Laboratory Report

Effect of polylactic acid (PLA) packaging on the preservation of mushroom (*Agaricus bisporus*)

1 Experimental purpose

The study of the preservation effects of polylactic acid (PLA, Zhejiang Aike Fans New Material Technology Co., LTD) packaging on mushroom (*Agaricus bisporus*) stored at ambient temperature provided a convenient preservation method for mushroom (*Agaricus bisporus*) stored at ambient temperature.

2 Materials and methods

2.1 Materials

Mushrooms (*Agaricus bisporus*) were purchased from supermarket at Yinzhou District, Ningbo City on May 8, 2023. The mushrooms with consistent size were picked and the decayed, moldy and soft mushrooms were removed. The picked mushrooms were placed in plastic packages for the experiment.

2.2 Methods

The selected mushroom were packaged as Table 1 and stored at temperature of 20-25℃, and the physicochemical indices were regularly measured.

Table 1 Experimental groups

Number	Group	Manner of packing	Experimental volume
1	CK	storage without package	12/package×6 repeat
2	PE	Packed with PE	12/package×6 repeat
3	PLA	Packed with PLA	12/package×6 repeat

2.3 Quality indexes

(1) Rate of weight loss

The rate of weight loss was determined by the weighing method.

(2) Mold index (%) = number of molded fruits x degree of molding / total number of fruits / highest degree of molding x 100%.

(3) Gas concentration (O_2 / CO_2 content) measurements

Gas concentration of mushroom were determined using PBI Dansensor (Denmark).

(4) Total soluble solid (TSS) and titratable acid (TA) measurements

The TSS and TA of mushroom were measured by using PAL-BX / ACID F5 glucometer (ATAGO Co., Ltd, Japan).

(5) TPA measurements

Six of mushroom (*Agaricus bisporus*) samples was used for TPA measurements using a texture analyser (TA.XT Plus texture analyser, Stable Micro Systems Co., Ltd, UK). The device and test parameters were a P/5 cylindrical probe, a pre-test velocity of 1 mm/s, a test velocity of 2 mm/s, a after-test velocity of 1 mm/s, a compression distance of 5 mm, and a trigger force of 5 g. The texture variables, including hardness and springiness were obtained from the Texture Expert software. Each fruit sample was measured in triplicate.

(6) Measurement of relative conductivity

A sample of 1 g mushroom (*Agaricus bisporus*) weighted from each treatment and control group was diluted and homogenized with 10 ml distilled water using a ZLD-300 homogeniser (Zonce Machinery Co., Ltd, Shanghai, China) at 8000 r/min for 1 min. The homogenate was used to determine the conductivity by using an DDSJ-308A conductivity meter (Shanghai INESA scientific instrument Co., Ltd, Shanghai, China) befor and after stewing for 10 min respectively, which seted as P_1 and P_2.

Relative conductivity (%)= $(P_1-P_0)/(P_2-P_0)\times 100\%$

where P_0 was the conductivity of distilled water

(7) Colour difference analysis

The colour difference of mushroom (*Agaricus bisporus*) samples was evaluated by using a CI-60 colour difference meter (X-rite Pantone Co., Ltd, USA). The colour differences were analysed by L^*, a^*, and b^*, where L^* denotes lightness, a^* denotes from green to red, and b^* denotes blue to yellow. All experiments were tested in triplicate.

2.4 Statistical analysis

All measurements were performed in triplicate and the results were reported as means ± standard deviations (SDs). Data were analyzed by one-way analysis of variance (ANOVA) and Tukey's pairwise comparisons tests by using SPSS v.20 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at the level of $p < 0.05$. The figures were plotted with Origin v. 8.0 (OriginLab, Northampton, MA, USA) software.

3 Results and discussion

3.1 Rate of weight loss of mushroom (*Agaricus bisporus*) under different package

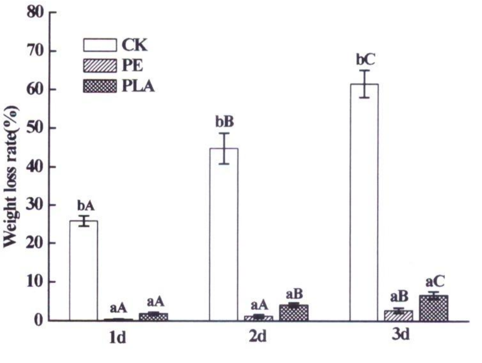

Figure 1 The effect of different package on the rate of weight loss of mushroom (*Agaricus bisporus*)

The rate of weight loss is the external expression of the loss of water, and it is an important indicator to measure fruit quality. Fresh mushrooms have sufficient moisture and are in a firm and plump state. Loss of weight will lead to it becoming dry and wilt, which significantly affect its commercial and economic value. As can be seen from Figure 1, the mushroom (CK) without packaged losed water quickly. After being stored at room temperature for 1 day, the rate of weight loss reached 25.8%, and after 2 days, it reached 44.8%, and after 3 days, it reached 61.6%. It can be seen that mushrooms were extremely susceptible to water loss, which could cause serious economic losses. The use of PLA and PE packages could significantly reduced the weight loss and delayed the dispersion of water. The water loss prevention effect of

PLA package was slightly lower than that of PE package, but the difference is not significant (*P>0.05*). Therefore, PLA package could significantly reduce the water loss and weight loss of mushrooms (*P<0.05*).

3.2 Moldy index of mushroom (*Agaricus bisporus*) under different package

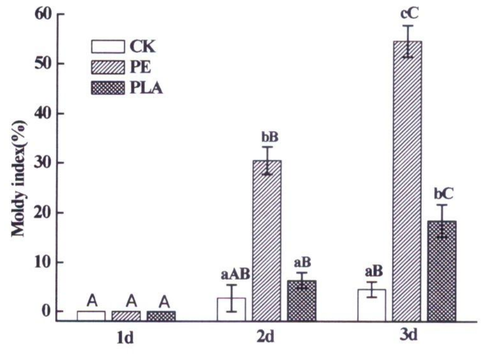

Figure 2 The effect of different package on the moldy index of mushroom

Moldy is one of the main appearance of deterioration during the storage of mushrooms. Once molded, it lost the commercial value. Moldy mainly starts from the root, and the soil-borne bacteria and wounds during harvesting provide a prerequisite for molding. The effect of different packages on the moldy index of mushrooms was shown in Figure 2. No molding occurred in all packaging methods after being stored for 1 day. The moldy index in the control group increased slightly after being stored for 2 and 3 days, but it was still lower than that in other treatments, which may be related to the rapid loss of water and reduced the relative humidity on the surface. The moldy index of mushrooms packaging by PE and PLA were significantly increased during storage, thereinto the index of PE package group was remarkably higher than that of PLA package group. Obvious condensation and increase of relative humidity occured inside of the PE package, which provided a good environment for microbial growth. This was an important factor leading to molding and deterioration of mushrooms. In compare with PE package, PLA package could significantly reduce the moldy index (*P<0.05*). Hence, preservation package could reduce the water loss but also increase the relative humidity, which promote the growth of mold. Therefore, the PLA package is more suitable for storing mushroom (*Agaricus bisporus*) because it provided suitable relative humidity for storage, no condensation in the package, and resulted in a smaller impact on the moldy.

3.3 Changes of gas concentration of mushroom (*Agaricus bisporus*) under different package

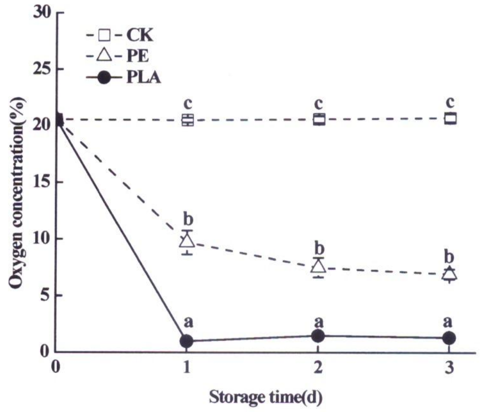

Figure 3 Effect of different packaging treatments on the oxygen concentration in the package

Suitable gas concentration can reduce the phenomenon of mold and decay, otherwise will affect the respiratory metabolism of the fruit, and produce the flavor of alcohol flavor under anaerobic respiration. The change of gas concentration in different packaging treatments was shown in Figure 3-4. Figure 3 showed the effect of different packaging treatments on the oxygen concentration in the package. For the control group, its gas concentration is the same to the air. For PLA and PE package, their oxygen concentration decreased rapidly and its rate of decrease for PLA package was faster. On the first day of storage, the oxygen concentration for PLA package was 1% and for PE package was 9.7%.

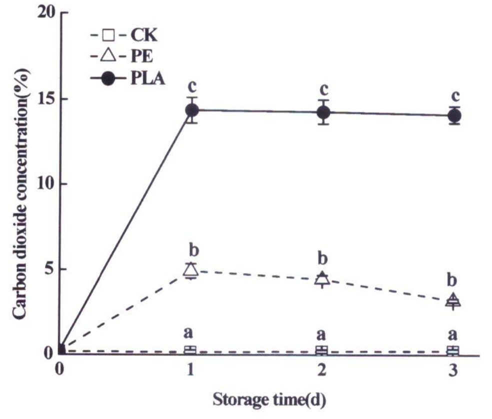

Figure 4 Effect of different packaging treatments on the carbon dioxide concentration in the package

Because the mushroom is live, it could consume O_2 and release CO_2 in the package. So the gas concentration changes in the PE and PLA package. The oxygen concentration descreases while the concentration of carbon dioxide increases in the package. Among the groups, the concentration of carbon dioxide in the PLA package increased faster. After being stored for 1 day, the carbon dioxide concentration of mushrooms in PLA package was 14.4%, while in PE package was 4.9%. Therefore, the PLA package could quickly increase the concentration of carbon dioxide, which has antibacterial effects. But it should pay an attention to the change of gas composition, and prevent the alcohol metabolism induced by anaerobic respiration, which resulting in the development of off-flavors.

3.4 Changes of TSS and TA of mushroom (*Agaricus bisporus*) under different package

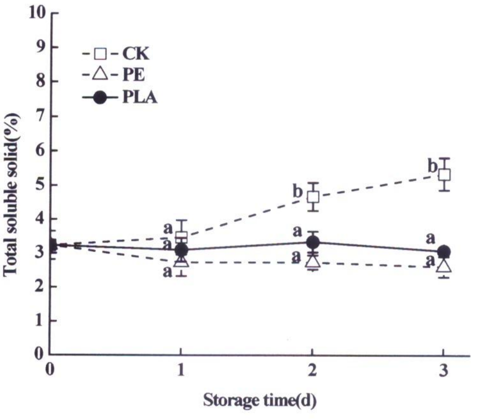

Figure 5 Effect of different packaging treatments on the total soluble solid of mushroom (*Agaricus bisporus*)

Total soluble solid (TSS) is an important evaluation index to measuring the sweetness of mushroom (*Agaricus bisporus*). As shown in Figure 5, there was significant difference in the effect of different packaging treatments on the total soluble solid of mushroom ($P>0.05$), mainly manifested by the gradual increase of TSS in the CK group. The rise of TSS may be related to the weight loss and water loss of mushroom. For the PLA and PE package groups, they have relatively little change in TSS due to less weight and water loss.

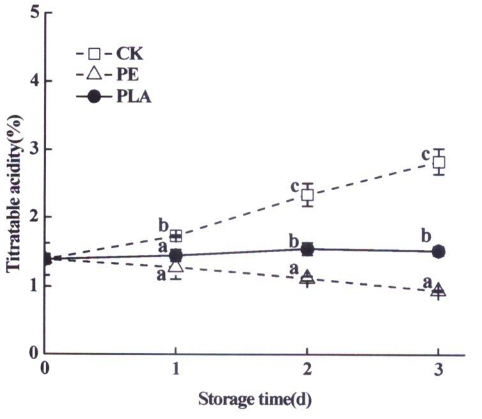

Figure 6 Effect of different packaging treatments on the titratable acid of mushroom (*Agaricus bisporus*)

Titratable acid (TA) is an important evaluation index to measuring the acidity of mushroom (*Agaricus bisporus*). As shown in Figure 6, the obvious increase in TA can be seen in the CK group, which was similar to the results of TSS. This phenomenon may be related to the loss of water. Both PLA package and PE package groups showed slower rise rate of TA compared with the CK group.

3.5 Changes of TPA of mushroom (*Agaricus bisporus*) under different package

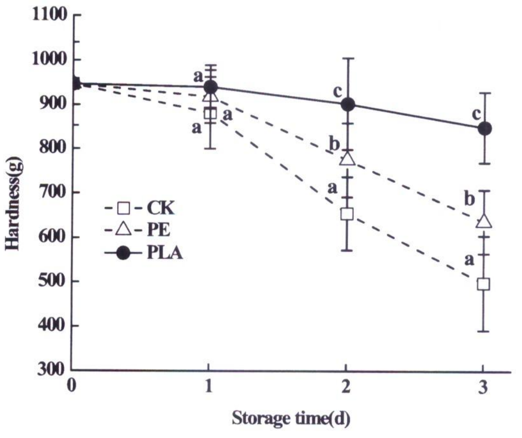

Figure 7 Effect of different packaging treatments on the hardness of mushroom (*Agaricus bisporus*)

TPA intuitively reflects the fruit edible quality, and hardness is one of the indexes of TPA. Figure 7 showed the change of the hardness of mushroom in different packages during storage. It can be seen that the hardness of button mushrooms under different treatments decreased during storage, thereinto the control group showed the fastest decrease rate. The hardness of fresh mushroom was 946.4 g, and it decreased to 881.3 g after being stored for 1 day, 654.8 g after being stored for 2 days, and 498.6 g after being stored for 3 days, which is almost a 50% decrease, indicating that the texture of mushroom was gradually softened. The mushroom packaged by PLA and PE packages were significantly delayed the decrease in hardness, and the delaying effect of PLA package was better than that of PE package ($P<0.05$). It was generally believed that the more the water lost, the more the wilting and shrinkage happened, and the faster the hardness decreased. However, based on the experiment, it showed that although PE package have the best control effect of water loss, they are not as good as PLA package in preventing texture softening. It was speculated that this may be related to the inherent texture characteristics of mushroom. High relative humidity may accelerated softening of mushroom.

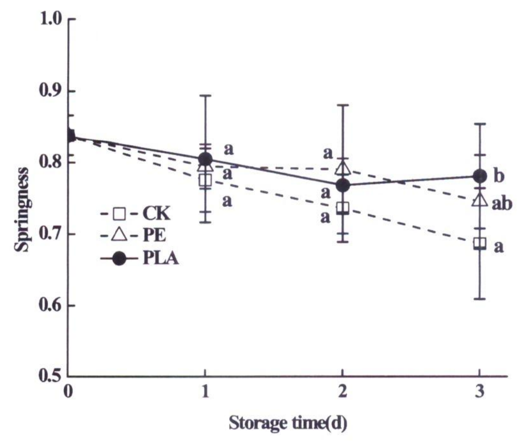

Figure 8 Effect of different packaging treatments on the springness of mushroom (*Agaricus bisporus*)

Springness is one of texture feature parameters, which demonstrated the ratio of the height that can be recovered between the end of the first bite and the beginning of the second bite. Figure 8 showed the effect of different packaging treatments on the springness of mushroom. Results showed that there is no significant difference among the groups during the first two days of storage($P>0.05$). But when stored for 3 days, the springness of mushroom in PLA package was higher than that without package, but it was no different from that in PE package. Although it also showed that PLA package has a certain role in maintaining the springness characteristics of mushroom, the change of springness is less than the structural difference of the hardness.

3.6 Changes of colour of mushroom (*Agaricus bisporus*) under different package

Table 2 Effect of different packaging treatments on the colour of mushroom (*Agaricus bisporus*)

	L^*	a^*	b^*	$\triangle E$
0 d	93.48 ± 1.03^{d}	-0.57 ± 0.14^{a}	10.77 ± 1.20^{a}	/
CK 1d	91.23 ± 0.90^{c}	0.53 ± 0.64^{b}	12.95 ± 1.00^{b}	3.326
PE 1d	93.73 ± 0.97^{d}	-0.43 ± 0.22^{a}	10.61 ± 0.98^{a}	0.331
PLA 1d	93.10 ± 0.82^{d}	-0.36 ± 0.28^{a}	12.94 ± 0.87^{b}	2.214
CK 2d	88.24 ± 1.38^{b}	2.06 ± 1.10^{c}	17.69 ± 1.15^{c}	9.071
PE 2d	91.25 ± 1.44^{c}	0.29 ± 0.42^{ab}	13.50 ± 1.05^{b}	3.631
PLA 2d	88.87 ± 1.54^{b}	1.71 ± 0.67^{c}	17.98 ± 0.97^{c}	8.859
CK 3d	86.44 ± 1.41^{a}	2.12 ± 0.64^{c}	18.45 ± 1.23^{c}	10.767
PE 3d	86.42 ± 1.50^{a}	2.01 ± 0.77^{c}	17.94 ± 0.75^{c}	10.393
PLA 3d	87.84 ± 1.58^{ab}	2.43 ± 0.84^{c}	19.03 ± 1.05^{c}	10.448

Color is an important evaluation index of fruit commodity. At present, Lab method is a commonly used method. This method uses the lightness $L*$ and chromatism coordinates $a*$ and $b*$ to represent the position of the color in the color space, where $L*$ indicates the lightness of the color. $a*$ positive value indicates red and negative value indicates green. Table 2 showed the effect of different packaging treatments on the color parameters of $L*$, $a*$ and $b*$ of mushroom. With the prolongation of storage time, $L*$ value of mushroom was decreased, $a*$ was increased, and $b*$ was increased. These indicated that the color of mushroom was gradually changed to darker, redder, and yellower, which was also described as a browning phenomenon. As can be seen from the color difference value ($\triangle E$), the color change of mushroom without package was largest after being stored for 1 day and 2 days, followed by PLA package group, and then PE package group. After being stored for 3 days, the color difference of all the groups were similar. The results showed that PLA package could delay the browning effect of mushroom to a certain extent.

3.7 Effect of different packaging treatments on the relative conductivity of mushroom (*Agaricus bisporus*)

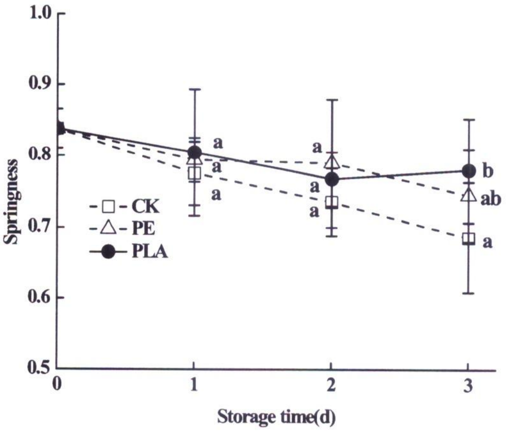

Figure 9 Effect of different packaging treatments on the relative conductivity of mushroom (*Agaricus bisporus*)

Relative conductivity is an important physiological and biochemical index to reflect the condition of plant membrane system. In aging or adversity, the cell membrane is easy to rupture and membrane proteins are damaged, which lead to the infiltration of the cytosol, so that the relative conductivity increase. Figure 9 showed the changes of relative conductivity of mushroom in different package. As can be seen from the figure, the relative conductivity of mushroom with different treatments increased during the storage period, thereinto the rising rate of CK group was fastest. Both PLA and PE package could delayed the increase in relative conductivity of mushroom, with PLA package showing slightly weaker effect than PE package. It indicated that PLA package has a similar effect to PE package in avoiding the cell membrane damage, and has a good regulation effect on the inhibition of mushroom aging.

4 Conclusion

(1) Through these experiments, it was found that the main storage problems of mushroom were the water loss, shrinkage, softening, and mold growth.

(2) Although the PE package could effectively inhibits the wilting and drying caused by water loss, it still existed the phenomenon of soften, condensation and mildew of mushroom.

(3) For the PLA package from Zhejiang Aike Fans New Material Technology Co., LTD, results showed that PLA package is better than PE package in the aspects of preventing the knot dew and water loss. Also it could effectively reduced the mildew rate of mushroom, delayed the decrease of the hardness, inhibited the aging process, and extended the shelf life. The commodity of mushroom was still good when stored for three days.

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2023-5-28

Attached: experimental pictures of the preservation effect of mushroom (*Agaricus bisporus*)

Overall figure of mushroom stored for 1 day

Overall figure of mushroom stored for 2 days

Overall figure of mushroom stored for 3 days

Figure of root of the mushroom stored for 2 days

Figure of root of the mushroom stored for 3 days

Figure of the condensation of the mushroom in the PE package

Figure of the condensation of the mushroom in the PE package

Figure of the condensation of the mushroom in the PLA package