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Oregano and Rosemary Essential Oils in Collagen Fiber and Polyvinyl Alcohol Films: Application as Biodegradable Active Packaging for Ready-to-Eat Meat Products

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Abstract

Developing packaging using biomaterials offers a solution to the problem of solid plastic waste in urban areas. However, selecting suitable materials remains challenging, particularly when the packaging is intended for food products with high moisture content, such as meat. In response to this challenge, a novel film comprising a blend of collagen fiber and polyvinyl alcohol, supplemented with varying concentrations of oregano and rosemary essential oils, was developed. The mechanical properties, *in vitro* antioxidant activity, and chemical composition of these collagen fiber and polyvinyl alcohol films, enhanced with oregano and rosemary essential oils, were thoroughly characterized. The compounds γ-terpinene and ρ-cymene played a significant role in the antioxidant activity of the packaging. The impact of this active packaging on the quality of sliced mortadella was evaluated over 0, 3, 6, and 9 days of refrigeration. Time significantly influenced most of the variables examined. The concentration of essential oils (0%, 2%, 4%, and 6%) significantly affected the moisture level, water activity, pH, and *Escherichia coli* count in the packaged product. For lipid oxidation analysis, the type of essential oil proved significant, with oregano essential oil demonstrating superior activity. A pro-oxidant effect was noted in products with 6% rosemary essential oil. Packages containing essential oils from oregano and rosemary exhibited lower *E. coli* counts. Films enriched with essential oils showed a higher rate of biodegradation.

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#In memoriam.

Keywords

Lipid Oxidation, *Escherichia coli*, Principal Component Analysis, Bioactive Compounds, Shelf Life, Mechanical Properties

1. Introduction

A global effort is underway to find materials that can replace non-biodegradable petroleum products in packaging applications. However, identifying suitable replacement materials poses a significant challenge [1]. Developing new packaging materials for foods, particularly meat products, is exceptionally challenging due to several intrinsic parameters critical to these products' sensory characteristics. These characteristics include humidity, water activity, pH, and color, which may change during storage depending on the packaging material, potentially rendering the product less acceptable.

Consumers increasingly prefer ready-to-eat meat products for convenience and practicality [2] [3]. These products are more prone to microbiological contamination since they are consumed without further heat treatment. Cross-contamination primarily occurs during the slicing stage through equipment, handlers, or the processing environment itself [4]. In Brazil, the Ministry of Health data between 2012 and 2021 identified *Escherichia coli* as the predominant etiological agent [5]. *E. coli*, a pathogen from the Enterobacteriaceae family, is most commonly found in the gastrointestinal tract of animals and humans and is used as an indicator of hygiene and health in food handling, with frequent reports in ready-to-eat products [6] [7]. Furthermore, complex chemical reactions, such as lipid oxidation, are more intense in these products due to the increased surface area for reactions, which can alter the products both nutritionally and sensorially [3].

Incorporating bioactive compounds into packaging matrices has garnered attention for controlling pathogenic bacteria and oxidation reactions and for the potential to increase the shelf life of products, thereby reducing food waste [6]. According to the 2021 *Food Waste Index Report*, almost one billion tons of food are wasted annually, a problem prevalent in countries of all income levels, including middle and low-income nations [8]. Reducing food waste aligns with one of the UN's Sustainable Development Goals, aiming for reduction at the retail and consumer levels and along production chains by 2030 [9]. Karwowska *et al.* [10] noted that losses and waste in the meat sector are highest at the consumption stage, including households and food services.

Brazil is one of the world's leading animal protein producers and was the largest exporter of beef and chicken in 2022 [11]. Animal slaughtering generates significant waste at various stages, including solid waste such as bones, hides, skins, and viscera [12]. Collagen, which accounts for approximately 25% - 30% of animal protein content, can be extracted from these by-products [13]. Collagen fibers offer high elasticity, strong mechanical properties, biocompatibility, biodegradability, a

fibrous structure, and partial insolubility—all vital characteristics for application in flexible food packaging [14] [15]. Polyvinyl alcohol, a synthetic, non-toxic, odorless, biocompatible, thermostable, semi-crystalline polymer, boasts excellent mechanical properties, chemical resistance, biodegradability, flexibility, and good oxygen barrier properties. However, its sensitivity to humidity and poor UV protection are limiting factors [16]-[18]. Its hydroxyl groups facilitate hydrogen bonding with other materials [17], making mixing polyvinyl alcohol with additional materials an effective method to offset some of its limitations.

Oregano and rosemary essential oils (EO) are recognized for their antimicrobial and antioxidant properties. Despite their confirmed biological activities, their direct application is restricted by the strong flavors and odors they may impart to food [19]. Thus, incorporating matrices presents an intriguing approach.

The study aimed to analyze the mechanical properties, antioxidant capabilities, and chemical composition of collagen fiber and polyvinyl alcohol films infused with oregano and rosemary EOs. The films were then applied as active packaging to sliced meat products, assessing their humidity, water activity, pH, color (L^*, a^*, and b^*), lipid oxidation, and antimicrobial activity against *E. coli* during refrigerated storage at 7˚C for 9 days.

2. Materials and Methods

2.1. Materials

The bovine collagen fiber was supplied by Novapron Food Ingredients (São Paulo, Brazil). Polyvinyl alcohol was purchased from Kuraray (São Paulo, Brazil). Essential oils were donated by doTERRA (São Paulo, Brazil). Other reagents, all of analytical quality, included brain heart infusion (BHI) agar (Kasvi, Spain), sodium chloride (Synth, Brazil), acetic acid (Vetec, Brazil), Tween 80 (InduLab, Brazil), glycerol (Dinâmica, Brazil), potassium nitrate (Dinâmica, Brazil), chloroform (Dinâmica, Brazil), peptone water (Kasvi, Spain), 1,1,3,3-Tetraethoxypropane (Sigma Aldrich, USA), thiorbituric acid (Merck, Germany), trichloroacetic acid (Dinâmica, Brazil), sulfanilamide (Vetec, Brazil), butylated hydroxytoluene (Vetec, Brazil), 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazin-1-yl (DPPH) (Sigma Aldrich, USA), and eosin methylene blue agar (Kasvi, Spain).

2.2. Essential Oils Compounds

The compounds in the EOs were characterized according to the method described by Thiel *et al.* [20]. The chemical characterization of the essential oils was performed by gas chromatography coupled to mass spectrometry (GC/MS Shimadzu QP2010 Plus; Shimadzu Corporation, Tokyo, JP). It used a ZB-5 MS capillary column (30 m × 0.25 m; 0.25 mm, flow rate 1 mL/min), and ramp conditions were 40˚C for 1 min and 230˚C at a rate of 4˚C/min for 3 min and the injector at 250˚C. Identification was made by comparing mass spectra from the National Institute of Standards and Technology (NIST) and experimental LRI with those available in the literature.

2.3. Film production

The films were produced using the method proposed by Thiel *et al.* [20]. Film-forming solution: collagen fiber (5.0% w/v), polyvinyl alcohol (5.0% w/v), Tween 80 (1.0%), and glycerol (1.30%). The pH is corrected to 3.0. The solution was homogenized and heated at 90˚C for 20 min. After the heating time, 2%, 4%, and 6% concentrations of the OEO or REO were added. Each film-forming solution was inverted in glass plates (29.7 × 21.0 cm) and placed in an oven with air circulation for roughly 24 h. The films were then stored until the respective analyses in desiccators (RH 60% and 25˚C).

2.4. Chemical Compounds Characterization, *in Vitro* Antioxidant Activity, and Film Mechanics

2.4.1. Essential Oil Compounds in the Films

Each film was cut into smaller pieces and weighed at 0.3 g, and 1 mL of chloroform was added. The vials were then sealed and placed in a water bath at 30˚C for 1 hour. After this period, the extract was removed and transferred to vials. After removing the extract, an additional 1 mL of chloroform was added, and the procedure was repeated. The chemical characterization was conducted in accordance with Section 2.2.

2.4.2. *In Vitro* Antioxidant Activity of the Films

The samples were cut, weighed to 0.5 g, and immersed in 50 mL of 95% ethyl alcohol for 4 days. Aliquots of 2 mL were then mixed with 2 mL of DPPH solution in 95% ethyl alcohol (75 μM). After stirring, the solutions were left in the dark for 30 minutes. The absorbance was subsequently measured at 570 nm using a UV–vis spectrophotometer (BEL-UV-M51, BEL Equipment, Italy) [21]. The antioxidant activity was quantified as the percentage inhibition of the DPPH radical (Equation (1)).

$$I\ (\%) = AC - Asample/AC \times 100 \quad (1)$$

where AC is the absorbance of the DPPH solution, and Asample is the absorbance of the sample solution with DPPH.

2.4.3. Film Mechanical Properties

Tensile strength (TS) (MPa), elongation at break (EB) (%), tenacity (T) (MJ/M^3), and Young's modulus (YM) (MPa) were determined according to the ASTM D882-18 method [22]. The TA.XT plus texture analyzer (Stable Micro Systems, UK) was utilized. The films were cut into dimensions of 20 × 80 mm, with a claw distance of 30 mm and a speed of 8.33 mm/s. The thickness of the films was measured at the time of analysis using a digital micrometer (Digimess, Brazil).

2.5. Application as Active Packaging in Ready-to-Eat Meat Products

2.5.1. Sample Packaging Preparation

The sliced mortadella was purchased in sealed 1 kg packages from a local shop in Santa Maria (RS/Brazil), and the same brand was consistently used throughout

the experiment. However, different batches were utilized for each repetition.

The films, measuring 310 × 200 mm, were folded in half and sealed at both ends; the slices were then placed inside, and the end was heat-sealed at 60˚C for 5 seconds using a Cetro PFS200 Manual Sealer. The width of the sealing area measured 0.6 mm, resulting in the final package dimensions of 155 × 100 mm (Figure 1). The slices, measuring 7.1 mm and 1.8 mm in thickness, were placed in the package, totaling 14 slices per package. The packages were stored at 7˚C for 9 days, and samples were collected on days 0, 3, 6, and 9 to analyze moisture content, water activity, pH, color, and thiobarbituric acid reactive substances (TBARS). Before conducting the analyses, the samples were ground for 10 seconds using a Philips Walita RI2622 mini processor.

Figure 1. Collagen fiber and polyvinyl alcohol packaging with sliced meat product samples.

2.5.2. Physicochemical Analysis of the Packaged Product

The moisture content [23] and water activity of the products were assessed using an Aqualab Series 4 TEV (Decagon Devices, USA) at a temperature of 20˚C. A digital potentiometer (Tecnopon Mpa 210) facilitated the electrometric analysis of pH. The analysis of color employed the CIE L^*, a^*, and b^* color space, with six readings taken using a CM700D colorimetric spectrophotometer (Konica Minolta Sensing, Japan). This analysis was conducted utilizing a D65 illuminant and a 10˚ observation angle, with the specular component excluded and the measurement area set to 8 mm. Lipid oxidation was evaluated through the TBARS assay, following the methodology described by Bruna *et al.* [24] with modifications. For that, 5 g samples were homogenized in 1 mL of 0.15% BHT, 1 mL of 0.5% Sulfanilamide, and 20 mL of 5% Trichloroacetic Acid. Homogenize 3 times for 20 seconds by vortex. The homogenate was centrifuged at 4˚C for 10 minutes at 3000 rpm, and filter the solution. An aliquot of 2 mL of the filtrate is placed in a tube with 2

mL of TBA. Homogenize in vortex for 20 seconds and heated at 40˚C/80 minutes in an ultra-thermostatic bath (Solab, Model SL 152) after cooling (20˚C) by immersion in water. Finally, the absorbance was measured at 532 nm (BEL-UV-M51, BEL Equipment, Italy). The results were expressed in milligrams of malondialdehyde per kilogram (mg MDA/kg). A standard curve using 1,1,3,3-tetraethoxypropane was established for each analysis.

2.5.3. Antimicrobial Activity of the Active Packaging *in Situ*

1) Preparation of the bacteria

The *E. coli* strain (ATCC 8739) was stored at freezing conditions (−18˚C) in a mixture of tryptone soy broth and glycerol, serving as stock cultures until required for the experiment. Approximately 24 hours prior to analysis, the strain was reactivated by inoculation into a tube containing BHI agar, followed by incubation in a microbiological oven at 37˚C. Subsequently, a cell suspension (10^8 CFU/mL) was introduced, achieving a final concentration of approximately 10^6 CFU/mL.

2) Sample preparation in the active packaging

The slices of mortadella were exposed for 20 minutes to UV light from a 30 W Germicidal Lamp (diameter 26.0 mm, length 893.0 mm) inside a laminar flow chamber (TROX TFL, Brazil), with the procedure repeated for both sides of the slices [25]. Following this, an aliquot of 100 µL from the adjusted solution containing 10^6 CFU/mL of the *E. coli* strain was inoculated onto each side of the meat product slices. After 20 minutes, the slices were placed in their respective packaging, which varied by EO (Essential Oil) concentration (0%, 2%, 4%, and 6% OEO (Oregano Essential Oil) or REO (Rosemary Essential Oil), were heat-sealed using a Cetro PFS200 Manual Sealer, and then immediately stored under refrigeration at 7˚C.

3) *E. coli* count

The samples were diluted in peptone water (0.1%), homogenized using a stomacher (BagMixer 400 CC, Interscience, France), and subjected to decimal dilutions ranging from 10^{-1} to 10^{-10}. Subsequently, aliquots were inoculated onto eosin methylene blue agar plates, and the resulting bacterial counts were expressed as log (CFU/g) [25]. This procedure was performed at 0, 3, 6, and 9 days of storage at 7˚C).

2.6. Biodegradability of the Films

The qualitative method proposed by Jaramillo *et al.* [26] was employed. Vegetable compost was poured into a tray measuring 60 cm by 60 cm. The films, each measuring 2 cm by 3 cm, were buried in the soil to a depth of 2 cm. They were maintained at room temperature, and approximately 250 mL of water was sprayed daily to preserve humidity. The samples were carefully removed and photographed on days 0, 3, 6, and 9.

2.7. Statistical Analysis

The experimental design was completely randomized with a factorial arrangement

of 2 × 4 × 4 (2 types of EOs × 4 levels of EOs × 4 storage times), totaling 32 treatments with three replicates each, according to Equation (2):

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl} \quad (2)$$

where Y_{ijkl} is the value observed in the *i*-th type, *j*-th level, *k*-th time, and *l*-th repetition, μ is the general average of the response variable; α_i is the fixed effect of the *i*-th type, β_j is the fixed effect of the *j*-th level, γ_k is the fixed effect of the *k*-th time; $(\alpha\beta)_{ij}$ = is the fixed effect of interaction between type and level; $(\alpha\gamma)_{ik}$ = is the fixed effect of interaction between type and time, $(\beta\gamma)_{jk}$ is the fixed effect of interaction between level and time $(\alpha\beta\gamma)_{ijk}$ = is the fixed effect of interaction between type, level, and time, and ε_{ijkl} = is the random effect associated with observation Y_{ijkl}, assuming $\varepsilon_{ijkl} \overset{iid}{\sim} N(0, \sigma^2)$.

For the mechanical analyses, antioxidant activity, and volatile compounds, the design was completely randomized with a factorial arrangement of 2 × 4 (2 types of EO × 4 levels of EO), resulting in a total of 8 treatments with five replicates for mechanical analyses and three replicates for antioxidant activity and volatile compounds for each treatment.

The data were subjected to a univariate analysis of variance (ANOVA) using the general linear model (GLM); their means were adjusted using the ordinary least squares method with the LSMEANS statement and compared using the Student Newman Keuls test. Additionally, the levels' and times' linear and quadratic trends were evaluated using orthogonal contrast analysis based on the coefficients for interpolating the orthogonal polynomials, estimated using the Interactive Matrix Language (IML) procedure.

Polynomial regression analysis was then conducted to investigate changes in dependent variables as a function of the quantitative independent variables (level and time). The parameters of the regression models were estimated using the GLM (simple) and response surface regression—RSREG (multiple) procedures. The values of the coefficient of determination (R^2) were expressed in relation to the source treatments (regression + lack of fit).

Subsequently, a multivariate analysis of variance (MANOVA) was conducted, wherein the matrices of sums of squares and products were obtained: *T*, *H*, and *E*, where *T* refers to total, *H* to treatments, and *E* to residual. The Wilks (λ), Pillai (*V*), Hotelling-Lawley (*U*), and Roy (F_0) tests were used to test the hypothesis that the treatment mean vectors were null.

In addition, a cluster analysis of the treatments was conducted using the DISTANCE, CLUSTER, and TREE procedures, employing the average Euclidean distance as a measure of dissimilarity and Ward's method as a clustering method. A cluster analysis of the dependent variables was also conducted using the VARCLUS and TREE procedures, using the correlation matrix as input.

Principal Component Analysis (PCA) was performed using the PRINQUAL, PRINCOMP, and FACTOR procedures [27]. Statistical analyses were performed on the SAS analytical platform Viya for Learners v. 4.0 (SAS Institute, USA) [28] at a 5% significance level.

3. Results and Discussion

3.1. Essential Oils Compounds

In the analysis of oregano EO (Essential Oil), 17 compounds were identified (Table 1). The predominant compound found was carvacrol, followed by p-cymene, a finding that corroborates the results reported by Lee *et al.* [29]. Notably, carvacrol, thymol, γ-terpinene, and linalool exhibit antioxidant activities. Furthermore, carvacrol is recognized in the literature for its comprehensive antimicrobial activity [30].

Table 1. Compounds identified in oregano essential oil (OEO) and rosemary essential oil (REO).

Compounds	OEO, %	REO, %
Carvacrol	75.27	-
ρ-Cymene	9.89	1.05
β-Caryophyllene	4.48	5.44
γ-Terpinene	4.43	1.29
Linalool	1.62	1.24
Myrcene	1.27	-
Thymol	1.01	-
α-Pinene	0.81	12.36
Terpinen-4-ol	0.31	0.26
β-Pinene	0.20	8.17
1,8-Cineole	0.18	48.30
Borneol	0.15	1.08
α-Terpineol	0.12	0.82
Caryophyllene oxide	0.12	0.05
α-Caryophyllene	0.07	-
α-Copaene	0.06	0.06
D-Limonene	0.01	-
Camphor	-	16.37
Camphene	-	1.85
β-Myrcene	-	0.59
Bornyl acetate	-	1.05
α-Terpinene	-	0.20
α-Humulene	-	0.14
α-Phellandrene	-	0.13
σ-Cadinene	-	0.09
Terpinolene	-	0.09
α-Thujene	-	0.06
α-Tricyclene	-	0.06

Continued

β-Bisabolene	-	0.03
γ-Cadinene	-	0.03
trans-Pinocarveol	-	0.02

For rosemary EO, 26 compounds were identified. This composition aligns with the findings of Giarratana *et al.* [31]. The literature presents varying accounts of the biological activities of rosemary EO, reporting intermediate antioxidant and antimicrobial activity [32]. The biological effects are primarily attributed to the compounds 1,8-cineole and camphor. However, a study by Ribeiro-Santos *et al.* [33] did not report any activity against *E. coli* and *Staphylococcus aureus*, with the primary compounds identified in the EO being α -pinene and 1,8-cineole.

3.2. Chemical Compounds Characterization, *in Vitro* Antioxidant Activity, and Film Mechanics

There was a significant effect ($p < 0.05$) of the level of EO (Essential Oil) on TS (Tensile strength), EB (Elongation at break), and YM (Young's modulus). Additionally, the type of EO significantly affected YM ($p < 0.05$). In contrast, no significant difference was observed for T (Tenacity) ($p > 0.05$). The increase in EOs led to a reduction ($p < 0.05$) in TS and YM and an increase ($p < 0.05$) in EB (**Figure 2**). Moreover, rosemary EO exhibited a significantly higher YM ($p < 0.05$) compared to oregano.

Zhang *et al.* [34] reported a reduction in TS and an increase in EB with the addition of thyme EO to polyvinyl alcohol and curdlan films. Incorporating EO promotes a reduction in polymer-polymer interactions, thereby facilitating the movement of the polymer chains and consequently increasing the film's flexibility [34]. Conversely, Shen *et al.* [35] observed decreased EB values upon adding oregano EO to starch-based films, highlighting that the film matrix and its interaction with the EO significantly influence EB enhancement. Typical food packaging materials generally possess a TS of 5 - 15 MPa and an EB of approximately 200%. The films examined in this study exhibited higher values, thereby satisfying the requirements for their application in packaging [34].

The reduction in YM renders the films more pliable, as suggested by Sánchez-Soto *et al.* [36]. A significant difference ($p < 0.05$) was noted in the average flexibility between the films incorporating oregano and rosemary EOs. Increasing the concentration of EOs in the films, composed of collagen fiber and polyvinyl alcohol, enhanced their flexibility; notably, films with oregano EO were more flexible than those with rosemary EO.

The interplay between the type and concentration markedly affected the DPPH free radical scavenging activity ($p < 0.05$). Films containing oregano exhibited the highest antioxidant activity, reaching a peak at approximately 4.2% EO (**Figure 3**). The addition of 2% - 6% oregano resulted in significantly greater ($p < 0.05$) antioxidant activity. Shen *et al.* [35] reported similar inhibition rates in films enhanced with oregano EO.

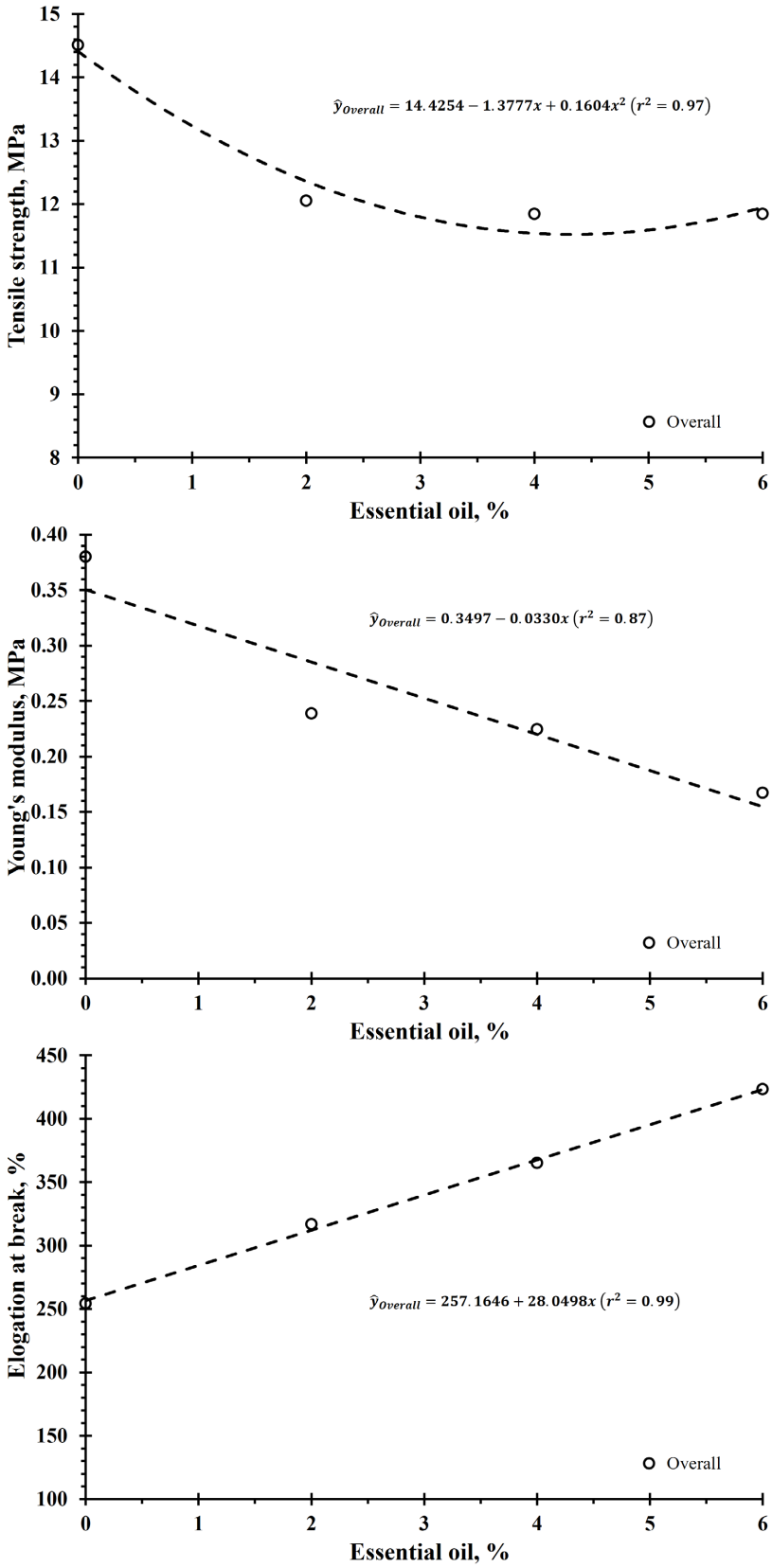

Figure 2. Mechanical properties of collagen fiber and polyvinyl alcohol films with different types and levels of essential oil.

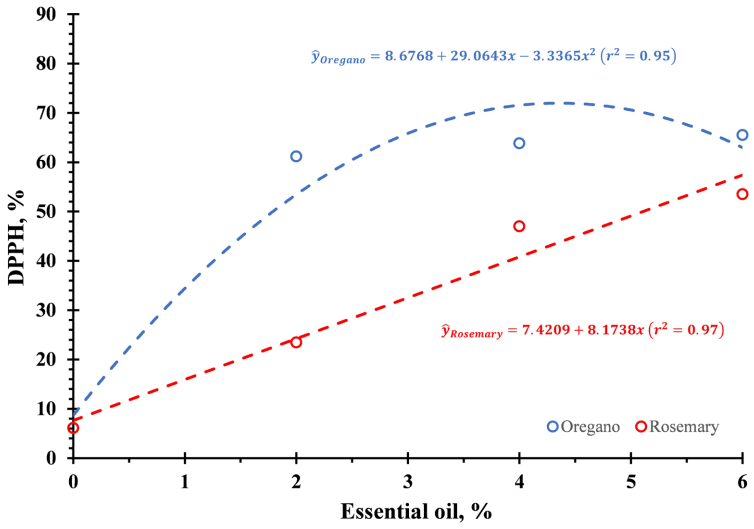

Figure 3. DPPH inhibitory activity in collagen fiber and polyvinyl alcohol films with different types and levels of essential oil.

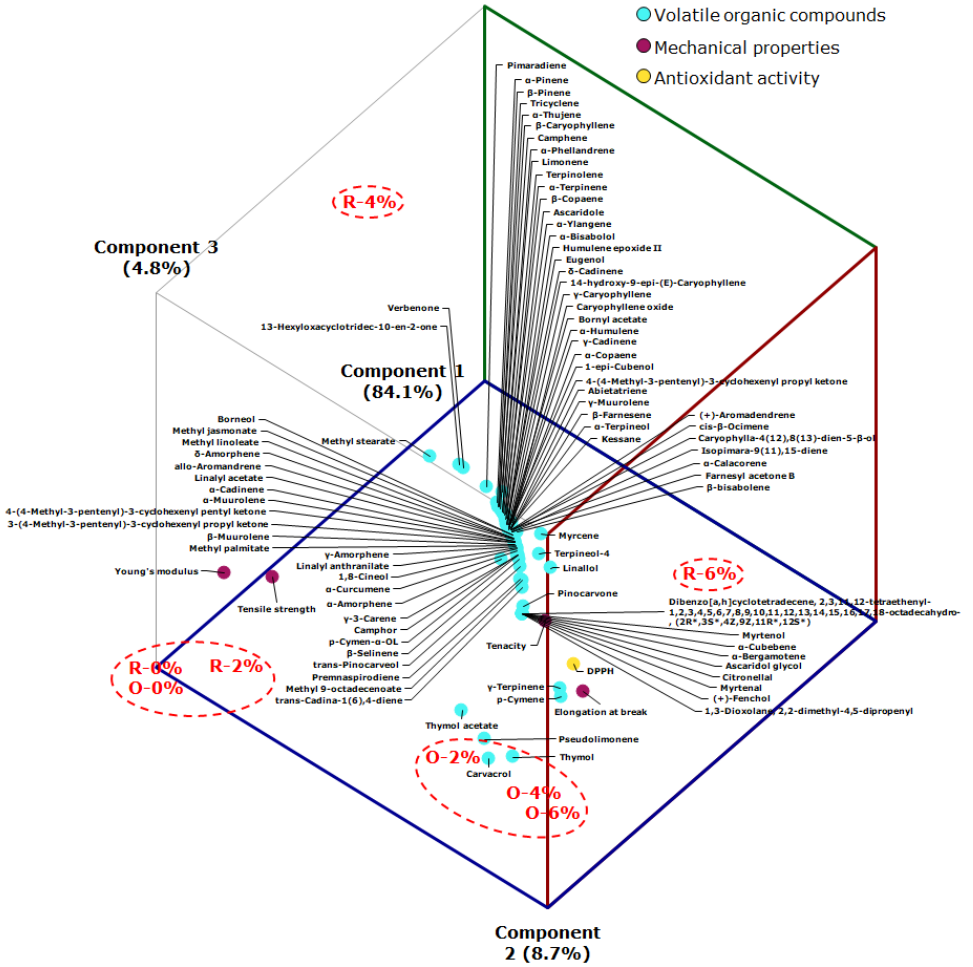

Figure 4. Tridimensional biplot from films with types (O = Oregano, R = Rosemary) and levels (0%, 2%, 4%, and 6%) of essential oil (scores) versus volatile organic compounds, mechanical properties, and antioxidant activity (loadings) in relation to the principal components.

The multivariate analysis of the films (Figure 4) revealed four distinct groups: I. control films without the inclusion of EOs and with 2% rosemary EO, characterized by the highest TS, YM, and the lowest antioxidant activity, EB, as well as practically no volatile organic compounds; II. films with 2%, 4%, and 6% oregano EO, characterized by the highest antioxidant activity (determined by DPPH), EB, and the lowest YM, as well as an association with the compounds carvacrol, thymol, thymol acetate, pseudolimonene, γ-terpinene, and ρ-cymene; III. film with 4% rosemary EO, characterized by an abundance of 1,8-cineole, camphor, and a scarcity of carvacrol and thymol, as well as intermediate antioxidant activity; IV. film with 6% rosemary EO, differing essentially from group III by the magnitude of the volatile organic compounds present.

The analysis of the film matrix is critical to ensure the incorporation of oils essentials, given that their production involves the use of temperatures above room temperature and that they are sensitive to heat, thus increasing their volatility with elevated temperatures. The composition of the films revealed that all compounds present in oregano EO were retained in their respective films to the extent that they formed a single, exclusive group (Figure 4) due to their similar chemical composition and mechanical properties. However, the films containing rosemary EO were found to contain additional compounds, which may be attributed to the film matrix, processing temperatures, and the thermal stability of rosemary EO.

3.3. Application of Films as Active Packaging in Sliced Meat Products

The type of EO (E) significantly affected ($p < 0.05$) the TBARS values of sliced mortadella. In contrast, the concentration of EO (L) significantly influenced ($p < 0.05$) the humidity, water activity (Aw), pH, and *E. coli* counts. Additionally, the storage duration (T) had a significant impact ($p < 0.05$) on the moisture content, Aw, pH, TBARS, and color attributes (L^*, a^*, b^*) of the sliced mortadella. Notably, there was a significant interaction ($p < 0.05$) between the type and concentration of (E × L) on TBARS values and between the concentration of and storage time (L × T) on Aw, TBARS, and *E. coli* counts.

The highest moisture content observed was 52.1%, corresponding to the addition of 3.1% EO, and the pH showed a significant decrease ($p < 0.05$) with an increase in the EO concentration (Figure 5). Over the storage period, there was a significant reduction ($p < 0.05$) in humidity, pH, brightness (L^*), and paleness (b^*), along with an increase in redness (a^*) (Figure 6).

The color of meat products is an important attribute and closely relates to the acceptability of the product [37]. According to Viuda-Martos *et al.* [38], the L^* and a^* parameters are associated with the water content present in the meat product, its surface availability, and a tendency for humidity to decrease during storage, resulting in a decrease in L^* and an increase in a^*. The behavior of b^* may depend on pH, degree of oxidation, and water activity [38]. The principal components analysis revealed a correlation between humidity, water activity, L^*, and b^*.

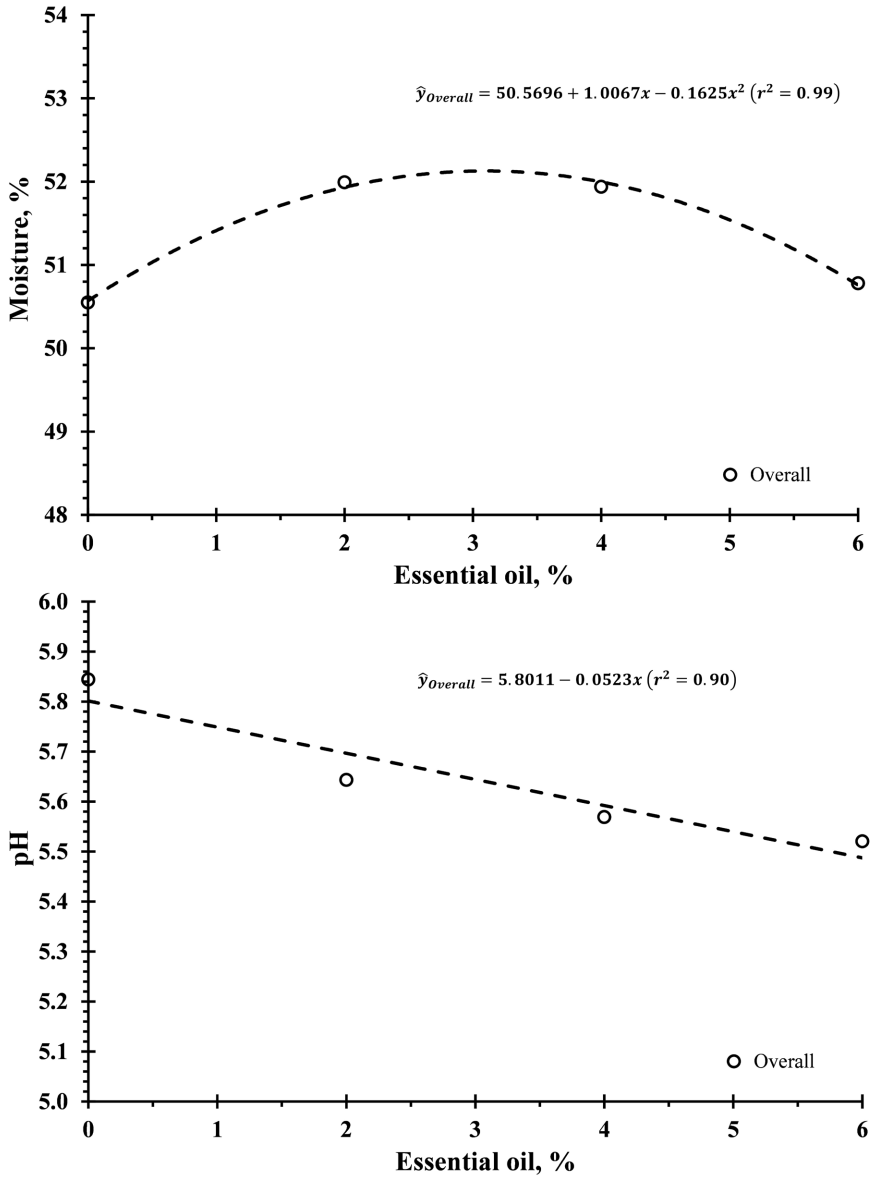

Figure 5. Moisture and pH in the application of active packaging of collagen fiber and polyvinyl alcohol with the addition of oregano and rosemary essential oils in sliced meat product under refrigeration (7˚C).

Changes in the pH of meat products can occur due to the metabolic activity of microorganisms and chemical reactions, such as protein breakdown, which increases the pH [39]. Meat products packed in active packaging composed of collagen fiber and polyvinyl alcohol prevented the pH from rising, which is attributable to the biological activity of the EOs and inhibited bacterial growth and the progression of chemical reactions. Gedikoglu [40] and Quesada *et al.* [19] observed lower pH values in meat products with coatings and chitosan films infused with EOs and extracts. During storage, a decrease in pH was observed until approximately the sixth day, after which there was a stabilization followed by a gradual increase in pH around the eighth day. Qiu *et al.* [3] reported a pH increase in

chicken burgers coated with gelatin-chitosan and infused with clove EO and rosemary extract from the fourth day of storage, with the increase becoming significant by the eighth day.

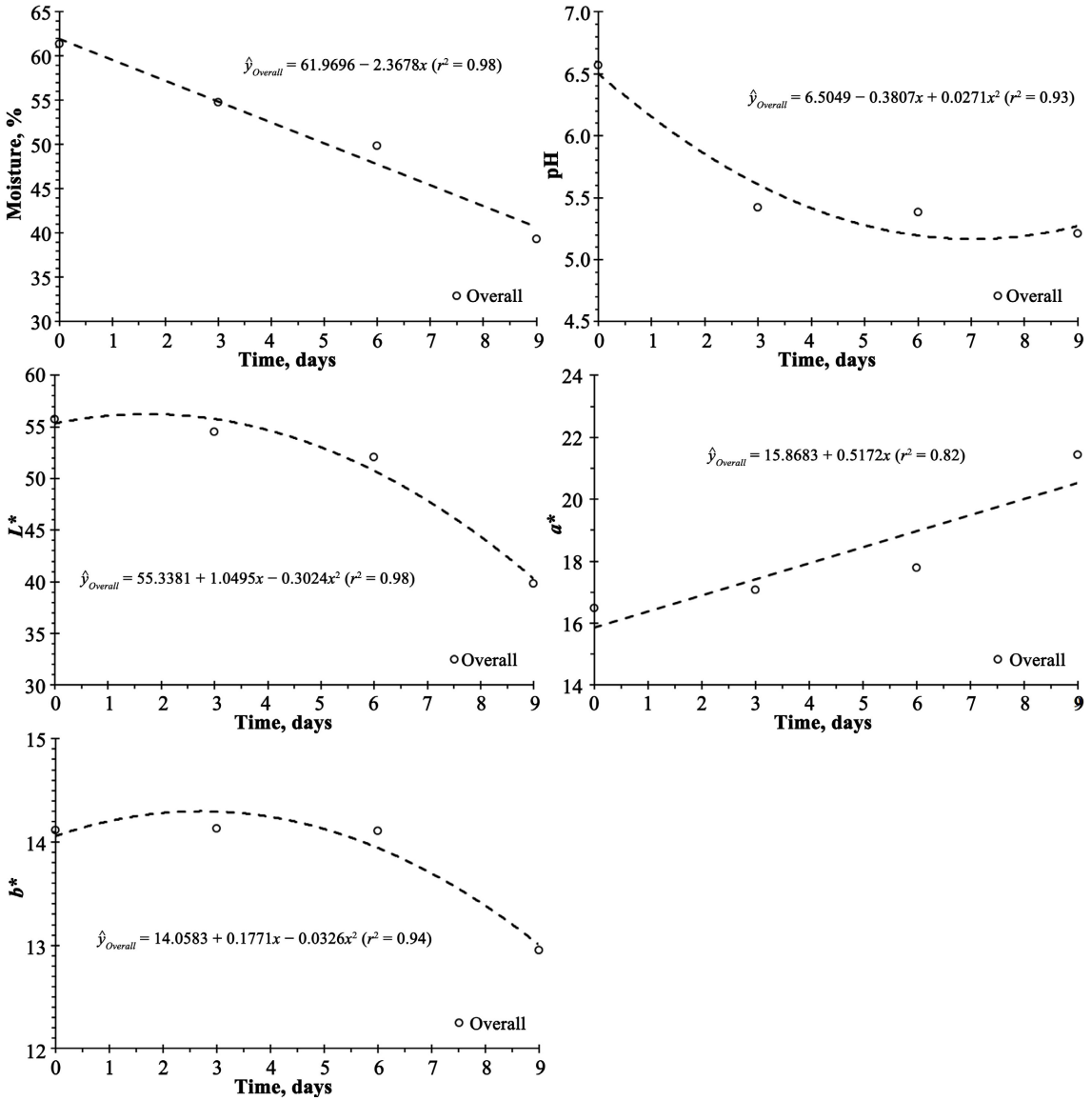

Figure 6. Moisture, pH, and instrumental color (L^*, a^*, and b^*) during storage time in the application of active packaging of collagen fiber and polyvinyl alcohol with the addition of oregano and rosemary essential oils in sliced meat product under refrigeration (7˚C).

The decline in Aw at 9 days of storage was significantly lower ($p < 0.05$) for films with 2% - 4% levels of EO. Packaging protection is pivotal in mitigating the exchange between the food storage environment and relative humidity, which directly influences Aw. Environments with low relative humidity are known to reduce the Aw of food. The refrigerator's internal environment is notably dry, leading to a tendency for refrigerated products to lose water to the environment [41] [42]. A significant effect ($p < 0.05$) was observed between the level of EO and time

on the Aw of the product in the packaging. At 9 days of storage, the packaging without the addition of EO (control) and with 6% EO obtained a lower Aw value, likely due to the greater loss of water in these products (Figure 7).

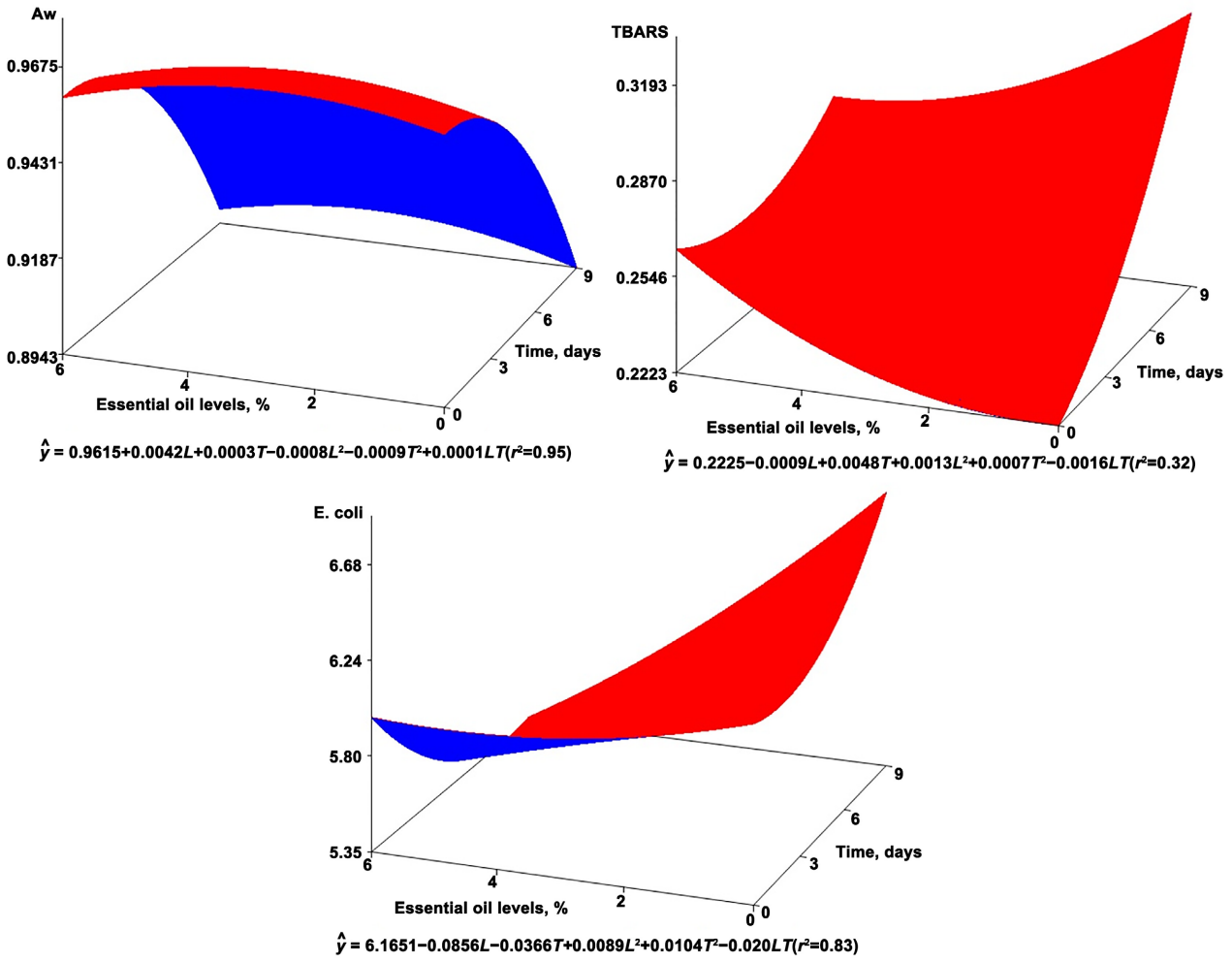

Figure 7. Response surface for water activity (Aw), TBARS, and *Escherichia coli* (log/CFU).

The response surface model for Aw as a function of the level and time variables is shown in Equation (3).

$$\hat{y} = 0.9615 + 0.0042L + 0.0003T - 0.0008L^2 - 0.0009T^2 + 0.0001LT \left(r^2 = 0.95\right) (3)$$

The incorporation of EOs into the collagen fiber and polyvinyl alcohol matrix contributes to the reduction of packaging permeability through the presence of various highly hydrophobic compounds, which aid in decreasing water passage. Nonetheless, excessive incorporation of EOs into the matrix (6%) may adversely affect permeability, attributed to the OE's agglomeration and heightened structure sensitivity [43].

Lipid oxidation plays a crucial role in determining the quality of meat products, being responsible for the loss of nutritional quality. The secondary products formed during storage alter sensory characteristics [44]. In this study, the observed values

were lower than those reported in the literature, which is known to impact sensory quality (2 - 2.5 MDA/kg) [45]. The inclusion of 6% rosemary EO in the films was associated with a greater ($p < 0.05$) susceptibility to lipid oxidation (TBARS) (**Figure 8**). In contrast, the addition of 2% - 6% oregano EO was found to reduce ($p < 0.05$) susceptibility to lipid oxidation.

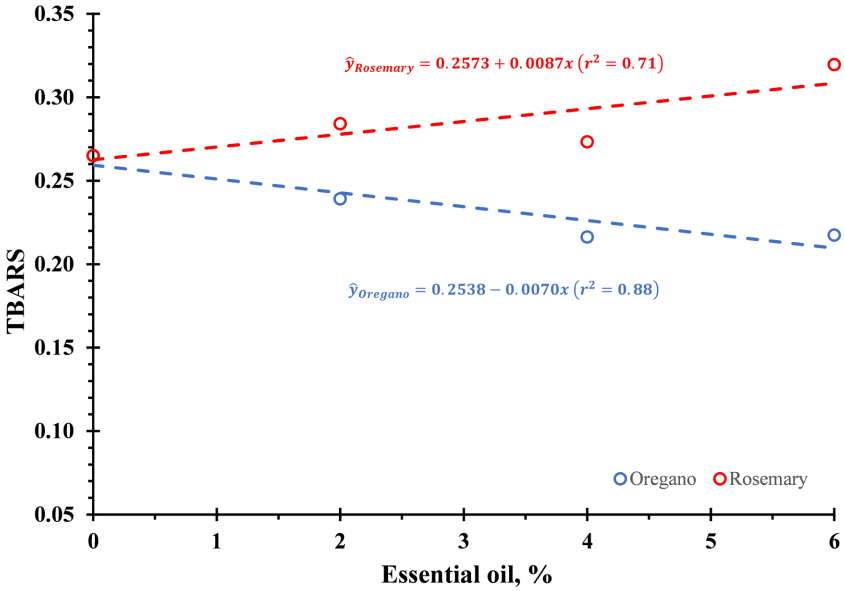

Figure 8. TBARS in the application of active packaging of collagen fiber and polyvinyl alcohol with the addition of different levels of oregano and rosemary essential oils in sliced meat products stored under refrigeration (7˚C).

The results of this study, along with the *in situ* application, corroborate the findings regarding the inactivation of the DPPH radical *in vitro*. As anticipated, excluding EO from the films and prolonged storage time resulted in a higher susceptibility to lipid oxidation, as indicated by TBARS measurements. In 0 - 3 days, adding EO at concentrations above 2% acted as a pro-oxidant. However, its inclusion proved effective in delaying lipid oxidation from day 3 onward (**Figure 7**). The response surface model for TBARS as a function of the level and storage time is presented in Equation (4).

$$\hat{y} = 0.2225 - 0.0009L + 0.0048T + 0.0013L^2 + 0.0007T^2 - 0.0016LT \left(r^2 = 0.32\right) \quad (4)$$

Similar outcomes were observed by Gedikoglu [40], with the application of pectin coatings enriched with EOs and extracts from *T. vulgaris* and *T. spicata* on slices of mortadella (0.235 - 0.392 μmol MDA/g) over 21 days. Moreover, Zheng *et al.* [46] reported values of 0.37 - 0.26 mg MDA/kg during a 6-day storage period of chicken breasts in chitosan and oregano EO films.

Oregano EO is renowned in the literature for its antimicrobial and antioxidant activities, attributed to phenolic compounds such as carvacrol, γ-terpinene, and thymol [29]. In the PCA (**Figure 4**), these compounds and ρ-cymene demonstrated a significant association with the DPPH free radical inactivation analysis.

The overall composition of the EO is crucial. As noted by Amorati *et al.* [47], synergistic or antagonistic behaviors can be anticipated depending on the specific makeup of the EO and experimental conditions. Non-phenolic terpenoids, particularly unsaturated ones, may self-oxidize similarly to lipids, a notable example being α-pinene. When these compounds are integrated with lipids from the food matrix, both are subject to auto-oxidation. There are exceptions, such as γ-terpinene, which can alter the propagation and termination phases of the chain reaction, thereby accelerating the termination process, shortening the chain, and reducing the overall oxidation rate [47] [48]. Research conducted by Guo *et al.* [48] has confirmed that in the presence of mono- or polyphenolic compounds, γ-terpinene exhibits a synergistic effect, thus enhancing antioxidant activity. In context with monophenolic compounds like carvacrol and thymol, the regeneration mechanism of the intermediate phenoxyl radical by γ-terpinene is suggested, which extends the inhibition period [48].

The increased oxidation observed at the 6% rosemary EO concentration may be ascribed to certain compounds in the EO that possibly promote co-oxidation within the product.

Discrepancies exist in the literature concerning the antimicrobial activity in food matrices with high-fat content. Some studies suggest that such activity against spoilage and pathogenic bacteria is diminished in higher-fat environments [49]. However, this study found the antimicrobial effect against *E. coli* to remain unaffected by the fat content in meat products, which may contain up to 30% - 35% fat in the formulation [50]. Elevating the concentration of EOs in the films lowered the microbial contamination (*E. coli*) throughout the storage period (**Figure 7**). With prolonged storage, the control packaging exhibited greater bacterial growth. The response surface model for the *E. coli* count as a function of EO concentration and time is performed using Equation (5).

$$\hat{y} = 6.1651 - 0.0856L - 0.0366T + 0.0089L^2 + 0.0104T^2 - 0.0207LT \left(r^2 = 0.83\right) \quad (5)$$

Ready-to-eat meat products, available in the refrigerated sections of supermarkets, can be sliced at the branding company or directly at bakeries or supermarkets before sale. Here, the cleanliness of the slicer, handler, and premises plays a critical role in preventing contamination [51]. Strains of *E. coli* O157:H7, known for their biofilm-forming capabilities, exhibit increased resistance to sanitizers when forming a biofilm network [52]. Sheen and Hwang [51] demonstrated that experimental contamination of slicing equipment blades with 8 log CFU of *E. coli* resulted in significant contamination transfers during ham slicing, ranging between 7 and 6 log CFU. Active packaging can serve as a protective barrier for these products by inhibiting pathogenic bacterial growth.

The PCA summarizes all analyses, accounting for 85.7% of the total variance, with 68.3% captured by the PC1 and 17.4% by PC2. Moisture content, Aw, L^*, b^*, and pH were positively correlated, with average EO content (2% - 4%) and shorter storage times. Conversely, TBARS, *E. coli* counts, and a^* were negatively correlated

with each other but positively related to longer storage times (6 days).

3.4. Film Biodegradability

A qualitative test of the films' biodegradability was carried out, and images were taken in 0, 3, 6, and 9 days (Figure 9).

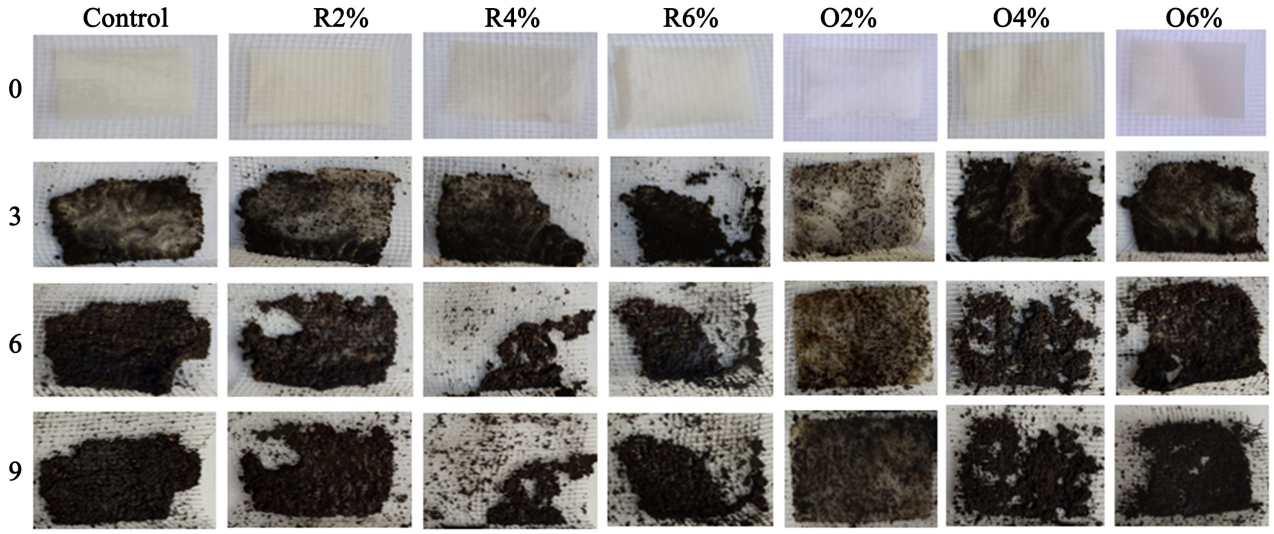

Figure 9. Qualitative analysis of biodegradability of collagen fiber and polyvinyl alcohol films with the addition of different levels of oregano (O2%, O4%, and O6%) and rosemary (R2%, R4%, and R6%) essential oils.

Assessing the visual aspect, on the third day, there were already changes in the appearance of the films, which were more wrinkled and fragile due to swelling. Generally, the films containing oregano or rosemary EO were more sensitive to the biodegradation process, which was more pronounced with higher EOs. Similar results were found by Wang *et al.* [43], starch films with higher additions of *Zanthoxylum armatum* EO showed a higher rate of biodegradation. The films with no or lower levels of EO presented a smoother surface, limiting water attack due to the increase in how it enters the polymer matrix. The greater incorporation of EOs leads to uneven dispersion due to weaker areas that promote water absorption, swelling, and microbial decomposition [43].

4. Conclusions

The collagen fiber and polyvinyl alcohol matrix is promising for packaging sliced meat products. The addition of essential oil improves the antimicrobial efficiency of the packaging. The addition of oregano essential oil reduces the lipid oxidation of the packaged product compared to rosemary essential oil. The addition of 2% - 4% oregano essential oil is recommended for packaging applications.

This study proves that it is possible to use biodegradable packaging for meat products. Beyond consumer awareness, the development of public policy that favors the production and consumption of these materials is important.

A suggestion for future studies is to improve film production on a large scale

and packaging migration analysis.

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Conflicts of Interest

The authors report no conflicts of interest.

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Abbreviations

BHI: Brain heart infusion
EB: Elongation at break
EO: Essential oil
PCA: Principal component analysis
T: Tenacity
TS: Tensile strength
YM: Young's modulus

Supplementary Materials

Table S1. Results of univariate analysis of variance (ANOVA) according to the essential oil type (E) and level (L).

Variables	Sources of variation (p-value)			Means	Contrast (L)	Tendency (L)	
	E	L	E × L		WO × W[1]	Linear	Quadratic
Tensile strength	0.0988	0.0074	0.4032	12.6	0.0007	0.0042	0.0490
Elongation at break	0.1122	0.0017	0.6369	340.6	0.0016	0.0001	0.9389
Tenacity	0.9500	0.1019	0.5979	21.9	0.5920	0.0753	0.0908
Young's modulus	0.0274	0.0001	0.1672	0.25	0.0001	0.0001	0.0753
DPPH	0.0001	0.0001	0.0001	40.6	0.0001	0.0001	0.0001

[1]WO × W = without (0%) × with (2%, 4% and 6%) essential oil.

Table S2. Results of Young's modulus and DPPH of films with types and levels of essential oil

Essential oil type	Essential oil level				Mean	SEM[1]	CV[2] (%)
	0	2	4	6			
	Young's modulus, MPa					0.04	28.2
Oregano	0.38	0.23	0.15	0.14	0.23^{B}		
Rosemary	0.38	0.25	0.29	0.20	0.28^{A}		
Mean	0.38^{a}	0.24^{b}	0.22^{b}	0.17^{b}			
	DPPH, %					2.35	8.2
Oregano	6.1^{b}	61.2^{Aa}	63.8^{Aa}	65.5^{Aa}	49.2		
Rosemary	6.1^{c}	23.4^{Bb}	47.0^{Ba}	53.5^{Ba}	32.5		
Mean	6.1	42.3	55.4	59.5			

Means followed by different capital letters within the same column and different small letters within the same row differ ($p < 0.05$), respectively, between types and levels of essential oils by the *Student Newman Keuls* test. [1]SEM = Standard Error of the Mean; [2]CV = Coefficient of Variation.

Table S3. Results of univariate analysis of variance (ANOVA) according to the essential oil (E), level (L) and time (T).

Variables	Sources of variation (*p*-value)						
	E	L	T	E × L	E × T	L × T	E × L × T
Moisture	0.2902	0.0008	0.0001	0.9145	0.9681	0.0904	0.8486
Aw	0.2300	0.0003	0.0001	0.2238	0.7048	0.0007	0.8137
pH	0.1833	0.0002	0.0001	0.7947	0.5114	0.4757	0.9886
TBARS	0.0001	0.1397	0.0001	0.0001	0.5736	0.0300	0.9994
Escherichia coli	0.7494	0.0001	0.0924	0.6923	0.7749	0.0379	0.9140
L^*	0.4233	0.0605	0.0001	0.6724	0.9312	0.2585	0.9787
a^*	0.4178	0.6263	0.0001	0.8831	0.6918	0.8805	0.9364
b^*	0.6576	0.1036	0.0001	0.9549	0.8895	0.3511	0.9999

Table S4. Results of sliced meat products packaged into films with levels of essential oil at different times.

Time	Essential oil level				Mean	SEM[1]	CV[2] (%)
	0	2	4	6			
	Aw					0.003	0.72
0	0.96^{A}	0.97^{A}	0.97^{A}	0.97^{A}	0.97		
3	0.95^{B}	0.95^{B}	0.95^{B}	0.95^{B}	0.95		
6	0.94^{C}	0.95^{B}	0.94^{C}	0.94^{C}	0.94		
9	0.88^{Dc}	0.91^{Ca}	0.90^{Da}	0.89^{Db}	0.90		
Mean	0.94	0.94	0.94	0.94			
	TBARS					0.01	13.3
0	0.22^{B}	0.23^{B}	0.24	0.26	0.24		
3	0.24^{B}	0.25^{AB}	0.24	0.26	0.25		
6	0.25^{B}	0.27^{AB}	0.25	0.27	0.26		
9	0.35^{Aa}	0.29^{Ab}	0.26^{b}	0.28^{b}	0.30		
Mean	0.27	0.26	0.24	0.27			
	Escherichia coli					0.15	6.24
0	6.02^{B}	6.02	6.02	6.02^{A}	6.02		
3	6.35^{ABa}	5.94^{ab}	5.72^{b}	5.49^{Bb}	5.87		
6	6.28^{ABa}	5.87^{ab}	5.65^{bc}	5.30^{Bc}	5.78		
9	6.75^{Aa}	5.93^{b}	5.87^{b}	5.49^{Bb}	6.01		
Mean	6.35	5.94	5.81	5.57			

Means followed by different capital letters within the same column and different small letters within the same row differ ($p < 0.05$), respectively, between types and levels of essential oils by the *Student Newman Keuls* test. [1]SEM = Standard Error of the Mean; [2]CV = Coefficient of Variation.

Table S5. Results of lipid oxidation susceptibility (TBARS = thiobarbituric acid-reactive substances) of sliced meat product packaged into films with types and levels of essential oil.

Essential oil type	Essential oil level				Mean
	0	2	4	6	
Oregano	0.27^{a}	0.24^{Bb}	0.22^{Bb}	0.22^{Bb}	0.23
Rosemary	0.27^{b}	0.28^{Ab}	0.27^{Ab}	0.32^{Aa}	0.29
Mean	0.27	0.26	0.24	0.27	

Means followed by different capital letters within the same column and different small letters within the same row differ ($p < 0.05$), respectively, between types and levels of essential oils by the *Student Newman Keuls* test. Coefficient of Variation (CV, %) = 13.3; Standard Error of the Mean (SEM) = 0.01.

Table S6. Results of multivariate analysis of variance (MANOVA) for sliced meat product packaged into films with types (E) and levels (L) of essential oil at different times (T) using likelihood ratio test (Wilks), Pillai, Hotelling-Lawley and Roy's tests.

Sources of variation	Tests			
	Wilks (λ)	Pillai (V)	Hotelling-Lawley (U)	Roy (F_0)
	p-value			
E	0.0001	0.0001	0.0001	0.0001
L	0.0001	0.0001	0.0001	0.0001
T	0.0001	0.0001	0.0001	0.0001
E × L	0.0447	0.0519	0.0437	0.0028
E × T	0.8127	0.8112	0.8049	0.1460
L × T	0.0001	0.0037	0.0001	0.0001
E × L × T	0.9612	0.9851	0.8756	0.0001

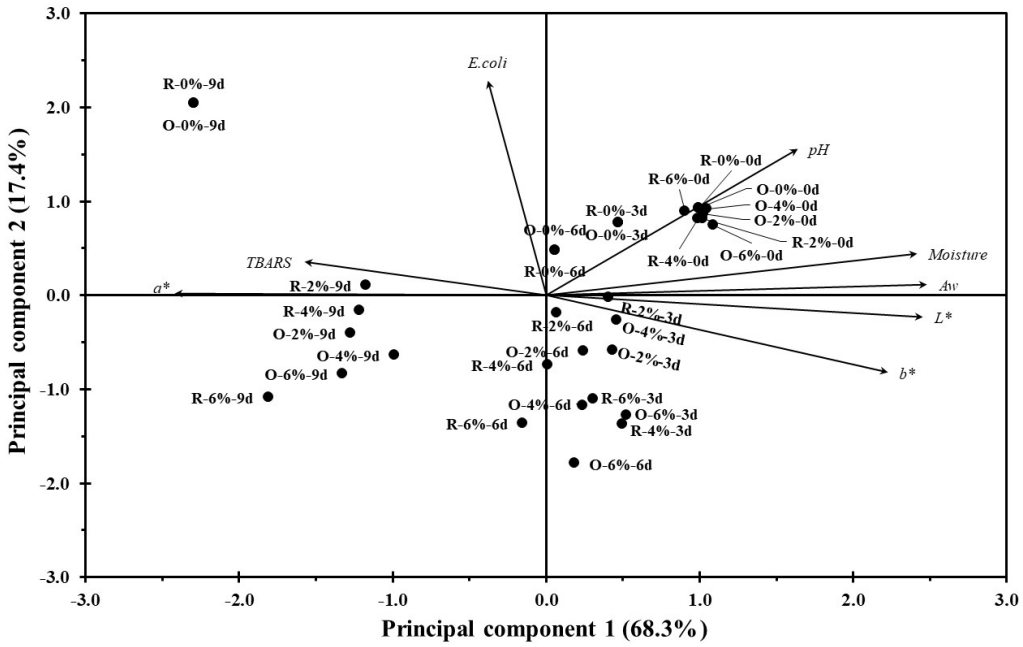

Figure S1. Bidimensional biplot from sliced meat product packaged into films with types (O = Oregano, R = Rosemary) and levels (0, 2, 4, and 6%) of essential oil at different times (0, 3, 6, and 9 days) versus physical-chemical traits (loadings) in relation to the principal components.

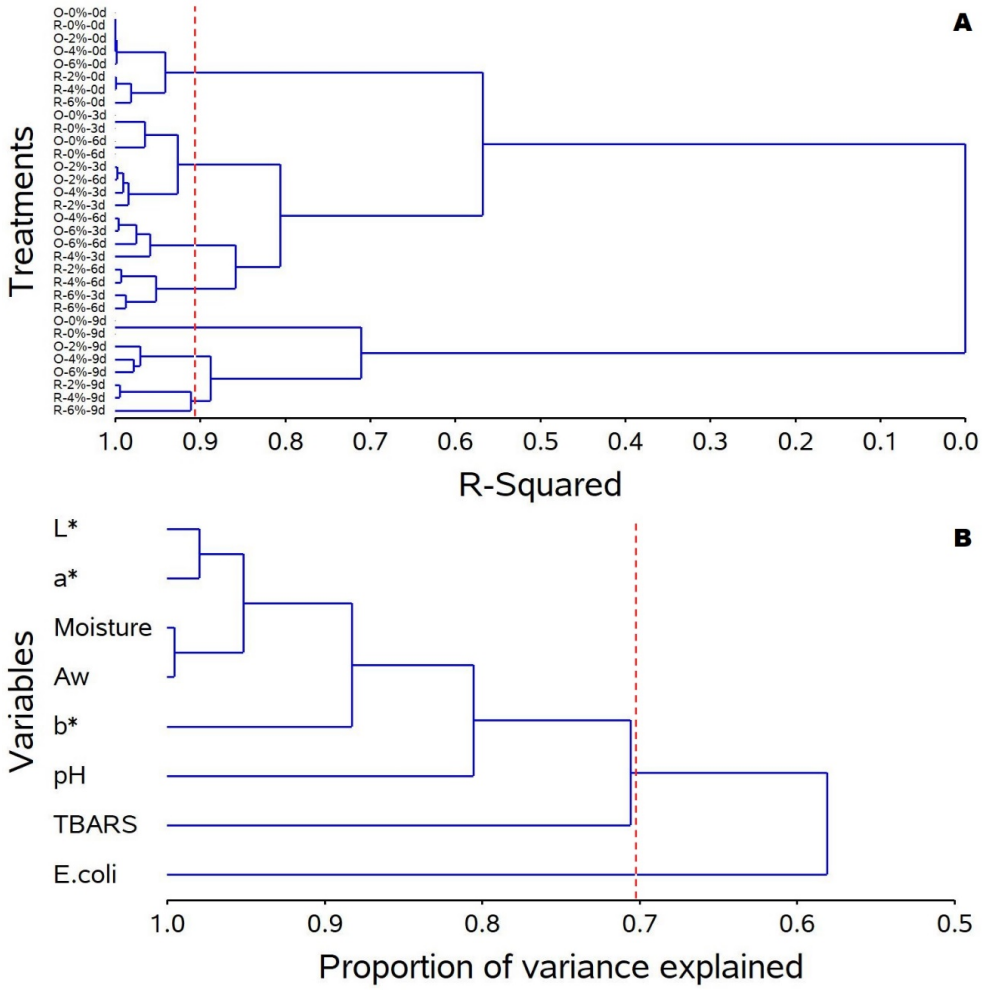

Figure S2. Dendrogram from sliced meat product packaged into films with types (O = Oregano, R = Rosemary) and levels (0, 2, 4, and 6%) of essential oil at different times (0, 3, 6, and 9 days) in relation to the coefficient of determination (r^2; abcissa axis) using Euclidean distance as dissimilarity measure and Ward's agglomerative hierarchical algorithm as clustering method (**A**); and dendrogram of physical-chemical traits (ordinate axis) in relation to the coefficient of determination (r^2; abcissa axis) using the correlation matrix as similarity measure and principal component as clustering method (**B**).