Innovative use of chitosan/ZnO NPs bio-nanocomposites for sustainable antimicrobial food packaging of poultry meat

Innovative Use of Chitosan/ZnO NPs Bio-nanocomposites for Sustainable Antimicrobial Food Packaging of Poultry Meat.

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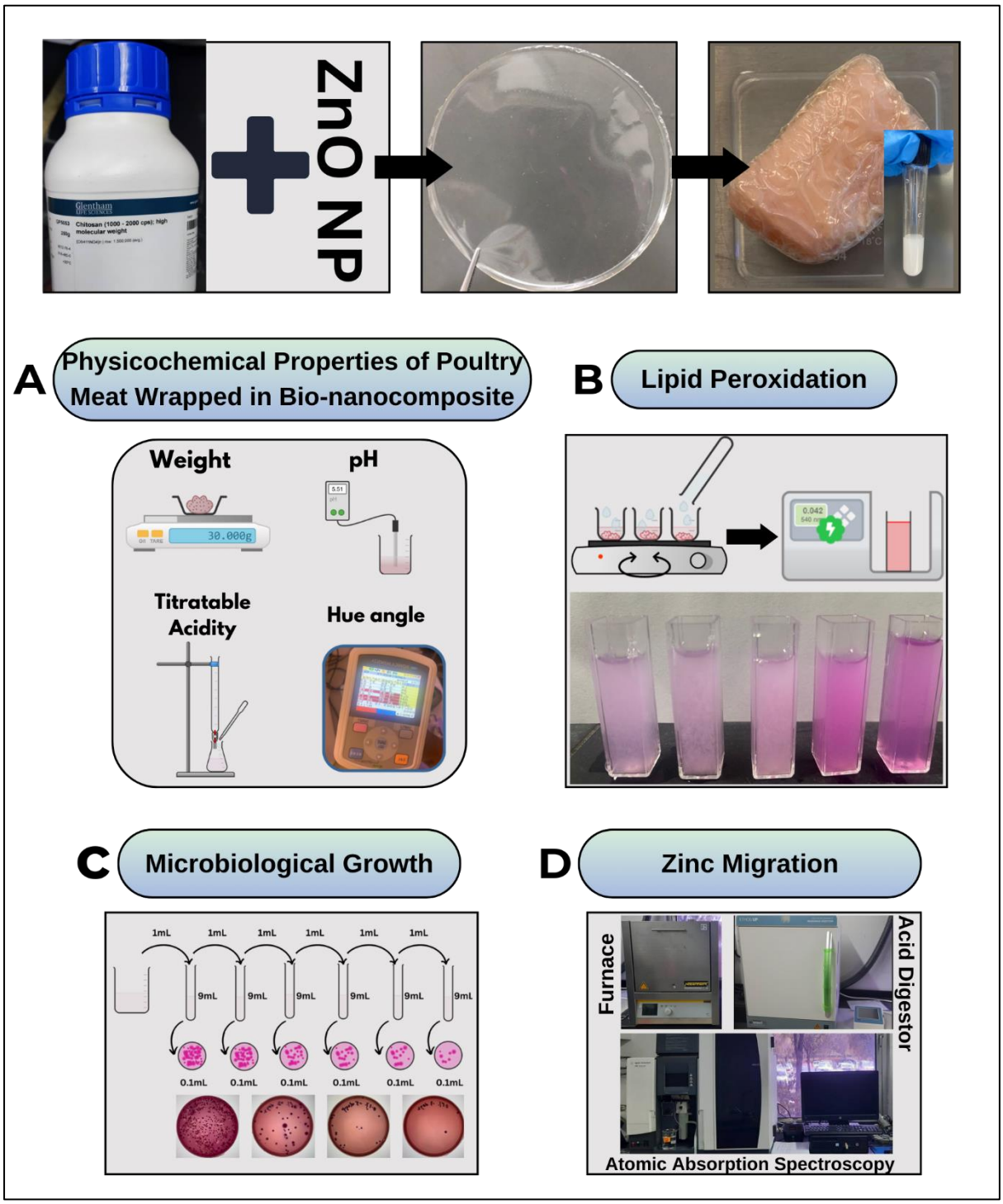
Abstract:

A novel nanocomposite was developed by integrating zinc oxide nanoparticles (ZnO NPs) into chitosan (CS) matrix and investigated for its impact on the quality and shelf life of refrigerated poultry meat over 11 days. Physicochemical properties including weight, pH, titratable acidity, color, thiobarbituric acid reactive substances assay, microbiological growth studies encompassed total psychotropic and mesophilic aerobic microorganisms, *Enterobacteriaceae* analyses, and zinc migration levels were conducted to determine the optimal nanocomposite concentration. Results revealed that bio-nanocomposite exhibited superior characteristics compared to chemogenic nanocomposite, chitosan, polyvinyl alcohol, and unwrapped meats. Bio-nanocomposite with reduced unsaturated lipid content extends poultry shelf life to 7 days in packaging, outperforming chemogenic-nanocomposites (5 days) and chitosan (4 days). This study proves that CS/ZnO NP nanocomposite is a promising active packaging material for meat, extending their shelf life without deteriorating its physicochemical characteristics and supporting sustainability, though further research on its toxicological properties is warranted.

Keywords: Biogenic Zinc oxide nanoparticles, chitosan, bio-nanocomposite, antimicrobial film, active packaging, Food security

Graphical Abstract



1. Introduction

Bio-based polymeric films in food packaging are growing rapidly as a sustainable alternative to traditional plastic materials (Marzlan et al., 2022). Biodegradable polymers, the next generation of plastics, have environmental advantages over petroleum-based counterparts (Souza et al., 2019). Biopolymer films, while holding promise as materials suitable for various packaging applications, are constrained by numerous limitations that hinder their widespread adoption and effectiveness (Kaya et al., 2022). These include insufficient mechanical strength, inferior barrier properties, water sensitivity, thermal stability, and shorter shelf life (Sobhan et al., 2021). To add value to these next-generation plastics, incorporating nanotechnology into active packaging is becoming important to improve antimicrobial and antioxidant properties and to enhance food safety and quality preservation (Souza et al., 2018). The combination of bio-based polymers and nanotechnology holds great promise for advancing sustainable food packaging (Montazer & Harifi, 2017).

Chitosan (CS), derived from chitin, is a widely available biopolymer with properties such as biocompatibility, degradability, non-toxicity, and antimicrobial activity (Freitas et al., 2022). Chitosan-based packaging, incorporating natural antimicrobial agents and metal nanoparticles like ZnO (CS/ZnO NP), is becoming popular in the food industry due to increasing demand for preservative-free options. ZnO NPs are known for their cost-effectiveness and strong antimicrobial properties (Darvishi et al., 2019). Surface modification techniques can address aggregation issues caused by their hydrophilic nature and high surface area, improving compatibility with the polymer matrix (Hajibeygi et al., 2018).

The study objective was to fabricate nanocomposites, innovatively synthesized for the first time, by employing chitosan and ZnO NPs utilizing waste banana (*Musa acuminata*) leaves. This novel circular economy approach minimizes waste generation, disconnecting economic growth from natural resource consumption. It utilizes alternative raw materials to produce high-value products like primary packaging for fresh poultry meat (Geueke et al., 2018). Nano-composite packaging by integrating biogenic and chemogenic ZnO NP into a chitosan matrix (B-CS/ZnO NP and C-CS/ZnO NP) separately offers promising potential for enhancing the preservation of poultry meat during storage. Through various physicochemical properties, including weight, pH, titratable acidity, hue angle, thiobarbituric acid reactive substances (TBARS), microbiological activity, and zinc migration. This study aims to identify the most effective combination of CS/ZnO NP active antimicrobial packaging among different concentrations. By storing the poultry meat at 4 ± 2 °C for an extended period of 11 days, insights can be gained into how each combination influences the meat's quality and safety over time. This comprehensive analysis will enable the selection of the optimal concentration of nano-composite packaging that consistently demonstrates superior performance across all evaluated parameters.

2. Materials and Methods

Sodium hydroxide (NaOH) was purchased from Labmedical Science (Malaysia), ethanol absolute was obtained from Synertec (Malaysia), trichloroacetic acid (TCA) was procured from Premier Diagnostics (Malaysia), malondialdehyde (MDA) was obtained from Medigene (Malaysia), 1,1,3,3-tetraethoxypropane (TEP) was provided from Synertec (Malaysia), Plate Count Agar (PCA) and Violet Red Bile Glucose (VRBG) were supplied by Fc. Bios Sdn Bhd (Malaysia), and nitric acid (HNO_3) was provided from GaiaScience (Malaysia). All chemicals used were either of analytical or reagent grade.

2.1 Brief Methodology on the Synthesis of Biogenic and Chemogenic ZnO NP and Development of Nanocomposite

In green synthesis, 2 g of zinc nitrate was mixed with 30 mL of *M. acuminata* leaf extract and heated at 60 °C on a hotplate until a dark brown paste formed. The paste was then calcinated at 400 °C for 2 hours using a furnace from Nabertherm, Germany, resulting in pale white ZnO NP powder. In chemical synthesis, a solution of 0.2 M zinc nitrate was combined with 0.4 M potassium hydroxide for an hour, followed by centrifugation at 5000 rpm for 20 minutes. After washing, the paste underwent calcination at 400 °C for 2 hours using a furnace from Nabertherm, Germany, yielding white ZnO NP powder (Sasidharan et al., 2023).

Preparation of CS/ZnO NP bio-nanocomposites involved dissolving 1.5% control film, chitosan (1.5 g in 100 mL deionized water from a deionizer (Favorit, Italy) in a 1% solution of glacial acetic acid under stirring for 1 hour. Biogenic and chemogenic ZnO NPs at concentrations of 0.5%, 1%, 1.5%, 2%, and 2.5% weight/weight of chitosan were then separately added, followed by degassing in an ultrasound bath (360 Watt) (Selecta, Spain) for 15 minutes. The resulting mixture was cast into a petri dish and oven-dried for 16 hours (Souza et al., 2020).

The detailed description of the methods for the synthesis, characterization, and antimicrobial properties of ZnO NPs using waste banana (*Musa acuminata*) leaves and the development of nanocomposite incorporating ZnO NP into a chitosan matrix, together with the physicochemical properties and antimicrobial efficacy has been reported elsewhere (Sasidharan et al., 2023).

2.2. Detailed Methodology on Shelf Life and Toxicological Studies of Poultry Meat Wrapped in CS/ZnO NP Nanocomposite

About 30 g of fresh poultry breast meat procured from a local wet market in Kampar district of Perak was wrapped using bio-nanocomposites (9 cm X 15 cm) prepared from different concentrations of biogenic (produced from waste *M. acuminata* leaves) and chemogenic ZnO NPs. Poultry samples were wrapped in

biogenic and chemogenic nanocomposites and stored in plastic containers, meanwhile refrigerated at 4 ± 2 °C for 11 days. The meat left unwrapped acted as the experimental control. The experiments were conducted in triplicate. Physicochemical properties including weight loss, pH, titratable acidity, and hue angle using the Association of Official Analytical Chemists method (AOAC, 2016), The thiobarbituric acid reactive substances (TBARS), microbiological growth of total psychotropic aerobic microorganisms (TPAM), total mesophilic aerobic microorganisms (TMAM), *Enterobacteriaceae* were conducted. Lastly, zinc migration analysis of the poultry meat was carried out at specified intervals (days 0, 2, 4, 7, and 11).

2.2.1. pH

2 g of the poultry sample was homogenized in 20 mL of deionized water from a deionizer (Favorit, Italy), then transferred to a beaker for pH measurement using a pH meter (Mettler Toledo, USA) at intervals of 0, 2, 4, 7, and 11-days during storage. The buffers used to calibrate pH meters typically included standard solutions at pH 4.00, pH 7.00, and pH 10.00. This procedure was replicated for poultry meats wrapped in C-CS/ZnO NP nanocomposite.

2.2.2. Titratable acidity

A 30 mL of homogenized chicken extract was titrated with 0.1 M NaOH to a light pink endpoint using phenolphthalein The measurement was taken every 0, 2, 4, 7, and 11 days of storage and the experiment was repeated on poultry meats wrapped in C-CS/ZnO NP nanocomposite. The percentage of titratable acidity was expressed as % citric acid on fresh poultry meat.

$$\text{TAC (\%)} = \frac{0.0064 \text{ X Titre x Volume made}}{\text{Volume of fresh chicken pulp x Volume of aliquot used for titration}} \text{ X } 100\%$$

2.2.3. Hue angle

The color of poultry meat was assessed using CIE-Lab* coordinates, where L ranges from 0 (black) to 100 (white), –a denotes greenness while +a indicates redness, and –b signifies blueness while +b represents yellowness using a CR 410 colorimeter from Minolta Co., Tokyo, Japan, equipped with a D65 illumination as well as a visual angle set at 10°. Measurements were performed at intervals of 0, 2-, 4-, 7-, and 11-days during storage. This procedure was also conducted for poultry meats wrapped in C-CS/ZnO NP nanocomposite. The hue angle was determined utilizing a specified equation.

$$\text{Hue angle} = \tan(b/a)^{-1}$$

2.2.4. TBARS Assay

10 g of poultry meat was mixed with 20 mL of 7.5% trichloroacetic Acid (TCA), agitated for 30 minutes, and filtrated. Then, 5 mL of the filtered solution was combined with an equal volume of 0.02 M thiobarbituric Acid (TBA) and subjected to heating in a water bath (Memmert, Büchenbach, Germany) for 30 minutes at 80 °C, and the absorbance at 530 nm was measured using GENESYS 180 UV–vis spectrophotometer (Thermo Scientific). The TBARS index was calculated using a calibration curve with known MDA concentrations dissolved in TEP solution (0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1.0 M). Measurements were conducted at intervals of 0, 2, 4, 7, and 11-days during storage, with the experiment repeated for poultry meats wrapped in CS/chemogenic ZnO NP nanocomposite. The findings were presented in terms of mg MDA/kg of meat (Souza et al., 2020).

2.2.5. Determination of the Microbiological Growth of Poultry meat wrapped in biogenic and chemogenic-nanocomposite using the Viable Cell Colony Count Method

1 mL of poultry samples wrapped with different concentrations of ZnO NPs was added into 9 mL of 0.8% NaCl solution and further proceeded for serial dilution. From the serial dilutions, samples of TMAM and TPAM were plated on PCA and incubated at 30 °C for 72 h or 7 °C for 168 h whereas *Enterobacteriaceae* was plated on VRBG and at 30 °C for 24 h. Measurements were conducted at intervals of 0, 2, 4, 7, and 11 days during storage, with the experiment repeated for poultry meats wrapped in C-CS/ZnO NP nanocomposite. The count of viable microbial colonies was determined, and findings were presented as log CFU (colony forming units)/g of meat (Souza et al., 2020).

2.2.6 Total Migration of Zinc

A 50 g sample of poultry meat was subjected to mineralization in a furnace (Nabertherm, Germany) at 550 °C for 90 minutes. The resulting 0.3 g of ash residue was then combined with 10 mL of concentrated nitric acid and subjected to acid digestion using a microwave digestor (Milestone Ethos Up, USA). The resulting yellow liquid was diluted by a factor of 100 and subsequently used to determine the zinc concentration in the packaged chicken meat employing atomic absorption spectrometry (Zeenit 700, Analytikjena, Jena, Germany) on both days 0 and 11. The zinc content was initially measured before wrapping the chicken, and then again on the 11th day after wrapping with nanocomposites. This procedure was replicated for poultry meats wrapped in C-CS/ZnO NP nanocomposite. The results were expressed in units of mg Zn/kg fresh meat (A.G. Soares Silva et al., 2023).

2.3. Statistical Analysis

One-way ANOVA analysis was conducted in SPSS 22, and Post hoc analysis was performed using the Tukey test to further examine any significant differences. Statistical significance was defined as $p < 0.05$.

3. Result and Discussion

The assessed meat exhibited an increase in weight loss, pH levels, and hue angle (H*) over its shelf life, accompanied by a decline in titratable acidity. The elevated presence of unsaturated fats, which are susceptible to oxidation, along with the proliferation of aerobic spoilage microorganisms (TPAM, TMAM, and *Enterobacteriaceae*), renders poultry meat highly susceptible to spoilage. Meat exposed without packaging exhibited more significant changes compared to biofilm-enclosed meat (Silva et al., 2018). The physical changes of the meat in different packaging for 11 days are shown in **Fig. 1**. The changes in weight loss, pH levels, hue angle (H*), titratable acidity, and TBARS of B-CS/ZnO NP, C-CS/ZnO NP, chitosan, polyvinyl alcohol (PVA), and unwrapped meat are stated in **Table 1** and **Table 2**.

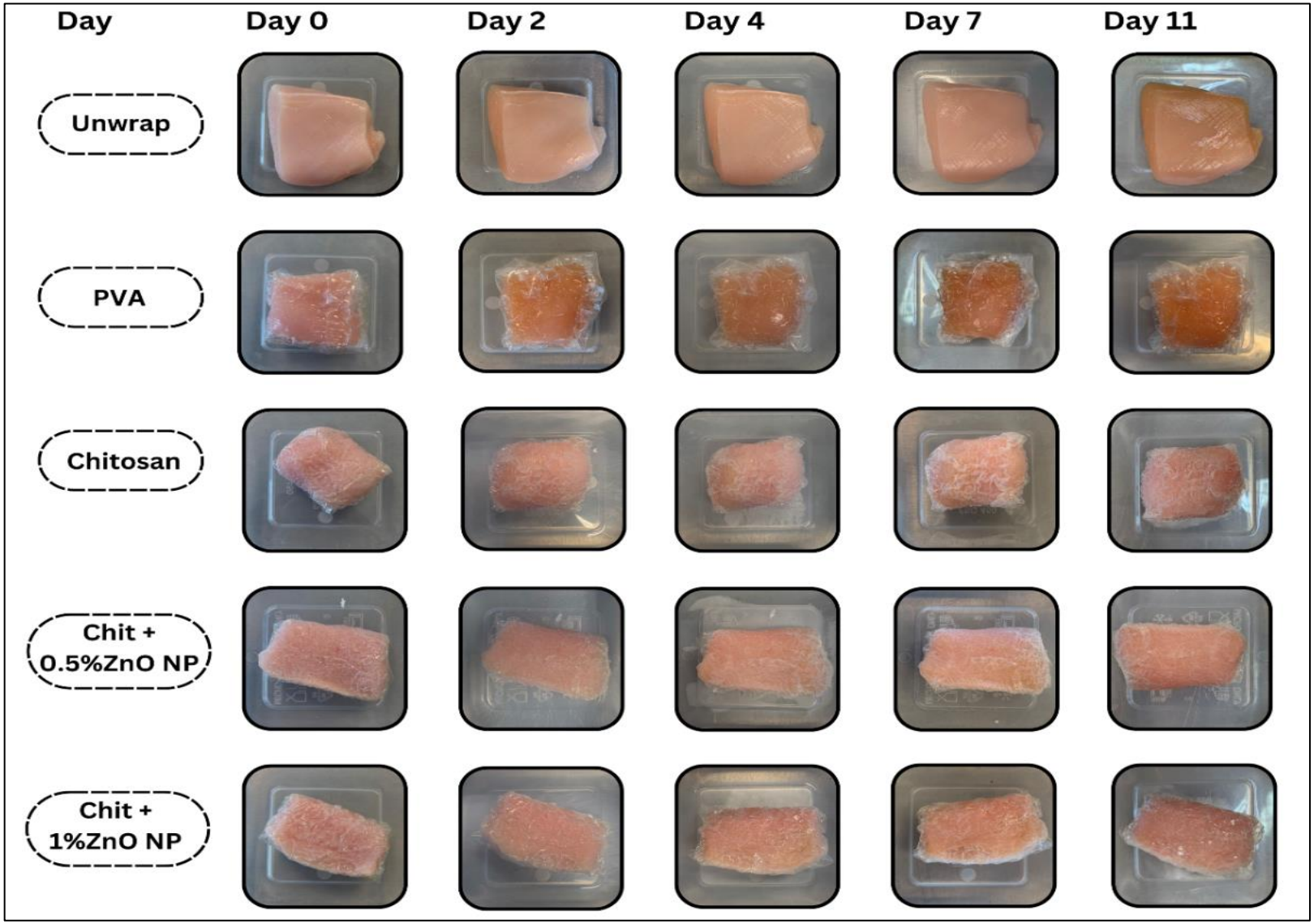
3.1. Weight loss

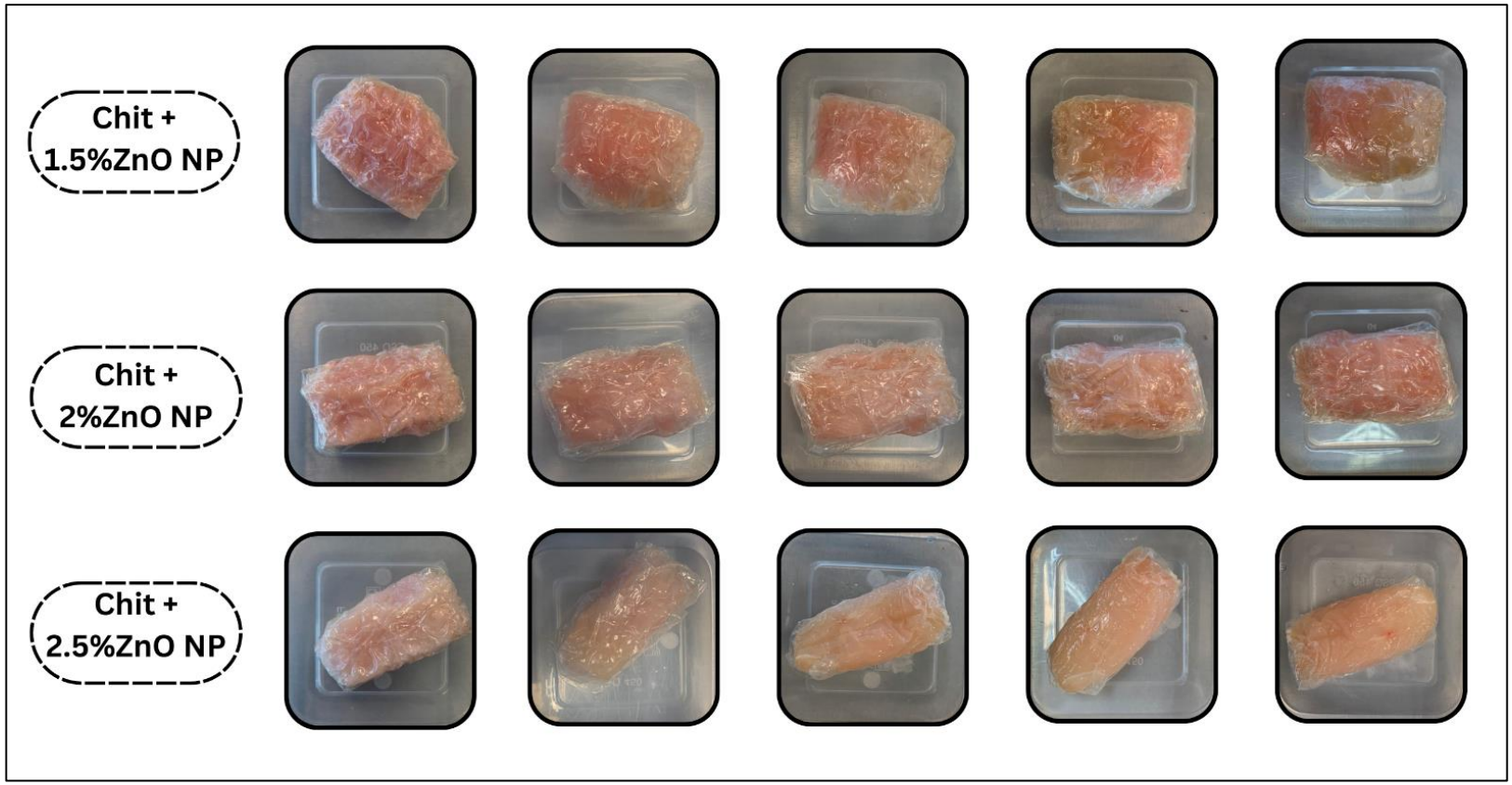
The initial weight of the poultry meat was 30 g. However, after the refrigerated storage period, this value exhibited a significant drop, peaking at 25.619 g for the unwrapped meat, and approximately 28 g and 27 g for samples wrapped with the biogenic and chemogenic films, respectively (**Table 3** and **Table 4**). Weight reduction was the lowest in the bio-nanocomposite, followed by the chemogenic-nanocomposite, chitosan, , and unwrapped samples. The film exhibited excellent water barrier attributes, characterized by its ability to have minimal absorption of moisture and permit the passage of water vapor, limiting moisture uptake by enhancing polymer chain interactions. This resulted in a more rigid and less flexible bio-nanocomposite, leading to significantly lower weight loss. The reduced spaces between polymer chains made it harder for water molecules to infiltrate the film (Song et al., 2022; Souza et al., 2019). Moreover, the interaction between water molecules and zinc ions or functional groups in ZnO NPs within the film structure suggests an interesting mechanism that enhances the mitigation of weight loss in poultry meat samples (Matei et al., 2023). Therefore, it was concluded that the most efficient formulation for reducing weight loss was the bio-nanocomposite with 2.5% ZnO NPs, primarily because of its greatest film thickness, which effectively obstructed the entry of water vapor into the meat.

3.2. pH & Titratable Acidity

The pH level is associated with multiple aspects of meat quality, like color, texture, flavor, moisture retention ability, and microbial resistance (Souza et al., 2020). Typically, the pH values of poultry meat fall within the range of 5.2 to 7 (Barbut, 2009; Pires et al., 2021). Initially, the pH value was 5.51, peaking at 7.83 for the unwrapped meat. However, the increase was less significant for samples wrapped with bio-based films. For instance, meat shielded with Chit+2.5% ZnO NPs displayed the lowest pH value of 6.29 and 6.50 in both

biogenic and chemogenic films, respectively, after 11 days of refrigerated storage, indicating it as the most effective treatment compared to others. The pH values were lowest in the bio-nanocomposite, followed by the chemogenic-nanocomposite, chitosan, PVA, and unwrapped samples (**Table 3** and **Table 4**). Poultry meat typically exhibits pH values between 5.2 and 7.0 (Souza et al., 2018). Over time, titratable acidity declined, corresponding with the rise in pH. Titratable acidity exhibited the highest levels in the bio-nanocomposite, followed by the chemogenic-nanocomposite, chitosan, PVA, and unwrapped samples. This trend is attributed to the alkaline response during the spoilage process, which results in the formation of amines and NH_3 due to the release of free amino acids, as demonstrated by Karabagias et al., (2011). This phenomenon is closely linked to the proliferation of microbes in meat products, as highlighted by Gomes et al., (2019). Previous research has established a relationship between the increase in pH levels in chicken meat and bacterial growth. The rise in pH can be ascribed to the denaturation of proteins and the buildup of amines and ammonia aided by psychotropic bacteria, which are commonly found in chicken meat, as emphasized by Ghollasi-Mood et al. (2017). Similar results were observed with ZnO NPs incorporated into carboxymethyl cellulose (CMC), showing a delay in the pH rise in refrigerated poultry meat (Suo et al., 2016). The initial acidity levels for freshly cut pork meat were approximately 5.70. After a 14-day storage period, the control film showed a pH of 8.85, whereas the meat coated with bio-nanocomposites exhibited a significantly lower pH of 6.12. The authors attributed these findings to the antibacterial properties of the films, which inhibited the generation of alkaline compounds responsible for pH elevation (Suo B. et al., 2016).


Day
Day 0
Day 2
Day 4
Day 7
Day 11
Unwrap
PVA
Chitosan
Chit + 0.5%ZnO NP
Chit + 1%ZnO NP


Chit +
1.5%ZnO NP
Chit +
2%ZnO NP
Chit +
2.5%ZnO NP

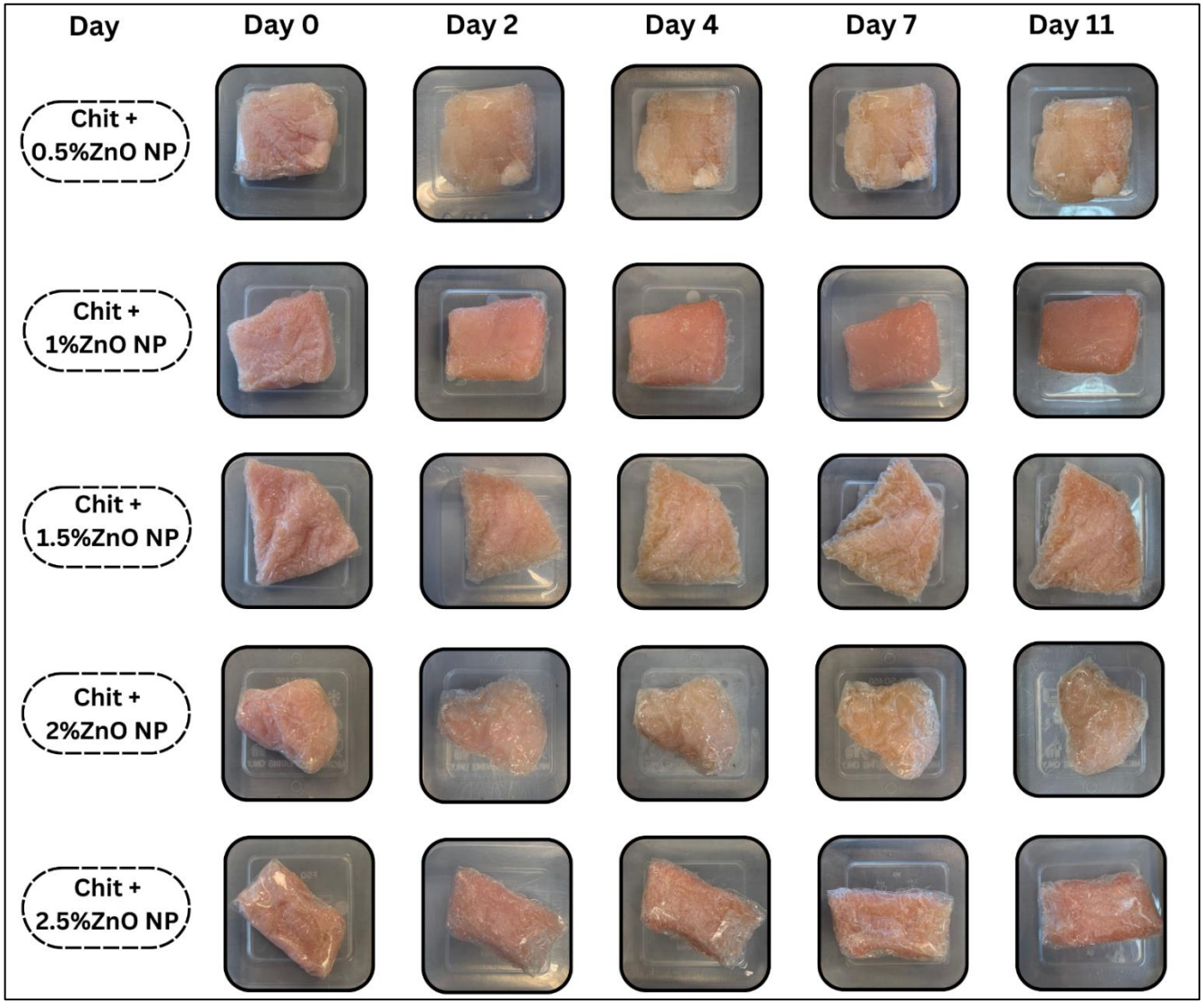


Fig. 1. The visual appearance of poultry wrapped with PVA, chitosan, and bio and chemogenic-nanocomposites.

352 **Table 1**

353 Overview of the physicochemical analysis of meat wrapped with bio-nanocomposites for 11 days of storage.

Parameter	Day	Unwrap	Chitosan	PVA	B-CS/0.5%ZnO NP	B-CS/1%ZnO NP	B-CS/1.5%ZnO NP	B-CS/2%ZnO NP	B-CS/2.5%ZnO NP
Weight loss (g)	0	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015
	2	27.262 ± 0.006	28.148 ± 0.025	27.506 ± 0.035	29.222 ± 0.015	29.403 ± 0.020	29.502 ± 0.025	29.724 ± 0.015	29.843 ± 0.025
	4	26.582 ± 0.025	27.785 ± 0.036	27.352 ± 0.020	29.047 ± 0.031	29.279 ± 0.036	29.447 ± 0.010	29.327 ± 0.020	29.475 ± 0.035
	7	25.835 ± 0.557	27.433 ± 0.006	27.189 ± 0.015	28.953 ± 0.026	28.875 ± 0.015	28.403 ± 0.026	28.819 ± 0.006	28.932 ± 0.042
	11	25.619 ± 0.025	27.369 ± 0.021	26.367 ± 0.020	28.331 ± 0.032	28.563 ± 0.021	28.538 ± 0.025	28.463 ± 0.025	28.326 ± 0.572
pH	0	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038
	2	6.93 ± 0.070	6.47 ± 0.056	6.81 ± 0.061	6.22 ± 0.096	5.90 ± 0.361	5.83 ± 0.045	5.76 ± 0.118	5.69 ± 0.053
	4	7.32 ± 0.062	6.61 ± 0.047	7.01 ±0.078	6.33 ± 0.053	6.21 ± 0.053	6.08 ± 0.026	5.93 ± 0.185	5.77 ± 0.071
	7	7.57 ±0.070	6.99 ± 0.036	7.25 ± 0.070	6.79 ± 0.046	6.63 ± 0.051	6.49 ± 0.165	6.31 ± 0.117	6.16 ± 0.140
	11	7.83 ± 0.060	7.39 ± 0.075	7.61 ± 0.059	7.01 ± 0.085	6.81 ± 0.025	6.55 ± 0.070	6.43 ± 0.017	6.29 ± 0.046
Hue angle (^{0})	0	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010
	2	76.21 ± 0.015	71.51 ± 0.020	74.60 ± 0.026	65.99 ± 0.142	66.64 ± 0.010	66.32 ± 0.010	67.78 ± 0.036	68.29 ± 0.015
	4	79.44 ± 0.010	73.16 ± 0.006	76.01 ± 0.061	67.64 ± 0.015	68.43 ± 0.015	69.16 ± 0.006	70.92 ± 0.010	71.96 ± 0.012
	7	81.56 ± 0.017	75.83 ± 0.010	79.39 ± 0.015	68.05 ± 0.041	68.90 ± 0l025	69.55 ± 0.015	71.46 ± 0.012	73.54 ± 0.010
	11	84.21 ± 0.032	79.33 ± 0.020	82.93 ± 0.015	68.93 ± 0.012	70.12 ± 0.031	73.56 ± 0.010	75.34 ± 0.010	76.44 ± 0.024
Titratable Acidity (% citric acid equivalent)	0	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002
	2	0.572 ± 0.010	0.594 ± 0.011	0.585 ± 0.004	0.613 ± 0.005	0.622 ± 0.014	0.646 ± 0.012	0.691 ± 0.011	0.704 ± 0.010
	4	0.451 ± 0.013	0.482 ± 0.005	0.472 ± 0.009	0.491 ± 0.011	0.503 ± 0.005	0.512 ± 0.011	0.587 ± 0.010	0.632 ± 0.007
	7	0.412 ± 0.013	0.439 ± 0.008	0.420 ± 0.005	0.451 ± 0.012	0.462 ± 0.010	0.479 ± 0.017	0.531 ± 0.003	0.569 ± 0.004
	11	0.402 ± 0.009	0.413 ± 0.010	0.405 ± 0.013	0.422 ± 0.005	0.427 ± 0.012	0.431 ± 0.006	0.523 ±0.009	0.526 ± 0.011
	0	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002

TBARS (mg	**2**	0.351 ± 0.010	0.301 ± 0.008	0.338 ± 0.004	0.282 ± 0.006	0.285 ± 0.007	0.288 ± 0.004	0.286 ± 0.007	0.290 ± 0.026
MDA/kg	**4**	0.543 ± 0.013	0.391 ± 0.011	0.493 ± 0.009	0.383 ± 0.006	0.392 ± 0.007	0.388 ± 0.004	0.391 ± 0.006	0.397 ± 0.018
meat)	**7**	0.632 ± 0.013	0.462 ± 0.008	0.521 ± 0.005	0.401 ± 0.003	0.411 ± 0.004	0.414 ± 0.007	0.422 ± 0.065	0.425 ± 0.011
	11	0.704 ± 0.009	0.533 ± 0.010	0.638 ± 0.013	0.423 ± 0.007	0.427 ± 0.003	0.433 ± 0.006	0.437 ± 0.006	0.444 ± 0.007

Table 2

Overview of the physicochemical analysis of meat wrapped with chemogenic-nanocomposites for 11 days of storage.

Parameter	Days	C-CS/0.5%ZnO NP	C-CS/1%ZnO NP	C-CS/1.5%ZnO NP	C-CS/2%ZnO NP	C-CS/2.5%ZnO NP
Weight loss (g)	**0**	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015	30.000 ± 0.015
	2	28.754 ± 0.030	28.881 ± 0.010	29.037 ± 0.021	29.322 ± 0.045	29.431 ± 0.025
	4	28.446 ± 0.040	28.372 ± 0.024	28.529 ± 0.029	28.617 ± 0.103	28.677 ± 0.017
	7	27.821 ± 0.022	27.955 ± 0.046	28.112 ± 0.051	28.255 ± 0.032	28.334 ± 0.033
	11	27.809 ± 0.042	27.813 ± 0.032	27.736 ± 0.043	28.243 ± 0.022	28.162 ± 0.022
pH	**0**	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038	5.51 ± 0.038
	2	6.32 ± 0.021	6.11 ± 0.040	6.01 ± 0.552	5.91 ± 0.031	5.89 ± 0.030
	4	6.74 ± 0.025	6.53 ±0.031	6.40 ± 0.035	6.27 ± 0.040	6.02 ± 0.051
	7	6.95 ± 0.031	6.82 ± 0.033	6.61 ± 0.050	6.45 ± 0.025	6.31 ± 0.036
	11	7.21 ± 0.032	7.15 ± 0.031	6.94 ± 0.015	6.73 ± 0.026	6.50 ± 0.095
Hue angle (^{0})	**0**	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010	63.66 ± 0.010
	2	66.92 ± 0.036	67.43 ± 0.025	68.32 ± 0.022	70.09 ± 0.024	72.04 ± 0.050
	4	67.89 ± 0.041	68.54 ± 0.036	69.74 ± 0.038	71.54 ± 0.032	72.76 ± 0.034
	7	68.61 ± 0.032	68.91 ± 0.015	70.54 ± 0.026	72.65 ± 0.041	74.55 ± 0.048
	11	69.14 ± 0.100	72.31 ± 0.042	75.76 ± 0.021	77.82 ± 0.017	78.09 ± 0.045

Titratable acidity (% citric acid equivalent)	**0**	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002	0.924 ± 0.002
	2	0.613 ± 0.003	0.735 ± 0.003	0.758 ± 0.004	0.791 ± 0.004	0.832 ± 0.003
	4	0.407 ± 0.003	0.559 ± 0.005	0.623 ± 0.004	0.697 ± 0.002	0.756 ± 0.021
	7	0.389 ± 0.003	0.502 ± 0.004	0.539 ± 0.003	0.583 ± 0.019	0.628 ± 0.042
	11	0.352 ± 0.004	0.417 ± 0.003	0.462 ± 0.003	0.566 ± 0.035	0.582 ± 0.003
TBARS (mg MDA/kg meat)	**0**	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002	0.0591 ± 0.002
	2	0.361 ± 0.004	0.365 ± 0.003	0.371 ± 0.004	0.359 ± 0.002	0.388 ± 0.002
	4	0.389 ± 0.003	0.374 ± 0.003	0.393 ± 0.004	0.403 ± 0.004	0.408 ± 0.003
	7	0.402 ± 0.002	0.397 ± 0.003	0.412 ± 0.003	0.417 ± 0.003	0.421 ± 0.003
	11	0.441 ± 0.003	0.453 ± 0.002	0.459 ± 0.004	0.481 ± 0.004	0.492 ± 0.004

358

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360

3.3. Hue angle

The hue angle is indicative of the color of the sample. An increase in hue* signifies a discoloration process, as the color shifts towards a more yellowish (90° and above) or greenish tone (Nouri A. et al., 2017). Initially, the H° values were approximately 63.66°, indicating a reddish tone. After 11 days of refrigerated storage, unwrapped meat showed values around 84.21°, signifying a color change. In contrast, the active films maintained the color within the range of 68° to 79° throughout the shelf-life period, effectively preserving the original reddish hue. Unlike the unwrapped sample, the typical red hue associated with poultry meat was absent. The hue angle was lowest in the bio-nanocomposite, followed by the chemogenic-nanocomposite, chitosan, PVA, and unwrapped samples (**Table 3** and **Table 4**). Chitosan's ability to bind and neutralize iron (Fe^{3+}) may have slowed down the oxidative process facilitated by this metal (Ghaderi et al., 2014). The bio-nanocomposites had a positive impact on meat color, with protected samples exhibiting higher brightness and redness, thereby enhancing visual appeal, and confirming sensory quality findings (Mulla et al., 2017). Out of the various concentrations tested, the most effective formulation for minimizing color alterations was found to be 0.5% biogenic ZnO NPs.

Suo B. et al. (2016) reported that ZnO NPs were integrated into carboxymethylcellulose (CMC) to create coating films applied to fresh pork meat. The bio-nanocomposites positively influenced the meat's color, with the protected samples showing increasing levels of brightness and redness throughout the storage duration. The decrease in the a* value (indicating a loss of redness) during cold storage is attributed to the oxidation process, which transforms oxymyoglobin into metmyoglobin (Ghaderi et al., 2014). In poultry meat, iron mainly exists as heme iron, responsible for the red color, and is a crucial component of hemoglobin, the protein that transports oxygen in red blood cells. The iron in heme is in the form of Fe^{2+} ions, with a positive charge of +2, easily absorbed by the human digestive system. Fe^{3+} ions with a charge of +3, are not abundant in chicken meat but may be present in minimal amounts due to factors such as oxidation from prolonged air exposure (Bahja et al., 2022). Chitosan incorporated with 2.5% of ZnO NPs exhibited the highest hue angle, indicating a reduction in a*, resulting in discoloration.

3.4. Lipid Peroxidation

Lipid oxidation in food causes flavor changes, including off-flavors and rancid odors (Shankar & Rhim, 2016). Malondialdehyde (MDA) concentration serves as a biomarker for oxidative stress, indicating lipid peroxidation extent (De Oliveira et al., 2020). These changes lead to consumer dissatisfaction and food rejection (Vilarinho et al., 2018). According to Souza et al. (2018), consumers detect off-odors when the TBARS value reaches 0.5 mg MDA/kg in pork patties. The unwrapped meat exhibited MDA levels of 0.5 mg/kg or higher starting from day 4, indicating the onset of rancidity (Souza et al., 2020). PVA surpassed this threshold from day 5 onwards. Conversely, by day 9, the meat preserved with chitosan film had also

reached the threshold for off-flavor. The efficacy of chitosan in slowing down the process of oxidation in meat can be credited to its outstanding ability to block oxygen and light (Al-Naamani et al., 2016). Additionally, chitosan's chelating ability, which prevents the initiation of oxidative reactions by interacting with metallic ions, particularly iron ions (Fe^{2+} to Fe^{3+}), effectively slows down the series of reactions responsible for deteriorating the flavor and taste of food (Petrou et al., 2012). Samples protected with both biogenic and chemogenic nanocomposites consistently maintained MDA levels below this threshold throughout the entire assessment period (**Table 3** and **Table 4**). Therefore, it can be concluded that the most effective formulation for reducing the oxidation reaction was found to be biogenic 0.5% ZnO NPs among the various concentrations tested.

An evaluation of chicken breast meat stored in LDPE-Ag NP and LDPE-ZnO NP films revealed a quality improvement (Panea et al., 2014). The findings indicated that films containing nanoparticles notably delayed lipid oxidation compared to the control film. Additionally, Baek S.K. et al. (2018) noted that the incorporation of ZnO NPs into films containing *Gracilaria vermiculophylla* extract exhibited antibacterial properties and effectively slowed down the oxidation process in smoked salmon. The researchers suggested that the reduced antimicrobial activity of the nanocomposite impacted lipid oxidation in the treated nano-packaging, consistent with the findings of this study where the 0.5% bio-nanocomposite exhibited the highest log reduction, while the 2.5% bio-nanocomposite exhibited the lowest log reduction (Sasidharan et al., 2023).

Table 3

Overview of the microbiological study of meat wrapped with bio-nanocomposite throughout the storage period.

Parameter	Days	Unwrap	Chitosan	PVA	B-CS/0.5%ZnO NP	B-CS/1%ZnO NP	B-CS/1.5%ZnO NP	B-CS/2%ZnO NP	B-CS/2.5%ZnO NP
Total psychotropic aerobic microbial count (Log CFU/g meat)	0	3.427 ± 0.003	3.427 ± 0.003	3.427 ± 0.003	3.427 ± 0.003	3.427 ± 0.003	3.427 ± 0.003	3.427 ± 0.003	3.427 ± 0.003
	2	6.172 ± 0.164	5.722 ± 0.127	5.764 ± 0.026	4.492 ± 0.060	5.253 ± 0.043	5.425 ± 0.064	5.842 ± 0.056	5.733 ± 0.018
	4	8.355 ± 0.100	6.354 ± 0.110	7.929 ± 0.081	5.871 ± 0.060	6.834 ± 0.086	6.986 ± 0.050	6.942 ± 0.075	6.882 ± 0.100
	7	8.794 ± 0.100	8.035 ± 0.105	8.207 ± 0.080	7.331 ± 0.056	7.546 ± 0.060	7.813 ± 0.052	7.534 ± 0.069	7.916 ± 0.030
	11	9.317 ± 0.115	8.327 ± 0.076	8.546 ± 0.065	7.576 ± 0.064	7.724 ± 0.075	7.946 ± 0.051	8.037 ± 0.009	8.151 ± 0.041
Total mesophilic aerobic microbial count (Log CFU/g meat)	0	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004
	2	5.283 ± 0.050	4.753 ± 0.040	5.130 ± 0.088	4.744 ± 0.037	4.627 ± 0.009	4.772 ± 0.021	4.749 ± 0.007	4.654 ± 0.045
	4	8.035 ± 0.157	6.848 ± 0.040	7.537 ± 0.072	6.119 ± 0.009	6.185 ± 0.005	6.326 ± 0.006	6.548 ± 0.005	6.767 ± 0.008
	7	8.556 ± 0.055	7.257 ± 0.044	8.013 ± 0.066	7.021 ± 0.082	7.064 ± 0.005	7.258 ± 0.008	7.325 ± 0.070	7.443 ± 0.026
	11	8.832 ± 0.054	7.879 ± 0.075	8.194 ± 0.073	7.552 ± 0.030	7.632 ± 0.014	7.913 ± 0.064	8.032 ± 0.061	8.115 ± 0.119
Enterobacteriaceae (Log CFU/g meat)	0	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004	3.545 ± 0.004
	2	5.014 ± 0.086	4.517 ± 0.049	4.835 ± 0.057	3.977 ± 0.026	4.172 ± 0.097	4.217 ± 0.010	4.429 ± 0.006	4.326 ± 0.032
	4	6.835 ± 0.021	5.944 ± 0.008	6.514 ± 0.006	4.624 ± 0.007	4.817 ± 0.044	5.034 ± 0.056	5.315 ± 0.018	5.718 ± 0.019
	7	7.557 ± 0.034	6.336 ± 0.078	7.327 ± 0.025	5.198 ± 0.046	5.328 ± 0.016	5.817 ± 0.016	5.923 ± 0.022	6.057 ± 0.075
	11	8.039 ± 0.058	7.419 ± 0.015	7.894 ± 0.029	6.034 ± 0.043	6.113 ± 0.088	6.215 ± 0.030	6.547 ± 0.019	6.834 ± 0.054

Table 4

Overview of the microbiological study of meat wrapped with chemogenic-nanocomposite throughout the storage period.

Parameter	Days	C-CS/0.5%ZnO NP	C-CS/1%ZnO NP	C-CS/1.5%ZnO NP	C-CS/2%ZnO NP	C-CS/2.5%ZnO NP
Total psychotropic aerobic microbial count (Log CFU/g meat)	**0**	3.558 ± 0.003	3.558 ± 0.003	3.558 ± 0.003	3.558 ± 0.003	3.558 ± 0.003
	2	4.882 ± 0.100	4.903 ± 0.032	5.603 ± 0.040	5.912 ± 0.016	5.501 ± 0.005
	4	6.339 ± 0.047	6.924 ± 0.032	7.201 ± 0.018	7.346 ± 0.055	6.981 ± 0.002
	7	7.329 ± 0.003	7.757 ± 0.045	7.938 ± 0.056	8.071 ± 0.021	8.145 ± 0.085
	11	7.618 ± 0.040	7.991 ± 0.068	8.173 ± 0.032	8.224 ± 0.023	8.367 ± 0.005
Total mesophilic aerobic microbial count (Log CFU/g meat)	**0**	3.661 ± 0.004	3.661 ± 0.004	3.661 ± 0.004	3.661 ± 0.004	3.661 ± 0.004
	2	4.710 ± 0.014	4.519 ± 0.011	5.132 ± 0.015	5.337 ± 0.019	5.459 ± 0.004
	4	6.032 ± 0.042	6.494 ± 0.050	6.554 ± 0.066	6.735 ± 0.029	7.880 ± 0.017
	7	7.118 ± 0.018	7.382 ± 0.026	7.618 ± 0.087	7.734 ± 0.007	7.923 ± 0.020
	11	7.457 ± 0.021	7.941 ± 0.026	8.119 ± 0.007	7.112 ± 0.007	8.215 ± 0.031
***Enterobacteriaceae* (Log CFU/g meat)**	**0**	3.661 ± 0.004	3.661 ± 0.004	3.661 ± 0.004	3.661 ± 0.004	3.661 ± 0.004
	2	4.032 ± 0.123	4.219 ± 0.153	4.338 ± 0.054	4.719 ± 0.013	4.832 ± 0.100
	4	4.773 ± 0.059	4.923 ± 0.023	5.386 ± 0.009	5.442 ± 0.029	5.892 ± 0.016
	7	5.271 ± 0.040	5.532 ± 0.011	5.905 ± 0.009	6.116 ± 0.010	6.328 ± 0.009
	11	6.242 ± 0.015	6.394 ± 0.023	6.477 ± 0.086	6.648 ± 0.018	6.943 ± 0.015

3.5. Microbiological Growth (TPAM, TMAM, and Enterobacteriaceae)

Microbial growth is a key factor influencing the shelf life of perishable items such as meat. The samples exhibited a natural degradation process, as indicated by an increase in bacterial counts for all strains analyzed (Silva et al., 2018). The nanocomposite-packaged poultry meat underwent an assessment for TPAM, TMAM as well as *Enterobacteriaceae* throughout the refrigerated preservation. Further information on the outcomes of microbial growth is provided in **Table 3** and **Table 4**.

Unwrapped poultry meat showed higher levels of contamination and more rapid microbial growth, as reported by Gutiérrez et al. (2017). Both total mesophilic and psychotropic aerobic microorganisms exhibited similar patterns, although the count of psychotropic microorganisms slightly exceeded that of mesophilic ones. This observation is attributed to the refrigerated storage conditions, which favor the proliferation of preexisting contaminants. It is essential to assess psychotropic microorganisms in poultry meat samples, especially during refrigerated storage, as many cold-adapted strains are associated with spoilage, and some may pose pathogenic risks (Souza et al., 2020).

According to Regulation No. 2073/2005 of the European Commission (EC), food products should not contain microorganisms or their toxins or metabolites in amounts that pose an unacceptable risk to human health. For noncooked minced meat, the maximum limit for aerobic colony counting is set at 6.70 log CFU/g of meat. Exceeding this threshold indicates a need to enhance hygienic production practices and/or select better raw materials (Souza et al., 2020).

Psychotropic aerobic microorganism contamination commenced at 3.427 log CFU/g of meat. The highest bacterial growth level recorded after 11 days of storage was 9.317 log CFU/g of meat in unwrapped samples. In contrast, poultry samples wrapped with PVA, and chitosan exhibited lower contamination levels of 8.546 and 8.327 log CFU/g of meat, respectively. Samples wrapped with biogenic and chemogenic films experienced a less pronounced increase, ranging from 7.5 to 8.2 and 7.6 to 8.4 log CFU/g of meat, respectively (**Table 3** and **Table 4**). Regarding TPAM, unwrapped and PVA-wrapped meat exceeded 7 log CFU/g by day 3, while meat protected with chitosan film reached this threshold value by day 5. The maximum limit for chemogenic-nanocomposites enveloped in 0.5% was reached by day 6, with 1% achieved by day 5, 1.5% by day 4, 2% by day 4, and 2.5% by day 5 (**Table 4**). However, meats wrapped in bio-nanocomposites reached the maximum limit by day 6 (**Table 3**). Among the concentrations tested, the optimal formulation for minimizing microbiological growth was determined to be biogenic 0.5% of ZnO NPs.

A similar pattern was observed in TMAM, with initial bacterial growth recorded at 3.545 log CFU/g of meat. The highest level of bacterial growth attained after 11 days of storage was 8.832 log CFU/g of meat in unwrapped samples. Conversely, poultry samples wrapped with PVA, and chitosan exhibited lower contamination levels of 8.194 and 7.879 log CFU/g of meat, respectively. Samples wrapped with biogenic and chemogenic films experienced a less notable increase, ranging from 7.5 to 8.1 and 7.5 to 8.2 log CFU/g of meat, respectively (**Table 3** and **Table 4**). Unwrapped and PVA-wrapped meats reached the maximum

limit by day 3, followed by chitosan-wrapped meats reaching this limit by day 5. The maximum limit for chemogenic-nanocomposites enveloped in concentrations of 0.5% to 1.5% was reached by day 7, while for 2% it was achieved by day 5, and for 2.5% by day 4 (**Table 4**). However, meats wrapped in bio-nanocomposites reached the maximum limit by day 7 (**Table 3**).

It was noted that 2.5% of biogenic biofilms and 1%, 1.5%, 2%, and 2.5% of chemogenic biofilms exhibited higher microbial growth compared to the chitosan film (**Table 3** and **Table 4**). This could be attributed to a positive interaction between chemogenic ZnO NPs and the chitosan matrix, resulting in a reduction in the availability of active groups, specifically the amino groups of chitosan, for microbial interaction. Consequently, this leads to a decrease in antimicrobial effectiveness (Souza et al., 2019). The film displayed diminished antimicrobial properties with the addition of 2.5% ZnO NPs. This finding is consistent with a prior study where the film containing 2.5% ZnO NP exhibited the lowest log reduction against microbial strains (Sasidharan et al., 2023). In summary, the most effective formulation for minimizing microbial growth was determined to be biogenic 0.5% of ZnO NPs among the various concentrations tested.

The initial contamination levels of both biogenic and chemogenic biofilms in mesophilic aerobic microorganisms were recorded at 3.545 and 3.661 log CFU/g, respectively. The initial bacterial growth for both mesophilic and psychotropic strains fell within the upper limits specified by European regulations for ground meat, namely 3.46 log CFU/g of meat and 3.81 log CFU/g of meat, correspondingly. These findings aligned with prior research, which reported contamination levels ranging from 4.0 log CFU/g (Noshirvani et al., 2017) to 4.85 log CFU/g (Petrou et al., 2012) for freshly sourced chicken breast meat.

This technology effectively preserves the food item, prolonging its shelf life by a minimum of 1–2 days, as shown in **Table 3** and **Table 4**. Natural polymers delay the growth of microbes in contrast to meat left unwrapped, however, they do not completely prevent it. Microbial proliferation likely occurs near the biofilm inhibition zone, as observed during antibacterial testing, while other microbes nearby continue to multiply. Similar patterns in film behavior have been seen in previous studies (Souza et al., 2019). For instance, Emamifar et al. (2010) noted that the total count of aerobic bacteria in orange juice stored with LDPE-1% ZnO NPs increased after a week, resembling the trend in the control group. Similarly, strawberries wrapped in LDPE containing 3% ZnO NPs showed a decrease in aerobic counts until day 4, followed by an increase on day 8 (Pires et al., 2021).

The European Food Safety Authority (EFSA) suggests monitoring and examining *Enterobacteriaceae* in both the production environment and the end food product is mandatory. It is crucial to understand that the *Enterobacteriaceae* family comprises both harmful and harmless species commonly encountered in food production environments. Harmless species in this family do not present health hazards. Consequently, routine monitoring of *Enterobacteriaceae* does not automatically imply a health risk, as it encompasses both harmless and potentially harmful members (Kanatt et al., 2012).

In *Enterobacteriaceae*, initial bacterial growth was recorded at 3.545 log CFU/g of meat. The highest contamination level reached after 11 days of storage was 8.039 log CFU/g of meat in unprotected samples. In contrast, poultry samples wrapped with PVA, and chitosan showed lower contamination levels of 7.894

and 7.419 log CFU/g of meat, respectively (**Table 3** and **Table 4**). Samples wrapped with biogenic and chemogenic films experienced a notably lesser increase, ranging from 6.0 to 6.8 and 6.2 to 6.9 log CFU/g of meat, respectively. Unwrapped and PVA-wrapped samples reached the maximum limit by day 6, whereas chitosan-wrapped samples reached it by day 10. Chemogenic and bio-nanocomposite-wrapped samples did not exceed the threshold until day 11 of the storage period (**Table 3** and **Table 4**). The protected samples consistently maintained a lower level of contamination compared to the unwrapped meat sample. Overall, the most effective formulation for minimizing microbiological growth was determined to be biogenic 0.5% of ZnO NPs among the various concentrations tested.

Petrou et al. (2012) reported a similar quantification of *Enterobacteriaceae* in chicken breast meat, indicating an initial contamination level of approximately 3 log CFU/g of meat. By the end of 12 days of modified atmosphere packaging (MAP) and refrigerated storage, the contamination level reached its peak at 6 log CFU/g of meat. The study investigated the impact of the dipping method on chitosan, either alone or combined with oregano essential oil, and found that both treatments reduced *Enterobacteriaceae* counts by approximately 3–4 logarithmic colony-forming units (CFU) per gram of meat. This discovery is consistent with the results of our study.

3.6. Zinc Migration

In the assessment of materials designed for direct contact with food, it is crucial to analyze both overall migration and potential toxic effects, as highlighted by Souza et al. (2020). According to the CEF Panel of the European Food Safety Authority (EFSA), responsible for evaluating food contact materials such as enzymes, flavorings, and processing aids, it was concluded by Souza et al. (2018) that nanoscale ZnO is unlikely to undergo migration. Consequently, the primary focus of safety assessment lies in the migration of Zn ions. In 2003, the CEF Panel recommended a maximum threshold of 25 mg per 100 grams per individual daily for food contact materials, enzymes, flavorings, and processing aids, as documented by Souza et al. (2020). Under these recommendations, the collective zinc levels in fresh poultry meat were evaluated both initially and after 11 days of refrigerated storage across all utilized bio-nanocomposites, with detailed results provided in **Table 5**.

Table 5

The migration of Zn of biogenic and chemogenic-nanocomposite into poultry meat.

Sample	Zinc Concentration (mg Zn/ kg Fresh Meat)	Percentage of zinc diffused (mg Zn Diffused/ Maximum Limit)
Initial Zinc Content – day 0	15.323 ± 0.004	-
Unwrapped – day 11	15.900 ± 0.003	-
Chitosan – day 11	17.481 ± 0.002	-
Bio-nanocomposite		
B-CS/0.5%ZnO NP – day 11	50.511 ± 0.003	75.69 ± 0.006
B-CS/1%ZnO NP – day 11	59.376 ± 0.004	59.67 ± 0.010
B-CS/1.5%ZnO NP – day 11	67.022 ± 0.003	50.46 ± 0.008
B-CS/2%ZnO NP – day 11	71.556 ± 0.004	46.23 ± 0.021
B-CS/2.5%ZnO NP – day 11	74.328 ± 0.002	43.98 ± 0.020
Chemogenic-nanocomposite		
C-CS/0.5%ZnO NP – day 11	66.581 ± 0.002	50.92 ± 0.007
C-CS/1%ZnO NP – day 11	73.781 ± 0.006	44.41 ± 0.011
C-CS/1.5%ZnO NP – day 11	89.215 ± 0.006	34.85 ± 0.015
C-CS/2%ZnO NP – day 11	99.668 ± 0.006	30.42 ± 0.018
C-CS/2.5%ZnO NP – day 11	103.553 ± 0.005	29.05 ± 0.027

The initial concentration of zinc ions was at 15.323 mg/kg of meat, which closely corresponds to the official guideline from the Portuguese database (PortFIR) suggesting 8 mg/kg for chicken breast (INSA PortFIR, 2020). Over time, there was a minimal increase observed in unwrapped meat and chitosan, while poultry meat enveloped by bio-nanocomposites showed a significant rise in zinc levels (**Table 5**), likely attributed to the transfer of zinc from the packaging material (ZnO NPs) into the food.

An observed concentration effect was noted, as the sample enveloped with CS/2.5% ZnO NP exhibited the highest zinc content. With increased levels of added zinc, the percentage diffusion of zinc content relative to the incorporated amount declined. The rise in total zinc content in the bio-nanocomposite varied from 33.030 to 56.847 mg Zn/kg, while in the chemogenic-nanocomposite, it ranged from 49.100 to 86.072 mg Zn/kg (**Table 5**). Since the suggested maximum daily intake of zinc at 25 mg per individual, a moderate portion of fresh poultry meat (100g) could contain 0.803 to 3.185 mg Zn/100g, constituting 3.21% to 12.74% of the daily maximum limit in the bio-nanocomposite, whereas the chemogenic-nanocomposite yields 2.411 to 6.107 mg Zn/100g, equivalent to 9.64% to 24.43% of the maximum limit (**Table 5**). Poultry meat wrapped in biogenic films contributed to a lower diffusion of zinc compared to chemogenic films. However, further research is necessary to assess its safety concerning consumer exposure.

While numerous studies have examined the antimicrobial characteristics of ZnO and its migration into food simulants, research on its migration into meat products, particularly raw meat, remains scarce (Abu-Thabit et al., 2020). The principal mechanism of zinc migration entails the dissolution of zinc ions from the material into the surrounding medium, with the solubility of zinc compounds like ZnO dictating the degree of ion dissolution. Once zinc ions are liberated into the surrounding medium, they can diffuse through the material matrix, moving from regions of higher concentration to lower concentration.

The introduction of ZnO NPs at a concentration of 0.5% led to high water vapor permeability and low tensile strength, facilitating improved processes involving zinc ions (Gasti et al., 2022). This corresponds with the in vitro antimicrobial efficacy of the bio-nanocomposites, where the 0.5% bio-nanocomposite demonstrated the most significant inhibition of bacterial growth due to exceptional diffusion of bioactive components into the medium (**Table 5**) (Rahman et al., 2017). While daily consumption of fresh poultry meat wrapped with these bio-nanocomposites seems safe according to current findings, further investigations into these biobased products are crucial to fully grasp the potential risks associated with consumer exposure.

Certainly, the quality of fresh meat is predominantly affected by the existence and proliferation of spoilage and harmful microorganisms, along with oxidation. Inadequate preservation and handling can lead to microbial contamination of these products (Shankar et al., 2015). Therefore, employing preservatives with antimicrobial characteristics and adopting active packaging techniques like thin films or coatings can be beneficial. This strategy aims to enhance both the excellence and security of the food (Khalid et al., 2017).

4. Conclusion and Recommendations

The study focused on incorporating ZnO NP into a chitosan matrix as an eco-friendly alternative to non-biodegradable packaging. The bio-nanocomposite showed enhanced physicochemical properties in terms of pH, reduction in titratable acidity, and discoloration process. The chicken sample wrapped with bio-nanocomposite exhibited the lowest colony count in TPAM and TMAM, as well as the decreased level of TBARS, followed by chemogenic-nanocomposites, chitosan, PVA, and unwrapped. Higher Zn concentration increased zinc content in poultry meat, but the percentage diffused relative to the incorporated amount decreased at higher levels. The primary method for assessing the preservation of meat involves measuring TBARS to determine the development of off-flavors and microbiological growth, which determines the shelf-life. Off-flavor begins on day 10 in chitosan-wrapped meat, exceeding the maximum limit of microbial growth on day 4. Additionally, chemogenic and biogenic-wrapped meats maintain the off-flavor limit until day 11; however, they exceed the maximum limit of microbial growth on day 5 and day 6, respectively. Therefore, biogenic nanocomposite showed the best in all parameters including weight, pH, titratable acidity, hue angle, TBARS, microbiological growth, and zinc migration, however, among all the concentrations, it can be chosen that biogenic 0.5% ZnO NP acted as the best formulation. While ZnO is an essential mineral and is listed as generally recognized as safe (GRAS), further studies are required to evaluate its safety concerning consumer exposure. In summary, conducting an MTT assay will reveal the

cytotoxic effects of ZnO NPs. Similarly, analyzing the gut microbiome will offer insights into the composition and function of microbial communities in the gastrointestinal tract. Together, these studies will provide valuable data to understand the risks of ZnO exposure for both consumers and the environment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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