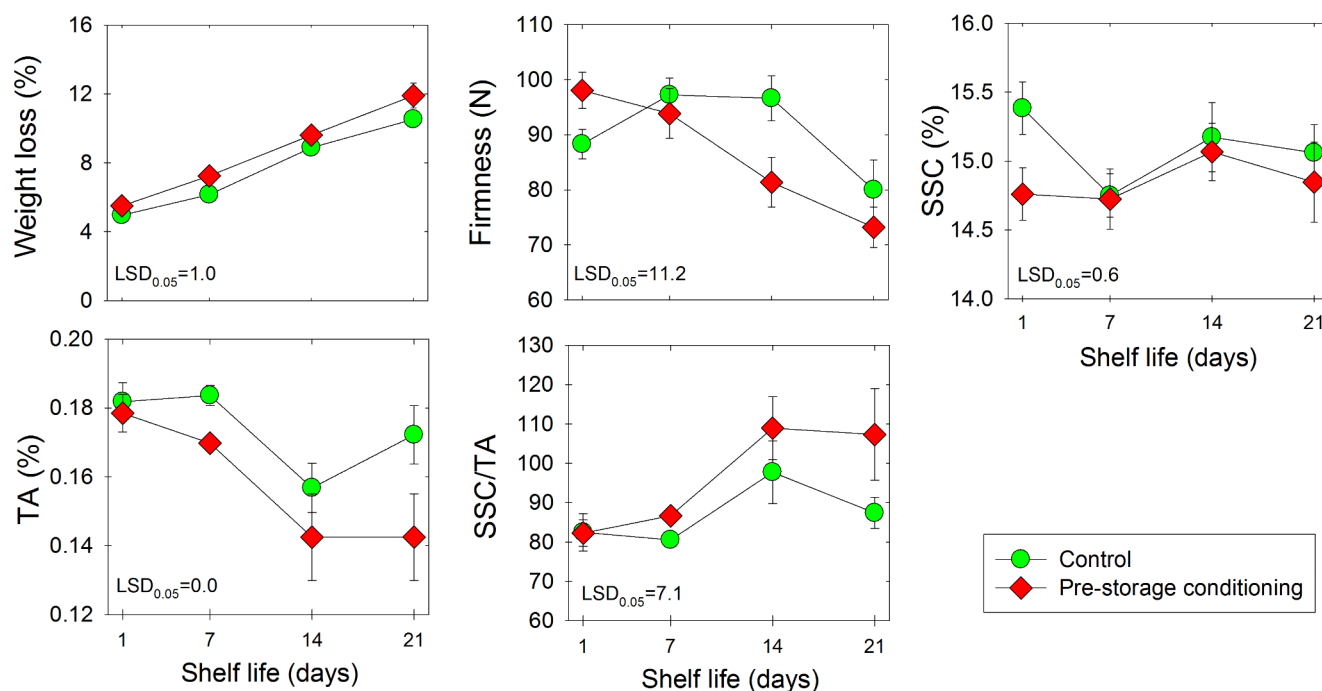


Fig. 2 Physiological responses of fruit fresh weight loss, flesh firmness, soluble solids content (SSC), titratable acidity (TA), and the ratio of SSC to TA to the pre-storage conditioning treatment at 22 °C for 7 days immediately after harvest in ‘Chuhwangbae’ pear fruit and stored

at 0.5 °C for up to 3 months followed by 21 days at 22 °C. Each datum point indicates the mean of fifteen fruit replicates ($n=15$) $\pm$ standard error, where larger than the symbols. The least significant difference (LSD) test was applied to examine significance at $p=0.05$

The treatment responses of the colour variables of the fruit peel, cortex, and core tissues were shown in Fig. 3. The lightness and chroma values of the peel, cortex, and core tissues were gradually declined during shelf life, although there was an inconsistent response to the pre-storage ambient temperature conditioning treatment. However, the hue angle value of the cortex tissue was higher in pre-storage conditioned fruit than in untreated control fruit, while the values of peel and core tissues did not consistently respond to pre-storage ambient temperature conditioning treatment during shelf life after short-term cold storage (Figs. 3 and 5).

3.3 Targeted primary metabolites

The metabolic responses of the pre-storage ambient temperature conditioning treatment were evaluated during the shelf life immediately after short-term cold storage (Fig. 4). Soluble carbohydrates, such as fructose, sucrose, glucose, and sorbitol were detected in the fruits of the control and treated groups. The levels of fructose and glucose were increased; however, sucrose was decreased during the shelf life in both the untreated control and treated fruits. The sorbitol content was increased in the control fruit until 14 days of shelf life, while the pre-storage ambient temperature conditioning-treated fruit remained constant until the end of the shelf life period. Similarly, the response to pre-storage

ambient temperature conditioning was not significantly affected during shelf life for major organic acid contents. Of the targeted primary metabolites, no significant effects were observed on free amino acid contents by pre-storage ambient temperature conditioning treatment during the shelf life period compared to the untreated control fruit. However, amino acid contents, such as glutamic acid, threonine, and alanine, were higher in the pre-storage ambient temperature conditioning treatment than in the untreated control fruit at the end of the shelf life period. Meanwhile, isoleucine and leucine contents were higher in the untreated control fruit than in the pre-storage-conditioned fruit at the end of the shelf life period. Overall, after short-term cold storage, the free amino acid contents in both control and pre-storage conditioned fruits were decreased in accordance with the shelf life period (Figs. 4 and 5).

3.4 Normalized heatmap matrix responses to pre-storage conditioning treatment

The normalized heatmap matrix was used to analyse the effects of pre-storage ambient temperature conditioning treatment on fruit quality attributes, physiological disorders, and targeted primary metabolites in the fruit of ‘Chuhwangbae’ pears during the shelf life period after short-term cold storage, together with the results of ANOVA with the treatment and interaction effects at 1, 7, 14, and 21 days shelf life

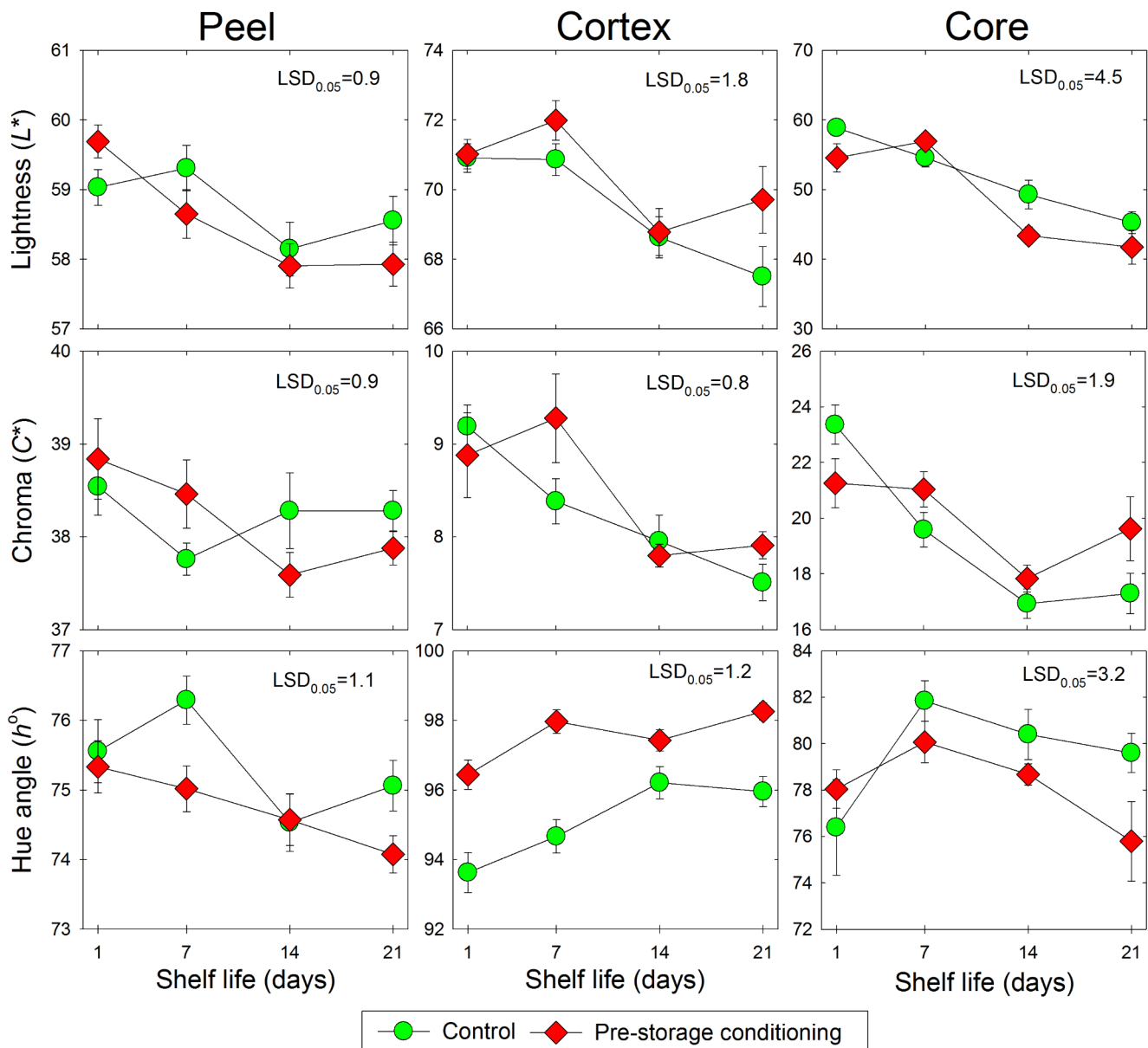


Fig. 3 Physiological responses of lightness (L^*), chroma (C^*), and hue angle (h°) from peel, cortex, or core tissues to pre-storage conditioning treatment at 22 °C for 7 days immediately after harvest in ‘Chuhwang-bae’ pear fruit and stored at 0.5 °C for 3 months followed by 21 days at

22 °C. Each datum point indicates the mean of fifteen fruit replicates ($n=15$) $\pm$ standard error, where larger than the symbols. The least significant difference (LSD) test was applied to examine significance at $p=0.05$

at 22 °C (Fig. 5). The results indicated that variables, such as fruit fresh weight loss, cortex h° , targeted primary metabolites, total amino acids, and threonine were highly affected by the pre-storage ambient temperature conditioning treatment. Physiological disorders, such as peel blackening, cortex browning, core browning and cavity, were significantly influenced by the application of the pre-storage ambient temperature conditioning treatment. However, the incidence and severity of physiological disorders were not influenced by the shelf life period or the interaction effects of the pre-storage ambient temperature conditioning treatment and

shelf life period. Moreover, flesh firmness, peel C^* , SSC, SSC/TA, TA, sorbitol, citric acid, shikimic acid, total amino acids, serine, aspartic acid, and lysine were not affected by shelf life duration, and the remaining variables showed significant responses to shelf life duration. Among the variables examined, only fresh fruit weight loss, flesh firmness, asparagine, and GABA were influenced by the interaction effect of pre-storage ambient temperature conditioning and shelf life duration factors (Fig. 5).

3.5 Correlation coefficient heatmap matrix

Pearson's correlation coefficient heatmap matrix was applied to determine the overall relationship among fruit quality attributes, physiological disorders, and targeted primary metabolites, irrespective of pre-storage ambient temperature conditioning treatment in the fruits of the late season 'Chuhwangbae' pears during the shelf life period immediately after short-term cold storage. The results were compared with all data obtained from untreated and conditioned fruits. According to the results of overall correlation coefficient heatmap matrix revealed that malic acid, fructose, cortex h° , peel C^* , SSC, core h° , fresh weight loss, peel h° , peel L^* , and cortex L^* were strongly and positively correlated with each other. Additionally, this positive correlation coefficient cluster was positively correlated with arginine, glucose, citric acid, fumaric acid, and SSC/TA at the lower left of the correlation heatmap matrix. In the upper-right corner of the correlation heatmap matrix, glutamic acid, aspartic acid, threonine, serine, glycine, cortex C^* , lysine, total amino acids, and asparagine were positively correlated. However, their relationship was relatively weak compared with the results from the previous cluster. Notably, cavity, peel blackening, cortex browning, and core browning were strongly and positively correlated. Furthermore, isoleucine was positively correlated with valine (Fig. 6).

3.6 Pearson's correlation coefficient network

The effects of pre-storage ambient temperature conditioning treatment were examined with a Pearson's correlation coefficient network, based on the threshold value of correlation coefficient ($r \geq |0.75|$) (Fig. 7). In the untreated control fruits, fruit weight loss was strongly and positively correlated with colour variables, such as peel L^* , peel C^* , peel h° , core h° , cortex L^* , cortex h° , shikimic acid, and aspartic acid. Moreover, fumaric acid was strongly and negatively associated with sucrose, glutamine, and tyrosine. Physiological disorders in untreated control fruit were positively correlated with some amino acids, such as isoleucine, valine, threonine, arginine, and glucose, which were positively correlated with organic acids, such as citric and malic acids (Fig. 7A). In contrast, for physiological disorders in pre-storage conditioned fruit, cortex browning and cavity were positively correlated with sorbitol, sucrose, and fumaric acid. In addition, fresh fruit weight loss was strongly and positively correlated with TA and flesh firmness and was positively correlated with amino acids, such as isoleucine, tyrosine, phenylalanine, and glycine. Cortex h° was positively correlated with glutamine and tryptophan; simultaneously, they were positively linked to fruit fresh weight loss and flesh firmness (Fig. 7B).

4 Discussion

Late-season Asian pear cultivars, such as 'Chuhwangbae' cultivar, are highly sensitive to long-term cold storage (Lwin and Lee 2021a; Lwin et al. 2021b). The symptoms of several physiological disorders, including skin blackening (Choi et al. 2020; Lee et al. 2018), peel staining (Latt et al. 2024a), internal browning (Lwin and Lee 2021a), core browning (Latt et al. 2024a), and cavity (Lwin et al. 2023b) could be detected and severely developed in the fruit of 'Chuhwangbae' Asian pears during cold storage and even shelf life after cold storage. Several potential technical approaches have been continuously performed and evaluated to confirm the effectiveness of controlling or suppressing the incidence of physiological disorders in cold-stored pear fruit. Of those technical applications, postharvest 1-MCP technology was not highly effective in controlling the incidence of internal browning and peel blackening in 'Chuhwangbae' pears during the shelf life after cold storage (Lwin and Lee 2021a; Lwin et al. 2021b). However, gradual cooling, pre-drying, storage temperature control, and CO₂ treatments could be fairly effective in reducing the incidence of physiological disorders during the shelf life or cold storage (Choi et al. 2020; Latt et al. 2024a; Lee et al. 2018). In addition, pre-storage ambient temperature conditioning treatment could be highly effective on controlling the development of physiological storage disorders in cold-stored 'Chuhwangbae' pears (Lwin et al. 2023b). In this study, pre-storage ambient temperature conditioning treatment at harvest reduced the incidence and severity of physiological disorders in the 'Chuhwangbae' cultivar during its shelf life after cold storage (Figs. 1 and 5). Previously, it was confirmed that pre-storage ambient temperature conditioning treatments could control physiological disorders only during cold storage (Lwin et al. 2023b). Furthermore, pre-drying treatment and delayed cooling after pre-conditioning at harvest reduce the incidence of skin blackening in cold-stored 'Chuhwangbae' pears (Lee et al. 2018). Pre-conditioning suppresses chilling injury symptoms by mediating ethylene biosynthesis and altering valine and isoleucine metabolism in cold-stored peaches, thereby enhancing the chilling injury tolerance (Tanou et al. 2017). Pre-storage ambient temperature conditioning treatment considerably mitigates the incidence and severity of chilling injury symptoms, such as soft scald and soggy breakdown, in long-term CA-stored 'Honeycrisp' apples by influencing the glycolysis/gluconeogenesis, propionate metabolism, and alanine, aspartate, and glutamate metabolism (Leisso et al. 2017). Therefore, the results indicated that pre-storage ambient temperature conditioning immediately before cold storage could be associated with the metabolic adjustment within pear fruit. This process potentially allows a physiological adaptation period

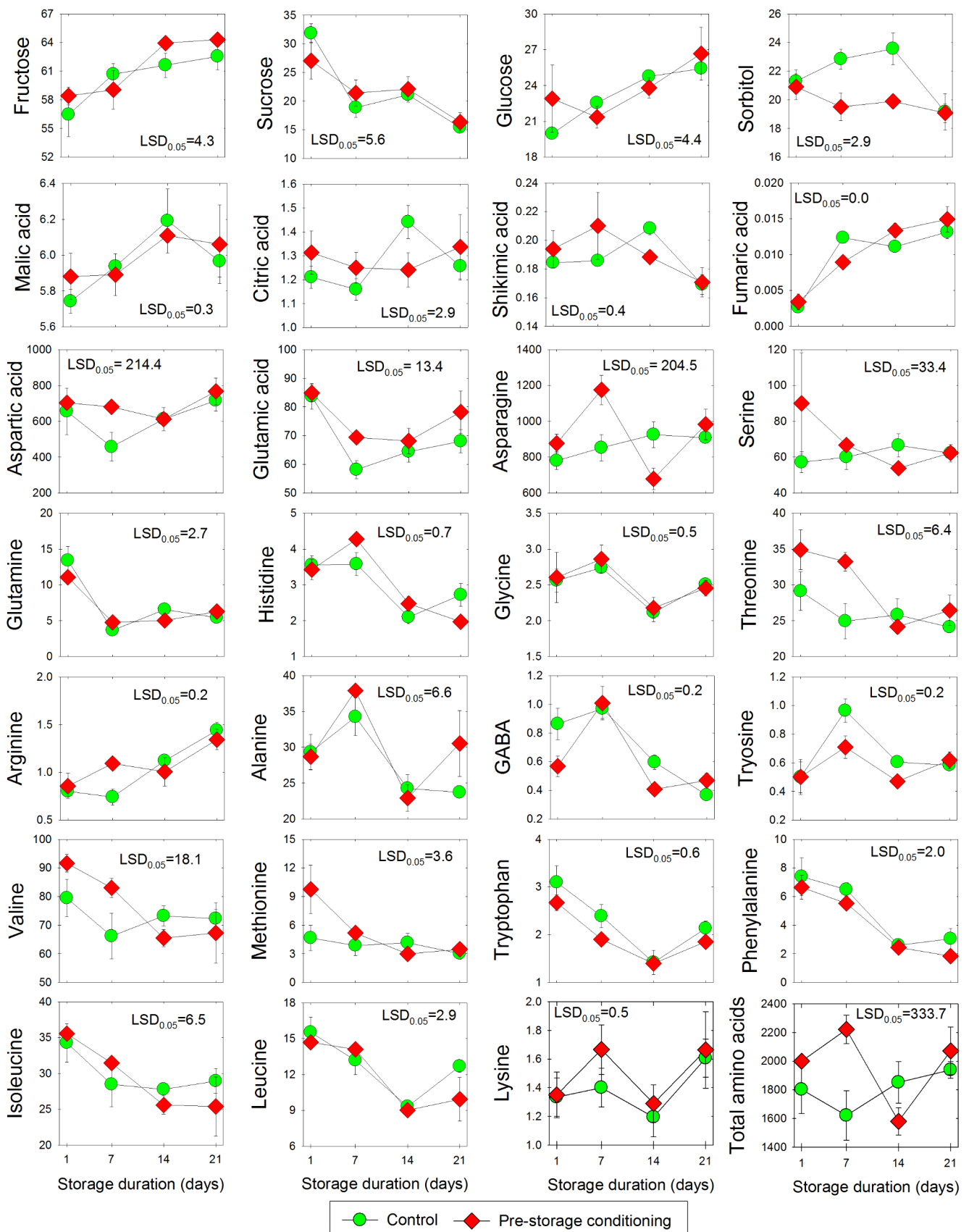


Fig. 4 Metabolic responses of soluble carbohydrates (fructose, sucrose, glucose, and sorbitol), major organic acids (malic, citric, shikimic, and fumaric acids), and free amino acids contents to pre-storage conditioning treatment at 22 °C for 7 days immediately after harvest in ‘Chuhwangbae’ pear fruit and stored at 0.5 °C for 3 months followed by 21 days at 22 °C. Each datum point is the mean of three replicates ($n=3$) $\pm$ standard error. Each data unit was mentioned as fresh weight basic and expressed as g kg⁻¹ FW for soluble carbohydrates and organic acids and mg kg⁻¹ FW for amino acids, respectively. The least significant difference (LSD) test was applied to examine significance at $p=0.05$

to the rapid cold storage, thereby reducing the incidence and severity of physiological disorders during the shelf life period. In contrast, pre-drying treatment immediately before cold storage gradually decreases the incidence of skin blackening during cold storage, as fruit fresh weight loss increases at harvest (Lee et al. 2018). Typically, late-season Asian pear cultivars would be more susceptible to the incidence of water soaking or watercore at harvest (Hayama et al. 2014; Liu et al. 2022) and even during cold storage (Latt et al. 2024b). The intercellular space of flesh tissues from the symptoms of water soaking or watercore would be fully saturated with water molecules within the pore space of the cortex tissues (He et al. 2024) and core regions (Chigwaya et al. 2021) in stored apples. With an increase in the delayed cooling air temperature, fruit fresh weight loss increases and flesh firmness decreases in apples (Li et al. 2015) and Asian pears (Lee et al. 2016b). However, ethylene production and fruit respiration rates are increased with increasing pre-conditioning temperature in Asian pears during the pre-conditioning treatment period (Lee et al. 2016b). This indicates that the pre-storage ambient temperature conditioning treatment likely increases the effectiveness of removing water molecules from the intercellular spaces of flesh tissues during that period. Thus, losing water molecules from the pithy connectivity of flesh tissues would reduce the incidence of physiological disorders by enhancing oxygen diffusivity within flesh tissues during cold storage.

Regarding fruit quality attributes, fruit fresh weight loss was higher in pre-storage temperature-conditioned fruit than in the untreated control fruit during the shelf life after short-term cold storage (Figs. 2 and 5). Although the fruit respiration rate was not determined in this study, the fruit fresh weight loss could be derived from fruit respiration and transpiration during the shelf life. In addition, fruit respiration and transpiration were significantly increased during the pre-storage ambient temperature conditioning period. Therefore, the fruit fresh weight loss was already sufficiently high in the pre-storage conditioned fruit compared to the untreated fruit at the removal time point after short-term cold storage (Fig. 2). Furthermore, the lower levels of SSC and TA in the pre-storage ambient temperature conditioning treatment immediately after cold storage could have

resulted from the effectiveness of the pre-storage ambient temperature conditioning treatment immediately after harvest for fruit respiration to provide or support the energy sources (Fig. 2). Fresh weight loss increases with increasing pre-storage ambient temperature during the conditioning period; thus, fruit respiration and ethylene production rates are proportional to the conditioned ambient temperature in early season Asian pears (Lee et al. 2016b). Internal ethylene concentration (IEC) is higher in conditioned fruit than in untreated control fruit, and thus, the levels of acetaldehyde and ethanol are lower in conditioned fruit of ‘Honeycrisp’ apples during the shelf life and cold storage periods (Al Shoffe et al. 2018). Therefore, the results indicated that the reduction in fruit fresh weight loss in pre-storage conditioned fruit could be derived from fruit respiration and transpiration during the conditioning period, based on the decrease in SSC and TA immediately after cold storage. In addition, the pre-storage ambient temperature conditioning treatment tended to reduce the levels of SSC and TA during the entire shelf life period, although the flesh firmness did not consistently respond to the conditioning treatment (Fig. 2). However, flesh firmness was positively correlated with TA and fruit fresh weight loss in conditioned fruit (Fig. 7B).

Of the fruit colour variables, cortex h° values were only higher in pre-storage conditioned fruit than in the untreated control fruit during the entire shelf life period immediately after short-term cold storage, although the other colour variables were not affected by conditioning treatment (Figs. 3 and 5). The fruit colour variables were highly affected during the shelf life period, except for peel C^* (Fig. 5). Notably, all the colour variables in the untreated control fruit were strongly correlated with each other and were placed between most amino acids and the other parameters (Fig. 7A). Cortex L^* could play a key role in connecting most amino acids and some of the parameters in pre-storage conditioned fruit (Fig. 7B). In particular, the pre-storage ambient temperature conditioning treatment could be assumed to differentially affect all the colour variables by mediating major metabolites during shelf life. The exterior colour parameters gradually respond to increased conditioning temperature in avocados (Arpaia et al. 2018). However, the exterior peel h° values respond differently to the corresponding fruits and vegetables as well as to the pre-storage temperature and delayed cooling period (Li et al. 2015). The cortex h° values were higher in pre-storage conditioned fruit than in untreated fruit during the shelf life (Fig. 3) and were also strongly affected by the shelf life period after cold storage (Fig. 5). Thus, the cortex h° was positively correlated with tryptophan, glutamine, flesh firmness, and fresh weight loss in the pre-storage ambient temperature conditioning treatment (Fig. 7B), but broadly correlated with numerous

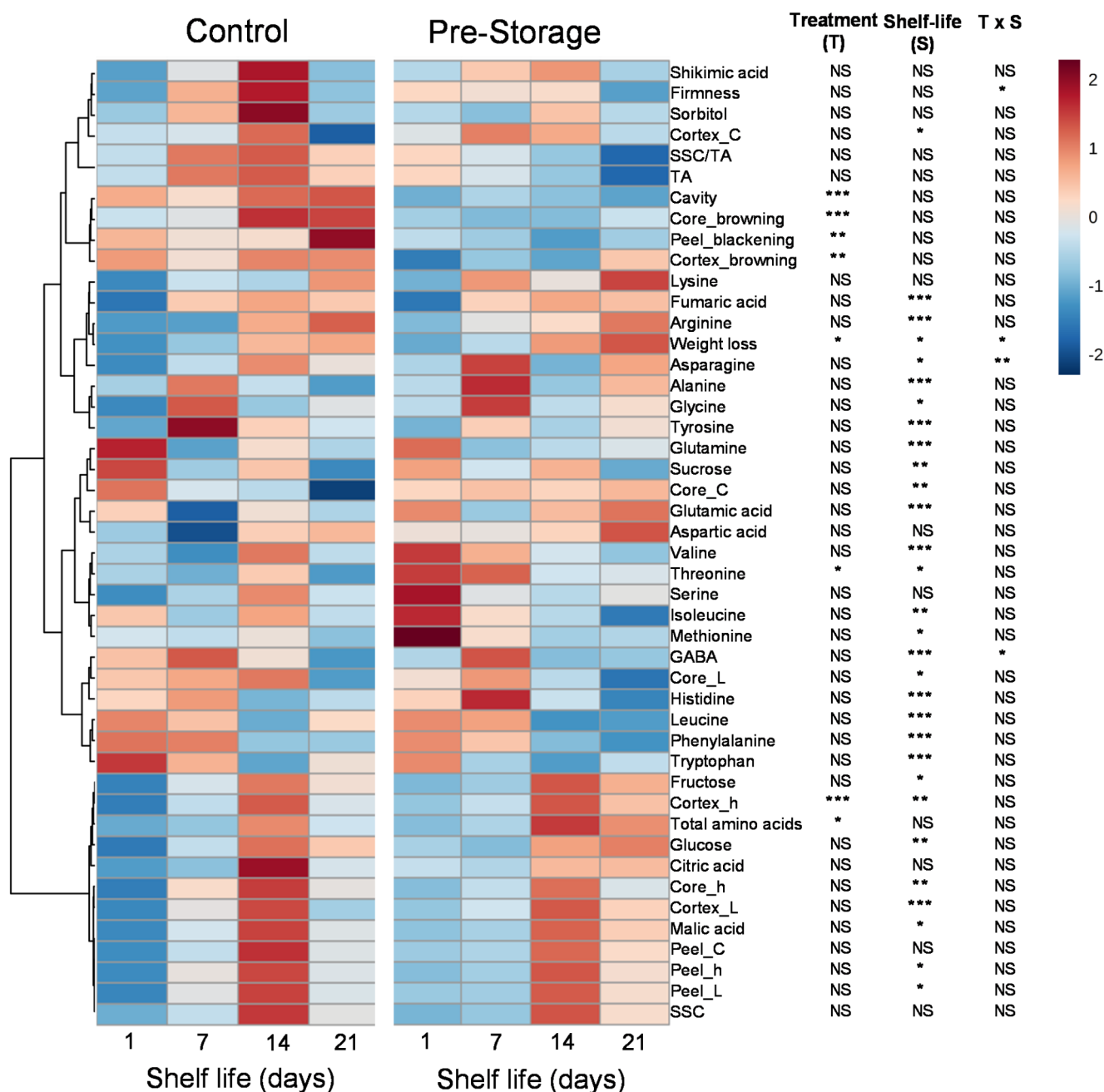


Fig. 5 The normalized heatmap matrix of physiological fruit quality attributes, severity of physiological disorders, and targeted primary metabolites in ‘Chuhwangbae’ pear fruit treated with pre-storage conditioning treatment at 22 °C for 7 days immediately after harvest and

stored at 0.5 °C for 3 months followed by 21 days at 22 °C. The figure legend indicates the results of relative responses. NS, *, **, ***, or ****= not significant or significant at $p < 0.05$, 0.01, 0.001, or 0.0001, respectively

variables, including colour parameters in untreated control fruit (Fig. 7A). High cortex h° values are associated with the incidence and severity of cortex browning during shelf life. The incidence and severity of cortex browning were higher in untreated control fruit than in pre-storage conditioned fruit, resulting in the colour alteration of the cortex tissue, thereby contributing to retaining the higher level of cortex h° values during shelf life. The alteration of flesh

tissue colour in long-term CA-stored ‘Empire’ apples is strongly associated with the development and severity of firm flesh browning (Lee et al. 2012b). The incidence and severity of internal browning in cold-stored ‘Royal Gala’ apples are highly linked with the reduction of h° values (Lee et al. 2013a), and also the incidence and severity of diffuse flesh breakdown are negatively correlated with h° values (Lee et al. 2016a). The incidence of flesh and core

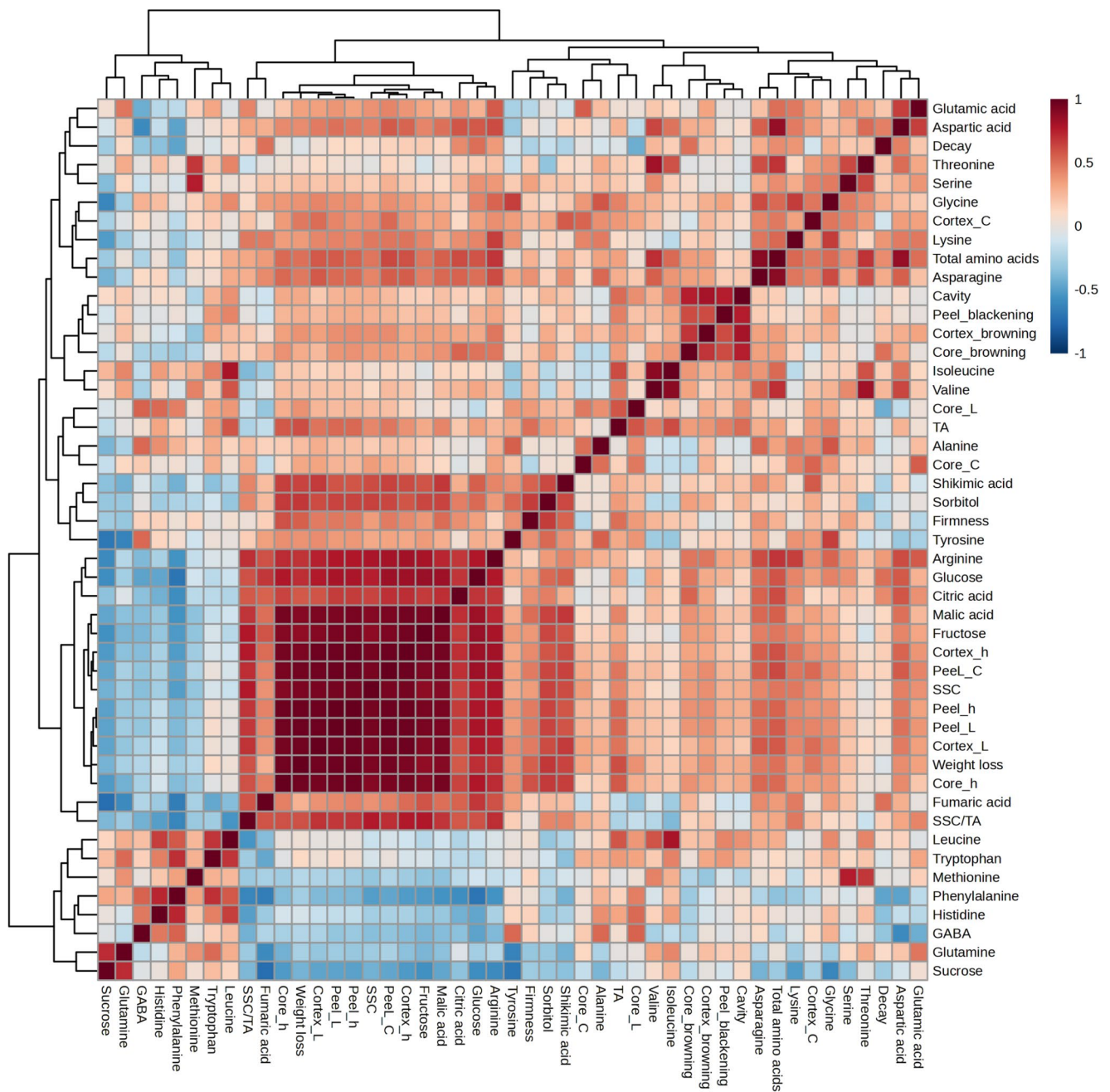


Fig. 6 Pearson's correlation coefficient (r) heatmap matrix among the variables of physiological fruit quality attributes, physiological disorders, and targeted primary metabolites in 'Chuhwangbae' pear fruit treated with pre-storage conditioning treatment at 22 °C for 7 days

browning is positively correlated with a^* value during shelf life in 'Fuji' apples (Lee et al. 2019). Therefore, the results provided that the alteration of fruit colour variables could be strongly resulted from the symptoms of physiological disorders during shelf life after cold storage. In addition, pre-storage ambient temperature conditioning treatment could play a pivotal role in delaying the reduction of cortex tissue coloration during shelf life after cold storage.

immediately after harvest and stored at 0.5 °C for 3 months followed by 21 days at 22 °C. The figure legend indicates the results of the correlation coefficient (r). The red and blue colours indicate positive and negative correlation coefficients between variables, respectively

Among the targeted primary metabolites, the contents of threonine and total amino acids were highly affected only by the pre-storage ambient temperature conditioning treatment during the shelf life after cold storage (Figs. 4 and 5). Threonine and total amino acid levels were higher during the first half of the shelf life period after pre-storage ambient temperature conditioning treatment. In addition, valine and isoleucine contents were higher in pre-storage

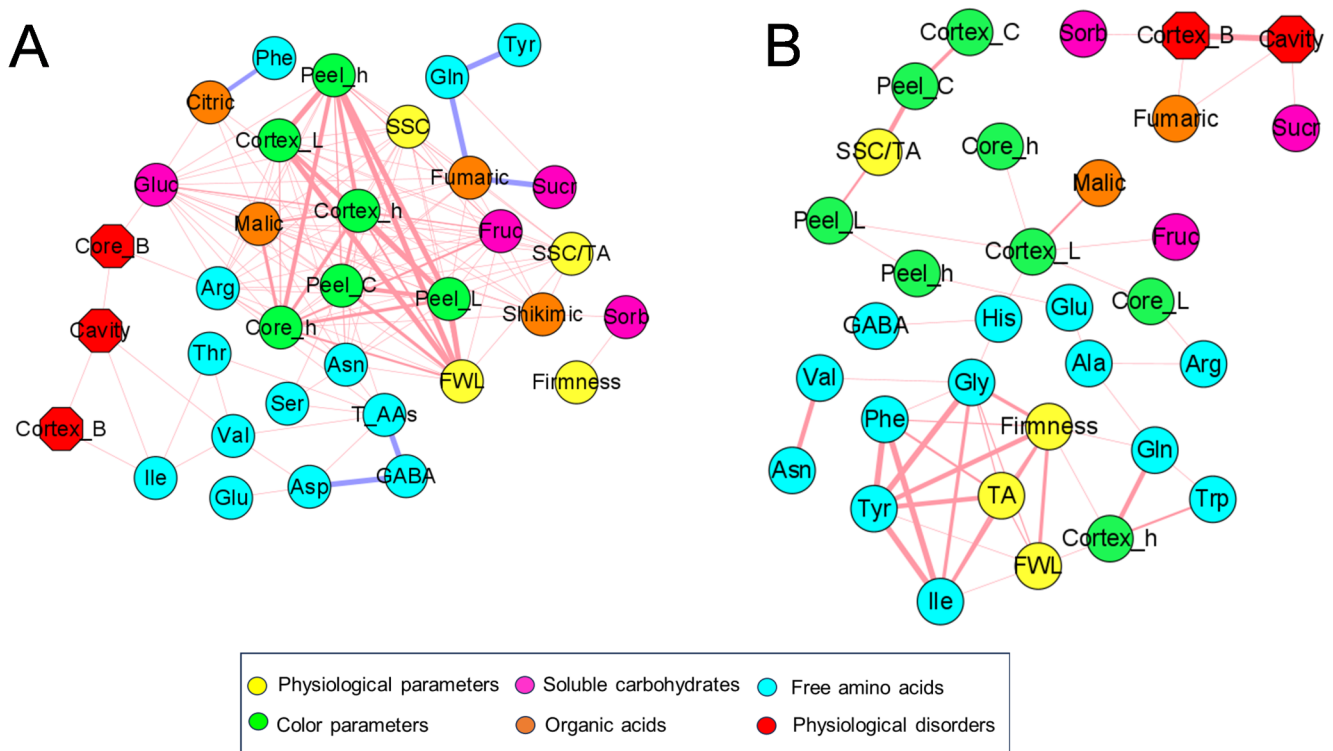


Fig. 7 Pearson's correlation coefficient network ($r \geq 0.75$) among the response variables in targeted primary metabolites and fruit quality attributes in 'Chuhwangbae' pear fruit in the control (**A**) and pre-storage conditioning treatment (**B**) at 22 °C for 7 days immediately after harvest and stored at 0.5 °C for 3 months followed by 21 days at 22 °C.

The red and blue lines indicate positive and negative correlation coefficients between variables, respectively. The thickness of correlation coefficient network lines indicates the significant degree of correlation coefficient (r) between variables

conditioned fruit than in the untreated fruit during the first half of the shelf life, whereas branched chain and aromatic amino acids, leucine, tryptophan, tyrosine, and phenylalanine, were not affected by pre-storage ambient temperature conditioning treatment (Fig. 4). Valine was strongly and positively correlated with isoleucine in the overall correlation coefficient heatmap matrix (Fig. 6). However, other primary metabolites were not consistently affected by the conditioning treatment or shelf life (Fig. 5). However, the correlation coefficient network results indicated that core browning, cavity, and cortex browning were positively correlated with glucose, arginine, valine, and isoleucine in the untreated control fruits during the shelf life (Fig. 7A), but cortex browning and cavity were positively correlated with sorbitol, fumaric acid, and sucrose in pre-storage conditioned fruits (Fig. 7B). Higher levels of valine and isoleucine, which play fundamental roles as substrates for the production of volatile organic compounds (VOCs), are associated with fruit respiration rather than VOCs generation during shelf life (Kochevenko et al. 2012). Furthermore, the differential responses of the targeted primary metabolites were associated with pre-storage ambient temperature conditioning treatment during shelf life. As several

symptoms of physiological disorders can occur during shelf life, the *de novo* production and accumulation of free amino acids and corresponding enzymes are associated with the collapse of cellular structure and thus contributed to aggravating cortex and core browning in flesh tissues during shelf life after cold storage. The incidence of blackheart, a chilling-induced internal browning disorder in pineapple fruit, increased the enzymatic activity of polyphenol oxidase (PPO) 10-fold than that of unaffected fruit, in which PPO was synthesized *de novo* in response to chilling injury (Stewart et al. 2001). In addition, pre-storage cold acclimation at 10 °C for 72 h induces the tolerance to chilling injury in cucumber fruit by mediating plant hormone signal transduction, carbon metabolism, and amino acids biosynthesis metabolism (Wang et al. 2021). The incidence of internal browning is also strongly associated with higher accumulation of amino acids in CA-stored apples (Lee et al. 2012a, b). Moreover, the incidence rate of peel blackening, cortex browning, and cavity was increased in pre-storage conditioning treated fruit particularly at 14 days of shelf life condition, similar trend with the sharp decrease in the contents of total amino acids. Otherwise, it could be assumed that the fluctuation of major amino acid contents could be associated

with the inconsistent responses of total amino acids content during shelf life. Therefore, the results indicated that the development of internal browning is affected by the alteration of cellular structure, thereby contributing to increasing *de novo* production or accumulation of amino acids during shelf life after cold storage.

5 Conclusion

The late-season Asian pear cultivar, ‘Chuhwangbae,’ is prone to the incidence of physiological disorders in terms of chilling injury symptoms during cold storage and further getting severe during the shelf life period after cold storage. Pre-storage ambient temperature conditioning treatment is highly recommended and applied to commercial orchards and storehouses to control or reduce the incidence and severity of physiological disorders. However, the impact of pre-storage ambient temperature conditioning on fruit quality and its efficacy in preventing the incidence of physiological disorders during shelf life after short-term cold storage remains uncertain. In this study, pre-storage ambient temperature conditioning treatment reduced the incidence and severity of physiological disorders, including peel blackening, cortex browning, core browning, and cavities. In addition, fruit fresh weight loss was higher in pre-storage conditioned fruit than in untreated fruit and gradually increased during the shelf life. However, flesh firmness and TA would be lower in the conditioned fruit than in the untreated fruit during the second half of the shelf life. Of the fruit colour variables, cortex h° values were higher in pre-storage conditioned fruit than in untreated fruit during the entire shelf life period after cold storage, whereas the other colour variables remained inconsistent. Among the targeted primary metabolites, threonine and total amino acids were highly affected by pre-storage ambient temperature conditioning during the shelf life, whereas the other metabolites responded inconsistently to the pre-storage ambient temperature conditioning treatment during the shelf life. However, physiological disorders were differentially correlated with the primary metabolites. Core browning, cavity, and cortex browning were positively correlated with glucose, arginine, valine, and isoleucine in untreated fruit. However, cortex browning and cavity formation were positively correlated with sorbitol, fumaric acid, and sucrose in pre-storage conditioned fruit. Overall, the results suggested that the pre-storage ambient temperature conditioning treatment could be involved in changing the biosynthesis metabolism of primary metabolites, such as soluble carbohydrates, organic acids, and amino acids, thereby contributing to reducing

the incidence and severity of chilling injury symptoms as observed and detected in the peel, cortex, and core tissues during the shelf life immediately after cold storage. Therefore, the pre-storage ambient temperature conditioning treatment could be highly effective in retaining fruit quality and reducing the physiological disorders in late-season ‘Chuhwangbae’ Asian pears during the shelf life following short-term cold storage.

Acknowledgements We deeply appreciate Dr. Ug-Yong Lee at the Pear Farms Company in Cheonan, Republic of Korea, for his cooperation and support.

Author contributions Theint Thandar Latt: Methodology, data curation, formal analysis, investigation, validation, original draft, writing, review, and editing. Jinhee Lee: Methodology, data curation, formal analysis, investigation, validation, review, and editing. Hyowon Park: Methodology, data curation, formal analysis, investigation, validation, review, and editing. Si-Eun Byeon: Methodology, data curation, formal analysis, investigation, validation, review, and editing. Seung Yeon Han: Methodology, data curation, formal analysis, investigation, validation, review, and editing. Hnin Phyu Lwin: Methodology, data curation, formal analysis, investigation, validation, review, and editing. Ho-Jin Seo: Conceptualization, methodology, validation, discussion, review, and editing. Seok Gyu Yun: Conceptualization, methodology, validation, discussion, review, and editing. Jinwook Lee: Conceptualization, data curation, investigation, writing, review and editing, supervision, funding acquisition, and project administration.

Funding Open Access funding enabled and organized by Chung-Ang University

This research was supported by the Chung-Ang University Research Grants in 2024. In addition, this work was supported by the Rural Development Administration (RDA) [grant number RS-2023-00222984].

Data availability All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests “Jinwook Lee” is the Associate Editor of the Horticulture, Environment, and Biotechnology while conducting this study. Editorial Board Member status has no bearing on editorial consideration. The authors declare that they have no conflict of interest.

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