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Extension of Blueberry Shelf-Life with Edible Coatings from Chlorella Vulgaris

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Berries are fruits with a particularly high nutritional value and content of bioactive components, which leads to an ever-increasing demand. However, their supply is hampered by their seasonality and sensitivity while being transported and stored. Typically, oxidative reactions, contamination by pathogenic microorganisms, bacteria that induce spoiling, respiratory processes, and poor storage conditions lead to degradation. In the food sector, it is still very difficult to extend the shelf life of berries while retaining their nutritional composition, physical properties, and appealing appearance. Edible coating is an emerging food protection procedure, as it can increase the shelf life of berries, is a sustainable process, and can be safely consumed as part of the product. The aim of this study is to investigate the effect of edible coatings developed components derived from microalgae (Chlorella vulgaris) on the shelf life of blueberries and compare them with conventional ones. Polysaccharides and proteins were extracted from Chlorella vulgaris and were incorporated into a membrane system that also contained plasticizers and other elements The creation of conventional membranes involved the use of chitosan and potato starch. The created coatings were applied to the berries, which were then analysed for colour, weight loss, content of Total Soluble Solids, Total Acidity, Antioxidant Activity and microbiological growth in order to establish the shelf-life of the product. The coatings made from algae extracts, according to the results of all tests, produced products with excellent quality and long shelf lives.

1. Introduction

Berries are known for their high-water content and delicate character, which makes them vulnerable to post-harvest degradation despite their antioxidant and nutritional richness. Berries' susceptibility to external elements, like temperature changes and humidity, can hasten berry deterioration and lower their quality and shelf life. In order to overcome this sensitivity, researchers have looked for creative solutions, and the creation of edible coatings is one such technique. When edible coatings are placed to berries, they reduce sensitivity and maintain the freshness of the fruit by functioning as a shield against outside factors. According to studies, these coatings may be able to preserve the quality of berries throughout handling and storage, which may assist lower post-harvest losses (Horvitz, 2016).

It is impossible to exaggerate the role edible coverings play in the preservation of fruits, especially berries. These coatings provide a barrier against moisture loss, microbial contamination, and other harmful elements; therefore, they are an efficient way to prolong the shelf life of perishable goods. The creation of edible coatings has attracted a lot of interest lately, and scientists are experimenting with different compositions to maximize their effectiveness. Edible coatings, which utilize natural polymers, proteins, or lipids, offer a sustainable and environmentally beneficial substitute for conventional packaging materials. This is in line with the increasing desire for food preservation techniques that are considerate of the environment (Nunes et al., 2023).

In an effort to find biobased and sustainable substitutes, scientists are looking at using materials obtained from microalgae to create edible coatings. Microalgae are intriguing possibilities for environmentally friendly coating solutions because of their special qualities, which include their capacity for biodegradability and the creation of useful chemicals. The use of substitute coatings derived from microalgae offers a promising approach to prolonging the shelf life of berries. In addition to addressing berries' sensitivity to environmental influences, this strategy supports the larger objective of encouraging sustainable practices in the food sector. These cutting-

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edge coatings have the potential to transform berry preservation and enhance the general sustainability of the food supply chain as long as they are investigated and improved (Madadi et al., 2021).
The scope of this work is to develop edible coatings from alternative sources, such as *Chlorella vulgaris*, and conventional sources and apply them on blueberries, in order to extend their shelf-life, intensify their characteristics and increase their nutritional value.

2. Materials and Methods

2.1 Materials

The materials used for the development of edible coatings were chitosan, starch, glycerol, Tween 20 and *Chlorella vulgaris*. Chitosan, starch, glycerol and *Chlorella vulgaris* were purchased from local markets and Tween 20 from Sigma-Aldrich (Steinheim, USA).

2.2 Preparation of edible coatings and application on berries

The extraction of proteins and starch from Chlorella vulgaris was conducted following Corrêa et al. (2021).and Wong et al. (2019), accordingly. The formulation of chitosan coating was based on Han et al., (2014) and contained chitosan, acetic acid, glycerol and Tween 20. The formulation of starch coating contained starch (conventional or extracted from *Chlorella vulgaris*), pectin and glycerol. Finally, the protein solution was based on Bamdad et al. (2006) and contained protein, glycerol and Tween 20, with pH 11.
After the preparation of the solutions, the berries were immersed in each solution for 2 min and afterwards, they were air dried, until the coating was dried fully on the berry.

2.3 Evaluation of coated berries

Weight Loss

Fresh and coated berries are weighed every 2 days for 21 days, with a 4-decimal precision scale and the percentage of the weight loss is calculated based on the weight of day 1.

Total Soluble Solids

Total soluble solids (TSS) are measured through the sweetness of the food, which indicates the degree of ripeness of the fruit. A refractometer is used to measure the TSS and they are measured in Brix degrees. The measurements are repeated every 7 days, until 21 days, in order to study the ripening of the fruit.

Total Acidity

The total acidity of foods is also an important indicator of ripeness. Total acidity is measured through a titration method. More specifically, berry juice is diluted in deionized water with a ratio of 1:10 v/v and titrated with 0.1M NaOH, after a small amount of phenolphthalein indicator has been added. Finally, total acidity is determined through Eq (1) expressed as % citric acid.

$$Acidity\ (\%) = \frac{V_{NaOH}\ \cdot C_{NaOH} \cdot Acidity\ Factor}{V_{sample}} \quad (1)$$

Where: V_{NaOH}: volume of caustic soda consumed (ml), C_{NaOH}: the concentration of caustic soda (N), Acidity factor: 0.064 for citric acid, V_{sample}: volume of sample (ml).

Antioxidant Activity

The antioxidant activity is determined by the DPPH method of Brand-Williams et al. (1995). A DPPH solution is prepared (2.9 mg of the active substance dissolved in 100 mL of methanol) and is 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 are added to a cuvette, in order to measure the absorbance in a UV-Vis spectrophotometer (UV-Vis Spectrophotometer UV-M51, BEL PHOTONICS) at a frequency of 515 nm for 20 min. During the reduction reaction the deep purple methanolic solution decolorizes and the light absorption is monitored. The free radical scavenging capacity %RSA is determined through Eq (2).

$$\%\mathrm{RSA} = \frac{1 - \mathrm{AE}}{\mathrm{AD}} \cdot 100 \quad (2)$$

Where: AE: the absorption of the antioxidant solution, AD: absorbance of DPPH sample.
Various dilutions of the sample solution are photometered to generate a calibration curve that relates the concentration to the amount of DPPH remaining. The remaining amount of DPPH (DPPHrem) is calculated using Eq (3):

$$DPPHrem\ (\%) = \frac{DPPH_t}{DPPH_{t=0}} \cdot 100 \quad (3)$$

The IC50 value (InhibitionConcentration 50%), the solution concentration at which 50% of DPPH is destroyed, is found using the calibration curve.

Microbial growth

The microbiological tests were carried out 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 measurement was carried out on the 14th day. For Total Count the sample was incubated at 35ºC for 48 h and its limit is 105 CFU/mL. and for, E. Coli bacteria, the sample was incubated at 35ºC for 24 h and its limit is 100 CFU/mL.
To calculate them, the colonies that developed on the tablets were measured and then calculated with Eq (4).

$$\frac{CFU}{mL} = \frac{CFU * dilution\ degree}{V} \quad (4)$$

Where: CFU: number of colonies on substrates, degree of dilution: 10n, n=1 for first dilution, n=2 for second etc. V: volume placed on substrate (mL).

Statistical Analysis

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

3. Results and discussion

3.1 Weight Loss

The weight loss of berries plays a crucial role in the determination of their shelf life and overall quality. The importance of monitoring weight loss in berries lies in its correlation with moisture content, texture changes, and the overall quality of the fruit. The results are presented in the following figure.

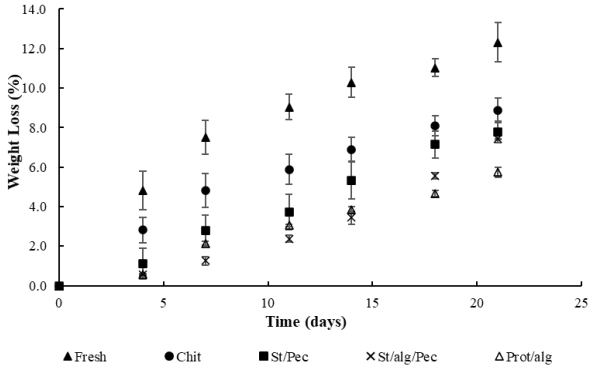

Figure 1: Weight Loss (%) of fresh and coated berries for 21 days.

The fresh berries seem to have lost a significant amount of weight over time, unlike the other coated samples. In particular, on day 21, the optimal coated sample has a 6% weight loss, which is reduced by 50%, compared to a nearly 13% loss for the fresh sample. The protein algae coating seems to provide the best outcomes when compared to the other coatings. This is due to the protein properties that contribute to improved moisture retention, protective barriers, and overall preservation of the berries. Algal proteins often have a hydrophilic nature, which enables to create strong barriers that prevent berries from losing moisture, by slowing down the dehydration process and improving moisture retention (Chen et al., 2019). The starch/ pectin coatings, also, present satisfactory results leading up to 8% weight loss. Starch and pectin are natural polymers that, when combined can form an effective barrier on the surface of berries, contributing to improved moisture retention and overall preservation, due to their hydrophilic nature (Kong et al., 2022). Chitosan leads to better results than the fresh berries, although the values are higher than other coatings. Chitosan, being a derivative of chitin, has a hydrophobic nature, which can present many advantages creating a protective barrier, but not in moisture (Kumar et al., 2020).

3.2 Total Soluble Solids

Total solids are an indicator of the ripeness of the fruit, so a lesser price increase corresponds to a later ripening period (Silva, Finkler and Finkler, 2018). The charts below detail the measurement of total soluble solids for all coatings.

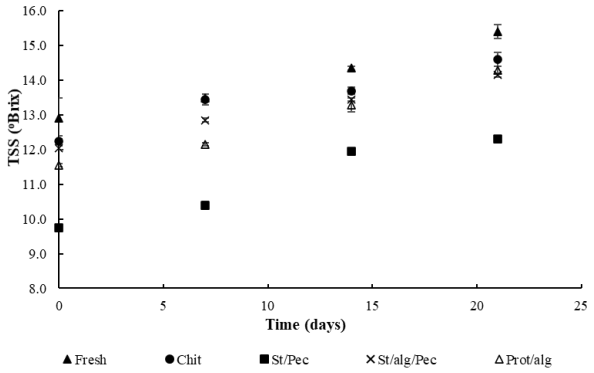

Figure 2: Total Soluble Solids (°Brix) of fresh and coated berries for 21 days.

It is observed that all of the coated samples show a lower rise in total solids than the fresh samples, demonstrating the coatings' efficacy in preventing blueberry ripening. The optimum results are presented in the samples coated with starch and pectin as they ensure consistent protection across the berry surface, contributing to consistent physiological responses (Ngo et al., 2021). The samples with the protein and algal starch coatings show a satisfactory decrease compared to the fresh samples, as they might act as barriers that reduce water loss, leading to concentration of soluble solids (Carpintero et al., 2023).

3.3 Total Acidity

The utilization of organic acids as respiratory substrates and as a carbon skeleton for the synthesis of new compounds during ripening is responsible for the decrease in acidity. Therefore, the more beneficial the edible coating's effect, the smaller the reduction in acidity (El Gaouth et al., 1991). The following figure presents the Total acidity of coated and fresh berries.

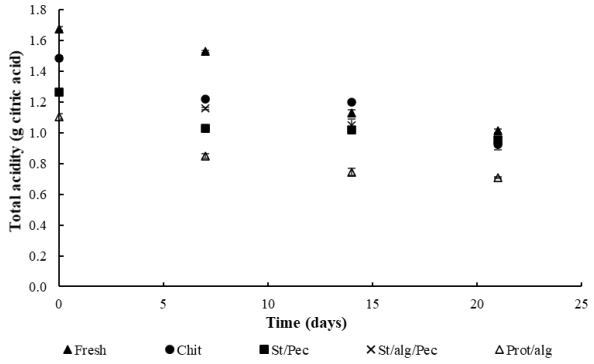

Figure 3: Total Acidity (g citric acid) of fresh and coated berries for 21 days.

The coated samples present a lower Total Acidity overall, even on day 0. It is observed that for all samples the total acidity decreases significant on the 7th day and after that there is a smaller reduction. The lower values are observed in the samples coated with Protein alg. and the Chitosan also present satisfactory results. These coatings can affect the production of organic acids, contributing to changes in Total acidity levels (Sun et al., 2018).

3.4 Antioxidant Activity

Coatings restrict oxygen and carbon dioxide from entering and exiting the blueberry, which leads to antioxidant retention. This has the effect of preventing the blueberry's antioxidants, like ascorbic acid and anthocyanins, from oxidizing and maintaining their potent effects. With this analysis method used, IC50 is measured. IC50 is

a measure of the concentration of an antioxidant at which it inhibits or scavenges 50% of a specific biological or chemical activity, so a lower IC50 is considered better. The results are displayed in the figure below.

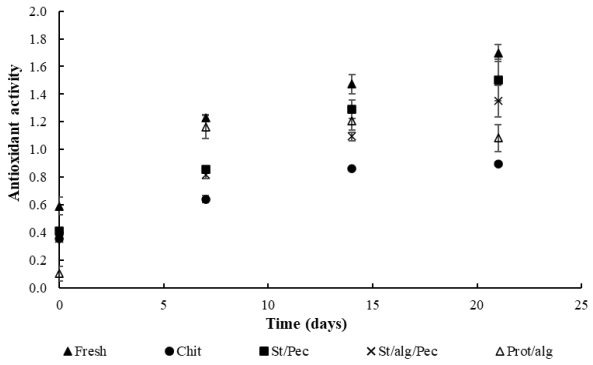

Figure 3: Antioxidant activity (IC50) of fresh and coated berries for 21 days.

The aforementioned diagrams demonstrate how the coatings help to preserve antioxidants through time. The antioxidant activity tends to decrease through time; however, the coatings lead to lower values of IC50 at day 21, compared to the fresh samples. The algae extracts contain additional natural antioxidants that contribute to the overall impact, there is a noticeable increase in antioxidant content in the algal extract coatings, even from day 0, compared to the other samples (Hamad et al., 2023). The samples coated with chitosan show the least loss in antioxidants throughout the course of the days. Chitosan itself has been shown to have free radical-scavenging activity in addition to antioxidant qualities. The coating's chitosan content may help to slow down oxidative processes that could otherwise cause antioxidants to degrade (Wang et al., 2013).

3.5 Microbial growth

The following table shows the results of the microbial tests, of all samples.

Table 1: Microbial analysis of berries fresh and coated.

Sample	Total Count (log_{10}CFU/mL)	E. Coli (log_{10}CFU/mL)
Fresh	4.16 ± 0.07	3.44 ± 0.09
Chit	n.d.	n.d.
St/Pec	3.87± 0.15	3.42 ± 0.10
St/alg/Pec	4.13 ± 0.03	2.44 ± 0.07
Prot/alg	4.18 **±** 0.02	2.15 ± 0.18

* Mean value ± standard deviation of three replicates, * n.d.: log10CFU/mL < 1.

It is observed that in the Total Counts and the E. coli there is a large difference in the number of microorganisms grown depending on the coating. Chitosan, which is known for its antimicrobial activity, creates an effective coating, while a certain number of microorganisms is not detected (Yilmaz Atay, 2020). On the contrary, the coatings with algae extracts, present a very small activity in Total Counts and a little higher in E. Coli compared to the Control sample. This may be due to a quantity of micro-organisms that the micro-algae may contain either before the extraction or acquire during it and thus increase the total load and due to their smaller growth.

In the limits found in the literature for the number of microbes you notice that in the Total Counts where the limit is 5 log10CFU/mL (European Commission 2012). all samples are within limits on day 14. On the contrary in E. Coli where the limit is 2 log10CFU/mL (European Commission 2012). you notice that the Control and the samples from algae extracts and starch-pectin are above the limit, which makes them inedible.

4. Conclusions

This work focused on the application of conventional and alternative edible coatings on blueberries and the evaluation of their shelf-life. Algae extract coatings were found to yield the greatest outcomes across nearly all parameters. More precisely, during the course of 21 days, the weight loss for the algal protein coatings were the lowest at roughly 6%, compared to 13% for the control sample. The blueberries coated with algal proteins had the best ripening characteristics, as measured by total solids and acidity. In addition, there was a significant variation in the antioxidant retention of the processed blueberries; over the course of the 21-day period, the antioxidant levels in the samples coated with algal components declined far less than those in the control and conventionally coated samples.

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