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Effects of chitosan/nano-silica on postharvest quality and antioxidant capacity of loquat fruit during cold storage

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ABSTRACT

Loquat (*Eriobotrya japonica* Lindl.) fruit has a very short shelf-life during ambient temperature storage, and is susceptible to cold storage appearing chilling injury symptoms. The effect of chitosan/nano-silica coating on chilling tolerance in white-flesh loquat fruit (cv Baiyu) stored at 5 °C for 40 d was investigated. In comparison with control fruit, internal browning and weight loss in chitosan/nano-silica treated fruit were significantly delayed. Likewise, the decrease of total soluble solids (TSS) and titratable acidity (TA) was also inhibited. Glucose and fructose contents increased from 11.31 to 12.76 g kg^{-1} and from 33.20 to 44.37 g kg^{-1}, respectively. The increase of reducing sugars contributed to the improvement of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities, reaching the peaks of 623510.12, 462371.21 and 41114.35 U kg^{-1}, respectively. Enhanced activities of antioxidant enzymes markedly alleviated the generation of superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Low levels of malondialdehyde (MDA) and membrane permeability in chitosan/nano-silica treated fruit were also detected, ranged from 4.35 to 8.31 mmol kg^{-1} and from 24.32 to 43.45%, respectively. Moreover, activities of phenylalanine ammonia-lyase (PAL), polyphenoloxidase (PPO) and lipoxidase (LOX) were also much lower than those in treatment alone and control fruit. Our results indicated that the use of chitosan/nano-silica coating was effective in enhancing chilling tolerance and providing a longer storage life with acceptable external and internal quality in loquat fruit.

1. Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical fruit popular for its flavor and nutritive value (Pareek et al., 2014). However, loquat fruit has very short shelf-life at ambient temperature due to flesh browning and fungi decay (Cao et al., 2008). Low-temperature storage (1–5 °C for 2–3 weeks) causes chilling injury symptoms such as flesh shrivel and internal browning. Although various methods such as low-temperature conditioning, hot air treatment, and application of 1-methylcyclopropene or methyl jasmonate have been demonstrated to reduce chilling injury of loquat fruit (Cai et al., 2006; Jin et al., 2014; Cao et al., 2009, 2011), few are used extensively in commercial situations at present (Cao et al., 2009). With the extensive use of cold chain storage during the postharvest transport of loquat fruit, there is still a need for developing more effective techniques to alleviate chilling injury (Jin et al., 2014).

Currently, some bio- or nano-based materials with improved mechanical barriers and antimicrobial properties provide a novel packaging technology in postharvest industry (Silvestre et al., 2011). We previously found that an integration of chitosan and

Abbreviations: TSS, total soluble solids; TA, titratable acidity; PPO, polyphenoloxidase; LOX, lipoxidase; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; ROS, reactive oxygen species; $O_2^{\bullet -}$, superoxide anion; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; TEOS, tetraorthosilicate; IB, internal browning; PAL, phenylalanine ammonia-lyase; AA, ascorbic acid.

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nano-silica greatly improved the quality of longan fruit during ambient temperature storage (Shi et al., 2013). However, effect of chitosan/nano-silica coating on alleviating chilling injury of perishable fruits has not been reported until now. Chilling injury induces fruit membrane damage and reactive oxygen species (ROS) production (Aghdam and Bodbodak, 2014). The injury alleviation is related to the ability to reduce and scavenge ROS by the increase of antioxidant enzyme activities (Cao et al., 2009). Partial antioxidant metabolism had been evaluated in enhancing fruit chilling tolerance during cold storage. Sugar metabolism is regarded as one of essential factors for biosynthesis of antioxidant molecules against oxidative chilling stress (Couée et al., 2006).

In our present study, a chitosan/nano-silica hybrid was successfully synthesized through the sol-gel approach. And its effects on fruit quality and chilling tolerance were evaluated using physiological indexes and antioxidant properties in white-flesh loquat (cv Baiyu) prone to quality recession and flesh browning at 5 °C. Sugar types and contents under chilling stress were also investigated. The objective is to understand that antioxidant system and sugar composition are associated with enhancing chilling tolerance in chitosan/nano-silica treated loquat fruit during cold storage.

2. Materials and methods

2.1. Materials

Loquat fruit (*Eriobotrya japonica* Lindl. cv Baiyu) were harvested from 12-year-old trees grown in the Loquat Germplasm Preservation Orchard of Jiangsu Province, Suzhou, China. The uniform fruit were selected in size, weight and color without signs of disease and pest damage. Chitosan (with 75.6% of deacetylation degree, CAS Number 9012-76-4, Batch 10305DD, Sigma-Aldrich, St. Louis, MO, USA) and glacial acetic acid (98%, v/v) were used to prepare the film-forming dispersion. Tetraorthosilicate [$(C_2H_5O)_4Si$, TEOS, 98% purity] was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation and detection of the chitosan/nano-silica coating

The chitosan/nano-silica coating was prepared according to the description of Yeh et al. (2007) and Lai et al. (2006) with some modification. Chitosan (0.75%, w/v) was dissolved in glacial acetic acid (1.0%, v/v) to form a viscous and pale yellow solution. The solution was stirred at 300 rpm for 8 h at room temperature. Silica solution was prepared at a molarratio of TEOS: deionized water: ethanol (1:4:1). After stirred at 200 rpm for 2 h, the solution was kept for 30 min at room temperature prior to mixing with the chitosan solution. The pH was adjusted to 5.6 with NaOH (1 mol L^{-1}). Afterwards, the silica solution was added into the chitosan solution followed by an ultrasonication (KQ-250E, Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, China) for 30 min under 300 W at 60 °C. Sucrose ester of fatty acid (0.5%, v/v) was finally added and repeatedly stirred with a magnetic stirrer until the mixture solution became clear. Morphology of chitosan or/and nano-silica coating was examined by a transmission electron microscope (TEM, Hitachi, Model H-800, Japan.).

2.3. Treatment and storage of loquat fruit

The selected fruit samples were distributed randomly into four groups. Four different treatments were used as following: (1) control (deionized water); (2) chitosan (0.75%, w/v); (3) nano-silica (30%, w/v) and (4) chitosan/nano-silica. The fruit samples from the same group were dipped into chitosan or/and nano-silica solutions for 5 min, respectively. The deionized water was used as a control. All fruit samples were placed in 0.02 millimeter polyethylene bags and were kept at 5 °C.

2.4. Measurement of fruit weight loss and browning index

Fresh weight loss was monitored at the interval of 5 d during storage at 5 °C. The internal browning (IB) index manifested as browning discoloration near the core was evaluated visually after cutting the fruit longitudinally in half (Cao et al., 2009). For each fruit, IB was scored according to a 5-grade scale, where 0 = none (excellent quality), 1 = browning area < 5% (slight), 2 = browning area 5%–25% (moderate), 3 = browning area 25%–50% (moderately severe) and 4 = browning area > 50% (severe). An IB index was expressed using the following formula: IB index (between 0 and 4) = $\sum$[(browning rating) × (fruit number at the browning rating)]/total fruit number.

2.5. Determination of sugar contents

5 g of flesh tissue were homogenized in 20 mL of re-cold ethanol (95%, v/v). The extract was evaporated under vacuum at 35 °C for removing the ethanol. The final volume was dilute with distilled water to 25 mL. After filtering with a 0.2 μm filter, 6 mL of the extract was passed through a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) activated with acetonitrile and water (Cao et al., 2013). A 20-μL aliquot was injected into a high-performance liquid chromatography system in a Waters chromatograph equipped with a refractive index detector (Waters, Mildford, MA) and a CarboSep CHO-682 carbohydrate analysis column (Transgenomic, San Jose, CA). The separation was operated at 80 °C with ultrapure water as a mobile-phase (0.5 mL min^{-1}). Individual sugar was identified and quantified by comparison with the retention times and peak areas of individual sugar standard. The results were expressed as g kg^{-1}.

2.6. Determination of total soluble solids (TSS) and titratable acidity (TA) contents

5 g of flesh tissue from each replicate were homogenized and then centrifuged at 13,000*g* for 20 min. The supernatant was used to analyze TSS and TA contents (Cao et al., 2008). TSS (%) was determined using a digital pocket refractometer (PAL-a, ATAGO, Tokyo, Japan). TA was determined by titration with NaOH (0.1 mol L^{-1}) until pH 8.1 and expressed as% malic acid (mass/mass).

2.7. Determination of superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), membrane permeability and malondialdehyde (MDA)

The rate of $O_2^{\bullet -}$ production was calculated against a standard curve with $NaNO_2$ as previously described by Cao et al. (2009). 5 g of flesh tissue were homogenized with 5 mL of phosphate buffer (50 mmol L^{-1}, pH 7.8) and then centrifuged at 12,000*g* for 20 min at 4 °C. 1 mL of hydroxyammonium chloride (1 mol L^{-1}) was added to 1 mL of the supernatant, and the mixture was incubated at 25 °C for 1 h. 2 mL of ether was added to prevent interference from chlorophyll. After centrifugation at 12,000*g* for 10 min, 1 mL of the supernatant was mixed with 1 mL of 17 mmol L^{-1} *p*-aminophenylsulfonic acid and 1 mL of 7 mmol L^{-1} α-naphthylamine at 25 °C for 20 min. This rate was measured using the absorbance at 530 nm, and expressed as mmol min^{-1} kg^{-1}.

H_2O_2 content was measured according to the method of Cao et al. (2009). 5 g of flesh tissue were homogenized with 30 mL of re-cold acetone, and centrifuged at 12,000*g* for 20 min at 4 °C. The supernatant was mixed with hydrochloric acid (containing 200 mL L^{-1} $TiCl_4$) and ammonia solution (17 mol L^{-1}). The