Research note

Effects of a composite chitosan–gelatin edible coating on postharvest quality and storability of red bell peppers

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ABSTRACT

For the first time, a composite chitosan–gelatin (CH–GL) coating was applied to peppers and its effects on fruit quality and storability were examined. Pure chitosan (CH) and gelatin (GL) coatings were studied for comparison. The CH coating inhibited microbial spoilage and prolonged the possible storage period. The GL coating contributed to fruit firmness, but did not allow for prolonged storage. The composite CH–GL coating was associated with a two-fold decrease in microbial decay, significantly (p ≤ 0.05) enhanced fruit texture and prolonged the possible period of cold storage up to 21 days and fruit shelf-life up to 14 days, without affecting the respiration or nutritional content of the fruit.

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1. Introduction

Red bell peppers (Capsicum annuum L.) are one of the most popular health-promoting crops traded on the global market (AgMRC, 2011). The crucial problem facing marketers of this crop is its relatively short shelf-life, which stands at about 2 weeks and limits exports to distant markets. The primary reasons for pepper quality deterioration are water loss and microbial decay that is mainly caused by Alternaria alternata and Botrytis cinerea (Fallik et al., 1999). Finding an effective approach to prolong pepper storage is a matter of great practical significance. Edible coatings based on natural materials are a promising safe and healthy tool for extending the shelf-life of fresh agricultural products (Dhall, 2013). Polysaccharide chitosan is widely used for the formation of edible coatings due to its inherent antimicrobial properties (Dutta et al., 2009). The addition of gelatin was reported to enhance the efficacy of chitosan formulations (Pereda et al., 2011). To the best of our knowledge, composite chitosan coatings have not been previously applied on pepper fruit. Moreover, the effect of pure chitosan coating on the quality and storability of peppers has received only a small amount of research attention (El Ghaouth et al., 1991).

The goal of the current work was to improve the physiological and microbial quality of red bell peppers and to prolong the period of time for which these fruit can be stored. For this purpose, a composite chitosan–gelatin (CH–GL) edible coating was utilized. The effects of pure chitosan (CH) and gelatin (GL) coatings on fruit quality were also studied and compared with those of the combined CH–GL coating.

2. Materials and methods

Red bell peppers (Capsicum annuum L. cv. Vergasa) were harvested from the Arava valley in the south of Israel in the winter season, then brushed and dried, as previously described (Fallik et al., 1999). The fruit were randomly packed in 3–5 kg cartons, 15–20 fruit in each. Four treatments were conducted: (a) chitosan coated peppers, (b) gelatin coated peppers, (c) chitosan–gelatin coated peppers and (d) uncoated peppers which served as a control. For each treatment three cartons were used for replicates. Three experiments were performed. The experiments differed in their storage conditions. (a) Regular storage (14/5) = 14 days at 7 °C and RH of 95%, then 5 days at 20 °C and RH of 75%. (b) Prolonged cold storage (21/5) = 21 days at 7 °C and RH of 95%, then 5 days at 20 °C and RH of 75%. (c) Long shelf storage (14) = 14 days at 20 °C and RH of 75% with no prior cold storage. Each of the described experiments included three coating treatments and control. For each experiment, all fruit were collected at the same time, treatments were applied at the same time, quality examinations were performed at the same time. The results were compared within each experiment.
Gelatin (GL) coating. Gelatin powder (Sigma–Aldrich) was dissolved in sterilized Double Distilled Water, DDW (1%, w/v) and the solution was stirred at 45 °C for 45 min. Chitosan (CH) coating. Chitosan powder (Sigma–Aldrich) was dissolved in sterilized DDW (2%, w/v) that included 0.7% of acetic acid (Sigma–Aldrich) and the solution was stirred at 30 °C for 2 h. Chitosan–gelatin (CH–GL) coating. To a chitosan (2%, w/v) solution prepared as described above, gelatin powder (1%, w/v) was added and the solution was stirred at 45 °C for 45 min. Peppers were hand-coated with the cold coating solutions by a paint brush and dried in a drying tunnel for 2 min at 38 °C.

Fruit firmness was measured at zero time and at the end of each storage period as previously described (Hamson, 1952) utilizing an Inpekt 5 dynamic firmness analyzer (Hegewald and Peschke, Germany). Fruit weight loss was evaluated by weighing fruit at zero time and at the end of each storage period and calculating the percentage weight loss. For ethanol, acetaldehyde and carbon dioxide measurements, 5 mL air samples were withdrawn from the fruit internal atmosphere using a gas-tight syringe and injected into the gas chromatograph (GC). The ethanol and acetaldehyde concentrations were analyzed with a Varian 3300 GC equipped with a flame ionization detector and 20% Carbowax 20 M packed column using helium as the carrier gas. Column, injector and detector temperatures were 80, 110 and 180 °C, respectively. The carbon dioxide concentration was analyzed by a Gow–Mac Series 580 GC equipped with a thermal conductivity detector and Alltech Chromosorb 80/100 (1/8 in. × 1.2 m) column after passage through a molecular sieve 5 Å 45/60 (1/8 in. × 1.2 m). The oven, injector and detector temperatures were 35, 110 and 150 °C, respectively. Total soluble solids (∼50 μL of the juice from 1 g of fruit sample) were measured by a digital Refractometer (Atago, Japan). To measure biochemical parameters, peppers were cut in a 2.5 cm × 2.5 cm, freeze-dried, frozen using liquid nitrogen and crushed. Each sample represented a blend of 15 different fruit from the same treatment. The ascorbic acid concentration was measured by the enzyme kit (Ascorbic Acid TEST Kit- lot- H11995 Hanna Instruments, USA) utilizing a previously reported method (Beutler and Beinstingl, 1980). Total phenol content was analyzed using the Folin–Ciocalteu colorimetric method (Remorini et al., 2008). Antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl DPPH radical scavenger (Sanchez-Moreno et al., 1998). Fruit decay is expressed as percentage of the infected fruit in a box. Fruit were considered decayed once fungal mycella appeared on pericarp or calyx. For fruit inoculation, B. cinerea inoculum was prepared as described by El Chaout et al. (1992). Fruit were washed with 70% ethanol and then punctured by a 1.5 mm diameter nail. Each wound site was inoculated with 40 μL of a spore suspension (10^6 spore/mL). Fruit were stored at 20 °C (RH 95%) for 24 h and then coated with CH, GL and CH–GL coatings. Control was coated with DDW. The experiment was maintained for 12 days.

For each test 15 randomly selected fruit from each treatment (=60 fruit for test) were tested. Microsoft office excel spreadsheets were used to calculate the means, standard deviations and standard errors. Statistical analysis was performed by JMP 7 (SAS Institute Inc., Cary, NC, USA), including a LS Means Differences Tukey HSD.

3. Results and discussion

3.1. Prolonging fruit storability

In the first experiment, the fruit were stored under typical pepper storage conditions (14 days at 7 °C and then 5 days at 20 °C) and it was confirmed that the coatings did not cause negative effects. In the second experiment, the cold-storage (7 °C) was extended to 21 days. In the third experiment, the shelf-storage (20 °C) was extended to 14 days.

Peppers firmness is shown in Fig. 1. After regular storage (14/5 days), slight texture enhancement caused by the composite CH–GL coating was observed. After the extended cold storage, uncoated fruit showed dramatic texture degradation, whereas significantly less degradation was observed among the fruit coated with CH or GL. Notably, the firmness of the fruit coated with the composite CH–GL coating remained practically unchanged from the zero-time measurement. After the prolonged shelf-life storage, uncoated fruit and fruit coated with GL only showed dramatic texture degradation, CH-coated peppers showed significantly less texture degradation and, as in previous experiment, the CH–GL coated fruit showed superior firmness. Since they are hollow, peppers are very sensitive to respiratory changes.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>14 days at 7 °C</th>
<th>5 days at 20 °C</th>
<th>21 days at 7 °C</th>
<th>5 days at 20 °C</th>
<th>14 days at 20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH–GL</td>
<td>4.33 ± 0.37 a</td>
<td>6.52 ± 0.15 b</td>
<td>8.27 ± 0.29 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>4.08 ± 0.31 a</td>
<td>5.89 ± 0.13 c</td>
<td>8.12 ± 0.27 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GL</td>
<td>3.99 ± 0.14 a</td>
<td>5.87 ± 0.13 c</td>
<td>10.60 ± 0.27 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.98 ± 0.13 a</td>
<td>7.28 ± 0.20 a</td>
<td>9.33 ± 0.24 b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2

CO₂ in the pepper internal atmospheres. The data represent means of fifteen replications, 95% t-based confidence intervals. The values in columns followed by the different letter are significantly (at p < 0.05) different according to Tukey–Kramer HSD.

<table>
<thead>
<tr>
<th></th>
<th>14 days at 7 °C</th>
<th>5 days at 20 °C</th>
<th>21 days at 7 °C</th>
<th>5 days at 20 °C</th>
<th>14 days at 20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH–GL</td>
<td>2.02 ± 0.24 a</td>
<td>2.39 ± 0.12 a</td>
<td>1.25 ± 0.04 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>1.99 ± 0.24 a</td>
<td>1.75 ± 0.14 bc</td>
<td>0.87 ± 0.05 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GL</td>
<td>2.02 ± 0.15 a</td>
<td>2.02 ± 0.14 ab</td>
<td>1.18 ± 0.11 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.76 ± 0.19 a</td>
<td>1.45 ± 0.09 c</td>
<td>0.88 ± 0.05 b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3

Decay incidence of the coated and uncoated peppers after 21 days at 7 °C and additional 5 days at 20 °C. An infection diameter of the peppers inoculated with B. cinerea measured after 12 days of storage at 7 °C. The data represent means of fifteen replications, 95% t-based confidence intervals. The values in columns followed by the different letter are significantly (at p < 0.05) different according to Tukey–Kramer HSD.

<table>
<thead>
<tr>
<th></th>
<th>Decay incidence (%)</th>
<th>21/5 days</th>
<th>Infection diameter (cm)</th>
<th>12 days post inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH–GL</td>
<td>10.62 ± 2.21 b</td>
<td>2.51 ± 0.14 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>7.42 ± 3.59 b</td>
<td>2.31 ± 0.19 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GL</td>
<td>17.34 ± 1.98 a</td>
<td>2.75 ± 0.15 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25.32 ± 7.06 a</td>
<td>3.08 ± 0.15 a</td>
<td></td>
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</tbody>
</table>
to structural deterioration. By utilizing a combination of the beneficial features of CH and GL coating components, we succeeded in achieving a notable enhancement of the pepper structure. Adhesive GL contributes to the formation of a homogeneous coating and helps to inhibit naturally occurring fruit senescence. Meanwhile, antimicrobial CH reduces damage caused by microorganisms (Dutta et al., 2009; Ali et al., 2011). The favorable influence of the two components was also clearly visible after the long shelf-life storage treatment; following that treatment, the fruit coated with the CH–GL coating had the highest level of firmness. The synergistic effect of various components on coating performance was also reported in our recent work (Poverenov et al., 2014).

The amount of weight lost by the peppers was also measured (Table 1). No significant differences in pepper weight loss were observed after the regular storage period. After prolonged cold storage (21/5), all of the coated peppers had retained more of their original weight than the uncoated fruit. In this regard, the individual CH and GL coatings were more effective than the CH–GL coating. On the other hand, after the prolonged shelf-life storage (14 days) treatment, the CH and CH–GL coatings significantly inhibited water loss; whereas the GL coating promoted water loss. The pattern observed among the weight loss data was similar, but not identical to that observed for fruit texture. For both weight loss and texture, the effect of an antimicrobial component became more important in the context of the long shelf-life storage treatment. However, the beneficial effect of the composite coating was more pronounced in the firmness studies. This could be due to the fact that the effect of cell wall damage (which is effectively reduced by a composite coating) is more significant in the context of fruit firmness than in the context of fruit weight loss.

CO₂ concentration was measured to follow the respiration process and ethanol and acetaldehyde concentrations were measured to follow fermentation. An increase in the concentration of CO₂ was observed following the application of the edible coatings (Table 2). However, in all of the examined treatments, CO₂ values were within the normal range of pepper fruit respiration (Villavicencio et al., 2001). Examination of ethanol or acetaldehyde content showed negligible (0–0.6 μL L⁻¹) concentrations in all treatments (data not shown). The gas-exchange problem is known to restrict the application of edible coatings on fresh products (Dhall, 2013). The coatings did not harm fruit respiration and did not cause fermentation. These findings strengthen their candidacy for use as coatings on fresh produce.

3.2. Inhibition of microbial spoilage of fruit

The incidence of fruit decay following prolonged storage is shown in Table 3. The CH and CH–GL coatings significantly inhibited the incidence of microbial decay, which was 7.4% among the CH-coated fruit and 10.6% among the fruit coated with CH–GL. This is a two- to three-fold inhibition as compared to the uncoated peppers, among which a 25.3% incidence of decay was observed. The GL coatings barely inhibited decay. In addition to the evaluation of general decay, antimicrobial studies involving specific pathogens were performed. The fruit were artificially inoculated with B. cinerea, an important pathogen of pepper plants (Fallik et al., 1999). To the best of our knowledge, this is the first study to examine the effect of an edible coating on the inhibition of microbial development in inoculated fruit. The diameter of the infection spots observed on peppers coated with CH or CH–GL was significantly less than that observed on the uncoated fruit (Table 3). The antimicrobial activity of chitosan is one of the main reasons for its widespread use as an edible coating on fresh fruit, with or without additional antimicrobial reagents (Dutta et al., 2009; Ali et al., 2011). It has been demonstrated that chitosan antifungal activity is due to an activation of defense enzymes (Edirisinghe et al., 2012). The CH and CH–GL coatings exhibited significant antimicrobial activity when applied alone, without any additional antimicrobial components. Adding gelatin to the coating did not diminish the antimicrobial effect.

3.3. Effect of the coatings on the biochemical parameters of the fruit

The fruit biochemical parameters, including ascorbic acid content, phenol content, antioxidant content and total soluble solids content were examined (Table 4). Neither the CH nor the CH–GL edible coatings affected any of the measured biochemical parameters. The peppers coated with GL showed enhanced phenol contents. This observation is important, since several edible coatings have been reported to affect biochemical parameters of fresh products. For instance, an increase in phenol and antioxidant content and a decrease in the ascorbic acid content of strawberries upon application of 1.5% chitosan coatings were recently reported (Wang and Gao, 2013).

4. Conclusions

For the first time, the effect of a composite CH–GL coating on the quality of pepper fruit was examined. Due to the combination of the beneficial features of the two components, the CH–GL coating resulted in notable fruit structure enhancement, inhibited decay and improved storability. The proposed coatings are biodegradable, easily applied and inexpensive. Currently, pepper can be kept for up to 2 weeks in cold storage and no more than 5 days under shelf conditions. Utilizing the composite coating described here, we succeeded in extending the pepper storage period to 3 weeks under cold-storage conditions and to 14 days on the shelf, with no loss of fruit quality. Since peppers are one of the most popular agricultural products, this achievement is of great practical importance for farmers, suppliers and consumers. We hope our findings will also contribute to edible coatings research, in general, and help to promote the application of composite coatings as a postharvest treatment of fresh agricultural produce.

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