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Challenge of nisin and its nanoparticles in eliminating *Listeria monocytogenes* inoculated in chilled minced meat

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ABSTRACT

Nisin, a bacteriocin produced by Lactococcus lactis subsp. lactis, has shown significant potential as a food preservative, particularly in inhibiting the growth of *Listeria monocytogenes*. This study aimed to characterize nisin nanoparticles (NNP) and evaluate their antibacterial activity in minced beef meat during refrigerated storage at 4°C. Fourier- transform infrared spectroscopy (FTIR) analysis revealed shifts in peaks, indicating increased hydrogen bonding in nisin nanoparticles compared to free nisin. Transmission electron microscopy (TEM) images showed spherical particles with an average size of 10.34±3.98 nm, exhibiting excellent stability. In antibacterial activity tests, minced meat treated with nisin nanoparticles at concentrations of 0.6 and 1.2 ml/100g showed a significant reduction in *L. monocytogenes* counts compared to the control ($p < 0.01$) on the 6th and 3rd days, respectively. However, samples treated with nisin at 1.2 ml/100g spoiled on the 6th day. Sensory evaluation revealed that nisin nanoparticles effectively maintained the sensory quality of minced meat throughout the storage period, with enhanced acceptability on the 6th day. The pH of treated samples remained lower than the control throughout storage, with no significant difference between nisin nanoparticle treatments on the 6th day. TBA values showed that nisin nanoparticles at 0.6 and 1.2 ml/100g inhibited lipid oxidation, with values below 0.78 mg/kg on the 6th day. Overall, nisin nanoparticles showed promising antibacterial and preservative effects in minced beef meat, highlighting their potential as a safe and effective food preservative.

Introduction

Ground beef is the primary ingredient used in many meals (Donsí *et al*., 2011). It is a high-protein and nutritious meat product, but it can quickly spoil if not stored under proper conditions. Minced beef becomes hazardous for consumers if pathogens are present, such as *Listeria monocytogenes*. Therefore, ensuring the safety of meat for consumers through stable transportation and storage is crucial (Rahman *et al*., 2023).

Foodborne outbreaks, especially cases of human listeriosis caused by *L. monocytogenes*, pose significant threats to public health due to their high mortality rates and wide distribution across raw meat products. Furthermore, these bacteria have the ability to flourish in cold conditions and create communities in a wide range of food items and within settings related to food processing (Ravindhiran *et al*., 2023).

The use of chemicals to prevent or delay food spoilage has become widespread. Concerns about the adverse effects of industrial chemicals, including arguments about their carcinogenicity and toxicity to humans, have been raised (Jangi *et al*., 2021). To harmonize consumer preferences with vital safety regulations, traditional approaches for managing microbial spoilage and food safety risks are being substituted with inventive technologies. These innovations encompass biological antimicrobial mechanisms like lactic acid bacteria and their bacteriocins (Salem, 2012).

Nisin, a bacteriocin produced by Lactococcus lactis subsp. lactis in fermented dairy and meat products, has demonstrated antibacterial effects against various microorganisms, particularly *L. monocytogenes* and other gram-positive organisms (Abts *et al*., 2011; Gharsallaoui *et al*., 2016).

Nisin is considered a safe substance and is approved for use in food without causing toxic effects when ingested in large quantities (Icer *et al*., 2023). However, the effectiveness of bacteriocins depends on the concentration of endogenous enzymes and other meat and non-meat additives (Karbowiak *et al*., 2023). Consequently, there is an agreeing trend in utilizing nanotechnology to regulate and enhance the properties of nisin, aiming to bolster its potential in the food industry. Nanotechnology plays a vital role in food science, particularly in nanoparticle delivery systems and safety applications (Elsherif *et al*., 2024). Nevertheless, several significant challenges exist in the use of nisin in the food industry, including its uncontrolled interaction with various food components, degradation, and issues related to electrostatic repulsion, which may limit its application (Khan and Oh, 2016).

Various nanoparticle techniques have been employed to enhance the preservative effectiveness of nisin; however, not all of these techniques are suitable for application in the food industry. Some involve the use of non-organic solvents and chemical compounds, making them expensive and complex (Kazemzadeh *et al*., 2022). As an alternative, there is a pressing need to develop chemical-free, organic solvent-free, and environmentally friendly nanoparticle-based systems. These systems can help overcome the inactivation of free nisin by various food components and enhance its preservative properties by protecting nisin and releasing it gradually (Abd-El Hameed and Elsherif, 2019).

Under such conditions, ensuring the hygienic quality and safety of minced beef meat becomes imperative. Thus, the objective of this study was to investigate the antibacterial activity of nisin and its nanoparticles against *L. monocytogenes*. Additionally, the study aimed to evaluate the preservative effects on chilled minced meat during refrigerated storage. This evaluation included assessing the potential impacts on organoleptic properties, pH, and lipid peroxidation in raw minced meat.

Materials and methods

Materials

Listeria monocytogenes (NCTC 13372\ ATCC® 7644) was obtained from a certified food lab at the Animal Health Research Institute (AHRI), Giza, Egypt as reference strain. Polyethylene glycolsorbitan monooleate

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(Tween* 80) was purchased from Sigma Aldrich. Deionized water was obtained from the Molecular Biology Unit, Assiut University. Buffered Listeria enrichment broth base (CM0897) was purchased from Oxoid LOT 1665116, and Harlequin™ Listeria Chromogenic Agar (ALOA) (ISO) HAL010 was purchased from the HiMedia Pvt. (India LOT 0000286672). Buffered peptone water (ISO) and LAB204 Neogen Company 0.5 McFarland Standard (8.2 log10cfu/ ml) (Cat. No. TM50) were purchased from Dalynn Biologicals Co. Acetic acid (Merck Co., Germany), and nisin (Sigma Aldrich, 1414-45-5) were purchased and used as received. 96-well plates and nano-filters were purchased from Dar-ElHekma Co. (Assiut City, Egypt). Four kilograms of raw minced beef were purchased from the local markets in Assiut city, Egypt and transported in an ice box as rapidly as possible to the laboratory.

Preparation of Nisin Nanoparticles

Nisin (obtained from Sigma Aldrich with code 1414-45-5) at a concentration of 2 mg/mL was completely dissolved in 100mL of an aqueous acetic acid solution (0.1 mol/L), under magnetic stirring at 100% amplitude of ultrasound probe sonication (UP100H Hielscher Ultrasound, 750 W). This process was carried out in an ice bath for 15 minutes. Subsequently, 50 mL of deionized water was added drop by drop to the nisin solution while maintaining the pH within the range 2.5–3 by using acetic acid (Chang *et al*., 2018). The dispersion was constantly stirred at 25°C for 7 hours. Finally, 0.01% Tween 80 was added as a stabilizer to the stability of the end solution, followed by sonication for 5 minutes. The prepared solution was stored at refrigeration temperature (4±2°C) and examined for size and shape of fabricated nanoparticles by using (DLS) using a Zetasizer ZS90 (3000 HS, Malvern Instruments, Malvern, UK) at the Nanotechnology Unit, Al-Azhar University, Assiut Branch, Egypt, and (HRTEM) (JEM2100, Jeol, Japan) at the Electronic Microscope Unit, National Research Center, Egypt.

Cytotoxicity of Prepared NNPs

Cell Culture

Vero cells, derived from Green monkey kidney, were obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). These cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units/ml of penicillin, and 10% heat-inactivated fetal bovine serum in humidified atmosphere with 5% (v/v) CO_2 at 37°C (Skehan *et al*., 1990).

Cytotoxicity Assay

Cell viability was assessed by using Sulforhodamine B (SRB) assay. Aliquots of 100 µl of cell suspension ($5x10^3$ cells) were plated in 96-well plates and incubated for 24 hours. Afterward, cells were treated with another 100 µl aliquot of media containing drugs at various concentrations. Following 72 hours of drug exposure, the cells were fixed by replacing media with 150 µl of 10% TCA and incubated at 4°C for 1 hour. The TCA solution was then removed, and the cells were washed 5 times with distilled water. Subsequently, 70 µl aliquots of SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 minutes. The plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Finally, 150 µl of TRIS (10 mm) was added to dissolve the protein-bound SRB stain, and the absorbance was measured at 540 nm using a BMG LABTECH®-FLUOSTAR Omega microplate reader (Ortenberg, Germany) (Allam *et al*., 2018).

Preparation of L. monocytogenes for inoculation

Following ISO 11290-2:2017 guidelines, the tested reference *L. monocytogenes* bacteria (NCTC 13372 ATCC® 7644) were cultured in selected broth and incubated before being plated on selective agar. Pure colonies of the bacterial strain (1–3 colonies) were injected into 5 mL of saline. The bacterial suspension was adjusted to a concentration equivalent to 0.5 McFarland Standard, as per McFarland (1907) and Gupta *et al*. (1992)

Preparation of minced meat samples

In the laboratory, minced meat samples were divided into two parts: The first part was allocated for assessing the antibacterial effectiveness of nisin and its nanoparticles in raw minced beef meat and was placed inside a laminar flow hood. The second was reversed to investigate the quality and the shelf life of the minced meat treated with prepared nisin and its nanoparticles during cold storage.

Assessment of nisin and nisin nanoparticles antibacterial efficacy in raw minced beef meat

Under aseptic conditions, four equal portions of minced meat (each 500 g) were prepared. To ensure the experiment adhered to rigorous aseptic standard, the all groups were inoculated with the same concentration of a suspension of *L. monocytogenes* at the level of 10^5 CFU/ml (1ml/100g).

One portion served as a positive control group without any treatment, while the other three portions of inoculated minced meat were treated as follows; one portions was treated with 1.2 ml of prepared nisin /100g of minced meat, another with 0.6 ml, and the third with 1.2 ml of prepared nisin nanoparticles per 100 g. Two hours after inoculation, the initial bacterial count was determined.

All experiments were carried out in triplicate. Each portion was sealed in a polyethylene bag inside a laminar flow hood and then stored at 4°C. The samples were periodically examined to determine the count of *L. monocytogenes* at 24-hour intervals.

For bacterial enumeration, 25 g of minced meat were aseptically added to 225 mL of 0.1 percent peptone water to create a tenfold serial dilution. Subsequently, 0.1 mL of this dilution was speeded on ALOA agar plates to count *L. monocytogenes*. The plates were then incubated for 24 hours at 37°C following ISO 11290-2:2017 guidelines.

Assessment of the minced meat quality and the shelf life when treated with prepared nisin and its nanoparticles during cold storage

Raw minced meat was divided into four equal portions labeled as I, II, III and IV (each weighing 500 g). The untreated portion served as the control group received no treatment. One portion was treated with 1.2 ml prepared nisin /100 g. Two parts were treated with prepared nisin nanoparticles, one receiving 0.6 ml, and other with 1.2 ml /100 g.

All experiments were conducted in triplicate and stored cool at 4°C. Over a specified interval (every 24 hours), the minced meat samples were subjected to sensory, physicochemical assessment, include:

Sensory Assessment

Minced meat samples from various groups were coded and evaluated by semi-trained panelists for color, odor, texture, all over acceptance. All panelists' experts had an experience in meat sensory evaluation at the Animal Health Research Institute, Agriculture Research Centre and Food Department of Assiut University Hospitals, and they gave their permission to participate within this sensory research study. In addition, they had previously participated in the research conducted by Tolba and Abdel-Aziz (2024) and our institution has permission and ethical approval to conduct sensory panel research focused on food.

Organoleptic assessment of the samples was conducted using 9-point hedonic scale, ranging from 1("extremely dislike") to 9 ("extremely like"), following the method outlined by Meilgaard *et al*. (2007) and in

accordance with UNE ISO 4121 /2006 and UNE ISO 6658 /2019 standards.

Physicochemical examination

pH evaluation

pH of the samples was measured at room temperature, following the procedure described by Sabikun *et al.* (2019) and based on method prescribed in ISO 2917 :1999. A portable pocket pH meter (AD11, Adwa pH-Tem waterproof, Romania) was used for pH measurement.

TBA determination (Thiobarbituric Acid Value)

Evaluation of lipid oxidation in minced meat samples was performed by determining the thiobarbituric acid value according to the procedure of Radha *et al.* (2014). The absorbance was measurement at 531 nm using (UV-160 A, UV-Visible Recording Spectrophotometer, Shimadzu, Tokyo, Japan) against a reagent blank. The TBA numbers were expressed in milligrams of malonaldehyde per kilogram samples.

Statistical analysis

Three replicates for each experiment were conducted. To assess the statistical significance of differences between samples, a one-way analysis of variance (ANOVA), was performed Using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Nisin nanoparticles characterization

The FTIR results of free nisin showed that the peak at 1620 cm^{-1}, corresponding to COO^- was, shifted to 1610 cm^{-1} in nisin nanoparticles, indicating an increase in hydrogen bonding in nisin nanoparticles. Conversely, the amid II band in free nisin, which appeared at 1530 cm^{-1}, became more prominent at 1549 cm^{-1} in nisin nanoparticles (Figure 1).

The size and morphology of the nisin nanoparticles (NNP) created using Nano precipitation was assessed through transmission electron microscopy (TEM), as shown in Figure 2. The TEM images displayed separate spherical particles with an average size of 10.34±3.98 nm, exhibiting no aggregation and excellent stability. In contrast, the dynamically measured average size was 76.34±23.2 nm with a PDI of 0.02.

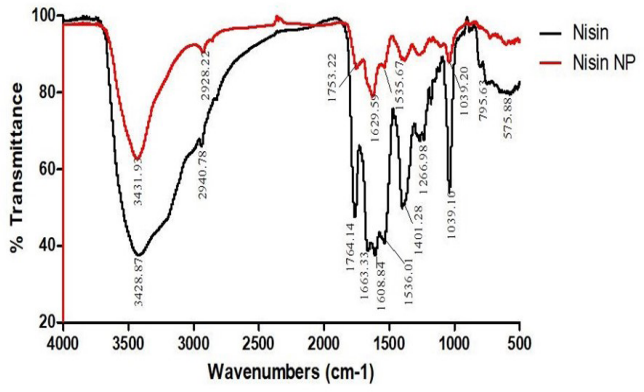

Fig. 1. FTIR of nisin and its nanoparticles.

Cytotoxicity of prepared NNPs

The cytotoxicity effect of NNP was determined using the SRB assay at different concentration, ranged from 100 to 0.01 µg /ml. notably; cell viability remained within the range of 85% to 99.9 % across all studied concentrations. After exposure to100 µg/ml of NNPs for 24 to 72 hours, the percentages of cell viability ranged from 98% to 85%, respectively (Figure 3).

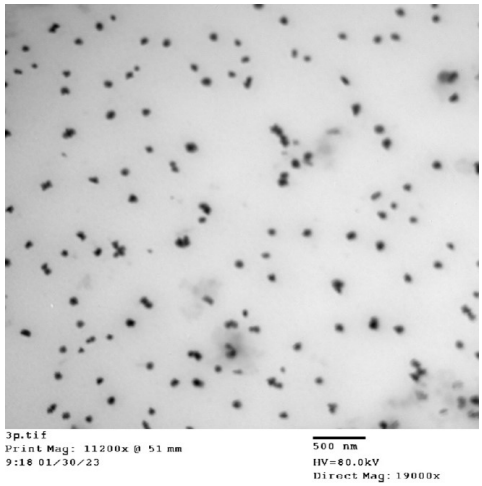

Fig. 2. HRTEM for fabricated NNPs with average size 10.34±3.98 nm.

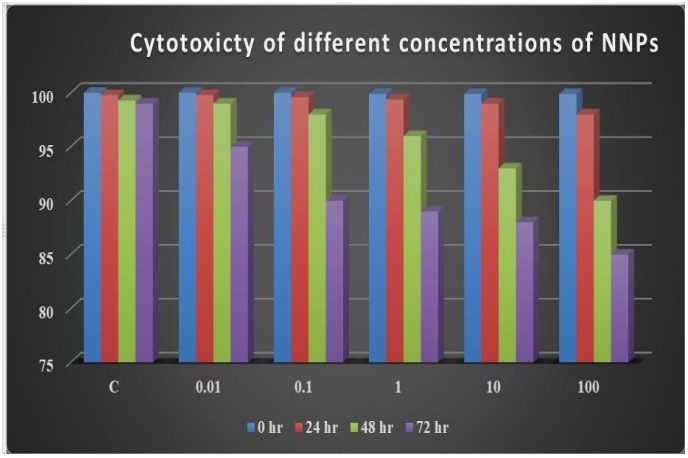

Fig. 3. Cytotoxicity of different conc. of NNPs.

Anti-listeria activity of nisin and its nano particles inoculated in minced meat during cold storage at 4°C

Figure 4 presents the results depicting the impact of different concentrations of nisin and nisin nanoparticles on the count of *L. monocytogenes* bacteria in minced beef meat. Initially, on the day zero, the total number of *L. monocytogenes* bacteria in minced meat was 3.3 x10^6 CFU/g. As time progressed, the number of bacterial count in the control samples increased. However on the first day of storage, the samples treated with nisin and its nanoparticles exhibited a significantly lower count of Listeria bacteria compared to the control samples ($p < 0.01$).

By the third day of storage, the number of Listeria bacteria in control samples had become uncountable. in contrast, the treated samples continued to show a meaningful inhibitory effect against Listeria bacteria. Specifically, nisin nanoparticles at concentrations of 0.6 and 1.2 ml/100g effectively inhibited bacterial growth, reducing the number of bacteria to below detectable levels (>10 zero) ($p < 0.01$) on the 6th and 3rd days, respectively. However, the sample treated with nisin (1.2ml/100gm) spoiled on the 6th day of storage.

Sensory evaluation of treated raw minced meat samples with nisin and its nanoparticles during cold storage at 4°C

Table 1 displays the sensory attributes of fresh chilled minced beef meat, encompassing aspects such as color, odor, texture, and overall acceptability, in relation to treatments involving nisin and its nanoparticles.

These sensory evaluations demonstrated significant effectiveness in mitigating sensory deterioration over the entire refrigerated storage period. Notably, a substantial divergence in sensory quality was observed from the 3rd to the 6th day ($p < 0.01$) when compared to the negative control sample. It's worth highlighting that, by the 6th day of storage, minced beef meat samples treated with nisin nanoparticles (1.2%) exhibited particularly enhanced sensory evaluations.

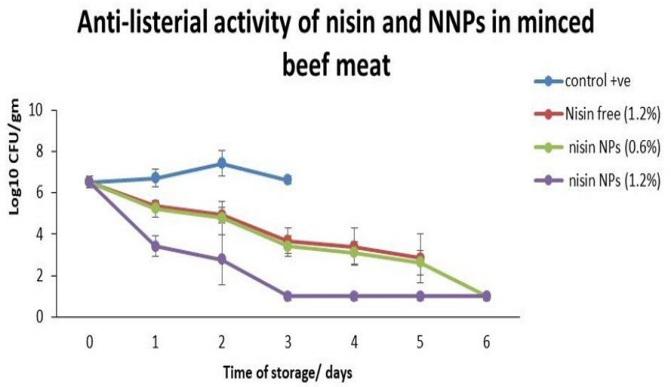

Fig. 4. Statistical analyses of *L. monocytogenes* count in treated raw minced beef with nisin and its nano particles during cold storage at 4°C. are presented as Mean±Standard error for triplicate, Significant at $P < 0.01$.

The pH of the chilled minced beef increased gradually with extended storage time. Initially, the pH values for control and various treatments (nisin at 1.2 ml/100g and nisin nanoparticles at 0.6/100g and 1.2 ml / 100g) were around 5.86, 5.81, 5.7, and 5.67, respectively. throughout the storage period, the final pH Values of the different treatments consistently remained lower than the control at day 4.

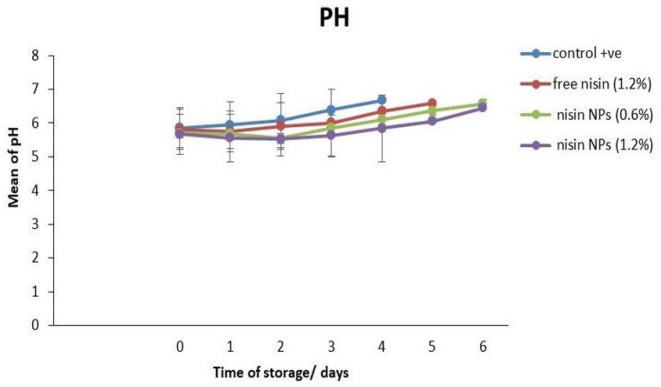

Fig. 5. Statistical analyses of pH of treated raw minced beef samples with nisin and its nanoparticles during cold storage at 4 °C. are presented as Mean±Standard error for triplicate, Significant at $P < 0.01$.

On the fourth day of storage, the oh values of control, nisin at 1.2 ml/100g, nisin nanoparticles at 0.6 and 1.2 ml /100g treatments were 6.68, 6.35, 6.11and 5.84 respectively. Treated minced meat samples showed a highly significant difference compared to the control.

Notably, there was no significant difference between samples treat-

Table 1. Sensory evaluation of treated raw minced beef meat samples with nisin and its nanoparticles during cold storage at 4°C.

Organoleptic parameters	days	Control	Nisin (1.2%)	Nisin NP (0.6%)	Nisin NP (1.2%)
Color	0 day	9.0±0.0	9±0.0	9.0±0.0	9.0±0.0
	1st	8.7±0.33	8.3±0.33	9.0±0.0	9.0±0.0
	2nd	8.7±0.33	8.7±0.33	9.0±0.0	9.0±0.0
	3rd	5.7±0.33	7.3±0.33*	8.3±0.33**	8.7±0.33***
	4th	2.3±0.33	7.6±0.33***	7.0±0.0***	7.3±0.33***
	5th	spoiled	6.0±0.5	5.3±0.33	6.0±0.0
	6th	spoiled	spoiled	3.7±0.33	3.7±0.33
Odor	0 day	9.0±0.0	9.0±0.0	9.0±0.0	9.0±0.0
	1st	9.0±0.0	9.0±0.0	9.0±0.0	9.0±0.0
	2nd	8.0±0.6	8.7±0.33	9.0±0.0	9.0±0.0
	3rd	6.3±0.333	8±0.0**	7.7±0.33*	8.0±0.0**
	4th	2.3±0.33	7.3±0.33***	7±0.0***	8.0±0.0***
	5th	spoiled	6.7±0.33	6±0.0	6.3±0.33
	6th	spoiled	spoiled	3.667±0.33	3.7±0.33
Texture	0 day	9.0±0.0	9±0.0	9.0±0.0	9.0±0.0
	1st	9.0±0.0	8.7±0.33	9.0±0.0	9.0±0.0
	2nd	8.3±0.33	8.6±0.33	9.0±0.0	9.0±0.0
	3rd	5.7±0.33	7.3±0.88	8.0±0.6	8.3±0.33*
	4th	1.3±0.33	6.66±0.33***	6.6±0.33***	6. 7±0.33***
	5th	spoiled	5.66±0.33	5.7±0.33	5.3±0.33
	6th	spoiled	spoiled	3.3±0.33	4.0±0.0
Overall acceptance	0 day	9.0±0.0	9.0±0.0	9.0±0.0	9.0±0.0
	1st	9.0±0.0	8.7±0.33	9.0±0.0	9.0±0.0
	2nd	8.7±0.33	8.33±0.33	9.0±0.0	9.0±0.0
	3rd	4.7±0.33	8.0±0.5**	7.7±0.33**	8.3±0.33***
	4th	1.7±0.33	7.0±0.6***	6.7±0.33***	7.3±0.33***
	5th	spoiled	5.7±0.33	5.7±0.33	6.0±0.0
	6th	spoiled	spoiled	3.33±0.33	4.0±0.0

Data are presented as means±standard error of triplicate measurements.
* indicates a significant difference ($p < 0.01$) between treated minced meat samples and the negative control starting at day 4. For the nisin 1.2% treatment, the difference was significant until day 6 (after which the negative control sample significantly deteriorated).

ed with nisin nanoparticles at 0.6 and 1.2 ml/100g ($p > 0.01$), resulting in final pH values of approximately 6.57 and 6.45 on the 6 day, respectively, this suggests that these treatments had the best inhibitory efficacy in preventing the increase in pH in chilled minced meat. In contrast, the minced meat samples treated with nisin at 1.2 ml/100g spoiled on the 6th day of storage.

As depicted in Figure 6, the TBA values in all the samples continuously increased over time. Various treatments exhibited a more effective role inhibiting the rise of TBA values in chilled minced beef meat during refrigerated storage compared to the control.

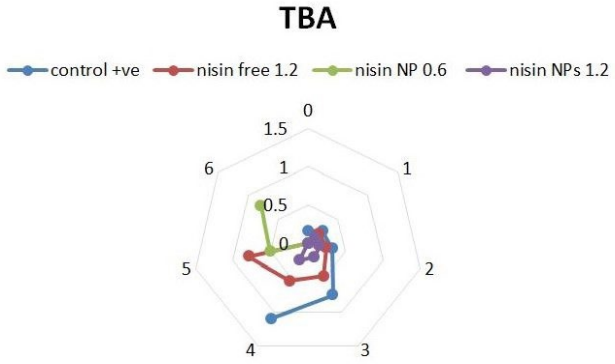

Fig. 6. Statistical analyses of TBA of treated raw minced beef samples with nisin and its nanoparticles during cold storage at 4 °C. Data are presented as Mean±Standard error for triplicate, Significant at $P < 0.01$.

The initial value for the controls on day 0 was 0.16 mg/kg, and by the 4th day of storage, it had reached 1.1mg/kg. The TBA values for samples treated with nisin nanoparticles at 0.6 and 1.2 ml/100g did not exceed 0.78 mg/kg and 0.61 mg/kg (indicating lower oxidation) on the 6th day, respectively. Samples treated with nisin 1.2 ml/100g reached 0.78 mg/kg on the 5th day and then spoiled on the 6th day of storage.

Discussion

The FTIR results presented in Figure 1 are consistent with findings by Flynn *et al.* (2020), wherein the -OH stretching peak of NNP exhibited greater intensity compared to free nisin. This heightened intensity suggests a stronger formation of hydrogen bonds within the nisin nanoparticles. Additionally, as shown in Figure 1, in agreement with Webber *et al.* (2021), a distinct amide I band appeared at wavenumber of 1632 cm^{-1}. This shift in the amide I band could be due to attributed to alterations in the structure of free nisin when it is converted into nano-nisin using natural acetic acid. This structural change underscores that enhanced antibacterial activity of prepared nano-nisin. FTIR analysis is a valuable tool for determining the molecular composition and structure of materials and is often likened to a molecular fingerprint (Nandiyanto *et al.* 2019).

The size of the nanoparticles, as determined by TEM, appeared smaller than the measurements obtained from the Zeta sizer, which provides hydrodynamic diameters of nanoparticles in a solution. This discrepancy is likely due to the nanoparticles undergoing shrinkage during the drying process in TEM sample preparation (Mittal *et al.*, 2014).

It's interesting to note that Chang *et al.* (2018) reported larger NNP sizes at a concentration of 0.5%, with spherical particles measuring approximately 15 nm by TEM and 180.3±10.2 nm by Zeta sizer, along with a PDI measurement of 0.236. Furthermore, Haider *et al.* (2022) discovered significantly larger spherical nanoparticles with a size of 289.09 nm, while nanoparticles prepared with nisin encapsulation in chitosan by Kazemzadeh *et al.* (2022) were even larger at 1003 nm (with PDI = 0.525).

The Cytotoxicity effect of nanomaterials on Vero SF cell, specifically the Vero SF monkey kidney epithelial cells, was recommended by EFSA as a standard cell line (Kourmentza *et al.*, 2021). The finding in figure 3 regarding NNPs is consistent with Moustafa *et al.* (2022), who confirmed material significant toxicity when cell viability value was less than 50% compared to the control cells. In light of these results, our prepared nano-materials demonstrated no cytotoxicity to the cells, underscoring their high safety profile as potential candidates for smart food preservative.

Several researchers have also assessed the biosafety of various nisin nano materials through in vitro cytotoxicity tests (Zhao and Kuipers, 2021; Haider *et al.*, 2022; Popa *et al.*, 2022). Our finding align with the conclusion of Goudarzi *et al.* (2018), who reported that nisin-loaded nanoparticles have no cytotoxic effects on Vero (non-tumor) cell line at the studied concentrations.

In the processing of ground beef, the application of nisin and its nanoparticles at various concentrations (illustrated in Figure 4), reveals a significantly reduction in the counts of *L. monocytogenes*, demonstrating antibacterial activity. This effect is dependent on the concentrations level of nisin nanoparticles and the specific strained use.

Notably, treating of minced beef with 1.2ml /100g nisin nanoparticles exhibited stronger inhibitory activity against *L. monocytogenes* compared to treatment with at 1.2 ml /100g of nisin and 0.6 ml /100g nisin nanoparticles when stored at 4°C. In the latter cases, the count of *L. monocytogenes* exceeded 10 on 3rd day of storage. In contrast, when treated with nisin nanoparticles at a concentration of 1.2 ml /100g, the count remained above 10 on the 6th day of refrigerated storage, while the samples treated with nisin at 1.2 ml /100g spoiled on the sixth day.

Nisin, in fact, poses challenges material exchange processes like ATP and ADP due to its impact on the cytoplasmic membrane of microorganisms, leading to the formation of membrane pores. It also induces pores in the cell membrane by affecting the positive charge of the cells, ultimately leading to the microorganism's death (De Arauza *et al.*, 2009; Jangi *et al.*, 2021). Transforming nisin into nanoparticles enhances its ability to penetrant the bacteria cells and may additionally increase the sensitivity of certain strains of *L. monocytogenes* that exhinit resistance to the pure form of nisin (Boziaris and Nychas, 2006).

Nisin's mode of action against gram- positive bacteria involves binding to the surface of the target cell and disrupting the structure of cytoplasmic membrane. This binding is facilitated by electrostatic interactions between nisin, which carries a positive charge, and the negatively charged phospholipids in the membrane. The result is the release of crucial cytoplasmic components and breakdown of the cell membrane, leading to the death of the bacteria. Nisin nanoparticles, possessing a large specific surface area, can easily adhere to the target cell surface, enhancing membrane permeability and ultimately causing cell death. Furthermore, in comparison to free nisin solution, nisin nanoparticles demonstrate thermotolerance due to internal non-covalent interaction, allowing them to maintain their effeteness even under elevated temperatures.

In a parallel manner, the β-D-xylosidase nanoparticles preserved around 60% of their intial activity when subjected to a one-hour incubation at 80°C, whereas the native β-D-xylosidase enzyme experienced a complete loss of activity under identical conditions within the same time frame (Hegedus & Nagy, 2015; Chang *et al.*, 2018).

It should be noted that *L. monocytogenes* has recently been reported to exhibit the ability to survive at high concentrations of nisin, possibly due to less few negative charges on bacterial cell surface (Wu *et al.*, 2017). This poses a risk of creating and spreading strains resistant to this bacteriocin (Ettayebi *et al.*, 2000). To address this issue, some researches have opted to use the nisin in combination with essential oil such as peppermint, carvacrol and thymol. In our research, we have transformation nisin into nisin nanoparticles to enhance the anti-Listeria effect, mitigating the need for additional additives or combinations.

Nisin presents various benefits compared to alternative food preservatives. These advantages encompass its non-toxic nature, quick digestibility by the enzyme α-chymotrypsin, stability under heat at low levels, and the absence of color and flavor (Pongtharangkul and Demirci, 2004).

Sensory evaluation is a crucial factor in assessing meat quality. Table 1 displays the sensory evaluation results of fresh chilled minced beef meat, including color, odor, and texture and overall acceptance, with var-

ious treatment involving nisin and its nano particles. The results indicate that different treatments had a positive impact on the sensory characteristics of minced beef meat.

The observed sensory changes can be ascribed to proteolysis and lipid oxidation in untreated samples (control), which became more noticeable over a shorter period due to progressive growth of microorganisms. However, the sensory panelists noted that the trends in sensory color and texture changes aligned with the instrumental color and texture of treatments involving nisin and its nanoparticles. The slower rate of decline in the sensory scores for color and could be attributed to inhibition of enzyme activity and microbial growth in child minced meat by nisin nanoparticles, thereby reducing sensory issues and extending shelf life compared to control and minced meat treated with nisin at 1.2ml/100gm. In the latter case, gross discoloration, strong off-odors, and the development of slime and stickiness became the primary qualitative criteria for meat rejection on the 4th and 5th days, respectively.

Alterations in sensory odor characteristics and identification of spoiled meat by based on odor may be linked to pH changes and microbial growth in chilled minced beef meat. After a storage period of four days, there was a decrease in sensory scores for color, odor, and texture. Given that meat is deemed acceptable when that sensory score is above 6.00, the chilled minced beef meat treatments with nisin nanoparticles remained acceptable after five days storage, except for the samples treated with nisin at 1.2ml/100gm, which were rejected.

To mitigate associated spoilage, the potential of nisin nanoparticles as biological preservatives could be explored to complement existing traditional preservation techniques.

The observed increase in pH of chilled minced meat during storage, as shown in Figure 5, can be attributed to the growth of the microorganisms in the meat. This microbial growth leads to the decomposition of various nutrients, such as fat and amino acid (Masniyom *et al*., 2002). In contrast, the lower pH observed in chilled beef minced meat treated with nisin and its nanoparticles might be attributed to the inhibitory and antimicrobial effects of these treatments on the growth of spoilage microorganisms.

Lipid oxidation stands as a significant factor that imposes limits on the shelf life of meat products. TBA values serve as indicators of lipid oxidation content and are commonly utilized as key metrics to evaluate the quality of meat products due to their relatively straightforward measurement.

Our finding, as depicted in Figure 6, demonstrated that samples treated with nisin at 1.2 ml/100g and nisin nanoparticles at 0.6 and 1.2 ml/ 100g effectively inhibited lipid oxidation in chilled minced meat during refrigerated storage. TBA values can be attributed to the antioxidant activity of these treatments. Nisin nanoparticles have demonstrated efficacy in controlling lipid oxidation in meat products.

These results align with previous reports. For instance, Wang *et al*. (2017) observed that treatments with nisin, potassium, and phages led to a reduction in TBA values in fresh chilled pork, thereby extending the shelf life to 14 days.

Antioxidants are potent compounds against free radicals, and this study indicates that even at low concentrations, nisin and its nanoparticles can impede the oxidation of easily biomolecules. Notably, nanoparticles exhibit enhanced efficacy, ultimately improving product shelf life by safeguarding against deterioration caused by oxidation.

Conclusion

In this study, it has been demonstrated that treating of minced meat samples with nisin and its nanoparticles exhibits superior the activity of *Listeria monocytogenes*, particularly with nisin nanoparticles at a concentration of 1.2ml/100g, and has the ability to extend shelf life compared to untreated samples (control). The treatment with nisin and nisin nanoparticles seems to have a synergistic effect on microbial inhibition, resulting in reduced pH values and lower TBA levels in raw minced meat during the 5th and 6th days of storage at 4°C, respectively. Furthermore, the treatment with nisin nanoparticles also provides a protective effect against the deterioration of texture properties and degradation of color. As a result, this treatment strategy has the potential to enhance both the shelf life and sensory quality of chilled raw minced meat during refrigerated storage.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abd-El Hameed, Z.M., Elsherif, W.M., 2019. Effect of nisin as a bio preservative on shelf life of pasteurized milk. Assiut Veterinary Medical Journal 65, 16-24.

Abts, A., Mavaro, A., Stindt, J., Bakkes, P.J. Metzger, S., Driessen, A.J.M., Smits, S.H.J., Schmitt, L., 2011. Easy and rapid purification of highly active nisin. International Journal of Peptides 157145.

Allam, R. M., Al-Abd, A.M., Khedr, A., Sharaf, O.A., Nofal, S.M., Khalifa, A.E., Mosli, H.A., Abdel-Naim, A.B., 2018. Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. Toxicology Letters 291, 77–85.

Boziaris, I.S., Nychas, G.J.E., 2006. Effect of nisin on growth boundaries of L.monocytogenes Scott A, at various temperatures, pH and water activities. Food Microbiology 8, 779 - 784.

Chang, R., Lu, H., Li, M., Zhang, S.h., Xiong, L., Sun, Q., 2018. Preparation of extra-small nisin nanoparticles for enhanced antibacterial activity after autoclave treatment. Food Chemistry 245, 756-760.

De Arauza, L.J., Jozalaa, A.F., Mazzola, P.G., VessoniPenna, T.C., 2009. Nisin biotechnological production and application: a review. Trends in Food Science and Technology 3 – 4, 146-154.

Donsí, F., Annunziata, M., Sessa, M., Ferrari, G., 2011. Nan encapsulation of essential oils to enhance their antimicrobial activity in foods. Food Science and Technology 44, 1908–1914.

Elsherif, W.M., Zayed, G.M., Tolba, A.O., 2024. Antimicrobial activity of chitosan- edible films containing a combination of carvacrol and rosemary nano-emulsion against Salmonella enterica serovar Typhimurium and *Listeria monocytogenes* for ground meat. International Journal of Food Microbiology 418, 110713.

Ettayebi, K., Yamani, E. I.J., Rossi-Hassani, B.D., 2000. Synergistic effects of nisin and thymol on antimicroibial activities in listeriamonocytogenes and Bacillus subtilis. FEMS Microbiology Letters 183, 191-195.

Flynn, J., Durack, E., Collins, M.N., Hudson, S.P., 2020. Tuning the strength and swelling of an injectable polysaccharide hydrogel and the subsequent release of a broad spectrum bacteriocin, nisin A. Journal of Materials Chemistry B. 8, 4029–4038.

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.

Goudarzi, F., Asadi, A., Afsharpour, M., Jamadi, R.H., 2018. In vitro characterization and evaluation of the cytotoxicity effects of nisin and nisin-loaded PLA-PEG-PLA nanoparticles on gastrointestinal (AGS and KYSE-30), hepatic (HepG2) and blood (K562) cancer cell lines. Aaps Pharmscitech. 19, 1554-1566.

Gupta, L.K., Jindal, R., Beri, H.K., Chhibber, S., 1992. Virulence of silver resistant mutant of Klebsiellapneumoniae in burn wound model. Folia Microbiologica (Praha). 37, 245-248.

Haider, T., Pandey, V., Behera, C., Kumar, P., Gupta, P.N., Soni, V., 2022. Nisin and nisin-loaded nanoparticles: a cytotoxicity investigation. Drug Development and Industrial Pharmacy 48, 310–321.

Hegedus, I., Nagy, E., 2015. Stabilization of activity of cellulase and hemicellulose enzymes by covering with polyacrylamide layer. Chemical Engineering and Processing: Process Intensification 95, 143–150.

Icer, M.A., Özbay, S., Ağagündüz, D., Kelle, B., Bartkiene, E., Rocha, J.M.F., Ozogul, F. 2023. The Impacts of Acidophilic Lactic Acid Bacteria on Food and Human Health: A Review of the Current Knowledge. Foods 12, 2965.

ISO 11290-2: 2017. Procedure steps for *L. monocytogenes* and Listeria spp. isolation and enumeration according to the revised EN ISO 11290-1, 2, 2017 standard. https: //www.sigmaaldrich.com/technical-documents/articles/microbiology/liste ria-detection-food-chain-iso-11290.html.

ISO 2917: 1999. Meat and Products-Measurement of pH-Reference Method. https://www.studocu.com/pt-br/document/universidade-federal-da-paraiba/engenharia-de-alimentos/iso-2917-1999-meat-and-meat-products-measurement-of-p-h-reference-method/42612116.

Jangi, A.N., Dooghikalaei, E.A., Eskandani, M.A., 2021. The Effect of Peppermint Essential Oil and Nisin on L.monocytogenes Inoculated in Minced Meat of Silver Carp. Aquatic Food Studies 1, AFS63.

Karbowiak, M., Szymański, P., Zielińska, D., 2023. Synergistic Effect of Combination of Various Microbial Hurdles in the Biopreservation of Meat and Meat Products—Systematic Review. Foods 12, 1430.

Kazemzadeh, S., Abed- Elmdoust, A., Mirvaghefi, A., Hosseni, S.V., Abdollahikhameneh, H., 2022. Physicochemical evaluations of chitosan/nisin nanocapsulation and its synergistic effects in quality preservation in tilapia fish sausage. Journal of Food Processing and Preservation 00, e16355.

Khan, I., Oh, D-H., 2016. Integration of nisin into nanoparticles for application in foods, Innovative Food Science and Emerging Technologies 34, 376-384.

Kourmentza, K., Gromada, X., Michael, N., Degraeve, C., Vanier, G., Ravallec, R., Coutte, F., Karatzas, K.A., Jauregi, P., 2021. Antimicrobial activity of lipopeptide biosurfactants against foodborne pathogen and food spoilage microorganisms

and their cytotoxicity. Frontiers in Microbiology 11, 561060

Masniyom, P., Benjakul, S., Visessanguan, W., 2002. Shelf-Life Extension of Refrigerated Seabass Slices under Modified Atmosphere packaging. Journal of the Science of Food and Agriculture 82, 873–880.

McFarland, J., 1907. Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. JAMA journal of the American Medical Association 14, 1176–1178.

Meilgaard, M., Civille, G.V., Carr, B.T., 2007. Sensory Evaluation Techniques, 4th Ed., CRC Press, Boca Raton, FL.

Mittal, A.K., Bhaumik, J., Kumar, S., Banerjee, U.C., 2014. Biosynthesis of silver nanoparticles: elucidation of prospective mechanism and therapeutic potential. Journal of Colloid and Interface Science 415, 39–47.

Moustafa, H., Nasr, H.E., Youssef, A.M., 2022. Development of antibacterial carboxymethyl cellulose/quaternized starch bio-nanocomposites based on cinnamon essential oil nano-emulsion for wound healing applications. Biomass Conversion and Biorefinery 1–13.

Nandiyanto, A.B.D., Oktiani, R., Ragadhita, R., 2019. How to read and interpret FTIR spectroscope of organic material. Indonesian Journal of Science and Technolog. 4, 97–118.

Pongtharangkul, T., Demirci, A., 2004. Evaluation of agar bioassay for nisin quantification. Applied Microbiology and Biotechnology. 65, 268-272.

Popa, E.E., Miteluț, A. C., Râpă, M., Popescu, P. A., Drăghici, M.C., Geicu-Cristea, M., Popa, M.E., 2022. Antimicrobial Active Packaging Containing Nisin for Preservation of Products of Animal Origin: An Overview. Foods 11, 3820.

Radha, K., Babuskin, S., Azhagu, S.B.P., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M., Sukumar, M., 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. International J. Food Microbiol. 171, 32–40.

Rahman, M.M., Hashem, M.A., Azad, M.A.K., Choudhury, M.S.H., Bhuiyan, M. K. J., 2023. Techniques of meat preservation-A review. Meat Research 3, 3.

Ravindhiran, R., Sivarajan, K., Sekar, J.N., Murugesan, R., Dhandapani, K., 2023. *L. monocytogenes* an Emerging Pathogen: a Comprehensive Overview on Listeriosis, Virulence Determinants, Detection, and Anti-Listerial Interventions. Microbial Ecology, 1-21.

Sabikun, N., Bakhsh, A., Ismail, I., Hwang, Y.H., Rahman, M.S., Joo, S.T., 2019. Changes in physicochemical characteristics and oxidative stability of pre- and post-rigor frozen chicken muscles during cold storage. Journal of Food Science and Technology 56, 4809-4816.

Salem, A.M., 2012. Bio-Preservation Challenge for Shelf-Life and Safety Improvement of Minced Beef. Global Journal of Biochemistry and Biotechnology 7, 50-60.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M. R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI: Journal of the National Cancer Institute 82, 1107–1112.

Tolba, A.O., Abdel-Aziz, N.M., 2024. Quality survey of frozen chicken meat consumed at government hospitals throughout different seasons in Assiut city, Egypt. Bulgarian Journal Veterinary Medicine 27, 130-142.

UNE ISO 4121: 2006. Sensory analysis-Guidelines for the use of quantitative response scales. https://www.en-standard.eu/une-iso-4121-2006-sensory-analysis-guidelines-for-the-use-of-quantitative-response-scales-iso-4121-2003/

UNE ISO 6658: 2019. Sensory analysis-- Methodology. General guidance. https://www.en-standard.eu/une-iso-6658-2019-sensory-analysis-methodology-general-guidance/

Wang, C., Yang, J., Zhu, X., Lu, Y., Xue, Y., Lu, Z., 2017. Effects of Salmonella Bacteriophage, Nisin and Potassium Sorbate and Their Combination on Safety and Shelf Life of Fresh Chilled pork [J]. Food Control 73, 869–877.

Webber, J.L., Namivandi-Zangeneh, R., Drozdek, S., Wilk, K.A., Boyer, C., Wong, E.H.H., Bradshaw-Hajek, B.H., Krasowska, M., Beattie, D.A., 2021. Incorporation and antimicrobial activity of nisin Z within carrageenan/chitosan multilayers. Scientific Reports 11, 1–15.

Wu, S., Yu, P.-L., Flint, S., 2017. Persister cell formation of L.monocytogenes in response to natural antimicrobial agent nisin. Food Control 77, 243–250.

Zhao, X., Kuipers, O.P., 2021. Synthesis of silver-nisin nanoparticles with low cytotoxicity as antimicrobials against biofilm-forming pathogens. Colloids and Surfaces B: Biointerfaces 206, 111965.