Journal of
Composites Science

MDPI

Article

Application of Biocomposite Films of Chitosan/Natural Active Compounds for Shelf Life Extension of Fresh Poultry Meat

João Ricardo Afonso Pires, Karen Miranda Almeida, Ana Sofia Augusto, Érica Torrido Vieira, Ana Luísa Fernando and Victor Gomes Lauriano Souza *,†

MEtRiCS, Departamento de Química, NOVA School of Science and Technology = FCT NOVA, Campus de Caparica, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
* Correspondence: v.souza@campus.fct.unl.pt
† Current affiliation: INL, International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga S/N, 4715-330 Braga, Portugal.

Abstract: Active packaging based on chitosan (Ch) incorporated with six different natural hydro-alcoholic extracts (HAE) (rosemary, green tea, black tea, ginger, kenaf, and sage) were developed and tested to extend the shelf life of fresh poultry meat. The quality of the meat packaged was assessed through physical-chemical and microbiological characterization over 15 days of refrigerated storage. In vitro antimicrobial activity of pure extracts and films against Gram-positive (*B. cereus*) and Gram-negative (*S. enterica*) foodborne bacteria was also addressed. Pure extracts and the films developed showed antimicrobial activity by the diffusion agar method only against the Gram-positive bacteria. Microbial analysis of the meat wrapped with films incorporated with HAE showed a reduction of 3.1–4.5 log CFU/g and 2.5–4.0 log CFU/g on the total viable microorganisms and total coliforms, respectively. Ch + Kenaf and Ch + Sage films presented the highest antimicrobial activity. Regarding the oxidation degradation, as expected, TBARS values increased for all samples over time. However, the meat wrapped in the biocomposites, except for CH + Sage, presented lower secondary oxidation metabolites (reduction of 75–93%) in the content of malonaldehyde. This protection was superior for the meat wrapped with Ch + Rosemary. Active film also showed promising results by retarding the discoloration process and the increase of pH over time. Thus, the biocomposites produced can pose as an alternative technology to enhance the shelf life of fresh poultry meat and maintain its quality.

Keywords: biodegradable films; active packaging; hydro-alcoholic extracts; natural additives; antimicrobial; antioxidant

Citation: Pires, J.R.A.; Almeida, K.M.; Augusto, A.S.; Vieira, É.T.; Fernando, A.L.; Souza, V.G.L. Application of Biocomposite Films of Chitosan/Natural Active Compounds for Shelf Life Extension of Fresh Poultry Meat. *J. Compos. Sci.* **2022**, *6*, 342. https://doi.org/10.3390/jcs6110342

Academic Editor: Ahmed Koubaa

Received: 16 September 2022
Accepted: 1 November 2022
Published: 5 November 2022

1. Introduction

The use of conventional plastics derived from petroleum as packaging materials is suitable for the preservation of food; nevertheless, with its overuse, sustainability problems have emerged which pose serious environmental concerns [1,2]. In fact, the high persistence and extreme distribution in the environment associated with the extremely slow degradation process and poor segregation and recycling options for traditional petroleum-based packaging materials are responsible for environmental damage associated with their presence and their accumulation in landfills [3–5]. The volume of plastic waste produced worldwide since the 1950s is nearly 6.3 billion tonnes, of which only 9% are recycled and another 12% incinerated, i.e., 79% of the waste has accumulated in landfills or the natural environment [6]. Moreover, in the natural environment, these non-biodegradable plastics are associated with the production of microplastics (<5 mm in size), which contaminate air, water, and soil [7], as well as posing detrimental health risks to humans [4].

In this scenario, eco-friendly food packaging has recently gained more attention, with the increasing interest in the development of new types of food packaging made from renewable and biodegradable materials [8,9]. Furthermore, in response to the consumer

demand for improved food safety, natural alternatives to synthetic functional substances have been sought in the development of new active food packaging [10–12]. The active packaging aims to improve the quality and safety of food through the interaction between the packaging, the product, and the environment, thus extending the shelf life [13]. The incorporation of active compounds, such as antioxidants and antimicrobial agents, is one of the most promising methods to develop active food packaging [14].

Chitosan (Ch), mainly produced from crustacean shells, is a linear cationic polysaccharide composed of glucosamine and acetylglucosamine with a high molecular weight and is derived by deacetylation of chitin, which is the second most abundant polysaccharide found in nature after cellulose [15,16]. This functional biopolymer has the advantages of being biofunctional, biocompatible, non-toxic, and has wound-healing properties, as well as hemostatic activity; consequently, it demonstrates a high potential to be used as a biodegradable film in a food-active packaging system [17]. Although chitosan is a promising biopolymer, low solubility in water, low water resistance, and poor mechanical and barrier properties that are attributed to the high molecular weight and hydrophilic nature of this material limit its industrial use [8,18]. Examples of strategies adopted to improve the flaws presented by bio-based films include their reinforcement with other materials (e.g., nanoparticles, plasticizers, natural compounds and extracts, to mention a few) to produce bionanocomposites/biocomposites [19,20], or the mixture of different biopolymers aiming to produce blends or bilayers with enhanced properties [21]. The incorporation of reinforcements that improve the antioxidant, antimicrobial, mechanical, and barrier properties have been intensively exploited in recent years [10].

Deterioration, rancidity, and discoloration are common problems resulting from the oxidative processes in food systems. Antioxidants are synthetic or natural substances used to overcome this issue [19]. As antioxidant activity is one of the main functions of active packaging, incorporation of natural active compounds, such as plant extracts, into the films is an alternative to the synthetic additives currently used, increasing the shelf life of the food without affecting its nutritional and sensorial quality [10,22,23]. Several extracts, mainly from fruits or plants due to the high content of phenolic contents, have been used in biodegradable chitosan films in order to enhance the antioxidant activity, such as mint extract [24], rosemary extract [25,26], green tea [27], black tea [28], grape seed extract [29,30], and propolis [31], showing that natural extracts combined with chitosan films are able to reduce and stabilize free radicals, hence avoiding the oxidative process.

Although some studies have been published in recent years on the beneficial effects of the application of chitosan films incorporated with natural extracts in food matrices [23,25,32–34], there are no relevant studies comparing the application and consequent effects of chitosan films incorporated with different types of natural extracts in food matrices. Therefore, the aim of this study was to develop active packaging based on chitosan, a biodegradable biopolymer from a natural resource, incorporated with six different natural hydro-alcoholic extracts (rosemary, green tea, black tea, ginger, kenaf, or sage) and use it as the primary active packaging to preserve fresh poultry meat.

2. Materials and Methods

2.1. Materials and Reagents

Chitosan (Poly(D-glucosamine)) with 75% deacetylation and high molecular weight (31–37 kDa) was used as a polymeric matrix and purchased from Sigma Aldrich (Germany). The hydro-alcoholic extracts were made from six different dried plants: Rosemary (*Rosmarinus officinalis* L.), ginger (*Zingiber officinale* Roscoe), and sage (*Salvia officinalis* L.) were purchased on a local commercial surface; black and green tea (*Camellia sinensis* (L.) Kuntze) were obtained from the Portuguese island of the Azores, in the Gorreana area; and the leaves of the kenaf (*Hibiscus cannabinus* L.) variety Everglades 41 were grown in pilot fields in the area of Caparica, near Lisbon (Portugal), and harvested in September 2005 before flowering.

Glacial acetic acid (purity ≥ 99%), glycerol, sodium hydroxide (NaOH), and tween 80 (polyethylene glycol sorbitan monolaurate) were obtained from Alfa Aesar (Kandel, Germany). 1,1,3,3-tetraethoxypropane (TEP) (purity ≥ 96%) and absolute ethanol (purity ≥ 99.8%) was purchased from Sigma Aldrich (Steinheim, Germany). Trichloroacetic acid (TCA) (purity ≥ 99%) and 2-thiobarbituric acid (TBA) (purity ≥ 98%) were obtained from PanReac (Barcelona, Spain). Plate count agar (PCA), mueller hinton agar (MHA), trypto-casein agar (TSA), and brilliant green agar were purchased from Biokar (Allonne, Beauvais, France). Tryptone was purchased from Difco Laboratiories (Detroit, USA). The water used during the experiment was purified using a Milli-Q system (Millipore, Bedford, MA, USA) and all chemicals were of analytical reagent grade.

2.2. Sample Preparation

2.2.1. Hydro-Alcoholic Extracts (HAE) Preparation

The extraction was performed according to Souza et al., 2017 [19]. Prior to the extraction, the dried plants were ground to a powder using an electric blender (ProfiCook model KSW 1021, Kempen, Germany), aiming to increase the bioactive compounds' extraction yield. Then, 5 g of each powder was mixed with 50 mL of 50% (v/v) ethanol using an electric mill ultra-turrax®(Model IKA®T18, Staufen, Germany). The mixture was kept refrigerated (4 °C ± 2 °C) in the dark for 24 h and then subjected to an ultrasound bath for 30 min at 50 Hz (Selecta, Barcelona, Spain) at room temperature (20 °C ± 2 °C). Subsequently, the mixture was centrifuged for 30 min at 4 °C and at 10,000 g force (Sigma model 4K15, Osterode am Harz, Germany), in which the supernatant was removed, and the extraction process was repeated. The supernatants of both extractions were pooled and filtered through Whatman n°4 filter paper, and the volume was corrected to 100 mL with ethanol 50% and concentrated 4 times in rotary vapour (Büchi Rotavapor R-200, Flawil, Switzerland) at 40 °C to evaporate the ethanol. In order to avoid degradations of the active compounds present in the extracts, they were stored at −18 °C until analysis and use.

2.2.2. Chitosan Film Preparation

Chitosan film-forming dispersion (FFD) was produced according to the method used by [19,35]. Briefly, 1.5% (w/v) of chitosan was dissolved in 1% (v/v) solution of glacial acetic acid for 24 h with continuous agitation at room temperature. To this FFD, 30% (w/w chitosan) of glycerol was added as a plasticizer to all treatments and the system was agitated for 5 more min to complete homogenization. The hydro-alcoholic extracts were then added to the system in 1% (v/v) of FFD and homogenized in a magnetic stirrer for a further 5 min at room temperature, and finally, the active antioxidant solution of chitosan was obtained. After homogenization, the mixture was degassed in an ultrasonic bath for 5 min. The resulting dispersion (140 mL) was poured into glass molds (18 × 25 cm^2) and dried for 72 h at room temperature. Dried films were sheered and stored (protected from light) at 25 °C and 50% relative humidity until use. All treatments were conducted with a random design with three replicates.

2.2.3. Fresh Poultry Meat Preparation

Fresh poultry meat was purchased at a local market. The meat was grounded, and for each treatment and analysis day, 30 g was wrapped in 5 × 18 cm^2 films. Unwrapped meat was also evaluated; all experiments were performed in triplicate. The poultry meat samples (wrapped and unwrapped) were stocked inside plastic boxes with a screw cap and placed under refrigeration (4 °C ± 2 °C). Each set was indiscriminately collected and characterized at 0, 3, 7, 10, and 15 days of storage. Even though the normal shelf life of fresh poultry meat stored under refrigeration is less than 7 days, in this work, we chose to evaluate the samples until the 15th day of storage to verify the shelf-life extension capacity of the active packaging.

2.3. In Vitro Antimicrobial Activity

In vitro antimicrobial activity of HAE and chitosan films incorporated with HAE was assessed by the agar diffusion method [31] against Gram-positive (*Bacillus cereus* (ATCC® 11778)) and Gram-negative (*Salmonella Enterica* (ATCC®10708)) foodborne bacteria. Microorganisms were made to grow in TSA overnight and isolated colonies of each strain were transferred to 0.85% NaCl solution; the turbidity of the suspension was adjusted to match a 0.5 McFarland turbidity standard (corresponding to 1×10^8 CFU/mL) using a McFarland densitometer (Model Den-1B, Grant Instruments, UK). The films were cut into disks of 6 mm diameter and placed on mueller hinton agar plates which had been previously seeded with inoculum containing indicator microorganisms. Subsequently, the plates were incubated at 37 °C or 30 °C for 20–24 h for *S. enterica* or *B. cereus*, respectively. To evaluate the pure HAE antimicrobial activity, wells of 6 mm diameter were cut in MHA and filled with 50 µL of each HAE. Clear zones below disk film or around the disks and wells were considered as inhibitory zones.

2.4. Meat Characterization

2.4.1. Moisture Content

Moisture content was determined gravimetrically using the oven-drying method at 103 °C ± 2 °C according to AOAC 2016 [36]. The assay was completed once the weight of the samples was stabilized.

2.4.2. PH and Titratable Acidity

For pH measurement, poultry meat (5 g) was homogenized in 50 mL of water (40 °C) for 15 min under constant stirring, and then measured with a pH glass electrode linked to a calibrated Crison MicropH 2001 potentiometer (Crison Instruments, Barcelona, Spain). Total titratable acidity was stated by titration with 0.1N NaOH solution, and the outcome expressed as % (w/w) of oleic acid equivalent [37].

2.4.3. Lipid Oxidation

The lipid oxidation of the samples was assessed by the determination of the thiobarbituric acid reactive substances (TBARS), according to Rosmini et al., 1996 [38]. Poultry meat samples of 10 g were weighed into Erlenmeyers and 20 mL of trichloroacetic acid (TCA) 7.5% (w/v) was added. The Erlenmeyers went through constant stirring for one hour. The supernatants were filtered, and 5 mL of the filtrate was mixed with 5 mL of 0.02 M 2-thiobarbituric acid (TBA) and heated in a water bath at 95 °C for 30 min. After cooling, the absorbance of the samples was measured using a UV/VIS spectrophotometer (Model Spekol 1500, Analytikjena, Germany) at 530 nm. The characterization of malonaldehyde (MDA) content was made from calibration curves constructed with known concentrations of MDA using 1,1,3,3-tetraethoxypropane (TEP). Values are expressed as mg of MDA/kg of sample.

2.4.4. Color

Instrumental color of the poultry meat was evaluated by the measurement of the coordinates CIE-L* a* b* (where L* represents the light, 0 for black and 100 for white; a* the chromaticity from green (−60) to red (+60); and b* the chromaticity from blue (−60) to yellow (+60)). This measurement was made using a CR 410 colorimeter (Minolta Co., Tokyo, Japan) with a D65 light source, and visual angle of 10°. Three measurements were taken at 3 different points of the meat for averaging purposes, and the shots were done on a standardized white background. The Hue angle was evaluated according to Equations (1) and (2) [19,39].

$$hue = arctan\left(\frac{b^*}{a^*}\right) \ (if\ a^* > 0) \ \text{or} \quad (1)$$

$$hue = arctan\left(\frac{b^*}{a^*}\right) + 180^\circ \ (if\ a^* < 0) \quad (2)$$

2.4.5. Microbiological Analysis

Microbiological quality of the sample over the refrigerated storage was evaluated by the determination of the total viable microorganisms (TVM) and total coliforms. Total viable microorganisms were counted on Petri dishes containing PCA agar after incubating for 72 h at 30 °C, according to ISO 4833-1 (2013) [40]. Total coliforms were evaluated using the most probable number (MNP) technique, after incubating in a liquid medium (Brilliant green lactose bile broth) for 48 h at 30 °C, according to ISO 4831 (2006) [41].

2.5. Statistical Analysis

The statistical analysis of data was done through a one-way ANOVA and, when suitable, the differences among means by the Tukey test. The analysis was carried out using the software Statistical Package for Social Sciences (SPSS), version 23, IBM, and the significance was defined at $p < 0.05$.

3. Results and Discussion

3.1. In Vitro Antimicrobial Activity

Pure HAE showed antimicrobial activity only against Gram-positive bacteria tested (Figure 1); no inhibition zone was observed for *S. enterica*. Green tea and rosemary HAE had the most activity against *B. cereus*, while kenaf and ginger had the least ($p < 0.05$). Non-polar components such as phenolic diterpenes, responsible for the antimicrobial properties, are generally considered to be more effective only against Gram-positive bacteria. According to Geonrgantelis et al. (2007) [25], this happens because phenolic diterpenes are organic molecules with high molecular weight bulky substituents, which may lead to a reduction in the ability to reach the cell membrane of Gram-negative bacteria such as coliforms. The difference in the activities between the HAE may be attributed to the content of total phenolic compounds (TPC) present in the extracts. In a previous work, Souza et al. (2017) [19] characterized those extracts and found the following TPC per g of dry plant: 68 mg acid gallic equivalent (AGE) (green tea); 33 mg AGE (rosemary); 30 mg AGE (black tea); 20 mg AGE (sage); 11.8 mg AGE (kenaf); and 7.4 mg AGE (ginger). The antimicrobial activity here observed was directly correlated to the values of TPC present in the extracts.

Regarding the films, only the bio-based films incorporated with HAE showed antimicrobial activity, but only on the contact surface underneath the disks and for *B. cereus*. No inhibition was observed against *S. enterica*, and pristine chitosan film did not show action against both bacteria. The results corroborate the test of pure HAE, where no inhibition zone was observed for Gram-negative bacteria. Similar results were previously reported by Siripatrawan and Vitchayakitti (2016) [31], who tested chitosan films incorporated with propolis extract, and only observed inhibition underneath the disk films. According to these authors, chitosan in the form of insoluble film is unable to diffuse through the adjacent agar media, which may cause the well-known antimicrobial activity of the polysaccharide to become negligible. Therefore, the incorporation of HAE enhanced the antimicrobial activity of the active films.

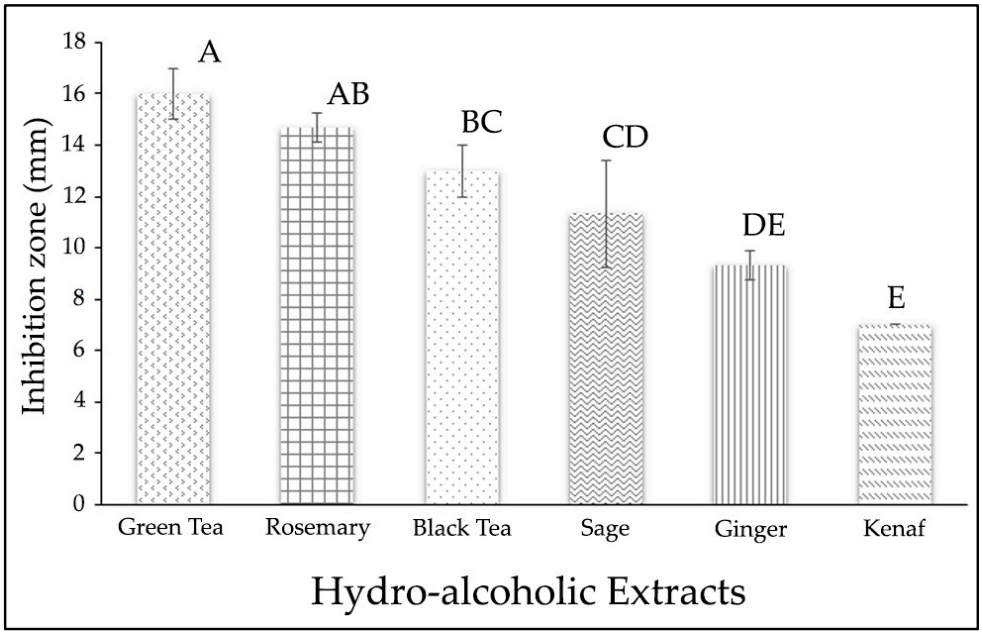

Figure 1. In vitro antimicrobial activity of pure HAE against *B. cereus*. A–E: Means with different letters indicate significant differences between HAE ($p < 0.05$).

3.2. Moisture Content

The sensory quality and stability of stored food products are influenced by moisture; many chemical, enzymatic, and microbiological deteriorating processes are directly linked to high food water content [42,43].

The moisture content of the unwrapped fresh poultry meat increased slightly but significantly ($p < 0.05$) over time, from 74.8% to a maximum of 77.5% after 15 days of storage (Table 1). In contrast, the moisture content of the protected samples showed lower ($p < 0.05$) (Ch + Sage; Ch + Green Tea; Ch + Black Tea) or similar values ($p > 0.05$) (Ch; Ch + Ginger; Ch + Kenaf; Ch + Rosemary) after 15 days of storage to that of fresh poultry meat at the beginning of the experiment. Chitosan is a hydrophilic polymer with good water adsorption capacity (high swelling degree) [44,45] and this may explain the loss of water from the meat when it is in contact with the films. Similar results can be observed in [46,47], where chitosan films absorbed water from the bolognese meat and patties, increasing their thickness. Furthermore, cured ham vacuum-packaged in a N_2-modified atmosphere presented reductions of around 3% of its moisture content after three weeks of storage [48].

Samples wrapped in Ch + HAE films showed a slight but significant decrease ($p < 0.05$) in moisture content after fifteen days of storage compared with the control sample, and no significant differences ($p > 0.05$) were observed between chitosan films incorporated with the different extracts. This reduction in moisture values suggests that the films incorporated with natural extracts adsorbed more water from the poultry meat, which supports the results presented in a previous work that characterized this film published by Souza et al., 2017 [19], in which an increase in water content and solubility occurred for Ch + HAE films. According to [31,49], hydrophobic interactions between polyphenols and hydrophobic regions of the chitosan matrix can occur. The polyphenolic compounds of the extracts should be able to fit into the chitosan matrix and establish interactions like hydrogen or covalent bonding with reactive groups of chitosan, thus limiting the availability of hydrogen groups to form hydrophilic bonding with water. On the other hand, the amount of extract incorporated into the films may not have been enough to make them more hydrophobic. Bof et al. (2016) [50] observed that the incorporation of 1% grapefruit seed extract into corn-starch-chitosan films was not sufficient to cause significant changes in moisture and solubility values.

Table 1. Physical and chemical results of the poultry meat over the shelf-life time.

Parameters	Storage Days	Samples							
		Unwrapped	**Ch**	**Ch + Ginger**	**Ch + Kenaf**	**Ch + Sage**	**Ch + Green Tea**	**Ch + Black Tea**	**Ch + Rosemary**
Moisture (% w/w)	0	$74.8 \pm 0.0^{a,C}$	$74.8 \pm 0.0^{a,A}$	$74.8 \pm 0.0^{a,A}$	$74.8 \pm 0.0^{a,A}$	$74.8 \pm 0.0^{a,A}$	$74.8 \pm 0.0^{a,A}$	$74.8 \pm 0.0^{a,A}$	$74.8 \pm 0.0^{a,A}$
	3	$75.8 \pm 0.0^{a,B}$	$73.6 \pm 0.0^{b,c,A}$	$72.6 \pm 0.7^{c,d,A}$	$72.6 \pm 0.6^{c,d,A}$	$71.6 \pm 0.0^{d,B}$	$73.8 \pm 0.3^{b,c,B,C}$	$72.5 \pm 0.4^{c,d,B,C}$	$74.5 \pm 0.4^{a,b,A}$
	7	$76.4 \pm 0.3^{a,B}$	$72.4 \pm 0.0^{b,A}$	$72.7 \pm 2.4^{b,A}$	$71.1 \pm 2.3^{b,A}$	$72.0 \pm 1.6^{b,A,B}$	$72.2 \pm 0.0^{b,B,C}$	$71.8 \pm 0.7^{b,B,C}$	$73.4 \pm 0.7^{b,A}$
	10	$76.1 \pm 0.2^{a,B}$	$74.7 \pm 0.0^{a,b,A}$	$72.0 \pm 2.6^{a,b,A}$	$71.7 \pm 0.6^{a,b,A}$	$70.5 \pm 0.1^{b,B}$	$71.4 \pm 1.3^{b,B,C}$	$70.9 \pm 1.0^{b,B,C}$	$73.5 \pm 0.6^{a,b,A}$
	15	$77.5 \pm 0.1^{a,A}$	$74.2 \pm 0.0^{b,A}$	$71.9 \pm 0.0^{c,A}$	$71.0 \pm 0.0^{c,A}$	$70.4 \pm 0.0^{c,B}$	$70.7 \pm 5.2^{b,c,C}$	$69.5 \pm 2.5^{c,C}$	$72.8 \pm 0.2^{c,A}$
Hue Angle	0	$55.7 \pm 0.5^{a,B}$	$55.7 \pm 0.5^{a,A}$	$55.7 \pm 0.5^{a,B}$	$55.7 \pm 0.5^{a,A}$	$55.7 \pm 0.5^{a,B}$	$55.7 \pm 0.5^{a,A}$	$55.7 \pm 0.5^{a,A,B}$	$55.7 \pm 0.5^{a,A,B}$
	3	$57.4 \pm 2.6^{a,B}$	$55.6 \pm 0.0^{a,b,A}$	$49.4 \pm 1.8^{c,C}$	$52.5 \pm 1.3^{a,b,c,A}$	$49.4 \pm 1.3^{c,C}$	$51.7 \pm 1.7^{a,b,c,A}$	$51.4 \pm 1.3^{b,c,C}$	$52.9 \pm 0.6^{a,b,c,C}$
	7	$57.9 \pm 1.2^{a,B}$	$52.3 \pm 0.0^{b,c,A}$	$51.0 \pm 0.7^{c,C}$	$51.7 \pm 1.3^{c,A}$	$53.3 \pm 1.2^{a,b,c,B}$	$49.4 \pm 1.8^{c,A}$	$53.0 \pm 0.7^{a,b,c,B,C}$	$57.4 \pm 2.1^{a,b,A,B}$
	10	$58.5 \pm 0.6^{a,B}$	$53.6 \pm 0.0^{a,A}$	$55.0 \pm 0.9^{a,B}$	$50.5 \pm 1.6^{a,A}$	$55.3 \pm 1.2^{a,B}$	$57.7 \pm 4.3^{a,A}$	$58.1 \pm 0.6^{a,A}$	$53.7 \pm 3.4^{a,A,B}$
	15	$69.0 \pm 0.0^{a,A}$	$55.6 \pm 0.0^{c,A}$	$60.6 \pm 0.0^{b,A}$	$52.1 \pm 0.0^{c,A}$	$60.6 \pm 0.0^{b,A}$	$57.9 \pm 0.7^{b,c,A}$	$57.0 \pm 0.7^{c,A}$	$61.0 \pm 2.0^{b,A}$
pH	0	$6.3 \pm 0.0^{a,B}$	$6.3 \pm 0.0^{a,B}$	$6.3 \pm 0.0^{a,A}$	$6.3 \pm 0.0^{a,A}$	$6.3 \pm 0.0^{a,A}$	$6.3 \pm 0.0^{a,A}$	$6.3 \pm 0.0^{a,A}$	$6.3 \pm 0.0^{a,A}$
	3	$6.1 \pm 0.3^{a,b,c,d,B}$	$6.2 \pm 0.0^{a,b,c,B}$	$6.2 \pm 0.0^{a,b,c,A}$	$5.6 \pm 0.0^{c,d,B}$	$5.6 \pm 0.0^{c,d,B}$	$5.7 \pm 0.1^{b,c,d,B}$	$6.3 \pm 0.0^{a,A}$	$5.5 \pm 0.0^{d,C}$
	7	$6.8 \pm 0.3^{a,A,B}$	$6.3 \pm 0.0^{b,B}$	$6.1 \pm 0.0^{b,c,A}$	$5.7 \pm 0.1^{c,B}$	$5.8 \pm 0.0^{c,B}$	$5.8 \pm 0.0^{c,B}$	$6.0 \pm 0.1^{b,c,A}$	$5.7 \pm 0.1^{c,B,C}$
	10	$6.7 \pm 0.1^{a,A,B}$	$6.3 \pm 0.0^{a,b,c,B}$	$6.1 \pm 0.4^{b,c,A}$	$5.8 \pm 0.2^{b,c,B}$	$5.9 \pm 0.1^{b,c,B}$	$6.3 \pm 0.4^{a,b,c,A}$	$5.8 \pm 0.0^{c,A}$	$5.9 \pm 0.0^{b,c,B}$
	15	$7.1 \pm 0.0^{a,A}$	$6.9 \pm 0.0^{b,A}$	$5.0 \pm 0.0^{d,B}$	$5.1 \pm 0.0^{d,C}$	$5.0 \pm 0.0^{d,C}$	$5.0 \pm 0.0^{d,C}$	$5.1 \pm 0.0^{d,B}$	$5.5 \pm 0.1^{c,C}$
Acidity % (w/w)	0	$1.9 \pm 0.0^{a,A}$	$1.9 \pm 0.0^{a,b,A}$	$1.9 \pm 0.0^{a,A}$	$1.9 \pm 0.0^{a,A}$	1.9 ± 0.0^{aA}	$1.9 \pm 0.0^{a,A}$	$1.9 \pm 0.0^{a,A}$	$1.9 \pm 0.0^{a,A}$
	3	$1.6 \pm 0.0^{a,B}$	$1.1 \pm 0.0^{b,C}$	$1.4 \pm 0.0^{a,b,B}$	$1.3 \pm 0.0^{a,b,B}$	$1.6 \pm 0.2^{a,A,B}$	$1.5 \pm 0.1^{a,B}$	$1.5 \pm 0.0^{a,B}$	$1.6 \pm 0.0^{a,B,C}$
	7	$0.9 \pm 0.5^{b,B,C}$	$0.9 \pm 0.0^{b,C}$	$1.3 \pm 0.0^{a,b,B}$	$1.2 \pm 0.0^{a,b,B}$	$1.4 \pm 0.1^{a,B}$	$1.4 \pm 0.1^{a,B}$	$1.2 \pm 0.0^{a,b,C}$	$1.6 \pm 0.1^{a,B,C}$
	10	$0.7 \pm 0.2^{c,C}$	$1.3 \pm 0.0^{a,b,B}$	$0.8 \pm 0.1^{c,C}$	$1.2 \pm 0.1^{a,b,c,B}$	$1.1 \pm 0.1^{b,c,C}$	$1.1 \pm 0.0^{b,c,C}$	$1.2 \pm 0.2^{b,c,C}$	$1.7 \pm 0.2^{a,A,B}$
	15	$0.6 \pm 0.1^{d,C}$	$1.5 \pm 0.0^{a,B}$	$1.2 \pm 0.0^{a,b,c,B}$	$1.0 \pm 0.0^{b,c,C}$	$1.1 \pm 0.0^{b,c,C}$	$1.1 \pm 0.4^{b,c,C}$	$0.9 \pm 0.4^{b,c,d,C}$	$1.3 \pm 0.2^{a,b,C}$

a–d: Different letters indicate significant differences between lines ($p < 0.05$). A–C: Different letters indicate significant differences between columns ($p < 0.05$). Ref: Ch: Chitosan.

3.3. PH and Titratable Acidity

In food science and technology, the pH value of foods is an important parameter to be evaluated. The degradation process of food is directly influenced by its pH once it interferes in the enzymatic reactions and the growth and survival of microorganisms [51]. Several attributes of meat quality, such as color, tenderness, water retention capacity, juiciness and microbial stability, have been associated with this important physicochemical parameter [52]. The pH in meat is a measure of its acidity, and the normal values found in fresh poultry meat are around 5.2–7 [53].

The poultry meat presented an initial pH value of 6.3 (Table 1). Over time, the deterioration process of the unprotected meat led to a rise in the pH to a maximum value of 7.1 on the last day of storage ($p < 0.05$). A high pH favors the proliferation of bacteria, while a low pH constrains them, and in some cases, completely inhibits them [51]. The sample wrapped in the control film (Ch) showed maintenance of pH until the tenth stored day ($p > 0.05$), probably due to the presence of residual acetic acid in the films that counterbalanced the basic compounds produced by microorganisms, and then increased significantly ($p < 0.05$) to 6.9 on the last day. Georgantelis et al. (2007) [25] obtained similar pH results in their study of cooked pork sausages packed with chitosan and clove essential oil films. These authors attributed this behavior to the increase of the bacterial population (such as Enterobacteriaceae and Pseudomonas, as well as fungi and molds) that caused the degradation of proteins and amino acids, resulting in the formation of ammonium compounds and consequently an increase in pH.

The meat wrapped with Ch + HAE had a significant decrease ($p < 0.05$) in pH values from day three until the end of the experiment, and these values are also significantly lower ($p < 0.05$) that the ones presented by the control in the last day of storage. Beyond different films, no difference was observed in the pH value at the 15th day of storage ($p > 0.05$), except for Ch + Rosemary that showed slightly higher pH ($p < 0.05$). These results have shown that chitosan films incorporated with natural extracts are more effective in preserving the poultry meat, indicating that the microbiological growth in the protected samples may have been lower. Several reports of pH decrease over time in meat samples treated with natural extracts that are in agreement with our results can be found in the literature [54–56].

Titratable acidity is a more accurate method for determining the total acid concentration in a food than pH, indicating more strictly the microbiological stability (USFDA, 2001). The titratable acidity of the unwrapped meat had a marked decrease ($p < 0.05$) from day zero (1.9% of oleic acid equivalent) until day fifteen (0.6% of oleic acid equivalent), which is in accordance with the results of raising the pH of the meat (Table 1). Over time, there is a degradation of the meat due to the microbiological contamination, in which the microorganisms degrade the proteins, producing amines and ammonia that neutralize the present acids, reducing their content [57]. Once again, as observed for the pH, there were no significant differences ($p < 0.05$) in the titratable acidity values of the meat wrapped with the Ch + HAE films at the end of the fifteen days of storage.

3.4. Color Variation

Color is one of the most relevant sensory attributes valued by the consumers when choosing a meat product [52]. Consumers prefer fresh meat with a bright red/pink color, which they associate with freshness, thus, discolored products are generally rejected [58]. The fresh poultry meat had an initial hue angle of 55.7° (Table 1). The unpackaged meat had an increase in the hue angle ($p < 0.05$) to a maximum of 69.0 after 15 days of refrigerated storage. There was a gradual discoloration of the unprotected meat toward a more brownish hue, therefore diminishing its market value. This change in color was mainly due to the accumulation of metmyoglobin [58]. Color retention can be achieved by decreasing metmyoglobin concentration and increasing oxymyoglobin values during storage [59]. There were significant differences ($p < 0.05$) between the color presented by the unprotected sample and the samples protected by the bio-based films.

At day 15, the samples wrapped in the Ch + Ginger and Ch + Sage films presented a slight increase in the values of the hue angle ($p < 0.05$), while the remaining protected samples maintained the original tonality ($p > 0.05$). It was also observed that the sample protected with Ch + Kenaf showed the best color retention capacity. The results presented by the sample involved in the Ch film without extracts was also positive and similar to the rest. Thus, the films were found to be effective in preserving the red tone of the meat. It was reported that chitosan affected the a* values of sausages, resulting in a red tone surface color [60]. Moreover, a blend of chitosan and rosemary extract was tested in the preservation of beef burgers and acted synergistically and refined the redness of the burgers during frozen storage, while the single use of chitosan or rosemary extract improved color stability as compared with the controls [61]. The mechanism of color retention by chitosan has not yet been fully understood but may be related to the chelating ability of chitosan. Iron (Fe^{3+}), which is a component of metmyoglobin and other meat components, can be absorbed by chitosan at a rate of 17.6 mg per gram in 30 min according to Knorr (1991) [62]. Iron is known to promote oxidation through the generation of free radicals. Free iron from meat tissue can be absorbed by chitosan and these interactions between chitosan and iron can stabilize the color of the meat surface over time, maintaining the good appearance of the meat [63]. In addition to the fact that chitosan has a metal chelating and intrinsic antioxidant activity, an addition of natural extracts to the polymer matrix helps to consolidate this property as well as to increase the oxygen barrier property of the film, better preventing oxidation [59,64]. Overall, both chitosan and natural extracts may act as preventive antioxidants in food products, including poultry meat.

3.5. Lipid Oxidation (TBARS Assays)

Together with microbial spoilage, lipid oxidation is the main factor influencing meat quality. TBARS assays are a good analytical method to determine the oxidation state of meat by the quantification of the malonaldehyde present in the sample; thus, it is considered a representative indicator of lipid rancidity [38].

Throughout the storage time, MDA concentration values of the unprotected poultry meat sample increased significantly ($p < 0.05$). At day zero, it had an MDA value of 0.10 mg/kg, and at the end of the fifteen days, it presented a value of 1.71 mg/kg (Figure 2). Fresh poultry meat, rich in unsaturated fatty acids, has a low oxidative stability and is very sensitive to rancidity during production and storage [34]; therefore, it is observable that fresh poultry meat is a perishable food, and if not properly protected, it will undergo lipid oxidation [65]. This assay showed that there was an increase in lipid oxidation in the sample coated with the control film over storage time but these values were lower than those presented by the unwrapped meat. This difference started to be significant ($p < 0.05$) since day 10 of evaluation. In addition to antimicrobial activity, chitosan also has an intrinsic antioxidant activity [66], but this activity is not significant [24]. When used as a food additive, the antioxidant activity of chitosan is generally assigned to its chelating efficiency, since when it binds with the metal ions, chitosan avoids the beginning of lipid oxidation, acting as a secondary antioxidant. In addition, due to the low oxygen permeability, chitosan films and coatings reduce the oxidation rate of packaged foods simply by preventing their contact with oxygen [20,67].

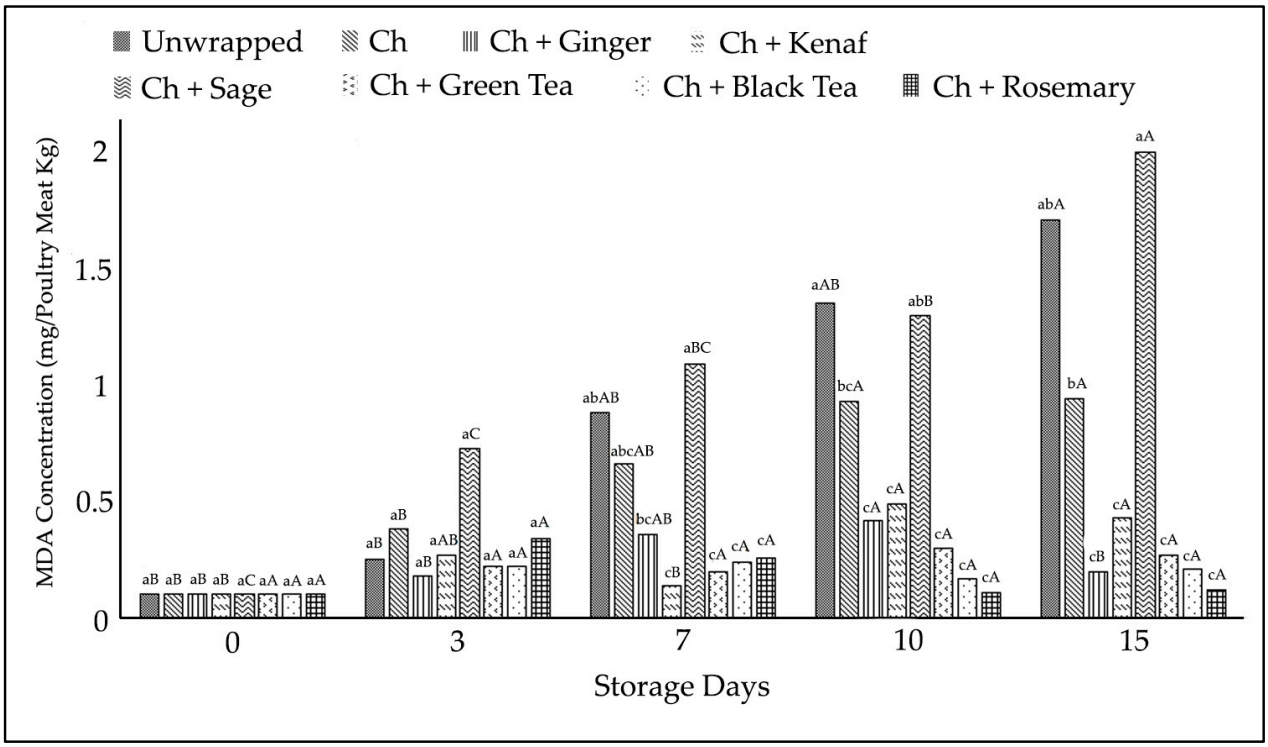

Figure 2. Lipid oxidation of the meat over refrigerated storage. a–c: Different letters indicate significant differences between treatments ($p < 0.05$). A–C: Different letters indicate significant differences between storage time ($p < 0.05$). Ch: Chitosan; MDA: Malonaldehyde.

The incorporation of extracts into the films controlled the increase in MDA concentrations over time, except for salvia that exceeded the values presented by the filmless sample and the control sample in the last day of assessment (behaved as a pro-oxidative compound). For ginger, green tea, black tea, and rosemary HAE, there were no significant changes ($p > 0.05$) from the first evaluation time to the last one, and the Ch + Rosemary wrapped sample showed the lowest increase in TBARS. Therefore, extracts together with chitosan films have been shown to be an asset in preventing the increase of lipid oxidation in fresh poultry meat, acting synergistically. This occurred because the phenolic compounds present in the natural extracts can terminate the propagation reactions induced by free radical intermediates either by reaction with the free radical or by preventing hydroperoxides from decomposing into free radicals [68]. Recently, [32] found that propolis extracts along with chitosan coatings decreased MDA concentration compared with controls, increasing the quality of refrigerated chicken fillet. The same results were recently observed by several other authors [31,37,69].

3.6. Microbiological Analysis

The total viable microorganisms count increased significantly ($p < 0.05$) over the 10 days of evaluation for all studied samples. As expected, the greater microbial growth was reported in the unwrapped samples (Table 2). In fact, bacterial contamination is one of the main factors determining the loss of quality of fresh meat, since these products are very likely to be contaminated with microorganisms if they are not properly preserved and handled, so it is desirable to use preservatives with antimicrobial properties [24].

Table 2. Mean values of the microbiological analysis demonstrated by the various samples over the evaluation time.

Parameters	Storage Days	Samples							
		Unwrapped	Ch	Ch + Ginger	Ch + Kenaf	Ch + Sage	Ch + Green Tea	Ch + Black Tea	Ch + Rosemary
TVM (Log CFU/g	0	$5.86 \pm 0.02^{a,D}$	$5.86 \pm 0.02^{a,D}$	$5.86 \pm 0.02^{a,C}$	$5.86 \pm 0.02^{a,B}$	$5.86 \pm 0.02^{a,B}$	$5.86 \pm 0.02^{a,C}$	$5.86 \pm 0.02^{a,B}$	$5.86 \pm 0.02^{a,D}$
	3	$9.78 \pm 0.02^{a,C}$	$7.79 \pm 0.02^{b,c,C}$	$7.68 \pm 0.29^{b,c,B}$	$7.75 \pm 0.13^{b,c,A}$	$6.92 \pm 0.95^{b,c,A}$	$7.61 \pm 0.05^{b,c,B}$	$8.05 \pm 0.28^{b,A}$	$6.56 \pm 0.18^{c,C}$
	7	$10.79 \pm 0.04^{a,B}$	$9.47 \pm 0.02^{b,B}$	$8.65 \pm 0.31^{b,c,A}$	$8.51 \pm 0.09^{b,c,A}$	$7.24 \pm 0.15^{c,A}$	$8.73 \pm 0\text{-}03^{b,c,A}$	$8.36 \pm 0.18^{b,c,A}$	$8.23 \pm 0.11^{b,c,A}$
	10	$11.71 \pm 0.04^{a,A}$	$10.14 \pm 0.02^{b,A}$	$8.46 \pm 0.01^{c,d,A}$	$7.72 \pm 0.40^{c,d,e,A}$	$7.45 \pm 0.15^{d,e,A}$	$8.60 \pm 0.08^{c,A}$	$8.47 \pm 0.60^{c,d,A}$	$7.22 \pm 0.12^{e,B}$
Total Coliforms (Log MPN/g)	0	$2.00 \pm 0.01^{a,C}$	$2.00 \pm 0.01^{a,D}$	$2.00 \pm 0.01^{a,C}$	$2.00 \pm 0.01^{a,C}$	$2.00 \pm 0.01^{a,B}$	$2.00 \pm 0.01^{a,B}$	$2.00 \pm 0.01^{a,B}$	$2.00 \pm 0.01^{a,C}$
	3	$4.38 \pm 0.02^{a,B}$	$2.30 \pm 0.01^{b,c,C}$	$3.21 \pm 0.24^{a,b,A,B}$	$2.85 \pm 0.75^{a,b,c,A,B}$	$1.88 \pm 0.62^{b,c,B}$	$2.86 \pm 0.25^{a,b,c,A}$	$2.68 \pm 0.51^{b,c,A}$	$1.44 \pm 0.01^{c,D}$
	7	$5.08 \pm 0.15^{a,A,B}$	$3.30 \pm 0.01^{b,B}$	$3.66 \pm 0.42^{a,b,A}$	$3.28 \pm 0.96^{b,A}$	$2.72 \pm 0.17^{b,A}$	$2.89 \pm 0.40^{b,A}$	$3.24 \pm 0.09^{b,A}$	$2.84 \pm 0.02^{b,B}$
	10	$5.68 \pm 0.51^{a,A}$	$4.17 \pm 0.02^{a,b,A}$	$2.67 \pm 0.71^{b,c,A,B}$	$2.17 \pm 0.01^{c,B}$	$1.72 \pm 0.17^{c,B}$	$2.57 \pm 0.71^{b,c,A}$	$2.67 \pm 0.71^{c,A}$	$3.17 \pm 0.02^{b,c,A}$

a–e: Different letters indicate significant differences between lines ($p < 0.05$). A–D: Different letters indicate significant differences between columns ($p < 0.05$). Ref: Ch: Chitosan; CFU: Colony forming-unit; TVM: Total viable microorganisms; MPN: Most probable number.

In comparison with unwrapped meat, all the samples protected with the chitosan films presented smaller TVM ($p < 0.05$), a reduction between 1.6 to 4.5 log CFU/g meat. Moreover, the incorporation of HAE into the biopolymer also conferred extra antimicrobial activity, thus the TVM of the samples wrapped in the Ch + HAE was smaller than the meat packaged in pristine chitosan film ($p < 0.05$). Kenaf, sage, and rosemary were the extracts that were more effective in controlling the growth of microorganisms. Chitosan presents an intrinsic antimicrobial activity, which is effectively expressed in aqueous systems [1]. One of the reasons for the antimicrobial character of Ch is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms [16]. The antimicrobial activity of chitosan can be enhanced with the incorporation of natural extracts and oils [46]. Terpenes and phenolic compounds are mainly responsible for the antimicrobial effects of HAE; however, the antimicrobial properties of these compounds cannot be attributed solely to the mixture of the main components that have this capacity [70]. Similar behavior is reported in the literature, where pure chitosan films reduced *Listeria monocytogenes* by 2 logs, while in films enriched with 1% and 2% of oregano oil, the number of *L. monocytogenes* was reduced by 3, 6, and 4 logs, and 3 logs in *E. coli* [46]. Additionally, [71] showed excellent compatibility between cinnamon essential oil and chitosan. Its incorporation improved the antibacterial capacity of the film, which is a good alternative for the coating of highly perishable foods such as fish and poultry.

The total coliform count in unwrapped meat showed a significant increase ($p < 0.05$) over time, reaching a maximum of 5.68 log on the tenth day (MPN/g) (Table 2). Throughout the storage time, the total coliform count remained more stable for the meat samples protected with films. Except for the control sample, on the last day, all samples wrapped with CH+HAE showed lower results ($p < 0.05$) than the unwrapped sample (log reductions between 1.5–4.0). The samples coated with Ch + Kenaf and Ch + Sage presented the lowest growth of total coliforms. Antimicrobial activity of HAE is related to their rich content in phenolic compounds, as previously discussed in the in vitro assay. This corroborates the study with fresh pork sausages refrigerated at 4 °C, where after twenty days of storage, researchers registered a decrease in Enterobacteriaceae count for the samples coated with chitosan films with rosemary in comparison to samples without film [25].

4. Conclusions

Chitosan films incorporated with HAE delayed chemical and microbiological changes of minced poultry meat by maintaining the pH value and the characteristic reddish color, retarding the lipid oxidation and microbial growth better than the pristine chitosan film or unwrapped samples. HAEs enhance the antimicrobial and antioxidant ability of chitosan films. Beyond the extracts tested, green tea, black tea, ginger, and rosemary HAE can be highlighted as the most efficient for protecting the poultry meat towards oxidation; however, sage, kenaf, and rosemary are the most efficient regarding microbiology proliferation. The developed active film could be used as active packaging to maintain quality and extend the shelf life of fresh poultry meat. Chitosan films incorporating plant and food extracts have the potential to meet consumer demand for foods with less chemical preservatives. Moreover, these extracts can be obtained from the food industry by-products, which is in line with the bio-circular economy concept for a more sustainable and environmentally friendly approach. The inherent biodegradability of chitosan is also a strong advantage of the developed film.

Author Contributions: Conceptualization, V.G.L.S. and A.L.F.; methodology, J.R.A.P., V.G.L.S., K.M.A., A.S.A. and É.T.V.; software, J.R.A.P. and V.G.L.S.; formal analysis, K.M.A., A.S.A. and É.T.V.; investigation, J.R.A.P. and V.G.L.S.; resources, A.L.F.; writing—Original draft preparation, J.R.A.P. and V.G.L.S.; writing—Review and editing, J.R.A.P., V.G.L.S. and A.L.F.; supervision, V.G.L.S. and A.L.F.; funding acquisition, A.L.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by national funding by FCT, Foundation for Science and Technology, MCTES, Portugal, through the individual PhD research grant (SFRH/BD/144346/2019) of J.R.A.P. This work was also supported by the MEtRICs unit which is funded by national funds from FCT/MCTES (UIDB/04077/2020 and UIDP/04077/2020).

Data Availability Statement: Not applicable.

Acknowledgments: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Aider, M. Chitosan application for active bio-based films production and potential in the food industry: Review. *LWT—Food Sci. Technol.* **2010**, *43*, 837–842. [CrossRef]
2. Pires, J.R.A.; Souza, V.G.L.; Fuciños, P.; Pastrana, L.; Fernando, A.L. Methodologies to Assess the Biodegradability of Bio-Based Polymers—Current Knowledge and Existing Gaps. *Polymers* **2022**, *14*, 1359. [CrossRef] [PubMed]
3. Thomas, K.V. Understanding the plastics cycle to minimize exposure. *Nat. Sustain.* **2022**, *5*, 282–284. [CrossRef]
4. Kumar, R.; Manna, C.; Padha, S.; Verma, A.; Sharma, P.; Dhar, A.; Ghosh, A.; Bhattacharya, P. Micro(nano)plastics pollution and human health: How plastics can induce carcinogenesis to humans? *Chemosphere* **2022**, *298*, 134267. [CrossRef] [PubMed]
5. Samantaray, P.K.; Ellingford, C.; Farris, S.; O'Sullivan, D.; Tan, B.; Sun, Z.; McNally, T.; Wan, C. Electron Beam-Mediated Cross-Linking of Blown Film-Extruded Biodegradable PGA/PBAT Blends toward High Toughness and Low Oxygen Permeation. *ACS Sustain. Chem. Eng.* **2022**, *10*, 1267–1276. [CrossRef]
6. Samantaray, P.K.; Little, A.; Haddleton, D.M.; McNally, T.; Tan, B.; Sun, Z.; Huang, W.; Ji, Y.; Wan, C. Poly(glycolic acid) (PGA): A versatile building block expanding high performance and sustainable bioplastic applications. *Green Chem.* **2020**, *22*, 4055–4081. [CrossRef]
7. Samantaray, P.K.; Little, A.; Wemyss, A.M.; Iacovidou, E.; Wan, C. Design and Control of Compostability in Synthetic Biopolyesters. *ACS Sustain. Chem. Eng.* **2021**, *9*, 9151–9164. [CrossRef]
8. Ashrafi, A.; Jokar, M.; Mohammadi Nafchi, A. Preparation and characterization of biocomposite film based on chitosan and kombucha tea as active food packaging. *Int. J. Biol. Macromol.* **2018**, *108*, 444–454. [CrossRef]
9. Andrade, M.A.; Barbosa, C.H.; Souza, V.G.L.; Coelhoso, I.M.; Reboleira, J.; Bernardino, S.; Ganhão, R.; Mendes, S.; Fernando, A.L.; Vilarinho, F.; et al. Novel Active Food Packaging Films Based on Whey Protein Incorporated with Seaweed Extract: Development, Characterization, and Application in Fresh Poultry Meat. *Coatings* **2021**, *11*, 229. [CrossRef]
10. Wang, Q.; Tian, F.; Feng, Z.; Fan, X.; Pan, Z.; Zhou, J. Antioxidant activity and physicochemical properties of chitosan films incorporated with Lycium barbarum fruit extract for active food packaging. *Int. J. Food Sci. Technol.* **2015**, *50*, 458–464. [CrossRef]
11. Santos, J.C.P.; Sousa, R.C.S.; Otoni, C.G.; Moraes, A.R.F.; Souza, V.G.L.; Medeiros, E.A.A.; Espitia, P.J.P.; Pires, A.C.S.; Coimbra, J.S.R.; Soares, N.F.F. Nisin and other antimicrobial peptides: Production, mechanisms of action, and application in active food packaging. *Innov. Food Sci. Emerg. Technol.* **2018**, *48*, 179–194. [CrossRef]
12. Rodrigues, C.; Souza, V.G.L.; Coelhoso, I.; Fernando, A.L. Bio-Based Sensors for Smart Food Packaging—Current Applications and Future Trends. *Sensors* **2021**, *21*, 2148. [CrossRef] [PubMed]
13. Fang, Z.; Zhao, Y.; Warner, R.D.; Johnson, S.K. Active and intelligent packaging in meat industry. *Trends Food Sci. Technol.* **2017**, *61*, 60–71. [CrossRef]
14. Bonilla, J.; Sobral, P.J.A. Investigation of the physicochemical, antimicrobial and antioxidant properties of gelatin-chitosan edible film mixed with plant ethanolic extracts. *Food Biosci.* **2016**, *16*, 17–25. [CrossRef]
15. Giannakas, A.; Grigoriadi, K.; Leontiou, A.; Barkoula, N.M.; Ladavos, A. Preparation, characterization, mechanical and barrier properties investigation of chitosan-clay nanocomposites. *Carbohydr. Polym.* **2014**, *108*, 103–111. [CrossRef]
16. Souza, V.G.L.; Pires, J.R.A.; Rodrigues, C.; Coelhoso, I.M.; Fernando, A.L. Chitosan Composites in Packaging Industry—Current Trends and Future Challenges. *Polymers* **2020**, *12*, 417. [CrossRef]
17. Lekjing, S. A chitosan-based coating with or without clove oil extends the shelf life of cooked pork sausages in refrigerated storage. *Meat Sci.* **2016**, *111*, 192–197. [CrossRef]
18. Beigzadeh Ghelejlu, S.; Esmaiili, M.; Almasi, H. Characterization of chitosan-nanoclay bionanocomposite active films containing milk thistle extract. *Int. J. Biol. Macromol.* **2016**, *86*, 613–621. [CrossRef]
19. Souza, V.G.L.; Fernando, A.L.; Pires, J.R.A.; Rodrigues, P.F.; Lopes, A.A.S.S.; Fernandes, F.M.B. Physical properties of chitosan films incorporated with natural antioxidants. *Ind. Crops Prod.* **2017**, *107*, 565–572. [CrossRef]
20. Pires, J.; de Paula, C.D.; Souza, V.G.L.; Fernando, A.L.; Coelhoso, I. Understanding the Barrier and Mechanical Behavior of Different Nanofillers in Chitosan Films for Food Packaging. *Polymers* **2021**, *13*, 721. [CrossRef]
21. Souza, V.G.L.; Mello, I.P.; Khalid, O.; Pires, J.R.A.; Rodrigues, C.; Alves, M.M.; Santos, C.; Fernando, A.L.; Coelhoso, I. Strategies to Improve the Barrier and Mechanical Properties of Pectin Films for Food Packaging: Comparing Nanocomposites with Bilayers. *Coatings* **2022**, *12*, 108. [CrossRef]
22. Ribeiro-Santos, R.; Andrade, M.; de Melo, N.R.; Sanches-Silva, A. Use of essential oils in active food packaging: Recent advances and future trends. *Trends Food Sci. Technol.* **2017**, *61*, 132–140. [CrossRef]

23. Jafari, A.; Jafarpour, A.; Safari, R. Influence of Chitosan Nanocomposite and Rosemary (*Rosmarinus officinalis* L.) Extract Coating on Quality of Huso huso Fillet Inoculated with Listeria monocytogenes During Refrigerated Storage. *J. Aquat. Food Prod. Technol.* **2017**, *26*, 675–685. [CrossRef]
24. Kanatt, S.R.; Chander, R.; Sharma, A. Chitosan and mint mixture: A new preservative for meat and meat products. *Food Chem.* **2008**, *107*, 845–852. [CrossRef]
25. Georgantelis, D.; Ambrosiadis, I.; Katikou, P.; Blekas, G.; Georgakis, S.A. Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 °C. *Meat Sci.* **2007**, *76*, 172–181. [CrossRef]
26. Li, T.; Hu, W.; Li, J.; Zhang, X.; Zhu, J.; Li, X. Coating effects of tea polyphenol and rosemary extract combined with chitosan on the storage quality of large yellow croaker (*Pseudosciaena crocea*). *Food Control* **2012**, *25*, 101–106. [CrossRef]
27. Siripatrawan, U.; Noipha, S. Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. *Food Hydrocoll.* **2012**, *27*, 102–108. [CrossRef]
28. Peng, Y.; Wu, Y.; Li, Y. Development of tea extracts and chitosan composite films for active packaging materials. *Int. J. Biol. Macromol.* **2013**, *59*, 282–289. [CrossRef]
29. Moradi, M.; Tajik, H.; Razavi Rohani, S.M.; Oromiehie, A.R.; Malekinejad, H.; Aliakbarlu, J.; Hadian, M. Characterization of antioxidant chitosan film incorporated with Zataria multiflora Boiss essential oil and grape seed extract. *LWT—Food Sci. Technol.* **2012**, *46*, 477–484. [CrossRef]
30. Rubilar, J.F.; Cruz, R.M.S.; Silva, H.D.; Vicente, A.A.; Khmelinskii, I.; Vieira, M.C. Physico-mechanical properties of chitosan films with carvacrol and grape seed extract. *J. Food Eng.* **2013**, *115*, 466–474. [CrossRef]
31. Siripatrawan, U.; Vitchayakitti, W. Improving functional properties of chitosan films as active food packaging by incorporating with propolis. *Food Hydrocoll.* **2016**, *61*, 695–702. [CrossRef]
32. Jonaidi Jafari, N.; Kargozari, M.; Ranjbar, R.; Rostami, H.; Hamedi, H. The effect of chitosan coating incorporated with ethanolic extract of propolis on the quality of refrigerated chicken fillet. *J. Food Process. Preserv.* **2018**, *42*, e13336. [CrossRef]
33. Mujeeb Rahman, P.; Abdul Mujeeb, V.M.; Muraleedharan, K. Chitosan–green tea extract powder composite pouches for extending the shelf life of raw meat. *Polym. Bull.* **2017**, *74*, 3399–3419. [CrossRef]
34. Souza, V.G.L.; Pires, J.R.; Vieira, É.T.; Coelhoso, I.M.; Duarte, M.P.; Fernando, A.L. Shelf Life Assessment of Fresh Poultry Meat Packaged in Novel Bionanocomposite of Chitosan/Montmorillonite Incorporated with Ginger Essential Oil. *Coatings* **2018**, *8*, 177. [CrossRef]
35. Souza, V.G.L.; Rodrigues, P.F.; Duarte, M.P.; Fernando, A.L. Antioxidant Migration Studies in Chitosan Films Incorporated with Plant Extracts. *J. Renew. Mater.* **2018**, *6*, 548–558. [CrossRef]
36. AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 20th ed.; AOAC: Rockville, MD, USA, 2016.
37. Souza, V.G.L.; Pires, J.R.A.; Vieira, É.T.; Coelhoso, I.M.; Duarte, M.P.; Fernando, A.L. Activity of chitosan-montmorillonite bionanocomposites incorporated with rosemary essential oil: From in vitro assays to application in fresh poultry meat. *Food Hydrocoll.* **2019**, *89*, 241–252. [CrossRef]
38. Rosmini, M.R.; Perlo, F.; Pérez-Alvarez, J.A.; Pagán-Moreno, M.J.; Gago-Gago, A.; López-Santoveña, F.; Aranda-Catalá, V. TBA test by an extractive method applied to "paté". *Meat Sci.* **1996**, *42*, 103–110. [CrossRef]
39. Pastor, C.; Sánchez-González, L.; Chiralt, A.; Cháfer, M.; González-Martínez, C. Physical and antioxidant properties of chitosan and methylcellulose based films containing resveratrol. *Food Hydrocoll.* **2013**, *30*, 272–280. [CrossRef]
40. *ISO 4833-1*; Microbiology of the Food Chain Horizontal Method for the Enumeration of Microorganisms Part 1: Colony Count at 30 °C by the Pour Plate Technique. ISO: Geneva, Switzerland, 2013.
41. *ISO 4831:2006(en)*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Detection and Enumeration of Coliforms—Most Probable Number Technique. ISO: Geneva, Switzerland, 2006.
42. Kester, J.J.; Fennema, O. An Edible Film of Lipids and Cellulose Ethers: Barrier Properties to Moisture Vapor Transmission and Structural Evaluation. *J. Food Sci.* **1989**, *54*, 1383–1389. [CrossRef]
43. Pandey, P.K.; Kass, P.H.; Soupir, M.L.; Biswas, S.; Singh, V.P. Contamination of water resources by pathogenic bacteria. *AMB Express* **2014**, *4*, 51. [CrossRef]
44. Crini, G. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. *Prog. Polym. Sci.* **2005**, *30*, 38–70. [CrossRef]
45. Souza, V.G.L.; Pires, J.R.A.; Rodrigues, P.F.; Lopes, A.A.S.; Fernandes, F.M.B.; Duarte, M.P.; Coelhoso, I.M.; Fernando, A.L. Bionanocomposites of chitosan/montmorillonite incorporated with Rosmarinus officinalis essential oil: Development and physical characterization. *Food Packag. Shelf Life* **2018**, *16*, 148–156. [CrossRef]
46. Zivanovic, S.; Chi, S.; Draughon, A. Antimicrobial Activity of Chitosan. *Science* **2005**, *70*, 45–51. [CrossRef]
47. Wu, Y.; Rhim, J.W.; Weller, C.L.; Hamouz, F.; Cuppett, S.; Schnepf, M. Moisture Loss and Lipid Oxidation for Precooked Beef Patties Stored in Edible Coatings and Films. *J. Food Sci.* **2000**, *65*, 300–304. [CrossRef]
48. García-Esteban, M.; Ansorena, D.; Astiasarán, I. Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: Effects on colour, texture and microbiological quality. *Meat Sci.* **2004**, *67*, 57–63. [CrossRef]
49. Wu, J.; Chen, S.; Ge, S.; Miao, J.; Li, J.; Zhang, Q. Preparation, properties and antioxidant activity of an active film from silver carp (Hypophthalmichthys molitrix) skin gelatin incorporated with green tea extract. *Food Hydrocoll.* **2013**, *32*, 42–51. [CrossRef]
50. Bof, M.J.; Jiménez, A.; Locaso, D.E.; García, M.A.; Chiralt, A. Grapefruit Seed Extract and Lemon Essential Oil as Active Agents in Corn Starch–Chitosan Blend Films. *Food Bioprocess Technol.* **2016**, *9*, 2033–2045. [CrossRef]

51. Mendes, J. Qualidade Nutricional e Microbiológica de Enchidos. Master's Thesis, Escola Superior Agrária, Instituto Politécnico de, Refóios do Lima, Portugal, 2013.
52. Fletcher, D.L. Poultry meat quality. *Worlds. Poult. Sci. J.* **2002**, *58*, 131–145. [CrossRef]
53. Barbut, S. Pale, soft, and exudative poultry meat—Reviewing ways to manage at the processing plant. *Poult. Sci.* **2009**, *88*, 1506–1512. [CrossRef]
54. Radha Krishnan, K.; Babuskin, S.; Azhagu Saravana Babu, P.; Sasikala, M.; Sabina, K.; Archana, G.; Sivarajan, M.; Sukumar, M. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *Int. J. Food Microbiol.* **2014**, *171*, 32–40. [CrossRef]
55. Contini, C.; Álvarez, R.; O'Sullivan, M.; Dowling, D.P.; Gargan, S.Ó.; Monahan, F.J. Effect of an active packaging with citrus extract on lipid oxidation and sensory quality of cooked turkey meat. *Meat Sci.* **2014**, *96*, 1171–1176. [CrossRef] [PubMed]
56. Souza, V.G.L.; Rodrigues, C.; Valente, S.; Pimenta, C.; Pires, J.R.A.; Alves, M.M.; Santos, C.F.; Coelhoso, I.M.; Fernando, A.L.L. Eco-Friendly ZnO/Chitosan Bionanocomposites Films for Packaging of Fresh Poultry Meat. *Coatings* **2020**, *10*, 110. [CrossRef]
57. Jay, J.M.; Loessner, M.J.; Golde, D.A. *Modern Food Microbiology*, 7th ed.; Springer: New York, NY, USA, 2005.
58. Hunt, M.C.; King, A.; Barbut, S.; Clause, J.; Cornforth, D.; Hanson, D.; Lindahl, G.; Mancini, R.; Milkowski, A.; Mohan, A. *AMSA Meat Color Measurement Guidelines*; AMSA: Champaign, IL, USA, 2012; Volume 61820.
59. Qin, Y.-Y.; Yang, J.-Y.; Lu, H.-B.; Wang, S.-S.; Yang, J.; Yang, X.-C.; Chai, M.; Li, L.; Cao, J.-X. Effect of chitosan film incorporated with tea polyphenol on quality and shelf life of pork meat patties. *Int. J. Biol. Macromol.* **2013**, *61*, 312–316. [CrossRef] [PubMed]
60. Youn, S.K.; Park, S.M.; Kim, Y.J.; Ahn, D.H. Effect on storage property and quality in meat sausage by added chitosan. *J. Chitin Chitosan* **1999**, *4*, 189–195.
61. Georgantelis, D.; Blekas, G.; Katikou, P.; Ambrosiadis, I.; Fletouris, D.J. Effect of rosemary extract, chitosan and α-tocopherol on lipid oxidation and colour stability during frozen storage of beef burgers. *Meat Sci.* **2007**, *75*, 256–264. [CrossRef]
62. Knorr, D. Recovery and utilization of chitin and chitosan in food processing waste management. *Food Technol.* **1991**, *45*, 114–122.
63. Park, S.I.; Marsh, K.S.; Dawson, P. Application of chitosan-incorporated LDPE film to sliced fresh red meats for shelf life extension. *Meat Sci.* **2010**, *85*, 493–499. [CrossRef]
64. Uddin, F. Clays, nanoclays, and montmorillonite minerals. *Metall. Mater. Trans. A Phys. Metall. Mater. Sci.* **2008**, *39*, 2804–2814. [CrossRef]
65. Khanjari, A.; Karabagias, I.K.; Kontominas, M.G. Combined effect of N,O-carboxymethyl chitosan and oregano essential oil to extend shelf life and control Listeria monocytogenes in raw chicken meat fillets. *LWT—Food Sci. Technol.* **2013**, *53*, 94–99. [CrossRef]
66. Siripatrawan, U.; Harte, B.R. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocoll.* **2010**, *24*, 770–775. [CrossRef]
67. Schreiber, S.B.; Bozell, J.J.; Hayes, D.G.; Zivanovic, S. Introduction of primary antioxidant activity to chitosan for application as a multifunctional food packaging material. *Food Hydrocoll.* **2013**, *33*, 207–214. [CrossRef]
68. Talón, E.; Trifkovic, K.T.; Nedovic, V.A.; Bugarski, B.M.; Vargas, M.; Chiralt, A.; González-Martínez, C. Antioxidant edible films based on chitosan and starch containing polyphenols from thyme extracts. *Carbohydr. Polym.* **2017**, *157*, 1153–1161. [CrossRef] [PubMed]
69. Wang, Y.; Xia, Y.; Zhang, P.; Ye, L.; Wu, L.; He, S. Physical Characterization and Pork Packaging Application of Chitosan Films Incorporated with Combined Essential Oils of Cinnamon and Ginger. *Food Bioprocess Technol.* **2017**, *10*, 503–511. [CrossRef]
70. Sánchez-González, L.; Cháfer, M.; Hernández, M.; Chiralt, A.; González-Martínez, C. Antimicrobial activity of polysaccharide films containing essential oils. *Food Control* **2011**, *22*, 1302–1310. [CrossRef]
71. Ojagh, S.M.; Rezaei, M.; Razavi, S.H.; Hosseini, S.M.H. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chem.* **2010**, *122*, 161–166. [CrossRef]