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High-Relative-Humidity Storage Reduces the Chilling Injury Symptoms of Red Sweet Peppers in the Breaker Stage

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Abstract: Water loss, interwoven with other factors, is identified as the cause of chilling injury to sweet peppers. The breaker stage is the most vulnerable of all maturity stages. The purpose of this study was to see if increasing the storage relative humidity (RH) reduces the chilling injury and to compare its effect on breaker-stage fruits to red-ripe fruits at a normal RH. The fruits were stored at 5 °C with a RH of 98 ± 2% and 70 ± 6% for high and low RH, respectively. After 15 days of cold storage, the fruits were moved to ambient conditions for 3–5 days for chilling injury symptoms to appear. The results showed that high RH storage reduced fruit water loss by 4–4.5% compared to low RH storage, resulting in fewer chilling injury symptoms regardless of fruit maturity stage. Due to the increased RH, cell membrane damage indicators such as electrolyte leakage, MDA, respiration, and ethylene production rates were shown to be reduced, while brix and color were well maintained, indicating reduced or stopped senescence. Furthermore, DPPH antioxidant activity and vitamin C were retained and optimized. The microbiological analyses also showed that a high RH may not promote the growth of microorganisms as quickly as may have been thought. Finally, the findings of this study indicate that breaker-stage peppers stored at a high RH may be less susceptible to chilling injury than red-ripe peppers stored at a low RH.

Keywords: breaker stage; cold storage; high RH; low RH; red-ripe

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1. Introduction

Sweet peppers (*Capsicum annuum* L.) are one of the most economically significant fruit vegetables, widely cultivated due to their nutritional value, taste, and color [1]. However, these peppers have a somewhat short shelf life when stored at ambient temperatures due to postharvest water loss. According to Maalekuu [2], postharvest water loss is the main physiological factor that affects their quality and shelf life. Cold storage, on the contrary, has been shown to be an effective method as it reduces the vapor pressure deficit [3]. However, Lim [1] similarly identified water loss as the cause of chilling injury (CI), as water exchange through the skin requires cellular disintegration, deterioration of membrane integrity, and loss of epicuticular wax. This type of injury tends to be more common for peppers stored at temperatures lower than 7 °C [4], with typical symptoms including surface pitting, shriveling due to water loss, and calyx browning [1]. Babellahi [4] estimated that this accounts for 25–35% of yearly production losses. Alleviating this disorder has been a serious problem, and at higher temperatures, water loss rates, respiration rates, ripening, and senescence processes increase faster, which eventually shorten the shelf life.

Several attempts have been made to lessen the severity of this damage. Intermittent warming [5], UV-C treatments [6], hot water treatments [7], and treatments with plant growth regulators such as methyl jasmonate [8], brassinolide [9], and diphenylamine [10] have all been used. Despite the fact that these various methods have been shown to

prevent CI, they are either expensive, require technical know-how, may have negative effects from their accumulation on the fruits, or, with the ever-increasing demand for fruits with zero compounds, treatment may be a limitation. Even though modified atmosphere packaging (MAP) has been found to minimize the increase in putrescine and abscisic acid levels attributed to the CI of peppers [11], it may be impossible to control the gas levels within the packages, affecting the fruit's quality and storability. In a previous report, decay and off-flavor development associated with some MAP films continued to be some of the impediments to their commercial acceptance [12]. This shows the importance of additional efforts.

In studies of chilled sensitive fruits, high relative humidity (RH) has been found to be effective in reducing the extent of chilling damage in zucchini [13], cucumbers [14], navel oranges [13], and lemons [13]. Meng [15] also discovered that it significantly reduces the weight loss rates in blooming cabbages, milk cabbages, papayas, and guavas when compared to fruits stored in a typical cold room without a dry fog-humidifier regulator. Even though excessive RH and subsequent water condensation can increase the risk of fruit rot [16], peppers are recommended to be kept at a RH of 90–95%. When Nunes [17] compared various humidity levels, they established that a 95% RH exhibits the least decay, the highest soluble solids, and the best quality curves. This implies its capability of improving CI tolerance at chilling temperatures. However, attempts to determine the outcome of its effect on sweet peppers have not been made. Previous studies have also shown that fruits in the breaker maturity stage are more sensitive to CI than in the mature green- and red-ripe stages, owing to higher water loss [1]. As a result, this study specifically compared the effects of high RH storage on the CI of breaker-stage fruits to that of red-ripe fruits at a normal cold storage RH (low RH).

2. Materials and Methods

2.1. Fruit Materials and Storage Conditions

The Nagno (Rijk Zwaan) red sweet pepper cultivar was used, and the fruits were harvested from a greenhouse at Kangwon National University. They weighed 227.7 ± 14.7 g and had a diameter of 83.9 ± 5.4 mm and a height of 105.7 ± 5.6 mm for the red-ripe ones, while the fruits in the breaker stage weighed 214.7 g and had a diameter of 80.73 ± 5.4 mm and a height of 97.3 ± 8.8 mm. The breaker stage was classified based on the formation of an incipient red color [1]. When the fruits arrived at the laboratory, they were thoroughly inspected for field damage and randomly categorized into two categories for both groups: One representing high RH and the other low RH. Each sample contained 10 fruits in a carton box, with high RH storage carried out in a humidified refrigerator with a RH of 98 ± 2% and low RH storage in a regular refrigerator with a RH of 70 ± 6%. The temperature of both refrigerators was kept at 5 °C, while the RH changes were monitored by an automated digital hygrometer thermometer humidity meter (UT330B Humidity/Temperature Data Logger).

The fruits were stored for 15 days before being moved to ambient conditions for another three to five days for chilling symptoms to develop. The weight loss rate, CI index, calyx browning index, and color were measured at a five-day interval during cold storage and checked on the third and fifth days of ambient storage. The respiration and ethylene production rates were measured before and after cold storage and on the third and fifth days of ambient storage. Electrolyte leakage and malondialdehyde were measured before storage and on the third and fifth days of ambient storage, because they require that such fruits be destroyed, and fruit quality as indicated soluble solids, vitamin C, DPPH, and firmness was measured before and on the last day. These parameters were focused on because they were shown to be good indicators of chilling damage [1,9,13]. Microbial analysis was also carried out to check how fast the humidity promoted the growth of microorganisms.

2.2. Weight Loss, Chilling Injury (CI) Index and Calyx Browning Index

According to Zuo [13], the fresh weight loss rate was calculated using the formula below:

$$\text{Weight loss rate } (\%) = \frac{\text{Initial fresh weight} - \text{Final fresh weight}}{\text{Initial fresh weight}} \times 100\% \quad (1)$$

The CI index was measured by visual observation by a three-member panel in reference to Zuo [13]. A four-point rating scale was used to score the fruits based on their level of surface pitting (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%). After this, the following formula was used to determine the CI index:

$$\text{CI} = \frac{\Sigma\ (\text{CI scale } (0\text{–}4) \times \text{the number of corresponding fruit within each class})}{\text{Total number of fruit estimated}} \quad (2)$$

Similar to the CI index, the calyx browning index was also measured by visual observation in reference to Li [18]. A five-point scale was used based on color changes of the calyxes from green to brown (1 = green; 2 = slightly yellow; 3 = moderately yellow; 4 = totally yellow; 5 = brown). The mean score was then calculated for all of the fruits in each sample.

2.3. Respiration and Ethylene Production Rates

After keeping five fruits in an airtight container (1050 mL) and leaving them at ambient conditions for 3 h, a syringe was used to collect 1.0 mL of the gas sample (81330, Hamilton, OH, USA), which was passed into a GC machine through its septum to measure the ethylene production rate, while the fruits' respiration rate was measured with an infrared CO_2/O_2 analyzer (Model Check Mate 9900, PBI-Dansensor, Ringsted, Denmark), and the corresponding respiration and ethylene production rates were then determined. The Shimadzu GC-2010 gas chromatograph (GC-2010, Shimadzu Corporation, Tokyo, Japan) had a BP 20 Wax column (30 m × 0.25 mm × 0.25 um, SGE Analytical Science, Ringwood, Australia) and a flame ionization detector (FID). The detector and injector were set to 127 °C, while the oven was at 50 °C, and the flow rate of the carrier gas (N_2) was 0.67 mL·s^{-1}.

2.4. Electrolyte Leakage and Malondialdehyde Content (MDA)

Electrolyte leakage (EL) was determined in relation to Wang's [19] guidelines. A disc of uniform thickness and size was made from the exocarp of the fruit samples by a stainless-steel puncher and was submerged in a test tube containing a 0.4 M mannitol (25 mL) solution. After shaking the mixture for 3 h on an orbital shaker (SH30) at ambient temperature (20 ± 1 °C), the EL was measured using a handheld meter (HI 9813-6 Portable pH/EC/TDS/°C Meter; Hanna Instruments, Padova, Italy). Subsequently, the samples were frozen at −20 °C and thawed at ambient temperature; this was carried out three times, and the values were recorded. The EL was calculated using the following formula:

$$\text{Relative electrolyte leakage } (\text{EL}) = \frac{\text{EL}_\text{i}}{\text{EL}_\text{f}} \times 100\% \quad (3)$$

where EL_i means initial electrolyte leakage, while EL_f means final electrolyte leakage

According to Wang [9], the malondialdehyde was measured using the thiobarbituric acid reactive substance (TBARS). 20 mL of 10% trichloroacetic acid were used to homogenize the 4.0 g of pulp tissue from 10 pepper fruits, which were subsequently centrifuged at 5000× *g* for 10 min. 3 mL of 0.5% thiobarbituric acid (TBA) that had previously been dissolved in 10% trichloroacetic acid were added to 1 mL of the supernatant. To clarify the precipitation, the reaction mixture solution was heated for 20 min at 95 °C, quickly cooled, and centrifuged for 10 min at 10,000× *g*. The non-specific absorbance at 600 nm was measured, and the absorbance at 532 nm was removed. Using an attenuation value

of 155 Mm^{-1} cm^{-1}, the quantity of MDA was estimated as micro mole per kilogram (μM kg^{-1}) of fresh weight (FW).

2.5. Color Change

A colorimeter (Color Reader CR-20) was used to obtain the red/green coordinate (a*) and hue angle (h°) modes of the fruit samples. Ten fruits were used as an initial replicate, but the number decreased to five on the last day because the measuring of MDA and electrolyte leakage required the destruction of some fruits.

2.6. Soluble Solids Content and Firmness

A pocket refractometer was used to determine the fruits' soluble solid content (PAL-1, Atago, Tokyo, Japan). Fruit juice was thrust out and made to drop directly onto the refractometer sensor by gauze-wrapping the chopped fruit sample pieces, and the results were shown as brix.

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

2.7. DPPH Radical Scavenging Activity and Vitamin C

The free radical scavenging activity of volatile oils and non-volatile extracts was determined using guidelines by Oboh [20]. Before the assay, a solution of DPPH methanol solution was immediately prepared. A 0.5 mL aliquot of a 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). The reaction mixture was kept in the dark for 30 min after being powerfully agitated. The samples' absorbance was then measured with a spectrophotometer at 516 nm. In order to validate the assay, methyl alcohol was used as a standard antioxidant. For accuracy's sake, the experiment was repeated three times.

The vitamin C content of the fruits was measured with reference to Afolabi [21]. The RQ-flex method was used, in which 18 mL of distilled water was mixed with 2 g of the chopped pepper sample, homogenized, and centrifuged in a test tube. Before taking readings, the instrument was calibrated with a test strip. Then, a Merck stick was dipped in the mixture for around 2 s and put in the instrument after 10 s while the reflectometer was on. The total vitamin C present in the sample then came up on the screen. This method was then used to calculate the vitamin C content of 100 g of fruit. As established from the reference, the line equation below was used. The x was replaced with the readings from the instrument, and the result shows the amount of vitamin C present in 100 g of fruit.

$$y = 3.9122x - 27.978 \quad (4)$$

2.8. Microbiological Analysis

Microbial analysis was conducted according to Wang [19]. Astomacher (Powermixer, B&F Korea, Gimpo-si, Republic of Korea) set at the highest speed (level 10, 200 rpm) was used to mix approximately 2.0 g of a pepper sample with 18 mL of diluent (sterile water) for 3 min. The mixture was then diluted, and 1 mL of the dilution was put onto 3 M microbiological count plate petrifilms (3M Co., St. Paul, MN, USA). The aerobic bacteria were then grown for 24–72 h at 35 °C, whereas yeast and molds were grown at 25 °C. The total aerobic bacterial growth, yeast, and molds were measured with a Petrifilm Plate Reader (3M Co., ST. Pau, MN, USA). Microorganism concentrations were reported in log colony-forming units (CFU).g^{-1}.

2.9. Statistical Analysis

GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) and Microsoft Excel 2016 were used to analyze the data. Each sample was replicated three or five times. The

means were compared using Tukey's multiple comparison test of two-way ANOVA, and statistical significance was considered at $p < 0.05$.

3. Results and Discussion

3.1. Fruit Water Loss Rate, Chilling Injury Index and Calyx Browning Index

Due to the high RH storage, it was discovered that the water loss rate, CI index, and calyx browning index were significantly lower for fruits stored at a high RH regardless of their maturity stages. Specifically, they were lower for breaker-stage fruits at a high RH than red-ripe fruits at a low RH for each sampling day (Figure 1). The results show that fruits at the same RH had nearly the same water loss rate as storage days passed (Figure 1A), contrary to what was formerly reported for breaker-stage fruits [1], and even appeared to be higher for red-ripe fruits at a low RH. The cause of this occurrence is most likely due to the fruits' firmness, as breaker-stage fruits were much firmer before storage (Table 1), and fruit firmness has been shown to affect water loss rates during storage on several occasions. Even though this observation was previously reported in another similarly designed study, the cause of this occurrence was not pinpointed, but fruit firmness was shown to be higher for breaker-stage sweet peppers [22]. Water loss control is important for sweet peppers, and previous studies have shown that peppers have a tolerable water loss rate of 8% during storage [21]. Our results show that high RH storage could be helpful in delaying this.

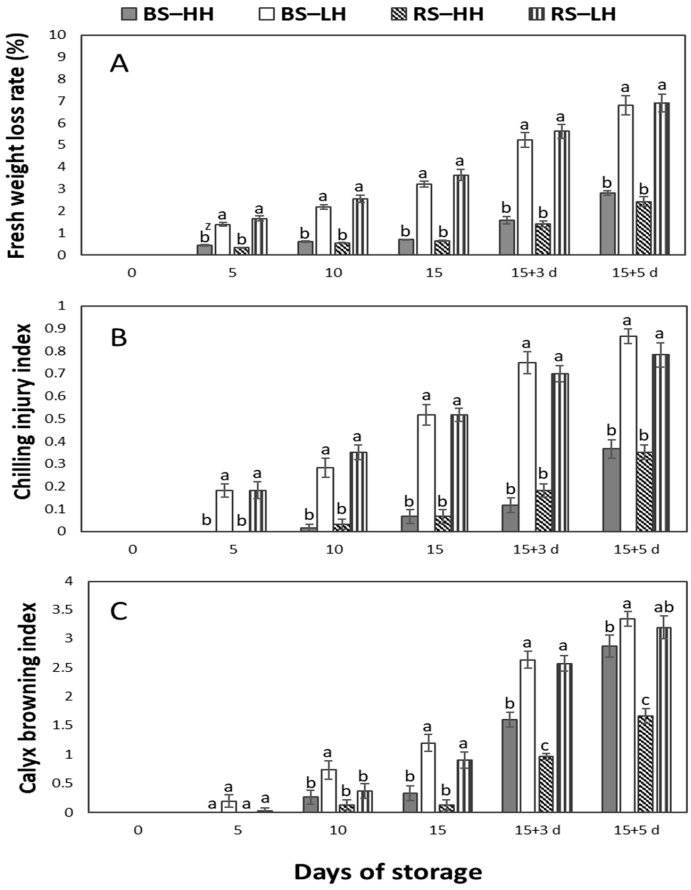

Figure 1. High and low RH effects on sweet peppers' weight loss (**A**), chilling injury (CI) index (**B**), and calyx browning index (**C**) during 15 days of cold storage at 5 °C and 5 days at ambient conditions. HH means fruit kept in a humidified refrigerator, and LH means fruit kept in a regular refrigerator, while BS (breaker stage) and RS (red-ripe) represent maturity stages. Vertical bars represent ± SE of the mean ($n = 5$). ^{Z} Different letters represent significant differences among treatments for each sampling day using Tukey's multiple comparison test at $p < 0.05$. Note: 15 + 3 d means the third day in ambient conditions, and 15 + 5 d means the fifth day in ambient conditions.

Table 1. The effect of high and low RH on the soluble solids content and firmness of sweet peppers before and after 15 days of cold storage at 5 °C and 5 days at ambient conditions.

Maturity Stage	Storage Conditions	Firmness (*n*)		Soluble Solids Content (°Brix)	
		Initial	Final	Initial	Final
BS	HH	50.17 ± 2.70 a [Z]	43.80 ± 1.07 a	6.84 ± 0.02 b	7.06 ± 0.06 c
	LH		39.52 ± 0.67 ab		7.08 ± 0.06 c
RS	HH	38.80 ± 0.66 b	38.52 ± 1.36 ab	7.48 ± 0.04 a	7.40± 0.03 b
	LH		36.11 ± 1.24 b		8.26 ± 0.27 a
Maturity stage (A)		***	***	***	***
Storage conditions (B)		NS	***	NS	***
A × B		NS	*	NS	***

[Z] Values are shown as the mean ± SE (n = 5), and different letters represent significant differences among treatments for each sampling day using Tukey's multiple comparison test. NS, *, ***: not significant, $p \leq 0.05$, 0.01, and 0.001.

Water loss affects peppers in several ways, which this study similarly confirms, as it greatly contributed to the chilling injury observed. Lim [1] previously showed the process at low temperatures to be a sign of CI because it involves cellular disintegration, membrane integrity deterioration, and the loss of epicuticular wax that allows water exchange to occur through the fruits' skin. Another study further supported this by also showing it involves carbohydrate breakdown, whereby water is produced alongside carbon dioxide during respiration [23]. As a result of the higher water loss rate for low RH-stored fruits, CI began to show starting from Day 5 of storage (Figure 1B), whereas high RH-stored fruits matched this rate after the 18th day of storage with indifference, regardless of the maturity stages for each sampling time. Moreover, no significant difference was found for fruits at similar humidities until the end of the experiment, and the injury severity increased over time for all fruits, with temperature stress becoming more visible at ambient conditions, where fruits that appeared to be healthy during cold storage developed typical CI symptoms. This result agrees with the positive correlation found between water loss and CI [13] and also shows CI as being highly dependent on the amount of fruit water loss during storage and not the stage of ripeness.

Similarly, high RH storage was shown to significantly reduce the calyx browning, as the difference became noticeable on the 10th day of storage, with the highest in the breaker stage at a low RH and the lowest in the red-ripe stage at a high RH on the last day (Figures 1C and 2). This is believed to be a result of the water loss, as a somewhat causal relationship between water loss and calyx browning existed. A previous study showed calyx as an important route for water loss [24], and browning was further concluded to probably be a result of surface growth stress in the calyx [25].

3.2. Storage Humidity Effect on Soluble Solids Content and Firmness of the Fruit

The initial soluble solid values differed significantly between the two maturation stages, as envisaged (Table 1), due to the conversion of starch to sugar during ripening. After storage, brix significantly increased only in the red-ripe fruits stored at a low RH, without any corresponding increase in the breaker stage at a low RH. The etiology of this is not fully understood, but the hypothesis for this significant increase is due to the dilution effect. Water loss in fruits sometimes results in a concentration of solutes, thereby increasing the fruits' total soluble solids, which is supported by another study's results [26]. It is possible that other factors prevented it from increasing in breaker-stage fruit.

Figure 2. Fruit's overhead image on the final day after 15 days of cold storage at 5 °C and 5 days at ambient conditions. HH means fruit kept in a humidified refrigerator, and LH means fruit kept in a regular refrigerator, while BS (breaker stage) and RS (red-ripe) represent maturity stages.

Firmness was higher before storage in the breaker stage (Table 1), as firmness showed a decline during maturation for sweet peppers due to cell wall softening with aging [22]. Firmness declined on the last day of storage for all fruits, with it being the highest in the breaker stage at a high RH. This general decline in firmness is certainly due to fruit water loss during storage. A link has already been established between firmness and water loss [21]. However, the higher firmness loss for low RH fruit is due to poor storage conditions, and as shown by Valenzuela [27], firmness loss at low temperatures is a good sign of CI. This same trend was reported for cucumbers [14] and zucchini [13] stored under similar conditions.

3.3. Effect of Storage Humidity on Fruit Respiration and Ethylene Production Rates

In accordance with Lim [1], the respiration and ethylene production rates for both maturity stages showed no significant differences before storage (Table 2). However, significant differences appeared after storage, with the ethylene decreasing more than the initial value at each sampling time after cold storage for all fruits, similarly in agreement with Lim [1], while the respiration rate increased during cold storage and decreases at ambient conditions. In contrast to what Lim [1] observed for higher respiration and ethylene production rates for breaker-stage fruits, no highly significant differences were shown in this study. They do, however, appear lower in the red-ripe stage and better for high RH-stored fruits. High respiration and ethylene production rates have been shown to be good signs of chilling damage before a visible, irreversible symptom appears, as high respiration also causes the accumulation of aberrant compounds such as malondialdehyde (MDA) in fruit tissue [27]. The production of these gases has been shown to be induced at low temperatures and to coincide with the degree of damage that occurs to fruits when they are transported to ambient conditions [27]. Water loss in sweet peppers contributes to the production of these gases [27], which may therefore be responsible for the source of the increase for fruits kept at a low RH, particularly on the last day of the experiment. Although fruits kept at a high RH initially showed high gas rates, which may also have resulted from external stress from high humidity because stress also contributes to gas production rates, these gas rates decreased at ambient conditions.

Table 2. High and low RH effects on the sweet pepper's respiration and ethylene production rates before, after, and during 5 days of ambient conditions after 15 days of cold storage at 5 °C.

Maturity	Storage Conditions	Respiration Rate (CO_2 mg kg^{-1} h^{-1})				Ethylene Production Rate (μL kg^{-1} h^{-1})h			
		0	15	15 + 3 d	15 + 5 d	0	15	15 + 3 d	15 + 5 d
BS	HH	9.52 ± 0.68 a [Z]	14.18 ± 0.40 a	7.87 ± 0.28 a	3.70 ± 0.30 a	2.37 ± 0.12 a	0.90 ± 0.07 a	0.53 ± 0.03 a	0.66 ± 0.10 a
	LH		11.24 ± 0.90 b	7.08 ± 0.44 a	4.82 ± 1.61 a		1.15 ± 0.12 ab	0.68 ± 0.07 a	0.76 ± 0.04 a
RS	HH	8.34 ± 0.76 a	9.63 ± 1.21 b	6.03 ± 0.03 a	2.30 ± 0.30 b	2.21 ± 0.09 a	0.87 ± 0.08 ab	0.67 ± 0.05 a	0.55 ± 0.03 a
	LH		9.84 ± 0.51 b	8.19 ± 0.58 a	3.71 ± 0.12 a		0.73 ± 0.03 b	0.63 ± 0.03 a	0.67 ± 0.06 a
Maturity stage (A)		NS	***	NS	*	NS	**	NS	NS
Storage conditions (B)		NS	***	*	***	NS	***	***	***
A × B		NS	*	*	*	NS	NS	NS	NS

[Z] Values are shown as the mean ± SE (n = 3), and different letters within columns represent significant differences among treatments for each sampling day using Tukey's multiple comparison test. NS, *, **, ***: not significant, $p \leq 0.05$, 0.01, and 0.001. Note: 15 + 3 d means the third day in ambient conditions, and 15 + 5 d means the fifth day in ambient conditions.

3.4. Storage Humidity Effect on Electrolyte Leakage and Malondialdehyde Content (MDA)

As shown in Figure 3A, fruit maturity did not significantly affect the fruits' electrolyte leakage, although fruit water loss did. Based on the comparison, a higher electrolyte leakage was observed for low RH-stored fruits, as was a significantly higher electrolyte leakage from the red-ripe stage at a low RH to the breaker-stage at a high RH. Moreover, the electrolyte leakage increased on the 20th day from that on the 18th day for all fruits. This result indicates a higher CI for low RH-stored fruits and conforms to a report on zucchini stored at high and low RHs [13]. This also shows that injuries progress with time. Previous monitoring of electrolyte leakage has demonstrated the presence of oxidative stress and has confirmed the plastid membrane as the site of chilling-induced peroxidation [27]. Electrolyte leakage has, however, been used during cold storage as an indirect qualitative measure of damage done to the cell membrane. Water loss, as shown in this result and as previously demonstrated by Kissinger [28], significantly contributes to this for sweet peppers, and our results show that a high RH may be helpful in reducing it.

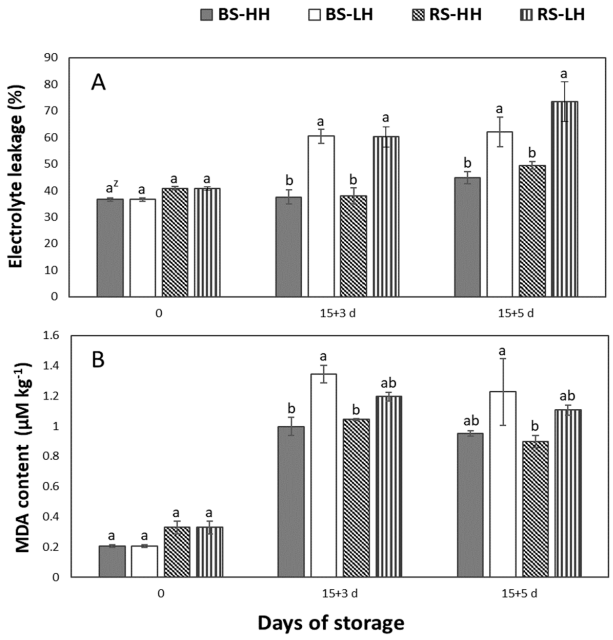

Figure 3. High and low RH effects on sweet pepper's electrolyte leakage (**A**) and MDA content (**B**) before and during ambient conditions after 15 days of cold storage at 5 °C. Vertical bars represent ± SE of the mean (n = 3). [Z] Different letters represent significant differences among treatments for each sampling day using Tukey's multiple comparison test at $p < 0.05$. Note: 15 + 3 d means the third day in ambient conditions, and 15 + 5 d means the fifth day in ambient conditions.

Malondialdehyde (MDA) followed a similar pattern and was almost the same value on the 18th and 20th days when checked. This indicates more membrane integrity degradation in fruits stored at a low RH, as MDA has been shown to be produced from lipid peroxidation [27]. It was shown to be highest and lowest on the last day for the low RH breaker-stage and high RH red-ripe fruits, respectively (Figure 3B). This could be a result of water loss, contributing to the increased gas production rates, thereby leading to the accumulation of MDA in fruits kept at a low RH. MDA has also been identified as an abnormal compound that accumulates during anaerobic respiration or oxidative damage [27]. This result is consistent with other research that shows lower MDA as a sign of reduced CI [9].

3.5. The Effect of Fruit Storage Humidity on Color

Prior to storage, a* and hue angle demonstrated a significant relationship with maturity stages, as expected (Table 3). The a* value increased during storage for all fruits, especially for those in the breaker stage at a higher RH than those at a low RH during cold storage, while the a* values for red-ripe fruits were indifferent, except that they were higher on the 5th and 10th days for low RH fruits, which could be due to the fruits' initial values. This result indicates better color formation during cold storage for fruits in the breaker stage at a high RH. CI affects the red coloration of fruit during storage, causing chilled, injured fruits to develop poor color. Peel pitting, as demonstrated in a previous study, inhibits chlorophyll degradation of breaker stage peppers during color development during cold storage [1], which means that the higher a* value observed during cold storage is a result of favorable high RH conditions. CI, as also seen for tomatoes, affects the color formation of fruits. The hue variation in this study was associated with fruit maturity stage and not storage conditions. However, slightly higher hue angles were observed for high RH-stored fruits at ambient conditions, indicating a slight addition of red color development [6].

Table 3. The effect of high and low RH on sweet peppers' a* and hue angle (h°) before, during, and after 15 days of cold storage at 5 °C and 5 days at ambient conditions.

	Maturity Stage	Storage Conditions	Storage Days					
			0	5	10	15	15 + 3 d	15 + 5 d
a*	BS	HH	3.28 ± 0.3 b [Z]	2.96 ± 1.4 b	6.82 ± 2.0 b	10.16 ± 2.1 b	33.60 ± 1.5 b	34.90 ± 1.4 a
		LH	3.38 ± 0.3 b	2.36 ± 0.8 b	3.90 ± 1.0 b	5.18 ± 1.1 c	33.08 ± 0.7 b	34.48 ± 3.2 a
	RS	HH	27.94 ± 0.4 a	39.78 ± 0.4 a	40.70 ± 0.4 a	38.94 ± 0.6 a	37.16 ± 0.3 a	36.38 ± 0.7 a
		LH	30.40 ± 0.6 a	44.02 ± 1.1 a	42.54 ± 1.1 a	39.60 ± 0.7 a	38.36 ± 0.6 a	34.14 ± 1.7 a
h°	BS	HH	66.14 ± 2.9 a	81.08 ± 4.6 a	75.62 ± 4.6 a	68.24 ± 4.9 a	41.08 ± 2.1 a	31.62 ± 1.3 a
		LH	64.08 ± 4.4 a	82.76 ± 3.7 a	74.92 ± 3.5 a	72.60 ± 4.2 a	39.04 ± 2.3 a	29.06 ± 2.4 a
	RS	HH	23.24 ± 0.6 b	28.70 ± 0.8 b	28.52 ± 0.8 b	27.20 ± 0.9 b	25.26 ± 0.4 b	23.06 ± 0.6 a
		LH	23.78 ± 0.7 b	31.42 ± 0.8 b	29.58 ± 0.6 b	27.08 ± 0.6 b	24.56 ± 0.3 b	21.52 ± 0.9 a
Maturity stage (A)			***	***	***	*	*	NS
Storage conditions (B)			NS	**	*	**	***	***
A × B			NS	***	***	***	***	***

[Z] Values are shown as the mean ± SE (n = 5), and different letters within columns represent significant differences among treatments for each sampling day using Tukey's multiple comparison test. NS, *, **, ***: not significant, $p \leq 0.05$, 0.01, and 0.001. Note: 15 + 3 d means the third day in ambient conditions, and 15 + 5 d means the fifth day in ambient conditions.

3.6. Storage Humidity Effect on DPPH Radical Scavenging Activity and Vitamin C of the Fruit

The DPPH test has been demonstrated to be one of the most effective methods for determining fruits' antioxidant activity. Prior to storage of the fruits, the DPPH was shown to be higher for the red-ripe fruits (Figure 4A), which follows the same pattern as previously reported results [29]. Following cold storage, antioxidant activity increased for breaker-stage fruits and reduced for red-ripe fruits. Comparing the high RH breaker stage with the low RH red-ripe stage, a significant difference was found, in favor of the breaker stage at a

high RH. Storage conditions are important for enhancing antioxidant activity, as revealed by this result. Moreover, higher DPPH readings for high RH fruits are linked to CI tolerance and correlate with antioxidant activity [30,31].

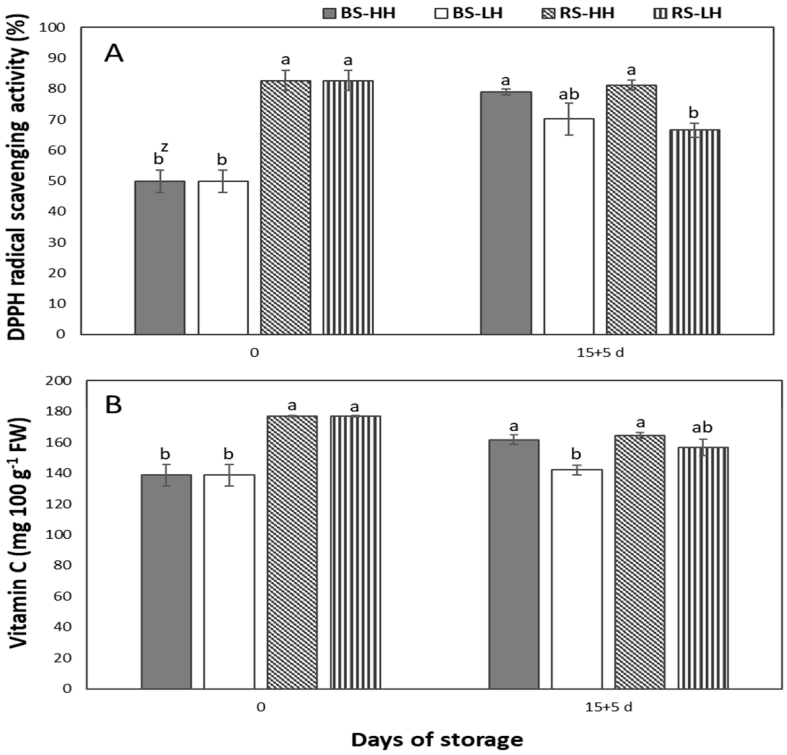

Figure 4. High and low RH effects on the sweet pepper's DPPH radical scavenging activity (**A**) and vitamin C (**B**) before and on the final day after 15 days of cold storage at 5 °C and 5 days at ambient conditions. Vertical bars represent ± SE of the mean (n = 3). ^{Z} Different letters represent significant differences among treatments for each sampling day using Tukey's multiple comparison test at $p < 0.05$. Note: 15 + 5 d means the fifth day in ambient conditions.

Because vitamin C is one of the most unstable vitamins, measuring it is an excellent way to determine the impact of storage conditions on fruit quality. Several factors, including storage temperature and days, have been shown to influence the vitamin C content of fruits [32]. Before storage, a significant difference was shown for the vitamin C content of both maturity stages. Red-ripe fruits showed a higher vitamin C content than break-stage fruits (Figure 4B). This is consistent with previous findings [29,33]. After 15 days of cold storage, a higher (albeit non-significant) level of vitamin C was found for red-stage fruits stored at a high RH than those stored at a low RH. A study on similar storage conditions corroborated this result [17]. Vitamin C increased in breaker-stage fruits; this is because vitamin C showed a 1.2 to 1.4 ratio increase for sweet peppers during ripening [33], but maybe due to the poor storage conditions at low RH, this increase was not maximized. A high vitamin C content in fruits has been speculated to be a sign of tolerance to oxidative damage, because vitamin C is highly susceptible to oxidation when there is damage [30,31]. Sweet peppers are known to be an excellent source of vitamin C. This shows that high RH storage can retain vitamin C and reduce CI.

3.7. Microbiological Analysis

Table 4 shows the total number of microorganisms counted. There was no significant difference in the yeast and mold counts, as well as the *Escherichia coli* (*E. coli*) counts, despite high RH storage, while only the total aerobic count showed differences, with the highest in the breaker stage kept at a low RH. The other observation was that the total number of these microorganisms was, in most cases, lower than the initial count, but showed no significant

differences. This reveals that a high level of RH may not promote the growth of disease as early as may have been thought, and the assumptions may have been blown out of proportion. Although this study does not deny that a high RH can make fruits vulnerable to microorganism attacks, it does suggest that it may take longer for this to happen as expected. As pointed out, consequent condensation promotes the risk of rot [16], which can only happen with frequent temperature changes. Apart from the results of this study, other studies have shown that a high RH is better than what may have been thought [13].

Table 4. High and low RH effects on the growth of microorganisms on sweet peppers before and on the final day after 15 days of cold storage at 5 °C and 5 days at ambient conditions.

Maturity Stage	Storage Conditions	Number of Microorganisms (log CFU g^{-1})					
		Aerobic Count		*E. coli*		Yeast and Mold	
		Initial	Final	Initial	Final	Initial	Final
BS	HH	3.86 ± 0.11 a [Z]	3.15 ± 0.15 c	3.52 ± 0.13 a	3.87 ± 0.09 a	3.27 ± 0.16 a	3.49 ± 0.12 a
	LH		4.57 ± 0.15 a		3.74 ± 0.16 a		3.00 ± 0.00 a
RS	HH	4.08 ± 0.06 a	3.90 ± 0.14 b	4.03 ± 0.08 a	3.64 ± 0.22 a	3.39 ± 0.05 a	3.00 ± 0.00 a
	LH		3.19 ± 0.12 c		3.87 ± 0.17 a		3.15 ± 0.15 a
Maturity (A)		NS	*	NS	NS	NS	NS
Storage conditions (B)		NS	***	NS	NS	NS	NS
A × B		NS	***	NS	NS	NS	NS

[Z] Values are shown as the mean ± SE (n = 3), and different letters within columns represent significant differences among treatments for each sampling day using Tukey's multiple comparison test. NS, *, ***: not significant, $p \leq 0.05$, 0.01, and 0.001.

4. Conclusions

This study compared the effects of increasing storage humidity on chilling injury in sweet peppers, specifically comparing its effect on the breaker stage at a high RH to the red-ripe stage at a normal refrigerator humidity. The results revealed that proper storage conditions are important regarding the water loss rate of peppers, which, in turn, affects the cold damage index (electrolyte leakage, MDA, respiration and ethylene production rates, chilling injury, and calyx browning index), quality index (firmness, color, and soluble solids), and antioxidant index (vitamin C and DPPH) of peppers. They also showed that the maturity stage of peppers is not the primary factor that affects the chilling injury, but rather the water loss rate. Hence, this study advocates for the harvesting of sweet peppers at the color-breaking stage for subsequent high-humidity cold preservation compared to the traditional cold preservation of sweet peppers at the red-ripening stage, for whatever reasons fruits are chosen to be harvested at this stage. Moreover, microbiological analysis also showed that this humidity level is not detrimental to the fast promotion of diseases. However, further studies are needed to see how long fruits can be kept under these conditions.

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