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Investigation the effect of using ZnO and Ag Nanoparticles and Nisin on the Microbial Contamination of yogurt

Afkar Y. Ahmed[1], Ahmed Ibrahim Salih[2], Nehan Bahaaldden Jafar[2], Ali Mohammed Saadi[3]*

[1]University of Mosul, Mosul, Iraq
[2]University of Kirkuk, Iraq.
[3]Northern Technical University, Iraq
*Corresponding author, e-mail: ali.mohammed@ntu.edu.iq

Abstract

The study aimed to evaluate the effect of silver (AgNPs) and zinc (ZnNPs) nanoparticles incorporated into edible gelatin coatings at concentrations of 20 and 30%, either alone or combined with 50% nisin, on the microbiological quality of yogurt stored for 20 days at 5 ± 2 °C. Total bacterial counts at the beginning of storage ranged from 12.00 to 16.33 Log/g among the nanoparticle treatments, whereas the control treatments T1 and T2 recorded 24.66 and 22.66 Log/g, respectively. During storage, treatment T11 showed the greatest antimicrobial effectiveness, recording a total bacterial count of 15.33 Log/g on day 20, compared with 34.33 and 26.00 Log/g for T1 and T2, respectively. No yeast or mold growth was detected in any treatment on the first day. After 20 days, no yeast or mold growth was observed in T11, while T7 recorded only 0.33 Log/g, compared with 3.33 and 2.00 Log/g in the controls. Lipolytic bacteria were also initially undetectable. At the end of storage, T11 exhibited the lowest lipolytic bacterial count (1.66 Log/g), compared with 12.33 and 9.33 Log/g in T1 and T2. Similarly, T11 showed the lowest proteolytic bacterial count on day 20 (1.00 Log/g), whereas the controls reached 4.66 and 2.66 Log/g. Overall, nanoparticle-containing gelatin coatings, particularly treatment T11, effectively limited microbial growth and improved the microbiological stability of refrigerated yogurt.

Keywords: Ag nanoparticles, microbial contamination, nisin, yogurt, ZnO

Introduction

Nanoscience is the study and characterisation of nanomaterials, including their physical, chemical, and mechanical properties, as well as understanding associated phenomena due to their small size (Abod *et al.*, 2017). Nanomaterials have diameters ranging from 1 to 100 nanometers and are defined as one per billion. Chemistry, physics, molecular biology, applied biology, and medical and therapeutic specializations have particular applications in human life and health, defining them apart from other disciplines and technology (Sondi and Salopek-Sondi, 2004). Nanotechnology describes goods produced from nanoparticles with broad applications, including tissue engineering, cancer treatment, sports equipment, solar cells, space materials, and cosmetics (Bachhav & Deore, 2015).

Because of its many uses, silver nanoparticles (AgNPs) are known as one of the most commonly marketed nanomaterials. They have several beneficial properties, such as high conductivity, chemical stability, and potent antibacterial activity (Kaler *et al.*, 2010). Additionally, silver particles can interact with sulfur-containing amino acids both inside and outside of cell membranes, which impacts how effective cells are (Yuan *et al.*, 2013) . Also, the thiol groups in the amino acids that comprise the respiratory chain enzymes of cells can interact with silver ions (Maurer & Meyer, 2016). The high physical and chemical properties of zinc nanoparticles and their oxides, such as conductivity, surface tension, and surface area increase due to the granules' small size, have made them more effective in a variety of applications, including gas sensors, biological and chemical sensors, and materials used to prepare paint and cosmetics (Vaseem *et al.*, 2010) . One of the most significant bacteriocins made by lactic acid bacteria is Nicin. It is commonly used for preservation of foods,

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particularly milk products, and is made up of 34 amino acids with a molecular weight of 3354 Dalton. It is known for its ability to inhibit bacteria (Gharsallaoui *et al.,* 2016).

Nanoparticles can be produced by a variety of techniques, such as chemical, physical, and biological Attention is being paid to preparing these nanoparticles using biological methods, which have been shown to be simple to implement, safe to use in their health and environment outcomes, inexpensive, and environmentally friendly (Zhang *et al.,* 2016). Chemical and physical methods are characterized by being economically costly and having negative effects if used for cases related to human health and the environment. In addition to their use requiring special conditions of chemicals, energy sources, pressure, and high temperature (Rai *et al.,* 2011).

The use of biological methods relies on the organisms' ability to produce metabolic compounds that can be used to create nanoparticles from their primary compounds, such as salts or chlorides of metallic elements like gold, silver, zinc, aluminum, and others. Each living organism used in the synthesis has unique dimensions and characteristics, and fungi are among the most important organisms used in the production of nanoparticles (Ahmed *et al.,* 2016).

The fungus Aspergillus niger is unique in that it can produce a lot of extracellular enzymes, which are primarily responsible for breaking down the base material into smaller pieces and making it easier for the fungus to absorb them. It can also produce a lot of conidia (Hoseinzadeh *et al.,* 2017).

Through their many methods of action, oxides of nanoparticles have been shown in numerous studies to have the capacity to inhibit and kill antibiotic-resistant bacteria (Singh *et al.,* 2016), because nanomaterials are employed in many fields, including biology, agriculture, the environment, and industry, interest in their manufacture has grown in recent year (Gahlawat *et al.,* 2016). The primary factor that influences the significance of nanomaterials is their small size, which increases their surface area relative to other substances (Abdullah *et al.,* 2023). The products of microorganisms (bacteria, algae, and yeast) or plant extracts are used in the biosynthesis of nanoparticles. This method's advantages include being beneficial to the environment and requiring no quick or cheaper energy (Amiri *et al.,* 2017). Reactive oxygen species (ROS) are generally produced by nanoparticle metal oxides, which are also what give them their antibacterial properties (Tewari *et al.,* 2019).

Lactic acid bacteria produce yoghurt, a fermentative dairy product made from milk. Lactobacillus bulgaricus and Streptococcus thermophilus are two starters used in the production of yogurt (Claeys *et al.,* 2019). The dairy industry has gone through an area of worldwide expansion and consolidation in recent years, which has led to a number of issues with safety, sustainability, and innovation in addition to several potential markets. Milk, an important raw material for the dairy sector, is particularly vulnerable to deterioration because of its comparatively high fat, protein, and sugar content (van den Oever & Mayer, 2021). For a long time, heat treatments have been used to stop the growth of microorganisms and some natural milk enzymes, like lipases. But greater concern is being raised by side effects of ultrahigh temperature processing, such as the production of acrylamide and HMF (5-hydroxymethylfurfural) in dairy products (Fusco *et al.,* 2020). There have been incidents of dairy products becoming contaminated again after post-pasteurization processing, even though pasteurization usually gets rid of harmful microorganisms (Deshwal *et al.,* 2021). The use of advanced methods, such as non-thermal processes like ohmic, high-pressure, ultrasound, membrane technology, and nanotechnology, can be an intriguing alternative to address these problems in light of consumers' growing desire for natural and minimally processed food products (Lopes & Brandelli, 2018). Specifically, nanotechnology has been regarded as an intriguing instrument, exhibiting considerable potential for the administration and regulated release of organic food preservatives (Poonia, 2019). This method has the potential to improve the quality and safety of dairy products by carrying and preserving antimicrobial chemicals from harmful environmental conditions that can occur during the production and storage of these foods. Overall, nanotechnology is an exciting approach to increasing the quality, shelf life, and safety of dairy products, as well as the creation of innovative packaging materials with improved mechanical, barrier, and antimicrobial properties (Pinilla *et al.,* 2021). Because of this, scientists and the dairy sector are becoming more interested in investigating how nanotechnology may be used to create active packaging or coatings with antibacterial nanostructures, as well as to encapsulate and transport antimicrobial substances like bacteriocins and essential oils. Numerous applications and encouraging results of nanostructured antimicrobials in dairy products are discussed in this review.

Nisin, pediocin, enterocins, and the antifungal natamycin are examples of antimicrobial drugs that have a microbiological origin. The most widely used natural antibacterial in the dairy industry is nisin, which

is especially used as a preservative in low-pH and heat-processed goods. The Food and Agriculture Organization/ World Health Organization (FAO/WHO) has approved this substance, and the FDA has classed it as a food additive (E234) that is generally recognized as safe (GRAS). Because of its capacity to inhibit a variety of Gram-positive bacteria, particularly foodborne pathogens, nisin has attracted particular attention. Compared to free antimicrobials, nanostructured antimicrobials offer a number of benefits, including improved properties to combat spoiling and pathogenic microorganisms, enhanced bioactivity, improved chemical stability, controlled release, and target delivery (Jafarzadeh *et al.,* 2021).

Applications of Antimicrobial Nanostructures in Packaging In the dairy manufacturing sector, the creation of packaging materials with enhanced qualities to ensure food safety and quality is a highly relevant topic. As a result, several types of nanocomposite active packaging have been identified that may find use in dairy products (Brandelli *et al.,* 2017). Several formulations of active films made with natural or petroleum-based polymers have been thoroughly studied and shown to suppress foodborne pathogens both in vitro and in situ (Gammariello *et al.,* 2011). Both actually dairy products and milk agar, ab model system, have demonstrated the efficacy of antimicrobial packaging formulations. It has been reported that antimicrobial coatings made of common polymers (like polyethylene and polypropylene) that include metallic nanoparticles like silver (Ag) and zinc oxide (ZnO) are an efficient way to regulate microbial development in cheeses (Carbone *et al.,* 2016; Li *et al.,* 2021). However, the use of biopolymers to create edible and/or biodegradable food packaging for dairy applications has also received more attention (Brandelli *et al.,* 2017). Based on the aforementioned, the study sought to identify if AgNPs or ZnNPs nanoparticles Which was produced biologically using the Aspergillus niger mold were effective with nicin and to evaluate their impact on the microbial load of yogurt.

Material and Method

Making a gelatin membrane that is loaded with nisin, ZnO, and Ag nanoparticles.

Gelatin film solutions were prepared using Carvalho and Grosso's (de Carvalho & Grosso, 2004). procedure, which included weighing 10 g of gelatin powder, diluting it with 80 ml of distilled water, and mixing all the ingredients for 15 minutes at 60 °C using a hot plate magnetic stirrer. Glycerol was then added at a rate of 30% by weight of dry gelatin after the volume was added to 100 milliliters of distilled water and the pH was brought to 7. In order to use a gelatin membrane with nanoparticles for the coating procedure, the membrane was prepared and then formed into a hollow to hold the yogurt samples.

Production of yogurt

The yogurt was made employing the procedure indicated by (Tamime & Robinson, 2007). After being pasteurized for 10 minutes at 90°C, *Lactobacillus bulgaricus and Streptococcus thermophiles*, which comprise the initial phase of culture, were used to cool a portion of the milk to 42±2°C. At 42±2°C, it was incubated for 4–6 hours until the pH decreased to 4.6 and the coagulation process was complete. After the coagulation step completed, The yogurt was stored at 5°C until tests that were required could be carried out, which was 1–10–20 days following the manufacturing date.

Microbiological Tests:
Estimating the Total Bacterial Counts in yogurt

Total bacterial counts were estimated using the method described by (Frank & Yousef, 2004). A sterile, clean spoon was used to take up 5 g of each yogurt sample. In a volumetric flask, the weighed quantity was combined with 45 milliliters of distilled water. To get a uniform solution, the mixture was vigorously shook. The first dilution was regarded as 1:10. Then, in order to achieve the best counts on plates, the proper dilutions were created. 0.1 ml of the suitable dilution was taken out and spread over nutrient agar using the spreading method. The plates were then incubated at 37°C for 24 hours. The total number of growing bacterial colonies was then determined.

Estimating the total number of (yeasts and molds) in yogurt

The total number of yeasts and molds was assessed after 0.1 ml of sample dilutions were distributed on Potato Dextrose Agar plates. After the plates were cultured for seven days at 25°C, the total number of colonies that formed on the surface of the culture media was tallied (Frank & Yousef, 2004).

Estimation of proteolytic bacteria in yogurt

Milk Agar Skimmed (100 ml of nutrient agar with 10% skimmed milk) was used for the estimation. 0.1 ml of dilutions made from yogurt samples were used to inoculate plates. After that, the plates were incubated for two to three days at 21±2°C. After adding 1% hydrochloric acid, it was left for a minute. After that, colonies encircled

by clear zones were recorded by Harrigan (2000).

Estimation of Lipolytic Bacteria in yogurt

Milk Agar Skimmed (100 ml of nutritional agar with 10% skimmed milk) was used for the estimation. 0.1 ml of dilutions made from yogurt samples were used to inoculate plates. After that, the plates were incubated for two to three days at 21±2°C. After adding 1% hydrochloric acid, it was left for a minute. After that, colonies encircled by clear zones were recorded by (Harrigan, 1998).

Results and discussion

Microbial evaluation of yogurt

Estimating total count of bacteria in yogurt

The results shown in **Table 1** indicate that the total bacterial counts on the first day of storage were 24.66, 22.66, 15.33, 14.33, 16.33, 14.33, 12.33, 16.33, 12.66, 14.00, and 12.00 Log/g, respectively. With the continuation of the storage period at 10 days, The greatest values were recorded by the control treatments T1 and T2., getting to 26.66 and 24.33 Log/g, while the lowest total number was for treatments T11 and T7, reaching 12.66 and 12.00 Log/g, which did not differ significantly from each other. With the extension of the storage period to 20 days, there was a gradual increase in the total bacterial numbers for the control treatments, reaching 34.33 and 26.00 Log/g. The lowest and best values in terms of microbial load were treatment T7, reaching 15.00 Log/g. As for the rest of the treatments T3, T4, T5, T6, T8, T9, T10, and T11 they recorded higher values with the continuation of storage, reaching 20.00, 17.33, and 21.33 and 17.66, 21.00, 16.33, 17.00, and 15.33 Log/g, respectively The inhibition might be the result of nanoparticles' ability to decrease or prevent the growth of germs by altering the cell wall, becoming more permeable, inhibiting respiratory enzymes, and preventing genetic material replication. The results agree with the findings published by (Kavas *et al.,* 2015). They reported that the protein membrane-coated samples had overall bacterial counts of 4.3–12 Log/g. The final results agree with what was suggested (Sondi and Salopek-Sondi, 2004; Saadi *et al.,* 2022). who shown that the small size of nanoparticles and their extensive surface area cause cell death because the smaller size builds up in greater quantities on the cell surface, influencing the permeability of the plasma membrane and increasing toxicity to microorganisms.

Estimation the number of molds and yeasts in yogurt

The results shown in **Table 2** show that there was no growth of yeasts and molds at time zero, which is a natural result of the pasteurization processes to which the milk prepared for yogurt was exposed. However, at time 10 days, mold growth was observed in the control sample not coated with gelatin at the highest values, reaching 1.00 Log/g. Treatments T3, T5, and T8 recorded growth of molds and yeasts at logarithmic rates of 0.33 Log/g, the results of which were identical to the control treatment T2. This result demonstrates the importance of oxygen retention by the membrane covering these samples compared to the control sample T1, which was not coated with a gelatin membrane .There was no noticeable distinction between these treatments and the control treatment T2., while no growth of molds and yeasts was observed for the rest of the studied treatments. The employment of antimicrobial agents with these coatings may be the cause, as they helped prevent the growth of molds in comparison to treatments that did not employ these agents. The results showed that the inclusion of nisin and active nanocomposites in gelatin membranes enabled them to suppress or eliminate both mold spores and hyphae, which was in agreement with

Table 1. Total bacterial Counts (log /g) that Contaminant of yoghurt stored for 20 days at a temperature (2 ± 5 °C).

Treatments		1 day	10 days	20 days	Mean of treatments
T1	Control 1	24.66 c	26.66 b	34.33 a	28.55 a
T2	Control 2	22.66 de	24.33 cd	26.00 bc	24.33 b
T3	Ag 20 mg	15.33 h-k	17.00 g-i	20.00 f	17.44 c
T4	Ag 30 mg	14.33 j-l	14.00 k-n	17.33 gh	15.22 de
T5	Nis 50 mg	16.33 g-j	16.33 g-j	21.33 ef	18.00 c
T6	Ag 20+50	14.33 j-l	15.66 g-k	17.66 g	15.88 d
T7	Ag 30 +50	12.33 l-m	12.66 l-m	15.00 i-k	13.33 f
T8	Zn 20 mg	16.33 g-j	16.33 g-j	21.00 ef	17.88 c
T9	Zn 30 mg	12.66 l-m	14.00 k-m	16.33 g-j	14.33 ef
T10	Zn 20 +50	14.00 k-m	13.66 k-m	17.00 g-i	14.88 de
T11	Zn 30 + 50	12.00 m	12.66 l-m	15.33 h-k	13.33 f
Mean of period		15.90 c	16.66 b	20.12a	

* Different letters on average in one column indicate significant differences equal to a substantial level of 0.05.

*T1 = control (yogurt only) , T2=yogurt + Gelatin film , T3 = Gelatin film + silver NPs 20 mg. , T4 =Gelatin film + silver NPs 30 mg. , T5= Gelatin film + Nis 50 mg. , T6= Gelatin film + silver NPs 20 mg + Nis 50 mg., T7= Gelatin film + silver NPs 30 mg + Nis 50 mg. , T8= Gelatin film + Zinc NPs 20 mg. , T9= Gelatin film + Zinc NPs 30 mg, T10= Zinc NPs 20 mg, + Nis 50 mg, T11= Zinc NPs 30 mg. + Nis 50 mg.

Table 2. Mold and yeast (log /g) that Contaminant of yoghurt stored for 20 days at a temperature (2 ± 5 °C).

Treatments		1 day	10 days	20 days	Mean of treatments
T1	Control 1	0.00 b	1.00 a	3.33 a	1.44 a
T2	Control 2	0.00 b	0.33 b	2.00 a	0.77 ab
T3	Ag 20 mg	0.00 b	0.33 b	1.00 b	0.44 ab
T4	Ag 30 mg	0.00 b	0.00 b	0.66 b	0.22 b
T5	Nis 50 mg	0.00 b	0.33 b	1.00 b	0.44 ab
T6	Ag 20+50	0.00 b	0.00 b	1.00 b	0.33 b
T7	Ag 30 +50	0.00 b	0.00 b	0.33 b	0.11 b
T8	Zn 20 mg	0.00 b	0.33 b	0.44 b	o.77ab
T9	Zn 30 mg	0.00 b	0.00 b	0.66 b	0.22 b
T10	Zn 20 +50	0.00 b	0.00 b	0.66 b	0.22 b
T11	Zn 30 +50	0.00 b	0.00 b	0.00b	0.22 b
Mean of period		0.00 b	0.00 b	1.00 a	0.00 b

* Different letters on average in one column indicate significant differences equal to a substantial level of 0.05.

*T1 = control (yogurt only) , T2=yogurt + Gelatin film , T3 = Gelatin film + silver NPs 20 mg. , T4 =Gelatin film + silver NPs 30 mg. , T5= Gelatin film + Nis 50 mg. , T6= Gelatin film + silver NPs 20 mg + Nis 50 mg., T7= Gelatin film + silver NPs 30 mg + Nis 50 mg. , T8= Gelatin film + Zinc NPs 20 mg. , T9= Gelatin film + Zinc NPs 30 mg. , T10= Zinc NPs 20 mg + Nis 50 mg. , T11= Zinc NPs 30 mg + Nis 50 mg.

Table 3. lipolytic bacteria (log/g) that Contaminant of yoghurt stored for 20 days at a temperature (2 ± 5 °C).

Treatments		1 day	10 days	20 days	Mean of treatments
T1	Control 1	0.00 j	7.33 c	12.33 a	6.55 a
T2	Control 2	0.00 j	4.33 ef	9.33 b	4.66 b
T3	Ag 20 mg	0.00 j	2.26 f-h	5.00 ed	2.55 c
T4	Ag 30 mg	0.00 j	0.66 ij	3.33 fg	1.33 d
T5	Nis 50 mg	0.00 j	2.66 f-h	6.33 cd	3.11 c
T6	Ag 20+50	0.00 j	0.66 ij	3.00 f-h	1.22 d
T7	Ag 30 +50	0.00 j	0.33 j	3.00 f-h	1.11 d
T8	Zn 20 mg	0.00 j	2.33 gi	7.00 c	3.22 c
T9	Zn 30 mg	0.00 j	1.66 g-j	6.00 cd	2.55 c
T10	Zn 20 +50	0.00 j	1.66 g-j	5.33 ed	2.33 c
T11	Zn 30 + 50	0.00 j	0.66 i-j	1.66 ji	0.77 d
Mean of period		0. 00 c	2.27 b	5.66 a	

* Different letters on average in one column indicate significant differences equal to a substantial level of 0.05.

*T1 = control (yogurt only) , T2=yogurt + Gelatin film , T3 = Gelatin film + silver NPs 20 mg. , T4 =Gelatin film + silver NPs 30 mg. , T5= Gelatin film + Nis 50 mg. , T6= Gelatin film + silver NPs 20 mg + Nis 50 mg., T7= Gelatin film + silver NPs 30 mg + Nis 50 mg. , T8= Gelatin film + Zinc NPs 20 mg. , T9= Gelatin film + Zinc NPs 30 mg , T10 = Zinc NPs 20 mg + Nis 50 mg. , T11= Zinc NPs 30 mg + Nis 50 mg

what (D'Amato & Sinigaglia, 2010), had suggested. When the 20-day storage period ends, the best and lowest treatments in terms of mold and yeast growth in yogurt samples were treatment T11, which recorded no growth. This was followed by treatment T7, with colony counts reaching 0.33 Log/g. The greatest results were obtained with control treatments T1 and T2., coming in at 3.33 and 2.00 Log/g respectively. The remaining treatments recorded varying rules that differed significantly from the control treatments. According to (Abbood *et al.,* 2020), the study's findings also supported what they said. The interaction of nanoparticles with yeast cells results in yeast death. Because synthetic nano oxides have a positive charge and microorganisms have a negative charge, the yeast and the particle surface are attracted to each other electromagnetically. Ions released by the nanoparticles interact with thiols to increase membrane permeability, which in turn causes cell death. The findings of the study support the conclusions drawn by (Brayner *et al.,* 2006; Xia *et al.,* 2008). Because zinc nanoparticles promote the production of reactive oxygen species (ROS) in cells, they have antifungal and/or fungicidal properties. Furthermore, it has been documented that they alter membrane potential by causing lipid peroxidation and general malfunction, which ultimately results in cell death.

Estimation Yogurt's lipolytic bacterial count

It is evident from **Table 3** data that no fat-dissolving bacterial growth occurred on the first day. In comparison to the two control treatments, T1 and T2, where the number of lipolytic bacteria reached 7.33 and 4.33 Log/g, respectively, growth of fat-dissolving bacteria was observed after 10 days of storage. The numbers were 2.26, 0.66, 2.66, 0.66, and 0.33, 2.33, 1.66, 1.66, and 0.66 Log/g. It was noted that the lowest growth of lipolytic bacteria was for treatment T7, as it reached 0.33 Log/g. When the storage period is complete 20 day, treatments, These include T3,T4,T5,T6,T7,T8,T9, T10, and T11. recorded values of 5.00, 3.33, 6.33, 3.00, 3.00, 7.00, 6.00, 5.33 and 1.66 Log/g respectively, which was significantly different

Table 4. proteolytic bacteria (log/g) that Contaminant of yoghurt stored for 20 days at a temperature (2 ± 5 °C).

Treatments		1 day	10 days	20 days	Mean of treatments
T 1	Control 1	0.00 h	2.00 c-e	4.66 a	2.22 a
T 2	Control 2	0.00 h	1.66 c-f	2.66 bc	1.44 b
T 3	Ag 20 mg	0.00 h	0.66 f-h	2.33 bcd	1.00 bcd
T 4	Ag 30 mg	0.00 h	0.33 gh	1.33 d-g	0.55 cd
T 5	Nis 50 mg	0.00 h	0.66 f-h	2.66 bc	1.11 bc
T 6	Ag 20+50	0.00 h	0.33 gh	2.66 bc	1.00 bcd
T 7	Ag 30 +50	0.00 h	0.00 h	1.66 c-f	0.55 cd
T 8	Zn 20 mg	0.00 h	1.00 e-h	3.33 b	1.44 b
T 9	Zn 30 mg	0.00 h	0.66 f-h	2.33 bcd	1.00 bcd
T 10	Zn 20 +50	0.00 h	0.33 gh	2.00 cde	0.77 bcd
T 11	Zn 30 +50	0.00 h	0.00 h	1.00 e-h	0.33 d
	Mean of period	0.00 c	0.69 b	2.24 a	

* Different letters on average in one column indicate significant differences equal to a substantial level of 0.05.

*T1 = control (yogurt only) , T2=yogurt + Gelatin film , T3 = Gelatin film + silver NPs 20 mg. , T4 =Gelatin film + silver NPs 30 mg. , T5= Gelatin film + Nis 50 mg. , T6= Gelatin film + silver NPs 20 mg + Nis 50 mg., T7= Gelatin film + silver NPs 30 mg + Nis 50 mg. , T8= Gelatin film + Zinc NPs 20 mg. , T9= Gelatin film + Zinc NPs 30 mg , T10 = Zinc NPs 20 mg + Nis 50 mg, T11= Zinc NPs 30 mg, + Nis 50 mg

from the values of the two control treatments, T2 and T1, which were 12.33 and 9.33 Log/g. and the decrease was clear for these treatments compared to the two control treatments. The results also showed that treatment T11 was the best and had the lowest rate of increase in lipolytic bacteria, which recorded 1.66 Log/g at the end of the storage period. In addition to the growth of spores of these bacteria, which may be of the type resistant to pasteurization temperature, the data show that the high percentage of fat resulting from the decrease in moisture content with prolonged storage is the cause of the increase in lipolytic bacteria. The new environmental conditions brought about by the packaging process—particularly the retention of gases, particularly oxygen—may be the cause of the decline in these bacteria's population. These conditions have a substantial effect on the respiration process and the proper water activity of these organisms, which in turn causes the extension of their adaptation phase (Lag phase) (Torres, 2017). These results agreed with the findings of (Helander *et al.,* 1997; Al-Bedrani *et al.,* 2023), who showed that nisin inhibits food-rotting microorganisms. This may be because nisin effectively breaks the glycosidic bond of type 1-4-***B*** that bonds the building blocks of the peptidoglycan layer, which includes N-acetyl muramic acids and acetyl glucose amine N-. This breaks and destroys the cell wall, which in turn inhibits the growth of these bacteria or lessens its effectiveness.

Estimation of proteolytic bacteria in yogurt

According to **Table 4,** the results indicate that there was no growth on the first day of storage. With continued storage, it was found that proteolytic bacteria grew in yogurt samples at 10 days of storage. The T3 , T4 , T5 , T6 , T7 , T8 , T9 , T10 and T11 samples had growth rates of 0.66, 0.33, 0.66, 0.33, 0.00, 1.00, 0.66, 0.33, and 0.00 Log/g, which varied significantly. from the control treatments T1and T2, as the value of each reached 2.00 and 1.66 Log/g, respectively. Treatments T7 and T11 were the best, as no growth of these types of bacteria was observed. At the time of 20 days, there was an increase in the growth of proteolytic bacteria, which reached 2.33, 1.33, 2.66, 2.66, 1.66, 3.33, 2.33, and 1.00 Log/g for all treatments, respectively. The control treatments T1and T2 recorded the highest values, which reached 4.66 and 2.66 Log/g, respectively, when the storage time is over. The best treatments, which had the lowest numbers of proteolytic bacteria, were for treatment T11, which recorded 1.00 Log/g. These findings align with those of (El-Sisi *et al.,* 2015; Rahi *et al.,* 2023), who observed that over the designated storage periods, the number of proteolytic bacteria rose at growth rates of 1.5-6.4 log/g. AgNPs, ZnNPs, and Nisin added to the gelatin membranes have been shown to decrease the growth of microorganisms, which explains the low growth rates of proteolytic bacteria. The outcomes align with the results of (Sondi and Salopek-Sondi, 2004; Hamid *et al.,* 2025). They noted that microbial cells that are exposed to silver nanoparticles develop perforations in their surfaces, which allows the particles to accumulate and affects the permeability of the cell membrane, ultimately resulting in cell death. Because of its limited penetration into cells and disruption of transport mechanisms, including ion flow, nanosilver has been shown to successfully infect microbial cells, meaning that low amounts are sufficient to combat microorganisms, especially species less sensitive to antibiotics (Morones *et al.,* 2005).

Conclusion

Results about this investigation demonstrated the importance of using bio-produced nanomaterials such

as silver and zinc alone or with nisin compound, which proved to be effective in reducing the microbial load of the yogurt samples under test compared to the control treatments with continued storage, which is a suitable alternative to treating functional foods such as dairy products, especially yogurt, in order to avoid the use of industrial compounds in packaging processes to produce healthy foods that meet the needs of consumers looking for natural foods that are not chemically treated, and to improve the properties of these nano-coatings and determine their precise effectiveness towards public health requirements, microbial activity and environmental impact. Therefore, we need more comprehensive and in-depth studies and continuous research to ensure their long-term use.

References

Abbood, H.A., Mohammed, B.L., Aboid, H.A. 2020. In vitro antifungal efficacy of Saccharomyces cerevisiae mediated nanoparticle against resistant Candida albicans isolates. Indian Journal of Forensic Medicine & Toxicology 14: 666-671.

Abdullah, E.A., Badawi, S.K., Jafar, N.B. 2023. Evaluation of antibacterial activity of selenium and copper oxide nanoparticles synthesized by Hibiscus sabdariffa L. extract. IOP Conference Series: Earth and Environmental Science 1262: 062043.

Abod, H.A., Bander, I., Zain-Al-Abddeen, S.S. 2017. The effect of silver nanoparticles prepared using Aspergillus niger in some pathogenic bacteria Aspergillus niger. Kirkuk University Journal of Scientific Studies 12: 1-16.

Ahmed, S., Ahmad, M., Swami, B.L., Ikram, S. 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. Journal of Advanced Research 7: 17-28.

Al-Bedrani, D.I., AlKaisy, Q.H., Rahi, A.K. 2023. Evaluation of milk source on physicochemical, texture, rheological and sensory properties of yogurts. Journal of Applied and Natural Science 15: 128.

Amiri, M., Etemadifar, Z., Daneshkazemi, A., Nateghi, M. 2017. Antimicrobial effect of copper oxide nanoparticles on some oral bacteria and Candida species. Journal of Dental Biomaterials 4: 347.

Bachhav, R.M., Deore, S.N. 2015. A review on nanomaterials. International Journal of Science and Research 4: 1451-1457.

Brandelli, A., Brum, L.F.W., dos Santos, J.H.Z. 2017. Nanostructured bioactive compounds for ecological food packaging. Environmental Chemistry Letters 15: 193-204.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M.F., Fiévet, F. 2006. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Letters 6: 866-870.

Carbone, M., Donia, D.T., Sabbatella, G., Antiochia, R. 2016. Silver nanoparticles in polymeric matrices for fresh food packaging. Journal of King Saud University - Science 28: 273-279.

Carvalho, R.A., Grosso, C.R.F. 2004. Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. Food Hydrocolloids 18: 717-726.

Claeys, W.L., Cardoen, S., Daube, G., De Block, J., Dewettinck, K., Dierick, K., et al. 2013. Raw or heated cow milk consumption: review of risks and benefits. Food Control 31: 251-262.

D'Amato, D., Sinigaglia, M. 2010. Antimicrobial agents of microbial origin: nisin. In: Application of alternative food-preservation technologies to enhance food safety and stability. Bentham Science Publishers. p. 83-91.

Deshwal, G.K., Tiwari, S., Kadyan, S. 2021. Applications of emerging processing technologies for quality and safety enhancement of non-bovine milk and milk products. LWT 149: 111845.

El-Sisi, A.S., Gapr, A.M., Kamaly, K.M. 2015. Use of chitosan as an edible coating in RAS cheese. Biolife 3: 564-570.

Frank, J.F., Yousef, A.E. 2004. Tests for groups of microorganisms. In: Standard methods for the examination of dairy products. 17th ed. p. 227-248.

Fusco, V., Chieffi, D., Fanelli, F., Logrieco, A.F., Cho, G.S., Kabisch, J., et al. 2020. Microbial quality and safety of milk and milk products in the 21st century. Comprehensive Reviews in Food Science and Food Safety 19: 2013-2049.

Gahlawat, G., Shikha, S., Chaddha, B.S., Chaudhuri, S.R., Mayilraj, S., Choudhury, A.R. 2016. Microbial glycolipoprotein-capped silver nanoparticles as emerging antibacterial agents against cholera. Microbial Cell Factories 15: 25.

Gammariello, D., Conte, A., Buonocore, G.G., Del Nobile, M.A. 2011. Bio-based nanocomposite coating to preserve quality of Fior di latte cheese. Journal of Dairy Science 94: 5298-5304.

Gharsallaoui, A., Oulahal, N., Joly, C., Degraeve, P. 2016. Nisin as a food preservative: part 1: physicochemical properties, antimicrobial activity, and main uses. Critical Reviews in Food Science and Nutrition 56: 1262-1274.

Hamid, R.A., Ahmed, A.Y., Ahmed, I.M., Saadi, A.M., Mohammed, T.M.T. 2025. Effect of adding mint, thyme, and Eruca sativa extracts on some of the health properties of yogurt. Assiut Veterinary Medical Journal 71: 546-555.

Harrigan, W. F. Laboratory Methods in Food Microbiology. 3. ed. San Diego: Academic Press, 1998. 532 p.

Helander, I., von Wright, A., Mattila-Sandholm, T.M. 1997. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends in Food Science & Technology 8: 146-150.

Hoseinzadeh, E., Makhdoumi, P., Taha, P., Hossini, H.,

Stelling, J., Amjad Kamal, M., Md. Ashraf, G. 2017. A review on nano-antimicrobials: metal nanoparticles, methods and mechanisms. Current Drug Metabolism 18: 120-128.

Jafarzadeh, S., Salehabadi, A., Nafchi, A.M., Oladzadabbasabadi, N., Jafari, S.M. 2021. Cheese packaging by edible coatings and biodegradable nanocomposites; improvement in shelf life, physicochemical and sensory properties. Trends in Food Science & Technology 116: 218-231.

Kaler, A., Patel, N., Banerjee, U.C. 2010. Green synthesis of silver nanoparticles. Current Research & Information on Pharmaceutical Sciences 11: 68-71.

Kavas, G., Kavas, N.A.Z.A.N., Saygili, D. 2015. The effects of thyme and clove essential oil fortified edible films on the physical, chemical and microbiological characteristics of kashar cheese. Journal of Food Quality 38: 405-412.

Li, Y., Chen, Z., Wu, K. 2021. Effect of antibacterial nanocomposite film on the preservation of cheese. Food Science and Technology 42: e93321.

Lopes, N.A., Brandelli, A. 2018. Nanostructures for delivery of natural antimicrobials in food. Critical Reviews in Food Science and Nutrition 58: 2202-2212.

Maurer, L.L., Meyer, J.N. 2016. A systematic review of evidence for silver nanoparticle-induced mitochondrial toxicity. Environmental Science: Nano 3: 311-322.

Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., Yacaman, M.J. 2005. The bactericidal effect of silver nanoparticles. Nanotechnology 16: 2346-2353.

Pinilla, C.M.B., Lopes, N.A., Brandelli, A. 2021. Lipid-based nanostructures for the delivery of natural antimicrobials. Molecules 26: 3587.

Poonia, A. 2019. Recent trends in nanomaterials used in dairy industry. In: Nanoscience for sustainable agriculture. p. 375-396.

Rahi, A.K., Al-Bedrani, D.I.J., Saadi, A.M., AlKaisy, Q.H. 2023. Effect of adding sodium caseinate, whey protein concentrate and milk protein concentrate on the physical, rheological and sensory properties of yogurt produced from goat milk. Journal of Applied and Natural Science 15: 1434.

Rai, M., Gade, A., Yadav, A. 2011. Biogenic nanoparticles: an introduction to what they are, how they are synthesized and their applications. In: Metal nanoparticles in microbiology. p. 1-14.

Saadi, A.M., Jafar, N.B., Ahmed, M. 2022. Effect of some types of stabilizers on the quality of yogurt during storage. Journal of Hygienic Engineering & Design 38: 125-130.

Singh, P., Kim, Y.J., Zhang, D., Yang, D.C. 2016. Biological synthesis of nanoparticles from plants and microorganisms. Trends in Biotechnology 34: 588-599.

Sondi, I., Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of

Tamime, A.Y., Robinson, R.K. 2007. Tamime and Robinson's yoghurt: science and technology. Elsevier, Cambridge, UK. 808p.

Tewari, S., David, J., Gautam, A. 2019. A review on probiotic dairy products and digestive health. Journal of Pharmacognosy and Phytochemistry 8: 368-372.

Torres, J.A. 2017. Microbial stabilization of intermediate moisture food surfaces. In: Water activity. Routledge. p. 329-368.

van den Oever, S.P., Mayer, H.K. 2021. Analytical assessment of the intensity of heat treatment of milk and dairy products. International Dairy Journal 121: 105097.

Vaseem, M., Umar, A., Hahn, Y.B. 2010. ZnO nanoparticles: growth, properties, and applications. In: Metal oxide nanostructures and their applications 5: 10-20.

Xia, T., Kovochich, M., Liong, M., Madler, L., Gilbert, B., Shi, H., et al. 2008. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2: 2121-2134.

Yuan, Z., Li, J., Cui, L., Xu, B., Zhang, H., Yu, C.P. 2013. Interaction of silver nanoparticles with pure nitrifying bacteria. Chemosphere 90: 1404-1411.

Zhang, X.F., Liu, Z.G., Shen, W., Gurunathan, S. 2016. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. International Journal of Molecular Sciences 17: 1534.

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