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DECREASING MICROBIAL CONTAMINATION OF DRY KIELBASA BY SILVER NANO PACKAGING BASE ON TITANIUM DIOXIDE

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ABSTRACT

Today, increasing safety and shelf life of food products via applying coats reducing microbial contamination is possible.

In this study, titanium based coats comprising nano silver particles with various percentage (1% , 3% , 5%) are applied for packaging kielbasa. first to evaluate microbe total count, packaging samples supplied in Tehran market (district 2) were sent to food microbiology and based on Iran National Standard organization number 5272, total count was done, then microbial test of staphylococcus aureus and E.coli with National Standard number of 68.6-3, 2946, was conducted on packaging of products with 1% , 3% and 5% nano silver coats and control samples with no nano coats, besides, to determine and evaluate the range of nano

particles release, chemical test of migration was done and finally the size of silver particles constructing the nano coats were measured and assessed with SEM (electron microscope). The analysis of results represent that microbial total count of common packaging coats in most of the treatment samples were reported uncountable, moreover, microbial count test of nano coats prove that in coats comprising 5% nano silver particles in comparison with 3% and 1% and control group, the number of staphylococcus aureus and E.coli was drastically dropped by %78. It should be remarked that the results of release with Pvalue was reported equal to 0/034.

5% nano coats was known as the most efficient and effective coats in decreasing the microbial contamination and increasing the shelf life in dry kielbasa.

Keywords: **Nano Silver Packaging, Microbial Contamination Reduction, Kielbasa, Residue**

INTRODUCTION

In the recent years, cellulosic materials have been replaced with plastics for packing as improvements in packaging with plastics for fast food, frozen food, dairy products, beverage, bread and chocolate have become morvital and consuming stand, in the first priority. In this row among the plastics, polyethylenes, and polypropylenes (mono and copolymers) are preferred in packaging of monolayer, multilayer and co-entroded structure as a contact layer for food since , these coats are chemical material with well-sustainability and have the least reaction with most of the foods, good protection against moisture and thermal sewing capability and small amount of residue of solvents, monomers, plastisizers and inhibitors or released materials from models may enter to packaged food where aromatic structure cannot penetrate into the package **[13 , 19]**.

Today plastic coats with the technology of nano particles are applied in food packaging industry. since oxygen is considered as the major concern for food packaging (this element causes lipidosis (decay of fat in food) and even changes their color), nano particles are loaded in a zinc oxide model inside the plastic coats to act like a barrier for oxygen penetration, in other word the distance for gas traveling to enter the pack will be elongated, therefore, food stuff in these new packaging can longer keep their freshness and in fact, the longer oxygen molecules move, the latter the food spoils **[7, 12]**. According to high ratio of surface to the volume and vest numbers at atoms of the metal on the surface per unit, nano silver particles used in the ordinary plastic will gain better.

Contact with microorganism and is used as a drastic antimicrobial matter for bacteria/viruses and other microorganism so we can produce sort of coats to increase shelf life and reduce their microbe count **[15]**.

Timothy in 2011, has considered usages food safety and some applications of nano in food packaging, for instance; soil-based nano composite polymer as inhibitor stuff; nano silver particles as strong antibacterial agents and thermal nano sensors and deliberating nano stuff to recognize the analytes relevant to food stuff (gases, tiny biotic molecules and transferred pathogens via food). As a results, these application seemed to be chosen for being customer, friendly in the market since nano particles are not directly loaded in consuming food **[20]**. According to FAO 30%-50% of food product in the world are annually discarded due to inappropriate packaging, raw material, transportation and poor maintenance condition. In Iran, based upon

Shahrokh Zahiri's speech (head of agriculture & food industry in Tehran trade room), Approximately 40% of food products are discarded that converting industry can help us to solve the problem **[22]**.

The major, share of discarded food is the result of poor packaging resulting in spoiling of food and consequently increases the spoilage, packaging is also associated with other factors of discarding, now that packages with high quality of maintenance can enhance the shelf life and reduce the spoilage, on the other side, today, food safety is a major concern to keep the consumers health so than antimicrobial packages with inhibitory properties are produced and supplied to improve the quality at food, besides they should have the ability of destroying or preventing micro organic pathogens by adding anti-microbial agents to the packaging system **[7]**. Thus, this mechanism (anti-microbial activities) will automatically increase the delay time and reduce microorganism life time. To stop microbe growth, moreover antimicrobial packages are designed to resist all attempts of microorganism which contradicts the aims of increasing shelf life, keeping the quality and health safety.

Hence, antimicrobial packaging is considered a good guarantee to accomplish food security goals while it has different effects on microorganism via various activities with their antimicrobial and physiologic properties **[6]**.

Gattesman & et al in 2011, coated some papers with AgNp nano particles; plunged samples in age NO3 solution

have been incubated in suspension of *E.coli and staphylococcus*. After 4 days samples had a promising reaction against both microorganism **[15]**.

Patiño & *et al* (2013) have studied antimicrobial activities of Ag-Zn particles in poly amid composite coats (PA-6) for sausage package. the aim of achieving a film with good mechanical and preventing property led us to make an active coat by adding 3% (w/w) of Ag-Zn particles inhibiting the growth of various microbe strains like *salmonella typhimorium* (ATCC 4028) on the other hand loading Ag-Zn particles in PA-6 films did not make any changes in mechanical property and reduces the oxygen transference of film **[16]**.

Kumar Anal & Akbar in 2014, have studies the effect of nano particles of zinc oxide in packaging against salmonella typhimorium and staphylococcus aureus in ready-to-eat poultry meat.

Nano films using zinc oxide to oppose two outstanding pathogens emerging from food, *salmonella typhimorium* and staphylococcus aureus had a very drastic effect where the number of targeted infectious bacteria

proved a declining slope from 7 to 0 log. Being kept in incubator in 118ºC for 10 min **[6]**.

Emami far & *et al* in 1390 prepared composite films with Ag ZnO and LDPE in package of fresh and steriled orange juice. This was inoculated with lacto bacillus plantarium. The packages were kept in 4 for 112 days microbe count was assessed, demonstrating the reduction at 5% ($P < 0/05$) was very significant **[9]**.

Foroughi & *et al* in 1390, investigated the effect of nano coats in increasing the shelf life for a meat product like cocktail sausage. Parameters as color, flavor, stability, appearance and smell were assessed in two sample groups namely; control and test. the results for samples with nano packaging even after expiring time had appropriate appearance and clear color with a good quality to be consumed while samples in control group, after the expiring time, were not suitable for use with undesirable smell and dark color and a wrinkled face **[11]**.

Sharifi Soltani & *et al* in 1391, studied the effect of antimicrobial nano silver packages in chicken meat in refrigerator and freeze (1+3 ċ).

The samples were assessed for *E.coli and staphylococcus aureus* on days 1, 3, 7, 10, 14 and microbe count proved that nano silver packages cause a reduction in microbial growth in slaughtered chicken and extended the life time from 2 days in common packaging to 7 days in nano silver ones with promising scores **[17]**. Although nano coats packaging likely causes to increase shelf life of product and consequently decreased the final price for producers and consumers, it is very vital not to compromise the health of consumers; this issue is considered with or without releasing nano particles in to the product **[13]**.

Ahari & *et al*. 1391, have represented the effect of nano silver particles on shelf life for Iranian saffron with nano coat packaging SNP 103.3 on microbial properties and releasing nano particles into final product. the result showed that one of the coats at 0, 3 and 5% equal to 4000 ppm could reduce microbe count by 98% moreover, having not released the nano particles to packaged product was also assessed, the rate of releasing for groups under examination was-reported equal to 0 ppm.in addition nano particles residues in packaged product for 3 months in a row was followed the result was less than 1ppm. nano silver particles used in coats has enhanced the microbial effectiveness due to being metric and it causes to increase shelf life for the product **[5]**.

Now at present, According to an increasing tendency of consumers to such products like sausage and kielbasa and the importance of food security, the concern in relation with

the approach of prevention of waste has been extremely declined due to using coats for these products which not only keeps the quality well, but they decreases the microbial count as well, besides, the major goals of food industries, particularly the increase of shelf life is accomplished. One of the incentine reason to start this research was the usage of coats in cluding nano silver particles in kielbasa to fulfill such requirements.

MATERIAL & METHODS

- Acid acetic solution
- Aceto nitrile solution
- Count agar plate
- Nutrient broth
- Mack conckey agar
- *Staphylococcus aureus* strain ATCC 6538 and E.coli ATCC 25922
- Samples of kielbasa of 60%, 70%, 80% (10 from each and totally 30 samples in two groups) was bought from stores of district 2 in Tehran.
- nano packaging coats 1,3,5% from nano nasb pars company.
- Sample diluting, preparing culture media, culturing bacteria, total bacteria count test, nano particles microbial test, nano particles migration to food test and evaluation of nano particles structures with electron microscope.

Microbial count test of common coats

According to 5272 National Standard, 10 mg sample of packaging coat is mixed with 90 ml diluting solution (physiologic serum) then incorporated into 2 petri dish and 1 ml of dilutions at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}transfered to them. Having prepared the plate count agar, it was put in autoclave, the culture was melted and added to plates at 44-46 ċ and cultured with pour plate method. Having closed the plate it was turned upside down and incubated at 29 – 31 ċ for about 48 hours.

Microbial count test of nano layers (coats)

For coating the bacteria on nano coats, first packaging covers including 1% , 3% and 5% of nano silver particles cut with scissor at 6×6 dimension equal to the numbers of treatment (4 cut from each sample for every bacterium of both sample) Were put in autoclave (121 ċ for 15 min) to be steriled. It is to say that filter papers have been used between packaging coats to prevent any clings or attachment, then according to National Standard 2946 and 68.6 strains of staphylococcus aureus and *E.coli* were cultured on mack conkey agar, incubated in 32 ±2 ċ for 12-24 hours, then they were sent to tubes (falcons) comprising nutrient broth and well-shaked. dilution of 10^{-2} was prepared from the first suspension equal to 1×10^{6}. 0.3 ml of the suspension of bacteria was separately poured (sprayed) on test

samples (nano coats at 6×6 dimensions). Samples including suspension of bacterium were settled in fully steriled condition for 24 hours and then 4-6 hours was allocated for the samples to be contacted with plate count agar in order to transfer the suspension on samples to the plated for a full absorbtions. Having finished that time, the samples were taken out from peteries including the culture and then peteries with closed cab and empty were ready to enter the next phase. Afterward peteries under the test condition at 37±2 ċ for 24 to 48 hours were incubated in a controlled way and finally, bacteria count was done.

Total count of microbe for packaged *Kielbasa* sample in nano coat

First, the surface of kielbasa was cleaned with cotton, moistened with alcohol and was cut into parts with scalpel, weighed and positioned next to the flame and were settled on coats comprising 1%, 3% and 5% nano silver particles with a steriled force and control sample was on coats with no nano particles, then packaged and kept in the refrigerator at 4 ċ after 7, 14, 21, 28, 32 days, Bacteria were counted.

Plate count agar with national standard of 8923-2 was provided to count bacteria. Coats with 1%, 3% and 5% nano silver particles (2 samples from each) including dry kielbasa was taken from refrigerator and 1gr kielbasa with 9 ml pepton and ether was incorporated. Expected dilutions were prepared and 1ml from each dilution was put in the plate and plate count ager was added with pour plate method. The bacteria count was done after 48 hours being incubated at 37 + 2ºC.

Chemical migration test by acid acetic test method

The principle of migration is EN-1186 standard demonstrating the migration of parts from polymer packaging into the food stuff in touch with polymer of packaging. It is just the amount of packaging material penetrated to food stuff measured in general migration **[13, 18]**.

This test needs a determined surface of 1%, 3% and 5% films sewed with thermal stitch and formed like sewed packaged pocket, next, acid acetic 3% (food similar one)was put in incubator at 40ċ for 10 days after having transferred to the pockets. The weight of one quartz crucible being in an oven at 105 ċ after cooling and fixed the weight was recorded in desiccator and that similar food from pockets was poured into crucible and it was slowly evaporated over the heater at 100 ċ as long as just 1ml of food similar was left in crucible.

Then crucible was taken from the heater and was put in the oven for 1,2 hours until that remained 1ml evaporated, reaching to the minimum level of moisture, and then the dried cooled weight of crucible was

recorded in dried cooled weight of crucible was recorded in desiccator. The difference of the weight of two crucible is the amount of the material migrated from polymer package into food similar which is the evaporated acid (formula 1). It is obtained based gram. The area of polymer which is adjaceutto similar food is measured and it is measured based on centimeter square. Gm/ cm^2 unit should be converted to mg/ dm^2 unit. The maximum migration is 10 mg/ dm^2 **[5]**.

$$\frac{\text{2nd weight of cruise-primary weightX 1000}}{\text{The touch surface of siuilar foods}}$$

The evaluation of particles distribution with electron microscope

First, making samples of package coats in 1%, 3 % and 5% through preparation of suspension in acetonitrile solvent in lab falcon is done and 3cc of obtained solution should be put on leg with glue to be evaporated and then settled in spotter coater comprising argon gas to stabilize (fix) golden cover on existing samples on leg, next samples in 10 minutes (prepared ones with golden cover) are taken inside the microscope chest and finally having set the SEM on 10X magnification, the sample pictures are appeared on monitor **[5]**.

Statistical analysis

In this study, the results achieved based on simple and random scheme with Spss 21 software are analyzed and for assessing the significancy of the test, Kruskal Wallis test and

Mann-Whitney test were applied.

RESULTS

The results of microbial total count of common coats in diagram 1 indicates that microbial community of these packages were high and increase in purity of kielbasa causes an increase in microbial count (it's likely due to contamination of meat).

Table 1 shows microbial total count of packaging coats of 1%, 3% and 5%nano silver particles for *staphylococcus aureus* and *E.coli*. The results imply that microbial count in coat at 1%, 3% and 5% has a significant difference (P < 0/05) between *staphylococcus aureus* and *E.coli*

Table 2 illustrates the evaluation of nano silver release from different coats and the residue in kielbasa of 60%, 70% and 80% findings represent that the amount of residue of nano particles in kielbasa with 1%, 3% and 5% coats has a significant difference (P <doesn't get impacted by kielbasa, so that this 0/05). 5% coat has the most release and 1% coat as this amount is dependent to percentage of should have the least effective materials in nano coats.

Diagram 2 & 3 show the residue of nano silver particles in samples, having been neighboring for 45 days comprising *staphylococcus aureus and E.coli* which had high microbial community the results show that the highest range of residue was

for 5% nano silver coats in comparison with 1% and 5% nano silver particles.

This result illustrates that increase in nano particles percent causes an increase in amount of the released particles into the packaged product. However, the residue nano silver particles reported for 5% nano coat does not leave had influence on human health.

Figures 1, 2 show the evaluation of packages coat of 3% and 5% of nano silver by electron microscope with 10 kx magnification at 1 nanometer scale that was seen completely incorporated and the distribution of particles can be totally seen incorporated the average size of silver particles were reported approximately 86 nanometer.

Table 1: microbe count of packages coat 3%, 5% of silver nanoparticles in *staphylococcus aureus* and *E.coli*

pvalue	Packing containing silver nanoparticles
0.025	packages coat 3%of silver nanoparticles
0.025	packages coat 5% of silver nanoparticles

Table 2: evaluation of residue of nano silver particle in different coats (60%, 70%, 80% meat kielbasa).

pvalue	Type of kielbasa
0.018	60% meat kielbasa
0.018	70% meat kielbasa
0.018	80% meat kielbasa

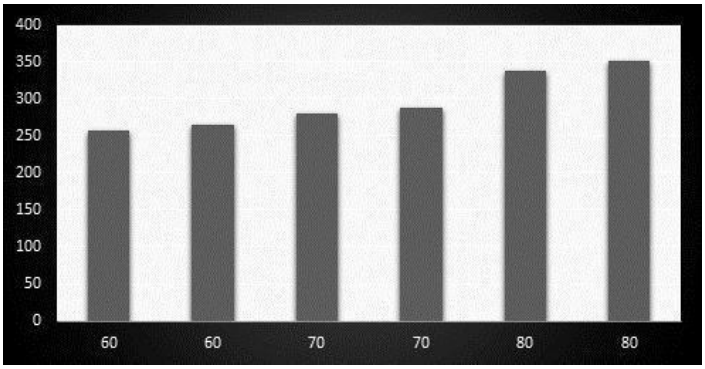

Diagram 1: Microbial total count in common coats for *kielbasa.*

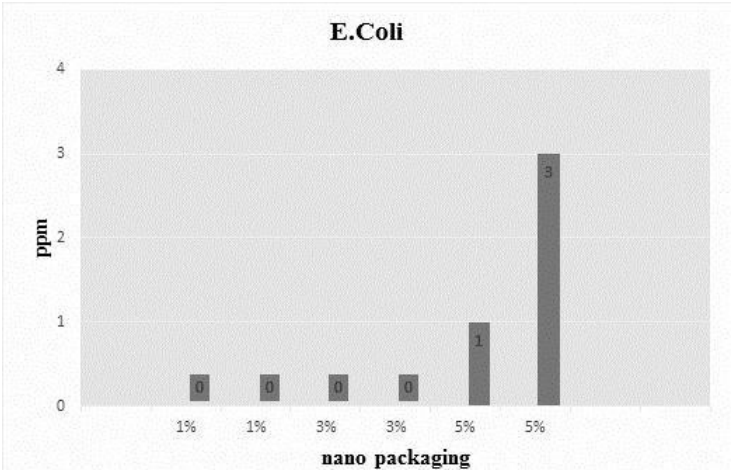

Diagram 2: comparison of nano coats in residue of nanoparticles for *E.coli*

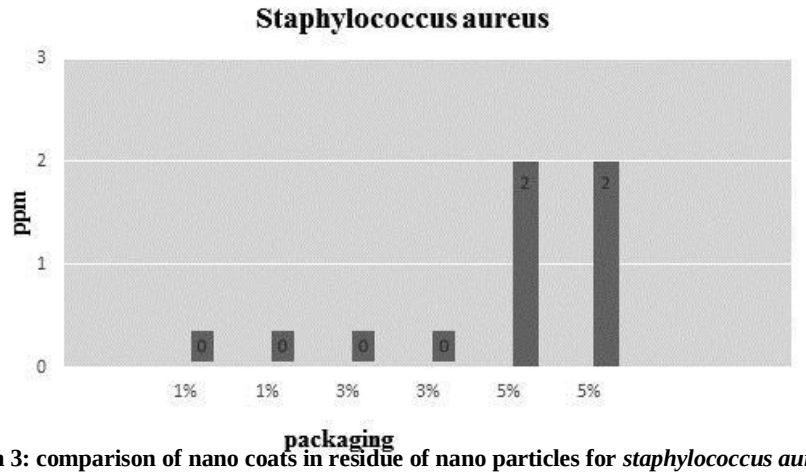

Diagram 3: comparison of nano coats in residue of nano particles for *staphylococcus aureus*

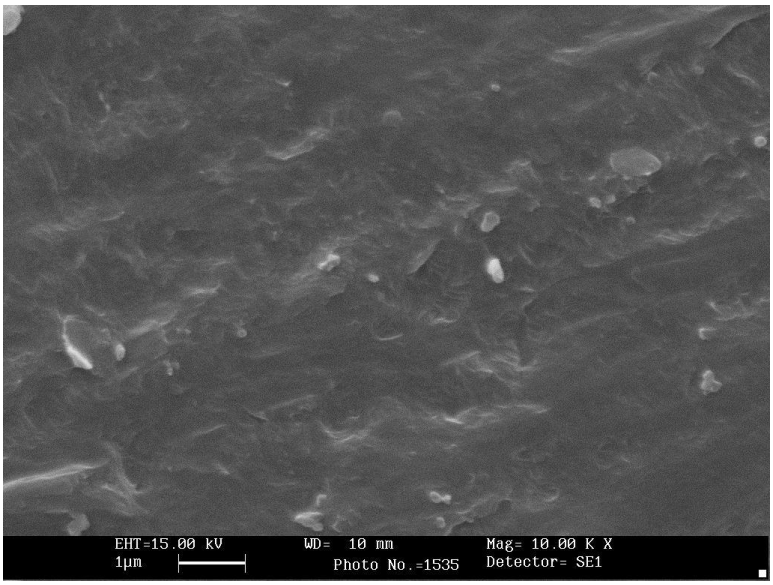

Figure 1: electron microscope for packaging coats of 3% nano silver particles

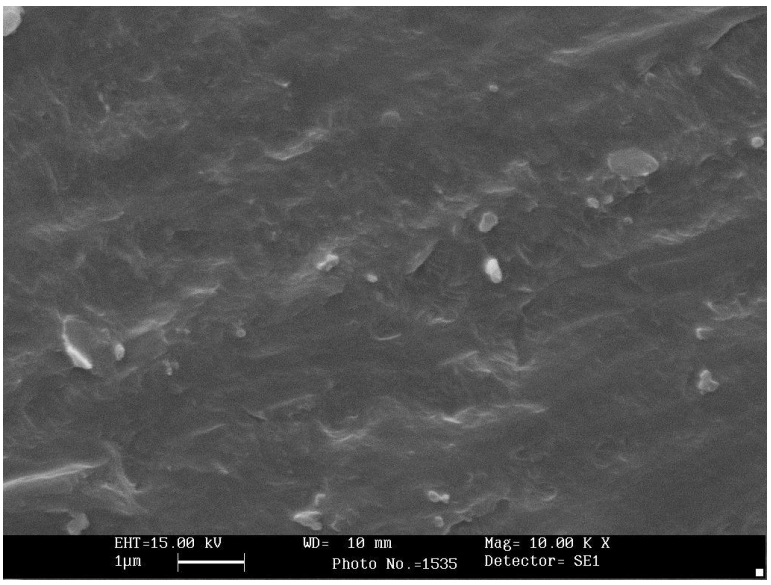

Figure 2: electron microscope for packaging coat of 5% nano silver particles

DISCUSSION

At present, food packaging is particularly focused on control and setting and modern technology packaging is done by smart nano materials, which are able to adapt with environment condition, recover themselves and inform (warm) the consumers about the infection of existing pathogens. Thus once any spoilage starts in food smart packaging will release preserving materials and recognize any changes in temperature moisture penetration liquid permeability from food to inform the consumer. It is taken for granted to apply nano in packaging since in these packages the major concern is absorbtion and consumption of oxygen, in addition, their antimicrobial property seems to be extremely vital that nano products can greatly accommodate this need **[20]**.

Nano films increase the shelf life of the product, hence they help to have less waste and help the environment too, on the other side these films are more flexible than common ones due to their slight thickness. This tenderness is associated with their transparency, improving its beatness though that does not mean to have less strength and residence they are even stranger than the common films **[13]**.

According to antimicrobial properties of nano composites, one of their main usages is in the structure of polymer package resulting in better maintenance (preservation), inhibiting properties and improving the oxygen and carbon dioxide permeability in polyethylene terephthalate and other plastic containers **[15, 19]**.

In our country, the usage of packaging coat with nano particles in food & drug industry has drawn attention due to health and hygiene advantages and this method of packaging coats comprising silver essence in food product has become very usual inas much as silver has antibacterial clearacter **[21]**.

Akbar & Kumar Anal In 2014, have investigated the effect of antibacterial one packaging with active nano particles of zinc oxide against *salmonella typhimorium* and *staphylococcus aureus* in ready-to-eat poultry meat and the size of nano particles structure with electron microscope. They were reported to be less than 100 nm. the antimicrobial property of nano silver particles opposing *E.coli and staphylococcus aureus*, the evaluation of particles size, the model of distribution and their morphologic structure with SEM (electron microscope) represents that the size of particles in 1% , 3% and 5% coats were less than 100 nm (nanometers) equal to 86 and 86 nm.

Patiño and *et al* in 2013, studies antibacterial activity of zinc-silver

particles in coats with polyamide composite including these particles as package for sausage to inhibit *salmonella typhimorium* (ATCC 4028) and the reported results were similar to what was achieved in antimicrobial effect of nano silver particles at present study. **Cachaldora & *et al* in 2013,** assessed the effect of packaging in modified atmosphere with various ratio of the mixture of three gas: O_2, N_2, $Co_2$and packaging in vacuum for shelf life of morcilla this was announced to be more than 8 weeks for all the above conditions (its shelf life is increased two times more than normal packaging). It was also reported the similar results for kielbasa packaging to two times more with nano coats on their shelf life, nevertheless, in a significant comparison, the rate in fluence of packaging in atmosphere and vacuum ($P < 0/05$) with nano silver particles coats ($P = 0/034$) were less efficient coats.

Marcos & *et al* in 2013, evaluated the effect of antimicrobial packaging films of PVOH including nicin on reducing the range of listeria monocytogenes in fermented sausage, and **Khajehali & *et al* 1391**, assessed the effect of nicin and modified atmosphere packaging in determining the growth of mesophilic, aero-psychrophilic bacteria and lactic acid in emulsion of sausage and increase in their shelf life it is to say that similar results at present research have been achieved that packaging including nano silver particles relating to their disinfectant property (microbe killing effect) on staphylococcus aureus and E.coli are introduced as antimicrobial package leading to keep the quality of dry kielbasa and good influence of nano silver particles on enhancing the shelf life.

Esmaeeli in 1392, evaluated the effect of 3% and 5% nano silver particles for coats of packages in poultry meat in 48 hours after slaughter on microbe count. It is reported to have similar results with present study on dry kielbasa indicating the effective ness of nano silver particles in enhancing the shelf life and keeping the quality of food product.

Sharifi Soltani & *et al* 1391, achieved similar results with our study on dry kielbasa in increasing the shelf life via nano silver films packaging in refrigerator (3±1ċ) and decreasing E.coli and staphylococcus and increasing the poultry meat shelf life from 2 days in common packaging to 7 days in nano silver packaging.

Foroughi & *et al* 1390, based upon the research on dry kielbasa and particularly the effects of nano coats in increasing shelf life of food stuff like sausage found it positively effective and generally the

results was reported significant (P= 0/018). **Emami Far & *et al* in 1390**, used nano composites of poly ethylene and nano silver particles for fresh orange juice and findings implies the significant effect of nano particles on reducing microbial colony (P< 0/05).the results of research for dry kielbasa were reported similar to these samples (P < 0/05).

The main object in this research is evaluation and comparison of the shelf life of kielbasa using these packaging coats with nano silver particles with common ones and the results indicate that fruitful life of preserving the kielbasa has increased from 1 month to two and microbe count was significantly dropped (P = 0/025).

CONCLUSION

The evaluations conducted in this research drew this conclusion that among the packaging comprising 3% and 5% nano silver, the 5% one proved more efficient so at the time applying 5% nano silver particles in comparison with 3% and control ones, the number of *staphylococcus aureus and E.coli* was reduces by 78%.

The 5% nano coats is known as the most efficient coat in decreasing microbe count and increasing the shelf life and the results proving that shelf life for dry kielbasa is two times enhances.

Packaging coat with 1% practically has no difference with control group, thus the results have not been considered.

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