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Effect of oligosaccharides derived from *Laminaria japonica*-incorporated pullulan coatings on preservation of cherry tomatoes

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ABSTRACT

Laminaria japonica-derived oligosaccharides (LJOs) exhibit antibacterial and antioxidant activities, and pullulan is a food thickener that can form impermeable films. The ability of pullulan coatings with various LJO concentrations (1% pullulan + 0.1%, 0.2% or 0.3% LJOs) to preserve cherry tomatoes during storage at room temperature was investigated. The LJO-incorporated pullulan coatings were found to effectively reduce respiratory intensity, vitamin C loss, weight loss and softening, as well as to increase the amount of titratable acid and the overall likeness of fruit compared with the control. These effects were observed to be dose-dependent. Therefore, using LJO-incorporated pullulan coatings can extend the shelf life of cherry tomatoes.

1. Introduction

The consumption of fresh, and naturally grown, fruit and vegetables is becoming increasingly popular to consumers. Cherry tomato (*Solanum lycopersicum* L) is rich in vitamins C and β-carotene and is thus one of the most popular vegetables worldwide (Zhao et al., 2010).

Cherry tomato is a seasonal fruit that is highly perishable due to transpiration, pathogenic invasion, and rapid ripening and aging (Zapata et al., 2008). Therefore, safe and effective methods for extending their shelf life are desirable. The optimum conditions for cold storage effectively extend the shelf life of cherry tomatoes, although the quality of fruit is not sufficiently preserved through this method (Fagundes, Palou, Monteiro, & Pérez-Gago, 2014).

A number of studies have reported that *Laminaria japonica*-derived oligosaccharides (LJOs) exhibit antioxidant and antibacterial activities (Chen, Tian, Deng, & Fang, 2012; Li, Li, & Guo, 2010; Peng, Liu, Fang, & Zhang, 2012; Wang, Zhang, Zhang, & Li, 2008; Wu, 2014; Xu et al., 2010). Meanwhile, pullulan can form thin films that are transparent, oil resistant and impermeable to oxygen, which can be used as coating and packaging material (Deshpande, Rale, & Lynch, 1992).

Therefore, the aim was to investigate the ability of pullulan coatings with LJOs to extend the shelf life of cherry tomatoes, at room temperature. In addition, we investigated the effects of pullulan film on the respiratory rate, vitamin C (V_C) content, amount of titratable acid, weight loss, firmness, and overall likeness to the control.

2. Methods and materials

2.1. Materials

Cherry tomatoes of uniform size, appearance and ripeness were purchased from a local market, and *L. japonica* was purchased from a local supermarket (Xinpu, China). The cold-adapted α-amylase produced by the *Escherichia coli* BL21/pEtac-amy and derived from marine *Pseudoalteromonas arctica* GS230 was prepared in our laboratory (Lu et al., 2010). Pullulan, with molecular weight 2.7×10^5, was purchased from Pharmacopoeia, Japan. H_2O_2 (30%, v/v) was purchased from the Laiyang Kant Chemical Co., Ltd. (Laiyang, China). All other chemicals were of reagent grade.

2.2. Preparation of LJOs

The shredded *L. japonica* was washed with tap water, dried in hot air oven (JK-OOI-240A, China) for 6 h at 70 °C, pulverised and then sifted through a 60-mesh sieve. The lipids in the dried powder

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were extracted using a Soxhlet extractor, light petroleum was the solvent. The extracted lipids were suspended in distilled water to yield a 1% suspension (w/v). About 12 U/g of cold-adapted α-amylase produced by *E. coli* BL21/pEtac-amy, derived from marine *P. arctica* GS230, was added to the reactor containing the suspension; this reactor was maintained in a thermostatic water bath for 4 h at 20 °C. An aliquot of the suspension was withdrawn and was tested for the presence of starch using an iodine solution. These experimental procedures were repeated to guarantee that the starch was completely removed from the suspension. Subsequently, H_2O_2 (4%) was added into the reactor containing 200 ml of the suspension, and then the reactor was incubated in a thermostatic water bath for 24 h at 75 °C. The hydrolysates were filtered and concentrated to approximately 15%. The proteins were collected using the Sevag method, precipitated using 5 volumes of absolute ethanol, filtered for the second time, and then freeze-dried.

2.3. LJO characterization

The ash, moisture, protein, and total sugar contents of the samples were determined according to standard methods (Hou, 2004). The amount of reducing sugars was estimated using the Somogyi method and was expressed as a dextrose equivalent (DE) value (Nelson, 1944).

2.4. Dipping of cherry tomato and storage conditions

The four dipping solutions include the (1) control (deionised water), (2) 0.1% LJOs + 1% pullulan, (3) 0.2% LJOs + 1% pullulan and (4) 0.3% LJOs + 1% pullulan. The cherry tomatoes were immersed into the dipping solutions for 3 min. The residual solutions on the cherry tomatoes were allowed to drip for 3 min. The cherry tomatoes were then stored at ~20 °C for 14 days.

2.5. Measurement of respiration rate

The effect of the pullulan coating on the respiration rate of the cherry tomatoes was measured by analyzing the headspace gas composition. The cherry tomatoes (100 g) were stored in a 100 ml tightly sealed glass container for 24 h at 25 °C. The headspace samples were withdrawn at various time intervals and were analyzed for CO_2 content using a Trace 2000 GC series gas chromatograph and a Thermo mass spectrometer. A SGE BPx70 column (60 m × 0.25 mm, 0.25 mm film thickness) was used. Helium was the carrier gas at 35 ml/min. The temperature of the injector, detector and column was 50, 100 and 50 °C, respectively (Qi, Hu, Jiang, Tian, & Li, 2010).

2.6. V_C analysis

V_C was analyzed as described previously (Fan, Sokorai, Engemann, Gurtler, & Liu, 2012). Briefly, four fruits were cut into pieces and 10 g of the mixed pieces was homogenized in 20 ml 5% metaphosphoric acid (62.5 mM), using a homogeniser (Virtishear, Virtis, Gardiner, NY, USA) at a speed of 70/min. The homogenate was subsequently filtered and centrifuged. Aliquots of the supernatant after filtration through a 0.45 μm membrane were injected into the Hewlett Packard Ti-series 1050 HPLC system (Agilent Technologies, Palo Alto, CA, USA), equipped with an Aminex HPX-87H organic acid column (300 mm × 7.8 mm). V_C was monitored at 245 nm, and the sample V_C content was calculated from a V_C standard curve.

2.7. Titratable acidity (TA) analysis

The TA of the tomato juice was determined by titrating 5 ml of the sample with 0.1 mol/l sodium hydroxide to an end point of pH 8.1; the results were expressed as gram of citric acid per liter.

2.8. Firmness measurements

The firmness of the tomatoes was evaluated using TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA), as described by Yun et al. (2015). A probe with 3 mm diameter was used to penetrate the fruit to a depth of 10 mm at a speed of 10 mm/s. Three fruits from the triplicate for a total of nine fruits were used for firmness measurements. The maximum force was recorded using the Texture Expert software (version 1.22, Texture Technologies Corp.).

2.9. Weight loss

The weight of the cherry tomatoes was determined during storage to evaluate the efficacy of pullulan coatings as moisture barriers. The percent weight loss was calculated by weighing the samples every 2 days.

2.10. Sensory evaluation

The cherry tomatoes were washed and their sensory characteristics were evaluated by 18 panellists (age range: 20–25 years old) from the Department of Food Science and Technology. The panellists used the 9-point hedonic scale to compare the likeness of the fruit to the control, where 9 = extremely like; 7 = moderately like; 5 = neither like or nor dislike; 3 = moderately dislike; 1 = extremely dislike (Meilgaard, Civille, & Carr, 2006). The panellists are regular consumers of cherry tomato.

2.11. Statistical analysis

All data are presented as mean ± S.D. Statistical analysis was performed using Statgraphics Centurion XV Version 15.1.02. A multifactor ANOVA with posterior multiple range tests was used to determine the differences in the effects of storage time and dipping condition on color, firmness and microbiological count.

3. Results and discussion

3.1. LJO characterization

The ash, moisture and total sugar contents of the LJOs were 3.19%, 2.03% and 94.76%, respectively. The LJOs did not contain any protein. The DE of the LJOs was 12.14, indicating that the average degree of polymerization was ~9. The light green water soluble LJO powder was similar to the previously obtained LJO powder (Wu, 2014).

3.2. Respiration rate of cherry tomato during storage

The cherry tomatoes mature and age during storage. Fruits and vegetables continuously consume their nutrients for energy and cellular respiration. Greater amounts of nutrients are consumed at higher respiration rate, leading to faster aging and consequently shorter shelf life. Fig. 1 shows the effects of various concentrations of the incorporated LJOs in pullulan coatings on the respiration of cherry tomatoes during storage. Cherry tomatoes are climacteric fruit; under normal temperature, the cherry tomato quickly undergoes through a respiratory climacteric period postharvest, wherein

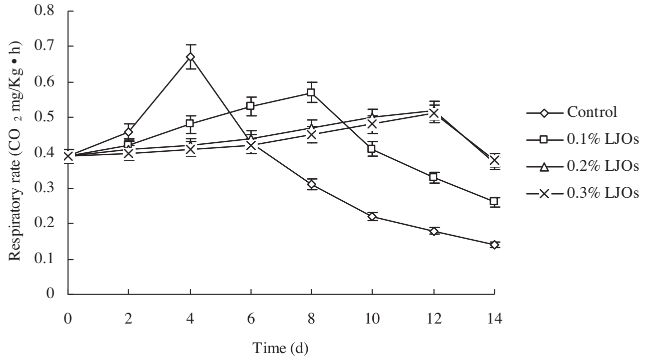

Fig. 1. Effect of pullulan-based coatings incorporated LJOs on respiration rate of cherry tomatoes during storage. Values are the mean of three replicates. Vertical bars represent standard deviation.

a respiration peak occurs, and then the respiratory intensity gradually decreases; consequently, the cherry tomato quickly becomes soft, undergoes senescence and decays thereafter (Yun et al., 2015). The LJO-incorporated pullulan coatings could delay the onset of the respiration peak and reduce the respiratory intensity in the fruit. The respiratory intensity after 8 days of storage was lower by about 50%, in the control group, than in the pullulan-coated group; thus, the coatings effectively inhibited respiration in the cherry tomatoes. The 0.1% LJO group showed a lower degree of inhibition of respiration intensity, although no significant difference was observed between the 0.1% and 0.3% LJO groups.

3.3. V_C content in cherry tomato

V_C maintains the physiological functions in the human body and is a measure of the freshness of fruits. V_C is higher in cherry tomatoes than in most fruits and vegetables, thus, high demands for cherry tomatoes exist. Fig. 2 shows the effect of various concentrations of LJO in pullulan coatings on V_C content of the tomatoes during storage. A similar trend was observed in the treatment and control groups, wherein the V_C content was initially high, and then gradually decreased. This observation may be attributed to the fact that the maximum amount of V_C was reached at the early stage of ripening, which further increased the nutritional value of the tomato. Thereafter, the amount of V_C decreased resulting from oxidation. Lower rate of V_C reduction occurred in the treatment group than in the control, indicating the role of LJO-incorporated pullulan coating on V_C content. The film could inhibit gas exchange between the tissues and the environment, which reduces the O_2 concentration that oxidizes V_C; thus, V_C oxidation was effectively prevented, thereby keeping a higher amount of V_C in the treated fruits.

3.4. TA content

The amount of TA in fruits and vegetables postharvest reflects the efficiency of the assimilation of their nutrients in the body. In addition, TA is an important indicator of the state of preservation of fruits and vegetables. Fig. 3 shows the changes in the amount of TA in the cherry tomatoes during storage. The amount of TA gradually decreased in each group with storage time, the rate of reduction was faster in the control group than in the treatment groups, which may be attributed to the metabolism in the fruit. Metabolic activities continuously occur in fruits and vegetables postharvest. Organic acids are not only the main source of ATP during respiration but also the source of intermediate metabolites of numerous biochemical reactions; thus, the amount of TA decreases during storage. After coating, the rate of respiration in cherry tomatoes was significantly inhibited, and the metabolism of the organic acid was reduced. These results indicate that the LJO-incorporated pullulan coatings could delay the consumption of the nutrients and extend the shelf life of the fruits. LJO (0.2%) significantly improved the preservation of cherry tomatoes as seen in the slow rate of reduction in TA content.

3.5. Weight loss in cherry tomatoes

Fruits and vegetables lose weight postharvest resulting from water loss via respiration and transpiration. In addition, water loss reduces the plumpness and gloss of fruits and vegetables, resulting in the loss of commercial value. Therefore, weight loss rate is an important indicator to measure the degree of preservation of fruits and vegetables. Fig. 4 shows the changes in weight loss rate in cherry tomatoes during storage. All groups exhibited gradual weight loss during storage. In addition, increased rate of weight loss was observed in the control compared with the treatment groups ($p < 0.05$), indicating that coating considerably delayed water loss, thereby extending the shelf life of the fruits. The concentration of LJOs affected the weight loss rate in cherry tomatoes, that is, higher LJO concentration reduced weight loss, although no significant effect on weight loss was observed at LJO concentrations higher than 0.2% ($p > 0.05$), indicating that LJO effectively inhibits water loss at certain amounts only. This observation may be ascribed to the fact that LJOs exhibit antibacterial activity, which prevents dehydration of the fruits caused by pathogens.

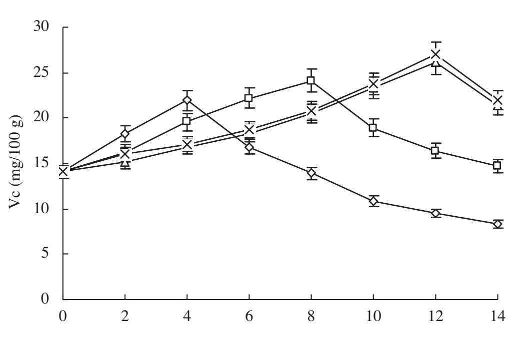

Fig. 2. Effect of pullulan-based coatings incorporated LJOs on vitamin C content of cherry tomatoes during storage. Values are the mean of three replicates. Vertical bars represent standard deviation.

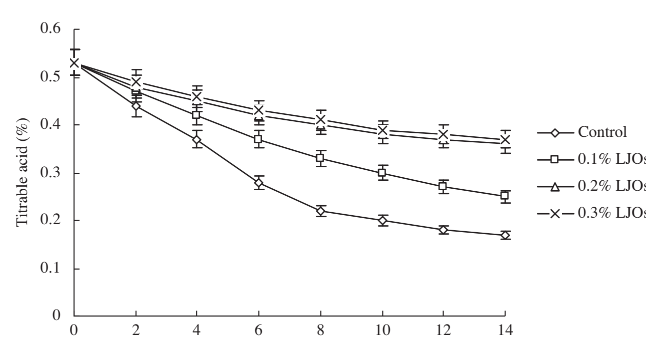

Fig. 3. Effect of pullulan-based coatings incorporated LJOs on titrable acid of cherry tomatoes during storage. Values are the mean of three replicates. Vertical bars represent standard deviation.

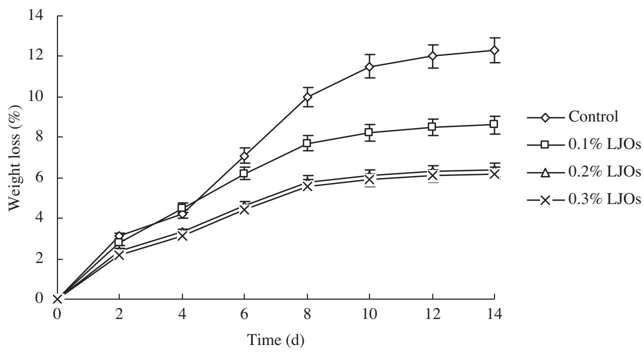

Fig. 4. Effect of pullulan-based coatings incorporated LJOs on weight loss of cherry tomatoes during storage. Values are the mean of three replicates. Vertical bars represent standard deviation.

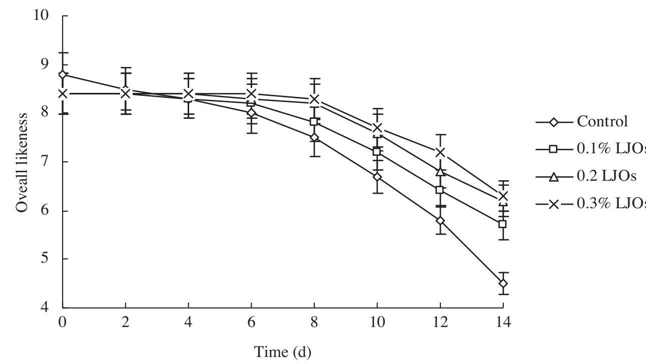

Fig. 6. Effect of pullulan-based coatings incorporated LJOs on overall likeness of cherry tomatoes during storage. Values are the mean of three replicates. Vertical bars represent standard deviation.

3.6. Changes in the firmness of cherry tomatoes

The firmness of the fruits in each group gradually decreased with storage time; reduction in firmness was faster in the control group than in the treatment groups, indicating that LJO-incorporated pullulan coatings effectively retarded tissue softening (Fig. 5). This result may be ascribed to the lower water loss rate caused by the surface coatings (Fig. 4). In this study, LJO-incorporated pullulan coatings maintained the firmness of cherry tomatoes, suggesting it could be a promising method to inhibit fruit softening.

3.7. Changes in fruit sensory quality

The overall likeness to control did not significantly decrease in all groups ($p < 0.05$), and no significant differences in the overall likeness between the control and treatment groups were observed in the first 2 days of storage (Fig. 6). However, the overall likeness in the control group decreased steadily after 2 days of storage, whereas the overall likeness for the 0.1% group did not change much during the first 4 days of storage, and overall likeness for 0.2% and 0.3% groups remained unchanged during the first 8 days of storage. In the present study, no decay was observed in any of the treatments during the first 10 days of storage.

4. Conclusions

The shelf life of cherry tomatoes is reduced by respiration, decreased amount of nutrient, reduced firmness and weight loss. LJO-incorporated pullulan coatings effectively inhibited respiration, retarded tissue softening and reduced both nutrient consumption and weight loss rate in cherry tomatoes during storage. Therefore, LJO-incorporated pullulan coatings extended the shelf life of cherry tomatoes.

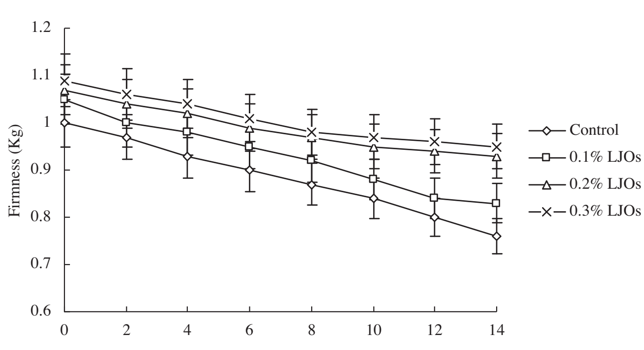

Fig. 5. Effect of pullulan-based coatings incorporated LJOs on firmness of cherry tomatoes during storage. Values are the mean of three replicates. Vertical bars represent standard deviation.

Acknowledgments

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