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Comparative Study of Polyethylene and Polyamide Packaging Containing Silver Nanoparticles in Reduction of Meat Products (Mince Meat) Microbial Load

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ABSTRACT: In order to measure the effect of antibacterial nano-covers, the direct contact of covers with meat products mixture was used as a control. Samples were contaminated with standard strains of gram-negative *Escherichia coli* and gram-positive bacteria *Staphylococcus* aureus. The samples were compared at specified times (0, 24, 48, and 72 hours). Despite the samples had large number of *Staphylococcus aureus*, except for one case, the confirmatory tests were found no other positive *Staphylococcus aureus* coagulase bacteria. This absence, despite the manual infection of two sections out of seven, raised the possibility that the domination of Escherichia coli bacteria prevented the growth of *Staphylococcus aureus* bacteria in the broth. The hypothesis was tested using a standard method (spot-on-lawn method), and no growth of positive *staphylococci coagulase* was observed on the Escherichia coli plate. However, the control plates, became turbid due to the growth of bacteria. Lack of growth can be attributed to the use of unsuitable bacteria.

Keywords: *Nanoparticle Cover, Polyethylene and Polyamide Covers, Reducing Microbial Load.*

Introduction

As the world population continues to rise, healthy food production is one of the major concerns of the food industry. Todays' tendency to minimize the process of food production, facilitate the long-term storage of food, and prevent food-borne outbreaks has led to the development of methods that impede the growth of microorganisms and reduce the use of antimicrobials in packed food products (SilvestreDuraccio and Cimmino, 2011). Antimicrobial substances are utilized in foods that are heat-sterilized or possess a self-controlling immune system to prevent their spoilage due to the secondary contamination during packing, distribution, or unpacking. Various antimicrobial substances are used in food packing, and each of these substances has its own properties and mechanism of action. These substances are selected and utilized based on factors such as type of food, type and growth rate of microorganisms, antimicrobial activity and spread, and antimicrobial chemical composition (Quintavalla and Vicini, 2002).

The effects of the antimicrobial properties of nanocomposite low silver films, low-density polyethylene, and modified

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atmosphere were examined, considering the shelf life of packed chicken breast fillets. The findings indicated a significant difference ($p<0.05$) between the shelf life of the chicken breast fillet and oxidation of the control film. The results showed that low density polyethylene films containing silver nanoparticles can be used as antimicrobial packaging for food. According to the research conducted, common packaging available in the market is made up of polyethylene and polyamide, which tend to be inappropriate for highly perishable meat products. However, owing to their antibacterial properties, nanoparticle silver covers can increase the shelf life of the products.

One method of using antimicrobial substances is Photon-Excited Nylon Film, i.e., polyamide nylon films that are exposed to ultraviolet radiation. Under such exposure, some of the amide groups convert into amine, which increases the amine concentration in the nylon and enables the surface to act as an antimicrobial cover; however, these films are only useful for preventing Staphylococcus aureus (Haghighi-Manesh and Azizi, 2017). From other types of antimicrobial agents in food packing, we might point to the use of silver compounds or antimicrobial enzymes, such as lactoperoxidase, in direct combination with the polymer. In such cases, the antimicrobial activity of metals depends on their ionic form (silver ion) (Appendini and Hotchkiss, 2002). Among metal ions, silver ions have the greatest impact and antimicrobial activity. In order to employ the antimicrobial activity of silver in food packing, zeolites are commonly utilized. Silver zeolite leads to the slow release of silver in the package, which is of crucial importance. One percent of silver zeolite in polyethylene packing cover is enough to reduce the amount of bacteria on the plastic surface. Therefore, using 1–3% will destroy all the bacteria within 1–2 days (CowanAbshireHouk and Evans, 2003).

While investigating the effects of physically manipulated packaging materials on the quality and safety of meat products, it was found that innovative measures such as active packaging—e.g., nanotechnologies—have certain problems that need to be considered and solved. Consideration should also be given to the consumers' expectations and to safety issues during the packaging process and storage of meat products. Given the importance of food safety and the research conducted, meat covers should be selected so that they not only maintain the quality of products, reduce the microbial load, and increase the products' shelf life, but also address the concerns related to preventing product waste (Duncan, 2011).

In recent years, the use of wrapping (film) has grown in the food packing industry. However, substances currently used for packing foods have a number of disadvantages that limit their use such as non-biodegradability in the environment, low resistance, low strength, and oxygen permeability, which results in the early spoilage of food. These disadvantages are among the reasons scientists began to develop new technologies for tackling these problems (Azeredo, 2009).

Due to the antimicrobial properties of nano products, they are extensively used in the packing industry. Therefore, the production of packages using nanotechnology, which can effectively and efficiently withstand a variety of microorganisms, might increase the shelf life and long-term durability of the products.

Hosseini *et al*. (2017) evaluated the antibacterial properties and migration of silver from nanocomposite packaging into 48 shrimp samples. They measured the diameter of inhibition zone for both Escherichia coli and staphylococcus aureus to assess antibacterial effects of nanoparticle

coatings. Normal and nano coatings were placed in contact with standard cultures and checked at 48, 72, 96, 120, 144 hours. They concluded that diameter of inhibition zone in the packaging containing nanosilver was significantly higher than that of PE, PET and PVC packagings (P< 0.05)

Wong and Goddard (2014) examined the effect of the antimicrobial mixture of nanocomposite low silver films, low-density polyethylene, and modifications to the atmosphere on the shelf life of packed chicken breast fillets. The findings indicated a significant difference ($p < 0.05$) between the shelf life of a chicken breast fillet and oxidation of the control film. Furthermore, the results showed that low density polyethylene films containing silver nanoparticles might be suitable in antimicrobial packaging for food. The data were analyzed using Tukey's test and one-way analysis of variance using SPSS. In this study, samples packed with 4,000 ppm nano-particle covers, based on titanium dioxide, indicated a significance level of $p<0.05$, even when samples of meat products were contaminated by Escherichia coli and Staphylococcus aureus bacteria in a controlled condition.

Foroughi *et al.* (2011) investigated the effects of nanoparticle covers on the shelf life of food products. First, 14 random samples of cocktail sausages of a certified brand were prepared and divided equally into control (A) and experimental (B) groups. The control samples were placed in normal conditions, without any special cover (usual sausages packing), while the experimental samples were covered with nano-covers containing silver particles on titanium dioxide. All the samples were placed in a fridge that was empty of any other food products. An examination of the shelf life of food products showed that the silver nano-cover technology positively influenced and increased the shelf life of sausages and cocktail meat products. In order to increase the shelf life and reduce the microbial load, the samples of packed *Thunnus tonggol* were covered with polyethylene, polyamide, and silver nanoparticles and examined at 0, 24, 48, and 72 hours for the existence of Escherichia coli and Staphylococcus aureus bacteria. The microbial growth in the samples packed with silver nanoparticle covers were compared with the control group and with the samples packed with conventional covers. The results indicated a significant decrease in microbial growth ($p<0.05$)

Patiño *et al.* (2014) investigated the production of polyamide composite covers containing silver nanoparticles for packing sausages. The results revealed that the cover is effective at inhibiting the growth of Salmonella Typhimurium (at the significance level of $p<0.05$) and reducing the transfer of oxygen from the cover. A statistical analysis was performed using Duncan's multi range test (ANOVA). In this study, the silver nanoparticle covers inhibited the growth of pathogenic bacteria at a significance level of $p<0.05$, thus highlighting the antibacterial properties of this type of cover as compared with other cover.

Wang *et al.* (2014) investigated the effect of a polyamide film releasing lactic acid bacteria on the growth of Escherichia coli, Enterobacteriaceae, and total aerobic bacteria in beef packed in a vacuum and found that the number of bacteria had been reduced using this method. The findings of this study showed that the covers containing silver nanoparticles made better contact with microorganisms due to the increase in the surface to volume ratio and a large number of metal atoms per surface area. These covers might thus be an effective antimicrobial agent against pathogenic bacteria, viruses, and other microorganisms. This property of the silver nanoparticle covers increases the shelf life of meat products.

El-Wakil Hassan *et al*. (2015) examined the microbial (biological) degradation of low density polyethylene by applying various analysis techniques in vitro to deal with some of the issues related to low-density polyethylene, such as fungal activity in LDPE, biological benefits and challenges of LDPE, and microorganisms involved in the degradation of LDPE. Major concerns were reported for the LDPE covers. In the samples that were packed with polyethylene and polyamide covers (without controlled contamination), the total bacteria count increased after a certain period. However, the samples packed with silver nanoparticle covers (controlled contamination) revealed a significant decrease in contamination after 48 hours ($p<0.05$).

Azlin-Hasim *et al*. (2016) investigated the antimicrobial activity of chitosan, organic acids, and nano-sized solvents for potential use in smart active antimicrobial packing and potential applications for food products. The results indicated that chitosan, with average molecular weight (MMW), benzoic acid, and sorbic acid nano-sized solvents indicated significantly ($p<0.05$) higher antimicrobial properties than their non-nano equivalents. The statistical analysis was performed using Tukey's test, with three repetitions, and one-way analysis of variance. Furthermore, studies comparing polyethylene and polyamide packages with a cover containing silver nanoparticles indicated that the control group sample significantly differed from the sample packed with silver nanoparticle covers ($p<.05$). The statistical analysis was carried out using Kruskal-Wallis and one-way analysis of variance.

Lee *et al*. (2015) studied the effect of active packing with citrus juice on fat oxidation and the quality of cooked turkey meat. The results indicated that oxidation influences the fat of the cooked turkey meat because significant differences were observed in their sensory properties when being kept in a refrigerator. The data were analyzed using Tukey's test and one-way analysis of variance (ANOVA). The studies carried out on meat products proved a significant difference between the control group sample and the contaminated (controlled) sample packed with silver nanoparticle covers ($p<.05$). This finding highlights the antibacterial properties of covers containing 4,000 ppm silver nanoparticles, which increase the shelf life of the products.

Materials and Methods

The followings were employed or obtained as listed below:

- Brain-Heart infusion (BHI) liquid broth manufactured by Quelab company with Lot: 404219
- Plate count agar (PCA) broth manufactured by Biolife company with Lot: DC7105
- Brad Parker agar (BPA) broth manufactured by Quelab company with Lot: 904304
- MacConkey broth manufactured by Quelab company with Lot: 904304
- Gram-negative Staphylococcus aureus bacteria ATCC 25923
- Gram-positive Escherichia coli bacteria ATCC 25992
- Cover containing nanosilver in the form of film
- PE packing cover in the form of film
- PA (pvc) packing cover in the form of film
- Freezer bags sterilized with UV rays
- Polystyrene Sample (e.g., foamed polystyrene cup)
- 40:60 Dichloromethane: diethyl ether solution
- Disposable dropping pipette
- Watch glass
- Paper towels
- Sample of meat products (Pooya protein brand)- (two samples of control and

contaminated meat, 33 samples in three iterations) purchased from the shops in the region 2 of Tehran

- Common microbiology laboratory utensils (for more information, refer to the Iranian National Standard No. 2747)
- Diluent, according to national standards 1-8923 and 2-8932, such as a solution with the brand name of Ringer (Merck, Germany)
- Egg yolk emulsion manufactured by iBresco company with Lot: Y2029 (containing potassium tellurite)

The current study examined the effect of nanosilver covers on the microbial effects of meat products in two microorganisms usually found in meat products: Escherichia coli and Staphylococcus aureus. Table 1 presents the maximum allowed amounts of each colony per pack. This study compared the nanosilver cover with other conventional covers (e.g., polyethylene and polyamide) and sought to reveal its ability to lower the microbial load of the two microorganisms, which, in turn, greatly influences the final product health.

An alternative method for making nano-composite film was carried out using casting technology. Polymer casting is a manufacturing process used to make flexible film components, which are typically in the shape of a single or multi-lumen tube commonly utilized in the food industry.

The synthesis of nanosilver packaging was carried out according to the procedure used by TiO_2 and silver mixed (nanosilver particle size is 22 nm), and the casting method was one of the more practical ways of composite packaging product.

First, the meat products were purchased in a one-kilogram package from a randomly selected shop in region 2 of Tehran. Second, to measure the effect of antibacterial nano-covers and compare the results with other conventional covers used in packing food products, the method of direct contact with the food products was used as a control method. The same samples were also infected with standard strains of gram-positive and gram-negative bacteria (Pure strains of “industrial bacteria and fungi collection center of Iran”) and compared at defined time intervals (at 0, 24, 28, and 72 hours). The whole sample sent to the laboratory was quite homogenized and divided into two equal parts. Half was used as control and the other half was manually mixed, contaminated, and homogenized with 1 ml of suspension containing approximately 10^6 CFU/ml of standard stains of Escherichia coli and Staphylococcus aureus bacteria.

- Total microbial count at 30 degrees Celsius (standard 5272)

One gram of the sample was carefully weighed in sterile conditions. After homogenization, it was transferred to a test tube containing 9 ml of sterile Ringer’s solution and was well mixed. The required dilutions were prepared and cultivated in plate count agar broth (pour plate mixed cultivation). After closing the broth, the prepared plates were incubated upside down in groups of less than six at a proper distance from each other, on the roof and walls of the incubator, at 30°C for 72 hours. After the incubation, plates with two successive dilutions containing at least 15 and at most 300 colonies were selected. All of the colonies in the plates were counted, the results were calculated using the standard formula (5,272 standard), and the number of microorganisms were reported based on two significant values (value between 1.1 and 9.9) multiplied by an appropriate power of 1. (Sampling was carried out according to the Iranian National Standard No. 690; samples, experiments, and dilutions were prepared based on Iran National Standards 1-8923 and 2-8932; and the broth was prepared and sterilized according to the manufacturer’s instructions in its brochure).

- *Search method for positive Staphylococcus aureus coagulase count based on the national standard 6806-1*

One gram of the sample was carefully weighed in sterile conditions. After homogenization, it was transferred to a test tube containing 9 ml of sterile Ringer's solution and was well mixed. Subsequent to preparing the requisite dilutions, 0.1 ml of the initial suspension and each prepared dilution were transferred to two sterile plates containing Baird-Parker agar broth (which was previously prepared with the emulsion of egg yolk and potassium tellurite) using a sterile pipette or a sampler. The dilutions were quickly spread on the surface of the plates using a glass rod. The plates were placed in vitro for 15 minutes (25°C) for the liquid to be absorbed into the broth. They were placed at 37°C for 24 hours and then incubated for 48 hours. At the end of each incubation, the plates were investigated for marking the colonies. The marked colonies could be seen as dark or light gray, convex, and with the diameter of 1 to 1.5 mm (after 24 hours of incubation) or 1.5 to 2.5 mm (after 48 hours of incubation) with a transparent halo; a sedimentary ring might also be clung to the colony, which was quite distinguishable. To confirm a number of selected specified and unspecified colonies (commonly five of each), they were cultivated in the Brain Heart Infusion broth, and after 24 hours of incubation in 25°C, confirmatory coagulate test (clot formation), using rabbit plasma, was conducted according to the manufacturer's instructions.

The results and the estimated number of positive Staphylococcus aureus coagulase were obtained using the formula and the results were reported as the number in each gram of sample. (Sampling was carried out according to the Iranian National Standard No. 690; the samples, experiments, and dilutions were prepared based on Iran National Standards 1-8923 and 2-8932; and the broth was prepared and sterilized according to the manufacturer's instructions in its brochure). (Standard 6806-1)

- *Escherichia coli count by estimating the most probable number (MPN)*

Escherichia coli are the bacteria that produce gas with lactose fermentation at 44°C and produce indole from tryptophan. Escherichia coli counting was carried out by using the selected agar MacConkey broth and Indole +. A confirmatory test was conducted on the suspicious colonies. (Standard 2946)

- *Test method*
- *Search method*

First, the presence or absence of Escherichia coli was examined. If positive (the presence of Escherichia coli in the sample), the counting method could be used to estimate the probable number of microorganisms per gram. A certain amount of sample or initial suspension was inoculated in the selected enriching liquid broth (Lauryl sulfate broth). The inoculated broth was incubated at 37°C for 48 hours and its gas content was measured at 24 and 48 hours. If turbidity or gas was observed in the tube, it was inoculated in the tube containing liquid EC broth. The inoculated EC tube was incubated at 44°C for up to 48 hours, and its gas content was measured at 24 and 48 hours. If gas was observed in the tube, it was inoculated in the indole-free peptone water tube, the tube was incubated at 44°C for up to 48 hours, and the production of indole was checked. Regarding the presence of Escherichia coli in a certain weight or volume, if the tubes caused turbidity or gas in the selected broth, the confirmed EC broth produced gas, and the peptone water produced indole at 44°C, the results were all considered affirmative .

- *Counting method*

1. Three tubes from the selected enriched broth were inoculated with double

concentration and a certain amount of the initial suspension.

2. Three tubes from the selected enriched broth were inoculated with a typical concentration and a certain amount of the initial suspension; then, in an identical condition, three other tubes from the selected enriched broth with typical concentrations were inoculated with a certain amount of decimal dilutions. The addition of other decimal dilutions to the selective broth with typical concentration continued until the tubes with the last dilution produced negative results.
3. The tube was incubated at 37°C for up to 48 hours, and the amount of gas production was investigated at 24 and 48 hours.
4. The EC broth was incubated with each tube of double concentration that had produced turbidity or gas and with each tube of typical concentration that had produced gas. The results of the analysis are presented in tables.

SEM was used to measure the height of the nano particles. The findings of the present study were analyzed based on a simple random plan using SPSS version 21, and a Kruskal Wallis test was performed to estimate the significance of the findings.

Results and Discussion

- Analysis of total bacterial count

Table 1 present the maximum number of colonies per pack of meat products.

Table 2 and Figures 1, 2, 3 and 4 present the results of the comparison of the polyethylene, polyamide, and silver nanoparticle covers in a total count test of microorganisms at four times of 0, 24, 48 and 72 hours.

A comparison of the total counts of the microorganisms showed that, at the initial time of zero hour, the contaminated sample covered with the nanosilver nano-particles, polyethylene, and polyamide covers significantly differed from the control group ($p<0.05$), which indicates the enhanced bacterial growth in meat (Figures 1, 2, 3 and 4).

At 72 hours, the results showed a reduction in the bacterial growth rate in the samples of polyethylene, polyamide, and silver nanoparticles as compared to the control sample ($p<0.05$). The fewest bacteria were observed with the group of silver nanoparticle covers as compared with the other groups. This finding was due to the antibacterial property of the silver nanoparticles covers preventing the growth of pathogenic bacteria.

Table 1. Maximum allowed number of colonies per pack of meat products

Product type	Experiment type	Maximum allowed
	Total count of microorganisms	$5^{\times} 10^{5}$ in gram
Fresh or frozen Meat products	Staphylococcus coagulase +	$5^{\times}10^{2}$ in gram
	Escherichia coli	50 gram

Table 2. Mean and standard deviation logarithm of seven treatments (mentioned packages) of total bacteria count at four times (zero, 24, 48, and 72 hours)

Time /Samples	Zero h	Time 24 h	Time 48 h	Time 72 h
Control	$5.71^{a} \pm 4.18$	$5.86^{a} \pm 4.31$	$5.97^{a} \pm 4.18$	$6.07^{a} \pm 5$
PE	$5.76^{b} \pm 4.18$	$5.06^{b} \pm 3.76$	$5.34^{b} \pm 3.76$	$5.12^{b} \pm 4.18$
PA	$5.68^{a} \pm 4.30$	$5.01^{b} \pm 3.76$	$5.64^{ab} \pm 4.30$	$5.67^{c} \pm 4.23$
NS	$5.69^{a} \pm 4.63$	$4.68^{c} \pm 3.18$	$4.20^{c} \pm 4.31$	$3.34^{d} \pm 2$
PEC	$6.04^{c} \pm 5$	$5.99^{d} \pm 4.18$	$5.82^{ab} \pm 4.18$	$5.88^{ae} \pm 3.76$
PAC	$6.06^{c} \pm 4.76$	$6.00^{ad} \pm 4.84$	$5.91^{ab} \pm 4.18$	$5.97^{af} \pm 4.18$
NSC	$6.06^{c} \pm 4.76$	$5.91^{a} \pm 4$	$5.53^{b} \pm 4.30$	$5.26^{b} \pm 4.18$

Owing to the controlled inoculation with bacterial strains, samples of contaminated polyethylene, contaminated polyamide, and contaminated silver nanoparticles showed an increase in bacterial growth as compared with the control sample. No significant difference was observed between the contaminated polyethylene and polyamide samples with the control group considering the bacterial growth rate ($p<0.05$). However, due to the use of silver nanoparticles with antibacterial properties, the contaminated sample of silver nanoparticles showed a slight decrease in bacterial growth as compared to the control sample and the contaminated polyethylene and polyamide samples ($p<0.05$).

- Total bacteria count at the four measurement times

According to Table 2 and Figure 5, significant differences were evident in the total count at zero, 24, 48, and 72 hours ($p<0.05$); however, no significant difference was observed in the total bacteria count between 24 and 48 hours ($p<0.05$).

- Analysis of microbial tests for Escherichia coli

Table 3 presents the mean logarithms of total bacteria count at 0, 24, 48 and 72 hours. Table 4 and Figures 6, 7, 8 and 9 present increases in the microbial load of Escherichia coli strain in the samples of contaminated polyethylene, contaminated polyamide, and contaminated silver nanoparticles as compared with the control sample. The difference between the contaminated sample and the control group was significant ($p<0.05$) because of the controlled inoculation of the sample (meat products) with the bacteria strain.

Given that p=.05, we conclude that the degree of significance of the total count variable differs in repeated measures. In other words, the passage of time significantly influences the total count. The graphs of each group indicate whether the difference is due to the decrease or increase in the total count.

Table 4 and Figure 10 indicate that the differences in the Escherichia coli bacteria count are significant at zero, 24, 48, and 72 hours ($p<0.05$).

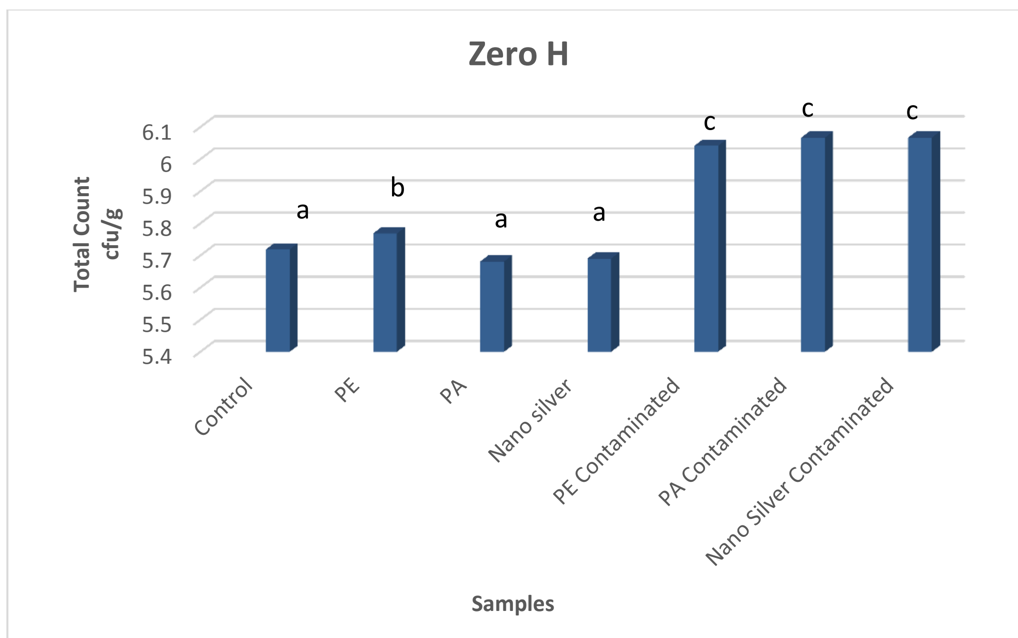

Fig. 1. Comparing mean logarithms of total bacteria count in 7 treatments at the hour of zero.

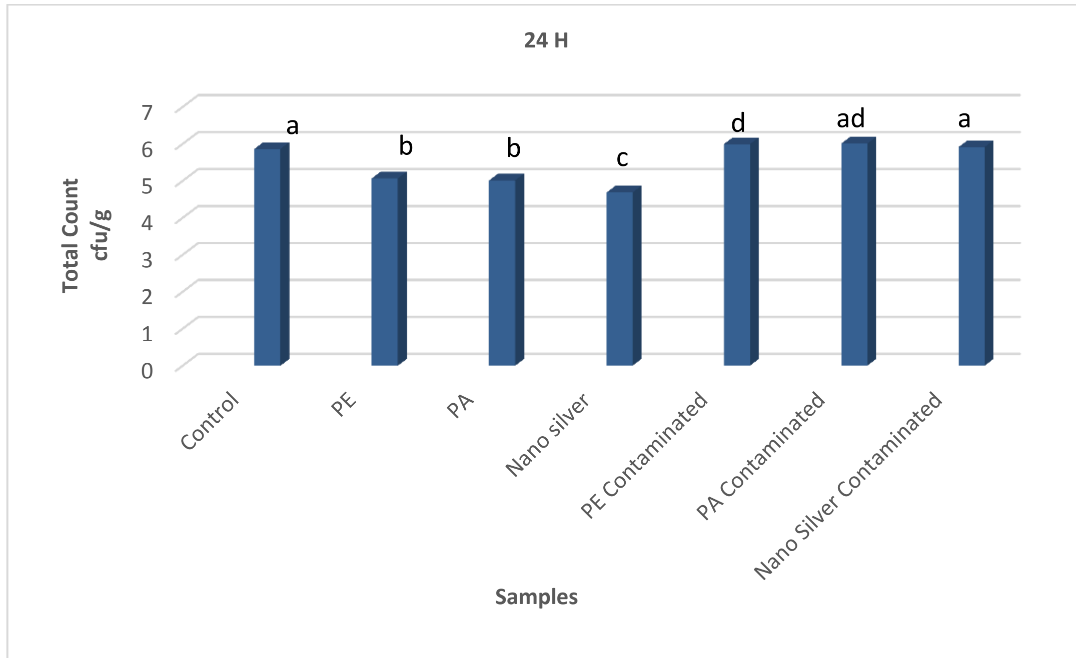

Fig. 2. Comparing mean logarithms of total bacteria count in 7 treatments at the hour of 24.

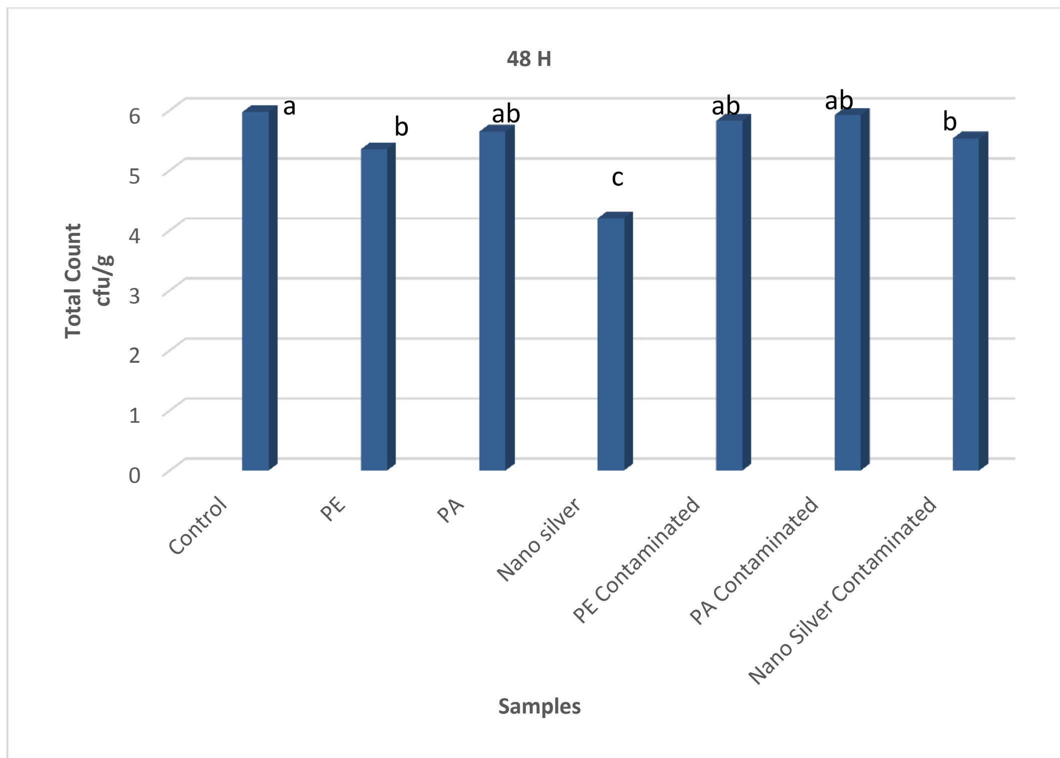

Fig. 3. Comparing mean logarithms of total bacteria count in 7 treatments at the hour of 48.

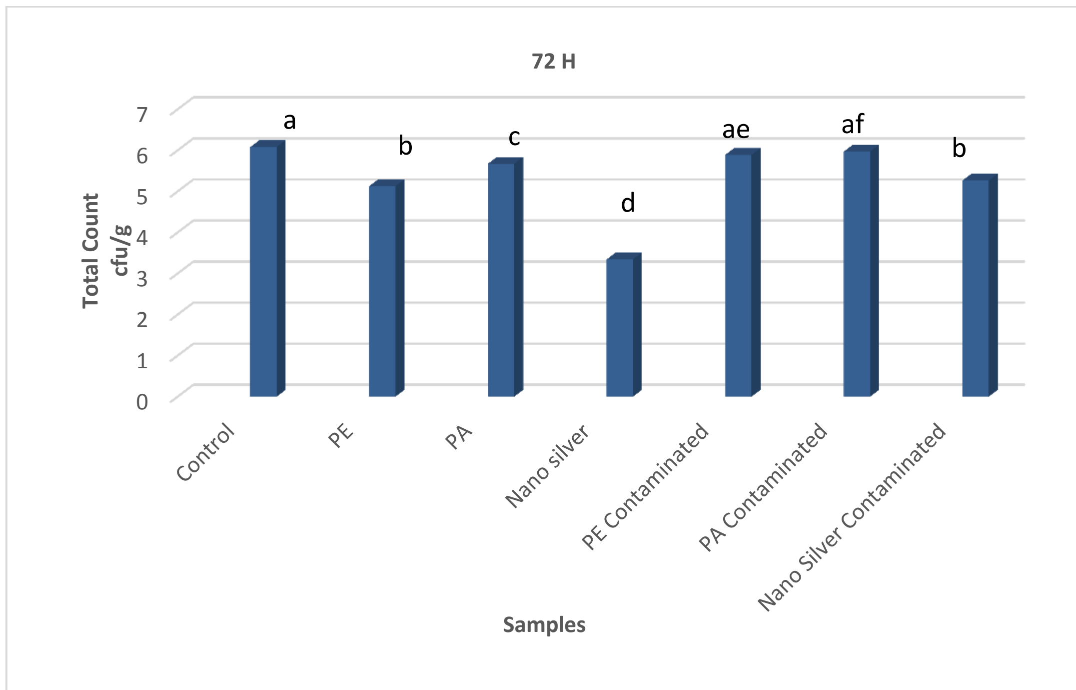

Fig. 4. Comparing mean logarithms of total bacteria count in 7 treatments at the hour of 72.

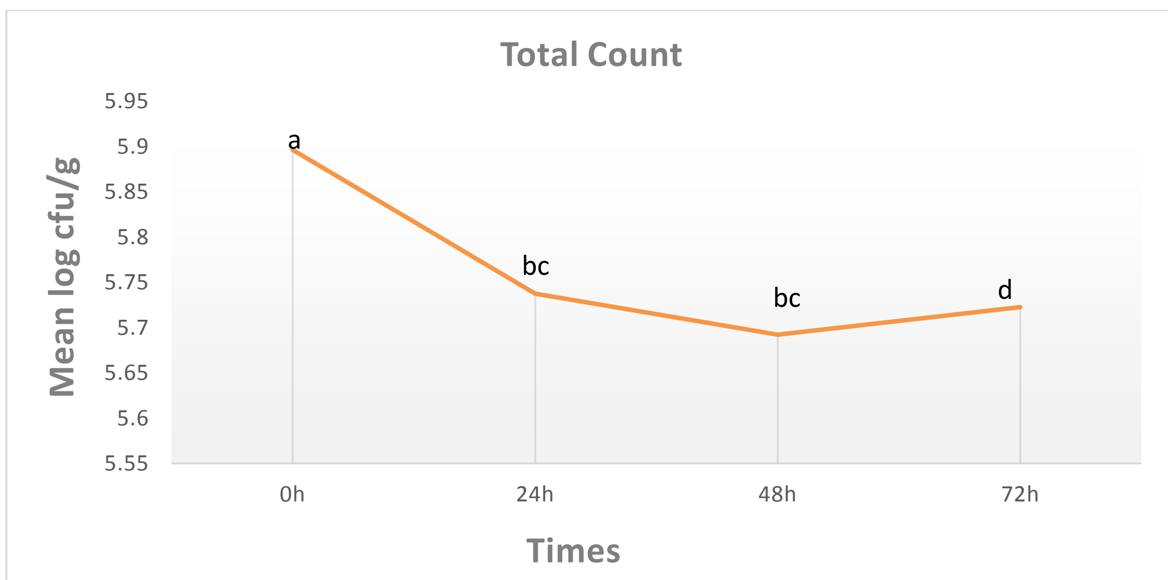

Fig. 5. Mean logarithms of total bacteria count at four times (zero, 24, 48 and 72 hours).

Table 3. Mean logarithms of total bacteria count at four times (zero, 24, 48, and 72 hours)

******	**Zero H**	**24 H**	**48 H**	**72 H**
Mean ± standard deviation	a 4.05± 5.9	bc 3.8± 5.75	bc 3.56± 5.7	d 3.93±5.75

Table 4. Mean and standard deviation logarithm of 7 treatments (mentioned packages) of total Escherichia coli count at four times (zero, 24, 48, and 72 hours)

Times / Samples	Zero H	24 H	48 H	72 H
control	1 ^{a} ± -0.23	5.04 ^{a} ±0.31	1.36 ^{a} ± 0	2.06 ^{a} ± 0.40
PE	0.97 ^{a} ± -0.23	0.86 ^{a} ± 0.31	1.25 ^{ab} ± 0	1.97 ^{ab} ± 0.67
PA	0.88 ^{a} ± -0.23	1 ^{a} ± 0	1.23 ^{ab} ± 0.63	2.06 ^{ab} ± 0.88
NS	1 ^{a} ± 0.30	0.84 ^{a} ± 0	1.19 ^{b} ±-0.23	1.93 ^{b} ± 0.40
PEC	4.75 ^{b} ± 3.55	4.92 ^{b} ± 3.78	6.31 ^{c} ± 4.76	6.55 ^{abc} ± 6.42
PAC	5.06 ^{c} ± 4.06	4.70 ^{b} ± 3	6.49 ^{d} ± 5	6.63 ^{c} ± 5.06
NSC	5.04 ^{c} ± 4	4.49 ^{c} ± 3	2.27 ^{e} ± 1.42	2.05 ^{ab} ± 0.76

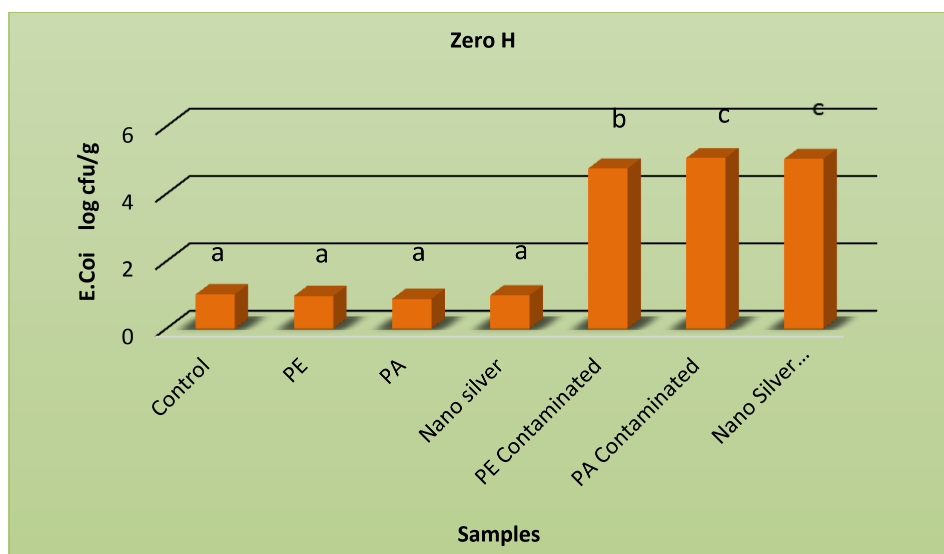

Fig. 6. Comparing mean logarithms of total Escherichia coli bacteria count in 7 treatments at the hour of zero.

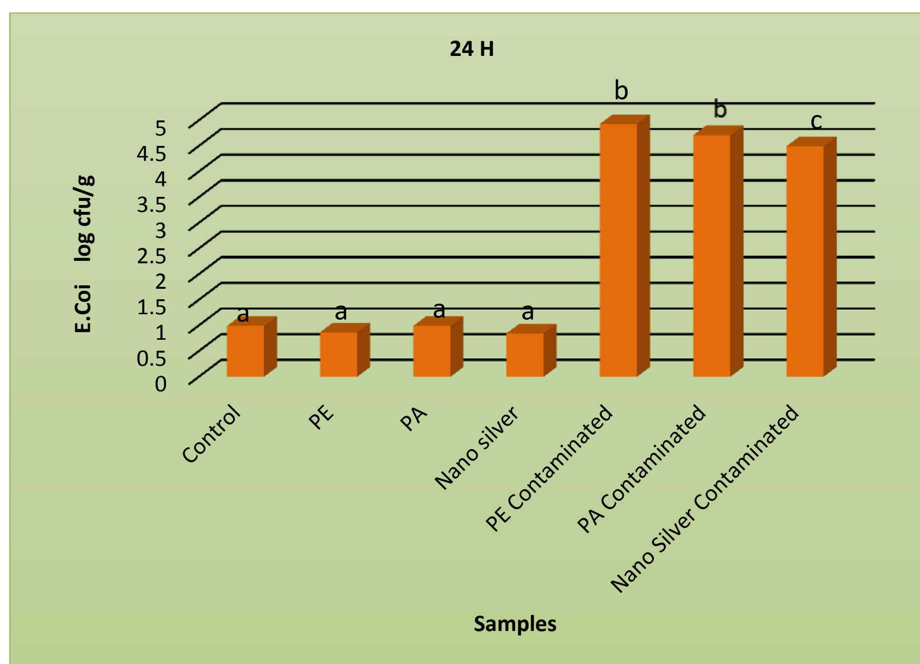

Fig. 7. Comparing mean logarithms of total Escherichia coli bacteria count in 7 treatments at the hour of 24

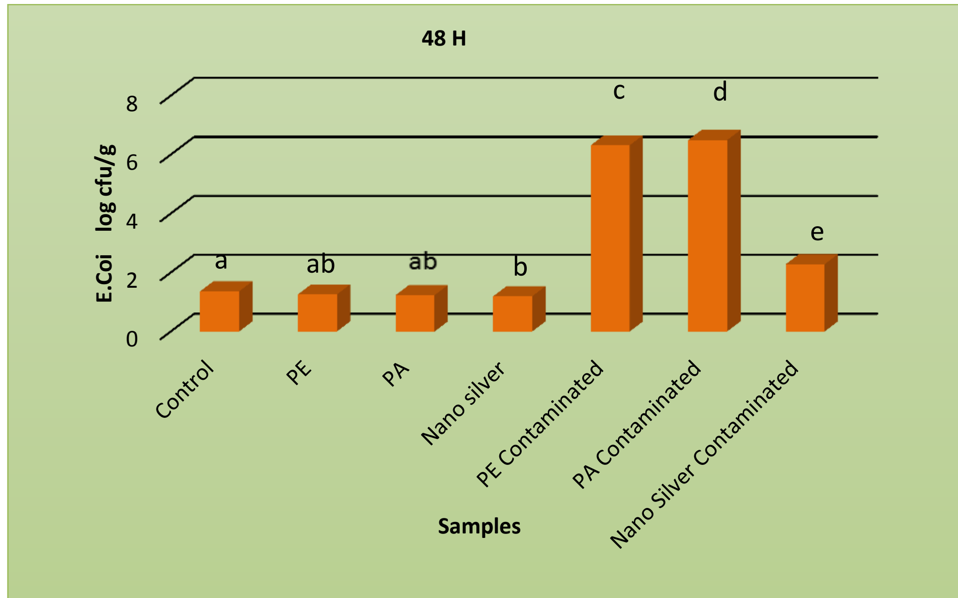

Fig. 8. Comparing mean logarithms of total Escherichia coli bacteria count in 7 treatments at the hour of 48.

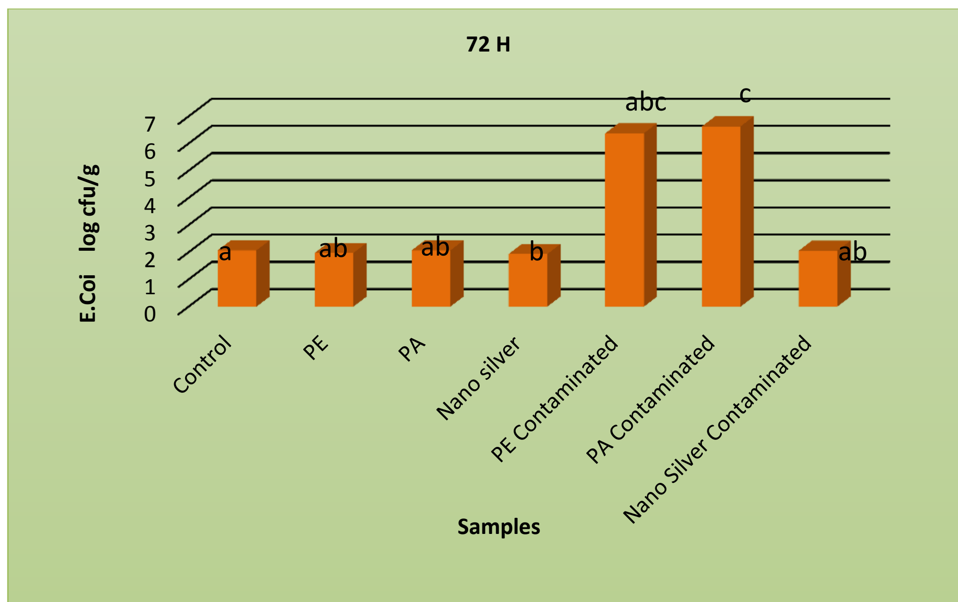

Fig. 9. Comparing mean logarithms of total Escherichia coli bacteria count in 7 treatments at the hour of 72.

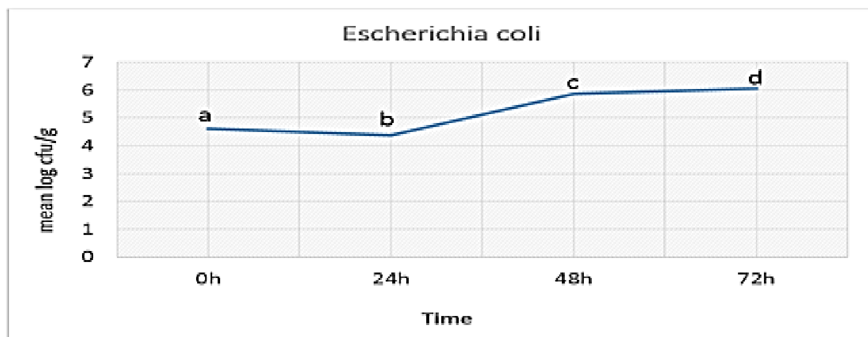

Fig. 10. Mean logarithms of total Escherichia coli bacteria count at four times (zero, 24, 48 and 72 houra).

- *Test for Staphylococcus aureus bacteria*

Despite counting a large number of Staphylococcus aureus colonies as normal flora in the meat tested, except for one case (the control sample not contaminated between two layers of disks made from polyethylene cover), the confirmatory tests found no other cases of positive Staphylococcus aureus coagulase growth. However, the absence of growth of even a colony of positive staphylococci coagulase, despite manual contamination (between two layers of a polyethylene covered disk, between two layers of a polyamide covered disk, and between two layers of a silver nanoparticle covered disk) raises the possibility of Escherichia coli bacteria dominance in the broth preventing the growth of Staphylococcus aureus bacteria. This hypothesis was tested using the standard spot-on-lawn method and no growth sign of positive Staphylococcus coagulase (mixed cultivation with plate count agar broth in the second layer) was observed in the plate containing Escherichia coli cultivated in MacConkey agar broth. However, in the suspension containing positive Staphylococcus aureus coagulase in the agar count broth plate, the turbidity caused by the bacterial growth observed after 24 hours or the lack of growth might be attributed to the use of unsuitable bacteria.

- *Scanning electronic microscope and FTIR*

The data shown in the Figures 11, 12, 13, 14 show that the average size of a nano particle is between 22 nm and 82 nm, which indicates that homogeneity is suitable.

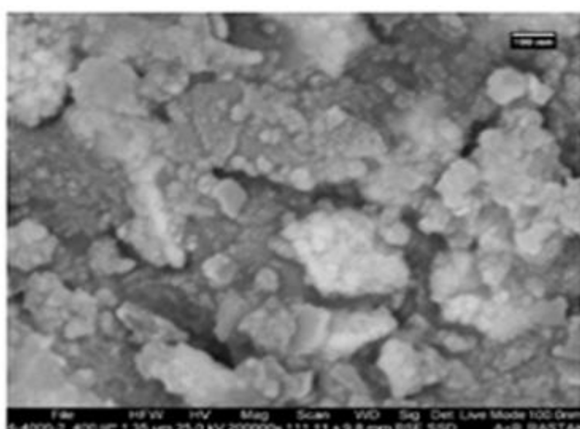

Fig. 11. Nanosilver scanning electronic microscope and Fouriertransform infrar spectroscopy (FTIR).

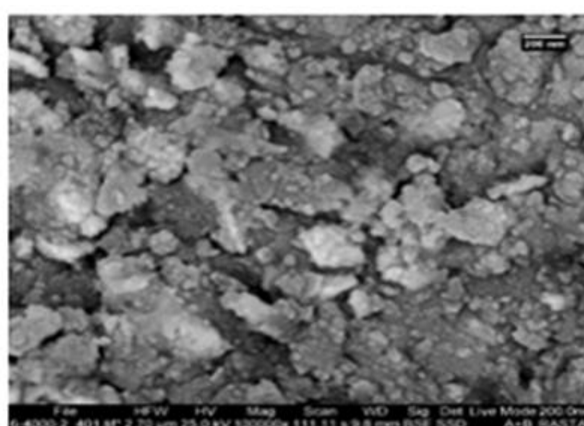

Fig. 12. Nanosilver scanning electronic microscope and Fouriertransform infrar spectroscopy (FTIR).

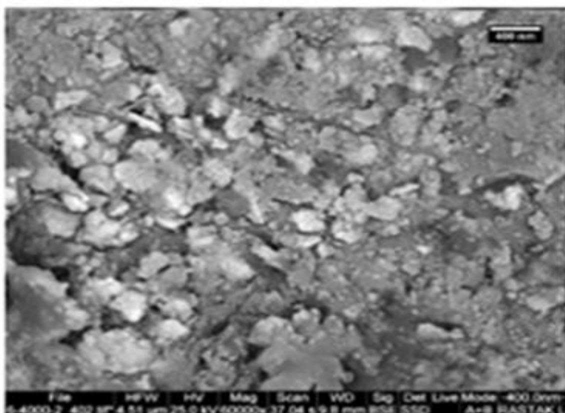

Fig. 13. Nanosilver scanning electronic microscope and Fouriertransform infrar spectroscopy (FTIR).

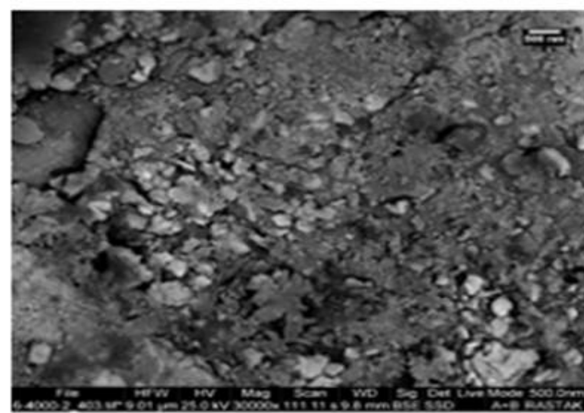

Fig. 14. Nanosilver scanning electronic microscope and Fouriertransform infrar spectroscopy (FTIR).

After preparing the samples at time intervals of zero, 24, 48, and 72 hours, microbial tests were performed on the samples, and the results were recorded. Despite counting a large number of colonies of Staphylococcus as normal flora in the meat tested, except for one case in one section, no growth sign of positive Staphylococcus aureus coagulase bacteria was observed or reported by the confirmatory tests. However, the absence of the growth of even a colony of positive staphylococcus coagulase, despite manual contamination of two sections from seven sections, offers the possibility of Escherichia coli bacteria dominance in the broth preventing the growth of Staphylococcus aureus bacteria. This hypothesis was tested using the standard spot-on-lawn method, and no growth sign of positive Staphylococcus coagulase (mixed cultivation with PCA broth in the second layer) was observed in the plate containing Escherichia coli cultivated in MacConkey agar broth . However, in the suspension containing positive Staphylococcus aureus coagulase in PCA broth, the turbidity caused by bacterial growth was observed after 24 hours. The lack of growth might be attributed to the use of unsuitable bacteria. Owing to the high surface to volume ratio and the large number of metal atoms, silver nano-particles have better contact with microorganisms and work as effective antimicrobial substances against bacteria, viruses, and other microorganisms. Therefore, we might be able to produce covers that increase the shelf life and reduce the microbial load of meat. Considering food safety, this study also emphasizes the necessity of using covers for meat products, which in addition to maintaining the quality of this type of products, reduce the microbial load, increase their shelf life, and reduce the concerns about how to prevent product waste (Iacobucci, 2015; Llana-Ruiz-Cabello *et al*., 2015; El-Wakil *et al*., 2015).

The synergistic antimicrobial effect of low silver nanocomposite films and low density polyethylene under modified atmosphere on the maintenance of packed chicken breast fillets was previously studied by Azlin-Hasim *et al*. (2015). The results showed the significant increase of chicken breast fillet maintenance ($p<0.05$) as compared to the amount of oxidation in the control film. The achieved results suggested that the low-density polyethylene films containing silver nanoparticles could be used as antimicrobial food packaging. According to the existing research, the commonly used packaging materials currently on the market are polyethylene and polyamide, which are suitable for perishable products such as meat products; however, silver nanoparticle covers with antibacterial activity can favorably increase the maintenance period of the product. Patino *et al*. (2014) reviewed the application of a polyamide composite cover containing nanosilver as sausage packaging and showed that the suggested cover can significantly inhibit the growth of bacteria Salmonella typhimurium and oxygen transfer through the cover ($p<0.05$). In the present study, covers containing silver nanoparticles prohibited the growth of pathogenic bacteria related to prior antibacterial characteristics of the cover as compared to other types. In a previous study by Smulders *et al*. (2013), using the effect of lactic acid releasing polyamide film on Escherichia coli and Enterobacteriaceae demonstrated the decrease of bacterial growth in the preservation of packed vacuum beef with this method. Considering the existing studies, covers containing silver nanoparticles could be implemented as a potential antimicrobial agent against pathogen bacteria, viruses, and other microorganisms because they provide a higher surface to volume ratio, which increases the possibility of contact between metal atoms and microorganisms. Hence, this effect of silver nanoparticle covers leads

to a prolonged preservation period of meat products.

For improved food safety, special covers are needed to maintain the quality, decrease the microbial load, and extend the preservation period of meat products. Concerns regarding product waste prevention were also motivating factors to accomplish the current project (El-Wakil *et al.,* 2015; Deus *et al*., 2016).

Conclusion

The results achieved from this project implicated the better efficacy of the covers that included silver nanoparticles (4,000 ppm) as compared to polyethylene and polyamide covers. In the experiment with covers containing silver nanoparticles, the growth of Escherichia coli remarkably decreased in the control samples, and the growth of Staphylococcus aureus decreased completely. The covers containing silver nanoparticles have been recognized as the most efficient cover for eliminating the microbial load of meat products; the preservation time can be extended from two days to 1 to 2 weeks in refrigerated temperatures. However, the results of the experiments using other types of covers for samples and control were not statistically significant ($p<0.05$), and the results were not consequently reported.

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