



# 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.

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## 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  $\beta$ -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 (Xinqu, China). The cold-adapted  $\alpha$ -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  $2.7 \times 10^5$ , 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  $\alpha$ -amylase produced by *E. coli* BL21/pE<sub>tac</sub>-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<sub>2</sub>O<sub>2</sub> (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<sub>2</sub> 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<sub>C</sub> analysis

V<sub>C</sub> 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  $\mu$ m 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<sub>C</sub> was monitored at 245 nm, and the sample V<sub>C</sub> content was calculated from a V<sub>C</sub> 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  $\pm$  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

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