



White Button Mushroom Storage in Trays with Cinnamaldehyde Sachets

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Abstract

White button mushrooms (*Agaricus bisporus*) were stored for up to 13 days at 7°C with microcapsules containing cinnamaldehyde. Along the storage period, samples were retrieved to determine respiration rates, fresh weight losses, pileus firmness and color, stem length, cap opening and total phenolic content. On the last day of storage, the mushrooms stored with cinnamaldehyde sachets presented the lowest percentage of fresh weight losses, had higher firmness and the stem length elongation and cap opening were also reduced in comparison to the control mushrooms stored without the cinnamaldehyde sachets. The mushrooms treated with cinnamaldehyde until the seventh day of storage had the lowest pileus browning index, accumulated the highest total phenolic content and the respiration rates were the lowest. Adding cinnamaldehyde microcapsules to white button mushroom storage trays lengthened mushroom shelf life and maintained the quality, evidencing the potential benefits of cinnamaldehyde for mushroom storage.

Keywords: *Agaricus bisporus*; Cinnamon Essential Oil; Ionic Gelation; Sodium Alginate Spheres

Introduction

The mushroom species *Agaricus bisporus* (J.E. Lange) Imbach represents about 15% of the global mushroom market [1]. Mushrooms are highly perishable and face many problems along the postharvest storage period: dehydration, browning, textural changes and overall losses of quality and nutritional value. And, furthermore, visual quality drives consumer acceptability.

Essential oils are rich sources of antioxidants and antimicrobial substances and are capable of triggering biological reactions in tissues that may alter the physicochemical parameters of horticultural crops [2]. Incorporating essential oils in methods to preserve fresh mushrooms inhibited browning [3], reduced microorganism counts, improved sensory quality and total antioxidant capacity [4]. An indication that essential oils improve the quality and prolong the shelf life of mushrooms.

Methods of varied complexity using essential oils are available for food preservation and, among those, the encapsulation of active ingredients in multifunctional spheres and of different sizes [5, 6]. Encapsulation may require more sophisticated equipment and, therefore, the need to evaluate less complex encapsulation methods such as ionic gelation. Ionic gelation consists in complexing species with opposite charges to form insoluble gels that retain water and active ingredients [7]. A variety of biopolymers that are of low cost, stable, low toxicity, and adaptable can be used in a range of applications and formulations.

To date, there is little information available on the response of mushrooms to storage with sachets of microcapsules containing essential oils. Therefore, microcapsules with or without cinnamaldehyde were developed and transferred to sachets. The objective of this work was to test the sachets for effects on shelf life and overall quality of white button mushrooms during refrigerated storage.

Materials and Methods

The microcapsules encapsulated with or without cinnamaldehyde were prepared with modifications via the ionic gelation method described by Masod and coworkers [8]. A 3% sodium alginate solution (w/v) with or without 0.06 mol.L⁻¹ natural cinnamaldehyde (≥95% of purity; Sigma-Aldrich) and 1% 80 PS polysorbate (w/v) were poured into a 20 mL syringe positioned about 10 cm above a 2% calcium chloride solution (w/v) under constant agitation. The dripping of the

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alginate solution into calcium chloride resulted in microcapsules. The microcapsules were agitated for 15 minutes and afterwards rinsed three times with distilled water. Immediately after that procedure, the microcapsules were transferred to sachets of a porous and non-toxic material.

White button mushrooms (*A. bisporus*) were acquired from a local vendor. The mushrooms harvested the day before were transported by car to the postharvest laboratory, where they were maintained overnight in cold storage at $7^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 90% relative humidity. The following morning the experimental units were prepared by placing the sachets of the respective treatments inside the polystyrene trays. Approximately 8 units of white button mushrooms (90 g) were positioned vertically and covered with a 10 micrometer stretch film. Mushroom stem length was about $1.4 \text{ cm} \pm 0.53 \text{ cm}$ and had an intact pileus. The following treatments: control, alginate microcapsules encapsulated with or without cinnamaldehyde with three replicates for every treatment and for every storage period of 3, 7, 10 or 13 days at $7^{\circ}\text{C} \pm 2^{\circ}\text{C}$ were applied. Two consecutive trials were conducted in completely randomized designs.

Respiration rates (CO_2 production) in a static system were determined according to the procedure of Li and coworkers [9]. Approximately 30 g of white button mushrooms along with 25 mL 0.4 mol.L^{-1} sodium hydroxide solution (w/v) were placed in hermetically closed glass flasks. After 90 minutes, the sodium hydroxide solutions were titrated a 0.1 mol.L^{-1} with oxalic acid solution adding a few drops of 1% phenolphthalein (w/v) as indicator. For every replicate, two measurements were performed to determine respiration rates, expressed as $\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, using equation 1 as indicated by Jiang [10]:

$$\text{Respiration rate} = [(V_1 - V_2) \cdot c \cdot 44] / W \cdot t \quad (\text{Equation 1})$$

where V_1 = oxalic acid volume dispensed for the control (mL), V_2 = oxalic acid volume dispensed for every replicate (mL), c = oxalic acid molarity (concentration), 44 = molar mass of CO_2 , W = fresh mass of the mushroom replicate (g), and t = time, expressed in hours, used to accumulate CO_2 inside the glass flasks.

The fresh weight losses of the white button mushrooms were determined by weighing the trays on the day of retrieval from storage. Fresh weight losses were expressed as the percentage of fresh mass losses compared to the initial weight at installment of the trials [11]. Firmness measurements were performed on one white button mushroom pileus selected at random from every replicate using a Texture Analyzer TA.XT (Stable Micro Systems, UK) to a depth of 5 mm with a 5 mm cylindrical probe at a speed of 2.0 mm s^{-1} . Data of kgf (kilogram force) were registered via the software Texture Expert (Version 1.0). From the curves of kgf versus time, firmness maximum force was determined and expressed in Newton (N).

The external color of white button mushroom pileus was determined with a model CR 400 Konica/Minolta colorimeter. Two white button mushrooms from every replicate were selected at random and the tristimulus values L^* , a^* and b^* under illuminant D65 measured at three equidistant spots of every pileus. Color differences (ΔE) were calculated using equation 2 as indicated by Xu and coworkers [11]:

$$\Delta E = \sqrt{(L_e - L_t)^2 + (a_e - a_t)^2 + (b_e - b_t)^2} \quad (\text{Equation 2})$$

where L_e , a_e and b_e are the tristimulus values from the day of installment of the trial and L_t , a_t and b_t the values measured on the

day of retrieval from storage.

The Browning Index (BI) was calculated according to Palou and coworkers [12] using equations 3 and 4:

$$\text{BI} = [100(x-0.31)]/0.172 \quad (\text{Equation 3})$$

$$X = (a+1.75L)/(5.645L+a-3.012b) \quad (\text{Equation 4})$$

where L = luminosity varies from 0 (100% reflection of the xenon light beam) to 100 (no reflection of the xenon light beam), a = the green-red component and b = the yellow-blue component of the color [13].

The stem lengths of three white button mushrooms from every replicate were determined using a pachymeter at day zero (installment of the trials) and on the day of retrieval from storage. The differences express the percentages of gains in length during storage periods. The opening of the cap was based on the development of the cap shape followed by the detachment of the veil [10]. All the mushrooms from every replicate were evaluated for cap opening, results were expressed as percentages and were calculated using equation 5:

$$\% \text{Open caps} = (\text{Noc/Nt}) \cdot 100 \quad (\text{Equation 5})$$

where Noc = number of mushrooms with an open cap and Nt = total number of mushrooms in every replicate.

Total phenolic content was quantified via the spectrophotometric Folin-Ciocalteu method based on Dias [14] with slight modifications: 3 g of pileus were homogenized in 8 mL extraction solvent (acetone 50% - v/v) in an ultrasonic bath for 30 minutes. Afterward, the homogenate was filtered in Whatman no. 1 filter paper and the filtrate transferred to amber glass flasks for storage at -18°C in a domestic freezer. To 0.25 mL of the filtered homogenate, 2.75 mL of 3% Folin-Ciocalteu (v/v) and 0.25 mL of a 10% sodium carbonate (w/v) solution were added. Absorbance was read on a T60U spectrophotometer (PG Instruments, UK) at 765 nm. Measurements were made in triplicate and compared to standard curve of gallic acid to calculate mg GAE.100g^{-1} fresh mass using equation 6:

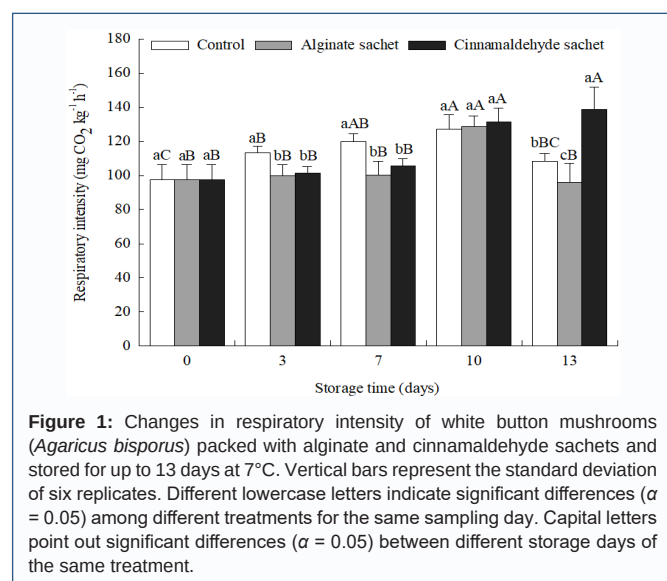
$$\text{GAE} = \{[(C \cdot V_e)/1000]/m\} \cdot 100 \quad (\text{Equation 6})$$

where C = mg.L^{-1} gallic acid, V_e = extract volume (mL), and m = fresh mass (g) of the extraction sample.

The trials were conducted in completely randomized designs with three replicates for every treatment and storage period. Two consecutive trials were conducted. Values from all experiments were expressed as the mean $\pm$ Standard Deviation (SD). A Box-Cox transformation was used. Data were submitted to ShapiroWilk's test, Bartlett's test and residuals plots for normality, homoscedasticity and independence of errors, respectively. The assumptions were met and the comparison between the treatments were performed using ANOVA (analysis of variance). When significant, averages separations were performed by Tukey test. Differences at $\alpha = 0.05$ were considered as significant. The data were analyzed with software R version 4.0.1 [15].

Results and Discussion

Respiration rates of white button mushroom increased from $97.5 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at the beginning of trial setup to beyond $120 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ on the tenth day of storage (Figure 1). Differences in between treatments were not significant. After 13 days of storage, only mushrooms from the cinnamaldehyde treatment had a significantly higher respiration rate ($138.7 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$). Xylia and coworkers



[16] observed an increase in respiration rates in apples after using eucalyptol essential oil, which according to Namiota and Bonikowski [2] originates from the fact that essential oils release volatile substances that diffuse through tissues and alter the biochemical, microbiological and physicochemical parameters of fresh produce.

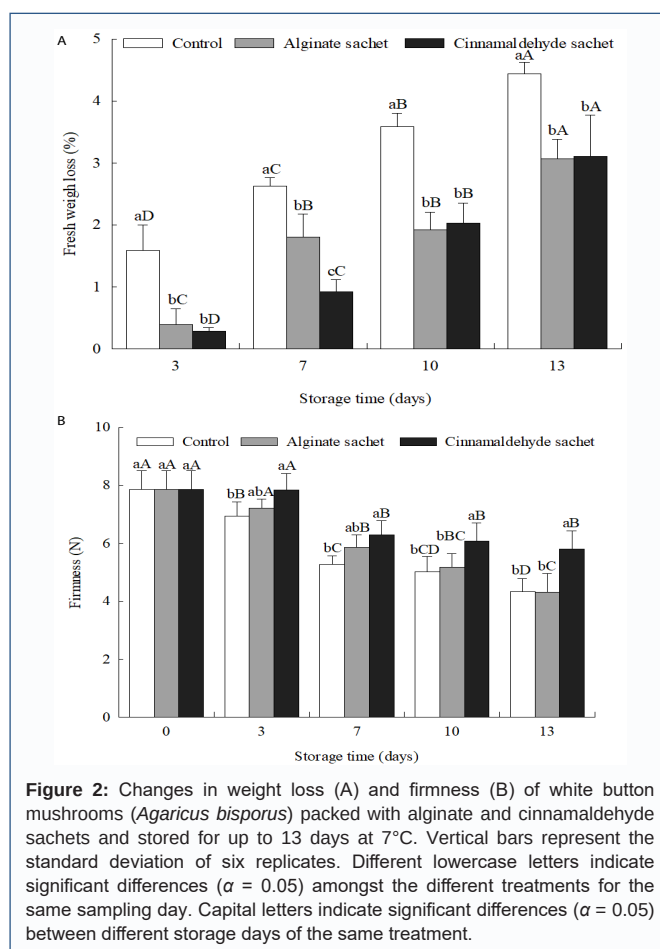
Martínez-Hernández and López-Gómez [17] attributed the reduction in respiration rates to the inhibition of some key enzymes of the primary metabolism. Increases in respiratory activities are, as a rule, related to senescence processes and cellular collapse [18]. A physiological stage that seems not be the circumstance as the cinnamaldehyde treatment did have a positive effect on mushroom preservation as will be further discussed.

The fresh weigh loss of the mushrooms after 13 days of storage varied from 3.11% up to 4.44% (Figure 2A). The control mushrooms showed the highest percentages of losses. Fresh weigh losses are attributable to dehydration of the mushrooms and to their intense respiratory activity favored by cap opening and veil detachment [4]. According to Diamantopoulou and Philippoussis [19], mushrooms have a high-water content and a thin epidermal structure, which also facilitates water loss. Louis and coworkers [20] indicate that fresh produce exhibit visible signs of wilting and surface shriveling when fresh mass losses are between 4% and 6%.

Mushroom cap firmness value at the beginning of the trials was of 7.87N and decreased along the storage period by 44.9% and 26.2% in the control mushrooms and cinnamaldehyde treated mushrooms, respectively (Figure 2B). An indication that cinnamaldehyde contributed to mushrooms to retain firmness and texture that are, according to Jiang [10] crucial for mushroom shelf life.

Freshness of the mushrooms in the present trials could be attributed to a higher relative humidity inside the storage trays, as the sachets lost about 25% of their fresh weight during the storage period. Maintaining a high relative humidity inside the packaging is a major factor in reducing dehydration processes. Echegoyen and Nerín [3] also observed significantly lower fresh weight losses in white button mushrooms in the presence of cinnamaldehyde. However, the authors did not provide the basis for those lower fresh weight losses.

Jiang *et al.*, [4] also noticed better mushroom firmness with



cinnamaldehyde treatments. Texture changes can occur quickly after harvest and are related to metabolic changes induced mushroom ripening processes [21].

Zivanovic and coworkers [22] visualized structural and compositional changes in *A. bisporus* after harvest: increments in intercellular spaces, hyphae contraction, vacuole rupture, protein and polysaccharide loss and cell wall breakdown. Ni and coworkers [23] reported differences in cellulose and chitin concentrations along the mushroom storage. According to the authors, this is evidence of the complexity of interactions between cell wall components and metabolism associated with texture changes, which are not yet well characterized in mushrooms.

Color is one of the most important traits in purchase decisions [10]. Before storage of the mushrooms, the lightness represented by the L^* values [20] of the chromameter were 91.4 (Table 1). After 13 days of storage, the index decreased to 78.3, 76.9 and 83.7 for the control treatment, the treatment with alginate microcapsules and the sachets containing cinnamaldehyde, respectively.

Briones and coworkers [24] suggested a classification procedure in which an L^* below 80 indicates low quality of the mushrooms and commercially improper for sale. According to that statement, after 13 days of cold storage, only the mushrooms in trays containing cinnamaldehyde remained above this marketable limit. Furthermore, lightness and firmness were highly and positively correlated (0.902), indicating that both are important for the consumer's decision.

Total color differences (ΔE) might be analytically classified

Table 1: Changes in luminosity (L*), total color difference (ΔE) and Browning Index (BI) of white button mushrooms (*A. bisporus*) packed with alginate and cinnamaldehyde sachets stored for up to 13 days at 7°C.

Days in storage	Control	Alginate sachet	Cinnamaldehyde sachet
Luminosity (L*)			
0	91.2 $\pm$ 1.18 a, A ¹	91.2 $\pm$ 1.18 a, A	91.2 $\pm$ 1.18 a, A
3	88.3 $\pm$ 0.77 b, B	87.6 $\pm$ 0.95 b, B	90.0 $\pm$ 1.44 a, A
7	83.7 $\pm$ 0.78 b, C	85.3 $\pm$ 0.51 b, C	87.6 $\pm$ 0.99 a, B
10	81.4 $\pm$ 1.02 b, D	82.4 $\pm$ 1.07 b, D	84.7 $\pm$ 1.01 a, C
13	78.3 $\pm$ 0.58 b, E	76.9 $\pm$ 1.20 b, E	83.6 $\pm$ 1.31 a, C
Color difference(ΔE)			
0	-	-	-
3	04.6 $\pm$ 1.36 a, D	05.4 $\pm$ 0.89 a, D	02.6 $\pm$ 0.62 b, D
7	09.4 $\pm$ 0.66 a, C	09.2 $\pm$ 0.84 a, C	05.7 $\pm$ 0.65 b, C
10	13.7 $\pm$ 1.38 a, B	11.7 $\pm$ 1.31 ab, B	11.4 $\pm$ 0.76 b, B
13	17.8 $\pm$ 3.88 a, A	18.0 $\pm$ 0.92 a, A	13.9 $\pm$ 1.43 b, A
Browning Index (BI)			
0	12.3 $\pm$ 0.95 a, E	12.3 $\pm$ 0.95 a, E	12.3 $\pm$ 0.95 a, E
3	16.9 $\pm$ 1.02 a, D	16.6 $\pm$ 0.58 a, D	15.2 $\pm$ 0.66 b, D
7	22.5 $\pm$ 1.32 b, C	24.3 $\pm$ 1.27 a, C	19.1 $\pm$ 0.96 c, C
10	26.3 $\pm$ 0.95 ab, B	27.5 $\pm$ 1.03 a, B	24.9 $\pm$ 1.01 b, B
13	31.7 $\pm$ 1.82 a, A	31.7 $\pm$ 1.35 a, A	27.5 $\pm$ 1.16 b, A

¹Averages of six replicates $\pm$ standard deviation.

Averages in the same line and within the same variable followed by different lowercase letters are significantly different at $p \leq 0.05$.

Averages in the same column within the same variable followed by different capital letters are significantly different at $p \leq 0.05$.

Table 2: Percentages of stem length elongation and cap opening of white button mushrooms (*Agaricus bisporus*) packed with alginate and cinnamaldehyde sachets and stored for up to 13 days at 7°C.

Days in storage	Control	Alginate sachet	Cinnamaldehyde sachet
Stem length (%)			
3	18.9 $\pm$ 3.30 a, D ¹	11.7 $\pm$ 2.41 b, C	05.3 $\pm$ 0.80 c, D
7	23.8 $\pm$ 2.36 b, C	29.4 $\pm$ 4.05 a, B	16.7 $\pm$ 2.83 c, C
10	30.2 $\pm$ 4.38 a, B	32.0 $\pm$ 2.26 a, AB	24.3 $\pm$ 3.11 b, B
13	59.9 $\pm$ 7.14 a, A	37.4 $\pm$ 4.43 b, A	34.0 $\pm$ 3.11 b, A
Percentages open caps			
3	39.4 $\pm$ 5.25 a, D	28.4 $\pm$ 2.02 b, C	21.1 $\pm$ 3.53 b, C
7	57.6 $\pm$ 2.80 a, C	53.3 $\pm$ 5.77 a, B	27.5 $\pm$ 3.96 b, C
10	75.9 $\pm$ 3.72 a, B	61.2 $\pm$ 2.10 b, B	52.4 $\pm$ 2.09 c, B
13	86.3 $\pm$ 4.05 a, A	78.1 $\pm$ 6.03 b, A	66.4 $\pm$ 5.56 c, A

¹Averages of six replicates $\pm$ standard deviation.

Averages in the same line within every storage period followed by different lowercase letters are significantly different at $p \leq 0.05$.

Averages columns within every treatment followed by different capital letters are significantly different at $p \leq 0.05$.

according to Pathare and coworkers [25]: when above 3, represent very distinguishable samples regarding color. When the ΔE is in the range of 1.5 to 3, samples are considered distinguishable and if ΔE is below 1.5, samples are no longer dissimilar. Mushrooms after three days of storage with cinnamaldehyde had a color difference of 2.6, which means consumer would only notice slight differences in color. After 13 days of storage, the mushrooms with cinnamaldehyde sachets presented the lowest ΔE values (13.9) among the treatments as presented in Table 1.

Browning Index (BI) values are an indication of enzymatic and non-enzymatic browning [12]. The initial BI value of control samples was 12.3 and at the end of the storage period, the control mushrooms

and mushrooms in trays with only alginate microcapsules had BI values of 31.7. Mushrooms treated with cinnamaldehyde showed the lowest BI value during storage (27.5).

Enzymatic browning of *A. bisporus* is the result of the activity of Polyphenoloxidase enzyme (PPO). The PPO oxidizes phenolic compounds to quinones and the subsequent reactions lead to the synthesis of melanins [26]. Echegoyen and Nerin [3] determined that 4.73 mmol.L⁻¹ was the average Inhibitory Concentration (IC₅₀) of cinnamaldehyde capable to promote a dose dependent inhibition of the oxidation of 3,4-Dihydroxy-L-phenylalanine (L-DOPA), i.e., the inhibition of PPO activity and reduction of browning of white button mushrooms. Louis and coworkers [20] observed that mushrooms

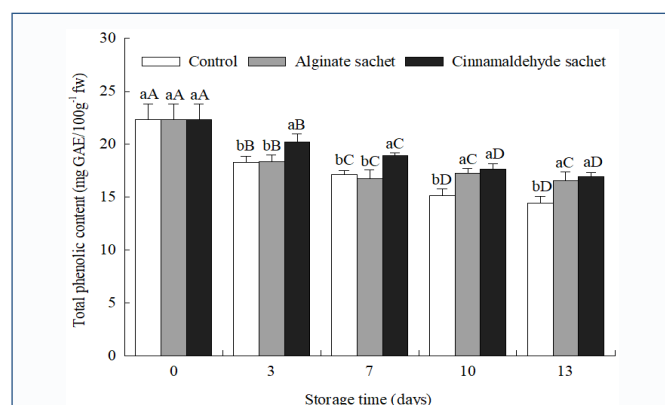


Figure 3: Changes in total phenolic content of white button mushrooms (*Agaricus bisporus*) packed with alginate and cinnamaldehyde sachets and stored for up to 13 days at 7°C. The vertical bars represent the standard deviation of six replicates. Different lowercase letters indicate significant differences at $\alpha = 0.05$ among the different treatment on the same sampling day. Capital letters show significant differences ($\alpha = 0.05$) between different storage days on the same treatment.

treated with cinnamaldehyde had higher L^* values and lower ΔE and BI values throughout the storage period. This suggests that cinnamaldehyde may have a positive effect in delaying browning of mushrooms and maintaining the quality and market value of white button mushrooms.

Changes in stem length and cap opening are related to mushroom maturity, spores release and storage conditions. White button mushrooms from all treatments showed an elongation of stem length over the 13 days of storage (Table 2). Variations in stem length could be a result of continued growth of the stem, as the mushrooms are harvested at an unripe stage and with cell expansion at the apical portion of the stem [27]. Qu and coworkers [28] observed that cinnamaldehyde reduces ergosterol synthesis and causes damage to cell membranes in *Aspergillus flavus*. Ramos and coworkers [1] described that ergosterol is high in most species of mushrooms and the *A. bisporus* is a good source of ergosterol and B-group vitamins. In this sense, cinnamaldehyde sachets may have induced biochemical reactions in mushroom tissues that were able to inhibit the stem growth of the mushrooms. Nevertheless, more studies are necessary to better elucidate the effects of essential oils use.

The percentage of open caps increased with storage time (Table 2). After 13 days of storage, the control samples and the sachets with cinnamaldehyde had 86.3% and 66.4% of open caps, respectively. Diamantopoulou and Philippoussis [19] suggest that cap opening is related to tissue dehydration. Mushrooms stored with cinnamaldehyde sachets delayed cap opening that could be related to the lower fresh mass losses determined in that treatment. Cap opening and fresh weight losses were highly and positively correlated (0.916), supporting that evidence.

The total phenolic content on day zero was 22.3 mg GAE.100g⁻¹ fresh weight and decreased during the storage period (Figure 3). Mushrooms treated with cinnamaldehyde had significantly higher phenolic content at the end of the storage period (16.9 mg GAE.100g⁻¹ FW) compared to control mushrooms. Jiang and coworkers [4] suggested that the accumulation of total phenolic content could be due to secondary metabolites as a defense mechanism, as essential oils trigger signals similar to stresses. On the other hand, Fattahifar and coworkers [29] and Nasiri and coworkers [30] concluded that

lower contents of phenolic compounds contribute to the synthesis of brown pigments, which is consistent with the total phenolic content in the control samples of the present study. Echegoyen and Nerin [3] showed that cinnamaldehyde has an inhibitory effect on PPO in white button mushrooms, avoiding oxidation of phenolic and browning of tissues.

Conclusion

The addition of sachets of cinnamaldehyde microcapsules to storage positively affects the respiration rates and overall quality of white button mushrooms for up to 13 days of storage at 7°C. Sachets containing cinnamaldehyde prolong the shelf life of white button mushrooms in comparison to control and alginate microcapsules treatments.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon request.

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Conflicts of Interest

The authors declare no conflict of interest.

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