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Effect of Titanium Dioxide Nanocomposite Material and Antimicrobial Agents on Mushrooms Shelf-Life Preservation

Rokayya Sami [1,*], Abeer Elhakem [2], Mona Alharbi [2], Nada Benajiba [3], Manal Almatrafi [1,4], Jing Jing [5] and Mahmoud Helal [6,7]

1 Department of Food Science and Nutrition, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; manal.almatrafi@uconn.edu
2 Department of Biology, College of Science and humanities in Al-Kharj, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia; a.elhakem@psau.edu.sa (A.E.); mh.alharbi@psau.edu.sa (M.A.)
3 Department of Basic Health Sciences, Deanship of Preparatory Year, Princess Nourah Bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; nabenajiba@pnu.edu.sa
4 Department of Nutritional Sciences, Connecticut University, Storrs, CT 06269, USA
5 School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin 150090, China; jingjing@hit.edu.cn
6 Department of Mechanical Engineering, Faculty of Engineering, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; helal.mo@tu.edu.sa
7 Production and Mechanical Design Department, Faculty of Engineering, Mansoura University, Mansoura 35516, Egypt
* Correspondence: rokayya.d@tu.edu.sa

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Abstract: Mushrooms have limited shelf-life and it can be prolonged if suitable conditions and treatments are effectively applied. In this study, nanocomposite material and antimicrobial agents with a combination of chitosan were used as novel packaging material for mushroom preservation. The microbiological analysis, physicochemical properties, headspace gas analysis, and polyphenol oxidase activity (PPO) during cold storage were investigated. As compared with control, coated mushrooms with chitosan (CHS), and nano-titanium dioxide CHSTiO$_2$ thymol + tween-80 CHSTiO$_2$/TT80 coating treatment showed significantly ($p \leq 0.05$) lower respiration rate, microbial contaminations (4.27 log CFU/g), and (5.93 log CFU/g) for total yeast/mold and aerobic plate counts, respectively. The weight loss ratio was the lowest for CHSTiO$_2$/TT80 (10.88% loss) followed by CHSTiO$_2$ (11.76% loss). CHSTiO$_2$/TT80 recorded a higher electrolyte leakage rate (25.84%) and acidity. While the lowest PPO activity was established for CHSTiO$_2$ (17.09 U mg^{-1} Protein), while the lowest values for total soluble solid concentrations were reported for CHSTiO$_2$/TT80 mushrooms (4.91%). These results indicated that CHSTiO$_2$/TT80 coating treatment might delay the aging degree of white button mushrooms and be investigated as a novel packaging material for other food products in the future.

Keywords: mushrooms; nanocomposite material; antimicrobial agents; shelf-life

1. Introduction

Mushrooms are highly perishable due to their high respiration, metabolic activities, and transpiration rates [1]. Themushroom's shelf-life can be prolonged if the suitable conditions and treatment are effectively applied, as chilling at 4 °C can prolong the shelf life up to 3–4 days compared to the ambient temperature (1–2 days) [2]. Singh et al. [3], reported that the properties,

quality, texture, and appearance can be influenced during the storage period. Mushrooms face several huge problems during storage which negatively affect marketing strategy as quality deterioration, color changes, tissue damages, cap opening, weight loss, turgidity, senescence, and bacterial contaminations [4]. The high demand for nutritious, fresh, healthy, cheap, and delicious protein has awakened the mushroom preservation industry to fulfill the needs of customers in several countries. Mushroom tissues are good sources of minerals, vitamins, phenolics, and oxidative enzymes. Inactivation of polyphenol oxidase is the main reason for browning reactions [5]. Effective packaging systems are applied for mushroom preservation and retaining quality to extend shelf-life, such as film wrap, vacuum, perforations, and modified atmosphere with the addition of several chemical treatments, especially polyethylene and polyvinyl chloride films [6,7]. Chemicals such as potassium metasulphite, calcium chloride, citric acid, sodium ethylene diamine tetra acetic acid, and sorbitol were applied for mushroom preservation due to the vital functions to reduce pH, increase antioxidant activities, and maintain the firmness [8]. Blanching and autoclaved methods are recently used for avoiding browning, weight loss, and nutritional leakage [5].Licciardello et al. [9] established that 6% O_2 with the addition of chitosan can efficiently decrease cap progress, respiration, and enzymatic browning. Consequently, the adoption of novel technologies is needed for commercial use. Titanium dioxide nanoemulsion (TiO_2) is an efficient photocatalyst, cheap, and toxic for several applications of coatings against microorganisms [10]. The American Food and Drug Administration (FDA) announced that Nano-TiO_2 with low concentrations is safe in the food industry and cosmetics [11]. Qiao et al. [12] established that thymol and tween are effective antimicrobials against microbes.

The research work focused on the effects of chitosan/titanium dioxide nanocomposite material with the addition of thymol and tween-80 agents on mushrooms shelf-life preservation along with the storage period.

2. Materials and Methods

2.1. Materials

Nano-titanium dioxide with a partial size of (15 nm), acetic acid, chitosan (85%), thymol, and tween-80 were from (Sigma-Aldrich, Shanghai, China).

White button mushrooms were purchased from a local orchard in Taif, Saudi Arabia. Mushroom samples were at the closed cap stage, about 3–4 cm in diameter. Injured, damaged, shriveled, and decadent samples were rejected. Mushroom samples were categorized as follows: control: Mushrooms were subjected to deionized water, placed on a trellis shelf, allowed to dry at ambient temperature, and stored without any coating treatment. CHS: Mushrooms washed with chitosan (1%) and acetic acid (1%). CHSTiO_2: Mushrooms were washed with the CHS solution and nano-titanium dioxide (15 nm) (1%). CHSTiO_2/TT80: Mushrooms were washed with the CHSTiO_2 solution with the addition of thymol (0.5%) and tween-80 (0.25%) as antimicrobial agents. All mushroom categories were washed for 2 min, allowed to dry, then packaged with a zipped lock polyethylene overwrapping bags with twelve perforations with a 5 mm diameter hole [13]. Three various trays of each mushroom treatment were prepared on each sampling day. All the mushroom samples were stored at 4 °C for 12 days to be detected at an interval of 3 days.

2.2. Microbiological Analysis

Approximately 30 g of mushroom samples were homogenized by using a stomacher (400 VW, Weymouth, MA, USA) for 5 min with 225 mLof (0.1%, *w*/*v*) Rose Bengal Medium. Serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were made and incubated for 5 days at 28 °C [12]. Total aerobic plate counts were incubated for 2 days by using 3 M petriflims at 37 °C [14]. Total aerobic counts, yeast/mold populations were evaluated and expressed as the average of the triplicate measurements as log CFU/g.

2.3. Color Analysis

The color analysis was detected by a CR-400 (Konica Co., Japan), where L^* value is the lightness that ranges from (0) black to (100) white; a^* value ranges from (−120) green to (+120) red, while b^* value ranges from (−120) blue to (+120) yellow at three different locations on mushroom samples [15].Total color difference (ΔE*) was calculated by Equation (1):

$$\Delta E^* = [(L^* - L_0)\ 2 + (a^* - a_0)2 + (b^* - b_0)\ 2]\ 0.5 \quad (1)$$

where L_0, a_0, and b_0 are the initial color values. Moreover, the color was evaluated in terms of the browning index that was calculated by Equation (2):

$$\text{Browning index} = \frac{100(x - 0.31)}{0.17} \quad (2)$$

where $x = (a - 1.75L)/(5.645L) + (a - 3.012L)$.

2.4. Weight Loss Ratio and Texture Measurements

The weight loss ratio (%) for each mushroom treatments was evaluated by dividing the weight change by using a digital balance with an accuracy of 0.01 g and calculated by Equation (3):

$$Weightloss(\%) = \left(\frac{w_i - w_f}{w_i}\right) \times 100 \quad (3)$$

where, w_i is the initial weight, while w_f is the weight during the storage period.

The firmness of mushroom samples was detected on the top side of nine mushroom pieces for each treatment by using an FHR-1 (1 kg) a texture analyzer with a speed of 2 mm/s, diameter 5 mm cylinder-type, 250 N load cell, and 0.5 Ncontact force (Nippon CO., Tokyo, Japan) [15].

2.5. Headspace Gas Analysis and pH

The gas compositions, carbon dioxide (CO_2) and oxygen (O_2), inside the headspace of packaged mushrooms were detected by using a gas chromatograph (GC) (Check Mate-II, Ringsted, Denmark) Propaq-Q and Molecular Sieve 5A columns (25 m × 0.5 mm i.d. × 1 pm)were used for CO_2 and O_2 determinations, respectively. Mushroom samples were detected by a gas-tight syringe septum and placed on the film exterior, while the carrier of the gas was the helium flow of (2.5 mL/min) and detection of FID (250 °C). The chromatography was applied in triplicate with a thermal conductivity detector [16]. The pH value of the mushroom juices was detected after homogenization, filtration, and the use of a digital pH meter (S20-K, Columbia, OH, USA).

2.6. Enzyme Activity Analysis and Total Soluble Solids

The polyphenol oxidase activity (PPO) was evaluated by using a kit (Solarbio, Beijing, China) according to the protocol method reported by the manufacturer's instructions by mixing 1 mL pyrocatechol (50 mM) and 1 mL of sodium phosphate buffer (100 mM, pH 7.0) with detection at 410 nm. (PPO) activity was expressed at U mg^{-1} Protein [17]. The total soluble solids (TSS) of mushroom samples were homogenized, filtered by a 40 µm filter pape, and evaluated by a hand-held refractometer with a resolution of 0.01in three replications (Krüss, Hamburg, Germany) [18].

2.7. Membrane Permeability Analysis and Open Cap Percents

Randomly selected mushroom bodies (5 g) were cut into several parts, leaving the pileus intact and suspended in a 50 mL beaker of deionized water. Electrical conductivity was detected and recorded (P0) then detected again after 10 min (P1) after the addition of deionized water several times and

soaking for 1 h. Mushroom tissues were boiled for 10 min then (P2) was detected after cooling [19]. Electrolyte leakage rate (%) was described by Equation (4):

$$(P1 - P0)/(P2 - P0) \quad (4)$$

The quality of white button mushrooms was detected according to the development of the opening cap and described by Equation (5) [20]:

$$\%Opencaps = \frac{N_{ac}}{N_t} \times 100 \quad (5)$$

where, N_t is the total mushroom numbers and N_{ac} is the open cap numbers.

2.8. Statistical Analyses

The normal test (Kolmogorov_Smirnov) was done to check the normal distribution of the samples. Analysis of variance (ANOVA) was used to compare the storage period for each treatment. Duncan's tests as post hoc were performed to investigate the differences between days interval at $p \leq 0.05$. The computer program SPSS software for windows version 22.0 was used for statistical analysis (Statistical Package for Social Science, Armonk, NY, USA: IBM Corp) at significant levels 0.05 (p-Value ≤ 0.5), while the charts were drawn by Origin 8 software.

3. Results and Discussion

3.1. Microbiological Analysis

The yeast and mold counts of CHSTiO_2 and CHSTiO_2/TT80 coating treatments did not vary significantly for the first 9 days but were raised by prolonging the storage time, Table 1. The yeast and mold counts of CHS coating (6.17 log CFU/g) and CHSTiO_2 coating (6.13 log CFU/g) treatments were higher than (control) samples on days 12. The lowest count was established in CHSTiO_2/TT80 coating treatment (4.27 log CFU/g).

Table 1. Microbiological analysis (log CFU/g).

Days	Control	CHS	CHSTiO_2	CHSTiO_2/TT80
Yeast and Mold counts				
0	1.23 ± 0.60 ^{c}	1.13 ± 0.49 ^{c}	1.10 ± 0.56 ^{c}	0.73 ± 0.21 ^{c}
3	2.50 ± 0.44 ^{bc}	2.57 ± 0.67 ^{bc}	2.70 ± 0.46 ^{b}	1.47 ± 0.55 ^{bc}
6	2.90 ± 0.75 ^{b}	2.87 ± 0.19 ^{b}	2.80 ± 0.14 ^{b}	1.80 ± 0.26 ^{bc}
9	4.03 ± 0.15 ^{b}	4.17 ± 0.06 ^{b}	3.90 ± 0.10 ^{b}	2.87 ± 0.15 ^{b}
12	6.30 ± 0.13 ^{a}	6.17 ± 0.55 ^{a}	6.13 ± 0.16 ^{a}	4.27 ± 0.12 ^{a}
Total aerobic plate counts				
0	3.50 ± 0.30 ^{d}	3.37 ± 0.71 ^{c}	3.40 ± 0.95 ^{c}	3.20 ± 0.61 ^{b}
3	4.47 ± 0.81 ^{cd}	4.27 ± 0.14 ^{bc}	4.20 ± 0.66 ^{bc}	3.93 ± 0.91 ^{b}
6	5.17 ± 0.51 ^{bc}	5.07 ± 0.91 ^{ab}	5.03 ± 0.51 ^{b}	4.70 ± 0.72 ^{ab}
9	5.73 ± 0.80 ^{ab}	5.53 ± 0.64 ^{ab}	5.33 ± 0.40 ^{ab}	5.03 ± 0.75 ^{ab}
12	6.80 ± 0.26 ^{a}	6.43 ± 0.72 ^{a}	6.43 ± 0.61 ^{a}	5.93 ± 0.12 ^{a}

Results in the same column as $^{a; b; c; d}$ mean significant differences between treatments at $p \leq 0.05$.

It was noticed that the addition of thymol and tween-80 as antimicrobial agents have suppressed the growth of total yeast and mold loads [12]. Rok [14] reported that the pH values and high sugar contents are the mean reasons for microbial growth enhancement.

According to Table 1, aerobic plate counts were efficiently obtained for CHSTiO_2/TT80 coating treatments (5.93 log CFU/g). Furthermore, CHS and CHSTiO_2 coating treatments established parallel

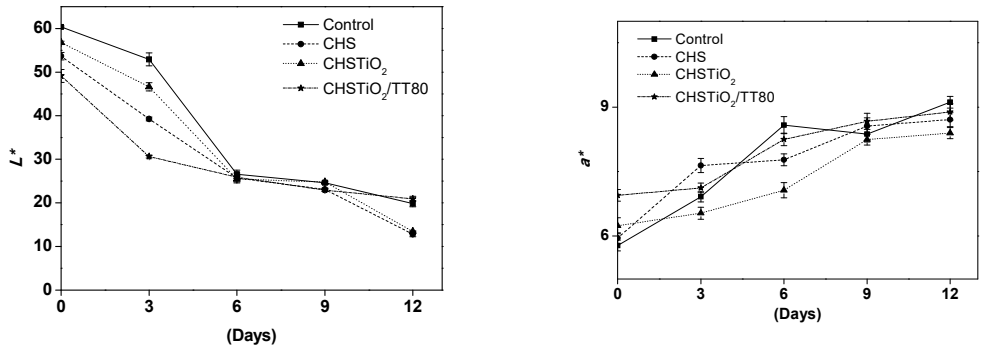
values (6.43 log CFU/g). The reduction of aerobic plate counts in CHSTiO$_2$/TT80 mushroom samples can be due to the presence of thymol and tween-80 as antimicrobial agents. In agreement with the aerobic plate counts, Karimirad et al. [21] reported the strong effect of chitosan nanoparticles on the mushroom contaminations and shelf life extension. The combination of nano-films with the antimicrobial agents leads to control the electronegative and polycationic on the surface of the mushrooms for the modification of cell permeability [12].

3.2. Color Attribute Changes

Mushrooms have a very short shelf-life due to turning brown and losing quality within a few days as the majority significant parameter for customer approval is the color [4]. The L^* value reduced and the browning index increased with the storage period onwards. The results for color attribute changes are shown in Figure 1. Although, on the sixth day, L^* value values were in parallel in all coating treatments compared with (control), Figure 1a. CHS coating treatment preserved lightness (12.81% loss) as compared to other coatings treatments, whereas CHSTiO$_2$/TT80 mushroom samples established the maximum (20.91% loss) on day 12. Weight loss and enzyme activities might be the major reasons for the optical reduction. Parameter L^*, depending on the mushroom reflectivity surface that can show the luminosity [22]. Gholami et al. [2] reported that the lower lightness values can be due to the coating films that can cause some changes on the mushroom surfaces.

a^* and b^* values of all treatments were raised for the duration of the storage period, while the increase in control samples (9.12–24.56% loss) was greater compared with the coated mushrooms, respectively Figure 1b,c. The larger a^* value linked to the enzymatic browning increase during the storage period [5].

The browning index can be influenced by L^* value decrease, as it is one of the major quality features for white mushroom deterioration measurements and freshness. As shown in Figure 1d, the browning index values raised with the upwards of storage days. Attractively, at day 12, CHSTiO$_2$/TT80 (0.71% loss) mushrooms significantly reduced the browning index compared with the control and other treatments. As a result, the presence of thymol and tween-80 could delay repining and preserve color. Lin et al. [23] established that modified atmosphere packages also can control the browning index, physiological injuries, and cell membrane damage.



(**a**) (**b**)

Figure 1. *Cont.*

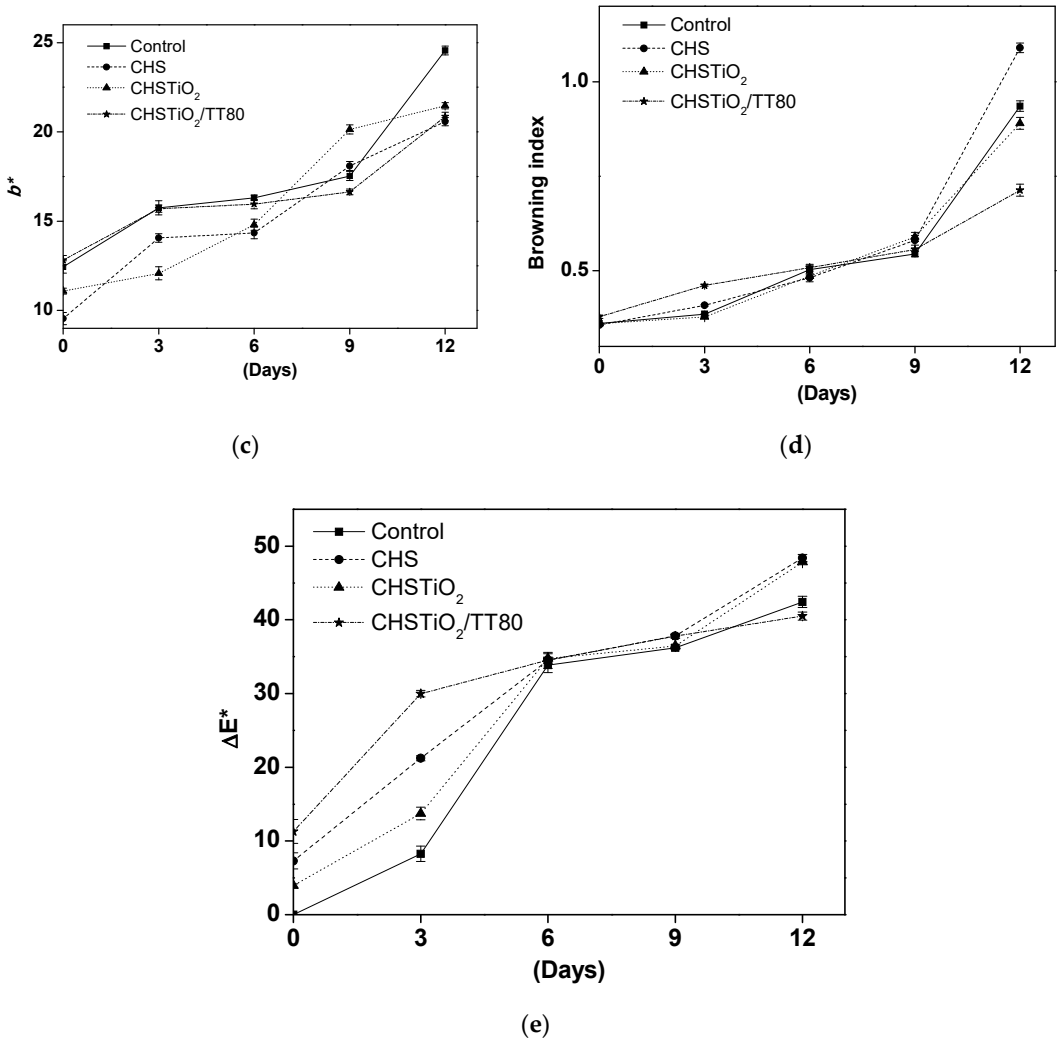


Figure 1. Effects of nanocomposite material and antimicrobial agents on color attribute changes, L^* (**a**), a^* (**b**), b^* (**c**), browning index (**d**), and ΔE*(**e**).

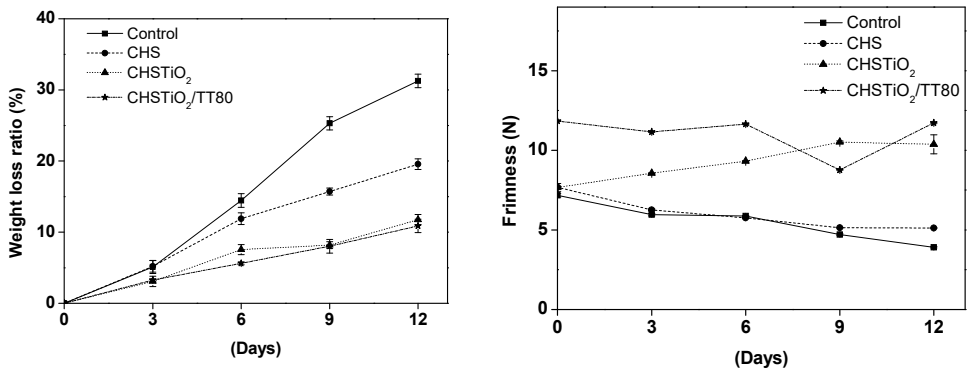
After 12 days of the storage period, the largest ΔE* values were established for the CHS (48.39), Figure 1e. Walkowiak-Tomczak et al. [24] confirmed that when the total color variation values were more than five it observed two colors impression. The minimum color differences were observed for $CHSTiO_2$/TT80 (ΔE* from 37.79 to 40.50), as a total color variation of more than two means a significant color difference. Tarlak et al. [15] confirmed that color can be influenced by several factors such as enzyme oxidations and microbial population. Rok et al. [25] confirmed that the reduction of the color attribute could be due to the polyphenolic compounds oxidation reactions.

3.3. Weight Loss Ratio and Texture Measurements

Weight loss can be occurred due to several factors such as respiration, microbial growth, and transpiration during the storage period. Figure 2a shows a significant ($p \leq 0.05$) weight loss during storage for all samples. As expected, the weight losses were significantly the lowest for $CHSTiO_2$/TT80 (10.88% loss) followed by $CHSTiO_2$ (11.76% loss) compared with the control samples due to the presence of nanocomposite material and antimicrobial agents which retained the respiration, inhibited the microbial growth, and delayed the enzyme activities [25].

The mushroom texture is a vital item for overall acceptance, which is influenced by the quality during the marketing [26]. Control samples were reduced rapidly during the storage onwards by 3.91 N from the initial value, Figure 2b. It was also obvious that the firmness of CHS mushrooms

was significantly ($p \leq 0.05$) suppressed on day 12 and had 5.11 N. Firmness loss can be influenced by several factors such as the biochemical and microbial processes [27]. However, $CHSTiO_2$ mushrooms established that the nanocomposite material might decrease cell-wall-degrading enzyme activities, due to its high anti-oxidation capacities during the storage time [14]. Our results were linked with the finding of Gholami et al. [2] who established that applying nanocomposite materials can enhance the firmness of mushroom samples.

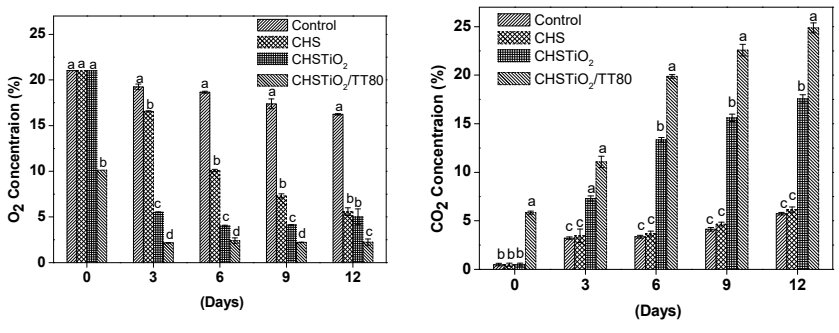


(**a**) (**b**)

Figure 2. Effects of nanocomposite material and antimicrobial agents on weight loss ratio (**a**) and texture measurements (**b**).

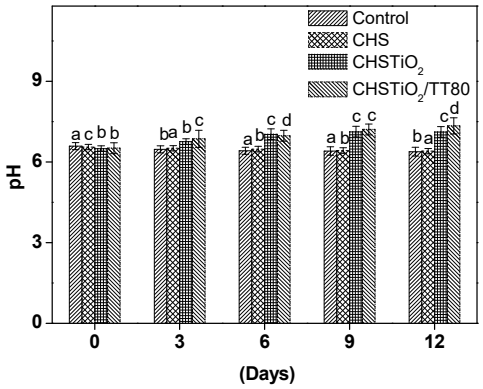
3.4. Headspace Gas Analysis and pH

The changes in headspace gas concentrations control and coated mushroom samples are presented in Figure 3. However, after three days of the storage period, O_2 concentration ratio was reduced in mushroom samples during the respiration process, Figure 3a. Decreases values differed according to the coating treatments. In detail, O_2 concentration in control samples (19.25%) + CHS (16.57%) decreased slightly, while in both $CHSTTiO_2$ (5.52%) and $CHSTiO_2$/TT80 (2.17%), the O_2 concentration consumptions were very low due to the effective oxygen barrier properties of nanocomposite material and antimicrobial agents. A subtle increase was detected from day 9 to day 12 for $CHSTiO_2$ and $CHSTiO_2$/TT80 mushrooms, which might be a sign of O_2 permeation through the mushroom packages. In contrast to oxygen, carbon dioxide concentration was raised to reach (5.73%) in (control) samples on day 12 due to the respiration and permeation, while the deposition of other coating treatments did not, Figure 3b. Qin et al. [28], reported similar values for carbon dioxide concentration as it may influence by white button mushroom quality.



(**a**) (**b**)

Figure 3. *Cont.*



(**c**)

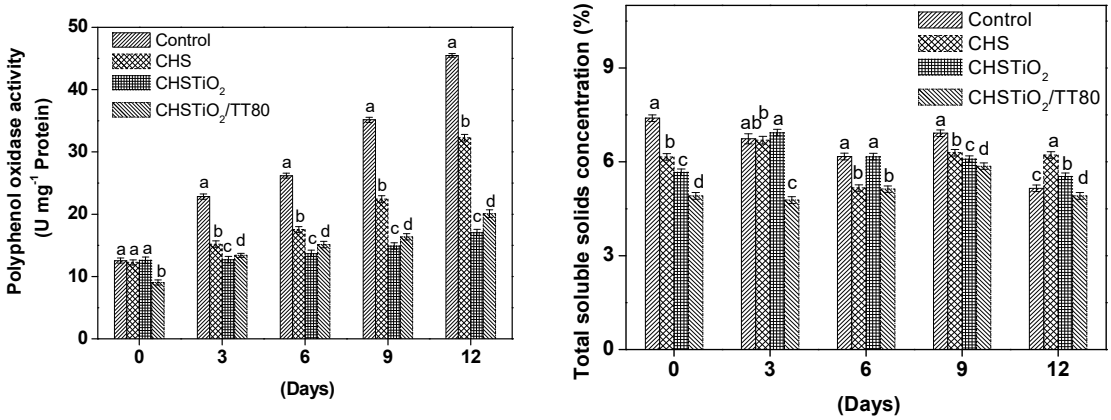
Figure 3. Effects of nanocomposite material and antimicrobial agents on headspace gas analysis, O_2 (**a**), CO_2 (**b**), and pH (**c**); $^{a; b; c; d}$ mean significant differences between treatments at $p \leq 0.05$.

Mushroom samples $CHSTiO_2$/TT80 followed by $CHESTiO_2$ established the best results as there were slightly higher acidity values from the initial pH value, Figure 3c. The decrement in pH was recorded for CHS mushroom samples 6.40 compared with the inertial ph 6.56 on day 12. Higher O_2 concentrations activate microorganisms on foods as visual bacteria growth was observed with the storage time on control and CHS samples. The pH of the coated mushroom with CHS decreased during storage can be due to the microbial growth population rate [29]. The evaluated pH values were in agreement with the results from the literature [3,30].

3.5. PPO Activity and TSS Concentration

In all the coating treatments, the PPO activity (Figure 4a) was raised with the progress of the storage time, while the maximum enzyme value was detected in control (45.49 U mg^{-1} Protein) after 12 days. The lowest PPO activity was established for$CHSTiO_2$ (17.09 U mg^{-1} Protein), while its initial value was (12.62 U mg^{-1} Protein). Besides, CHS obtained lower PPO activity (32.29 U mg^{-1} Protein) compared with the control (45.49 U mg^{-1} Protein) could be due to the chitosan component. Karimirad et al. [21] explained that mushroom browning is a result of phenolic oxidation by polyphenol oxidase activity. Meanwhile, Wei et al. [6] reported that PPO enzyme is the main reason for browning in mushrooms as it catalyzes the polyphenolic matrix to create dyes, which reduces the marketability. Consequently, nanocomposite material inhibited color changes and the ability to retain antioxidant phenolics in mushroom bodies.

TSS concentration decreased with the storage period due to higher respiration and ripening rates [8]. $CHSTiO_2$/TT80 mushrooms (4.91%) showed the best results as compared to the control (5.15%), Figure 4b. This study shows that the senescence rate was the lowest in the case of nanocomposite material with the addition of thymol and tween-80 as antimicrobial agents [6].

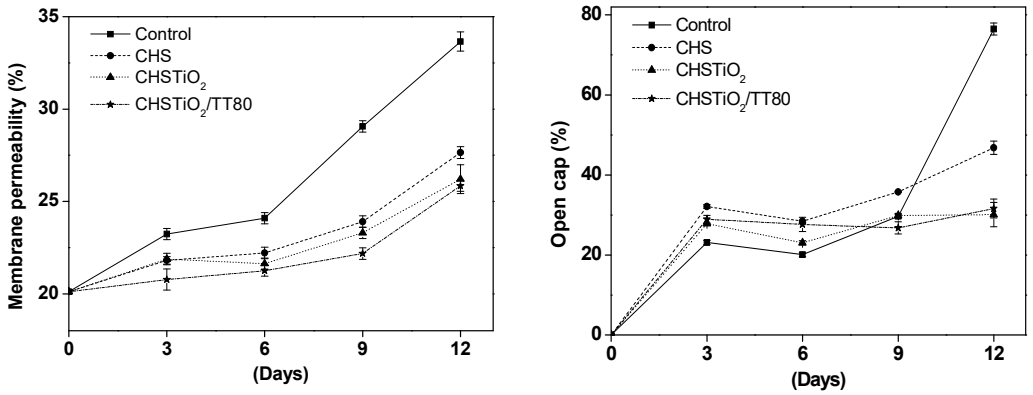


(**a**) (**b**)

Figure 4. Effects of nanocomposite material and antimicrobial agents on PPO activity (**a**) and TSS concentration (**b**); $^{a;\,b;\,c;\,d}$ mean significant differences between treatments at $p \leq 0.05$.

3.6. Membrane Permeability and Open Cap Percents

Membrane permeability percentage reflected frequently by the electrolyte leakage rate [31]. According to Figure 5a, the electrolyte leakage rate increased as long as the storage time in all coated samples, indicating a decrease in the mushroom membrane integrity. Furthermore, $CHSTiO_2$/TT80 mushrooms (25.84%) exhibited a significantly ($p \leq 0.05$) lower electrolyte leakage rate than the other samples, which might be attributed to the presence of thymol and tween-80. Other reports suggested the lower value of mushroom membrane integrity was directly related to the mushroom browning and lipid peroxidation [32–34].



(**a**) (**b**)

Figure 5. Effects of nanocomposite material and antimicrobial agents on membrane permeability (**a**) and open cap percents (**b**).

Cap opening was raised in all coating treatments with the duration of the storage period, and the highest value was detected for uncoated samples (76.49%) after 12 days, Figure 5b. At the end of the experiment, the value of cap opening was in-between 30.08 and 31.61% in $CHSTiO_2$ + $CHSTiO_2$/TT80 samples, respectively, which prevented the water vapor from affecting the packaged mushrooms. The cap opening percentile is regarded as the maturity indicator and refers to moisture content loss and mushroom aging [35,36]. In addition, according to our findings, high CO_2 and low O_2 concentrations have a positive effect on the cap opening reduction and preventing repining.

4. Conclusions

The results of this research work have established that $CHSTiO_2$/TT80 coating treatment has a positive effect on button mushroom preservation. The preservation effect might be attributed to the combination of chitosan, nanocomposite material, and antimicrobial agents (thymol and tween-80) $CHSTiO_2$/TT80. Thus, it showed lower respiration rate, weight loss, browning degree, and microbial contaminations, and higher electrolyte leakage rate and acidity than those coated with CHS and $CHSTiO_2$. Meanwhile, $CHSTiO_2$ coating treatment established the lowest polyphenol oxidase activity andCHS maintained lightness. These results indicated that $CHSTiO_2$/TT80 coating treatment might be investigated as a novel packaging material for other consumable vegetables and fruit products in the future. Semi nano-films mainly with the addition of (thymol-tween) is suggested for nanotechnology application researches and preservation manufacturing.

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