The Effect of Nano-Silver Packaging on Quality Maintenance of Fresh Strawberry

Naser Valipour Motlagh, Majid Aliabadi, Elnaz Rahmani, Samira Ghorbanpour

***Abstract*—**Strawberry is one of the most favored fruits all along the world. But due to its vulnerability to microbial contamination and short life storage, there are lots of problems in industrial production and transportation of this fruit. Therefore, lots of ideas have tried to increase the storage life of strawberries especially through proper packaging. This paper works on efficient packaging as well. The primary material used is produced through simple mixing of low-density polyethylene (LDPE) and silver nanoparticles in different weight fractions of 0.5 and 1% in presence of dicumyl peroxide as a cross-linking agent. Final packages were made in a twin-screw extruder. Then, their effect on the quality maintenance of strawberry is evaluated. The SEM images of nano-silver packages show the distribution of silver nanoparticles in the packages. Total bacteria count, mold, yeast and *E. coli* are measured for microbial evaluation of all samples. Texture, color, appearance, odor, taste and total acceptance of various samples are evaluated by trained panelists and based on 9-point hedonic scale method. The results show a decrease in total bacteria count and mold in nano-silver packages compared to the samples packed in polyethylene packages for the same storage time. The optimum concentration of silver nanoparticles for the lowest bacteria count and mold is predicted to be around 0.5% which has attained the most acceptance from the panelist as well. Moreover, organoleptic properties of strawberry are preserved for a longer period in nano-silver packages. It can be concluded that using nano-silver particles in strawberry packages has improved the storage life and quality maintenance of the fruit.

***Keywords*—**Antimicrobial properties, polyethylene, silver nanoparticles, strawberry.

I. Introduction

FRESH strawberry fruit (Fragaria× ananassa Duch.) is known to have a high level of antioxidant and be a good source of potassium and calcium. High water content (90%), a smooth texture and high level of respiration make strawberry vulnerable to microbial contamination and induce a very short storage-life [1], [2]. Therefore, alternative strategies have been developed to keep strawberry fresh for a longer storage time. Nowadays polymeric packages are widely used in food packaging industries. Polyethylene plastic is one of the most important plastics which are used in food packaging. However, most of the polymeric plastics used for food packaging do not include the ability to prevent the mass transfer of oxygen, carbon dioxide, water vapor and aromatic compounds. Therefore, the polymeric packages containing antimicrobial nanoparticles have been introduced as a suitable strategy in order to keep fruits fresh, extend their storage-life, and prevent or delay their spoilage [3], [4].

Food packages containing antimicrobial nanofillers are known in active packaging systems. The use of nanoparticles in plastic packaging materials can improve the impermeability properties of the packaging stuff [5], [6]. Moreover, some metals or metal oxides nanoparticles such as gold, silver, copper, nickel, copper oxide, titanium dioxide, magnesium oxide and zinc oxide have antimicrobial properties and thus can keep food fresher for a longer time [7]-[11]. Among them, silver nanoparticles (AgNs) have the most effective bactericidal properties against a wide range of microorganisms, including bacteria, fungi, yeasts and viruses [12]-[14]. In addition, silver has high thermal stability and low volatility and can tolerate the processing conditions [13]. This element in larger scales is a metal with low reactivity property, but turned to small sizes of the nanometer, its antibacterial property increases over 99%. It also affects the metabolism, respiration and reproduction of microorganisms at this scale [15]. The AgN has higher surface to volume ratio and slower release rate than the larger size of silver particles. So, there is an inverse relationship between the sizes of AgNs with their anti-bacterial properties [13], [16]-[18]. Various methods exist for the production of polymer nanocomposites containing nanoparticles such as polyethylene packages containing AgNs. The most common method is direct mixing along with melting.

Donglu et al. [19] tested the effects of polyethylene packages containing AgN, titanium and silica dioxide on the storage-life of a kind of mushroom. Nanocomposite packages were able to adjust the amount of oxygen and carbon dioxide, eliminate ethylene and prevent the microbial growth. Therefore, maintaining good sensory properties, reducing degradation of nutritional components, preventing physiologic changes and thus prolonging storage-life of mushroom were obtained using the nano-silver packages. In another study, Iranian Saffron was packed in the polyethylene packages containing 0, 400, 800 and 1200 ppm AgNs for more than 6 months. Nano-silver packages accelerated the reduction of microbial growth in Saffron. Packages containing 400 ppm AgNs were more effective compared to others [20]. Also, polyvinyl alcohol starch containing AgNs showed antimicrobial activity against both *E. coli* and *Listeria innocua* and two types of fungus *Aspergillus niger* and *Penicillium expansum* [21]. Furthermore, the cheese packed in nanocomposite packages prepared by incorporating AgNs into

N. Valipour Motlagh is with Department of Chemistry, Faculty of Science, University of Birjand, Birjand, Iran (Corresponding author, phone: +989153636841; fax: +985632202515; e-mail: n.valipour.m@birjand.ac.ir, nasservalipoormotlagh@gmail.com)

M. Aliabadi is and E. Rahmani was with Department of chemical engineering, Islamic Azad University of Birjand, Birjand, Iran.

S. Ghorbanpour was with Department of chemical engineering, Islamic Azad University of Quchan, Quchan, Iran.

poly(lactic acid) (PLA) matrix showed better retention in pH value, lactic acid bacteria count, sensory quality, and antimicrobial activity compared with those packed in PLA with LDPE film [22].

In this study, nano-silver packages are produced with two different concentrations of AgNs. The samples of fresh strawberry are packed in several produced packages and evaluated at specified intervals based on different microbiological and organoleptic characteristics.

II. Materials and Methods

A. Production of Packages

The preparation of the packages was carried out based on the detailed procedure reported in previous works [23], [24]. Briefly, the LDPE containing AgN (Ag-LDPE) and conventional LDPE were prepared under the same circumstances using a twin-screw extruder. In all produced packages, the LDPE was used as the base material in the preparation of packages. 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 for fabrication of the main granules containing AgNs. Then, the aforementioned main granolas and LDPE granolas were directly mixed. The final nanocomposite films containing 0.5 and 1 weight percent (wt%) of AgNs were prepared using a blow extruder machine.

B. Packaging of Strawberries

Strawberry samples were picked completely random from a garden in Birjand, an eastern city of Iran. After evaluating microbial and visual quality of strawberry samples, 100 gr of collected samples were randomly selected and packaged in each of the normal cellophane pure LDPE package (sample A), nanocomposite package containing 0.5% (sample B) and 1% (sample C) AgNs. The packaged samples were stored at 4 °C and analyzed at specified intervals (5, 10 and 15 days after the packaging). The experiment was designed based on the response surface methodology with two variable of storage time and the concentration of AgNs.

C. Scanning Electron Microscope

To investigate the presence, distribution and size of nanoparticles in LDPE, a field emission scanning electron microscope (MIRA3TESCAN-XMU, Czech Republic) was implemented. Prior to imaging, samples were coated by a thin layer of gold using sputtering method. Imaging was performed at a voltage of 15 kv.

D. Microbial Test

The total bacteria count, mold and yeast, *E. coli* were done according to the standards of the international organization for standardization (ISO) and the institute of standards and industrial research of Iran (ISIRI) (ISO 6887–1, 6887–4, 6887–3, 7251, 4831, 4832, 7218, 21527–2 and ISIRI 1-5272, 1-10899, 2946).

E. Sensory Test

The texture, color, appearance, odor, taste and total acceptance of samples were evaluated with the help of 20 trained panelists and were ranked based on 9-point Hedonic scale method. Table I shows the words or sentences used in order to describe the mentioned qualitative 9 points.

TABLE I
NINE POINT HEDONIC SCALE

I like it a lot	1	I hate it a little	6
I like it	2	I moderately hate it	7
I moderately like it	3	I hate it	8
I like it a little	4	I hate it a lot	9
I do not like it	5		

F. Assessment of Apparent Color

The sample's color was evaluated using L*a*b* model. In this system, the images of samples were taken in a chamber with constant photon and magnification. After improvement of background contrast and segmentation of images using Adobe Photoshop, the image conversion of RGB chromatic space into L*, a*, b* units was carried out. In this study, image processing was performed using Image J software.

G. Assessment of Apparent Color

Design of experiment and statistical evaluation of results were done based on response surface method with two variables of the concentration of AgNs (A) and the storage time (B) using design expert software.

In this study, a central composite design with two independent variables mentioned was employed. The effect of concentration of nanoparticles in the polyethylene packages on the different characteristics was evaluated at 5, 10 and 15 days after packaging. A P-value of less than 0.05 was considered significant in all analyses.

III. Results and Discussion

A. SEM Images

As shown in Figs. 1 and 2, AgNs were present in both nanocomposite packages. The images showed an agglomeration of particles in sample C (1% AgNs). This agglomeration can be due to higher weight fraction of nanoparticles in this package compared to sample B (0.5% AgNs).

The agglomeration of nanoparticles makes the nanoparticles distributed unevenly in the package. It seems that one reason of following different results is the non-homogenous distribution sample A (1% AgNs) in comparison with sample B (0.5% AgNs).

B. Evaluation of Microbial Growth

In the microbial tests, the *E. coli* was not observed in any of samples. The results related to the growth of other microorganisms in different samples were obtained as follows.

The design expert software suggested a quadratic model to fit the results of mold and total bacteria count (P value < 0.05). The ANOVA table showed that the parameters A, B, A^2 and B^2 in the proposed models for the results of total bacteria count and mold have P-values less than 0.05 and therefore are significant.

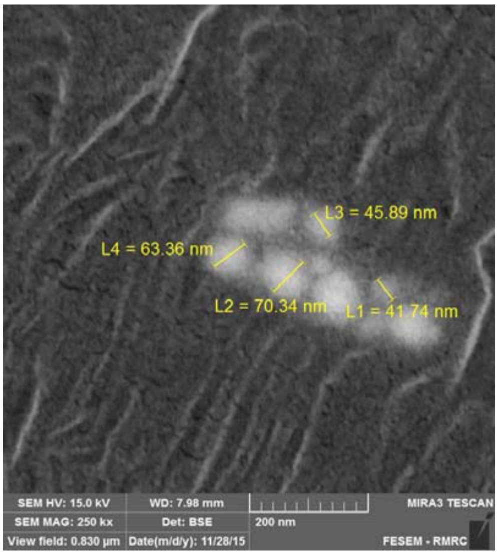


Fig. 1 SEM image of polyethylene packages containing 0.5% of AgN

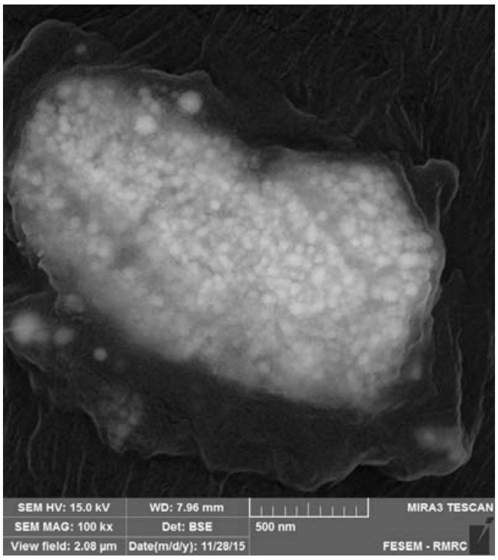


Fig. 2 SEM image of polyethylene packages containing 1% of AgN

As represented in Fig. 3, as the concentration of AgNs increased from zero to 0.5%, the mold growth and total bacteria count reduced. Also, these microbial parameters went up as the concentration of AgNs increased from 0.5 to 1%, although the increase rate in this period was not as high and the microbial growth of sample C was less than sample A. The optimum concentration of nanoparticles in the graph was in the range of 0.5 and 0.75%. In samples B and C, the results of mold growth and total bacteria count changed in an incremental trend from the fifth day to the tenth day of storage and did not change from day 10 onwards. In sample A, the mold growth and total bacteria count increased throughout the time period, although the increasing trend of microbial growth in the range of 5 to 10 days was more severe than the range of 10 to 15 days.

The numerical optimization of microbial parameters was carried out using design expert software. Design expert uses an optimization method developed by [25]. First, the lower limit, upper limit and goal of all parameters were set as Table II. For these goals and limitations, the optimal conditions for the best results were an AgNs concentration of 0.7% and the storage time of 15 days. Under these conditions, the mold, total bacteria count and desirability were 111.319, 274.019 and 0.987, respectively.

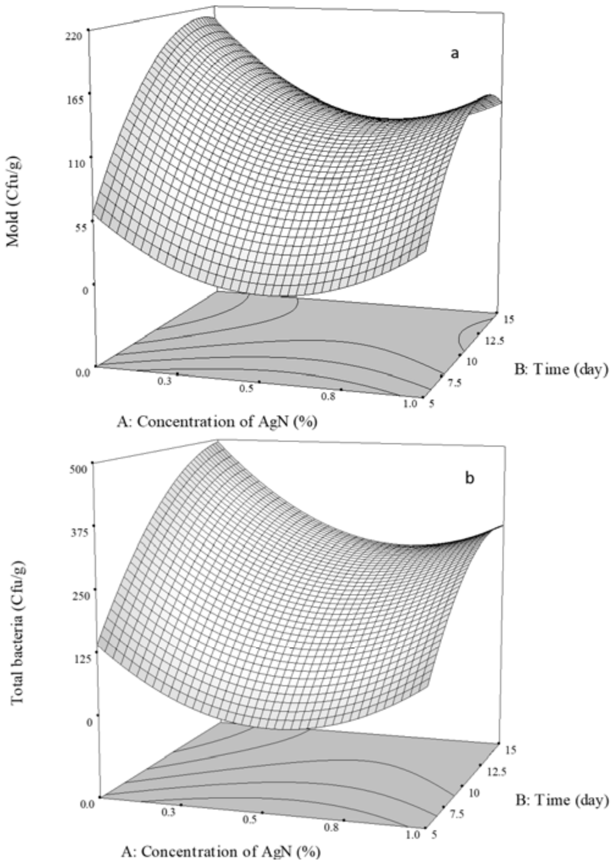


Fig. 3 Three-dimensional graph of results related to Mold (a) and total bacteria count (b) for the different samples

TABLE II
THE LOWER LIMIT, UPPER LIMIT AND GOAL OF ALL MICROBIAL PARAMETERS FOR NUMERICAL OPTIMIZATION

Parameter	Goal	Lower limit	Upper limit
AgNs concentration	In range	0%	3%
Storage time	Maximize	5 days	15 days
Mold	Minimize	10 Cfu/g	3000 Cfu/g
Total bacteria count	Minimize	10 Cfu/g	70000 Cfu/g

The general trend of the microbial growth in various samples showed that the microbial load increased in sample A as the storage time increased from five to 10 days. It seems that this result has been obtained due to improvement of suitable conditions of microorganism growth in storage time. Passing from 10 to 15 days in storage, the microbial load in different samples showed a relative stable trend. This trend was probably observed because the moisture content of the samples reduced as the storage time increased.

The results showed that the presence of AgNs in the packages induced the reduction of the microbial growth in the samples. This issue is related to the mechanism of AgNs performance. Ha et al. found similar results for samples of ground beef packed in the nano-silver packages [26]. Antibacterial mechanisms of AgNs are still unclear. According to the studies, the possible mechanisms have been identified up to this date for the AgNs are [8], [27]-[30]:

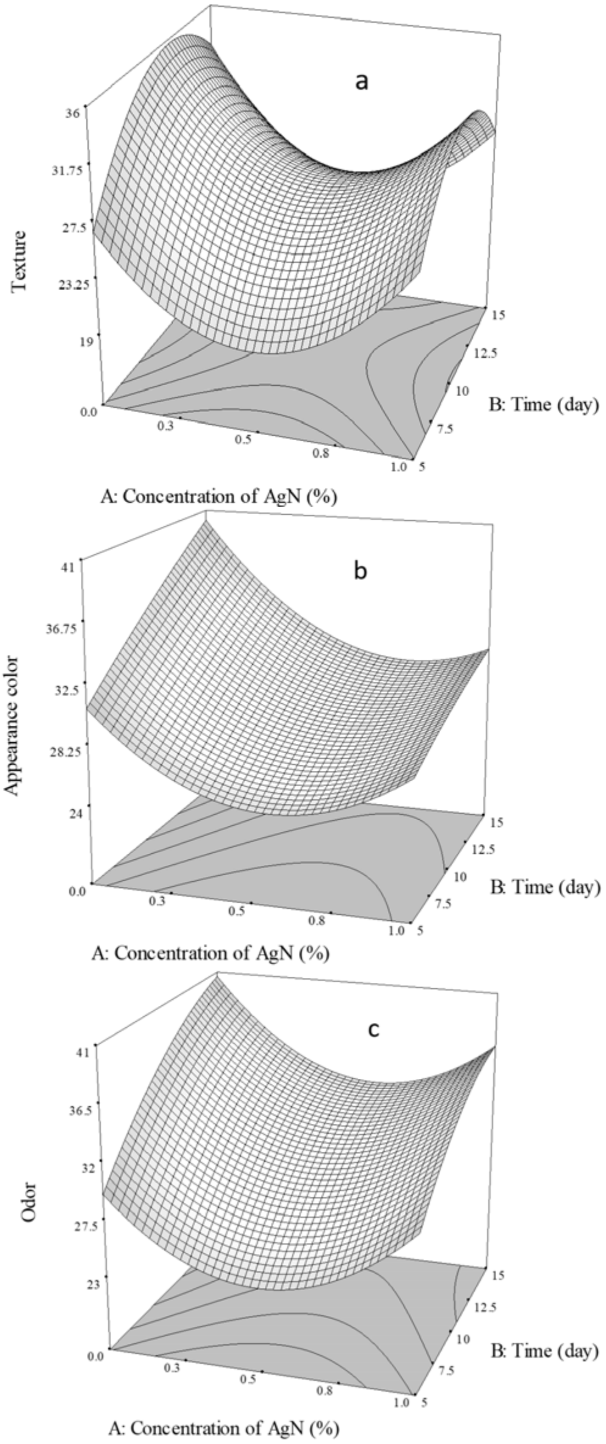
- The release of silver ions disrupts the production of adenosine triphosphate and proliferation of deoxyribonucleic acid, interacts with membrane proteins and influences their function.
- Induced oxidative stress in the microbial cell membrane due to the release of reactive oxygen types or accumulation in the cell membrane affects the membrane permeability and disturbs the common tasks of cells such as respiration and material transportation and thereby damages the cell wall.
- The particles penetrate the bacteria cells.
- The presence of nanoparticles in the polymer packaging creates an indirect and longer path for the penetration of air and moisture and this factor makes appropriate conditions for a fewer growth of microorganisms in nano-silver packages compared to the LDPE packages.

The results also illustrated that there were no statistically significant differences in the changes of microbial load between samples A and B. Even microbial load of sample B was slightly more than sample A. It can be related to agglomeration and lack of appropriate nanoparticles distribution in the packages containing 1% of AgNs.

C. Evaluation of Organoleptic Properties

Statistical evaluation of the results related to organoleptic properties showed that the quadratic model is suitable for fitting the data of texture, color, appearance, odor, taste and total acceptance ($P < 0.05$). Moreover, from parameters of the quadratic models related to the organoleptic properties, B^2 and A^2 for texture, A^2 for apparent color, A^2 and B for odor and taste, and A^2, A and B for the total acceptance were significant ($P < 0.05$).

As indicated in Fig. 4, at a fixed time period, all organoleptic characteristics (texture, color, appearance, odor, taste and total acceptance) of the samples were improved as the concentration of AgNs increased from zero to 0.5%. Organoleptic characteristics showed a constant trend as the concentration of AgNs increased from 0.5 to 1%. Besides, at constant concentration of AgNs, the quality of all the organoleptic characteristics showed a decreasing trend as the storage time increased. In this state, the reducing trend of organoleptic properties related to sample A had a steeper slope than samples B and C. Emamifar et al. [31] obtained similar results for fresh orange juice packed in the nano-silver packages. Generally, the best results for all organoleptic properties were observed in samples with 0.5% concentration of AgNs. Furthermore, organoleptic characteristics of the sample with 1% concentration of nanoparticles were better than the sample without AgNs.



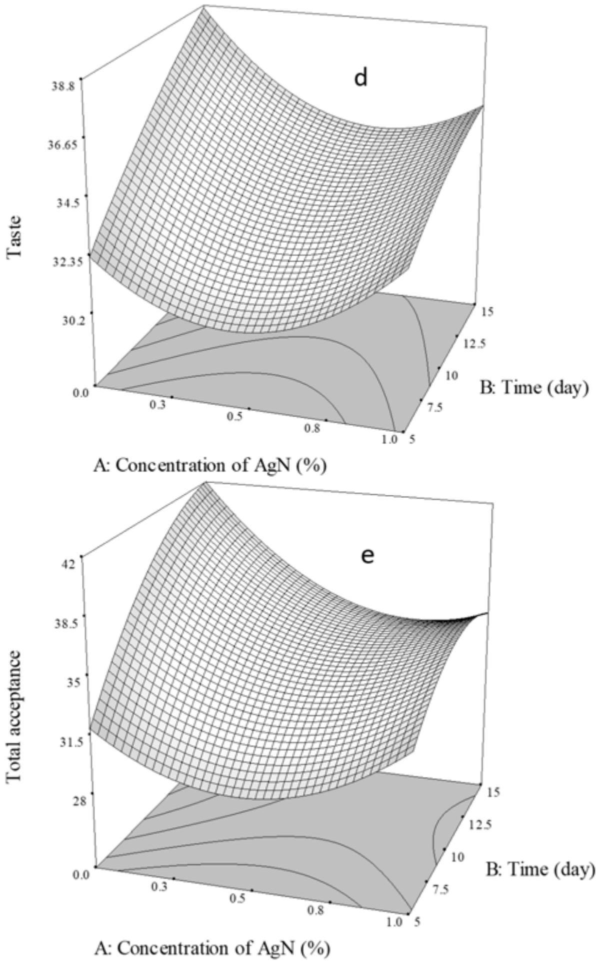


Fig. 4 Three-dimensional diagrams related to the results of the texture (a), apparent color (b), odor (c), taste (d) and total acceptance (e) for various samples

As shown, change in the most of organoleptic characteristics is so similar to the microbial changes trend in the samples, and this showed that the organoleptic changes of several samples were affected by microbial load and changes in the growth conditions of the microorganisms. In addition, it appears that the reduced quality of some organoleptic characteristics such as odor, color, appearance, taste and total acceptance in sample C compared to sample B can be related to higher migration of silver ions from the package to the sample. Furthermore, during the period of 10 to 15 days after the packaging, despite the unchanged trend of microbial characteristics in various samples, the samples showed decreased organoleptic properties which can be related to the effect of migration of metal ions into the strawberries.

D. Visual Color Evaluation

Statistical evaluation of the results obtained by the Image J software showed that the quadratic model is suitable for fitting different color characteristics of samples ($P < 0.05$). Also, the statistical analysis showed that A^2, A and B have the P value less than 0.05 for each of three color characteristics (L*, a* and b*) and are therefore significant. Also, from a statistical view, the results showed that B^2 has a significant effect on a* and b*.

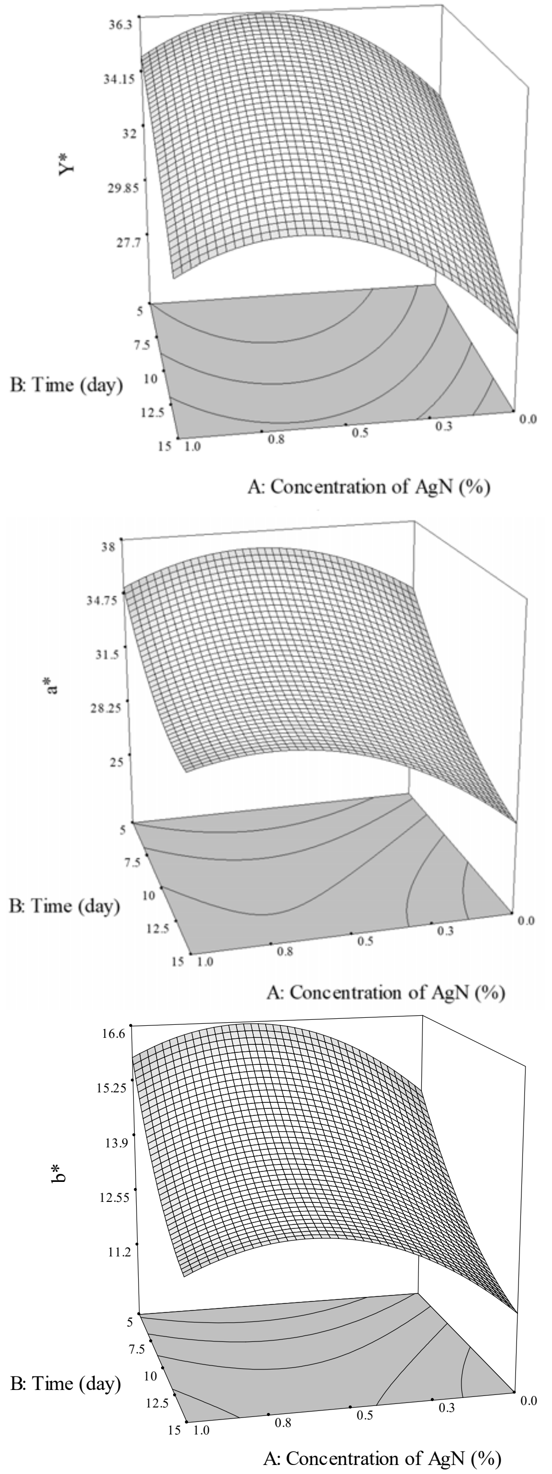


Fig. 5 The three-dimensional graph of the results of three color characteristics obtained by the Image J software

As shown in Fig. 5, L*, a* and b* of different samples decreased with an increase in storage time. The intensity of this reduction was higher for sample A comparing to samples B and C. Donglu et al. also found similar results for a type of fungus packed in nano-silver packages [19]. With increasing the storage time, it seems that an increase in the migration of silver ions to samples B and C as well as more microbial growth induce the following results. In all characteristics of L*, a* and b*, the results related to sample B were slightly better than sample C. This can be related to the higher migration of silver particles into the food in samples containing more AgNs. As it is apparent, the results of visual color evaluation obtained from the Image J software confirmed the appearance results of sensory tests.

IV. Conclusion

In this study, fresh strawberry was packed in the LDPE packages and Ag-LDPE packages containing different concentrations of AgNs (0.5 and 1%). These samples were selected from the packages at specified intervals (5, 10 and 15 days after the packaging) and were evaluated for microbial growth, sensory properties and apparent color. Total bacteria count and mold in the samples containing AgNs were less than samples without nanoparticles. As time passed in storage, the results showed that all desirable organoleptic features of strawberry packed in Ag-LDPE packages were destroyed less than samples packed in LDPE packages.

Acknowledgment

The authors would like to thank Mrs. Ansarifar for the assistance in the evaluation of apparent color.

References

[1] Kash , A. and J. Hekmati, *strawberry cultivation*. 1st ed. 1991.
[2] Samadi, S., A. Ghasemnezhad, and J. Imani, *Extending shelf life of strawberry using some pre-storage treatments.* Acta Hortic, 2017. 1156: p. 643-652.
[3] Ayala-Zavala, J.F., et al., *Natural antimicrobial agents incorporated in active packaging to preserve the quality of fresh fruits and vegetables.* Stewart Postharvest Review, 2008. 4(3): p. 1-9.
[4] Pareek, S., *Fresh-Cut Fruits and Vegetables: Technology, Physiology, and Safety*. 2016, Boca Raton: CRC Press.
[5] Siracusa, V., *Food packaging permeability behaviour: a report.* International Journal of Polymer Science, 2012. 2012: p. 1-11.
[6] Rhim, J.-W. and Y.-T. Kim, *Biopolymer-Based Composite Packaging Materials with Nanoparticles*, in *Innovations in Food Packaging*, J.H. Han, Editor. 2014, Academic Press. p. 413-442.
[7] Argueta-Figueroa, L., Morales-Luckie, R.A., Scougall-Vilchis, R.J., Olea-Mejía, O.F., *Synthesis, characterization and antibacterial activity of copper, nickel and bimetallic Cu–Ni nanoparticles for potential use in dental materials.* Progress in Natural Science: Materials International, 2014. 24: p. 321-328.
[8] Chernousova, S. and M. Epple, *Silver as Antibacterial Agent: Ion, Nanoparticle, and Metal.* Angewandte Chemie International Edition, 2012. 52(6): p. 1636–1653.
[9] Duncan, T.V., *Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors.* Journal of Colloid and Interface Science, 2011. 363(1): p. 1-24.
[10] Radusin, T.I., et al., *Antimicrobial nanomaterials for food packaging applications.* Food and Feed Research, 2016. 43(2): p. 119-126.
[11] Zhou, Y., et al., *Antibacterial activities of gold and silver nanoparticles against Escherichia coli and bacillus Calmette-Guérin.* Journal of Nanobiotechnology, 2012. 10: p. 19.
[12] Orsuwan, A., et al., *One-step preparation of banana powder/silver nanoparticles composite films.* Journal of Food Science and Technology, 2017. 54(2): p. 497-506.
[13] Rigi, M., *Antimicrobial activities of gold and silver nanoparticles against Vibrio cholera.* International Journal of Advanced Biological and Biomedical Research, 2016. 4(1): p. 104-107.
[14] Carbone, M., et al., *Silver nanoparticles in polymeric matrices for fresh food packaging.* Journal of King Saud University - Science, 2016. 28(4): p. 273-279.
[15] Cho, K.-H., et al., *The study of antimicrobial activity and preservative effects of nanosilver ingredient.* Electrochimica Acta, 2005. 51(5): p. 956-960.
[16] Khodashenas, B. and H.R. Ghorbani, *Synthesis of silver nanoparticles with different shapes.* Arabian Journal of Chemistry, 2015.
[17] Reidy, B., et al., *Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications.* Materials, 2013. 6(6): p. 2295-2350.
[18] Raza, M.A., et al., *Size-and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes.* Nanomaterials, 2016. 6(4): p. 74.
[19] Donglu, F., et al., *Effect of nanocomposite-based packaging on storage stability of mushrooms (Flammulina velutipes).* Innovative Food Science & Emerging Technologies, 2016. 33: p. 489-497.
[20] Eslami, M., et al., *Effect of polymer/nanosilver composite packaging on long-term microbiological status of Iranian saffron (Crocus sativus L.).* Saudi journal of biological sciences, 2016. 23(3): p. 341-347.
[21] Cano, A., et al., *Development and characterization of active films based on starch-PVA, containing silver nanoparticles.* Food Packaging and Shelf Life, 2016. 10: p. 16-24.
[22] Li, W., et al., *Evaluation of PLA nanocomposite films on physicochemical and microbiological properties of refrigerated cottage cheese.* Journal of Food Processing and Preservation, 2017. e13362.
[23] Valipoor-Motlagh, N., M.-T. Hamed-Mosavian, and S.-A. Mortazavi, *Effect of Polyethylene Packaging Modified with Silver Particles on the Microbial, Sensory and Appearance of Dried Barberry.* Packaging Technology and Science, 2013. 26(1): p. 39-49.
[24] Valipoor-Motlagh, N., et al., *Beneficial Effects of Polyethylene Packages Containing Micrometer-Sized Silver Particles on the Quality and Shelf Life of Dried Barberry (Berberis vulgaris).* Journal of Food Science, 2012. 77(1): p. E2–E9.
[25] Derringer, G. and R. Suich, *Simultaneous Optimization of Several Response Variables.* Journal of quality technology, 1980. 12(4): p. 214-219.
[26] Ha, J.U., Y.M. Kim, and D.S. Lee, *Multilayered antimicrobial polyethylene films applied to the packaging of ground beef.* Packaging Technology and Science, 2001. 14(2): p. 55-62.
[27] Damm, C., H. Münstedt, and A. Rösch, *The antimicrobial efficacy of polyamide 6/silver-nano-and microcomposites.* Materials Chemistry and Physics, 2008. 108(1): p. 61-66.
[28] Neal, A.L., *What can be inferred from bacterium–nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles?* Ecotoxicology, 2008. 17(5): p. 362.
[29] Shrivastava, S., et al., *Characterization of enhanced antibacterial effects of novel silver nanoparticles.* Nanotechnology, 2007. 18(22): p. 225103 (9pp).
[30] Jung, W.K., et al., *Antibacterial Activity and Mechanism of Action of the Silver Ion in Staphylococcus aureus and Escherichia coli.* Applied and Environmental Microbiology, 2008. 74(7): p. 2171-2178.
[31] Emamifar, A., et al., *Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice.* Innovative Food Science & Emerging Technologies, 2010. 11(4): p. 742-748.