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Original article

Improved post-harvest preservation effects of mushroom (*Agaricus bisporus*) using bacterial cellulose nanocrystals-gelatin/cinnamon essential oil emulsion coatings

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Summary This work investigated the effect of bacterial cellulose nanocrystals (BCNCs)-gelatin (GelA)/cinnamon essential oil (CEO) emulsion coatings (BCNCs-GelA/CEO) on the shelf-life extension of button mushrooms (*Agaricus bisporus*). CEO loadings between 1200 and 2400 μL/L were used in the coatings applied on the mushrooms' surface. The overall quality of coated mushrooms was monitored over 15 days of cold storage (4 ± 0.5 °C). Regardless of the storage time, using the highest amount of CEO within the coating formulation minimised the impact on tested parameters (weight loss, firmness, percentage of opened caps, total soluble solids, content of ascorbic acid and soluble protein, PPO/POD enzymatic activity) as compared to control samples. Interestingly, a linear relationship (R^2 = 0.96 on average) between CEO concentration and the respiration rate of coated mushrooms was disclosed. Overall, this study pinpointed the capability of BCNCs-GelA/CEO coatings to delay the aging process of button mushrooms under cold storage. Our findings could be applied to address the issue of food losses, highlighting the positive role of coating technology in enhancing the efficiency of the early stages of the food supply chain, especially in the case of button mushrooms. However, an assessment of the impact of the concentration of other coating components (BCNC and fish gelatin) on the shelf-life extension of button mushrooms, as well as an evaluation of the coating's effectiveness in prolonging the shelf-life of other food items, particularly non-respiring products, is necessary to widespread the applicability of the proposed technology.

Keywords Edible coatings, mushrooms, nanotechnology, shelf life.

Introduction

Agaricus bisporus mushrooms (also known as button mushrooms) represent the most popular and extensively consumed variety of mushrooms worldwide, constituting approximately 30% of the global production of edible mushrooms (Gholami *et al.*, 2017). Unfortunately, the commercial availability of button mushrooms is dramatically limited by their short shelf-life, which ranges between 1–3 days and 5–8 days when stored at ambient or refrigerated conditions, respectively (Castellano-Reyes *et al.*, 2021). This is mainly due to the absence of a protective cuticle, which makes button mushrooms susceptible to physical damage and microbial attacks, thus resulting in substantial post-harvest wastage (Donglu *et al.*, 2016). Degradation of this product occurs through browning, water loss, textural alteration, microbial attack, enzyme activity changes, loss of nutrients/flavour, and cap opening (Wang *et al.*, 2021a, 2021b). The short shelf-life of this product poses a significant challenge to its distribution and marketing (Akram & Kwon, 2010). Consequently, the button mushroom industry heavily relies on post-harvest technologies to optimise its economic viability. These technologies are designed to slow down the rate of product aging and encompass strategies such as modified atmosphere packaging (MAP), low-temperature storage, active/intelligent packaging, and chemical preservative treatments (Choi *et al.*, 2016; Kumar *et al.*, 2017). While these approaches have proven effective in extending the shelf-life of products, they have some inherent

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drawbacks (Thakur *et al.*, 2018). For instance, the use of chemical preservatives (e.g., ammonium compounds, halogen compounds, oxidising agents, and reducing agents) in these processes is considered unfavourable (Tavassoli-Kafrani *et al.*, 2016). Plus, conventional packaging methods have involved so far the use of large amounts of non-biodegradable materials, such as plastic bags, trays, and covers (Arnon-Rips & Poverenov, 2018).

In 2022, the European Commission discussed the possibility of banning all packaging for fruit and vegetables with a content of less than 1.5 kg, proposing to sell them in bulk (Papadaki *et al.*, 2022). In this context, the implementation of edible coatings can complement conventional packaging solutions by extending shelf life, reducing food losses, and minimising plastic waste. Remarkably, the positive effect of edible coatings of disparate nature, such as chitosan and guar gum (Huang *et al.*, 2019), pectin, chitosan, sodium alginate, and carboxymethyl cellulose individually and/or in combination with N-acetyl cysteine (Ple oianu & Nour, 2022), chitosan/zein containing lemon essential oil (Wang *et al.*, 2021a, 2021b), and chitosan nanoparticles containing cashew nut essential oil (Chaudhari *et al.*, 2023) on mushroom shelf-life have been successfully demonstrated.

Gelatin is a water-soluble, biodegradable, and edible polymer that has been widely used for the fabrication of edible coatings mainly due to its film-forming capacity, good optical properties, and outstanding oxygen-barrier performance (Nilsuwan *et al.*, 2017; Razavi *et al.*, 2022a). Nevertheless, coatings solely based on pure gelatin face some limitations due to their intrinsic hydrophilic nature, such as poor moisture barrier capability (Xia *et al.*, 2019). Hence, gelatin can represent a suitable substrate for microbial growth, thus leading to food spoilage e.g. due to fungi and/or bacteria. Incorporating hydrophobic and antimicrobial components into the main gelatin polymer (such as essential oils and plant extracts) has been proposed as a viable strategy. This approach aims to preserve the quality of perishable foods while preventing contamination of the edible coating itself (Tripathi *et al.*, 2008). Nevertheless, gelatin-based coatings have been shown to exhibit limited flexibility due to strong intermolecular forces along their polymeric chains. Consequently, exposure to low temperatures can lead to moisture absorption, weight loss, and product shrinkage or wrinkling, potentially resulting in blistering, flaking, or cracking of the coating (Riva *et al.*, 2020).

Recently, antimicrobial emulsion coatings using cinnamon essential oil (CEO), bacterial cellulose nanocrystals (BCNCs), and fish gelatin (GelA) were successfully developed (Razavi *et al.*, 2020, 2022b, 2022c; Golmohammadi *et al.*, 2023). In all these works, the use of CNCs in combination with gelatin has been emphasised as a key aspect to increase the shelf-life of fresh products (e.g., fruit and vegetables). In particular, it was pointed out that BCNCs offer superior performance over insoluble spherical nanoparticles in stabilising emulsions, mainly due to their mechanical and interface properties. However, the combination of CNCs and hydrocolloids for coating formulations remains relatively new and only a few studies have been conducted in this field. This may be due to the drawbacks of BCNCs in scaled-up production, which is time-consuming and not economically favourable (Akbari *et al.*, 2024). Additionally, because of their size, CNCs are still considered a potential hazard to humans' health. However, a very recent study has outlined that cellulose nanoparticles pose no immediate safety concerns, though their application seems to be hindered mostly by the lack of specific regulation for their use as food ingredients (Cañas-Gutiérrez *et al.*, 2024).

The latest investigations include the application of a CNC emulsion with chitosan on Bartlett pears (Deng *et al.*, 2018; Jung *et al.*, 2020), apple pectin, CNCs, and lemongrass essential oil coatings on strawberries (Da Silva *et al.*, 2019), and gelatin-based edible coatings containing chitosan-nanocellulose composites (Dong *et al.*, 2015) on acerola fruit. Broadly speaking, these works suggest that CNCs can be advantageously used in the formulation of edible coatings for the extension of the post-harvest shelf-life of fruits.

This study aimed to evaluate the potential of a gelatin-based coating loaded with cellulose nanocrystals and cinnamon essential oil to extend the shelf-life of button mushrooms. The novelty of this work lies in the use of a previously assessed coating (the gelatin-based coating loaded with cellulose nanocrystals and cinnamon essential oil) on a food item that, in comparison with foods tested in our previous works, has not cuticle and thus is very delicate to undergo these kinds of surface treatments. Several quality attributes of mushrooms coated with different loads of CEO (1200–2400 μL/L) were investigated and systematically compared with those pertaining to uncoated samples over 15 days of cold storage.

Materials and methods

Raw materials and chemicals

Button mushrooms with an approximate mass of 20 ± 2 g each were purchased from Sablan Mushroom Company (Ardabi, Iran). Only mushrooms free from pathogens at the closed cap stage were selected according to a uniform colour, shape, and size and subsequently stored in a refrigerator ($T = 4$ °C) before analyses. Fish gelatin (GelA) of type A, 200 Bloom, Halal certified, and of high purity (>90%) was obtained from Weishardt (Graulhet, France). Cinnamon essential

oil (CEO) containing E-cinnamaldehyde (70.6%), E-cinnamyl acetate (5.3%), β-caryophyllene (5.1%), linalool (4.2%), eugenol (3.7%), and 1,8-cineole + β-phellandrene (1.2%) was acquired from Plant Therapy (Twin Falls, USA). Bacterial cellulose (BC) was produced from *Komagataeibacter sucrofermentans DSM 15973* (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) under static conditions (Rovera *et al.*, 2018). Sulfuric acid (99% v/v), ethanol (96% v/v), and cellulose dialysis tubing membrane (12 kDa, average width 43 mm) were purchased from Sigma-Aldrich-Merck, (Milan, Italy).

Production of emulsion coatings

BCNCs-GelA/CEO emulsion coatings were obtained via a three-step methodology, as already specified elsewhere (Razavi *et al.*, 2022c). Briefly, different amounts of cinnamon essential oil (600–1200 μL) were added to a BCNCs aqueous suspension prepared at pH 5 at a concentration of 0.4% (w/w) (Rovera *et al.*, 2018), so as to obtain a series of CEO concentrations within the range of 0.8–1.6% (v/w). Then, 75 g of the CEO/BCNCs dispersion was added dropwise into the fish gelatin solution (Razavi *et al.*, 2020). The so-obtained BCNC-GelA/CEO emulsions were composed of BCNCs and gelatin at final concentrations of 0.06% (w/w) and 3% (w/w), respectively, while the achieved concentrations of cinnamon essential oil stood between 1200 and 2400 μL/L.

Coating deposition on mushrooms

Only mushrooms with no clear sign of microbial deterioration or mechanical harm were chosen and then thoroughly rinsed with distilled water. Precautionary, the samples were also disinfected with a 0.05% (w/v) sodium hypochlorite solution, in order to reset any potential difference in terms of possible contamination. This allows to highlight the differences between samples solely due to the coating treatments. Sodium hypochlorite is by far one of the most widely used and effective sanitising agent for vegetables, also due to its low cost, large availability, and no significant impact on the nutritional and sensorial quality of fresh products. Although a high concentration of sodium hypochlorite may pose some concerns due to the generation of disinfection by-products (DPBs) arising from chlorine, studies have demonstrated that, in washing processes, effective disinfection is obtained by reaching free chlorine concentrations in the range of 50–200 mg/L, which are far below the allowed DBP concentrations in drinking water (Arienzo *et al.*, 2023).

After disinfection, mushrooms were rinsed with sterile distilled water and lastly dried under a chemical hood at room temperature for 2 h. Afterward, mushrooms were blotted and immediately dipped in the coating solutions for 15 s at 25 °C to yield 3 different batches, namely C1200, C1800, and C2400 (coated sample with a CEO load of 1200, 1800, and 2400 μL/L, respectively). The coated samples were then left at ambient conditions for 12 h to enable the coating to dry (Fig. 1). Control samples followed the above-described preparation procedure but without the application of coating (uncoated samples). Then, the mushrooms were placed in a plastic container and kept under controlled conditions (4 ± 0.5 °C, 80–85% RH) for 15 days. Sampling occurred every 5 days and all the analyses were performed on at least 15 samples randomly chosen from each batch. Any coating cycle involved a minimal amount of BCNCs-GelA/CEO solution, that is, ≈10 mL kg^{-1} of button mushrooms.

Physicochemical characterisation of mushrooms

Weight loss (WL), firmness, percentage of opened caps, and respiration rate (R_{CO2})

WL was calculated employing a digital scale with a precision of 0.001 g (GF-600, A&D Weighing, Tokyo, Japan) according to (AOAC, 2000).

Firmness was assessed by a puncture test using a stainless steel probe (6 mm diameter) connected to an Instron Universal Testing Machine (STM-20). Load cell nominal capacity, probe speed, and penetration depth during the test were 100 N, 20 mm/min, and 5 mm, respectively. During each test, samples were secured on a steel plate while being punctured by the probe. The firmness was identified as the peak force in the final force-deformation plot (Louis *et al.*, 2021).

The determination of the percentage of opened caps is indicative of the progress towards an umbrella-like shape of the cap, typically followed by the detachment of the membrane. The quantification of open caps was conducted as reported by Nasiri *et al.* (2019).

The respiration rate of mushrooms was evaluated according to a method described in our previous work (Razavi *et al.*, 2022b). After sampling from each batch, mushrooms with a known mass and volume were equilibrated at ambient conditions and subsequently put in a 2 L glass jar. A CO_2/O_2 detector device (OXYBABY6, WITT, Witten, Germany), equipped with a needle sensor, was inserted inside the jars at intervals of 30 min for a total of 4 h to monitor the evolution of CO_2 concentration (%). The respiration rate (R_{CO2}) of mushrooms was then calculated using the eqn (1):

$$R_{CO_2}\left(mL_{CO_2}\ kg^{-1}\ h^{-1}\right) = \frac{y_{f,CO_2} - y_{i,CO_2}}{t_f - t_i} \times \frac{V_f}{m} \quad (1)$$

where y_{i,CO_2} and y_{f,CO_2} represent the initial and final gas concentration in the jar (% v/v), respectively, t_i is

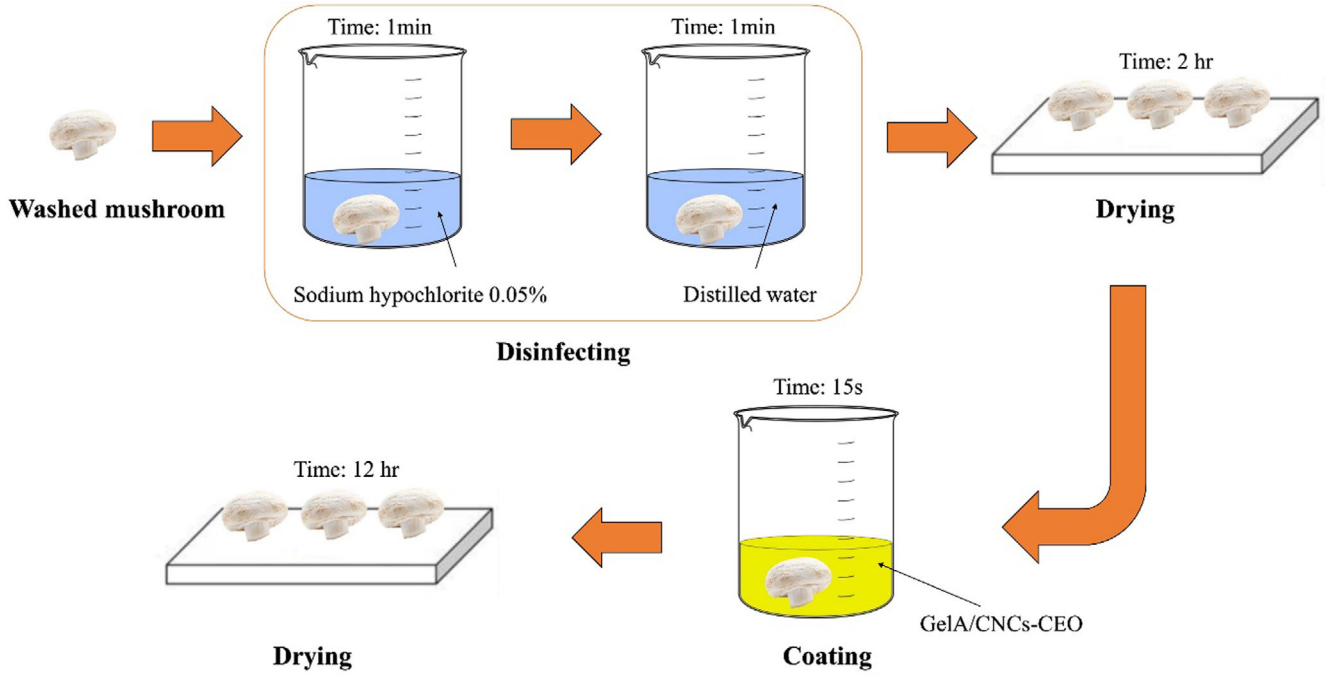

Figure 1 Schematisation of the BCNCs/GelA-CEO coating deposition process on mushrooms.

the initial time and t_f is the final time (4 h), m is the total mass of mushrooms inside the jar (kg), and V_f is the free volume in the jar (mL).

Total soluble solids (TSS), ascorbic acid (AA), and soluble proteins (SP)

TSS (in ° Brix) of the mushroom extract was measured using a digital refractometer (RHB-32/ATC, Brix 0–32%, 0.2% accuracy; China), according to the procedure proposed in our previous work (Razavi *et al.*, 2022c).

Ascorbic acid (AA) was quantified through a titration process using a starch solution (0.5 mL, 5% w/w) as an indicator. For each batch, 100 g of mushroom tissue was blended to yield an extract. The latter underwent filtration using a clean cloth, and the resultant clear juice was diluted with distilled water to a total volume of 100 mL, 20 mL of which was transferred to an Erlenmeyer flask. Approximately 150 mL of distilled water was added to the flask, followed by the addition of 5 mL of a 6 mM potassium iodide solution and 5 mL of 1 M hydrochloric acid solution. Titration was performed utilising a 2 mM potassium iodate solution until it reached a dark blue-black hue colour. The ascorbic acid content, expressed in mg 100 g^{-1} of mushrooms, was calculated taking into account the stoichiometry of the redox reactions as reported in (Razavi *et al.*, 2022c).

The concentration of soluble protein (SP, in g of equivalent bovine serum albumin 100 g^{-1} mushrooms, that is, in %) was determined by the Bradford method as illustrated elsewhere (Moosavi-Nasab *et al.*, 2023).

Polyphenol oxidase (PPO) and peroxidase (POD) activity

The enzyme extracts were first prepared from the sample tissue. The enzyme extraction solution comprised 0.2 M sodium phosphate buffer (pH = 6.5) containing 4% (w/v) polyvinyl polypyrrolidone (PVPP) and 1% (v/v) Triton X-100. After homogenisation of the mushrooms, 10 g of the homogenate was mixed with 20 mL of the enzyme extraction solution. The mixture was then centrifuged (LISA, France) at 4 °C for 10 min. The supernatant was finally harnessed for the subsequent PPO and POD activity (abs min^{-1} g^{-1}) (Eldib *et al.*, 2020; Moosavi-Nasab *et al.*, 2023).

Colour measurement

To characterise the colour properties of the samples, measurements were conducted using a PS-100 spectroradiometer (Apogee Instruments, INC, Logan, USA). Colour data were collected in the $L^*a^*b^*$ colour space from 10 different positions on 5 replicate samples per treatment at regular intervals during storage. The obtained values were averaged, and the overall colour difference (ΔE) was calculated based on eqn (2).

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (2)$$

where ΔL^*, Δa^*, and Δb^* are the differences between L^*, a^*, and b^* of samples every 5 days of storage time

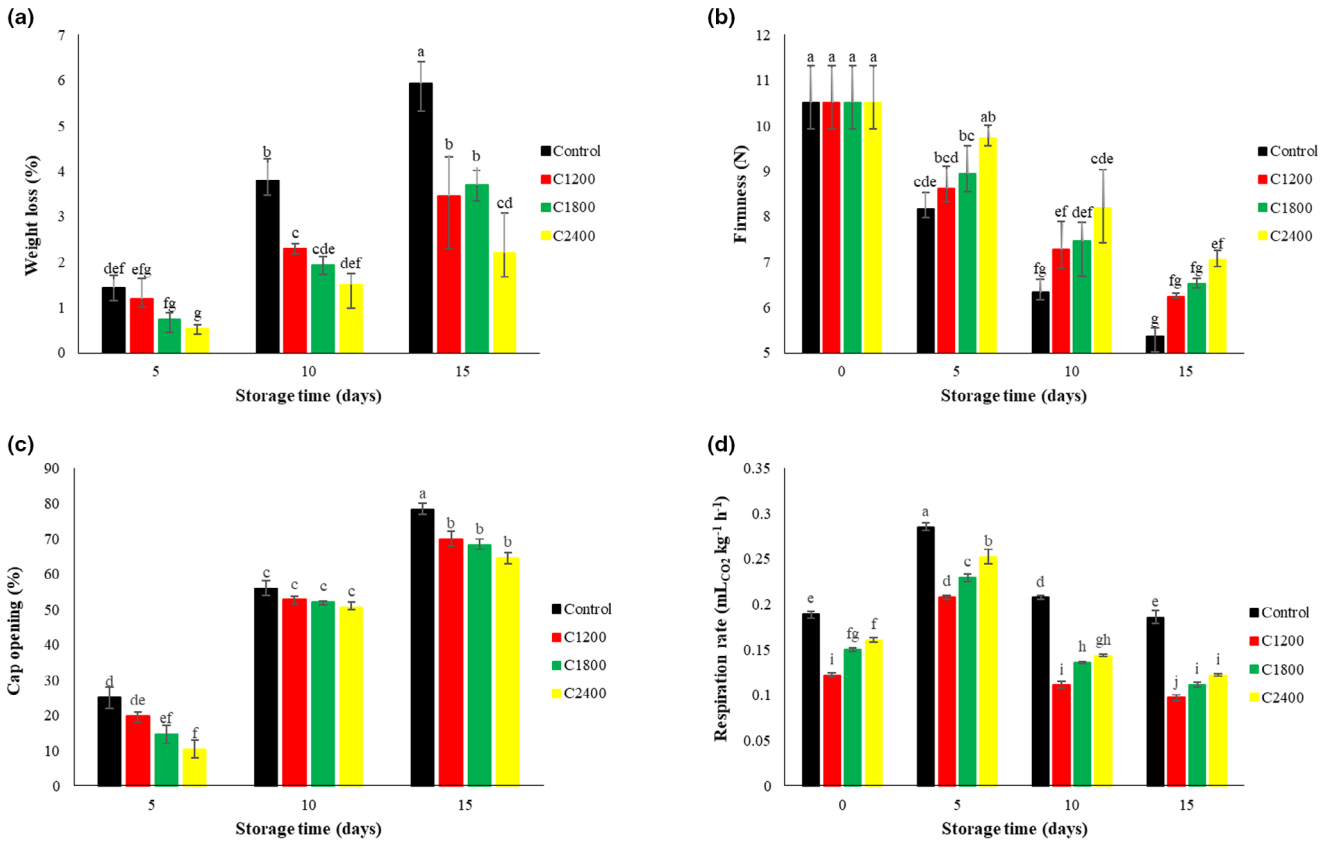

Figure 2 Values of weight loss (a), firmness (b), percentage of open caps (c), and respiration rate (d) of untreated (control) and coated (CEO load = 1200–2400 μL/L) mushrooms during 15 days of cold storage ($T = 4$ °C). Standard deviations were used as error bars. Different letters above the bars denote significant ($P < 0.05$) differences among mean values.

in comparison to the first day of storage (Razavi *et al.*, 2022b).

Sensory evaluation

The sensory characteristics of mushrooms, including odour, gill colour, gill surface, cap uniformity, and the appearance of dark areas on the cap were evaluated using a 9-point hedonic test (1: very weak, 3: weak, 5: acceptable (marketability limitation), 7: good, and 9: excellent) (Jiang *et al.*, 2012). The samples were evaluated by ten members of a trained panel. Before evaluation, the mushrooms were stored in sealed, odourless plastic containers at room temperature and evaluated within 2 h to prevent off-odours.

Statistical analysis

A factorial experimental design based on a completely randomised setup featuring a minimum of three replicates was used throughout the investigation. The experimental data underwent scrutiny using SAS 9.1 software, while the analysis was further supplemented by Duncan's multi-range test. This comparative analysis of means was conducted at a maximum acceptable error threshold of 5% ($P < 0.05$).

Results and discussion

Weight loss

Figure 2a shows that a substantial weight loss occurred for both control and coated samples, irrespective of the observation time. However, this phenomenon was more pronounced when the coating was not applied ($P < 0.05$). Control samples displayed a maximum WL of 5.93% after 15 days of cold storage. As a general trend, the increase in storage time and weight loss had the same evolution for all the treatments, with some deviation likely due to sample variability. The best WL mitigation effect was recorded for the C2400 sample. This can be attributed to the larger presence within the coating of hydrophobic molecules (i.e., main components of cinnamon essential oil), which behaved as a semipermeable membrane,

thus increasing the resistance against the moisture transfer to the surface of the mushrooms. In other words, the great water-repellent effect pertaining to the coating system dramatically limits moisture evaporation phenomena (Razavi *et al.*, 2022c). This is in good agreement with previous findings on the involvement of natural coatings to boost the shelf-life of button mushrooms. For instance, using chitosan as a coating agent for mushrooms enabled the reduction of the WL values well below those shown by control samples over 21 days of storage (Najafian-Jazi *et al.*, 2020). The authors attributed these findings to the semi-permeable property of chitosan coating against gases, which in turn is due to its polycationic feature. In another study, Moosavi-Nasab *et al.* (2023) explored the protective effect of aloe vera-gelatin composite coatings when deposited onto button mushrooms throughout an observation window of 16 days. The developed composite coatings led to better quality preservation of mushrooms, with a consistent reduction in WL as compared to the application of aloe vera- and gelatin-based coatings alone. In a recent study, coatings based on sodium alginate, pectin, carboxymethyl cellulose, and chitosan were applied to fresh button mushrooms. The lowest WL values were observed for sodium alginate-coated samples (Ple oianu & Nour, 2022).

Overall, the results of Fig. 2a align with those of previous works focusing on the implementation of nanocrystalline cellulose (CNCs)-based coatings on several fruits, such as apples, pears, and strawberries (Fakhouri *et al.*, 2014; Dong *et al.*, 2015; Deng *et al.*, 2017; Da Silva *et al.*, 2019). An opposite behaviour was disclosed by Sapper and coworkers (Sapper *et al.*, 2019, 2020), who reported the coating's inability to curb apple fruit weight loss. The authors explained these results considering both the highly hydrophilic nature of the polymers employed in the coating and the thinness of the coating due to the uneven distribution of solid particles on the surface.

Firmness

The tissue firmness values measured for both control and coated mushrooms during storage are depicted in the histograms of Fig. 2b. As can be seen, a reduction in firmness was detected for all samples as a function of the sampling time. However, the coating on the product surface relented the decay of mushrooms' textural properties by preserving the integrity of the tissues, in agreement with the trend of Fig. 2a, though no statistically significant differences ($P > 0.05$) were detected among treatments.

Firmness loss during vegetable ripening and storage is attributed to the degradation of parenchymal cell walls (Wang *et al.*, 2021b). Moreover, firmness is influenced by variables like cell wall strength, intercellular contact, and cellular turgidity (Toivonen & Brummell, 2008). Post-harvest processing may introduce cell wall-degrading enzymes such as chitinase, cellulase, and β-1,3 glucanase, which affect cell-to-cell adhesion and weaken cell wall mechanical robustness, thus eventually leading to firmness reduction (Maftoonazad *et al.*, 2008). Gelatin and other biopolymers mitigate physiological degradation by curbing respiration rates and consequently the activity of cell wall-degrading enzymes (Li *et al.*, 2022).

Overall, consistent with the findings of other similar studies (Nasiri *et al.*, 2019; Louis *et al.*, 2021; Razavi *et al.*, 2022a), the process of firmness deterioration is decelerated by the deposition of the coating on the food items.

Percentage of open caps

In our work, a progressive increase in the percentage of cap opening was detected as a function of the storage time (Fig. 2c), with only slightly higher values ($P < 0.05$) shown by control samples as compared to coated samples after 5 and 15 days of storage. During storage, moisture loss reduces the cohesive forces between water molecules and hydrophilic compounds, such as proteins. Consequently, a void emerges between the caps and the veil within the mushrooms (Wang *et al.*, 2015). The reduced cap opening observed in the coated samples (Fig. 2c) is likely due to the coating's obstructive effect on some skin pores and openings, which hinders moisture from escaping the product. Our results are supported by previous literature evidence. In particular, Nasiri and coworkers (Nasiri *et al.*, 2019) observed a reduction in the cap opening of mushrooms when treated with tragacanth gum-based coatings enriched with marjoram and thyme essential oils (100–1000 mg/L). Similarly, Zhu *et al.* (2019) demonstrated the potential of sodium alginate-based coatings containing thymol (1% v/v), L-cysteine (0.3 g/L), and nisin (0.4 g/L) to inhibit the failure of the veil in *P. nameko* mushrooms.

Respiration rate

During the storage period, the respiration rate exhibited an initial rise up to 5 days followed by a decline for all treatments (Fig. 2d). This indicates the occurrence of product senescence caused by intense physiological activities and the partial loss of its edible value due to an inhibition of electron transfer in the mitochondria and an increase in the production of ROS during storage (Li *et al.*, 2013). However, the respiration rate of the coated samples remained significantly lower ($P < 0.05$) than that of the control samples, regardless of the observation time. This finding

highlights the potential of coating to act as a gas-barrier system, thereby controlling the oxygen consumption of mushrooms. Noticeably, a positive linear correlation between CEO concentration and respiration rate was found ($0.92 < R^2 < 0.99$, Fig. S1). This trend can be ascribed to the increase in intermolecular free volume within the main polymer phase (i.e., fish gelatin) brought about by the CEO plasticising effect, thus speeding up CO_2 transport phenomena (Razavi *et al.*, 2022b, 2022c). Similarly, a substantial reduction in CO_2 production rate, that is, a delay in the ripening and aging processes, for mushrooms protected with agar-lecithin and chitosan-based coatings was recently noted (Cavusoglu *et al.*, 2021; Khojah *et al.*, 2021).

Total soluble solids

An upward trend in TSS was observed across all tested samples as storage time increased (Fig. 3a). In particular, maximum and minimum increases in the TSS content of mushrooms occurred in correspondence with the control group and C2400 group, respectively, after 15 days of storage. Figure 3a also shows that, for each storage time, the higher the CEO concentration, the lower the TSS values. The increase in TSS can be explained by first considering the increased weight loss of the product during storage (Fig. 2a). Second, the rise in TSS can be attributed to more intense catabolic processes (e.g., respiration and the conversion of more complex polysaccharides, such as starch and hemicellulose into simpler sugars) as a consequence of product aging (Mahfoudhi & Hamdi, 2015). The positive effect of the coating to control the respiration rate by relenting the CO_2/O_2 exchanges between the product and the external environment explains the decelerated metabolic progression linked to aging. Parallel outcomes have been documented in the coating of button mushrooms using tragacanth gum loaded with marjoram and thyme essential oils (Nasiri *et al.*, 2019), as well as in the cherry coating process utilising carboxymethyl chitosan-gelatin (Zhang *et al.*, 2021).

Ascorbic acid and soluble proteins

Figure 3b illustrates that, over the storage period, the ascorbic acid content decreased for all treatments; however, the control samples exhibited a more pronounced reduction ($P < 0.05$) compared to the coated samples below the original levels ($t = 0$ days). Furthermore, higher concentrations of CEO within the coating resulted in better preservation of ascorbic acid. Given that soluble protein content indicates tissue degradation caused by ROS (Bajgai *et al.*, 2006), a similar trend to that observed for ascorbic acid was revealed (Fig. 3c). Previous works reported the positive effect of reduced oxygen transfer phenomena on the oxidation

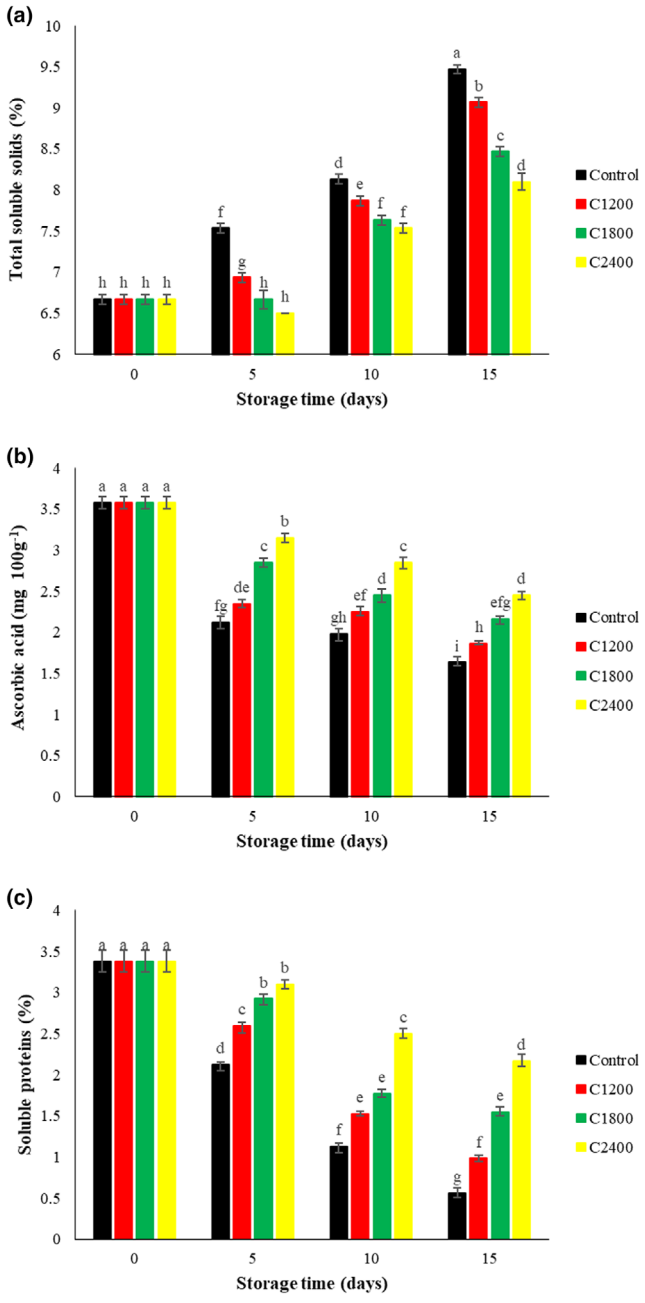

Figure 3 Values of total soluble solids (a), ascorbic acid (b), and soluble proteins (SP) content (c) of untreated (control) and coated (CEO load = 1200–2400 μL/L) mushrooms during 15 days of cold storage ($T = 4$ °C). Standard deviations were used as error bars. Different letters above the bars denote significant ($P < 0.05$) differences among mean values.

of ascorbic acid (Sena *et al.*, 2019). Moreover, cinnamaldehyde, the most representative compound of CEO, has outstanding antioxidant attributes (Subash-Babu *et al.*, 2014). Hence, the effect of the coatings

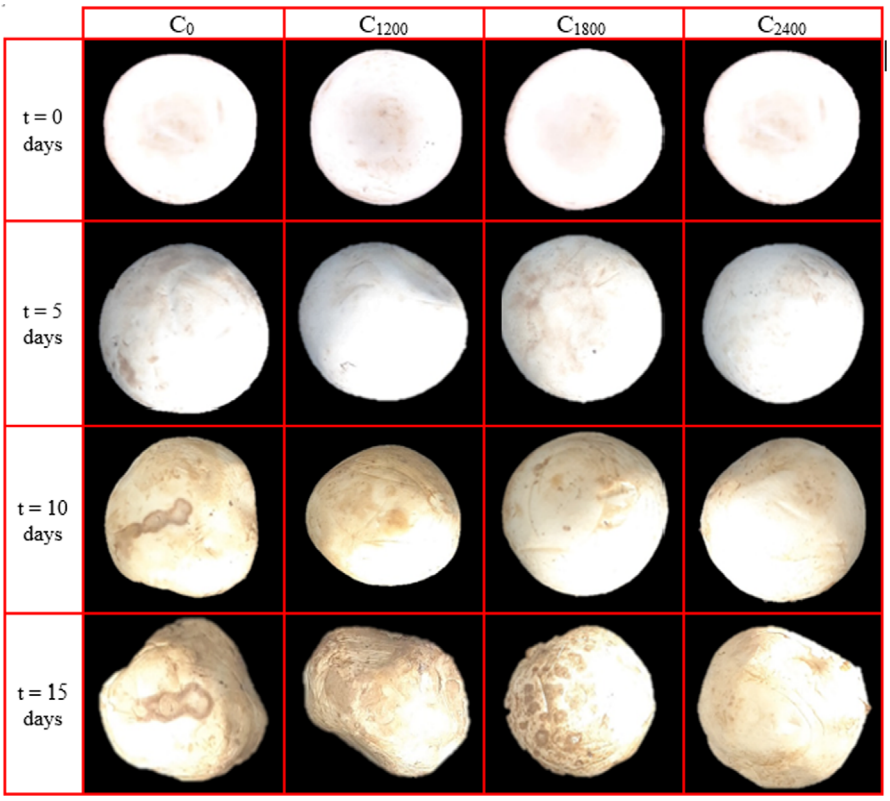

Figure 4 Digital images of mushrooms' surface treated with BCNCs/GelA-CEO emulsion coatings at different CEO loads (1200–2400 μL/L) as a function of the storage time ($T = 4$ °C).

prepared in this work on ascorbic acid preservation is due to two primary factors, namely the reduction in O_2 permeability and the inherent antioxidant property of CEO. This dual mechanism appears to both suppress oxidative damage and retard the aging process in button mushrooms. Once again, the results shown in Fig. 3b and Fig. 3c are in good agreement with previous research focused on the preservation of (i) apples protected using gelatin coatings loaded with BCNCs and cinnamon essential oil (Razavi *et al.*, 2022b), (ii) mushroom packed utilising a bionanocomposite film embedding nanosilica and oregano essential oil (Yan *et al.*, 2023), and (iii) *Pholiota nameko* mushrooms coated using sodium alginate (Zhu *et al.*, 2019).

Polyphenol oxidase (PPO) and peroxidase (POD) activity

Fresh mushrooms are naturally white (Fig. 4) but, after storage, their surface becomes brown because of the oxidation of phenolic compounds by PPO, especially tyrosinase (Ple oianu & Nour, 2022). The PPO activity exhibited an increasing trend during the cold storage period for all treatments (Fig. 5a). A similar behaviour was exhibited by the POD activity, but only up to the intermediate observation time, above which a marked decline took place (Fig. 5b). In both cases, the application of BCNCs-GelA/CEO coatings on the surface of button mushrooms attenuated the enzyme activity as a function of the CEO loading.

The ascorbic acid content significantly influences the activity of the oxidation enzymes and can mitigate the browning effect caused by these enzymes (Othman, 2012; Can *et al.*, 2014). Based on the previous considerations on the evolution of AA, its decrease during the storage period may presumably contribute to the increase in PPO activity caused by the absence of an inhibitory effect. However, it should be noted that ascorbic acid efficacy in reducing the PPO activity hinges on its concentration in the product, thus potentially leading to instances where its inhibitory influence may be neglected (Othman, 2012).

Ultimately, the results of Fig. 5 fit with those obtained by other scientists delving into the application of gum Arabic/roselle extract-based coating on blueberries (Yang *et al.*, 2019), chitosan and guar gum-based composite edible coating for mushrooms (Huang *et al.*, 2019) and carboxymethyl cellulose-pectin coatings on plums (Panahirad *et al.*, 2020).

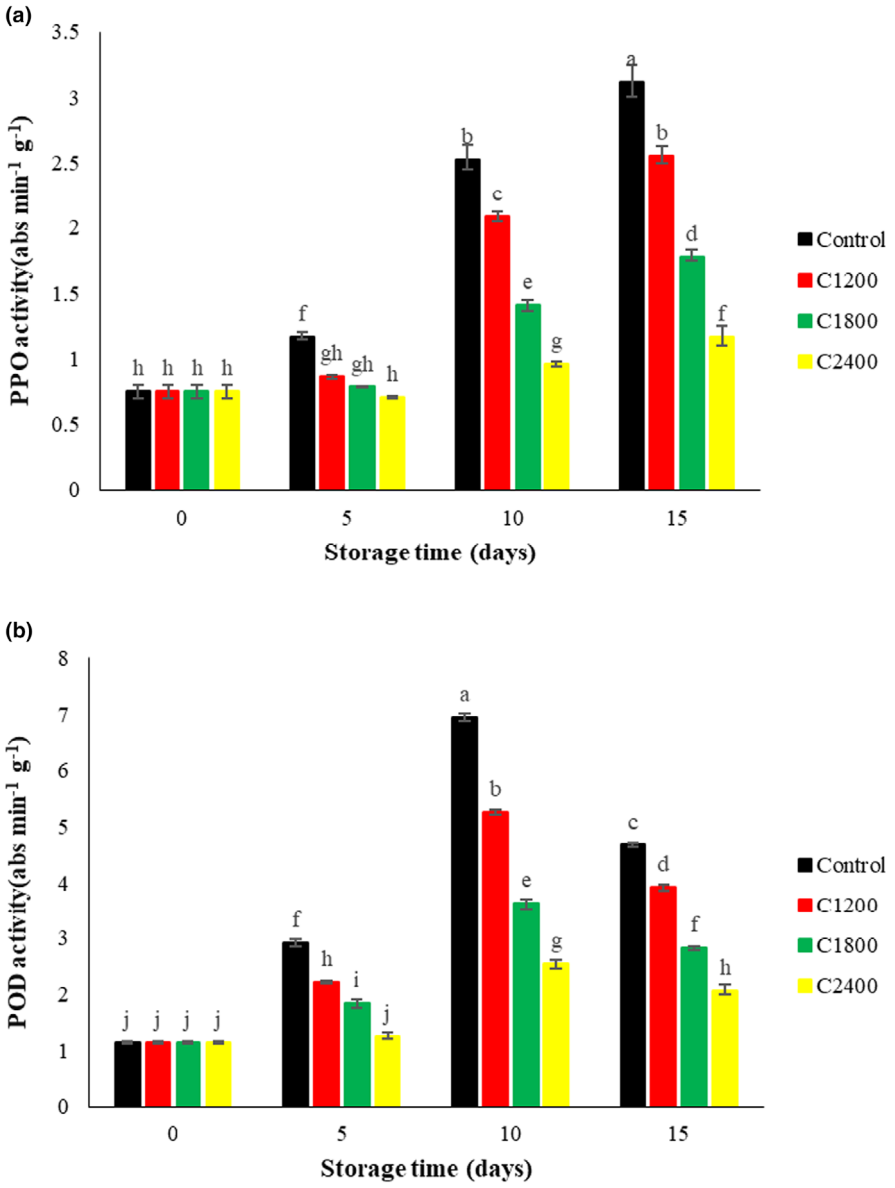

Figure 5 Values of PPO (a) and POD (b) activity of untreated (control) and coated (CEO load = 1200–2400 μL/L) mushrooms during 15 days of cold storage ($T = 4$ °C). Standard deviations were used as error bars. Different letters above the bars denote significant ($P < 0.05$) differences among mean values.

Colour

Colour is a critical parameter for assessing the freshness and quality of fruits and vegetables during post-harvest storage. In button mushrooms, discoloration to brown tones is a common phenomenon due to oxidative processes and microbial spoilage, leading to deterioration in nutritional quality and overall shelf-life (Moosavi-Nasab *et al.*, 2023). As illustrated in Fig. 6a, the L^* values of coated samples were significantly higher than those of the control throughout the storage period. This suggests that the applied coatings, owing to the light-scattering properties of the BCNC-based gelatin polymer system (Razavi *et al.*, 2022b), altered the optical properties of the samples. Moreover, a decline in lightness (L^*) was observed in all treatments over time, being indicative of browning. However, while increasing essential oil concentration slowed down the browning process, significant differences in L^* values were only found

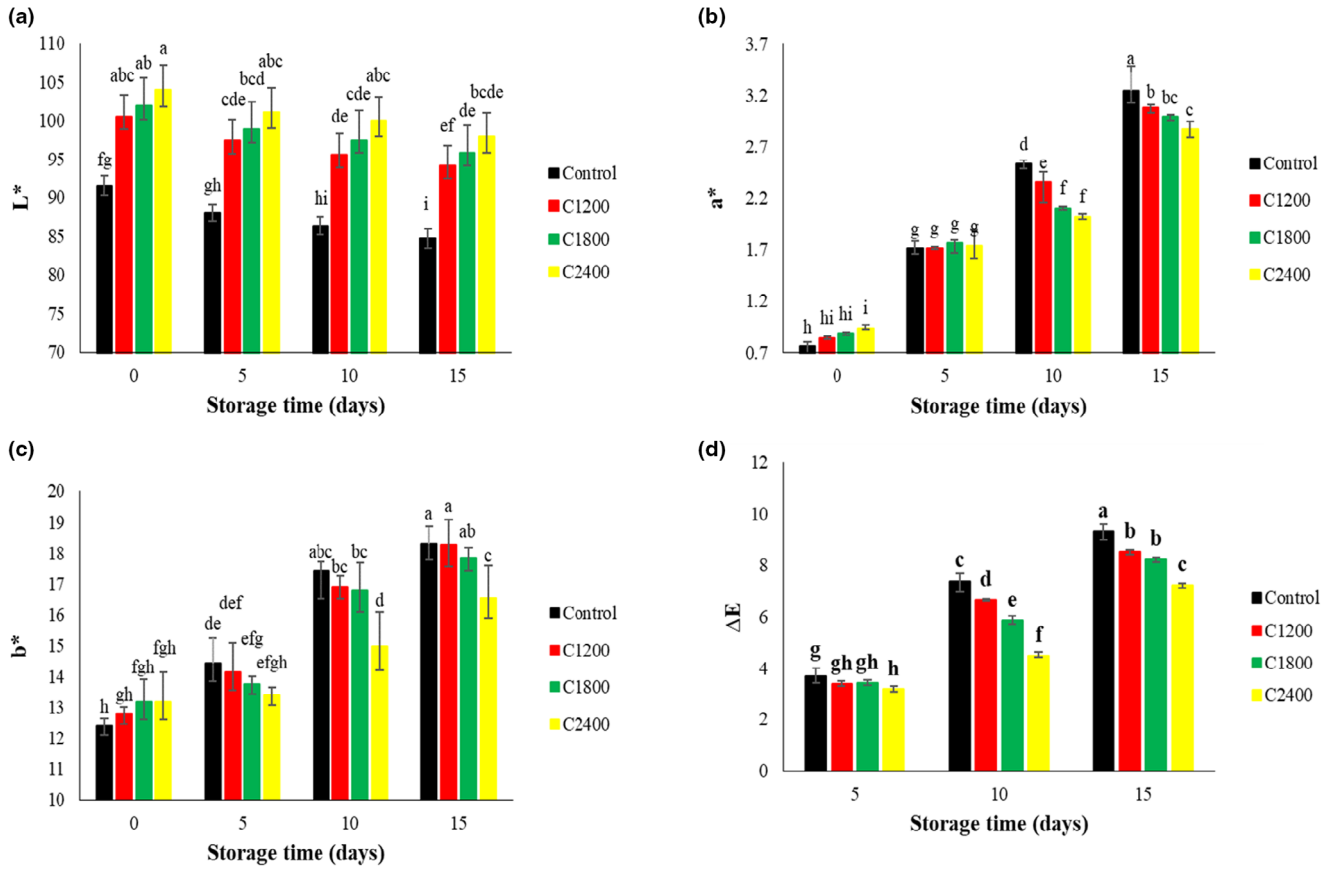

Figure 6 Values of total L^* (a), a^* (b), b^* (c), and ΔE (d) of untreated (control) and coated (CEO load = 1200–2400 μL/L) mushrooms during 15 days of cold storage ($T = 4$ °C). Standard deviations were used as error bars. Different letters above the bars denote significant ($P < 0.05$) differences among mean values.

between treatments C1200 and C1800 on days 10 and 15 ($P > 0.05$). Given the correlation between changes in L^* and the activity of oxidative enzymes, particularly PPO (Zambrano-Zaragoza *et al.*, 2014), the coatings likely mitigated browning by reducing respiration rate and inhibiting enzymatic activity (Fig. 5).

The analysis of a^* and b^* colour parameters revealed that, initially, these values were slightly higher in coated samples, despite being not significantly different from control samples ($P > 0.05$). This chromatic variation can be attributed to the presence of phenolic compounds in cinnamon essential oil, which exhibit red-yellow hues and absorb light at lower wavelengths (Sivarooban *et al.*, 2008). Over time, both a^* and b^* values increased in all samples (Fig 6b,c), indicating intensified yellow and red tones and leading to browning. However, these changes were less pronounced in coated samples, especially C2400. As shown in Fig. 6d, ΔE increased with storage duration in all treatments. Nevertheless, the colour differences in coated samples were significantly lower, particularly on days 10 and 15. Moreover, higher essential oil concentrations in the coating mitigated colour changes. Given that ΔE is influenced by all three-colour parameters (L^*, a^*, and b^*), its increase suggests that browning, primarily driven by a^* and b^*, was the predominant colour alteration. The coatings suppressed respiration and limited the mushroom's oxygen access, thereby reducing oxidative enzyme activity and subsequent browning pigment formation (Choi *et al.*, 2016). Additionally, the coatings inhibited the growth of spoilage microorganisms and melanin formation, especially at higher cinnamon essential oil concentrations (Jiang *et al.*, 2012). These factors collectively accounted for the reduced colour changes in coated samples. Similar protective effects of coatings such as gum, agar, sodium alginate, egg white protein, lecithin (Cavusoglu *et al.*, 2021), pectin, chitosan, sodium alginate, and CMC (Ple oianu & Nour, 2022), and gelatin and aloe vera gel (Moosavi-Nasab *et al.*, 2023) against colour changes in mushrooms have been previously reported. Our findings suggest

Figure 7 Spider web charts depicting the effect of BCNCs/GelA-CEO coatings loaded with different CEO concentrations (1200–2400 μL/L) on the sensory attributes of button mushrooms after 15 days of cold storage at 4 °C. Numbers indicate the average score attributed by panellists to each parameter upon the hedonic test.

that the applied coating, particularly C2400, holds promise as a potential strategy to mitigate visual deterioration (Fig. 4) and extend the shelf life of button mushrooms.

Sensory evaluation

The results related to the sensory characteristics of mushrooms after 15-day cold storage are shown in Fig. 7. As a general trend, the higher the CEO concentration within the coating formulation, the greater the averaged score assigned to the sensory characteristics of the samples, with the lowest score being assigned to control samples. This highlights that the coating not only had no negative impact on the colour of the product but also allowed the samples to retain odour and appearance better than reference samples. Browning of mushrooms is attributed to the action of oxidative enzymes and microorganisms on their tissue (Huang *et al.*, 2019). Accordingly, it is plausible that the higher concentration of essential oil in the coating helped reduce the population of spoilage microorganisms, such as *Escherichia coli* and *Staphylococcus aureus*, which are known to cause the oxidation of phenolic compounds, leading to the formation of brown melanins, as reported by Jiang *et al.* (2012). In addition, the lower activity of PPO and POD enzymes in coated samples prevents the formation of brown spots and consequently better preserves the sensory characteristics of button mushrooms.

Conclusions

In this research, the effect of BCNCs-GelA/CEO emulsion coating on the shelf-life of button mushrooms during cold storage was evaluated. The overall quality of all samples decreased during storage, despite this trend being significantly slower in coated samples as compared to control ones. Increasing the concentration of essential oil also led to better preservation of the quality characteristics of the samples during storage. In addition, the inability of the coating used, especially at higher concentrations of essential oil, to impair the sensory attributes of mushrooms was demonstrated. Therefore, BCNCs-CEO/GelA nanoemulsion coatings can be used to reduce post-harvest waste and extend the shelf-life of button mushrooms.

Declaration of competing interest

The authors declare that they have no known competing interests.

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Author contributions

Abdollah Golmohammadi: Conceptualization; methodology; writing – review and editing; supervision; project administration; funding acquisition. **Mohammad Tahmasebi:** Conceptualization; methodology; investigation; formal analysis; writing – original draft. **Mahsa Sadat Razavi:** Methodology; writing – review and editing. **Vahid Neysari-Fam:** Investigation; writing – review and editing. **Daniele Carullo:** Writing – review and editing. **Stefano Farris:** Writing – review and editing; supervision; project administration.

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Data availability statement

Data will be made available on request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Linear correlation between respiration rate of mushrooms and CEO load employed within the coating formulation. For each sampling time, the correlation coefficient (R2) was reported.