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Sustainable Edible Coatings Enriched with Bioactive Extracts from Exhausted Olive Pomace, *Fucus Spiralis*, and *Limnospira* sp. for the Postharvest Preservation of Strawberries

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Abstract

Exhausted olive oil pomace (EOP), *Fucus spiralis*, and *Limnospira* sp. extracts—rich in bioactives, polysaccharides, or proteins—were incorporated into alginate-based edible coatings and applied to strawberries to evaluate their effects on postharvest quality parameters, including decay, weight loss, color, antioxidant activity, and microbial growth. Among the tested formulations, the EOP-based coating (0.25% bioactive rich-extract) was the most effective, reducing weight loss to approximately 18% after 10 days at 10 °C, compared with higher losses in the control and other coatings, while also better preserving color through higher hue and chroma retention. Antioxidant activity, measured by ABTS and DPPH assays, was consistently higher in EOP-coated strawberries, despite a general decline in total phenolic content across treatments. Specifically, ABTS values decreased from 21.43 ± 0.90 (day 0) to 12.88 ± 0.39 (day 10) mmol TE/100 mg DW, while DPPH values declined from 10.23 ± 1.39 (day 0) to 5.96 ± 1.03 (day 10) mmol TE/100 mg DW. Microbial analyses further showed that the EOP coating strongly inhibited spoilage fungi, yeasts, and bacteria, whereas coatings containing *Fucus spiralis* or *Limnospira* sp. extracts (0.25% bioactive rich-extract plus 0.5% polysaccharide- or protein-rich extract) offered only moderate protection and, in some cases, promoted microbial growth. Overall, the EOP coating demonstrated superior performance in maintaining freshness, delaying microbial spoilage, and extending the shelf life of strawberries, highlighting its potential as a sustainable and functional strategy for fruit preservation.

Keywords: edible coatings; exhausted olive oil pomace; *Fucus spiralis*; *Limnospira*; antioxidant activity; antimicrobial activity; total phenolic content; decay; water loss; color

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1. Introduction

Fruit and vegetable spoilage remains a major challenge across the food supply chain, with global losses estimated between 45% and 50% [1]. According to the United Nations Environment Program (UNEP), approximately 913 million tons of food were lost in 2019 alone [2]. In Europe, fruits and vegetables account for 50% of domestic food waste [3], representing around 22 million tons of fresh produce. In certain EU countries, up to 30% of fruits and vegetables are lost before reaching consumers, primarily due to moisture loss, discoloration, and microbial spoilage [4,5].

Edible coatings—thin layers composed of polysaccharides, proteins, or lipids, often enhanced with bioactive compounds—act as barriers against oxygen, carbon dioxide, water

vapor, and microbial invasion. Their main benefits include reducing weight and water loss, slowing respiration and enzymatic activity, and preserving color and texture. When enriched with bioactives, such coatings additionally provide antioxidant and antimicrobial functions, thereby extending shelf life. Other advantages include enhancing visual quality (e.g., brightness), preserving flavor, and reducing reliance on non-degradable plastic packaging, thereby contributing to environmental sustainability [6].

Strawberries are highly perishable fruits, prone to rapid weight loss and fungal decay. Numerous edible coatings have been studied to mitigate these challenges. Chitosan-based coatings, for example, are widely recognized for their effectiveness in reducing fungal spoilage and maintaining firmness and color of strawberries. Incorporating glycerol into chitosan formulations enhanced antimicrobial activity, extended shelf life, and preserved sensory qualities [7]. Nanostructured chitosan coatings have further proved effective in extending strawberry storage up to 15 days at 4 °C while delaying both discoloration and mold growth [8]. Alginate-based coatings, usually cross-linked with calcium, also contribute to shelf-life extension by reducing respiration and transpiration rates. For instance, sodium alginate–calcium chloride formulations extended the mold-free storage of cut strawberries to 15 days at 4 °C [9]. Other polysaccharides, including carboxymethyl cellulose (CMC), pectin, and tragacanth gum have shown similar promise. In one comparative study, both CMC and tragacanth coatings reduced weight loss and decay during 16 days of storage at 4 °C, with CMC providing the highest sensory acceptance [10]. Enrichment of coatings with essential oils or bioactive compounds offers additional benefits; for example, alginate and pectin coatings containing citral or eugenol improved firmness, preserved color, maintained antioxidant activity, and reduced spoilage during 14 days of storage [11]. Collectively, these findings highlight the potential of polysaccharide-based coatings in improving the postharvest quality of strawberries.

By-products such as olive pomace and renewable sources like algae (macroalgae and microalgae) are rich in nutrients and bioactive compounds beneficial to human health. Notably, algae cultivation does not compete with arable land. In this study, exhausted olive oil pomace (EOP), the brown macroalga *Fucus spiralis*, and the microalga *Limnospira* sp. were selected as bioactive sources, based on previous findings [12–14]. Extracts from *Fucus spiralis* and *Limnospira* sp. have been successfully incorporated into alginate-based edible films, demonstrating strong antioxidant and antimicrobial properties [12,13]. Among seven tested microalgae, *Limnospira* sp. showed the highest antioxidant capacity (DPPH and ORAC assays) and notable antimicrobial activity [13]. EOP extracts also exhibited strong antioxidant activity (ABTS = 63.528 ± 0.34 µmol Trolox equivalent (TE)/100 mg DW; DPPH = 25.099 ± 2.161 µmol TE/100 mg DW) and inhibited more than 50% of *Bacillus cereus* and *Listeria monocytogenes* growth [14]. A preliminary study using EOP-derived polysaccharides and bioactives in edible coatings also suggested their potential for extending strawberry shelf life [15].

EOP, a by-product of olive pomace processing, is particularly rich in phenolic compounds such as hydroxytyrosol, tyrosol, and catechol [14]. These bioactives are linked to a wide range of health-promoting properties, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, and antiviral activities (e.g., anti-HIV) [16].

Among brown macroalgae, *Fucus spiralis* stands out as a valuable source of diverse bioactive compounds, including phlorotannins, fucoxanthin, phenolic compounds, vitamins, lipids, and sulfated polysaccharides such as fucoidan [17,18]. These metabolites are associated with antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, anticoagulant, and immunomodulatory properties [18–23]. Fucoidan, in particular, has attracted attention for its application in biodegradable films and edible coatings designed to prolong the shelf life of fruits and other perishable goods [23,24].

Limnospira sp. (formerly *Arthrospira* sp., commonly known as Spirulina) is a filamentous cyanobacterium widely recognized for its nutritional and functional benefits [25]. With a protein content of approximately 60%–70% of its dry weight, it contains all essential amino acids and is rich in B-complex vitamins, vitamin E, iron, calcium, and polyunsaturated fatty acids—particularly γ-linolenic acid [26]. This unique macro- and micronutrient composition makes *Limnospira* sp. an attractive candidate for functional edible coatings.

Alginate, a naturally derived polysaccharide extracted from brown algae, is non-toxic, colorless, odorless, and biodegradable. It forms a semipermeable barrier against moisture and gases, and is compatible with a wide range of bioactive compounds. However, alginate has some limitations: alginate lacks inherent antimicrobial properties, is water-sensitive, and requires cross-linking with calcium to gain structural integrity [6].

Information on the thickness of alginate coatings remains limited. Parreidt et al. [27] reported a thickness of 235 ± 77 µm for a 1.25% (*w*/*v*) alginate coating applied to strawberries. Coating thickness generally increases with alginate concentration; for example, fresh-cut watermelon coated with 1% and 2% alginate exhibited thicknesses of 180 ± 2.0 µm and 412 ± 0.8 µm, respectively [28]. Incorporation of proteins or carbohydrates into alginate matrices further increases viscosity and solid content, typically yielding thicker dip-coated films of about 0.02 mm when 0.5% protein or polysaccharide is included in the formulation [29,30].

Regarding microbiome modulation, edible coatings may act as prebiotics, or postbiotics. Formulation containing sulfated propylene glycol ester of low-molecular-weight alginate exhibits a slight prebiotic effect but is resistant to degradation by gut microbiota [31]. Formulations with EOP extracts function as a functional ingredient with both prebiotic and postbiotic properties, as polyphenols promote beneficial microbes that produce short-chain fatty acids (SCFAs) through fermentation [32]. Formulations with *Fucus* spiralis extracts also have significant prebiotic potential, with fucoidans and laminarans fermented by gut bacteria to generate SCFAs that beneficially modulate the microbiota [33]. Finally, formulations with *Limnospira* sp. extracts demonstrate strong prebiotic and postbiotic effects, as polysaccharides and proteins from this microalga modulate beneficial bacteria and release bioactive peptides with antioxidative and anti-inflammatory properties [34–36].

In this study, alginate-based coatings were developed by incorporating bioactive-rich extract (0.25%) from EOP, bioactive-rich extract (0.25%) and polysaccharide-rich extract (0.5%) from *Fucus spiralis*, and bioactive-rich extract (0.25%) plus protein-rich extract (0.5%) from *Limnospira* sp. These coatings were applied to strawberries and evaluated during 10 days of storage at 10 °C for their effects on weight loss, color preservation, decay, antioxidant capacity, and antimicrobial activity.

2. Materials and Methods

All chemical compounds used were pro analysis (p.a.) grade.

2.1. Plant Material

The exhausted olive oil pomace (EOP) was supplied in powder by Casa Alta—Sociedade Transformadora de Bagaços, a company from Ferreira do Alentejo, Beja, Portugal. It was obtained by processing olives harvested in 2021, in olive oil extraction units, and after olive pomace drying and residual olive oil extraction with hexane. The olive oil pomace was stored in hermetic containers in the dark and in a dry air ambience (55% of relative humidity (RH)). The container was opened to weigh the samples just before use to avoid phenolic degradation.

Fucus spiralis alga was collected from a beach called "Memória" located in the region between Vila do Conde and Matosinhos, Portugal, on 10 September 2024. It was quickly transported to the laboratory, where it was cleaned with water, identified based on its

morphological characteristics. The identification was performed by Prof. Dr. Isabel Sousa Pinto (CIIMAR, Porto, Portugal), and an exemplar was kept at CIIMAR. Subsequently, samples of 200 g were vacuum-sealed, frozen, and stored at −20 °C. For the extraction of bioactive compounds and polysaccharides, samples were defrosted overnight at 10 °C.

The *Limnospira* sp. biomass was kindly donated frozen by A4F—Algae for Future, Portugal. It was stored at −20 °C until it was necessary for the assay of the extraction of bioactive compounds and protein, at which point it was defrosted and freeze-dried.

Organic strawberries (*Fragaria ananassa*) with similar dimensions and good visual quality were purchased from the local market. To prepare strawberries before the coating application, they were rinsed with 50 ppm chlorinated water for three minutes, followed by immersion in distilled water for one minute and subsequently drained is. This treatment is a sanitization procedure aimed at reducing surface contamination.

2.2. Extraction of Bioactive Compounds

2.2.1. Extraction of Bioactive Compounds from the EOP and *Limnospira* sp.

A classical solvent extraction method was employed using a hydroalcoholic solution (water/ethanol, 1:9 *v*/*v*). One gram of sample powder (for EOP and *Limnospira* sp.) was immersed in 30 mL of the solution and incubated at 50 °C with continuous stirring at 120 rpm (Orbital Shaker, MaxQ6000, Thermo Scientific, Waltham, MA, USA) for 120 min, with the process repeated twice. The mixture was then subjected to ultrasonic homogenization using a probe sonicator (Sonics, Vibra-Cell, Newtown, CT, USA) with 20 kHz pulses applied for 30 s at a time over 10 min. Following homogenization, the solution was filtered, and the ethanol was removed through rotary evaporation (Buchi R-210, Buchi Labortechnik AG, Flawil, Switzerland). Finally, the extract was lyophilized to obtain a dry powder [26]. The resulting bioactive-rich extract was used to formulate an edible coating and it was applied to strawberries (Section 2.5). The residual solid phase (from *Limnospira* sp. microalgae biomass) was stored in the dark at −20 °C for later protein extraction.

2.2.2. Extraction of Bioactive Compounds from *Fucus spiralis*

Microwave hydrodiffusion and gravity (MGH) extraction was performed on *Fucus spiralis* using an advanced microwave system (Milestone S.r.l., Sorisole, Italy), equipped with a 1.5 L vessel capable of accommodating up to 200 g of algal biomass. Extraction conditions were adapted based on methodologies reported by Pérez et al. [37] and Flórez-Fernández et al. [38]. The seaweed was subjected to 300 W for 20 min at a frequency of 50 Hz, according to Martins et al.'s findings [12]. The liquid extract was collected by gravity and subsequently stored at 4 °C either for immediate analysis or before freeze-drying. The residual solid phase (hereafter referred to as the first residue) was stored in the dark at −20 °C for later polysaccharide extraction.

2.3. Extraction of Polysaccharides from Fucus spiralis

The solid-phase residues obtained from *Fucus spiralis* in Section 2.2.2 were suspended in distilled water at a concentration of 50 g/L and subjected to thermal treatment at approximately 90 °C (near boiling point) under continuous magnetic stirring (500 rpm) using an IKA Labortechnik RCT basic system (IKA Werke Gmbh & Co.KG, Staufen, Germany) for 60 min. The resulting mixture was filtered through qualitative filter paper, and the extraction procedure was repeated to maximize yield. Polysaccharides (PS) were subsequently precipitated by the gradual addition of ethanol to achieve a 1:1 water-to-ethanol volume ratio, followed by centrifugation at 5000 rpm for 20 min. The precipitate was redissolved in distilled water and freeze-dried, as described in literature [39,40]. The PS-rich extract was used in the edible coating formulation (Section 2.5).

2.4. Extraction of Protein from Limnospira sp.

A protein-rich extract from *Limnospira* sp. solid-phase residues (Section 2.2.1) was obtained following the methodology described by Cunha et al. [41], with sequential hydrolysis steps. Initially, acid hydrolysis was carried out using 2% (*v*/*v*) acetic acid at 50 °C with a solid-to-liquid ratio of 1:3 (*w*/*v*), under agitation at 125 rpm for one hour. Subsequently, enzymatic hydrolysis was performed in two phases: first, with cellulase (5% *w*/*w*) at 50 °C and pH 7.5, using a biomass-to-buffer ratio of 1:10 (*w*/*v*) under agitation (125 rpm) for 2 h; then, with protease (3.9% *w*/*w*) at 40 °C and pH 7.5, also under agitation (125 rpm) for 2 h. Enzymes were inactivated by heating the mixture to 90 °C for 10 min. The resulting suspension was centrifuged at 5000× *g* for 20 min, and the supernatant—containing the bioactive protein hydrolysates—was subsequently freeze-dried for further use.

2.5. Edible Coating Formulations and Their Application on Strawberries

Edible coating solutions were prepared following Martins et al. [42], with some modifications. To prepare the edible coating solution with bioactives from EOP (formulation B), sodium alginate (2% *w*/*v*) (Sigma, Aldrich Chemie GmbH, Steinheim, Germany) was dissolved in hot distilled water, and the bioactive-rich extract was added (0.25% *w*/*v*) under stirring until completely dissolved. To prepare the edible coating solutions with bioactives and nutrients from *Fucus spiralis* (formulation C) and *Limnospira* sp. (formulation D), sodium alginate (2% *w*/*v*) was dissolved in hot distilled water, and then, 0.5% of the polysaccharide-rich extract (Section 2.3) and 0.5% of the protein-rich extract (Section 2.4) were added, respectively, until total dissolution, followed by continuous stirring for one hour. Bioactive-rich extract was added (0.25% *w*/*v*) under stirring until completely dissolved. Subsequently, diacetyllauroyl glycerol (0.6% *v*/*v*) (Tokyo Chemical Industry, Toshima, Kita-ku, Tokyo, Japan) was incorporated into each solution and stirred for another hour at room temperature (25 °C).

To apply the coating solution, the following methodology was used [43]. All fruits were dipped in coating solutions for 30 sec, and the solutions in excess were dripped off for five min. The environmental conditions in the laboratory were 26 °C and 45% RH. The coated fruits were dried at room temperature for 30 min and then placed in plastic baskets and stored at 10 °C for ten days.

Three fruits of each replicate were periodically sampled every two days for decay, moisture content, color, antioxidant activity, and antimicrobial activity. The control sample was coated with 2% alginate. In summary, the different coating formulations were the following:

A. Alginate (2%)
B. Alginate (2%) + EOP bioactive-rich extract (0.25%)
C. Alginate (2%) + *Fucus spiralis* bioactive-rich extract (0.25%) + *Fucus spiralis* polysaccharide-rich extract (0.5%)
D. Alginate (2%) + *Limnospira* sp. bioactive-rich extract (0.25%) + *Limnospira* sp. protein-rich extract (0.5%)

2.6. Evaluation of Coated Strawberries

2.6.1. Decay

Decay visual observation was registered photographically at any sign of decay, lesion, or mycelia development on the fruit surface during storage.

2.6.2. Moisture Loss

The moisture loss was determined every two days for 10 days. For this the strawberries were weighed. Three replicates were prepared for each sample and the moisture loss was calculated using the formula [44]:

$$\text{Moisture loss} = ((\text{initial weight (day 0)} - \text{current weight (day D)})/\text{initial weight (day 0)}) \times 100 \quad (1)$$

2.6.3. Color Analysis

The color of each strawberry was analyzed using a Chroma Meter CR 400 (Konica Minolta Sensing, Osaka, Japan), which was calibrated with a white standard color plate. The color parameters evaluated are in the CIE mode color space [45], including L^* (lightness), a^* (green–red coordinate), and b^* (blue–yellow coordinate), as well as the hue angle and the saturation index (chroma).

Hue and Chroma values were calculated using the following equations:

$$\text{Hue} = \arctan(b^*/a^*) \quad (2)$$

$$\text{Chroma} = \sqrt{a*^2 + b*^2} \quad (3)$$

2.6.4. Determination of Total Phenolic Content Antioxidant Activity

The total phenolic content (TPC) antioxidant capacity of the extracts (20 mg/mL) was evaluated using three methods: TPC, ABTS and DPPH assays. Each assay was performed in triplicate across three independent extractions.

TPC

The TPC assay was performed using the Folin–Ciocalteu method, originally described by Martins et al. [14], In brief, 30 µL of the extract suspension was mixed with 100 µL of Folin–Ciocalteu reagent (20%, v/v), followed by the addition of 100 µL of sodium carbonate solution (7.4%, w/v). The mixture was incubated at room temperature (≈25 °C) for 30 min in the dark. Absorbance was recorded at 765 nm using a microplate reader (Synergy H1, Biotek, Winooski, VT, USA) in 96-well plates (Sarstedt, Nümbrecht, Germany). A calibration curve was constructed with gallic acid, and results were expressed as milligrams of gallic acid equivalents per 100 mg of dry weight extract (mg GAE/100 mg DW).

ABTS Radical Scavenging Assay

The ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) assay was performed according to the method by Martins et al. [14]. The ABTS radical was generated by reacting ABTS (0.0384 g in 10 mL water) with potassium persulfate (0.0066 g in 10 mL water) and adjusted to an absorbance of 0.700 ± 0.020 at 734 nm. An aliquot of extract (20 µL) was reacted with 180 µL of the ABTS solution in the dark at room temperature for 5 min. Absorbance was read at 734 nm. Trolox was used as the standard, and results were expressed as µmol Trolox equivalents per 100 mg of dry extract (µmol TE/100 mg DW). Inhibition (%) was calculated as:

$$\text{I (\%)} = [(\text{Abs A0} - \text{Abs sample}) \div \text{Abs A0}] \times 100 \quad (4)$$

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay followed the protocol by Martins et al. [14]. A 600 µM stock solution of DPPH (23.6592 mg/100 mL methanol) was diluted to 90 µM working solution to yield an absorbance of 0.600 ± 0.100 at 515 nm. Extract (25 µL) was added to 175 µL of DPPH solution in a 96-well plate and incubated for 30 min in the

dark. Absorbance was recorded at 515 nm. Trolox served as the calibration standard, and antioxidant activity was reported in μmol TE/100 mg DW.

2.6.5. Determination of Antimicrobial Activity

The assay was performed every two days for ten days, and strawberries were analyzed for mesophilic aerobic bacteria, psychrophilic bacteria, *Enterobacteriaceae*, and total coliforms, and for molds and yeasts. These microbiological analyses followed Moreira et al.' s and Treviño-Garza et al. 's methodologies [46,47]. Approximately 10 g of strawberry from each sample was macerated in 90 mL of 1 g/L peptonized water and homogenized using a Stomacher homogenizer (Seward, West Sussex, UK). Serial 1:10 dilutions of each homogenized sample were prepared and plated in duplicate. Enumeration and differentiation of microorganisms were conducted using the appropriate media and conditions. Mesophilic aerobic bacteria were cultured on plate count agar (PCA) and incubated at 30–32 °C for 48–72 h. Psychrophilic bacteria were incubated on PCA at 5–7 °C for 5–7 days. *Enterobacteriaceae* and total coliforms were incubated in Mac Conkey agar at 30–32 °C for 24 h. Molds and yeasts were quantified on potato dextrose agar (PDA) and incubated at 25 °C for five days. Microbial counts were performed in duplicate across three independent experimental replicates.

2.7. Statistical Analysis

All data are expressed as mean ± standard deviation (n = 3). Statistical analysis was performed using one-way repeated measures ANOVA with three conditions (a significance level $p < 0.05$). Analyses were conducted using IBM SPSS Statistics v22 (IBM, Armonk, NY, USA). Assumptions of normality and homogeneity of the variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Mauchly's test was used to evaluate the sphericity assumption. If the sphericity assumption was violated, the Greenhouse–Geisser correction was applied; otherwise, the standard F-test was used. Post hoc comparisons were conducted using Fisher's LSD test for within-subject comparisons and Bonferroni correction for between-subject comparisons.

3. Results and Discussion

3.1. Effects of Coating on the Strawberry Moisture Loss

Weight loss (WL) during the 10-day storage period, measured at six time points, showed a significant dependence on the type of coating applied. Marginal or main effects pairwise comparisons revealed statistically significant differences across nearly all coatings' average performance during the storage time, except for the control vs. EOP coating and *Fucus spiralis* vs. *Limnospira* sp. coatings. Marginal or main effects pairwise comparisons also showed that all time points' average performance across all coatings differed significantly.

After 10 days of storage, strawberries coated with EOP exhibited the lowest WL (17.87 ± 1.32%), followed by the uncoated control (33.79 ± 5.88%), *Fucus spiralis* (54.18 ± 0.84%), and *Limnospira* sp. (57.86 ± 7.39%) (Figure 1) with pairwise significant differences between most coatings, except between control and EOP coatings, and between *Fucus spiralis* and *Limonspira* sp. The superior performance of the EOP formulation is likely due to the high lipid content in the EOP bioactive-rich extract, obtained using 90% ethanol. Lipids, being hydrophobic, reduce water vapor permeability when incorporated into edible coatings, thereby limiting moisture loss [27,48].

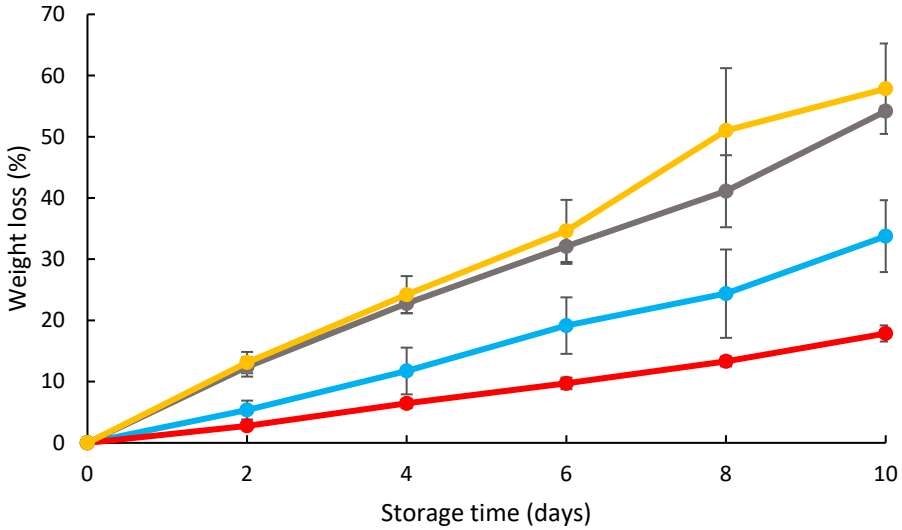

Figure 1. Weight loss of strawberry for ten days storage (control in blue; exhausted olive oil pomace (EOP) coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

In contrast, strawberries coated with *Fucus spiralis* or *Limnospira* sp. extracts experienced greater weight loss than the control. Although these extracts contain bioactive compounds with potential barrier function, their high content of hydrophilic polysaccharides and proteins may have compromised the water vapor resistance of the alginate matrix. The incorporation of such hydrophilic components likely increased permeability, thereby accelerating water loss [42,49].

3.2. Effects of Coating on the Strawberry Color

Strawberry color varied significantly with both storage duration and coating formulation. The lightness parameter (L^*) (Figure 2) showed significant differences across all coatings and at all six time points, confirming that L^* was influenced by both the coating formulation and storage time. Marginal pairwise comparisons between coatings showed significant differences for all coatings and also revealed that all time points differed significantly, except for the comparisons between day 0 and days 2, 4, 6, and 8; between day 2 and day 6; and between day 4 and day 8.

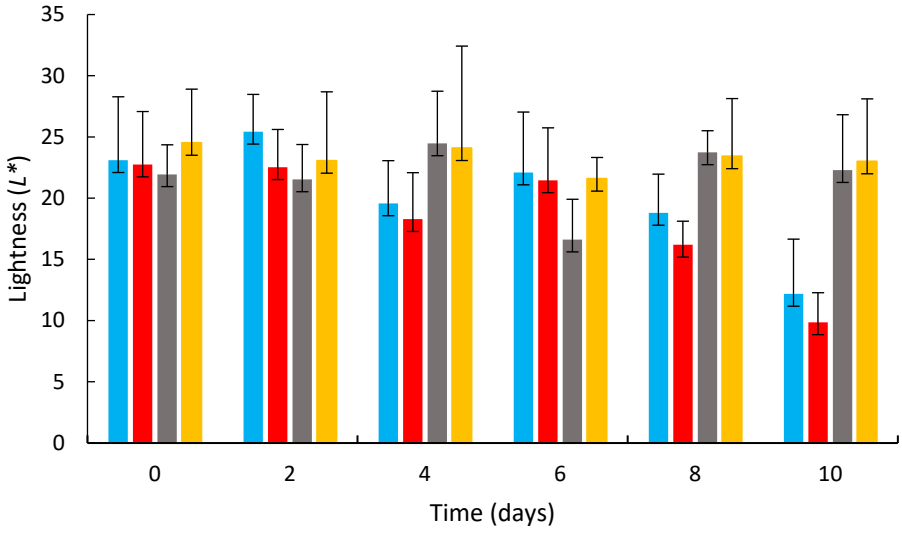

Figure 2. L^* color parameter of strawberry for ten days storage (control in blue; EOP coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

A significant difference in the L^* parameter was also observed among all coating formulations. At the end of storage (day 10), pairwise comparisons showed no significant differences between L^* values of the control and EOP coating, nor between *Fucus spiralis* and *Limnospira* sp. coatings. However, significant differences were found between the control and *Fucus spiralis* or *Limnospira* sp., and between EOP and *Fucus spiralis* or *Limnospira* sp.

At day 10, L^* values were 12.18 ± 4.48 for the control, 9.85 ± 2.43 for the EOP, 22.29 ± 4.53 for *Fucus spiralis*, and 23.00 ± 5.12 for *Limnospira* sp, corresponding to decreases of 47.2%, 56.7%, −1.6% and 6.2%, respectively, compared to day 0. The greater decreases in L^* observed for the control and EOP coatings indicate a marked darkening of these samples during storage. In contrast, *Fucus spiralis* and *Limnospira* sp. coatings preserved fruit lightness. Overall, lightness progressively declined in the control and EOP-coated strawberries, whereas *Fucus spiralis* and *Limnospira* sp. coatings were more effective in maintaining brightness through 10 days of storage.

The a^* values (red–green axis) (Figure 3) changed significantly in a time- and formulation-dependent manner, indicating that red color intensity was influenced by both storage duration and coating type. Marginal pairwise comparisons revealed significant differences between most time points, except between day 0 and day 10, day 2 and day 6, and day 4 and day 10. For strawberries coated with *Fucus spiralis* and *Limnospira* sp., the a^* values decreased from day 0 to day 2, meaning a decrease in the red color intensity.

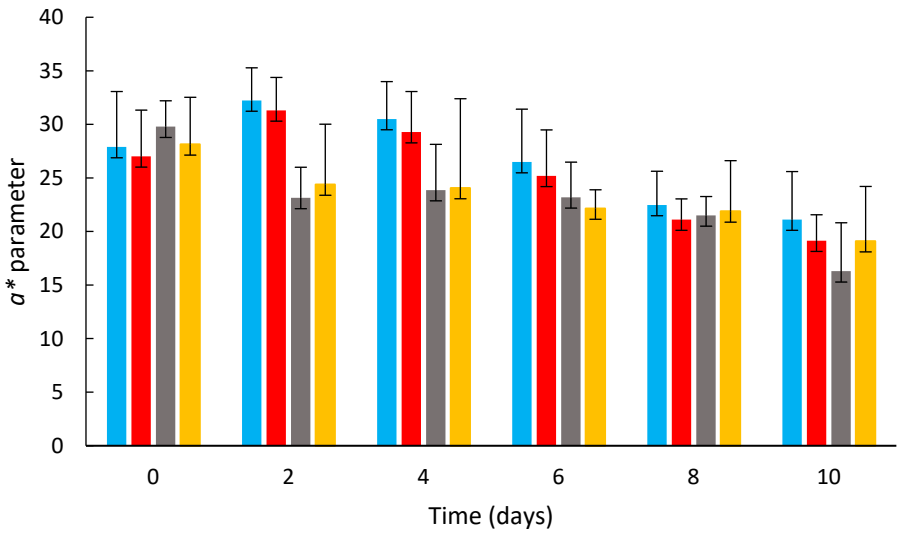

Figure 3. a^* color parameter of strawberry for ten days storage (control in blue; EOP coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

A significant main effect of coating formulation was also observed, although no significant differences were found between *Fucus spiralis* and *Limnospira* sp. At day 10, pairwise comparisons revealed no significant differences between the control vs. EOP, control vs. *Limnospira* sp., or EOP vs. *Limnospira* sp. However, significant differences were detected between the control vs. *Fucus spiralis*, EOP vs. *Fucus spiralis*, and *Fucus spiralis* vs. *Limnospira* sp.

At day 10, a^* values were 21.12 ± 2.81 for the control, 19.14 ± 2.19 for EOP, 16.30 ± 2.03 for *Fucus spiralis*, and 19.10 ± 2.40 for *Limnospira* sp., corresponding to decreases of 24.3%, 29.1%, 45.3%, and 28.6%, respectively, compared with day 0. The greater decrease observed in *Fucus spiralis*-coated strawberries indicates a more pronounced loss of red color intensity relative to the other treatments.

The b^* values (yellow–blue axis) (Figure 4) also showed clear effects of storage time and coating type, generally decreasing as storage progressed. Post hoc marginal pairwise

comparisons showed significant differences between coatings and also revealed significant differences between most time points, except for day 2 vs. day 4, day 2 vs. day 10, and day 4 vs. day 10, which were not statistically different.

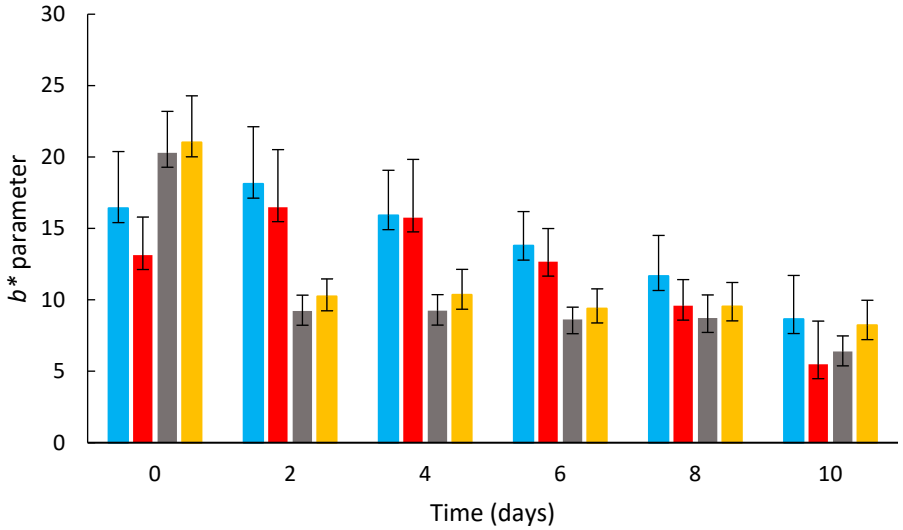

Figure 4. *b** color parameter of strawberry for ten days storage (control in blue; EOP coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

For strawberries coated with *Fucus spiralis* and *Limnospira* sp., the *b** values decreased from day 0 to day 2 and then remained relatively stable thereafter. At day 10, pairwise comparisons between coating formulations showed significant differences for control vs. EOP, control vs. *Fucus spiralis*, and EOP vs. *Limnospira* sp. By contrast, no significant differences were found between control vs. *Limnospira* sp., EOP vs. *Fucus spiralis*, or *Fucus spiralis* vs. *Limnospira* sp.

At day 10, *b** values were 8.65 ± 3.07 for the control, 5.49 ± 3.03 for EOP, 6.38 ± 1.09 for *Fucus spiralis*, and 8.22 ± 1.75 for *Limnospira* sp., corresponding to decreases of 47.3%, 58.2%, 68.6%, and 60.9%, respectively, compared with day 0. These results confirm that coating formulation significantly influenced the *b** parameter. Overall, b* values declined throughout storage, with the greatest reduction observed in *Fucus spiralis*-coated sample.

Hue angle (Figure 5) significantly decreased with storage (main effect). Post hoc marginal pairwise comparisons revealed that day 0 differed significantly from all subsequent time points (days 2, 6, 8, and 10). For strawberries coated with *Fucus spiralis* and *Limnospira* sp., the hue angle decreased from day 0 to day 2.

A significant main effect of coating formulation was also detected, although no significant differences were found between EOP and *Limnospira* sp., or between *Fucus spiralis* and *Limnospira* sp. At day 10, pairwise comparisons revealed significant differences between the control vs. *Fucus spiralis* and *Limnospira* sp., and between EOP vs. *Fucus spiralis* and *Limnospira* sp. In contrast, no significant differences were observed between the control vs. EOP or between *Fucus spiralis* and *Limnospira* sp.

At day 10, hue values were 29.28 ± 5.54 for the control, 27.48 ± 4.77 for EOP, 21.73 ± 1.89 for *Fucus spiralis*, and 22.92 ± 2.94 for *Limnospira* sp., corresponding to decreases of 2.9%, −6.5%, 36.4%, and 37.3%, respectively, compared with day 0. These results follow a pattern similar to the *b** parameter, with greater reductions observed in *Fucus spiralis* and *Limnospira* sp. coatings, indicating more pronounced shifts in fruit coloration during storage.

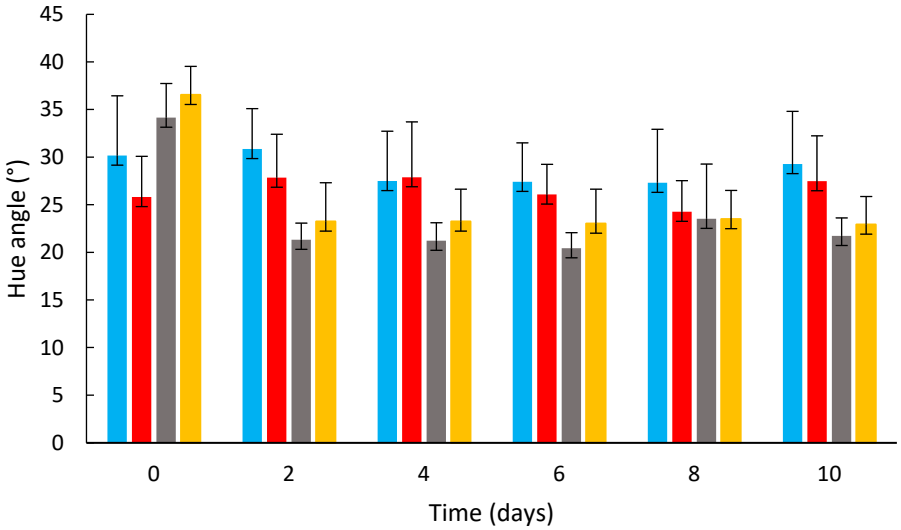

Figure 5. Hue angle of strawberries for ten days storage (control in blue; EOP coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

Chroma (saturation index, Figure 6) was similarly affected by the storage time and coating type, except for *Fucus spiralis* vs. *Limnospira* sp., which did not differ significantly. Post hoc marginal pairwise comparisons revealed significant differences between coatings, except for *Fucus spiralis* vs. *Limnospira* sp. Marginal pairwise comparisons also showed that most time points differed significantly, with the exception of day 2 vs. day 6, and day 4 vs. day 10.

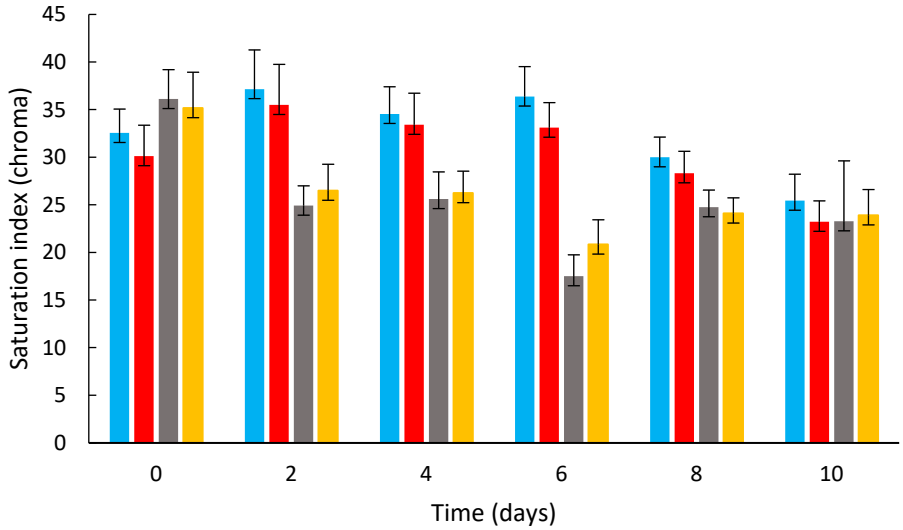

Figure 6. Saturation index (chroma) of strawberry for ten days storage (control in blue; EOP coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

Similar to the hue angle, chroma values decreased from day 0 to day 2 for strawberries coated with *Fucus spiralis* and *Limnospira* sp., consistent with the trends observed in the a^* and b^* parameters, indicating a reduction in red color intensity. A comparable decrease was also observed for EOP-coated strawberries, although this occurred after day 6.

At day 10, pairwise comparisons between coating formulations revealed no significant differences among treatments. Chroma values were 25.44 ± 2.78 for the control, 23.22 ± 2.20 for EOP, 23.26 ± 6.36 for *Fucus spiralis*, and 23.90 ± 2.70 for *Limnospira* sp., corresponding to decreases of 21.2%, 22.9%, 35.6%, and 32.0%, respectively, compared with

day 0. These results align with the patterns observed in the a^* and b^* parameters, with the largest decreases in hue and chroma recorded for *Fucus spiralis* and *Limnospira* sp. coatings.

Overall, these color parameter changes reflect a decline in red color intensity, likely associated with the degradation of carotenoids and anthocyanins, as well as pH fluctuations and enzymatic activity. Carotenoids are particularly susceptible to degradation by light, oxygen, or enzymes, while anthocyanins are highly sensitive to pH [50,51]. Acidic conditions help stabilize anthocyanins and other pigments, whereas alkaline conditions promote degradation. For edible coatings, pH affects not only pigment stability but also the structural integrity of the coating matrix and its interaction with fruit tissues [52,53].

Edible coatings can mitigate these effects by protecting fruit pigments, though their efficacy depends on their biochemical composition. Alginate coatings, for example, do not modify fruit pH and lacks intrinsic antioxidant or anti-browning activity, but they can enhance surface brightness and gloss [9].

The EOP-based coating, enriched with polyphenols, enhanced color retention, likely by inhibiting polyphenol oxidase (PPO), the main enzyme responsible for enzymatic browning [54,55]. Similar results were reported by Natalia et al. [54], who showed that starch-based coatings with olive pomace effectively reduced browning and maintained firmness of fresh-cut apples. Arnold and Gramza-Michałowska [55] also found that phenol-enriched coatings containing hexylresorcinol or pink pepper extracts lowered PPO activity and reduced browning.

Fucus spiralis-based coatings also promoted color stability, partly due to their buffering capacity, which helps maintain a stable microenvironment and delays pigment degradation. In addition, their phlorotannin content contributes potent antioxidant and enzyme-inhibitory activity. Catarino et al. [56] reported that phlorotannins extracted from *Fucus vesiculosus* inhibited α-glucosidase, α-amylase, and lipase, with particularly strong activity against α-glucosidase. Phlorotannins are also effective inhibitors of PPO, peroxidase, and other enzymes involved in fruit deterioration [57].

Coatings with *Limnospira* sp. extracts contribute to color stability through their protein- and peptide-rich composition, which provides buffering effects and antioxidant activity. Phycocyanin, a major pigment in this microalga, also supports antioxidant protection but is highly sensitive to pH fluctuations, which may limit its long-term stability [58,59].

3.3. Effects of Coating on the Strawberry Antioxidant Activity

There was a significant main effect of storage time on the total phenolic content (TPC) levels (Figure 7); however, no significant interaction was observed between time and coating formulation. Post hoc marginal pairwise comparisons showed no significant differences between coatings and also revealed that most time points did not differ significantly, except for comparisons between day 0 and days 4 and 10, between day 2 and day 10, and between day 8 and day 10, where significant differences were observed. Overall, TPC declined progressively over storage, with the greatest reduction occurring in the control samples, particularly during the first two days. From day 0 to day 10, TPC losses were 54.0% for the control, 34.0% for EOP, 21.2% for *Fucus spiralis*, and 29.8% for *Limnospira* sp., confirming that the control sample suffered the most pronounced degradation. However, post hoc pairwise comparisons between coatings at day 10 showed no significant differences among formulations.

Both ABTS and DPPH assays (Figures 8 and 9) confirmed a significant decline in antioxidant capacity during storage. Coatings, however, consistently influenced the extent of this decline.

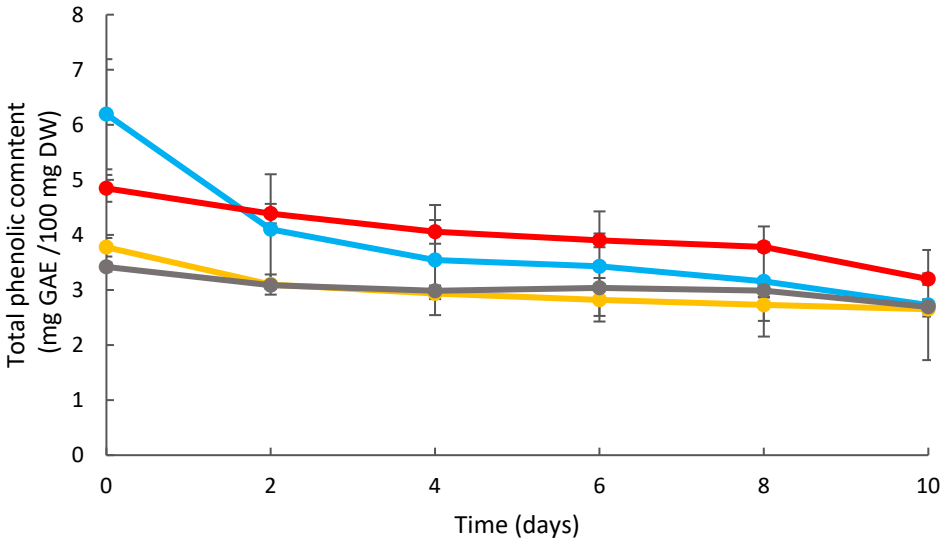

Figure 7. Total phenolic content of strawberry for ten days storage (control in blue; EOP coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

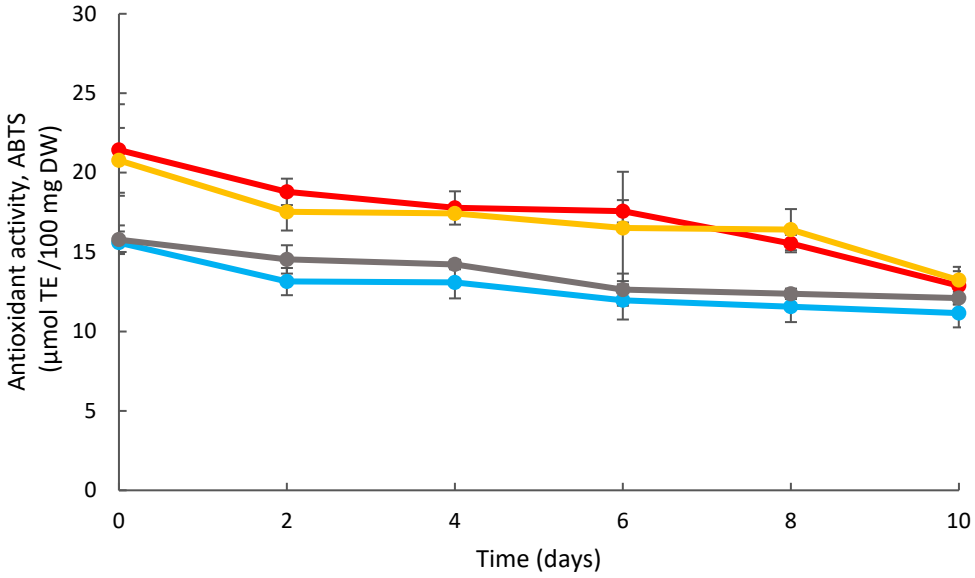

Figure 8. Antioxidant activity (ABTS assay) of strawberry for ten days storage (control in blue; EOP coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

Post hoc marginal pairwise comparisons of ABTS values revealed that most time points differed significantly, except for days 2 vs. 4, 2 vs. 6, 4 and 6, and 6 vs. 8, which showed no statistical differences. Significant differences in marginal pairwise comparisons of coating formulations were also observed, except between the control and EOP coatings, and between *Fucus spiralis* and *Limnospira* sp. coatings.

Post hoc pairwise comparisons at day 10 showed no significant differences in ABTS values between control vs. EOP, EOP vs. *Fucus spiralis* or *Limnospira* sp., and *Fucus spiralis* vs. *Limnospira* sp. Significant differences were observed between control vs. *Fucus spiralis* or *Limnospira* sp.

Overall, ABTS values declined over time, with the most notable decrease occurring during the first two days in strawberries coated with EOP and *Limnospira* sp. Despite this early reduction, these two coatings maintained higher ABTS values throughout storage compared to the control and *Fucus spiralis* coatings. From day 0 to day 10, ABTS values

decreased by 28.4% for the control, 39.9% for EOP, 23.3% for *Fucus spiralis* and 36.3% *Limnospira* sp.

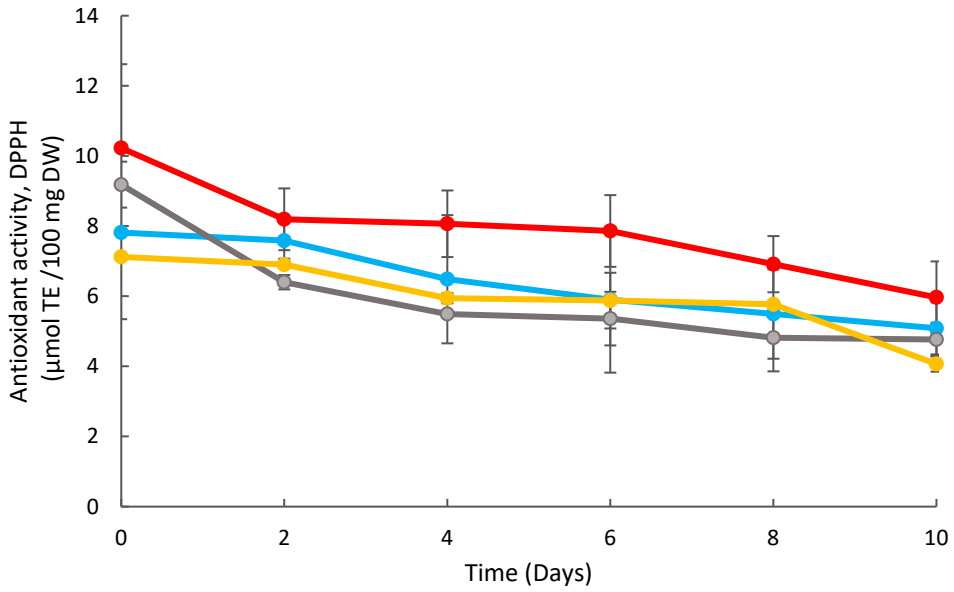

Figure 9. Antioxidant activity (DPPH) of strawberry for ten days storage (control in blue; EOP coating in red; *Fucus spiralis* coating in grey, *Limnospira* sp. coating in yellow).

Similarly, DPPH values (Figure 9) decreased over time (significant main effect of storage time), but EOP consistently outperformed other treatments. Post hoc marginal pairwise comparisons showed that most time points differed significantly, except between days 4 and 6, which showed no statistical difference. Overall, DPPH values declined over time, with the sharpest decrease occurring within the first two days for the EOP-coated samples. Despite this early drop, EOP coating consistently showed a trend toward higher DPPH values compared to the other coatings. A significant main effect of coating formulation on DPPH was also found, except between the control and both *Fucus spiralis* and *Limnospira* sp., as well as between *Fucus spiralis* and *Limnospira* sp.

Post hoc pairwise comparisons at day 10 showed no significant differences in DPPH values between control vs. EOP or *Fucus spiralis*, and *Fucus spiralis* vs. *Limnospira* sp. Post hoc comparisons at day 10 revealed no significant differences in DPPH values between control vs. EOP or *Fucus spiralis*, and *Fucus spiralis* vs. *Limnospira* sp. Significant differences were found between control vs. *Limnospira* sp. and EOP vs. *Fucus spiralis* or *Limnospira* sp.

From day 0 to day 10, DPPH values decreased by 34.95% for the control, 41.7% for EOP, 48.1% for *Fucus spiralis*; and 42.9% for *Limnospira* sp.

The EOP coating demonstrated superior preservation of antioxidant activity, as shown by both ABTS and DPPH assays, compared to the control. This aligns with the role of phenolic compounds in EOP extracts, which enhance the antioxidant capacity of alginate-based coatings. Although coating formulation did not significantly affect total phenolic content (TPC), the increased radical scavenging activity indicates that bioactive compounds from EOP retained their functional properties despite partial degradation during storage. These results agree with Valero and Serrano [60], who noted that antioxidant effectiveness can persist even when phenolic levels decline. Similarly, coatings enriched with *Fucus spiralis* or *Limnospira* sp. extracts did not significantly increase TPC compared to the control, yet they still enhanced antioxidant activity. This supports previous findings that alginate matrices enriched with marine extracts act as effective carriers, stabilizing phenolic compounds and maintaining antioxidant properties during storage [61,62]. Overall, olive pomace extracts tend to promote both phenolic retention and antioxidant activity in edible coatings, whereas

alginate formulations with algal extracts mainly boost antioxidant responses without significantly increasing total phenolics. These findings are supported by previous studies, which showed that ABTS and DPPH values were remarkably higher for the bioactive-rich EOP extracts [14] than for similar extracts from *Fucus spiralis* [12] and *Limnospira* sp. [13].

3.4. Effects of Coating on the Strawberry Decay

Visible decay was most pronounced in the control and *Fucus spiralis* coatings, with fungi appearing by day 2 and day 8, respectively. In contrast, EOP and *Limnospira* sp. coatings effectively delayed visible spoilage until the end of storage (Figure 10).

Figure 10. Visible decay of strawberries during ten days of storage ((**a**) control; (**b**) EOP coating; (**c**) *Fucus spiralis* coating; (**d**) *Limnospira* sp. coating).

3.5. Effects of Coating on the Strawberry Antimicrobial Activity

Microbial growth patterns further supported the observations on visible decay.

Since it was not possible to macroscopically distinguish all bacterial colonies from yeast and fungi, the total number of mesophilic aerobic microorganisms (mesophilic aerobic bacteria plus yeast and molds growth results) was determined (Figure 11).

The growth of mesophilic aerobic microorganisms during 10 days of storage was significantly influenced by storage time, coating type, and their interaction. Marginal pairwise comparisons showed significant differences between most time points, except for days 4 vs. 6, 4 vs. 8, and 8 vs. 10.

Regarding coatings, significant differences were observed except between the control and *Fucus spiralis* or *Limnospira* sp. coatings, and between *Fucus spiralis* and *Limnospira* sp. coatings. Post hoc pairwise comparisons at day 10 showed no significant differences in mesophilic microorganisms counts between EOP vs. *Fucus spiralis* or *Limnospira* sp. For all pairwise comparisons on day 10 differences were not statistically significant.

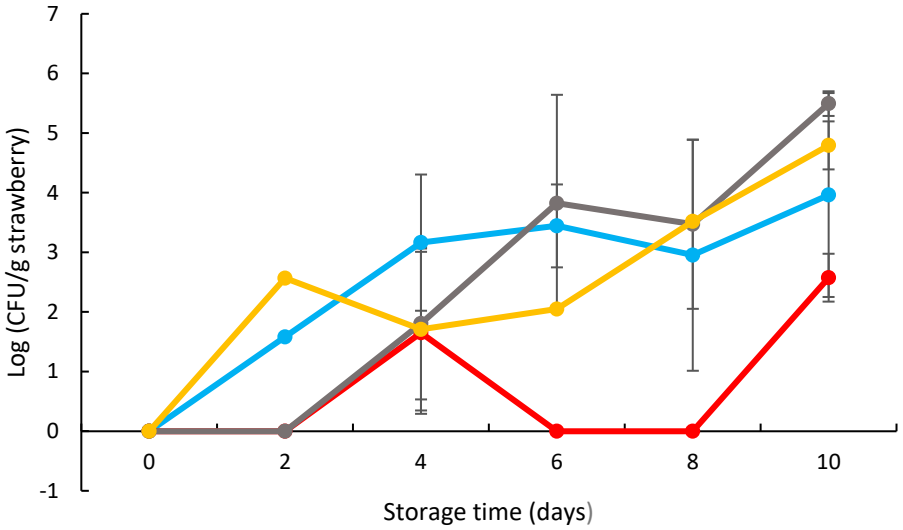

Figure 11. Mesophilic aerobic microorganism growth in strawberries during ten days of storage using different coatings. Control—blue; EOP coating—red; *Fucus spiralis*—grey; *Limnospira* sp. coating—yellow.

At day 10, mesophilic microorganisms counts (log(CFU/g strawberries)) were 3.96 ± 1.68 for the control, 2.58 ± 0.69 for EOP, 5.49 ± 0.19 for *Fucus spiralis*, and 4.79 ± 0.36 for *Limnospira* sp. The EOP-coated sample, which performed best, had approximately 53% lower mesophilic aerobic microorganisms counts than the *Fucus spiralis*-coated sample, which showed the highest growth.

The growth of psychrophilic bacteria over six time points during 10 days of storage was significantly affected by storage time, coating type, and their interaction. Marginal pairwise comparisons revealed significant differences between most time points, except for days 2 vs. 4, 2 vs. 6, 2 vs. 8, 4 vs. 6, 4 vs. 8, and 6 vs. 8.

Significant differences were also observed between coatings in marginal pairwise comparisons, except between the control with *Limnospira* sp., and between EOP and *Limnospira* sp. Up to day 8, *Limnospira* sp. and EOP coatings effectively controlled psychrophilic bacterial growth on strawberries (Figure 12). Post hoc pairwise comparisons at day 10 revealed that psychrophilic bacteria counts were significantly different for control vs. EOP, and EOP vs. *Fucus spiralis* or *Limnospira* sp., but not for control vs. *Fucus spiralis* or *Limnospira* sp., or *Fucus spiralis* vs. *Limnospira* sp.

At the end of storage (day 10), psychrophilic bacteria counts (log(CFU/g strawberries)) were 3.89 ± 1.35 for the control, 2.42 ± 0.28 for EOP, 4.94 ± 0.24 for *Fucus spiralis*, and 4.09 ± 0.19 for *Limnospira* sp. The EOP-coated sample, which performed best, had approximately 51% lower psychrophilic bacterial counts than the *Fucus spiralis*-coated sample, which showed the highest growth.

Coating efficiency may vary depending on microbial sensitivity. For example, mesophilic bacteria are more susceptible to phenolic compounds and organic acids, while psychrophilic bacteria and yeasts may display higher tolerance [63], possibly explaining the observed differences. Yeasts and molds generally take longer to grow than bacteria [64], which is why they were only detected on PDA after day 2. Variability among fruit samples should also be considered when interpreting differences in microbial growth patterns.

Among all tested coatings, the EOP formulation demonstrated the strongest antimicrobial activity, reducing microbial counts after 10 days of storage (Figures 11 and 12). This effect is likely due to phenolic compounds with known antimicrobial properties, such as hydroxytyrosol [14]. In contrast, coatings containing *Fucus spiralis* or *Limnospira* sp. extracts showed higher microbial growth than the control. This may result from the reduced

antimicrobial efficacy of phenolics in these formulations, as the added polysaccharides and proteins could serve as substrates for microbial growth on the strawberry surface (epicarp) [12,26,65,66]. *Enterobacteriaceae* and total coliforms, commonly found on strawberries [67], were not detected in any sample, likely because the fruits had been previously washed with chlorinated water.

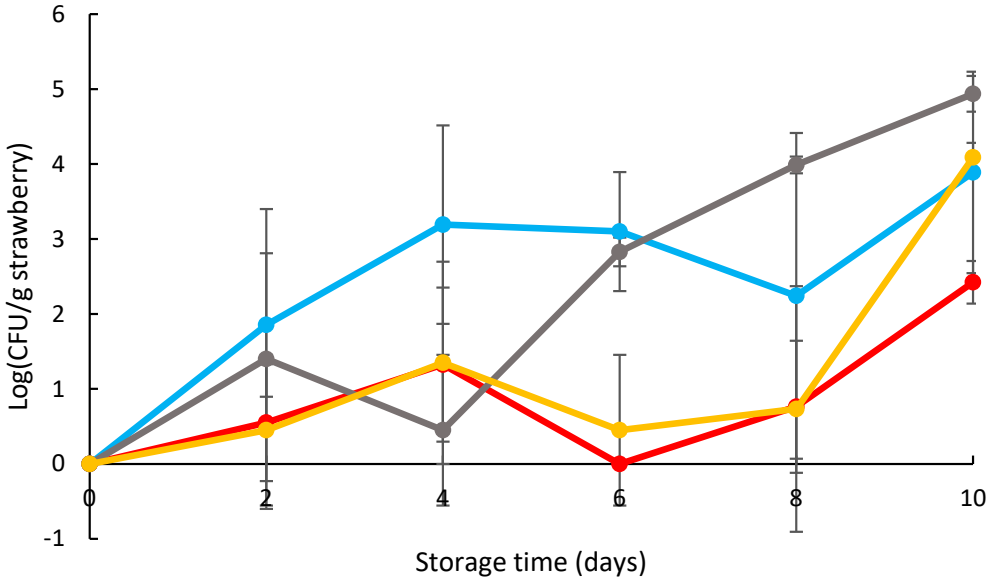

Figure 12. Psychrophilic bacteria growth in strawberries during ten days of storage using different coatings. Control—blue; EOP coating—red; *Fucus spiralis*—grey; and *Limnospira* sp. coating—yellow.

These microbial findings are consistent with decay observations (Section 3.4). The EOP formulation (B) exhibited minimal visible decay (Figure 10b), whereas the other coatings showed more pronounced spoilage. In particular, formulation C (with *Fucus spiralis* extracts) was associated with increased decay and microbial counts (Figures 10c and 11).

4. Conclusions

This study evaluated the effects of different alginate-based edible coatings enriched with bioactive extracts—olive oil pomace (EOP), *Fucus spiralis*, and *Limnospira* sp.—on the postharvest quality of strawberries during 10 days of storage at 10 °C. The coatings varied in their ability to modulate moisture loss, color stability, antioxidant activity, microbial growth, and overall fruit decay, depending on their composition.

Among all formulations, the EOP coating consistently outperformed the others across multiple parameters. It significantly reduced weight loss. Additionally, EOP coating best preserved fruit color and antioxidant activity, as evidenced by higher ABTS and DPPH values. In contrast, coatings with *Fucus spiralis* and *Limnospira* sp. provided lower protection. Their polysaccharide and protein content, while offering some antioxidant buffering, contributed to higher weight loss and suboptimal color retention. Furthermore, microbial analyses revealed that EOP-coated strawberries exhibited the least microbial growth and decay, supporting their antimicrobial effectiveness. By contrast, *Fucus spiralis* and *Limnospira* sp. coatings sometimes performed worse than the control, possibly because their nutrient-rich matrices served as substrates for microbial proliferation. Although TPC decreased in all treatments over time, coatings, especially EOP, sustained antioxidant functionality.

Therefore, while all coatings offered some degree of protection, the EOP coating emerged as the most effective in preserving physical quality, bioactivity, and microbiologi-

cal safety of strawberries over a 10-day storage period at 10 °C. These findings support the further development and commercial application of EOP-based edible coatings in postharvest fruit preservation. These coatings also have potential in microbiome modulation. Bioactive coatings, especially those containing marine or microalgal extracts, can function as prebiotics or postbiotics, highlighting a dual role in food preservation and functional food development.

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Abbreviations

The following abbreviations are used in this manuscript:

EOP	Exhausted olive oil pomace
TPC	Total phenolic content
ABTS	2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
UNEP	United Nations Environment Program
TE	Trolox equivalent
HIV	Human immunodeficiency virus
RH	Relative humidity
CIIMAR	Interdisciplinary Center of Marine and Environmental Research
MGH	Microwave hydrodiffusion and gravity
PS	Polysaccharides
CIE	Commission Internationale de l'Eclairage
PCA	Plate count agar
PDA	Potato dextrose agar
ANOVA	Analysis of variance
WL	Weight loss
SCFA	Short-chain fatty acids
PPO	Polyphenol oxidase

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