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ORIGINAL PAPER

Improved Postharvest Preservation Effects of *Pholiota nameko* Mushroom by Sodium Alginate–Based Edible Composite Coating

Dan Zhu[1,2] · Rui Guo[1] · Wenxiang Li[1] · Jingya Song[1] · Fansheng Cheng[1,3]

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Abstract

The mushroom *Pholiota nameko* is extensively cultivated for its nutritional and health benefits in many countries. However, senescence and decay in fruiting bodies occur instantly after harvest, reducing their nutritional value and edible nature. In this study, edible composite coating materials were prepared using sodium alginate, enriched with 1% (*v*/*v*) thyme essential oil, 0.3 g/L *L*-cysteine, and 0.4 g/L nisin. The effects of these coatings on the postharvest quality of *P. nameko* were compared with that of low temperature (4 °C). It was shown that the edible coatings significantly inhibited the weight loss, cap opening percentage, degree of browning, malondialdehyde content, polyphenol oxygenase (PPO), peroxidase (POD), and cellulase activity of *P. nameko*. In addition, edible coatings effectively preserved the soluble sugar, ascorbic acid, and soluble protein contents. The peaks of superoxide dismutase, catalase, POD, and PPO activities were delayed by edible coatings. Moreover, edible composite coatings exerted better antibacterial efficiency compared with low-temperature treatment. Our study suggests that the edible coatings might be a promising candidate for maintaining the postharvest quality of *P. nameko*.

Keywords *Pholiota nameko* · Edible composite coating · Postharvest quality · Sodium alginate · Thyme essential oil · *L*-cysteine

Introduction

The edible mushroom *Pholiota nameko* has drawn increasing attention in recent years for its nutritive value and unique taste (Zhang et al. 2014). The antitumor, antioxidant, antihyperlipidemic, and hepatoprotective active substances of *P. nameko* have been isolated, and the health benefits of this particular mushroom as a traditional medicine have been verified (Rodrigues et al. 2017). *P. nameko* is cultivated in many countries and consumed in fresh. The *P. nameko* fruiting body is prone to immediate senescence, decay, and browning after harvest owing to the high water content, strong respiration rate, and delicate architecture. Most importantly, the fruiting body of this type of mushroom is covered with thick, translucent, and glutinous slime on the cap. Bacterial infection of this mucilaginous coat can easily pose risks of spoilage and threaten food safety during storage or transport. Conventionally, *P. nameko* should be consumed immediately within 1 day after harvest, and quick freezing is the main strategy for long-term preservation. In many developing countries, canning preservation techniques coupled with sodium hydrosulfite are applied as chemical food antiseptics. Although this method is a low-cost approach to inhibit mushroom rotting, the release of sulfur dioxide involves issues in public food safety. Thus, developing a new *P. nameko* postharvest preservation technique is crucial for this rare type of mushroom (Nerya et al. 2006; Ye et al. 2012).

Edible coatings, based on biopolymers, such as chitosan and sodium alginate have shown potential for maintaining the quality and extending the shelf life of horticultural products because they hinder water loss and gas exchange, creating a micro-modified atmosphere for the products (Duan et al. 2011). For instance, chitosan-coating treatment significantly extended the postharvest life, improved the storability, and enhanced the nutraceutical value of sweet cherry (Petriccione et al. 2015).

Dan Zhu and Rui Guo contributed equally to this work.

✉ Fansheng Cheng
fscheng@qau.edu.cn

[1] College of Food Science and Engineering, Qingdao Agricultural University, No. 700 Changcheng Road, Chengyang District, Qingdao 266109, China

[2] College of Life Science, Qingdao Agricultural University, Qingdao 266109, China

[3] Shandong Province Key Laboratory of Applied Mycology, Qingdao 266109, China

Moreover, numerous naturally occurring chemicals are proved effective in maintaining the postharvest quality of fruits and vegetables. The combination of chitosan or sodium alginate with specific substances has been widely documented in food preservation technology. Pagliarulo et al. (2016) prepared an edible active coating with a basis of peony extracts in chitosan, which effectively prolongs the shelf life and slows down the growth of the native fungal microflora on strawberries. Liu et al. (2016) and Jiang et al. (2013) developed protocatechuic acid–grafted chitosan and alginate/nano-Ag coatings for the postharvest storage of *Pleurotus eryngii* and *Lentinus edodes*, respectively. However, postharvest preservation of *P. nameko* mushrooms by edible coatings was rarely conducted.

In addition, browning and microbial spoilage caused by postharvest senescence lead to severe quality deterioration of mushroom. Thyme essential oil and nisin are food-grade agents with broad antimicrobial activity against pathogenic bacteria, such as *Listeria monocytogenes* in minced fish and *Escherichia coli* O157:H7 in minced beef (Abdollahzadeh et al. 2014; Solomakos et al. 2008). Jiang et al. (2012) demonstrated that treatment with chitosan–thyme oil inhibited the increase in respiration rate, reduced the microorganism count, and maintained the overall quality of shiitake mushroom during storage. Moreover, numerous studies have reported on *L*-cysteine as one of the most effective anti-browning agents for various fresh-cut fruits and vegetables. *L*-cysteine-treated (0.25%, *w*/*v*) litchi fruit exhibited a significant reduction in weight loss, disease incidence, disease severity, browning index, and membrane leakage; in addition, the fruit maintained its antioxidative system for up to 28 days (Ali et al. 2016).

To explore an efficient and safe preservation method that can be used to maintain the postharvest quality of *P. nameko*, sodium alginate–based composite coatings enriched with or without thyme essential oil, nisin, and *L*-cysteine were developed in the current study. Their effects on the postharvest quality and antioxidant enzyme activities of *P. nameko* were also evaluated.

Materials and Methods

Materials and Treatments

Food-grade sodium alginate, glycerin, thyme essential oil, *L*-cysteine, and nisin were purchased from China National Pharmaceutical Group Corporation. The other reagents were of analytical grade. To prepare the edible coating, 0.5 g sodium alginate was dissolved in 100 mL sterile distilled water at 60 °C. After stirring for complete dissolution and recovery at room temperature, 1.0 g glycerol was added as a plasticizer. The edible composite coating material was prepared by adding the 1% (*v*/*v*) thyme essential oil, 0.3 g/L *L*-cysteine, and 0.4 g/L nisin to the aforementioned sodium alginate solution in a vortex mixer. All chemical mixtures were used immediately after preparation.

P. nameko mushrooms were harvested from a local farm in Chengyang (Qingdao, China) and brought to the laboratory within 1 h. The mushrooms free of mechanical damage were screened for uniformity and maturity. The selected mushrooms were divided into four different groups, with each group containing three replicates, and each replicates weighing 1 kg. The sodium alginate coating (SC) group was immersed in the aforementioned sodium alginate coating solution for 30 s and drained in a plastic sieve for 10 min. Subsequently, the mushrooms were dipped in 5% (*w*/*v*) $CaCl_2$ liquor for 10 s to strengthen the coating structure and subjected to drying on a plastic sieve for 30 min under low-speed air. The composite coating (CC) group was treated using the edible composite coating material following the SC procedures. The SC group, CC group, and untreated control group (CK) were stored under 20 °C with 85% relative humidity for 9 days, whereas the low-temperature (LT) group was stored under 4 °C with the same relative humidity. Weight loss, open-cap percentage, and degree of browning were analyzed using the same sample, consisting of about 100 g mushrooms per replicate (approximately 40 mushrooms). Other mushrooms, around 100 g per replicate per treatment, were taken each time and cut into small pieces, frozen in liquid nitrogen, and mixed to provide one sample for each treatment replicate for further analysis.

Determination of Weight Loss, Open-Cap Percentage, and Degree of Browning

The weight loss percentage and the open-cap percentage were determined in accordance with the Wang et al. (2015) method. The weight loss percentage was expressed as the ratio of the reduction in weight to the initial weight. The open-cap percentage was determined as follows:

$$\text{Open cap rate} = \frac{\text{Noc}}{\text{Nt}} \times 100\%$$

where Nt is the total number of mushrooms, and Noc is the number of open-capped mushrooms.

The degree of browning was determined according to the method described by Liu et al. (2013) with modifications. Frozen *P. nameko* mushroom sample (4 g) was grounded by the addition of 20 mL of sodium phosphate buffer (0.2 M, pH 6.8), containing 2.5% (*w*/*v*) polyvinylpolypyrrolidone and 0.15 M NaCl under ice bath. The obtained homogenate was then centrifuged at 10,000*g* under 4 °C for 10 min. The resulting supernatant was determined by measuring the absorbance at 420 nm with UV–visible spectrophotometer.

Determination of Soluble Sugar, Soluble Protein, and Ascorbic Acid

Soluble sugar content was determined according to the Ye et al. (2012), on the basis of anthrone-sulfuric acid colorimetry. For protein extraction, 3 g of frozen mushrooms was homogenized with 6 mL of PBS buffer (50 mM, pH 7.8). The homogenized mixture was centrifuged at 10,000*g* for 20 min at 4 °C. Soluble protein content was measured in accordance with the Bradford method using bovine serum albumin as the standard (Bradford 1976).

For the quantification of ascorbic acid, 5 g samples were homogenized in 50 mL 5% (*w*/*v*) metaphosphoric acid solution under ice bath. After centrifugation of the homogenate at 5000*g* for 5 min, the supernatant was used for ascorbic acid determination with the 2, 4-dinitrophenylhydrazine method as described by Bajgai et al. (2006).

Determination of Malondialdehyde, Total Soluble Phenol, Flavonoid, and Total Bacterial Counts

The malondialdehyde (MDA) level reflects the degree of lipid peroxidation. One gram sample was homogenized in 5 mL of 5% (*w*/*v*) trichloroacetic acid and centrifuged for 20 min. Subsequently, 1 mL supernatant was added with 1 mL 0.6% (*w*/*v*) 2-thiobarbituric acid, and incubated in boiling water for 20 min. After cooled to room temperature, the reaction mixture was finally centrifuged at 10,000*g* at 4 °C for 10 min. The MDA content was determined and calculated by measuring the absorbance of the supernatant at three different wavelengths (450, 532, and 600 nm) as described by Liu and Wang (2012).

Total soluble phenol and flavonoid contents in mushrooms were measured as described in the study by Meng et al. (2012). The total soluble phenol content was measured based on the method of Folin-Ciocalteu reaction using gallic acid as a standard, while the flavonoid content was determined using a colorimetric assay using vitexin as the standard. The total bacterial counts (TBCs, $\log_{10}$ CFU/g) of aerobic mesophilic bacteria were determined using 3M aerobic count plates as described in the study by Jiang (2012).

Determination of Enzyme Activities

Enzymes were extracted from the *P. nameko* fruiting body according to the methods used by Zhang et al. (2013) and Meng et al. (2012). Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using the method described by Rao et al. (1996). One unit of SOD activity was defined as a change of 1 per min at 560 nm, and the results were expressed as $U \cdot g^{-1}$ fresh weight. Catalase (CAT, EC 1.11.1.6) activity was analyzed based on the Wang et al. (2005) method with several modifications. The reaction mixture consisted of 1.4 mL sodium phosphate buffer (50 mM, pH 7.0), 0.6 mL enzyme extract, and 1 mL H_2O_2 (40 mM) was added ultimately. One unit of CAT activity was defined as an absorbance change of 0.01 per min at 240 nm, and the results were expressed as $U \cdot mg^{-1}$ of fresh weight.

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity was assayed following the Assis et al. (2001) method. Briefly, 1 mL crude enzyme extraction solution was incubated with 2 mL sodium borate buffer (50 mM, pH 8.8, 1 mM β-mercaptoethanol, and 2 mM EDTA) and 0.5 mL of 20 mM *L*-phenylalanine, for 30 min at 37 °C. The reaction was stopped by adding 0.1 mL of 5 M HCl. One unit of PAL enzymatic activity is defined as an increment of 0.01 at 290 nm, and expressed as $U \cdot mg^{-1}$ of fresh weight. Peroxidase (POD, EC1.11.1.7) activity was assayed following the method described by Moerschbacher et al. (1988). The reaction mixture consisted of 2 mL sodium phosphate buffer (50 mM, pH 6.0, 5 mM guaiacol, 5 mM H_2O_2) and 1 mL crude enzyme extraction solution. One unit of POD activity was defined as an increase of 0.01 in absorbance per min at 470 nm, per gram of fresh weight. Polyphenol oxidase (PPO, EC 1.10.3.2) activity was measured according to the method by Sun et al. (2013). Subsequently, 0.1 mL crude enzyme extract was added to 4.0 mL PBS buffer (0.05 M, pH 6.0, 0.1 M catechol), and the absorbance was measured immediately. One unit of PPO activity was expressed as an increase of 0.01 in absorbance at 410 nm per min, per microgram fresh weight. Glutathione reductase (GR, EC 1.6.4.2) and cellulase (EC 3.2.1.4) activities were determined according to the method by Smith et al. (1988) and Chen et al. (2017), respectively.

Statistical Analysis

Experimental mushrooms were evaluated using a completely randomized design. All experiments were run in triplicate. Data were presented as mean and performed by one-way analysis of variance (ANOVA) with SPSS ver. 17 (SPSS Inc., Chicago, IL). Differences were considered significant if $P < 0.05$. Correlations were estimated using Pearson's correlation coefficient (r). Figures were generated using Origin 9.0.

Results and Discussion

Changes of Weight Loss, Open-Cap Percentage, and Degree of Browning

Dehydration distinctly occurs in mushrooms exhibiting quality deterioration during postharvest storage because mushrooms are generally protected by a thin epidermal structure (Ye et al. 2012). However, coatings can act as alternatives to prevent superficial dehydration. Figure 1a shows a continuous increase in weight loss in all treatment groups during storage. After being stored for 9 days, the CK group showed the

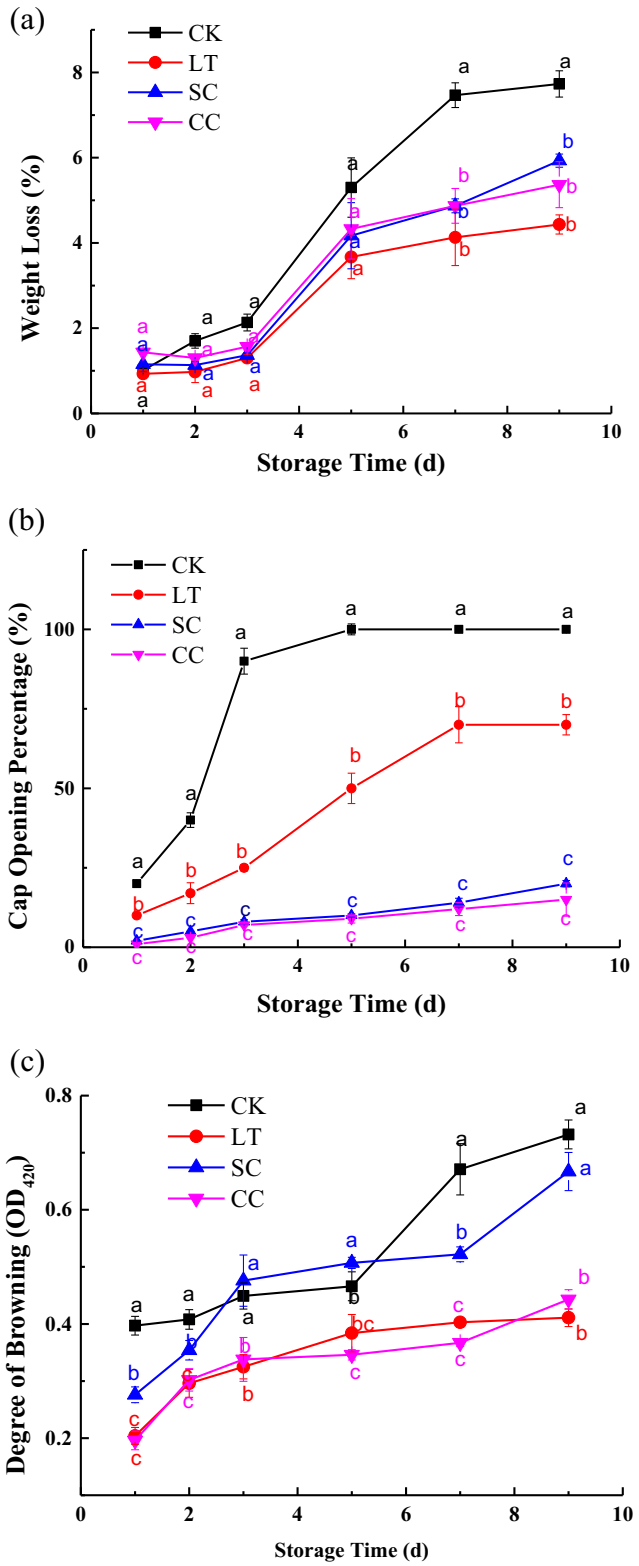


Fig. 1 The weight loss (**a**), cap opening percentage (**b**), and the degree of browning (**c**) of *P. nameko* during the storage. The black, red, blue, and purple represent control (CK), low temperature (LT), sodium alginate (SC), and composite treatment (CC), respectively. Experimental values are mean ± standard deviation (SD) of $n = 3$, and different letters (a, b, c, and d) indicate significant differences at $P < 0.05$

highest weight loss (7.73%), which was significantly different ($P < 0.05$) from those of the other groups. Notably, the LT group exhibited no significant difference from the SC and CC groups ($P < 0.05$). The results indicated that the coating effectively delayed the shriveling and quality deterioration of *P. nameko* mushroom. This finding could be attributed to the effect of coatings on osmotic pressure and moisture balance in mushroom tissues (Ashraf and Foolad 2007; Wang et al. 2015).

The open-cap percentage is an indicator of freshness. Studies exploring the inhibition of the percentage of cap opening by chemicals such as glycine betaine and 4-methoxycinnamic acid have been widely conducted (Hu et al. 2015; Wang et al. 2015). In the present study, the percentage of cap opening continuously increased in all treatment groups during storage. The percentage of cap opening in the CK group markedly increased in the first 3 days (Fig. 1b), whereas those in the other groups were significantly ($P < 0.05$) inhibited. The SC and CC groups, comprising only 15–20% of the CK group, were found to be most effective throughout the storage period of 9 days. Jiang (2013) also found that alginate coatings delayed the browning and cap opening percentage of *Agaricus bisporus*, which was in agreement with the present study. Cap opening in mushrooms is related to dryness resulting from water loss during storage. The reason for this study may be the increase in water loss during storage, which decreases the binding force of water and other hydrophilic molecules, such as proteins, to which cause the intact position of the caps and veil in mushrooms (Wang et al. 2015).

Browning is one of the biochemical reactions affecting the nutritive value and quality of mushroom during storage. Browning is often used to evaluate deterioration and reflect the freshness of mushroom (Liu et al. 2013). Figure 1c shows that all treatments displayed a steady increase in degree of browning during storage. The CK group showed the highest degree of browning at the end of storage, whereas the CC and LT groups were the lowest, and reflected the lowest rate of increase during storage (Fig. 2). Meanwhile, the composite coating exhibited the best effectiveness, which might result from the *L*-cysteine added in coating material, as an anti-browning substance (Ali et al. 2016). Low temperature has been proved an effective way to inhibit browning of horticultural products by decreasing the PPO and POD activities (Gao et al. 2014), which was also found in our study ("Changes in Antioxidant Enzyme Activities" section).

Changes in Soluble Protein, Ascorbic Acid, and Soluble Sugar Contents

As indicated in Fig. 3a, the total soluble protein presents a steady and apparent decrease in all treatments during storage, which was also described in a previous study on button

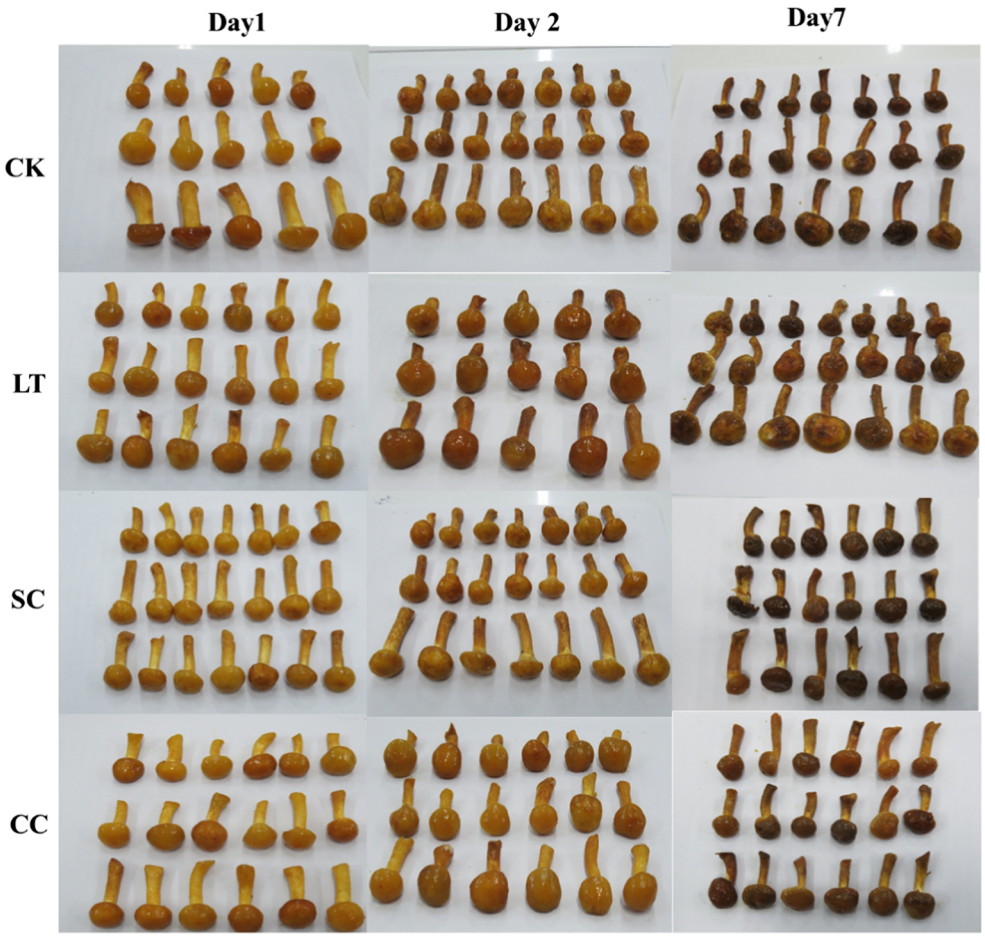


Fig. 2 Effects of coatings and low temperature on *P. nameko* mushroom during the storage time at day 1, day 2, and day 7. CK, LT, SC, and CC represent control, low temperature, sodium alginate, and composite treatment, respectively

mushrooms (Khan et al. 2015). However, the CC, LT, and SC groups showed a significant difference in the reduction rate of soluble protein content ($P < 0.05$), compared with the CK group. As soluble protein content is considered a sensitive indicator of tissue destruction and reactive oxygen species (ROS) damage (Bajgai et al. 2006), these results indicated that coating and low-temperature treatment could delay the senescence of *P. nameko*.

The ascorbic acid content can be used as an index of anti-aging because it is an antioxidant substance. Figure 3b shows a steady and apparent decrease in ascorbic acid content during storage. The level of ascorbic acid content in all treatment samples was significantly higher than that in the CK group ($P < 0.05$). Notably, the CC and SC groups exhibited no significant difference during the first 7 days of storage. The result indicated that low temperature and edible coating could maintain high ascorbic acid content, reduce the accumulation of intracellular ROS, and delay senescence in fruits (Wang et al. 2015).

The soluble sugar content in harvested plant products is also considered an important index of nutritive value and postharvest deterioration (Ye et al. 2012). Figure 3c demonstrated an apparent decreasing tendency in soluble sugar content during storage for all treated samples. The soluble sugar contents of the CC, SC, and LT groups were significantly higher than that of the CK group ($P < 0.05$). The CC group showed the lowest rate of decrease and the highest soluble sugar content, without marked difference from those of SC and LT groups ($P < 0.05$). These results indicated that coating and low-temperature treatment could probably slow down respiration and metabolic activity, hence retarding the polysaccharide and hemicellulose hydrolysis process to sugars in the cell wall (Jiang et al. 2012).

Changes of Total Soluble Phenol and Flavonoid Contents

Figure 4 shows that total soluble phenol and flavonoid continuously accumulate in all samples during storage, which is consistent with a previous study (Meng et al. 2017). The flavonoid contents of the CC and LT groups were significantly lower than that of the CK group ($P < 0.05$). The total soluble phenol content of the CK group was highest during storage and reflected the largest increment. As previously mentioned, the CC and LT groups showed markedly lower degrees of

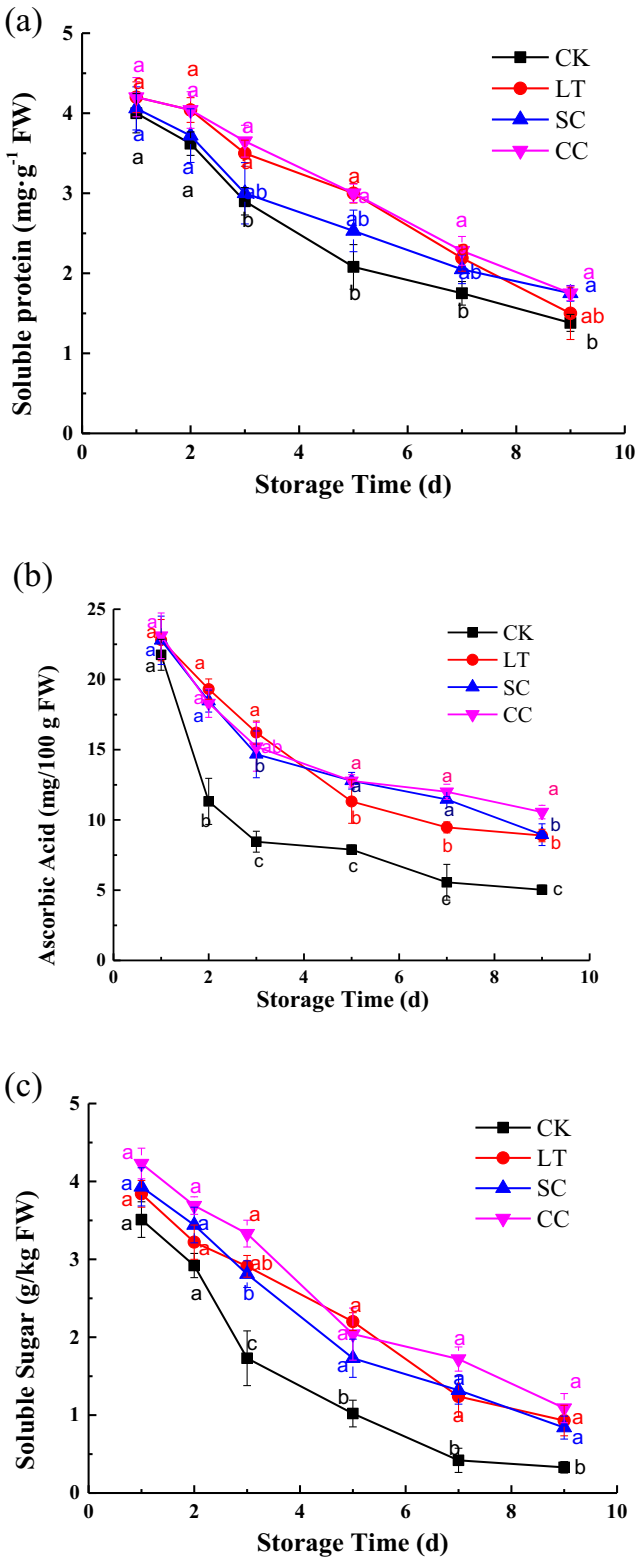


Fig. 3 The soluble protein (**a**), ascorbic acid (**b**), and soluble sugar (**c**) content of *P. nameko* during the storage. The black, red, blue, and purple represent control (CK), low temperature (LT), sodium alginate (SC), and composite treatment (CC), respectively. Experimental values are mean ± standard deviation (SD) of $n = 3$, and different letters (a, b, c, and d) indicate significant differences at $P < 0.05$

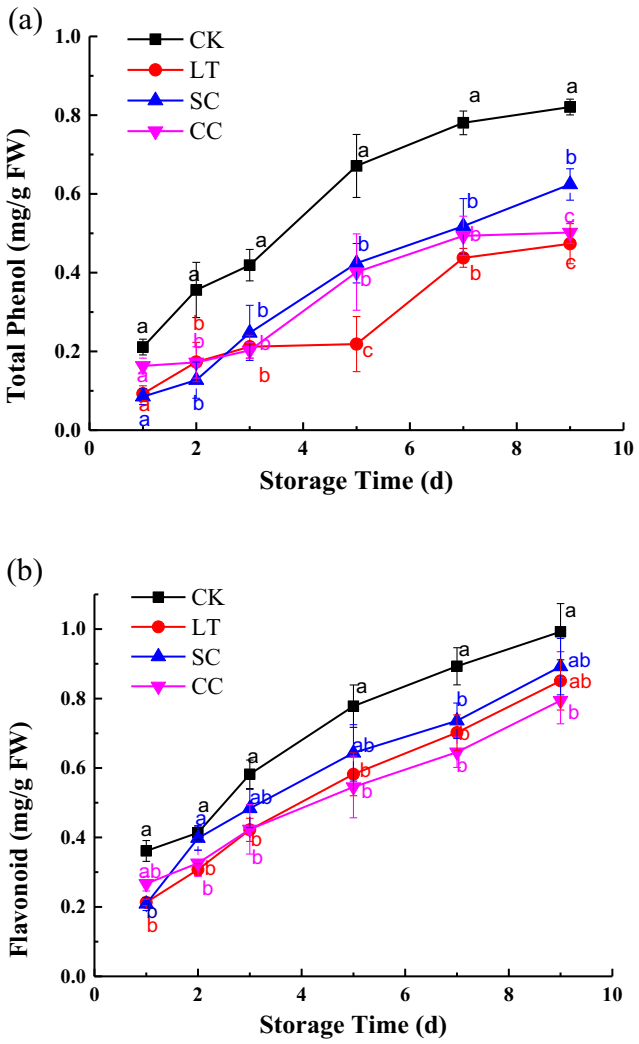


Fig. 4 The total soluble phenol (**a**) and the flavonoid (**b**) content change during the *P. nameko* storage. The black, red, blue, and purple represent control (CK), low temperature (LT), sodium alginate (SC), and composite treatment (CC), respectively. Experimental values are mean ± standard deviation (SD) of $n = 3$, and different letters (a, b, c, and d) indicate significant differences at $P < 0.05$

browning compared with the other groups. Analysis of Pearson's correlation coefficient indicated that total soluble phenol was significantly related to the degree of browning in button mushrooms ($r = 0.861$), which was consistent with the results obtained by Gao et al. (2014) and Meng et al. (2017).

Phenolic compounds in mushrooms have been regarded as functional substances that scavenge free radicals and terminate radical chain reactions, as well as the major substrate of enzymatic browning reaction (Lu et al. 2016). Meanwhile, flavonoids are identified as key factors affecting mushroom antioxidant properties (Meng et al. 2017). The present results indicated that the flavonoid and phenolic compounds participated in balancing ROS stress and the browning synthesis process during *P. nameko* storage (Gao et al. 2014).

Change of MDA Content

MDA is the main product of membrane lipid peroxidation caused by mechanical damage or postharvest senescence. MDA accumulation is expected to damage the plasma membrane and organelle of the mushroom via reaction or cross-linking with protein, nucleic acid, or cellulose chain to inhibit the corresponding functions (Gürbüz and Heinonen 2015). In Fig. 5, MDA in the CK and other treatment groups increased during storage markedly. The MDA content of the CC and SC groups obtained the lowest level during storage. After storage for 9 days, the MDA content of the CC group comprised only half that of the CK group and was significantly different from those of the other groups ($P < 0.05$). The decreases in MDA content in both the CC and SC groups were probably due to the enhancement of the antioxidant enzymes by edible coatings, such as PAL and CAT, as shown in the "Changes in Antioxidant Enzyme Activities" section (Khan et al. 2015).

Changes in Antioxidant Enzyme Activities

During mushroom storage, SOD, POD, CAT, and the other antioxidant enzymes play a key role in scavenging $O^{2-}\cdot$ and H_2O_2, which have been reported to induce membrane peroxidation and accelerate cell senescence (Wang et al. 2015).

SOD is regarded as the key enzymatic antioxidant in plant response to stress factors to eliminate superoxide radicals and is the first line of defense from damage caused by oxygen radicals (Starzyńska et al. 2003). SOD activity is induced by the substrate level–superoxide radical and correlated with tolerance against different environmental stresses. Figure 6a shows that SOD activity generally fluctuates regardless of the treatment. The CK group reached the peak on day 5, whereas the other treatment measures postponed the peak for about 2 days. The SOD activity of the CK group was significantly lower compared with the other groups at the end of the storage period ($P < 0.05$), with the CC group showing the highest SOD activity. Combined with the MDA data in this study, the SOD result indicated severe oxidative injury in the CK group. Similar SOD activity was observed in button mushrooms (Khan et al. 2015).

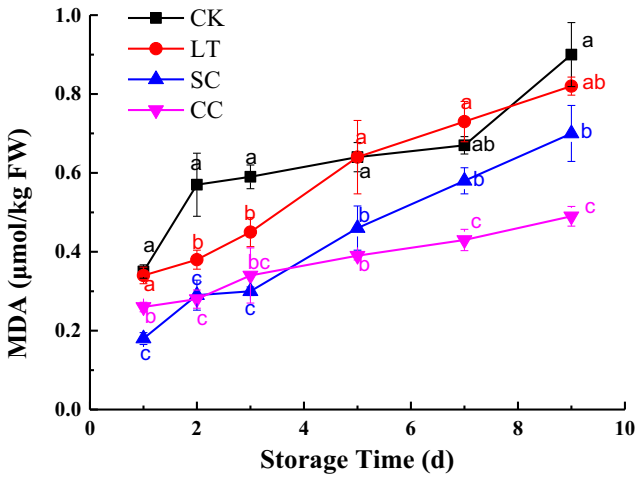
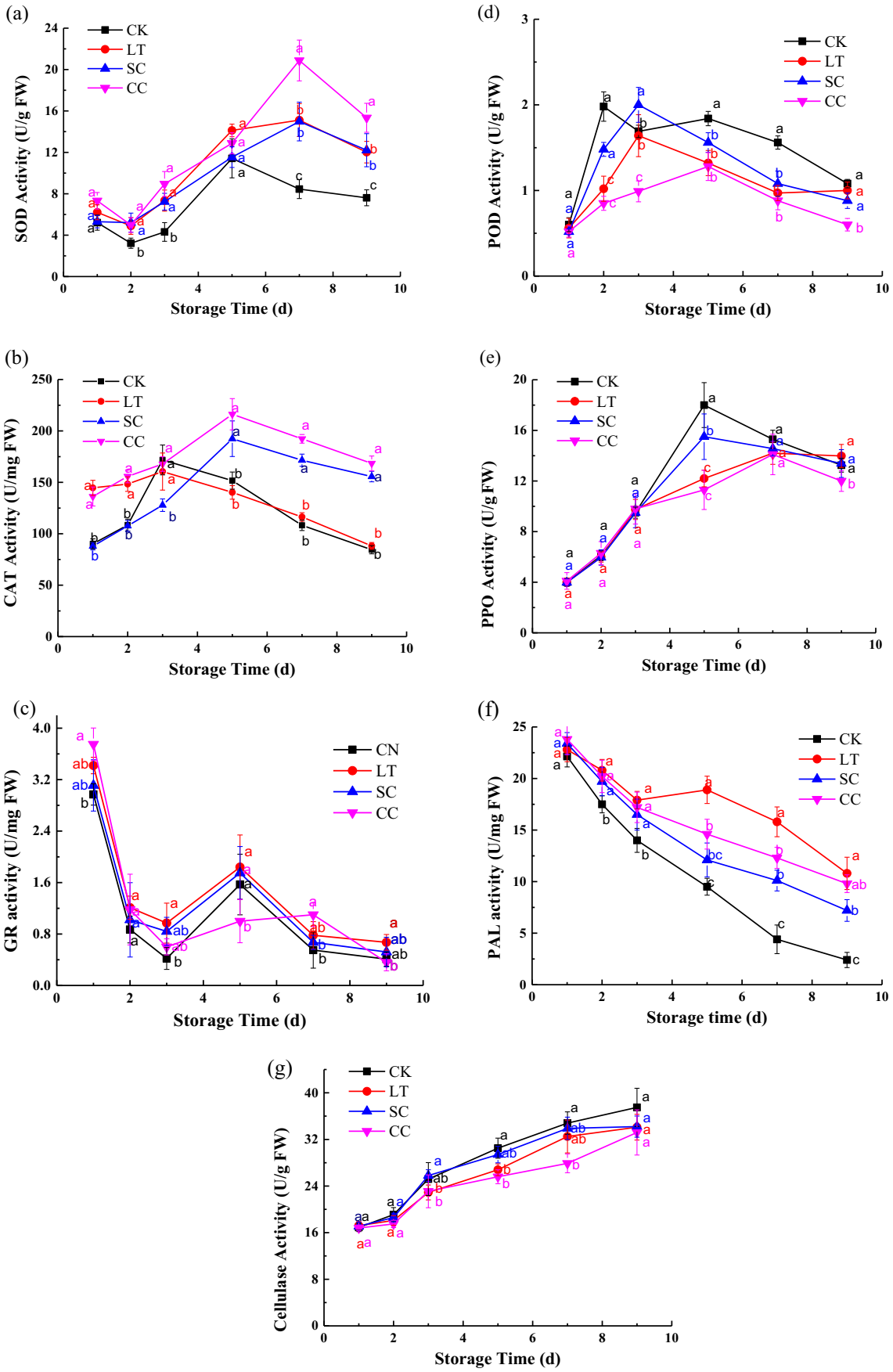


Fig. 5 The MDA content change of *P. nameko* during the storage. The black square, red cycle, blue up triangle, and purple down triangle represent control (CK), low temperature (LT), sodium alginate (SC), and composite treatment (CC) respectively. Experimental values are mean ± standard deviation (SD) of $n = 3$, and different letters (a, b, c, and d) indicate significant differences at $P < 0.05$

CAT is one of the most important enzymes scavenging active oxygen species. This enzyme participates in the main defense system against accumulation and toxicity of hydrogen peroxide and can help control the H_2O_2 level in cells. The generation of superoxide anion, which induced oxidative injury, as well as SOD and CAT scavenged the active oxygen species to alleviate these injuries (Khan et al. 2015; Wang et al. 2015). In Fig. 6b, the CAT activities increase with storage and peaked on day 3 and day 5 in the coated and non-coated samples, respectively; this activity then declines until storage ends. Compared with those in the CC and SC groups, the CAT activities in the other groups peaked much earlier; however, the peaks were lower, ranging only from 162.30 to 176.12 U/mg FW. Regarding to the coating treatment, significant differences ($P < 0.05$) in CAT activity were found between the CC and SC groups. The CC group exhibited the highest CAT activities, about 214.28 U/mg FW on day 5. The peak of CAT activity appeared after treatment. The composite coating could maintain the high SOD and CAT activities in the latter part of the storage period. These high activities scavenged the active oxygen species in the mushroom cell and maintained the quality of mushrooms. Senescence decreased the CAT activity during the latter stage of storage; meanwhile, the CAT activity of the coated samples remained higher than those of the other samples. All of these aforementioned observations indicated that coating positively influenced the delay of senescence. The same results were observed in the other samples. Khan et al. (2015) found that both SOD and CAT activities exhibited similar changing patterns and that coating-treated button mushrooms maintained significantly higher levels of CAT activity ($P < 0.05$).

GR also participates in scavenging the ROS and has been implicated in senescence-related stress responses in plants (Jimenez et al. 2002). In Fig. 6c, GR activity in all groups exhibited similar trends—decreasing in the first few days, increasing until it reaches its peak, and then decreasing. The CC group delayed the GR activity peak, compared with the other groups.

POD has diverse biochemical functions and participates in the growth, development, and senescence of plants. It is an important active free radical scavenging enzyme. Figure 6d


(a)
SOD Activity (U/g FW)
Storage Time (d)
CK
LT
SC
CC
(d)
POD Activity (U/g FW)
Storage Time (d)
(b)
CAT Activity (U/mg FW)
Storage Time (d)
(e)
PPO Activity (U/g FW)
Storage Time (d)
(c)
GR activity (U/mg FW)
Storage Time (d)
CN
(f)
PAL activity (U/mg FW)
Storage time (d)
(g)
Cellulase Activity (U/g FW)
Storage Time (d)

◀ **Fig. 6** The SOD (**a**) ,CAT (**b**), GR (**c**), POD (**d**), PPO (**e**), PAL (**f**), and cellulase (**g**) activity change of the *P. nameko* during storage. The black, red, blue, and purple represent control (CK), low temperature (LT), sodium alginate (SC), and composite treatment (CC), respectively. Experimental values are mean ± standard deviation (SD) of $n = 3$, and different letters (a, b, c, and d) indicate significant differences at $P < 0.05$

shows that the POD activity of the CK group reaches the peak on day 2, the LT group on day 5, and the SC and CC groups on day 3. Gao et al. (2013) also found that the chitosan–glucose complex coating could delay the appearance of POD peak during table grapes storage; however, the POD activity level of the chitosan–glucose complex treatment group was higher than that of the control group. In the present study, the POD activity level in the CC group was the lowest, indicating that the composite coating provided a more suitable environment for storage, compared with other treatments.

Browning in fruit and vegetables results from oxidation and polymerization of phenolic compounds and is associated with PAL, PPO, and POD activity levels. Mushroom browning occurring mainly results from tyrosinase activity, an enzyme belonging to the PPO family and is regarded as a key enzyme in melanin biosynthesis (Cheng et al. 2015; Nerya et al. 2006). Figure 6e shows that PPO activity increases with storage time, peaks, and then decreases. The PPO activity levels of the CC and LT groups were significantly ($P < 0.05$) lower than that of the CK group during storage, verifying that the composite coating and treatment at 4 °C controlled the browning process.

In Fig. 6f, the PAL activity levels of all treated and untreated samples decrease significantly ($P < 0.05$) during storage. The PAL activity level of the LT and CC groups was significantly higher than those of the CK and other groups. This result contradicted the previous consensus that PAL activity was related to tissue browning of fresh fruits and vegetables during storage (Duan et al. 2007). However, other studies also found that browning accompanies the activation or suppression of PAL. Gao et al. (2014) and Jiang et al. (2012) demonstrated that treatment with natamycin and pure oxygen or fumigation with essential oil (clove, cinnamaldehyde, and thyme essential oil) could delay browning during storage of *A. bisporus* and the respective PPO and POD activity levels were significantly inhibited. However, PAL activity was decreased by natamycin and pure oxygen, but activated by essential oil. This activation trend was also observed in salicylic acid treatment of *A. bisporus* (Dokhanieh and Aghdam 2016).

Moreover, comparisons between the CC and SC treatments verified that *L*-cysteine was effective as an anti-browning agent and can maintain the postharvest quality and antioxidative system on different fresh-cut fruits and vegetables, either alone or in combination with different organic acid/edible coating treatments (Ali et al. 2016). The present study also proved that essential oil, combined with the edible coating, was as effective as fumigation treatments in preserving button mushrooms (Gao et al. 2014).

Changes in Cellulase Activity

Mushrooms may soften and change in texture *via* degradation of the cell wall by bacterial enzymes and endogenous autolysins during storage (Khan et al. 2017). Cellulase is a complex enzyme, consisting of exoglucanase, endoglucanase, and β-glucosidase. Cellulase can degrade the mushroom tissue and decrease its firmness (Wang et al. 2011). Figure 6g shows an apparent increase in cellulase activity in all treatments during the storage. The cellulase activity levels of the SC, CC, and LT groups were lower than that of the CK group during storage, whereas that of the CC group exhibited the lowest cellulase activity. The results indicated that coating and treatment at 4 °C may delay the quality degradation of *P. nameko* by preventing cellulose hydrolysis. Notably, $CaCl_2$ in the present study acted not only to strengthen the coating, as the $CaCl_2$ with citric acid reportedly suppressed the activity of cell wall–degrading enzymes, gene expression, and senescence (Khan et al. 2015, 2017).

The TBCs of Aerobic Mesophilic Bacteria During Storage

Pathogenic microbial species to which spoilage of mushrooms is attributed might be present during processing, transport, distribution, storage, and handling (Wu et al. 2018). The effects of the edible coating on TBCs were evaluated. At the initial stage of storage, the TBCs of *P. nameko* mushroom were 2.95 ± 0.05, rising dramatically at the end of the storage. In addition, the TBCs of the CC (4.24 ± 0.12), SC (4.52 ± 0.04), and LT (5.06 ± 0.04) groups were lower than that of the CK (5.35 ± 0.03), with significant differences between the groups ($P < 0.05$). These results indicated that edible coating containing thyme essential oil and nisin exerted a higher bacteriostatic activity compared with low-temperature treatment. The effectiveness of thyme essential oil and nisin in the present study was in accordance with previous studies in minced fish and beef (Abdollahzadeh et al. 2014; Solomakos et al. 2008).

Interestingly, the present result indicated that the sodium alginate–based coating material also exhibited bacteriostatic activity. Abdel Aziz et al. (2018) found that alginate possessed no antibacterial activity toward *Staphylococcus aureus* (RCMB 010010) and *Bacillus subtilis* (RCMB 010067), *E. coli* (RCMB 010052), and *Salmonella Typhi*, using the agar well diffusion method, which was in accordance with the research by Liu et al. (2018). By contrast, 1% sodium alginate coating significantly inhibited the growth of *Pseudomonas* spp. and total viable counts in the first 3 days of storage of

fresh-cut apple ($P < 0.05$). Sodium alginate is presumed to provide nutrition for microbial growth and reproduction, leading to the quick growth of mold, yeast, and *Pseudomonas* spp. at the later stage of the storage (Liu et al. 2018). In addition, the sodium alginate coating was strengthened by $CaCl_2$ treatment in the present study. Osmokrovic et al. (2018) reported that Ca-alginate hydrogels exhibited bacteriostatic activity against *S. aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 27853, which was in accordance with our research. The discrepancies in the result of different studies may be attributed to the tested *Pseudomonas* spp., which is the main pollution bacteria in mushroom (Tsukamoto et al. 1998) and may be sensitive to alginate.

Conclusion

In summary, the special and precious *P. nameko* mushroom undergoes senescence, browning, and decay immediately after harvest. The application of sodium alginate–based coatings effectively reduced the weight loss, cap opening percentage, degree of browning, MDA content, and total soluble phenol content relative to those of the untreated control group. In addition, the appearance of the peaks of SOD, POD, PPO, and CAT activity levels was delayed after the coating treatment. The ascorbic acid, soluble sugar, protein, and PAL activity of the treated mushrooms were higher than those of the untreated samples during storage. In addition, sodium alginate–based composite coating, containing 1% (*v*/*v*) thyme essential oil, 0.3 g/L *L*-cysteine, and 0.4 g/L nisin, can potentially control the senescence process, aerobic mesophilic bacteria growth, and quality deterioration of *P. nameko* mushroom.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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