Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (10) Issue (6) (2025), (21-35) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg
Veterinary Medical Sciences

Enhancing the Chilling Stability of Chicken Fillets: The Role of Capsulated vs. Nanoparticle forms of Propolis-Infused Pectin Edible Coating.

MennatAllah H.Elsheshtawy, Saad M.Saad, Abu Bakr M.Edris and Hemmat M.Ibrahim
Food Hygiene and Control Dept., Faculty of Veterinary Medicine, Benha University, Egypt
E-mail: menna.hatem@fvtm.bu.edu.eg

Abstract
Poultry meat is highly perishable and characterized by short shelf life. Due to growing concerns regarding the safety and side effects of synthetic preservatives, this study investigated the effect of propolis-infused pectin-based edible coating on the chicken fillets chilling stability. This research explored the application of propolis in different forms, ethanolic extract, capsulated, and nanoparticles, within pectin edible coatings. Chicken fillets were coated, stored under refrigeration, and evaluated for chemical, microbiological, and sensory properties over 18 days. Results demonstrated that all coated samples exhibited significant reductions in Total Volatile Based Nitrogen (TVMN), Thiobarbituric Acid Reactive Substances (TBARS), pH, and microbial growth fluctuations compared to uncoated controls. The propolis was analyzed for phenolic and flavonoid content by using Gas Chromatography–Mass Spectrometry (GC-MS) device and surprisingly the phenolic compounds were much higher than flavonoid ones indicating high antibacterial effect, additionally, the High-Resolution Transmission Electron Microscopy (HRTEM) was used for the nanoparticles to measure size (19.7nm) while functional substances measured by Fourier Transform Infrared Spectroscopy (FTIR) device. The coatings effectively suppressed total aerobic plate count, Enterobacteriaceae, mold and yeast count with the nanoparticle formulation. Sensory evaluations revealed superior color, texture, and odor stability in treated samples, with the Propolis nanoparticles (PN2%) retaining the best organoleptic properties. Cooking loss analysis further supported the protective role of propolis coatings in moisture retention. Among the treatments, the nanoparticle form showed the most potent preservation effects, maintaining acceptable sensory attributes and microbial safety during storage duration, outperforming Propolis capsule (PC5%) and Propolis Ethanolic Extract (PEE10%) formulations.

Keywords: Propolis, Capsulated, Nanoparticles, Pectin and Edible coating

Introduction

Due to its high nutritional content and lower cost when compared to other meats, the consumption of poultry meat has steadily increased over the past few decades **(1).** However, raw chicken exhibits a limited shelf life attributable to its protein and moisture content, elevated pH levels, and aerobic storage conditions, which facilitate rapid microbial proliferation and lipid oxidation **(2).** Preventing microbial growth and delaying lipid oxidation can increase the shelf life of poultry meat. To achieve prolonged safety and extend shelflife approaches, the poultry industry has employed various packaging strategies . In response to manufacturer and consumer demands to increase the shelf life of food products, active edible coatings have garnered increased attention recently **(3).** Coating meat with edible ingredients can improve organoleptic qualities, decreased cooking losses, and preserve meat quality throughout storage time. Furthermore, many coating materials are widely available, reasonably priced, and biodegradable **(4).** Biopolymers from biomass (chitosan, starch, casein, pectin, and collagen), biobased monomers (polylactide as well as different polyesters), and polymers of origin from bacteria (marine algae cellulose, microbial cellulose, curdlan,and xanthine) can all be used to create edible coatings. Proteins from animal or plant sources, lipids, and polysaccharides continue to be the most widely used ingredients in the creation of edible packaging **(5).** Recently, it has been noted that adding bioactive compounds to edible biopolymers may enhance product's functional quality and increase its shelf life **(6).** Pectin is a water-soluble polysaccharide found in plant cell walls that is used extensively in the food industry as an emulsifier, gelling agent, and stabilizer **(7).** Pectin is a great option for creating edible coatings that prolong the shelf life of food because it is widely available in nature, has low toxicity, and selectively permeabilizes oxygen and carbon dioxide **(8).** Additionally, pectin-based coatings have the ability to preserve hydrophilic materials, immobilize water molecules, and incorporate active ingredients like extracts and essential oils to improve nutritional value **(9),** besides its cost-effectiveness, easy accessibility, and superior biodegradability **(10).** Utilizing the antimicrobial qualities of pectin in biodegradable coatings represents a health-conscious strategy, offering an alternative to preservatives that may have adverse effects **(11).** Propolis is one of the natural preservatives used in biopreservation, which represents a potent tool in sustainable food preservation **(12).** Propolis, a resinous substance produced by Apismellifera L. bees, is used to keep insects and microbes out of the hive **(13).** About 60% of it, is made up of resin,

with the remainder being broken up into waxes, essential oils, vitamins, along with microelements **(14).** Propolis is created by the accumulation of resinous and balsamic materials found in pollen, flours, branches, and leaves. These materials combine to form enzymes and salivary secretions in bees **(15).** Ethanolic extracts, often prepared at concentrations such as 10% (w/v), are the most commonly utilized form of propolis **(16).** The remaining co-product is typically thrown away or used to improve animal feed. Although adding propolis extract to food is an alternative method to prolong shelf life, its strong flavor poses a challenge because of the phenolic compounds' disagreeable taste, which restricts their use at higher concentrations, and their lipophilic nature, which is evident in their limited solubility in water **(17).** Therefore, to effectively utilize propolis as a food additive, strategies are needed to mitigate its strong flavor **(18)** and address its poor water solubility. Encapsulating propolis in different organic wall materials to conceal flavors and aromas while preserving their qualities is one of the most widely used and commercially successful techniques for this kind of application **(19).** Another cutting-edge method for resolving these issues is nanotechnology. A natural nanomaterial called nano-propolis has potential applications in food preservation . Due to their smaller size and increased surface area, nanoparticles can exhibit enhanced efficacy; for instance, the antibacterial and antifungal properties of nano-propolis are superior to those of propolis extract **(20).**

Despite extensive studies on propolis as a natural preservative, limited research has compared the effects of encapsulated versus nanoparticle forms of propolis when incorporated into pectin-based edible coatings for meat preservation. Therefore, the objective of the present study was to evaluate and compare the impact of ethanolic extract, capsulated, and nanoparticle forms of propolis incorporated into pectin edible coatings on the physiochemical, microbial and sensory properties of chilled chicken fillets.

2. Material and Methods

2.1. Propolis samples

Propolis samples were collected from honey bee hives in Benha, Al-Qaluobia, Egypt, during the spring and summer of 2024. Propolis samples were cleaned, stripped of wax, paint, and wood, then crushed into small pieces and placed in a sterile container.

2.2. Preparation of propolis ethanolic extract (PEE)

A shaker incubator was used to shake a volumetric flask with 100 milliliters (ml) of 70% ethanol and 10 grams (g) of propolis vigorously for five hours at room temperature **(21).** The supernatant was transferred to a new container for future use after the final extract was allowed to cool to room temperature and centrifuged for five minutes at 1500 rpm. The excerpt was filtered through the Whatman paper filter No. 1 after being stored at -20ºC until the experiments started. The samples were filtered and sterilized using a 0.45 µm filter before the extracts were added **(22).**

2.3. Preparation of propolis nanoparticles (PN)

Propolis nanoparticles (PNs) were produced at the Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Egypt's Nanomaterials Research and Synthesis Unit. For 30 minutes, 10 mL of Propolis Extract PEE), 10 mL of tween 80, and 80 mL of distilled deionized water were homogenized in a 1500-watt homogeneous blender (Model: 400ELPC, PRO Scientific Inc., 01-02411ELPC HOMOGENIZER, USA) at 18,000 rpm for 30 min in the presence of an ice water bath to reduce the temperature of the mixture.The mixed oil phase was then gradually supplemented with distilled water **(23).**

2.4. Preparation of Propolis capsule (PC)

A homogenizer was used to thoroughly mix the 80 ml alginate solution with 20 mL of propolis extract after 1% (w/v) sodium alginate had been dissolved in 30% (v/v) ethanol solution. The ionic gelation process was used to create the microcapsules. By completely dissolving 80 ml sodium alginate (SA) in ultrapure water, a certain concentration of SA was created. After that, it was uniformly combined with 20 ml PEE at a specific ratio. The aforesaid solution was then dripped into a specific calcium chloride concentration using an encapsulator (B-390, BUCHI Co., Ltd., Flawil, Switzerland) at a frequency of 150 Hz and an electrode voltage of 250 V while the pressure was 150 mbar. After fully gelating, the resulting beads were filtered and dried for five hours at 40 °C after being cleaned to eliminate any remaining ionized water from the surface" after that we dissolve the obtained capsule in 30% ethanol to form solution **(24)**

2.5. Identification and Quantification of Bioactive Compounds of PEE Using GC-MS

The analysis took place in Center of Excellence in Screening of Environmental Contaminants, faculty of Veterinary Medicine, Benha University. The Agilent GC-MS/MS-7000D (Agilent Technologies, Santa Clara, CA, USA) with Agilent J&W, GC-MS column HP-5Ms (15 m × 250 mm × 0.25 µm) was used to perform the GC-MS analysis. In split-less mode, the injector temperature was 280 °C, and the carrier gas was helium flowing at a rate of 1 mL/min. After being maintained at 60 °C for 4

minutes, the oven temperature was raised to 150 °C for 15 minutes at a rate of 10 °C per minute. The mass spectra were optimized using the following parameters: the transfer temperature was set to 150 °C and the source temperature was set to 280 °C. The scan range was 35–500 Da, and the solvent delay time was 2 min. Ultimately, the temperature was increased to 310 °C. The GC ran for 40.5 minutes in total. The chemicals were identified by matching their mass spectra to information from the National Institute of Standards and Technology (NIST) library.

2.6. Determination of propolis nanoparticles characterization

Propolis nanoparticles were characterized at the Faculty of Agriculture, Cairo University. High-resolution transmission electron microscopy (HRTEM; JEM 1400F, JEOL Ltd., Japan) was used to evaluate particle size, morphology, and distribution. Fourier-transform infrared spectroscopy (FTIR; Thermo Nicolet 380, USA) equipped with Attenuated Total Reflectance (ATR) was utilized to analyze the chemical composition **(25).**

2.7. Purchasing of chicken fillets samples

About 3 kg of the chicken breast fillets (CBF) were purchased from the local market in Benha, Al-Qaluobia, Egypt, which sells freshly slaughtered chicken carcasses. They were transferred hygienically and rapidly to the laboratory in which they were divided aseptically into pieces.

2.8. Preparation of coating formulas

The coating was prepared using a modified version of the **(26)** method. Three grams of pectin (E440) were dissolved in 99 milliliters of distilled water with 0.5% (v/v) acetic acid solution (pH 5.8) with continuous stirring at 95 °C for 2 hours. . The solution was filtered through Whatman No. 3 filter paper, followed by the addition of 1 mL polyethylene glycol (PEG; MW 400, Sigma–Aldrich). After homogenization, respective propolis forms (PEE10%, PC5%, or PN2%) were incorporated into the coating solutions.

2.9. Coating and storage of chicken fillet.

The samples were divided into four groups, each weighing roughly 700±5 g, and each group was divided into pieces weighting about 30 g, 2 × 2.5 × 2 cm chicken cubes. The first group is known as control one (C), Propolis extract (PEE10%) was administered to the second group, propolis capsule (PC5%) to the third, and propolis nanoparticles (PN2%) to the fourth. For one minute, the samples in each treated group were submerged in the treated coating solutions. To achieve a consistent coating on the muscle's surface, the pieces were dipped in the various coating solutions for an additional minute. The samples were then permitted to drip extra solution. After being coated, the samples were dried for 15 minutes at 25 °C. For fifteen minutes, the coated samples were air-dried at 25°C. Each group's samples were put into sterile plastic bags, hermetically sealed, and kept chilled for up to 18 days (until spoiled) with examination every 3 days. **Each experiment was conducted in triplicate.**

2.10. Quality indexes

2.10.Chemical assessment

2.10.1. Total Volatile Based Nitrogen (TVB-N)

TVB-N was estimated using the microtitration method **(27).** Results were expressed as mg N/100 g of meat.

2.10.2. Lipid Oxidation

Thiobarbituric Acid Reactive Substances (TBARS) were determined by measuring malondialdehyde (MDA) formation using spectrophotometry at 532 nm **(28).** Results were expressed as mg MDA/kg sample.

2.10.3. pH

Five grams of homogenized sample were mixed with 50 mL of distilled water, and pH was measured using a calibrated digital pH meter (Hanna Instruments HI4522).

2.11. Microbiological analysis

The samples were analysed on the zero, 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, and 18^{th} days of storage to determine total aerobic plate count (APC) (using Plate Count Agar), Enterobacteriaceae count (using Violet Red Bile Glucose Agar), and total count of mold and yeast (using Sabouraud Dextrose Agar media) according to **(29).** Serial dilutions were prepared using 1% peptone water, and 1 mL was plated in duplicate.

2.12.Sensory Evaluation

Each sample was evaluated by a panel of five experts. Each group received 10 g of chicken breast fillet, and they were asked to score the fillet's color, texture, and odor. The panelists were not informed of the experiment's methodology, and the samples were coded using random numbers., Sensory evaluation of the samples was carried out using a 9-point hedonic scale, where 1 represented 'dislike extremely' and 9 represented 'like extremely'. Each sensory parameter—including color, texture, flavor, aroma, and overall acceptability—was scored individually by the panelists using this scale. according to **(30).**

2.13.Cooking loss

The cooking loss of a sample of chicken fillets was determined following **(29)** method. After being weighed prior to cooking, the chicken sample (15 g) was heated in a water bath for 20 minutes at 80 °C until the internal temperature reached 70 °C, then cooled, and

reweighed. . The percentage weight loss was calculated to determine the cooking loss.

2.14.Statistical Analyses

The data was statistically analyzed using Graph Pad Prism 8.0.2 and two-way analysis of variance (ANOVA) at $p < 0.05$ with Tukey's HSD post-hoc test was performed. **(31).** Results are presented as mean ± SD of three triplicates.

3. Results

3.1. Identification and quantification of bioactive compounds of PEE using GC-MS

Fig. 1. Gas chromatography–mass spectrometry (GC-MS) chromatogram of significant propolis compounds.

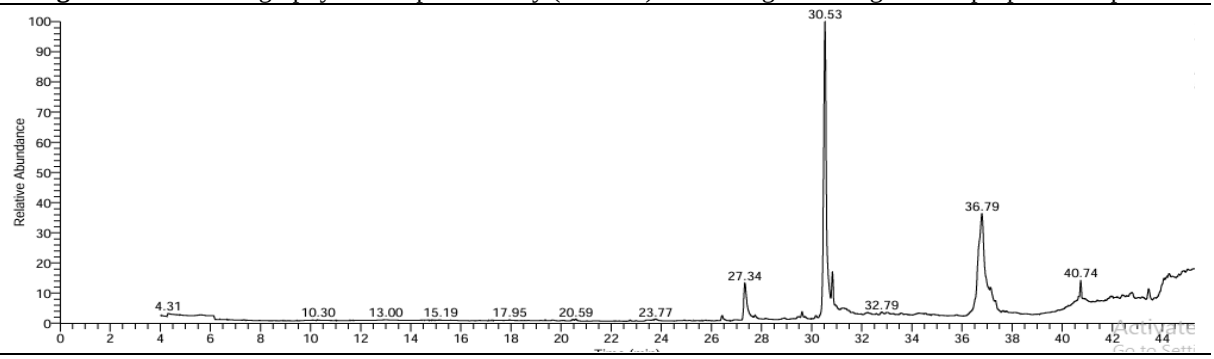


Table 1. GC-MS analysis used to identify bioactive compounds in propolis extract (mean ± SD, n = 3.0)

Retention Time (min)	Compound Name	Chemical Class	Molecular Formula	Area %	Known Bioactivity
30.53	Oleic acid (cis-9-octadecenoic acid)	Unsaturated fatty acid	C18H34O2	50.22%	Antibacterial, anti-inflammatory, antioxidant
36.8	Lupeol	Triterpenoid	C30H50O	25.80%	Anti-inflammatory, antimicrobial, antioxidant
27.33	Palmitic acid (n-hexadecanoic acid)	Saturated fatty acid	C16H32O2	7.76%	Antibacterial, emollient, antioxidant
30.83	9-Octadecenoic acid, methyl ester	Fatty acid ester	C19H36O2	2.77%	Antioxidant, emollient
40.74	Cholestan-3-ol, 2-methylene-, (3α,5α)-	Sterol derivative	C28H48O	2.48%	Membrane stability, cholesterol metabolism
29.62	9,12-Octadecadienoyl chloride (Z,Z)-	Fatty acid derivative	C18H31ClO	0.90%	Preservative, antimicrobial potential
26.42	Methyl 14-methylpentadecanoate	Fatty acid ester	C17H34O2	0.82%	Antioxidant
6.04	Benzenemethanol, α-[1-(methylamino)ethyl]-	Phenolic-like aromatic alcohol	C10H15N	0.87%	Potential antibacterial, phenolic activity
40.85	Arachidonic acid, methyl ester	Polyunsaturated fatty acid	C21H34O2	0.91%	Anti-inflammatory, antioxidant

Figure 1. illustrates Gas chromatography-mass spectrometry (GC-MS) analysis of the ethanolic propolis extract identified a complex mixture dominated by unsaturated fatty acids, triterpenoids, and a small fraction of aromatic phenolic-like compounds. **Table 1.** Shows the bioactive compounds of the propolis as the most abundant component was oleic acid (cis-9-octadecenoic acid), accounting for 50.22% of the total ion chromatogram at a retention time (RT) of 30.53

minutes. The second most prominent compound was lupeol, a bioactive triterpenoid, representing 25.80% of the extract (RT = 36.80 min). Additional fatty acids such as palmitic acid (n-hexadecanoic acid, 7.76%), linoleic acid methyl ester (~2.77%), and arachidonic acid methyl ester (0.91%) were also present.

Minor constituents included aromatic and phenolic-like compounds such as benzenemethanol, α-[1-(methylamino)ethyl]- (0.87%) and other low-abundance heterocyclic or substituted benzenoid structures detected between RT 6.04 and 6.13 minutes. However, no classical flavonoid compounds (e.g., quercetin, apigenin, chrysin) were detected in this GC-MS profile—likely due to their non-volatility and thermal instability under the conditions used.

The low flavonoid content in this analysis suggests that the antioxidant activity of this particular propolis extract may be less dependent on polyphenols and more attributed to the presence of unsaturated fatty acids and terpenoids, such as oleic acid and lupeol. While these compounds possess moderate antioxidant properties, they are generally less potent than typical flavonoids in free radical scavenging and metal ion chelation

3.2. Characterization of propolis nanoparticles

Fig. 2: (a) The droplet size was determined by the use of high-resolution transmission electron microscopy (HRTEM). (b): FTIR of 2% propolis nanoparticles.

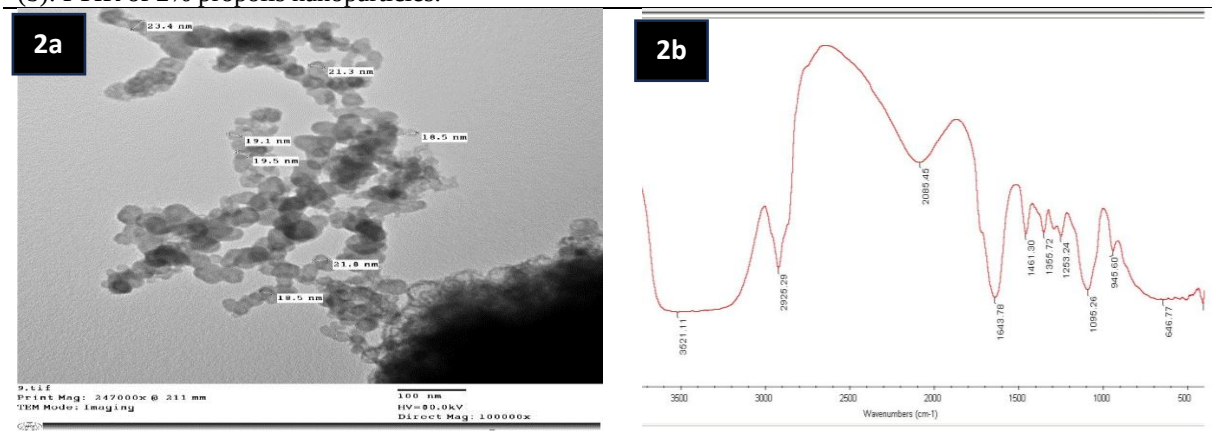
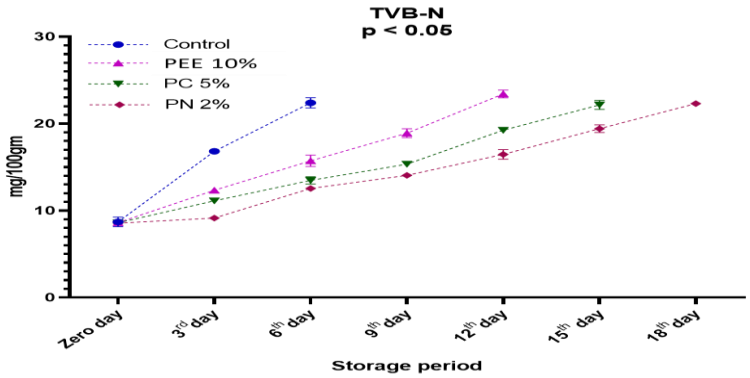


Figure 2a. illustrated that propolis nanoparticles had a mean size of 19.783 nm with a polydispersity index of 0.253, indicating high homogeneity. High-Resolution Transmission Electron Microscopy (HRTEM) analysis confirmed their spherical shape with no aggregation. **Figure 2b.** illustrated Fourier Transform Infrared Spectroscopy (FT-IR) analysis which revealed characteristic functional groups such as hydroxyls, carbonyls, and alkanes, indicating the presence of flavonoids, phenolic compounds, and esters. These findings confirm the bioactive potential of nanopropolis due to its rich organic composition and structural properties.

3.3. Chemical analysis

3.3.1. Total Volatile Based Nitrogen (TVB-N)

Fig. 3. illustrates the values of TVB-N of chicken breast fillets during chill storage



Total Volatile Based Nitrogen (TVB-N) results for coated chicken breast fillets during 18 days of chill storage has been shown in Figure 2. Based on Egyptian Organization for Standardization (EOS), the acceptable value for TVN is 20 mg/100g. On zero day, there was no significant differences between groups as all the samples are fresh ($p>0.05$). On the 3rd day, the TVB-N value of control group showed significant increase (16.82±0.25) while coated groups remained lower and scored 12.34, 11.12, 9.14 for PEE10%, PC5% and PN2%, respectively. On day 6, the control group exceeded the acceptable limit for TVBN and scored 22.4 mg/100g, whereas PN2% remained the most stable, showing the strongest preservative effect. By day 9, PC5% and PN2% exhibit significantly lower TVB-N levels compared to control and PEE10% ($p < 0.05$). .

3.3.2. Lipid Oxidation

Fig. 4. illustrates the values of TBARS of chicken breast fillets during chill storage

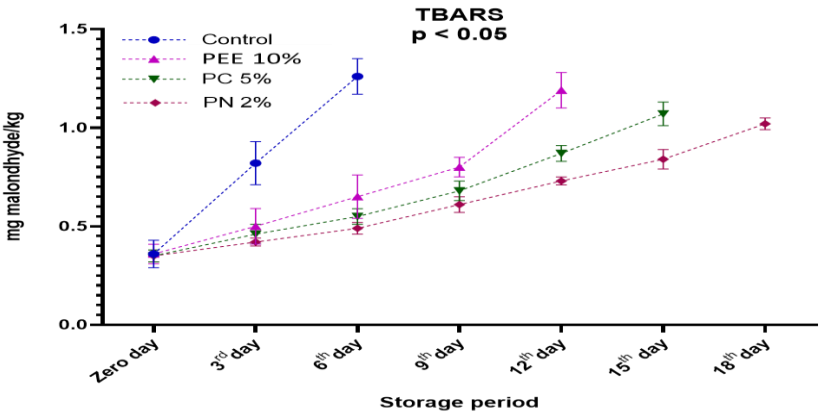
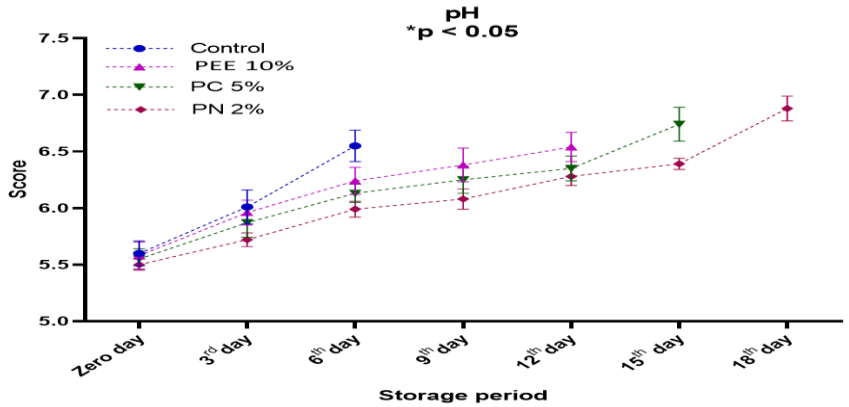


Figure 3. illustrate the oxidative stability of the treated groups. According to the Egyptian standards, the acceptable limit of TBA shouldn't exceed 0.9 mg malondhyde/kg. TBARS values increased in all samples during storage, with the control group exceeding the acceptable limit of 0.9 mg MDA/kg by day 6. Coated samples showed delayed oxidation: PEE10% and PC5% exceeded the limit by days 9 and 12, respectively, while PN2% maintained values below the limit up to day 15 (0.84 mg MDA/kg) and reached 1.02 mg MDA/kg by day 18.

3.3.3. pH

Fig. 5. illustrates the values of pH of chicken breast fillets during chill storage

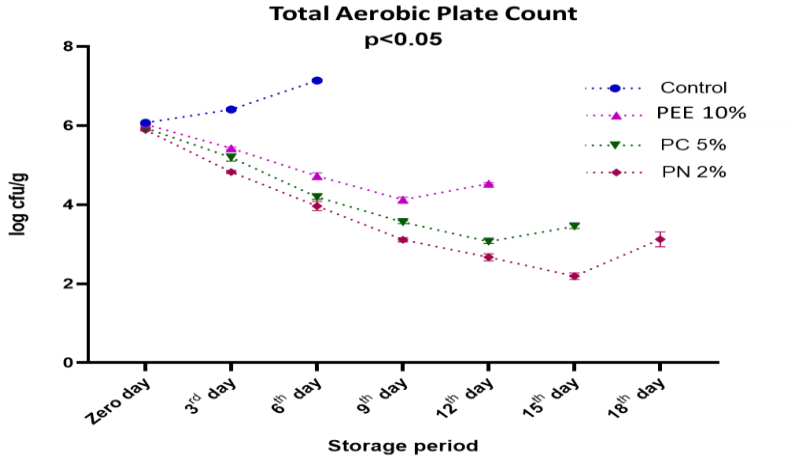


All groups started with comparable pH values on day 0. By day 3, the control group reached 6.01 ± 0.15, while PEE10%, PC5%, and PN2% remained at lower pH values (5.96, 5.87, and 5.72, respectively). The control spoiled by day 9, while PEE10% and PC5% maintained acceptable pH until days 12 and 15, respectively. PN2% showed superior stability, remaining within acceptable pH limits throughout the 18-day storage period. confirming its superior preservative effect. At every time point, the significant differences ($p < 0.05$) revealed that PN (2%) was the most successful in preserving pH stability.

3.4. Microbiological analysis

3.4.1. Total Aerobic Plate Count

Fig.6. APC in chicken breast fillets over 18 days of refrigerated storage. Data are presented as mean ± SD.



The statistical analysis's findings demonstrated that the main effects (treatment × storage) had a significant ($P \leq 0.05$) impact on the Total Aerobic Plate Count (TAPC). The International Commission on Microbiological Specifications for Foods (ICMFS) states that the Total Aerobic Plate Count (TAPC) changes of chicken fillet meat samples during chilling should not be greater than 7 $\log_{10}$ CFU/g. The initial TAPC ($\log_{10}$ CFU/g) of all samples, which includes the control and treatments, ranged from 6.07 to 3.12, as can be seen from the results in **Figure 6.** The TAPC of coated chicken fillet samples dropped considerably ($p \leq 0.05$) over the course of the chill storage period, and the values stayed within the advised range. On day 9 of chill storage, only the count of the control samples got close to the spoil limit. However, PEE10% and PC5% coated groups had gone bad on the 15th and 18th days of chill storage. Furthermore, by the 12th day, the chicken fillet samples coated with pectin fortified with PN2% achieved a log reduction of over 3 compared to initial Control values, indicating strong bacterial suppression. At the end of the chill storage period, these samples had the lowest ($p \leq 0.05$) TAPC of all the treatments. However, compared to all other treated groups, the control samples had the highest TAPC ($p \leq 0.05$).

3.4.2. Enterobacteriaceae

Fig. 7. Enterobacteriaceae of chicken breast fillets during refrigerated storage. Data are presented as mean ± SD.

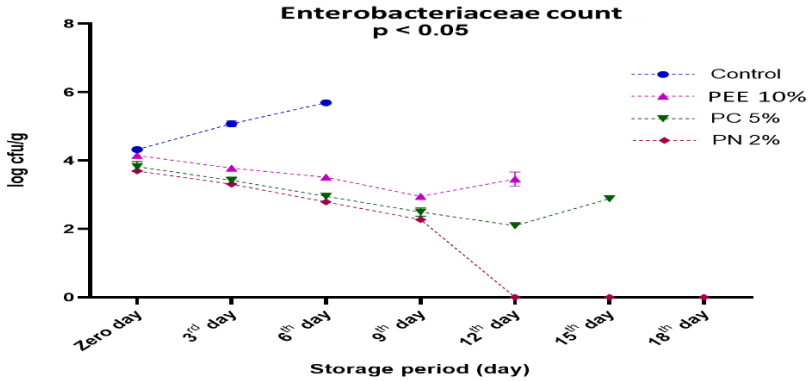
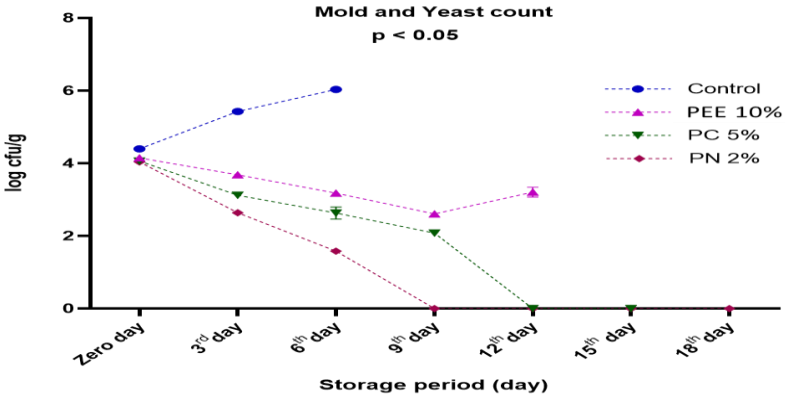


Figure 7. shows the total Enterobacteriaceae count of chicken meat fillets. Enterobacteriaceae are known as hygiene indicators and spoilage microorganisms in food. The initial number of Enterobacteriaceae was 4.32 $\log_{10}$ CFU / gm. The Enterobacteriaceae count decreased in all groups except the control on the third day of chill storage, and the control group had spoiled by the 9th day of storage. The control group consistently had the greatest Enterobacteriaceae numbers at earlier time points, substantially larger than all experimental groups at ($p < 0.05$ to $p < 0.001$), indicating the power of treatments, PEE10%, PC5% and PN2% coated groups, to suppress Enterobacteriaceae growth. PEE's10%Enterobacteriaceae count shows moderate reductions, from 3.77 ± 0.08 log CFU/g on day three to 3.45 ± 0.21 $\log_{10}$ CFU/g by day nine. In contrast, PC5% is a more effective form of treatment than PEE10%, showing reductions ranging from 3.42 ± 0.09 $\log_{10}$CFU/g on day three, to 2.88 ± 0.02 $\log_{10}$CFU/g on day 12. Lastly, recorded 3.31± 0.04 log CFU/g on day three and suppressed dramatically (non-detectable levels) by the 12th day until the end of the experiment ($p < 0.001$).

3.4.3. Mold and yeast count

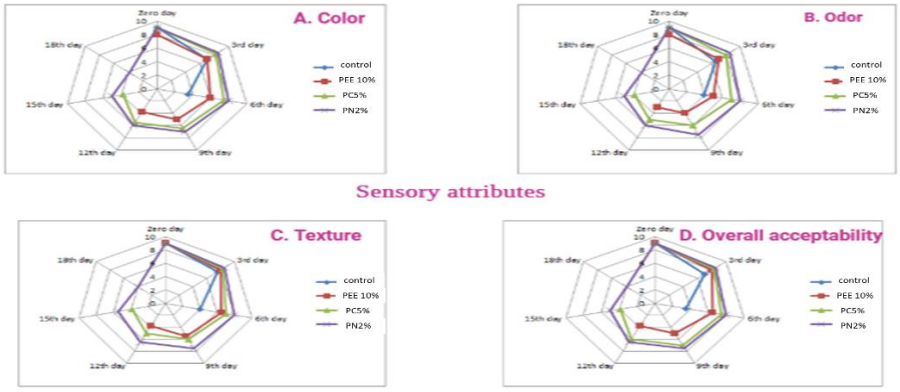
Fig. 8. Mold and Yeast count of chicken breast fillets during chill storage. Data are presented as mean ± SD



According to the figure 8, the untreated chicken fillet samples (control) had the highest mold and yeast counts ($P < 0.05$) after being chilled stored for 0, 3, and 6 days, recorded 4.39, 5.42, and 6.03 $\log_{10}$cfu/g, respectively, until spoiling. This suggests that the control group had the largest significant difference among all treated groups ($p < 0.05$ to $p < 0.001$). With the exception of the PE10% group, there was a notable difference on day zero between the control and the other treated coated samples. The group treated with propolis ethanolic extract (10%) demonstrated a significant suppression of mold and yeast from day 0 (4.25 $\log_{10}$cfu/g) till day 9 (2.60 $\log_{10}$cfu/g) and then increased slightly by day 12 (3.20 log10cfu/g), possibly as a result of microbial regrowth ($p < 0.05$), until spoilage on the 15th day of chill storage. From day 0 (4.06 log10cfu/g) to day 9 (2.08 log10cfu/g), the Propolis Capsule (5%) coated group exhibits a consistent inhibition effect on mold and yeast growth ($p < 0.05$), until they are no longer detectable by day 12, at which point they spoiled on day 18. Lastly, from the ninth day until the end of the chill storage period, the PN2% treated group exhibits the most notable and quick inhibition effect on fungal growth, eventually becoming undetectable.

3.5. Sensory evaluation

Fig. 9. Sensory evaluation



Significant variations in color, odor, texture, and general acceptability over the course of the storage period were found in the sensory evaluation results between the groups (Figure. 9). The control group experienced the most noticeable color deterioration, going from 9±0.2 on day 0 to 3.5 ± 0.2 on day 6 then becoming visually unacceptable by day 9. Conversely, PEE10%and PC5% demonstrated moderate protection, scoring 5± 0.7 and 6.5± 0.3 by day 9, while PN2% maintained noticeably better color stability, achieving 8± 0.3on day 6 and 7± 0.4 on day 9. The control group's odor scores fell sharply from 9± 0.3 on day 0 to 3.9± 0.4 by day 6, but PEE10% andPC5% maintained scores between 5.0 and 6.0 until day 12. The best odor retention was shown by PN2%, which outperformed the control by a significant margin, maintaining scores at 7.5± 0.2 by day 9 and 6.5± 0.2 by day 12. The control group's texture deterioration trended similarly, going from 9± 0.2 to 4± 0.2 by day 6, while PC5% and PEE 10%scored between 5± 0.2 and 7± 0.2 on day 9, indicating moderate preservation. With a score of 7.5± 0.2 on day 6 and 6.5± 0.5 on day 12, PN2% notably showed superior texture stability, indicating its improved capacity to postpone structural degradation. In terms of overall acceptability, PEE10% andPC5% scored about 6 until day 12, while the control group dropped below the acceptability threshold by day 6 (4.0). Propolis nanoparticles (PN2%) achieved scores of 8.5± 0.2 on day 3, 7.5± 0.2 on day 9, and 6.5± 0.2 on day 12, , on the other hand, consistently outperformed all other treatments, indicating prolonged sensory stability.

3.6. Cooking loss

Fig. 10. The cooking loss

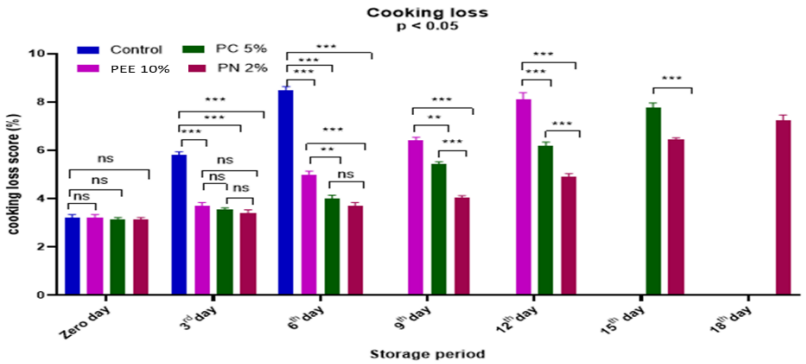


Figure 10. illustrates the outcome of each group's cooking loss over the chill storage period. Since all of the samples were fresh, there were no significant differences (p>0.05p) between the groups on zero day. On the third day of chill storage, all groups showed significant differences, with the exception of PC and PN, which were statistically similar. On day 6, PC and PN started to statistically differ (p<0.05), while the control group increased dramatically to 8.5±0.11 and remained significantly higher than all treatments (p<0.001). At every time point from day 9 until the experiment's conclusion, PN2% cooking loss was significantly lower than PEE10% and PC5% (p<0.05).

4. Discussion

Food safety is a major issue for the economy and public health. According to **(32)**, one out of every ten people contract food poisoning each year as a result of contaminated food. **The European Commission (33)** predicted that providing high-quality, safe, and healthy food will become more

difficult in the coming decades, as nutrition and food safety have an impact on food systems' health outcomes. As a result, natural preservatives with antibacterial activity are increasingly being used in food processing plants **(34).**

The study's findings indicate that propolis-infused pectin edible coatings, particularly in nanoparticle form, effectively enhanced the chilling stability of chicken fillets by improving chemical, microbiological, and sensory quality over 18 days of refrigerated storage. The TVBN values for the control group exceeded the acceptable limit on day 6 of the experiment, while those of the other treated groups remained stable, particularly the PE2% group, which began to decline on day 18. The metabolism of bacteria in food causes enzymatic autolysis as well as lipid and protein oxidation, resulting in the decomposition of protein and non-nitrogen components of meat Protein degradation over time leads to an increase in organic amines like TVB-N **(35)**. These findings are consistent with **(36)**, who found that coating chicken fillets with an ethanolic extract of propolis combined with chitosan coating might delay the rise in TVBN content. There was also a substantial positive relationship between the TVN and pH, as an increase in volatile amine generated an increase in pH **(37)**, which is revealed in this study. **(38)** found that adding propolis extract lowers TVN levels of both chicken patties and marinated chicken, pointing to improve preservation and freshness during storage. This can be explained as Propolis' antimicrobial properties help to reduce TVN by inhibiting the growth of spoilage bacteria, which are responsible for the increase **(39)**.

Additionally, the findings found that propolis treatment at any form could significantly reduce TBARS formation which confirmed the findings of **(36)** study on the effect of various concentrations of propolis in combination with chitosan on the quality of chicken breast fillets. **(39)** found that chicken coated with Carboxymethyl Cellulose (CMC) and ethanolicpropolis extract at 2%, 3%, and 4% levels had lower ($p \leq 0.05$) TBARS values than chicken breast meat coated with CMC alone at each storage interval. Based on earlier research, adding propolis extract at various concentrations (200–400 mg/kg) significantly decreased TBARS results and improved the meat's oxidative stability **(40)**. In a similar vein, propolis extracts have been successfully used to stop oxidative alterations in meat products. This decrease might result from propolis's phenolic and flavonoid components and their consequent impact on TBARS and free radicals. As a result, lipid oxidation secondary products such TBARS dropped **(37)**. The fact that coated chicken breast flesh does not oxidize lipids when stored in a cold environment may also be explained by the pectin coating's poor permeability. Despite this, the interaction between pectin molecules and water in high-moisture foods, such meat, causes the coating film's oxygen permeability and penetration to increase during storage. In these situations, preventing oxidation in the coated samples may depend on the proper concentration of phenolic compounds and free radical scavengers added to pectin solutions.

On the other hand, the pH variations between the coated and uncoated samples (controls) may be explained by the narrow range of proteolytic changes in muscle proteins that cause the chilled chicken to gradually become alkaline,while the other coated treatment groups remained stable for a longer period of time after that, with variations depending on the kind of propolis used, the pH of the control samples surpasses the allowable limit and becomes undesirable organoleptically by day 9. In contrast to chicken patties without propolis extract, a prior study by **(41)** discovered that adding propolis extract to the patties improved their storage qualities by lowering their total bacterial count and total volatile basic nitrogen (TVBN) concentration. The decrease in microbial growth and TVBN content, which inhibit proteolysis and alter microbiological growth, indicates that propolis aids in keeping a more constant pH during chill storage, which is essential for preserving the safety and quality of meat, even though the study did not evaluate pH directly. The observed pH variations in stored chicken breast flesh may be caused by propolis's antibacterial and antioxidant qualities, which inhibit proteolysis and alter microbiological growth. Additionally, as the length of storage rose, so did the pH values of the coated and uncoated chicken breast flesh samples. The pH readings of the uncoated samples (control) increased more over the storage period than those of the coated samples treated with PEE10%. This may occur as a result of ammonia buildup and the breakdown products of amino acids, which raise pH **(٤٢)**. Throughout the chill storage period, the pH was significantly affected ($p \leq 0.05$) by the addition of ethanolicpropolis extract (EPE) to the carboxymethyl cellulose (CMC) coating as compared to uncoated control samples **(٤١)**. The creation of peptides, amino acids, and ammonia as a result of elevated protease activity (cathepsins B, calpains, and L peptidases) or microbial development may be connected to an increase in the pH values of kept meat (**٤٣**). It showed be mentioned that the antioxidant effect of the propolis in this study is limited and less than the antibacterial ones due to the low flavonoid contents approved by the chemical profile of the propolis carried on by the GC-M.

The antimicrobial performance of propolis-based edible coatings—particularly in their nanoparticle form—was clearly demonstrated by the suppression of total aerobic count,

Enterobacteriaceae, and mold/yeast during chilled storage. This preservation correlates with the chemical profile of the propolis extract revealed by GC-MS analysis, which identified **oleic acid (50.22%)**, **lupeol (25.80%)**, and other unsaturated fatty acids and triterpenoids as dominant constituents. These compounds are widely reported to possess membrane-disrupting, enzyme-inhibiting, and anti-metabolic properties against a broad spectrum of microorganisms **(44).** These findings largely concurred with the work of **(39)**, which verified that the total aerobic plate count (TAPC) in chicken breast meat samples was not significantly impacted by the coating treatment with ethanolic propolis extract (EPE). However, as compared to uncoated control samples, EPE was successful in preventing microbial growth. **(39)** justified their findings by pointing out that all coating materials have limited gas permeability, which can effectively prevent the formation of aerobic organisms. Not only does propolis extract have antibacterial qualities due to its polyphenolic content, but it also inhibits the synthesis of DNA and RNA by changing the membranes of microorganisms **(٤٤)**. This theory is supported by propolis' ability to hydrolyze the peptidoglycan layer that encircles bacteria's cytoplasmic membrane **(36)**. These effects are driven by the synergistic action of lipophilic fatty acids (e.g., oleic and palmitic acid) and terpenoids (e.g., lupeol), which target bacterial membranes, disrupt ergosterol synthesis in fungi, and inhibit microbial DNA replication **(45).**

Additionally, our results are in line with those of **(46)**, who found that minced beef coated with a polylactic acid (PLA) film containing propolis ethanolic extract continued to be edible for at least 11 days without exhibiting any adverse organoleptic characteristics.

Salmonella species, Escherichia coli, fecal coliforms, and coliforms are all members of the Enterobacteriaceae family. High quantities of Enterobacteriaceae could be utilized to assess hygiene standards and serve as an indicator for fecal contamination **(47)**. In his research on the impact of propolis on meatball quality, **(48)** found that the initial Enterobacteriaceae count was high for both the propolis treatments (5.58 log CFU/g) and the control (5.77 log CFU/g), and that there was no significant difference ($P>0.05$) between the treatments. The propolis treatment, however, demonstrated a significant ($P<0.05$) decrease in the Enterobacteriaceae count later on, with 1.18 log CFU/g (17.8%) on day 5 and 2.24 log CFU/g (31.9%) on day 7. According to **(36)**, chitosan-coated beef patties with 2% propolis extract demonstrated antibacterial efficacy against the coliform bacteria. **(38)** found that marinating chicken kebap with water propolis extract inhibits the growth of Escherichia coli.

According to (45), propolis has strong antifungal activity against a variety of yeast strains, including Candida albicans, with minimum inhibitory concentrations (MIC) ranging from 1.56 to 400 µg/mL. The **antifungal activity** observed, especially in PN2%, aligns with literature describing the effects of **propolis-derived polyphenols and terpenoids** on **mold and yeast growth**, by targeting fungal mitochondria and blocking respiratory enzymes **(49).** According to their research, this study demonstrates the powerful effect of propolis, particularly the nanoparticles, on the inhibition of mold and yeast. Propolis's polyphenol content helps it interfere with the metabolism of microorganisms, which successfully lowers the amount of mold and yeast in food products **(50)**. Another study published by **(49)** found that introducing propolis into chicken diets can drastically modify the microbial flora in the gastrointestinal tract, resulting in lower yeast and mold levels in meat.

The organoleptic evaluation of the study samples indicated that PN2%-treated fillets retained superior color, odor, and texture scores compared to PC5% and PEE10%, and remained acceptable throughout the storage period. previous reports showing that using propolis in conjunction with the pectin coating is superior to using it alone **(٥٠, ٤٦).** Additionally, cooking loss was lowest in PN2%-treated samples, indicating better water retention and muscle integrity.

These findings can be explained by the fact that there is a positive correlation between the cooking loss and the degree of myofibrillar protein deterioration, as denaturation of myofibrillar proteins during the thermal process causes structural mutations and the loss of sarcoplasmic fluid from muscle fibers. In particular, the temperature range of 70°C to 100°C caused structural alterations and the removal of sarcoplasmic fluid from muscle fibers **(29)**, which contributed to the development of cooking loss in chicken meat.

Various compounds and mixtures can be prepared using capsules to improve their stability and shelf life, cover up their taste and odor, solubilize otherwise insoluble compounds, and release the encapsulated compound or mixture gradually over a longer period of time or at the desired time, among other benefits **(51)**. Nevertheless, despite all of these benefits, encapsulation frequently prevents some of the compounds' actions, such as lowering their cytotoxicity, antibacterial activity, and immediate antioxidant activity **(52)**. As a result, the encapsulation of somse substances needs to be precisely tailored to the intended use. Several researchers discovered that propolis capsules exhibited higher inhibitory effects than their extracts **(53)**. The antibacterial activity of

encapsulated propolis was dependent on the mass ratio of propolis to wall material, which in turn depended on the fraction of wall material utilized for encapsulation **(54)**.

Despite all these advantages of propolis capsule (PC), propolis nanoparticles (PN) are still the most effective form **(55)**. The superior efficacy of propolis nanoparticles (PN2%) over encapsulated (PC5%) and ethanolic extract (PEE10%) forms is attributed to their nanoscale size (~19.78 nm) and high surface area-to-volume ratio, which enhances the delivery and local concentration of bioactives at microbial contact points. This facilitates rapid membrane permeabilization, intracellular leakage, and interaction with nucleic acids and ATP-dependent processes **(55).** The nano-form not only improves dispersibility in aqueous systems but also protects active compounds from oxidative degradation during storage **(52).**

These findings highlight that the combination of pectin with propolis nanoparticles provides both preservation and functional benefits, consistent with prior work using bioactive edible coatings

5. Conclusions

This study demonstrated that pectin-based edible coatings infused with propolis, particularly in nanoparticle form (PN2%), significantly enhanced the chilling stability of chicken fillets during refrigerated storage. The application of propolis coatings resulted in reduced lipid oxidation (TBARS), lower pH increases, and suppressed microbial proliferation, including aerobic bacteria, Enterobacteriaceae, molds, and yeasts. Sensory attributes such as color, odor, and texture were better preserved in treated samples, with PN2% consistently maintaining the highest acceptability scores throughout the 18-day storage period. Furthermore, GC-MS analysis of the ethanolic extract confirmed the dominance of bioactive compounds such as oleic acid and lupeol, which contributed to the antimicrobial performance observed. The HRTEM and FTIR analyses supported the nanoscale structure and functional integrity of the PN2% coating, affirming its superior preservative efficiency.

Given these results, this work highlights the potential of propolis-infused pectin coatings particularly in nanoparticle form as a natural and effective alternative to synthetic preservatives in poultry preservation. Future studies are recommended to evaluate the application of this coating system under commercial processing conditions and against a wider range of foodborne pathogens.

6. Acknowledgement

The authors extend their appreciation to Mohebat A. Abd El- Aziz, Researcher of Food Hygiene Department, Animal Health Research Institute, Shibin-Elkom branch, Agriculture Research Centre, Egypt for her efforts during research conceptualization.

7. Conflict of interest

The authors declare no conflicts of interest.

8. Authors contributed

Authors contribution equally to the study.

9. Ethical approval

BUFVTM 03-05-25

10. References

[1] Noori, S., Zeynali, F., &Almasi, H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (Zingiberofficinale) essential oil and its effect on safety and quality attributes of chicken breast fillets. *Journal of Food Control*, 84, 312–320.2018

[2] Hakeem, M. J., Feng, J., Nilghaz, A., Ma, L., Seah, H. C., Konkel, M. E., & Lu, X. Active packaging of immobilized zinc oxide nanoparticles controls Campylobacter jejuni in raw chicken meat. *Journal of Applied and Environmental Microbiology*, 86(22). e01195-01120. 2023

[3] Panea, B., Ripoll, G., Gonzalez, ´ J., Fern´ andez-Cuello, A., ´ &Albertí, P.Effect of nanocomposite packaging containing different proportions of ZnO and Ag on chicken breast meat quality. *Journal of Food Engineering*, 123, 104–112.2014

[4] Abdollahzadeh, M., Elhamirad, A. H., Shariatifar, N., Saeidiasl, M., & Armin, M. Effects of nano-chitosan coatings incorporating with free/nano-encapsulated essential oil of Golpar (Heracleumpersicum L.) on quality characteristics and safety of rainbow trout (Oncorhynchusmykiss). *International Journal of Food Microbiology*, 385, Article 109996.2023

[5] Hassan, B., Chatha, S. A. S., Hussain, A. I., Zia, K. M., & Akhtar, N. Recent advances on polysaccharides, lipids, and protein-based edible films and coatings: A review. International *Journal of Biological Macromolecule*s, 109, 1095–1107. 2018

[6] Quirós-Sauceda, A. E., Zavala, J. F. A., Olivas, G., & González-Aguilar, G. A. Edible coatings as encapsulating matrices for bioactive compounds: A review. *Journal of Food Science and Technology*, 51(8), 1674–1685. 2014

[7] Nguyen, T. T. T., Ho, H. T., Hoang, D., Nguyen, Q. A. P., & Tran, T. Van. Novel films of pectin extracted from ambarella fruit peel and jackfruit seed slimy sheath: Effect of ionic crosslinking on the properties of pectin film. *Journal of Carbohydrate Polymers*, 334, 122043. 2024

[8] Panahirad, S., Dadpour, M., Peighambardoust, S. H., Soltanzadeh, M., Gullon, ´ B., Alirezalu, K., & Lorenzo, J. M. Applications of carboxymethyl cellulose-and pectin-based

active edible coatings in preservation of fruits and vegetables: A review. *Journal of Trends in Food Science & Technology*, 110, 663–673. 2021

[9] Wei, H., &Pascall, M. A. Evaluation of structural and functional properties of citrus pectin film enriched with green tea extract. *Journal of Polymer Engineering & Science*, 63, 2522–2533.2023

[10] Dong, M., Tian, L., Li, J., Jia, J., Dong, Y., Tu, Y., and Duan, X. Improving physicochemical properties of edible wheat gluten protein films with proteins, polysaccharides and organic acid. *Journal of Lebensmittel-Wissenschaft&Technologie*, 154, Article 112868. 2022

[11] Aala, J., Ahmadi, M., Golestan, L., Shahidi, S.-A., &Shariatifar, N. Effect of multifactorial free and liposome-coated of bay laurel (Laurusnobilis) and rosemary (Salvia rosmarinus) extracts on the behavior of Listeria monocytogenes and Vibrio parahaemolyticus in silver carp (Hypophthalmichthysmolitrix) stored at 4◦C. *Journal of Environmental Research*, 216, Article 114478.2023

[12] Muthuvelu, K. S., Ethiraj, B., Pramnik, S., Raj, N. K., Venkataraman, S., Rajendran, D. S., &Muthusamy, S. Biopreservative technologies of food: An alternative to chemical preservation and recent developments. *Journal of Food Science and Biotechnology*, 32(10), 1337-1350.2023

[13] Freitas, A. S., Barth, O. M., & Luz, C. F. P. Brownish propolis from the Atlantic coastal areas in the state of Rio de Janeiro. Brazil: a palynological approach. *Journal of Revista Brasileira de Botanica*, 33 , 343-354.2010

[14] Matos, V. R., Alencar, S. M., & Santos, F. A. R. Pollen types and levels of total phenolic compounds in propolis produced by Apismellifera L. (Apidae) in an area of the semiarid region of Bahia, Brazil. *Journal of Anais da Academia Brasileira de Ciências*. 86(1):407-418.2014 -

[15] Lustosa, S. R., Galindo, A. B., Nunes, L. C. C., Randau, K. P., &Neto, P. J. R. Divulgaç~ aoPropolis: atualizaçoessobre a química e a farmacologia. ~ Brazilian *Journal of Pharmacognosy*, 18, 447-454.2008

[16] Heimbach, N. S., Itavo, C. C. B. F., Itavo, L. C. V., Franco, G. L., Leal, C. R. B., Leal, E. S., et al. Residue from extraction of brown propolis in the diet to ruminants: in vitro digestibility and gas production. *Journal of Archivos de Zootecnia*, 63, 259- 267.2014

[17] Sforcin, J.M. Biological Properties and Therapeutic Applications of Propolis. *Journal of Phytotherapy Research*, 30, 894–905.2016

[18] Mello, B.C.B.S.; Petrus, J.C.C.; Hubinger, M.D. Concentration of flavonoids and phenolic compounds in aqueous and ethanolicpropolis extracts through nanofiltration. *Journal of Food Engineering*., 96, 533–539.2010

[19] Šturm, L.; OsojnikCrnivec, I.G.; Isteniˇc, K.; Ota, A.; Megušar, P.; Slukan, A.; Humar, M.; Levic, S.; Nedovi´c, V.; Kopinˇc, R. Encapsulation of non-dewaxed propolis by freeze-drying and spray-drying using gum Arabic, maltodextrin and inulin as coating materials. *Journal of Food Bioproducts. Process*. 116, 196–211.2019

[20] Salama, S.A.; Atta, R.R.; Khalil, E.M.; Abdelaleim, Y.F.; Abd-Eltawab, S.; Farghali, A.A.; Essam, D.; Alkhalifah, D.H.M.; Hozzein, W.N.; Mahmoud, R. Nano Propolis, Zinc Oxide Nanoparticles, and Their Composites: A Novel Green Synthesis with Synergistic Antioxidant and Anticancer Properties. *Journal of Composites Science.* 7, 480.2023

[21] Ghavidel, F., Javadi, A., Anarjan, N., &Jafarizadeh-Malmiri H. New approach in process intensification based on subcritical water, as green solvent, in propolis oil in water nanoemulsion preparation. *Journal of Green Processing and Synthesing*,10(1), 208-218. 2021

[22] Said, S. A., Khan, S. A., Ahmad, I., & Ali, H. S. Chemical composition of Egyptian and UAE propolis. *Pakistan journal of pharmaceutical sciences*, 19(1), 58-61.2006

[23] Rao, J., &McClements, D. J. Formation of flavor oil microemulsions, nanoemulsions and emulsions: influence of composition and preparation method. *Journal of agricultural and food chemistry*, 59(9), 5026-5035.2011

[24] Deng L, Xu R, Zhang S, Lu J, Wang H, Zhou J, Zhang C, Golding J, Jiang W, Wang B. Calcium alginate-encapsulated propolis microcapsules: Optimization, characterization, and preservation effects on postharvest sweet cherry. *International Journal of Biological Macromolecules*. 282(6):137473. 2024

[25] Emil, A. B., Hassan, N. H., Ibrahim, S., Hassanen, E. I., Eldin, Z. E., & Ali, S. E. Propolis extract nanoparticles alleviate diabetes-induced reproductive dysfunction in male rats: antidiabetic, antioxidant, and steroidogenesis modulatory role. *Scientific Reports*, 14(1), 30607. 2024.

[26] Mahardiani, L., Larasati, R., Susilowati, E., Hastuti, B., &Azizah, N. L. Potential edible coating of pectin obtained from banana peel for fruit preservation. *Journal of Physics*: Conference Series,1912,1-012019.2021

[27] Abelti, A. L. Microbiological and chemical changes of Nile Tilapia (*Oreochromis niloticus* L.) fillet during ice storage: Effect of age and sex. *Advance Journal of Food Science and Technology*, 5, 1260–1265. 2013.

[28] Li, R., Cai, L., Gao, T., Li, C., Zhou, G., & Ye, K. Comparing the quality characteristics and bacterial communities in meatballs with or without blown pack spoilage. *Journal of Food Science and Technology*, 130, Article 109529.2020

[29] Li, C., Wang, D., Xu, W., Gao, F., & Zhou, G. Effect of final cooked temperature on tenderness, protein solubility and microstructure of duck breast muscle. *Journal of Food Science and Technology*, 51(1), 266–274.2013

[30] Kandil, A. A., Aly-Aldin, M. M., &Allam, A. Y. Quality characteristics of processed low-fat beef sausage as affected by chickpea protein isolates prolonged cold storage. *Journal of Food and Dairy Sciences*, 11(12), 363-368.2020

[31] Greenhouse, S. W. &Geisser, S. "On methods in the analysis of profile data". *Journal of Psychometrika*. 24: 95–112.1959

[32] Lee, H., & Yoon, Y. Etiological agents implicated in foodborne illness worldwide. *Journal of Food Science of Animal Resources*, 41(1), 1–7.2021

[33] The European Commission. Farm to Fork Strategy for a fair, healthy and environmentally friendly food system. Retrieved June 14, 2022, from https://ec.europa.eu/food/horizontal-topics/farm-fork-strategy

[34] Elsabagh, R., Ibrahim, S. S., Abd-Elaaty, E. M., Abdeen, A., Rayan, A. M., Ibrahim, S. F., &Morsy, M. K. Chitosan edible coating: a potential control of toxic biogenic amines and enhancing the quality and shelf life of chilled tuna filets. *Journal of Frontiers in Sustainable Food Systems*, 7, 1177010.2023

[35] Ruelas-Chacon, X., Aguilar-González, A., de la Luz Reyes-Vega, M., Peralta-Rodríguez, R. D., Corona-Flores, J., Rebolloso-Padilla, O. N., & Aguilera-Carbo, A. F. Bioactive protecting coating of guar gum with thyme oil to extend shelf life of tilapia (Oreoschromisniloticus) fillets. *Journal of Polymers*, 12(12), 3019.2020

[36] Jafari, N., Kargozari, M., Ranjbar, R., Rostami, H., &Hamedi, H. The effect of chitosan coating incorporated with ethanolic extract of propolis on the quality of refrigerated chicken fillet. *Journal of Food Processing and Preservation*, 42(1), e13336.2018

[37] Mehdizadeh, T., &Langroodi, A. M. Chitosan coatings incorporated with propolis extract and Zataria multiflora Boiss oil for active packaging of chicken breast meat. International *Journal of Biological Macromolecules*, 141, 401–409.2019

[38] Mahdavi-Roshan, M., Gheibi, S., &Pourfarzad, A. Effect of propolis extract as a natural preservative on quality and shelf life of marinated chicken breast (chicken Kebab). *Journal of Lwt, Food Science and Technology, 155*, 112942.2022

[39] El Sheikha, A. F., Allam, A. Y., ElObeid, T., Basiouny, E. A., Abdelaal, A. A., Amarowicz, R., & Oz, F. Impact of a carboxymethyl cellulose coating incorporated with an ethanolicpropolis extract on the quality criteria of chicken breast meat. *Journal of Antioxidants*, 11(6), 1191.2022

[40] Haščík, P., Elimam, I. O., Kročko, M., Bobko, M., Kačániová, M., Garlík, J., ... & Saleh, A. A. The influence of propolis as supplement diet on broiler meat growth performance, carcass body weight, chemical composition and lipid oxidation stability. Acta Univ. Agric. et Silvic. Mendel. Brun, 63, 411-418.2015

[41] Lim, Y. H., Park, G. T., & Choi, J. S. Effects of Propolis Extract on Quality and Storage Characteristics of Chicken Patty. *Korean Journal of Poultry Science*, 50(4), 251-260.2023

[42] Chew, S. F., &Ip, Y. K. Excretory nitrogen metabolism and defence against ammonia toxicity in air-breathing fishes. *Journal of fish biology*, 84(3), 603-638.2014

[43] Singh, P. K., Shrivastava, N., &Ojha, B. K. Enzymes in the meat industry. In Enzymes in food biotechnology (pp. 111-128). Academic Press.2019

[44] Almuhayawi, M. S. Propolis as a novel antibacterial agent. *Saudi journal of biological sciences*, 27(11), 3079-3086.2020

[45] Kerek, Á., Csanády, P., Tuska-Szalay, B., Kovács, L., &Jerzsele, Á. In Vitro Efficacy of Hungarian Propolis against Bacteria, Yeast, and Trichomonasgallinae Isolated from Pigeons—A Possible Antibiotic Alternative?.*Journal of Resources*, 12(9), 101. 2023

[46] Shavisi, N., Khanjari, A., Basti, A. A., Misaghi, A., &Shahbazi, Y. Effect of PLA films containing propolisethanolic extract, cellulose nanoparticle and Ziziphoraclinopodioides essential oil on chemical, microbial and sensory properties of minced beef. Meat science, *Journal of Meat Science*,124, 95-104.2017

[47] Halkman, H., & Halkman, A. Indicator organisms. In: Batt, C.A., Tortorello, M.L. (Eds.), *Encyclopedia of Food Microbiology*. 2nd ed., pp. 358–363. Academic Press. 2014.

[48] Gedikoğlu, A. Antimicrobial and antioxidant activities of commercialized Turkish propolis extract, and application to beef meatballs. *Turkish Journal of Agriculture - Food Science and Technology*, 10, 2021–2029. 2022.

[49] Saeed, M., Arain, M. A., Kamboh, A. A., Memon, S. A., Umar, M. M., Rashid, M.,

Babazadeh, D., Abd El-Hack, M. E., &Alagawany, M. Raw Propolis as a Promising Feed Additive in Poultry Nutrition: Trends and Advances. *Journal of Animal Health Production*, 5(4).2017

[50] Duman, M., &Ozpolat, E. Effects of water extract of propolis on fresh shibuta (Barbusgrypus) fillets during chilled storage. *Journal of Food Chemistry*, 189, 80–85.2015

[51] Munin, A., & Edwards-Lévy, F. Encapsulation of natural polyphenolic compounds; a review. *Journal of Pharmaceutics*, 3(4), 793-829.2011

[52] Ilhan-Ayisigi, E., Ulucan, F., Saygili, E., Saglam-Metiner, P., Gulce-Iz, S., &Yesil-Celiktas, O. Nano-vesicular formulation of propolis and cytotoxic effects in a 3D spheroid model of lung cancer. *Journal of the Science of Food and Agriculture*, 100(8), 3525-3535.2020

[53] Hegazi, A. G., El-Houssiny, A. S., & Fouad, E. A. Egyptian propolis 14: Potential antibacterial activity of propolis-encapsulated alginate nanoparticles against different pathogenic bacteria strains. *Journal of Advances in Natural Sciences: Nanoscience and Nanotechnology*, 10(4), 045019.2019

[54] Jansen-Alves, C., Maia, D. S., Krumreich, F. D., Crizel-Cardoso, M. M., Fioravante, J. B., da Silva, W. P., &Zambiazi, R. C. Propolismicroparticles produced with pea protein: Characterization and evaluation of antioxidant and antimicrobial activities. *Journal of Food Hydrocolloids*, 87, 703-711.2019

[55] Afrasiabi, S., Pourhajibagher, M., Chiniforush, N., Bahador, A. Propolis nanoparticle enhances the potency of antimicrobial photodynamic therapy against Streptococcus mutans in a synergistic manner. Scientific Reports., 10:15560. 2020