

Research Paper

Maturity-dependent response of 1-MCP treatment on fruit quality and antioxidant scavenging metabolism in cold-stored ‘Changjo’ Asian pears

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ABSTRACT

The early-season cultivar of ‘Changjo’ Asian pear is popular in commercial cultivation for its premium fruit quality at harvest and after cold storage. However, the effects of fruit maturity at harvest time and postharvest 1-methylcyclopropene (1-MCP) treatment have not yet been determined. The fruit quality attributes, targeted metabolites, and non-enzymatic and enzymatic antioxidant scavenging metabolism by the combination effects of fruit maturity and postharvest 1-MCP treatment, were evaluated in the fruit of ‘Changjo’ Asian pears stored at 0.5°C and 90% relative humidity for 6 months. The fruits were harvested at two maturity stages: immature and mature and treated with 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at 22°C for 16 h. Fruit fresh weight loss, soluble solids content, and ethylene production rate were highest in 1-MCP-treated mature fruit during cold storage, compared with the other treatment groups. Peel lightness and yellowness increased, and hue angle decreased during storage, despite 1-MCP treatment and fruit maturity. However, the cortex colour variables were mostly higher in 1-MCP-treated fruit than in untreated fruit during cold storage. Fructose and glucose increased during cold storage, irrespective of 1-MCP treatment or maturity; however, sorbitol was higher in immature than in mature fruits, even with 1-MCP treatment. Levels of malic and shikimic acids were higher in immature than in mature fruit, regardless of 1-MCP treatment during cold storage. In immature fruit, arbutin, ferulic acid, quercetin, epicatechin, gallic acid, caffeic acid, and total phenolic compounds increased in untreated fruit but catechin, rutin, and *p*-coumaric acid increased in 1-MCP-treated fruit. In mature fruit, the level of quercitrin was highest in 1-MCP-treated fruit during cold storage. Superoxide anion radicals and dehydroascorbic acid increased in untreated immature fruit, whereas hydrogen peroxide was higher in untreated mature fruit. Malondialdehyde, reduced glutathione, and glutathione oxidized contents increased in 1-MCP-treated mature fruit during cold storage. The enzymatic activities such as phenylalanine ammonia-lyase, polyphenol oxidase, superoxide dismutase, catalase, glutathione peroxidase, and glutathione *S*-transferase showed higher activity in 1-MCP-treated immature fruit, whereas ascorbate peroxidase, monodehydroascorbate reductase, and glutathione reductase activities increased in 1-MCP-treated mature fruit. Overall, the quality of ‘Changjo’ Asian pear in long-term cold storage is more strongly affected by fruit maturity at harvest rather than by postharvest 1-MCP treatment.

1. Introduction

Owing to persistent consumer interest in Asian pear fruit, numerous

cultivars have been bred, registered, and cultivated in Asia and other continents, meanwhile, suitability for cultivation, high pest and disease tolerance, high yield, and premium fruit quality became the important

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criteria. Thus, based on higher satisfaction across all the Asian pear fruit acceptance categories, the ‘Changjo’ cultivar is officially registered in 2011 (Shin et al., 2011). The cultivar has since gained significant interest among orchardists and the fruit industry. Nonetheless, in terms of fruit quality attributes, the incidence of physiological disorders, such as flesh browning, core browning, and mealiness, has been increasingly reported during the shelf-life period immediately after harvest (Choi et al., 2017).

Fruit quality varies to a great degree, both between cultivars and within the same cultivar, due to the wide variation between harvest years, orchards, and growing regions (Leisso et al., 2019). In the case of Asian pears, fruit quality attributes, including flesh firmness, ethylene production rate, fruit respiration rate, and other parameters differ between cultivars in their response after harvest (Zhang et al., 2023). The fruit quality parameters, such as firmness, soluble solids content (SSC), titratable acidity (TA), and weight loss in European pear cultivars, are variable during shelf life and cold storage (Pasquariello et al., 2013). In general, fruit quality of Asian pears has been differed from European pear cultivars in fruit quality attributes, such as fruit colour, flesh firmness, titratable acidity, soluble solids content, and dry weight under long-term cold stored fruit (Arzani et al., 2008). Fruit maturity, as determined by the harvest date, also affects fruit quality and the composition of phenolic compounds in cold-stored fruits, as has been shown in European pear cultivars (Amiot et al., 1995). Although the incidence of internal browning disorder varies among pear cultivars, the severity of internal browning generally increases as the cold storage period progresses (Koushesh Saba and Moradi, 2016). Fruit physiological quality attributes, such as ethylene production rate or the incidence of superficial scald, respond differently to cold storage periods depending on the cultivar (in the case of European pears) (Larrigaudière et al., 2016; Lindo-García et al., 2021). Furthermore, fruit maturity based on harvest date has been shown to be highly correlated with fruit quality during cold storage and varies among European pear cultivars (Blanckenberg et al., 2016).

To maintain fruit quality both after harvest and during long-term cold storage, several postharvest quality management technologies have been developed and actively applied in the pear industry, including the use of 1-methylcyclopropene (1-MCP) as a postharvest preservative (Latt et al., 2023; Lwin and Lee, 2022; Lwin et al., 2021b; Moya-Leon et al., 2006). Nevertheless, the effectiveness of postharvest 1-MCP treatment is not consistent, as it varies depending on the pear cultivar, storage duration, fruit maturity, and storage regimes. For example, fruit flesh firmness does not maintain throughout the entire cold storage period by postharvest 1-MCP treatment in either the ‘Chuhwangbae’ (Lwin et al., 2021b) or ‘Greensis’ (Latt et al., 2023) pear cultivars; however, flesh firmness of the ‘Wonhwang’ cultivar is found to be higher in postharvest 1-MCP-treated fruit than in untreated fruit (Lwin et al., 2021a; Seo et al., 2019). In addition, flesh firmness following postharvest 1-MCP treatment is also differently affected by fruit maturity at harvest in long-term cold-stored ‘Bartlett’ European pear (Villalobos-Acuña et al., 2011). Moreover, there are numerous research findings in controlling fruit ripening and quality by using 1-MCP treatments, accounting for many factors, such as the cultivar, maturity, concentration, and treatment exposure duration (Zhang et al., 2020; Dias et al., 2021), as well as the storage temperature, storage conditions, and storage duration (Lee et al., 2017; Jia et al., 2018; Wang et al., 2020). Therefore, the responsiveness of Asian pear fruit to postharvest 1-MCP treatment would be expected to vary depending on the cultivar and harvest maturity.

The level of primary metabolites, in particular soluble carbohydrates such as fructose, glucose, sucrose, and sorbitol, and organic acids, including malic, citric, shikimic, and fumaric acids, are greatly affected by postharvest 1-MCP treatment in both shelf-stored (Lwin et al., 2021a) and cold-stored Asian pear fruit (Lwin and Lee, 2022; Lwin et al., 2021b). As fruit maturity also affects the SSC, TA, and starch iodine pattern index in long-term cold-stored pear fruit (Hendges et al., 2018) it

would be expected that changes in the levels of these primary metabolites would be influenced by harvest maturity during cold storage; for example, in ‘Conference’ pears, the levels of sugars, phosphorylated sugar, organic acids, and amino acids are found to respond to differences in fruit maturity (Terzoudis et al., 2022). Furthermore, the contents of individual phenolic compounds have been found to fluctuate with fruit maturity in numerous European pear cultivars during storage (Amiot et al., 1995). In ‘Wonhwang’ Asian pears, postharvest 1-MCP treatment affects the responses of total phenolic compounds during shelf storage after harvest (Lwin et al., 2021a). However, in a study of non-enzymatic and enzymatic antioxidant scavenging metabolism in cold-stored ‘Rocha’ European pears, neither the levels of reduced and oxidized ascorbic acids and glutathione nor free radical scavenging activity are affected by fruit maturity at harvest (Silva et al., 2010).

Postharvest 1-MCP treatment affects the levels of ascorbic acid (AsA) and lipid peroxidation, however, the response of the enzymatic activity of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) is found to be inconsistent in 1-MCP-treated ‘Passe-Crassane’ European pears during cold storage (Cocetta et al., 2016). Likewise, the activity of catalase (CAT), guaiacol peroxidase (POD), and superoxide dismutase (SOD) responds differently to postharvest 1-MCP treatment in cold-stored Asian pear fruit (Yazdani et al., 2011). The combined effects of fruit maturity and postharvest 1-MCP treatment on fruit quality attributes, primary and secondary metabolites, along with both non-enzymatic and enzymatic antioxidant scavenging metabolism, using cold-stored fruit of the ‘Changjo’ Asian pear cultivar, became the research subject based on the above literature. In addition, the previous study indicated that the commercial harvest time of ‘Changjo’ pears should be before 153 DAFB and late harvest times would not be preferable for quality control during shelf-life (Lee et al., 2017). Therefore, we tested the hypothesis that fruit maturity: immature [149 days after full bloom (DAFB)] and mature (156 DAFB) and postharvest 1-MCP treatment would differentially affect fruit quality attributes, primary and secondary metabolites, as well as non-enzymatic and enzymatic antioxidant scavenging metabolism of the ‘Changjo’ Asian pear cultivar under cold storage.

2. Materials and methods

2.1. Fruit material, treatment, and storage conditions

Fruits of the ‘Changjo’ Asian pear cultivar, representing two maturity levels: immature [149 days after full bloom (DAFB)] and mature (156 DAFB), were harvested from a commercial orchard in Naju, Jeollanam-do, Republic of Korea (35.02° N, 126.71° E), on September 1st and 8th, 2023. Uniform fruits without defects or injuries were treated with 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (SmartFresh™ powder, 3.8% active ingredient, AgroFresh Inc., Philadelphia, PA, USA) at 22°C for 16 h at a laboratory at Chung-Ang University in Anseong, Republic of Korea. The 1-MCP treated fruit were put in airtight chamber, in which 1-MCP powder was dissolved in water to liberate the 1-MCP as gas fumigation. Both the untreated (control) and 1-MCP-treated fruit in different maturity stages were stored at 0.5°C and 90% relative humidity for 6 months.

2.2. Determination of fruit quality parameters

For each of the two harvest times, the starch pattern index of the fruit was evaluated by staining the equator cuts of the fruit with iodine solution; and determined the starch-iodine index. The starch pattern index (SPI) is the scores determining the iodine-stained area of fruit flesh (0 to 100%), which explaining the conversion of starch to soluble carbohydrates in accordance with the maturity levels of the fruit (Blanpied and Silsby, 1992). Then, the stained area was quantified using the ImageJ software (Ver 1.53a, NIH, Bethesda, MD, USA). After storage at 0.5°C for six months and 1-day shelf life at 22°C, the fruit quality attributes were

evaluated, and sampling was performed. The fruit fresh weight loss (%), flesh firmness (N), SSC, and TA, were determined as described in a previous study of Latt et al. (2024a). The fruit peel, cortex, and core colour variables were measured with a colorimeter (Minolta CR 200 Chroma Meter, Konica Minolta Co., Osaka, Japan) three times each at equator portions of the fruit (Lwin et al., 2023b). The CO₂ production rate for fruit respiration and C₂H₄ production rate were measured, using the protocol as described previously by Latt et al. (2023). For further analysis, the cortex fruit tissues were flash frozen with liquid nitrogen and stored immediately at -80°C.

2.3. Determination of soluble carbohydrates and organic acids

The sample extraction process of soluble carbohydrates and organic acids were similar to the protocol used by Latt et al. (2024a). In sample extraction, 1 g frozen sample was extracted with 10 mL of high performance liquid chromatography (HPLC)-grade distilled water and filtered through 0.45-µm hydrophilic filter before analysis in HPLC which was used with same machine and program as in previous study (Latt et al., 2024b). The data were reported in g kg⁻¹ fresh weight.

2.4. Determination of total antioxidant scavenging capacities and phenolic compounds

Total antioxidant scavenging capacity, the DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), were extracted using 1 g of frozen sample and their activities were measured at 734 and 517 nm, respectively. The experiment protocols and data calculation were followed as in previous study (Latt et al., 2023). The Folin-Ciocalteu method was applied to determine the contents of total phenolic compounds (TPC), using the following buffers, as described by Lwin et al. (2023b) in which Folin-Ciocalteu's phenol reagent (Sigma-Aldrich Co., St. Louis, MO, USA), HPLC-grade water, and sodium carbonate (Na₂CO₃) were used (Lwin et al., 2023b). The absorbance was 750 nm, and data were expressed in dry weight basis of mg GAE kg⁻¹. In determining the content of total flavonoids, the 5% sodium nitrate and HPLC grade water were used, following the protocols established by previous study (Latt et al., 2023).

To measure the contents of individual phenolic compounds, 1 g frozen sample and 10 mL of ice-cold 80% methanol were homogenized for extraction. Then, 1 mL volume of extracted sample was evaporated in a vacuum dryer (HyperVac VC2200, LaboGene, Lillerød, Denmark), and the concentrated sample was dissolved with 200 µL of 80% methanol buffer. In analysing individual phenolic compounds, the same procedure and Ultimate 3000 HPLC system was adopted from a previous study (Latt et al., 2023). The measurement of phenylalanine ammonia-lyase (PAL) activity was carried out based on the methods of the previous research (Assis et al., 2001). The polyphenol oxidase (PPO) activity was measured by adopting the protocols of Zhou et al. (1993).

2.5. Determination of superoxide anion radical, hydrogen peroxide, and lipid peroxidation

The protocols of previous studies were applied in determining the content of superoxide anion radical (O₂⁻) by measuring the reducing activity of nitroblue tetrazolium (NBT), based on the procedure described by Doke (1983). Absorbance was measured at 580 nm using a spectrophotometer (Epoch 2; BioTek, Winooski, VT, USA). The reducing ability of NBT was expressed as OD₅₈₀ kg⁻¹s⁻¹ (Chen and Cheng, 2004). Hydrogen peroxide (H₂O₂) content was determined using the method in the study by Patterson et al. (1984). The assay mixture was read at 508 nm with a spectrophotometer (Epoch 2, BioTek, Winooski, VT, USA). The standard H₂O₂ curve, ranging from 0 to 100 µM, was used to calculate the amount of H₂O₂. The malondialdehyde (MDA) content, an indicator of lipid peroxidation in fruit flesh samples was determined by measuring at 440, 532, and 600 nm using an Epoch 2

spectrophotometer (BioTek, Winooski, VT, USA) as in the protocols of the previous study and calculated (Hodges et al., 1999).

2.6. Measurement of non-enzymatic antioxidant metabolites

The ascorbic acid (AsA) and dehydroascorbic acid (DHA) were determined based on the methods described in the study by previous research (Logan et al., 1998). The DHA content was calculated by the difference between total ascorbate and AsA content. The total glutathione (GSH) and oxidised glutathione (GSSG) were determined through enzymatic cycling as described by Griffith (1980). The standard regression curves of GSH and GSSG contents were ranged from 0 to 50 µM for total GSH and from 0 to 10 µM for GSSG. Reduced GSH was obtained by calculating the difference between the total GSH and GSSG.

2.7. Determining the antioxidant enzyme activities

The analysis of antioxidant enzymes, namely SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), POD (EC 1.11.1.7), MDHAR (EC 1.6.5.4), DHAR (EC 1.8.5.1), and GR (EC 1.6.4.2), were used the same protocol described by Kochhar et al. (2003). The SOD assay was performed by the inhibition of the photochemical reduction of NBT, following a method adapted from Ginnopolitis and Ries (1977) and Frei et al. (2010). SOD activity was calculated as NBT reduction in light with extraction buffer (control) minus NBT reduction with sample extraction (sample), which was monitored at 560 nm. APX activity was determined following the protocol of Grace and Logan (1996) and Aebi (1984) as referred to determine the level of CAT activity. MDHAR activity, due to NADH oxidation, was assayed by recording the decrease in absorbance at 340 nm over 3 min as described in the previous study (Miyake and Asada, 1992). The activity of DHAR was measured following the procedure described by Nakano and Asada (1981). The GR activity assay was performed using the method described by Grace and Logan (1996) with some modifications. POD activity was measured using the method described by Hammerschmidt et al. (1982). Protein concentration was determined using the Bradford assay according to the manufacturer's micro-assay protocol (Sigma-Aldrich Co., St. Louis, MO, USA) and quantified by comparing the bovine serum albumin (BSA) standard curve ranging from 0 to 10 mg L⁻¹ (Bradford, 1976).

2.8. Statistical analyses

The results were presented as the mean of three replicates ± standard error (SE). All data were analysed using two-way analysis of variance (ANOVA) with two main factors, fruit maturity and 1-MCP treatment. The interaction of these two factors was assessed using SAS Ver. 9.3 (SAS Institute Inc., Cary, NC, USA). The mean difference between two factors was assessed using the least significant difference (LSD) test. All measurements of treatment effects such as heatmap analysis, VIP scores plot analysis and volcano plot analysis were generated using a MetaboAnalyst Ver. 5.0 (Chong et al., 2018; Lwin et al., 2021a). The network of Pearson's correlation coefficients was constructed using the MetScape plugin in the Cytoscape software Ver. 3.8.2 (Basu et al., 2017; Lwin and Lee, 2021; Lwin et al., 2021b).

3. Results

3.1. Fruit quality attributes

The ratio of iodine starch-stained area at harvest, the maturity index, was higher in immature fruit than in mature fruit (Fig. 1A). During cold storage, fruit fresh weight loss was highest in 1-MCP-treated mature fruit, compared with the control fruit. Flesh firmness was not affected by fruit maturity or 1-MCP treatment during cold storage but declined during the storage period. The level of SSC increased during cold storage, compared with harvest, and was highest in 1-MCP-treated mature

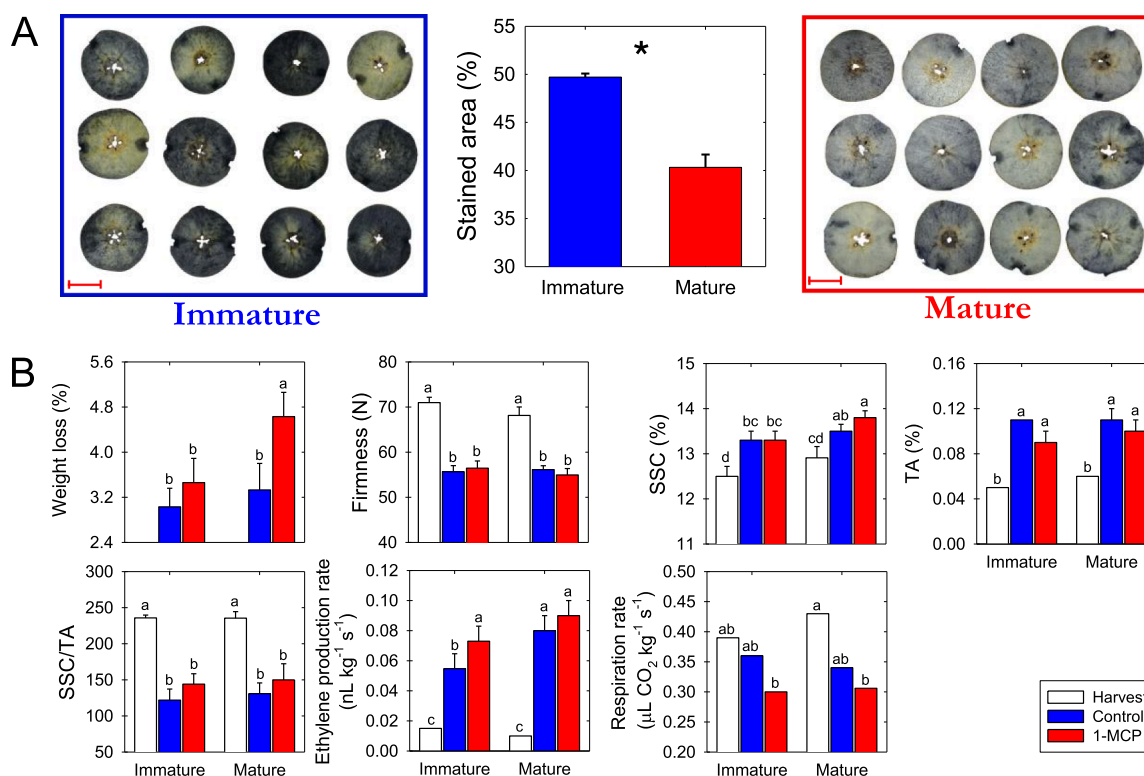


Fig. 1. Starch iodine pattern index at two maturity levels (A) and physiological responses of fruit fresh weight loss, flesh firmness, soluble solids content (SSC), titratable acidity (TA), ratio of SSC to TA, ethylene production rate, and respiration rate in the fruit of ‘Chango’ Asian pears at different maturity treated with 1 $\mu\text{L L}^{-1}$ 1-methylcyclopropene (1-MCP) at harvest and then, stored at 0.5°C for 6 months before transferred to 22°C for one-day shelf life. Each datum point indicates the mean of twelve fruit replicates ($n = 12$) $\pm$ standard error for fruit fresh weight loss, SSC, and flesh firmness, and the mean of three replicates ($n = 3$) $\pm$ standard error for TA, ethylene production rate, and respiration rate. Statistical difference in the starch iodine pattern index was ascertained from the results of Student’s *t*-test at $p < 0.05$. * indicates significant difference at $p < 0.05$. Different letters above the bars indicate significant differences according to Duncan’s multiple range test at $p = 0.05$. Red scale bar represents 3 cm.

fruit compared with the immature fruit. TA was higher in cold-stored fruit than in harvest, irrespective of fruit maturity or 1-MCP treatment. Thus, the ratio of SSC to TA was significantly reduced during cold storage, without regard to fruit maturity and 1-MCP treatment. Ethylene production rate was higher in cold-stored fruit than in harvested fruits. Although the ethylene production rate was not affected by 1-MCP treatment in mature fruit after long-term cold storage, it was higher in the 1-MCP treatment group than in untreated immature fruit. However, the fruit respiration rate was significantly reduced by 1-MCP treatment after cold storage compared with that at harvest (Fig. 1B).

Peel lightness (L^*) and yellowness (b^*) were higher in cold-stored fruit than in harvest, irrespective of fruit maturity or 1-MCP treatment, but oppositely in peel hue angle (h°) (Fig. 2). In the cold-stored fruits, the cortex and core L^* were only higher in 1-MCP-treated fruit, compared to the corresponding values at harvest, irrespective of fruit maturity; the contents of L^* values were lowest in harvest time. The cortex and core b^* responded with same trends in different maturity levels, in which the untreated control fruit was higher than in 1-MCP treated fruit in immature, but oppositely responded in mature fruit, respectively. The peel chroma (C^*) was higher in 1-MCP treated fruit than in untreated immature fruit, whereas it was unaffected in mature fruit. In contrast, the cortex C^* was not consistently affected by fruit maturity or 1-MCP treatment, the fruit in control were significantly lower in control, compared to 1-MCP treated fruit in mature but, not significant in immature fruit. Core C^* was lower in cold-stored fruit than in 1-MCP treatment at harvest fruit in both maturities. Nonetheless, core C^* responded inversely to 1-MCP treatment by fruit maturity during cold storage. The value of cortex h° responded inconsistently to fruit maturity and 1-MCP treatment during cold storage compared to the harvest time

point (Fig. 2).

3.2. Targeted metabolites

Fructose and glucose contents were higher in cold-stored fruit than in harvested fruit, despite fruit maturity or 1-MCP treatment. However, the sucrose content was lower in cold-stored fruit than in harvested fruit in both immature and mature fruit without considering the 1-MCP treatment. Although the level of sorbitol in immature fruit was not affected by 1-MCP treatment, the sorbitol levels in 1-MCP-treated fruit decreased than harvest in mature (Fig. 3A). The malic acid content was higher in cold-stored fruit than at harvest, despite 1-MCP treatment. Citric acid levels were not affected by fruit maturity or 1-MCP treatment during cold storage. Shikimic acid tended to be lower in mature than immature fruit, irrespective of 1-MCP treatment. However, the level of fumaric acid was higher in cold-stored fruit than in fruit at harvest, despite fruit maturity and 1-MCP treatment (Fig. 3A).

Chlorogenic acid was higher in immature fruit at harvest but decreased with 1-MCP during storage. Arbutin was higher in cold-stored fruit than in harvested fruit, regardless of maturity, but 1-MCP reduced arbutin only in immature fruit. In immature fruit, the catechin was lower in cold-stored fruit than in harvested fruit at both maturities level. Quercitrin levels increased in 1-MCP-treated fruit regardless of maturity during cold storage. Ferulic acid levels were lower in 1-MCP-treated fruit than in untreated immature fruit during cold storage. Quercetin and epicatechin were lower in 1-MCP in immature but not mature fruit. Syringic acid was higher in mature fruit, and rutin and *p*-coumaric acid were higher in 1-MCP treatment of immature fruit during storage. Gallic and caffeic acids were highest at harvest in immature fruit (Fig. 3B).

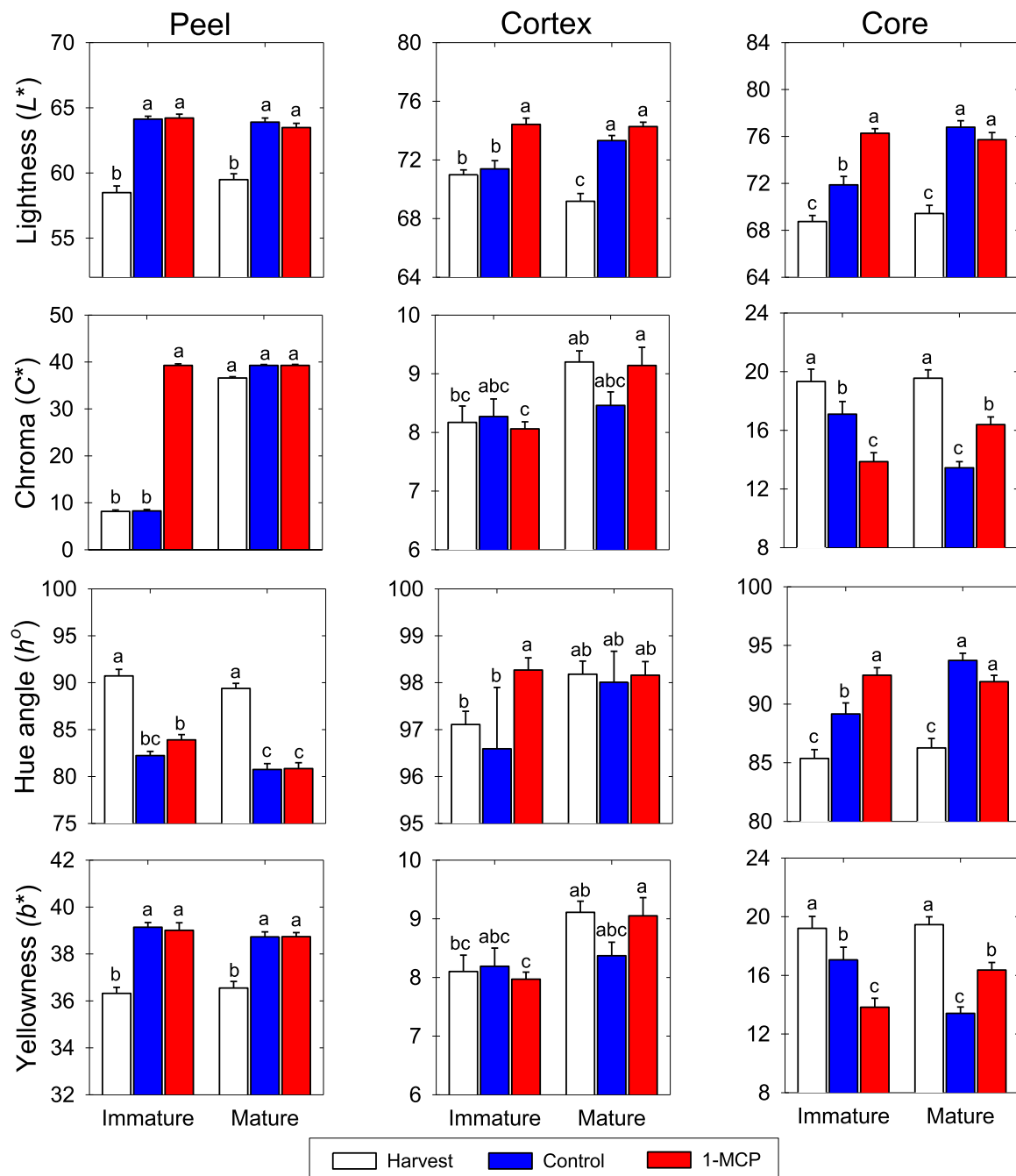


Fig. 2. Fruit color responses in lightness (L^*), chroma (C^*), hue angle (h°), and yellowness (b^*) of peel, cortex, and core tissues in the fruit of 'Changjo' Asian pears at different maturity and treated with $1 \mu\text{L L}^{-1}$ 1-methylcyclopropene (1-MCP) treatment at harvest followed by storage at 0.5°C for 6 months before transferred to 22°C for one-day shelf life. Each datum point indicates the mean of twelve fruit replicates ($n = 12$) $\pm$ standard error. Different letters above the bars indicate significant differences according to Duncan's multiple range test at $p = 0.05$.

The level of TPC was highest in untreated fruit but lowest in 1-MCP-treated fruit in immature fruit. However, the TFC was not affected by fruit maturity or 1-MCP treatment during cold storage. In comparing all treatment groups, DPPH activity was found to be highest at harvest in immature fruit and lowest at harvest in mature fruit. However, ABTS activity was higher in mature fruit than in immature fruit, irrespective of 1-MCP treatment (Fig. 3B).

3.3. Antioxidant metabolites, enzymatic antioxidant metabolism and reactive oxygen species

The level of superoxide anion radicals (O_2^-) was found to be highest in

untreated fruit during cold storage, irrespective of fruit maturity. The level of H_2O_2 was lowest in harvest at immature but higher than 1-MCP treatment in mature (Fig. 4. A). As a result of oxidative stress, MDA content was lowest in immature fruit at harvest, but highest in 1-MCP-treated mature fruit during cold storage. In terms of non-enzymatic antioxidant scavenging metabolism, AsA content was highest at harvest in immature fruit, but lowest in 1-MCP-treated mature fruit after cold storage. The content of DHA was highest in untreated immature fruit but lowest in untreated mature fruit, compared with the other treatments. The GSH content was lowest in mature fruit at harvest, but highest in 1-MCP treatment in mature fruit, compared with the control treatment. In contrast, the GSSG content was highest at harvest in

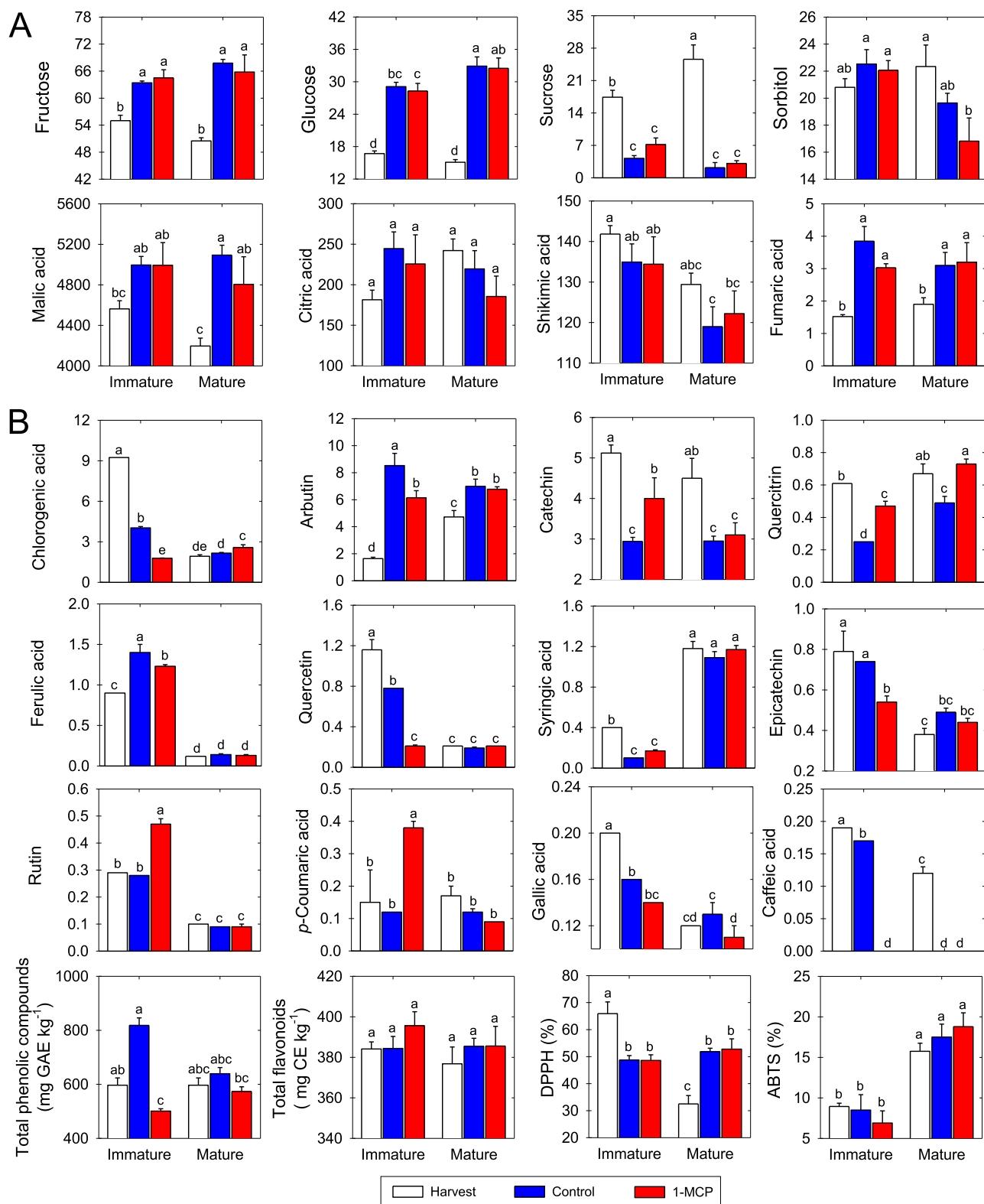


Fig. 3. Responses of soluble carbohydrates (g kg⁻¹ FW) and organic acids (mg kg⁻¹ FW) (A), and the contents of individual phenolic compounds (g kg⁻¹ FW), total phenolic compounds, total flavonoids, and total antioxidant scavenging capacities (B) in the fruit of 'Changjo' Asian pears at different maturity and treated with 1 μ L L⁻¹1-methylcyclopropene (1-MCP) treatment at harvest followed by storage at 0.5°C for 6 months before transferred to 22°C for one-day shelf life. Each datum point indicates the mean of three replicates ($n = 3$) $\pm$ standard error. Different letters above the bars indicate significant differences according to Duncan's multiple range test at $p = 0.05$.

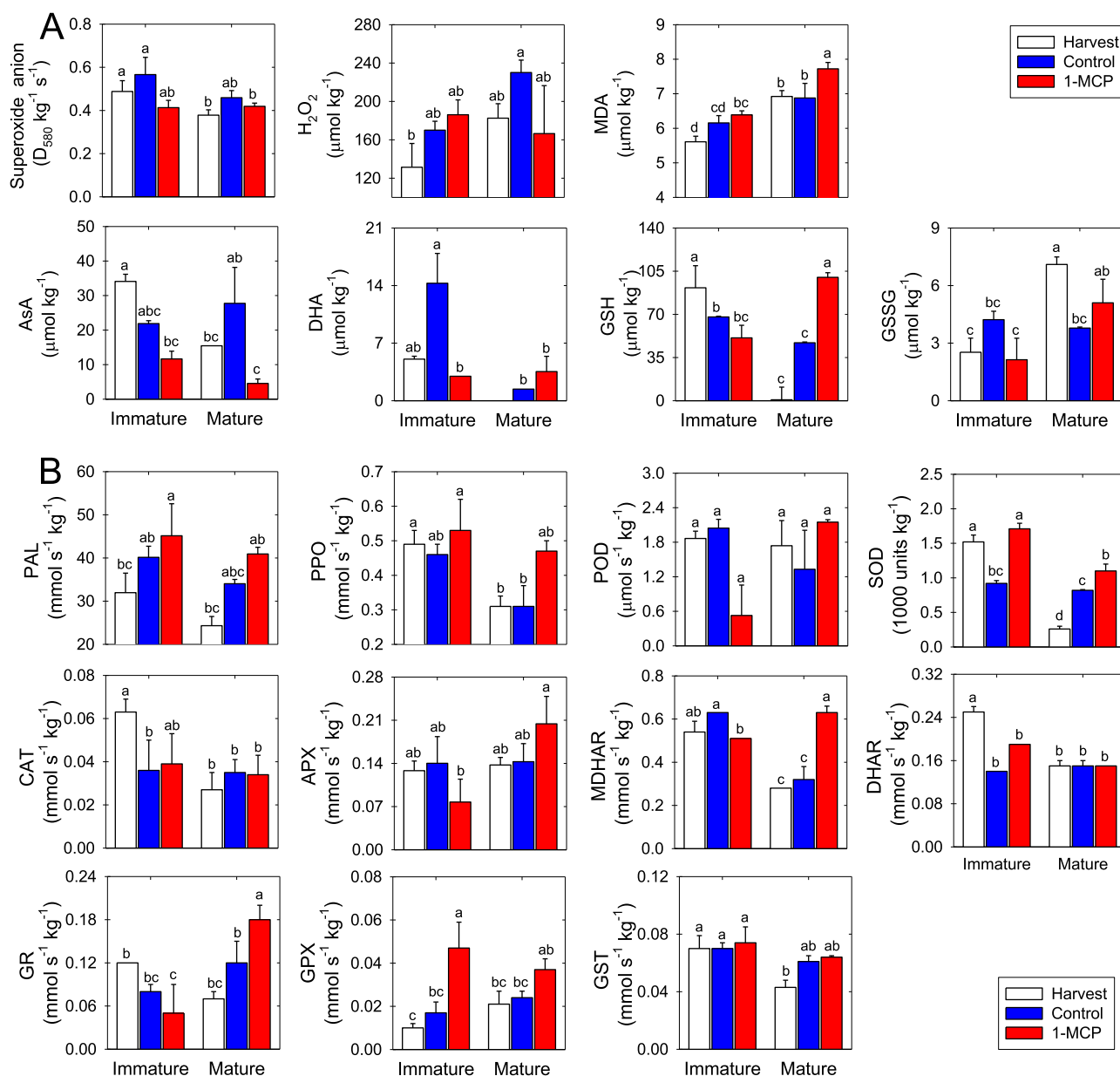


Fig. 4. Contents of superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), ascorbic acid (AsA), dehydroascorbic acid (DHA), reduced glutathione (GSH), and glutathione oxidized (GSSG) (A), and enzymatic activities of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), guaiacol peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione peroxidase (GPX), and glutathione S-transferase (GST) (B) in the fruit of 'Changjio' Asian pears at different maturity and treated with $1 \mu\text{L L}^{-1}$ 1-methylcyclopropene (1-MCP) treatment at harvest followed by storage at 0.5°C for 6 months before transferred to 22°C for one-day shelf life. Different letters above the bars indicate significant differences according to the Duncan's multiple range test at $p = 0.05$.

mature fruit but lowest in 1-MCP-treated immature fruit during cold storage (Fig. 4A).

Among the enzymatic activities, PAL activity was highest in 1-MCP-treated fruit, while PPO and POD activities were not affected by fruit maturity or 1-MCP treatment (Fig. 4B). In enzymatic antioxidant scavenging metabolism, SOD activity was highest in 1-MCP-treated immature fruit, whereas 1-MCP treatment was highest following control and harvest time in mature fruit. The CAT activity was highest at harvest in immature fruit, but lowest in mature fruit. The levels of APX, MDHAR, and GR activities were highest in 1-MCP treatment of mature fruit after six months cold storage, but DHAR was lowest at harvest in mature fruit (Fig. 4B). GR activity was lowest in 1-MCP-treated immature fruit, but highest in 1-MCP-treated mature fruit. GPX activity was lowest at harvest, but highest in 1-MCP-treated immature fruit. GST activity was not

affected by fruit maturity or 1-MCP treatment during cold storage (Fig. 4B).

In terms of the overall antioxidant scavenging metabolism, all response variables were separated at harvest and during cold storage, as shown in Fig. 5. At harvest, superoxide anion radical (O_2^-), however, H_2O_2 was higher in 1-MCP treatment of mature fruit. SOD activity was higher in immature fruit than in mature fruit, whereas MDA level was lower. The activities of POD, CAT, APX, GPX, and GST did not differ among fruit at different maturity stages. AsA and DHA levels, and the enzymatic activities of MDHAR, DHAR, and GR were higher in immature fruit than in mature fruit. During cold storage, the level of superoxide anion radical was highest in untreated immature fruit, compared to all treatment groups, whereas H_2O_2 levels were not affected. SOD activity was highest in 1-MCP-treated immature fruit, but MDA levels were

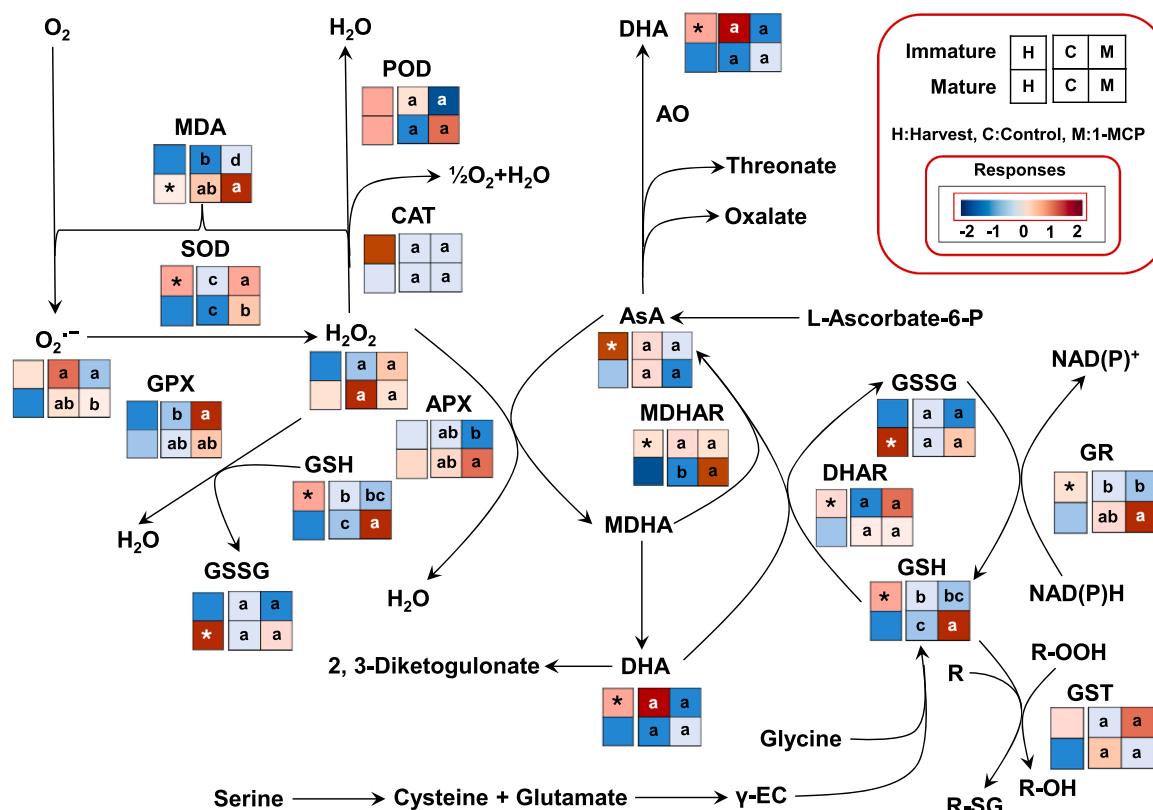


Fig. 5. Heatmap diagram of enzymatic and non-enzymatic antioxidant scavenging metabolism (antioxidant metabolites and enzymatic activities) in response to different maturity of ‘Changjo’ Asian pears treated with 1 $\mu\text{L L}^{-1}$ 1-MCP treatment at harvest and then stored at 0.5°C for 6 months before transferred to 22°C for one-day of shelf life. Statistical difference at harvest was determined from the results of Student’s *t*-test at $p < 0.05$ * indicates significant difference at $p < 0.05$. Different letters on the boxes indicate significant differences according to Duncan’s multiple range test at $p = 0.05$. AO, ascorbate oxidase; APX, ascorbate peroxidase; AsA, L-ascorbate; CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; γ -EC, γ -glutamylcysteine; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; GST, glutathione-S-transferase; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; POX, peroxidase; SOD, superoxide dismutase.

highest in 1-MCP-treated mature fruit. The level of POD, CAT, DHAR, and GST activities did not differ among treatments. The levels of AsA, DHA, and GSSG were not affected by fruit maturity or the 1-MCP treatment. In contrast, the level of GSH was highest in 1-MCP-treated mature fruit. Interestingly, the level of activity of APX, MDHAR, and GR was highest in 1-MCP-treated mature fruit; in contrast, GPX activity was highest in 1-MCP-treated immature fruit during cold storage (Fig. 5).

3.4. Volcano plot analysis

To compare the effects of fruit maturity and 1-MCP treatment, a volcano plot analysis was performed, as shown in Fig. 6. In terms of fruit maturity at harvest, SOD, chlorogenic acid, ferulic acid, rutin, DHA, quercetin, MDHAR, AsA, ABTS, GSH, GST, and CAT were highly up-regulated in immature fruit whereas GSSG, DPPH, syringic acid, arbutin, sucrose, GPX, and H_2O_2 were highly up-regulated in mature fruit (Fig. 6A). In terms of the effect of fruit maturity during cold storage, the levels of ferulic acid, rutin, quercetin, chlorogenic acid, AsA, DHA, SOD, caffeic acid, and CAT were all highly up-regulated in immature fruit, whereas syringic acid, DPPH, GSSG, quercitrin, APX, arbutin, GR, and C_2H_4 were highly up-regulated in mature fruit (Fig. 6B). With respect to the 1-MCP treatment effect, AsA and DHA were only highly up-regulated in untreated fruit, and GPX was only up-regulated in 1-MCP-treated fruit during cold storage (Fig. 6C).

3.5. Variable importance in projection (VIP) scores

The explanatory power of the model by dependent variables, i.e., explain with the VIP scores plots analysis, as shown in Fig. 7. Usually, the variables with VIP values above 1 were considered important contributors to the model and were responsible for the separation among maturity levels (Fig. 7A and B) and 1-MCP treatments (Fig. 7C and D). In total, 22 variables were detected either in immature or mature fruit after cold storage. In immature fruit, caffeic acid, quercitrin, chlorogenic acid, peel b^* , arbutin, AsA, TPC, epicatechin, POD, DHA, fumaric acid, and core C* were important in the control fruit, whereas *p*-coumaric acid, SOD, rutin, quercetin, syringic acid, GPX, sucrose, catechin, cortex C*, and H_2O_2 were found to be important variables in 1-MCP-treated fruit during cold storage (Fig. 7A). In mature fruit, sorbitol, *p*-coumaric acid, H_2O_2 , AsA, and gallic acid were important in untreated fruit, whereas GSH, MDHAR, quercetin, core b^* , core C*, PPO, SOD, GPX, PAL, GR, fresh weight loss, C_2H_4 , quercitrin, MDA, APX, GST, and chlorogenic acid were important in 1-MCP-treated fruit during cold storage (Fig. 7B). In untreated fruit, quercetin, rutin, ferulic acid, chlorogenic acid, caffeic acid, DHA, GSH, epicatechin, SOD, and MDHAR were considered important variables in immature fruit, whereas syringic acid, DPPH, GSSG, quercitrin, H_2O_2 , MDA, GPX, cortex b^* , cortex C*, firmness, CO_2 , and peel h^* were important in mature fruit during cold storage (Fig. 7C). In contrast, in the 1-MCP treatment, ferulic acid, rutin, ascorbic acid, SOD, epicatechin, quercetin, and *p*-coumaric acid were important in immature fruit, whereas SA, GSSG, DPPH, peel b^* , core b^* , quercetin, core C*, APX, MDA, CO_2 , cortex b^* , POD, cortex C*, firmness, and arbutin were only important in mature fruit during

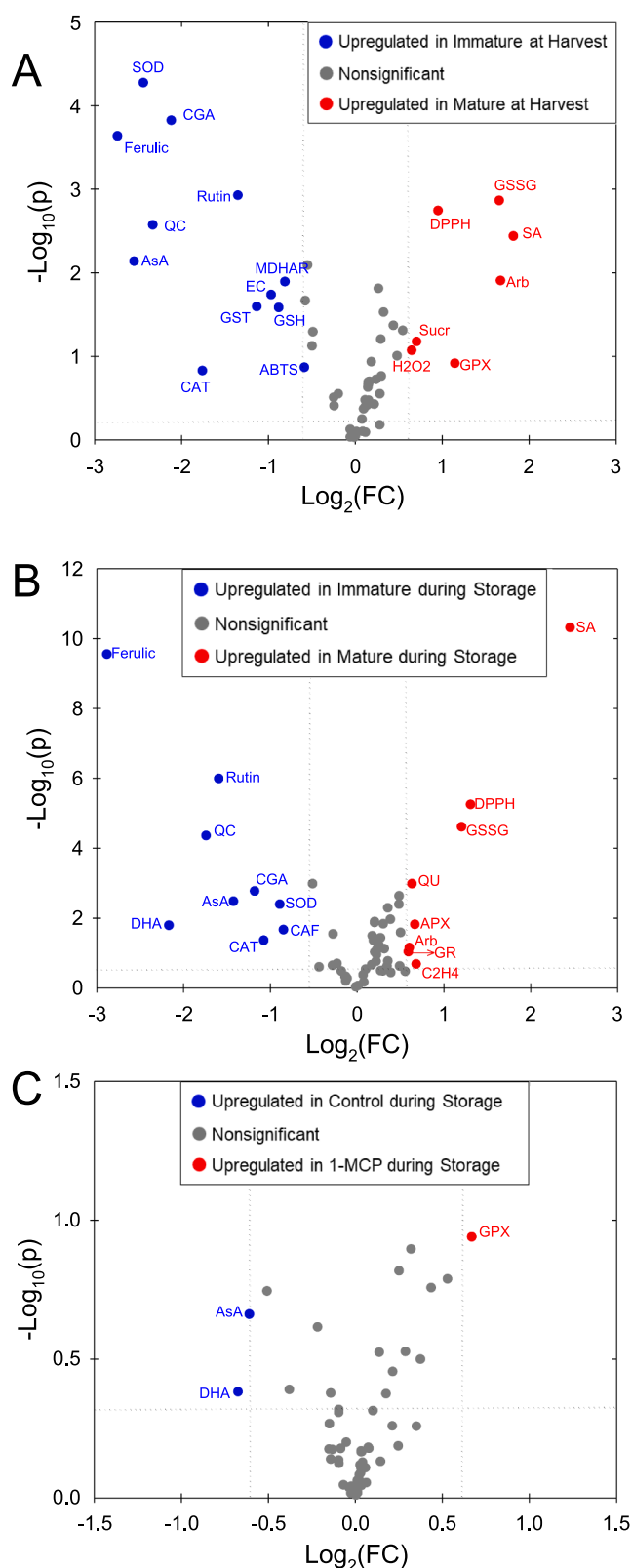


Fig. 6. Volcano plot analysis of immature vs. mature fruit at harvest (A), immature vs. mature fruit during cold storage (B), and control vs. 1-MCP-treated fruit during cold storage (C). In the volcano plots, the X-axis indicates $\log_2$ fold changes (FC) between two groups, and the Y-axis indicates $-\log_{10} p$ -value of variations. The red and blue dots represent the upregulated variables of each respective group, and grey dots indicate non-significant variables in figure legends.

cold storage (Fig. 7D).

3.6. Pearson's correlation coefficient network

The effects of fruit maturity and postharvest 1-MCP treatment were examined using a Pearson's correlation coefficient network, based on the threshold value of correlation coefficient ($r \geq |0.85|$) (Fig. 8). Regarding fruit maturity during cold storage, peel h° , sorbitol, cortex L^* , malic acid, core L^* , core h° , TFC, cortex C^* , cortex b^* , SSC, peel C^* , cortex h° , peel L^* , shikimic acid, fructose, glucose, ethylene production rate (C_2H_4), fruit fresh weight loss (FWL), ferulic acid, fumaric acid, and TA were positively correlated in immature fruit. In addition, cortex C^* was positively correlated with rutin, which was positively correlated with GPX and *p*-coumaric acid. H_2O_2 was positively correlated with cortex b^* , ethylene production rate (C_2H_4), and fruit fresh weight loss but negatively correlated with AsA and chlorogenic acid. FWL was positively correlated with H_2O_2 , C_2H_4 , glucose, ferulic acid, fumaric acid, TA, and arbutin but negatively correlated with sucrose, chlorogenic acid, and AsA. Sucrose was only positively correlated with syringic acid and negatively correlated with TA, fumaric acid, ferulic acid, FWL, and arbutin. At the same time, syringic acid was positively correlated with SSC/TA but negatively correlated with TA, fumaric acid, ferulic acid, and arbutin. AsA and chlorogenic acid were negatively correlated with H_2O_2 and FWL but positively correlated with quercitrin, which was positively correlated with caffeic acid, peel b^* , and TPC in immature fruit during cold storage (Fig. 8A). However, most variables were positively correlated. Caffeic acid and sucrose were only negatively correlated with C_2H_4 and FWL, which were closely and positively correlated with most variables in mature fruit during cold storage (Fig. 8B).

In terms of the effect of postharvest 1-MCP treatment during cold storage, most variables were tightly and positively correlated among themselves in the untreated control fruit. Among all variables, the greatest number of positive correlations was found with DPPH, which was also positively correlated with syringic acid content. However, the content of syringic acid was negatively correlated with that of ferulic acid, which was positively correlated with rutin, quercitrin, and chlorogenic acid (in that order) in the untreated control fruit during cold storage (Fig. 9A). Most variables were positively correlated among themselves; however, FWL and fumaric acid were negatively correlated with caffeic acid in 1-MCP-treated fruit during cold storage (Fig. 9B).

4. Discussion

Fruit quality is generally affected by maturity at harvest and continues to be affected by cold storage and shelf life. However, in the present study, we found that the prominent significant effects by two maturities were not significantly prominent among the response variables, at both at harvest and after cold storage, but the starch hydrolysis rate was strongly affected at harvest time. Our findings are in contrast to what has been reported in European pear cultivars; i.e., that fruit quality attributes, such as melting texture and juiciness after storage differ depending on fruit maturity at harvest (Dong et al., 2023; Hendges et al., 2018). That difference maturity responses may be due to the variations in harvest timing of the growing season or cultivar differences. Nonetheless, postharvest 1-MCP treatment affected fruit weight loss, SSC, ethylene production, and respiration rates during cold storage. Ethylene production rate in cold-stored 'Yali' pear responded to 1-MCP treatment in a dose-dependent manner, whereas firmness, SSC and TA showed no clear differences at all (Cheng et al., 2019). Our finding revealed that the ethylene production in fruit with 1-MCP treatment was lower and respiration rate was higher than control fruit (Fig. 1. B). These results would be related to the binding ability of the ethylene receptors by 1-MCP, which reducing as storage period continues. In addition, the newly synthesized ethylene receptors would not be bound by the 1-MCP, consequently, ethylene signalling capacity could be returned after long term storage condition. Although the ethylene production rate induce by

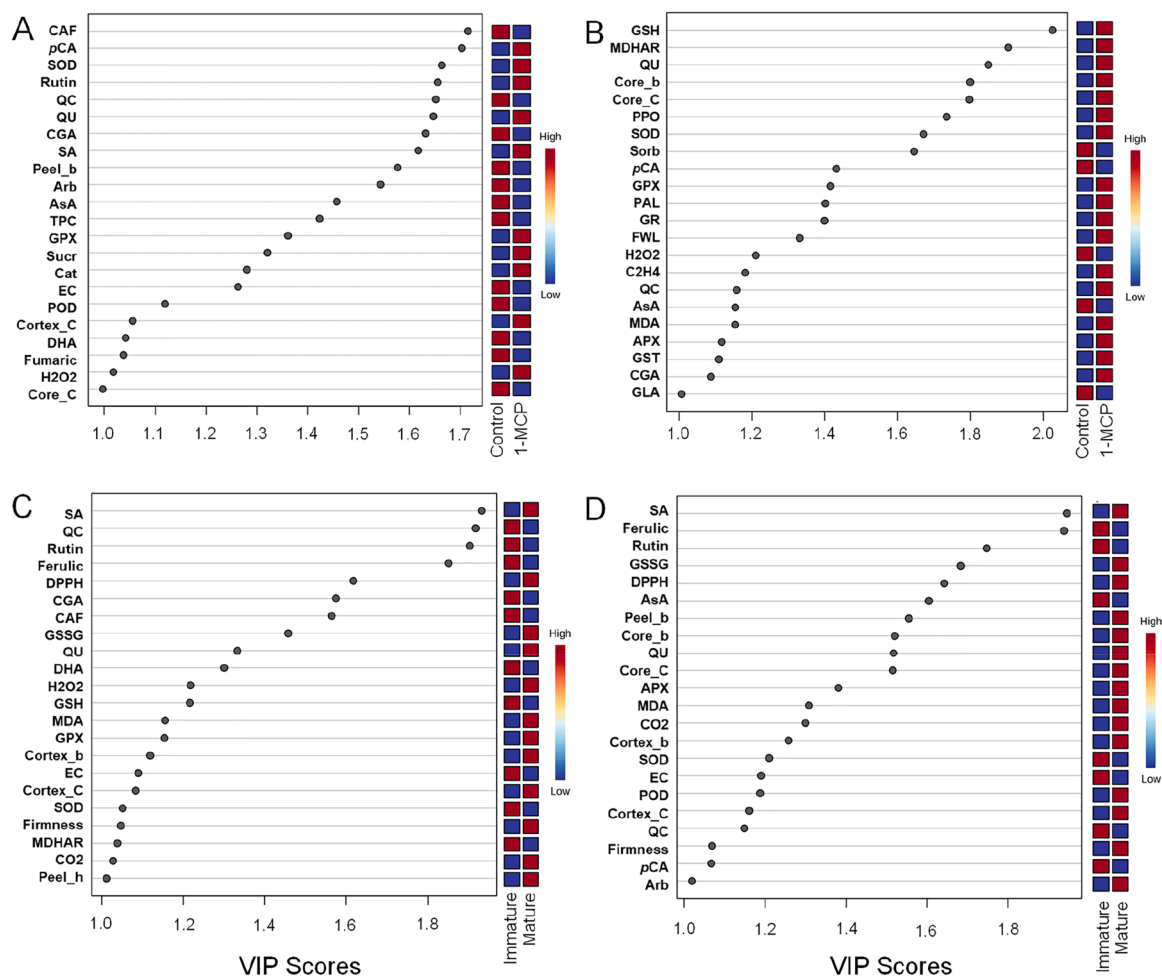


Fig. 7. Variable importance in projection (VIP) scores of response variables in the fruit of ‘Changjo’ Asian pears at different maturity and treated with 1 $\mu\text{L L}^{-1}$ 1-methylcyclopropene (1-MCP) treatment at harvest followed by storage at 0.5°C for 6 months before transferred to 22°C for one-day shelf life. Each figure represents immature (A) and mature (B), and control (C) and 1-MCP-treated (D) fruit during cold storage. Variables with VIP scores > 1 were defined as the important indicators, and the values were derived using a PLS-DA model.

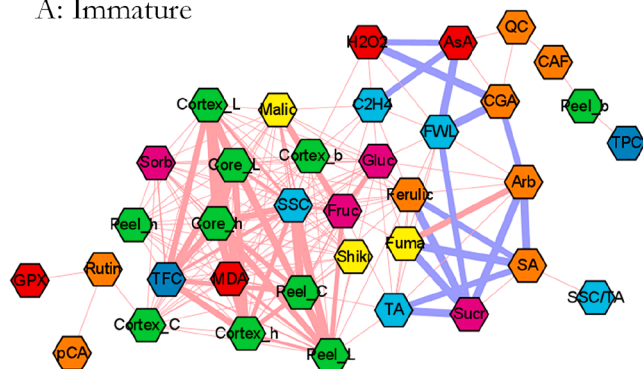
the ripening in climacteric fruit could be inhibited by AVG or 1-MCP treatment, the effectiveness could be stable for a short period because of the fruit genotypes, ethylene sensitivity and hormonal recovery (Sisler and Serek, 1997). Conversely, flesh firmness in ‘Wonhwang’ Asian pear is found to respond to postharvest 1-MCP treatment during short-term international transport, although fresh weight loss and SSC are minimally affected (Seo et al., 2019). In the present study, all the variables responded more significantly to fruit maturity than to postharvest 1-MCP treatment during cold storage.

In terms of soluble carbohydrates, the fructose and glucose levels were higher during cold storage than at harvest, whereas sucrose levels were lower. Meanwhile, sorbitol levels were not affected differently by 1-MCP treatment but were influenced by fruit maturity. In contrast, fructose and sucrose levels were unaffected by fruit maturity or postharvest 1-MCP treatment. However, glucose and sorbitol were highly affected by maturity but not by 1-MCP treatment. In comparison, in ‘Chuhwangbae’ Asian pears in cold storage, the levels of fructose and glucose are found to gradually increase, while the level of sucrose decreases (Latt et al., 2025; Latt et al., 2024a). In comparing pear varieties, the responses of soluble carbohydrates vary during shelf life after cold storage in ‘Nanguo’ pears (Wang et al., 2020) and during long-term cold storage in ‘Yali’ pears (Chen et al., 2006); the response of carbohydrate content during cold storage has been found to vary between cultivars (Zhao et al., 2019). In the present study, sucrose was found to be a highly important variable (based on VIP scores) in immature fruit, whereas

sorbitol was only detected as an important variable in mature fruit during cold storage. Furthermore, sucrose was strongly correlated with some fruit quality attributes and phenolic compounds during cold storage, irrespective of fruit maturity and was unaffected by postharvest 1-MCP treatment, which suggests that the differential responses of soluble carbohydrates may be highly affected by fruit maturity rather than postharvest 1-MCP treatment. On the other hand, the metabolic responses of malic and fumaric acids were similar, but shikimic acid was affected more by fruit maturity than by postharvest 1-MCP treatment. Thus, TA was higher during cold storage than at harvest, although fruit maturity and 1-MCP treatment, because malic acid plays a pivotal role in organic acid metabolism. However, postharvest 1-MCP treatment has been reported to reduce TA levels in long-term cold-stored ‘Xenia’ pears (Lugaresi et al., 2025). Nevertheless, we identified that fumaric acid in immature fruit is involved in a strong relationship among the other variables in immature fruit and postharvest 1-MCP treatment during cold storage. The level of fumaric acid has been shown to respond to preharvest storage conditioning treatment in cold-stored ‘Chuhwangbae’ Asian pears (Lwin et al., 2023a) and to postharvest 1-MCP treatment in long-term cold-stored ‘AC Harrow Crisp’ European pears (Flaherty et al., 2018). These findings demonstrate that minor organic acids can be more strongly influenced and associated with fruit maturity and postharvest 1-MCP treatment than are major organic acids.

As secondary metabolites, individual phenolic compounds responded differently to fruit maturity and postharvest 1-MCP treatment

A: Immature



B: Mature

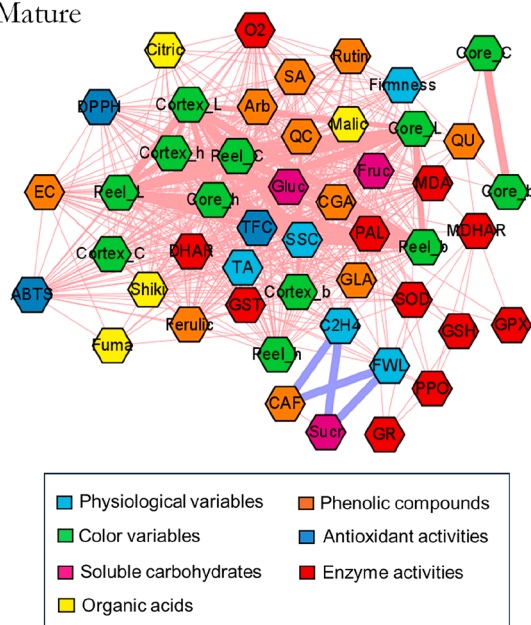
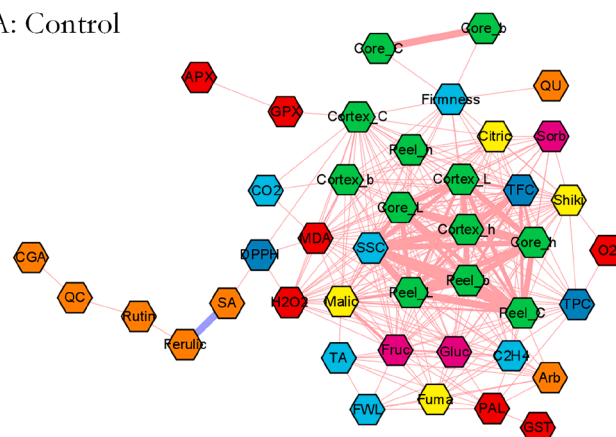


Fig. 8. Pairwise correlation coefficient network ($r \geq |0.85|$) constructed from the response variables in the fruit of ‘Changjo’ Asian pears at different maturity and treated with $1 \mu\text{L L}^{-1}$ 1-methylcyclopropene (1-MCP) treatment at harvest followed by storage at 0.5°C for 6 months before transferred to 22°C for one-day shelf life. Each figure represents the correlation network constructed from the relative changes in immature (A) and mature (B) fruit. Red and blue lines indicate positive and negative correlations among variables, respectively. The thickness of the correlation lines indicates the significance of the correlation coefficient (r) among variables.

during cold storage. Although TFs levels remained unchanged, level of TPC was highly affected by fruit maturity and postharvest 1-MCP treatment. As shown in the volcano plot, individual phenolic compounds and total antioxidant scavenging capacity were more responsive to fruit maturity than to postharvest 1-MCP treatment, both at harvest and during cold storage. Additionally, individual and TPC were detected at higher levels in immature fruit than in mature fruit during cold storage, considering as a strong correlation with variables related to fruit maturity and postharvest 1-MCP treatment. Typically, the content of individual phenolic compounds gradually declines in cold-stored pear fruit (Kou et al., 2015; Ogurlu et al., 2024; Zhai et al., 2021). Because phenolic compounds are water-soluble (Haminiuk et al., 2012; Tomás-Barberán and Espin, 2001), they may naturally degrade during cold storage due to fruit respiration and skin transpiration, leading to increased fresh weight loss. Nonetheless, quercitrin, ferulic acid, syringic acid, rutin, and gallic acid content responded differentially to fruit maturity. That is, higher responses of those individual phenolic

A: Control



B: 1-MCP

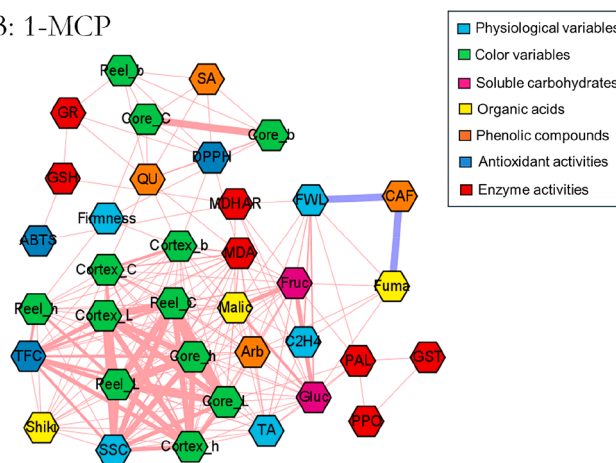


Fig. 9. Pairwise correlation coefficient network ($r \geq |0.85|$) constructed from the response variables in the fruit of ‘Changjo’ Asian pears at different maturity and treated with $1 \mu\text{L L}^{-1}$ 1-methylcyclopropene (1-MCP) treatment at harvest followed by storage at 0.5°C for 6 months before transferred to 22°C for one-day shelf life. Each figure represents the correlation network constructed from the relative changes at untreated control fruit (A) and 1-MCP-treated fruit (B) during cold storage. Red and blue lines indicate positive and negative correlations among variables, respectively. The thickness of the correlation lines indicates the significance of the correlation coefficient (r) among variables.

compounds, which are classified as hydroxycinnamates, hydroquinones, and flavanols, may be involved in the upstream metabolic pathways of phenolic compound metabolism (Sun et al., 2021). Interestingly, among individual phenolic compounds, catechin, quercitrin, rutin, and *p*-coumaric acid were much higher in 1-MCP-treated immature fruit compared to the corresponding results in control fruit during cold storage. Postharvest 1-MCP treatment is shown to elevate levels of both individual and TPC in long-term stored ‘Williams’ pears (Aubert et al., 2025). In general, postharvest 1-MCP treatment plays a key role in reducing fruit respiration and maintaining flesh firmness during shelf life and cold storage (Li et al., 2023; Xiang et al., 2022). The effectiveness of postharvest 1-MCP treatment is typically less in Asian pears than in apples due to considerable differences in the fruit ripening process and peel characteristics. Thus, flesh firmness and fresh weight loss as fruit quality attributes are not clearly affected by postharvest 1-MCP treatment during cold storage (Latt et al., 2023). In this study, postharvest 1-MCP treatment may result in retaining or delaying the degradation of certain individual phenolic compounds during cold storage; however, it is less effective in mature fruit than in immature fruit. Overall, the different fruit characteristics of Asian pears may affect the differential metabolism of phenolic compounds during cold storage.

In the present study, we found that the level of AsA was higher in immature fruit than in mature fruit at harvest and was reduced by postharvest 1-MCP treatment during cold storage, whereas DHA levels remained inconsistent. The levels of GSH and GSSG responded inversely at harvest; however, in cold storage, GSH was only higher in 1-MCP treatment (compared to control fruit). Notably, AsA and DHA were detected only in immature control fruit during cold storage, as indicated by the volcano plots, and were identified as important variables in the VIP scores plot. The contents of AsA and DHA have been reported to respond inversely to fruit maturity at harvest in ‘Conference’ pears (Lentheric et al., 1999). However, in another cultivar, ‘Passa Crassane’, the level of AsA does not consistently respond to fruit maturity (Spinardi, 2005). It could be that the differences between results arise from differences in methodology. The contents of AsA and GSH gradually decline in pear fruit during cold storage (Adhikary et al., 2021; Cheng et al., 2019; Spinardi, 2005; Wei et al., 2019). As mentioned above, the ripening characteristics of Asian pears are very different from those of typical apples and European pears. In particular, peel morphology is completely different, as the cuticle and epicuticular wax layers found on apple peels are absent in pear peel tissues, which contributed to the inconsistent responses of water-soluble compounds, such as AsA and GSH, during cold storage. Thus, AsA was strongly and negatively correlated with fresh weight loss and H₂O₂ levels in immature fruit during cold storage.

The activity of numerous antioxidant-scavenging enzymes is generally higher in immature fruit than in mature fruit at harvest. In ‘Conference’ pears, SOD and CAT activity is found to decrease, whereas POD and APX activities increase as harvest date advances (Lentheric et al., 1999). However, APX activity is not affected by harvest date in ‘Passa Crassane’ pears (Spinardi, 2005). The difference in these results may be attributed either to the length of the harvest window or to differences among pear cultivars. Nevertheless, the antioxidant enzyme parameters were more important in mature fruit during cold storage, as indicated by the VIP scores plot. In the case of the enzymes, APX, MDHAR, DHAR, and GR enzymatic activity does not consistently respond to postharvest 1-MCP treatment in cold-stored ‘Passe-Crassane’ pears (Cocetta et al., 2016). However, in the same study, postharvest 1-MCP treatment increases the activity of APX, MDHAR, and DHAR but reduces GR activity during shelf life after cold storage (Cocetta et al., 2016). In cold-stored ‘Wujiuxiang’ pears, postharvest 1-MCP treatment increases the activity of APX, SOD, and CAT but reduces LOX activity (Gao et al., 2015). In addition, the level of activity of both SOD and CAT is found to be higher in 1-MCP-treated fruit than in untreated fruit of cold-stored ‘Conference’ pears, although POD activity remains inconsistent (Chiriboga et al., 2013). Postharvest 1-MCP treatment does not have a clear effect on the antioxidant scavenging enzyme activities in long-term stored ‘Empire’ apples (Lee et al., 2019; Lee et al., 2012). Overall, the findings reported in the literature demonstrate that antioxidant scavenging activity of enzymes during cold storage of pears is influenced to a greater degree by fruit maturity at harvest than by postharvest 1-MCP treatment.

5. Conclusion

Fruit maturity was found to affect the starch iodine pattern index of ‘Changjo’ pears, cortex L*, and peel C* at harvest; however, it did not affect other fruit quality attributes, including firmness, SSC, TA, and respiration rate. Although fructose and sucrose levels were unaffected by fruit maturity or 1-MCP treatment, glucose levels were higher in mature fruit than in immature fruit, despite 1-MCP treatment. Malic acid concentration increased in mature fruit during cold storage, irrespective of 1-MCP treatment. However, fumaric acid increased during cold storage than at harvest, despite fruit maturity or 1-MCP treatment. Individual phenolic compounds responded differently to fruit maturity at harvest and to 1-MCP treatment during cold storage. Immature fruit contained more phenolic acids and flavonoids both at harvest and after cold storage than in mature fruit, regardless of 1-MCP treatment. AsA,

DHA, and GSH levels were higher in immature fruit than in mature fruit at harvest, whereas GSSG levels were lower. DHA was highest in untreated immature fruit, while GSH and GSSG were highest in 1-MCP-treated mature fruit during cold storage. PPO, SOD, CAT, MDHAR, DHAR, and GST enzyme activities were higher in the immature fruit than in the mature fruit at the time of harvest. During cold storage, the enzymatic activity of PAL, PPO, SOD, GPX, and GST was highest in 1-MCP treated immature fruit but APX, MDHAR, and GR activity was highest in mature fruit. Volcano and VIP scores plots indicated that all variables were strongly influenced by fruit maturity but relatively less influenced by 1-MCP treatment. In addition, all variables exhibited more differential correlations in immature than in mature fruit during cold storage, but relatively fewer differences were observed under 1-MCP treatment. Overall, these findings suggested that fruit quality attributes, soluble carbohydrates, organic acids, phenolic compounds, non-enzymatic and enzymatic antioxidant scavenging metabolism-related parameters could be more strongly affected by fruit maturity than by 1-MCP treatment in cold-stored ‘Changjo’ Asian pears.

CRedit authorship contribution statement

Theint Thandar Latt: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Hyowon Park:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Jiwon Park:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Si-Eun Byeon:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Jinhee Lee:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Hnin Phyu Lwin:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Ho-Jin Seo:** Writing – review & editing, Validation, Methodology, Conceptualization. **Seok Kyu Yun:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Jinwook Lee:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2026.114761](https://doi.org/10.1016/j.scienta.2026.114761).

Data availability

Data will be made available on request.

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