International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

International Journal of Food Science and Technology 2024, **59**, 3245–3257

Original article

Enhancing cold tolerance and quality characteristics of *Carica papaya* Linn through the application of 1-methylcyclopropene, geranium and lavender oil

Meng Yi Loh,[1] Noranizan Mohd Adzahan,[1] Ezzat Mohamad Azman,[1] Soo Peng Koh[2] & Noor Liyana Yusof[1,3]*

1 Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan 43400, Malaysia
2 Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor 43400, Malaysia
3 Halal Products Research Institute, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan 43400, Malaysia

(Received 22 November 2023; Accepted in revised form 28 February 2024)

Summary Postharvest application of 1-methylcyclopropene (1-MCP), lavender oil (LO) and geranium oil (GO) was investigated in alleviating chilling injury (CI) by regulating the cold tolerance of papaya cold stored at 4 °C for 16 days. The papaya was assessed on the attributes of CI, weight loss, firmness, colour, respiration rate, total soluble solids (TSS), soluble sugar (SS), proline and malondialdehyde content. The antagonistic effect of 1-MCP against ethylene proved highly effective in delaying fruit ripening and metabolic processes. Notably, a 1-h misting treatment with 600 mg L^{-1} 1-MCP (active ingredient, a.i.) solution significantly enhanced papaya's resistance to chilling conditions and reported a remarkable 2.08-fold reduction in CI damage compared to the control. Furthermore, 1-MCP-treated papayas exhibited the highest proline concentration (61.59 μg/g FW) and the lowest malondialdehyde content (39.65 μmol/g FW). Additionally, 1-MCP-treated papayas displayed a 1.1-fold reduction in oxygen consumption compared to the control. They also exhibited minimal changes in weight loss (3.6%), the least increase in TSS and SS compared to the control and better retention of fruit colour and firmness. Comparatively, GO known for its robust antioxidant and antimicrobial properties played a pivotal role in enhancing papaya's cold tolerance and providing superior protection against chilling-induced damage when compared to the control.

Keywords Cold storage, essential oil, physicochemical properties, proline, respiration rate.

Introduction

In 2020, papaya achieved an impressive self-sufficiency ratio of 156%, marking a 2.9% increase compared to the previous year within the fruit category (Department of Statistics Malaysia Official Portal, 2021). Nonetheless, the high perishability of papaya, primarily attributed to its elevated respiration rate (RR), poses a substantial challenge as its short shelf life makes marketing challenging, leading to significant postharvest losses. To mitigate the high RR and extend the shelf life of papaya, cold storage (CS) has emerged as a widely employed technique. This approach effectively preserves the postharvest quality of fresh produce by retarding their metabolic activities. However, it is worth noting that low-temperature storage may not be suitable for certain tropical and subtropical fruits, as these fruits often require specific CS temperatures tailored to their unique characteristics. Deviating from these optimal temperatures can result in physiological disorders and a decline in postharvest quality, as highlighted by Sevillano *et al.* (2009). In the case of papaya, these physiological disorders are collectively referred to as chilling injury (CI). CI becomes apparent in papayas when they are exposed to prolonged chilling stress or stored below their critical chilling temperature. The recommended critical temperature for papayas is no less than 10 °C, as temperatures lower than this threshold can induce CI symptoms, including the formation of pits, scalds, hard lumps in the pulp and surrounding vascular bundles, a loss of firmness in the flesh, abnormal ripening and increased susceptibility to microbial decay (Sevillano *et al.*, 2009).

Numerous studies have been dedicated to exploring the application of 1-methylcyclopropene (1-MCP) as a means to mitigate CI in postharvest production.

*Correspondent: E-mail: noorliyana@upm.edu.my

doi:10.1111/ijfs.17070

Researchers have shown growing interest in the 1-MCP compound due to its remarkable antagonistic effect on ethylene. Ethylene is closely interlinked with plant response to abiotic stresses such as low temperature, including chilling and freezing (Yang & Hoffman, 1984). Ethylene production is stimulated in tandem with various induced stresses including chilling, freezing, salinity stress, pathogen attack and wounding. The mechanism of action of 1-MCP in alleviating chilling injury in fruit and delaying senescence primarily involves its inhibition of ethylene perception and signalling pathways. Due to its planar molecular structure and higher affinity for ethylene receptors, 1-MCP competitively binds to ethylene receptors, disrupting ethylene response, slowing fruit respiration and organic matter consumption and extending fruit shelf life (Wang *et al.*, 2023). The 1-MCP treatment also potentially alters the oxidative status of fruits by enhancing the activities of enzymes involved in scavenging reactive oxygen species (ROS), reducing lipid peroxidation and electrolyte leakage and alleviating membrane damage during chilling stress (Zhang *et al.*, 2010; Li *et al.*, 2018). The effectiveness of 1-MCP treatment in controlling and alleviating the CI severity has been documented in various fruits, including mango (Li *et al.*, 2020), peach (Qian *et al.*, 2021) and persimmon (Besada *et al.*, 2015).

A variety of edible coatings are commonly employed in fruits and vegetables as a viable alternative to chemical preservatives, primarily owing to their cost-effectiveness and their ability to effectively manage the physiological processes of these produce items. Essential oils (EO) are generally recognised as safe (GRAS) and are considered potential candidates for use as antioxidants and antimicrobial agents. Consequently, there is a growing interest in integrating EO into edible coatings to explore their effectiveness in preserving postharvest quality. Postharvest treatment of essential oils has therefore been proposed to reduce the incidence of chilling injury in fruit. Research in this area has focused on assessing the ability of EO-infused coatings to mitigate CI in guava (Etemadipoor *et al.*, 2020) and loquat (Bahadirli *et al.*, 2020). The effectiveness of essential oils in alleviating the chilling injury in fruits, possibly owing to the presence of natural volatile compounds that augment the antioxidant activity and free radical scavenging capacity in fruit tissues (Wang, 2006). The elevated antioxidant enzyme activity and radical scavenging capacity in turn increase the fruit's resistance to oxidative stress during cold storage. For instance, the antioxidant content, namely catechin, chlorogenic and caffeic acids, in red- and yellow-skinned peaches, increased after 24 h postharvest thyme oil vaporisation and chilling injury symptoms, manifested as brown rot on the fruit skin, were alleviated (Khumalo *et al.*, 2017).

Nonetheless, to the best of the author's knowledge, little is known about the effectiveness of 1-MCP and EO through misting treatment in reducing CI. Very little attention has been paid to the consequences of the 1-MCP and EO treatment on the metabolism of papaya. This is an important aspect because the product shelf life is strongly dependent on the tissue metabolic activity. Thus, the main purpose of this study was to explore the metabolic consequences of treating papaya with 1-MCP and EO as a means to alleviate CI.

Material and methods

Plant material and sampling

Carica papaya Linn fruits were sourced from a commercial farm situated in Pahang, Malaysia. Subsequently, these fruits were harvested and transported to the laboratory located within the Faculty of Food Science and Technology at Universiti Putra Malaysia (UPM). The selection criteria for the papaya specimens adhered to standardised guidelines, which included uniform fruit size (ranging from 1300 to 1500 g) at stage 2 of ripeness, characterised by a light green hue with 5% yellowing of the skin and an absence of visible defects on the surface. The assessment of the fruit's skin colour was conducted following the ripening/maturating stage classification outlined by the Federal Agricultural Marketing Authority Malaysia (FAMA), as per their 2008 guidelines. To prepare the papaya for experiment, an initial wash with tap water was performed, followed by immersion in a 2% (w/v) sodium chloride solution for disinfection. Subsequently, the papaya underwent a thorough rinse with distilled water and was left to air dry for 1 h until completely moisture free.

The papaya was divided into four groups, namely the control, 600 mg L^{-1} 1-methlcylcopropene, 1-MCP (active ingredient, a.i.; Panpan Industry Co. Limited, Henan, China), 2% (v/v) pure geranium oil, GO (NOW®, Bloomingdale, Illinois, USA), and pure lavender oil, LO (NOW®, Bloomingdale, Illinois, USA) by misting treatment. The 600 mg L^{-1} 1-MCP (a.i.) solution was prepared and calculated from 3.5 per cent active ingredient according to manufacturer's instruction. The misting treatment lasted for 1 h at 20 ± 2 °C, in a known volume of camping tent (180 × 120 × 110 cm) to simulate a closed controlled chamber, with a relative humidity of 85–90%. To prevent vaporisation, all camping closures were sealed with cellophane tape. The misting effect was generated by using an ultrasonic diffuser (REDBUZZ, Malaysia) operating at a frequency of 2.4 MHz and producing a mist of 30 mL h^{-1}. A total of four sets of papaya samples were utilised, with each set comprising 36 fruits, arranged in triplicate. Following the designated treatments, the papayas were stored at a temperature

of 4 °C for a duration of 16 days, with analyses conducted at 4-day intervals. All assessments were carried out after the fruits had returned to the ambient room temperature, which was maintained at 20 ± 2 °C.

Measurement of weight loss (WL)

The initial weight of each papaya was recorded prior to placing them in cold storage. Subsequently, the fruits' weights were measured again at 4-day intervals to assess the WL that occurred during storage. The percentage of fruit WL was calculated using eqn (1) as follows:

$$\mathrm{WL}(\%) = \frac{\text{Initial fruit weight} - \text{Final fruit weight}}{\text{Initial fruit weight}} \times 100\% \quad (1)$$

Measurement of colour

Colour measurements were conducted at 4-day intervals using a Chroma Meter (CM-700d, Minolta Corporation, Japan) within an environment maintained at 20 ± 2 °C. The L^*, a^* and b^* values of the papaya were measured at the top, middle and bottom regions, and the final colour representation for both the control and treated papayas was determined by averaging these readings. Throughout the cold storage period, the same fruit was consistently used for tracking colour changes, and all measurements were carried out in triplicate.

Measurement of firmness

The firmness of the papaya flesh was assessed at three specific locations: the top, equatorial and bottom regions. After removing a 0.3 cm^2-thick section of the fruit's skin, the papaya was evenly divided into three segments. The firmness of each section was then quantified using a texture analyser (TA HA plus, Stable Micro Systems, Surrey, UK) equipped with an SMS P/2 N needle probe, with a penetration depth set at 10 mm. The ultimate measurement was derived as the average reading, and the firmness values were expressed in grammes.

Measurement of chilling injury index (CII)

Typically, papayas exhibit chilling injury (CI) symptoms such as the appearance of pits, darkening with a watery texture, sunken areas and the development of whitish spots. To assess and quantify these CI symptoms, we employed open-source image processing software called 'ImageJ' (v1.53i, NIH Image, National Institutes of Health, Bethesda, MD, USA). Each papaya fruit's skin surface was evenly divided into four distinct sections, each clearly labelled for reference. At 4-day intervals, images of these four sections per fruit were captured and saved as JPEG files for subsequent analysis. The CII was calculated according to eqn (2), which involves determining the ratio between the total area affected by CI and the total surface area of the fruit. The resulting value was expressed as a percentage.

$$\mathrm{CII}(\%) = (\text{Total chilling injured area} / \text{Total fruit surface area}) \times 100\% \quad (2)$$

Measurement of gas composition

Changes in the gaseous composition of the space surrounding the papaya were quantified within a sealed environment using a headspace gas analyser (Gaspace advance G3, Systech Instrument, UK). Each fruit was positioned within an air-tight container of known volume (6.0 L). To maintain a sealed environment, the container's lid featured a hole of identical diameter to the probe, ensuring no gas could escape while it was connected to the gas analyser. The concentrations of O_2 and CO_2 within the closed container's headspace were assessed at 4-day intervals and subsequently expressed as percentages.

Measurement of total soluble solids (TSS)

A 100 g portion of fruit pulp was sliced and processed using a blender (MX900M, Panasonic, Malaysia). A single drop of the resulting filtrate was utilised to ascertain the TSS content via a refractometer (N1, Atago Co. Ltd., Tokyo, Japan). The TSS measurement for papaya was conducted at three distinct regions: top, equatorial and bottom, and the results were presented as Brix values.

Measurement of soluble sugar content

A 30 g sample of fruit pulp was extracted and processed into juice. The resulting filtrate was then collected for the analysis of soluble sugar content. The levels of glucose, fructose and sucrose within the fruit pulp were determined enzymatically following the Megazyme K-SUFRG 05/20 Assay Procedure (Megazyme, Megazyme International, Ireland). These assays were conducted with measurements taken at an absorbance of 340 nm using a UV–visible spectrophotometer (Genesys™ 10S UV–Vis, Thermo Scientific, Waltham, MA, USA). All analyses were carried out in triplicate, and the results were expressed as grammes of sugar per 100 grammes of fresh weight (g/100 g FW).

Measurement of proline content

The determination of papaya's proline content was conducted following the method established by Sun *et al*. (2020), with some modifications. Initially, 2.0 g of thawed fruit pulp were subjected to extraction using a 10 mL solution of 30 g L^{-1} sulfosalicylic acid, heated to 100 °C and agitated continuously for 15 min. After boiling, the mixture was allowed to cool, followed by centrifugation at 6000 rpm for 10 min. The resultant supernatant was collected for proline content assessment.

Subsequently, 2 mL of this supernatant was combined with 2 mL of glacial acetic acid and 3 mL of acidic ninhydrin, followed by a 30-min boiling period. After cooling, the mixture was extracted using 4 mL of toluene, shaken for 30 s and set aside until distinct layers formed. The upper layer, containing the proline–toluene solution, was collected and its absorbance was measured at 520 nm using a spectrophotometer (Genesys™ 10S UV–Vis, Thermo Scientific, Waltham, MA, USA). Toluene's absorbance at 520 nm served as a blank control, and a standard curve for L-proline with concentrations ranging from 0 to 100 μg mL^{-1} was prepared. The proline content was subsequently calculated, as described in eqn (3) below:

$$\text{Proline content}\left(\mu g\, g^{-1}\right) = \frac{\text{proline mass of standard curve} \times \text{total volume sample extract solution}}{\text{sample extract solution volume during determination} \times \text{sample mass}} \quad (3)$$

Malondialdehyde (MDA) content

The MDA content was analysed following the method described by Heath & Packer (1968) with some modifications. To measure the MDA content, 2 g of fruit pulp was homogenised with 10 mL of 10% (w/v) trichloroacetic acid (TCA) and 0.25% (w/v) thiobarbituric acid (TBA). The mixture was then heated in a water bath at 95 °C for 25 min and rapidly cooled in an ice bath to halt the reaction. After centrifugation at 5000 × ***g*** for 15 min, the supernatant was assessed for absorbance at 532 and 600 nm. The concentration of TBA-reactive compounds was determined by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm. For the calculation, an extinction coefficient of 155 mM^{-1} cm^{-1} was used, and the MDA content was expressed in μmol MDA g^{-1} FW. The blank consisted of 10% (w/v) TCA and 0.25% (w/v) TBA.

Statistical analysis

Statistical analyses were conducted using Minitab Version 19 Statistical Software (Minitab Inc., Coventry, UK). A two-way analysis of variance was employed and tested for statistical significance at a threshold of $P < 0.05$. To assess genuine differences in means across various treatments and storage durations, Tukey–Kramer multiple-comparison tests were utilised. All analyses were carried out in triplicate, and the outcomes were presented as the mean value along with its corresponding standard deviation.

Result

CI index and changes in proline and malondialdehyde content in fruit

During the initial 4 days, a notable rise in chilling injury (CI) was observed in both the control and LO-treated groups, as illustrated in Fig. 1a. This manifested as the development of scalds on the peel, with scald occurrence in the control group substantially increasing from day 8 onward (as shown in Fig. 2). On day 8, there was a significant escalation in the severity of CI across all treatment groups. Notably, the control group exhibited the most pronounced CI symptoms, reaching 17.1%, while the 1-MCP-treated group demonstrated the lowest CI symptoms at 8.2% after 16 days of storage. The application of 1-MCP proved to be the most effective in mitigating CI, resulting in a significant 2.08-fold reduction in CI symptoms compared to the control group. There was an inverse relationship observed between proline content and the CI severity. Proline content exhibited a significant increase during the storage period, showing a nearly twofold rise compared to day 4 (as depicted in Fig. 1b). Subsequently, proline content continued to steadily increase throughout storage, ranging from a 1.18- to 1.36-fold increment. On day 16, the 1-MCP treatment demonstrated the highest proline content at 61.59 μg/g FW, followed closely by GO (59.12 μg/g FW) and LO (56.19 μg/g FW), and the lowest proline content was observed in the control group (50.23 μg/g FW). Notably, the 1-MCP treatment led to a 1.23-fold increase in proline content compared to the control, while the EO groups exhibited a 1.1-fold rise.

Conversely, MDA content exhibited a consistent rise throughout the storage period, displaying a positive correlation with the CI severity (as illustrated in Fig. 1c). The MDA content experienced a sharp increase on day 4 and subsequently saw a significant surge by day 16. Notably, on day 16, the application of 1-MCP led to a significant reduction in MDA

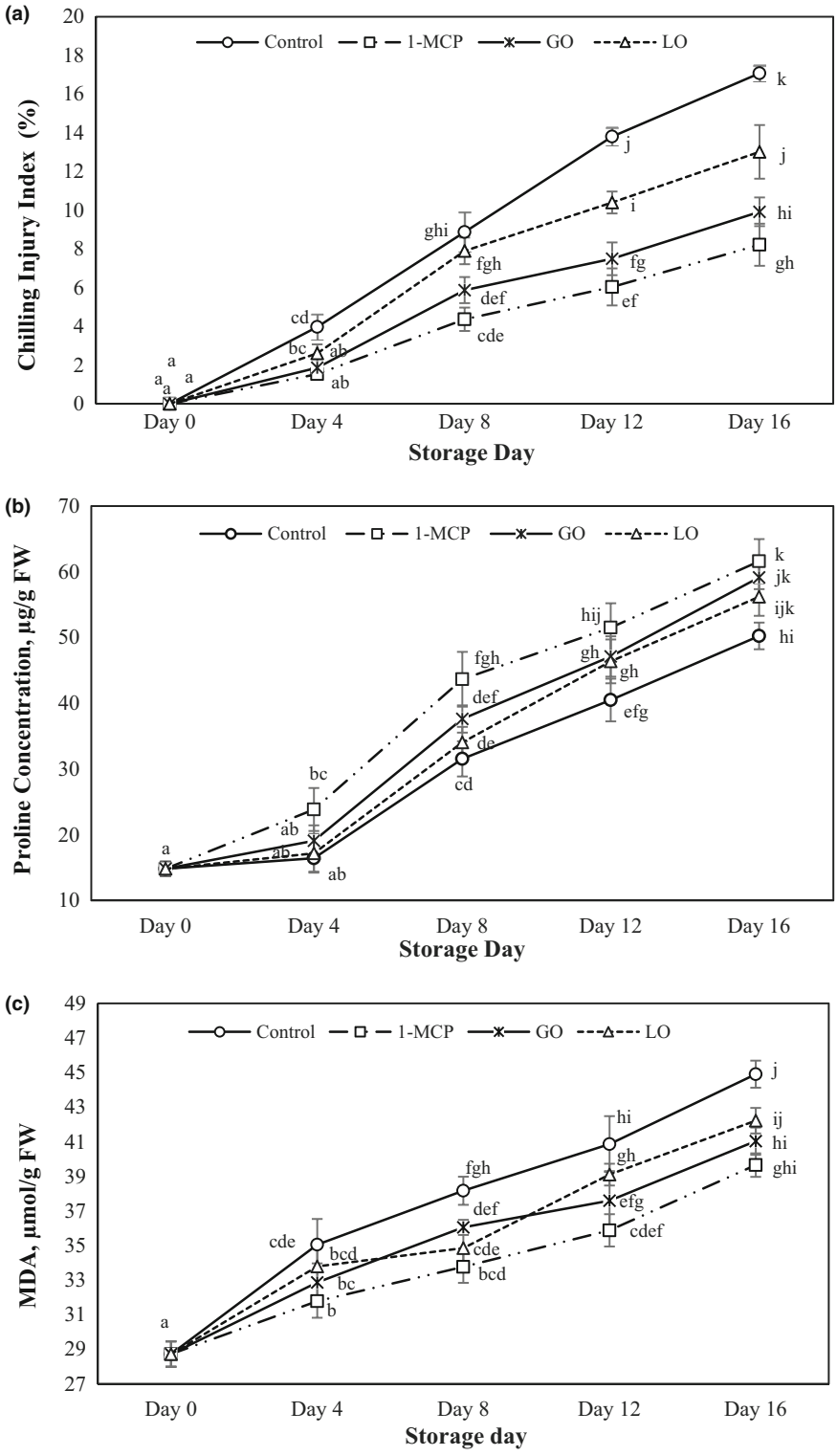

Figure 1 The changes in (a) CI index, (b) proline content and (c) MDA content of control and treated papaya fruit during 16 storage days at 4 °C. All the data are presented as mean value ± SD and the different letters denote the significant differences among fruits by using Tukey's test ($P < 0.05$). The error bars represented the standard deviation of the means of triplicate measurements.

Figure 2 The visual changes in both control and treated papaya were monitored every 4 days throughout a 16-day storage period at 4 °C.

content, registering a 15.9% decrease (7.1 μmol/g FW) compared to the control group. Furthermore, no significant differences were observed between the EO groups and the 1-MCP treatment in terms of MDA content.

Weight loss (WL) and respiratory gas composition

The changes in weight loss (WL) and the composition of respiratory gases in papayas were closely monitored throughout the storage period, as depicted in Fig. 3. On day 16, the control group exhibited the highest WL, registering at 6.3%, followed by the LO-treated group (4.9%), GO-treated group (4.3%) and the 1-MCP-treated group (3.6%). Notably, there was a significant increase in WL from day 12 to day 16, with a 2.09-fold rise in the control group and respective increases of 1.91-fold, 1.95-fold and 1.84-fold in the LO-, GO- and 1-MCP-treated fruits respectively. The WL exhibited a strong correlation with the fruit's respiration rate (RR). In a closed system, the O_2 concentration declined while the CO_2 concentration increased during cold storage. The highest RR, indicated by the highest O_2 consumption and CO_2 release, corresponded to the greatest WL observed in the control group. Notably, significant differences were observed between the control and 1-MCP-treated fruits, with disparities becoming evident at day 8 and day 12 for O_2 and CO_2 concentrations respectively. The application of 1-MCP led to a reduction in RR, decreasing it by at least 1.1-fold in both O_2 consumption and CO_2 release.

Fruit firmness and colour changes

The changes in papaya's firmness and colour (L^*, a^* and b^*) over the course of 16 days of storage at 4 °C are visually presented in Fig. 4. By day 16, there was a

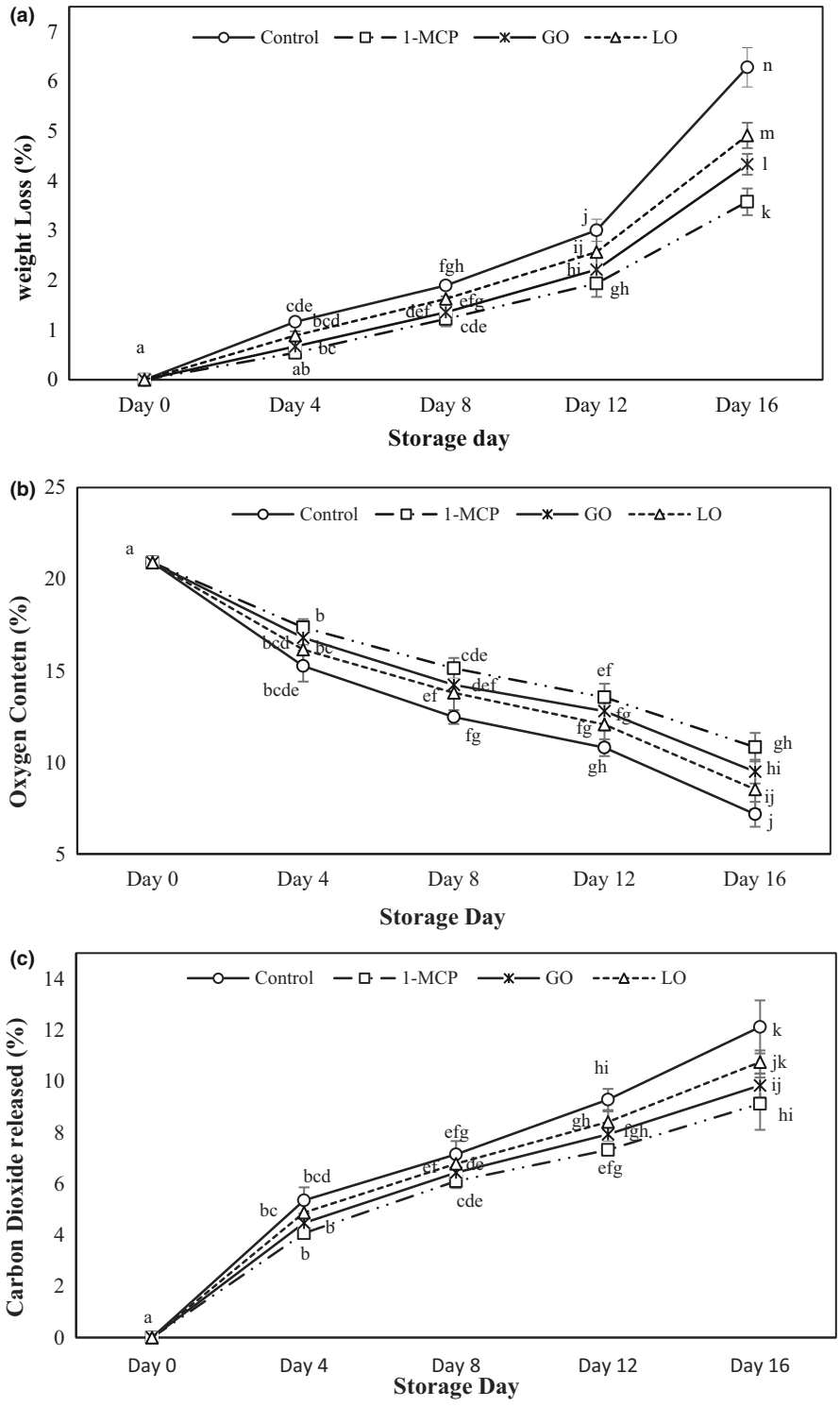

Figure 3 Effect of 1-MCP and EO treatment on (a) weight loss, WL and respiration gas changes in (b) oxygen concentration and (c) carbon dioxide concentration in papayas cold stored at 4 °C for 16 days. Different letters above the error bars represented statistically significant differences ($P < 0.05$) within each curve by using Tukey's test. Bars represent the standard deviation of the means of triplicate measurements.

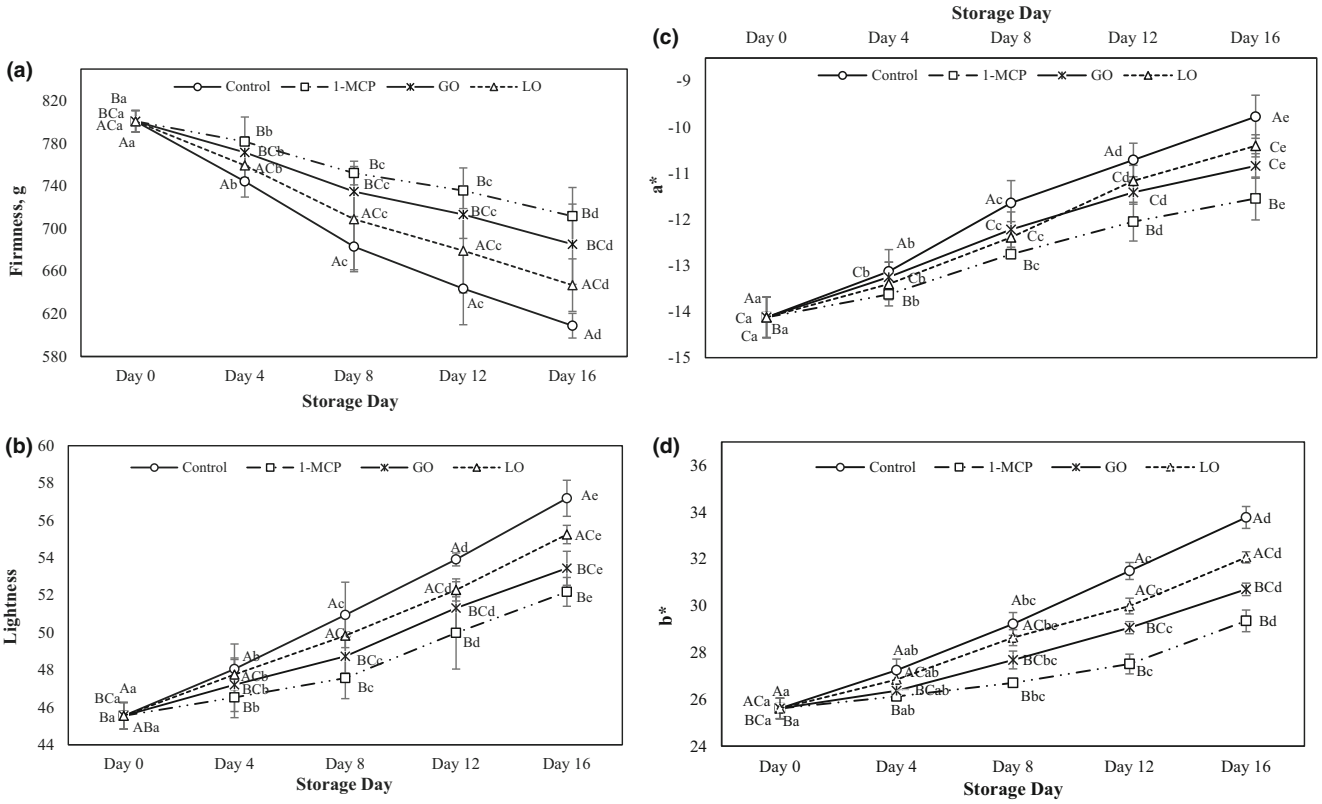

Figure 4 The changes in (a) firmness, (b) lightness, (c) redness/greenness, a^* value (d) yellowness/blueness and b^* value of papaya at every 4 days of storage at 4 °C. The data expressed as mean value ($n = 3$) and capital and lowercase superscript letters denote significant differences ($P < 0.05$) among treatments and storage days respectively.

24.0% reduction in firmness for the control group, 19.2% for LO-treated papayas, 14.4% for GO-treated papayas and 11.1% for those treated with 1-MCP. On day 16, the lightness (L^*) increased by 25.6% in the control group, equivalent to being 1.10-fold, 1.07-fold and 1.04-fold higher than in the 1-MCP, GO and LO treatments respectively. Over the storage period, the yellowness value exhibited a 1.15-fold increase in the control group, compared to 1.1-fold and 1.05-fold increases in the 1-MCP, GO and LO treatments respectively. Conversely, the greenness value decreased by 30.8%, 26.3%, 23.3% and 18.2% in the control, LO, GO and 1-MCP treatments respectively. Notably, the control group displayed lower colour retention and underwent more substantial colour changes during cold storage compared to the treated groups.

Total soluble solid content at selected regions and soluble sugar content

The total soluble solids (TSS) content demonstrated a proportional increase in all fruits and regions as the storage period progressed, as illustrated in Fig. 5a–c. After 16 days, regardless of the region, the highest TSS content was recorded in the control group (ranging from 29.8% to 46.8%), followed by LO-treated papayas (ranging from 23.4% to 40.5%), GO-treated papayas (ranging from 17.7% to 36.5%) and the 1-MCP treatment group (ranging from 13.5% to 31.8%). Throughout the storage period, the highest TSS values were consistently observed in the bottom region of the fruit, while the lowest values were recorded in the top region. There was a noticeable relocation of accumulated sugar content, initially starting in the top region (for the first 4 days), transitioning across the equatorial region (from day 8 onwards) and finally settling in the bottom regions (from day 12 onwards). The equatorial region consistently exhibited the highest TSS values. Notably, only the 1-MCP treatment group reported the highest TSS in the equatorial region on both day 8 and day 16. During CS, a significant increase in sucrose and glucose content was observed from day 0 to day 8 (as depicted in Fig. 5d, e), while the fructose content displayed a significant increase between day 8 and day 16 (as shown in Fig. 5f). From statistical analysis, notable differences

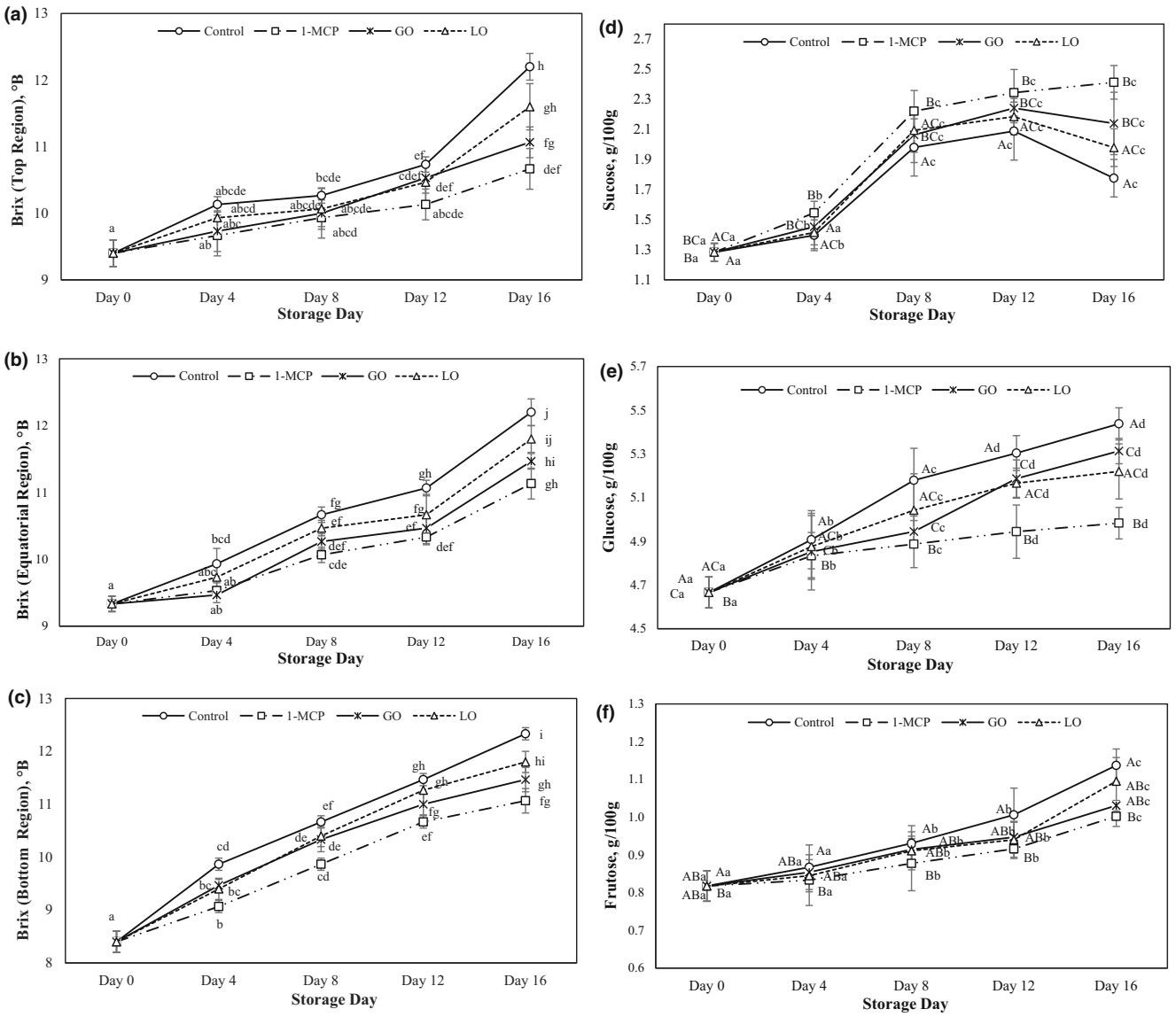

Figure 5 The changes in total soluble solids content at (a) top, (b) equatorial and (c) bottom regions and the changes of the soluble sugar content in (d) sucrose, (e) glucose and (f) fructose content in papaya cold stored at 4 °C. The data were expressed in mean value ($n = 3$) and the bars represented the standard deviations for the means of triplicate measurement. The superscript letter denoted difference ($P < 0.05$) between interaction effects among treatments and storage days for the total soluble sugar content measurements, whereas the capital and small case superscript letter in the sucrose, glucose and fructose content measurement denoted difference ($P < 0.05$) for the treatments and storage days respectively.

emerged between the control and 1-MCP-treated papayas in terms of sucrose, glucose and fructose. However, when considering sucrose, the statistical difference was solely observed between the control and GO treatments, while for glucose, it was only reported between the control and EO groups. It is worth noting that sucrose comprised the highest proportion of soluble sugar in papayas, followed by glucose and fructose. During CS, the most substantial increase in sucrose content was observed in the 1-MCP treatment group, which was 1.36-fold higher than in the control group, 1.13-fold higher than in the GO treatment group and 1.22-fold higher than in the LO treatment group. However, by day 16, a decline of 15.0%, 9.4% and 4.6% in sucrose content was noted in the control, LO and GO groups respectively. Conversely, the control group exhibited the highest glucose content (ranging from 1.02- to 1.09-fold higher) and fructose content

(ranging from 1.03- to 1.23-fold higher) throughout the storage period.

Discussion

Chilling injury (CI) in papaya is characterised by scald and pitting skin, soggy flesh and failure to ripen. Ethylene regulates and mediates a diverse array of biochemical, physiological and developmental processes during biotic and abiotic stresses, including postharvest handling, particularly in fruit storage at chilling temperatures. Studies have shown that CI development in fruit is closely linked to its maturity stage and ethylene level. The severity of CI is determined by the fruit's sensitivity to ethylene, which triggers signal cascades leading to secondary events and changes in firmness and membrane lipid structure (Besada *et al.*, 2015). Ethylene biosynthesis is involved in the elevated ethylene levels in climacteric fruit. Ethylene biosynthesis is interlinked with the increased transcription of key enzymes acyl-coenzyme A synthetases (ACS) and acyl-CoA oxidase, and a significant up-regulation of ACS (isoform II) has been reported during the cold storage of papaya (Zou *et al.*, 2014). 1-MCP is acclaimed for its suppressing effect on ethylene biosynthesis and signalling genes by actively binding to receptors, potentially reducing receptor protein degradation and rendering fruit less sensitive to ethylene signalling (Megías *et al.*, 2016). Thus, the insensitivity to ethylene induced by 1-MCP exposure could contribute to the alleviation of CI in fruit. Treatment with 1-MCP has been found to have a protective effect by enhancing the activity of ROS-scavenging enzymes in fruit and stabilising the redox state alteration caused by chilling stress (Zhang *et al.*, 2010). Similar results have been reported, indicating that 1-MCP treatment effectively reduces CI symptoms and makes them less noticeable during cold storage compared to untreated fruit (Ahmad *et al.*, 2013).

Essential oils possess an analogous effect to 1-MCP, regulating and modulating ethylene biosynthesis. The intervention in ethylene biosynthesis is attributed to alcohol and aldehyde groups present in essential oils including linalool, citronellol, geraniol, citral, citronellal and 1-octanal, which exerts inhibitory effect on 1-aminocyclopropane-1-carboxylic acid synthesis, subsequently lowering the ethylene level present in fruit (Rabbany & Mizutani, 1996; López-Gómez *et al.*, 2023). The major constituents in lavender and geranium oil, namely linalool, citronellol, geraniol, linalyl acetate and citronellyl formate, can be attributed to the regulation of papaya fruit's sensitivity to ethylene, unequivocally further improving its cold tolerance. Furthermore, incorporating essential oil (EO) into fruit coatings not only enhances the fruit's antioxidant and antimicrobial properties but also improves the complexity of the polymer matrix and enhances the bioactive action of the peel (Etemadipoor *et al.*, 2020). EO is recognised for its strong antioxidant capabilities, acting as a chain-breaking antioxidant due to its high phenolic content, actively transferring hydrogen atoms to peroxyl radicals (Amorati *et al.*, 2013). Additionally, EO exhibits a favourable antibacterial effect due to its hydrophobic nature, which increases bacterial cell permeability by disrupting the lipid structure of the cell membrane and mitochondria, leading to cell death (Chen *et al.*, 2021). Pathogen attack triggers elevated ethylene levels in fruit owing to rapid increases in ethylene biosynthesis during plant–pathogen interactions, driven by the activated plant stress adaptation response to fend off the pathogen (Jhalegar *et al.*, 2015; Shekhawat *et al.*, 2023). EO treatments such as lemongrass, eucalyptus, clove and neem oil have been demonstrated to reduce the rise in ethylene in pathogen-attacked mandarin fruit (Jhalegar *et al.*, 2015). Conceivably, EO may help dampen the abrupt rise of ethylene which is associated with CI development in fruit.

In response to the intensified severity of CI, proline accumulation initiates to aid the plant in recovering from stress by stabilising cellular homeostasis, activating specific gene expression and preserving protein integrity (Szabados & Savoure, 2010). It has been reported that treatments with 1-MCP and EO resulted in increased proline accumulation during storage. Our findings align with those of Qian *et al.* (2021), demonstrating that 1-MCP treatment enhances the fruit's ability to tolerate cold temperatures by promoting proline synthesis and accumulation during cold stress, particularly before and at the early stage of CI. Moreover, the MDA content in fruits serves as a biomarker that indicates the extent of stress-induced lipid peroxidation in the plasma membrane and reflects changes in cell membrane rigidity. During CS, the MDA content increases in parallel with the CI incidence and the fruit ripening process. Ripening triggers ethylene synthesis, which leads to degradation and damage of cell wall materials. Prolonged exposure to cold stress results in physiological disorders and related reactions that can harm the cell membrane (Liu *et al.*, 2021). The efficacy of 1-MCP in suppressing MDA content has also been reported in mandarins (Liu *et al.*, 2021) and plums (Bi *et al.*, 2022). The reduced accumulation of MDA in 1-MCP-treated fruits may be attributed to decreased respiratory rate, activated antioxidative enzyme systems and increased peroxidase enzyme activity, which further inhibit lipid peroxidation and senescence (Bi *et al.*, 2022). Additionally, the phenolic compounds found in flower EO contribute to its strong antioxidant effect by donating electrons to combat free radicals and inhibit oxidative reactions (Chen *et al.*, 2021). Therefore, misting treatment with EO could potentially minimise the accumulation of MDA in papaya.

Consumers consider papaya's size, colour, maturity and firmness when purchasing it. Both 1-MCP- and EO-treated papaya show lower WL and RR compared to the control group. WL causes papaya to shrink,

deform and negatively affect its appearance, resulting in lower marketability and consumer acceptance. WL in papaya is caused by transpiration (water and soluble substances removal) and respiration (carbon loss as CO_2). EO in food coating acts as a protective barrier, preventing moisture loss, oxygen exposure and microbial damage. It also resists lipid oxidation and protein degradation and maintains fruit moisture. All treatments exhibit a significant increase in WL from day 12 onwards, linked to increased cell metabolism, ripening, cell wall degradation and enhanced cell membrane permeability, causing rapid water evaporation (Lee *et al.*, 1995; Parven *et al.*, 2020; Chen *et al.*, 2021). A positive correlation between ethylene production rate and fruit respiration is proposed. An increase in fruit respiration is always observed either coinciding with or following an increase in ethylene production rate (Lelièvre *et al.*, 1997). Correspondingly, suppression of respiration and delayed ripening are primarily driven by low fruit ethylene and oxygen/carbon dioxide ratios (Paul & Pandey, 2014). In our study, lower fruit respiration is demonstrated in 1-MCP- and EO-treated fruits, most likely in response to the efficacy in suppressing ethylene biosynthesis. A similar response is reported in the study applying pummelo EO, turmeric EO and neem EO on papaya cv. Red Lady, where the fruit respiration exhibited a pattern resembling that of the ethylene evolution rate (Prasad *et al.*, 2022). Furthermore, during misting, volatile compounds liberated in fine mist adhere and adsorb to the fruit surface, possibly forming a thin film covering entire fruit. This mimics similar effects observed in a study of thyme oil and savoury oil fumigation on peaches and nectarines, suggesting that EO fumigation develops a coating on the fruit surface that modifies gas permeability, subsequently reducing fruit respiration rate and preventing moisture loss (Santoro *et al.*, 2018).

Over time, the fruit's rigidity decreases, and the pulp softens due to the depolymerisation of polysaccharides and solubilisation of pectin. The degradation of hemicellulose, cellulose and middle lamella of the cell wall leads to pectin release and solubilisation, causing softening (Perkins-Veazie, 1995). RR greatly affects pectin solubility and depolymerisation (Lazan *et al.*, 1995). Thus, 1-MCP and EO treatments effectively slow down RR and metabolic activity, resulting in higher fruit pulp rigidity. Similar findings were observed in *Carica papaya* L. cv Sekaki, where 1-MCP treatment reduced cell wall degradation enzymes and maintained rigidity (Ahmad *et al.*, 2013). The decline in fruit firmness is slower with 1-MCP treatment due to reduced activity of softening enzymes such as pectin esterase, endo-1,4-β-d-glucanase and endopolygalacturonase, preserving fruit integrity (Li *et al.*, 2020). The coating also helps maintain fruit firmness and provides an appropriate internal atmosphere (Carrillo-Lopez *et al.*, 2000).

As the papaya fruit ripens, the pulp softens and the skin undergoes colour changes, transitioning from green to pale green and yellowish-orange. The peel colour change is a result of chlorophyll degradation and the transformation of green pigment into other pigments (Carrillo-Lopez *et al.*, 2000). After chlorophyll degradation, carotenoid synthesis begins, making carotenoid pigments visible (Hashim *et al.*, 2012). Over time, the fruit becomes lighter and more yellow, while the greenness decreases. 1-MCP has an antagonistic effect against ethylene, which delays de-greening and slows down the ripening process. It achieves this by irreversibly binding to the ethylene receptor site, reducing the activities of acetyl-CoA synthetase and 1-aminocyclopropane-1-carboxylic acid oxidase enzymes that contribute to fruit ripening and senescence (Sisler & Serek, 1997). As a result, there are fewer colour changes in the group treated with 1-MCP. This can be attributed to its ability to decrease chlorophyll breakdown by downregulating genes and enzymes involved in the chlorophyll degradation pathway (Satekge & Magwaza, 2020). Similar effects have been observed in Solo papaya (cv. 'Gold' and Rainbow), Sekaki papaya and mango (Manenoi *et al.*, 2007; Ahmad *et al.*, 2013; Li *et al.*, 2020). Delayed de-greening in EO-treated papaya is probably due to diminished chlorophyll degradation. This phenomenon aligns with the findings from research employing an EO mixture comprising eugenol, thymol and carvacrol, which revealed a delay in chlorophyllase activity responsible for chlorophyll degradation, ultimately resulting in the preservation of lightness and chroma value in table grapes (Guillén *et al.*, 2007).

During CS, both treated and untreated papaya showed an increase in TSS and soluble sugar levels due to sugar accumulation and moisture loss (Etemadipoor *et al.*, 2020). However, the sugar content in papaya treated with 1-MCP and EO was lower than in the control, possibly because the coating retained moisture levels in the fruit (Etemadipoor *et al.*, 2020). Soluble sugars play a vital role in osmotic regulation and protecting plants from oxidative damage under low-temperature stress (Guy *et al.*, 1992). Sucrose, being a storage carbohydrate, accumulates and can be rapidly mobilised and used in respiration during times of need (Guy *et al.*, 1992). Glucose and fructose accumulate to a lesser extent than sucrose under cold stress, consistent with our results showing higher sucrose content compared to glucose and fructose (Guy *et al.*, 1992). Soluble sugar also replenishes water loss, improving water-holding capacity, chilling tolerance and maintaining cell membrane integrity (Liu *et al.*, 2019). Furthermore, the increase in sugar content is related to fruit ripening, as starch breaks down into sugars, contributing to a unique aroma profile (Parven *et al.*, 2020), making the fruit sweeter.

Conclusion

In this study, we investigated the effectiveness of misting treatments involving 1-MCP, GO and LO in alleviating CI in papaya. Our findings highlight that both 1-MCP and EO treatments have the potential to enhance the chilling tolerance of papaya by promoting proline synthesis and sugar metabolism. Additionally, both 1-MCP and EO treatments effectively suppressed the metabolic activity of papaya, leading to better preservation of postharvest quality. To further advance our understanding, future research endeavours should delve into the influence of 1-MCP and essential oils on antioxidant enzymes in papaya and the production of free radicals induced by CI.

Acknowledgment

This study was supported by grants from Universiti Putra Malaysia (Grant number UPM/800/3/3/1/GP-IPM/2018/9657000).

Author contributions

Meng Yi Loh: Conceptualization; investigation; writing – review and editing; writing – original draft; methodology; validation; software; formal analysis; project administration; data curation; visualization. **Noranizan Mohd Adzahan:** Conceptualization; supervision; resources. **Ezzat Mohamad Azman:** Conceptualization; supervision; resources. **Soo Peng Koh:** Conceptualization; resources; supervision. **Noor Liyana Yusof:** Conceptualization; writing – review and editing; funding acquisition; supervision; resources; validation.

Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

Ethics approval was not required for this research.

Data availability statement

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

References

Ahmad, A., Ali, Z.M. & Zainal, Z. (2013). Delayed softening of papaya (*Carica papaya* L. cv. Sekaki) fruit by 1-methylcyclopropene (1-MCP) during ripening at ambient and low temperature storage conditions. *Australian Journal of Crop Science*, **7**, 750–757.

This reference offers supportive evidence regarding the papaya fruit's reactions to 1-methylcyclopropene treatment through vaporisation in both ambient and cold storage conditions. The study's presented discoveries offer guidance and references for ongoing research utilising the misting technique. This reference study serves as a basis for comparing the effects and evaluating the effectiveness of misting treatment with 1-methylcyclopropene.

Amorati, R., Foti, M.C. & Valgimigli, L. (2013). Antioxidant activity of essential oils. *Journal of Agricultural and Food Chemistry*, **61**, 10835–10847.

Bahadirli, N.P., Kahramanoğlu, I. & Wan, C. (2020). Exposure to volatile essential oils of myrtle (*Myrtus communis* L.) leaves for improving the postharvest storability of fresh loquat fruits. *Journal of Food Quality*, **2020**, 8857669.

Besada, C., Llorca, E., Novillo, P., Hernando, I. & Salvador, A. (2015). Short-term high CO_2 treatment alleviates chilling injury of persimmon cv. Fuyu by preserving the parenchyma structure. *Food Control*, **51**, 163–170.

Bi, X., Dai, Y., Zhou, Z., Xing, Y. & Che, Z. (2022). Combining natamycin and 1-methylcyclopropene with modified atmosphere packaging to evaluate plum (*Prunus salicina* cv. 'Cuihongli') quality. *Postharvest Biology and Technology*, **183**, 111749.

Carrillo-Lopez, A., Ramirez-Bustamante, F., Valdez-Torres, J.B., Rojas-Villegas, R. & Yahia, E.M. (2000). Ripening and quality changes in mango fruit as affected by coating with an edible film. *Journal of Food Quality*, **23**, 479–486.

Chen, K., Zhang, M., Bhandari, B. & Mujumdar, A.S. (2021). Edible flower essential oils: a review of chemical compositions, bioactivities, safety and applications in food preservation. *Food Research International*, **139**, 109809.

This source offers context and foundational information regarding essential oils and their prospective uses in enhancing postharvest fruit quality. The research aids readers in comprehending the potential mechanisms by which essential oils function as natural antioxidants and antimicrobial agents.

Department of Statistics Malaysia Official Portal. (2021). Supply and Utilization Account Selected Agricultural Commodities, Malaysia 2016-2020. Retrieved from https://www.dosm.gov.my/v1/index.php?r=column/cthemeByCat&cat=164&bul_id=cHgwanhNdU4vWXRvc3pnZU9xSjZTUT09&menu_id=Z0VTZGU1UHBUT1VJMFlpaXRRR0xpdz09

Etemadipoor, R., Dastjerdi, A.M., Ramezanian, A. & Ehteshami, S. (2020). Ameliorative effect of gum arabic, oleic acid and/or cinnamon essential oil on chilling injury and quality loss of guava fruit. *Scientia Horticulturae*, **266**, 109255.

This study served as supporting evidence of employing essential oil in ameliorating chilling injury in fruit. The presented findings in this study provided benchmarks and guidelines for the current study, elucidating the potential responses of fruits following essential oil treatment during cold stress.

Guillén, F., Zapata, P.J., Martínez-Romero, D., Castillo, S., Serrano, M. & Valero, D. (2007). Improvement of the overall quality of table grapes stored under modified atmosphere packaging in combination with natural antimicrobial compounds. *Journal of Food Science*, **72**, S185–S190.

Guy, C.L., Huber, J.L.A. & Huber, S.C. (1992). Sucrose phosphate synthase and sucrose accumulation at low temperature. *Plant Physiology*, **100**, 502–508.

Hashim, N., Janius, R.B., Baranyai, L., Rahman, R.A., Osman, A. & Zude, M. (2012). Kinetic model for colour changes in bananas during the appearance of chilling injury symptoms. *Food and Bioprocess Technology*, **5**, 2952–2963.

Heath, L.R. & Packer, L. (1968). Photoperoxidation in isolated chloroplast I. Kinetics and stoichiometry of fatty acid peroxidation. *Achieves of Biochemistry and Biophysics*, **125**, 189–198.

Jhalegar, M.J., Sharma, R.R. & Singh, D. (2015). In vitro and in vivo activity of essential oils against major postharvest pathogens of Kinnow (*Citrus nobilis* × *C. deliciosa*) mandarin. *Journal of Food Science and Technology*, **52**, 2229–2237.

Khumalo, K.N., Tinyane, P., Soundy, P., Romanazzi, G., Glowacz, M. & Sivakumar, D. (2017). Effect of thyme oil vapour exposure

on the brown rot infection, phenylalanine ammonia-lyase (PAL) activity, phenolic content and antioxidant activity in red and yellow skin peach cultivars. *Scientia Horticulturae*, **214**, 195–199.

Lazan, H., Selamat, M.K. & Ali, Z.M. (1995). β-Galactosidase, polygalacturonase and pectinesterase in differential softening and cell wall modification during papaya fruit ripening. *Physiologia Plantarum*, **95**, 106–112.

Lee, L., Arul, J., Lencki, R. & Castaigne, F. (1995). A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: physiological basis and practical aspects—part I. *Packaging Technology and Science*, **8**, 315–331.

Lelièvre, J., Latchè, A., Jones, B., Bouzayen, M. & Pech, J. (1997). Ethylene and fruit ripening. *Physiologia Plantarum*, **101**, 727–739.

Li, J., Han, Y., Hu, M., Jin, M. & Rao, J. (2018). Oxalic acid and 1-methylcyclopropene alleviate chilling injury of "Youhou" sweet persimmon during cold storage. *Postharvest Biology and Technology*, **137**, 134–141.

Li, L., Li, C., Sun, J. *et al*. (2020). The effects of 1-methylcyclopropene in the regulation of antioxidative system and softening of mango fruit during storage. *Journal of Food Quality*, **2020**, 1–11.

Liu, H.H., Pei, H., Jiao, J. *et al*. (2021). 1-methylcyclopropene treatment followed with ethylene treatment alleviates postharvest chilling injury of 'Xuxiang' kiwifruit during low-temperature storage. *Food Control*, **130**, 108340.

This reference served as corroborative evidence for the postharvest utilisation of 1-methylcyclopropene and the resulting metabolic alterations in fruits, with a specific focus on lipid peroxidation. It offered valuable insights into the mechanisms through which 1-methylcyclopropene operates to preserve cell membrane rigidity and mitigate the occurrence of lipid peroxidation.

Liu, J., Li, F., Li, T., Yun, Z., Duan, X. & Jiang, Y. (2019). Fibroin treatment inhibits chilling injury of banana fruit via energy regulation. *Scientia Horticulturae*, **248**, 8–13.

López-Gómez, A., Navarro-Martínez, A., Garre, A., Iguaz, A. & Martínez-Hernández, G.B. (2023). The potential of essential oils from active packaging to reduce ethylene biosynthesis in plant products. Part 2: fruits (blueberries and blackberries). *Plants*, **12**, 3418.

Manenoi, A., Bayogan, E.R.V., Thumdee, S. & Paull, R.E. (2007). Utility of 1-methylcyclopropene as a papaya postharvest treatment. *Postharvest Biology and Technology*, **44**, 55–62.

Megías, Z., Martínez, C., Manzano, S. *et al*. (2016). Ethylene biosynthesis and signaling elements involved in chilling injury and other postharvest quality traits in the non-climacteric fruit of zucchini (*Cucurbita pepo*). *Postharvest Biology and Technology*, **113**, 48–57.

Parven, A., Sarker, M.R., Megharaj, M. & Meftaul, M.I. (2020). Prolonging the shelf life of papaya (*Carica papaya* L.) using aloe vera gel at ambient temperature. *Scientia Horticulturae*, **265**, 109228.

Paul, V. & Pandey, R. (2014). Role of internal atmosphere on fruit ripening and storability—a review. *Journal of Food Science and Technology*, **51**, 1223–1250.

Perkins-Veazie, P. (1995). Growth and ripening of strawberry fruit. *Horticultural Reviews*, **17**, 267–297.

Prasad, K., Singh, G., Singh, S.K., Pradhan, J., Kumar, U. & Singh, H. (2022). Plant extract and essential oil coating prolongs shelf life and maintains keeping quality of papaya fruit during storage. *Journal of Food Processing and Preservation*, **46**, e17015.

Qian, C., Ji, Z., Zhu, Q. *et al*. (2021). Effects of 1-MCP on proline, polyamine, and nitric oxide metabolism in postharvest peach fruit under chilling stress. *Horticultural Plant Journal*, **7**, 188–196.

This source supplied evidence and support for the ongoing research. The application of 1-methylcyclopropene through misting yielded a comparable effect to the vaporisation technique, demonstrating the feasibility of adopting this novel application method for 1-methylcyclopropene on different fruits in a more time-efficient manner.

Rabbany, A.B.M.G. & Mizutani, F. (1996). Effect of essential oils on ethylene production and ACC content in apple fruit and peach seed tissues. *Journal of the Japanese Society for Horticultural Science*, **65**, 7–13.

Santoro, K., Maghenzani, M., Chiabrando, V. *et al*. (2018). Thyme and savory essential oil vapor treatments control brown rot and improve the storage quality of peaches and nectarines, but could favor gray Mold. *Food*, **7**, 7.

Satekge, T.K. & Magwaza, L.S. (2020). The combined effect of 1-methylcyclopropene (1-MCP) and ethylene on green-life and postharvest quality of banana fruit. *International Journal of Fruit Science*, **20**, S1539–S1551.

Sevillano, L., Sanchez-Ballest, M.T., Romojaro, F. & Flores, F.B. (2009). Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. *Journal of the Science of Food and Agriculture*, **89**, 555–573.

Shekhawat, K., Fröhlich, K., García-Ramírez, G.X., Trapp, M.A. & Hirt, H. (2023). Ethylene: a master regulator of plant–microbe interactions under abiotic stresses. *Cells*, **12**, 31.

Sisler, E.C. & Serek, M. (1997). Inhibitors of ethylene responses in plants at the receptor level: recent developments. *Physiologia Plantarum*, **100**, 577–582.

Sun, H., Luo, M., Zhou, X. *et al*. (2020). Exogenous glycine betaine treatment alleviates low temperature-induced pericarp browning of 'Nanguo' pears by regulating antioxidant enzymes and proline metabolism. *Food Chemistry*, **306**, 125626.

Szabados, L. & Savoure, A. (2010). Proline: a multifunctional amino acid. *Trends in Plant Science*, **15**, 89–97.

Wang, C.Y. (2006). Reducing chilling injury and maintaining quality of horticultural crops with natural products and their derivatives. *Acta Horticulturae*, **712**, 285–290.

Wang, S., Guo, X., Wang, Z., Xu, H., Ding, W. & Kou, L. (2023). Shelf life quality retention and decay inhibition of winter jujube (*Zizyphus jujuba mill*. cv. Dalidongzao) using 1-methylcyclopropene. *Journal of Agriculture and Food Research*, **14**, 100841.

Yang, S.F. & Hoffman, N.E. (1984). Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology*, **35**, 155–189.

Zhang, Z., Zhang, Y., Huber, D.J., Rao, J., Sun, Y. & Li, S. (2010). Changes in prooxidant and antioxidant enzymes and reduction of chilling injury symptoms during low-temperature storage of 'Fuyu' persimmon treated with 1-methylcyclopropene. *HortScience*, **45**, 1713–1718.

Zou, Y., Zhang, L., Rao, S. *et al*. (2014). The relationship between the expression of ethylene-related genes and papaya fruit ripening disorder caused by chilling injury. *PLoS One*, **9**, e116002.