Beneficial Effects of Polyethylene Packages Containing Micrometer-Sized Silver Particles on the Quality and Shelf Life of Dried Barberry (*Berberis vulgaris*)

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Abstract: In this research, the effects of low-density polyethylene (LDPE) packages containing micrometer-sized silver particles (LDPE-Ag) on microbial and sensory factors of dried barberry were investigated in comparison with the pure LDPE packages. LDPE-Ag packages with 1% and 2% concentrations of silver particles statistically caused a decrease in the microbial growth of barberry, especially in the case of mold and total bacteria count, compared with the pure LDPE packages. The taste, aroma, appearance, and total acceptance were evaluated by trained panelists using the 9-point hedonic scale. This test showed improvement of all these factors in the samples related to packages containing 1% and 2% concentrations of silver particles in comparison with other samples.

Keywords: antimicrobial effects, Barberry (*Berberis vulgaris*), low-density polyethylene package, micrometer-sized silver particle, sensory properties

Practical Application: Low-density polyethylene package containing micrometer-sized silver particles had beneficial effects on the sensory and microbial quality of barberry when compared with normal packing material.

Introduction

Seedless barberries have food usages and drug benefits. Apart from using dry barberry as seasoning of foods, barberry is used in the production of some food stuffs, such as sauce, jelly, candy, concentrate, juice, gassy drinks, jam, marmalade, barberry sherbet, barberry powder, polaki, honey candy, and barberry fruit roll. In addition to the applications of barberry in traditional medicine, antihistaminic and anticholinergic (Shamsa and others 1999), antioxidant (Tomosaka and others 2008; Qadir and others 2009), anticancer (Duke 2002; Ho and others 2009; Qadir and others 2009), antigout, antiphlogosis, antiacne, and antispasm properties (Kafi and Balendari 2002) of this plant have been proved. Moreover, barberry decreases blood sugar and blood cholesterol (Kafi and Balendari 2002; Schmandke 2007).

Water and enzyme activities, microbial growth, and change in the pigment color are some of the most important factors that affect the quality of barberry. One problem related to these damaging factors is barberry packaging (Kafi and Balendari 2002). Nowadays, more attention is paid to the nanocomposite packages than other ones. Nanocomposites improve the mechanical, thermal, and oxidation stability and also the barrier properties of polymers (Azeredo 2009; Arora and Padua 2010; Mahalik and Nambiar 2010). Meanwhile, biodegradable biopolymers as polysaccharides, proteins and so on are expected to gradually replace poorly degradable plastics but these materials, generally have serious disadvantages, such as poor mechanical characteristics and high water permeability. Combination of biopolymers with some nanoparticles seems to solve this problem (Popov and others 2010). In addition to the mentioned properties, some nanocomposites containing antimicrobial agents have antimicrobial properties. Different types of metal antimicrobial agents, like copper, zinc, titanium dioxide, magnesium, gold, and silver are available; however, silver particles have proved to be the most effective. This is due to their antimicrobial activity against a wide range of microorganisms. Micrometer-sized silver particles have larger surface area available for interaction with microbial cells in comparison with larger particles. This improves their antimicrobial property. Among antibacterial metals, silver is a safe and broad-spectrum antibacterial agent (Gong and others 2007; Azeredo 2009; Chudasama and others 2009; Rai and others 2009). It should be noted that particles of 50 nm can penetrate to cells, of 70 nm to lungs, and of 30 nm even to blood and brain cells (Chau and others 2007; Mortimer and others 2008). Thus, they can migrate from lungs to the circulatory system, spread over the whole body, and further enter some organs (liver, spleen, marrow, heart, brain, and so on) (Popov and others 2010). Scientific Committee on Emerging and Newly Identified Health Risk (2006) reported that expected consumer exposure remains low as long as the inert silver nanoparticles (Ag-NPs) are bound in the packaging materials or in the coatings on surfaces of packaging materials and food preparation devices. A scientific opinion of European Food Safety

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doi: 10.1111/j.1750-3841.2011.02497.x

Journal of Food Science A Publication of the Institute of Food Technologists

Authority (EFSA) (EFSA Panel on food contact materials, enzymes, flavourings and processing aids (CEF) 2011) presents that there is no safety concern for the consumer if migration of silver ion does not exceed the group specific migration limit of 0.05 mg Ag/kg food. Also, The permissible exposure limit (PEL) recommended by the Occupational Safety and Health Administration and the Mine Safety and Health Administration (OSHA 1989) and the Natl. Inst. for Occupational Safety and Health (NIOSH 1992, NIOSH 2003) is 0.01 mg/m^3 for all forms of silver.

The effect of LDPE-Ag packages on barberry has not yet been studied. However, in some studies related to properties of silver nanocomposites, antimicrobial activity of poly lactic acid (PLA) containing Ag-NPs has been observed 98.5% against *Staphylococcus aureus* and 94.2% against *Escherichia coli* (Xu and others 2006). Fernández and others (2010) produced PLA/silver zeolite films. They showed antimicrobial activity of these films against *S. aureus* and *E. coli*. Also, Fresher longer storage containers contain Ag-NPs infused into polypropylene-based material. These storage containers keep food, especially fruits and vegetables, up to 3 or 4 times longer by reducing the growth of mold and fungus (Brody and others 2008). Yang and others (2010) blended polyethylene with silver powder. They showed that these packages were able to maintain the sensory, physicochemical, and physiological quality of strawberry fruits. In another work, Ag^+-based antimicrobial film was obtained by depositing an Ag-containing polyethylenoxide-like coating on a polyethylene layer. This active film successfully inhibited the growth of *Alicyclobacillus acidoterrestris* in acidified malt extract broth and apple juice (Nobile and others 2004). An and others (2008) investigated the effect of Polyvinylpyrrolidone packages containing Ag-NPs on green asparagus spears. These packages reduced the growth of microorganisms. Asparagus spears coated by Ag-NPs could be kept in good quality for 25 d at 2 °C, and for 20 d at 10 °C. Also, the microbiological tests of a low-density polyethylene (LDPE) film containing Ag-NPs indicated reduction against mold and yeast (Brody 2008). Li and others (2009) showed that the nanocomposite PE film with Ag-NPs had quite a beneficial effect on the physicochemical and sensory quality of Chinese jujube when compared with normal packaging material. In another work, silver-impregnated chitosan film was produced. It was more effective against *E. coli* than pure chitosan film while chitosan itself has antimicrobial activity due to its cationic properties. This film demonstrated high antibacterial activity against Gram-positive and Gram-negative bacteria (Wei and others 2009).

In this research, we studied the effects of LDPE package containing micrometer-sized silver particles and the concentration of silver particles on the quality and shelf life of dried barberry.

Materials and Methods

Materials

The barberry used in this study was *berberis vulgaris*. *Berberis vulgaris var. asperma* was the variety of barberry used in this study. It was harvested in the south of Khorasan, an eastern province of Iran, at a mature stage in 2010.

First, the barberry was chosen randomly from a farm, as far as possible maintaining similar conditions during all the stages before packaging. They were packaged in LDPE packages containing micrometer-sized silver particles with a concentration of 0.02% (sample Nr. 1), 1% (sample Nr. 2), and 2% (sample Nr. 3) of silver particles, and in normal cellophane pure LDPE package (sample Nr. 4). These packages were produced at Ferdowsi Univ. of Mashhad. Their thickness was about 0.25 mm. Preservation of barberry was done during room temperature storage.

The cultures used in microbiological tests were: Yeast Extract Glucose Chloramphenicole agar (YGC agar) to detect and account for mold and yeast, Mac-conkey agar to detect and account for coliform and *E. coli*, Plate count agar (PCA) to total mesophilic aerobic bacteria count and Baird–Parker agar to detect and account *S. aureus*.

Methods

LDPE packages containing micrometer-sized silver particles were produced by extrusion method. In this experimental study,

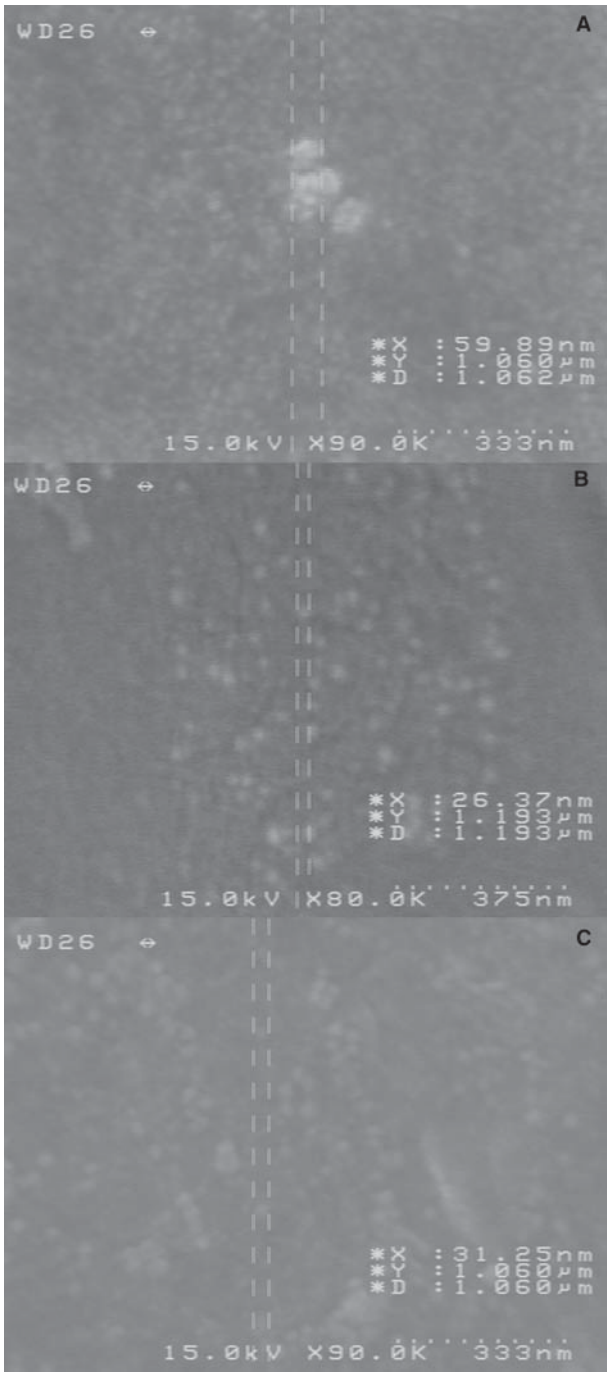

Figure 1–SEM image of polyethylene films with: (A) 0.02%; (B) 1% and 2% concentration of silver particles.

1 pack of each sample was studied weekly. The actual time to conduct this study from the initial packaging up to opening the last package took approximately 5 mo. The microbial, sensory, and apparent color investigations were done in 1st 13 wk for all the samples. The microbial tests were continued up to 22 wk for samples Nr. 2 and 4. It was done in order to compare the effect of LDPE-Ag packages with common packages in a much improved method.

Preparation of films

First of all, silver powder (33% wt), polyethylene (50% wt), and dicumyl peroxide as cross-linking agent (17% wt) were mixed for 1 h and then extruded by a twin-screw extruder. Then the produced granules and polyethylene granules were mixed. Finally, polyethylene films containing 0.02%, 1%, and 2% of micrometer-sized silver particles were produced by a blow extruder machine. Polyethylene packages of the same thickness without silver powder were used as controls.

Microstructure observation

Distribution and size of particles in polyethylene films were investigated by a TESCAN MV2300T/40 (TS 5136MM) scanning electron microscopy (SEM). For this investigation, films were precoated with a thin gold layer using a sputter coater. After gold coating, SEM images were taken at an acceleration voltage of 10 kV.

Microbial tests

The microbial tests were performed on mold, yeast, *E. coli, coliform, S. aureus, and mesophilic aerobic bacteria* based on standards

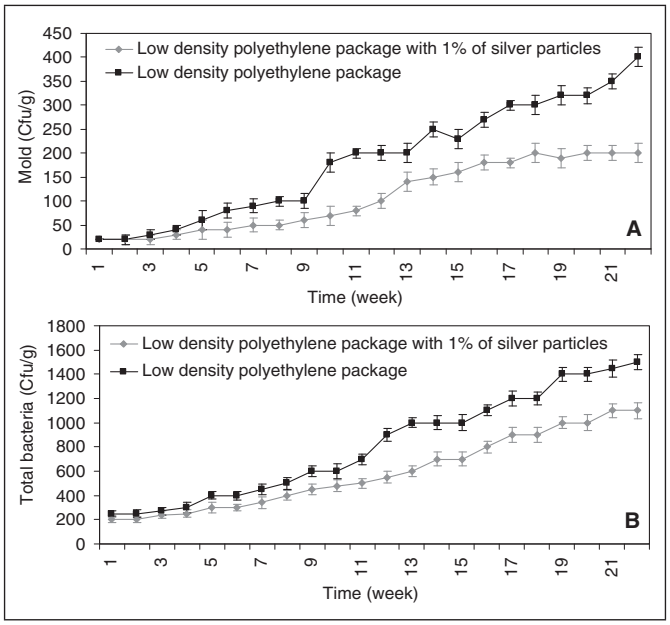

Figure 2–Average results (±SD) up to 22 wk: (A) mold; (B) total bacteria count.

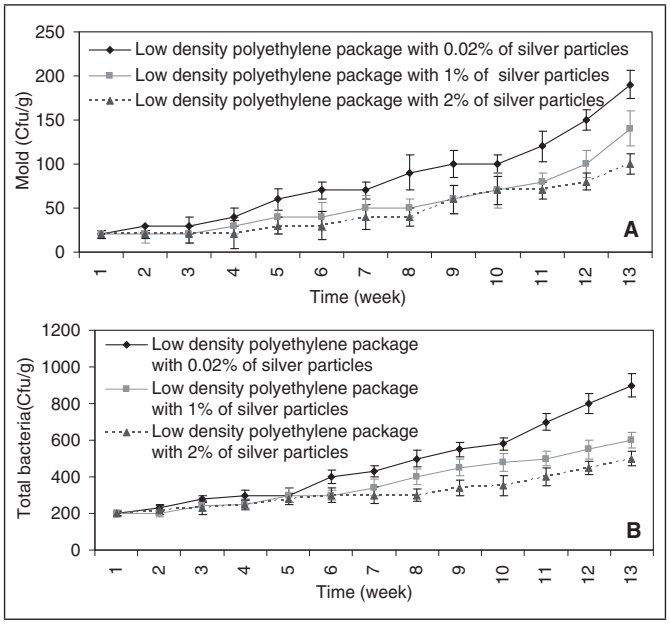

Figure 3–Average results (±SD) up to 13 wk: (A) mold; (B) total bacteria count.

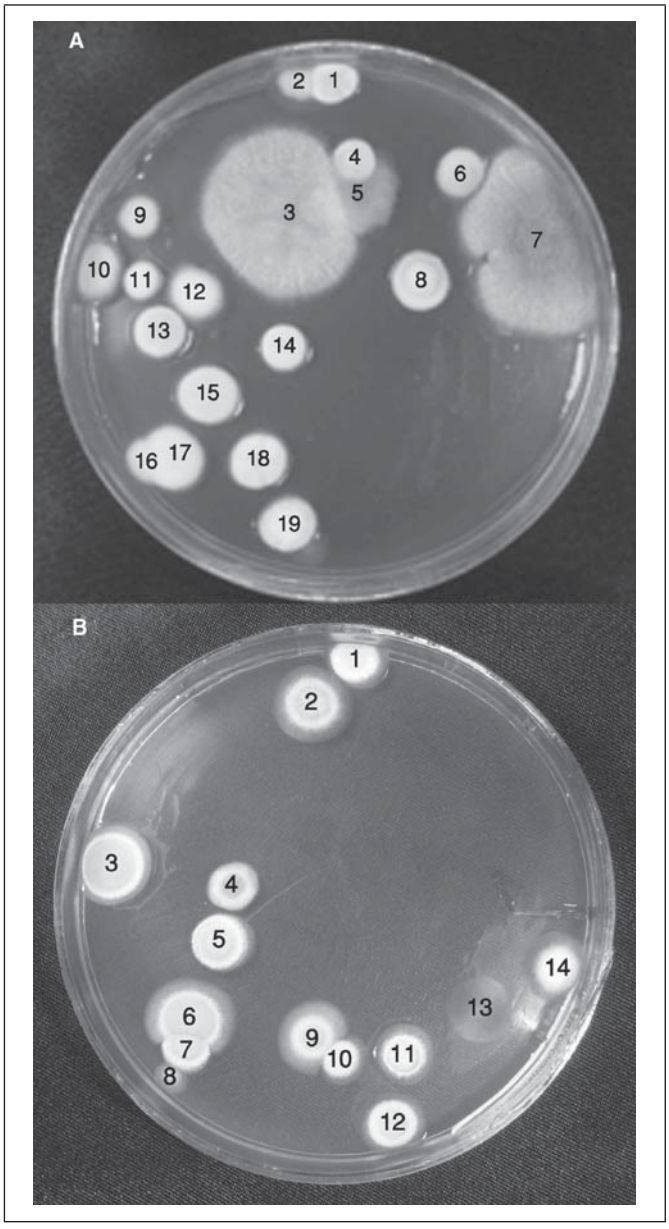

Figure 4–Mold growth in the 13th wk in decimal dilution 0.1 of samples: (A) LDPE-Ag package with 0.02% concentration of silver particles; (B) LDPE package with 1% concentration of silver particles.

of international organization for standardization (ISO) and Inst. of Standards and Industrial Research of Iran (ISIRI) (ISO 6887–1, 6887–4, 6887–3, 7251, 4831, 4832, 7218, 21527–2 and ISIRI 2836, 326, 2946, 6597, 6806–3, 8663–2, 8663–1, 8923–1, 8923–4, 9899, 10899–2). Main parts of these tests were similar. First, decimal dilutions (*DC*) were provided from samples with dilutor Ringer (decimal dilution is concentration of sample in dilutor Ringer [g/mL]). Then, 1 mL of each produced liquid was transferred to sterile empty plates. After that, 15 to 20 mL of selective agar culture for elective bacteria was added to these plates and was cultured by pure plate method. In this method, the plates should be rotated like an 8 on horizontal face. Afterwards, the plates were incubated at suitable temperature after coagulation of agar (For total bacteria count in 35 to 37 °C during 24 to 48 h, and for mold in 25 °C during 3 to 5 d). Finally, the number of colonies in all plates for each sample (*a*) was counted. In the case of necessity, confirm tests were performed. The number of bacteria per gram of sample for all plates (Colony forming unit [CFU]/g) is obtained from Eq. 1. The final average of the results in all plates is known as the number of colonies related to the microbial factor per gram for each sample.

$$\frac{\text{CFU}}{\text{g}} = \frac{1}{V} \times \frac{1}{DC} \times a \quad (1)$$

where V is the volume of produced solution that was used to culture. In this research, it is noteworthy that confirm tests were not needed unless the tests were related to *S. aureus*.

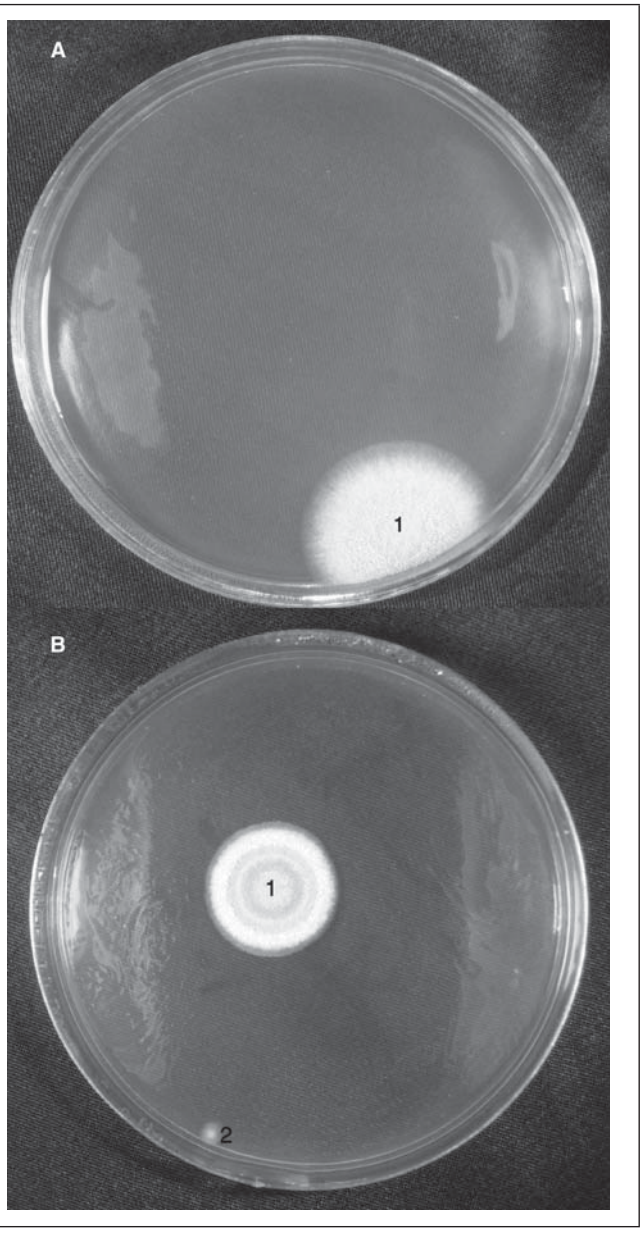

Figure 5–Mold growth in the 13th wk in decimal dilution 0.01 of samples: (A) LDPE-Ag package with 2% concentration of silver particles; (B) pure LDPE package.

Nine-point hedonic scale

The trained panelists who had the highest accuracy to evaluate sensory characteristics evaluated the appearance and some sensory characteristics of samples. For this purpose, 20 students from the food sciences and technology department were selected. Six students who had the most accuracy during the investigation of a similar sample were selected for the major test. Trained panelists selected a point from 1 to 9 for each of the taste, aroma, appearance, and total acceptance by the 9-point hedonic scale method. Responses on this scale were assigned numbers from 1 to 9 (1 = dislike extremely and 9 = like extremely) (Larmond 1970; Watls and others 1989).

Statistical analysis

A full factorial experimental design with 3 replicates was used for all experiments. A 1-way analysis of variance (ANOVA) was performed to detect significant differences between the treatment means. Multiple comparisons of means were performed by Duncan and Tukey's tests (P value < 0.05). The data obtained from all tests and investigations statistically were analyzed by the SPSS software (version 15). The results were presented as mean ± standard deviation (SD) of 3 replicates.

Results and Discussion

SEM image of films

SEM images of films (Figure 1) revealed uniform distribution of the silver particles in films. Also, most of silver particles were in the range of 500 nm and 1.2 μm.

Antimicrobial effects

In all the samples, *S. aureus, coliform, E. coli, and* yeast were not observed in all weeks. As you see in Figure 2 and 3, the number of colonies of molds and also the number of colonies related to experiment of *mesophilic aerobic bacteria* in most of the weeks for samples packaged in LDPE-Ag film were less than the samples packaged in pure LDPE film. The number of colonies related to these 2 factors increased with the passage of time for all the samples. No great differences were observed up to 4 wk. Over time, more difference among the number of the colonies related to the samples packaged in LDPE-Ag film with the samples packaged in pure LDPE film in comparison with previous weeks was observed.

LDPE-Ag packages with 1% and 2% concentrations of silver particles statistically showed a significant positive effect on all microbial factors compared with the packages of this kind with 0.02% concentration of silver particles and also the pure LDPE. Studied LDPE-Ag packages with 0.02% concentration of silver particles statistically had a significant positive effect on the mold compared with the pure LDPE packages.

In addition, by the increase of the concentration of silver particles in LDPE-Ag packages (samples 1, 2, and 3), a decrease in the number of colonies was obtained.

The samples packaged in LDPE-Ag film with 0.02% concentration of silver particles did not show a great difference in the number of the colonies in comparison with the samples packaged in pure LDPE film. Figure 4 to 6 are some of the plates related to microbial culture medium as sample in the 13th wk of the experiments that confirm the above results.

Sensory and appearance properties

Nine-point hedonic scale was done according to mentioned factorial design. Average results related to the 9-point hedonic scale are shown in Figure 7. All factors decreased with the passage of time for all the samples. In most of the weeks and for all the factors that were studied in this test, the results related to LDPE-Ag packages with 1% and 2% concentration of silver particles were more than other samples.

In other words, there was a significant statistical difference in taste, aroma, and total acceptance when comparing the samples packaged in LDPE-Ag film with 1% and 2% concentration of silver particles together, and also each of them with other samples.

Discussion

The action of O_2 (Azeredo 2009), water and enzymatic activities, and microbial growth are some of the most important factors that affect the quality of barberry (Kafi and Balendari 2002). As you see in results, LDPE-Ag packages decrease growth of microorganisms in barberry. This is due to antimicrobial activity of silver particles and ions.

There is no specific mechanism for the antimicrobial activity of silver particles and ions (Chudasama and others 2009). Different mechanisms have already been proposed for the antimicrobial activity of silver particles and ions:

(1) They Inhibit cross-linking of polysaccharide chains with tetra-peptides in the cell walls of bacteria (Sondi and Salopek-Sondi 2004; Chung and others 2008; Azeredo 2009).

(2) The antimicrobial Ag^+ ions are released by silver particles dissolution (Kumar and Münstedt 2005; Morones and others 2005; Azeredo 2009; Chudasama and others 2009; Fernández and others 2010). The interaction of silver ions with the ribosome causes the suppression of enzymes and proteins essential to adenosinetriphosphate production (Rai and others 2009).

(3) Silver particles adsorb to the microbial cells, then they disturb the normal mechanisms of cells, such as the respiration process and mass transfer between cells (Sondi and Salopek-Sondi 2004; Chung and others 2008; Azeredo 2009; Rai and others 2009; Wei and others 2009).

(4) Small silver particles may also enter into the microorganism and cause damage to the cell (Song and others 2006; Chung and others 2008; Li and others 2008; Azeredo 2009; Chudasama and others 2009; Rai and others 2009). Also, they interact with phosphorus-containing compounds (Wei and others 2009; Chen and others 2010).

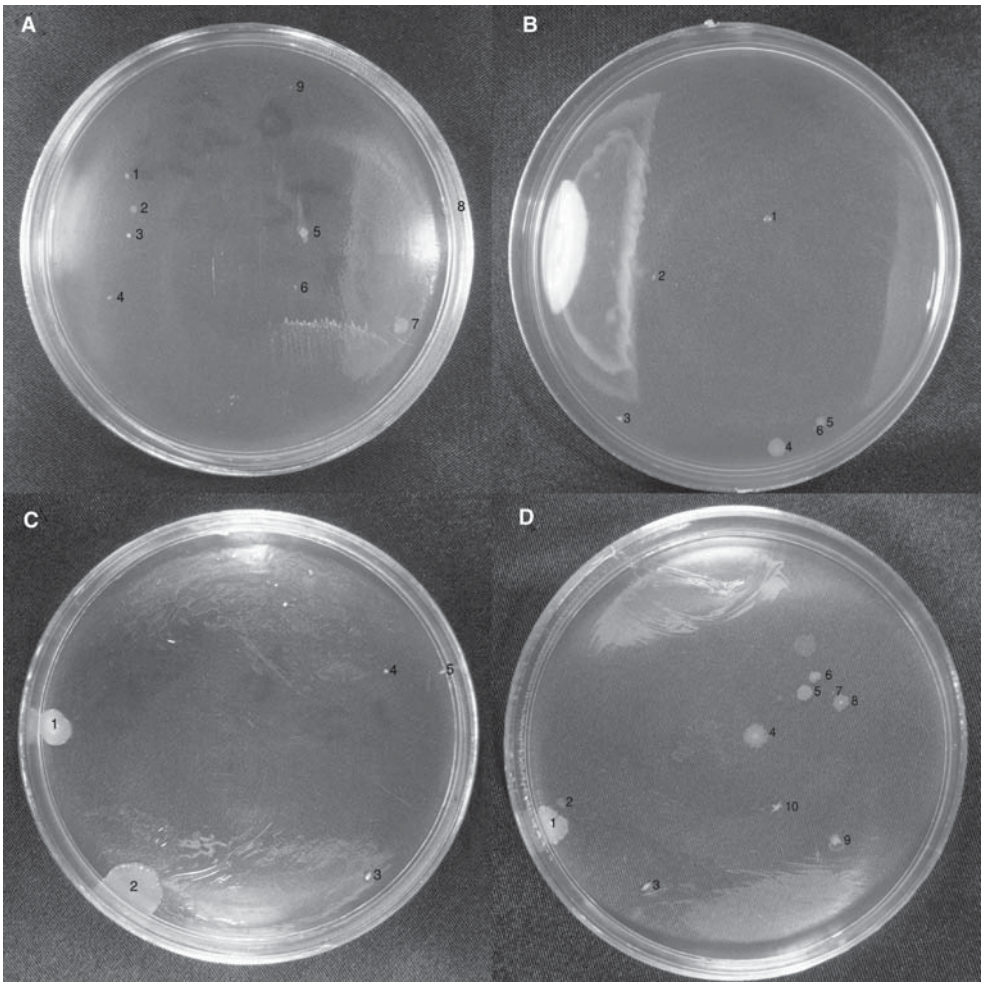

Figure 6–Total bacteria count in the 13th week in decimal dilution 0.01 of samples: (A) LDPE-Ag package with 0.02% concentration of silver particles; (B) LDPE-Ag package with 1% concentration of silver particles; (C) LDPE-Ag package with 2% concentration of silver particles; (D) pure LDPE package.

(5) Silver particles can attach to the compounds of the thiol groups found in the proteins, chloride ions, nucleic acids, or respiratory enzymes of bacterial cells, and thereby block metabolism and lead to a decrease in protease activity (Zheng and others 2006; Chung and others 2008; Chudasama and others 2009; Rai and others 2009).

(6) Silver particles may also interact with the building elements of the bacterial membrane such as sulfur-containing proteins and cause damage to the cell wall and cell membrane. It could cause an increase in cell permeability and lead to an uncontrolled transport through the membrane and ultimately cell death (Sondi and Salopek-Sondi 2004; Song and others 2006; Chung and others 2008; Azeredo 2009; Rai and others 2009; Chen and others 2010).

(7) Other mechanism for the antimicrobial activity of silver particles is related to DNA damage (Chung and others 2008; Li and others 2008; Azeredo 2009; Chudasama and others 2009). When the silver ions penetrate into a bacterial cell, the DNA molecule becomes condensed and loses its replication ability and lead to cell death (Rai and others 2009; Yang and others 2009).

Although we did not measure the oxygen and humidity permeation, barrier properties of polyethylene packages containing silver particles have been observed in some studies (Li and others 2009; Sánchez-Valdes and others 2009). Silver particles are able to delay the molecule pathway making the diffusive path more tortuous (Sorrentino and others 2007; Azeredo 2009; Li and others 2009; Arora and Padua 2010).

In addition to antimicrobial activity of silver particles, obtained results especially about sensory characteristics may be related to barrier properties of LDPE-Ag packages. Fresh barberry has a bright red color that gradually turns to dark red by dehydration. The action of enzymes, oxidation, and light and heat effects may change the pigments into other components and decrease the appearance quality of barberry (Kafi and Balendari 2002). Also, oxidation increases odors loss and flavors loss (Brody and others 2008). Prevention against humidity permeation causes a decrease in water and enzyme activity in LDPE-Ag packages. Decreasing water activity improves the physicochemical status of barberry and affects the growth of microorganisms. Also, mentioned barrier properties of LDPE-Ag packages against O_2 permeation cause a decrease in oxidation. This may decrease the odors loss, flavors loss,

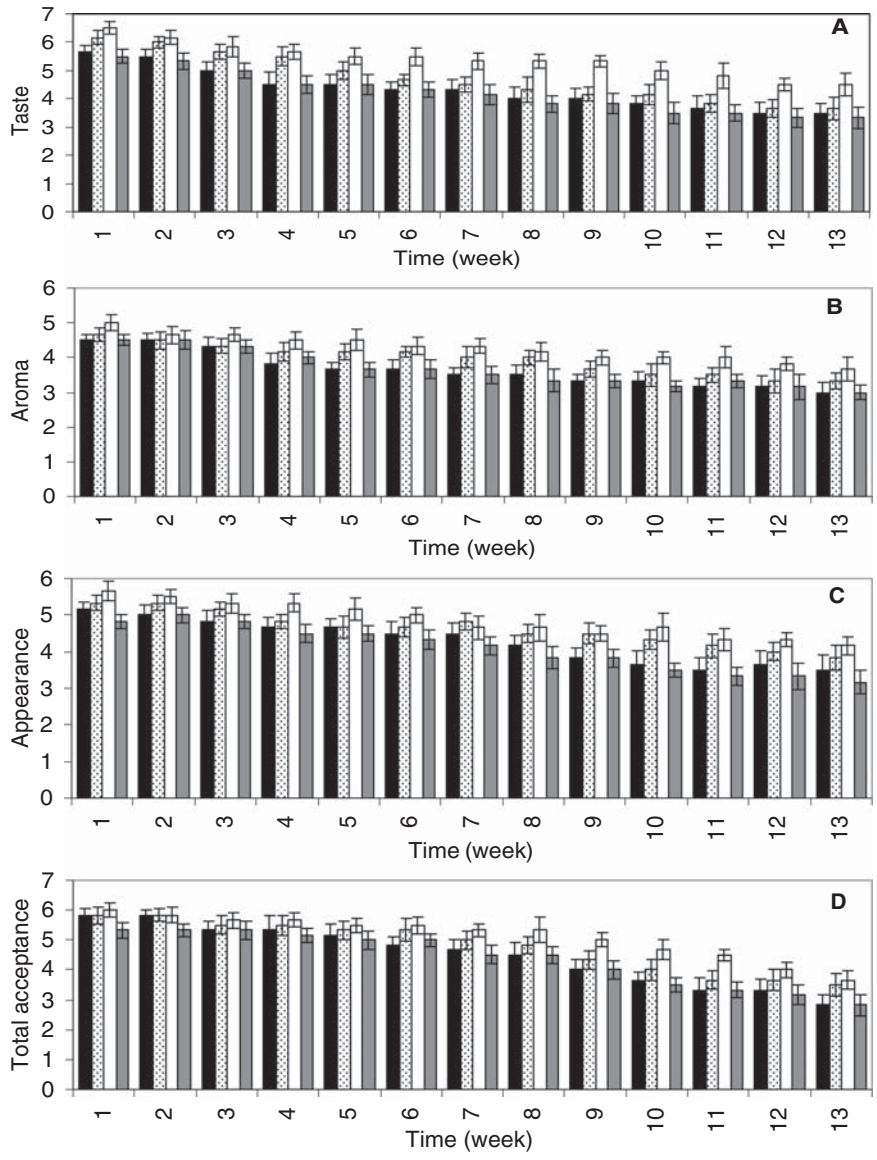

Figure 7–Average results (±SD) for samples packaged in LDPE-Ag films with 0.02% ■, 1% ▫ and 2% □ concentration of silver particles and pure LDPE packages up ▪ to 13 wk: (A) taste; (B) aroma; (C) appearance; (D) total acceptance of barberry.

the growth of aerobic microorganisms, and change in the pigment color and thereby preserve the appearance quality of barberry. Also, barrier properties of LDPE-Ag packages cause a resistance to volatile ingredient permeation. This may cause a decrease in the odors loss and flavors loss of barberry.

Conclusion

Water and enzyme activities, microbial growth, and change in the pigment color are some of the most important factors that affect the quality of barberry. One problem related to these damaging factors is barberry packaging. We studied the effects of LDPE-Ag packages on the several factors of dried barberry. All in all the following results were achieved:

(1) LDPE-Ag packages showed antimicrobial effects on barberry compared with pure LDPE packages except in the low concentration of silver particles of about 0.02%.

(2) By the increase of the concentration of silver particles in LDPE-Ag packages, a decrease in microbial growth in the barberry was obtained.

(3) LDPE-Ag packages helped to keep the aroma, taste and total acceptance factors of the barberry in comparison with pure LDPE packages except in the low concentration of silver particles of about 0.02%.

So, LDPE-Ag packages with more than 1% concentration of silver particles well-preserved the quality of barberry and increased its shelf life in comparison with pure LDPE packages. LDPE-Ag packages will probably solve the problem of barberry packaging. However, further research is needed to investigate the effect of packages containing higher concentration and smaller size of silver particles on the microbiological and physiological properties of barberry. It should be noted that further studies are still required to understand the barrier properties of LDPE-Ag packages and the possible health problems caused by silver particles penetration into barberry.

Acknowledgment

The authors would also like to thank the food laboratory of Birjand Univ. of Medical Sciences for supporting laboratory space. We are also thankful for Atefeh Nakheyi's cooperation in this research.

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