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Combination of nisin and ε-polylysine with chitosan coating inhibits the white blush of fresh-cut carrots

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ABSTRACT

Effects of the combination of nisin and ε-polylysine with chitosan coating on quality maintenance and white blush inhibition were investigated in fresh-cut carrots. Fresh-cut carrots were treated with 1% lactic acid solution (v/v), 1% chitosan solution (w/v), or 1% chitosan solution containing 64 μg/mL nisin and 250 μg/mL ε-polylysine (LA + CH + Nisin + ε-PL). The samples were packed in polyethylene plastic bags and stored at 4 °C for 9 days. Changes in sensory attributes, physicochemical indices, respiration rate, microbiological counts and white blush were measured. Results showed that LA + CH + Nisin + ε-PL significantly ($P < 0.05$) inhibited respiration rate, decline of ascorbic acid and growth of microorganism (yeast and mold, total viable counts, total coliforms counts, *Staphylococcus aureus and Pseudomonas* spp.), and increased total phenol content and phenylalanine ammonialyse (PAL) activity compared with the control after 9-day storage. It was also strongly effective in inhibiting the white blush of fresh-cut carrots. Furthermore, LA + CH + Nisin + ε-PL significantly ($P < 0.05$) reduced the lignin synthesis in fresh-cut carrots by inhibiting the cinnamate-4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL) activity, as well as *Dc4CL* and *DcC4H* gene expression. Our results may provide some basis for the use of the combination of nisin and ε-polylysine with chitosan coating as an alternative preservation method for fresh-cut carrots.

1. Introduction

Increasing consumer demand for high-quality, fresh, natural, nutritive and conveniently prepared foods has dramatically stimulated the development of the fresh-cut fruit and vegetable market over the past decades (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). Fresh-cut carrots are one of the most widely consumed vegetables in ready-to-eat salads (Vargas, Chiralt, Albors, & González-Martínez, 2009), which accounted for up to 47% in US fresh-cut vegetable category shares of dollar sales in 2012 (Cook, 2012). Moreover, it is also becoming more and more popular in developing countries due to its convenience and freshness. However, mechanical operations during processing of fresh-cut carrots, such as peeling, shredding, cutting and slicing, usually cause tissues damages, and subsequently lead to enzymatic browning, cut-surface discoloration, texture softening, off-flavor development and microbial spoilage (Pushkala, Parvathy, & Srividya, 2012). Therefore, fresh-cut carrots have a shorter shelf-life compared to the intact vegetable, and there is a need to develop suitable technologies to extend the shelf-life of fresh-cut carrots.

White blush usually occurs in fresh-cut carrots as a consequence of exposure of damaged cell wall materials on processed or cut edges to drying conditions. It greatly affects the acceptance of consumers towards fresh-cut carrots. On the one hand, some authors attributed the formation of while blush to the dehydration of external damaged cells during processing (Tatsumi, Watada, & Wergin, 1991). On the other hand, lignin synthesis is believed to be related to the development of white blush (Lavelli, Pagliarini, Ambrosoli, Minati, & Zanoni, 2006). Post-processing accumulation of lignified material intensified the incidence and severity of white brush (Hennion, Anthony Little, & Hartmann, 1992; Howard, Griffin, & Lee, 1994). Lignification is an enzyme-stimulated reaction, in which several enzymes are involved, including phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL) (Hamada et al., 2004). It is evidenced

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that inhibition of these enzymes was correlated with reduced accumulation of lignin and white blush in minimally processed carrots (Howard et al., 1994).

Various strategies have been attempted to improve the quality of fresh-cut carrots during storage, one of which is the use of edible coatings. Chitosan is an attractive coating material for perishable fruits and vegetables due to its edible feature, non-toxic property, film-forming capacity and antimicrobial activities (Shahidi, Arachchi, & Jeon, 1999). However, hydrophilic character and weak water resistance are major limitations of chitosan coatings because effective moisture transfer control is a desirable property in most foods (Butler, Vergano, Testin, Bunn, & Wiles, 1996). It has shown the effectiveness in inhibiting the growth of pathogenic and spoilage microorganisms in minimally processed carrots (Durango, Soares, & Andrade, 2006). Besides, it was able to delay or inhibit the white brush of fresh-cut carrots (Vargas et al., 2009). Moreover, the functional properties of chitosan films can be improved by combining chitosan with some bioactive compounds or plant extracts. For instance, Santiago-Silva et al. (2009) reported that combined use of the film and pediocin had higher antimicrobial efficiency in sliced ham. Addition of 0.03% rosemary extracts into chitosan coating significantly improved preservative quality of fresh-cut pears (Xiao, Zhu, Luo, Song, & Deng, 2010). Nisin is a well-known antimicrobial agent, which has been widely used for dairy products, canned vegetables and fruits in almost 50 countries due to its efficiency against some gram-positive bacteria (de Arauz, Jozala, Mazzola, & Vessoni Penna, 2009). ε-Polylysine (ε-PL) is an FDA-approved natural antimicrobial agent that is effective against a wide range of microorganisms, including most gram-positive and gram-negative bacteria, fungi, yeasts and some viruses (Shih, Shen, & Van, 2006; Yoshida & Nagasawa, 2003). Although previous studies reported the effectiveness of nisin or ε-PL in extending the shelf-life of numerous fruits and vegetables, there is little information available regarding the effect of combination of these antimicrobial agents and chitosan coating on shelf-life of fresh-cut carrots. The objective of this study was to assess the potential of combination of nisin and ε-PL and chitosan coating for while blush control and quality maintenance during storage of fresh-cut carrots. Our results may contribute some valuable information to the control of white blush of fresh-cut carrots during storage.

2. Materials and methods

2.1. Chemicals

Chitosan with a deacetylation degree of ≥95% was provided by Aladdin Reagent Company (Shanghai, China). Lactic acid, glycerol and Tween-80 were purchased from Yongda Chemical Reagent Co., Ltd. (Tianjin, China). Nisin and ε-PL were obtained from Sigma-Aldrich (St. Louis, MI, USA). All other chemicals were of analytical grade and obtained from Shanghai Reagent Co. (Shanghai, China).

2.2. Preparation and packaging of fresh-cut carrots

Fresh carrots (*Daucus carota* L. cv. *Tino*) were obtained from Tianlv Agricultural Co., Ltd (Taian, China). They were harvested at 120 days after planting, and immediately transported to our laboratory. Damaged carrots and those with defects were discarded, and only carrots with an average diameter of 4 cm and a length of 15 cm were selected. All samples were washed, peeled and sliced into 1 cm thickness pieces prior to coating. The slices were randomly divided into four groups. In the preliminary experiment, different concentrations of chitosan solution (0.1%, 0.5%, 1%, 2% and 5%, w/v) were used for coating of fresh-cut carrots. After storage for 9 days, sensory acceptability was evaluated by a sensory panel with ten female assessors from our department according to the methods of Pushkala et al. (2012). The panelists were asked to rate the samples in terms of surface dryness, fresh orange-red color, bitter aftertaste and flavor. Results showed that the samples treated with 1% chitosan had higher sensory score (data not shown), thus 1% chitosan solution was used in the subsequent experiments. The coating solutions were prepared by dissolving chitosan in 1% lactic acid solution (v/v). After stirring overnight at room temperature, 0.1% glycerol (w/v), 0.1% tween-80 (v/v), 64 μg/mL nisin and 250 μg/mL ε-PL were added into the solution. Two kilogram of the carrot slices were randomly immersed in 2 L of different coating solutions, namely 1% lactic acid solution (LA), 1% chitosan solution (LA + CH), and 1% chitosan solution containing nisin and ε-PL (LA + CH + Nisin+ε-PL) for 30 s. After drying at 20 °C in a forced air convection oven for 3 h, the slices were placed into trays (25 cm × 40 cm) over-wrapped with 0.02 mm thickness polyethylene plastic films, and stored at 4 °C in the dark for 9 days. The fresh-cut carrots submerged in distilled water instead of various coating solution were served as the control (CK). For each treatment, three replicates were used. Determination of each index was performed on 0, 1, 3, 5, 7 and 9 day of storage.

2.3. Determination of headspace composition

The O_2 and CO_2 concentrations in the headspace of the package bags were determined according to the procedures described previously (Zhang, Shi, Zhu, Li, & Wang, 2014). Briefly, three trays were taken from each treatment at each sampling interval, and 5 cm^3 of headspace gas was subjected to an O_2 and CO_2 Analyzer (PBI-940437B, PBID Ansensor, Denmark). The headspace composition was expressed as percentage of O_2 and CO_2, and the respiration rate was calculated according to the method of Castelló et al. (2006).

2.4. Determination of weight loss

Weight loss of the carrot samples was measured according the previous literature (Yingsanga, Srilaong, Kanlayanarat, Noichinda, & McGlasson, 2008). The initial weight of each tray, and the weight at each sampling interval were monitored using an F1004A analytical balance (Hengping Experimental Instrument Inc., Shanghai, China).

2.5. Determination of ascorbic acid and total phenolic content

The pulp tissue (0.5 g) was homogenized with 20 mL of 0.06 g/mL metaphosphoric acid solution, and then centrifuged at 13,000 × *g* and 4 °C for 10 min. Afterwards, 10 mL of the supernatant was used to measure ascorbic acid content by the 2,4-dinitrophenylhydrazine method (Terada, Watanabe, Kunitomo, & Hayashi, 1978).

Total phenolic content was assessed by the Folin-Ciocalteau method as reported previously (Rocha & Morais, 2002). Frozen tissue (0.5 g) was extracted with 70% methanol (v/v) for 1 min, and then centrifuged at 12,000 × *g* and 4 °C for 15 min. One milliliter of the supernatant was mixed with 2 mL of 0.35 M NaOH and 1 mL of Folin-Ciocalteau reagent. The reaction mixture was kept at room temperature for 3 min, and the absorbance was immediately measured at 760 nm on a UNICO UV-2000 spectrophotometer (Shanghai Instruments Co. Ltd., Shanghai, China). The results were expressed as milligrams of gallic acid equivalent per kilogram of sample.

2.6. Sensory analysis

Sensory analysis was carried out according to the methods of