Research Article

Impact of Propolis Nanoemulsion on *Listeria monocytogenes* Contaminating Chilled Stored Breaded Chicken Panne

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Abstract | Nanotechnology involves using minuscule particles and substances at a highly reduced scale. Its quick and precise movements and high bioavailability provide a scientific advantage in dealing with foodborne pathogens. The number of *Listeria monocytogenes* outbreaks caused by eating food is increasing worldwide, and people are increasingly looking for natural ways to stop the development of *L. monocytogenes*. In this context, propolis, a natural substance produced by bees, looks like an attractive option owing to its antibacterial action against many pathogens in food. The aim of this study is to investigate the effect of propolis extract (PE) and propolis nanoemulsion (PN) on *L. monocytogenes*, its toxins' expression, and the sensory qualities of breaded chicken panne during chilled storage. The 30% PN concentration had a size of 19.783 nm and a narrow size distribution (polydispersity index: 0.253) with a distinct chemical composition. PE's minimum inhibitory concentration (MIC) was 50 and 25 mg/mL, while PN's was 150, 75, and 37.5 mg/mL. The number of viable *L. monocytogenes* in the breaded chicken panne with PE and PNs gradually decreased significantly ($P < 0.05$) on chilling compared to the control. Moreover, in the PE and PN-treated groups, the relative expression of hemolysin A (*hly*A) decreased until the 9th day, reaching 0.251 and 0.125, respectively, and the relative expression of *IAP* reached 0.293 and 0.125, respectively. Additionally, treating the chicken panne with either PE or PN did not appear to affect the average sensory score of the breaded chicken panne. Our study has demonstrated that natural product-based biocontrol techniques have the potential to be developed to control the development of *L. monocytogenes* in chicken and chicken products. This result indicates that using natural and bio-based products can effectively manage diseases in the food industry.

Keywords | Breaded chicken panne; *L. monocytogenes*; Gene expression; Food safety; Bee glue; Nanotechnology

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INTRODUCTION

Nanotechnology involves using tiny materials that can undergo new changes. Nano-propolis comprises small propolis particles that are more easily absorbed by the body. It is expected to have a more substantial antibacterial effect than regular propolis (Barsola and Kumari, 2022).

Propolis, alternatively referred to as bee glue, is an adhe-

sive compound that resembles wax manufactured by bees through the combination of plant resins, saliva, wax, and pollen. Honeybees exploit propolis to protect the inside of their hives. Propolis in its raw form and the ethanolic and aqueous extracts have been demonstrated to exhibit antimicrobial properties, owing to the presence of polar phenolic substances, particularly the flavonoid portion (El-Sakhawy et al., 2023). The most widespread foodborne diseases in developed and developing nations are caused by Salmonella, *Clostridium perfringens*, Campylobacter, *Staphylococcus aureus*, and *L. monocytogenes* (CDC, 2019). *L. monocytogenes* is an intracellular facultative Gram-positive organism. Since *L. monocytogenes* can live on equipment and in manufacturing facilities, food items may become contaminated throughout the manufacturing process. In addition, it has been found that *L. monocytogenes* may survive challenging harsh environments, including low temperatures, high acidity, and salt content (Martín et al., 2022).

Chicken meat products are becoming increasingly popular due to their convenience in preparing quick meat meals and their ability to offer a solution for the shortage of fresh meat, which is often too expensive for many families with limited incomes. However, the extensive handling of chicken nuggets may heighten the risk of cross-contamination with pathogenic microorganisms (Fayed and Saad, 2021).

The *hly* gene produces listeriolysin O (LLO), the primary *L. monocytogenes* virulence factor (Quereda et al., 2021). LLO works by helping *L. monocytogenes* escape from intracellular phagocytic vesicles. LLO inactivation can cause hemolytic action depletion, phagosomal evasion obstruction, and virulence reduction, as the bacterium cannot reach the cytoplasm (Li et al., 2022).

The *IAP* gene produces the extracellular protein p60, the invasion-associated protein (*IAP*). As a crucial mouse hydrolase enzyme, it aids in separating the septum during the last stage of cell division. Additionally, it participates in *L. monocytogenes*' attachment to the host cell and is crucial to the bacterium's virulence and pathogenicity (Matle et al., 2020).

Based on the available information, there have been no previous studies on the effectiveness of PE and/or PNs in combating *L. monocytogenes* in breaded chicken panne or their influence on the expression of their toxin genes. Therefore, this study aims to investigate the antibacterial properties of PE and/or PNs against *L. monocytogenes*-contaminated breaded chicken panne during chilled storage and evaluate the expression of the toxin genes *hly*A and *IAP*.

MATERIALS AND METHODS

Bacterial strain

The *Listeria monocytogenes* (NLQP 333) strain used in this experiment was acquired from the National Laboratory for Veterinary Quality Control on Poultry Production (NLQP) and stored at -80°C. The frozen (glycerol stock) *L. monocytogenes* culture was activated by inoculation into Tryptone soya yeast extract broth (TSYEB; HiMedia), and a final concentration of around 10^8 CFU/mL was achieved through overnight incubation at 37°C. A sequence dilution was performed on 0.1% sterile buffered peptone water (pH = 7) to provide roughly 10^7 CFU/mL in the inoculated fluid (Lianou et al., 2018).

Preparation of PE

10 g of propolis and 70% ethanol to 100 mL in a volumetric flask at room temperature in shaker incubation for 5 hours (Ghavidel et al., 2021) while shaking vigorously. The supernatant was transferred to a new container for further use after the final extract was cooled to room temperature and centrifuged at 1500 rpm for five minutes. The Whatman paper filter No. 1 was utilized to filter the excerpt, which was stored at -20°C until the start of the experiments. Before adding the extracts to the samples, a 0.45-m filter was used to filtrate and sterilize (Said et al., 2006).

Preparation and characterization of PNs

PNs were prepared in the Nanomaterials Research and Synthesis Unit. Then, PE (30 mL), tween 80 (30 mL), and distilled deionized water (40 mL) were homogenized for thirty minutes utilizing a 1500-watt homogeneous blender. Subsequently, distilled water was added gradually to the mixed oil phase in accordance with Rao (2011). Distinguish the nanoemulsion and assess the electrical conductivity, zeta potential (surface charge), and both size droplet and distribution polydispersity indexes (PDI), a measure of sample size-dependent heterogeneity. Due to the sample size distribution, accumulation, or aggregation of the example during isolation or analysis can result in the polydispersity of nanoemulsion using the Microtrac FLEX (12.0.1.0) instrument.

The nanoemulsion's electrical conductivity and characterization were measured via high-resolution transmission electron microscopy (HRTEM) monitoring at the Faculty of Agriculture, Cairo University, using a JEM 1400F HR-TEM equipped with a 300 keV beam energy. The chemical fingerprint of PNs was conducted by the Thermo Nicolet 380 Fourier Transform Infrared (FT-IR) spectrometer supplied with the Smart SplitPea horizontal Attenuated Total Reflectance (ATR) microsampling device. Additionally, it is accompanied by the Thermo Electron Nicolet Centaurus FT-IR Contact Alert microscope, which has a

Triton 10x objective with a numerical aperture of 0.71. The system is operated using the Omnic PC workstation.

MTT cytotoxicity assay

Stock solutions of the drug under investigation were prepared using 10% DMSO in ddH2O and additional dilutions to working solutions using DMEM to determine the half-maximum cytotoxic concentration (IC_{50}). The sample's cytotoxic activity was identified in BNL cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) technique with minimal modifications. Briefly, the cells were sown in 96-well plates (100 uL/well) at 3105 cells/mL density and incubated for 24 hours at 37˚C in 5% CO_2. Cells were given varied concentrations of the tested substances in triplicate after 24 hours.

After 72 hours, the supernatant was discarded. Cell monolayers were subsequently rinsed three times with sterile 1x phosphate-buffered saline (PBS). MTT solution, amounting to 20 l of a stock solution containing 5 mg/mL, was added to each well. The wells were incubated at 37˚C for 4 hours before medium aspiration.

The generated formazan crystals were melted in each well with 200 l of acidified isopropanol (0.04 M HCl in 100% isopropanol = 0.073 mL HCL in 50 mL isopropanol).

The absorbance of formazan solutions was quantified at a maximum wavelength of 540 nm utilizing a multi-well plate reader (BMG LABTECH®FLUO star Omega, Germany) with a reference wavelength of 620 nm. The proportion of cytotoxicity relative to untreated cells was computed using the subsequent equation. Using a plot of per cent cytotoxicity against sample concentration, the cytotoxic 50% concentration (TC_{50}) was determined (Mostafa et al., 2020).

Antimicrobial efficacy of PE and PNs

The standard broth dilution method was used to determine PE and PNs' antimicrobial effectiveness by assessing the microorganisms' observable growth within the broth medium (CLSI, 2019). The MIC was determined by serial double dilutions for each examined material obtained in tryptone soy yeast extract broth (TSYEB) medium using a sterile 96-well round-bottom polystyrene microtiter plate (12 columns x 8 rows) as described by Markowska et al. (2018). Two-fold serial dilutions were prepared from PE and PN to acquire the final dilutions of 1/1, 1/4, 1/8, 1/16, 1/32, 1/64, and 1/128 with an *L. monocytogenes* concentration of 10^7/mL CFU (adjusted by 0.5 McFarland's) in tryptone soya yeast extract broth (TSYEB; HiMedia), while the control solely comprised inoculated broth. Plate incubation lasted for twenty-four hours at 37˚C. At the MIC endpoint, the minimum concentration is reached at which no discernible growth is observed in the tubes. The MIC value was confirmed by observing the wells' visual clouding both before and after incubation.

Preparation of breaded chicken panne and inoculation

1500 g of boneless, skinless chicken breast were purchased from markets. The boneless, skinless chicken breast was examined to ensure it was free from *L. monocytogenes* contamination. After that, the boneless, skinless chicken breast was cut into thin, 25-g pieces and marinated in salt and black pepper (Moschonas et al., 2012). After marinating, the parts were immersed in an equivalent amount of 2×10^7 CFU/mL overnight *L. monocytogenes* working culture, maintained at 4ºC for 20 minutes to allow for bacterial adhesion, and dried for fifteen minutes at laminar airflow (model NuAire). After that, all inoculated chicken pieces were divided into three groups (Moschonas et al., 2012):

Control group: marinated boneless skinless chicken breast parts immersed in sterile PBS without any treatment corresponding to PE and PN dipping treatment.

PE-group: marinated boneless skinless chicken breast pieces were treated (dipped) into PE and let to dry at laminar air flow for 15 minutes.

Lastly, the **PNs-group** marinated boneless skinless chicken breast pieces were treated (immersed) into PN and left to dry for 15 minutes at laminar airflow.

The dried groups were dipped in egg emulsion before breading. Each group was packaged in sterile plastic bags aseptically. For *L. monocytogenes* enumeration, samples were collected from each group following inoculation (zero time) and relative *hly*A and *IAP* expressions, and then each group was saved at 4ºC in a refrigerator. The samples were examined every three days until spoilage. The experiment was repeated three times.

L. monocytogenes viable count

According to the FDA (2017), 25 g of every group were homogenized utilizing a stomacher (Seward stomacher 80 Biomaster, England) with 225 mL of sterile BLEB (Buffered Listeria Enrichment Broth) without the selective agent to prepare homogenate. The samples were stomached, and a ten-fold serial dilution was performed. Based on the work of Ottaviani and Agosti (AlOA) agar media, 1 mL of each sample was put directly on three plates of Listeria chromogenic agar base. The inoculum was applied using a sterile bent glass streaking rod. For quantifying *L. monocytogenes*, the plates were inverted and incubated at 35 degrees Celsius for 48 hours.

Sensory evaluation

Twelve experts from the Food Hygiene Department of the Animal Health Research Institute (ARC) joined as the reviewers. The color, flavor, taste, and overall approval of breaded chicken panne were compared with PE and PN to plain breaded chicken (neither contained PE nor PNs). For each of the four descriptors, the experts were requested to evaluate the samples in order of intensity on a 9-point hedonic scale, with nine being the mean (Pimentel et al., 2016). The examiner judged the model rejected if the stated findings fell below point five.

Gene expression assessment

Following the manufacturer's instructions, mRNA was extracted using a FastPure® DNA/RNA Mini kit. 200 µL of the sample and 500 µL of Lysis Solution were added to a 1.5 mL RNase-free tube and vigorously vortexed. Following its addition to the adsorption column, the mixture underwent a one-minute high-speed centrifugation. Once the filtrate was discarded, 600 µL of rinsing buffer was introduced into the adsorption column, which was then centrifuged at high speed for 30 seconds. One more washing cycle was performed. The adsorption column was moved to a new 1.5 mL centrifuge tube, 50 µL of elution buffer was introduced, and the mixture was incubated for one minute at room temperature before rapid centrifugation for 1 minute. The extract was kept until use at -70°C.

Table 1: Primer used in this study

Gene	Primer sequence (5' to 3')	Reference
16S *rRNA*	F: CCTACGGGAGGCAGCAG	(Gou et al., 2010)
	R: GTATTACCGCGGCTGCTG	
*hly*A	F: GCAGTTGCAAGCGCTTG-GAGTGAA	(Swetha et al., 2015)
	R: GCAACGTATCCTCCAGAGT-GATCG	
iap	F: CAAACTGCTAACACAGC-TACT	(Bubert et al., 1999)
	R: TTATACGCGACCGAAGC-CAAC	

The expression levels of *L. monocytogenes* toxin genes (*hly*A and *IAP*) (Table 1) were determined by qPCR utilizing a HERA SYBR® Green RT-qPCR Kit (Willowfort) and a real-time PCR detection system (Applied Biosystems). Amplification was performed using 10 µL reaction volumes containing 0.5 µL of each primer and 1 µL of RNA. The subsequent conditions for thermal cycling were applied: reverse transcription at 55°C for 30 minutes followed by activation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 10 seconds, annealing (60°C for 16s*rRNA* and *hly*A and 58°C for *IAP*) for 30 seconds, and extension at 60°C for 30 seconds. The synthesized oligonucleotide primers (Oligo™) were utilized in this study (Table 1).

Statistical analysis

The imaging and statistical analyses were conducted utilizing Microsoft Excel software. To assess the comparative expression of target genes, the $2^{-\Delta\Delta CT}$ method was utilized in comparison with the control group. To determine the ΔCT values, the average CT values of the target genes were subtracted from those of the endogenous control gene 16s*rRNA* (Livak, Schmittgen, 2001).

RESULTS AND DISCUSSION

Preparation and characterization of PNs

The process of producing nanoemulsion involves various factors that affect the shape and size of the particles, including particle size, morphology, and size distribution. The shape and size of the particles are typically measured using HRTEM. The ProN particles, for instance, had an average length of 19.783 nm, with a narrow size distribution (polydispersity index: 0.253), indicating high homogeneity (Figure 1a). Further analysis revealed the absence of particle aggregation and uniform spherical morphology. According to the FT-IR analysis, the nanoemulsion that contained 30% propolis had vibrational frequencies ranging from 3500 to 1500 cm^{-1}. The medium C-H bending alkane vibration at 1458.50 cm-1 indicated the presence of a methylene group. Vibrations at 1364.92 cm^{-1} were attributed to phenol and alcohol groups, and vibrations at 1091.17 cm^{-1} represented secondary alcohol and showed the C-O-C bonding.

The H-bending and O-H group-extending areas vibrated at 3451.97 cm^{-1}, indicating the presence of phenol and alcohol groups. The symmetric CH_3 vibration at 2922.79 cm^{-1} suggested the presence of alkanes. These variances were primarily due to concentrations of phenol, flavonoids, and esters, and the accompanying spectrum variations were most noticeable in the fingerprint area (1800 - 600 cm^{-1}). Additionally, the N-H stretching at 1640.50 cm^{-1} indicated the presence of primary amines.

The spectral features of phenol were identified through the interaction of C-O stretching vibrations and O-H deformation in the spectral range of 1405 to 1220 cm^{-1} (with maximum absorbance at 1375 cm^{-1}). Additionally, a sequence of feeble pulses was observed between 1260 and 1180 cm^{-1}, as illustrated in Figure 1C.

PE was characterized by its self-emulsifying properties, resulting in the production of a transparent nanoemulsion. The globule size of the nanoemulsion ranged from 13 to 45 nm, while the zeta potential was measured to be less than –38 mV. The thermodynamic stability of the PESE formulation, which consists of 150 mg/mL PE, 20% castor oil, 40%–70% Kolliphor EL, and 10%–40% polyethylene

glycol (PEG) 400, was observed by Syukri et al. (2021).

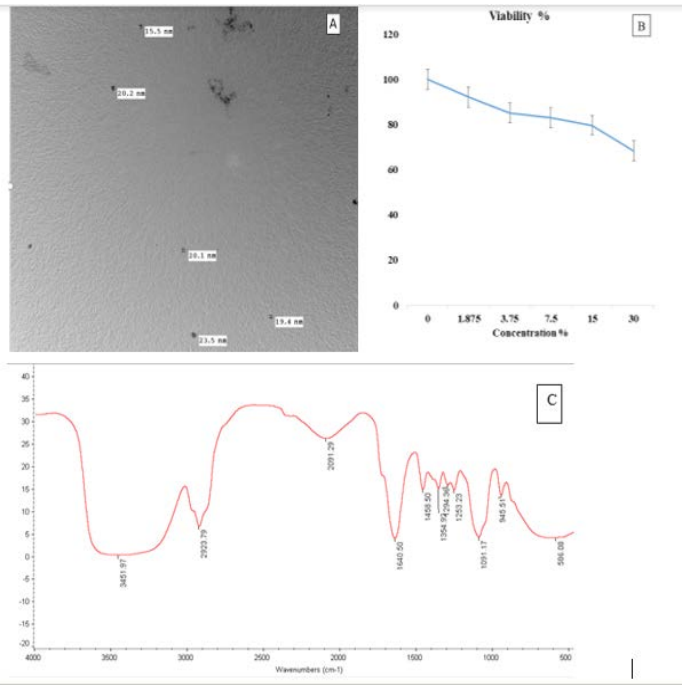

Figure 1: (a) The droplet size was determined to be 19.78 nm by the use of high-resolution transmission electron microscopy (HRTEM).)b): Cell viability % of 30% PNs effect on Vero cells. (C): FTIR of 30% propolis nanoparticles.

Toledo et al. (2015) found significant similarities in the chemical composition of propolis between both entities. The spectral peaks at 3300, 2917, 2849, 1735, 1630, 1530, 1462, 1376, 1265, 1196, 1172, and 1030 cm^{-1} were particularly noteworthy.

Antimicrobial activity of PE and PNs

After being incubated aerobically at 37°C for 24 hours, PE with a 0.1 g/mL concentration demonstrated antibacterial properties against *L. monocytogenes* in the 1/1 and 1/4 dilutions (50 and 25 mg/mL concentrations, respectively). This finding aligns with the study performed by Vică et al. (2022), which reported that 1/1, 1/4, and 1/8 dilutions exhibited inhibitory effects on *L. monocytogenes*.

Additionally, the study found that dilutions of PNs (1/1, 1/4, and 1/8) with concentrations of 150, 75, and 37.5 mg/mL, respectively, exhibited an inhibitory effect on *L. monocytogenes*.

The activity of PE and PNs on *L. monocytogenes* inoculated in breaded chicken panne

It is crucial to acknowledge that *L. monocytogenes* poses a significant risk as a processing contaminant for refrigerated food items due to its pervasive occurrence in the natural environment and its ability to thrive in cold temperatures. This result proves the relation between the risk of foodborne listeriosis and processed meat and poultry products. Figure 2 displays the effect of PE and its nanoemulsion on *L. monocytogenes* in breaded chicken panne stored in a chilled environment. The results are presented as log CFU/g. The initial counts (after 1 hour on day 0) of the control group (Group 1), chicken panne treated with PE (Group 2), and breaded chicken panne treated with PNs (Group 3) were 5.7, 5.6, and 5.5 log CFU/g, respectively.

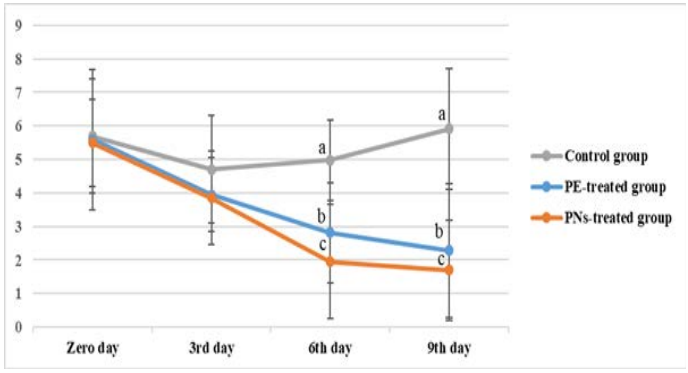

Figure 2: *L. monocytogenes* viable count (log CFU/g) of breaded chicken panni treated with PE and PNs and the control (untreated) at chilling preservation. Values are expressed as the mean ±SD. There are significant differences between points that have different letters on the same day of preservation.

During chilled storage, viable *L. monocytogenes* reduced significantly ($p < 0.05$) over time in breaded chicken panne treated with PE and PNs. On the ninth day of preservation, the count went down to 2.2 log CFU/g in the PE group and 1.6 log CFU/g (50 CFU/g) in the PNs group. It's interesting to note that using PE and PNs not only increased the shelf life of breaded chicken panne but also reduced the risk of *L. monocytogenes* (50 colony-forming units) (CFU/g or mL). This reduction brought the levels within the acceptable limits for *L. monocytogenes* in ready-to-eat foods. In the UK and European Union, the maximum limit is lower than 100 CFU/g (end-of-shelf life testing) for products on the market (El-Shenawy et al., 2011).

Additionally, the PE treatment group differed significantly from the group that received PNs on all experimental days ($p < 0.05$). Propolis exhibits greater efficacy against Gram-positive bacteria in comparison to Gram-negative bacteria as a result of the hydrolytic enzymes that are secreted by the outer membrane proteins of the latter. Consequently, these enzymes can decrease the effectiveness of propolis' active components (Sforcin and Bankova, 2011; Kędzia and Hołderna-Kędzia, 2013).

The antimicrobial traits of propolis are attributed to its chemical constituents. Resin, composed of flavonoids and

phenolic compounds, is a significant component of propolis. Candles and oils containing oleic acid, palmitic acid, and fiber of necessary oils and aromatics are also present. Pollen, which is low in amount, contains protein, free amino acids, vitamins, and minerals. Other substances, such as ketones, lactones, steroids, and sugars, are also in propolis (Değirmencioğlu, 2013). Propolis's antibacterial activity is due to specific polar and phenolic lipophilic molecules, especially flavonoid compounds. These molecules interact with bacterial cells, causing damage to their cell walls and membranes, ultimately leading to cell lysis and death. Propolis contains various highly polar and lipophilic groups such as carbonyl, electronegative, amine, imine, sulfide, thiol, methoxy, and hydroxyl groups that contribute to its antibacterial properties (Echeverría et al., 2017, Sanpa et al., 2013).

It has been found that nanoparticles (NPs) exhibit more significant antibacterial activity than propolis extract (PE) owing to their smaller size and higher surface area-to-volume ratio. These properties make them highly reactive, allowing them to avoid some of the limitations of raw propolis. NPs are generally utilized to enhance the conveyance of pharmaceutical substances to their intended targets (Afrasiabi et al., 2020).

*hly*A and *IAP* genes expression assay

The findings from the study on the comparative expression of *L. monocytogenes hly*A and *IAP* genes in breaded chicken panne with PE and PNs at inhibitory levels then preserved by chilling have been presented in Figure 3. The expression of both genes was notably decreased ($p < 0.05$) in the groups that received treatment in comparison with the untreated (control) group. Additionally, the PNs group showed a more significant decrease in gene expression than the PE group ($p < 0.05$). On day 0, the relative expression of the *hly*A gene was 0.90 and 0.80 in the PE and PNs groups, respectively. By the ninth day, the expression levels were 0.25 in the PE group and 0.12 in the PNs group. Similarly, on day 0, the relative expression of the *IAP* gene in the PE and PNs groups was 0.91 and 0.81, respectively. By the ninth day, it had decreased to 0.29 in the PE group and 0.12 in the PNs group.

The study suggests that propolis reduces gene expression by binding to bacterial proteins and peptides, changing their 3D designs, inhibiting cell division, enzymatic inactivation, and protein synthesis (Bouarab-Chibane et al., 2019). In addition, the flavonoids' B ring may impair bacterial systems and reduce their function, inhibiting nucleic acid synthesis (Uzel and Sorkun K, 2005; Uzel et al., 2005).

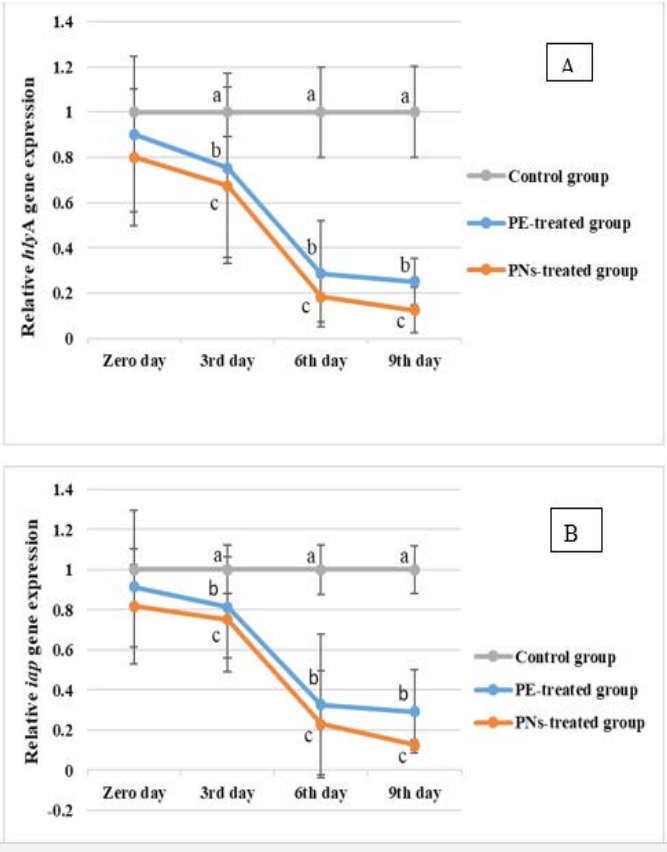

Figure 3: (A). Relative *hly*A gene expression of *L. monocytogenes* in breaded chicken panni treated with PE and PNs groups and control group at chilling preservation. Values are expressed as the mean ±SD. There are significant differences between points that have different letters on the same day of preservation. (B). Relative *iap* gene expression of *L. monocytogenes* in breaded chicken panni treated with PE and PNs groups and control group at chilling preservation. Values are expressed as the mean ±SD. There are significant differences between points that have different letters on the same day of preservation.

Sensory evaluation

Figure 4 exhibits the findings of the sensory evaluation of chilled, stored, breaded chicken panne treated with PE and PNs. The assessment criteria included color, taste, flavor, and overall acceptability. The scores for PE were 8.3, 7.75, 7.8, and 7.5 for color, taste, flavor, and overall acceptability, respectively. Similarly, the scores for PNs were 7.9, 6.3, 6, and 6.1, respectively. The treatment of chicken fillets with either PE or PNs did not significantly affect the mean rating for overall product acceptability, color, flavor, and taste relative to the breaded chicken panne. This result aligns with the results of Mahdavi-Roshan et al. (2022), who discovered that marinated chicken breast samples treated with propolis extract and refrigerated were not qualitatively different from fresh specimens. On the 6th day, the odor changed faster in the control group than in the experimental groups, possibly due to protein degradation. The use of PE and PNs prolonged this process. This result follows a

study by Mahdavi-Roshan et al. (2022), which found that PE can increase the longevity of marinated chicken breast when preserved at a chilled temperature.

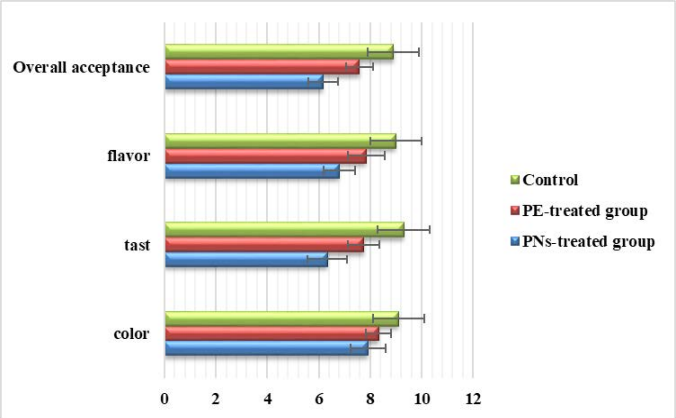

Figure 4: The average score of taste, color, flavor, and overall acceptability of breaded chicken panni treated with PE and PNs groups and control group at chilling preservation. Values are expressed as the mean ±SD.

CONCLUSION

Controlling the existence of *L. monocytogenes* in food products is of the highest priority for ensuring food safety. Natural bioactive substances such as PE and PNs effectively inhibit *L. monocytogenes* growth and toxin production. These promising results offer the potential for developing natural product-based biocontrol techniques to control *L. monocytogenes* growth in chicken and chicken products. Therefore, our research supports using natural and bio-based products as adequate disease controls in the food industry.

RECOMMENDATION

Additional research is necessary to ensure that propolis is considered a clean-label product, maintains its shelf life, and does not compromise consumer health.

ACKNOWLEDGMENTS

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NOVELTY STATEMENT

Propolis exhibited antimicrobial properties against several pathogenic microorganisms. However, its application is limited in food systems owing to its strong taste and low solubility. Numerous studies have demonstrated that, with some modifications, it could be utilized in food systems. The current research found that PE and PNs are potent inhibitors of *L. monocytogenes* growth and/or toxin generation. At the same time, PNs were more potent against *L. monocytogenes* growth and its toxin expression.

AUTHORS CONTRIBUTION

Aalaa Saad designed the experiment and the gene expression calculation. Taghreed Ali performed the bacteriological work of the investigation. Nayerah Alatfeehy performed the MIC. Dalia Elmasry performed the preparation and characterization of nanoemulsions. All authors participated in writing and revising the entire manuscript.

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