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Effect of poly-ε-lysine incorporated into alginate-based edible coatings on microbial and physicochemical properties of fresh-cut kiwifruit

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ABSTRACT

This work investigates the effectiveness of different concentrations (0.05%, 0.10% and 0.15%) of poly-ε- lysine (ε-PL) incorporated into an alginate-based edible coating to inhibit microbial proliferation and maintain physicochemical properties of fresh-cut kiwifruit stored at 4 ± 0.5 °C for 14 d. Changes in O_2 and CO_2 concentration, physicochemical indices, microbiological counts and morphological properties were measured. Results showed that low levels of ε-PL (0.05% and 0.10%) led to lower CO_2 and higher O_2 concentrations inside the packages compared with samples with control and 0.15% ε-PL treatments. Moreover, the alginate-based edible coating containing 0.05% ε-PL significantly reduced electrolyte leakage and MDA content while maintained the green color, total chlorophylls content, ascorbic acid, antioxidant capacity and morphological properties of fresh-cut kiwifruit. In addition, 0.05% ε-PL treatment reduced aerobic plate counts and yeast and mould counts by 3.5 and 2.5 log CFU/g, respectively, at the end of evaluation. Our results show that there is promise for the use of edible coating incorporated with ε-PL to preserve the quality of sliced kiwifruit.

1. Introduction

Regular intake of kiwifruit is associated with health-promoting properties *in vivo*, which may contribute to lowering the risk of cardiovascular disease (Chang and Liu, 2009) and certain types of cancer (Chang and Liu, 2009; Motohashi et al., 2002; Rush et al., 2006), and provides high concentration of bioactive compounds (Du et al., 2009; Giangrieco et al., 2016). Nowadays, the demand for ready-to-eat products, such as fresh-cut kiwifruit has increased (Meng et al., 2013), which is attributed to an excellent characteristic with the combination of the freshness, convenience and nutrition, compared to the intact fruit. However, fresh-cut produce causes an increase in respiration intensity, microbial spoilage, biochemical changes, cut-surface discoloration, softening, and off-flavor (Agar et al., 1999; Benítez et al., 2015; Mastromatteo et al., 2011).

Today, various approaches such as edible coating (Benítez et al., 2015, 2013), essential oil (Roller and Seedhar, 2002; Wang and Buta, 2003), H_2S (Gao et al., 2013), cold plasma (Ramazzina et al., 2015), MAP packaging (Mastromatteo et al., 2011; Rocculi et al., 2005) or combination treatments (Beirão-da-Costa et al., 2013; Meng et al., 2013) are reported to keep quality of fresh-cut products. Among these treatments, edible film packaging is considered to be one of the most cost-effective ways to maintain the quality and safety for fresh-cut fruit and vegetable, because it not only provides a barrier to gas permeation and water vapor, but has the characteristic of serving as a carrier of food additives such as anti-browning, antimicrobials agents, colorants, flavors, nutrients and spices to prolong the shelf-life of fresh-cut products (Koushesh Saba and Sogvar, 2016; Rojas-Graü et al., 2007; Tapia et al., 2007).

Poly-ε-lysine (ε-PL) is an FDA-approved natural antimicrobial agent, consisted by a homo-poly-amino acid characterized by the peptide bond between carboxyl and ε-amino groups of L-lysine, and which is water soluble, biodegradable, edible and non-toxic and shows a wide spectrum of antimicrobial activity. Recently, some studies were focused on the microbial synthesis, production enhancement and antibacterial mechanisms of ε-PL (Bo et al., 2016; Kawai et al., 2003; Li et al., 2014; Ye et al., 2013). However, to our knowledge, there are a few reports about the effectiveness of ε-PL incorporated into edible coating applied to fresh-cut products (Song et al., 2017; Wang et al., 2014). The objective of our research was to assess the efficiency of different concentration of ε-PL incorporated into alginate-based edible coatings on microbial growth and quality maintenance of fresh-cut kiwifruit during 14 d storage at 4 ± 0.5 °C.

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2. Materials and methods

2.1. Chemicals

Food grade sodium alginate was provided as a polysaccharide-based edible coating from Bright Moon Seaweed Group Co., Ltd (Qingdao, China). Food grade poly-ε-lysine (ε-PL) was purchased from Xilaike Chemical Products Co., Ltd, (Henan, China). Glycerol was applied as a plasticizer provided by Yangdong Chemical Industrial Co., Ltd (Guangdong, China). Sunflower oil was applied as a lipid source purchased by Yihai Kerry Co., Ltd (Shanghai, China).

2.2. Preparation and packaging of fresh-cut kiwifruit

Fresh kiwifruit (*A.diliciosa* cv. Harward) was obtained from local market (Zhengzhou, China). They were selected for uniformity in shape and size, defected fruit were discarded. The initial weight (90 ± 5.1 g) and soluble solids content (8.6 ± 2.2%), analyzed on a sample of 20 fruit. The kiwifruits were washed with tap water, drained for 20 min, then hand peeled and sliced into 1 cm thickness slices (about 10–15 g each) with a stainless steel knives prior to coating. The slices were randomly distributed among treatments.

The alginate-based coating solutions were prepared by dissolving sodium alginate (1%, w/v) powder in distilled water at 70 °C until the mixture became clear. Then 1.5% glycerol (v/v), 0.03% sunflower oil (v/v) and different concentration of ε-PL (0.05%, 0.10% and 0.15%, w/v) were added into the solution. The kiwifruit slices were randomly divided into four groups for the following treatments: (a) control (submerged in distilled water); (b) 0.05% (ε-PL 0.05% incorporated into alginate-based coating solution); (c) 0.10% (ε-PL 0.10% incorporated into alginate-based coating solution); (d) 0.15% (ε-PL 0.15% incorporated into alginate-based coating solution). Three kilograms of the kiwifruit slices were randomly immersed in 6 L of different coating solutions for 30 s. After drying at room temperature in a forced air convection oven for 3.0 h, the kiwifruit slices (55 g) were placed into plastic trays (18.7 cm × 14.0 cm) wrapped with 0.03 mm thickness polyethylene plastic films (the water vapor permeability of the film was 62 ± 12.4 g m^{-2} d^{-1}, the O_2 and CO_2 permeability were 0.24 ± 0.05 cm^3 m^{-2} d^{-1} Pa^{-1} and 0.52 ± 0.11 cm^3 m^{-2} d^{-1} Pa^{-1}, respectively), and then stored at 4 ± 0.5 °C in the dark for 14 d. For each treatment 48 trays were used, from which 6 trays were sampled on 0, 2, 4, 6, 8, 10, 12 and 14 d of storage at 4 ± 0.5 °C for analytical determinations, respectively. Of which, three trays from each treatment for O_2/CO_2 concentration determination, three trays for microbial and other indexes determination.

2.3. Determination of O_2 and CO_2 concentrations

The O_2 and CO_2 concentrations in the package bags were determined using Oxybaby M_+ O_2/CO_2 (WITT-Gasetechnik). The results were expressed in kPa.

2.4. Determination of soluble solid content and titratable acid content

Soluble solid content (SSC) was measured using a hand-held refractometer (PAL-1, Atago, Japan) to determine the juice after pressing the flesh. SSC was expressed as a percentage. Titratable acid (TA) was determined according to ISO 750-1998. Briefly, ten grams of kiwifruit slices were homogenized and diluted to 100 mL with distilled water. The mixture was then filtered, and approximately 3–5 drops of phenolphthalein (1% in 95% ethanol, w/v) added to 25 mL of the filtered solution, stirred and titrated with 0.1 N NaOH. TA content was expressed as percent of citric acid.

2.5. Determination of color and total chlorophylls content

The flesh color scores were assessed by Color Flex EZ colorimeter (Hunterlab, USA). The a^* parameter was measured at six thinly pared sides with the average used to record.

Total chlorophylls were extracted by grinding 5 g of kiwifruit flesh in 10 mL of 80% (v/v) cold acetone with a mortar and pestle at 4 °C, sodium carbonate (0.3 g) was added to prevent the chlorophylls from degrading to pheophytin during extraction. The residue was then re-washed with 80% (v/v) cold acetone until the residue was colorless, and diluting the final volume to 25 mL. The mixture was extracted for 30 min at 4 °C, then centrifuged at 10,000 × *g* for 10 min at 4 °C and the supernatant was used for determination. The total chlorophylls content was determined by measuring the absorbance at 663 and 645 nm simultaneously and calculated according to Arnon (1949). The results were expressed as total chlorophylls content on a fresh weight basis, mg kg^{-1}.

2.6. Determination of ascorbic acid content and antioxidant capacity

Sliced kiwifruits (2 g) were ground in a mortar and pestle in 10 mL of 6% (w/v) trichloroacetic acid (pre-cooled on ice) and centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant was used for analysis of the ascorbic acid content (AsA) and antioxidant capacity. AsA content was determined according to Kampfenkel et al. (1995). The results were expressed as ascorbic acid content on a fresh weight basis, g kg^{-1}.

The antioxidant activity of the samples was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma, USA) and ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) diammonium salt, Sigma, USA) free radical scavenging assays according to the method of Du et al. (2009) with some modifications. For DPPH assay, 4 mL of different extracts were added to 1 mL of methanolic solution containing DPPH radical, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then recorded at 517 nm against a blank. For ABTS assay, the stock solutions included 7 mM $ABTS^{\bullet+}$ solution and 2.45 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions at a ratio of 1:0.5 and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing $ABTS^{\bullet+}$ solution with ethanol to obtain an absorbance of 0.70 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. Different sample extracts (100 μL) were allowed to react with 3 mL of the $ABTS^{\bullet+}$ solution for 6 min in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer.

2.7. Determination of electrolyte leakage and malonadehyde content

Electrolyte leakage was determined as described by Zhang et al. (2014) with slight modification. Kiwifruit slices were cut into small discs (0.05 cm thick, use a razor blade) and washed three times with deionized water to remove surface-adhered electrolytes. After drying with filter paper, 10 discs were placed in closed vials containing 40 mL deionized water. After the vials were shaken slowly for 5 min, the conductivity was measured as C1 with a Conductivity Meter (DDS-307A, Shanghai REX Instrument Factory, Shanghai, China). The vials were boiled for 10 min and cooled quickly. Afterward, the conductivity was measured as C2. Electrolyte leakage was calculated as electrolyte leakage (%) = C1/C2 × 100.

The malonaldehyde content (MDA) was done according to the method of Liu and Wang (2012). Kiwifruit slices (2.0 g) were homogenized with 10 mL of trichloroacetic acid (10%, w/v) and centrifuged at 10,000 × *g* for 20 min at 4 °C. Two mililiter of supernatant was mixed with 2 mL of 0.67% (w/v) 2-thiobarbituric acid, heated in boiling water bath for 20 min, then cooled and centrifuged at 3,000 × *g* for 10 min. The absorbance was read at 450 nm, 532 nm and 600 nm.