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Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice

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ABSTRACT

Nanocomposite LDPE films containing Ag and ZnO nanoparticles were prepared by melt mixing in a twin-screw extruder. Packages prepared from the films were then filled with fresh orange juice and stored at 4 °C. Microbial stability, ascorbic acid (AA) content, browning index, color value, and sensory attributes of them were evaluated after 7, 28, and 56 days of storage. Packages containing the nanomaterials, expect 1% nano-ZnO, kept the microbial load of fresh juice below the limit of microbial shelf life (6 log cfu/ml) up to 28 days The least degradation of AA (80.50 mg/100 g), development of brown pigments (OD = 0.23) and losing of color ($\Delta E = 6.0$) were observed in pouches containing 0.25% nano-ZnO, after the same time. Sensory attributes were also ranked highest for the juice thus packed in the recent packages after 28 days ($p < 0.05$). Packages containing nanosilver increased shelf life of fresh juice although part of its sensory attributes were lost.

Industrial relevance: Compared with pure packaging materials, antimicrobial nanocomposite packages containing Ag and ZnO as an alternative non-thermal technology can extend the shelf life of fresh orange juice up to 28 days. However, a certain concentration of nano-ZnO in the packages showed less adverse effects on sensory characteristics.

1. Introduction

Orange juice is one of the most globally accepted fruit products (Meléndez-Martínez, Vicario, & Heredia, 2007). Demand for natural orange juice with high quality in terms of nutritional value, physico-chemical properties and sensory characteristics with minimal or no heat treatment has increased considerably (Bull et al., 2004; Souza, Benassi, Meneghel, & Silva, 2004). Natural orange juice, even kept under refrigeration, has a short shelf life due to increasing microbial spoilage (Souza et al., 2004). Recently, extensive studies have been conducted to develop non-thermal processing techniques (PEF, HHP, IR, UV, and US) as replacements for thermal processing in order to keep the freshness of the juice along with extending its shelf life (Baxter, Easton, Schneebeli, & Whitfield, 2005; Elez-Martínez, Soliva-Fortuny, & Martín-Belloso, 2006; Foley et al., 2002; Tran & Farid, 2004; Valero et al., 2007). Although some of these technologies are capable of decontaminating orange juice, they are energy-intensive and require costly equipment; hence, their yet relatively limited commercial applications (Han, 2007). Nanotechnology recently introduced in the food packaging industry can potentially provide solutions to food packaging challenges such as short shelf life (Chaudhry et al., 2008; Joseph & Morrison, 2006). Antimicrobially active packaging is a new generation of nano food packaging based on metal nanocomposites which are made by incorporating metal nanoparticles into polymer films (Chaudhry et al., 2008). The high performance of nanoparticles is due to their high surface area/volume ratio, which is the main reason for increasing antimicrobial activity of metal nanoparticles (Damm, Neumann, & Münstedt, 2006).

Nanoparticels (NP) of Ag and ZnO are being used industrially for several purposes (Gajjar et al., 2009). ZnO has found many applications in daily life such as in drug delivery, cosmetics, and medical devices (Yan et al., 2009) due to its strong antimicrobial effect on a board spectrum of microorganisms (Jones, Ray, Ranjit, & Manna, 2008). Moreover, it is currently listed by FDA as a generally recognized as safe (GRAS) material (Jin, Sun, Su, Zhang, & Sue, 2009). Silver has also been long known to have microbial inhibition (Lok et al., 2006). The antimicrobial activity of these nanoparticles may be related to several mechanisms including, induction of oxidative stress due to generation of reactive oxygen species (ROS) which may cause the degradation of the membrane structure of the cell (Sawai, 2003; Sawai & Yoshikawa, 2004; Sawai et al., 1998), release of ions from the surface of nanoparticles that has been reported to cause bacterial death due to binding to cell membrane (Feng et al., 2000; Sondi & Salopek-Sondi, 2004). However, the mechanism of toxicity is still only partially understood (Li et al., 2008).

Several methods are generally used to produce antimicrobial polymer nanocomposites. Because of the thermal stability of metal nanoparticles and the thermal processing method used for producing the LDPE film as a contacting juice layer in the package, melt mixing is

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a good approach for this nanocomposite (Appendini & Hotchkiss, 2002; Damm et al., 2006).

The main objectives of this study is to evaluate the capabilities of ZnO and Ag nanoparticles filled LDPE nanocomposite packaging as a new approach to preservation and prolonging shelf life of orange juice.

2. Experimental

2.1. Preparation of antimicrobial nanocomposite films

Film grade LDPE resin pellets (LF0200, MFI 2 g/10 min, density 0.92 g/ml, softening point 94 °C) and antimicrobial agents including P105 powder (a combination of 95% TiO_2 powder which provided a base for doping of nanosilver, plus 5% metal nanosilver with particle diameters of about 10 nm) and ZnO nanoparticle powder with an average particle diameter of about 70 nm (Fig. 1a, b) were obtained from Pars Nanonasb Tehran, Iran. Film grade LDPE resin pellets (0.9 kg) were directly mixed with each of the antimicrobial agents (P105 and nano-ZnO particles) (0.1 kg) separately and the mixture was fed into a twin-screw extruder machine (Cincinnati Milacron, Batavia, OH) with a screw diameter of 55 mm and a screw length/diameter ratio of 30 mm to be cut into masterbatch nano-granules. The mass fraction of the filler for each antimicrobial agent was 10%. The heating profile was set to six heating zones of the twin-screw extruder including 160 °C, 160 °C, 175 °C, 150 °C, 150 °C, and 140 °C. Proper amounts of masterbatch resins were then added to pure LDPE resin pellets into a single-screw blowing machine with a screw diameter of 45 mm and a length/diameter ratio of 28 mm (Venus Plastic Machinery, Taiwan) to fabricate the final nanocomposite film (50 μm thick) with the desired nanomaterial concentrations (0.25 and 1% for nano-ZnO and 1.5 and 5% for P105). The temperature profile for the single extruder was maintained at 190 °C in the two barrel zones.

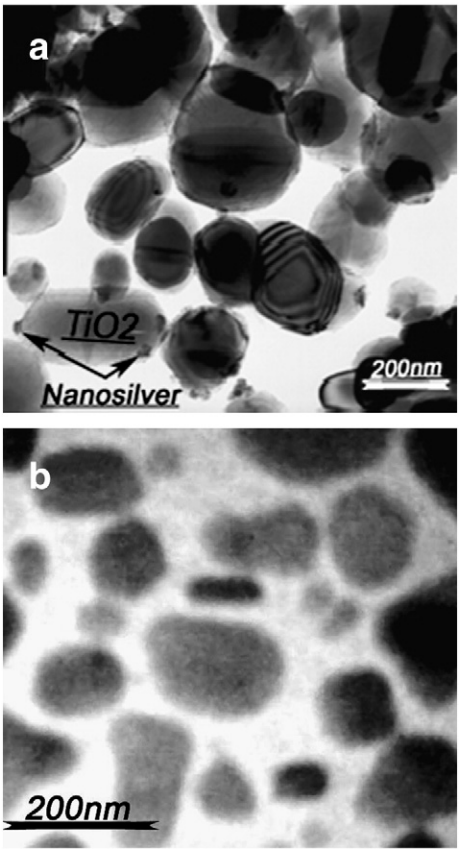

Fig. 1. TEM micrograph of a: P105 and b: nano-ZnO.

Film thickness was measured using a micrometer (Mitutoyo, Japan) and reported as the average of five readings taken at five different points on the film sample.

2.2. Transmission electron microscopy analysis

Dispersion quality of nanomaterials into the polymer matrix film was monitored using the Transmission Electron Microscope (PHILIPS CM 200 kV, The Netherlands).

2.3. Orange juice production

To prepare natural orange juice, 30 kg of oranges (*Citrus sinensis cv. Khaf)* were purchased from the local market in Isfahan, Iran. They were juiced using a semi-industrial juice extractor (M2000A-1, CMEC food machinery, China) equipped with a central fruit halving knife and a pair of holding cups, 90 mm in diameter, thoroughly washed with detergent and hot water. The juice (with an efficiency of 25.8%) was passed through a 1 mm mesh filter and immediately transferred into a sterile glass container under sanitized conditions. Packages were prepared by a hand heat sealer using antimicrobial nanocomposite and pure LDPE films 15×10 cm in size, similar to Doypack packaging commonly used for packaging fruit juice. The packages were immediately wrapped in aluminum foil and sanitized at 95 °C for 2 min. After cooling and under a sterile laboratory hood, 175 ml of fresh orange juice was poured into each package and sealed by the heat sealer.

2.4. Storage

Packages containing orange juice were stored in dark and cool conditions (4 °C). The samples were evaluated in duplicate for their microbiological, physicochemical, and sensory characteristics immediately after packaging and after 7, 28, and 56 days of storage.

2.5. Microbiological evaluations

Decimal dilutions were prepared from orange juice samples with sterile peptone water (0.1%). Volumes of dilution samples (0.1 ml) were then used. Total aerobic plate counts were enumerated using the pour plate method on the plate count agar (PCA, Scharlau Chemie, S.A., Barcelona, Spain). Incubation was performed at 30 °C for 3 days. Total yeast and moulds were enumerated using the surface plate method on the potato dextrose agar (PDA, Scharlau Chemie, SA., Barcelona, Spain) + 10% tartaric acid. Incubation for total yeast and mould counts was performed at 25 °C for 5 days. Each test was performed in duplicate and results were expressed as colony-forming units (CFU) per milliliter.

2.6. Ascorbic acid degradation

Most chemical analyses are based on the fact that ascorbic acid is easily oxidized. The most common method relies on the reduction of 2, 6 dichlorophenolindophenol reagent. Ascorbic acid degradation was determined using the titrimetric method (A.O.A.C., 967.21, 2002a).

2.7. Color measurement

Color was measured using a digital imaging method that used a combination of a digital camera (Panasonic, Japan), a computer, and a graphics software. A Petri dish containing 25 ml of orange juice was placed into the lighting system that consisted of two CIE source D65 lamps 45.0 cm long, mounted on the two sides of a frame installed on either side of the Petri dish, 30.5 cm above and at an angle of 45° to the orange juice sample plane. Images of the bottom surface of the orange juice were taken and saved using the digital camera that was placed 30.5 cm above the sample with its lens facing downwards towards the

orange juice. The color was analyzed using the Photoshop software. By turning on the grid feature in Photoshop, a grid was superimposed on the sample. As the computer pointer was placed at a grid point along the x or y axis, L, a, and b values corresponding to the pixels of that grid point were obtained from the Info Palette. The total color difference ($\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)]^{1/2}$) was determined in duplicate using CIE L, a, and b values (Yam & Papadakis, 2004).

2.8. Browning index measurement

To determine the browning index of the samples, 10 ml of the sample orange juice was centrifuged (10 min, 7800 ×*g* at 4 °C), and 5 ml of ethyl alcohol (95%) was added to 5 ml of the juice supernatant followed by centrifuging the mixture again under the same conditions. The absorbance of the supernatant was read at 420 nm using a spectrophotometer (2100-UV, Unico, NJ) according to the method described by Meydav, Saguy, and Kopelman (1977).

2.9. Sensory evaluations

Duplicate sensory panels comprising 10 experienced panelists were applied using a multiple comparison test of all packaging treatments (1.5%, 5% P105 and 0.25%, 1% nano-ZnO LDPE nanocomposite and pure LDPE) for each storage time. The samples were coded using random 3-digit figures and then served in transparent plastic glasses after opening each package. Orange juice was evaluated on a 5-point scale for four quality attributes, namely color, odor, taste, and overall acceptance. This scale comprised the expressions 'extremely liked' to 'extremely disliked' corresponding to the highest and lowest scores of 5 and 1, respectively. Data were analyzed by analysis variance and Duncan test. Significance was represented as $p<0.05$.

2.10. Metal ions releasing measurement

Silver and Zinc ions releasing into orange juice were determined using standard methods (A.O.A.C. 974.27, 2002b) slightly modified by Bings, Bogaerts, and Broekaert (2006) using a graphic furnace atomic absorption spectrometer (AA800, Perkin-Elmer, Shelton, CT) operated at 328.1 and 213.9 nm wavelengths.

2.11. Statistical analysis

Analysis of variance was carried out using the SAS statistical software release 6.12 (SAS Institute, Cray, NC) based on completely randomized designs. Significant differences among the data were represented as $p<0.05$.

3. Results

3.1. Transmission electron microscopy

Fig. 1(a, b) indicates that P105 particles are almost spherical in shape and that the diameter of silver nanoparticles doped on TiO_2 powder, is about 10 nm, whereas nano-ZnO particles have a hexagonal shape. Fig. 2(a,b,c,d) shows TEM images of nanocomposites LDPE containing different concentrations of P105 and ZnO nanoparticles. As shown in

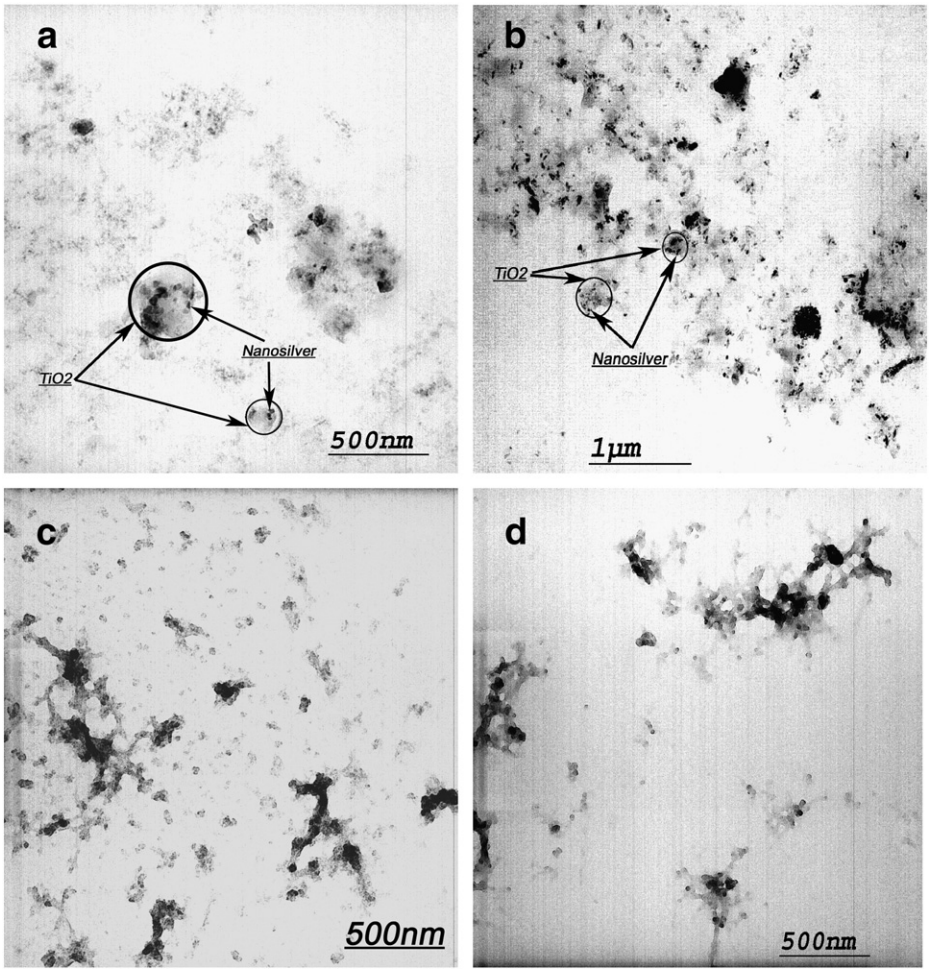

Fig. 2. TEM micrograph of antimicrobial nanocomposites LDPE film. a: LDPE + 1.5% P105, b: LDPE + 5% P105, c: LDPE + 0.25%nano-ZnO, d: LDPE + 1%nano-ZnO.

Fig. 2(a, b), P105 particles are well distributed in the polymer matrix. However, a slight agglomeration is observed by increasing the concentration of particle powders to 5%. The TEM image of nanocomposite LDPE + 0.25% nano-ZnO indicates that the particles are well dispersed in the polymer matrix exhibiting nanometer-scale aggregates ranging from 10 to 200 nm with an average size of 70 nm (Fig. 2c). As the nano-ZnO content increases to 1%, the quantity of the agglomerates increases and their size becomes more uneven (Fig. 2d).

3.2. Microbiological analysis

Mean initial population immediately after packaging was determined to be 4.93 log cfu/ml for yeast and moulds and 4.83 log cfu/ml for total aerobic bacteria in orange juice. The variations in the population of yeast and moulds and total aerobic bacteria are shown in Table 1. In packages made from pure LDPE, the mean population of yeast and moulds increased whereas that of total aerobic bacteria decreased after 7 days of storage. It can be observed that yeast and moulds are better adapted to orange juice under refrigeration than bacteria, which is in agreement with the findings of Sadler, Parish, and Wicker (1992). In the LDPE + 5% P105 sample, significance decreases were observed over 7 days of storage in total count and yeast and moulds population compared with LDPE + 0.25% nano-ZnO packages and LDPE pure packages containing the same concentration of nano-ZnO .

Table 1 shows that the level of population of yeast and moulds and total count increased to 6.47 log cfu/ml and 6.37 log cfu/ml, respectively, after 28 days of storage in LDPE pure packages. The shelf life of fresh orange juice is defined as the time to reach a microbial population of 6 log cfu/ml (Raccach & Mellatdoust, 2007). The mean population of total aerobic bacteria and yeast and moulds remained below 6 log cfu/ml after identical storage times in all the packages expect for the LDPE + 1% nano-ZnO one. By increasing nano-ZnO concentration to 1%, the antimicrobial activity of the film decreased (Table 1). This is due to the agglomeration of nanoparticles during the processing of the film (Fig. 2d). In contrast, by increasing the nanosilver concentration, the antimicrobial activity of the film increased (Table 1). The reduced antimicrobial activity of ZnO powder might be related to the increasing particle size, which might decrease the generation of H_2O_2 from the surface of ZnO powder (Yamamoto, 2001).

No significant differences were observed in total aerobic bacteria between LDPE + 1.5% P105 and LDPE + 0.25% nano-ZnO packages, whereas LDPE + 1.5% P105 showed a higher antifungal activity compared with LDPE + 0.25% nano-ZnO after 28 days of storage. It appears that antifungal activity of nanocomposites containing nanosilver is significantly ($p<0.05$) higher than that of nano-ZnO. Sawai and Yoshikawa (2004) have concluded that ZnO, CaO, and MgO powders have satisfactory antimicrobial effects against broad spectrum microorganisms but that ZnO has a poor antimicrobial effect on *Saccharomyces cerevisiae* and other yeast and moulds compared with bacteria. Based on our results (Table 1), the antimicrobial effect of Ag nanoparticles is much higher than that of ZnO nanoparticles. However, it seems that LDPE + 5% P105 has a significantly ($p<0.05$) higher antimicrobial activity compared with other nanocomposites over 28 days of storage for orange juice at 4 °C. This is while previous studies have shown a shelf life of up to 14 days for natural, cold orange juice (4 °C) (Bull et al., 2004; Fellers, 1988; Zanoni, Pagliarinib, Galli, & Laureati, 2005). Yeast, moulds and bacteria exhibit different levels of susceptibility to antimicrobial nanoparticles. The shelf life of natural orange juice has been observed to depend mainly on yeast growth in cold storage (Souza et al., 2004; Zanoni et al., 2005).

Microbial population increased with increasing storage time to 56 days in all the test packages, indicating the limited effect of long storage time on natural orange juice preservation. However, LDPE + 5% P105 packaging has a significantly less loading level at this storage time than other packaging materials.

Silver nanoparticles can damage cell membranes of microorganisms by forming "pits" on their surfaces. Moreover, they may penetrate into the cells to cause DNA damage (Sondi & Salopek-Sondi, 2004; Morones et al., 2005). Silver ions released from the surface of these nanoparticles can interact with thiol groups in protein to induce bacterial inactivation, condensation of DNA molecules, and loss of their replication ability (Feng et al., 2000). Based on electron spin resonance (ESR) measurements, Kim et al. (2007) observed that the antimicrobial mechanism of Ag nanoparticles is related to the formation of free radicals and the subsequent free radical-induced membrane damage. However, Ag/TiO_2 shows great promise as a photocatalytic material due to its photoreactivity and visible light

Table 1
Effect of packaging containing Ag and ZnO nanoparticles (mean ± SD)[a] on the fungi, and total aerobic bacteria population, ascorbic acid, browning index, and total color differences (ΔE) during 56 days storage at 4 °C.

Film type	Storage time (day)	(Yeast and moulds) (log cfu/ml)	Total aerobic bacteria (log cfu /ml)	Ascorbic acid (mg/100 g)	Browning Index (OD)	ΔE
LDPE pure	0	$4.94^{ij} \pm 0.05$	$4.84^{fg} \pm 0.07$	$85.67^{a} \pm 0.58$	$0.15^{h} \pm 0.0006$	$0.00^{j} \pm 0.00$
	7	$5.08^{h} \pm 0.08$	$4.65^{hi} \pm 0.05$	$83.07^{b} \pm 0.21$	$0.22^{g} \pm 0.003$	$4.70^{h} \pm 0.08$
	28	$6.26^{cd} \pm 0.02$	$5.27^{d} \pm 0.06$	$81.23^{c} \pm 0.32$	$0.24^{e} \pm 0.003$	$5.56^{g} \pm 0.10$
	56	$6.47^{b} \pm 0.14$	$6.35^{a} \pm 0.06$	$78.40^{ef} \pm 0.55$	$0.24^{de} \pm 0.003$	$7.41^{c} \pm 0.09$
LDPE + 1.5%P105	0	$4.94^{ij} \pm 0.03$	$4.83^{fg} \pm 0.02$	$85.83^{a} \pm 0.29$	$0.15^{h} \pm 0.001$	$0.00^{j} \pm 0.00$
	7	$4.51^{k} \pm 0.07$	$4.65^{hi} \pm 0.05$	$82.87^{b} \pm 0.15$	$0.23^{ef} \pm 0.002$	$5.59^{g} \pm 0.25$
	28	$5.74^{f} \pm 0.04$	$4.85^{fg} \pm 0.05$	$79.87^{d} \pm 0.42$	$0.24^{de} \pm 0.002$	$6.66^{d} \pm 0.32$
	56	$6.16^{d} \pm 0.05$	$5.76^{c} \pm 0.16$	$77.73^{f} \pm 0.38$	$0.25^{c} \pm 0.006$	$7.61^{b} \pm 0.01$
LDPE + 5%P105	0	$4.94^{ij} \pm 0.05$	$4.84^{fg} \pm 0.06$	$85.67^{a} \pm 0.58$	$0.15^{h} \pm 0.002$	$0.00^{j} \pm 0.00$
	7	$4.36^{l} \pm 0.05$	$4.16^{k} \pm 0.05$	$80.01^{d} \pm 0.98$	$0.25^{c} \pm 0.004$	$6.43^{e} \pm 0.06$
	28	$5.43^{g} \pm 0.08$	$4.54^{j} \pm 0.05$	$74.37^{h} \pm 0.40$	$0.27^{b} \pm 0.003$	$6.53^{de} \pm 0.09$
	56	$6.02^{e} \pm 0.02$	$5.66^{c} \pm 0.06$	$63.90^{i} \pm 0.66$	$0.28^{a} \pm 0.009$	$7.99^{a} \pm 0.01$
LDPE + 0.25%nano-ZnO	0	$4.95^{ij} \pm 0.03$	$4.83^{fg} \pm 0.05$	$86.33^{a} \pm 0.58$	$0.15^{h} \pm 0.001$	$0.00^{j} \pm 0.00$
	7	$4.85^{j} \pm 0.03$	$4.62^{ij} \pm 0.05$	$83.17^{b} \pm 0.38$	$0.23^{fg} \pm 0.006$	$3.69^{i} \pm 0.04$
	28	$5.97^{e} \pm 0.03$	$4.90^{f} \pm 0.10$	$80.50^{cd} \pm 0.46$	$0.23^{ef} \pm 0.003$	$6.00^{f} \pm 0.02$
	56	$6.30^{c} \pm 0.04$	$5.72^{c} \pm 0.08$	$78.33^{ef} \pm 0.29$	$0.24^{d} \pm 0.004$	$7.48^{bc} \pm 0.12$
LDPE + 1%nano-ZnO	0	$4.90^{ij} \pm 0.03$	$4.83^{fg} \pm 0.01$	$86.00^{a} \pm 0.06$	$0.15^{h} \pm 0.0001$	$0.00^{j} \pm 0.00$
	7	$4.93^{ij} \pm 0.03$	$4.75^{gh} \pm 0.05$	$83.63^{b} \pm 0.55$	$0.23^{fg} \pm 0.002$	$5.72^{g} \pm 0.04$
	28	$6.26^{c} \pm 0.03$	$5.100^{e} \pm 0.01$	$78.80^{e} \pm 0.26$	$0.25^{c} \pm 0.003$	$5.90^{f} \pm 0.06$
	56	$6.59^{a} \pm 0.12$	$6.150^{b} \pm 0.01$	$76.67^{g} \pm 0.58$	$0.25^{c} \pm 0.008$	$8.06^{a} \pm 0.09$

[a] Values followed by the same letter in the same row are not significantly different ($p<0.05$).

response (Li et al., 2008). Zhang and Chen (2009) showed that doping TiO_2 with a metallic form of nanosilver, enhanced its bactericidal activity due to the unique structural feature of nanosilver dispersed on TiO_2 surface. This indicated that TiO_2 serves as a solid antiaggregation support to maintain the dispersion of nanosilver, which could also contribute to its antibacterial performance. Kubacka et al. (2009) maintained that ethylene-vinyl alcohol copolymer (EVOH) nanocomposite containing mixed Ag–TiO_2 has a good antimicrobial activity against yeast and moulds and bacteria through a plasmonic effect. This interaction not only optimizes UV\visible photon handling by the film but also makes the whole surface of the nanomaterial biocidal while also eliminating the necessity for the contact between the primary biocidal inorganic agent and the microorganism.

An, Zhang, Wang, and Tang (2008) found an optimal preservation of 0.06 mgl⁻for asparagus spears by coating silver nanoparticles-PVPwas. Damm, Münstedt, and Rösch (2008) reported that polyamide 6 filled with 2% (w/w) nanosilver was effective against *E. coli* even after being immersed in water for 100 days. Fernández et al. (2009) reported that absorbent pads containing nanosilver were the common component in packaging to persevere poultry meat up to consumption and that they could yield a log reduction of up to 40% in aerobic mesophilic bacteria.

Antimicrobial effects of ZnO nanoparticles may be attributed to several mechanisms: 1) induction of oxidative stress due to ROS generation especially interior or out of cell H_2O_2 which leads to interaction with proteins, DNA, and lipids causing death (Adams, Lyon, & Alvarez, 2006; Sawai et al., 1998; Sawai, 2003; Sawai & Yoshikawa, 2004); 2) membrane disorganization due to accumulation of ZnO nanoparticles in the bacterial membrane and also their cellular internalization (Brayner et al., 2006); 3) release of Zn ions that may be responsible for antimicrobial activity by binding to the membrane of microorganisms (Gajjar et al., 2009). However, the toxicity of ZnO nanoparticles is not directly related to their entering into the cell, rather their intimate contact onto the cell causes changes in the microenvironment in the vicinity of the organism–particle contact area to either increase metal solubilization or to generate ROS, that may ultimately damage cell membrane (Heinlaan, Ivask, Blinova, & Dubourguier, 2008). Moreover, the toxicity of ZnO nanoparticles is not only affected by the light via ROS production, but may also happen in the dark although its mechanism is not yet defined (Adams et al., 2006).

Jin et al. (2009) studied several approaches (powder, film, PVP capped and coating) for the application of nano-ZnO in food systems and concluded that nano-ZnO exhibits antimicrobial effects against *L. monocytogenes* and *S. enteritidis* in liquid egg white and in culture media.

3.3. Ascorbic acid and browning index

The ascorbic acid content of orange juice is in the range of 26 to 84 $mg/_{100\ g}$ (Supraditareporn & Pinthong, 2007), which decreases during storage (Plaza et al., 2006). The values of browning index in fresh orange juice immediately after packaging were measured at 0.15. However, Leizerson and Shimoni (2005) reported that increased values of browning index by up to 0.367 are still invisible. Increasing temperature has a major effect on the increased rate of browning reaction in fruit juice (Koca, Burdurlu, & Karadeniz, 2003). According to Table 1, a significant decrease is observed in the ascorbic acid content of all the experimental packages during storage at 4 °C while the browning index increased significantly in all the test packages. This overall AA reduction might be due to the nonbarrier properties of packaging against oxygen (Fellers, 1988; Sadler et al., 1992) and the duration of storage time (Martín-Diana, Rico, Barat, & Barry-Ryan, 2009). Based on the same table, loss of ascorbic acid and development of brown pigments are significantly higher in LDPE + 5% P105 than in other packages while the rates for these changes decrease with decreasing nanosilver concentration in LDPE + 1.5% P105. This indicates that ROS which might be responsible for antimicrobial activity may also increase ascorbic acid losses (Choe, Huang, & Min, 2005). After storage for 28 days in LDPE + 5% P105, AA content decreased in the juice by about 13% (74.37 $mg/_{100\ g}$), while the values for its degradation in other nanopackages including LDPE + 1.5% P105, LDPE + 0.25% nano-ZnO, and LDPE + 1% nano-ZnO were 79.87 $mg/_{100\ g}$, 80.5 $mg/_{100\ g}$, and 78.8 $mg/_{100\ g}$, respectively. Table 1 shows that the rates of degradation of AA and creation of brown pigments in LDPE + 0.25% nano-ZnO packages were significantly lower than those in other cases. Ascorbic acid is usually degraded by the oxidative process which is stimulated in the presence of light, oxygen, heat, peroxides and enzymes (Plaza et al., 2006).

3.4. Color

Orange juice color is mainly due to the presence of carotenoid pigments and is influenced by product ripening, processing treatments, storage conditions, and browning reactions (Cortés, Esteve, & Frígola, 2008). Table 1 shows the changes in total color differences ($\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)]^{1/2}$) for all the test packages compared with pure LDPE. Statistical results show significant differences ($p<0.05$) in ΔE after 7 days of storage, indicating that storage time is an important factor influencing color value and ΔE (Tiwari, Odonnell, Muthukumarappan, & Cullen, 2009). ΔE changed in LDPE + 0.25% nano-ZnO after 7 days at a much lower rate than it did in LDPE + 5% P105 . It can be observed all the nanopackages tested had a significant difference in their ΔE values after 28 days compared with pure LDPE. It is clear that ΔE values are lower for nanopackages containing ZnO than for those containing nanosilver. These changes correlated well with the reduction of ascorbic acid and production of brown pigments during storage (Table 1). Bleaching effect in orange juice might be due to the oxidative degradation of carotenoids (Haugaard, Weber, Danielsen, & Bertelsen, 2002); thus, the free radical in antimicrobial orange juice packaging might be responsible for the change in ΔE. Bull et al. (2004) reported an increase in the total color differences with time in fresh orange juice during storage, regardless of the treatment.

3.5. Sensory evaluation

Fig. 3 shows the change of sensory attributes of natural orange juice packed in different packages. The high similarity observed in color

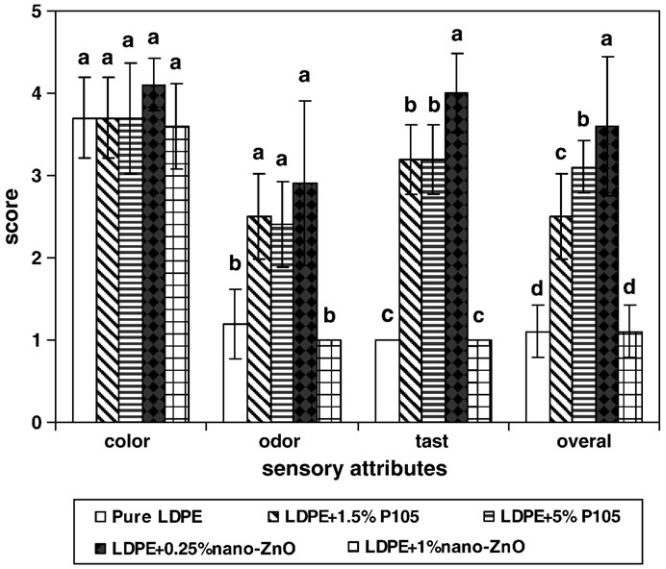

Fig. 3. Sensory attributes of orange juice that is stored in antimicrobial nanocomposite and pure packages.

attribute scores of the packages after 28 days of cold storage ($p<0.05$) indicates that the change in the color of the samples is still invisible. These results correlated well with the values of browning index presented in Table 1. Odor attribute is greatly influenced by microbial growth and may lead to fermentation in orange juice during storage (Haugaard et al., 2002; Parish & Higgins, 1989). After 28 days of storage, a significant difference is observed between the odor of orange juice packed in the test packages and that in pure package except for the one containing 1% nanoZnO. Changes in the taste of packed orange juice during 28 days of storage show the positive effect of nanoantimicrobial packaging. It is obvious that there is a significant difference between LDPE +0.25% nano-ZnO packaging and other nanosilver packaging materials, the lowest score being associated with LDPE + 1% nano-ZnO and pure LDPE. The sensory panelists recognized LDPE +0.25% nano-ZnO film followed by LDPE + 5% P105 and LDPE + 1.5% P105 as the best packaging material in terms of overall acceptability. It is noteworthy that changing orange juice flavor during storage is not only due to the growth of microorganisms but also to heating, storage time, and the common chemical interactions that occur in stored juices (Haugaard et al., 2002; Parish, 1998). Souza et al. (2004) reported that lower storage temperatures of unpasteurized orange juice gave rise to a higher sensory acceptance than the higher temperatures for 72 h. Leizerson and Shimoni (2005) reported that the sensorial shelf life of orange juice is equal to half its microbial and 2/3 its chemical shelf life.

3.6. Metal ions releasing measurement

The quantities of silver and Zinc ions in orange juice after 28 days of storage are shown in Table 2. The quantity of silver ions migrating into orange juice is less than its allowable concentration (10 ppm). It has been reported that silver ions at as low concentrations as 10^{-9} molesl^{-1} have an antimicrobial effect in water (Damm et al., 2006). Moreover, the quantity of Zinc ions indicated a higher rate of Zn migration than that of silver but as Zinc is proved to be a GRAS compound for food applications, its low concentration is in the acceptable range for food consumers (Jin et al., 2009).

4. Conclusion

This study showed that application of LDPE nanocomposite packaging materials containing Ag and ZnO nanoparticles is a new approach for preserving and extending the shelf life of fresh orange juice at 4 °C. The quality of the packaging film including good dispersion of nanomaterials in the polymer matrix free from agglomeration was shown to be very effective on the antimicrobial effects of these packaging materials. Application of packages containing nano-ZnO prolonged the shelf life of fresh orange juice up to 28 days without any negative effects on sensorial parameters. Nanosilver had a higher antimicrobial activity on yeast and moulds compared with ZnO nanoparticles. It was also shown that application of this nanopackaging for storage of fresh orange juice, was not sufficient for long time storage. The study revealed that for prolonged shelf life of fresh orange juice, a mild heat treatment is necessary in addition to the nanopackages.

Table 2
The quantity of Ag and Zn ions (mean ± SD) released from nanocomposite LDPE films containing Ag and ZnO nanoparticles in orange juice after 28 days.

Concentration (μgl^{-1})	Film type			
	LDPE + 1.5% P105	LDPE + 5% P105	LDPE + 0.25% nano-ZnO	LDPE + 1% nano-ZnO
Silver	0	0.1 ± 0.003		
Zinc			0.16 ± 0.007	0.11 ± 0.003

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References

A.O.A.C. (2002a). Vitamin C (ascorbic acid) in vitamin preparations and juices: 2, 6 dichloroindophenol titrimetric method final action. In W. Horwitz (Ed.), *Official Methods of Analysis of AOAC International*, 17 th ed. . Gaithersburg, MD: Association of Official Analytical Chemists Official Method 967.21.

A.O.A.C. (2002b). Cadmium, Chromium, Copper, Iron, Lead, Magnesium, Manganese, Silver, and Zinc in water. In W. Horwitz (Ed.), *Official Methods of Analysis of AOAC International*, 17 th ed. . Gaithersburg, MD: Association of Official Analytical Chemists Official Method 974.27.

Adams, L. K., Lyon, D. Y., & Alvarez, P. J. J. (2006). Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. *Water Research*, *40*(19), 3527−3532.

An, J., Zhang, M., Wang, S., & Tang, J. (2008). Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP. *LWT—Food Science and Technology*, *41*, 1100−1107.

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies*, *3*, 113−126.

Baxter, I. A., Easton, K., Schneebeli, K., & Whitfield, F. B. (2005). High pressure processing of Australian navel orange juices: Sensory analysis and volatile flavor profiling. *Innovative Food Science and Emerging Technologies*, *6*, 372−387.

Bings, N. H., Bogaerts, A., & Broekaert, J. A. C. (2006). Atomic spectroscopy. *Analytical Chemistry*, *78*(12), 3917−3945.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M. F., & Fiévet, F. (2006). Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine zno nanoparticles colloidal medium. *Nano Letters*, *6*(4), 866−870.

Bull, M. K., Zerdin, K., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., et al. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovative Food Science and Emerging Technologies*, *5*, 135−149.

Chaudhry, Q., Scotte, M., Blackburn, J., Ross, B., Boxall, A., & Castle, L. (2008). Applications and implications of nanotechnologies for the food sector. *Food Additives and Contaminants*, *25*(3), 241−258.

Choe, E., Huang, R., & Min, D. B. (2005). Chemical reactions and stability of riboflavin in foods. *Jounnal of Food Science*, *70*(1), 28−36.

Cortés, C., Esteve, M. J., & Frígola, A. (2008). Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice. *Food Control*, *19*, 151−158.

Damm, C., Münstedt, H., & Rösch, A. (2008). The antimicrobial efficacy of polyamide 6/silver-nano- and microcomposites. *Materials Chemistry and Physics*, *108*(1), 61−66.

Damm, C., Neumann, M., & Münstedt, H. (2006). Properties of nanosilver coatings on polymethyl methacrylate. *Soft Materials*, *3*(2), 71−88.

Elez-Martínez, P., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2006). Comparative study on shelf life of orange juice processed by high intensity pulsed electric fields or heat treatment. *European Food Research and Technology*, *222*, 321−329.

Fellers, P. J. (1988). Shelf life and quality of freshly squeezed, unpasteurized, polyethylene-bottled citrus juice. *Journal of Food Science*, *53*, 1699−1702.

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *Journal of Biomedical Materials Research*, *52*(4), 662−668.

Fernández, A., Soriano, E., López-Carballo, G., Picouet, P., Lloret, E., Gavara, R., et al. (2009). Preservation of aseptic conditions in absorbent pads by using silver nanotechnology. *Food Research International*, *42*(8), 1105−1112.

Foley, D. M., Pickett, K., Varon, J., Lee, J., Min, D. B., Caporaso, F., et al. (2002). Pasteurization of fresh orange juice using gamma irradiation: microbiological, flavor, and sensory analyses. *Jounnal of Food Science*, *67*(4), 1495−1501.

Gajjar, P., Pettee, B., Britt, D. W., Huang, W., Johnson, W., & Anderson, A. J. (2009). Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida KT2440*. *Jounnal of Biological Engineering*, *3*(9), 1183−1189.

Han, J. H. (2007). Packaging for nonthermally processed foods. In J. H. Han (Ed.), *Packaging for nonthermal processing of food* (pp. 213−225). Ames, Iowa: Wiley-blackwell.

Haugaard, V. K., Weber, C. J., Danielsen, B., & Bertelsen, G. (2002). Quality changes in orange juice packed in materials based on polylactate. *European Food Research and Technology*, *214*, 423−428.

Heinlaan, M., Ivask, A., Blinova, I., & Dubourguier, H. C. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere*, *71*, 1308−1316.

Jin, T., Sun, D., Su, J. Y., Zhang, H., & Sue, H. J. (2009). Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes*, *Salmonella enteritidis*, and *Escherichia coli* O157:H7. *Journal of Food Science*, *74*(1), 46−52.

Jones, N., Ray, B., Ranjit, K. T., & Manna, A. C. (2008). Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiology Letters*, *279*, 71−76.

Joseph, T., & Morrison, M. (2006). *Nanotechnology in agriculture and food*.A Nanoforum report www.nanoforum.org, 14 pp..

Kim, J. S., Kuk, E., Yu, K., Kim, J. H., Park, S. J., Lee, S. J., et al. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *3*, 95−101.

Koca, N., Burdurlu, H. S., & Karadeniz, F. (2003). Kinetics of nonenzymatic browning reaction in citrus juice concentrates during storage. *Turkish Journal of Agriculture and Forestry*, *27*, 353−360.

Kubacka, A., Cerrada, M. L., Serrano, C., Fernández-García, M., Ferre, M., & Fernández-García, M. (2009). Plasmonic nanoparticle/polymer nanocomposites with enhanced photocatalytic antimicrobial properties. *The Journal of Physical Chemistry, C*, *113*, 9182−9190.

Leizerson, S., & Shimoni, E. (2005). Stability and sensory shelf life of orange juice pasteurized by continuous ohmic heating. *Jounnal of Agriculture and Food Chemistry*, *53*, 4012−4018.

Li, Q., Mahendra, S., Lyon, D. Y., Brunet, L., Liga, M. V., & Li, D. (2008). Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. *Water Research*, *42*, 4591−4602.

Lok, C. N., Ho, C. M., Chen, R., He, Q. Y., Yu, W. Y., Sun, H., et al. (2006). Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *Journal of Proteome Research*, *5*, 916−924.

Martín-Diana, A. B., Rico, D., Barat, J. M., & Barry-Ryan, C. (2009). Orange juices enriched with chitosan: Optimisation for extending the shelf-life. *Innovative Food Science and Emerging Technologies*, *10*(4), 590−600.

Meléndez-Martínez, A. J., Vicario, I. M., & Heredia, F. J. (2007). Review: Analysis of carotenoids in orange juice. *Journal of Food Composition and Analysis*, *20*, 638−649.

Meydav, S., Saguy, I., & Kopelman, I. J. (1977). Browning determination in citrus products. *Jounnal of Agriculture and Food Chemistry*, *25*(3), 602−604.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., et al. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, *16*, 2346−2353.

Parish, M. E. (1998). Orange juice quality after treatment by thermal pasteurization or isostatic high pressure. *Lebensmittel Wissenschaft und Tecnhnologie*, *31*, 439−442.

Parish, M. E., & Higgins, D. P. (1989). Yeasts and mold isolated from spoiling citrus products and by-products. *Journal of Food Protection*, *52*(4), 261−263.

Plaza, L., Sánchez-Moreno, C., Elez-Martínez, P., De Ancos, B., Martín-Belloso, O., & Cano, M. P. (2006). Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. *European Food Research and Technology*, *223*, 487−493.

Raccach, M. M., & Mellatdoust, M. (2007). The effect of temprature on microbial growth in orange juice. *Journal of Food Processing and Preservation*, *31*, 129−142.

Sadler, G. D., Parish, M. E., & Wicker, L. (1992). Microbial, enzymatic, and chemical challenges during storage of fresh and processed orange juice. *Jounnal of Food Science*, *57*(5), 1187−1192.

Sawai, J. (2003). Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. *Journal of Microbiological Methods*, *54*, 177−182.

Sawai, J., Shoji, S., Igarashi, H., Hashimato, A., Kokugan, T., & Shimizu, M. (1998). Hydrogen peroxide as an antibacterial factor in zinc oxide powder slurry. *Jounnal of Fermentation and Bioengineering*, *86*(5), 521−522.

Sawai, J., & Yoshikawa, T. (2004). Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conductimetric assay. *Journal of Applied Microbiology*, *96*, 803−809.

Sondi, I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *Journal of Colloid and Interface Science*, *275*(1), 177−182.

Souza, M. C. C., Benassi, M. T., Meneghel, R. F. A., & Silva, R. S. S. F. (2004). Stability of unpasteurized and refrigerated orange juice. *Brazilian Archives of Biology and Technology*, *47*(3), 391−397.

Supraditareporn, W., & Pinthong, R. (2007). Physical, chemical and microbiological changes during storage of orange juices cv. Sai Nam Pung and cv. Khieo Waan in Northern Thailand. *International Journal of Agriculture & Biology*, *9*(5), 726−730.

Tiwari, B. K., Odonnell, C. P., Muthukumarappan, K., & Cullen, P. J. (2009). Effect of low temperature sonication on orange juice quality parameters using response surface methodology. *Food and Bioprocess Technology*, *2*, 109−114.

Tran, M., & Farid, M. (2004). Ultraviolet treatment of orange juice. *Innovative Food Science and Emerging Technologies*, *5*, 495−502.

Valero, M., Recrosio, N., Saura, D., Muñoz, N., Martí, N., & Lizama, V. (2007). Effects of ultrasonic treatments in orange juice processing. *Journal of Food Engineering*, *80*, 509−516.

Yam, K. L., & Papadakis, S. E. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. *Jounnal of Food Engineering*, *61*, 137−142.

Yamamoto, O. (2001). Influence of particle size on the antibacterial activity of zinc oxide. *International Journal of Inorganic Materials*, *3*, 643−646.

Yan, D., Yin, G., Huang, Z., Yang, M., Liao, X., Kang, Y., et al. (2009). Characterization and bacterial response of zinc oxide particles prepared by a biomineralization process. *The Journal of Physical Chemistry*, *113*, 6047−6053.

Zanoni, A., Pagliarinib, E., Galli, A., & Laureati, M. (2005). Shelf-life prediction of fresh blood orange juice. *Jounnal of Food Engineering*, *70*(4).

Zhang, H., & Chen, G. (2009). Potent antimicrobial activity of Ag/TiO2 nanocomposite powder synthesized by a one-pot sol–gel method. *Environmental Science & Technology*, *43*, 2905−2910.