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Innovative Microalgae-Based Edible Coatings with Encapsulated Bioactives: Enhancing Fresh Raspberry Shelf Life and Quality

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Abstract: Raspberries are increasingly popular due to their high nutritional value. However, oxidative reactions, respiration, spoilage bacteria, and improper storage conditions throughout the supply chain can lead to rapid quality degradation and a short shelf life. Extending the shelf life of raspberries with minimal processing, so as not to compromise their nutritional content, physical characteristics, or sensory attributes, remains a significant challenge in the food industry. Edible coatings offer a promising solution for extending the commercial shelf life of raspberries, while enriching these coatings with encapsulated bioactive compounds can further enhance their nutritional value. The objective of this study was to develop *Chlorella vulgaris* protein-based edible coatings, enriched with encapsulated bioactive compounds from rosemary (via electrospinning), to extend the shelf life of fresh raspberries. The berries were immersed in the coating solutions and air-dried until the coatings were fully set. The shelf life of the coated raspberries was then evaluated, with samples stored at 4 °C. Key quality attributes, including color, weight loss, antioxidant activity, and spoilage microorganism levels, were monitored at predetermined time intervals. The results demonstrated that the application of *Chlorella vulgaris* protein-based coatings enriched with bioactive compounds significantly extended the shelf life of raspberries and improved their overall quality.

Keywords: edible coating; berries; shelf life; microalgae; enhanced quality

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1. Introduction

Raspberries, with their high water content and delicate nature, are particularly prone to post-harvest degradation despite their rich antioxidant and nutritional profile. Their vulnerability to external factors such as temperature fluctuations and humidity accelerates deterioration, impacting both their quality and shelf life. To address these challenges, researchers have explored innovative solutions, including the development of edible coatings. These coatings act as protective barriers, helping to maintain the freshness of berries by shielding them from environmental stressors. Studies suggest that such coatings can significantly preserve berry quality during handling and storage, potentially reducing post-harvest losses [1].

The importance of edible coatings in extending the shelf life of fruits, especially raspberries, cannot be overstated. These coatings help prevent moisture loss, microbial contamination, and other detrimental effects, offering an effective means to prolong the freshness of perishable produce [2]. Recent research has focused on enhancing the performance of these coatings by experimenting with various formulations. Utilizing natural polymers, proteins, or lipids, edible coatings present a sustainable and environmentally friendly alternative to traditional packaging materials, aligning with the growing demand for eco-conscious food preservation methods [3,4].

In the quest for biobased and sustainable alternatives, researchers are exploring microalgae-derived materials for edible coatings. Unlike traditional coatings based on polysaccharides or animal-derived proteins, this study focuses on *Chlorella vulgaris*, a microalgae-based protein source, as a sustainable and bioactive material for edible coatings. Microalgae, renowned for their biodegradability and the presence of valuable bioactive compounds, present promising alternatives for sustainable coating solutions. The proteins derived from microalgae form hydrophilic, antioxidant-rich films, offering an eco-friendly alternative to conventional synthetic food preservation methods. These natural coatings provide not only enhanced environmental sustainability, but also potential health benefits due to their bioactive properties [3]. Applying coatings made from microalgae to raspberries could effectively extend their shelf life while supporting broader sustainability goals in the food industry [4]. This innovative approach has the potential to transform raspberry preservation and contribute to a more sustainable food supply chain.

Chlorella vulgaris can be cultivated through various methods, including small-scale photobioreactors and outdoor open pond systems, each offering distinct advantages depending on the target product [5]. Photobioreactors provide controlled conditions that optimize growth parameters such as light, CO_2, and nutrient supply, making them suitable for high-value bioproducts like pigments, antioxidants, and proteins. In contrast, open pond systems are more cost-effective for large-scale biomass production, especially when the focus is on biofuel precursors, such as lipids and methyl esters [6]. Research has shown that harvesting microalgae at different growth phases significantly impacts the biochemical composition of the biomass. For instance, harvesting during the log (exponential) phase typically yields higher protein content due to active cell division and metabolic activity, while the stationary phase often correlates with increased lipid accumulation, making it more favorable for biodiesel production [7–9]. Studies have demonstrated that nutrient-deprived conditions during the stationary phase of *Chlorella vulgaris* cultivation result in higher lipid and methyl ester yields.

Despite these promising advantages, several limitations must be considered when using microalgae-based proteins for edible coatings. First, the economic feasibility of large-scale production is influenced by the costs associated with cultivation, harvesting, and processing, with harvesting alone contributing 20–30% of the total production expenses [9]. Furthermore, microalgae proteins can introduce strong flavors, odors, and colors to food products, potentially affecting consumer acceptance [10]. Managing these sensory attributes is crucial for the successful adoption of microalgae-based coatings in food applications. Additionally, the stability and quality of microalgae-based coatings can be compromised during processing, particularly with drying methods like spray drying. Addressing these challenges is vital for ensuring the successful and widespread use of microalgae-based coatings in food preservation.

The use of natural extracts in developing edible coatings to extend the shelf life of fruits has gained significant attention as a sustainable alternative. Naturally occurring substances, such as essential oils from medicinal plants and herbs, have long been utilized for various purposes, including food preservation, due to their antimicrobial properties. A novel antifungal coating, using a gelatin-based formulation with encapsulated propolis extract, has been employed to extend the shelf life of raspberries [3,11]. In this study, electrospinning technology is utilized to encapsulate rosmarinic acid in zein nanostructures, ensuring controlled release and enhanced stability of bioactive compounds within the coating. This approach represents a novel application for raspberry preservation.

This study is motivated by the growing need for sustainable solutions to extend the shelf life and improve the quality of fresh produce, especially in light of global food waste. Raspberries, being highly perishable, require innovative preservation methods to maintain their freshness, nutritional value, and safety. The use of *Chlorella vulgaris* proteins as a base for edible coatings offers a renewable, eco-friendly alternative to conventional materials. By incorporating bioactive compounds like rosmarinic acid, the coatings provide additional antimicrobial and antioxidant benefits. This research aims to explore the potential of microalgae-based proteins and bioactive compounds for enhancing fruit preservation, addressing both food waste and the demand for natural, sustainable food technologies.

2. Materials and Methods

2.1. Materials

Fresh raspberries were supplied from local markets. The materials selected for the development of the edible coatings included glycerol, Tween 20, and Chlorella vulgaris. Glycerol and Chlorella vulgaris were purchased from local markets, and Tween 20 from Sigma-Aldrich (Merck KGaA), Darmstadt, Germany.

2.2. Extraction and Characterization of Proteins from Chlorella vulgaris

Proteins were extracted using a modified aqueous extraction method with ammonium sulfate, based on Corrêa et al. (2021) [12]. Initially, 1 g of *Chlorella vulgaris* was dissolved in 18 mL of water and stirred until fully homogeneous. Sodium hydroxide (1 M) was then added dropwise until the pH reached 11. The solution was gently stirred with a magnetic stirrer for 30 min, with pH monitored and adjusted as needed. The solution was centrifuged at 2550 rpm for 10 min, and the supernatant was collected for further processing. Ammonium sulfate was gradually added to the supernatant until saturation, while stirring for 3 h. After stirring, the mixture was refrigerated for at least 8 h, then centrifuged again under the same conditions. The supernatant was collected using a pipette, filtered under vacuum, and lyophilized to a powder form, using a Freeze Dryer Tabletop Freeze Dryer BK-FD10S (BIOBASE Group, Jinan, China).

Protein determination was performed using the Bradford method [13] to assess extraction yield and method effectiveness, with a calibration curve constructed for concentration determination. To verify the protein measurements, the Kjeldahl method [14] was applied to the lyophilized protein powder. The extraction yield obtained from the Bradford method was 3.85%, while the Kjeldahl method indicated a yield of 99.65%. The protein content of *Chlorella vulgaris* typically does not exceed 58%, with higher values often requiring chemical treatments such as Trichloroacetic Acid (TCA), which are not permissible in the food industry. The lower yield from the Bradford method is due to its specificity, ensuring the presence of only proteins in both liquid and dry extracts, thus excluding other substances that may increase yield, but compromise protein purity. This specificity maintains the integrity of the protein powder for subsequent applications and analyses.

Chlorella vulgaris was incorporated into an edible coating formulation, and the process was designed to minimize waste generation. However, during the preparation of the coating material—particularly during the extraction of *Chlorella* biomass—small amounts of residual biomass and processing water were generated as byproducts. While these residues were minimal, it is important to consider their potential for valorization, especially within a circular bioeconomy framework. Recent literature [15] highlights the growing interest in converting biowastes into renewable energy sources such as hydrogen gas through methods like dark fermentation and anaerobic digestion. The nutrient-rich residues from microalgal processing, including proteins, carbohydrates, and other organics, are promising substrates for biological hydrogen production [10]. Their biodegradability and high energy content make them suitable candidates for waste-to-energy conversion processes. Future work could explore the integration of microalgal coating production with waste valorization strategies, thereby enhancing the sustainability and economic feasibility of such biotechnological applications.

2.3. Preparation of Edible Coatings and Application on Berries

The preparation of the coating followed the method outlined by Mari et al. (2024) [16]. To ensure uniform protein dispersion, a 6% *w*/*v* protein solution was prepared in deionized water under continuous stirring. The addition of glycerol (50% *w*/*w*) acted as a plasticizer, enhancing the flexibility of the resulting film, while Tween 20 (0.4% *v*/*v*) facilitated emulsification and improved the stability of the mixture. The pH was adjusted to 11 ± 0.1 with 1 N NaOH to increase protein solubility, as the high pH supports better protein dissolution in the aqueous solution. The mixture was then heated to 70 °C for 20 min to encourage crosslinking and improve the mechanical properties of the final coating.

To incorporate bioactive components, encapsulated structures produced via electrospraying were added to achieve a final concentration of 5 g of rosmarinic acid per liter of coating solution. These structures were encapsulated in zein under optimized electrospraying conditions, with a flow rate of 0.5 mL/h, a voltage of 20–20.4 kV, and a distance of 10 cm. The encapsulation process involved an ethanol–water rosemary extract solution at an 80:20 ratio, with a zein concentration of 10% *w*/*v*.

Zein is not water-insoluble, but dissolves in the coating solution due to the pH adjustment to 11. While rosmarinic acid stability is known to be pH-dependent, with optimal retention observed at more neutral pH values, at pH 11, its solubility in the protein matrix was enhanced, facilitating its uniform incorporation into the coating solution. The solubility enhancement at this pH level ensured effective encapsulation and stability of the bioactive compound in the final product.

After preparation, the berries were immersed in the coating solution for 2 min, then air-dried until the coating had fully dried onto the berries.

2.4. Evaluation of Coated Berries

The evaluation of the coated berries was based on a comprehensive set of parameters. This includes an assessment of weight loss, color change, total soluble solids, total acidity, antioxidant activity, and organoleptic properties, and an in-depth analysis of microbial inhibition, providing a holistic overview of the coating's effectiveness.

2.4.1. Weight Loss

To evaluate the impact of coating on berry shelf life, fresh and coated berries are weighed every 2 days over a 14-day period. The measurements are taken using a high-precision scale with 4-decimal accuracy. The percentage of weight loss is then calculated relative to the initial weight recorded on day 1, based on Equation (1). This approach allows for a detailed assessment of how coating affects berry weight retention throughout the storage period.

$$Weight\ Loss = \frac{W_t - W_i}{W_i}\%, \quad (1)$$

where W_t is the weight of the berries at day t (g), and W_i is the weight of the berries at day 0 (g).

2.4.2. Color Change

To determine the color of the berry samples, the CIE *L** *a** *b** color scale was used with a portable spectrophotometer (MiniScan XE, Hunter Associates Laboratory Inc., Reston, VA, USA), featuring a 4 mm measuring head. The color metrics include *L** (lightness), which ranges from 0 (black) to 100 (perfect white), a^* (indicating the balance between red and green), and b^* (indicating the balance between yellow and blue). Measurements for L^*, a^*, and b^* were taken in triplicate to ensure accuracy [17].

2.4.3. Total Soluble Solids

Total soluble solids (TSS) serve as an indicator of fruit ripeness by reflecting its sweetness. TSS is measured using a refractometer, with results expressed in degrees Brix. Measurements are taken initially and repeated 2 times for 15 days of storage, to monitor changes in fruit ripeness over time [18].

2.4.4. Total Acidity

Total acidity is a crucial indicator of fruit ripeness. Total acidity was measured through a titration method on day 0 and repeated 2 times for 15 days of storage [18]. More specifically, berry juice was diluted in deionized water with a ratio of 1:10 v/v and titrated with 0.1 M NaOH, after a small amount of phenolphthalein indicator was added. Finally, total acidity was determined through Equation (2), expressed as % citric acid.

$$Acidity(\%) = \frac{V_{NaOH} \cdot C_{NaOH} \cdot Acidity\ Factor}{V_{sample}}, \quad (2)$$

where V_{NaOH} is the volume of sodium hydroxide consumed (mL), C_{NaOH} is the concentration of sodium hydroxide (N), *Acidity factor*: 0.064 for citric acid, and V_{sample} is the volume of sample (mL).

2.4.5. Antioxidant Activity

The antioxidant activity was determined by the DPPH method of Brand-Williams et al. (1995) [19]. A DPPH solution was prepared (2.9 mg of the active substance dissolved in 100 mL of methanol) and was stirred at room temperature for 45 min in the absence of light. Then, 3.9 mL of the DPPH solution and 0.1 mL of the test sample were added to a cuvette in order to measure the absorbance in a UV-Vis spectrophotometer (UV-Vis Spectrophotometer UV-M51, BEL PHOTONICS, Monza, Italy) at a frequency of 515 nm for 20 min. During the reduction reaction, the deep purple methanolic solution decolorized and the light absorption was monitored. The free radical scavenging capacity %RSA was determined through Equation (3).

$$\%\mathrm{RSA} = \frac{1 - \mathrm{AE}}{\mathrm{AD}} \cdot 100, \quad (3)$$

where *AE* is the absorption of the antioxidant solution, and *AD* is the absorbance of DPPH sample.

Various dilutions of the sample solution were photometered to generate a calibration curve that relates the concentration to the amount of DPPH remaining. The remaining amount of DPPH (DPPHrem) was calculated using Equation (4):

$$DPPHrem(\%) = \frac{DPPH_t}{DPPH_{t=0}} \cdot 100, \quad (4)$$

The IC_{50} value (InhibitionConcentration 50%), the solution concentration at which 50% of DPPH is destroyed, is found using the calibration curve.

2.4.6. Microbial Growth

The quantitative assessment of microbial counts on coated and uncoated berry samples was conducted using a serial dilution technique, adapted from the method described by [20]. This procedure involved employing nutrient agar, Yeast and Mold agar, and eosin methylene blue (EMB) agar to quantify microorganisms on the berry samples.

The microbiological tests were performed on the samples that had the best overall results, and the limits for their risk control were chosen from the European Commission of 2012. The berries were weighed, chopped, and placed in sterile filter bags with Ringer's solution (1:9 ratio). Ringer's solution was made by dissolving a Ringer tablet (Merck KGaA, Darmstadt, Germany) in 500 mL of deionized water. The samples were then subjected to serial dilution using a Stomacher for about 1.5 min.

For the preparation, 10 g of both the coated and uncoated berries were homogenized with a sterile pestle and mortar. The homogenates were diluted with 90 mL of sterile distilled water to create a 10^{-1} dilution, with further tenfold dilutions up to 10^{-7}. Each dilution was plated onto sterile Petri dishes with sterile blank medium, following the method of [21].

Nutrient agar was sterilized by autoclaving at 121 °C for 15 min. A 1 mL aliquot from each dilution was plated, covered with 15–20 mL of sterile nutrient agar, and mixed by swirling. Similar procedures were used for detecting Yeasts and Molds.

Microbiological tests were performed on the 5th and the 8th days, with Total Count (TC) incubated at 35 ± 2 °C for 48 h (limit: 5 log CFU/mL) and Yeasts and Molds (YM) incubated at 28 ± 2 °C for 24 h (limit: 2 log CFU/mL).

To calculate them, the colonies that developed on the tablets were measured and then calculated with Equation (5).

$$Microbial\ colonies\ (\frac{\mathrm{CFU}}{\mathrm{mL}}) = \frac{CFU * dilution\ degree}{V}, \tag{5}$$

where *CFU* is the number of colonies on substrates, the degree of dilution is 10^{n}, n = 1 for the first dilution, n = 2 for the second, etc., and *V* is the volume placed on substrate (mL).

2.4.7. Sensory Evaluation

A sensory evaluation was conducted to assess the overall acceptability of the raspberry samples. A panel of 10 trained individuals, including both male and female members, participated in the sensory testing. The panelists were chosen for their experience and expertise in evaluating food products. Panel details included a balanced gender distribution and an age range of 22–45 years, and all individuals were trained in sensory analysis. Each panelist was provided with coded raspberry samples: one coated with conventional edible coating, one coated with edible coating containing bioactive compounds, and one fresh control sample. The serving order of the samples was randomized to minimize order bias, and a palate cleanser (water) was provided between samples to prevent flavor carryover. The evaluation took place in a sensory laboratory with controlled lighting and temperature conditions to ensure consistency across evaluations. Panelists rated each sample using a 9-point hedonic scale, where 1 represented "dislike extremely" and 9 represented "like extremely". The attributes evaluated included flavor, texture, color, and overall acceptability. The results were collected and subjected to statistical analysis to assess the impact of the different treatments on the sensory quality of the raspberry samples.

2.5. Statistical Analysis

A one-way and factorial analysis of variance (ANOVA) was applied in order to analyze the differences. Tukey's range test (a = 0.05) was applied, and all the statistical tests were performed with SPSS 21 software.

3. Results and Discussion

3.1. Weight Loss

One quality indicator used to study fruit's shelf life is weight loss. Fruit undergoes weight loss during preservation even when it is done under ideal conditions and reduces the overall quality of the fruit. The two physiological processes contributing to weight loss are respiration and transpiration [22]. In addition, the presence of coating contributes to less weight loss during preservation [23].

Figure 1 demonstrates the relationship between raspberry weight loss and storage time. In particular, it is evident that uncoated fresh berries exhibit a substantial increase in weight loss as storage time progresses. In contrast, raspberries coated with either a standard coating or one enriched with bioactive compounds show a significantly lower weight loss over time. Notably, there is no substantial difference between the berries coated with or without bioactive substances. This indicates that the application of the coating itself contributes to better preservation and an extended shelf life of the raspberries.

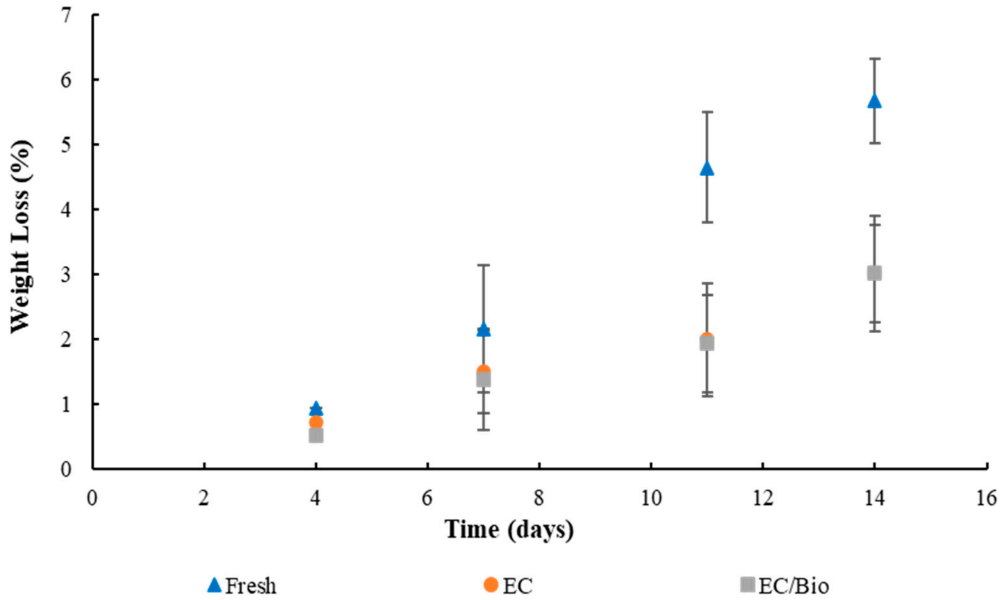

Figure 1. Weight loss of raspberries in 14 days.

Similar results were observed by Han et al. (2004) [24], where chitosan-based coatings reduced weight loss in red raspberries stored at 2 °C for 21 days. The protective barrier provided by the coatings in this study, whether enhanced with bioactive compounds or not, appears to be the primary factor in reducing moisture loss, leading to improved preservation.

Seaweed-derived protein coatings have demonstrated superior performance in maintaining the quality of raspberries compared to other coating materials. The hydrophilic nature of algal proteins facilitates the formation of strong, moisture-retentive barriers, which help to slow moisture loss, contributing to an extended shelf life of the fruit [18]. These coatings help to reduce transpiration, maintain the fruit's weight, and regulate respiration, further supporting their role in weight retention during storage.

Alginate coatings, derived from seaweed, are particularly effective in creating a barrier against oxygen, which also helps to slow down oxidative processes that could contribute to weight loss. Compared to other edible coatings, such as those based on polysaccharides like cellulose or proteins like whey and soy, seaweed-derived coatings excel in moisture retention due to their superior film-forming properties.

While coatings enriched with bioactive compounds have been explored for enhanced preservation, the difference in weight loss between bioactive and non-bioactive coatings is minimal over time. This suggests that the primary benefit in terms of weight retention comes from the moisture barrier provided by the coating, rather than the inclusion of bioactive compounds. Regardless, the application of these coatings—whether enriched with bioactive substances or not—significantly extends the shelf life of raspberries by reducing weight loss during storage.

3.2. Color Change

The color is a decisive factor for the freshness of the product and for consumer acceptance, as it indicates the ripening stage of the fruit. The results for the color variation, based on the *L**, *a**, and *b** indices of fresh raspberries, edible-coated raspberries, and, finally, those with edible coating and bioactive substance are presented below.

Figure 2 demonstrates the color change of the raspberries up to the 14th day of storage as measured by the *L** parameter (which represents lightness, where 0 is black and 100 is white). The *L** values do not present major changes over time, but there is a slight tendency for a decrease, indicating a gradual darkening of the raspberries during the storage period. This slight reduction in lightness suggests that as raspberries ripen and begin to age, their overall brightness diminishes, although the effect is subtle.

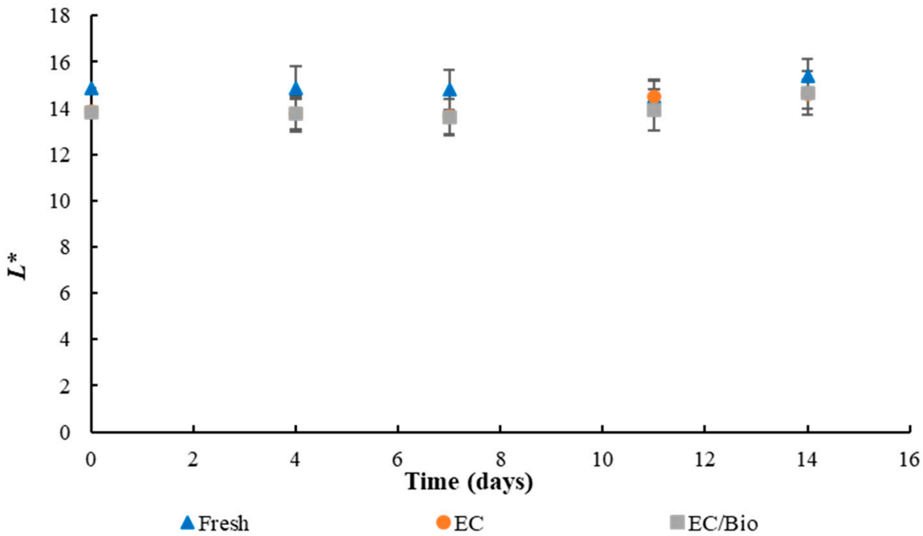

Figure 2. Parameter *L* of the color change of raspberries over a period of 14 days.

Figure 3 presents the changes in the *a** parameter, which measures the red–green color spectrum, with positive values indicating red. The *a** values also present a slight decrease during the preservation period across all treatments, especially for raspberries that were coated with and without the addition of bioactive substances. The gradual decline in the *a** parameter indicates a loss of redness in the raspberries, meaning they become less vibrant in terms of their red coloration over time. This is in line with typical ripening and degradation processes in fruits, where anthocyanins, the pigments responsible for red color, degrade or change structure as the fruit ages.

Similarly, Figure 4 displays the variation in the *b** parameter, which measures the blue–yellow spectrum, with positive values representing yellow. The *b** parameter tends to decrease until the 11th day of storage, followed by a slight increase on the 14th day. The decrease in *b** suggests a loss of yellow tones and a slight shift towards the blue spectrum during the earlier stages of preservation, which may correspond to the accumulation of secondary pigments or the degradation of carotenoids, which contribute to yellow hues.

The small increase in b^* on the 14th day might suggest a slight rebalancing of the color spectrum as the raspberries further age, although the changes are relatively minor.

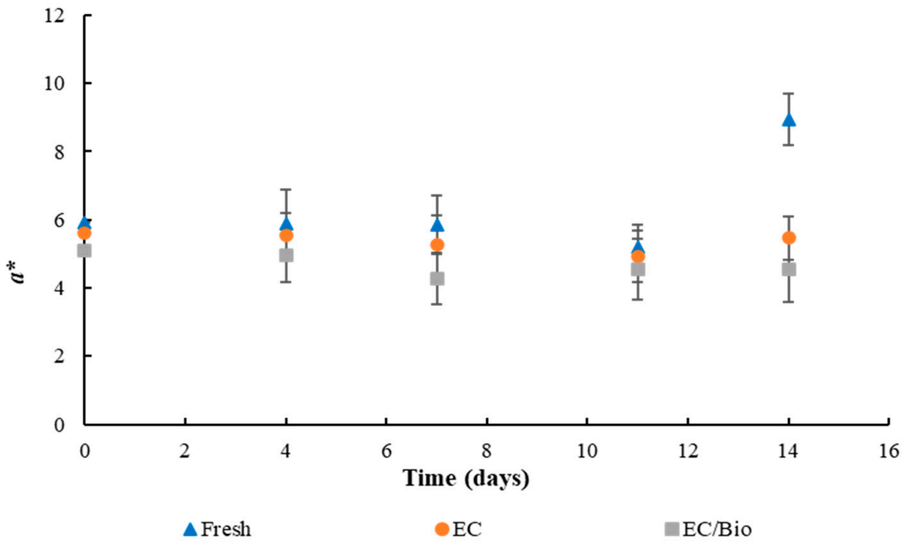

Figure 3. Parameter *a* of the color change of raspberries over a period of 14 days.

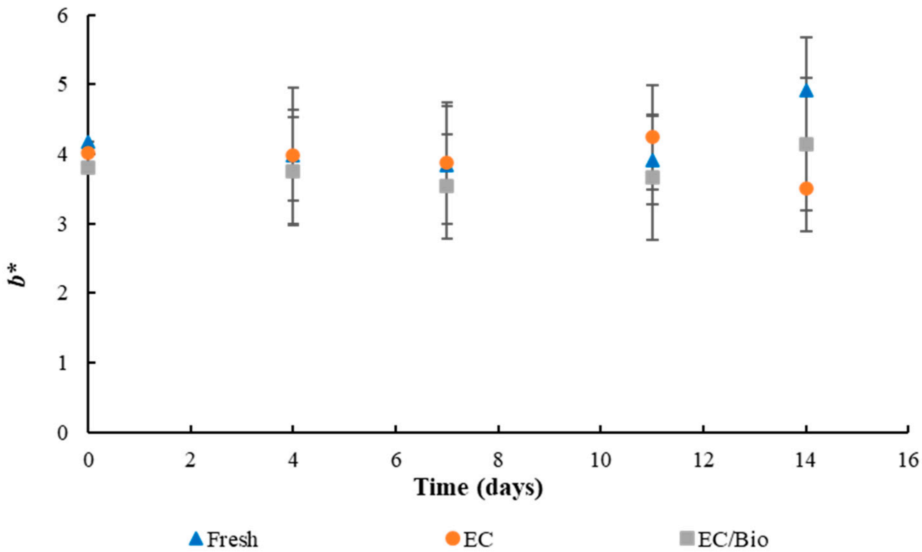

Figure 4. Parameter *b* of the color change of raspberries over a period of 14 days.

From the observations in Figures 3–5, it can be concluded that raspberries exhibit a gradual shift in color as they ripen during storage, becoming less red and more blue over time, and their brightness (L^*) decreases slightly. These changes align with natural ripening processes, where the breakdown of pigments, particularly anthocyanins, results in color shifts from red towards blue or purple tones. The reduction in brightness is likely due to moisture loss and pigment degradation, which are common in fruits undergoing aging and prolonged preservation.

This behavior is particularly notable in coated raspberries, including those coated with a bioactive substance, as they still undergo color changes, albeit at a reduced rate. The coating helps maintain the structural integrity and delays some of the visual signs of ripening, but does not completely stop the natural progression of these changes. Coated raspberries, while experiencing less drastic changes in the a^* (red) and b^* (blue–yellow) parameters compared to uncoated raspberries, still follow the general trend of becoming less red, more blue, and slightly darker over time.

Overall, the use of coatings appears to slow down the visual changes associated with raspberry ripening, such as color degradation and darkening, although it cannot entirely prevent them. This suggests that while edible coatings offer benefits in extending the shelf life and quality of raspberries, they do not entirely stop the natural processes that occur during prolonged storage [25–27].

3.3. Total Soluble Solids (TSS)

The total soluble solids (TSS) value affects the taste and increases over time as the fruit ripens and its sugar content increases. The variation in total soluble solids for the three raspberry samples is shown in Figure 5.

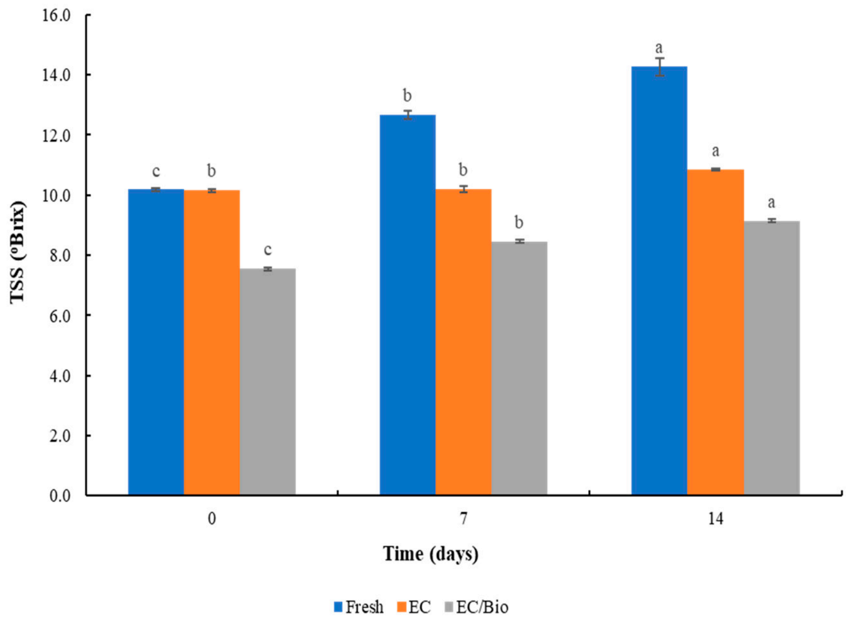

Figure 5. Total soluble solids of raspberries over a period of 15 days (different letters indicate significant difference $p < 0.05$).

Fresh (uncoated) berries exhibit a significant increase ($p < 0.05$) in TSS during storage from 10.2 ± 0.1 to 14.3 ± 0.3. This increase can be attributed to the ongoing metabolic respiration process, in which oxygen and soluble solids, particularly sugars, are consumed to generate energy. During respiration, the berries consume sugars to generate energy, but in the absence of a coating, moisture loss accelerates, leading to an increase in the concentration of total soluble solids (TSS). The reduction in fruit mass due to water loss results in a higher concentration of sugars and other soluble solids, causing the TSS to increase over time [28]. This increase in TSS is more pronounced in uncoated berries, as they are more exposed to environmental factors like oxygen, which can accelerate the respiration process and, subsequently, the loss of moisture.

The observed increase in TSS for fresh fruit during storage can, therefore, be linked to the high metabolic activity of the fruit following harvest. As the berries ripen and begin to age, their metabolic rate remains elevated, particularly in the absence of a coating that could slow down these processes. While respiration continues, moisture loss accelerates, leading to the concentration of sugars, organic acids, and other soluble solids within the fruit, which increases the TSS [29]. This is a typical response in fresh berries as they progress through the later stages of post-harvest ripening, where the loss of water causes an increase in the concentration of soluble solids rather than a reduction.

In contrast, raspberries with edible coatings maintain their TSS at more stable levels throughout storage (from 10.2 ± 0.1 to 10.9 ± 0.1). Edible coatings function as effective barriers to moisture and gas exchange, which helps slow the rate of water evaporation from the berries. This moisture retention preserves the sugar content, thereby delaying the degradation of soluble solids and contributing to a longer shelf life and improved overall fruit quality. As observed in Figure 1, the TSS of the coated berries did not show a significant increase ($p < 0.05$) over the course of 14 days of storage.

Raspberries with bioactive coatings, on the other hand, exhibited a significant ($p < 0.05$) increase in TSS during storage, although the increase was lower compared to the fresh uncoated samples (from 7.6 ± 0.1 to 9.2 ± 0.1). This reduced increase in TSS can be attributed to the concentration of soluble solids due to minimized moisture loss facilitated by the coating [22,30]. The slight rise in TSS values is consistent with the protective effect of the coating, which reduces water loss and slows down the metabolic processes that would typically contribute to a reduction in soluble solids.

The application of edible coatings effectively maintained the TSS levels of raspberries in a stable range throughout the storage period. While bioactive coatings also showed an increase in TSS, the increase was less than that observed in fresh berries, and it did not significantly enhance the preservation of TSS compared to the non-bioactive coatings. This suggests that while the bioactive compounds may provide some benefits in terms of preserving the fruit's overall quality, they do not offer additional advantages in terms of TSS preservation.

3.4. Total Acidity (TA)

The total acidity (TA) of the raspberries is shown in Figure 6.

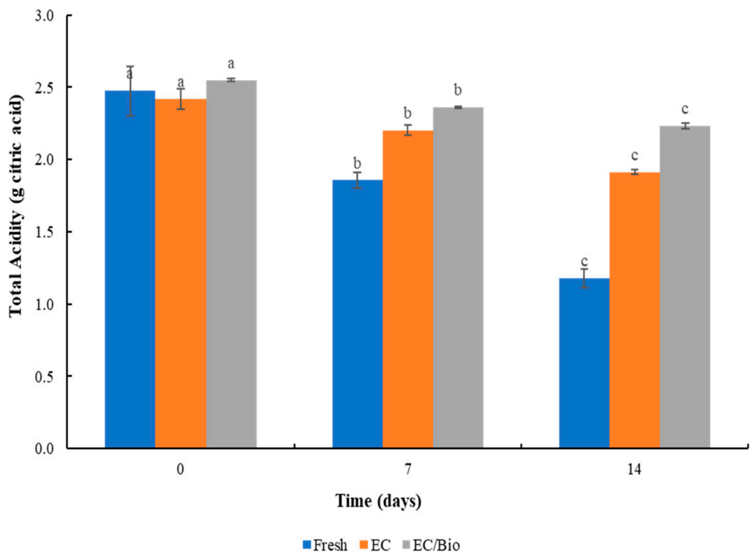

Figure 6. Total acidity of raspberries over a period of 15 days (different letters indicate significant difference $p < 0.05$).

Total acidity in raspberries typically decreases over time during storage. The decline is attributed to the consumption of organic acids during respiration and their conversion into new compounds via metabolic pathways, particularly the citric acid cycle [22,29]. As raspberries ripen, organic acids such as citric and malic acid are broken down, leading to a reduction in TA [30].

In the case of fresh (uncoated) berries, the decline in TA was most pronounced, with the value decreasing significantly from 2.47 ± 0.17 to 1.18 ± 0.06. This sharp reduction can be explained by the high respiration rates in uncoated berries, which accelerate the breakdown of organic acids [26]. Without the protective barrier provided by a coating, fresh berries are more susceptible to environmental factors such as oxygen exposure, further promoting metabolic processes that deplete organic acids.

Raspberries coated with edible films exhibited a more gradual decrease in total acidity (TA) compared to fresh (uncoated) berries. Specifically, the TA of berries with a plain edible coating ranged from 2.42 ± 0.07 to 1.91 ± 0.02, while berries with bioactive coatings showed a slightly higher preservation, from 2.55 ± 0.01 to 2.23 ± 0.02. Edible coatings act as barriers, reducing respiration rates and limiting exposure to oxygen, which helps preserve the organic acids and slows their degradation. While a decrease in TA is still observed, it is significantly less than that seen in fresh berries, highlighting the protective effects of the coatings.

This observed stability in TA can be attributed to the bioactive properties of the coatings. Beyond providing a physical barrier, bioactive coatings often contain antioxidant and antimicrobial compounds that actively contribute to the preservation of fruit quality. These compounds help to delay the degradation of organic acids and other bioactive substances, maintaining the acidity of the fruit over extended periods of storage.

Notably, coatings derived from proteins of the microalga *Chlorella vulgaris* have demonstrated particularly strong effectiveness in preserving the TA of berries [18]. The hydrophilic nature of *Chlorella vulgaris* proteins allows for the formation of a durable, moisture-regulating film that reduces gas exchange and prevents excessive moisture loss. This barrier helps protect the fruit from oxidative and metabolic processes, thereby preserving its structural and chemical integrity, including its acidity.

Therefore, the application of edible coatings, particularly those enriched with bioactive properties, results in minimal changes in TA and better overall preservation of the berry. By slowing down the metabolic consumption of organic acids and providing a protective barrier against environmental factors, these coatings help maintain the freshness and shelf life of raspberries. The differential decline in TA between fresh, edible-coated, and bioactive-coated berries further emphasizes the effectiveness of these coatings in maintaining the key quality attributes of raspberries during storage.

3.5. Antioxidant Activity

The antioxidant capacity of raspberries can be expressed by a parameter called IC_{50}, which indicates the concentration of the extract required to capture 50% of free radicals. More specifically, the IC_{50} value is related to the antioxidant capacity of the fruit, and the lower the value, the higher the antioxidant capacity [31]. The results are presented in Figure 7.

As shown in Figure 7, the antioxidant capacity of fresh raspberries significantly increased ($p < 0.05$) during the storage period, from 0.492 ± 0.019 to 0.677 ± 0.018. This increase in antioxidant capacity during the initial stages of preservation can be attributed to the natural antioxidants present in the fresh fruit, such as vitamin C, anthocyanins, and other polyphenolic compounds. However, these compounds are prone to degradation during storage, particularly in the early stages, as the fruit undergoes metabolic processes and oxidative stress.

In contrast, raspberries with edible coatings, both plain and bioactive, maintained a more stable antioxidant capacity over the 14-day storage period. Specifically, the antioxidant capacity of raspberries with plain edible coatings remained stable, showing a slight reduction from 0.417 ± 0.005 to 0.441 ± 0.008. On the other hand, raspberries coated with bioactive substances exhibited a decrease in antioxidant capacity from 0.096 ± 0.001 to 0.073 ± 0.002. The stability observed in these coatings can be attributed to the protective barrier they form, which reduces oxidative stress by limiting exposure to oxygen and moisture loss. This helps preserve the antioxidant compounds in the fruit.

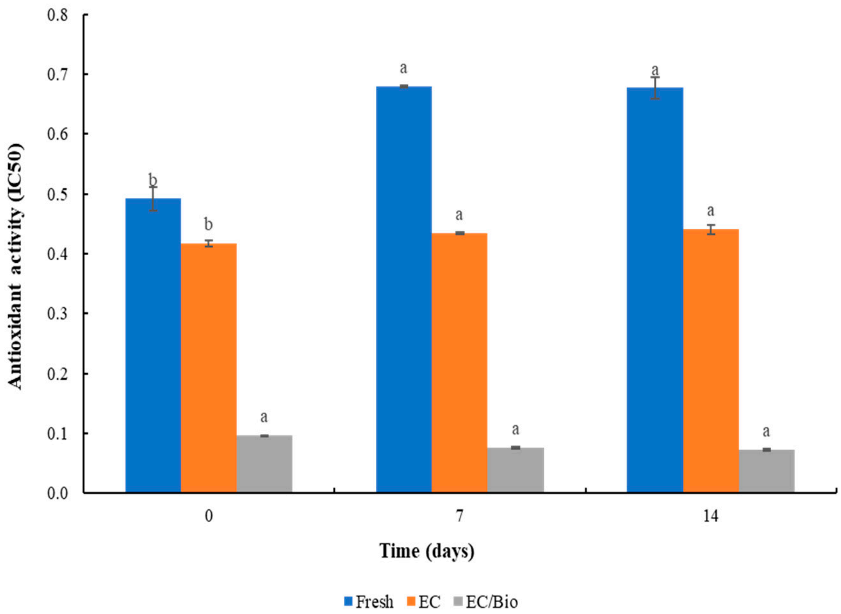

Figure 7. Antioxidant activity of raspberries over a period of 15 days (different letters indicate significant difference $p < 0.05$).

Notably, bioactive coatings containing natural antioxidants or antimicrobial agents actively contribute to enhancing the fruit's antioxidant capacity. These bioactive compounds are incorporated into the coating matrix, which not only protects the fruit from environmental stressors, but also directly improves its ability to neutralize free radicals and resist oxidative damage. The bioactive coatings demonstrated a decreased IC_{50} value, indicating an improved antioxidant activity from the beginning of the storage period, compared to uncoated fresh raspberries.

Additionally, the proteins derived from *Chlorella vulgaris*, a microalga known for its bioactive properties, further contribute to the antioxidant activity of the coatings. These proteins, with their hydrophilic nature, help to form a robust and stable film on the surface of the fruit, reducing oxidative damage by controlling the moisture and gas exchange. This protective layer helps maintain the integrity of the fruit's natural antioxidant compounds, enhancing the overall antioxidant capacity of the coated raspberries.

These findings align with previous studies that report the positive effects of edible coatings, such as Aloe Vera gel and seaweed extracts, on the antioxidant properties of coated fruits [18,23,29]. For example, Aloe Vera gel, known for its bioactive compounds, not only slows down moisture loss and delays ripening, but also boosts the antioxidant defense system of the fruit. Similarly, seaweed-derived coatings, particularly those from *Chlorella vulgaris* and *Laminaria*, are rich in natural antioxidants like phenolic compounds, carotenoids, and vitamins, which contribute to enhanced antioxidant activity and prolonged shelf life.

In conclusion, edible coatings, especially those containing bioactive compounds such as *Chlorella vulgaris* proteins and other natural bioactive substances, provide a dual benefit: They protect the fruit from environmental factors while simultaneously enhancing the fruit's antioxidant capacity. The improved antioxidant activity and decreased IC_{50} observed in raspberries with bioactive coatings demonstrate the effectiveness of these coatings in preserving and even enhancing the nutritional and functional qualities of the fruit during storage. This makes bioactive coatings a promising strategy for extending the shelf life of raspberries while maintaining or improving their health benefits.

3.6. Microbial Growth

Microbiological analysis was performed on all three raspberry samples, and the growth of Yeast and Mold (YM), Escherichia Coli (ECand Total Count (TC) microorganisms were studied. Table 1 presents their growth on the 14th day of storage.

Table 1. Total Count and Yeasts and Molds of raspberries over a period of 8 days (different letters indicate significant difference $p < 0.05$).

Sample	Total Count log (CFU/g)	Yeasts and Molds log (CFU/g)
Fresh	6.72 ± 0.12 [a]	6.60 ± 0.03 [a]
EC	4.42 ± 0.02 [b]	3.87 ± 0.03 [b]
EC/Bio	4.37 ± 0.02 [b]	3.78 ± 0.03 [b]

Mean value ± standard deviation (sdv). Different letters indicate statistically significant differences ($p < 0.05$).

The accepted microbial growth limit from the literature for safe consumption is 5 log CFU/mL for Total Counts (TC) and 3 log CFU/mL for Yeast and Mold (YM). Raspberries exceeding these thresholds are considered unsafe for consumption [32]. Microbiological analysis over the storage period revealed no *E. coli* growth in any of the raspberry samples, indicating that both the coated and uncoated preservation methods were effective in preventing *E. coli* contamination throughout the storage period, ensuring the safety of the fruit from this pathogen.

As shown in Table 1, the YM growth in the coated raspberry samples remained below the permissible 4 log CFU/g threshold, unlike in fresh raspberries. Notably, the raspberries coated with an edible coating containing bioactive compounds presented the lowest levels of YM growth, demonstrating that the inclusion of the bioactive compound contributed to more effective suppression of *YM* growth. This suggests that the bioactive coating, which includes antimicrobial agents like rosmarinic acid, provided additional protection against microbial activity, helping to preserve the microbiological quality of the fruit.

The antimicrobial properties of rosmarinic acid, known for its strong ability to disrupt bacterial and fungal cell membranes, significantly enhanced the bioactive coating's effectiveness. This, in turn, helped to inhibit the growth of Yeast and Mold, ensuring the raspberries remained microbiologically safe throughout the preservation period [33].

Regarding Total Counts (TC), the growth values for the coated raspberries remained below the 5 log CFU/g threshold, unlike fresh raspberries. The faster increase in microbial load for fresh raspberries can be attributed to the lack of a protective barrier, which allowed bacteria to proliferate more rapidly. However, there was no significant difference between the plain edible-coated raspberries and the bioactive-coated raspberries in terms of TC growth.

Based on these results, the bioactive coating containing rosmarinic acid was shown to be highly effective in suppressing both TC and YM growth to levels well below the safety thresholds. The bioactive compound not only enhanced the antimicrobial capacity of the coating, but also helped extend the shelf life of raspberries by preventing the excessive growth of harmful microorganisms. These findings suggest that bioactive coatings can be a valuable strategy for improving the microbiological safety of fresh produce, allowing raspberries to be stored for longer periods without compromising their safety for consumption.

In conclusion, the presence of rosmarinic acid in the bioactive coating significantly improved its antimicrobial efficacy. By inhibiting the growth of Total Counts and Yeasts and Molds to levels well below the permissible limits, the bioactive coating ensures that raspberries remain microbiologically safe for a longer period. This makes bioactive coatings a promising solution for extending the shelf life of perishable fruits like raspberries, ensuring their safety and quality for extended consumption.

3.7. Sensory Evaluation

Sensory evaluation is crucial as it provides insight into the quality and consumer acceptability of food products by directly assessing attributes like taste, texture, appearance, and aroma. Table 2 presents the evaluation of fresh and treated raspberries, highlighting differences in sensory characteristics such as color, flavor, sweetness, tartness, and texture. These variations can result from treatment methods affecting attributes like firmness, flavor intensity, and overall appeal. Understanding these changes helps in refining processing techniques to retain the natural qualities of raspberries, ensuring that the treated products meet consumer expectations. Figure 8 presents the total acceptance and aftertaste of raspberries.

Table 2. Sensory analysis of raspberries (different letters indicate significant difference $p < 0.05$).

	Fresh	**EC**	**EC/Bio**
Color	7.71 ± 0.70^{a}	7.84 ± 0.83^{c}	$8 \pm 1.77^{a,b}$
Hardness	5.71 ± 1.16^{a}	6.14 ± 1.46^{c}	6.29 ± 1.58^{b}
Astringent taste	$3.00 \pm 1.77^{a,b}$	3.00 ± 1.85^{a}	2.71 ± 1.48^{b}
Sweet taste	4.71 ± 1.58^{b}	$4.00 \pm 1.77^{a,b}$	$4.71 \pm 1.28^{a,b}$
Sour taste	5.43 ± 2.32^{a}	6.00 ± 1.07^{a}	5.57 ± 1.18^{a}
Bitter taste	1.29 ± 0.45^{a}	1.43 ± 0.49^{a}	1.71 ± 1.03^{a}
Aroma intensity	8.20 ± 1.16^{c}	7.60 ± 1.01^{a}	$6.80 \pm 0.97^{a,b}$

Mean value ± standard deviation (sdv). Different letters indicate statistically significant differences ($p < 0.05$).

The application of edible coatings was specifically designed to preserve the natural organoleptic characteristics of the raspberries, including taste, texture, appearance, and aroma. This is crucial in ensuring that the fruit maintains its appealing sensory properties throughout storage.

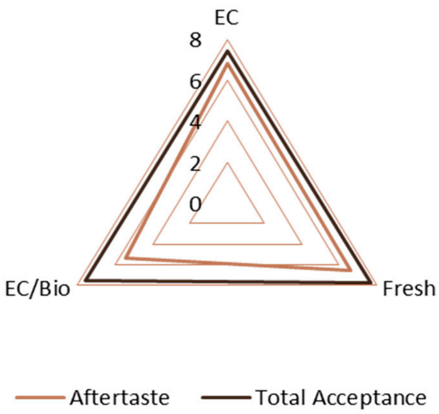

Figure 8. Total acceptance and aftertaste of raspberries.

The analysis of the results showed that there were no significant statistical differences between the three raspberry samples in terms of their organoleptic characteristics. Organoleptic attributes, which include taste, texture, appearance, and aroma, are critical factors in determining consumer acceptability of the fruit. In this study, it was found that the presence of an edible coating, whether with or without a bioactive compound, did not significantly affect these sensory properties of the raspberries. This suggests that the application of coatings, including bioactive ones, maintained the raspberries' natural qualities without imparting any negative sensory changes.

The organoleptic evaluation revealed that the raspberries retained their characteristic texture and flavor throughout the preservation period, regardless of the treatment they underwent. The coatings used, particularly those containing bioactive substances, were designed to protect the fruit without compromising its natural sensory attributes. This is crucial in ensuring consumer satisfaction, as alterations to taste, texture, or appearance could reduce the overall acceptability of the fruit. The fact that no significant differences were detected in organoleptic properties indicates that the coatings were successful in preserving the raspberries without negatively influencing the consumer experience.

One of the key findings was that the edible coatings created an effective protective barrier, which not only prevented dehydration and texture degradation, but also helped retain the raspberries' natural moisture content and crispness. This ensured that the raspberries remained firm and fresh throughout the storage period, maintaining their appealing texture and juiciness. The coatings' ability to shield the fruit from environmental factors, such as moisture loss and oxidation, was essential for extending shelf life, while simultaneously preserving the fruit's natural flavor profile and overall sensory appeal. This protection was critical in preventing any undesirable alterations, thereby maintaining the raspberries' inherent qualities and ensuring that they remained highly attractive to consumers.

Finally, the overall acceptability of the berries (Figure 8), as evaluated by sensory panels, was not altered by the application of edible coatings or bioactive substances. The evaluation showed that there was no significant difference in consumer preference or acceptability between the three raspberry samples—whether they were fresh, coated, or coated with bioactive compounds. This consistency in consumer acceptability is a positive outcome, demonstrating that the coatings effectively extended the shelf life and protected the fruit while maintaining the qualities that make raspberries appealing to consumers.

The results suggest that edible coatings, particularly those enriched with bioactive compounds like rosmarinic acid, can be used to extend the shelf life of raspberries and improve their preservation without negatively affecting their organoleptic properties. This makes bioactive coatings a promising technology for the fresh produce industry, as they provide functional benefits, such as antimicrobial protection and enhanced shelf life, without compromising the taste, texture, or overall consumer appeal of the fruit.

In conclusion, the analysis demonstrated that the presence of the coating and bioactive compounds did not significantly affect the organoleptic characteristics of the raspberries. The coatings were effective in preserving the fruit without altering its natural sensory qualities, and the overall acceptability of the berries remained consistent across all samples. This highlights the potential of bioactive coatings as an effective preservation technique that maintains the fruit's sensory integrity while offering added protection against spoilage.

4. Conclusions

In conclusion, this study successfully developed and applied innovative edible coatings derived from *Chlorella vulgaris* proteins, demonstrating their effectiveness in extending the shelf life of raspberries. The coatings provided a protective barrier, significantly reducing oxidation, moisture loss, and volatile compound transfer, thereby maintaining the fruit's freshness throughout the storage period. The incorporation of bioactive compounds, particularly rosmarinic acid, further enhanced the coatings' abilities to inhibit microbial growth and provide antioxidant benefits.

Over a 14-day storage period, coated raspberries exhibited a significantly lower weight loss (approximately 3%) compared to fresh, uncoated berries, which lost nearly twice as much weight. The total soluble solids (TSS) increased in the coated raspberries, while it decreased in fresh fruit, likely due to the higher metabolic activity in uncoated berries. Similarly, although total acidity (TA) declined in all samples, coated raspberries—especially those with bioactive compounds—retained more acidity, indicating a slower ripening process. This highlights the protective effect of the coatings in prolonging freshness.

The antioxidant capacity, measured via DPPH assays, was notably enhanced in the coated raspberries, particularly those with rosmarinic acid, by day 14 of storage. The incorporation of rosmarinic acid into the coating helped not only maintain, but also improve the antioxidant activity, thereby enriching the fruit's overall nutritional profile. Microbiological testing confirmed that uncoated raspberries became unsafe for consumption after 14 days due to microbial growth, whereas coated raspberries, particularly those with bioactive compounds, remained microbiologically safe beyond 14 days, highlighting the importance of rosmarinic acid's antimicrobial properties.

Organoleptic evaluations revealed no significant differences in taste, texture, or overall acceptability between coated and fresh raspberries, confirming that the coatings did not negatively affect the sensory qualities of the fruit.

In summary, the findings of this study demonstrate that edible coatings, particularly those containing bioactive compounds like rosmarinic acid, can effectively extend the shelf life of raspberries by reducing weight loss, slowing ripening, enhancing antioxidant content, and inhibiting microbial growth. These coatings maintain the sensory integrity of the fruit while improving its nutritional and safety attributes, offering a viable solution for enhancing the preservation of fresh produce.

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Abbreviations

The following abbreviations are used in this manuscript:

TSS	Total Soluble Solids
TA	Total Acidity
NaOH	Sodium Hydroxide
% RSA	Free Radical Scavenging Capacity
DPPH	2,2-Diphenyl-1-picrylhydrazyl
IC_50	Half Maximal Inhibitory Concentration
EMB	Eosin Methylene Blue
TC	Total Count
YM	Yeasts and Molds
E. coli	Escherichia coli
CFU	Colony-Forming Unit
ANOVA	One-Way and Factorial Analysis of Variance
SPSS	Statistical Package for the Social Sciences

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