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Influence of nano-ZnO on microbial growth, bioactive content and postharvest quality of strawberries during storage

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ABSTRACT

A rapid decline of quality causes economic loss of strawberries after harvest. Treatments based on nano-ZnO at different concentrations (0.03%, 0.07% and 0.5%) were used to prolong the shelf-life of strawberry fruit in cold storage. After treatments microbial growth, weight loss, firmness, titratable acidity, soluble solid content, pH value, vitamin C, anthocyanin and total phenolics and antioxidant activity were evaluated at 0, 3, 6, 9, 12, 15 and 18 days of storage. Furthermore, the levels of zinc and the sensory attributes of treated strawberries were evaluated three days after treatment. Nano-ZnO treatments decreased the microbial load during fruit storage (total mesophilic bacteria in control and 0.5% nano-ZnO treated strawberries were 4.35 and 3.67 Log CFU g^{-1}, respectively). Treatments delayed weight loss, retained fruit firmness and maintained anthocyanin, vitamin C, phenol content and antioxidant activity at higher concentration than those of untreated fruit. Fruit sweetness and aftertaste attributes were not affected by treatments but the 0.5% ZnO treated fruit was less luminous (1.6) compared to control (5.6). 0.5% nano-ZnO was the most effective in delaying changes in the ripening and reducing microbial populations among the treatments. These findings indicated that the nano-ZnO might provide an alternative to maintain quality and control decay of fresh strawberries during extended storage.
Industrial relevance: Strawberries are a highly perishable fruit and postharvest life is limited to 4 days or even shorter at room temperature or 2 weeks at cold storage, therefore finding a method to extend the shelf life of strawberries is important. Modified atmosphere packaging is a useful method but control of spoilage microorganisms is still a problem. Fruit coating has great potential to extend fruit postharvest life and maintain nutritional quality. Nano-ZnO may be an effective alternative.

1. Introduction

Strawberries (*Fragaria* × *ananassa* Duch.), one of the most popular berries worldwide, are characterized by unique and highly desirable taste and flavor, and are rich in polyphenols and anthocyanins, vitamins and other bioactive compounds (Campaniello, Bevilacqua, Sinigaglia, & Corbo, 2008; Koyuncu & Dilmacunal, 2010; Pinto, Lajolo, & Genovese, 2008; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Van De Velde, Tarola, Güemes, & Pirovani, 2013). However, strawberries are highly perishable resulting in a short shelf-life due to mechanical injury, physiological deterioration, water loss, fungal decay and high respiration rate (Aday, Buyukcan, & Caner, 2013; Del-Valle, Hernandez-Munoz, Guarda, & Galotto, 2005; Perkins-Veazie, 1995; Satin & 94, 1996; Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2006). Cold temperatures and modified atmospheres increase the storage life of strawberries (Aday et al., 2013; Martinez Romero, Guillen, Castillo, Valero, & Serrano, 2003), but additional methods of maintaining quality are still under investigation.

Post-harvest losses of strawberry are more serious and its shelf-life in room temperature or cold storage is limited to 4 days or 2 weeks, respectively (Romanazzi, Gabler, Margosan, Mackey, & Smilanick, 2009). The strawberry losses have been estimated between 80 to 85%, especially when conditions are favorable for disease development (Hernandez-Munoz, Almenar, Valle, Velez, & Gavara, 2008; Hong, Michailides, & Holtz, 1998; Larena et al., 2005). *Botrytis* fruit rot, also known as gray mold, is caused by the fungus *Botrytis cinerea* and is one of the most important diseases of strawberries worldwide. Because of high perishability of fruit, many strategies have been developed to reduce strawberry losses. One such approach is nano-science application in fruit post-harvest.

Recent advances in nanotechnology, particularly the ability to produce nanoparticles in different shapes and sizes led to the creation of a wide range of nanostructured compounds with antimicrobial properties. The greater surface area per mass compared with larger-sized particles of the same chemistry renders nano-sized particles more active biologically (Damm, Neumann, & Munstedt, 2005; Oberdorster,

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Oberdorster, & Oberdorster, 2005). As one of the multifunctional inorganic nanoparticles, zinc oxide (ZnO) nanoparticles are known to inhibit microbial growth (Aydin Sevinc & Hanley, 2010; Jin, Sun, Su, Zhang, & Sue, 2009; Jones, Ray, Ranjit, & Manna, 2008) that recently has been reviewed extensively (Oprea et al., 2014; Sirelkhatim et al., 2015; Zhang & Xiong, 2015). ZnO is found to have many applications in daily life such as in drug delivery, cosmetics, and medical devices (Yan, Salley, & Ng, 2009), due to its strong antimicrobial effect on a board spectrum of microorganisms (Jones et al., 2008). Moreover, it has been listed as generally recognized as safe by the U.S. Food and Drug Administration (21CFR182.8991; Xie, He, Irwin, Jin, & Shi, 2011). Lepot et al. (2011) found that polypropylene films containing nano-ZnO had good mechanical and oxygen barrier properties. It has been reported that low-density polyethylene nano-composite packaging materials containing silver and ZnO nanoparticles improved the shelf-life of fresh orange juice during cold storage (4 °C) (Emamifar, Kadivar, Shahedi, & Soleimanian-Zad, 2010). Li et al. (2011) successfully developed a novel polyvinyl chloride film coated with nano-ZnO particles as active packaging to improve the shelf-life of fresh-cut 'Fuji' apples.

Although nano-ZnO has been used as an additive to packaging films and an antibacterial against various bacteria (Tam et al., 2008; Jin et al., 2009), there is no information on direct application for fruit coatings. Therefore, the main objective of this research was to evaluate the effect of nano-ZnO coatings on the quality and shelf-life of fresh strawberries during storage.

2. Materials and methods

2.1. Plant material

Strawberries (*Fragaria × anannasa* Duch), cv. 'Parous', were harvested from commercial farm located near Kurdistan University, Sanandaj, Iran. The maturity stage of the fruit was 80% red color on the fruit surface. Fruit were selected for uniformity in size, shape and color without signs of mechanical damage, blemishes and disease. A total of 30 fruit were sampled for immediate analysis to monitor fruit characteristics at harvest before application of treatments (day 0). Also, sufficient harvested fruit were randomly distributed into 4 groups and each treated as described below. After treatments fruit of each group were randomly distributed into 18 boxes and stored at cold room. Sampling of either treated or control was carried out at 0, 3, 6, 9, 12, 15, and 18 days. Three boxes were sampled at each sampling times as three replications.

2.2. Coating and storage conditions

Antimicrobial activity of nanoparticles depends on particle size and concentration, nano-ZnO activity has been tested in the range of 0.002 to 0.5% (Brayner et al., 2006; Chitra & Annadurai, 2013; Reddy et al., 2007). Four different concentrations (0, 0.03, 0.07, and 0.5%) of nano zinc oxide (30–100 nm) suspension were prepared by ultrasonically-assisted dispersing of ZnO nanoparticle in distilled water. Strawberries were dipped in nano-ZnO solutions or distilled water at 20 °C for 5 min. All strawberries were air-dried at room temperature for 1 h then placed in polystyrene boxes and stored at 1 °C with 95% relative humidity.

2.3. Scanning electron microscopy (SEM) of strawberry fruit epidermis

The particle size of the coating suspensions applied in this study was determined using a scanning electron microscope (FESEM, TESCAN, Mira 3) at a voltage of 30 kV. Three strawberries were randomly removed from each treatment, and two flakes (8-mm long and 8-mm wide) of fruit epidermis from the equatorial region of each strawberry were cut with a razor blade, and then quickly immersed into liquid nitrogen and dried in a vacuum oven for 1 h. Prepared samples were observed by scanning electron microscopy.

2.4. Microbiological evaluations

A combined sample of all fruit with 1/8th of each fruit per replicate was homogenized and diluted with sterile 0.1% peptone water to obtain the microbial count. Serial dilutions were performed in triplicate. Total aerobic mesophilic bacteria counts were enumerated using the pour plate method on Plate Count Agar (PCA, Scharlau Chemie, S.A., Barcelona, Spain) after incubation at 32 °C for 2 days. Total yeasts and molds were enumerated using the surface plate method on a potato dextrose agar (PDA, Scharlau Chemie, SA., Barcelona, Spain). Incubation for total yeast and mold counts was performed at 25 °C for 2 days. Each test was performed in duplicate and results were expressed as Log colony-forming units (CFU) per g (Sogvar, Koushesh Saba, & Emamifar, 2016).

2.5. Weight loss (WL)

Fruit weight of each sample (box) was recorded on the day of treatment and at each sampling time. Cumulative weight loss was stated as percentage loss of the original fresh weight.

2.6. Firmness

Firmness was evaluated using a texture analyzer (Santam, STM-1), fitted with an 8-mm probe with constant speed of 20 mm min^{-1}. Two different measurements were carried out on two opposite sides of central zone of 10 berries. Values were expressed as newton (N).

2.7. Titratable acidity (TA), soluble solid concentration (SSC) and pH

A combined sample of all strawberries with two wedged-shaped slices of fresh tissue from two opposite sides of each fruit per replicate was juiced together and was used for TA, SSC, pH and vitamin C measurements. Aliquots of 10 mL were titrated to pH 8.1 with 0.1 N NaOH and expressed as % citric acid. The juice was also used to measure SSC using an Atago Digital Refractometer (Brix 0–32%, Atago, Japan). The pH of fruit juice was measured using a pH meter (Metrohm, 827).

2.8. Determination of waste

Waste percentage was assessed by visual observation. Strawberries with decay symptoms, brown spots and unmarketable appearance were assumed spoiled and weighted. Cumulative waste was expressed as percentage loss of the fresh weight.

2.9. Vitamin C assay

Vitamin C content was determined by titration with 2,6-dichlorophenolindophenol (DCPIP) (AOAC, 2000), using different ascorbic acid concentrations for the standard curve, and expressed in mg of vitamin C per 100 g of fresh weight.

2.10. Total anthocyanin and total phenolic (TP) concentrations

Total anthocyanin concentrations (TACs) were determined using the pH differential method of Cheng and Breen (1991). Absorbance was measured with a spectrophotometer (UV-2100, New Jersey) at 510 nm and 700 nm in buffers at pH 1.0 and 4.5. Results were expressed as mg of pelargonidin-3-glucoside equivalent, in the strawberry extract, per 100 g of FW. The absorbance difference between the buffer systems was calculated according to Eq. (1):

$$A = (A510 - A700\ nm)\ pH\ 1.0 - (A510 - A700\ nm)\ pH\ 4.5. \quad (1)$$