



## Article

# Can Edible Coatings Maintain Sweet Pepper Quality after Prolonged Storage at Sub-Optimal Temperatures?

Shani Kehila <sup>1,2</sup>, Sharon Alkalai-Tuvia <sup>1</sup>, Daniel Chalupowicz <sup>1</sup>, Elena Poverenov <sup>1</sup> and Elazar Fallik <sup>1,\*</sup>

<sup>1</sup> Agricultural Research Organization—The Volcani Center, Institute of Postharvest and Food Sciences, Rishon LeZiyyon 7505101, Israel; shani.kehila@mail.huji.ac.il (S.K.); sharon@volcani.agri.gov.il (S.A.-T.); chalu@volcani.agri.gov.il (D.C.); elenap@volcani.agri.gov.il (E.P.)

<sup>2</sup> Robert H. Smith—Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 9112102, Israel

\* Correspondence: efallik@volcani.agri.gov.il; Tel.: +972-3-9683665; Fax: +972-3-9683622

**Abstract:** This work evaluated the efficacies of different coatings: chitosan, gelatin and chitosan-gelatin applied layer-by-layer (LbL); for maintaining the quality of sweet peppers that were stored for 3 weeks at a sub-optimal temperature (1.5 °C) and at an optimal storage temperature (7 °C). After the cold-storage period, fruits were kept under marketing conditions (21 °C) for 3 more days. An edible chitosan coating (2%) effectively alleviated chilling injury and the incidence of decay, and also preserved the nutritional quality of sweet peppers that were kept for 3 weeks at 1.5 °C plus 3 more days at 21 °C. The chitosan coating was more effective than the two other coatings. All three coating treatments significantly reduced external CO<sub>2</sub> production, as compared to uncoated control fruit. Storage temperatures did not significantly affect external CO<sub>2</sub> production, although CO<sub>2</sub> production was slightly higher at 1.5 °C. The chitosan coating exhibited good CO<sub>2</sub> gas permeability properties and the peppers coated with that material had lower respiration rates than those in the other two experimental treatments or the control. From a practical point of view, chitosan coating could replace the plastic bags previously found to alleviate chilling injury in peppers that are stored at 1.5 °C as a quarantine treatment.

**Keywords:** marketing; quarantine; postharvest; shelf life



**Citation:** Kehila, S.; Alkalai-Tuvia, S.; Chalupowicz, D.; Poverenov, E.; Fallik, E. Can Edible Coatings Maintain Sweet Pepper Quality after Prolonged Storage at Sub-Optimal Temperatures? *Horticulturae* **2021**, *7*, 387. <https://doi.org/10.3390/horticulturae7100387>

Academic Editor: Tong Chen

Received: 10 September 2021

Accepted: 8 October 2021

Published: 10 October 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Sweet bell pepper (*Capsicum annuum* L.) is a very important fruit of the Solanaceae family with excellent nutritional qualities, including high levels of ascorbic acid, antioxidants and vitamins [1]. When they are kept at 7 °C, bell peppers have a relatively short storage and marketing period of less than two weeks, due to their susceptibility to flaccidity, wilting, shriveling, fungal diseases and decay [2]. However, keeping peppers at temperatures below 7 °C enhances physiological and pathological deterioration, mainly due to chilling injury [3]. To overcome chilling injury, before storage at sub-optimal storage temperatures, fruit can be rinsed in hot water over brushes and then wrapped in plastic film. Peppers treated in this manner can be kept at temperatures below 4 °C for up to 3 weeks [3].

The performance of different edible coatings has been investigated in many fruit crops [4]. Some coating materials have been found to reduce decay development and help to maintain fresh produce quality after prolonged storage [5]. The application of edible coatings has been shown to improve the physical appearance of freshly harvested produce by creating a barrier against respiration and moisture loss [6]. These coatings develop a modified atmosphere, which can induce diverse alterations in fruit and vegetables in terms of antioxidant properties, microbial growth, color, sensory quality, firmness, ethylene production and volatile compounds, as a result of anaerobic processes [6]. Fresh produce continues to respire after harvest. Therefore, edible coatings need to have precisely balanced

gas-permeability properties, to ensure normal CO<sub>2</sub>/O<sub>2</sub> exchange. At the same time, fresh commodities are also very sensitive to water loss after harvest, which can cause shrinkage and impair the texture of the produce. For this reason, the coatings need to have precisely balanced gas permeability and water-vapor permeability, to limit the loss of moisture [7]. Edible coatings must possess a number of traits such as sufficient antimicrobial activity, the ability to protect against environmental damage and exceptionally good adhesion. A single coating material cannot always satisfy all of these different requirements. Recently, the layer-by-layer (LbL) method was established to promote prolonged storability, enhanced physiological quality and improved appearance, and provide antimicrobial protection [8]. This approach is based on the alternate deposition of different edible coating materials and allows for more effective control over the physicochemical properties and activities of these materials [7,8].

In a previous study, Poverenov et al. [9] reported that coating pepper fruits with a composition of chitosan and gelatin reduced decay development 2-fold after 21 d storage at 7 °C plus 14 d at 20 °C, without affecting the respiration or nutritional content of the peppers. However, to the best of our knowledge, there is no information available as to whether edible coating materials can reduce or prevent pepper fruit deterioration and chilling injury during storage at a sub-optimal temperature of ~2 °C for 3 weeks. This sub-optimal temperature can be used as a quarantine treatment for pepper against the Mediterranean fruit fly, following hot water treatment and packing in plastic bags [3]. Therefore, the aim of this work was to evaluate the effectiveness of different coating materials in maintaining the quality of peppers stored for 3 weeks at a sub-optimal temperature.

## 2. Materials and Methods

### 2.1. Plant and Coating Materials

Red bell peppers (*Capsicum annum* L., cv. Kannon (Zeraim Gedera—Syngenta, Revadim, Israel)) were harvested from the Arava Valley in southern Israel. Fruits were cleaned and disinfected by hot-water rinsing and brushing before treatment [10]. Two cartons containing 20 fruits each were used for each treatment and four treatments were applied: (a) no coating (control), (b) a chitosan coating, (c) a gelatin coating and (d) a coating of chitosan and gelatin, applied in a layer-by-layer (LbL) manner. Three experiments (harvests) were conducted, once every 4 weeks between December to February, and each experiment contained the four mentioned treatments. Fruits were stored for 21 d at 7 °C or 1.5 °C and 95% RH, followed by an additional 3 days at 21 °C and 65% RH, to simulate marketing conditions.

Based on previous experiments, gelatin powder (Sigma–Aldrich, Rehovot, Israel) was dissolved (1% *w/v*) in sterilized, double-distilled water (DDW) and the solution was stirred at 45 °C for 45 min. Chitosan (2% *w/v*, degree of deacetylation: 85%) powder (Sigma–Aldrich, Rehovot, Israel) was dissolved in sterilized DDW that included 0.7% acetic acid (Sigma–Aldrich, Rehovot, Israel) and that solution was stirred at 30 °C for 2 h. Peppers were hand-coated with the cold coating solutions using a paint brush and dried in a drying tunnel for 2 min at 38 °C. The chitosan–gelatin coating was applied layer by layer: The fruits were first coated with chitosan, then dried for 1 min inside a hot drying tunnel (38 °C) and then recoated with gelatin, as described above. Uncoated fruit served as a control.

### 2.2. Quality Parameters

External quality, internal quality and nutritional content were evaluated after 21 d of storage at 7 or 1.5 °C plus 3 d at 20 °C, based on Lama et al. [2]. The following parameters were measured: Weight loss, elasticity and TSS (total soluble solids) were measured on 10 fruits. Fruit color development was evaluated on a scale of 1 to 5, in which 1 = less than 50% of red color of the total fruit surface skin area; 2 = red color between 50 and 75%; 3 = red color between 75 and 85%; 4 = red color between 85–95% and 5 = fruit is totally red. Percent of decay incidence and percentage of chilling injury (CI) were calculated from the 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 and the chilling index was calculated based on Lama et al. [2].

### 2.3. Nutritional Quality

Ascorbic acid (AsA) was measured and calculated based on Lama et al. [2]. Ten fruits were analyzed for each treatment and the results were obtained as mg AsA per 100 g fresh weight. Antioxidant activity (AOX) was measured from 10 fruits by extracting 350 mg of freeze-dried fruit powder. Activity was measured using the TEAC (Trolox equivalent antioxidant capacity) method [11] and the TE antioxidant capacity (TEAC) was calculated per unit weight of plant tissue using the following equation:  $TEAC \text{ (mmol TE/mg)} = (TE \times V)/(1000 \times M)$ ; in which V is the final extract volume and M is the amount of tissue extracted.

### 2.4. External and Internal CO<sub>2</sub> Production

External and internal CO<sub>2</sub> were measured at the end of third day of the shelf-life simulation (20 °C) by placing one fruit in a 2-L glass jar, with six jars per treatment. Jars were sealed for 3 h and the accumulated headspace gases (external CO<sub>2</sub>) were sampled using a syringe inserted through a septum in the jar lid. Two-ml samples of the fruit internal atmospheres were withdrawn through a syringe inserted through the blossom ends of the fruits. Three experiments were conducted as mentioned above (2.1). The CO<sub>2</sub> concentrations in the samples were measured with a Gow-Mac Series 580 gas chromatograph (Gow-Mac, Canton, MA, USA) as described by Poverenov et al. [9]. Results were calculated based on the following equation where 1.84 was the factor to convert volume to gram, C—the percentage of CO<sub>2</sub> measured by the GC, V—free volume in the jar in liter, 100—a factor to express the results in gram, W—fruit weight. H—the number of hours the jars were sealed. The results are expressed as µg/g FW/h of CO<sub>2</sub>.

$$\frac{1.84 \times C \times V}{100 \times W \times h} = \text{CO}_2 \text{ mg/gr/h}$$

### 2.5. Statistical Analysis

All data were analyzed with the JMP 14 statistical-analysis software program (SAS Institute, Cary, NC, USA). The results are the means of data from three experiments. A two-way factorial design, analysis of variance (ANOVA) and Tukey's HSD tests were used to analyze data from fruits kept at two storage temperatures and coated with three different coating materials and to compare that data with data collected from uncoated control fruit. Differences were considered significant at  $p = 0.05$ .

## 3. Results

Coating materials and storage temperature significantly affected fruit weight loss during the storage and the shelf-life simulation (Table 1). The chitosan coating significantly reduced weight loss, as compared to the control, but did not perform significantly different from the other two coating treatments. Storing the fruit at 1.5 °C instead of 7 °C significantly reduced weight loss (4.8% and 5.9%, respectively). No interaction was found between the coating treatments and the storage temperatures (Table 1).

**Table 1.** The effects of different coating materials and storage temperatures on red pepper fruit quality, in terms of weight loss (% of initial weight), elasticity (mm deformation), color index (1–5), total soluble solids (TSS; %) and the incidence of decay (%) after 21 d at 7 °C or 1.5 °C, followed by 3 d at 21 °C. Mean data from three harvests are presented; each harvest consisted of two boxes with 20 fruit in each box.

Treatment	Weight Loss (%)	Elasticity (mm)	Color Index (1–5)	TSS (%)	Decay Incidence (%)
Control-7 °C	6.4 a	5.4 ab	5.0 a	6.8 b	15.1 b
Chitosan-7 °C	5.3 ab	4.8 bc	4.7 ab	7.0 ab	3.7 c
Gelatin-7 °C	6.1 ab	5.7 a	5.0 a	7.1 ab	15.3 b
Chitosan+gelatin-7 °C (LbL)	5.6 ab	5.1 abc	4.9 ab	7.2 a	6.4 c
Control-1.5 °C	5.9 ab	4.9 abc	4.8 ab	6.9 ab	26.0 a
Chitosan-1.5 °C	4.0 b	3.3 e	4.3 b	7.1 ab	4.4 c
Gelatin-1.5 °C	5.1 ab	4.4 cd	4.6 ab	7.0 ab	15.4 b
Chitosan+gelatin-1.5 °C (LbL)	4.2 b	3.6 de	4.3 b	7.1 ab	7.5 bc
LSD	0.63	0.27	0.19	0.09	2.42
Mean of coating materials data					
Control	6.2 a	5.1 a	4.9 a	6.9 b	20.6 a
Chitosan	4.6 b	4.1 b	4.5 a	7.1 a	4.0 c
Gelatin	5.6 ab	5.1 a	4.8 a	7.0 ab	15.4 b
Chitosan+gelatin (LbL)	4.9 ab	4.4 b	4.6 a	7.1 a	7.0 c
LSD	0.45	0.19	0.14	0.05	1.71
Mean of storage temperatures data					
7 °C	5.9 a	5.3 a	4.9 a	7.0 a	13.3 a
1.5 °C	4.8 b	4.0 b	4.5 b	7.0 a	10.1 b
LSD	0.31	0.13	0.1	0.04	1.21
Table of variance (F-values)					
Coating (C)	*	****	NS	*	****
Temperature (T)	**	****	***	NS	*
C × T	NS	NS	NS	NS	*

\* Means within columns followed by the same letter do not differ according to Tukey's HSD test at  $p = 0.05$ . \*, \*\*, \*\*\* and \*\*\*\* indicate significance at  $\alpha$ -levels of 0.05, 0.01, 0.001 and 0.0001, respectively. NS—not significant.

Both coating materials and storage temperatures significantly affected fruit elasticity ( $F = 0.0001$ ). The firmer fruits were found in the chitosan and LbL treatments (4.1 mm and 4.4 mm, respectively). The average elasticity of fruit kept at 1.5 °C was 4 mm, as compared to 5.3 mm among fruit stored at 7 °C. No interaction was found between the coatings and the storage temperatures (Table 1).

Keeping the fruit at 1.5 °C instead of 7 °C significantly inhibited color development (color indices of 4.5 and 4.9, respectively). A slight inhibition of color development was observed among the coated fruits, especially among the chitosan-coated fruit, but no significant differences were observed between the treatments (Table 1).

A higher and significant TSS content was measured in fruit coated with chitosan and LbL, as compared to the uncoated control fruit. However, no significant differences were found between the three coating materials. Storage temperature did not affect TSS content (Table 1).

The coating materials and storage temperatures significantly affected the incidence of decay. At both storage temperatures, the lowest incidence of decay was observed for the chitosan-treated fruit (3.7% and 4.4% at 7 °C and 1.5 °C, respectively); whereas the highest incidence of decay was observed among the control fruits kept at both storage temperatures and among the gelatin-coated fruits stored at 7 °C. An interaction between coating materials and storage temperature was found to affect decay incidence (Table 1).

The lowest incidence and severity of chilling injury (CI), particularly at 1.5 °C, were observed among the chitosan-treated fruit; whereas the greatest incidence and severity of CI were observed among gelatin-treated fruit (13.7% CI/0.43 CINX and 56.3% CI/1.3

CINX, respectively). Even at 7 °C, CI was observed after 3 weeks of storage and shelf-life simulation; however, the CINX was very low. No interaction was observed between the two parameters (Table 2).

**Table 2.** The effects of different coating materials and storage temperatures on red pepper fruit quality, in terms of chilling injury (%) and chilling index (0–3) after 21 d at 7 °C or 1.5 °C, followed by 3 d at 21 °C. Mean data from three harvests are presented; each harvest consisted of two boxes with 20 fruit in each box.

Treatment	Chilling Injury (%)	Chilling Index (CINX; 1–3)
Control-7 °C	8.0 cd	0.13 bcd
Chitosan-7 °C	1.7 d	0.03 d
Gelatin-7 °C	8.3 cd	0.07 cd
Chitosan+gelatin-7 °C (LbL)	5.0 d	0.07 cd
Control-1.5 °C	47.7 ab	0.83 abc
Chitosan-1.5 °C	13.7 bcd	0.43 bcd
Gelatin-1.5 °C	56.3 a	1.30 a
Chitosan+gelatin-1.5 °C (LbL)	43.3 abc	0.89 ab
LSD	10.53	0.22
Mean of coating materials data		
Control	27.8 ab	0.48 ab
Chitosan	7.7 b	0.23 b
Gelatin	32.3 a	0.68 a
Chitosan+gelatin (LbL)	24.2 ab	0.47 ab
LSD	7.44	0.15
Mean of storage temperature data		
7 °C	5.8 b	0.08 b
1.5 °C	40.3 a	0.86 a
LSD	5.27	0.11
Table of variance ( <i>F</i> -value)		
Coating (C)	*	*
Temperature (T)	****	****
C × T	NS	NS

\* Means within columns followed by the same letter do not differ according to Tukey's HSD test at  $P = 0.05$ . \* and \*\*\*\* indicate significance at  $\alpha$ -levels of 0.05 and 0.0001, respectively. NS—not significant.

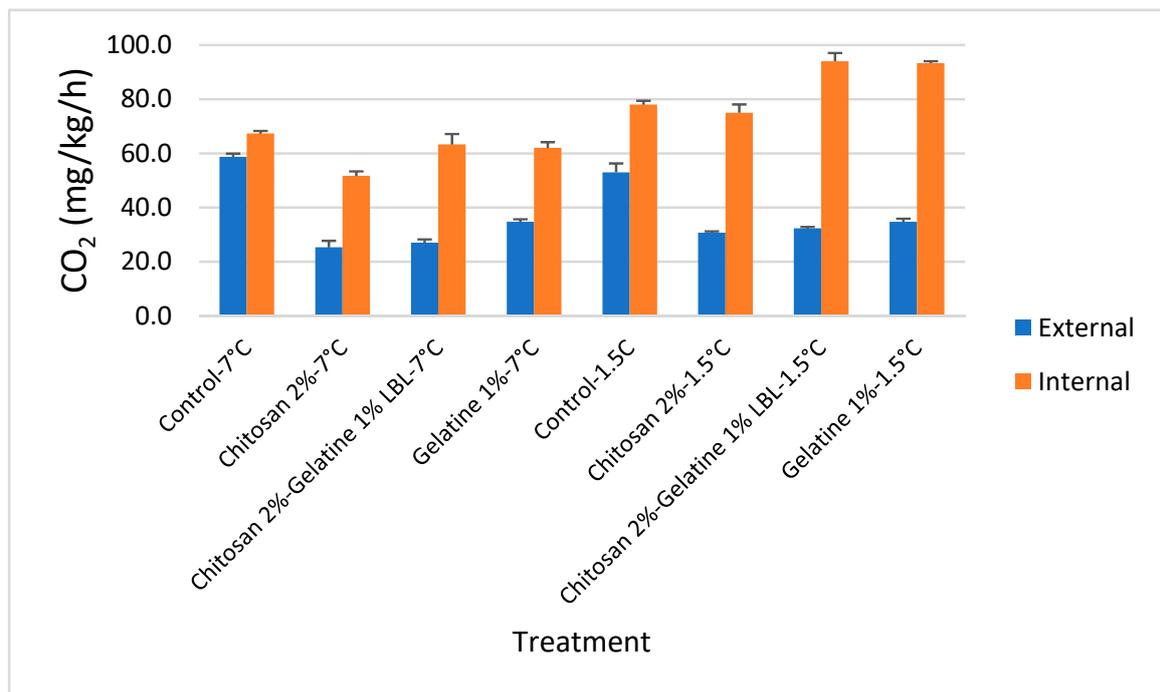
The effects of the different coatings and storage temperatures on the nutritional quality of pepper fruits are presented in Table 3. AsA was not affected by storage temperatures and coating treatments after cold storage and the shelf-life simulation (Table 3). The coating materials and storage temperature significantly affected AOX activity at the end of the storage and shelf-life test period. The highest AOX was measured among the chitosan-treated fruit, at both 7 °C and 1.5 °C (12.5 and 16.8  $\mu\text{M TE}$ , respectively); whereas the lowest activity was measured among gelatin-treated fruit kept at both storage temperatures. AOX activity was significantly higher at 1.5 °C than at 7 °C (Table 3).

**Table 3.** The effects of different coatings and storage temperatures on red pepper fruit quality, in terms of ascorbic acid (AsA) content (mg/100 g FW) and total antioxidant activity ( $\mu\text{M TE}$ ) after 21 d at 7 °C or 1.5 °C, followed by 3 d at 21 °C. Mean data from three harvests are presented; each harvest consisted of two boxes with 20 fruit in each box.

Treatment	Ascorbic Acid (AsA) (mg/100 g FW)	Antioxidant Activity (AOX) ( $\mu\text{M TE/g DW}$ )
Control-7 °C	105 a	11.6 c
Chitosan-7 °C	117 a	12.5 c
Gelatin-7 °C	116 a	9.6 c
Chitosan+gelatin-7 °C (LbL)	112 a	10.7 c
Control-1.5 °C	108 a	16.5 ab
Chitosan-1.5 °C	110 a	16.8 a
Gelatin-1.5 °C	109 a	12.5 bc
Chitosan+gelatin-1.5 °C (LbL)	106 a	13.3 abc
LSD	5.82	1.16
Mean of coating materials data		
Control	107 a	14.0 ab
Chitosan	113 a	14.6 a
Gelatin	112 a	11.0 c
Chitosan+gelatin (LbL)	109 a	12.0 bc
LSD	4.12	0.82
Mean of storage temperature data		
7 °C	112 a	11.1 b
1.5 °C	108 a	17.8 a
LSD	2.91	0.58
Table of variance ( <i>F</i> -value)		
Coating (C)	NS	**
Temperature (T)	NS	****
C $\times$ T	NS	NS

\* Means within columns followed by the same letter do not differ according to Tukey's HSD test at  $p = 0.05$ . \*\* and \*\*\*\* indicate significance at  $\alpha$ -levels of 0.01 and 0.0001, respectively. NS—not significant.

All three coating materials significantly reduced external  $\text{CO}_2$  production, compared to the uncoated control fruit. The different storage temperatures did not significantly affect external  $\text{CO}_2$  production, although at 1.5 °C,  $\text{CO}_2$  production was slightly higher (Figure 1). In contrast, internal  $\text{CO}_2$  (measured inside the fruits) was significantly affected by the coating materials and storage temperatures. The lowest internal  $\text{CO}_2$  was measured among the chitosan-treated fruits kept at 7 °C (52  $\mu\text{g}$ ), while the highest level was measured among fruits subjected to the 1.5 °C-LbL and 1.5 °C-gelatin treatments (94 and 93  $\mu\text{g}$ , respectively). All coating materials reduced the external production of  $\text{CO}_2$ , compared to the control. In general, the internal  $\text{CO}_2$  of fruits kept at 1.5 °C was significant higher (84  $\mu\text{g}$ ) than that of the fruits kept at 7 °C (60  $\mu\text{g}$ ). An interaction was found between the coating materials and the storage temperatures ( $F = 0.01$ ; Table 4).



**Figure 1.** The effects of the different coatings and storage temperatures on external and internal CO<sub>2</sub> production of pepper fruits after 21 d at 7 or 1.5 °C, followed by 3 d at 21 °C. Mean data from three harvests are presented, with 5 replications for each harvest ± S.E.

**Table 4.** External and internal CO<sub>2</sub> as means of coating materials and storage temperatures, and table of variance.

Treatment	External CO <sub>2</sub> (µg/g FW/h)	Internal CO <sub>2</sub> (µg/g FW/h)
Mean of coating materials data		
Control	56 a	73 ab
Chitosan	28 c	63 b
Gelatin	35 b	74 a
Chitosan+gelatin (LbL)	30 bc	79 a
LSD	2.06	2.89
Mean of storage temperature data		
7 °C	36 a	60 b
1.5 °C	38 a	84 a
LSD	1.45	2.04
Table of variance ( <i>F</i> -value)		
Coating (C)	****	***
Temperature (T)	NS	****
C × T	NS	**

\* Means within columns followed by the same letter do not differ according to Tukey's HSD test at  $p = 0.05$ . \*\*, \*\*\* and \*\*\*\* indicate significance at  $\alpha$ -levels of 0.01, 0.001 and 0.0001, respectively. NS—not significant.

#### 4. Discussion

Today's consumers demand high-quality, fresh produce that contains essential, health-promoting compounds. Sweet pepper is considered a very healthy fruit with high levels of vitamin C and antioxidants [1]. However, this fruit has a relatively short shelf life. The

optimal storage temperature for sweet colored pepper is 7 °C. Below this temperature, pepper will suffer from severe chilling injury and decay development [2]. However, subjecting harvested pepper fruits to a hot-water treatment and then keeping them in plastic bags has been shown to allow the fruit to keep for 3 weeks at 1.5 °C (as a quarantine treatment) [3]. Finding an effective way to prolong pepper storage, especially at temperatures below 4 °C, without plastic materials, is a matter of great practical significance. Edible coatings are a new, sustainable and safe approach for extending the marketing period for food products [12,13]. They delay spoilage by inhibiting oxidation, protecting against pathogens, and reducing water loss [6].

Among the studied coating materials and treatment combinations, chitosan applied alone was associated with the lowest weight loss, the greatest fruit firmness, the lowest incidence of decay and the lowest incidence and severity of chilling injury after 3 weeks storage at 7 °C or 1.5 °C. In previous work, Poverenov et al. [9] found that coating peppers with chitosan or a mixture of chitosan and gelatin could allow those fruits to maintain their external and internal qualities after 3 weeks of storage at 7 °C. However, a gelatin coating was not as effective in extending peppers' storability and shelf-life. It is possible that the 1% gelatin treatment may have been phytotoxic and damaged the fruit, based on the external quality parameters (Tables 1, 2 and 4). In a previous study, a chitosan-based coating was found to control fungal decay, alleviate weight loss and preserve the postharvest quality of fruits stored at an optimal temperature [5,14].

The low weight loss of chitosan-coated fruit (Table 1) is most likely due to the fact that chitosan impedes the transfer of water vapors, seals minor cracks and protects the fruit surface from mechanical injury, all of which reduce water loss during prolonged storage [14]. It is also possible that the relatively low weight loss among chitosan-coated fruit was due to the formation of a semi-permeable layer between the pepper fruit and the storage environment, as reported by Poverenov et al. [9].

Fruit coated with chitosan exhibited the lowest incidence of decay at both storage temperatures (Table 1). The chitosan coating may act as a barrier between the treated produce and pathogens, suppressing the incidence of disease [14]. Moreover, coating compounds might have intrinsic antioxidant and antimicrobial properties [15]. Chitosan is known to regulate several genes in plants, including genes involved in the activation of plant defense-signaling pathways. It is also known to indirectly inhibit fungal development and the incidence of decay. Chitosan can also act directly on pathogens through surface interactions that cause leaks to form in the pathogens' cell walls or by penetrating the nuclei of pathogenic microorganisms and inhibiting their synthesis of proteins and mRNA [5]. In addition, chitosan might increase antioxidant activity, as suggested by the data presented in Table 3, especially at 1.5 °C, which could further reduce decay development [16,17].

In previous studies, keeping pepper fruits below 4 °C for 3 weeks was found to be an effective way to eliminate quarantine insects [3,18]. However, to reduce fruit susceptibility to chilling injury at temperatures of 4 °C and below, the fruit was first treated with hot water, to trigger the formation of heat-shock proteins, which induced defense barriers against CI. That fruit was then packed in plastic bags that reduced the rate of water loss, which helped to maintain the integrity of the fruits' cell membranes [19]. The manifestation of CI symptoms has mainly been associated with problems such as cell-membrane alterations, physiological disorders and elevated levels of reactive oxygen species (ROS) [20]. Chitosan may act as an exogenous agent to induce several defense mechanisms, such as antioxidant metabolism and the accumulation of antifungal compounds.

At both temperatures, color development was slightly delayed by the chitosan coating, suggesting a delay in the rate of ripening due to suppression of metabolic activities that ultimately led to the inhibition of carotenoid synthesis (Table 1) [21,22]. Delaying ripening, even slightly, can extend storability and marketing by a few more days. Moreover, coatings act as a semipermeable barrier that restricts the movement of gases and reduces the rate of fruit respiration [23]. The 2% chitosan coating demonstrated good CO<sub>2</sub> gas-permeability properties and was associated with a lower fruit respiration rate than the control and the

two other coating treatments (Table 4), as also reported by others [23,24]. It is also possible that the chitosan coating reduced the respiration rate by limiting exposure to ambient O<sub>2</sub>. The higher levels of CO<sub>2</sub> in the gelatin- and LbL-coated fruits that were kept at 1.5 °C were due to highly prevalent and severe CI, as recently reported [25].

## 5. Conclusions

Chitosan is a polysaccharide carbohydrate with a high molecular weight that is soluble in organic acids. It is considered to be a natural biodegradable polymer and a non-toxic material [26]. Chitosan could be considered eco-friendly or a possible potential alternative to the synthetic chemicals that can no longer be used for the control of different postharvest pathogens on many types of fresh harvested produce [15]. In addition, the natural wax coating on the surface of pepper fruits is damaged during handling and transportation, resulting in damage to the fruits. The application of edible coatings can play a significant role in preventing mechanical damage and subsequent losses during postharvest handling and transportation.

Chitosan was found to maintain the quality of sweet peppers stored even at 1.5 °C, by limiting water loss to keep the fruit firm and reducing decay development and, especially, chilling injury, without affecting the peppers' nutritional content. Chitosan slightly inhibited ripening as shown by delayed color development, which could be favorable for prolonging storage life and maintaining pepper quality during 3 weeks of storage at 1.5 °C. However, more cheap edible coating materials should be examined individually or in combination (e.g., LbL), in order to find the most useful material for maintaining the quality of fresh produce. Those coating materials should be easy to apply automatically on the sorting and grading lines in packinghouses. From a practical point of view, fruit coatings may replace the plastic materials that are currently used to reduce water loss and chilling injury during storage at sub-optimal temperatures.

**Author Contributions:** S.K., conducted the research as part of her Master's degree program; S.A.-T. and D.C., research engineers that helped analyze some of the experiments; E.P. and E.F., supervised S.K. in her degree program, validated the results, wrote, edited and reviewed the article. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded, in part, by the Vegetable Division of the Israeli Plant Production and Marketing Board #430-0693-18.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

## References

1. Wahyuni, Y.; Ballester, A.R.; Sudarmonowati, E.; Bino, R.J.; Bovy, A.G. Secondary metabolites of *Capsicum* species and their importance in the human diet. *J. Nat. Prod.* **2013**, *76*, 783–793. [[CrossRef](#)] [[PubMed](#)]
2. Lama, K.; Alkalai-Tuvia, S.; Chalupowicz, D.; Fallik, E. Extended storage of yellow pepper fruits at suboptimal temperatures may alter their physical and nutritional quality. *Agronomy* **2020**, *10*, 1109. [[CrossRef](#)]
3. Fallik, E.; Perzelan, Y.; Alkalai-Tuvia, S.; Nemny-Lavy, E.; Nestel, D. Development of cold quarantine protocols to arrest the development of the Mediterranean fruit fly (*Ceratitidis capitata*) in pepper (*Capsicum annuum* L.) fruit after harvest. *Postharvest Biol. Technol.* **2012**, *70*, 7–12. [[CrossRef](#)]
4. Sharma, P.; Kehinde, B.A.; Kaur, S.; Vyas, P. Application of edible coatings on fresh and minimally processed fruits: A review. *Nutr. Food Sci.* **2019**, *49*, 713–738. [[CrossRef](#)]
5. Sharif, M.; Mujtaba, M.; Rahman, M.U.; Shalmani, A.; Ahmad, H.; Anwar, T.; Tianchan, D.; Wang, X. The multifunctional role of chitosan in horticultural crops: A review. *Molecules* **2018**, *23*, 872. [[CrossRef](#)] [[PubMed](#)]
6. Ncama, K.; Magwaza, L.S.; Mditshwa, A.; Tesfay, S.Z. Plant-based edible coatings for managing postharvest quality of fresh horticultural produce: A review. *Food Packag. Shelf Life* **2018**, *16*, 157–167. [[CrossRef](#)]

7. Arnon-Rips, H.; Poverenov, E. Improving food products' quality and storability by using layer by layer edible coatings. *Trends Food Sci. Technol.* **2018**, *75*, 81–92. [[CrossRef](#)]
8. Arnon, H.; Granit, R.; Porat, R.; Poverenov, E. Development of polysaccharide-based edible coatings for citrus fruits: A layer-by-layer approach. *Food Chem.* **2015**, *166*, 465–472. [[CrossRef](#)]
9. Poverenov, E.; Zaitsev, Y.; Arnon, H.; Granit, R.; Alkalai-Tuvia, S.; Perzelan, Y.; Weinberg, T.; Fallik, E. Effects of a composite chitosan-gelatin edible coating on postharvest quality and storability of red bell peppers. *Postharvest Biol. Technol.* **2014**, *96*, 106–109. [[CrossRef](#)]
10. Fallik, E.; Grinberg, S.; Alkalai, S.; Yekutieli, O.; Wiseblum, A.; Regev, R.; Bar-Lev, E. A unique rapid hot water treatment to improve storage quality of sweet pepper. *Postharvest Biol. Technol.* **1999**, *15*, 25–32. [[CrossRef](#)]
11. Vinokur, Y.; Rodov, V. Method for determining total (hydrophilic and lipophilic) radical-scavenging activity in the same sample of fresh produce. *Acta Hortic.* **2006**, *709*, 53–61. [[CrossRef](#)]
12. Shi, Z.; Wang, F.; Lu, Y.; Deng, J. Combination of chitosan and salicylic acid to control postharvest green mold caused by *Penicillium digitatum* in grapefruit fruit. *Sci. Hort.* **2018**, *233*, 54–60. [[CrossRef](#)]
13. Zhou, Y.; Ma, J.; Xie, J.; Deng, L.; Yao, S.; Zeng, K. Transcriptomic and biochemical analysis of highlighted induction of phenylpropanoid pathway metabolism of citrus fruit in response to salicylic acid, *Pichia membranaefaciens* and oligochitosan. *Postharvest Biol. Technol.* **2018**, *142*, 81–92. [[CrossRef](#)]
14. Galus, S.; Kibar, E.A.A.; Gniewosz, M.; Krasniewska, K. Novel materials in preparation of edible films and coatings—A review. *Coating* **2020**, *10*, 674. [[CrossRef](#)]
15. Vieira, J.M.; Flores-López, M.L.; de Rodríguez, D.J.; Sousa, M.C.; Vicente, A.A.; Martins, J.T. Effect of chitosan–aloe vera coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. *Postharvest Biol. Technol.* **2016**, *116*, 88–97. [[CrossRef](#)]
16. Khorram, K.; Ramezani, A. A cinnamon essential oil incorporated in shellac, a novel bio-product to maintain quality of 'Thomson navel' orange fruit. *J. Food Sci. Technol.* **2020**, *58*, 2963–2972. [[CrossRef](#)]
17. Shah, S.; Hashmi, M.S. Chitosan–aloe vera gel coating delays postharvest decay of mango fruit. *Hortic. Environ. Biotechnol.* **2020**, *61*, 279–289. [[CrossRef](#)]
18. Bar-Yosef, A.; Alkalai-Tuvia, S.; Perzelan, Y.; Aharon, Z.; Ilić, Z.; Lurie, S.; Fallik, E. Effect of shrink packaging in combination with rinsing and brushing treatment on chilling injury and decay of sweet pepper during storage. *Adv. Hortic. Sci.* **2009**, *23*, 225–230.
19. Fallik, E.; Bar-Yosef, A.; Alkalai-Tuvia, S.; Aharon, Z.; Perzelan, Y.; Ilić, Z.; Lurie, S. Prevention of chilling injury in sweet bell pepper stored at 1.5 °C by heat treatments and individual shrink packaging—3 years of research. *Folia Hortic.* **2009**, *21*, 87–97. [[CrossRef](#)]
20. Hashim, N.F.A.; Ahmad, A.; Bordoh, P.K. Effects of chitosan coating on chilling injury, antioxidant status and postharvest quality of Japanese cucumber during cold storage. *Sains Malays.* **2017**, *45*, 287–294.
21. Petriccione, M.; de Sanctis, F.; Pasquariello, M.S.; Mastrobuoni, F.; Rega, P.; Scortichini, M.; Mencarelli, F. The effect of chitosan coating on the quality and nutraceutical traits of sweet cherry during post-harvest life. *Food Bioprocess Technol.* **2015**, *8*, 394–408. [[CrossRef](#)]
22. Gayed, A.A.N.A.; Shaarawi, S.A.M.A.; Elkhishen, M.A.; Elsherbini, N.R.M. Pre-harvest application of calcium chloride and chitosan on fruit quality and storability of 'Early Swelling' peach during cold storage. *Ciência Agrotecnol.* **2017**, *41*, 220–231. [[CrossRef](#)]
23. Lin, B.; Du, Y.; Liang, X.; Wang, X.; Wang, X.; Yang, J. Effect of chitosan coating on respiratory behavior and quality of stored litchi under ambient temperature. *J. Food Eng.* **2011**, *102*, 94–99. [[CrossRef](#)]
24. Kumar, P.; Sethi, S.; Sharma, R.R.; Srivastav, M.; Varghese, E. Effect of chitosan coating on postharvest life and quality of plum during storage at low temperature. *Sci. Hortic.* **2017**, *226*, 104–109. [[CrossRef](#)]
25. Madhav, J.V.; Sethi, S.; Sharma, R.R.; Nagaraja, A.; Arora, A.; Varghese, E. Influence of bilayer coating of salicylic acid and edible wax on chilling injury and functional attributes of guava. *J. Food Process. Preserv.* **2021**, *45*, e15601. [[CrossRef](#)]
26. Drevinskas, T.; Naujokaitytė, G.; Maruška, A.; Kaya, M.; Sargin, I.; Daubaras, R.; Česonienė, L. Effect of molecular weight of chitosan on the shelf life and other quality parameters of three different cultivars of *Actinidia kolomikta* (kiwifruit). *Carbohydr. Polym.* **2017**, *173*, 269–275. [[CrossRef](#)] [[PubMed](#)]