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Utilisation of waste *Agaricus bisporus* and *Torreya grandis* as potential natural additive coating in *Agaricus bisporus* preservation

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Abstract: Natural additives obtained from vegetable and fruit wastes can potentially be used as coatings to enhance the shelf life of button mushrooms (*Agaricus bisporus*). The present study was therefore conducted to assess the effects of natural additive coatings *A. bisporus* polysaccharides (ABP) and *Torreya grandis* essential oils (TEO) on the post-harvest quality of button mushrooms up to 16 days under cold storage (4 °C). Different concentrations for coatings were used including control (no additives), 500, 1 000, 5 000 ppm or a combination of ABP and TEO. Changes in weight loss, firmness, polyphenol oxidase (PPO) activity and microbial biomass were investigated as well as the sensory evaluation was carried out. Results show a significant decrease in the weight loss rate (decreased up to 2.34%), down regulated the PPO activities (reduced up to 30.15%) and maintained tissue firmness in ABP/TEO treatments compared to the control, whereas TEO inhibited especially the growth of microorganisms. Natural additive coatings can better protect the inner mushroom colour and flavour compared to the control.

Keywords: polysaccharides; *Torreya grandis* essential oil; reuse; nature film; button mushroom; keep fresh

Button mushrooms (*Agaricus bisporus* L.) are susceptible to mechanical damage and microbial infections in case of improper storage (Rokni and Goltapeh 2019). Generally, after 3-4 days of storage at room temperature, the mushroom begins to lose firmness, colour and moisture, leading to a reduced market value. Due to the short shelf life, mushrooms cannot be transported over a long distance, which is easy to cause waste and limits the development of the mushroom industry (Alpuche-Solís and Paredes-López 2000). Such short life issues cause product spoilage resulting in a significant economic loss. In a recently published review

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about the browning of button mushrooms and its controlling methods, it was found that coating (chemical and natural additives) was frequently used in keeping button mushrooms fresh (Lin and Sun 2019). Considering the safety of chemical additives, researchers are more interested in applying natural additives to button mushrooms (Jiang et al. 2012). Polysaccharides, proteins, and lipids are the sources for the production of edible films and coatings with particular characteristics (Raeisi et al. 2015). It is therefore possible to use fungi as an important source of several biologically active compounds including polysaccharides (β-glucans, chitin and heteropolysaccharides), terpenes, phenols and so on (Kozarski et al. 2014). Recovered button mushroom crude polysaccharides [*A. bisporus* polysaccharides (ABP)] contain 11.57% of α-glucans, 16.37% of β-glucans and some proteins (16.24%). Such active compounds obtained from mushroom waste exhibit extraordinary antioxidant capacity (Cebin et al. 2018).

Torreya grandis essential oil (TEO) has been proved to have a capacity to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Yu et al. 2016). There are 24 kinds of ingredients in TEO, including limonene (40.78%), α-pinene (7.59%), (E)-β-farnesene (6.16%), and δ-cadinene (6.60%) as described by Shu et al. (1995). The volatile compounds of TEO are well-known antimicrobial and antioxidant agents which endow their high potential as natural additives for fruit and vegetables to enhance the shelf life.

The main objective of this study was to investigate the effects of polysaccharide coating, individually and/or in combination with TEO as a natural additive in mushroom storage. This will make it more efficient to keep mushrooms fresh and provide the possibility for the reuse of industrial mushroom factory waste.

MATERIAL AND METHODS

Raw material collection and pre-treatment. Mushrooms were obtained from Lian-Zhong Edible Fungi Cooperative Company (Shanghai, China). TEO (≥95% purity) was provided by Zhejiang Huamulai Biotechnology Co., Ltd. (Zhejiang, China). Crude polysaccharides from waste button mushrooms were extracted using hot water (Zhang et al. 2011). Briefly, 1 kg of button mushroom waste and 10 g of citric acid were cooked for 1 h with 10 L pure water at 100 °C. The solubilised material was recovered by filtration (filter with medical gauze) and the above extraction method was repeated thrice. The solution was evaporated until one-tenth of the original solution remained. The recovered solution was combined with a threefold volume of ethanol. The precipitate was then deproteinised by the Sevage method (Szwengiel and Stachowiak 2016).

Five different natural additive coating solutions were used to treat mushrooms: *i*) control (CK; water only), *ii*) ABP (0.5% ABP coating), *iii*) ABP/TEO5 (0.5% ABP + 1.0% sorbitol + 500 ppm TEO), *iv*) ABP/TEO10 (0.5% ABP + 1.0% sorbitol + 1 000 ppm TEO), and *v*) ABP/TEO50 (0.5% ABP + 1.0% sorbitol + 5 000 ppm TEO). Samples were soaked in the respective natural additive coating solutions for 5 min, then placed on a strainer for 30 min and dried with an electric fan at low-temperature wind. After treatment applications, the samples were packed in a sealed crisper with 0.06 mm thickness polyethylene (PE) film on the top (Shanghai Caiyang Food Co., Ltd., Shanghai, China). They were kept at 4 ± 1 °C in the dark (low temperature warehouse; Shanghai Lian-Zhong Edible Fungi Cooperative Company, Shanghai, China) until further use [technology roadmap of experiment refers to Figure S1 in electronic supplementary material (ESM); for ESM see the electronic version].

Weight loss rate. Changes in sample weight loss were recorded at 0, 4, 8, 12, and 16 days after storage. The weight loss rate was expressed as the weight difference before and after storage divided by the initial weight of the button mushrooms.

Texture analysis. Textural studies of the mushrooms were performed using a texture analyser (TA-XT plus; SMS, London, United Kingdom) equipped with a P/2 stainless probe. The speed of the probe was set at 1.00 mm s^{-1}. Force and time data were recorded using Texture Exponent 6.1.12 from Stable Micro Systems. Nine replicates were analysed for each treatment and the average data set is presented.

Polyphenol oxidase (PPO) activity. Polyphenol oxidase (PPO) activity was measured based on the method described by Wei et al. (2009). Briefly, three randomly selected samples were pooled and ground for 2 min in a freeze-grinder (JXFSTPRP-96; Shanghai Jingxi Industrial Development Co., Ltd., Shanghai, China). One-gram sample was then added into a 5 mL enzyme extraction buffer containing 1 mM polyethylene glycol (PEG), 4% polyvinyl polypyrrolidone (PVPP) and 1% Triton X-100 (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The suspension was centrifuged (12 000 rpm, 4 °C, 20 min) (TGL-16M; Cence Co., Ltd, Hunan, China) and the supernatant was collected. Acetic acid-sodium acetate (3 mL, 50 mM) and catechol (1 mL) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) were added into 100 μL supernatant and

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mixed for 3 min (Vortex-Genie 2; Scientific Industries, New York, USA) before the measurement of absorbance at 420 nm. Defined unit (U) as PPO activity, under certain conditions, the amount of the enzyme that can convert 1 μmol of catechol in 1 min, that is, 1 international unit (IU) = U = 1 μmol min^{-1}.

Microbiological analysis. Changes in the number of microorganisms (yeast, mould, and *Pseudomonas*) during the storage period were recorded. Aliquots of 25 g of samples were removed aseptically from each treatment group and mixed (Vortex-Genie 2; Scientific Industries, New York, USA) with 225 mL of 0.9% (w/v) saline solution. The mixture was shaken for 2 min (Vortex-Genie 2; Scientific Industries, New York, USA) and serially diluted (10^{-1}–10^{-9}) with the saline solution. *Pseudomonas* counts were determined on CN agar medium (Guangdong Dayuan Food Safety Technology Co. Ltd., Guangdong, China) after incubation at 36 ± 1 °C for 24 h (LRH-250; Yiheng, Shanghai, China). Yeasts and moulds were observed on potato dextrose agar (PDA) medium (Merck, Darmstadt, Germany). One mL solution of serial dilution (10^{-1}–10^{-9}) ware added into PDA medium and they were incubated at 28 ± 1 °C for 5 days (LRH-250; Yiheng, Shanghai, China) (Wells et al. 1996).

Sensory evaluation. The effects of different treatments on the sensory characteristics of button mushrooms were investigated after a preliminary judgment of mushroom quality during the preservation period. Ten people aged from 25 years to 50 years, of good health and with no smoking habits, were chosen as volunteers to evaluate sensory. The quality of mushrooms was assessed from five different aspects (colour, cap uniformity, texture, odour, and acceptability) at the end of storage. The defined full score for each aspect was 10.

Statistical analysis and software application. Experiments were conducted in triplicates; the results are reported as mean ± standard deviation (SD). All data were analysed statistically using SPSS 24.0 software (IBM SPSS Statistics, USA). One-way analysis of variance (ANOVA) with the Fisher least significant difference (LSD) test was conducted to measure the significance ($P < 0.05$) of the main effects between different groups. Figures were designed by Origin 9.0 (OriginLab Corporation, USA).

RESULTS AND DISCUSSION

Weight loss rate. The main reason behind the weight loss reduction is the high respiratory rate of button mushrooms. It is a simple chemical reaction in mushroom storage ($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + energy) which results in weight loss (Joshi et al. 2018). The highest weight loss was recorded in the control (CK), where it reached 5.85% at the end of storage (Figure 1). Literature is replete with reports that the fruits and vegetables lose their market value when the weight loss rate exceeds 4% (Bico et al. 2009). Generally, the button mushroom shelf life can be extended up to 9 days approximately without any treatment by storing at 4 °C, which is consistent with other reports found in the literature (Yang et al. 2019). Significant effects were observed on a reduction of weight loss due to natural additive coating treatments. The weight loss values decreased significantly to 2.45% or even less after treatment with mushroom polysaccharides and/or essential oil. ABP reached 2.98% at the 16th day after storage. Such results showed the importance of ABP as a film substrate to avoid the moisture loss from mushroom samples.

Texture analysis. Changes in the firmness of mushrooms are shown in Figure 2. During the preservation period, the surface firmness in CK decreased by 53.24% on the 16th day. The firmness of ABP and ABP/TEO maintained better than CK obviously, and there was a difference between ABP and ABP/TEO ($P < 0.05$). At the end of the cold storage period, the decrease in the firmness of different treatments was slowed down to different degrees compared to CK, suggesting that ABP/TEOs have affection for maintaining firm-

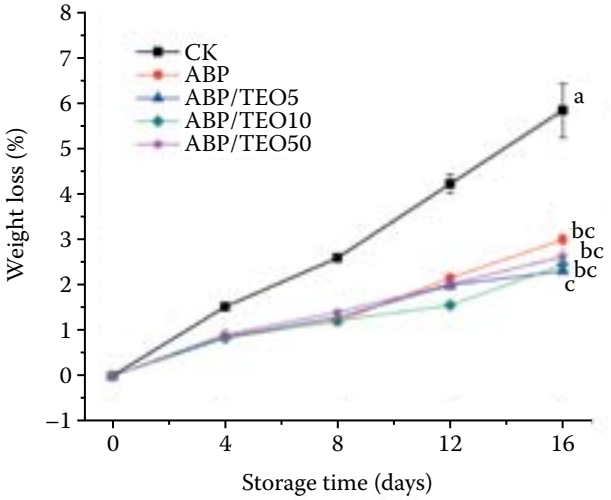

Figure 1. Effects of natural additives in reducing the weight loss in button mushroom when stored at 4 °C

a, b, c – different letters indicate the significant difference between groups ($P < 0.05$); CK – control; ABP – *Agaricus bisporus* polysaccharides; ABP/TEO5 – 0.5% ABP + 1.0% sorbitol + 500 ppm *Torreya grandis* essential oils (TEO); ABP/TEO10 – 0.5% ABP + 1.0% sorbitol + 1 000 ppm TEO; ABP/TEO50 – 0.5% ABP + 1.0% sorbitol + 5 000 ppm TEO

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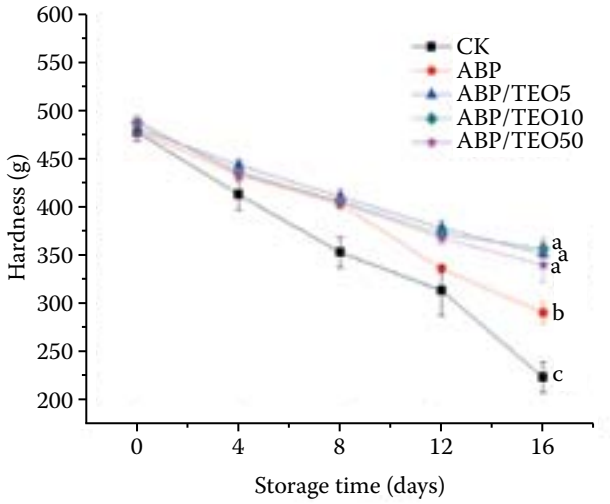

Figure 2. Effect of natural additives on surface hardness of button mushroom stored at 4 °C for 16 days

a, b, c – different letters indicate the significant difference between groups ($P < 0.05$); CK – control; ABP – *Agaricus bisporus* polysaccharides; ABP/TEO5 – 0.5% ABP + + 1.0% sorbitol + 500 ppm *Torreya grandis* essential oils (TEO); ABP/TEO10 – 0.5% ABP + 1.0% sorbitol + + 1 000 ppm TEO; ABP/TEO50 – 0.5% ABP + 1.0% sorbitol + 5 000 ppm TEO

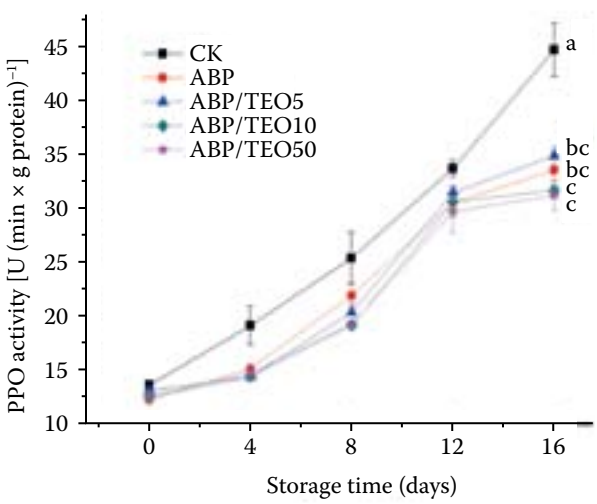

Figure 3. Effect of natural additives coating on PPO activity of button mushroom stored at 4 °C for 16 days

a, b, c – different letters indicate the significant difference between groups (P < 0.05); PPO – polyphenol oxidase; U – unit; CK – control; ABP – *Agaricus bisporus* polysaccharides; ABP/TEO5 – 0.5% ABP + 1.0% sorbitol + 500 ppm *Torreya grandis* essential oils (TEO); ABP/TEO10 – 0.5% ABP + 1.0% sorbitol + 1 000 ppm TEO; ABP/TEO50 – 0.5% ABP + 1.0% sorbitol + 5 000 ppm TEO

ness. It is also an obvious fact that microorganisms are among the factors affecting the firmness of mushroom tissue. ABP could be taken as a film to prevent moisture and oxygen exchange with the outer environment. Our results are in line with other researchers who reported the maintenance of firmness in mushroom samples by film-forming of gum (Nasiri et al. 2019). It also shows the potential of ABP as a film-forming material. TEO inhibited the growth of microorganisms. The possible mechanism of action is presumably as follows: TEO includes limonene and other content, which most likely disrupt the cell wall and the cell membrane integrity of microbial cells, which leads to the release of intracellular components and causes the electron transfer at the membrane, the repression of nucleotide synthesis and ATP activity, thereby inhibiting the growth of microorganisms. Therefore, ABP mixed with 500 ppm TEO could effectively maintain the firmness of mushrooms.

PPO activity. Melanin is synthesised in mushrooms and causes their browning, leading to the lower quality of mushrooms (Sapers et al. 2010). As depicted in Figure 3, the PPO activities increased gradually during the storage at 4 °C. There is an inflection point when the activity rate changes steeply on the 12th day, subsequently, the change rate was slowed down. But there is no significant difference within different treatments ($P \geq 0.05$). This clearly reveals that ABP could inhibit PPO activities instead of essential oils. At the end of storage, ABP reduced the PPO activity up to 20%. Lei et al. (2018) used ultraviolet-C (UV-C) to influence PPO activity in mushrooms, and it was found that the strip of mushrooms was more susceptible to its effects. The UV-C-treated group showed a 15–18% decrease in PPO activity on the 14th day compared to CK. Therefore, it can be concluded that ABP has some antioxidant effects reducing the PPO activities.

Microbial analysis. Button mushrooms are susceptible to yeast, mould (Koorapati et al. 2010) and the genus *Pseudomonas* (Hassanzadeh 2014) contaminations. Figure 4 depicts the antimicrobial effects of ABP/TEO coating against *Pseudomonas*, yeasts, and moulds. In the storage period, ABP/TEO groups inhibited the counts of *Pseudomonas*, yeast, and mould, with the increase of concentration. However, after 8 days, the effect was not obvious for *Pseudomonas*, which may be due to the rapid growth of *Pseudomonas* as the dominant bacteria and the volatilisation of essential oil. ABP had no obvious effects on microorganisms. In other studies, it was found that microorganisms belong to the factors affecting the weight loss and firmness of mushrooms, which proved the previ-

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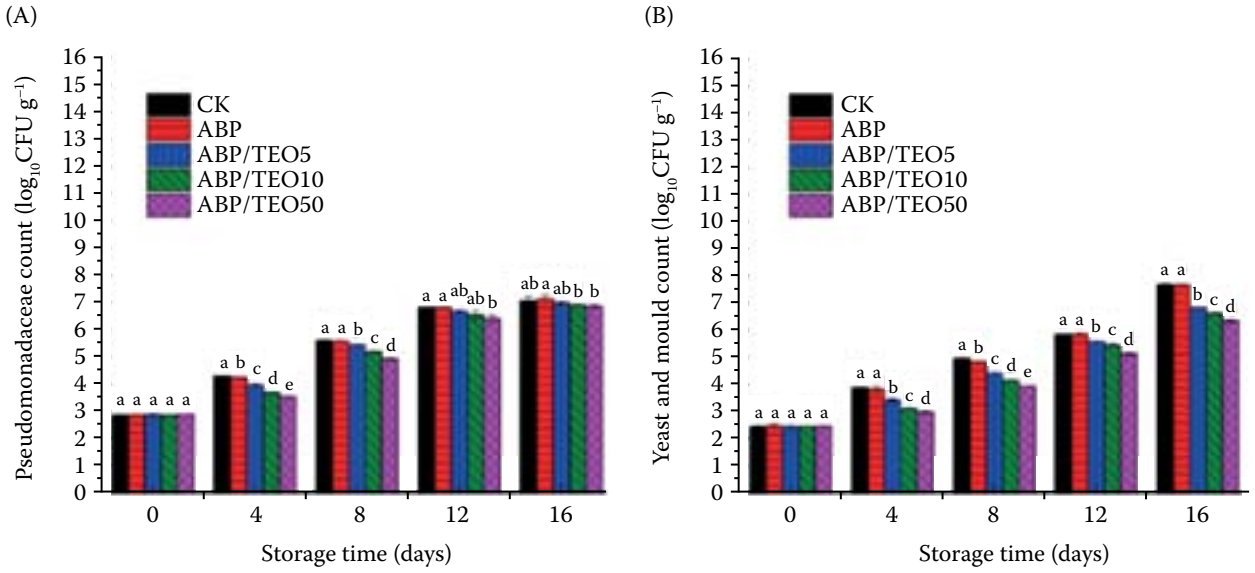

Figure 4. Effect of natural additives coating on the change of (A) genus Pseudomonadaceae count and (B) yeast/mould count ($\log_{10}$ CFU g^{-1}) of button mushroom stored at 4 °C for 16 days

a–e – Different letters on top of bars denote the significant difference at α = 0.05 among treatment means at same storage time ($P < 0.05$); CFU – colony forming unit; CK – control; ABP – *Agaricus bisporus* polysaccharides; ABP/TEO5 – 0.5% ABP + 1.0% sorbitol + 500 ppm *Torreya grandis* essential oils (TEO); ABP/TEO10 – 0.5% ABP + 1.0% sorbitol + 1 000 ppm TEO; ABP/TEO50 – 0.5% ABP + 1.0% sorbitol + 5 000 ppm TEO

ous experimental phenomenon. Various mechanisms have been suggested for the antibacterial activity of essential oils, such as attacking the phospholipid bilayer of the cell membrane or damage to lipids and proteins, disturbance of the proton motive force, and breaking the genetic material of bacteria (Dashipour et al. 2014). Therefore, the release mode and concentration of essential oil need to be optimised in the future.

Sensory evaluation. Sensory attributes of coated mushrooms in 16-day storage are shown in Figure 5. CK, ABP, and ABP/TEO5 performed best in the surface colour which showed no significant difference. Uncoated samples lost their mushroom special flavour when the mushrooms were cut in half. ABP/TEO10 and ABP/TEO50 performed inferior compared to ABP and ABP/TEO5. This may be due to the fact that the high concentration of essential oil masks the flavour of mushrooms. ABP and ABP/TEO5 retained the complete mushroom flavour. In terms of texture, the CK group became softer than others and was slightly sticky on the surface. ABP/TEO5 appeared better in flavour and inner mushroom tissue colour compared to other experimental treatments. Finally, it can also be seen from the inner and outer section of CK and ABP/TEO5 (Figure S2 in ESM; for ESM see the electronic version); ABP containing 500 ppm essential oils had no effects on the surface at the end of the 16-day storage period. In addition, the ABP/TEO5-treated mushrooms are brighter in colour and lighter in folds; they retained the original

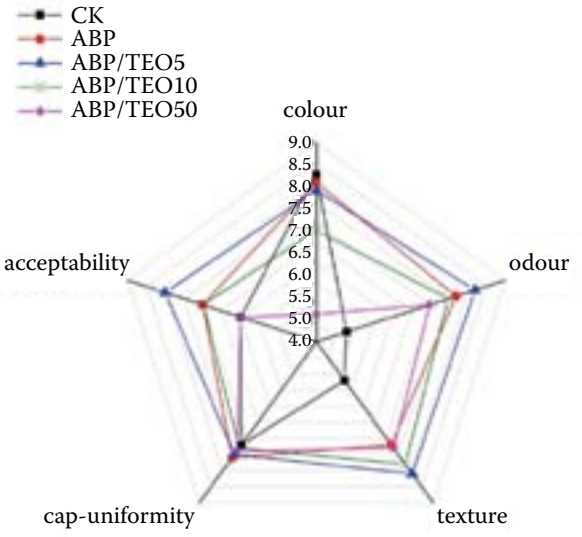

Figure 5. Effect of natural additives coating on sensory evaluation of button mushroom stored at 4 °C on the 16th day

CK – control; ABP – *Agaricus bisporus* polysaccharides; ABP/TEO5 – 0.5% ABP + 1.0% sorbitol + 500 ppm *Torreya grandis* essential oils (TEO); ABP/TEO10 – 0.5% ABP + + 1.0% sorbitol + 1 000 ppm TEO; ABP/TEO50 – 0.5% ABP + 1.0% sorbitol + 5 000 ppm TEO

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colour in the interior of the mushroom compared to CK. It was proved that the effects of film-forming ABP and anti-microbial TEO as potential materials could be applied in button mushroom preservation. But the film-forming needs to be improved by modification and the essential oils need to be optimised without affecting the unique flavour of mushrooms.

CONCLUSION

In the present study, ABP and TEO coating was shown as a green approach to extend the shelf life of mushrooms during storage, especially ABP/TEO5. It was obvious that ABP as a natural film over the mushroom reduces weight loss, inhibits PPO activity and maintains tissue firmness. TEO could inhibit dominant microorganisms. This type of coating (ABP/TEOs) could protect the mushrooms' inner colour and flavour while a certain high concentration of essential oils in the coatings would bring darker colour to the mushrooms, thus, would have a negative effect on flavour. Combined with the sensory evaluation, ABP and 500 ppm TEO combination might be ideal for the overall acceptance of the mushrooms during storage as a natural coating. The monitored parameters (without and with the addition of TEO/ABP) which most limit the shelf life were the amount of TEO/ABP addition, their critical values should be 0.5% ABP + 1.0% sorbitol + 500 ppm TEO coating. In such a condition, the application of the proposed ABP/TEO5 coating will extend the shelf life of button mushrooms by 16 days at 4 °C.

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