Effect of precooling temperature on physiological quality of cold stored *Agaricus bisporus*

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Abstract: Effect of precooling temperatures (2℃, 6℃, 10℃ and 14℃) on the physiological quality of postharvest *Agaricus bisporus* during cold storage was investigated. After six hours' precooling, *Agaricus bisporus* was stored at 3℃ and sampled on day 3, 6 and 9, respectively, for physiological quality analysis. Results showed that physiological quality of the *Agaricus bisporus* increased with the decrease of precooling temperature in the range of 2-14℃. Precooling at 2℃ before cold storage had a positive impact on the storage quality of *Agaricus bisporus.* The decrease of hardness, whiteness and pH value was delayed, while the increase of cell membrane permeability and PPO and POD activities was restrained. Whiteness value of the *Agaricus bisporus* precooled at 2℃ was above 80 on day 9, which means it was still acceptable, but the *Agaricus bisporus* precooled at 6℃ and 10℃ lost their commercial values.

Keywords: precooling, *Agaricus bisporus*, cold storage, precooling temperature, quality

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1 Introduction

Agaricus bisporus is the most widely cultivated species of edible mushrooms due to the delicate texture and abundant nutrients such as high quality proteins, amino acids, carbohydrates, vitamins and minerals[1]. However, fresh *Agaricus bisporus* is highly perishable and has a short shelf life of 3-5 days at 2℃ and around 1-2 days under ambient conditions (Temp. 25℃, RH70%) due to the active postharvest ripening process, which caused great economic losses[2]. Temperature, humidity, gas composition of the storage environment and the preservative treatment are the four key factors that affect storage and preservation quality of fruits and vegetables[3], among which temperature is the most important factor[4-8]. Shi Q L et al. studied the effect of storage temperature on postharvest physiological characteristics of *Agaricus bisporus*[4]. The results showed that temperature is a key factor for the postharvest ripening and quality change. Low temperature could slow down the quality deterioration and the aging process of *Agaricus bisporus*.

Precooling can quickly remove field heat[9-10], lower respiration and delay the after-ripening of the postharvest fruits and vegetables, which is beneficial to their freshness and the activity of nutrients. Precooling to the postharvest products before cold storage can also improve low temperature tolerance, reduce or postpone chilling injury occurrence, and decrease the cooling load of cold storage. During cold-chain transportation and logistics, fruits and vegetables without precooling suffer a 25%-30% loss[11,12], while the loss rate of precooled fruits and vegetables is only 5%-10%[13]. Commonly used precooling methods include pressure ventilation precooling[14,15], convection ventilation precooling[16-18] and vacuum precooling[19]. Cold air pre-cooling is a type of ventilation precooling methods. Because of its simple operation, economical investment and operating costs, it is currently a relatively practical and effective pre-cooling

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method.

The objective of this study was to investigate the impacts of different precooling temperature (2℃, 6℃, 10℃, and 14℃) on the quality of *Agaricus bisporus* during cold storage. The variations of whiteness, hardness, cell membrane permeability, and the activities of PPO and POD of the precooled *Agaricus Bisporus* were analyzed during cold storage at 3℃.

2 Materials and methods

2.1 Materials

Agaricus bisporus was collected from Nanshubei Village, Zibo City, China. The criteria of sampling included white color, whole shape, free mechanical injury, no open veil, free of insect damage and similar fruiting body size. *Agaricus bisporus* was shipped to a test-use refrigerator in Shandong University of Technology in 1 hour after harvest. The initial moisture content and temperature of the sample was 90% and 16°C, respectively. Five kilograms of samples were put in a plastic box (492 mm ×324 mm ×284 mm) and placed at a fixed position in the refrigerator. An eight-channel multi-point thermometer device (purchased from Nanjing Maijie Science and Technology Limited, China) was selected to measure the temperature.

2.2 Methods

Agaricus bisporus was placed in refrigerators with different temperatures (2℃, 6℃, 10 ℃, and 14 ℃) for precooling. To ensure a thorough precooling, a preliminary test was carried out to investigate the time needed to achieve certain precooling temperature (as shown in Figure 1). The result showed that it took about 5 to 6 hours to cool down the sample to 2℃. Therefore, it could be predicted that the whole amount of mushroom used in the test could be fully precooled within 6 hours.

The results of Shi Qilong et al. showed that the suitable storage temperature for *Agaricus bisporus* was 3℃[4]. Therefore, *Agaricus bisporus* pre-cooled for 6 hours was placed into refrigerator of 3℃ and sampled on day 3, 6 and 9, respectively, for quality indicator analysis. Each indicator was measured three times in parallel on randomly selected five pieces of mushroom at each time point to get average value.

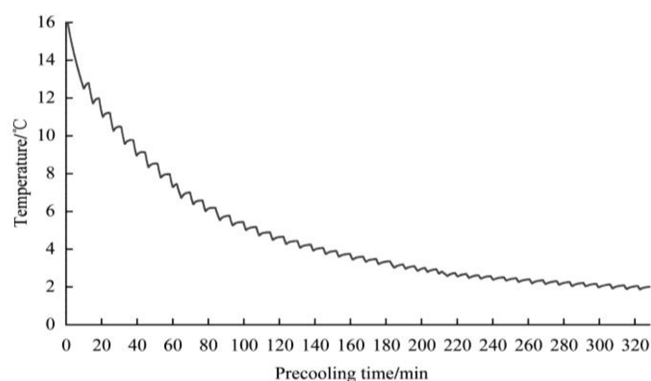

Figure 1 Variation curve of temperature and time precooling of *Agaricus bisporus* in refrigerator set at 2℃

2.3 Indicators and determination methods

Hardness was measured by using a GY-1 fruit hardness meter (Beijing Time Ricon Technology Co., Ltd, Beijing, China). Detecting probe of the hardness meter was perpendicularly poked into the pulp of peeled mushroom with the uniform force by 5 mm. The pointer reading at this moment was recorded as the hardness.

SC-80C Automatic Color Meter (Beijing Kangguang Instrument Co. Ltd., Beijing, China) was employed to measure whiteness. Whiteness of mushroom surface and sliced flesh, expressed by L value, was determined upon the standard ceramic plate (X = 81.75, Y = 86.40, Z = 90.89) L = 0 stood for pure black, and L = 100 stood for pure white. The higher the L value represents the higher the whiteness and the less browning.

Five grams of mushroom was grounded and the juice was acquired by squeezing with three layers of gauze wrapping around. The pH value of the juice measured with a pH meter was defined as the pH value of the samples.

Peroxidase (POD) activity was measured by the guaiacol method[20]. Activity of polyphenol oxidase (PPO) was determined using the method created by Shi Qilong et al.[4]. Membrane permeability was expressed by conductivity[21]. All the indicators were measured in triple repetition.

2.4 Statistical analysis

Use Excel 2003 for data analysis and figure creation.

3 Results and discussion

3.1 Effect of precooling temperature on hardness

Fresh *Agaricus bisporus* has high moisture content

and its surface has no obvious conservation tissue. Water loss easily occurs through transpiration after harvest, causing tissue wilting and hardness decrease. However, the decline of mushroom hardness helps prolong storage time. Figure 2 showed the hardness variations of the *Agaricus bisporus* precooled at different temperatures. Table 1 indicates that both pre-cooling temperature and storage time are important factors that affected hardness. Figure 2 showed that with the extension of storage time, hardness of all the samples precooled with test temperatures declined, but at different degrees. The hardness declining of samples at 2℃, 6℃, and 10℃ in the first six days was mild and slow, but after that the curve tended to go sharply downward. The results showed that the lower the precooling temperature, the slower the decrease in hardness. From day 0 to day 9, the hardness of the sample precooled at 2℃ decreased by 16.27%, from 11.98×10^5 Pa to 10.03×10^5 Pa, while those of the samples precooled at 6℃, 10℃ and 14℃decreased by 45.07%, 28.05%, and 49.08%, respectively. The results of Tao Fei et al.[22-23] and Zhu Jiying et al.[24] indicated that during the early stage after harvest *Agaricus bisporus* has sufficient water and pectin, closely-structured cell wall, and therefore has firm and hard mushroom body. With the extension of storage time, the pectin degraded to soluble pectin under the catalysis of pectinase. Pectin dissolves in the cytosol, which leads to the decrease of mushroom hardness. In this sense, precooling at low temperature (2℃) can inhibit respiration and reduce pectinase activity of the postharvest *Agaricus bisporus*, which in turn inhibit pectin metabolism and help to keep the mushroom hardness during storage.

3.2 Effect of precooling temperature on whiteness

The curve in Figure 3 showed the whiteness changes of mushroom flesh and peel at different pre-cooling temperature over storage time. Table 2 and Table 3 were double-factor ANOVA results of flesh whiteness and peel whiteness, respectively, during storage. As could be seen from Table 2, storage time significantly affected the whiteness of *Agaricus bisporus*. While the *p* value of pre-cooling temperature was greater than the significant level of 0.05, indicating the effect of pre-cooling temperature on hardness was not significant. Therefore, a single-factor ANOVA was conducted on the whiteness of each sample treatment on Day 3, 6, and 9. The *p* value of flesh whiteness in Table 3 indicated that the pre-cooling temperature was a significant factor for the peel whiteness.

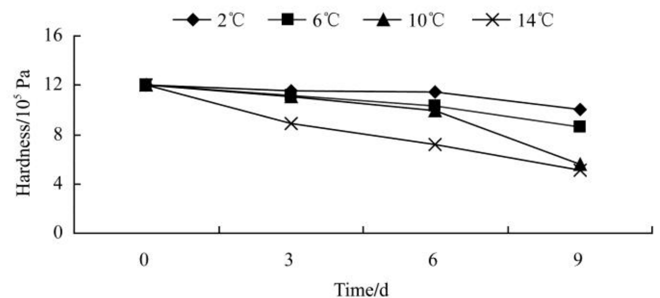

Figure 2 Effect of precooling temperature on hardness

Table 1 ANVOA analysis of precooling temperature affecting hardness

Factor	Degree of freedom	Mean Square	*F*	*p*
Precooling temperature	3	6.635	5.293	0.040
Storage time	2	10.132	8.083	0.019
Random factor	6	1.253		

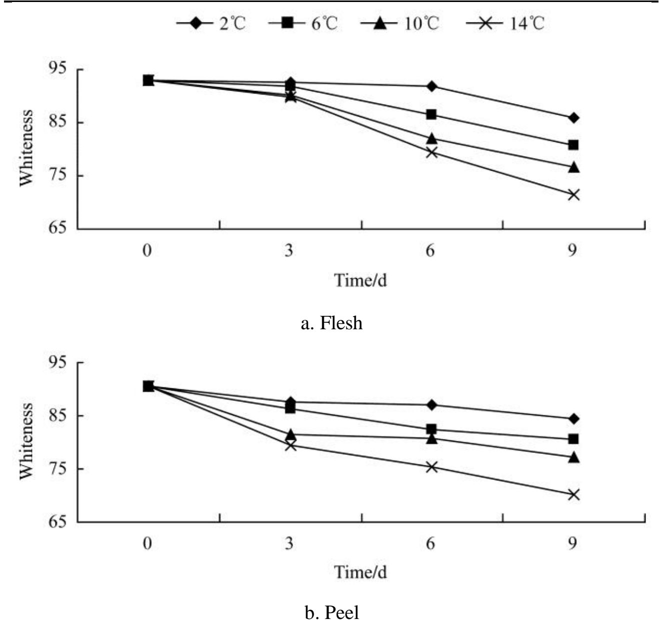

Figure 3 Effect of precooling temperature on *Agaricus bisporus* whiteness

Table 2 ANVOA of precooling temperature affecting *Agaricus bisporus* flesh whiteness

Factor	Degree of freedom	Mean Square	*F*	*p*
Precooling temperature	3	11.753	0.568	0.656
Storage time	2	175.215	8.472	0.018
Random factor	6	20.681		

Table 3 ANVOA of precooling temperature on *Agaricus bisporus* peel whiteness

Factor	Degree of freedom	Mean square	*F*	*p*
Precooling temperature	3	72.623	9.652	0.010
Storage time	2	14.191	1.886	0.231
Random factor	6	7.523		

As seen from Figure 3, whiteness of all the samples precooled at different temperatures declined with the extension of storage time. Browning degree increased with the increase of the precooling temperature. Gormley divided *Agaricus bisporus* into two quality levels: L≥86 is of good quality and 85≥L≥80 is of acceptable quality. According to Figure 3a, during the first 6 days the whiteness changed slowly. On Day 6, the whiteness values of *Agaricus bisporus* at 2℃, 6℃, 10℃, and 14℃ were 91, 84.71, 81.95 and 79.41, respectively, indicating that the sample pre-cooled at 2℃ was of good quality, those precooled at 6℃ and 10℃ were of acceptable quality, while the sample precooled at 14℃ was unacceptable. By day 9, the whiteness values of all treatments had dropped significantly. Only the sample pre-cooled at 2℃ was of acceptable quality (83.4) and all others lost commercial values.

Before storage, the flesh whiteness of each treatment was higher than that of the peel. As seen in Figure 3b, during the whole storage period, the mushroom peel had higher browning rate than the flesh. This might be due to the different tissue structure and enzyme activities. The browning degree was mainly associated with the metabolism and relevant enzyme activities. The mushroom peel was directly in contact with the storage environment. Browning was related to the environmental temperature and humidity. During the precooling process the influence of temperature on peel was greater than on flesh.

3.3 Effect of precooling temperature on pH

The optimal pH value range of the mycelium of *Agaricus bisporus* in the growth process is between 6.8 and 7.0. Due to the occurrence and growth of certain fungi, the mushroom body generates acid substances during post- harvesting storage, which lowers the pH and not conductive to long-term storage. Figure 4 showed the pH value changes over storage time of the *Agaricus bisporus* precooled at different temperatures. Table 4 was the ANOVA results of precooling effect on pH value. As seen from Table 4, the p values of both the precooling temperature and storage time were less than 0.05, indicating both factors had significant effect on pH value.

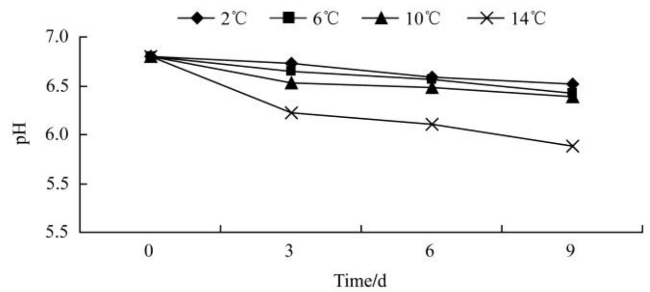

Figure 4 Effect of precooling temperature on *Agaricus bisporus* pH value

Table 4 ANVOA of precooling temperature on *Agaricus bisporus* pH value

Factor	Degree of freedom	Mean square	*F*	*p*
Precooling temperature	3	0.178	78.929	0
Storage time	2	0.053	23.689	0.001
Random factor	6	0.002		

From Figure 4, it could be seen that the pH value in the sampled *Agaricus bisporus* decreased over storage time. The pH value in the *Agaricus bisporus* precooled at 2℃ decreased more slowly compared to those precooled at other temperatures. The higher the precooling temperature was, the faster the pH decreased. The pH of mushroom precooled at 14℃ declined below 6.0 after 9 days of storage. Meanwhile, the skin of the mushroom appeared wilting and losing edibility. Therefore precooling at low temperature could effectively slow down pH decrease.

3.4 Effect of precooling temperature on cell membrane permeability

During the storage of *Agaricus bisporus*, the balance of free radical metabolism in cells is damaged. The radicals can result in or aggravate membrane lipids peroxidation, which results in the cell membrane fluidity reduction and membrane protein denaturation, and in turn causes damage to the cell membrane system and structure[25]. In this sense, the cell membrane permeability reflects the degree of tissue aging or

adversity injury. Figure 5 was the changing curve of cell membrane permeability of the *Agaricus bisporus* during storage period. Table 5 showed the ANOVA result of cell membrane permeability at different precooling temperatures. As seen in Table 5, the *p* values of precooling temperature and storage time were less than 0.05, indicating that both of the factors had significant effect on cell membrane permeability. Figure 5 showed that the permeability increased with the extension of storage time. On day 3, all the permeability values of the *Agaricus bisporus* precooled at 6℃, 10℃ and 14℃ were around 46%. On day 6 and day 9, the cell membrane permeability increased with the rise of precooling temperatures. The cell membrane permeability precooled of the *Agaricus bisporus* precooled at 2℃ was significantly lower than that of the others within the testing period. Therefore, precooling at 2℃ could inhibit the increase of permeability. This might be because the low precooling temperature at 2℃ could quickly remove field heat, reduce respiratory level, slow down metabolism, and in turn lower the oxidation of membrane lipid. To the opposite, cell membrane of the samples precooled at higher temperatures degraded fast, which increased cell permeability and aggravated deterioration of mushroom quality.

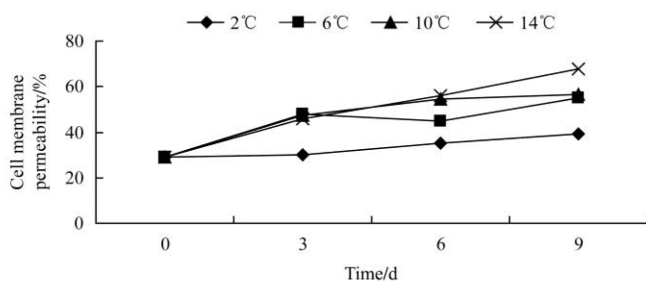

Figure 5 Effect of precooling temperature on *Agaricus bisporus*' cell membrane permeability

Table 5 ANVOA of precooling temperature on *Agaricus bisporus'* relative electrical conductivity

Factor	Degree of freedom	Mean square	*F*	*p*
Precooling temperature	3	0.021	15.501	0.003
Storage time	2	0.019	14.236	0.005
Random factor	6	0.001		

3.5 Effect of precooling temperature on POD activity

Peroxidase (POD) widely exists in plants as a rather active enzyme. It is associated with respiration, photosynthesis and the oxidation of auxin. In the process of plant growth and development its activity keeps changing, generally higher in aging tissues and lower in young tissues. This is because POD can transfer some carbohydrates in the tissues into lignin, promoting lignifications. Therefore the activity of POD can be viewed as a physiological indicator of tissue aging. Figure 6 was the POD activity changing curve of the *Agaricus bisporus* precooled at different temperatures. Table 6 was the ANOVA result of the impact of precooling temperature on POD activity. As seen from Table 6, storage time significantly affected the activity of POD, while the precooling temperature was not a significant factor. The results on day 3 and day 6 indicated that precooling temperature had significant impact on POD activity. As seen from Figure 6, the POD activity of mushroom precooled at 2℃ maintained at a low level during the whole testing period. Mushroom precooled at other temperatures POD activity tended to experience a sharp increase from day 6, which indicated tissue aging. By day 9, POD activities of all treatments quickly decreased, probably because all the tissue had aged and POD lost activity. Therefore precooling at 2℃ could significantly inhibit POD activity and prolong shelf life.

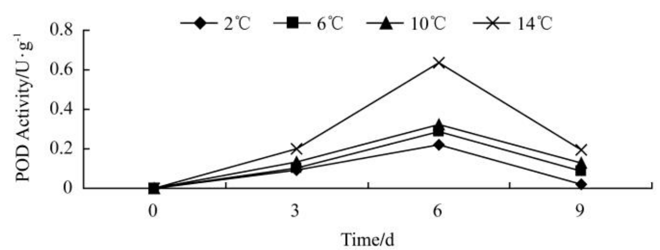

Figure 6 Effect of precooling temperature on *Agaricus bisporus'* POD activity

Table 6 ANVOA of precooling temperature on *Agaricus bisporus* POD activivity

Factor	Degree of freedom	Mean square	*F*	*p*
Precooling temperature	3	0.101	2.011	0.214
Storage time	2	0.451	8.986	0.016
Random factor	6	0.050		

3.6 Effect of precooling temperature on PPO activity

Figure 7 was the changing curve of PPO activity over

storage time. Table 7 was the ANOVA result of the effect of precooling temperature on *Agaricus bisporus*. As seen from Table 7, storage time was a significant factor for PPO activity, while the effect of precooling temperature on it was not significant (p=0.569>0.05). After running ANOVA on PPO activity on day 3, 6, and 9, the p values indicated that the PPO activity of the samples precooled at different temperatures had significant differences. As could be seen from Figure 7, the PPO activity of mushroom precooled at 6℃, 10℃, and 14℃ kept increasing until day 6, and then decreasing on day 9. PPO activity of the *Agaricus bisporus* precooled at 2℃ kept lower during the 9 days cool-storage. Therefore, precooling at 2℃ could significantly inhibit the PPO activity of *Agaricus bisporus*, and in turn reduce the rate and degree of enzymatic browning during storage.

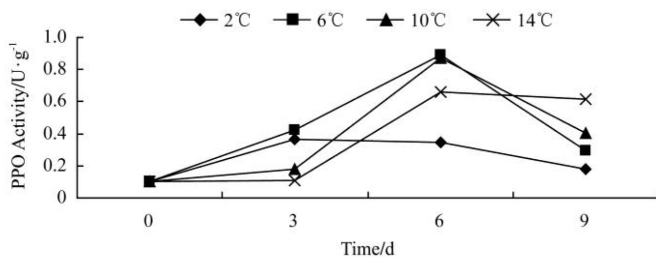

Figure 7 Effect of precooling temperature on *Agaricus bisporus* PPO activity

Table 7 ANVOA of precooling temperature on *Agaricus bisporus* PPO activity

Factor	Degree of freedom	Mean square	*F*	*p*
Precooling temperature	3	0.032	0.732	0.569
Storage time	2	0.196	4.435	0.046
Random factor	6	0.044		

4 Conclusions

Suffering the influence of field heat, respiration and transpiration, the quality of *Agaricus bisporus* is susceptible to decline and aging during postharvest storage due to its vulnerable tissue and lack of effective protective structure at surface. The hardness, whiteness and pH value decrease rapidly. The PPO and POD activities quickly increase to the highest value. The membrane permeability keeps rising as the storage time increases. A number of operating parameters can influence the precooling effect[26-32]. Precooling temperature is one of the most influential factors. The results showed that within tested temperature range, low temperature precooling was helpful to keep the storage quality of *Agaricus bisporus*. Precooled at 2℃ before refrigeration, the drop of physiological characteristics and quality deterioration of *Agaricus bisporus* was postponed, which meant that precooling at 2℃ could rapidly reduce the effect of respiration and transpiration on the storage quality of Agaricus bisporus, lower the activities of enzymes including POD and PPO, and inhibit the change of pH value, whiteness, and hardness.

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