

## Article

# Effect of Coatings Using Titanium Dioxide Nanoparticles and Chitosan Films on Oxidation during Storage on White Button Mushroom

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**Citation:** Khojah, E.; Sami, R.; Helal, M.; Elhakem, A.; Benajiba, N.; Alharbi, M.; Alkaltham, M.S. Effect of Coatings Using Titanium Dioxide Nanoparticles and Chitosan Films on Oxidation during Storage on White Button Mushroom. *Crystals* **2021**, *11*, 603. <https://doi.org/10.3390/cryst11060603>

Academic Editors:  
Alessandro Chiasera and Haitao Ye

Received: 22 March 2021

Accepted: 25 May 2021

Published: 27 May 2021

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**Abstract:** White button mushroom or (*Agaricus bisporus*) is known as a healthy foodstuff with several nutrients, polyphenols, proteins, and dietary fibers. Mushrooms have a short shelf-life, approximately three to four days at commercial storage and about eight days under chilling conditions. In the current study, titanium dioxide nanoparticles and chitosan films were used as novel active coating materials with the addition of thymol and tween (T and T) as food preservatives to prolong mushroom shelf life up to 12 days. Chitosan, Chitosan-Nano, and Chitosan-Nano/TT were used as coating materials, while water was used as control. Chitosan-Nano/TT film reported the lowest peroxidase activity (0.005 U kg<sup>-1</sup> FW) and the highest superoxide dismutase activity (4.033 U kg<sup>-1</sup> FW), while catalase activity in Chitosan-Nano film was (0.45 U kg<sup>-1</sup> FW). Chitosan-Nano film enhanced the reactive oxygen species production levels, DPPH radicals (74.70%), and malondialdehyde content (1.68 μmol kg<sup>-1</sup>FW). Chitosan-Nano/TT film preserved the respiration rates (O<sub>2</sub> consumption −0.026 mmol s<sup>-1</sup>kg<sup>-1</sup>, CO<sub>2</sub> production −0.004 mg CO<sub>2</sub> kg<sup>-1</sup>s<sup>-1</sup>) and increased the phenolic contents (0.38 g kg<sup>-1</sup>). The results suggested that nano-coating films can increase the oxidation processes which enhanced the quality of the mushrooms.

**Keywords:** *Agaricus bisporus*; coatings; film; storage; reactive oxygen species; total phenols; antioxidant; enzymes

## 1. Introduction

The natural bioactive compounds and oxidation processes research has increased tremendously due to the safety and health-promoting properties of the compounds [1–4]. *Agaricus bisporus*, the commercially cultivated button mushrooms, are known worldwide for their anticancer therapies, antitumor, pharmacological, cholesterol-lowering, immunostimulating, antimicrobial, anti-inflammatory, and antioxidant activities [5,6]. Mushrooms were used as folk medicines, they act as a valuable non-toxic medicine against several diseases such as cirrhosis, Alzheimer's, atherosclerosis, Parkinsonism, diabetes, and hypertension [7]. Mushroom methanolic extracts can capture minerals, reduce lipoxygenase, cell death, tissue damage, and remove free radical intermediates [8]. In addition, ease of harvesting, high nutrients, and lower prices are the major reasons for the common

cultivation [9]. Mushrooms have an extremely short shelf life, approximately 3 to 4 days at commercial storage and about eight days under chilling conditions due to high respiration rate, water loss, mechanical damage, loss of turgor, and microbial spoilage which can negatively affect the marketability and customer's acceptance. Oxidative reactions during the storage period can lower the quality of the mushrooms such as enzymatic browning, off-flavor, cap opening, and senescence [10]. Various techniques have been applied to manage quality during storage such as chilling conditions, chemical washing, high-pressure, argon, UV-c,  $\gamma$ -irradiation, pulsed light, ultrasound waves, gaseous ozone, and active and modified atmosphere packaging, though undesirable changes in the mushroom quality and appearance can occur [11]. One such technique is coating with chitosan, apple peel, aloe vera gel, peppermint oils, and nano-coating [12–16]. Titanium dioxide nanoparticles with low concentrations of less than 1% by weight of the food is non-toxic. It is well known for the chemical stability and suitable cost according to Food and Drug Administration (FDA) recommendations [17]. The nano-titanium dioxide is safe, non-toxic, has high rigidity and strength with a white color, and it can be colored with all colors to suit all food products. Chitosan can enhance the gas barrier by the interaction in-between chitosan chains and other chemical substances such as polysaccharides. Thymol and tween are considered effective antimicrobial agents for microbial contamination [5,6]. The combination between nano-titanium dioxide, chitosan, thymol, and tween-80 can be used in a lot of fields such as antireflection coating and the packaging industry against the permeation of gases that prevent spoilage, reduce the volatile compounds, and preserve the sensory evaluations.

For that reason, the current research work aims to evaluate the effect of coating on white button mushrooms quality characteristics such as antioxidant activities, browning enzymes, reactive oxygen species, respiration rates, malondialdehyde, and phenolic contents during storage to prolong the shelf-life.

## 2. Materials and Methods

### 2.1. Materials

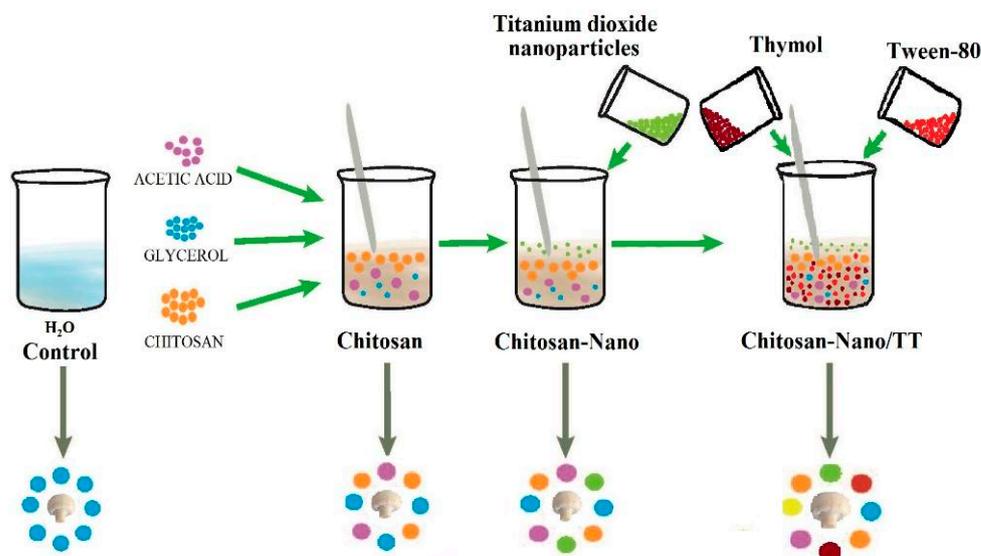
Titanium dioxide nanoparticles (15 nm with purity more than 99 wet %), chitosan (85% deacetylation), thymol and tween-80, Folin–Ciocalteu, and other reagents were from Sigma, Co., St. Louis, MO, USA.

### 2.2. Coating Film Preparations

Chitosan 1% (*w/v*) was prepared by mixing acetic acid 1% (*v/v*) and glycerol 0.5% (*v/v*) and stirring overnight at 300 rpm. Approximately 1% of titanium dioxide nanoparticles was added and named with Chitosan-Nano. Thymol and tween-80 1% (*w/v*) were added to Chitosan-Nano as antimicrobial preservatives and named with Chitosan-Nano/TT.

### 2.3. Mushroom Samples and Treatments

Fresh white button mushrooms were from the Food Science and Nutrition Department, Taif University, Taif City with closed caps (diameter of  $4 \pm 00$  cm), uniform size, mature sporophores, and free from any sign of physical weaknesses. They were immediately pre-cooled for 12 h at 4 °C. A total number of 120 mushroom caps with gills (thirtypieceper basket, three baskets per group). Four groups were prepared and then coated as follows: Control (distilled water), Chitosan, Chitosan Nano, and Chitosan-Nano/TT were prepared for coating treatments. Mushroom samples were spray-coated continually for five minutes [5,6]. Figure 1 presents film preparation and mushroom treatments. Mushrooms were allowed to dry for 1 h by using an industrial fan at ambient temperature. Mushroom samples were placed in 0.05 mm thickness bags, sealed, and stored at  $4 \pm 1$  °C for 12 days. Evaluations including browning enzyme activities, total phenols, malondialdehyde, antioxidant activities, reactive oxygen species production, O<sub>2</sub> consumption, and CO<sub>2</sub> production rates in all the mushroom treatments were carried out at 0, 3, 6, 9, and 12 days of storage under chilling.



**Figure 1.** Film preparation and mushroom treatments.

#### 2.4. Determination of Browning Enzyme Activities

Enzyme activities are responsible for browning during the storage period as they catalyze the polyphenolic matrix to create dyes. The determination method for peroxidase, superoxide dismutase activities measurements were reported from Sami et al. [18] and Mirshekari et al. [12], and catalase activity was measured by a titrimetric method reported according to Eissa [19]. Fresh mushrooms (10 g) were blended with 20 mL 50 mM mol phosphate buffer for the enzymatic measurements. The experiments were designed with three replications and expressed as  $\text{U kg}^{-1}$  fresh weight (FW).

#### 2.5. Determination of Total Phenols

Mushroom samples (10 g) were taken and ground with 10 ml of 80% methanol (MeOH), centrifuged for 15 min at 2000 r/min, and then filtered [4,10]. The color was measured by blending 2.0 mL aliquot filtrate, 1 mL distilled water, 1 mL Folin–Ciocalteu, and 3 mL  $\text{Na}_2\text{CO}_3$  (20 %). After 2 h, the absorbance was evaluated at 650 nm with the help of the spectrophotometer (Spectronic 20D, City, China). Total phenol concentration was measured according to the catechol concentrations ( $8\text{--}32 \mu\text{g/mL}$ ) as a standard and expressed in  $\text{g kg}^{-1}$  FW.

#### 2.6. Determination of Malondialdehyde Contents

The malondialdehyde contents were evaluated according to the described method in Eldib et al. [20]. Mushroom samples (2 g) were ground, homogenized with 5 mL of 5% trichloroacetic acid (TCA) using a mortar and pestle, cooled, and centrifuged at 8000 r/min for 10 min. Approximately 2 mL of the supernatant were blended with 2 mL of 0.67 % thiobarbituric acid, heated at  $100^\circ\text{C}$  for 30 min, centrifuged again, and measured at 450 nm, 532 nm, and 600 nm, respectively:

$$\text{MDA } (\mu\text{mol kg}^{-1} \text{FW}) = 6.45 \times (D_{532} - D_{600}) - 0.56 \times D_{450} \quad (1)$$

#### 2.7. Determination of Antioxidant Activities

##### 2.7.1. Scavenging Effect of DPPH Radicals

A liguete of 1 ml of 0.5 mmol/L DPPH solution in MeOH, 0.05 mol/L acetate buffer (pH = 5.5), an equal volume of methanolic mushroom extracts were shaken and incubated in the dark for 30 minutes and 1 ml of MeOH was served as a standard [8,10]. The absorbance was evaluated at 517 nm and expressed as a percentage against various concentrations of stock solution of ascorbic acid (1, 0.5, 0.25, 0.125, and 0.062).

### 2.7.2. Scavenging Effect of ABTS Radicals

Antioxidant activity evaluated by using the ABTS radicals was reported by Rokayya et al. [3]. Mushroom extracts (10  $\mu\text{L}$ ) were blended with 90  $\mu\text{L}$  of 7 mM ABTS solution, evaluated at 734 nm, and expressed as a percentage against MeOH as a blank.

### 2.8. Measurements of Respiration Rates

Mushroom samples were kept in glass jars with 2 valves and a rubber septum.  $\text{O}_2$  consumption and  $\text{CO}_2$  production rates were evaluated by the gas analyzer (PBI Dansensor Checkmate, 9900) during the whole storage time. The rates were evaluated according to the following equations [8,21]:

$$y_{\text{O}_2} = y_{\text{O}_2}^i - \frac{R_{\text{O}_2} \times W}{V_f} (t - t_i) \quad (2)$$

$$y_{\text{CO}_2} = y_{\text{CO}_2}^i - \frac{R_{\text{CO}_2} \times W}{V_f} (t - t_i) \quad (3)$$

where  $y^i_{\text{O}_2}$ ,  $y^i_{\text{CO}_2}$ ,  $y_{\text{O}_2}$ , and  $y_{\text{CO}_2}$  are, respectively, the  $\text{O}_2$  and  $\text{CO}_2$  concentrations at the initial time  $t_i$  (hour) and at time  $t$  (hour).  $R_{\text{O}_2}$  and  $R_{\text{CO}_2}$  are the respiration rates and  $W$  is the weight, and  $V_f$  is the free volume.

### 2.9. Determination of Reactive Oxygen Species Productions

Hydrogen peroxide content was evaluated according to the described method [8,10]. Mushroom samples (1 g) were blended with 5 mL of 0.1% TCA and centrifuged at 10,000 r/min for 15 min. The supernatant (1 mL) was added to 1 mL of 10 mmol/L potassium phosphate buffers (pH = 7) and 1 mL of 10 mol  $\text{L}^{-1}$  potassium iodide, evaluated at 390 nm after incubation for 1 hour and expressed as  $\mu\text{mol g}^{-1}$  (FW).

Hydroxyl radical was evaluated according to the described method [8,10]. Mushroom samples (50 mg) were blended with 1 mL of 10 mmol  $\text{L}^{-1}$  Na-phosphate buffer (pH = 7.4) with 15 mmol  $\text{L}^{-1}$  2-deoxy-d-ribose. After incubation at 37  $^\circ\text{C}$ , 0.7 mL of that solution was added to 3 mL of 0.5% TBA, 1 mL of glacial acetic acid, evaluated at 532 nm, and expressed as  $\mu\text{mol g}^{-1}$  (FW).

### 2.10. Statistical Analyses

All of the results were expressed as mean (+/−) standard deviation. One-way ANOVA (parametric data) with three replications was used. Duncan's test as a posthoc was used as multiple ranges at a significant level  $p$ -value < 0.05.

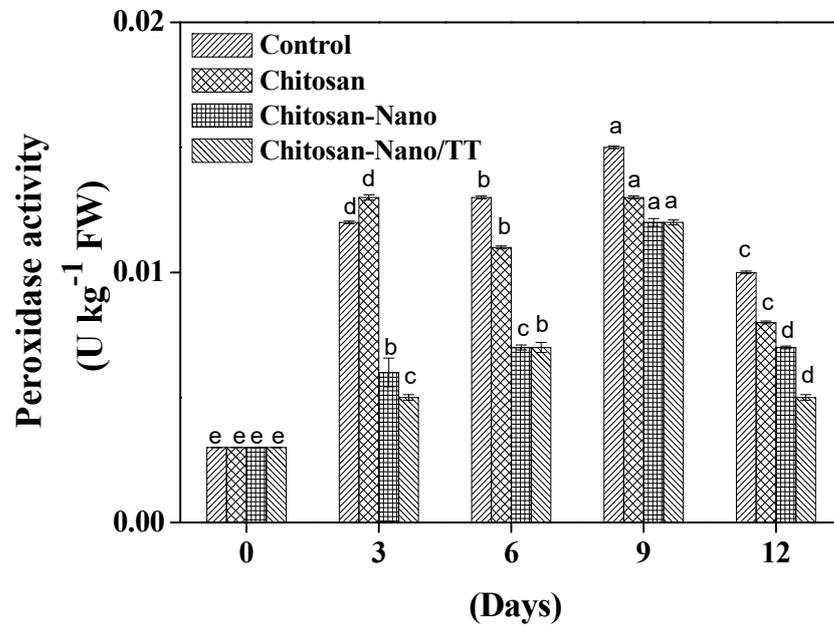
## 3. Results and Discussion

### 3.1. Browning Enzyme Activities

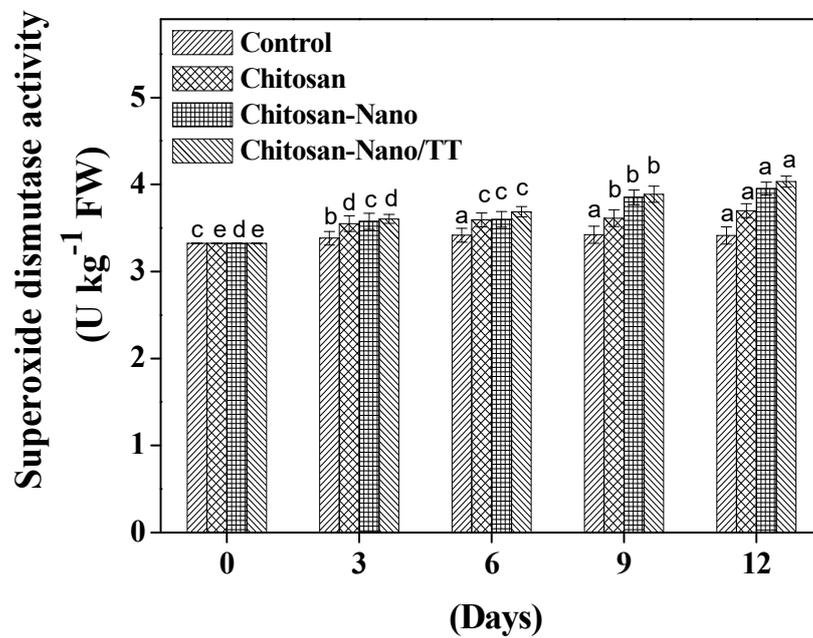
Antioxidant enzymes such as peroxidase, superoxide dismutase, and catalase play a vital role in the defense of the mushroom oxidation [22,23]. The mushroom quality started to deteriorate after the third day of storage due to oxidative enzymes that negatively influence taste, color, and smell. Figure 2 presents the quality characteristics of the chemical constituents during the storage period. Clear differences were noticed between various coating films. In Figure 2a, the highest activity of peroxidase was for the control samples (0.010  $\text{U kg}^{-1}$  FW) and followed by Chitosan as (0.008  $\text{U kg}^{-1}$  FW), while Chitosan-Nano/TT film reported the lowest activity (0.005  $\text{U kg}^{-1}$  FW) on day 12. Sami et al. [18] and Li et al. [24] reported that oxygen is required for the oxidation activities' occurrence; thus, the use of nano-films on blueberries could explain the peroxidase activity reduction.

As shown in Figure 2b, a minor increase in superoxide dismutase level was detected in treated mushrooms with Chitosan-Nano and Chitosan-Nano/TT films after three days of the storage period. Chitosan-Nano/TT mushrooms exhibited the highest superoxide dismutase activity (4.033  $\text{U kg}^{-1}$  FW) ( $p < 0.05$ ). High superoxide dismutase activity can

decrease the free radical aggregation by hydrogen peroxide formation as the superfluous hydrogen peroxide could convert into non-toxic molecules. Nano-coating with the addition of the antimicrobial agents can effectively catalyze the dismutation of oxygen to produce hydrogen peroxide contents [8,10,25].

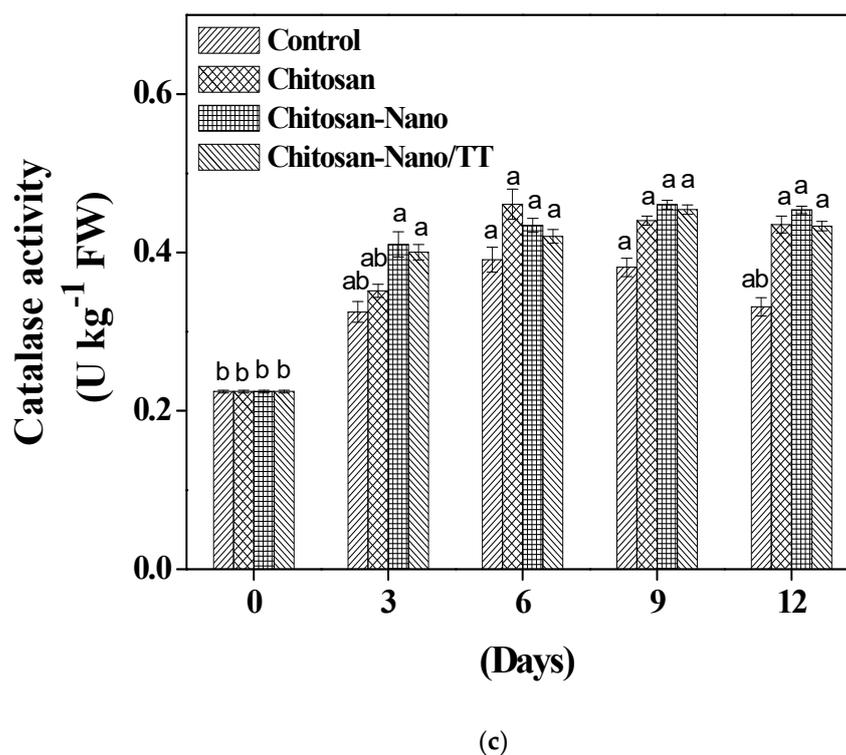


(a)



(b)

Figure 2. Cont.



**Figure 2.** Effect oxidoreductase enzyme activities measured in mushroom during storage at 4 °C; peroxidase (a), superoxide dismutase (b), and catalase (c) activities; different small letters <sup>a,b</sup> mean significant differences between treatments at  $p \leq 0.05$ .

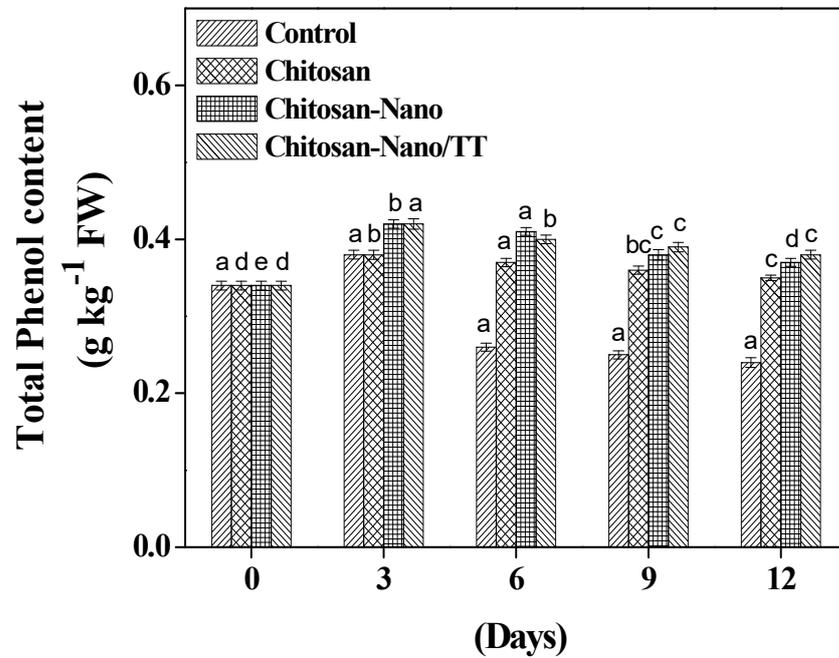
Catalase activity can decrease the oxidative damage caused by hydrogen peroxide contents in mushroom samples [8,10,26]. In Figure 2c, catalase activity in Chitosan-Nano ( $0.45 \text{ U kg}^{-1} \text{ FW}$ ) and Chitosan ( $0.44 \text{ U kg}^{-1} \text{ FW}$ ) films reported similar values at the end of the storage period. In general, chitosan combination with titanium dioxide nanoparticles coating films can fill the groove between hydrogen bonds and  $\Pi$ -sigma, or even  $\Pi$ - $\Pi$  interactions which extend the shelf-life of mushroom samples during storage [6,18,23,24]. In addition, many indexes such as season, type, maturation, and environmental conditions can affect the browning enzyme activities [6,24,27].

### 3.2. Total Phenol and Malondialdehyde Contents

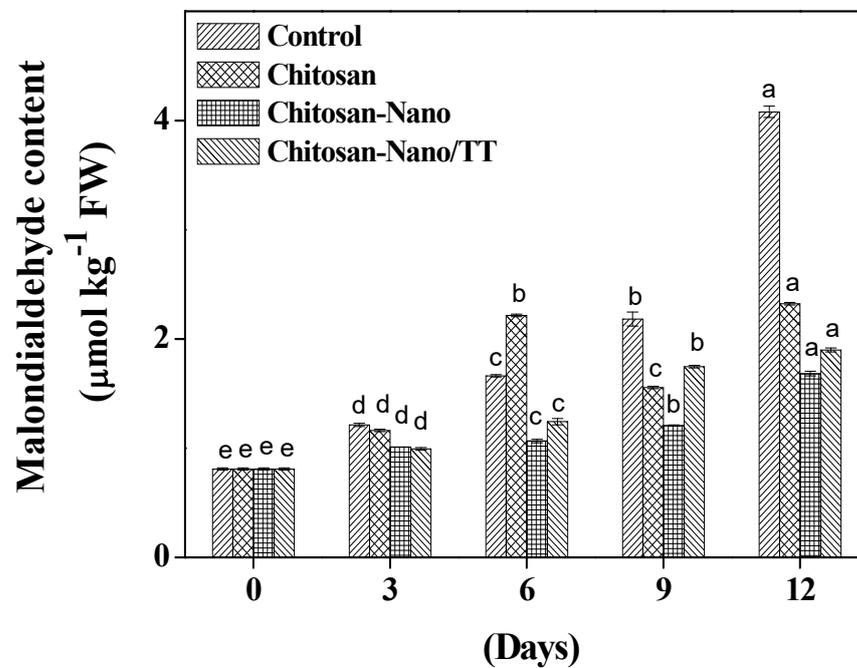
Figure 3a shows that the total phenol content of mushroom samples reported an increasing trend during the storage period after coating treatments, while the control samples were stable during the third day of the entire storage ( $0.38 \text{ g kg}^{-1}$ ). Chitosan-Nano/TT treated mushrooms had a significantly increased total phenol content ( $p < 0.05$ ) at the end of the storage period. Phenylalanine ammonia-lyase enzymes in mushrooms have an important role in raising the phenolic contents [10,28]. Cold conditions also help in raising the total phenol content by changing the phenolic metabolism [6]. Results recommend that Chitosan-Nano/TT treatment may maintain the total phenol contents' accumulation in the mushroom that can alleviate the browning processes.

Malondialdehyde accumulation content has been considered as a key index for the degree of membrane polyunsaturated fatty acid oxidation [8,27,29]. In Figure 3b, malondialdehyde contents increased continuously during the storage for 12 days in mushroom samples at 4 °C, which indicates a cell membrane integrity reduction [8,25]. In addition, coating with Chitosan-Nano film resulted in significantly ( $p < 0.05$ ) lower malondialdehyde contents formation ( $1.68 \text{ } \mu\text{mol kg}^{-1} \text{ FW}$ ) compared to the untreated mushrooms after 12 days of storage. Nano-coating probably preserved the mushrooms from peroxidation by reactive oxygen species and lipoxygenase [9,10,27]. The difference in the

malondialdehyde contents may be due to the dissolution power and polarity of the coating materials [20,27]. The current research indicated that nano-coating treatment decreased the membrane lipid peroxidation, in that manner prolonging the shelf life and delaying the aging processes [30,31].



(a)



(b)

**Figure 3.** Effect of total phenol (a) and malondialdehyde (b) contents on mushrooms during storage; \* Different small letters <sup>a,b,c;d</sup> mean significant differences between treatments at  $p \leq 0.05$ .

### 3.3. Antioxidant Activities

The scavenging effect of mushroom extracts on DPPH radicals is presented in (Table 1). Chitosan-Nano (74.70%) followed by Chitosan-Nano/TT (72.60%) exhibited a significantly different ( $p < 0.05$ ). Cheung et al. [32] reported lower DPPH activity results (37.9%, 55.4 %) for mushrooms.

**Table 1.** Effect of the different coatings on antioxidant activities in mushrooms during storage.

Days	Control	Chitosan	Chitosan-Nano	Chitosan-Nano/TT
<b>DPPH Radical Scavenging Capacity (%)</b>				
0	52.14 ± 0.02 <sup>b</sup>	52.14 ± 0.02 <sup>e</sup>	52.14 ± 0.02 <sup>e</sup>	52.14 ± 0.02 <sup>e</sup>
3	53.73 ± 0.05 <sup>a</sup>	59.90 ± 0.11 <sup>d</sup>	62.19 ± 0.04 <sup>d</sup>	62.26 ± 0.25 <sup>d</sup>
6	48.66 ± 0.23 <sup>c</sup>	61.39 ± 0.20 <sup>c</sup>	69.88 ± 0.14 <sup>c</sup>	66.88 ± 0.08 <sup>c</sup>
9	41.22 ± 0.10 <sup>d</sup>	63.92 ± 0.31 <sup>b</sup>	71.50 ± 0.07 <sup>b</sup>	71.03 ± 0.03 <sup>b</sup>
12	37.52 ± 0.37 <sup>e</sup>	69.74 ± 0.03 <sup>a</sup>	74.70 ± 0.08 <sup>a</sup>	72.60 ± 0.07 <sup>a</sup>
<b>ABTS Radical Scavenging Capacity (%)</b>				
0	52.03 ± 0.01 <sup>c</sup>	52.26 ± 0.12 <sup>d</sup>	52.26 ± 0.12 <sup>e</sup>	52.26 ± 0.12 <sup>d</sup>
3	52.22 ± 0.01 <sup>b</sup>	58.66 ± 0.01 <sup>c</sup>	66.24 ± 0.02 <sup>d</sup>	66.58 ± 0.61 <sup>c</sup>
6	53.29 ± 0.06 <sup>a</sup>	61.62 ± 0.06 <sup>b</sup>	67.19 ± 0.12 <sup>c</sup>	68.46 ± 0.36 <sup>b</sup>
9	51.65 ± 0.01 <sup>d</sup>	65.15 ± 0.01 <sup>a</sup>	68.20 ± 0.30 <sup>b</sup>	70.04 ± 0.15 <sup>a</sup>
12	45.55 ± 0.10 <sup>e</sup>	65.24 ± 0.02 <sup>a</sup>	69.84 ± 0.75 <sup>a</sup>	70.39 ± 0.24 <sup>a</sup>

\* Values within a column lowercase are significantly different  $p < 0.05$ . The values in parentheses indicate SD ± standard deviation; \* different small letters within the same line <sup>a,b,c,d</sup> mean significant differences between treatments at  $p \leq 0.05$ .

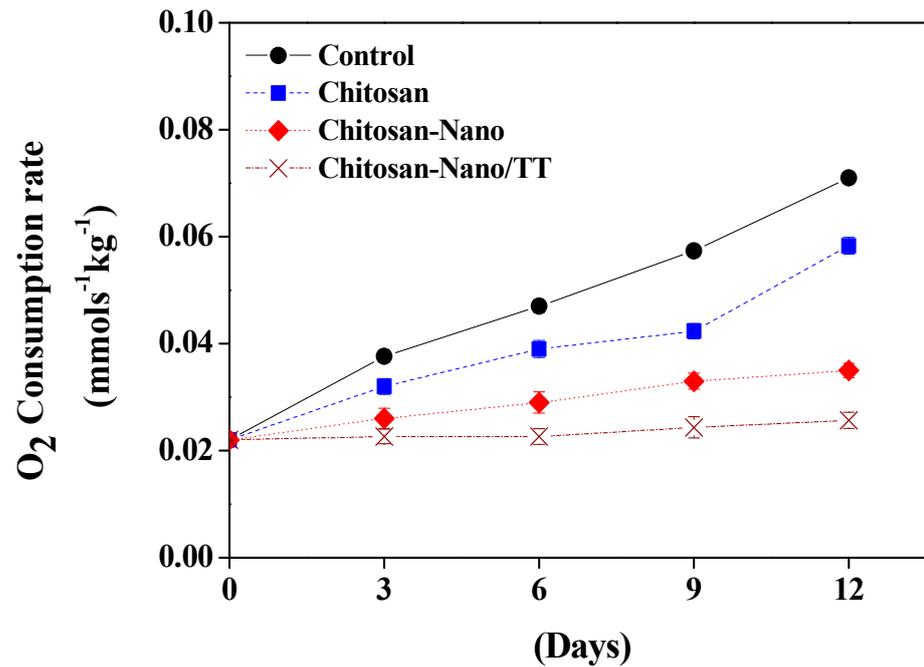
The rapid accumulation of antioxidant capacity might be attributed to cold conditions, DPPH radical scavenging capacity, and physically powerful maintenance of phenolic compounds of nano-coated mushroom samples that reached the saturation level in *Agaricus bisporus* bodies.

Antioxidant activity on ABTS radical results of coated mushrooms is expressed as a percentage in (Table 1). The scavenging effect of mushroom extracts was between 45.55% for control and 70.39% for Chitosan-Nano/TT mushrooms at the end of the whole storage period. The greatest impact of the ABTS radical enhancement was due to the presence of nano-coating with the addition of the antimicrobial agents, which possibly reduce the oxidoreductase enzyme activities and damage on tissues [10,33].

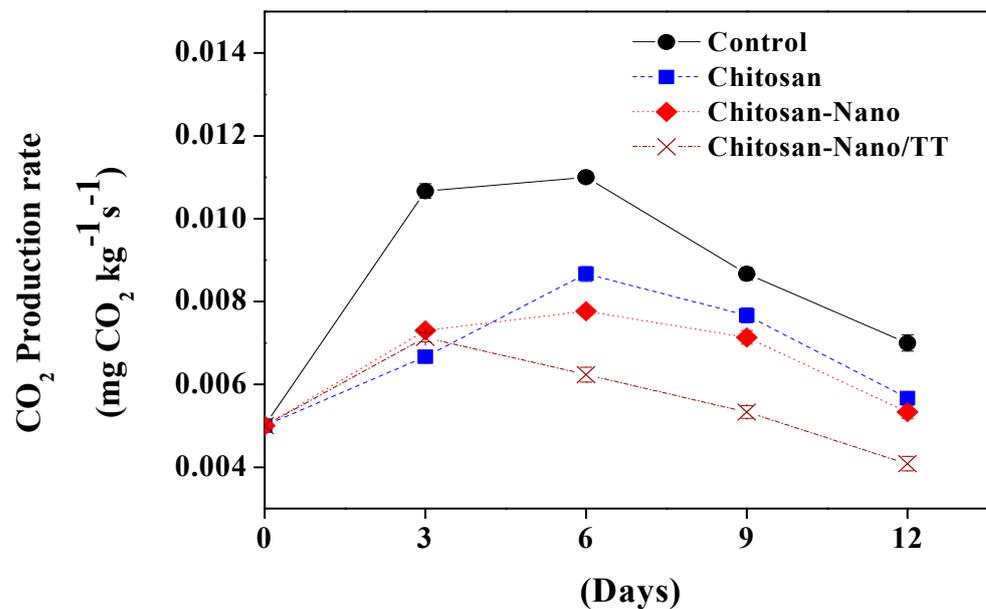
### 3.4. Respiration Rates

The reduced respiratory metabolism increases the shelf-life of the stored products. The changes in respiration rates after coating treatments are presented in Figure 4. Figure 3a,b show that O<sub>2</sub> consumption and CO<sub>2</sub> production rates increased in response to the storage period at 4 °C. The effect was further noticed with the increase of the storage time. The O<sub>2</sub> consumption rate continued to increase especially for uncoated samples and Chitosan films to reach (0.071, 0.058 mmol s<sup>-1</sup>kg<sup>-1</sup>, respectively), while Chitosan-Nano/TT and Chitosan-Nano films remarkably reached the lowest values (0.026, 0.035 mmol s<sup>-1</sup>kg<sup>-1</sup>, respectively) at the end of the storage period. Mild heat was recently used for decreasing the O<sub>2</sub> production rate on fresh-cut *Agaricus bisporus* mushrooms [34].

CO<sub>2</sub> production rates were raised conversely and Chitosan-Nano/TT film recorded the lowest CO<sub>2</sub> rate (0.004 mg CO<sub>2</sub> kg<sup>-1</sup>s<sup>-1</sup>) on the 12th day of the storage time. Hu et al. [35] reported that the high respiration rate can be due to the anaerobic respiration confirmed by high respiratory quotient values on uncoated samples and Chitosan samples, while, for nano-coated samples, the anaerobic behavior was not reversed. Similar results for respiration rates have been reported for mushrooms that could be due to the microbial attack [6,10,11].



(a)



(b)

**Figure 4.** Effect of respiration rates on mushrooms during storage; O<sub>2</sub> consumption (a) and CO<sub>2</sub> production rates (b).

### 3.5. Reactive Oxygen Species

The reactive oxygen species production levels, hydrogen peroxide, and hydroxyl radical contents in mushroom samples are shown in Table 2. Only low quantities of hydrogen peroxide contents were observed for Chitosan-Nano (22.40  $\mu\text{mol g}^{-1}$  FW), while the control was observed to be nearly double the content (41.23  $\mu\text{mol g}^{-1}$  FW) at the end of the storage period. Khan et al. [36] reported that a high level of hydrogen peroxide content can oxidize the membrane lipids, which leads to cell integrity destruction and the aging of mushrooms.

**Table 2.** Effect of the different coatings on hydrogen peroxide and hydroxyl radical levels in mushrooms during storage.

Days	Control	Chitosan	Chitosan-Nano	Chitosan-Nano/TT
<b>Hydrogen Peroxide (<math>\mu\text{mol g}^{-1}</math> FW)</b>				
0	25.27 $\pm$ 0.06 <sup>e</sup>	25.95 $\pm$ 0.07 <sup>b</sup>	25.95 $\pm$ 0.07 <sup>b</sup>	25.57 $\pm$ 0.02 <sup>b</sup>
3	35.43 $\pm$ 0.12 <sup>b</sup>	31.38 $\pm$ 0.06 <sup>a</sup>	29.22 $\pm$ 0.28 <sup>a</sup>	27.80 $\pm$ 0.06 <sup>a</sup>
6	31.50 $\pm$ 0.06 <sup>c</sup>	27.91 $\pm$ 0.05 <sup>ab</sup>	18.62 $\pm$ 0.22 <sup>d</sup>	22.14 $\pm$ 0.02 <sup>c</sup>
9	29.63 $\pm$ 0.42 <sup>d</sup>	26.36 $\pm$ 0.08 <sup>b</sup>	13.39 $\pm$ 0.05 <sup>e</sup>	15.25 $\pm$ 0.01 <sup>d</sup>
12	41.23 $\pm$ 0.17 <sup>a</sup>	30.44 $\pm$ 0.21 <sup>ab</sup>	22.40 $\pm$ 0.07 <sup>c</sup>	25.58 $\pm$ 0.06 <sup>b</sup>
<b>Hydroxyl Radical (<math>\mu\text{mol g}^{-1}</math> FW)</b>				
0	0.33 $\pm$ 0.01 <sup>a</sup>	0.32 $\pm$ 0.02 <sup>a</sup>	0.29 $\pm$ 0.02 <sup>a</sup>	0.27 $\pm$ 0.02 <sup>a</sup>
3	0.28 $\pm$ 0.02 <sup>b</sup>	0.20 $\pm$ 0.03 <sup>c</sup>	0.21 $\pm$ 0.01 <sup>b</sup>	0.20 $\pm$ 0.01 <sup>b</sup>
6	0.24 $\pm$ 0.01 <sup>c</sup>	0.15 $\pm$ 0.01 <sup>e</sup>	0.18 $\pm$ 0.01 <sup>c</sup>	0.17 $\pm$ 0.02 <sup>c</sup>
9	0.21 $\pm$ 0.02 <sup>d</sup>	0.19 $\pm$ 0.02 <sup>d</sup>	0.13 $\pm$ 0.02 <sup>e</sup>	0.14 $\pm$ 0.03 <sup>d</sup>
12	0.33 $\pm$ 0.03 <sup>a</sup>	0.21 $\pm$ 0.03 <sup>b</sup>	0.16 $\pm$ 0.03 <sup>d</sup>	0.19 $\pm$ 0.01 <sup>b</sup>

\* Values within a column lowercase are significantly different  $p < 0.05$ . The values in parentheses indicate SD  $\pm$  standard deviation.

Hydroxyl radical contents play a vital role in the oxidation process [8,10]. In the current work, Chitosan-Nano mushroom samples achieved the lowest values ( $0.16 \mu\text{mol g}^{-1}$ ) followed by Chitosan-Nano/TT ( $0.19 \mu\text{mol g}^{-1}$ ), while Chitosan reported ( $0.21 \mu\text{mol g}^{-1}$ ). Chomkitichai et al. [37] reported a proportional relationship between the accumulation of reactive oxygen species and the browning degrees. Higher reactive oxygen species could disrupt cell membrane structures, browning, and quality during storage [37]. The decrease in oxygen production rate has been occurred in mushrooms treated with 4-methoxy cinnamic acid, while the browning increase may lead to hydrogen peroxide and hydroxyl radical increase [8,10]. In a word, nano-titanium with the combination of the chitosan coating film can reduce the oxidation processes of white button mushrooms by maintaining the lowest hydrogen peroxide and hydroxyl radical activities during the storage period by reducing the cell degradation and oxidation processes [18,21].

#### 4. Conclusions

The current results present some insights into the oxidation processes and quality deterioration of white button mushrooms after the post-harvest methods to extend the shelf life. Reducing the oxidation is essential to provide more appropriate recommendations for the edible fungus industry with maintaining the sensory and nutritive values of fresh mushrooms by promoting antioxidant production. Coating nano-titanium with the combination of chitosan enhanced the color, reactive oxygen species, antioxidant activity, and malondialdehyde accumulation contents, while the addition of antimicrobial agents such as thymol and tween reduced the respiration rates and increased the phenolic contents. As a result, nano-coating with the combination of antimicrobial agents in food preservation is recommended for increasing the shelf-life of mushrooms with other fruits and vegetables.

**Author Contributions:** Funding acquisition, E.K. and R.S.; Methodology, E.K., R.S., M.H., N.B. and M.A.; Software, M.H., N.B., M.A. and M.S.A.; Visualization, E.K., R.S., M.H., N.B. and M.A.; Writing—review & editing, E.K., R.S., M.H., A.E., N.B., M.A. and M.S.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** Financial support was provided from the Deanship of Scientific Research by Taif University, award number (102-441-1).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Available upon request from the corresponding author.

**Acknowledgments:** Financial support was provided from the Deanship of Scientific Research by Taif University, award number (102-441-1).

**Conflicts of Interest:** The authors declared no conflicts of interest.

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