

Article

Packaging of Fresh Poultry Meat with Innovative and Sustainable ZnO/Pectin Bionanocomposite Films—A Contribution to the Bio and Circular Economy

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Abstract: The development of innovative/sustainable materials capable of enlarging the shelf-life of food products has lately been a focus of research, aiming to reduce food waste. Due to their good antimicrobial properties, zinc oxide nanoparticles (ZnO NPs) can add activity to food packaging, improving its performance. Furthermore, these nanoparticles are considered GRAS by the Food and Drug Administration (FDA), which represents an advantage in their application. Through an innovative and sustainable approach using tomato and passionfruit extracts, ZnO NPs were produced and incorporated into pectin films. The resulting bionanocomposites were tested for their activity via in situ studies, using fresh poultry meat as a food matrix. Overall, the bionanocomposites presented good antimicrobial activity, with the intrinsic antimicrobial properties of pectin having shown to be enhanced by the incorporated ZnO NPs. When used as primary packaging for the meat, the deterioration rate of the poultry meat, measured through microbiological growth and total volatile basic nitrogen content, was reduced. However, the nanoparticles contributed to the increment of discoloration and meat oxidation processes. Nonetheless, it can be concluded that fresh poultry meat protected with the bionanocomposites presented an extension of its shelf-life time, and it was confirmed that this eco-friendly packaging has potential to be employed by the food industry.

Keywords: active packaging; biodegradable material; shelf-life extension; antimicrobial activity; antioxidant activity; zinc oxide nanoparticles

1. Introduction

The population is constantly increasing. At the end of 2022, the number had surpassed 8 billion, and by the end of 2050 it will increase by almost another 2 billion. As a result, food technology is faced with an increasing challenge to provide each person with safe and healthy food. To cope with this situation, food industries are obliged to increase food production, which may result in the generation of huge amounts of food waste, of which

a significant part is composed of organic waste if the food is not properly preserved and packed. Indeed, food waste represents an inadequacy of the food systems and logistics, which contributes to environmental pollution and resource depletion and represents a considerable input to the human population threat [1]. Consequently, the United Nations Sustainable Development Goals (<https://sdgs.un.org/>, accessed on 9 October 2022) preconized a target to reduce food waste by 50% along the food supply chain by 2030.

Food packaging is an important element in the food supply chain because it is designed to provide protection against external factors and mechanical damage, reducing the amount of food wasted. Packaged food retains its nutritional and sensory properties as well as chemical and microbiological durability for longer [2]. Yet, the use of non-biodegradable plastics by the food packaging sector has had serious environmental impacts on the ecosystem, mainly due to its recalcitrance to degradation [3,4]. Their unsustainable use highlights the need to find innovative solutions (renewable, biodegradable) for their substitution. Bio-based polymers are promising solutions to that problem. The production of biopolymeric films uses a variety of sources, such as proteins (collagen, gelatin, zein), polysaccharides (cellulose, starch, pectin, alginate), fats (waxes or vegetable oils), and composite mixtures (for example, a mixture of lipids and hydrocolloids) [5]. Moreover, the switch to bioplastics will allow for the partial or total abandonment of fossil resources, thereby reducing the carbon footprint, and a production process that uses fewer toxic reagents [6]. Pectin is an edible and safe substance and is often used as a raw material in the production of coatings due to its barrier properties to lipids, oxygen, and aromatic compounds [7]. Pectin can be easily obtained from by-products from the fruit and vegetable industry, and its extraction and use from those wastes also contribute to the circular and bioeconomy [8]. However, this plant-derived polymer is also hydrophilic and susceptible to tearing, limitations that need to be surpassed [5,9,10], for example, with the addition of reinforcements or other substances that can improve pectin's properties [11].

The addition of zinc oxide nanoparticles (ZnO NPs), which improve the mechanical properties of film by strengthening its structure, is a promising solution to overcoming the limitations presented by pectin-based polymers [12,13]. Moreover, ZnO NPs are considered safe for human health, and they have been granted GRAS (Generally Recognized as Safe) status [10]. Numerous studies also show their positive effect on extending the microbiological and chemical durability of packaged food [9,14] without affecting the organoleptic characteristics of the product [15]. Ngo et al. [16] showed that the addition of 5% nanoparticles increased the elongation at break and the tensile strength, and also decreased the permeability of water vapor, oxygen, UV radiation, water absorption, and solubility. At the same time, the antibacterial properties were also increased [16]. On the other hand, in the study by El Fawal and coworkers [17], in which films with hydroxy cellulose with the addition of nanoparticles of zinc oxide and citric acid were produced, the films showed good antibacterial properties but also high hydrophilicity and the ability to swell. In these works, it was mentioned that one of the problems faced when incorporating these NPs into films was that the particles dispersed in the film in a non-homogeneous way and tended to form agglomerates. Interestingly, Dwivedi et al. [18] showed that ZnO nanoparticles added to oxidized sodium alginate coatings were more effective than pure ZnO due to the larger surface area associated with the oxidized sodium alginate-ZnO nanostructures. Yet, some other works have reported the opposite. This was referred to in the work by Singh and coworkers [19], in which coatings made of oxidized guar gum and ZnO NPs showed antibacterial properties but to a lesser extent than those of pure ZnO. This was explained by the fact that the raw material blocked the nanoparticles' access to the product, limiting its antibacterial action.

There are several techniques to obtain nanoparticles, including those of zinc oxide. These techniques can be divided into three main categories. The first is the physical method, which uses mechanical forces, hot steam, or ultrasound [20,21]. The second category deals with chemical techniques, in which reactions take place at lower temperatures than in physical ones. With these methods, the NPs produced can have various shapes and

sizes. Electrochemical and sonochemical methods, precipitation reactions, and sol-gel transformations are the main chemical methods used [22,23]. Zinc oxide NPs can also be obtained through biological techniques, which have gained popularity recently because of their many benefits, including their sustainable character (less demand for energy and less demand for non-renewable resources, including synthetic chemical substances). In this case, NP production is carried out using natural resources such as plant extracts and/or through the use of organisms such as microorganisms, and the pressure and temperature applied can be lower than with traditional processes, making it more environmentally friendly. Moreover, due to the modelling nature of natural compounds during these nanocrystal growth, a lower pH can also be used in the processing stage. The obtained nanoparticles are less toxic, repeatable, at low cost, and simple to manufacture [20,24,25]. Indeed, phytochemicals can modify the surface of the nanoparticles and create a protective layer that reduces NPs' toxicity. Additionally, plant extracts contain natural compounds that are often biocompatible and non-toxic to living organisms. Furthermore, the combination of different phytochemicals present in plant extracts can exhibit synergistic effects among them and with ZnO to enhance antibacterial activity while reducing toxicity. These synergistic interactions may lead to a targeted and selective effect against bacterial cells, minimizing adverse effects on human cells [20,24,26].

Formerly, in previous work [24], a green synthesis of ZnO NPs using apple peel wastes was performed, and the resulting NPs were tested in chitosan bionanocomposites. The activity of these films was characterized via *in vitro* and *in situ* studies, and the results obtained showed that the ZnO NPs added enhanced the chitosan intrinsic antimicrobial properties. The nanoparticles also improved the antioxidant properties of the films and proved to have the potential to be used as food preservative agents in active food packaging. Nonetheless, more research and studies are needed to understand the potential of other food by-products to synthesize ZnO NP through an eco-friendly route and to understand its potential in active bionanocomposites. Therefore, in this work, tomato and passionfruit extracts were tested to synthesize ZnO nanoparticles for the first time by the biological method (so-called "green synthesis"). These extracts contain phytochemicals, which can enhance the formation of nanoparticles and their activity. The created nanoparticles were incorporated into pectin films to test their microbiological and antioxidant properties in poultry meat during its shelf life. The performance of the films with these nanoparticles was compared with films using commercially available zinc oxide nanoparticles to understand the potential of those eco-friendly ZnO NPs.

2. Materials and Methods

2.1. Materials and Reagents

To synthesize nanoparticles of zinc oxide, zinc nitrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, Sigma-Aldrich, Schnelldorf, Germany) and potassium hydroxide (KOH, Sigma-Aldrich, Schnelldorf, Germany), red tomatoes (*Solanum lycopersicum*) and purple passionfruit (*Passiflora edulis* f. *edulis*) were used. The red tomatoes and the purple passion fruit were purchased in a local market in Lisbon, Portugal. To produce the films, apple pectin (ZPOW PEK-TOWIN S.A., Jasło, Poland) was used, along with glycerol (Avantor Performance Materials Poland S.A. Gliwice, Poland) and commercial nanoparticles Roti[®] nanoMETIC 25 nm (Carl Roth GmbH+ Co KG, Karlsruhe, Germany). All reagents used were of analytical reagent grade and were used as purchased. The water used was purified using the Milli-Q system (Millipore, Billerica, MA, USA). Ethanol absolute, potassium hydroxide, and 1,1,3,3-tetraethoxypropane (TEP) were acquired from Sigma-Aldrich (Steinheim, Germany). Glacial acetic acid and sodium hydroxide (NaOH) were purchased from Alfa Aesar (Kandel, Germany), whereas sodium chloride (NaCl), 2-thiobarbituric acid (TBA), nitric acid, hydrochloric acid, sodium carbonate anhydrous, and trichloroacetic acid (TCA) were obtained from PanReac (Barcelona, Spain). All microbiological reagents were purchased from Biokar (Allonne, Beauvais, France): violet red bile glucose (VRBG), tryptone, and plate count agar (PCA).

2.2. Preparation of Passionfruit and Tomato Extract

Firstly, commercially mature purple passionfruit (*Passiflora edulis*) peels were washed and dried. After that, pieces of passionfruit peels (20 g) were mixed with 100 mL of distilled water and boiled for 20 min. Likewise, mature red commercial tomatoes (*Solanum lycopersicum*) were washed, cut into small pieces, and squeezed to retrieve the juice. Then, 100 mL of tomato juice were added to 100 mL of water and boiled for 15 min. The obtained passionfruit and tomatoes extract were filtered with gauze, followed by a Whatmann No. 1 filter (Cytiva Life Sciences, Amersham, UK)

2.3. Synthesis of ZnO Nanoparticles

Zinc nitrate was used as a precursor to the synthesis of ZnO-P (NPs obtained from passionfruit extract) and ZnO-T (NPs obtained from tomato extract). A total of 2% (*w/v*) of zinc nitrate was added to both extract solutions. Then, to the resulting solutions, a (1:6 (*v/v*)) KOH solution (1 M) was added to precipitate ZnO NPs. The precipitated NPs were filtered with a PM UC500 filter (Microdyn Nadir, Wiesbaden, Germany) and were repeatedly washed with distilled water. Finally, both ZnO-P and ZnO-T NPs were dried overnight in an oven (WTB binder, Munich, Germany) at 50 °C. The eco-friendly production of ZnO nanoparticles followed a previous methodology already used for apples [26].

2.4. Physicochemical Characterization of ZnO NPs

The morphological characteristics and the elemental composition of the synthesized ZnO-P and ZnO-T NPs were studied using scanning electron microscopy (SEM- JEOL-JSM7001F apparatus, JEOL, Tokyo, Japan) combined with an X-ray energy dispersion spectrometer (EDS). For the studies of transmission electron microscopy (TEM) a Hitachi H-9000-NA microscope (Hitachi, Tokyo, Japan) was operated at 200 kV; samples were loaded in copper-carbon grids. An FTIR-ATR (Nicolet Thermo Electron, Horsham, UK) spectrometer in transmittance mode was used to identify the functional groups present in the synthesized ZnO NPs in the range of 600–4000 cm^{-1} .

2.5. Preparation of Pectin Films

Aqueous film-forming solutions were produced using apple pectin at a concentration of 5% and zinc oxide nanoparticles at 5% relative to pectin (0.25 g mixed with 5 g pectin powder) with glycerol as a plasticizer at 30% relative to pectin (1.5 g per 100 g water). The solutions were heated at 60 °C for 20 min at 250 rpm using an RCT basic IKAMAG magnetic stirrer (IKA Poland, Warsaw, Poland) to obtain a uniform film-forming solution. The final step in film production was to pour the films onto sheets at a speed of 10 mm/s and a film thickness of 1500 μm using a Zehntner ZAA 2300 automatic film applicator (Zehntner GmbH Testing Instruments, Sissach, Swiss), followed by drying at 30 °C for approximately 24 h (SUP-65 WG, WAMED S.A., Warsaw, Poland).

2.6. Physicochemical Characterization of Films

The film surface was analyzed by scanning electron microscopy (SEM) using a JEOL JSM-7001F (Tokyo, Japan) device with a 15 kV energy beam and a working distance of 10 mm.

2.7. Use of Coatings in Fresh Poultry Meat

Fresh ground poultry was purchased from a supermarket. Its samples (about 20 g of meat) were wrapped in previously produced film and stored in plastic boxes with caps in a refrigerator (5 ± 2 °C) for 15 days. Unpacked meat served as the control, and the experiment was carried out in triplicate. The meat was characterized periodically (storage days 0, 4, 8, 11, and 15).

2.7.1. Antibacterial Properties of Films

The number of microorganisms in the meat was determined to evaluate its quality, which was used to determine the effectiveness of the produced films. Appropriate dilutions were produced for each meat sample and applied to Petri dishes with a suitable medium. Total aerobic mesophilic microorganisms (TAMM) [27], total aerobic psychotropic microorganisms (TAPM) [28], and *Enterobacteriaceae* [29] were tested. PCA was used to test the first two types of microorganisms, whereas VRBG was used for the third. The seeded Petri dishes were incubated appropriately at 30 °C for 72 h (TAMM), 7 °C for 168 h (TAPM), or 37 °C for 24 h (*Enterobacteriaceae*). Results are expressed as log CFU (colony forming units)/g meat.

2.7.2. Physicochemical Characterization of Poultry Meat

Poultry meat was analyzed in terms of pH, titratable acidity, and moisture according to AOAC methods [30]. The total volatile basic nitrogen (TVB-N) was determined according to the method described by Malle and Poumeyrol [31]. Briefly, the sample was homogenized with 7.5% trichloroacetic acid and then filtered through Whatman No.1 filter paper. Then the solution was diluted based on the expected amount of nitrogen. Phenolphthalein at 0.1% was used as an indicator, and sodium hydroxide (NaOH 6 N) was added to alkalinize the mixture. Subsequently, 50 mL of 2% boric acid (20 g/L) and 0.5 mL of indicator solution were added to an Erlenmeyer flask, which collected the distillate, where the color changed from purple to green. Finally, the distillate solution was titrated with hydrochloric acid (HCl 0.02 N) until the solution turned purple again. The result is expressed as grams of nitrogen per 100 g of meat (g N/100 g). TBARS were used to monitor the lipid oxidation. To extract the malonaldehyde (MDA), the same extract obtained was used for the determination of TVB-N. The filtrate was combined with 5 mL of TBA 0.02 M and heated (95 °C/30 min) in a water bath (Memmert, Buechenbach, Germany). After cooling, the absorbance was measured at 530 nm in UV/VIS spectrophotometer (Spekol 1500, Analytikjena, Jena, Germany). A calibration curve using known concentrations of MDA (from TEP solution) was used to calculate the TBARS index. Results are expressed as mg of MDA/kg of meat [32].

2.7.3. Migration of Zinc Oxide Nanoparticles to Poultry Meat

The migration of zinc released into the poultry meat from the coatings was also investigated. The samples were mineralized using the dry method at 550 °C. Zinc concentration was checked by atomic absorption spectrometry (Zeenit 700, Analytikjena, Jena, Germany), after digesting the ash residue with nitric acid [33]. Results are presented as mg Zn/kg fresh meat.

2.7.4. Poultry Meat Color

The determination of poultry meat color was carried out using the CIELAB instrumental color measurement system ($L^*a^*b^*$) using a CR 410 colorimeter (Minolta Co., Tokyo, Japan) with a D65 light source and a visual angle of 10°. The determination was carried out on the surface of the poultry meat. The test temperature was equal to the ambient temperature of approximately 20 °C. The color was measured five times in each sample, and the average color components of the measurements (L^* , a^* , and b^*) were determined. The L^* color component measures perceptual lightness and takes values from 0 (black) to 100 (white), the a^* color component represents the color contribution from green (−) to red (+), and the b^* color component represents the color contribution from blue (−) to yellow (+).

The hue angle was calculated based on Equation (1) [34]:

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

where b^* and a^* are the coordinates measured from the samples.

2.8. Statistical Analysis

The experiments were run using a completely randomized design with three replications. Statistical analysis of the data was performed through a one-way analysis of variance (ANOVA) using Software OriginLab (version 8.5, Northampton, MA, USA), and when ANOVA was significant ($p < 0.05$), differences among mean values were processed by the Tukey test. Significance was defined at $p < 0.05$.

3. Results and Discussion

3.1. ZnO Nanocomposite Film Characterization

The morphologies of the ZnO nanoparticles precipitated from solutions containing purple passionfruit or tomato extracts by a green synthesis method are illustrated in Figure 1a,b, respectively. First, it can be observed that ZnO nanoparticles synthesized with different extracts had different morphologies. Additionally, it can be seen that the extract used in the synthesis had a strong impact on the size of the nanoparticles. It can be observed that the morphology of the ZnO-P precipitated in the presence of the passionfruit extract (Figure 1a) resulted in nanoparticles with a cotton-like morphology, whereas the ZnO-T formed in the presence of tomato extract (Figure 1b) presented a spherical-like morphology. The average size of the ZnO-P NPs was in the range of 300–500 nm, with a thickness of around 30 nm, whereas the average size of ZnO-T NPs was closer to 80 nm. It is known that there are different phytochemicals present in purple passionfruit [35] and tomato [36] extracts that can bind selectively to specific crystallographic facets [26], resulting in ZnO nanoparticles with different nanostructures.

The strong influence of phytochemicals on the chemical composition of the ZnO nanoparticles was examined through ATR-FTIR. Figure 1c,d shows the ATR-FTIR spectra of ZnO synthesized from solutions with purple passionfruit (Figure 1c) and tomato (Figure 1d). Both spectra showed the characteristic bands of Zn-O stretching at $\sim 840\text{ cm}^{-1}$ [26], as well as the broadening band attributed to absorbed water at around 3440 cm^{-1} . Despite the common features, an additional band was detected at 1100 cm^{-1} and the two bands that were present in the region of $1300\text{--}1700\text{ cm}^{-1}$ became more intense for the ZnO nanoparticles precipitated in the presence of tomato extract, suggesting that more organic compounds derived from the extracts remained adsorbed to the ZnO-T NP surface than to that of ZnO-P. It is known that tomatoes have antioxidant and anti-inflammatory activities and that the bioavailability of phytoconstituents in tomatoes is not affected by increased temperature [36]. This stability means that tomato phytoconstituents may have conferred the same properties to ZnO-T nanoparticles, playing a preponderant role in the antioxidant response.

The surface morphology of the apple pectin films with ZnO-P and ZnO-T NPs is shown in Figure 1e,f, respectively. It can be observed that the presence of ZnO-P and ZnO-T did not cause any significant modification in the morphology of the films. To avoid agglomeration of the particles, the amount of ZnO used in the production of the films was relatively low. No isolated particles could be detected in either film due to the small amounts used and the successful absence of agglomerates.

3.2. Application of the Bionanocoatings in Fresh Poultry Meat

The development of nanocomposites with ZnO NPs has been widely studied, especially by *in vitro* studies [37,38]. Nevertheless, not many studies have explored these materials in contact with food, especially those synthesized with eco-friendly routes [37]. Thus, the information retrieved from the contact of these bionanocoatings with poultry meat may contribute to clarifying the function of these nano-based materials in the preservation of foodstuffs.

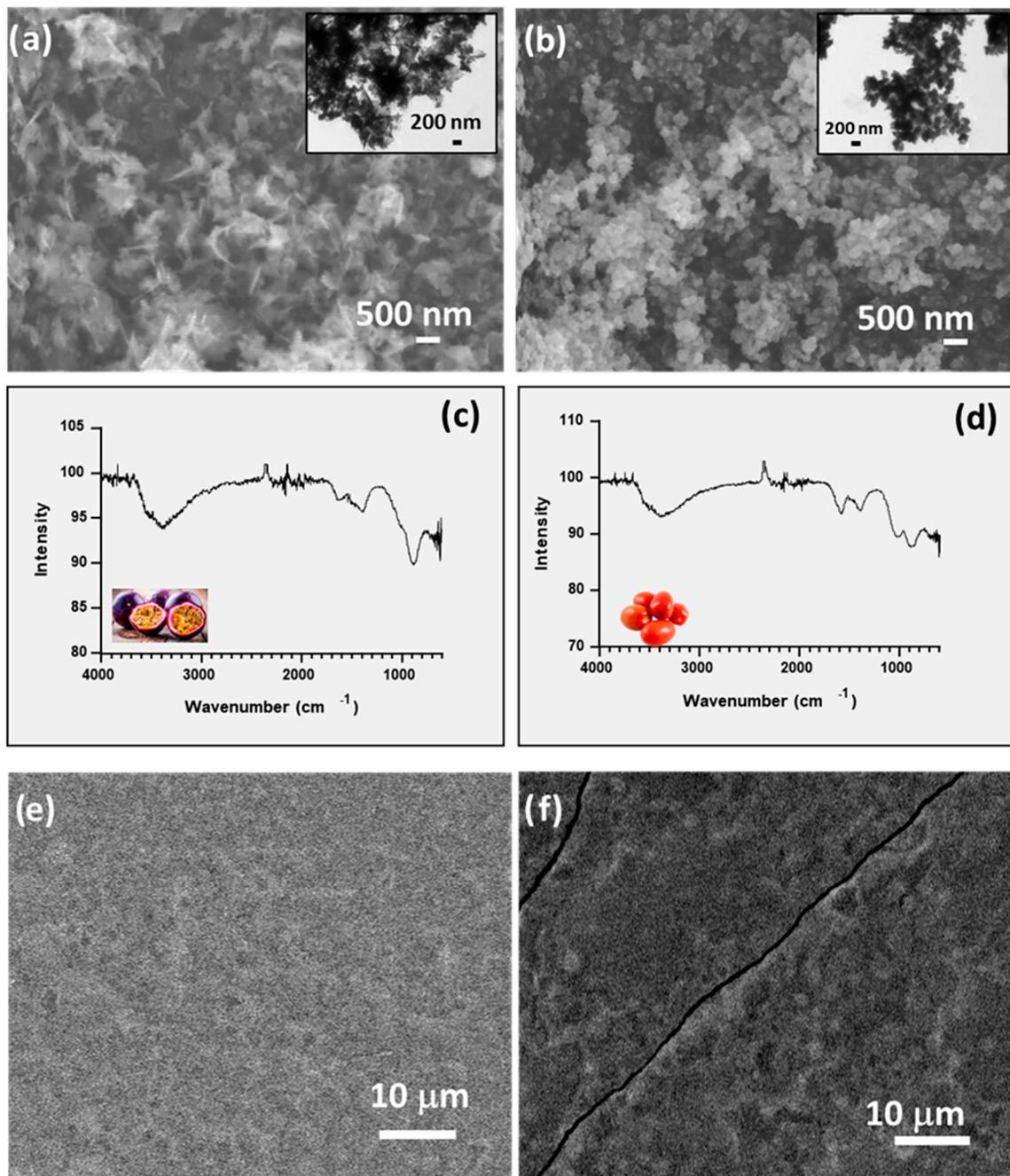


Figure 1. Physicochemical characterization of the ZnO nanocomposites. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) inset images of the ZnO-P NPs (a) and ZnO-T NPs (b) and respective ATR-FTIR analysis (c,d), and SEM top-view images of composite films loaded with ZnO-P (e) or loaded with ZnO-T (f).

3.2.1. Microbiological Growth

Fresh grounded poultry meat packaged in the bionanocomposites was evaluated in terms of total aerobic mesophilic (TAM) microorganisms, total aerobic psychotropic (TAP) microorganisms, and *Enterobacteriaceae* during the refrigerated storage time. The microbiological growth results are shown in Table 1.

Table 1. Microbiological results of the wrapped and unwrapped poultry meat during storage time.

Parameter	Day	Unwrapped	Pec	Pec + ZnO-C NPs	Pec + ZnO-T NPs	Pec + ZnO-P NPs
Total mesophilic aerobic microorganisms (Log CFU/g meat)	0	4.86 ± 0.06 ^{aD}	4.86 ± 0.06 ^{aC}	4.86 ± 0.06 ^{aC}	4.86 ± 0.06 ^{aC}	4.86 ± 0.06 ^{aC}
	4	7.69 ± 0.08 ^{aC}	7.29 ± 0.53 ^{abcB}	7.02 ± 0.04 ^{bB}	6.87 ± 0.02 ^{cB}	6.93 ± 0.23 ^{bcB}
	8	10.55 ± 0.78 ^{aB}	9.59 ± 0.66 ^{aA}	10.71 ± 1.49 ^{aA}	10.49 ± 1.47 ^{aA}	10.76 ± 1.55 ^{aA}
	11	13.33 ± 0.57 ^{aA}	9.60 ± 0.31 ^{bA}	8.96 ± 0.33 ^{bcA}	8.71 ± 0.29 ^{cA}	8.94 ± 0.30 ^{bcA}
	15	12.17 ± 1.18 ^{aAB}	9.70 ± 1.37 ^{aA}	9.74 ± 1.36 ^{aA}	9.69 ± 1.29 ^{aA}	10.00 ± 1.42 ^{aA}
Total psychrotropic aerobic microorganisms (Log CFU/g meat)	0	3.43 ± 0.20 ^{aC}	3.43 ± 0.20 ^{aC}	3.43 ± 0.20 ^{aD}	3.43 ± 0.20 ^{aC}	3.43 ± 0.20 ^{aD}
	4	6.99 ± 0.16 ^{aB}	6.72 ± 1.29 ^{aB}	7.09 ± 0.38 ^{aC}	6.83 ± 0.46 ^{aB}	7.05 ± 0.68 ^{aC}
	8	10.51 ± 0.90 ^{aA}	9.22 ± 0.78 ^{abA}	9.82 ± 1.11 ^{abAB}	9.84 ± 1.33 ^{abA}	8.55 ± 0.19 ^{bB}
	11	12.06 ± 0.63 ^{aA}	8.22 ± 1.04 ^{bAB}	8.48 ± 0.95 ^{bB}	8.88 ± 0.08 ^{bA}	8.91 ± 0.34 ^{bB}
	15	11.51 ± 0.18 ^{aA}	9.61 ± 1.34 ^{bA}	10.43 ± 0.78 ^{bA}	9.93 ± 1.32 ^{bA}	10.50 ± 0.70 ^{bA}
<i>Enterobacteriaceae</i> (Log CFU/g meat)	0	2.36 ± 0.17 ^{aE}	2.36 ± 0.17 ^{aC}	2.36 ± 0.17 ^{aD}	2.36 ± 0.17 ^{aC}	2.36 ± 0.17 ^{aD}
	4	5.71 ± 0.10 ^{aD}	5.86 ± 0.89 ^{abB}	5.62 ± 0.03 ^{aC}	5.34 ± 0.44 ^{abB}	5.08 ± 0.35 ^{bc}
	8	9.54 ± 0.06 ^{aB}	8.17 ± 0.25 ^{cA}	8.64 ± 0.01 ^{bA}	7.85 ± 0.71 ^{cdA}	7.57 ± 0.01 ^{dA}
	11	11.19 ± 0.54 ^{aA}	8.15 ± 0.11 ^{bA}	7.80 ± 0.17 ^{cB}	7.81 ± 0.33 ^{bcA}	7.00 ± 0.20 ^{dB}
	15	8.38 ± 0.38 ^{abC}	8.36 ± 0.58 ^{abA}	8.22 ± 0.72 ^{abAB}	8.31 ± 0.21 ^{aA}	7.39 ± 0.66 ^{bAB}

(A–E): Within each parameter, values in the same column not sharing uppercase superscript letters indicate statistically significant differences among days ($p < 0.05$) (a–d): Within each parameter, values on the same line not sharing lowercase superscript letters indicate statistically significant differences among formulations ($p < 0.05$). Pectin (Pec), zinc oxide commercial nanoparticles (ZnO-C NPs), and zinc oxide nanoparticles obtained from tomato extract (ZnO-T NPs) and passionfruit extract (ZnO-P NPs).

Poultry meat is considered a highly perishable food, in which rapid microbial growth and lipid oxidation take place [39,40]. These phenomena are responsible for the deteriorative process of meat and meat products, with losses in their quality and safety by changing their organoleptic properties (color, texture, and flavor) and nutrition value [41–43]. All samples presented this natural deteriorative process, which can be observed by the increase ($p < 0.05$) in the count for all microorganisms assessed over the refrigerated storage time (Table 1).

According to Regulation (EC) No. 2073/2005, uncooked minced meat is considered inappropriate for consumption when the TAM count reaches 6.69 log CFU/g meat [44]. The initial condition of the meat studied was below this limit, and over time the total mesophilic aerobic bacteria increased ($p < 0.05$), reaching a final count at day 15 varying from 9.69 to 12.17 log CFU/g meat. Unprotected samples presented the highest contamination, whereas the films retarded the proliferation of bacteria, reducing the TAM up to 2.48 log CFU/g meat with the Pec + ZnO-T NPs at day 15 of storage. Overall, no statistically significant differences were found between the samples in each of the assessed days ($p > 0.05$), except for days 4 and 7, when the unwrapped samples were always more contaminated than those protected with the films produced ($p < 0.05$). These results highlight the potential of this packaging as a tool to extend the shelf life of fresh poultry meat.

Regarding the psychotropic bacteria count, similar behavior was observed; however, there was a statistical difference between treatments starting only at day 8 of storage ($p < 0.05$) (Table 1). On day 15, unwrapped meat presented a TAP count of 11.51 log CFU/g meat, whereas for the samples protected with the pectin films that value was found to range from 9.61 to 10.50 log CFU/g meat, depending on the film used. This represents reductions varying from $\cong 1$ to 2 log CFU/g meat. Again, among the different pectin films, no statistical differences were observed ($p > 0.05$), but the lowest contamination found within the ZnO NP incorporated films was for those synthesized with tomato peels (ZnO-T NPs). Once most of the psychophilic strains are spoilage microorganisms and some are pathogenic (e.g., *Listeria monocytogenes*) [45], the capacity to reduce the TAP count enables these types of bio-based packaging to enhance the microbiological security while retarding the deterioration process of the packaged food products.

According to Econmou et al. [46], at TAM counts greater than 7 log CFU/g meat consumers start rejecting poultry meat, and this can be used as a limit for the sensory acceptance of this class of food product. Mesophilic bacteria, which may be responsible for the processes of spoilage and putrefaction and thus reduce the quality and cause deterioration of the taste and smell of meat, include, among others, *Bacillus*, *Micrococcus*, *Lactobacillus*, and *Streptococcus* bacteria. In fact, this level of contamination is in accordance with the safety standards established in European Regulation (EC) No. 2073/2005, as previously mentioned [44]. Taking this into consideration, at day 4 of the shelf-life assessment only the meat protected with the bionanocomposites with ZnO NPs synthesized with tomato or passionfruit by-products were below this limit, whereas unwrapped meat and meat wrapped with pectin film or pectin + commercial ZnO NPs exceeded it. It is noticeable that all samples protected with the bionanocomposite presented slightly smaller contamination, which may be related to the antimicrobial action of ZnO. This effect started to be neglected from day 8 of storage, indicating that ZnO NPs might have been released from the films in the first few days of the trials.

Polysaccharide polymers are known for their good oxygen barrier [47]. In fact, pectin film prepared similarly as in this work presented an oxygen permeability of 1.12×10^{-16} mol·m/m²·s·Pa [12], which is the same magnitude as the permeability reported for EVOH (0.24×10^{-16} mol m/m²·s·Pa [48]), a food-packaging material that is considered one of the best hydrophilic gas barriers used in the industry [13]. Moreover, the inclusion of nanoparticles (such as metal oxides) in polymeric matrices in general enhances the composite barrier properties, as the NPs create a more tortuous path that slows the permeation of gases through the polymeric chain [11]. Thus, the reduction in the microbial contamination of the meat protected with the bio-based films could also be related to the good O₂ barrier properties of pectin film once the growth of aerobic microorganisms is conditioned to the presence of oxygen [11,24].

The EFSA recommends the enumeration of the family *Enterobacteriaceae* in food-manufacturing environments and finished products as a routine for monitoring contaminations across the food chain [44]. Overall, the *Enterobacteriaceae* count increased over time for all treatments ($p < 0.05$) but with less intensity for those samples wrapped in pectin films (Table 1). The inclusion of ZnO NPs demonstrated the tendency to reduce the contamination even further, especially for the ZnO NPs synthesized with passionfruit by-products, which presented a smaller count for this family of microorganisms among the ZnO NPs tested. The ZnO NPs synthesized using food industry by-products also have the advantage of containing polyphenols [24] found in food (in this case tomato and passionfruit). Since these are compounds known for their antimicrobial and antioxidant properties [49], a synergistic antimicrobial effect of ZnO NPs and polyphenols may have occurred, explaining the enhanced protective property. Moreover, ZnO NPs are reported to be more effective against Gram-negative bacteria [37,50], and as *Enterobacteriaceae* are a group of psychotropic facultative anaerobic bacteria, better protective properties against this group of microorganisms were expected to be found compared to the TAM and TAP.

However, the antimicrobial mechanism of action of ZnO NPs is not fully understood. The most accepted and proposed one is based on the release of Zn²⁺, which affects active transport inhibition, amino acid metabolism, and enzyme system disruption. Through electrostatic interactions with the bacterial membrane, the ions are internalized and generate reactive oxygen species (ROS), which are responsible for oxidative stress and cell death [50,51].

Similar behavior was observed in fresh poultry meat wrapped in bionanocomposites based on chitosan and ZnO NPs synthesized with apple peels [24]. In this work, the intrinsic antimicrobial properties of chitosan were confirmed by delaying the growth of deteriorative microorganisms, which was enhanced by the zinc oxide nanoparticles added. The authors also reported the maintenance of the initial reddish color and a reduction in the oxidation process when compared to unwrapped meat [24]. In the recent work by Sharaby et al. [52], composite films of pectin/ZnONP/CNC (cellulose nanocrystals) were

evaluated in the preservation of sliced cheese after contamination with *Staphylococcus aureus*. The samples protected in the active films presented reductions of up to 1.2 log CFU/g after 5 days in refrigerated conditions (7 °C), and the small antimicrobial activity of the films was attributed to the small release of Zn²⁺ to the cheese [52]. Another shelf-life assessment of fresh poultry meat protection in active films incorporated with ZnO NPs was conducted by Mohammadi et al. [53]. The authors also observed an increased count for all bacteria assessed over the refrigerated storage, but all microbial counts were higher in the samples protected with the control films, i.e., carboxymethylcellulose (CMC) without the incorporation of either the nanoparticles or okra extract. A synergic effect of the okra and ZnO NPs was observed, demonstrating that the use of other compounds with known antimicrobial properties is a good strategy to enhance the activity of bionanocomposites with zinc oxide nanoparticles [53].

3.2.2. Physicochemical Characterization of Poultry Meat

Results obtained on the physicochemical characterization of poultry meat are displayed in Table 2 and Figure 2.

Table 2. Physicochemical results of the wrapped and unwrapped poultry meat during storage time.

Parameter	Day	Unwrapped	Pec	Pec + ZnO-C NPs	Pec + ZnO-T NPs	Pec + ZnO-P NPs
Moisture (%)	0	75.3 ± 0.3 ^{aB}	75.3 ± 0.3 ^{aA}	75.3 ± 0.3 ^{aA}	75.3 ± 0.3 ^{aA}	75.3 ± 0.3 ^{aA}
	4	75.0 ± 0.4 ^{aB}	69.5 ± 1.5 ^{cB}	71.0 ± 0.9 ^{bcB}	72.2 ± 0.7 ^{bB}	72.4 ± 1.6 ^{bcB}
	8	75.2 ± 0.6 ^{aB}	67.3 ± 2.6 ^{bB}	70.1 ± 1.2 ^{bbC}	70.2 ± 1.0 ^{bC}	70.3 ± 1.3 ^{bBC}
	11	76.9 ± 0.5 ^{aA}	68.5 ± 1.4 ^{bB}	68.8 ± 0.4 ^{bC}	68.9 ± 1.4 ^{bC}	68.9 ± 1.5 ^{bC}
	15	76.2 ± 0.8 ^{aAB}	66.5 ± 2.8 ^{bcB}	65.6 ± 1.8 ^{bcD}	65.9 ± 0.8 ^{cD}	69.5 ± 2.3 ^{bBC}
pH	0	5.96 ± 0.03 ^{aC}	5.96 ± 0.03 ^{aA}	5.96 ± 0.03 ^{aA}	5.96 ± 0.03 ^{aA}	5.96 ± 0.03 ^{aA}
	4	6.07 ± 0.03 ^{aB}	5.90 ± 0.06 ^{bcA}	5.76 ± 0.03 ^{dB}	5.83 ± 0.02 ^{cB}	5.94 ± 0.02 ^{aA}
	8	6.16 ± 0.06 ^{aB}	5.39 ± 0.06 ^{cB}	5.40 ± 0.07 ^{cC}	5.60 ± 0.04 ^{bC}	5.67 ± 0.13 ^{bB}
	11	6.50 ± 0.40 ^{aB}	5.30 ± 0.04 ^{bB}	5.19 ± 0.02 ^{cD}	5.29 ± 0.03 ^{bD}	5.30 ± 0.06 ^{bC}
	15	7.62 ± 0.15 ^{aA}	5.32 ± 0.06 ^{bB}	5.17 ± 0.07 ^{cdD}	5.16 ± 0.02 ^{dE}	5.25 ± 0.04 ^{bcC}
Titratable acidity (% oleic acid equivalent)	0	3.80 ± 0.03 ^{aC}	3.80 ± 0.03 ^{aC}	3.80 ± 0.03 ^{aC}	3.80 ± 0.03 ^{aE}	3.80 ± 0.03 ^{aC}
	4	4.10 ± 0.05 ^{cB}	4.65 ± 0.33 ^{aB}	4.02 ± 0.01 ^{cB}	4.26 ± 0.03 ^{bD}	3.93 ± 0.03 ^{dB}
	8	4.27 ± 0.03 ^{bA}	5.02 ± 0.73 ^{abB}	4.31 ± 0.38 ^{abAB}	4.60 ± 0.02 ^{aC}	4.14 ± 0.54 ^{abAB}
	11	3.26 ± 0.09 ^{cD}	4.84 ± 0.66 ^{abB}	4.88 ± 0.48 ^{abA}	4.66 ± 0.00 ^{aB}	4.27 ± 0.13 ^{bA}
	15	2.88 ± 0.04 ^{cE}	5.85 ± 0.02 ^{aA}	4.71 ± 0.52 ^{bA}	4.78 ± 0.05 ^{bA}	4.71 ± 0.43 ^{bA}
TBARS (mg malonaldehyde/kg meat)	0	0.26 ± 0.14 ^{aBC}	0.26 ± 0.14 ^{aC}	0.26 ± 0.14 ^{aB}	0.26 ± 0.14 ^{aC}	0.26 ± 0.14 ^{aC}
	4	0.21 ± 0.05 ^{cC}	0.53 ± 0.15 ^{abC}	0.36 ± 0.07 ^{abB}	0.41 ± 0.06 ^{aC}	0.31 ± 0.00 ^{bC}
	8	0.27 ± 0.12 ^{bBC}	0.42 ± 0.02 ^{bC}	0.42 ± 0.04 ^{bB}	0.37 ± 0.01 ^{bC}	0.75 ± 0.20 ^{aB}
	11	0.56 ± 0.23 ^{bAB}	0.99 ± 0.00 ^{aA}	0.44 ± 0.11 ^{bB}	0.52 ± 0.03 ^{bB}	0.59 ± 0.08 ^{bB}
	15	0.75 ± 0.13 ^{cA}	0.78 ± 0.11 ^{cB}	0.94 ± 0.02 ^{bA}	1.25 ± 0.30 ^{abA}	1.68 ± 0.18 ^{aA}
Hue angle (°)	0	55 ± 2 ^{aA}	55 ± 2 ^{aAB}	55 ± 2 ^{aB}	55 ± 2 ^{aB}	55 ± 2 ^{aC}
	4	57 ± 2 ^{abA}	54 ± 1 ^{bB}	60 ± 3 ^{aAB}	58 ± 3 ^{abAB}	58 ± 3 ^{abBC}
	8	54 ± 3 ^{bA}	58 ± 1 ^{abAB}	60 ± 2 ^{abAB}	61 ± 3 ^{abAB}	60 ± 1 ^{aB}
	11	61 ± 5 ^{abA}	59 ± 3 ^{bAB}	63 ± 2 ^{abA}	65 ± 3 ^{abA}	66 ± 2 ^{aA}
	15	54 ± 4 ^{bA}	60 ± 4 ^{abA}	62 ± 2 ^{abA}	63 ± 2 ^{aA}	62 ± 2 ^{abAB}

(A–E): Within each parameter, values in the same column not sharing uppercase superscript letters indicate statistically significant differences among days ($p < 0.05$) (a–d): Within each parameter, values on the same line not sharing lowercase superscript letters indicate statistically significant differences among formulations ($p < 0.05$). Pectin (Pec), zinc oxide commercial nanoparticles (ZnO-C NPs), and zinc oxide nanoparticles obtained from tomato extract (ZnO-T NPs) and passionfruit extract (ZnO-P NPs).

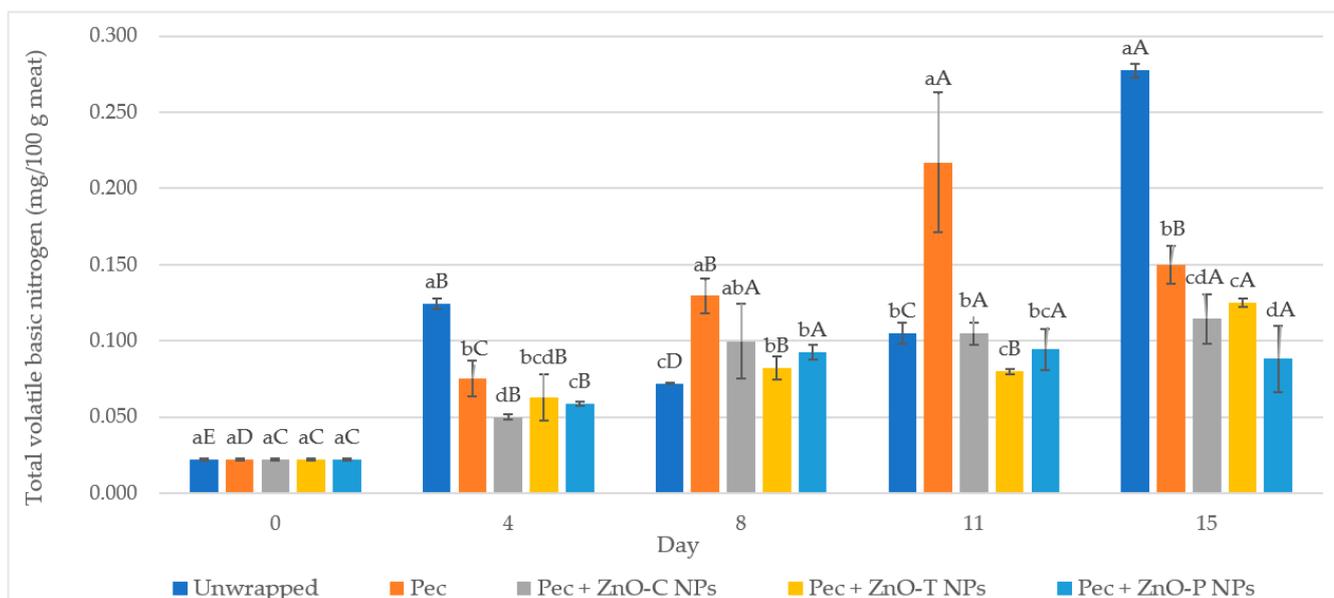


Figure 2. Total volatile basic nitrogen (mg/100 g meat) results of the wrapped and unwrapped poultry meat during storage time. ^(A–E): For each type of packaging treatment, values not sharing upper case superscript letters indicate statistically significant differences among days ($p < 0.05$); ^(a–d): At each day, values not sharing lower case superscript letters indicate statistically significant differences among formulations ($p < 0.05$). Pectin (Pec), Zinc oxide commercial nanoparticles (ZnO-C NPs) and Zinc Oxide nanoparticles obtained from tomato extract (ZnO-T NPs) and passion fruit extract (ZnO-P NPs).

The moisture content in unwrapped control meat stayed constant from day 0 to day 8, but an increase was observed after days 11 and 15. However, the differences were not significant ($p < 0.05$). The values were from 75.3 to 76.9% and indicated a fresh, high-moisture product (Table 2). Biobased packaging resulted in decreasing values of moisture content for all determinants. This can be attributed to the hydrophilic nature of pectin films [12,54], which resulted in the adsorption of moisture from poultry meat during storage. The values ranged from 75.3 to 66.5% for pectin films without nanoparticles, and similar ranges were observed for films containing commercial nanoparticles and those prepared from tomato extract. There was less reduction in moisture when films incorporated with ZnO synthesized with passionfruit extract were used (75.3–69.5%) compared with the ones with ZnO from tomato extract but not with pristine pectin or with pectin incorporated with commercial ZnO NPs. The differences between the samples were due to the interactions and compatibility of the components, which may vary based on the nanoparticle origin and characteristics.

There was a significant difference in pH values ($p < 0.05$) in the unwrapped poultry meat samples (Table 2) during storage. From day 0 to day 15, pH values gradually increased from 5.96 to 7.62. This was the most dynamic change in the pH values among all analyzed samples, as the surface of the control samples was easily exposed to water vapor, dirt, and microorganisms. The pH increase can be associated with the production of volatile basic components due to microbial growth, the denaturation process, and the consequential release of amines and ammonia [55]. The obtained results concerning the increase in pH values in the analyzed poultry meat (unwrapped) were in agreement with those reported by Eldaly et al. [56] and Amjadi et al. [57]. Moreover, the initial pH of the poultry meat is in agreement with results presented in the literature of values ranging from 5.2 to 7 [24]. Biobased packaging limited the pH increase over time, probably due to inhibition of the growth of bacteria and protein denaturation (in agreement with the results presented in Table 1 and discussed in Section 3.2.1). Pectin films both without and with the addition of ZnO nanoparticles showed a significant effect in reducing pH values and making the

samples more acidic, which can be related to the production of lactic acid by lactic acid bacteria. On each day, the pH values of control were higher than the pH of the wrapped meat. The lowest pH values (5.16–5.17 by 15 days of storage) were observed for poultry meat wrapped with films incorporated with zinc oxide commercial nanoparticles and those obtained from tomato extract. The intensity of changes in the pH values of chicken meat was somewhat different from those obtained by others. Meindrawan et al. [58] reported that the applications of biocomposite edible coating based on bovine gelatin incorporated with ZnO nanoparticles maintained the pH of fillets at 6. These observations were also confirmed by Naveen et al. [59] for chicken meat when using polymer composite packaging films embedded with zinc oxide nanoparticles. However, Souza et al. [12] noted an increase in pH values for control and wrapped chicken meat with chitosan-based films incorporated with zinc oxide nanoparticles during storage. The authors observed that the biobased packaging delayed the increase in pH values, which was greater at higher concentrations of ZnO nanoparticles.

The titratable acidity, expressed in % of oleic acid equivalent, increased over time for all samples of chicken meat except on days 11 and 15 for control. For these samples, an increase from 3.80 to 4.10 and 4.27 on days 4 and 8, respectively, was observed, and then a reduction to 3.26 and 2.88, respectively (Table 2). This decrease was expected, as it correlates with the increment observed in the pH values. The meat protected with biobased packaging presented higher values of titratable acidity, which was the most noticeable for pectin films without ZnO nanoparticles. These results also agree with the reduction in pH values observed for the wrapped poultry meat. The results also indicate that the rate of acidity increment was lower when ZnO was added to pectin.

The results of the total volatile basic nitrogen (TVB-N) content in poultry meat (Figure 2) are also in agreement with the results reported for pH, acidity, and microbiological growth. Indeed, TVB-N is an indicator of meat spoilage, which is related to protein breakdown by microbial development and enzymatic action, which produces ammonia and amines, among other alkaline substances containing nitrogen [55,60]. Therefore, the increment in TVB-N is associated with the degradation and decomposition of poultry meat. This increment was observed for all the samples, both unwrapped and wrapped, with significance ($p < 0.05$) and with an initial value of 22 mg/100 g poultry meat (Table 2). Yet, the release of TVB-N throughout storage time (15 days) was significantly slower for the wrapped meat. At 15 days of storage, unwrapped poultry meat presented a value of 278 mg/100 g poultry meat, a much higher result than the results presented by wrapped meat (88–150 mg/100 g). Interestingly, the protected meat with pristine pectin films also showed a significantly higher ($p < 0.05$) TVB-N value than the pectin films with ZnO nanoparticles (Table 2), thus indicating the capacity of ZnO to reduce the degradation process of poultry meat and to enlarge poultry meat shelf life. The same conclusion was reported by Suo et al. in their work, wherein fresh pork meat protected with carboxymethylcellulose (CMC) film either incorporated with ZnO NP or not was monitored for a long time in cold storage [55]. The same trend was also observed by Souza et al. [24], who incorporated ZnO NPs synthesized with apple peels into chitosan films to protect poultry meat. Among the different NPs, passionfruit bionanocomposites presented the lowest TVB-N value at 15 days of storage, in accordance with the data observed for the *Enterobacteriaceae* (Table 1), where poultry meat wrapped with pectin incorporated with zinc oxide nanoparticles obtained from passionfruit extracts (ZnO-P NPs) also presented the lowest values at day 15.

The high content of unsaturated fatty acid in poultry meat makes it susceptible to oxidation processes [61]. During this oxidative degradation, lipids, vitamins, and pigments are oxidized, leading to food odor and flavor alterations, with possible concurrent production of toxic compounds that may cause a threat to food safety [62]. These processes, together with microbiological growth, contribute to the deterioration of fresh poultry meat, reducing its shelf life. The Thiobarbituric Acid Reactive Substances (TBARS) Index and poultry meat color were two parameters analyzed to assess the oxidative processes in the poultry meat

during the 15 days of storage. The results of the TBARS (Table 2) showed an increment over time for all the samples ($p < 0.05$), a result that was expected given the nature of poultry meat. For the majority of the samples, from day 11, the results were higher than the off-flavor trash-hold value (0.5 mg MDA/kg), which is rated as the indicator of rancidity [32]. Yet, poultry meat wrapped with pectin incorporated with zinc oxide nanoparticles obtained from passionfruit extracts (ZnO-P NPs), reached this value earlier (day 8), and poultry meat wrapped with pectin incorporated with commercial ZnO nanoparticles reached this value only on day 15. In this case, the oxidative process was delayed for several days in this bionanocomposite compared with the other biobased films and with unwrapped samples. This result can be attributed to the higher barrier of pectin to UV light and O₂ caused by the incorporation of these ZnO commercial nanoparticles, as was observed by Baek and Song [63] with films based on *Gracilaria vermiculophylla* extract incorporated with ZnO NPs, which delayed the oxidation process of smoked salmon. The same trend was also registered when poultry meat was wrapped with low-density polyethylene (LPDE) incorporated with Ag and ZnO NPs [64]. Pristine pectin film did not retard the oxidation processes of the meat (Table 2) compared with unwrapped meat, although some authors mentioned the good barrier properties of pectin to oxygen [47]. When testing chitosan in a similar assay with poultry meat, chitosan showed a deceleration of the oxidative process [24], which was not observed with pectin in this assay (Table 2). The differences in the behavior of chitosan and pectin can be attributed to differences in O₂ permeabilities. According to Souza et al. [12], chitosan O₂ permeability reached a value of 0.28×10^{-16} mol.m/m².s.Pa and pectin had a much higher value of 1.12×10^{-16} mol.m/m².s.Pa. In addition, contrary to what was observed in terms of microbial growth, the incorporation of ZnO NPs synthesized from tomato extracts and passionfruit extracts also did not contribute to reducing the oxidative processes in poultry meat compared with unwrapped meat and meat wrapped with pectin only (Table 2). On the contrary, the results indicate that the incorporation of those ZnO nanoparticles induced significantly higher values of TBARS compared with unwrapped and pristine pectin-wrapped poultry meat ($p < 0.05$) (Table 2). It was expected that the bionanocomposites made from tomato and passionfruit extracts would improve the polymer's antioxidant activity, associated with the presence of phenolic compounds from those extracts [65,66], as was reported for apple peel extracts [24]. However, the opposite was observed, and tomato extracts and passionfruit extracts showed a pro-oxidant effect. The same was also reported by other authors. Kenar et al. [67] showed that sardine fillets treated with sage tea extracts showed significantly higher MDA values compared to the control, thus indicating that sage tea extracts presented pro-oxidant activity.

Concerning the hue angle, the initial color was 55°, which represents a reddish-orange color. This poultry meat presented a value that was more reddish than the value of poultry meat presented by Souza et al. [24], which was more orange than reddish. During refrigerated storage, unwrapped poultry meat maintained the same value, indicating that the characteristic reddish-orange color of poultry meat was maintained in spite of its deterioration. Concerning wrapped meat, all the samples increased ($p < 0.05$) the hue angle to 60–63° (Table 2), which indicates a change in the poultry meat color to a more orange color than red. Yet, at day 15, only hue angle values of poultry meat wrapped with pectin incorporated with zinc oxide nanoparticles obtained from tomato extracts (ZnO-TNPs) were significantly ($p < 0.05$) higher than the hue angle of unwrapped poultry meat (Table 2). The other wrapped samples presented a higher hue angle than unwrapped poultry meat, but the difference was not significant ($p > 0.05$) (Table 2). At day 11, no significant differences were observed between wrapped and unwrapped poultry meat in terms of color. Hue angle is a parameter that translates the CIELab coordinators a* and b* into color, and values near 0° correspond to red, whereas higher values of about 90° correspond to yellow [68]. Therefore, the change to a more orange than red color in the wrapped meat can be attributed to a decrease in the a* value during cold storage, which may have resulted from the oxymyoglobin oxidation to metmyoglobin [55]. This oxidative process is also well correlated with the TBARS results, which showed higher values in

poultry meat wrapped with pectin incorporated with ZnO nanoparticles synthesized with tomato and passionfruit extracts, which exhibited pro-oxidant activity. A comparison made with other studies reported in the literature shows that this pro-oxidant behavior is not the typical trend observed when meat is being wrapped with a biobased film incorporated with ZnO nanoparticles. Indeed, in the study by Suo et al. [55], fresh pork meat wrapped with ZnO nanoparticles inserted in CMC protected samples from discoloration. The same results were reported when fresh poultry meat was enveloped with chitosan incorporated with ZnO nanoparticles synthesized from apple peels [24]. Therefore, poultry meat color preservation can be related to the antioxidant or pro-oxidant activity of the applied biobased films. Even so, and although wrapped poultry meat presented a more orange color than red than unwrapped poultry meat, the difference observed is not relevant enough to influence consumer choice once the color is in the usual range of poultry meat color (60°) [24].

3.2.3. Total Zinc Migration to Poultry Meat

Concerning the application of ZnO nanoparticles in the biobased films, an evaluation of the migration of soluble ionic zinc to the food matrices should be made in order to evaluate the food safety associated with this type of novel material. This feature is mandatory since novel materials designed to be used in direct contact with foodstuffs should be evaluated in terms of the toxicologic effects that might result from their application [69], which includes migration studies. The European Food Safety Authority (EFSA) panel on food contact materials, enzymes, flavorings, and processing aids (CEF Panel) recommended a no observed adverse effect level of 50 mg/person per day and an upper limit of 25 mg/person per day for zinc [70].

Taking these recommendations into consideration, the total zinc content of the fresh poultry meat at day 0 and after 15 days of refrigerated storage was analyzed in all the samples (wrapped and unwrapped), and the results are displayed in Table 3.

Table 3. Total zinc migration to the wrapped and unwrapped poultry meat at day 0 and after 15 days of storage.

Sample	Zinc Content (mg Zn/kg Fresh Meat)
Initial zinc content—day 0	11.2 ± 2.4^B
Unwrapped—day 15	10.3 ± 2.7^B
Pec—day 15	15.2 ± 3.1^B
Pec + ZnO-CNPs—day 15	30.3 ± 4.1^A
Pec + ZnO-TNPs—day 15	35.2 ± 3.7^A
Pec + ZnO-PNPs—day 15	38.4 ± 4.8^A

(A,B): Values not sharing uppercase superscript letters indicate statistically significant differences among formulations ($p < 0.05$); pectin (Pec), zinc oxide commercial nanoparticles (ZnO-C NPs), and zinc oxide nanoparticles obtained from tomato extract (ZnO-T NPs) and passionfruit extract (ZnO-P NPs).

The content of zinc ions at day 0 was 11.2 mg/kg meat, which is in accordance with the value of 8 mg/kg meat reported by the official Portuguese database (PortFIR) for chicken breast [71]. After 15 days, no significant differences in fresh poultry meat at day 0 were reported for either unwrapped meat or the samples protected with pristine pectin film ($p > 0.05$) (Table 3). On the contrary, poultry meat protected with pectin incorporated with ZnO nanoparticles showed higher zinc content ($p < 0.05$) (Table 3). This can be credited to the migration of ZnO nanoparticles from the bionanobased films to poultry meat. No differences in the Zn migration were observed among the different NPs. The same pattern was also reported in the work of Souza et al. [24], who also tested the effect of ZnO nanoparticles on chitosan films as the packaging on chicken meat. There are several reasons for the diffusion process of ZnO nanoparticles in poultry meat: the association of NPs in the polymeric matrix, the morphologies of the incorporated NPs, the adsorption of phytochemicals to the ZnO NP surface, the polymer-bonding organization resulting from the incorporation of NPs, and the water absorbed [24,26]. However, as no differences

in the Zn migration were reported among the different NPs, it can be assumed that the facilitated diffusion process of ZnO NPs from the pectin matrix was similar to all and possibly linked with pectin's hydrophilic nature [12,54]. The absorption of moisture from the poultry meat by the pectin matrix may have contributed to the diffusion process of ZnO NPs to the poultry meat. The migration of ZnO NPs to poultry meat can also help to explain the antimicrobial activity observed in the bionanocomposites (discussed previously in Section 3.2.1).

Concerning the risks associated with zinc migration to poultry meat, in the bionanofilms tested in the current work, the increment in total Zn was in the range of 19–27 mg/kg poultry meat compared with unwrapped poultry meat at day 0. Considering the recommended upper intake limit of zinc (25 mg/person per day) [69], a medium portion of fresh poultry meat (100 g) may transport 3.0–3.8 mg of Zn, which is 12–15% of the upper intake limit per day. Given these results, it can be assumed that the consumption per day of fresh poultry meat wrapped with those bionanocomposites does not represent a risk. Yet, more studies are needed with these biobased products to better understand the risks associated with consumer exposure.

4. Conclusions

ZnO nanocomposite films were produced with success and the bionanocomposites presented good antimicrobial properties, especially against *Enterobacteriaceae* growth during refrigerated storage of fresh poultry meat. Indeed, pristine pectin-based films protected poultry meat and reduced the growth rate of microorganisms compared to unwrapped meat. However, the incorporation of ZnO NPs in the pectin matrix reduced this growth rate even further. This was attributed to the improvement in the barrier properties (O₂, UV light) derived from the incorporation of the NPs in the polymeric matrix. This improvement was also reflected in the pH, acidity, and total volatile basic nitrogen results. The results obtained for the poultry meat wrapped with pectin-ZnO NPs showed a higher reduction in the degradation process than pristine pectin films compared with unwrapped meat. Despite the reduction in the degradation of poultry meat, the application of the eco-friendly ZnO NPs (synthesized from tomato and passionfruit extracts) in the pectin matrix did not favor the bionanocomposites' antioxidant activity, evaluated by the TBARS values and meat color. In fact, only commercial ZnO NPs were able to retard the oxidative processes of meat, compared to unwrapped poultry meat and meat wrapped with pristine pectin. On the contrary, the results obtained for the poultry meat wrapped with pectin-ZnO NPs derived from tomato and passionfruit showed an increment in TBARS values and meat discoloration. This was attributed to the pro-oxidant activity of the tomato and passionfruit extracts.

Yet, it can be affirmed that fresh poultry meat protected with the bionanocomposites presented an extension of its shelf-life time, and it was confirmed that this eco-friendly packaging has the potential to be employed by the food industry. Comparing the effects of commercial ZnO NPs with ZnO NPs obtained via tomato and passionfruit extracts, it can be concluded that ZnO NPs synthesized from passionfruit extracts resulted in lower *Enterobacteriaceae* growth and lower TVB-N content in poultry meat, but the incorporation of those ZnO NPs also resulted in a higher TBARS content (with no statistical differences compared to poultry meat wrapped with tomato-derived pectin-ZnO NPs). In order to better clarify which formulation would be optimal for poultry meat, it is also important to characterize the properties of the bionanocomposites (mechanical, optical, barrier). Concerning safety issues, the migration of Zn from the films to the poultry meat should also be taken into consideration. Although zinc oxide is listed as a GRAS, further studies should be conducted in order to assess its safety for consumer exposure.

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