

Study on Nanosilver-TiO₂ photocatalytic nanocomposite coating with extrusion technique for increasing shelf life of Nile Tilapia (*Oreochromis niloticus*)

Azari A.^{1,a}; Anvar S.A.A.^{1*}; Ahari H.^{2,a}; Sharifan A.²; Motallebi Moghanjoghi A.A.¹

Received: February 2020

Accepted: July 2020

Abstract

Utilizing present-day advancements is a well-considered methodology. In such manner, the combination of nanotechnology and food science has prompted the development of numerous capacities in the nutrition section. This study was conducted to evaluate the antimicrobial effect of nano-silver packaging at two refrigerator temperatures (4 and 8°C) in Tilapia to evaluate the total viable count of microorganisms, *Escherichia coli*, *Staphylococcal aureus*, and psychrophilic bacteria. Ten grams of washed fish were divided into 5 groups treated with A: 1%: B 3%, C: 5%, D: 7% nano silver coating (E was the control group) and stored for 0, 3, 7, 14 and 30 days. The biochemical analysis of total volatile nitrogen and peroxide composition was investigated, and the best nano silver percentage was 7%. The result of this study demonstrates that the antimicrobial properties of nano silver for various bacterial species are exclusive and we believe that nano-silver packaging improved the quality and shelf life of Nile Tilapia.

Keywords: AgNPs, Nanosilver-TiO₂ nanocomposites, Extrusion technique, Scanning electron microscope, Total viable microorganisms count, *Staphylococcal aureus*, *Psychrophilic bacteria*, *Escherichia coli*.

1-Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran

2-Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran

a: contributed equally as a first author

*Corresponding author's Email: Saaa4824@gmail.com

Introduction

The developing interest for expanded fresh food shelf life and also the need of security against foodborne infections encouraged the improvement of antimicrobial food packaging (Bumbudsanpharoke and Ko, 2015; Valdés *et al.*, 2017).

Nanotechnology is useful information and innovation that covers boundless accomplishments, the primary concern being the capacity to make, control and utilize cases or gadgets in dimensions of less than one micron, around 1-100 nm. The principle contrast between nanotechnology and different advancements is on the size of materials and structures used in this innovation, since when the size of the material is turned to nanoscale, its natural properties including coloring, quality, erosion opposition, etc. (Khodanazary *et al.* 2019; Cushen *et al.*, 2012). Similar to the other new innovations, nanotechnology has also discovered its way into the food business. This modern science can be coordinated into all sections of food industry to enhance security and quality of food including reducing additives, protecting the feed ingredients and taste of food, as well as expanding its shelf life (Dimitrijevic *et al.*, 2015). Metal nanoparticles with their powerful antimicrobial properties are utilized as "dynamic packaging". Rising metal nanoparticles with biocidal properties are Cu, Zn, Au, Ti, and Ag (Toker *et al.*, 2013). One of the attributes of silver particle is its low harmfulness for humans (Fernandez *et al.*, 2010).

Silver nanoparticles (AgNPs), specifically, have antimicrobial, anti-

fungal, anti-yeast, and anti-viral activities, and can be joined with both non-degradable and edible polymers for dynamic food packaging. AgNPs indicated better antimicrobial effects contrasted with metallic silver because of a vast surface region which can be in contact with the surface of microorganisms (Toker *et al.*, 2013).

Titanium dioxide is broadly used as a disinfectant for surface coatings. The antimicrobial property of titanium dioxide is due to the photocatalytic properties and is affirmed by the food and drug administration for use in the food and medicine industry. The photochemical reaction of titanium dioxide is utilized to inactivate an extensive variety of microorganisms and its antimicrobial efficiency increments emphatically because of silver (Fernandez *et al.*, 2010).

Up to this point, there is no assessment of silver nanoparticles for Tilapia fish packaging against various pathogens. Fish are more defenseless to oxidation and hydrolysis of lipids and microbial degradation than the other meat products so the techniques for sustainment are more sensitive. The most commercial types of fish are the *Oreochromis niloticus* which is a standout amongst the most well-known oceanic creatures, because of its simplicity of spread and market advancement. Some food storage techniques lead to unfortunate consequences for it, so applying proper packaging would not only prolong food shelf-life, but also preserve its quality and freshness (Domínguez and Carballo, 2017).

In this examination, anti-microbial properties, and growth inhibitory effects of Ag NPs /TiO₂ photocatalytic nanocomposites were examined on the sensitivity of fish against spoilage. An extruder with minimal release was used for the first time on *Oreochromis niloticus*.

Materials and methods

Silver nano composite synthesise

Generation of coatings utilizing expulsion strategy: The extrusion technique (Brabender, DSE 20) was used to deliver coatings. Initially, the polymer granule (low density polyethylene: with 99.9% purity for food grade, Bandar Imam Petrochemical Complex) with pure nano-silver powder (Sigma: 99.9% purity and 60 nm average) was saved in the accompanying fixations: A: containing 1% silver, B: containing 3% silver, C: containing 5% silver, and D: containing 7% silver. At that point the nanosized granules entered the extruder amid the development of the helices inside the extruder, the granules soaked with nano silver were first dissolved, then expelled and afterward, according to the die type the extruder joined in different shapes was expelled from the machine. The extruder was set at 195°C and a speed of 80 rpm. For control coating (control), pure polyethylene without nano-silver was used. The melted granules were converted to film using the film blowing process. The film thickness was 50 microns and they were immediately put up in hygienic packaging and kept away from sunlight and moisture for better quality.

Determination of the properties of nanoscale materials in synthesized nanocomposite

Scanning electron microscope: To explore the transmission of nano-silver nanoparticles at the surface of the packaging coatings, the field emission examining electron magnifying lens was used. At first, the coatings were sliced in 2 times 2 dimensions, then dissolved in glutaraldehyde solvent and afterward disintegrated in acetonitrile solvent for 34 minutes, then each coating was broken up into 3 metal pots to dissipate the solvent and then fixed with special adhesives. Finally, it was exchanged to the Spotter Coater to expand the measure of electrical conductivity in the argon gas region with the gold covering on the samples. Afterward, the electron magnifying chamber for electronic barrage was transmitted and in an amplification of 5, 10 and 15 k with a voltage of 2500 KW, it was assessed as far as molecule morphology and homogeneity of the dispersion level (Moradi *et al.*, 2015).

Transmission electron microscope (TEM): The TEM (Zeiss Libra 200) instrument with a voltage range of 80 to 200 KV was used to determine the size of particles (Jackson *et al.*, 1998).

X-Ray diffraction: The X-ray diffraction method was used to identify the chemical composition and crystalline structure of the coating.

Energy-dispersive X-ray spectroscopy (E-DAX): The Energy-dispersive X-ray spectroscopy method was used to identify the chemical composition structure of the coating and to measure the purity of the nanoparticles in the coating.

Growth inhibitory zone test: In order to investigate the antimicrobial effect of the growth inhibitory zone, the coatings were first cut into 2 cm dimensions and placed in 20 ml of an antimicrobial agent (n hexane), afterward they were placed on a sonicator for 30 minutes and then exposed to ambient temperature for 24 hours. Finally, by preparing the appropriate concentration of two bacteria, *Staphylococcus* 1431 (ATCC25923) and *Escherichia coli* 1399 (ATCC25922) and cultivating them on a medium of Muller Hinton culture using a swab culture method, a drop of solvent coating composition was placed on the medium. For the control group, no solvent was used. After 24 hours, inhibited growth zone was evaluated on the medium (Bauer *et al.*, 1966).

Evaluation of microbial and chemical parameters during days of storage

Packaging and storage time: At first, Tilapias were provided and after the sacrificing process, the organs were extracted and washed with cold water and then transferred to the laboratory on ice. Afterward, surgery was performed with scalpel and 10 grams of fish meat in 5x5 cm² pieces placed in 5 group packages (A-E) by sterile pins. The packages contained A: 1%, B 3%, C: 5%, D: 7% silver coating and E: control group (silver-free package, in a typical freezer bag), and they were stored at 4 °C and 8 ° C for 0, 3, 7, 14 and 30 days.

Samples preparation: First, 1 g of the sample was homogenized in sterile condition for 1 min in a stomacher blender (Lab Blender 400. Seward, Worthing, UK)

and added into a test tube containing 7 ml of sterile ringer solution and mixed well. The resulting mixture was used as an initial suspension for further decimal dilutions.

Total viable microorganisms count: After preparing the dilutions, 1 ml of each dilution was added to sterilized plates using a sterile pipette, then the count agar culture medium was added and mixed by rotary motion and incubated at 30°C for 72 hours. At the end of the incubation period, plates with two consecutive dilutions containing at least 15 and a maximum of 300 colonies were selected and all colonies in the plates were counted according to the standard method (number ISO 4833-2; Mari and Antonini, 2011).

Coliform count: One ml of each dilution was added to marked duplicate Petri dishes using a pipette. Fifteen-twenty ml of VRB (violet red bile agar) was poured into each dish, which had been cooled to 45°C. Then the plates were swirled, left to solidify and overlaid with 3-4 mL of VRB. Afterwards the plates were inverted and incubated at 35-37°C for 18-24 hours. Incubation of more than 24 hours must be avoided. Next, the dark-red colonies having an estimated diameter of 0.5 mm or more were counted (colony size may be affected by the number of colonies per plate) which have a reddish zone of precipitated bile. Finally, the results were recorded and the number of coliforms per gram of each sample was calculated.

Staphylococcal coagulase-positive bacteria count: 0.1 ml of each dilution was added to the surface of baird parker agar medium and at the end of incubation period colonies were investigated. To

confirm specified colonies, some of them were cultured in BHIB medium to do a coagulase confirmation test (Clot formation) using the rabbit plasma. Estimates of the number of coagulase positive staphylococci were analyzed and the results were reported as numbers per gram of sample.

Total psychrophilic bacteria count: 0.1 ml of each dilution was cultured on count agar plate and the plates were incubated and inverted at 6.5 °C for 10 days. At the end of the incubation, the number of colonies in each plate was counted.

Measuring of total volatile nitrogen (TVN): First, 10 grams of meat, magnesium oxide and water, and a few pieces of boiling stone were added to the distillation balloons. In the next step, boric acid and a few droplets of methyl red were added and placed under the cooling unit in the distillation apparatus. Then, the distillation balloon was heated to boil and continued to distill. Afterwards, 0.1 g of normal sulfuric acid was added. To calculate the final value, the amount of sulfuric acid consumed was multiplied by 14 and the amount of volatile nitrogen was calculated (Maghami *et al.*, 2019).

Measuring of peroxide value: First, 1 gram of oil or fatty material was put in a clean and dry test tube and potassium iodide was added in powder form and the solvent solution. The test tube was then placed in boiling water. Afterwards, the test tube contents were immediately discharged into Erlenmeyer containing 5% potassium iodide solution and were finally titrated with 0.2% normal sodium hyposulfite solution and used as a marker for starch

glue. For chemical testing, the level of peroxide was expressed in milliequivalents per milligram equivalent per 1000 grams of fat (Barriuso *et al.*, 2013).

Statistical analysis

The results were analyzed using SPSS 20 software. The analysis of variance (ANOVA), Levene and Duncan's tests were used for normality of the data with homogeneous variance, and the ANOVA and the significance of the data were measured.

Results

Nanoscale materials characteristics in synthesized nanocomposite

High purity nano-silver coatings were prepared by combining low-density polyethylene granules using the extrusion method in 1, 3, 5 and 7% and for the control group, low-density polyethylene nylon was used. As indicated by TEM micrographs the silver stores on TiO₂ have nodular or round shape and the average size of silver nano droplets was about 30 to 40 nanometers. These nanocomposites are made by Ag NPs with tight size conveyance.

Scanning electron microscope and energy-dispersive X-ray

Scanning electron microscope (SEM, Hitachi, Japan) analysis showed that the average nanoparticle size was between 38-42 nm (500 nm) and was observed in some parts of the agglomeration of nanoparticles. The white points are silver nanoparticles (Fig. 1).

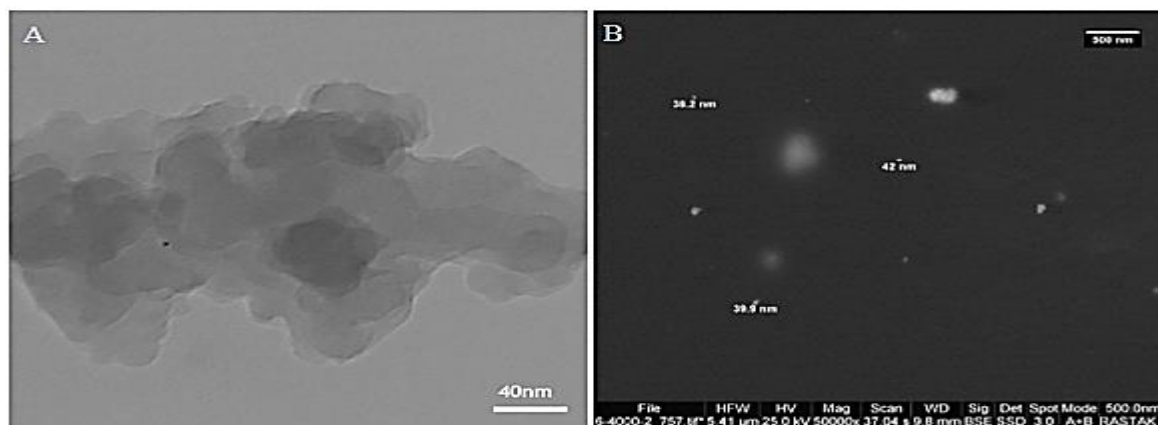


Figure 1: (A) TEM micrograph of silver nano particle, (B) Scanning electron microscope pictures of silver nano film in 5 k magnify with evaluating of silver nano size, the average size of nano particles was confirmed between 38-42 nm.

X-ray spectroscopy analysis

The presence and purity of silver in the film were evaluated by energy-dispersive X-ray spectroscopy analysis. Energy-dispersive X-ray spectroscopic results of the 7% nano-silver package silver particle displayed high concentrations of silver

particles. However, gold was detected, which is probably due to the covering gold layer in the scanning electronic microscope preparation (Figs. 2A-B and 2C-D).

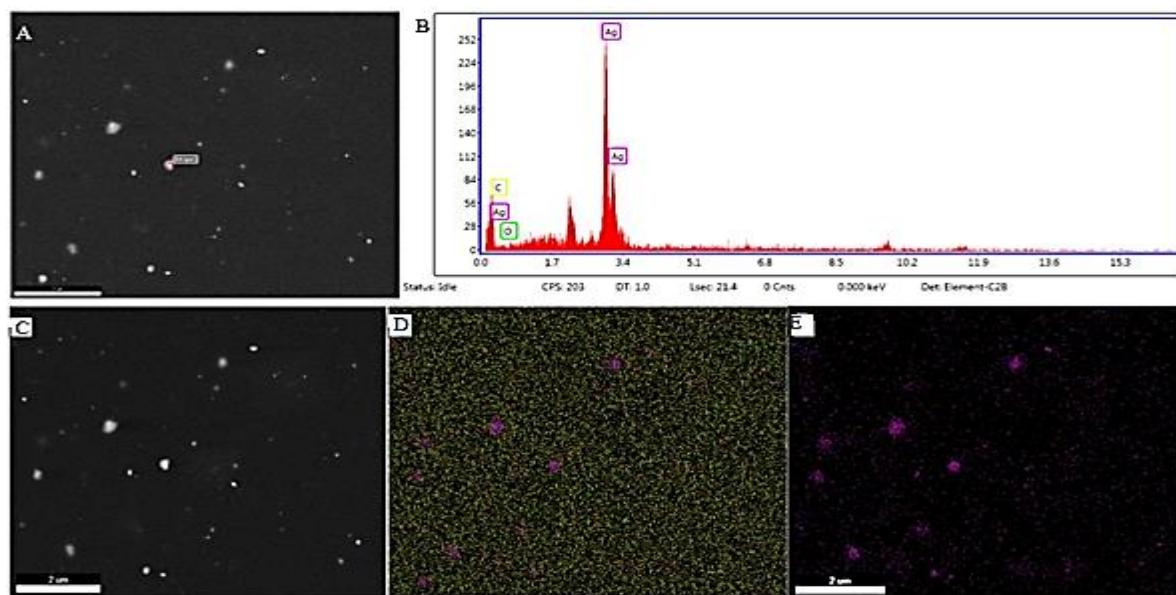


Figure 2: (A) scanning electron microscope 1k amplification from 7% nano silver film, (B) Energy-dispersive X-ray spectroscopy analysis of the silver nano film had shown film composition (Ag as silver and C as carbon in polyethylene), (C) scanning electron microscope in 1k amplification from 7% nano silver film, (D) carbon and oxygen that marked yellow and green respectively, (E) silver particles (Ag) that marked with violet color. The elements in the test separated by colors with percentage of the elements, as yellow: 78% C K, green: 8% O K, purple: 14% AgL.

X-ray diffraction

The content of the nano films was determined from the XRD patterns

recorded by Cu K α radiation (K-alpha1=1.54060 Å) (Fig. 3).

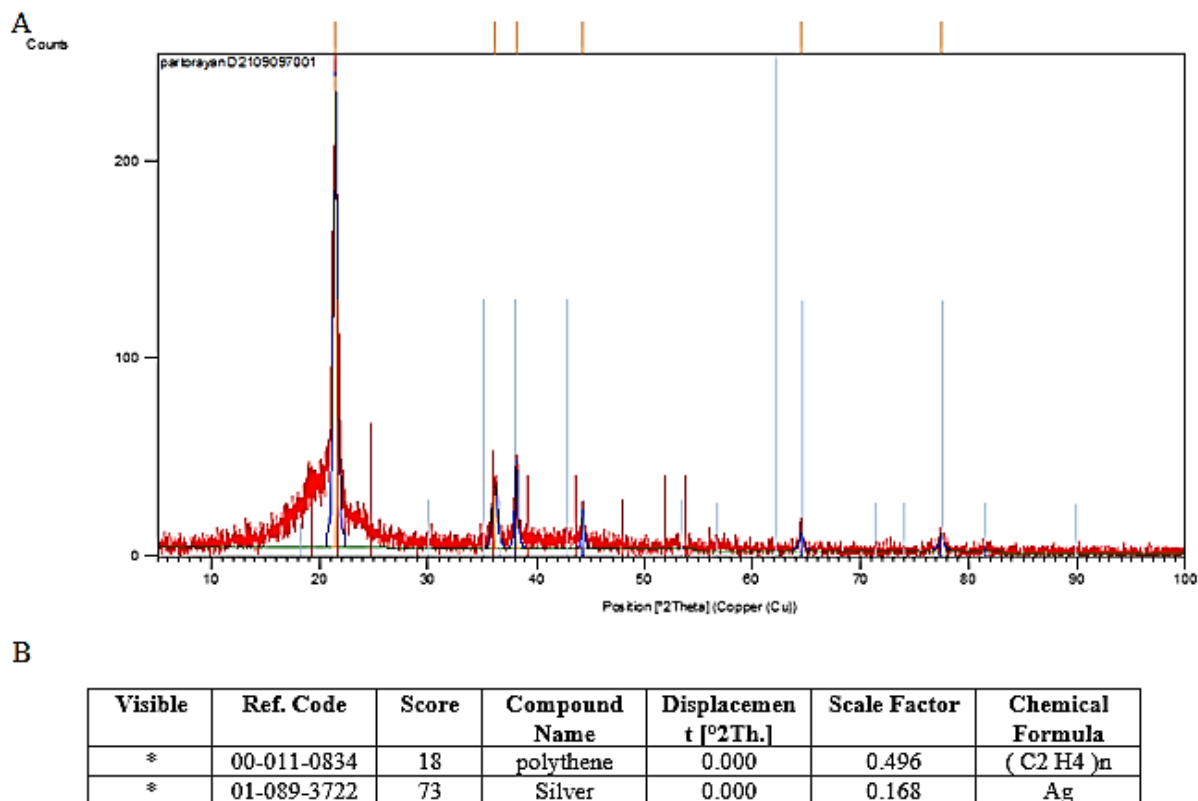


Figure 3: (A) XRD graph (B) identified pattern list

Strong and distinct couriers were seen at the theatrical angles of 38, 44, 64 and 77 degrees, which correspond to the crystalline levels of 111, 200, 220, and 311, respectively. As a result, silver nanotubes with a cubic structure of surface centers were confirmed in the sample.

Microbial and chemical parameters

The growth inhibitory zone test result illustrated in Fig. 4 showed that the higher nano-silver percentage raised, the more growth inhibition zone expands. There was a significant difference between the mean changes in the total number of bacteria counted in different nano-shell coatings on days 3 and 14 at 4°C. The third and

seventh days at 8°C showed a significant difference. On the third, seventh and fourteenth days, the total number of bacteria was decreased by increasing the dose of silver coating to 7% (Fig. 5A). The results of Duncan's test in Fig. 5B showed that there was a significant difference between the mean growth rates of coliform bacteria in different nano-silver coatings at 4°C and on the third, seventh and fourteenth days, by increasing the dose of silver coating to 7%, the number of bacteria decreased. At 8°C on the third and seventh days, with increasing dose of silver coating to 7%, the number of bacteria decreased, and on the fourteenth day there was no significant difference in

the number of bacteria. There was a significant difference between the mean changes in the number of *S. aureus* in different nano-shell coatings on the third and fourteenth day at 4°C and on the seventh day there was no significant difference. The number of *S. aureus* in different coatings of nano-silver was significant on the third and seventh day at 8°C, while on the fourteenth day there was no significant difference. In general, at both temperatures, on the third, seventh and fourteenth days, by increasing the dose of silver coating to 7% the number of bacteria decreased (Fig. 5C). There was a significant difference between the means of psychrophilic bacteria counted in different nano-shell coatings on days 3 and 14 at 4°C, and on the seventh day there was no significant difference. There was a significant difference between the means of psychrophilic bacteria counted in different nano-shell coatings on days 3, 7 and 14 at 8°C. On the third, seventh and fourteenth days, by increasing the dose of silver coating to 7%, the number of

phytochemical bacteria decreased at both temperatures (Fig. 5D).

The results of chemical tests indicated a significant difference between the mean changes in peroxide (mg/m²) in different nano-silver coatings on different days at 4°C and 8°C. On the third, seventh and fourteenth days, the amount of silver peroxide decreased by 7%. The mean changes in total volatile nitrogen in different nano-shell coatings indicated a significant difference between the third, seventh and fourteenth days of storage at 4°C, and at 8°C on the seventh and fourteenth days, with an increase in the dose of silver coating to 7% and the total amount of volatile nitrogen reduced (Figs. 6A and 6B). The results of comparison in measured chemical microbial index changes among coatings having different percentages of silver nanoparticles at 4 and 8°C during different days showed a significant difference between two temperatures. In general, the range of indices at 4°C was lower than at 8°C on different days.

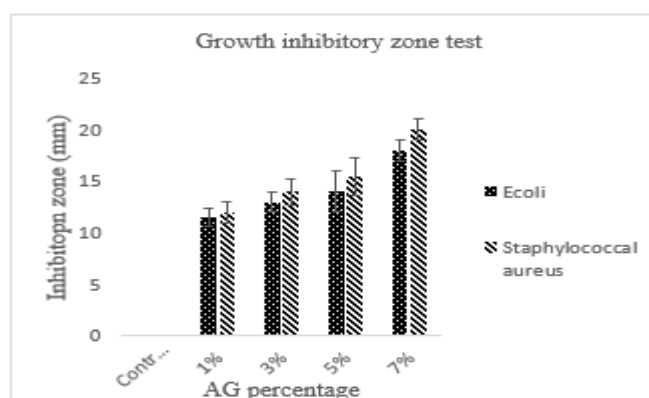


Figure 4: Growth inhibitory zone test

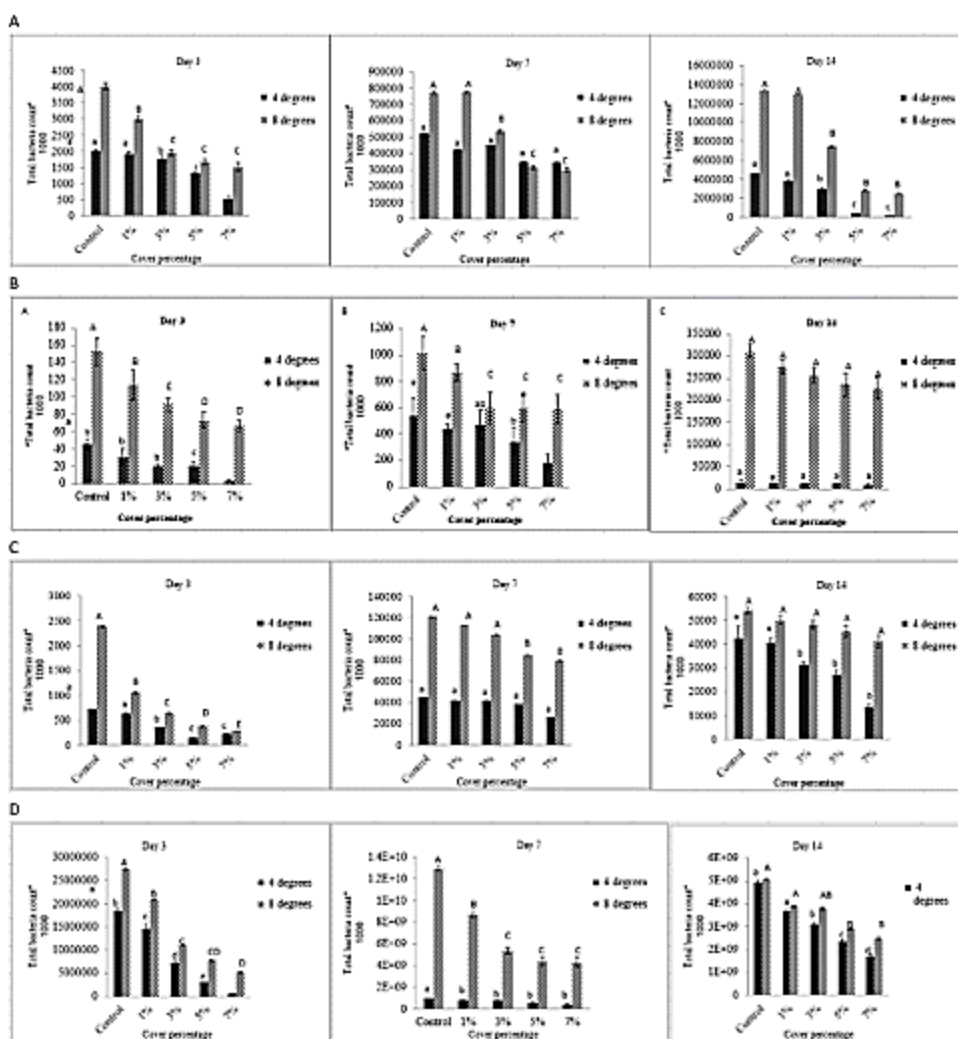


Figure 5: Comparing the average growth rate of (A) total bacteria count, (B) *E. coli* bacteria, (C) *Staphylococcus aureus*, (D) *Psychrophilic* bacteria in different nano-silver coatings percentage in different days at 4 and 8 °C

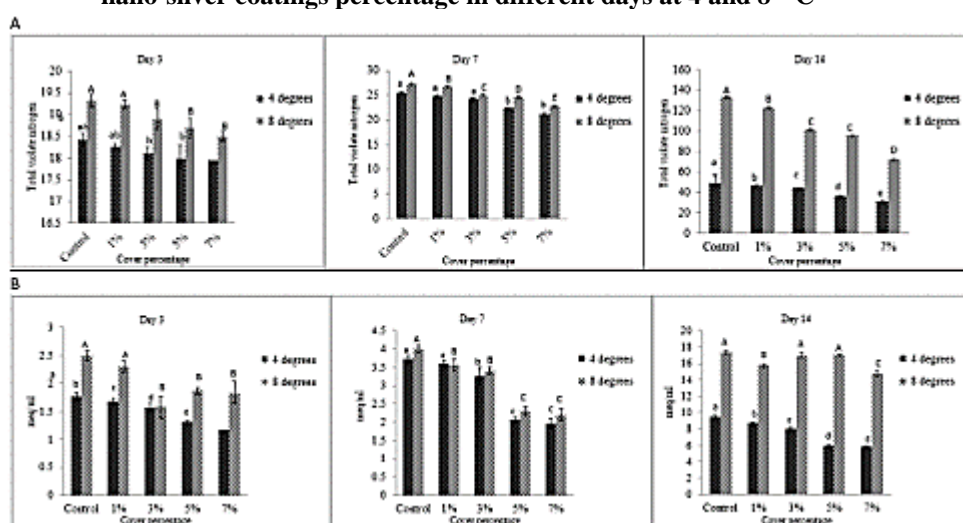


Figure 6: Comparing (A) the average of total volatile nitrogen level, (B) Peroxide changes (meq ml^{-1}) in different nano-silver coatings percentage in different days at 4 and 8 °C

Discussion

Recently, Tilapia is imported to Iran from China in a complete frozen fish or fillet packaging. After establishing a local farming for Tilapia fish in Yazd (Iran), and increasing the production of this valuable seafood, there is a need for proper packaging to prolong the shelf life of fish and keep its pleasant fresh taste for the domestic market as well as exports in the near future. The results of this study provide evidence that the synthesized Ag NPs /TiO₂ nanocomposite can expand shelf life of Tilapia's fresh fillet due to the inhibitory effect of Ag NPs against variety of pathogens.

The size and morphology of silver nanoparticles have prompted antimicrobial action by harming and degrading the bacterial film (Russell and Hugo, 1994). Because of this, nanoparticles have been utilized in drugs and sanitation. Nanoparticles can be utilized in sanitizing drinking water to expect pollution and the spread of oceanic pathogens in carbon channels to take out existing microorganisms noticeable all around. In addition, nanoparticles as antimicrobial coatings in therapeutic and orthopedic equipment can produce anti-gel germs used to treat burns. The utilization of silver nanoparticles to provide cloth in order to prohibition the transmission of contamination to patients is successfully used (Asadi *et al.*, 2014; Fernandez *et al.*, 2010).

The proficiency of nanotechnology is endorsed in creating packaging with enhanced mechanical properties for gas and penetration of heat, and additionally in the generation of dynamic antibacterial

packaging and in the creation of nano sensors in intelligent packaging framework for consumer alarms of food products with no long shelf-life (Dimitrijevic *et al.*, 2015). Metallic silver with nuclear number 43 and nuclear mass 143 have a solid antimicrobial impact on an extensive variety of microorganisms including microscopic organisms, growth and infection (Dimitrijevic *et al.*, 2015).

AgNPs based antimicrobial packaging is a promising type of dynamic food packaging which assumes an imperative job in expanding shelf-life of food and decreasing the danger of pathogens. Furthermore, no measurable amount of released silver nano-particles in food or food simulators have been reported, which means packages containing silver nano-particles are safe for foods (Dimitrijevic *et al.*, 2015; He and Hwang, 2016). A few investigations have been done on productivity of Ag polymer matrix on prevention and control of high microorganism probability on various kinds of foods except for fish items.

For example, the investigations carried out by Emamifar *et al.* (2010) which assessed the impact of LDPE+Ag, ZnO NPs on orange juice to control yeast, molds, total aerobic bacteria compared with pure packaging materials, antimicrobial nanocomposite bundles containing Ag and ZnO as an option of non-thermal innovation can expand the shelf life of fresh orange juice up to 28 days.

A study by Valipour Motlagh *et al.* (2013) assessed total aerobic bacteria through packaging by use of LDPE + AgNPs on barberry. It was proven in this study that the taste, aroma and appearance

of barberries in Ag-LDPE film packaging with 1 wt% silver nano-particles were preserved for approximately 2 to 3, 1 to 4, 2 to 5 and 2 to 4 weeks, respectively, more than barberries in pure polystyrene films packaging. According to previous studies, silver nanoparticles demonstrated the most antimicrobial impacts in comparison with other metal nanoparticles of metal oxide (Dimitrijevic *et al.*, 2015; He and Hwang, 2016).

Martinez-Abad *et al.* (2012) checked the impact of EVOH + AgNPs on chicken, pork, cheddar, lettuce, apples, strips and eggshell packaging to assess the *Salmonella* spp., *L. monocytogenes* and came to positive outcomes and its application in the food packaging industry, as food coatings (Martínez-Abad *et al.*, 2012). Toker *et al.* (2013) revealed the antibacterial movement of polyurethane + AgNPs against *E. coli* and *S. aureus* (Toker *et al.*, 2013). Ag/PS nanocomposite significantly repressed the development of pathogenic gram-positive microscopic organisms, such as *Bacillus subtilis* and *Enterococcus faecalis* besides gram-negative microbes, such as *E. Coli*, *Salmonella typhimurium* and yeast (*Candida albicans*) (Youssef and Abdel-Aziz, 2013).

The impacts of Ag and TiO₂ nanoparticles in PE polymer packaging were examined by Metak *et al.* on solid, fluid, high fat containing and high acidic nourishment tests, comparing with traditional containers. AgNPs holders demonstrated high antifungal activity by inhibiting the microorganism development following a few days of capacity (Metak *et al.*, 2013; Metak, 2015). Ag, TiO₂ NPs

was tested on food package of beef meat to research total aerobic bacteria, lactic acid bacteria, *Pseudomonas spp*; fresh-sliced melon to look the impacts on aggregate mesophilic high-impact microorganisms, psychrotrophic microscopic organisms, yeasts, molds, turkey shop meat to discover *L. monocytogenes*, *S. aureus* restraint, pears and carrots to explore *E. coli*, *S. aureus* separately by Fernandez *et al.* (2010), Khalaf *et al.* (2013), and Mohammed Fayaz *et al.* (2009) which all indicated beneficial outcomes (Mohammed Fayaz *et al.*, 2009; Fernandez *et al.*, 2010; Khalaf *et al.*, 2013). Because of the expansion in the measure of relative silver nanoscale to silver, its antimicrobial impact is higher because silver has better efficiency to enter into the microorganisms (Fernandez *et al.*, 2010). In this study, the growth of various tested bacteria reduced in terms of different percentages of silver coating, which was done in various days at two temperatures. According to our results, it tends to be reasoned that total microbial count at 8°C on day 14 and 7% of Ag shield was the most minimal bacterial growth. The result of our study was in agreement with Mahdi *et al.* (2012). The low *E.coli* count in the nano-silver packaging group compared with control group which were reliable with the results of research done by Sondi, Raffi and Lkhagvajav (Sondi and Salopek- Sondi, 2004; Raffi *et al.*, 2010; Lkhagvajav *et al.*, 2011). It was identified that those were gram negative and for the most parts could be susceptible to ionic bonding with Ag NPs (Lkhagvajav *et al.*, 2011).

Similarly, the count of *S. aureus* demonstrated that the temperature of 4°C

on the third day and 7% of Ag shield was the least appropriate culture conditions. These outcomes were predictable from past examinations (Metak and Ajaal, 2013; Toker *et al.*, 2013; Youssef and Abdel-Aziz, 2013).

As indicated by the standard association, microorganisms that can develop at a refrigerated temperature are situated in the group of psychrophilic bacterial, and the term was utilized for life forms whose base temperature of development is zero degrees Celsius and less, at a most extreme of 20 degrees Celsius and the ideal temperatures is 15 degrees Celsius (Metak and Ajaal, 2013; Youssef and Abdel-Aziz, 2013). The appearance or turbidity is generally present in food and in the correct condition causing them to end up corrupted or pathogenic. Psychrophilic bacteria have been recognized in marine situations, and it is important to investigate their presence in aquatic foods.

The action of silver nanoparticles depended on the dosage and was more articulated for gram-negative microscopic organisms than gram-positive bacteria. These examinations have revealed that the distinction between gram-negative and gram-positive microbes against silver nanoparticles is identified with their cell wall structure (Shrivastava *et al.*, 2007; Guzman *et al.*, 2012). Gram-negative bacteria have a thinner cell wall that has a slight solidness and there is a layer of lipopolysaccharide that has a negative charge. The presence of a negative charge at the cell surface of the bacteria makes it less demanding for the bacterial cell to collaborate between silver nanoparticles

that have weak positive charged load. This connecting action at first makes an opening in the cell wall, and afterward, by entering the nanoparticles into the cell, bacteria cooperate with the bacterial cell development and at last a reason for the microscopic organisms to decrease (Yun'an Qing *et al.*, 2018).

Total volatile nitrogen is created by the deterioration of proteins. As indicated by the executive order of health control and supervision of raw animal products of the Iranian Veterinary Organization as well as The International Fishmeal and Oil Manufacturers Association (IFOMA), the possible measure of TVN for fresh frozen fish is 20 mg 100g⁻¹, and the usable range is somewhere between 21 and 25 mg and its unusable sum is in excess of 25 mg per 100 g (Ricque-Marie *et al.*, 1998). Peroxide is the result of oxidation of fatty materials. Different changes happen when peroxide levels reach a specific range, and aldehyde ester materials with short-chain unsaturated fats, cause the smell and taste of fatty materials. The measurements of peroxide and aggregate unstable nitrogen value on various days with various silver shield rates demonstrated that these chemical parameters decreased by increasing the percentage of nano-silver in each group. The result of this research demonstrates that the antimicrobial properties of nano silver for various bacterial species are unique and particular. The amount of silver nanoparticles utilized in nanoscale coatings should be resolved regarding the obstruction of various types of microorganisms against silver, with the goal that the bacterial heap of the foodstuff did not exceed the permitted limit after a

given period of time. Moreover, the synthesized Ag NPs/TiO₂ nanocomposite has a bright creamy color in lower percentages of Ag nanoparticles, while it turns to a dark creamy shade in higher percentages. In other words, this packaging presents a lighter appearance as follows: 1% > 3% > 5% > 7%.

References

- Asadi, M., Khosravi-Darani, K., Mortazavi, A., Hajseyed Javadi, N., Azadnia, E., Kiani Harchegani, A. and Ahmadi, N., 2014.** Antimicrobial effect of silver nanoparticles produced by chemical reduction on *Staphylococcus aureus* and *Escheirchia coli*. *Iranian Journal of Nutrition Sciences and Food Technology*, 8(4), 83-92.
- Barriuso, B., Astiasarán, I. and Ansorena, D., 2013.** A review of analytical methods measuring lipid oxidation status in foods: a challenging task. *European Food Rresearch and Technology*, 236(1), 1-15. Doi:10.1007/s00217-012-1866-9
- Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turck, M., 1966.** Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4), 493-496.
- Bumbudsanpharoke, N. and Ko, S., 2015.** Nano-food packaging: an overview of market, migration research, and safety regulations. *Journal of Food Science*, 80(5), R910-923. Doi: 10.1111/1750-3841.12861
- Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M. and Cummins, E., 2012.** Nanotechnologies in the food industry – Recent developments, risks and regulation. *Trends in Food Science and Technology*, 24(1), 30-46. Doi:10.1016/j.tifs.2011.10.006
- Dimitrijevic, M., Karabasil, N., Boskovic, M., Teodorovic, V., Vasilev, D., Djordjevic, V., Kilibarda N. and Cobanovic, N., 2015.** Safety Aspects of Nanotechnology Applications in Food Packaging. *Procedia Food Science*, 5, 57-60. Doi: 10.1016/j.profoo.2015.09.015
- Domínguez, R. and Carballo, J., 2017.** Control of lipid oxidation in muscle food by active packaging technology. pp. 343-382.
- Emamifar, A., Kadivar, M., Shahedi, M. and Soleimanian-Zad, S., 2010.** Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice. *Innovative Food Science and Emerging Technologies*, 11(4), 742-748. Doi: 10.1016/j.ifset.2010.06.003
- Fernandez, A., Picouet, P. and Lloret, E., 2010.** Cellulose-silver nanoparticle hybrid materials to control spoilage-related microflora in absorbent pads located in trays of fresh-cut melon. *International Journal of Food Microbiology*, 142(1-2), 222-228. Doi: 10.1016/j.ijfoodmicro.2010.07.001
- Fernandez, A., Picouet, P. and Lloret, E., 2010.** Reduction of the spoilage-related microflora in absorbent pads by silver nanotechnology during modified atmosphere packaging of beef meat. *Journal of Food Protection*, 73(12),

- 2263-2269. Doi:10.4315/0362-028X-73.12.2263
- Guzman, M., Dille, J. and Godet, S., 2012.** Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. *Nanomedicine: Nanotechnology, Biology and Medicine*, 8(1), 37-45. Doi:10.1016/j.nano.2011.05.007
- He, X. and Hwang, H.M., 2016.** Nanotechnology in food science: Functionality, applicability, and safety assessment. *Journal of Food and Drug Analysis*, 24(4), 671-681. Doi:10.1016/j.jfda.2016.06.001
- Jackson, C.L., Chanzy, H. D., Booy, F.P., Drake, B.J., Tomalia, D.A., Bauer, B.J. and Amis, E.J., 1998.** Visualization of dendrimer molecules by transmission electron microscopy (TEM): Staining methods and cryo-TEM of vitrified solutions. *Macromolecules*, 31(18), 6259-6265. Doi:10.1021/ma9806155
- Khalaf, H., Sharoba, A., El-Tanahi, H. and Morsy, M., 2013.** Stability of antimicrobial activity of pullulan edible films incorporated with nanoparticles and essential oils and their impact on turkey deli meat quality. *Journal of Food and Dairy Sciences*, 4, 557-573. DOI: 10.21608/jfds.2013.72104
- Khodanazary, A., Salati, A. P., Bohlouli, S., Mohammadi, M., & Sadeghi, E. 2019.** Quality enhancement in refrigerated tiger tooth croaker (*Otolithes ruber*) fillets using chitosan coating containing green tea extract. *Iranian Journal of Fisheries Sciences*, 18 (2), 224-241. DOI: 10.22092/ijfs.2018.117758
- Lkhagvajav, N., Yasa, I., Celik, E., Koizhaiganova, M. and Sari, O., 2011.** Antimicrobial activity of colloidal silver nanoparticles prepared by sol-gel method. *Digest Journal of Nanomaterials and Biostructures*, 6(1), 149-154.
- Maghami, M., Motalebi, A.A. and Anvar, S.A.A., 2019.** Influence of chitosan nanoparticles and fennel essential oils (*Foeniculum vulgare*) on the shelf life of *Huso huso* fish fillets during the storage. *Food Science and Nutrition*, 7(9), 3030-3041. Doi:10.1002/fsn3.1161
- Mahdi, S., Vadood, R. and Nourdahr, R., 2012.** Study on the antimicrobial effect of nanosilver tray packaging of minced beef at refrigerator temperature. *Global Veterinary*, 9, 284-289.
- Mari, A. and Antonini, G., 2011.** Validation of the micro biological survey method for total viable count and *E. coli* in food samples. *American Journal of Food Technology*, 6(11), 951-962. DOI: 10.3923/ajft.2011.951.962
- Martínez-Abad, A., Lagaron, J.M. and Ocio, M.J., 2012.** Development and characterization of silver-based antimicrobial ethylene-vinyl alcohol copolymer (EVOH) films for food-packaging applications. *Journal of Agricultural and Food Chemistry*, 60(21), 5350-5359. Doi: 10.1021/jf300334z
- Metak, A., and Ajaal, T., 2013.** Investigation on polymer based nano-silver as food packaging materials.

- International Journal of Agricultural and Biological Engineering*, 7, 772-777.
- Metak, A.M., 2015.** Effects of nanocomposite based nano-silver and nano-titanium dioxide on food packaging materials. *International Journal of Applied Science and Technology*, 5(2), 26-40.
- Mohammed Fayaz, A., Balaji, K., Girilal, M., Kalaichelvan, P.T., and Venkatesan, R., 2009.** Mycobased synthesis of silver nanoparticles and their incorporation into sodium alginate films for vegetable and fruit preservation. *Journal of Agricultural and Food Chemistry*, 57(14), 6246-6252. Doi: 10.1021/jf900337h
- Moradi S., Motallebi A., Anvar A., Ahari H., Beheshtiha Shirazi H., Rokni N., Sohrabi Haghdooost N., Mokhtari A., Vahid S., Rahmannia H., Alimohammadi N., 2015.** Increasing the shelf life of Iranian caviar by nano silver packaging basis TiO_2 and determination of residue by titration. *Journal of Comparative Pathobiology Iran*, 12, 1(48), 1549-1560.
- Raffi, M., Mehrwan, S., Bhatti, T.M., Akhter, J.I., Hameed, A., Yawar, W. and ul Hasan, M.M., 2010.** Investigations into the antibacterial behavior of copper nanoparticles against *Escherichia coli*. *Annals of Microbiology*, 60(1), 75-80. Doi:10.1007/s13213-010-0015-6
- Ricque-Marie, D., Abdo-de La Parra, M.I., Cruz-Suárez, L.E., Cuzon, G., Cousin, M. and Pike, I.H., 1998.** Raw material freshness, a quality criterion for fish meal fed to shrimp. *Aquaculture*, 165(1-2), 95-109. Doi:10.1016/S0044-8486(98)00229-4
- Russell, A.D. and Hugo, W.B., 1994.** Antimicrobial activity and action of silver. *Progress in Medicinal Chemistry*, 31, 351-370. Doi: 10.1016/s0079-6468(08)70024-9
- Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P. and Dash, D., 2007.** Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*, 18(22), 225103. Doi: 10.1088/0957-4484/18/22/225103
- Sondi, I. and 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. Doi:10.1016/j.jcis.2004.02.012
- Toker, R.D., Kayaman-Apohan, N., and Kahraman, M.V., 2013.** UV-curable nano-silver containing polyurethane based organic-inorganic hybrid coatings. *Progress in Organic Coatings*, 76(9), 1243-1250. Doi: 10.1016/j.porgcoat.2013.03.023
- Valdés, A., Ramos, M., Beltrán, A., Jiménez, A., and Garrigós, M., 2017.** State of the art of antimicrobial edible coatings for food packaging applications. *Coating*, 7(56), 1-23. Doi:10.3390/coatings7040056
- Valipoor Motlagh, N., Hamed Mosavian, M. T., and Mortazavi, S.A., 2013.** Effect of polyethylene packaging modified with silver particles on the microbial, sensory and

appearance of dried barberry. *Packaging Technology and Science*, 26(1), 39-49. Doi: 10.1002/pts.1966

Youssef, A., and Abdel-Aziz, M.S., 2013. Preparation of polystyrene nanocomposites based on silver nanoparticles using marine bacterium for packaging. *Polymer-Plastics Technology and Engineering*, 52(6), 607-613. Doi: 10.1080/03602559.2012.762658

Yun'an Qing, L.C., Li, R., Liu, G., Zhang, Y., Tang, X., Wang, J., Liu, H., Qin, Y., 2018. Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. *International journal of nanomedicine*, 13, 3311. Doi: 10.2147/IJN.S165125