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The Effect of Whey Protein Films with Ginger and Rosemary Essential Oils on Microbiological Quality and Physicochemical Properties of Minced Lamb Meat

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Abstract: Consumers' constant search for high-quality and safe products, with the least possible preservatives and additives, as well as extended shelf life, has led industries to research and develop alternative forms of food preservation and packaging. The purpose of this research was the study of the effect of natural antimicrobials and, in particular, the essential oils of ginger (*Zingiber Officinale Roscoe*) and rosemary (*Rosmarinus officinalis* L.) on strengthening whey protein films' properties. Whey protein isolate (WPI) films, alone and with incorporated essential oils (WPI + EO) at different concentrations were prepared and then examined for their possible effect on delaying the deterioration of minced lamb meat. Microbiological and physicochemical measurements were carried out to examine the meat's shelf life. Results showed that films with 1% EO significantly improved the microbiological quality of meat. On day 11, total viable counts, *Pseudomonas* spp., *Br. thermosphacta*, lactic acid bacteria, *Enterobacteriaceae*, and yeasts remained low for films with 1% concentration of essential oil compared with 0.5%. Regarding, physicochemical properties the same pattern was observed for pH while oxidation degree was significantly reduced. Finally, color attributes measurements recorded fluctuations between samples, but overall, no considerable discoloration was observed.

Keywords: edible films; whey protein isolate; essential oils; rosemary; ginger; lamb minced meat; mechanical properties; microbiology

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1. Introduction

One of the important issues of the food industry for the maintenance, storage, handling, and promotion of safe and high-quality products is the design and selection of packaging materials with the appropriate specifications. The use of new technologies and new methods of food processing and preservation has led to a new packaging, which not only provides passive protection for packaged food but also plays an active role in preserving it by providing high-quality food and longer shelf life, compared with the classic packaging. Bioplastics have become a potentially environmentally friendly replacement for conventional petrochemical plastics.

The development of edible coatings for food packaging has increasingly gained the research interest for preserving quality, extending the product's shelf life, and being environmentally friendly [1]. Edible films and coatings can function as barriers to moisture, gases, etc., they contribute to the protection of lipids, prevent the loss of moisture and aroma from food, and are ideal substitutes for petroleum-derived polymers [1]. Furthermore, edible films and coatings can also function as carriers for antimicrobial and antioxidant agents, to control the diffusion rate of preservatives to the food interior, and as a part of a multilayer food packaging along with non-edible films [2]. Edible films and coatings can be applied to many different products, such as fruits, vegetables, meat products, and others [3]. In cheeses, for example, the edible packaging is primarily used to control microbiological deterioration on the surface of the cheese, to minimize the risk of contamination with

pathogenic microorganisms, to prolong the quality of the cheese, as well as to manage the taste, color, and nutritional value [4]. Cerqueira et al. [5] applied membranes from mixtures of chitosan, galactomannan, and corn oil to semi-hard cheeses. This prevented mold growth and reduced water evaporation. The handling of water and water activity (a_w) of food determines its microbiological-physicochemical stability and its organoleptic characteristics. Meat and meat products must also avoid the loss of moisture when packaging fresh or frozen meat, reduce the rate of oxidation, retain freshly cut meat juices, and reduce the loss of volatile aromatic compounds, and the uptake of unwanted odors [6]. A film with low oxygen, moisture, and gas permeability can be used to extend the shelf life of meat and meat products. Edible films and coatings do not, in any way, replace the need to package food with non-edible packaging materials; they help them improve the quality of the product, and extend its life. The new packaging coatings consist mainly of milk proteins, and are considered 500 times more effective in keeping oxygen away from food. Furthermore, protein can be easily broken down and even consumed. For the additional strengthening of the membranes, it is necessary to use additives, such as antioxidants and antimicrobial agents, vitamins, probiotics, and minerals. This way, the packaging will have nutritional value on its own.

Whey protein is a material that can be used in the production of biodegradable and edible food packaging. The positive environmental footprint of such food packaging has led the scientific community to research into the production of alternative and environmentally friendly biologically-based materials. In addition, the development of such active bioplastic and edible packaging not only effectively extends the shelf life of products but is also an effective solution to reduce food waste. Their enhanced functions through the incorporation of antioxidants and antimicrobials, along with the good film-forming capacity, safety, and fast biocompatibility and biodegradability rates are an important development in the field of biodegradable and/or edible packaging films [3,7,8].

Plant extracts are rich sources of active compounds with strong antioxidant and antimicrobial activity. Essential oils, as natural compounds, can be used to produce active packaging that exhibits antimicrobial activity against a variety of microorganisms, including Gram-positive and Gram-negative bacteria, yeasts, and molds [8–10]. There is a growing interest in incorporating essential oils into membranes to improve shelf life and microbiological food safety [11]. Among other things, oregano, rosemary, thyme, and sage essential oils are the ones that show the highest effectiveness against microorganisms. Although many of them are considered safe for consumption, their use as food preservatives is often limited as in some cases, to exhibit antimicrobial activity, they must be present in high concentrations, and as a result, it exceeds levels accepted by consumers [12], while due to their high variability, they can be lost during storage, reducing their antibacterial effectiveness [11].

In this perspective, the aim of the present study (conducted between April and May 2021) was to investigate the effectiveness of whey protein films (alone and with incorporated ginger and rosemary essential oils at different concentrations) for the package of lamb minced meat. The prepared films were applied on burger size samples and were tested during their storage time for microbiological and physicochemical properties. Additionally, the prepared WPI films were tested for their mechanical properties.

2. Materials and Methods

2.1. Preparation of Films

Whey protein isolate (WPI), 90% (Arla Foods Ingredients, Greece) was dissolved in distilled water at room temperature in a final concentration of 8% (*w/w*), stirring constantly, until the solution was homogeneous. The solution was then placed in a water bath at 90 °C for 30 min under constant stirring to denature the proteins and immediately afterward in a water bath with ice water to prevent further denaturation. Glycerol (50%) was added [glycerol/(WPI + glycerol)] on a dry basis as a plasticizer, to overcome the fragility of the membranes and to achieve easier handling for various measurements. To enhance the

antimicrobial properties of films, essential oils were added to the solution in appropriate amounts [0.5% and 1% essential oils of ginger (*Zingiber Officinale Roscoe*) and rosemary (*Rosmarinus officinalis* L.) (Vögele Ingredients, Germany)], followed by refrigeration for 24 h to remove the bubbles. The above amounts of essential oils are the results of preliminary tests based on sensory evaluation (data not shown). Finally, they were poured into glass molds (internal dimensions 40 cm × 20 cm) and were let at room temperature under a hood to dry. Five types of coatings were prepared (Table 1) including whey protein films (without the addition of essential oils) which were used as the control (WPI), whey protein films with ginger (WPI + GEO), and rosemary essential oil (WPI + REO).

Table 1. Types and number of films prepared.

Type of Coating	Essential Oil (%)	Abbreviation	Number of Films
Whey protein film	-	WPI	16
Whey protein film + ginger essential oil	0.5	WPI + 0.5%GEO	16
Whey protein film + ginger essential oil	1	WPI + 1%GEO	16
Whey protein film + rosemary essential oil	0.5	WPI + 0.5%REO	16
Whey protein film + rosemary essential oil	1	WPI + 1%REO	16

2.2. Film Characterization

2.2.1. Determination of Film Thickness

The film thickness was determined with a portable digital micrometer (IS 13,109 INSIZE CO., LTD, Japan). The film was measured at six different, random points on their surface. The measurements are provided as mean values ±standard deviations.

2.2.2. Mechanical Tests

The determination of the mechanical properties of the test specimens was performed using a Model 4411 Instron Dynamometer (Instron Engineering Corp., Canton, MA, USA). The tests were performed according to method D882 of the American Society for Testing and Materials (ASTM) [13]. The film samples were prepared in the form of rectangular dimensions (1.5 cm × 10 cm). The tests were performed at a temperature of 25 °C, with a transverse head velocity of 50 mm/min. From the measurements and the stress-strain diagrams, information was collected about the properties of the materials, such as the modulus of elasticity E, the leakage limit σy, the maximum stress σmax, and the percentage deformation at the break-off.

2.2.3. FT-IR Analysis

Infrared spectra of films were collected using attenuated total reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy Cary 630 (Agilent, Santa Clara, CA, USA). Each film was subjected to 16 scans at 4 cm^{-1} resolution from 4000 to 400 cm^{-1} at room temperature.

2.3. Samples Preparation

Fresh lamb of Greek origin was obtained immediately after grinding by a local butcher shop and transported to the laboratory in polystyrene boxes within 30 min. The minced meat was divided into portions of approximately 100 g, in the shape of a burger (8.5 cm diameter and 1.5 cm width), and after being wrapped with the WPI and WP + EO films, were placed in polystyrene trays and wrapped in a transparent oxygen-permeable household polyethylene film. The samples were then stored in 4 °C (±0.5 °C) refrigerators

until spoiled. The main goal of the above process was to simulate the packaged portions of minced meat with the corresponding ones available in retail stores. Sampling was performed on 0, 2, 5, 8, and 11 days.

2.4. Microbiological Analyzes

The microbiological analysis of the samples was performed based on official analysis methods [14]. The following groups of microorganisms were studied: total viable counts (TVC), *Pseudomonas* spp., *Enterobacteriaceae*, lactic acid bacteria (LAB), *Brochothrix thermosphacta*, and yeasts. The TVC was determined using a non-selective tryptic glucose yeast agar substrate [(TGYA) Biolife, Italiana S.r.l., Milano, Italy] which was incubated at 30 °C for 2 to 3 days. Accordingly, *Pseudomonas* spp.: on the selective pseudomonas agar base substrate (Oxoid, Basingstoke, UK), with the addition of the antibiotics cetrimide-fucidin-cephaloridine (C.F.C., Oxoid, Basingstoke, UK) was incubated at 25 °C for 2 to 3 days. *Brochothrix thermosphacta*: on the selective substrate streptomycin thallous acetate-actidione agar base (OXOID, Basingstoke, UK) with the addition of antibiotic (SR0151, OXOID, Basingstoke, UK) was incubated at 25 °C for 2 to 3 days. *Enterobacteriaceae*: on the selective violet red bile glucose agar substrate (Biolife, Italiana S.r.l., Milano, Italy) was incubated at 37 °C for 18 to 24 h. Lactic acid bacteria (LAB): on the selective substrate de Man–Rogosa–Sharpe agar (MRS, Biolife, Italiana S.r.l., Milano, Italy) was incubated at 25 °C for 3 to 5 days. Yeasts: on the selective substrate rose bengal chloramphenicol agar base (RBC, Biolife, Italiana S.r.l., Milano, Italy) was incubated at 25 °C for 5 days.

2.5. Physicochemical Analyses

Measurement of pH, Color Attributes, and Lipid Oxidation/2-thiobarbituric Acid Reactive Substances (TBARS) Assay

The pH was measured using a pH-meter model HD 3456.2 (Delta OHM Srl, Selvazzano Dentro, Italy) as follows: meat samples (20 g) were completely homogenized with 10 mL of distilled water, followed by immersion of the electrode and determination of pH.

Color attributes were measured to assess the color changes during the shelf-life of the samples. For that purpose, a Hunter Lab colorimeter model DP-9000 (Reston, VA, USA) was used. Approximately 70 g of minced the meat sample was placed on a glass plate and the parameters L* (brightness), a* (redness), and b* (yellowness) were measured. For each value, the plate was rotated approximately 60° to determine the color on all sides of the meat mass. The ΔE was calculated by the following equation:

$$\Delta E = \sqrt{(L_s^* - L_c^*)^2 + (a_s^* - a_c^*)^2 + (b_s^* - b_c^*)^2}$$

where L_s^* is the brightness value for each sample, L_c^* is the brightness value for the respective control sample, a_s^* is the redness value for each sample, a_c^* is the redness value for the respective control sample, b_s^* is the yellowness value for each sample, b_c^* is the yellowness value for the respective control sample [15].

Finally, the TBARS value was measured according to the method described by Karabagias et al. [16].

2.6. Sensory Evaluation

After each sampling, meat samples were frozen (−30 °C) until sensory evaluation. The attributes of cooked minced lamb meat on each sampling day were evaluated by a panel of eleven untrained judges (age range 25–60), graduate students, and faculty of the Laboratory of Food Chemistry, University of Ioannina. Panelists were asked to evaluate sensory attributes of cooked samples (ca. 100 g), which were prepared by steaming for ca. 10 min to an internal temperature of 85 °C. Sensory evaluation was conducted in individual booths under controlled conditions of temperature, light, and humidity. A set of five samples (corresponding to five different treatments) with random code numbers were presented to panelists. Along with the test samples, a freshly thawed and cooked meat

sample, stored at −30 °C throughout the experiment, was served to the panelists as the master control sample. Panelists were asked to score odor, taste, and overall perception of minced lamb meat using a 1–5 acceptability scale, with 5 corresponding to the most liked sample and 1 corresponding to the least liked sample. A score of 3 was taken as the lower limit of acceptability.

2.7. Statistical Analysis

Experiments were replicated twice while analyses were run in triplicate for each sampling day per treatment (n = 4 × 3 = 12). All analyses data were expressed as mean values ± standard deviations along with the microbiological counts which were converted to log CFU/g and subjected to analysis of variance (ANOVA) with Tukey's multiple range tests using the MINITAB software package version 18.0 [17]. Differences between means of multiple groups were analyzed by three-way ANOVA with Tukey's multiple range test. The main effects plots were constructed to assess the relative significance of various parameters on the response of the system.

3. Results and Discussion

3.1. Film Characterization

3.1.1. Film Thickness

Whey films with and without added essential oils were generally homogeneous, transparent, and yellowish. Membranes incorporated with higher concentrations of essential oils (1.0%) were visually more elastic than the WPI films. Similar visual characteristics with those found in the present study were recorded by Ramos et al. [18] who studied membranes produced from isolated whey protein or protein concentrate, and by Galus and Lenart [19], who studied whey protein membranes fortified with almond and walnut oils.

The characteristics of WPI films as well as those fortified with essential oils are presented in Table 2. The film thickness ranged from 0.090 ± 0.010 mm in WPI to 0.148 ± 0.020 mm in WPI + 1%REO. In general, the films fortified with 1% of essential oil were found to be thicker. Those differences in thickness between the control films (WPI) and the fortified ones can be caused by the addition of essential oils. Bertan et al. [20] observed that the addition of hydrophobic substances promoted an increase in the thickness of the biofilm, as it was necessary to use different ratios for each composition aimed at controlling the thickness for repeatability of measurements and validity of comparisons between properties. In the present study, it can be assumed that the percentage of hydrophobic substances (e.g., GEO and REO) was too low to cause such a variation, and the addition of essential oils did not show significant differences in films thickness other than that the higher concentration of both essential oils results in higher film thickness.

Table 2. Mechanical properties of WPI films alone and with incorporated EOs.

Treatment	Thickness (mm)	% Elongation at Break	Tensile Strength at Break (MPa)	Young's Modulus (MPa)
WPI	0.090 ± 0.01 ^{a}	243.10 ± 50.50 ^{a}	16.83 ± 2.10 ^{a}	160.8
WPI + 0.5%GEO	0.129 ± 0.01 ^{b}	300.66 ± 43.40 ^{a}	17.06 ± 2.90 ^{a}	60.80
WPI + 1%GEO	0.141 ± 0.00 ^{b}	415.20 ± 29.60 ^{b}	13.11 ± 1.70 ^{a}	45.90
WPI + 0.5%REO	0.131 ± 0.01 ^{b}	311.10 ± 33.60 ^{a}	15.99 ± 2.20 ^{a}	63.97
WPI + 1%REO	0.148 ± 0.02 ^{b}	399.70 ± 14.40 ^{b}	13.69 ± 0.90 ^{a}	44.77

Means with different letters in the same column indicate statistically significant differences ($p < 0.05$, Tukey's test).

3.1.2. Mechanical Properties

In terms of mechanical properties and the uniaxial tensile test, films with increased essential oil content (1% for both EOs) have statistically higher % elongation values compared with other films (WPI and WPI + 0.5% EOs) and the two EOs behaved similarly (Table 2).

The addition of any type and concentration of EOs did not significantly affect the values of tensile strength. However, the addition of 1% EOs slightly decreased the tensile strength at break (13.11 ± 1.70 for WPI + 1%GEO and 13.69 ± 0.90 for WPI + 1%REO).

The meaning of Young Modulus is an indication of films' elasticity and lower values show higher elasticity. The addition of EOs improved the films' elasticity compared with WPI films (160.8 MPa). Specifically, 1% concentration of EOs improved elasticity by 3.6 times and 0.5% 2.6 times.

Ma et al. [21] reported an increase in tensile strength and elasticity modulus at lower olive oil concentrations (5–15%) and a decrease in higher oil addition (20%) for gelatin films. However, Fang et al. [22] reported a decrease in tensile strength for whey protein membranes with increasing soybean oil content. Similar results were obtained for whey membranes containing olive oil [23] and quinoa-chitosan protein membranes incorporated with sunflower oil [24].

3.1.3. FT-IR Analysis

The ATR-FTIR spectra of produced films showed no differences among them (Figure S6). Specifically, approximately bands at 3500–3100 cm^{-1} and 2974–2800 cm^{-1} were attributed to O-H/N-H and C-H stretching vibrations, respectively. Major absorption bands of protein were peptide linkages of amide I and II and located approximately 1620 cm^{-1} and 1540 cm^{-1}, respectively. The amide I region is related to the stretching vibrations of C=O and C-N bonding while amide II to the stretching of the C-N. The strong band peaks at 1100 cm^{-1} and 1032 cm^{-1} attributed to C-O stretching of the C-O-H and C-O-C groups of the glucose ring [3,8,25–27].

3.2. Microbiological Analyses

The microbiological analysis showed that the initial microflora of minced lamb meat consisted of *Pseudomonas* spp., *Br. thermosphacta*, LAB, and yeasts. The dynamics of these microorganisms as well as their contribution to the final microflora was influenced by various factors, including the type of packaging used.

The initial TVC value (Figure 1a) of fresh minced lamb meat was 3.65 log CFU/g leading to an acceptable quality of fresh meat [28]. The maximum acceptable level for TVC (7 log CFU/g) [29] was reached on day 8 for WPI films (7.17 log CFU/g), between the 8th and 11th day for WPI + 0.5%EO, and on day 11 for WPI + 1%EO (ginger and rosemary for both cases). The fact that WPI + 1%EO reached the maximum acceptable level on day 11 indicates the possible antimicrobial effect of the tested films. Literature data are in accordance with the results of the present study [30–32] regarding WPI films incorporated with rosemary EO.

Pseudomonas spp. is an indicator of psychrotrophic bacteria, absolutely aerobic and sensitive to CO_2, and is considered as one of the main microorganisms responsible for meat spoilage [33]. The initial *Pseudomonas* spp., value was 1.69 log CFU/g, lower compared with literature [31,34], and reached the maximum on day 11 for WPI + 0.5%EO (8.39 log CFU/g for GEO and 8.13 log CFU/g for REO). The *Pseudomonas* spp., from day 2 to day 11 ranged between 2.6 and 8.4 log CFU/g, and according to Figure 1b the addition of essential oil did not hinder their development. Compared with the concentration of EOs added, films containing 1% EO appear to be more effective than 0.5%. Specifically, for WPI + 1%EO the samples also reached their maximum on day 11 (7.34 log CFU/g for GEO and 7.85 log CFU/g for REO); however, their values were lower compared with WPI + 0.5%EO samples, indicating that by increasing the concentration of EOs incorporated in the films, their inhibitory effect also increased.

Br. thermosphacta is a Gram-positive facultative anaerobe bacterium, constituting part of the natural microflora of fresh packaged meat, and one of the spoilage microorganisms, especially, in pork and lamb meat, as they combine different chemical and biochemical parameters that favor its growth [35,36]. Initial counts of *Br. thermosphacta* (Figure 1c) were 3.28 log CFU/g and reached the maximum on day 11 for WPI films (8.01 log CFU/g). On

day 2 a reduction was observed for WPI + 1%GEO (3.22 log CFU/g), and WPI + REO (3.14 log CFU/g for 0.5% and 3.05 log CFU/g for 1%).

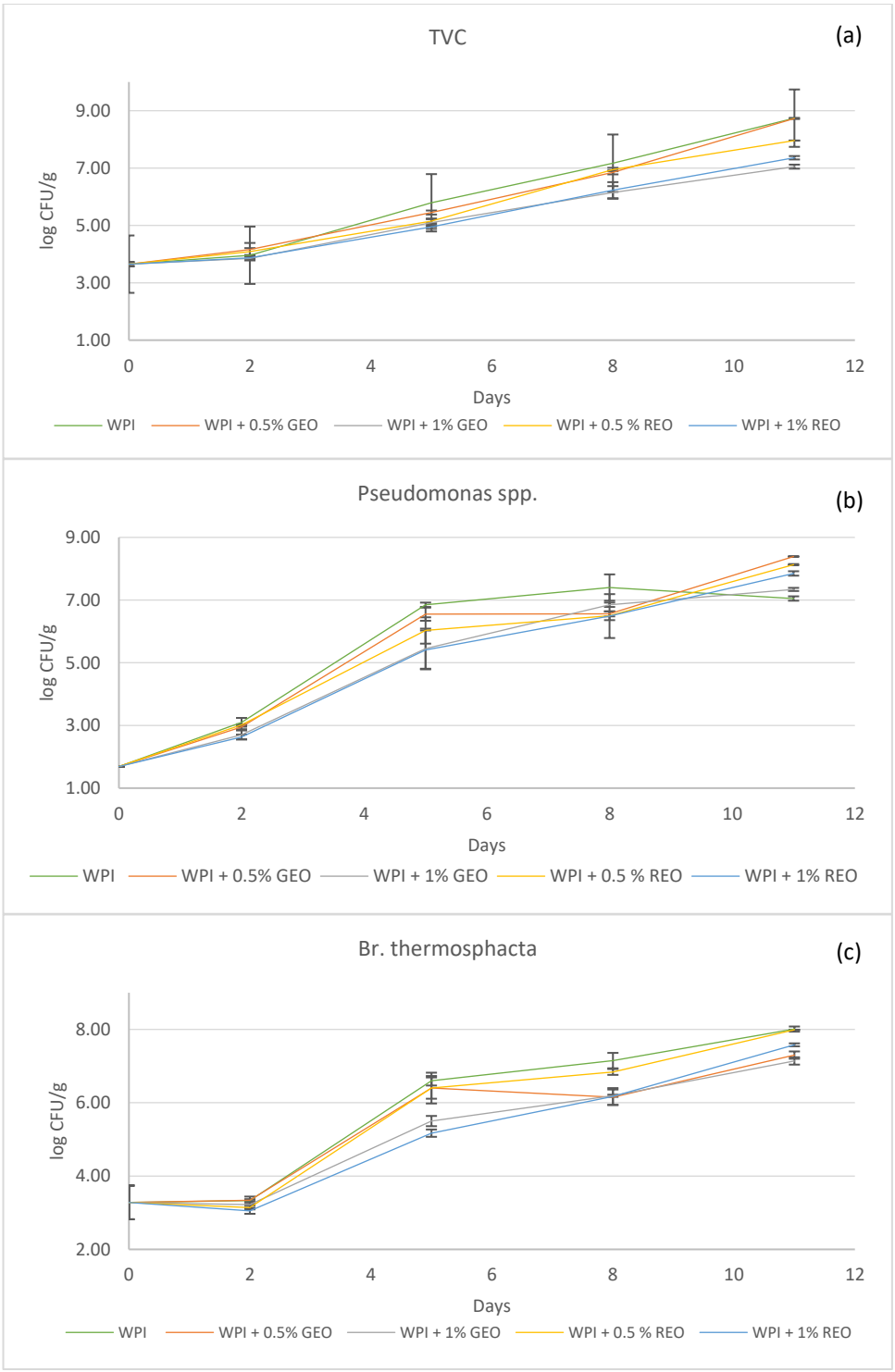

Figure 1. Effect of WPI alone and with incorporated EOs on the growth of (**a**) TVC, (**b**) *Pseudomonas* spp., and (**c**) *Br. thermosphacta*.

LAB are facultative anaerobic bacteria and comprise a significant part of meat microflora, as they can grow under low O_2 concentrations [37]. The growth of LAB during the storage time of the samples ranged from 2.8 to 7.5 log CFU/g. The concentration of the essential oil seems to contribute to the prolongation of the shelf life while the WPI + 1%EO

seems to prevent the growth of LAB. Regarding the activity of the two essential oils, rosemary is presented as more active as it has a positive effect on LAB growth. Specifically, the initial population of LAB was 2.81 log CFU/g and reached its maximum on day 11 (7.50 log CFU/g) for WPI, and WPI + 0.5%GEO films. On day 2, samples with films WPI + 1%GEO, and WPI + REO (for both concentrations) recorded a reduction of LAB population over 0.5 log CFU/g (Figure 2a). Over time though, the LAB growth seemed to be suppressed by the incorporated EOs in WPI films as their population remained low, and especially, for WPI + 1%EO where they recorded LAB population under 7 log CFU/g (6.60 log CFU/g for GEO and 6.21 log CFU/g for REO). The results of the present study are in accordance with literature data regarding the reduction of LAB growth by EOs [31,36,38].

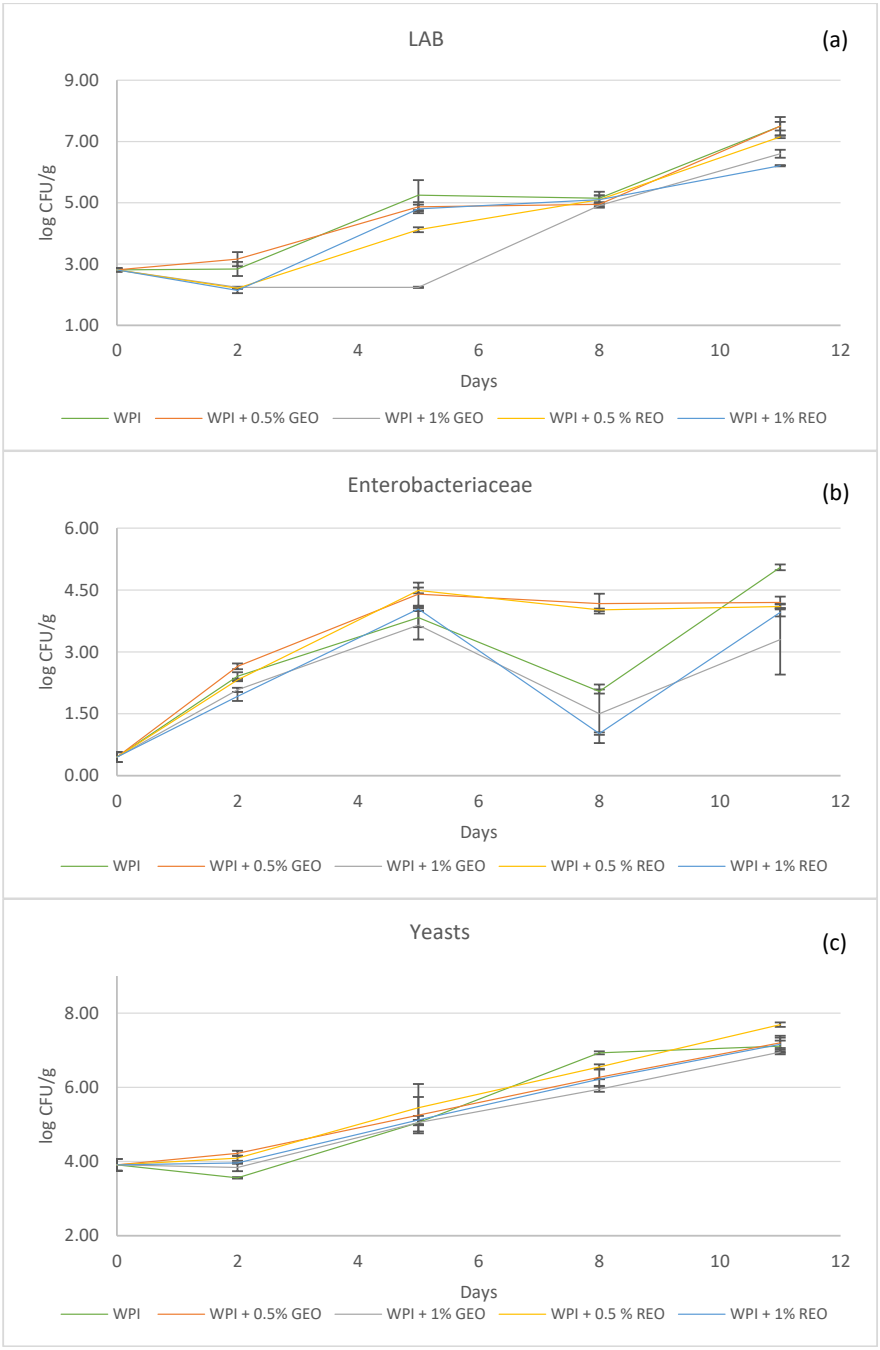

Figure 2. Effect of WPI alone and with incorporated EOs on the growth of (**a**) LAB, (**b**) *Enterobacteriaceae*, and (**c**) yeasts.

Concerning *Enterobacteriaceae*, which is usually considered a hygiene indicator [30] their growth ranged from 0.45 log CFU/g on day 0 to 5.05 log CFU/g on day 11 indicating a good quality of minced lamb meat. Although fluctuations were observed (Figure 2b) during the storage period, WPI + 1%EO samples seem to be more effective against *Enterobacteriaceae* development recording the highest values on day 11 (3.30 log CFU/g for GEO and 3.96 log CFU/g for REO), though lower than those of WPI + 0.5%EO (4.20 log CFU/g for GEO and 4.10 log CFU/g for REO). The final *Enterobacteriaceae* values of the present study are lower than those reported by Soldatou et al. [28] who studied the *Enterobacteriaceae* counts' changes during the storage time of lamb meat products under different package conditions (vacuum and modified atmosphere packaging). Alizadeh Sani et al. [31] also reported higher initial and final values for *Enterobacteriaceae* counts of lamb meat packaged with WPI films, although, as in the present study the inhibitory effect of REO was highlighted.

The yeasts' evolution during minced lamb meat storage is an important factor for its evaluation. The initial and final values (Figure 2c) of the yeasts' counts were higher compared with literature data (3.91 log CFU/g on day 0 and 7.69 log CFU/g on day 11) [30,32]. However, the WPI + 1%EO seemed to be more effective against yeast growth, as their final counts for the respective samples were lower (6.95 log CFU/g for GEO and 7.16 log CFU/g for REO) compared WPI + 0.5%EO (7.20 log CFU/g for GEO and 7.69 log CFU/g for REO).

3.3. Physicochemical Analyses (pH, Color Attributes, and TBARS)

Data of physicochemical analyses are shown in Table 3. The initial pH value of minced lamb meat was 5.72 ± 0.01, which is within the normal range for fresh, raw meat. It seems that the coating films, regardless of the concentration of the essential oils, show an increase in the pH value with the values reaching up to 6.37 ± 0.02 on the last day of storage for WPI + 0.5%REO, while the opposite was observed for the samples with WPI where the values reach marginally at pH = 6. The different types of membranes seem to influence the pH, as the values vary widely both in terms of essential oils and their concentration ($p = 0.034 < 0.05$) as well as in terms of storage time. Fluctuations in pH values during storage are also associated with various changes in the microbial profile of the samples. In general, the main parameters that seem to affect the pH value were found to be the essential oil ($p = 0.014 < 0.05$) and the storage time ($p = 0.000 < 0.05$) (Figure S1).

Table 3. Mean values and SD of physicochemical analyses tested.

Physicochemical Analyses	Days of Storage	Treatment					
		WPI	**WPI + 0.5% GEO**	**WPI + 1% GEO**	**WPI + 0.5% REO**	**WPI + 1% REO**	
pH	0	5.72 ± 0.01 [a]					
	2	5.73 ± 0.04	5.95 ± 0.04	5.91 ± 0.01	5.82 ± 0.01	5.81 ± 0.00	$p = 0.034 < 0.05$ [b]
	5	5.92 ± 0.02	5.92 ± 0.02	6.02 ± 0.02	6.01 ± 0.01	6.05 ± 0.04	$p = 0.014 < 0.05$ [c]
	8	6.05 ± 0.07	5.80 ± 0.01	5.94 ± 0.05	6.15 ± 0.07	6.05 ± 0.07	$p = 0.000 < 0.05$ [d]
	11	5.99 ± 0.00	6.32 ± 0.02	6.22 ± 0.01	6.37 ± 0.02	6.21 ± 0.01	
TBARS (mg MDA/kg)	0	0.88 ± 0.98 [a]					
	2	0.93 ± 0.00	2.19 ± 0.00	0.37 ± 0.00	1.94 ± 0.35	0.25 ± 0.01	$p = 0.027 < 0.05$ [b]
	5	0.28 ± 0.00	0.52 ± 0.00	0.35 ± 0.00	0.35 ± 0.00	0.24 ± 0.00	$p = 0.875 > 0.05$ [c]
	8	0.54 ± 0.00	0.52 ± 0.00	0.35 ± 0.00	0.45 ± 0.00	0.34 ± 0.00	$p = 0.000 < 0.05$ [d]
	11	0.37 ± 0.00	0.30 ± 0.00	0.45 ± 0.00	0.55 ± 0.00	0.57 ± 0.00	
L*	0	44.40 ± 0.40 [a]					
	2	44.13 ± 0.18	44.25 ± 0.23	44.60 ± 0.02	43.01 ± 0.01	42.84 ± 0.05	$p = 0.033 < 0.05$ [b]
	5	42.48 ± 0.01	42.01 ± 0.72	42.72 ± 0.02	42.91 ± 0.00	41.99 ± 0.02	$p = 0.003 < 0.05$ [c]
	8	44.89 ± 0.02	42.25 ± 0.21	43.50 ± 0.02	42.21 ± 0.03	42.50 ± 0.05	$p = 0.000 < 0.05$ [d]
	11	46.71 ± 0.14	48.36 ± 0.05	44.49 ± 0.02	45.35 ± 0.04	44.17 ± 0.08	

Table 3. *Cont.*

Physicochemical Analyses	Days of Storage	Treatment					
		WPI	WPI + 0.5% GEO	WPI + 1% GEO	WPI + 0.5% REO	WPI + 1% REO	
a*	0	14.73 ± 0.24 [a]					
	2	12.00 ± 0.00	12.65 ± 0.01	13.71 ± 0.02	13.60 ± 0.74	13.65 ± 0.04	$p = 0.109 > 0.05$ [b]
	5	15.34 ± 0.02	15.33 ± 0.04	14.12 ± 0.02	15.51 ± 0.01	16.02 ± 0.02	$p = 0.082 > 0.05$ [c]
	8	15.54 ± 0.04	15.43 ± 0.02	16.21 ± 0.04	14.82 ± 0.04	15.05 ± 0.06	$p = 0.000 < 0.05$ [d]
	11	13.33 ± 0.03	12.32 ± 0.00	14.76 ± 0.06	14.58 ± 0.01	15.17 ± 0.08	
b*	0	13.08 ± 0.24 [a]					
	2	13.33 ± 0.43	13.64 ± 0.00	12.92 ± 0.02	13.41 ± 0.01	13.56 ± 0.01	$p = 0.492 > 0.05$ [b]
	5	12.37 ± 0.01	12.39 ± 0.01	13.10 ± 0.13	13.12 ± 0.01	13.01 ± 0.01	$p = 0.217 > 0.05$ [c]
	8	14.19 ± 0.02	13.73 ± 0.03	14.63 ± 0.04	13.13 ± 0.04	13.83 ± 0.05	$p = 0.000 < 0.05$ [d]
	11	13.93 ± 0.00	13.32 ± 0.01	13.07 ± 0.04	14.89 ± 0.08	14.29 ± 0.04	
ΔE	0	-					
	2	-	1.72 ± 0.09	1.85 ± 0.10	2.02 ± 0.45	2.13 ± 0.16	$p = 0.001 < 0.05$ [b]
	5	-	0.51 ± 0.65	1.45 ± 0.04	0.87 ± 0.03	1.05 ± 0.61	$p = 0.000 < 0.05$ [c]
	8	-	2.69 ± 0.24	1.61 ± 0.03	2.97 ± 0.02	2.47 ± 0.56	$p = 0.000 < 0.05$ [d]
	11	-	2.02 ± 0.07	2.78 ± 0.09	2.08 ± 0.02	3.16 ± 0.02	

[a] Day 0 is the same for all samples, three-way ANOVA results, *p*-value for each physicochemical analysis between groups of: [b] concentration of essential oil, [c] type of essential oil, and [d] storage time.

The TBARS values are reported in milligrams of malondialdehyde (MDA) per kilogram of the sample (mg MDA/kg). MDA values above 1.5 mg/kg are associated with noticeable and unacceptable organoleptic changes in the meat [39]. The MDA values of meat samples did not show significant antioxidant activity for WPI + EO films compared with the WPI film, as the WPI films appear to have antioxidant activity throughout the storage period of the samples, as the oxidation degree values remained low. Specifically, the values ranged from 0.88 ± 0.98 (1st day) to 2.19 ± 0.00 (2nd day). It is important to note that on the 2nd day an increase in MDA values of WPI + 0.5%GEO and WPI + 0.5%REO was observed, while for the corresponding films with 1% concentration of essential oils the MDA values seem to be more than 50% lower. A similar picture is observed on the 5th day; however, upon comparing the 2nd and 5th days, a decrease in the degree of oxidation is observed mainly for the control films and the WPI + 0.5%EO films (both the GEO and REO). The oxidation degree was found to be statistically significant (Figure S2) as it was affected by the concentration of essential oils ($p = 0.027 < 0.05$), and the storage time ($p = 0.000 < 0.05$) of the samples but did not affect the essential oil ($p = 0.875 > 0.05$). Regarding the TBARS values, the findings of the present study seem to be in contrast with those of Siripatrawan and Noipha [40] who examined the oxidation grade of chitosan films incorporated with green tea extract for 20 days of storage of beefsteaks and reported a reduction in TBARS values for the untreated chitosan film as well as for the film incorporated with the natural antioxidant. A decrease in TBARS values was also observed by Rimini et al. [41] who studied the package conditions for fresh and stored chicken cuts for 12 and 90 days in the presence of a blend of thyme and orange essential oil compared with the control.

Fluctuations were observed in all color attributes' values in all types of films. Specifically, the brightness values (L* parameter), ranged from 44.40 ± 0.40 (1st day) to 48.36 ± 0.05 (11th day). For WPI films, the values increased from the 8th day onwards, while for the WPI + 0.5%EO and WPI + 1%EO films (both the GEO and REO) fluctuations were observed from the 2nd day of sampling onwards, which can be related to the denaturation of proteins in minced lamb meat. Regarding the brightness values, the findings of the present study are in agreement with those of Carvalho et al. [42] who investigated the antioxidant properties of thyme essential oil and whey protein isolate/cellulose nanofiber, nano biopolymers films containing TEO (20%, 30%, and 40% *w/w*) applied on ground beef. The researchers

recorded values of the same order of magnitude as those of the present study. In general, the brightness values were affected significantly (Figure S3) by the examined factors [essential oils ($p = 0.003 < 0.05$), concentration ($p = 0.033 < 0.05$), and storage time ($p = 0.000 < 0.05$)].

Regarding the redness values (parameter a*), they were statistically significantly affected only by storage time ($p = 0.000 < 0.05$) [essential oil ($p = 0.082 > 0.05$), and concentration ($p = 0.109 > 0.05$)] (Figure S4). In general, an increase was observed on the 8th day (from 14.82 ± 0.04 for the WPI + 0.5%REO to 16.21 ± 0.04 for the WPI + 1%GEO) followed by a decrease on the 11th day (from 12.32 ± 0.00 for the WPI + 0.5%GEO to 14.58 ± 0.01 for the WPI + 0.5%REO). This phenomenon is evident in all types of films and can be attributed to changes in myoglobin and the accumulation of meta-myoglobin over storage time of the samples. Higher redness values imply the contribution of membranes to the preservation and/or improvement of the meat's red color. However, the redness values of the present study were lower compared with those reported by Carvalho et al. [42] who, in addition, observed a decrease in redness values for all treatments tested.

Regarding the values of yellowness (parameter b*), they ranged from 13.08 ± 0.24 to 14.89 ± 0.08, with fluctuations observed for the WPI and the WPI + GEO films where they recorded their maximum value on day 8. On the other hand, for the WPI + REO films the maximum yellowness values were observed on the 11th day. The results of the present study are in contrast with literature data [42,43] where a decrease in yellowness was observed through storage time. In general, the values of yellowness were statistically significantly affected (Figure S5) by storage time ($p = 0.000 < 0.05$), but not by the concentration ($p = 0.492 > 0.05$) and the type of essential oil ($p = 0.217 > 0.05$).

The ΔE values indicate that there was no significant discoloration between samples and their respective controls (WPI samples) during the days 2 (1.72 ± 0.09 for WPI + 0.5%GEO to 2.13 ± 0.16 for WPI + 1%REO) and 5 (0.51 ± 0.65 for WPI + 0.5%GEO to 1.45 ± 0.04 for WPI + 1%GEO) of storage while this value moderately increased for days 8 (1.61 ± 0.03 for WPI + 1%GEO to 2.97 ± 0.02 for WPI + 0.5%REO) and 11 (2.02 ± 0.07 for WPI + 0.5%GEO to 3.16 ± 0.02 for WPI + 1%REO). The ΔE values between 0 and 1 indicate discoloration not perceptible by the human eye, while the ΔE values between 1 and 2 indicate discoloration perceptible by close observation or only obvious to a trained eye. The ΔE values between 2 and 3.5 that were measured in the sample stored for 11 days indicate discolorations that can be obvious to an untrained eye [44]. These results indicate that most of the prepared films can retain the color of lamb for almost 8 days of storage.

3.4. Sensory Evaluation

The results of sensory (odor, taste, and overall perception) evaluation of cooked minced lamb meat are presented in Figure 3a–c. All three sensory evaluation scores decreased significantly ($p < 0.05$) with storage time. Taste and odor proved to be more sensitive sensory attributes compared with the overall perception. The lower acceptability limit of 3 was reached for taste after day 5 for WPI samples, between day 5 and 8 for WPI + GEO, and after day 8 for WPI + REO samples. A similar pattern was observed for odor scores, the limit of 3 was reached after day 5 for WPI and WPI + GEO samples, and between days 5 and 8 for WPI + REO samples. For both attributes, WPI samples were found unacceptable on day 11 and for that reason, panelists were unable to taste them. The overall perception included the color and the general picture of each sample before consumption. For WPI samples the lower limit of acceptability was reached between days 5 and 8, while for WPI + GEO and WPI + 0.5%REO this limit was reached after day 8, and for WPI + 1%REO on day 11.

The use of EOs in both concentrations retained the sensory properties of lamb meat for almost 5 to 8 days. Specifically, WPI + REO samples reached the limit of acceptability for taste and odor after day 8. At this point, it should be mentioned that REO has a delicate taste compatible with the taste of cooked lamb, while the panelists found it more familiar than GEO.

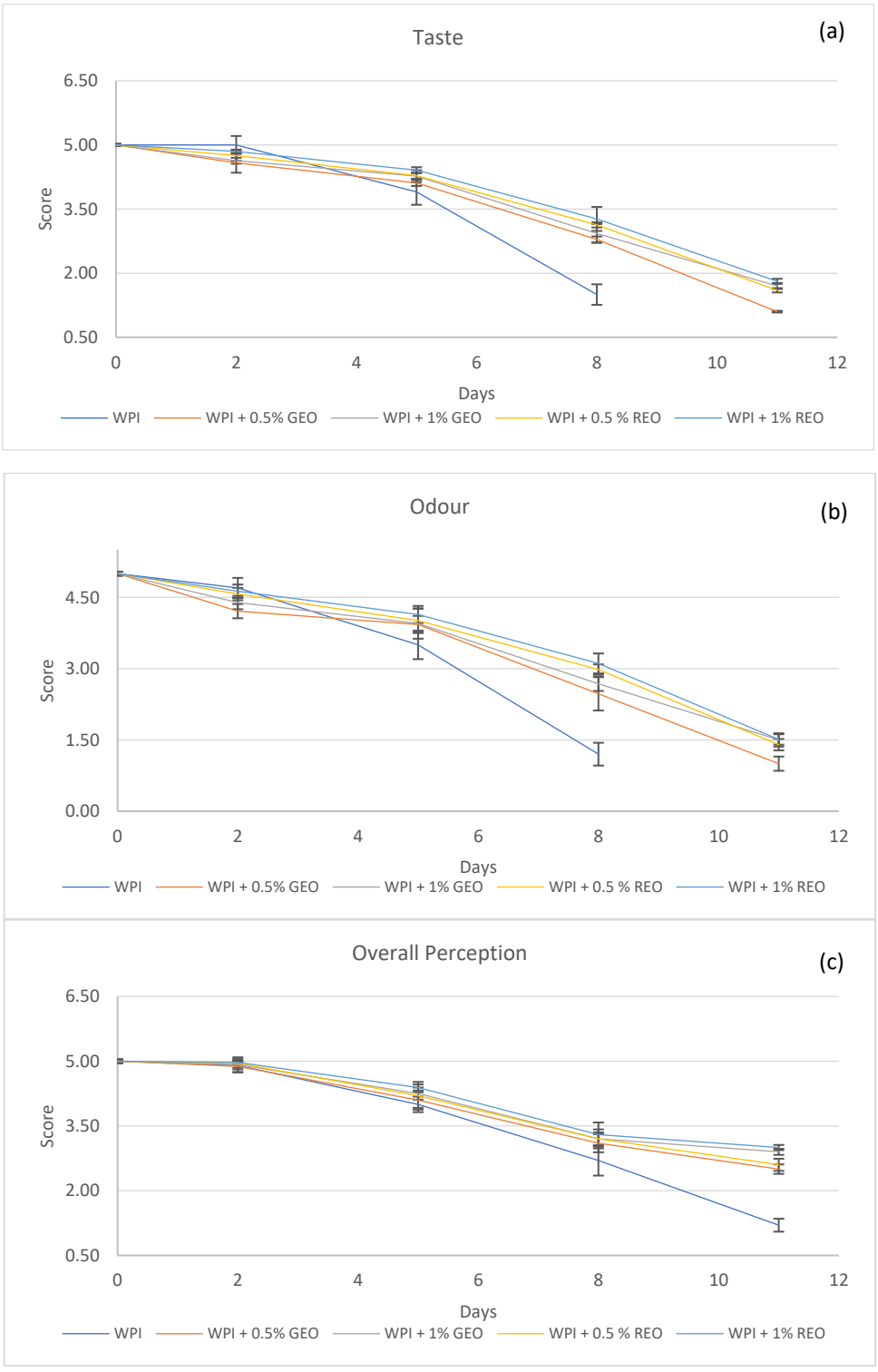

Figure 3. Sensory evaluation scores, taste (**a**), odor (**b**), and overall perception (**c**) of minced lamb meat packaged with WPI films with and without EOs at different concentrations.

Present sensory data were in reasonable agreement with microbiological data (TVC). Differences observed between the two may be attributed to the fact that it is not the total number of microorganisms but rather the number of specific spoilage organisms that are responsible for product deterioration [45]. Alizadeh Sani et al. [31] reported that the use of REO in biodegradable nanocomposite films containing TiO_2 nanoparticles increased

significantly the shelf life of lamb meat compared with control samples (for almost 15 days regarding texture, color, and overall acceptability).

4. Conclusions

Edible films/coatings are a great way to diversify the functional food market and a substitute for the packaging and prevailing products. These are promising ways to improve food quality, extend shelf life, ensure safety, maintain functionality, and reduce environmental impact. In addition, these films and coatings can be used as separate bags of homogeneous substances and carriers of the active ingredient. The WPI films prepared in the present study showed a significant delay in microbiological deterioration of minced lamb meat, and especially, the films with 1% incorporated EO (both GEO and REO), while the TBARS values remained low indicating a significant delay in oxidation degree of meat samples. Results showed no significant differences between the GEO and REO 1% films. Furthermore, the color attributes tested as well as the ΔE value showed no significant discoloration of the samples for almost 8 days of storage, while the sensory evaluation test showed that, in terms of taste, and odor, samples packaged with WPI + REO in both concentrations were sensory acceptable for almost 8 days.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su14063434/s1, Figure S1: Main effects plot for essential oil, their concentration and storage time on the pH values of minced lamb meat samples packaged with different types of WPI films. Figure S2: Main effects plot for essential oil, their concentration and storage time on TBARS values of minced lamb meat samples packaged with different types of WPI films. Figure S3: Main effects plot for essential oil, their concentration and storage time on L* parameter (brightness) values of minced lamb meat samples packaged with different types of WPI films. Figure S4: Main effects plot for essential oil, their concentration and storage time on a* parameter (redness) values of minced lamb meat samples packaged with different types of WPI films. Figure S5: Main effects plot for essential oil, their concentration and storage time on b* parameter (yellowness) values of minced lamb meat samples packaged with different types of WPI films. Figure S6: FTIR-ATR spectra of WPI films with and without EOs at different concentrations. Table S1: Compositional analysis (%) of ginger and rosemary essential oils.

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