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Research Article

Response of different cultivation substrates on the chilling injury symptom of sweet pepper grown in hydroponics

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Abstract

This study determined the chilling injury interactions of sweet peppers with their hydroponic growth substrate. The treatments were cocopeat, perlite, and a mixture of 50:50 cocopeat and perlite (coco-perlite). The fruits, when harvested, were stored for 50 days using the modified atmosphere package (MAP) at 5 °C. The results revealed no significant interactions between the growth substrate and the chilling injury indicators (respiration and ethylene production rates, electrolyte leakage, malondialdehyde), even though a significant interaction existed with the chilling injury index (a water-soaked area). This is believed to be due to the growth substrate's significant interactions with soluble solids and dry matter, which aided cellular balance and increased chilling injury tolerance in perlite and coco-perlite treatment. Weight loss rate and firmness loss were insignificant in all treatments, and cocopeat treatment may be considered the worst of all treatments.

Keywords: chilling injury; cocopeat; coco-perlite; modified atmosphere packaging; perlite

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Introduction

The production of sweet peppers has been increasing significantly over the past decade. FAOSTAT data (FAOSTAT, 2017) showed a periodic increase from 2007-2017 of 14.49 million tons, which was a 48.67% increase over previous level (Sobczak and Sobczak, 2019). More recently, it further increased by 4.13 million tons in 2021 (FAOSTAT, 2021). However, as important as production growth is, postharvest management should be similarly integral. Unfortunately, chilling injuries account for 25-35% of yearly production losses (Afolabi *et al.*, 2023). Aside from storage conditions and fruit cultivars, several variables point to farming conditions being essential in fruit susceptibility (Valenzuela *et al.*, 2017). Now, times are changing, and hydroponics is substantially replacing traditional farming methods. This method involves a soilless culture used to achieve maximum product and good quality in the shortest time possible (Majid *et al.*, 2012). As projected,

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a compound annual growth rate of 20.7% is envisaged between 2021 and 2028 (Velazquez-Gonzalez *et al.*, 2022). This is probably because, in addition to its advantages, it is sustainable and can partially solve the prevalent global warming problem and the generalized earth's pollution issues (Velazquez-Gonzalez *et al.*, 2022). Despite this, the fact that farming methods affect chilling remains and interactions may exist between their growth substrate and chilling injuries.

The plant's nature heavily influences the hydroponic cultivation technique. Drip irrigation is the most commonly used for fruiting vegetables like peppers, which require a substrate. This substrate utilized in supporting the plant's stem additionally interacts with the yield and productivity (Majid *et al.*, 2012) as well as the quality (Afolabi *et al.*, 2022). Hence, this study was designed to compare the effects of three substrates based on some of their uniqueness to determine which may reduce the pepper's chilling injury for future and commercial considerations. The first factor the study considered was something that could fit with the United Nations' 2030 agenda (Velazquez-Gonzalez *et al.*, 2022). That is, something sustainable and organic, for which cocopeat was selected (Islam, 2008). The next was the productivity and high yield aspect, for which a mixture of cocopeat and perlite was, selected (Majid *et al.*, 2012). Finally, for the quality and sugar aspects, perlite was selected (Afolabi *et al.*, 2022). Additionally, sugars may also help prevent chilling injury by assisting with cellular membrane stabilization and acting as reactive oxygen species (ROS) scavengers or osmoprotectants in the ROS balancing process (Valenzuela *et al.*, 2017).

In addition to suitable growth conditions, proper storage methods are also important. Modified atmosphere packaging (MAP) is an effective method for the postharvest handling of perishable produce. For instance, it worked for guava (Murmu and Mishra, 2018), sweet peppers (Afolabi *et al.*, 2022), and some other fruit. Furthermore, it is a promising method for alleviating chilling injury in sensitive produce (Fahmy *et al.*, 2019). This may be due to the presence of CO_2 and high humidity (Afolabi *et al.*, 2023). Aside from that, it is beneficial in the sense that it reduces the respiration rate and slows down ripening (Edusei *et al.*, 2012). Moreover, it was reported to reduce putrescine and abscisic acid levels attributed to the pepper's chilling injury (Serrano *et al.*, 1997; Edusei *et al.*, 2012). Therefore, the study decided to focus on MAP. However, packaging design needs appropriate material selection based on water vapor permeability, the fruit's respiration rate, and transpiration while considering the storage temperature, packaging size, amount of product, and the fruit's optimum O_2 and CO_2 conditions (Mahajanand Lee, 2023). Fruit exposed to levels above their CO_2 may suffer physiological damage (Fahmy *et al.*, 2019), and lower temperature storage is preferable. Considering all this information, packaging film was selected in accordance with Choi *et al.* (2011) report on 20,000 $cc{\cdot}m^{-2}{\cdot}day^{-1}{\cdot}atm^{-1}$ OTR film. Although it does not perform the best overall, it has a lower fungal incidence and may be more cost-effective commercially than 100,000 $cc{\cdot}m^{-2}{\cdot}day^{-1}{\cdot}atm^{-1}$ OTR film, which outperforms it in some areas.

Materials and Methods

Growing conditions and media properties

A plastic greenhouse at Kangwon National University, Republic of Kores was used for the growing phase of the experiment. The drip irrigation technique was used with cocopeat, perlite, and coco-perlite (a mixture of cocopeat and perlite) as substrates, while the mixing ratio for coco-perlite was 50:50. On June 15, 2022, 9-week-old pepper (Nagano, RijkZwaan) seedlings were transplanted. Then the Dutch PBG (Proefstation voor Bloemisterij en Glasgroente/ Research Station for Floriculture and Glasshouse Vegetables) nutrient solution was supplied throughout their growing phase (Afolabi *et al.*, 2022). The greenhouse was equipped with a cooling fan, which helped to maintain the optimum growth temperatures. Furthermore, the substrate properties, such as moisture content, temperature, and electrical conductivity (EC), were measured and shown in Table 1. The moisture content and medium temperature were determined with time domain reflectometry

(TDR), as described by Afolabi *et al.* (2022), while EC and PH were measured with a Hanna HI9813-51 waterproof pH/EC/TDS/°C meter. The PH range was around 5.8-6.0.

Table 1. Properties of the growth substrate on the August 8, 2022

Media property	Substrate		
	Cocopeat	Perlite	Coco-perlite
EC ($mS \cdot cm^{-1}$)	2.3 ± 0.3a	1.7 ± 0.1a	2.0 ± 0.2a
Moisture content (%)	24.6 ± 5.6a	4.8 ±1.5c	13.0 ± 4.5b
Temperature (°C)	25.7 ± 1.8a	26.2 ± 1.8a	25.9 ± 1.8a

The values are represented as ± SE of (n = 5).

Storage conditions, and parameters measured

On September 8, 2022, peppers of nearly similar sizes and uniform red ripeness were harvested and transferred to the laboratory. Before pre-cooling, they were thoroughly inspected for mechanical injuries or other types of defects. Thereafter, they were packed in pairs with 20,000 $cc \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$ OTR film and stored at 5 °C and 85% RH for 50 days. The average weight of the packages was 359.1, 329.4, and 347.4 g for cocopeat, perlite, and coco-perlite, respectively.

The pepper's firmness, soluble solids content, DPPH, vitamin C, malondialdehyde, respiration, and ethylene production rates were measured before storage. During storage, weight loss, visual quality, ethylene, oxygen, and carbon dioxide contents were measured at five-day intervals. On the last day, the same parameters as before storage were remeasured, in addition to the chilling injury index, off-odour, mould, and electrolyte leakage.

Firmness, soluble solids content, dry matter, and weight loss

The pepper's firmness was determined using a rheometer (Compac-100, Sun Scientific Co., Tokyo, Japan) with a probe (8.0 mm) moving at a speed of 1.0 mm/s and a distance of 15 mm.

A pocket refractometer (PAL-1, Atago, Tokyo, Japan) was used to measure the fruits' soluble solid content. By gauze-wrapping the chopped fruit sample pieces, the fruit juice was thrust out and made to drop directly onto the refractometer sensor. The results were displayed and recorded as °Brix.

Approximately 4 g of the pepper samples were weighed before oven drying at 80 °C for 3 days to determine the dry matter. The dried samples were then reweighed and expressed as a percentage using the following formula:

$$\text{Dry matter (\%)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100 \quad (1)$$

The fruits were weighed immediately after packing (harvest weight, HW) and at five-day intervals when they were removed from cold storage (storage weight, SW). The following formula was then used to get the weight loss rate:

$$\text{Weight loss rate (\%)} = (1 - \frac{\text{SW}}{\text{HW}}) \times 100 \quad (2)$$

Chilling injury index and visual quality

The chilling injury and visual quality scores were assigned after visual inspections by a well-trained three-member panel. A four-point rating scale was used for the chilling injury index based on surface pitting and water-soaked areas, and a five-point rating scale was used for the visual quality. The rating score was as follows: for the chilling injury index, 0 points were given to fruits without injury, 1 point for less than 5% injury, 2 points for 5% to 25% injury, 3 points for 26 to 50% injury, and 4 points for more than 50% injury (Afolabi *et al.*, 2023; Yao *et al.*, 2021). In addition, 5 points were given to fruits in the best conditions, 3 points to marketable fruits, and 1 point to fruits in worse conditions for visual quality (Afolabi *et al.*, 2022). The chilling injury index was expressed using the formula below:

$$\mathrm{CI} = \frac{\Sigma\ (\text{CI scale } (0\text{–}4) \times \text{the number of corresponding fruit within each class})}{\text{Total number of fruit estimated}} \qquad (3)$$

Gas production and concentrations

After keeping the fruits at room temperature (20 ± 1 °C) for no less than 4 h, the respiration and ethylene production rates were determined by keeping the fruits in an airtight container (1050 mL) and leaving them for 3–4 h at room temperature (20 ± 1 °C). The Shimadzu GC-2010 gas chromatograph (GC-2010, Shimadzu Corporation, Japan) machine was used to measure the ethylene concentration. The machine was outfitted with a BP 20 wax column (30 m × 0.25 mm × 0.25 μm, SGE analytical science, Australia) and a flame ionization detector (FID). The gas detector and injector were set to 200 °C, the oven to 50 °C, and the gas (N_2) carrier flow rate was 1.76 mL^{-1} min (Afolabi *et al.*, 2022). The respiration rate was measured with a CO_2/O_2 infrared analyser (Model Check Mate 9900, PBI-Dansensor, Ringsted, Denmark). A 1.0 mL gas sample was collected with a syringe (81330, Hamilton, OH, USA) from the headspace of the containers and MAP packages, which was passed through the septum into the GC machine. Also, the carbon dioxide and oxygen content were similarly measured from the headspace of the containers and MAP packages. The corresponding rate and concentrations were then calculated.

Off-odour and mould

The three-member panel evaluated the off-odour by smelling the packages after slightly opening them on the final day. The rating score was based on the presence of a foul smell, and points were given as follows: 5 points for fruits with a good smell, and 1 point for fruits with a foul smell. The mean was then calculated (Wang *et al.*, 2020).

Mould growth analysis was also done by visual observation. Mould's appearance on fruit was graded on a 5-point scale, with 1 indicating absence and 5 indicating severity, according to Murmu and Mishra (2018).

Cell membrane damage

Cell membrane damage was measured using the electrolyte leakage and malondialdehyde methods. The electrolyte leakage was checked according to a previous method (Afolabi et al., 2023). A stainless-steel punch was used to make a disc of uniform thickness and size from the exocarp of the fruit samples, which was then placed in a test tube containing a 0.4 M mannitol (25 mL) solution. The EL was measured using a handheld meter (HI 9813-6 Portable pH/EC/TDS/°C Meter; Hanna Instruments, Padova, Italy) after shaking the mixture for 3 hours on an orbital shaker (SH30) at ambient temperature (20 ± 1 °C). Afterwards, the samples were frozen at −20 °C and thawed at room temperature (20 ± 1 °C) twice, with the results recorded. The EL was determined using the formula below:

$$\text{Relative electrolyte leakage (\%)} = \frac{\mathrm{EL_i}}{\mathrm{EL_f}} \times 100 \qquad (4)$$

Similarly, malondialdehyde was also measured according to Afolabi *et al.* (2023). An amount of 4.0 g of chopped pepper samples were homogenized in 20 mL of 10% trichloroacetic acid before being centrifuged at 5000 × g for 10 min. To 1 mL of supernatant, 3 mL of 0.5% thiobarbituric acid (TBA) previously dissolved in 10% trichloroacetic acid were added. The reaction mixture solution was then heated for 20 minutes at 95 °C, quickly cooled in ice, and then centrifuged for 10 minutes at 10,000 × g to clear the precipitate. The absorbance at 532 nm was subtracted from the non-specific absorbance at 600 nm obtained with a spectrophotometer. The MDA content was expressed in micromoles per kilogram ($\mu M \cdot kg^{-1}$) of fresh weight (FW) using an attenuation value of 155 $m \cdot M^{-1} \cdot cm^{-1}$.

Vitamin C and DPPH measurement

The fruit's vitamin C content was determined by a previously described method (Afolabi *et al.*, 2022) using an RQ flex reflectometer (Merck, Germany). An amount of 2 g of the chopped samples was mixed with 18 mL of distilled water before being homogenized and centrifuged. The instrument was calibrated with a test strip prior to taking readings. Then, a Merck stick was dipped in the mixture for about 2 seconds, and placed in the instrument while the reflectometer was turned on. The vitamin content of the entire fruit was represented by the value on the screen. The following procedure was then used to determine the vitamin C content in 100 g of fruit: the line equation below was then used. The instrumental reading is substituted as y in the line equation, and the corresponding x, representing the vitamin C content in 100 g of fruit, is found.

$$y = 3.9122x - 27.978 \tag{5}$$

A DPPH methanol solution was immediately prepared prior to the measurement of the DPPH. A 0.5 mL aliquot of bell pepper (0.5 g of the bell pepper homogenized with 20 mL of methanol) was added to a 0.5 mL DPPH solution (0.4 mM methanolic solution containing 2,2-diphenyl-1-picrylhydrazyl radicals) (Afolabi *et al.*, 2023). After vigorously agitating the reaction mixture, it was kept in the dark for 30 min. The absorbance of the samples was then measured using a spectrophotometer at 516 nm (Afolabi *et al.*, 2023).

Statistical analysis

The statistical analysis was performed with GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA), and Microsoft Excel 2016. Tukey's multiple comparison test of two-way ANOVA was used to compare the means, and statistical significance was taken at $p < 0.05$.

Results

General characteristics at harvest time

The general characteristics of the fruit at harvest are shown in Table 2. Fruit with uniform characteristics was preferred to limit any effects from differences in the study. Furthermore, fruits of a similar size are more likely to be sorted together during storage, but little interaction still existed with the growth substrates. However, the dry matter was as expected; the perlite treatment had the highest concentrations due to differences in the amount of water available to plants during their growth stage. Then, highly significant differences were observed in the hue. No statistically significant differences were found in fruit appearance in terms of height and width. However, at $p < 0.05$, differences were found in the fruit weights, L* (lightness), a* (redness), and C* (chroma).

Table 2. The general fruit's characteristics at harvest before storage

Fruit characteristics	Substrate			
	Coco-perlite	Perlite	Cocopeat	Treatments
Height (mm)	84.0 ± 1.7a	84.4 ± 1.1a	89.5 ± 0.3a	NS
Width (mm)	74.0 ± 0.3a	74.8 ± 0.6a	76.5 ± 0.9a	NS
Weight (g)	161.4 ± 3.5b	150.3 ± 1.8c	174.1 ± 1.7a	*
Dry matter (%)	7.8 ± 0.3b	8.6 ± 0.2a	7.4 ±0.1c	**
L*	39.6 ± 0.1a	37.8 ± 0.1b	39.3 ± 0.2a	*
a*	33.3 ±0.1a	32.2 ±0.3a	30.8 ± 0.6b	*
C*	36.9 ± 0.3a	34.8 ± 0.3b	35.8 ± 0.6b	*
h°	25.0 ± 0.7b	21.2 ± 0.3c	29.1 ± 0.4a	***

Values are represented as the ± SE of (*n* = 5). Different letters among rows show significant difference among treatments. NS, *, ***: not significant, and significant at p≤ 0.05, 0.01, and 0.001.

Weight loss, firmness, and soluble solids

The stored fruits were packed in pairs with average harvest weights of 359.1, 329.4, and 347.4 g for cocopeat, perlite, and coco-perlite, respectively, which showed no statistical difference. The weight loss rate was insignificant, and no interactions were found with the growth substrate during storage (Figure 1A). However, the perlite treatment had the highest weight loss of all the treatments. Furthermore, no significant relationship existed between the growth substrate and the fruit's firmness at harvest, which was the same after storage (Figure 1B). Although the cocopeat treatment showed a greater loss than the other two treatments, as envisaged before the start of the experiment, interactions existed with the growth substrate and soluble solids; the highest was in the perlite treatment (Figure 1C). The observations following storage were almost identical to those at harvest.

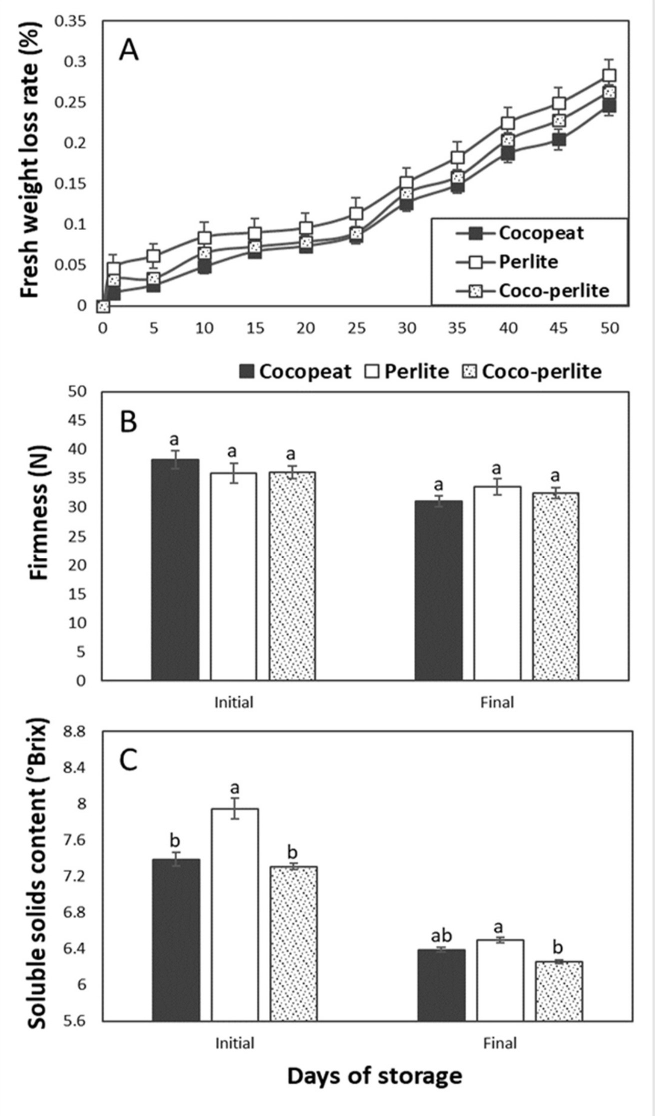

Figure 1. The effect of growth substrate on fresh weight loss (A), firmness (B), and soluble solids (C) of sweet pepper fruits stored at 5 °C using 20,000 $cc{\cdot}m^{-2}{\cdot}day^{-1}{\cdot}atm^{-1}$ OTR film modified atmosphere packaging (MAP) before, during, and on the final day of storage

The vertical bar represents ± SE ($n = 5$), and different letters show a significant difference with Tukey's multiple comparison test at $p < 0.05$.

Respiration and ethylene production rates

The respiration and ethylene production rates showed no interaction at harvest (Figure 2). Although there was no significance at $p < 0.05$ after storage, they both greatly increased for the cocopeat treatment compared to the other treatments. Furthermore, the after-storage values of both the respiration and ethylene production rates for all treatments significantly increased from their initial values. The increase in respiration rate was 3.7 times for cocopeat treatment, 2.7 times for perlite treatment, and 2.9 times for coco-perlite treatment. Similarly, the ethylene production rates increased by 2.4, 2.6, and 2.7 times for cocopeat, perlite, and coco-perlite treatments, respectively.

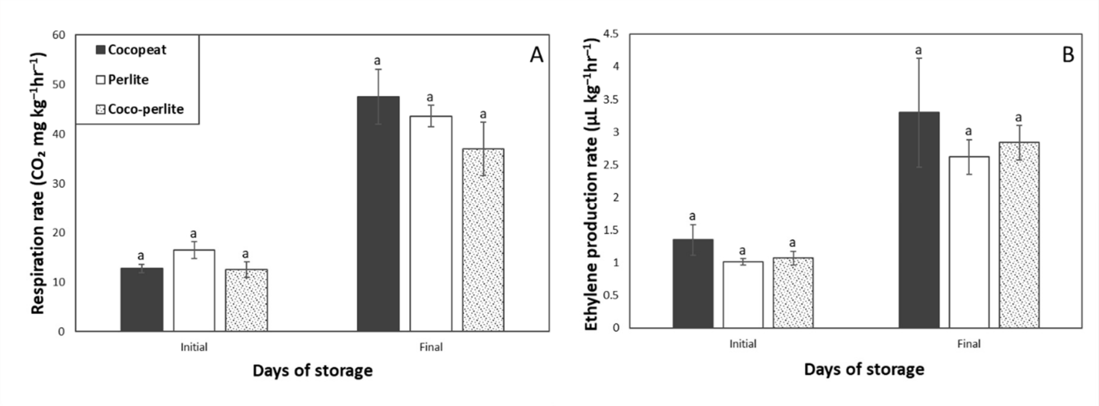

Figure 2. The effect of growth substrate on respiration (A) and ethylene production rates (B) of sweet pepper fruits stored at 5 °C using 20,000cc·m^{-2}·day^{-1}·atm^{-1}OTR film modified atmosphere packaging (MAP) before and on the final day

The vertical bar represents ± SE ($n = 5$), and different letters show a significant difference with Tukey's multiple comparison test at $p < 0.05$.

As shown in Figures 3A and B, CO_2 and O_2 have a reversed relationship in MAP conditions, which is consistent with current knowledge. The cocopeat and coco-perlite treatments maintained an average CO_2 content of less than 5% until day 30 of storage, while the perlite treatment extended until day 40 (Figure 3B). By day 50, all treatments had an average of 11.1± 2.4%, 7.3 ± 1.4%, and 8.5 ± 3.1% CO_2 for cocopeat, perlite, and coco-perlite, respectively, and the O_2 ranges were 8.45 ± 2.7%, 12.3 ± 1.7%, and 11.0 ± 3.5%, in the same order. Similarly, ethylene content varied between 0.92, 0.91, and 1.05 µL·L^{-1} for cocopeat, perlite, and coco-perlite, respectively, on the following day it was packed (Figure 3C), and peaked on the 30th day with values of 2.10, 2.23, and 2.10 µL·L^{-1}, respectively.

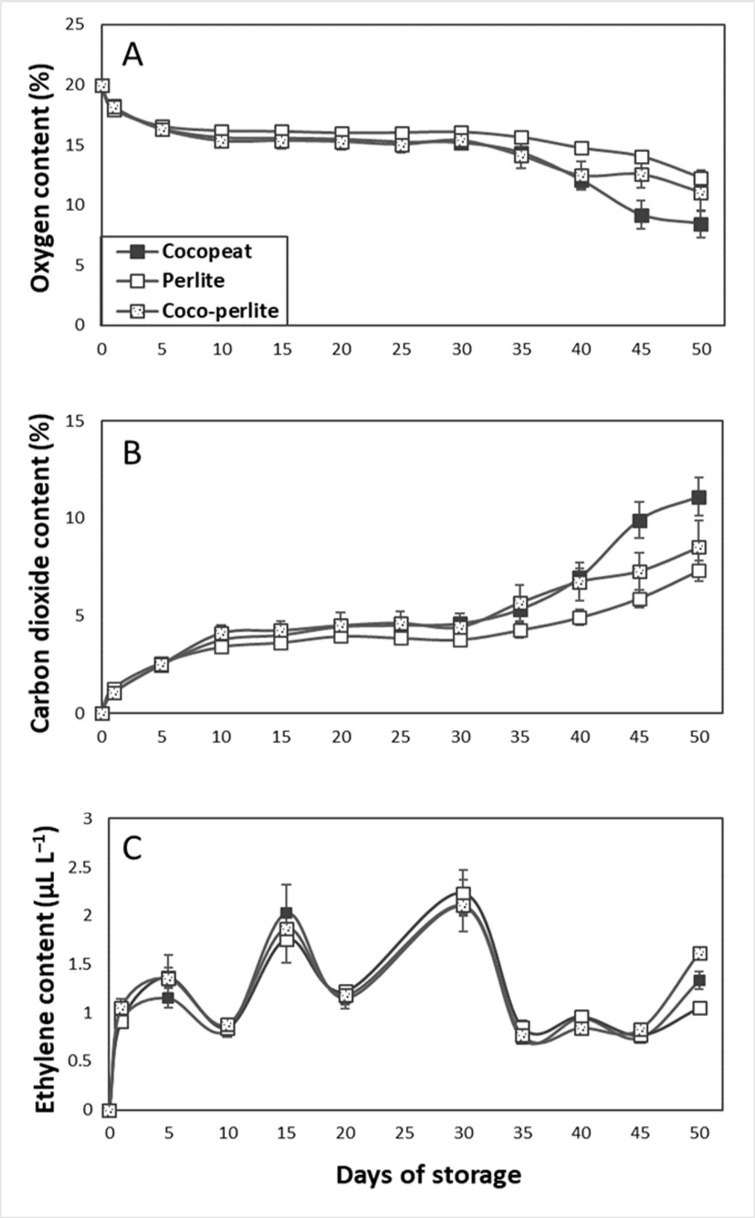

Figure 3. The effect of growth substrate on the oxygen (A), carbon dioxide (B), and ethylene (C) contents of sweet pepper fruits stored at 5 °C using 20,000 $cc{\cdot}m^{-2}{\cdot}day^{-1}{\cdot}atm^{-1}$ OTR film modified atmosphere packaging (MAP) during storage

The vertical bar represents ± SE (n = 5), and different letters show a significant difference with Tukey's multiple comparison test at $p < 0.05$.

Effect of growth substrate on chilling injury and visual quality

As shown in Figures 4 and 5, the growth substrate affected the chilling injury and visual quality. Aside from the water-soaked area, no other typical chilling injury symptoms were observed. The highest water-soaked area was found in the cocopeat treatment (Figures 4A and 5). It was difficult to determine when it started, as chilling injuries require careful observation, but the last day's extent was recorded after unpacking. Furthermore, the worst visual quality was this treatment, with an average score of 2.4 ± 0.4, while the perlite treatment had the overall best average visual quality of 3.4 ± 0.4 (Figure 4B), and cocopeat had 2.8 ± 0.3.

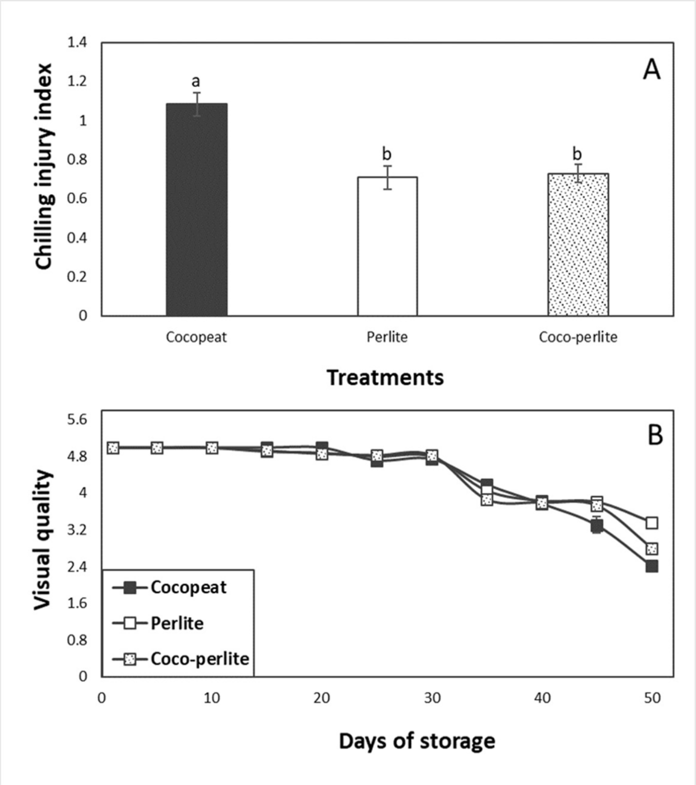

Figure 4. The effect of growth substrate on chilling injury (A) and visual quality (B) of sweet pepper fruits stored at 5 °C using 20,000 cc·m^{-2}·day^{-1}·atm^{-1} OTR film modified atmosphere packaging (MAP) during storage and on the final day

The vertical bar represents ± SE (*n* = 5), and different letters show a significant difference with Tukey's multiple comparison test at $p < 0.05$. NB: Chilling injury scoring was a 4-point rating (0 = no chilling injury; 1 = a chilling injury area less than 5%; 2 = a chilling injury area between 5% and 25%; 3 = a chilling injury area between 26% and 50%; 4 = a chilling injury area greater than 50%).

Figure 5. Photograph of fruits on day 50 after 5 °C storage using 20,000 cc·m^{-2}·day^{-1}·atm^{-1} OTR film modified atmosphere packaging (MAP)

Water-soaked areas are indicated with the grey arrow.

Electrolyte leakage and malondialdehyde (MDA) content as affected by storage

The extent of cell damage was determined through the electrolyte leakage and MDA methods, and the results are shown in Figures 6 and 7, respectively. The coco-perlite treatment had the highest ion leakage, contrary to expectations. Figure 7 shows the existence of interactions between the growth substrate and malondialdehyde at harvest. However, the growth substrate does not affect the fruit's MDA during storage. The cocopeat treatment had the highest MDA before storage; conversely, it had the lowest MDA after storage, even though these differences were insignificant. Perlite and coco-perlite treatments-maintained indifference in both cases. Also, the MDA value was similar to the initial values, except for the coco-perlite treatment, which decreased in value.

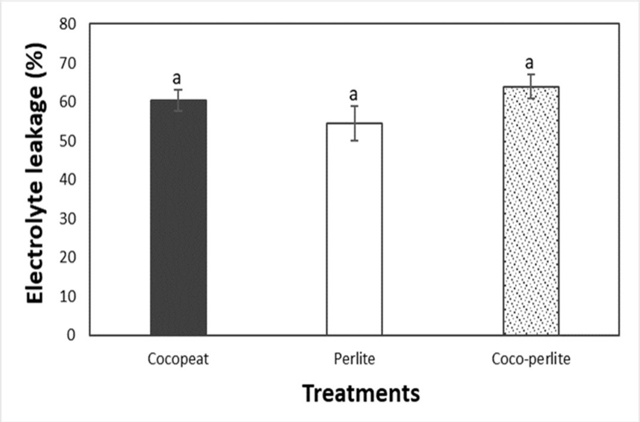

Figure 6. The effect of growth substrate on electrolyte leakage of sweet pepper fruits stored at 5 °C using 20,000 cc·m^{-2}·day^{-1}·atm^{-1} OTR film modified atmosphere packaging (MAP) on the final day

The vertical bar represents ± SE (n = 5), and different letters show a significant difference with Tukey's multiple comparison test at $p < 0.05$.

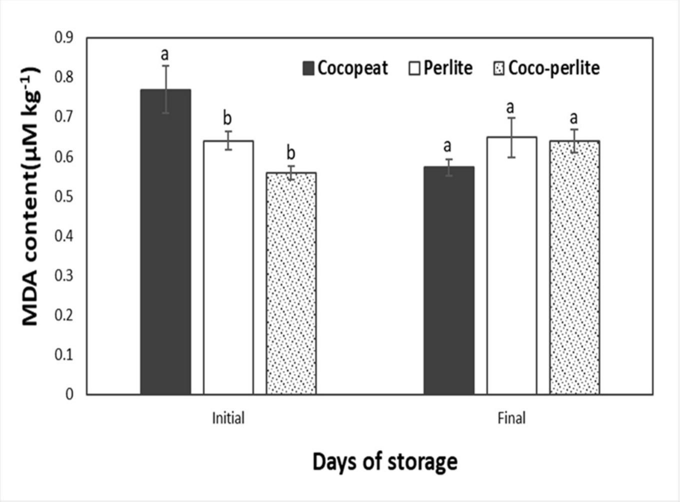

Figure 7. The effect of growth substrate on malondialdehyde (MDA) of sweet pepper fruits stored at 5 °C using 20,000 cc·m^{-2}·day^{-1}·atm^{-1} OTR film modified atmosphere packaging (MAP) during storage and on the final day

The vertical bar represents ± SE (n = 5), and different letters show a significant difference with Tukey's multiple comparison test at $p < 0.05$.

Antioxidant activities

No interaction existed between the growth substrate and DPPH before storage, but interactions were observed after storage (Figure 8A). Although no significance was shown for the cocopeat and perlite treatments at $p \leq 0.05$, the coco-perlite treatment had the lowest level after storage. Vitamin C showed a similar pattern, with the final values being almost as high as the initial vitamin C value. There was no growth substrate interaction with vitamin C at harvest. In contrast, the harvest for the coco-perlite treatment was higher. Additionally, the perlite treatment rose significantly after storage.

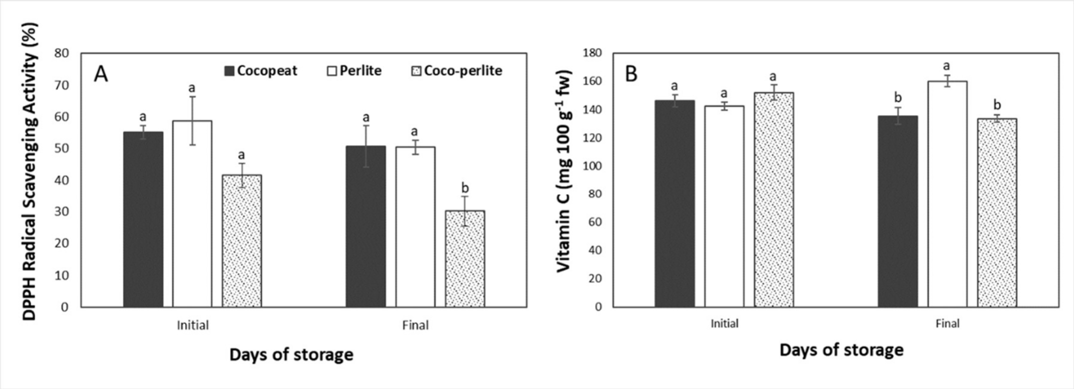

Figure 8. The effect of growth substrate on DPPH radical scavenging activity (A) and vitamin C (B) of sweet pepper fruits stored at 5 °C using 20,000 $cc{\cdot}m^{-2}{\cdot}day^{-1}{\cdot}atm^{-1}$ OTR film modified atmosphere packaging (MAP) during storage and on the final day

The vertical bar represents ± SE ($n = 5$), and different letters show a significant difference with Tukey's multiple comparison test at $p < 0.05$.

Mould and off-odour

The mould and off-odour results are shown in Figure 9. On the final day, significant interactions were observed between the growth substrate and both mould growth and off-odour. Furthermore, the cocopeat treatment had the highest levels of mould and the least off-odour (Figures 8A and B). The level of mould growth for the coco-perlite and perlite treatments was indifferent, while perlite treatments performed better in terms of off-odour despite being significantly indifferent.

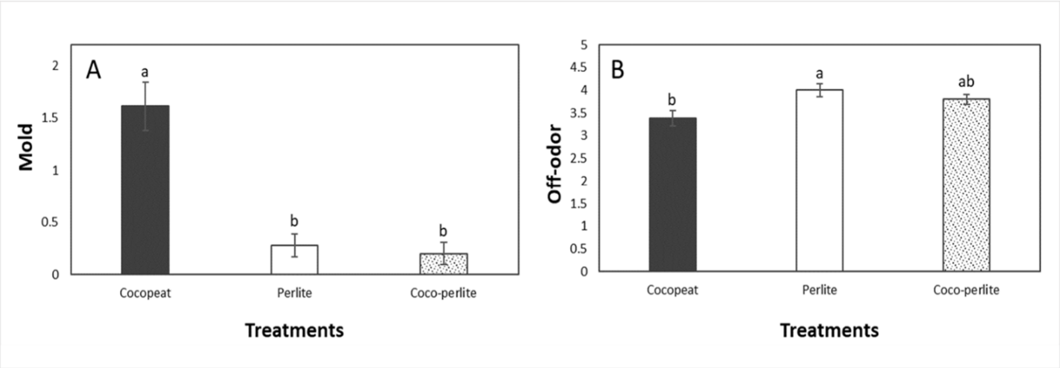

Figure 9. The effect of growth substrate on mould growth (A) and off-odour development (B) of sweet pepper fruits stored at 5 °C using 20,000 $cc{\cdot}m^{-2}{\cdot}day^{-1}{\cdot}atm^{-1}$ OTR film modified atmosphere packaging (MAP) during storage and on the final day

The vertical bar represents ± SE ($n = 5$), and different letters show a significant difference with Tukey's multiple comparison test at $p < 0.05$.

Discussion

Chilling injury is a serious problem when it comes to the postharvest handling of sweet peppers and accounts for 25-35% of the total yearly production losses (Afolabi *et al.*, 2003). The method of a fruit's growth is an important factor that can contribute to its susceptibility (Valenzuela *et al.*, 2017). For that reason, the interactions between hydroponic growth substrates and chilling injuries in sweet peppers were determined in this study. Furthermore, these growth substrates are related to current and future agricultural trends and practices.

The results show no interactions between the growth substrates and the weight of the stored fruits. Although highly significant interactions may exist with the growth substrates, this is because the moisture content of the substrate determines the readily available water for the plant's uptake and absorption. Meanwhile, peppers are considered to be sensitive plants (Afolabi *et al.*, 2022). The highest weights for stored fruits were in cocopeat, followed by coco-perlite, even though fruits were selectively chosen for storage. During storage, no significance was seen among all treatments for weight loss, which may be due to the fruit's similar firmness (Figure 1B), while the higher rate for perlite treatment is likely due to the size factor. This is because a surface area-to-volume ratio relationship exists with water loss for small fruits (Islam *et al.*, 2019). Furthermore, the MAP technique employed was beneficial in the sense that it reduced weight loss and shrinkage during storage and reduced respiration and ripening, thereby extending the shelf life (Edulsei *et al.*, 2012). Additionally, it kept the weight loss rate insignificant, which was far below the 8% permissible weight loss due to the high humidity (Afolabi *et al.*, 2023).

The insignificant differences shown in the fruit's firmness, both at harvest and after storage, are due to their uniformity in size, since it is known that pepper size can affect their firmness (Afolabi *et al.*, 2022). Meanwhile, the firmness reported in this study is lower than what was reported in the previous study (Afolabi *et al.*, 2022), which is likely due to maturation since cell wall softening during fruit maturation reduces firmness (Afolabi *et al.*, 2023). Also, after storage, due to the MAP's high humidity, the firmness was almost as high as the initial level and indifferent among the treatments. Furthermore, the higher losses for the cocopeat treatment are most likely due to their water content, which is not favourable in terms of chilling injuries (Valenzuela *et al.*, 2017; Afolabi *et al.*, 2023).

According to a previous study, the growth substrate interacted with the soluble solids, which was the case in this study. Inadequate availability of water for absorption causes electrolyte concentrations, which increase soluble solids (Afolabi *et al.*, 2022). Meanwhile, soluble sugars play a vital role in the stabilization of the cellular membrane. Furthermore, they are important in the balancing of ROS under stress conditions by acting as osmoprotectants (Valenzuela *et al.*, 2017). This suggests that the high amount of sugar in the perlite treatment may be helpful to protect the fruits from chilling injuries, and vice versa in cocopeat. Then, the soluble solid content decreased due to depletion during respiration.

The lack of interaction with either respiration or ethylene production rates at harvest was due to size and contradicts the previous report. Small-sized fruits have a large surface area, which increases their respiration (Afolabi *et al.*, 2022). In this case, similar-sized fruits were used and would likely have the same surface area. Moreover, perlite treatment had a higher respiration rate at harvest, and the general significant increase in their production rates could be due to cellular stress as a result of the high CO_2 environment. Besides, the higher respiration in the cocopeat treatment suggests that the impact of cold storage was greater, which may be a good indicator of more chilling injury.

The endogenous ethylene level and harvest respiration rate are correlated (Afolabi *et al.*, 2023), which explains the lack of a significant difference in this study (Afolabi *et al.*, 2022). This further suggests that the fruit size could be primarily responsible for the interactions. Due to the high CO_2 environment, the ethylene production rate increased after storage (Figure 2B). This is because CO_2 above tolerance can trigger an increase in ethylene production (Serrano *et al.*, 1997). The highest ethylene production was in the cocopeat treatment, which confirms more oxidative stress in this treatment. Also, ethylene production is a good modulator of chilling injury (Valenzuela *et al.*, 2017).

The changes in CO_2 and O_2 content were due to the retention of the respiration rate (Edusei *et al.*, 2012). Peppers have a 5% maximum CO_2 tolerance level in MAP conditions (Afolabi *et al.*, 2022), and levels higher than that can cause physiological damage (Fahmy et al., 2019). All treatments exceeded that level before day 50, which could explain some of the unexpected results (Serrano et al., 1997). Contrary to the previous report (Afolabi *et al.*, 2022), this result confirms that lowering the storage temperature reduces the fruit's respiration rate. The results reveal that fruit from all treatments may be storable for 30 days at 5 °C, and fruits from perlite treatment may last a little longer. In addition, the presence of more CO_2 likely reduced the ethylene content of coco-perlite and cocopeat, as well as the general decline in ethylene content after the peak. This is because O_2 is involved in the conversion of 1-amino-cyclopropane-1-carbonxylic acid to ethylene, and the presence of low O_2 restricts ethylene formation (Chitravathi *et al.*, 2015).

Aside from the water-soaked area, none of the other common symptoms of pepper chilling injury, such as surface pitting, decay, and calyx browning, were typically observed (Valenzuela *et al.*, 2017; Yao *et al.*, 2021). Other symptoms were likely prevented due to the presence of CO_2. MAP technology has been shown to protect sensitive fruits from chilling injuries. Aside from this result, it was reported to help alleviate chilling injuries in persimmon, melon, mango, and peach (Fahmy *et al.*, 2019). The differences in the chilling injuries are thought to be due to the soluble solids and the dry matter (Table 2 and Figure 1C).

The best average overall visual quality score was 3.4 ± 0.4 and was for perlite treatment (Figure 4B). This implies that fruits were marketable, while the cocopeat and coco-perlite treatments had 2.4 ± 0.4 and 2.8 ± 0.3, respectively, and may be unmarketable. The reason for these differences is due to the fruit's respiration rate. The average CO_2 content was less than 5% up until day 30, which extended up until day 40 for perlite treatment. As previously stated, higher levels of CO_2 above the fruit's tolerance limit cause physiological damage to fruits (Fahmy *et al.*, 2019), from which sweet peppers are not exempt (Afolabi *et al.*, 2022). This finding also highlights the significance of the fruit storage temperature under MAP conditions. Contrary to the previous study, due to the storage temperature, the rapid rise of CO_2 was delayed in this study, which enhanced the fruit's storability compared to a previous report (Afolabi *et al.*, 2022). A proper equilibrium in the atmosphere ensures that respiration, senescence, and deterioration rates are slow (Mahajan and Lee, 2023). The lowest CO_2 level of 7.3 ± 1.3 may also have favored the visual quality observed for perlite treatments.

Electrolyte leakage is a reliable and common way to check the extent of cell membrane damage. Chilling injury disrupts the metabolic activity of the cell, affecting the enzymatic activity of membrane lipids as well as protein synthesis and degradation (Edusei *et al.*, 2012). The results of this study negate this (Figure 5). This is probably due to the fruit part used for the measurement or the initial electrolyte content of the treatment. There are high chances of this treatment having the lowest electrolyte level before storage. Other studies have shown an increase in electrolyte leakage for chilling injury alongside water-soaked areas, which a study reported to be due to increased solute leakage, pectin polymer degradation, cell separation, and loss of cell wall rigidity for watermelon (Mao *et al.*, 2004). Moreover, it appears to be lowest in the perlite treatment due to the soluble sugars that act as osmoprotectants in the stabilization of ROS (Valenzuela *et al.*, 2017).

Even though there was a significant difference before storage, malondialdehyde levels between the cocopeat and the other treatments were not different after storage. This significance was due to the soluble sugars. The pattern of this result was similar to Zhang *et al.* (2020) report on peaches. Fruits with higher sugar content had lower MDA at harvest, whereas fruits with lower sugar content had a lower MDA after storage. MDA is a by-product of lipid oxidation in the cell membrane, and its content in fruit has been utilized to assess the extent of CI under chilling stress.

Interactions did not exist between the growth substrate and DPPH antioxidant activity before storage, but they did after. This is similar to the results obtained by Aslani *et al.* (2016) for harvesting DPPH and indicates that the growth substrate may not have significant interactions with DPPH. After storage, interaction existed, with perlite showing the highest value, and high values of DPPH are associated with chilling injury tolerance (Afolabi *et al*., 2023). This was the case in this study, where perlite treatment, which had the highest DPPH, also showed the least chilling injuries. In agreement with our previous study, which found no significant difference in vitamin C levels between fruits grown on perlite and cocopeat substrates, this study also found no interactions with the growth substrate. However, vitamin C content was higher for perlite treatment after storage, probably due to reversed oxidation (Afolabi *et al*., 2022). The high content of vitamin C in cocopeat treatment is also thought to be due to its high EC (Table 1). A study has shown that an increase in EC could be used to improve the fruit's taste and increase its vitamin C content (Aslani *et al*., 2016).

The presence of mould and an off-odour were believed to be reduced and delayed due to the presence of CO_2 (Cantwell and Suslow, 1999). The cocopeat treatment had the highest levels in both cases (Figures 9A and B). The greater mould growth was due to the higher moisture content of the fruits, while mould growth is generally believed to be due to the high MAP humidity. The highest average harvest weight was for this treatment, and water accounts for about 95.47% of the pepper's total weight (Guiné and Barroca, 2011). Mould growth is triggered and thrives in environments with high humidity, with produce with a high-water content encouraging it. Similarly, odours are due to fermentation, especially with the cocopeat treatment. Although the presence of CO_2, as mentioned, was initially beneficial in delaying this, the lower O_2 content toward the end of the experiment promoted odour development, likely due to fermentation. Fermentation is initiated in a CO_2-rich environment, resulting in aroma biosynthesis and off-flavour possibilities even though they were not focused on (Fahmy *et al*., 2019). Moreover, anaerobic metabolism is reported to occur in the absence of oxygen, resulting in off-odours and other quality issues (Cantwell and Suslow, 1999). Additionally, the water-soaked area and mould may have contributed to the odour of the cocopeat treatment.

Conclusions

The experiment evaluated the interactions between the chilling injury symptoms of sweet pepper and their growth substrates. The findings show that using cocopeat, perlite, or coco-perlite as a growth substrate does not significantly affect the chilling injury of sweet peppers. However, the fruit's sugar content, aided by some growth substrate, helps maintain cellular balance. Even though chilling indicator indexes were somewhat indifferent, perlite treatment proved to be the best in this study. Its higher sugar content enhanced its tolerance to chilling injuries, improving its visual quality. In addition, the higher water content in the cocopeat treatment increased its susceptibility to water soaking, mould growth, and the off-odour. The MAP technology is helpful in delaying the chilling injury index.

Authors' Contributions

Conceptualization and methodology, A.S.A., I.-L.C., and H.-M.K.; Experiment performance and data curation, A.S.A., J.H.L., Y.B.K., and I.-L.C.; software and formal analysis, A.S.A., Y.B.K., J.H.L., and I.-L.C.; resources, H.-M.K.; original draft preparation, A.S.A., I.-L.C., J.W.L., Y.B.K., and H.-M.K.; review and editing, A.S.A., I.-L.C., H.S.Y., and H.-M.K.; supervision, I.-LC. and H.-M.K.; project administration, A.S.A.; funding acquisition, I.-L.C. and H.-M.K. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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