



Article

Extended Storage of Yellow Pepper Fruits at Suboptimal Temperatures May Alter Their Physical and Nutritional Quality

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Abstract: Yellow sweet peppers (*Capsicum annuum* L. cv. Dinamo) are generally more susceptible than red sweet peppers to physiological and pathological deterioration after harvest. Yellow peppers also fetch higher prices at market. In this study, we examined the external and nutritional quality attributes of yellow pepper fruits stored at suboptimal temperatures of 1.5 and 4 °C for 3 weeks, followed by a 3-day shelf-life simulation. Notably, yellow peppers kept in plastic (Xtend®, Stepac, Tefen, Israel) bags at 4 °C maintained their external quality just as well as peppers stored at the optimum temperature of 7 °C. In addition, nutrient content (namely ascorbic acid) and total phenolic and hydrophilic antioxidant contents were not reduced when the peppers were kept at suboptimal storage temperatures of 4 or 1.5 °C in Xtend® plastic packaging. Thus, the external and nutritional qualities of yellow pepper fruits can be preserved at suboptimal temperatures of 4 °C with Xtend® plastic packaging. This is particularly significant in light of the fact that storage at such temperatures is sometimes used as a quarantine method to eliminate pests on produce imported into Israel.

Keywords: Capsicum annuum; postharvest; quality; shelf-life

1. Introduction

Sweet bell pepper (*Capsicum annuum* L.) is an important fruit crop worldwide. It is available mainly in red, yellow and orange, but also in green when harvested unripe. These fruits are rich in vitamin C and A and are a good source of polyphenols and antioxidants, which contribute to human nutrition and health [1]. All of the nutraceutical properties of pepper fruits are largely influenced by genotype, environmental conditions and production system [2,3]. After prolonged storage, red pepper cultivars were found to be more resilient to physiological and pathological deterioration than yellow and orange cultivars [3]. However, yellow and orange fruits fetch higher prices at market than the red ones [4].

Bell peppers are generally regarded as a non-climacteric fruit [5], although there is some variation between cultivars [3,6,7]. The storage life of pepper fruits is limited by rapid water loss during prolonged storage [8] and pathological deterioration [9]. The optimal storage temperature for ripe fruit is 7 °C. At temperatures below 7 °C, chilling injuries will appear within several days, in the form of severe pitting, weight loss and calyx darkening, accompanied by the development of decay [10].

Cold-based quarantine packaging of red bell pepper fruits in plastic film following hot water rinsing over brushes (HWRB) at 55 °C for 15 s enabled peppers to be stored for 3 weeks at 1.5 or 4 °C, without affecting the overall fruit quality [11]. However, there are a few reports regarding changes in

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pepper fruit quality and nutritional status during prolonged storage at 1.5 °C. Recently, Lama et al. [12] reported that storage of bagged red pepper fruits at a suboptimal temperature significantly influenced the fruits' nutritional qualities, including the quantity of aroma volatiles. However, vitamin C, total phenolic and antioxidant contents were not significantly reduced among peppers stored at suboptimal temperatures, as compared with those stored at the optimum storage temperature of 7 °C.

The variety of fruits and vegetables on display in today's supermarkets is enormous. Diversity within each species has also increased considerably, with differences in size, shape, color, aroma and flavor. These parameters may influence consumers' acceptance of fresh produce [1,13,14]. In the present study, we investigated how suboptimal storage temperatures used as quarantine treatments [11] may affect the external quality parameters and nutrient content of yellow peppers during prolonged storage.

2. Materials and Methods

2.1. Plant Material and Bagging

Yellow pepper fruits, cv. Dinamo (A.B. Seeds, Ltd., Airport City, Israel), were grown in a plastic house located in the southern part of Israel, in the Arava valley. The plastic house was covered with translucent plastic (0.12 mm thick, "IR—Ginegar Plastic Ltd., Kibbutz Ginegar, Israel). Transplants, approximately six weeks old, were transferred to a non-heated plastic house in the middle of August and were grown at a density of 33,000 plants ha⁻¹. Local common plastic house plant maintenance practiced for high-wire cultivation system, including leaf pruning, removing side shoots, vine-training (two main stems), and adjusting canopy height was conducted on a weekly basis, Plants were irrigated by drip irrigation with water quality at 2.8 dS/m, based on daily evapo-transpiration (ET) rate during the growing season. Fruits of uniform size (about 200 ± 10 g each) without defects or any signs of disease were harvested at nearly 90% coloration, with the calyx. Fruit was harvested three times during two growing seasons during two consecutive years (end of December—Harvest 1, beginning of February—Harvest 2, end of March—Harvest 3). Immediately after harvest, fruits were washed in a hot-water rinsing and brushing (HWRB) machine at 55 °C for 15 s, according to commercial practice [15]. Fruit were then packed inside micro-perforated Xtend® bags (Xtend®, Stepac, Tefen, Israel) [12] and stored at 1.5, 4, or 7 °C for 21 days, followed by a 3-day shelf-life simulation at 21 °C. Bags were folded shut before cold storage and the level of CO₂ did not exceed 1.5–2%, while the level of O₂ was ~19.5% during the 3 weeks of storage and the shelf-life simulation. Unbagged, HWRB-treated fruits served as a control. Each treatment consisted of four cartons of 5 kg each (22 fruits/carton).

2.2. External Quality Parameters

The following external quality parameters were evaluated after the shelf-life simulation: weight loss, elasticity, decay, chilling injury and chilling index (CI). Weight loss: This was expressed as the percentage of weight lost (relative to the initial weight) of 10 previously marked fruits per treatment. Elasticity: This was evaluated on 10 fruits per carton using a pressure gauge, as described by Lama et al. [12]. Fruits were considered very firm (very low elasticity) with 0–1.5 mm deformation; firm (low elasticity) with 1.6–3 mm deformation; soft (moderate elasticity) with 3.1–4.5 mm deformation and very soft (very high elasticity) with more than 4.6 mm deformation. Decay: Fruit was considered decayed once fungal mycelia appeared on the peel or calyx. Decay was expressed as the percentage of decayed fruit from total fruit in the treatment. Chilling injury (CI): A fruit with a sunken pitting of more than 2 mm on the skin or calyx was considered to be damaged. CI was expressed as the percentage of the fruits that showed damage from total fruit in the treatment. Chilling index (CINX): The severity of the chilling injury was expressed on a scale of 0 to 3 with 0 = No chilling injury; 1 = minor damage of less than 10% of the fruit peel; 2 = moderate, 10% to 30% of damage covering the peel and 3 = severe, more than 30% chilling damage. The index was calculated as follows (Bar-Yosef et al., 2009): CINX = $((N_u \times 0) + (N_{mi} \times 1) + (N_{mo} \times 2) + (N_s \times 3))/N_t$ in which: N_u , N_{mi} , N_{mo} , N_s , and

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N_t, are numbers of fruits with no, minor, moderate, severe damage, and total number of fruit in each treatment, respectively.

2.3. Nutritional Quality Parameters

2.3.1. Ascorbic Acid (AA)

Ascorbic acid content of the fruits was measured and calculated in terms of mg per 100 g fresh weight with the HI3850 Ascorbic Acid Test Kit (Hanna Instruments, Smithfield, RI, USA). This was done using 2 mL of juice homogenized from three 10 g fruit slices, as in Lama et al. [12].

2.3.2. Hydrophilic Antioxidant (HAOX) Content

Antioxidant activity was measured using the discoloration method, with 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate; ABTS; Sigma-Aldrich, Rehovot, Israel) in a 96-well microplate, in which 5 μ L of test sample were added to 300 μ L of ABTS⁺ at 734 nm after 15 min of incubation at room temperature. The hydrophilic fractions were extracted from 100 mg of freeze-dried fruit powder with acetate buffer, acetone and hexane, followed by repeated partitioning of the water-soluble and water-insoluble portions. The results were calculated by comparing the absorbance of the samples with that of the standard, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Sigma-Aldrich, Rehovot, Israel). The antioxidant levels in the samples were determined as Trolox equivalents (TE) according to the formula:

$$TE = (A_{sample} - A_{blank})/(A_{standard} - A_{blank}) \times C_{standard}$$
 (1)

where A is the absorbance at 734 nm and C is the concentration of Trolox (mmol).

The TE antioxidant capacity (TEAC) was calculated per unit weight of plant tissue, using the following equation:

TEAC (mmol TE/mg) =
$$(TE \times V)/(1000 \times M)$$
 (2)

in which V is the final extract volume and M is the amount of tissue extracted.

2.3.3. Total Phenolic (Polyphenol) Content

The total phenolic contents of the fruits were determined using Folin–Ciocalteu reagent (Sigma-Aldrich, Rehovot, Israel). One mL of double-distilled water (DDW) was added to a vial containing 150 mg of lyophilized pepper tissue. Following incubation for 1 h at 37 °C on an orbital shaker (Thermo Fisher Scientific, Waltham, MA, USA) at 250 rpm, vials were centrifuged for 15 min at $18,000 \times g$ and 4 °C (Sorvall RC 6+, Thermo Fisher Scientific, Waltham, MA, USA). The upper layer was removed from the vial and centrifuged again, as before, for another 15 min. The total-phenolics test was performed in a 96-well microplate (Thermo Fisher Scientific, Waltham, MA, USA) by adding $20~\mu$ L of the pepper extract, $850~\mu$ L of DDW, $25~\mu$ L of Fortin–Ciocalteu reagent and $100~\mu$ L of 20%~(w/v) Na₂CO₃. Following incubation for 1 h at $25~^{\circ}$ C, the intensity of blue coloration in plate was measured with an Enspire2300 multi-label reader (Perkin Elmer, Boston, MA, USA) at a wavelength of $765~\rm nm$. Those readings were compared with those for a 20 mmol solution of gallic acid that was used as a standard. The results were expressed as mmol gallic acid equivalents (GAE).

The levels of ascorbic acid, hydrophilic antioxidants and total phenolics were measured in five different composite samples, each taken from three fruits, with a total of 15 fruits in each treatment for every harvest.

2.4. Statistical Analysis

All data were analyzed with the JMP 11 statistical analysis software program (SAS Institute, Cary, NC, USA). The results are the means of figures from two growing seasons. A 2-way factorial design by Tukey-Karmer tests were used to apply analysis of variance (ANOVA) from 0.05 to 0.0001 to data

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from fruits of three different harvests kept at three storage temperatures, with and without packaging. Pearson's correlation analysis was done to determine significant level of correlation between the parameters of interest.

3. Results

3.1. Effect of Suboptimal Temperature on the External Quality of Yellow Pepper Fruit

From the investigation of external quality traits of yellow pepper fruit, we found that weight loss was significantly influenced by the storage temperature and the Xtend $^{\circledR}$ bag, and those findings were consistent across fruit from all three harvests (Table 1). In particular, at all storage temperatures (i.e., 7, 4 and 1.5 °C), the weight loss of unbagged fruits was significantly greater than that observed for bagged fruits stored at the same temperature. In terms of the storage temperatures, significantly greater weight loss was observed at 7 °C for both bagged and unbagged fruits from the first harvest-time, as compared to bagged and unbagged fruits from that harvest-time that were stored at 4 and 1.5 °C. The analysis of variance revealed a significant interaction between storage temperature and bagging among the fruits from the second and third harvests. In general, fruit weight loss was noticeably higher at the beginning of the growing season (first harvest) than at the end of the season (third harvest).

The elasticity of the yellow pepper fruits from the first harvest-time was not significantly affected by the different storage temperatures or by bagging. For the second harvest-time, significantly greater elasticity was noted among the unbagged fruits kept at 7 °C, as compared to the bagged fruits stored at that temperature. In addition, for the fruits from the third harvest-time, significantly greater elasticity was observed among the unbagged fruits stored at 1.5 °C than among the bagged fruits stored at that temperature. The analysis of variance revealed a significant effect of the Xtend® bag on the elasticity of peppers from all three harvests; whereas an interaction between temperature and bagging was observed only for the third harvest-time (Table 1).

As the growing and harvest season continued, from December to March, the level of decay increased among both unbagged and bagged fruits, at all of the examined temperatures (Table 1). The analysis of variance showed that neither temperature nor bagging affected the incidence of decay and there was no temperature \times bag effect on decay (Table 1).

Table 1. The effects of different temperatures and packaging (Xtend[®] bag) on yellow pepper fruit, cv. Dinamo, in terms of weight loss (% of initial weight), elasticity (mm deformation) and decay (%) after 21 days of storage at different temperatures, followed by 3 days of storage at 21°C.

Treatment	Weight Loss (%)			Elasticity (mm Deformation)			Decay (%)		
	Harv1	Harv2	Harv3	Harv1	Harv2	Harv3	Harv1	Harv2	Harv3
7 °C-control	5.9 A *	4.6 B	3.2 B	2.8 A	2.3 AB	2.1 B	9.9 A	19.4 A	21.2 B
7 °C-Xtend®	2.9 C	1.7 D	1.7 D	2.2 A	1.5 D	1.4 B	4.5 A	17.1 A	46.0 A
4 °C-control	4.2 B	6.0 A	4.5 A	2.3 A	3.0 A	2.3 B	2.7 A	19.8 A	30.5 AB
4 °C-Xtend®	2.0 D	2.4 CD	1.9 CD	1.9 A	2.3 ABC	2.1 B	2.7 A	10.9 A	20.5 AB
1.5 °C-control	4.2 B	3.1 C	2.6 BC	2.1 A	2.1 BCD	3.3 A	5.2 A	18.3 A	35.4 AB
1.5 °C-Xtend®	1.5 D	1.5 D	1.3 D	1.9 A	1.6 CD	1.3 B	4.4 A	10.3 A	22.0 B
Analysis of Variance (p-value)									
Temp. (T) ²	****	****	****	NS	****	NS	NS	NS	NS
$X tend^{\textcircled{R}} (X t)^{1}$	****	****	****	*	****	****	NS	NS	NS
$T \times Xt$	NS	**	**	NS	NS	**	NS	NS	NS

Control—without Xtend® bag; Xtend®-bagged; Temp.—Temperature. * Means from the same harvest-time that are followed by the same uppercase letter are not significantly different from one another, according to the Tukey-Kramer test; p < 0.05. $^{1.2} = 1$ and 2 degrees of freedom, respectively. *, **, and **** = significant at α levels of 0.05, 0.01, and 0.0001, respectively. NS—not significant.

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CI and CINX were not noticed among the fruit stored at 7 °C, but were observed among both unbagged and bagged fruits kept at the suboptimal storage temperatures (i.e., 4 and 1.5 °C; Table 2). The lower the storage temperature, the greater the CI. In particular, for the first and third harvest-times, CI was significantly higher among the unbagged fruit stored at 4 and 1.5 °C, as compared to bagged fruits stored at those temperatures. For the third harvest-time, CINX was significantly higher among the unbagged fruits stored at 1.5 °C, as compared to all of the other fruits from that harvest-time. The analysis of variance showed that the storage temperature and keeping the fruit inside the Xtend® bag both significantly affected CI and CINX (with the exception of the effect of bagging on CI among fruit at the second harvest-time; Table 2). For the fruits from the first and third harvest-times, a significant interaction was noticed between the storage temperature and the use of the Xtend® bags (Table 2).

Table 2. The effects of different temperatures and packaging (Xtend[®] bag) on yellow pepper fruit, cv. Dinamo, in terms of chilling injury and chilling index (0–3) after 21 days of storage at different low temperatures, followed by 3 days of storage at 21 °C.

Treatment	Ch	illing Injury ((%)	Chilling Index (CINX) (0-3)					
Treatment.	Harv1	Harv2	Harv3	Harv1	Harv2	Harv3			
7 °C-control	0	0	0	0	0	0			
7 °C-Xtend®	0	0	0	0	0	0			
4 °C-control	29 B *	16 A	21 A	0.5 B	0.3 AB	0.09 C			
4 °C-Xtend®	13 C	9 A	4 B	0.1 C	0.1 B	0.07 C			
1.5 °C-control	46 A	13 A	24 A	2.0 A	0.4 A	1.9 A			
1.5 °C-Xtend®	14 C	7 A	5 B	0.4 AB	0.2 AB	0.2 B			
Analysis of Variance (p-value)									
Temp. (T) ²	****	***	****	***	****	***			
$X tend^{\textcircled{R}} (X t)^{1}$	****	NS	****	***	***	***			
$T \times Xt$	****	NS	****	***	NS	***			

Control—without Xtend® bag; Xtend®-bagged; Temp.—Temperature. * Means from the same harvest-time that are followed by the same uppercase letter are not significantly different from one another, according to Tukey-Kramer test; p < 0.05. $^{1.2} = 1$ and 2 degrees of freedom, respectively. *, ***, and **** = significant at α levels of 0.05, 0.001 and 0.0001, respectively. NS—not significant.

3.2. Effects of Suboptimal Temperatures on the Nutritional Quality of Yellow Peppers

In the analysis of nutritional traits, for all harvest-times, total phenolic content was found to be higher among fruits that were kept inside the Xtend $^{\circledR}$ bags, as compared with non-bagged fruits (Table 3). Specifically, for the first harvest, bagged peppers stored at 7 and 4 $^{\circ}$ C showed significantly higher total phenolic contents than unbagged peppers stored at those same temperatures. A similar trend of significant differences was observed among the bagged vs. unbagged fruits from the second harvest-time that were stored at 4 and 1.5 $^{\circ}$ C and the bagged vs. unbagged fruits from the third harvest-time that were stored at 7 and 1.5 $^{\circ}$ C. The analysis of variance revealed a significant interaction between temperature and Xtend $^{\circledR}$ bag among the fruits from all of the harvests (Table 3).

Hydrophilic antioxidant levels increased from the first harvest in December to the last harvest in March, across all treatments (Table 3). In particular, for the third harvest-time, significantly higher levels of hydrophilic antioxidants were found among the bagged fruits, as compared to the unbagged fruits kept at each temperature. Among the fruits from the first harvest-time, neither storage temperature nor the use of Xtend[®] bags influenced the level of hydrophilic antioxidants. For the fruits from all harvests, no interaction between the storage temperature and bagging was observed.

Levels of AA were hardly affected by the storage temperatures or the use of Xtend[®] bags (Table 3). The storage temperature significantly influenced AA content only among the fruits from the first harvest-time (December). Furthermore, the AA content of the yellow pepper fruits was lower at the beginning of the season (first harvest-time) than it was at the second and third harvest-times (February and March, respectively).

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Table 3. Effects of cold treatments on the total phenolic content (mM GAE/100 mg dry weight), hydrophilic antioxidant content (μ M TE/g dry weight) and ascorbic acid (AA) content (μ M TE/g dry weight) and ascorbic acid (AA) content (μ M TE/g dry weight) of yellow peppers, cv. Dinamo, after 21 days of storage at different low temperatures, followed by 3 days of storage at 21 °C.

Treatment	Total Phenolics (mM GAE/100 mg Dry Weight)			,	Hydrophilic Antioxidants (μM TE/g Dry Weight)			Ascorbic Acid (AA) (mg/100 g Fresh Weight)		
	Harv1	Harv2	Harv3	Harv1	Harv2	Harv3	Harv1	Harv2	Harv3	
7 °C-control	380 C *	393 BC	302 C	81 A	87 CD	106 C	179 AB	193 B	211 A	
7 °C-Xtend®	474 A	410 BC	339 B	82 A	102 A	119 AB	179 AB	208 AB	202 A	
4 °C-control	390 C	380 C	386 A	70 A	92 BC	114 BC	184 A	214 AB	204 A	
4 °C-Xtend®	433 B	419 B	417 A	83 A	98 AB	128 A	182 AB	210 AB	214 A	
1.5 °C-control	440 AB	387 BC	324 BC	75 A	81 D	122 AB	165 BC	225 A	195 A	
$1.5~^{\circ}\text{C-Xtend}^{\circledR}$	446 AB	470 A	412 A	76 A	96 ABC	128 A	172 C	201 AB	222 A	
Analysis of Variance (p-value)										
Temp. (T) ²	NS	***	NS	NS	*	***	****	NS	NS	
Xtend® (Xt) ¹	****	****	****	NS	****	****	NS	NS	NS	
$T \times Xt$	****	****	**	NS	NS	NS	NS	*	*	

Control—without Xtend® bag; Xtend®-bagged; Temp.—Temperature. * Means from the same harvest-time that are followed by the same uppercase letter do not differ significantly from one another, according to the Tukey-Kramer test; p < 0.05. $^{1,2} = 1$ and 2 degrees of freedom, respectively *, ***, ****, and **** = significant at α levels of 0.05, 0.01, 0.001 and 0.0001, respectively. NS—not significant.

3.3. Correlations between the Quality Parameters

The relationships between the quality parameters of yellow pepper fruits stored at 1.5 °C in Xtend® bags were examined using Pearson's correlation analysis (Table 4). The correlation coefficients revealed significant positive relationships between weight loss and CI and between weight loss and CINX (p = 0.05). Similarly, significant positive relationships were also found between CI and CINX, and between CINX and elasticity (p = 0.05). The incidence of decay was also significantly and positively correlated with hydrophilic–antioxidant activity (p = 0.01).

Table 4. Correlation coefficients for the relationships between weight loss (WL), chilling injury (CI), chilling index (CINX), decay (DE), elasticity (EL), ascorbic acid content (AA), hydrophilic antioxidant (HAOX) content and total phenolic (TP) content in yellow pepper, cv. Dinamo, after 21 days of storage at 1.5 °C, followed by 3 days of storage at 21 °C, in combination with Xtend[®] packaging.

	CI	CINX	DE	EL	AA	HAOX	TP
	CI	CINA	DE	EL	AA	ПАОХ	11
WL	0.86 *	0.72 *	-0.15	0.41	-0.24	-0.20	0.67
CI		0.89 **	-0.34	0.45	-0.38	-0.25	0.50
CINX			0.04	0.74 *	-0.05	0.14	0.32
DE				0.37	0.65	0.96 ***	-0.40
EL					0.29	0.37	0.42
AA						0.52	-0.31
HAOX							-0.51

*, **, *** = significant at α levels of 0.05, 0.01, 0.001 and 0.0001, respectively.

4. Discussion

The attractiveness of yellow pepper fruit is largely determined by its physical quality (external appearance), which is a priority in consumers' purchasing decisions. During prolonged cold storage, especially storage at suboptimal temperatures, the physical quality of the fruits deteriorates, in terms of weight loss, CI and decay [16]. However, the importance of nutritional attributes was neglected [17]. In recent work conducted by Lama et al. [12], the external and internal qualities (including the aroma) of red sweet bell pepper fruits were maintained during storage at suboptimal temperatures of 4 or 1.5 °C when the peppers were kept in plastic bags. In fact, those fruits fared better than those stored

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inside plastic bags at the optimum storage temperature of 7 $^{\circ}$ C. To the best of our knowledge, there is very little information about the effects of suboptimal storage temperatures on the quality and nutrient levels of yellow peppers, which fetch a higher market price than red peppers.

In the present study, the external and internal quality parameters of yellow peppers stored at suboptimal temperatures were acceptable and most of the bagged fruits were marketable due to relatively low CI and minimal decay development, even after 3 weeks of storage. The best treatment for preserving those quality parameters was 4 °C, once the fruit was packed inside Xtend® bag (Tables 1 and 2). The time of harvest affected fruit quality, as has also been reported by Maalekuu et al. [2] and Bar-Yosef et al. [10]. At the beginning of the harvest season (first harvest-time), the incidence of decay was relatively low, due to young resilient plants with a less dense canopy and, therefore, better ventilation and fewer decay-causing agents in the plastic house, as reported by Barkai-Golan [18]. However, those fruits exhibited greater CI and CINX. This was probably due to the high day and night temperatures in the growing region between August to November, during fruit development and ripening, as previously reported by Bar-Yosef et al. [10]. As the harvest season continued, the incidence of decay on the harvested fruit increased as the plants senesced and the crop developed a thicker canopy, which led to less ventilation in the plastic house, which, in turn, made the fruit more susceptible to pathogen infection [18]. In contrast, CI and CINX were lower among the fruits from the second harvest-time (February). Those fruits were more resilient to suboptimal temperature storage, thanks to their exposure to low day and night temperatures as they were developing and ripening during December and January. Harvest date affected the fruit stored at very low temperature in a similar manner. Late-harvested fruits maintained their quality for longer than early-harvested fruits [19,20]. We found a strong positive correlation between CI and the incidence of decay (Table 4). It has been well documented that skin pitting due to chilling injury encourages decay on fresh produce during prolonged storage [18]. The lowest incidence of decay was observed among the bagged fruit that were stored at 4 °C (Table 1). This can be explained, in part, by the fact that the CI of the bagged fruit was reduced, thanks to better cell membrane integrity in the face of the hot-water treatment and the reduced water loss among bagged fruit, as previously reported by Fallik et al. [11].

Among the different types of fresh produce, pepper fruits have one of the highest levels of ascorbic acid [21]. Generally, freshly harvested fruits and vegetables show a gradual decrease in ascorbic acid content as the storage temperature decreases and the duration of the storage period increases [22]. Despite the known negative effects of cold storage on the ascorbic acid contents of different kinds of fruits, ascorbic acid levels did not decrease in the present study, even at suboptimal storage temperatures; this was especially true for the bagged fruits (Table 3). This might be associated with the low level of water loss among the bagged fruits, as increased water loss has been reported to cause the loss of ascorbic acid from leafy vegetables [23]. This finding could also be related to the slow rate of fruit senescence at suboptimal storage temperatures.

Bell pepper fruits contain a wide range of antioxidant compounds [24,25]. Although in the present study we did not measure any other antioxidants, such as vitamin E, carotenoids or flavonoids, we did evaluate overall hydrophilic–antioxidant activity, since mechanisms of antioxidant action can include the reduction of oxidative damage related to free-radical scavenging due to abiotic stress [26]. Measurement of antioxidant activity enables the evaluation of this nutritional quality of fruits stored at suboptimal temperatures [21].

In the present study, total phenolic content was higher among the bagged fruits and there was a significant storage temperature × packaging effect on total phenolic content. Rai et al. [27] reported similar results for pepper, mango and avocado fruits. An increase in phenolic compounds is considered a positive outcome and is thought to enhance the nutritional value of the plant product [27]. Hydrophilic antioxidants also showed the same pattern as phenolics; hydrophilic antioxidant content was enhanced by packaging fruits in Xtend[®] bags, similar to what was observed for total phenolics, but there was no significant difference among all of the treatments at the first harvest-time. The enhanced antioxidant activity among fruits that were bagged and exposed to suboptimal temperatures can help

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to maintain membrane integrity by reducing oxidative damage related to free-radical scavenging, thereby maintaining the balance between the production and elimination of activated oxygen [26,28], which would lead to less postharvest water loss. In addition, the higher antioxidant levels in fruits grown at lower temperatures might delay the onset of fruit senescence by reducing damage to the membrane system and/or reducing water loss and thereby better maintain external and internal quality, as was found for the bagged fruit kept at 4 °C [28]. Yet, a negative correlation between total phenolic content and antioxidants and ascorbic acids contents was found (Table 4). This could be explained, in part, by the ripening process of the pepper fruit at sub-optimal temperature, which increased total phenol content, while antioxidants activity and ascorbic acid production were reduced [29], or by the difference according to the cultivar types besides various variable factors include maturity of fruit and the analytical methods used in various studies for estimation antioxidant power [30].

Similar to our results, following HWRB treatment of bell pepper fruits, Ilić et al. [31] observed significantly higher hydrophilic–antioxidant contents among individually shrink-wrapped fruits than among fruits that were not shrink-wrapped. The higher antioxidant levels among these fruits, probably due to cold stress, might contribute to their resistance to postharvest pathogen infection, which would indirectly result in the decreased incidence of decay. Ghasemnezhad and Javaherdashti [17] proved that strong antioxidant activity was responsible for a low level of postharvest decay among raspberries. Moreover, abundant antioxidants are believed to protect living organisms from oxidative damage and thereby prevent disease [25].

In conclusion, a pre-storage HWRB treatment of yellow pepper fruits followed by storage at the suboptimal temperature of 4 $^{\circ}$ C in plastic bags (Xtend $^{\otimes}$) significantly maintained external and nutritional qualities, with a significant interaction between temperature and packaging, as compared to the traditional optimal storage temperature of 7 $^{\circ}$ C. Thus, keeping yellow peppers at 4 $^{\circ}$ C, as in the cold-quarantine treatment used to control the Mediterranean fruit fly [11], preserved the overall quality of the fruits. In the future, the relationships between suboptimal low temperatures, nutritional quality and levels of aromatic volatiles should be studied in several different cultivars, to enable the formation of firmer conclusions. In addition, more research is required to understand the molecular and biochemical mode(s) of action inside the fruits.

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