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Edible Coatings Based on Bacterial Nanocellulose and Its Functionalization Extend Postharvest Strawberry Conservation

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Abstract

Strawberry is a non-climacteric fruit with a short postharvest shelf life. Recently, edible coatings have attracted the attention of the food industry. Cellulose is the most abundant carbohydrate polymer on Earth, and is also a renewable natural material, biocompatible with food. This work aimed to evaluate the postharvest quality of strawberries coated with edible coatings based on hydroxypropylmethylcellulose (HPMC) and bacterial nanocellulose (BNC) and its functionalization, using vegetal extracts with reported antifungal activity. Five treatments were applied on postharvest strawberries: C (control, with no coating); Cel (HPMC:BNC in a 95:5 ratio); EPAC (cellulose + *Persicaria acuminata* extract); EO (cellulose + *Pelargonium graveolens* essential oil) and CBZ (cellulose + carbendazim). Weight, firmness, total soluble solids, titratable acidity, ripe index, respiration rate, ethylene production rate, and natural fungal incidence were measured. Furthermore, the C and Cel fruit surface was observed by SEM. Cel and EPAC treatments proved to be beneficial in maintaining the quality of the treated fruit during storage. Both coatings contributed to a lower weight loss and firmness. They also decreased the respiratory rate and the natural fungal incidence, delaying the senescence of the treated strawberries. These treatments can be alternatives to extend strawberry life postharvest.

Keywords: coatings; cellulose; bacterial nanocellulose; strawberry; vegetal extracts

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Academic Editor: Mayker Lazaro Dantas Miranda

Received: 22 November 2025
Revised: 14 January 2026
Accepted: 17 January 2026
Published: 26 January 2026

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1. Introduction

Strawberry is a non-climacteric fruit with a very short postharvest shelf life. Its quality loss is mainly due to its relatively high metabolic activity and sensitivity to fungal decay [1]. During ripening, strawberries suffer a significant loss of firmness, which determines the deterioration of the fruit during the postharvest period. Excessive softening increases susceptibility to physical or microbial injury, water loss, and bruising, resulting in constraints on their trade and consumption [2].

Several preservation techniques, such as heat treatments [3], freezing [4], modified atmosphere packaging [5], osmotic treatments [6], ultrasound [7], chemical treat-

ments [8,9], and edible coatings [10,11], have been investigated to maximize the shelf life of fresh strawberries.

For decades, synthetic fungicides have been applied immediately before or after harvest to manage postharvest fruit deterioration. Nevertheless, chemical treatments do not provide an efficient reduction of microbial load in the medium and long term, because of the occurrence of fungicide-resistant strains of fungal pathogens [8,12]. Furthermore, they are perceived negatively by consumers and considered dangerous to human health and harmful to the environment, for which reasons their usage is restricted [8,12].

Recently, edible coatings have attracted the attention of the food industry, as consumers increasingly demand toxin-free products and fewer artificial additives. This trend toward "green" consumerism promotes health safety, high nutritional value, overall product quality, and environmental sustainability [2,13].

Edible coatings work as a semi-permeable membrane around the fruit and therefore do not readily allow gas and moisture interchange, improving visual appearance and conserving the postharvest quality of coated fruits and vegetables [14,15]. Edible coatings based on natural polymeric materials include proteins, lipids, and polysaccharides or their combinations [16].

Cellulose is the most abundant carbohydrate polymer on Earth. This renewable natural biopolymer and its derived materials are relevant for application in environmentally friendly and biocompatible products and foods [17], considering its special structure and properties. Cellulose is classified as "generally recognized as safe" (GRAS), and has been approved by the FDA [18]. There is great potential in the use of cellulose derivatives as edible coatings. Films of methylcellulose (MC), hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), and carboxymethylcellulose (CMC) tend to have moderate strength, are resistant to oils and fats, and are flexible, transparent, odorless, tasteless, water-soluble, and moderate barriers to oxygen and moisture [19]. Cellulose is synthesized by plants, certain animals, and numerous microorganisms. Some bacterial species are capable of producing it, including *Pseudomonas fluorescens*, *Gluconacetobacter xylinus*, and *Gluconacetobacter hansenii*, among others [20]. In recent decades, bacterial nanocellulose (BNC) has attracted growing global interest due to its unique properties, such as hydrophilicity, favorable mechanical performance, excellent biocompatibility, and biodegradability, which make it an ideal material for green processing [21].

Furthermore, it is important to highlight that cellulose presents low production costs. In the food packaging industry, the most extended use of cellulose is as a reinforcing agent in microfiber or nanofiber form. The 3D structure of BNC facilitates combinations of cellulose with other soluble components to modify the film's structure, simplifying the process. It is possible to modify the properties of the BNC films while keeping intact their unique structure [18].

Plant extracts have been used in the food industry for a wide variety of purposes. During the last decades, research on the biological activity of plant extracts has endorsed several innovative applications, such as the development of natural coatings for food preservation [11]. Natural products from plants offer a set of structurally different fungicidal compounds that could represent an alternative to synthetic fungicides for the control of phytopathogenic fungi [1,22,23]. The use of antifungal packaging is a possible solution to control the growth of phytopathogens in fruits during postharvest storage, and to extend the safety and shelf-life of ready-to-eat foods [24–26]. It is noticeable that an extended postharvest life is also relevant for consumers, since it facilitates long-term storage at home [27]. In this sense, our research group has assessed the bioactivity of different species, obtaining a high fungicide effect against *Botrytis cinerea* from *P. acuminata* extracts and *P. graveolens* essential oil [22,23].

This work aimed to evaluate the postharvest quality of strawberries coated with edible coatings based on HPMC:NCB and functionalized using vegetal extracts with reported antifungal activity.

2. Materials and Methods

2.1. Fruit Material

Strawberries (*Fragaria* × *ananassa* [Weston] Duchesne) of cv. San Andrea were harvested from a production field in Coronda, Santa Fe province, Argentina, at a 100% fruit color coverage ripe stage. The selected fruit were uniform in size, color, shape, and ripeness, and all of them were free of visible damage on the surface. The day of harvest was denominated as D1.

2.2. Treatments

2.2.1. Preparation of Edible Coatings

A group of uncoated fruit were kept as a control treatment (C). Four film-forming suspensions (FFS) were prepared using HPMC (Methocel K4M) donated by Cicloquimica SAC (Buenos Aires, Argentina) and BNC, obtained at the Institute of Pharmaceutical and Food Biotechnology (INBIOFAL-Tucumán, Argentina), according to the static cultivation procedure developed in our laboratory [20]. To obtain BNC, a *P. fluorescens* WS strain (wrinkly spreader) was used (donated by PhD Andrew Spears, School of Science, Engineering and Technology, Abertay University). It was grown in LB broth (Britania—C.A.B.A., Argentina) and kept at 25 °C. All growing tests were conducted on petri dishes with 20 mL of fresh medium, for 72 h at 28 ± 1 °C. For BNC production estimation, all cellulose pellicles were washed with distillate water to remove medium. BNC was centrifuged 20 min at 8000 rpm. Then 1 mL of NaOH 2% v/v was added and autoclaved for 15 min at 121 °C. This procedure is done in order detach some bacterial cells that could be immersed on BNC. The FFS were obtained following a method optimized by our research group. It consisted of mixing HPMC and BNC (95:5 ratio) with distilled water in a thermostatted magnetic stirrer (Numak GL-3250, London, UK) until homogenization. The total cellulose mixture was 1.5% (w/v). Polyethylene glycol 400 (PEG-400) at 1% (v/v) was added as a plasticizer into the FFS and then stirred for more than 10 min. This filmogenic suspension was categorized as Cel treatment. Finally, antifungal substances were added: 2000 ppm extract of *P. acuminata* (EPAC), 2000 ppm essential oil of *P. graveolens* (EO), and 500 ppm CBZ (Gleba, Vicente López, Argentina), which is a commercial fungicide. These substances were incorporated into different filmogenic suspensions using a shaker for 30 min, to achieve the three remaining treatments. The concentrations of natural products were optimized by the research group through ex vivo assays using aqueous solutions of these extracts. To ensure the complete dispersion of the *P. acuminata extract*, the LSM adjuvant (2%) was used.

The treatments are detailed below:

- C (control, with no coating)
- Cel (cellulose)
- EPAC (cellulose + *P. acuminata* extract)
- EO (cellulose + *P. graveolens* essential oil)
- CBZ (cellulose + CBZ)

After obtention, each FFS was degassed using an ultrasonic cleaner. *P. acuminata* extract and *P. graveolens* essential oil were obtained according to Di Liberto et al. (2021) [22] and Stegmayer et al. (2022) [23], respectively.

2.2.2. Application of Edible Coatings on Strawberry Fruit

The coating treatments were applied by immersing the fruit in the suspensions for 2 min. Then, each fruit was drained and dried for 2 h under environmental conditions. Coated (Cel, EPAC, EO, and CBZ) fruit and C fruit were packed in polypropylene plastic trays and stored at 24 °C. All the fruit were analyzed up to 6 days of storage. The determinations were carried out on days 1, 3, and 6 (D1, D3, and D6, respectively).

2.3. Postharvest Quality Parameter Determinations

2.3.1. Weight and Weight Loss

The fruit were weighed using an analytical scale (PREC EHB-300, Shenzhen, China). The results were expressed as absolute daily weight and percentage of weight loss. Weight loss percentage (%) was obtained from the initial weight on D1, and the sampling day weight (D3 or D6).

2.3.2. Firmness and Firmness Loss

Strawberry firmness was determined using a non-destructive durometer (T.R. Turoni, Forlì, Italy). The penetration depth was 5 mm. Firmness was determined as the opposition force offered by the surface of the fruit in elastic modulus, measured as a percentage in relation to the maximum hardness measured by the equipment. Firmness loss percentage (%) was obtained from the initial firmness value on D1 and the firmness value on the sampling day (D3 or D6).

2.3.3. Total Soluble Solids, Titratable Acidity, and Ripe Index

For the total soluble solids (TSS), the juice from three fruit per measurement was extracted using a mixer and then filtered. TSS of fruit juice were estimated using a handheld refractometer (ZGRB-32ATC, Shenzhen, China), and the data were expressed as a percentage. For the titratable acidity (TA), aliquots of 4 mL of juice were titrated with 0.1 N NaOH, according to AOCC (1970) [28]. Phenolphthalein was used as a pH indicator, and results were expressed as citric acid equivalents. Later, the ripeness index (RI) was calculated as the TSS and TA ratio.

2.3.4. Respiration Rate and Ethylene Production Rate

Respiration rate and ethylene production were measured according to the method presented by Elnagar et al. (2021) [29]. To determine both variables, a closed and static system connected to a gas analyzer (Felix instrument F-950, Camas, WA, USA) was used. For each measurement, one fruit was placed in an airtight container and the accumulation of gases (carbon dioxide -CO_2- and ethylene -C_2H_4-) produced over 20 min was determined. Each measurement was made by placing the sensor inside the system, through a sealed hole at the beginning of the measurement (T1) and 20 min later (T2). Each result was obtained from the measurement of five determinations. The respiration rate was expressed as mL CO_2 g FW^{-1}·min^{-1} and the ethylene production rate produced was expressed as mL C_2H_4 g FW^{-1}·min^{-1}, where FW is the fresh weigh.

2.3.5. Natural Fungal Incidence

Natural fungal incidence was determined by dividing decayed fruit by the total number of fruit. The results were expressed as a percentage.

2.3.6. Observation of Fruit Surface by SEM

In order to compare C fruit and Cel treated fruit surfaces, a characterization was carried out by observing with a scanning electron microscope (Zeiss SUPRA 55VP, Jena, Germany) at a 3 kV magnification. For this, 1 cm thickness samples were taken using a scalpel and placed in an Eppendorff (Hamburg, Germany) tube with 5 mL of glutaraldehyde as a fixative, until its observation.

2.4. Statistical Analysis

All experiments were conducted following a completely randomized design, with 42 fruit evaluated per treatment. All measurements were performed at least in triplicate, and the results are presented as mean ± standard deviation. Statistical analyses were carried out using INFOSTAT 2020 software [30]. An analysis of variance (ANOVA) with an adjustment of the data to a linear mixed model was performed, and mean values were compared using Fisher's test at a significance level of $p < 0.05$. The comparisons of the means were made among the days of the measurements and among the treatments. A multifactorial model was fitted and the interactions between factors were evaluated: day (3 levels) and treatment (5 levels). Different letters in the graphics and the table in the Section 3 indicate statistically significant differences.

3. Results

3.1. Weight

The results of the weight evolution and the percentage of weight loss of strawberries under the different treatments are shown in Figures 1 and 2, respectively. On D6 of storage, the C fruit presented a significantly higher weight loss percentage (34.79%), compared to 28.58% in Cel and 27.02% in EPAC. Meanwhile, EO (32.2%) and CBZ (31.9%) showed no significant differences between them, but they were slightly though significantly lower when compared to C.

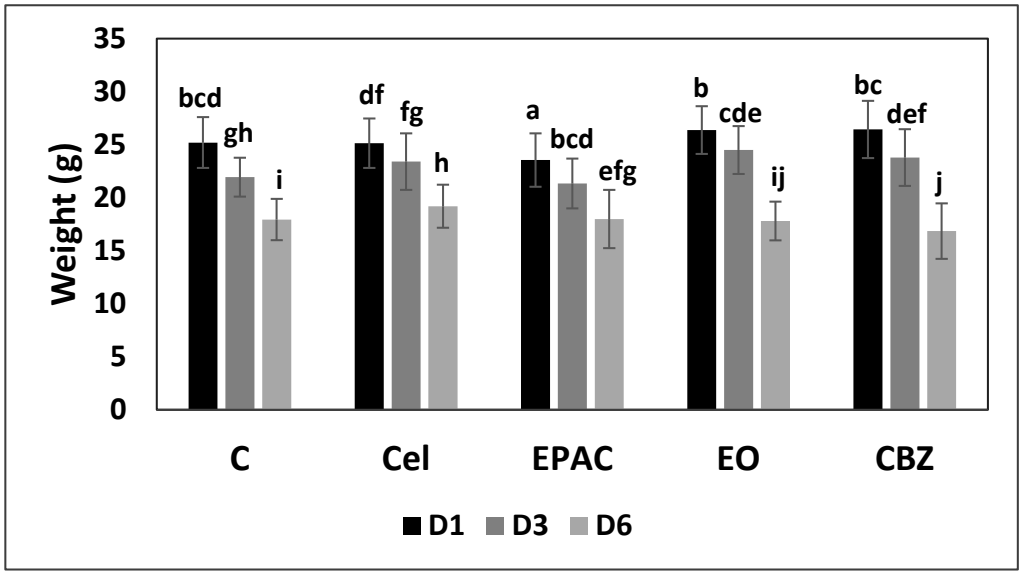


Figure 1. Fruit weight of treated and untreated strawberries on days 1, 3, and 6 (D1, D3, and D6, respectively) of storage. Different letters indicate significant differences. Treatments were: C (control, with no coating), Cel (cellulose), EPAC (cellulose + *P. acuminata* extract), EO (cellulose + *P. graveolens* essential oil), CBZ (cellulose + CBZ).

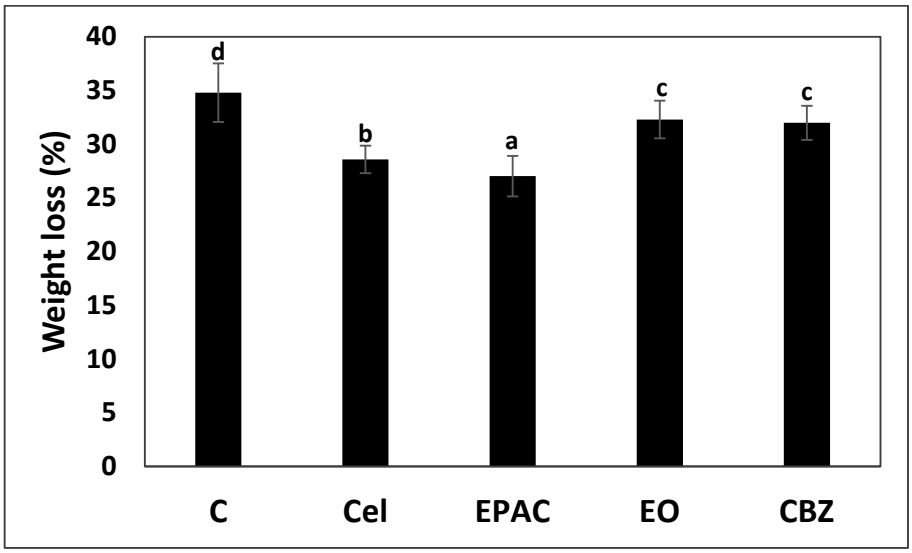


Figure 2. Fruit weight loss of treated and untreated strawberries on day 6 (D6) of storage. Different letters indicate statistically significant differences. Treatments were: C (control, with no coating), Cel (cellulose), EPAC (cellulose + *P. acuminata* extract), EO (cellulose + *P. graveolens* essential oil), CBZ (cellulose + CBZ).

3.2. Firmness

The firmness loss is shown in Figure 3. In Figure 4, the progressive decrease in fruit firmness can be observed throughout the days of storage. There were no significant differences between strawberries treated with Cel and those treated with EPAC (around 17.37% of firmness loss in both cases). At the same time, these treatments significantly reduced the loss of firmness compared to the control (60.71%). The EO treatment also showed a noticeably lower decrease compared to C, but significantly higher compared to Cel and EPAC. CBZ showed no significant difference compared to Cel, EPAC, and EO treatments.

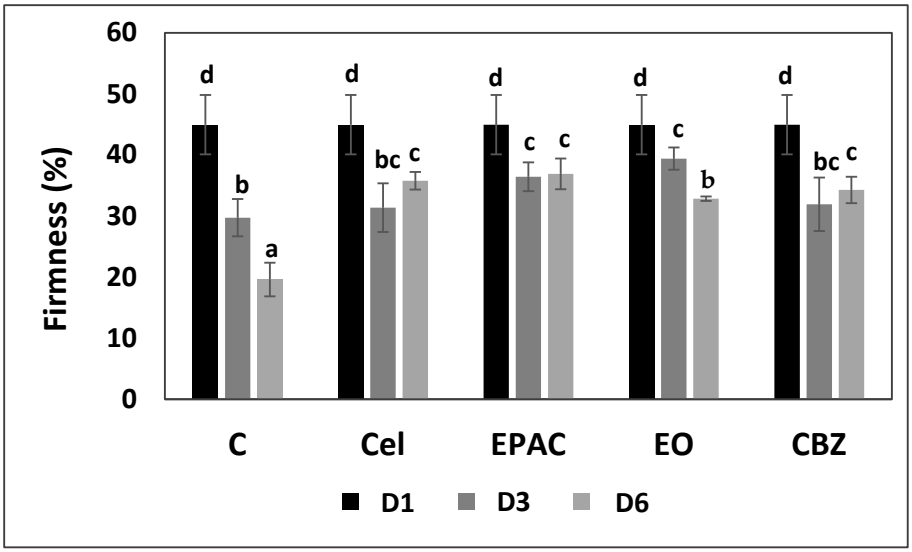


Figure 3. Fruit firmness of treated and untreated strawberries on days 1, 3, and 6 (D1, D3, and D6, respectively) of storage. Different letters indicate significant differences. Treatments were: C (control, with no coating), Cel (cellulose), EPAC (cellulose + *P. acuminata* extract), EO (cellulose + *P. graveolens* essential oil), CBZ (cellulose + CBZ).

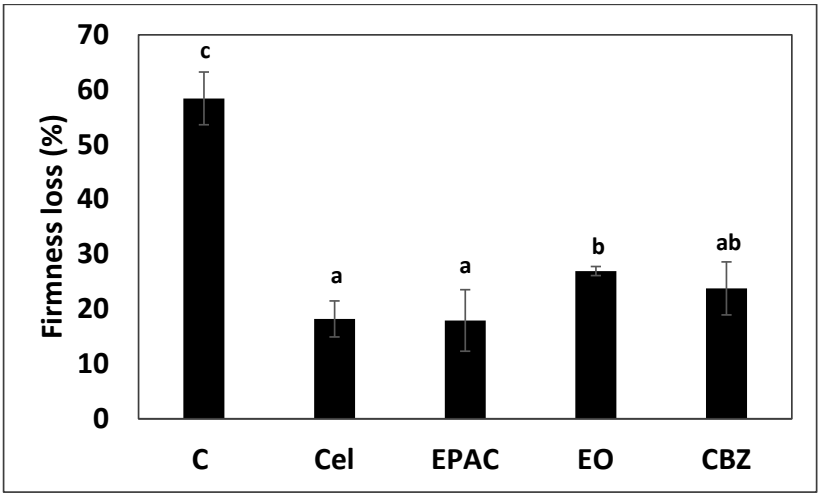


Figure 4. Fruit firmness loss of treated and untreated strawberries on day 6 (D6) of storage. Different letters indicate significant differences. Treatments were: C (control, with no coating), Cel (cellulose), EPAC (cellulose + *P. acuminata* extract), EO (cellulose + *P. graveolens* essential oil), CBZ (cellulose + CBZ).

3.3. Total Soluble Solids (TSS), Titratable Acidity (TA) and Ripeness Index (RI)

Table 1 shows the results of TSS, TA, and RI. Senescence during storage is responsible for the reduction of several components present in strawberries, such as TA and TSS, which alter the characteristics of the fruit. The results for TSS show an increase on D6 of storage in the Cel, EPAC, OE, and CBZ treatments compared to D0. The lowest TSS values were observed in the control (8.13%) and Cel (9.08%) on D6, with no significant difference between them. It was also observed that TSS significantly decreased in the control fruit on D6 compared to D0, while no differences were found between D0 and D6 in Cel fruit. In this study, TA showed a trend to increase throughout the experiment, except in the EPAC and OE treatments. Fruit under EPAC showed a decrease in TA by D6 after an increase observed on D3. Fruit under OE showed an increase in TA on D3 and remained steady through D6. RI progressively decreased during storage in the control, Cel, and CBZ treatments, whereas EPAC and OE treatments maintained the same TA compared to D0.

Table 1. Effect of strawberry coatings on total soluble solids (TSS), titratable acidity (TA), and ripeness index (RI) on days 1, 3, and 6 (D1, D3, and D6, respectively) of storage. Different letters indicate statistically significant differences. Treatments were: C (control, with no coating), Cel (cellulose), EPAC (cellulose + *P. acuminata* extract), EO (cellulose + *P. graveolens* essential oil), CBZ (cellulose + CBZ).

Variable	Days from Harvest (D)	C	Cel	EPAC	EO	CBZ
TSS (%)	D1	8.58 ± 0.43 efg	8.58 ± 0.43 efg	8.58 ± 0.43 efg	8.58 ± 0.43 efg	8.58 ± 0.43 efg
	D3	9.27 ± 0.25 cde	9.48 ± 0.54 cd	8.33 ± 0.58 fg	9.20 ± 0.54 def	8.10 ± 0.17 g
	D6	8.13 ± 0.23 g	9.08 ± 0.83 cd	12.20 ± 1.15 a	9.93 ± 0.61 bc	11.03 ± 0.91 b
TA (%)	D1	0.68 ± 0.10 e	0.68 ± 0.10 e	0.68 ± 0.10 e	0.68 ± 0.10 e	0.68 ± 0.10 e
	D3	0.88 ± 0.05 bcd	0.80 ± 0.12 de	0.98 ± 0.16 abc	0.88 ± 0.09 cd	0.83 ± 0.11 d
	D6	1.00 ± 0.10 ab	1.06 ± 0.06 ab	0.87 ± 0.07 cd	0.85 ± 0.08 cd	1.08 ± 0.15 a
RI	D1	13.38 ± 1.97 a	13.38 ± 1.97 a	13.38 ± 1.97 a	13.38 ± 1.97 a	13.38 ± 1.97 a
	D3	10.99 ± 0.71 bc	12.04 ± 2.16 ab	10.78 ± 0.35 bcd	11.38 ± 0.41 abc	9.11 ± 1.02 cd
	D6	8.80 ± 0.24 d	9.03 ± 0.64 cd	14.07 ± 1.32 a	11.45 ± 1.52 bc	10.14 ± 0.72 bcd

3.4. Respiration Rate and Ethylene Production Rate

Figure 5 shows the respiration rate. Through D6 of storage the respiration rate of strawberries under Cel and EPAC treatments was lower compared to the C treatment, with

the lowest values observed in Cel strawberries. In contrast, EO treatment increased the respiration rate compared to the control. Meanwhile, CBZ treatment showed a similar response in comparison to C treatment.

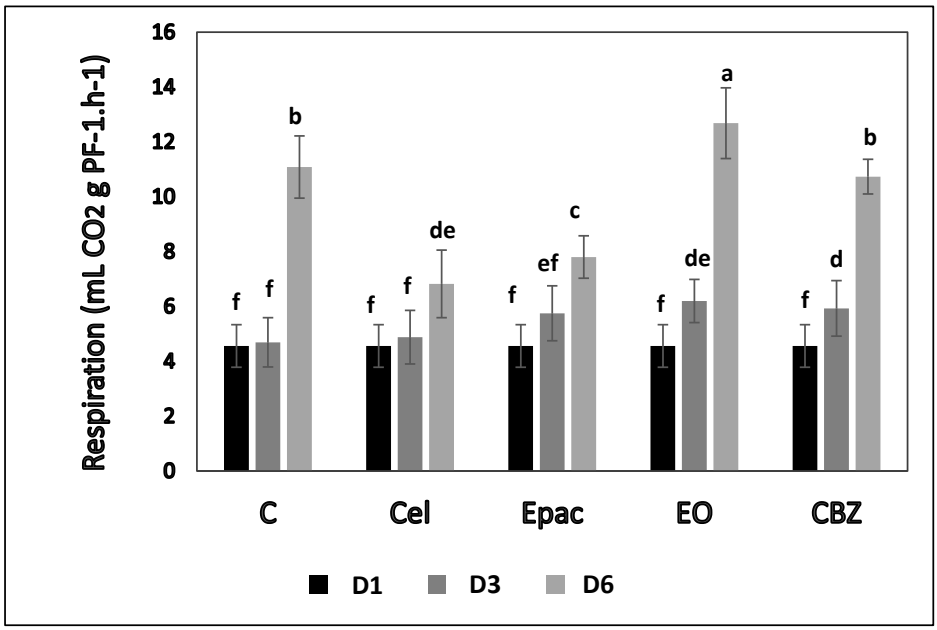


Figure 5. Fruit respiration rate of treated and untreated strawberries on days 1, 3, and 6 (D1, D3, and D6, respectively) of storage. Different letters indicate statistically significant differences. Treatments were: C (control, with no coating), Cel (cellulose), EPAC (cellulose + *P. acuminata* extract), EO (cellulose + *P. graveolens* essential oil), CBZ (cellulose + CBZ).

Figure 6 shows the ethylene production rate through D6. All treatments reduced ethylene production compared to C. Through D6, only Cel treatment showed a significantly lower ethylene production rate in comparison to C. EPAC and EO treatments presented similar values to C treatment. It is noticeable that CBZ treatment markedly increased ethylene production through D6 (fivefold) compared to C.

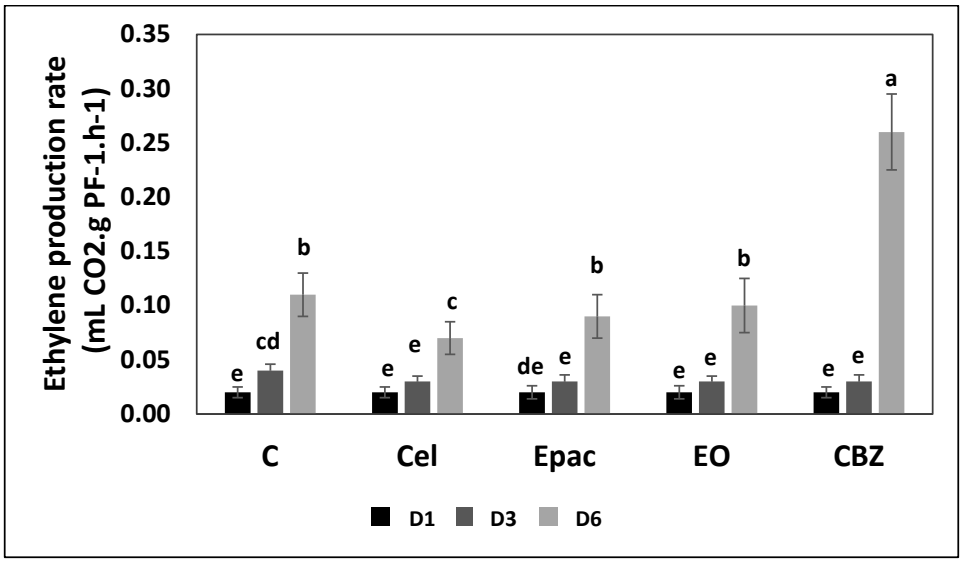


Figure 6. Fruit ethylene production rate of treated and untreated strawberries on days 1, 3, and 6 (D1, D3, and D6, respectively) of storage. Different letters indicate statistically significant differences. Treatments were: C (control, with no coating), Cel (cellulose), EPAC (cellulose + *P. acuminata* extract), EO (cellulose + *P. graveolens* essential oil), CBZ (cellulose + CBZ).

3.5. Natural Fungal Incidence

Figure 7 shows natural fungal incidence results through D6 of storage. There is a significantly higher incidence in the C treatment (88.46%) compared to the Cel (14.39%) and EPAC treatments (18.09%). EO and CBZ showed a significantly higher percentage of incidence than the C treatment.

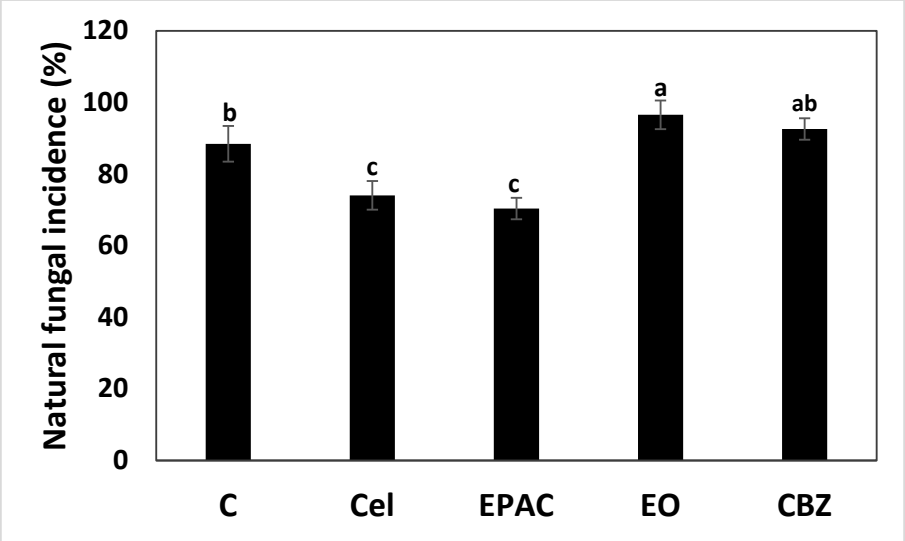


Figure 7. Fruit natural fungal incidence of treated and untreated strawberries on day 6 (D6) of storage. Different letters indicate statistically significant differences. Treatments were: C (control, with no coating), Cel (cellulose), EPAC (cellulose + *P. acuminata* extract), EO (cellulose + *P. graveolens* essential oil), CBZ (cellulose + CBZ).

Figure 8 shows the surface morphology of uncoated strawberries (C) and those coated with the Cel treatment. Figure 8A shows a porous structure observed in C fruit, while in Figure 8B it is observed that these pores were clearly covered by the Cel treatment. The Cell coating showed irregularities and fractures.

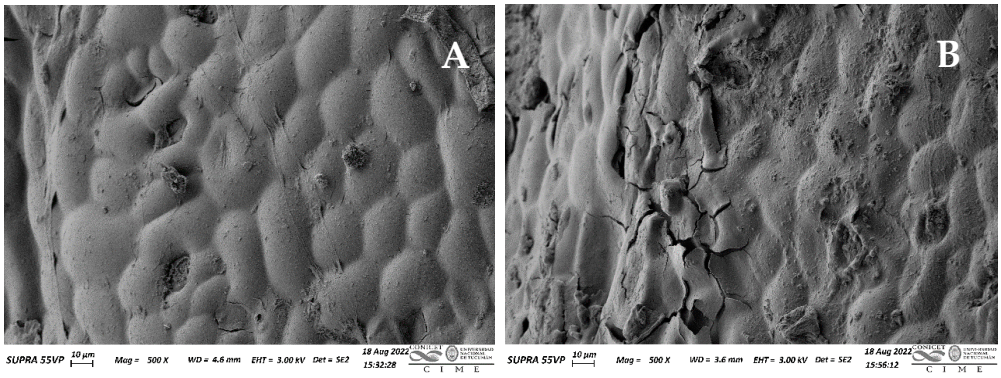


Figure 8. SEM micrograph of strawberry surface under: (**A**) C treatment and (**B**) Cel treatment observed at 500× magnification.

4. Discussion

Strawberry presents a short postharvest shelf life, since it rapidly loses water through transpiration, and hence, loses weight and firmness. This makes it susceptible to physical injury and fungal decay [1,2]. In this study we evaluated the postharvest quality of strawberries coated with edible coatings based on HPMC:NCB and functionalized using vegetal extracts against reported antifungal activity.

Firstly, it is important to highlight that the SEM analysis showed that the HPMC:NCB coating formed a continuous layer, although some fractures were observed. These fractures could be attributed to the covering thin thickness of 314.4 nm. Popescu et al. [31] attributed the variability in coating thickness to the high humidity of the fruit tissue. This issue could be improved by applying a double coating to achieve a thicker and more uniform layer.

Among the treatments, Cel and EPAC were more effective in reducing weight loss. Similar behavior was observed in Saleem et al. [15], who detected significantly lower weight loss in strawberries coated with chitosan and chitosan with ascorbic acid added (AA), compared to control fruit or fruit treated only with AA solution. In the study conducted by Kwak et al. [32], strawberries coated with carboxymethylated cellulose nanofibrils (NFC-CM) showed a higher water retention capacity (more than 21.22%). Weight loss is mainly caused by water evaporation through transpiration and carbon loss through respiration [33]. Edible coatings act as a barrier by forming a semipermeable layer on the fruit surface, reducing transpiration losses [15,33]. It is important to highlight that fruit and vegetable weight loss has a direct impact on profitability in each link of the commercial value chain. As described before, Cel and EPAC treatments showed similar behavior, indicating that the addition of the *P. acuminata* extract represents no advantage in reducing weight loss. At the same time, Cel and EPAC treatments similarly reduce the firmness loss. It indicates that all the treatments enhanced fruit quality preservation. Strawberries suffer high firmness loss during ripening [16] and postharvest storage. In our study, a significant decrease in firmness was observed in both control and coated fruit during storage. However, coated strawberries showed lower percentages of firmness loss compared to the control on D6 of storage. According to Kwak et al. [32] this decrease occurs due to the destruction of the cell wall structure by degradation enzymes. Weight loss and firmness loss are closely linked since water loss implies the reduction of cell turgor. Hence, by reducing weight loss, firmness will be less affected.

The results showed that all treatments, except C treatment, increased the TSS content. The retention of TSS in strawberries coated with Cel could be attributed to a reduction in internal O_2 concentration and/or an increase in CO_2, which leads to decreased respiration and reduced ethylene production rates. Hernández-Muñoz et al. [34] reported similar results, stating that the increase in TSS could be due to the solubilization of hemicelluloses present in the cell wall, as well as water loss through transpiration. At the same time, there was a slight but significant increase in TA in all treatments by D6. Other coatings applied to strawberries, such as arabic gum, xanthan gum, and carrageenan, produced opposite results [16], generating a decrease in TA. This could be due to the natural consumption of organic acids during the respiratory process [29,35]. The TSS/TA ratio is directly related to flavor; a higher TSS/TA ratio implies a better balance between sweetness and acidity.

The results suggest that both Cel and EPAC coatings are effective in delaying fruit CO_2 production. The respiration rate in coated fruit is mainly reduced by preventing gas exchange through the covered stomata. Consequently, this leads to a decrease in O_2 consumption and an increase in CO_2 production from internal gas [36]. Other authors reported similar results in strawberries coated with aloe vera gel enriched with basil essential oil [2] and cellulose nanofibers [32].

The strawberry is characterized by moderate respiratory rate and low ethylene production less than 0.1 $\mu L \cdot kg^{-1} \cdot h^{-1}$ [37]. A reduction of ethylene production indicates a delay in the tissue senescence pathways. Control treatment shows the natural behavior of strawberry under our experimental conditions. During the postharvest stage, fruits and vegetables become senescent naturally and unavoidably, because they are not generating CO_2 fixation and/or receiving primary metabolites, therefore, they start to consume their reserves, breaking the balance between O_2 capture and O_2 release. The start of this

unbalanced context is expressed as an increase in respiration and ethylene production rates, observed between D3 and D6 in our study. When some external factor speeds or delay tissue deterioration (for instance, the application of a treatment) it is expressed as an increase or decrease, respectively, in the respiration and ethylene production rates [38,39]. At the same time, an increase of ethylene production is related to an increase in the signals activating the plant defense pathways [38,39]. Although it is not the aim of this study, our results showed that the incorporation of the synthetic fungicide CBZ triggers ethylene synthesis. It would be relevant for understanding physiology to investigate whether the plant defense profile is modified or not when CBZ is applied to tissues.

Even though DNA recognition of fungal species was not carried out, the morphological features of the fungal incidence mainly indicated infections by *Botrytis cinerea*. It has been reported that gray mold infection accelerates ethylene production by the tissues of the affected host plant and by the fungus itself, and ethylene promotes the decay of nearby tissues or fruits [40].

The antimicrobial properties of *P. acuminata* extract have been reported [41–44]. Due to these properties, in addition to the formation of the cellulose film around the surface of the strawberries restricting the invasion of pathogens, a decrease in fruit rot is achieved [15,16]). However, the other two treatments (EO and CBZ) concluded the trial with a significantly higher incidence when compared to the untreated group. Van Long et al. [26]. concluded that CBZ carried by polymers was not suitable for controlling mold development, due to the high weight percentages of CBZ required for activity and due to the sensitivity of the films to moisture, which prevents long-term applications. In general, the results obtained under in vivo conditions show a significant decrease in the efficiency of the EOs compared to in vitro conditions. This decrease in efficiency is probably due to a higher volatility of EOs in vivo than in vitro [45,46], interactions between film components and fruit tissue [47], and a weaker contact area between EOs and pathogens for in vivo conditions, resulting in lower effectiveness [46].

5. Conclusions

The cellulose itself and the cellulose functionalized with *P. acuminate* extract coatings proved to be beneficial in maintaining the quality of the treated fruit during storage. Both coatings contributed to a lower weight loss and firmness. They also decreased the respiratory rate. The natural fungal incidence in the fruit coated with both treatments was observably lower than that of untreated fruit. The coating with bacterial nanocellulose and commercial cellulose mixture maintained the initial soluble solid content. It also obtained the lowest ethylene production, thus delaying the senescence of the treated strawberries. In addition to the advantages detailed above, the proposed coatings are biodegradable, edible, and easy to prepare, as alternatives to chemically synthesized phytosanitary products.

From a affordability point of view, Cel treatment would be the best choice, considering that it represents lower costs in return for similar storage results. Nevertheless, a biochemical study of plant defense parameters linked to antioxidant substance production could complement information to define the nutritional features and advantages of the treatments. Furthermore, it would be relevant to assess the combination of Cel treatments and different temperature storage conditions.

Author Contributions: Conceptualization, M.J.M., V.E.R., E.E.G., M.G.D. and M.E.S.C.; Methodology, V.E.R., E.E.G., M.G.D. and M.E.S.C.; Validation, V.E.R. and M.E.S.C.; Formal analysis, M.J.M., V.E.R., M.G.D. and M.E.S.C.; Investigation, M.J.M., V.E.R., E.E.G., M.G.D. and M.E.S.C.; Resources, V.E.R., M.G.D. and M.E.S.C.; Data curation, M.J.M.; Writing—original draft, M.J.M. and V.E.R.; Writing—review & editing, M.J.M., V.E.R., E.E.G., M.G.D. and M.E.S.C.; Supervision, V.E.R., M.G.D.

and M.E.S.C.; Project administration, M.E.S.C.; Funding acquisition, M.G.D., V.E.R. and M.E.S.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by FONCyT (Fondo para la Investigación Científica y Tecnológica): PICT2018-04682 Start-Up; PICT2019-01032; PICT2020-SerieA-02504; PICT2021-CAT-II-00097.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

C	control, with no coating
Cel	cellulose
EPAC	cellulose + *P. acuminata* extract
EO	cellulose + *P. graveolens* essential oil
CBZ	cellulose + carbendazim
D1	day 1
D3	day 3
D6	day 6
TSS	total soluble solids
TA	titratable acidy
SEM	scanning electron microscope

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