Postharvest biochemical characteristics and ultrastructure of *Coprinus comatus*

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Abstract

Background. *Coprinus comatus* is a novel cultivated edible fungus, hailed as a new preeminent breed of mushroom. However, *C. comatus* is difficult to keep fresh at room temperature after harvest due to high respiration, browning, self-dissolve and lack of physical protection.

Methods. In order to extend the shelf life of *C. comatus* and reduce its loss in storage, changes in quality, biochemical content, cell wall metabolism and ultrastructure of *C. comatus* (*C.c*77) under 4°C and 90% RH storage regimes were investigated in this study.

Results. The results showed that: (1) After 10 days of storage, mushrooms appeared acutely browning, cap opening and flowing black juice, rendering the mushrooms commercially unacceptable. (2) The activity of SOD, CAT, POD gradually increased, peaked at the day 10, up to 31.62 U·g^{-1}·FW、16.51 U·g^{-1}·FW、0.33 U·g^{-1} FW, respectively. High SOD, CAT, POD activity could be beneficial in protecting cells from ROS-induced injuries, alleviating lipid peroxidation and stabilizing membrane integrity. (3) The activities of chitinase and β-1,3-glucanase were significantly increased. Higher degrees of cell wall degradation observed during storage might be due to those enzymes' high activities. (4) The fresh *C. comatus* had dense tissue and every single cell had the number of intracellular organelles which structure can be observed clearly. After 10d storage, the number of intracellular organelles was declined and the structure was fuzzy, the nucleus disappeared. After 20d storage, *C. comatus*'s organization was completely lost, many cells were stacked together and the cell wall was badly damaged.

Introduction

40
41 *Coprinus comatus*, also called as shaggy ink cap, is a novel cultivated edible fungus with
42 significantly commercial potential, hailed as a new preeminent breed of mushroom (Bo *et al.*,
43 2010). Moreover, it is also a delicious and highly nutritious edible fungus, with numerous
44 valuable medicinal compounds (Fan *et al.*, 2006), including various bioactive functions, such as
45 hypoglycemic, hypolipidemic, antibacterial effects (Bailey *et al.*, 1985), immunomodulation,
46 antitumor (Jiang *et al.*, 2013) and antioxidant potential (Tsai & Mau, 2009). However, *C.*
47 *comatus* is difficult to keep fresh at room temperature after harvest due to high respiration,
48 browning, self-dissolve and lack of physical protection (Rui & Feng, 2006). The extremely
49 active metabolic process and rapid deterioration of quality have seriously affected the
50 commodity quality and shelf life of *C. comatus*, caused great losses to production and storage.
51   Under normal growing conditions, the production and clearance of intracellular free radicals
52 and reactive oxygen species (ROS) are in a dynamic balance, however, this balance is destroyed
53 with prolonged storage because the storage conditions are a sort of abiotic stress (Huang *et al.*,
54 2007; Gill & Tuteja, 2010). Followed by high generation capacity of ROS and low enzymatic
55 antioxidant defense. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were
56 important parts of enzymatic antioxidant defense system, which can efficiently eliminate ROS
57 (Duan *et al.*, 2011). SOD is one of the key enzymes for scavenging free radicals in mushroom,
58 which can transform superoxide anion radicals ($O_2^{\bullet-}$) to H_2O_2 and non-toxic molecular oxygen
59 by catalyzing the disproportionation reaction. The CAT and POD could transform H_2O_2 into H_2O
60 and O_2 through different actions, all of which are crucial for ROS detoxification (Prasad,
61 Rosangkima & Kharbangar, 2009).
62   During postharvest storage of edible fungi, changes in cell wall structure and composition
63 directly contribute to cell separation, resulting in loose organizational structure and decreased
64 hardness of fruiting body, finally, decreased storability of edible fungi (Zivanovic, Buescher &
65 Kim, 2003; Qi *et al.*, 2015). Studies have addressed that the content of chitin decreased during
66 postharvest storage due to the effect of chitinase by hydrolyzing the β-1, 4-glycoside bond in
67 chitin and producing n-acetylglucosamine oligomer or monomer (Buitimea *et al.*, 2013; Jiang *et*
68 *al.*, 2010). Lim & Choi (2009) revealed that the *Agaricus bisporus* could rapidly produce black
69 juice at 25°C for 15h after harvest and confirmed that the amount of chitinase synthesis in the
70 autolysis process was significantly higher than that of unpicked mushrooms. Xue (2012) studied
71 the effect of postharvest calcium treatment on cell stability of *Lentinula edodes* and found that
72 the stability of cell wall was correlated with β-1, 3-glucan and β-1, 3-glucanase, by inhibiting the
73 activity of β-1, 3-glucanase, the aging of *L. edodes* could be delayed. Liu *et al.* (2015) suggests
74 that β-1,3-glucanases plays a major role in the autolysis of *Coprinopsis* fruiting bodies.
75 However, the accurate role of these enzymes in altering the cell wall of *C. comatus* after harvest
76 were little known.
77   The degradation of cell wall substances and membrane lipid peroxidation led to changes in the
78 ultrastructure of cells. Ultrastructure is also called submicroscopic structure. Electron microscope
79 can well show the changes of tissue structure, cell structure, organelle function and

80   differentiation (Hu *et al*., 2015). By observing the ultrastructure of *C. comatus*, we can better
81   understand the degradation mechanism of *C. comatus* after harvest. However, there was no
82   report on the ultrastructure of *C. comatus*.
83   Therefore, it is necessary to conduct in-depth and systematic research on the physiological and
84   biochemical changes of *C. comatus* after harvesting and try to find influencing factors and
85   possible degradation mechanism of texture deterioration. The objective of this work was to
86   investigate sensory characteristics, chemical properties, functional components, cell wall
87   metabolic enzymes activity and ultrastructure of *C. comatus* during postharvest storage.
88

Materials & Methods

Materials

91   *C. comatus* used in this study were harvested from an edible fungus planting base of Chengdu
92   normal university in Sichuan, China (no. *C.c*77). *C. comatus* were transported to the laboratory
93   in one hour after picking. Then, they (60±5g) were screened for uniform size and maturity and
94   absence of mechanical damage, packaged in low density polyethylene sealing bags (0.04mm
95   thickness, size 18cm×20cm). After that, samples were stored at 4°C and 90% relative humidity
96   (RH) for 20d. Subsequently every 5 days, three replicates were randomly selected and analyzed
97   for each biochemical characteristic.

Browning analysis

99   We used a NR110 (Shenzhen 3nh technology co. LTD) type color difference meter (L, a, b) to
100 determine the chromatism of *C. comatus*, and evaluated ΔE ,$\Delta E =$
101 $\left[(L - 97)^2 + (a - (-2))^2 + b^2\right]^{1/2}$. L represents luminosity, which corresponds to brightness, a
102 represents the range from magenta to green, b represents the range from yellow to blue. ΔE
103 represents the overall color difference compared with the ideal or target color of the mushroom
104 (L=97, a=-2, b=0). The larger the L, the smaller the ΔE , indicating that the color of *C. comatus*
105 is white and the degree of browning is lower.

Weight loss analysis

107 According to the method of Jiang, Feng & Li (2012). It was expressed as the percentage of loss
108 of weight with respect to the initial weight.

Analysis of membrane permeability

110 According to the method of Liu *et al*. (2010). Randomly selected 3-4 *C. comatus*, used a 5mm
111 diameter puncher to punch holes in different parts, mixed and sampled 2g in 50ml beaker,
112 washed with no ion water several times, then add 30mL without ionized water to soak 1h,
113 conductivity of the surrounding solution was determined with a conductivity meter. The tissue
114 was then boiled for 10min and the total conductivity was recorded. Electrolyte leakage was
115 expressed as a percentage of total electrolytes in the tissue.

Analysis of chemical properties

117 Soluble protein was determined according to the method of Bradford (1976) using bovine serum
118 albumin as standard. Malondialdehyde (MDA) content was determined according to the method
119 of Jayakumar (Jayakumar, Ramesh & Geraldine, 2006). 2g of the fruit body of *C. comatus* was

120   ground in 5mL 5%TCA using a mortar and pestle, and quickly cooled in an ice bath and
121   centrifuged at 8,000r/min for 10min. Then 2mL supernatant was ground in 2mL 0.67%TBA,
122   after heating at 100°C for 30min, the mixture was centrifuged again. The absorbance of the
123   supernatant was read at 450nm, 532nm and 600nm respectively. Concentration of MDA:
124   C(μmol/L) =6.45(A532-A600)-0.56 A450.
125    $O_2^{\bullet-}$ was determined according to the methods of Jiang $et\ al.$ (2010). Weighed 2g of the
126   mushroom, grinded with 6mL of 65mmol/L (pH7.8) PBS, centrifuged the filtrate at 7000 r/min
127   for 5min at 4°C, and took the supernatant. Determination of $O_2^{\bullet-}$content: 1mL of the supernatant
128   was taken, and 0.9mL (pH7.8) of PBS, 0.1mL 10mmol/L of hydroxylamine hydrochloride
129   (distilled water instead of the sample supernatant was used as a blank) was added. After mixing,
130   it was incubated at 25°C for 20min. Took 0.5mL of the above culture solution, added 0.5mL
131   17mmol/L p-aminobenzenesulfonic acid, 0.5mL 7mmol/L a-naphthylamine, and kept the
132   reaction in a constant temperature water bath at 25°C for 20min, added 1.5mL of n-butanol, after
133   homogenization, the n-butanol phase was taken to determine the OD value at 530nm. Using
134    $NaNO_2$ as the standard, according to the measured OD530, check the NO_2^- standard curve,
135   convert OD530 into $[NO_2^-]$, and $[NO_2^-] \times 2$ to obtain $[O_2^{\bullet-}]$ content.
136   **Analysis of functional components**
137   Total phenolics: mushroom tissues (2g) were homogenized with 80mL water, boiled at 100°C
138   for 30min, settled to permit after cooling, then filtered. Color was developed by mixing 1ml
139   filtrate, 5mL water, 1mL Folin-Ciocalteu (Sigma–Aldrich Chemical Co., St. Louis, Mo, USA)
140   and 3ml 7.5% sodium carbonate solution. After 2h, the absorbance of solution was measured at
141   765nm wavelength. Total phenolics concentration was calculated according to Singleton & Rossi
142   (1964) and expressed as gallic acid equivalents, in mg/g fresh sample (FW).
143   Extraction crude extract of SOD and CAT: homogenized frozen tissue (2g) with 8mL of 50mM
144   sodium phosphate buffer (pH7.0), then centrifuged at 10,000g for 10min at 4°C and took the
145   supernatant. SOD activity was determined according to Jiang $et\ al$. (2010). The reaction mixture
146   contained: 1mL crude extract + 750μmol/L nitro-blue tetrazolium (NBT)+130mmol/L
147   methionine + 0.1mmol/L EDTA+ 20mmol/L riboflavin in 0.05mol/L K-phosphate buffer
148   (pH7.8). The reaction was started by adding riboflavin and placing the tubes of reaction mixture
149   under 4000lx irradiance at 25°C for 1h. The absorbance was recorded at 560nm. The 50%
150   inhibition of NBT photoreduction was expressed as one SOD activity unit.
151   CAT activity was assayed according to the method of Candan &Tarhan (2003). The reaction
152   mixture contained: 1mL of 50mM sodium phosphate buffer (pH7.0) + 1mL of 0.2% H_2O_2 + 1mL
153   of CAT extract. One unit of CAT activity was defined as the amount of enzyme that decomposed
154   1μmol H_2O_2 per min at 30°C.
155   POD was extracted and determined according to the methods of Bi $et\ al$. (2011).Weighed 2g of
156   shredded and mixed mushrooms, a little quartz sand, 7mL of pre-cooled 0.1mol/L pH5.5 sodium
157   acetate monoacetate buffer (containing 4% polyvinylpyrrolidone), grinded on ice bath, then
158   centrifuged (10000r/min 4°C) for 30min, and took the supernatant. The enzyme reaction system
159   includes: 3mL 25mmol/L guaiacol + 0.5mL the above supernatant +200uL 0.5mol/L H_2O_2

160 solution, quickly mixed and simultaneously recorded the absorbance at 470nm when the reaction
161 was 15s, as the initial value. Record every 30s and recorded continuously for 3 minutes. The
162 absorbance value was plotted against time, and the initial straight line portion of the reaction was
163 taken to calculate the change value per minute. One viability unit (U) was defined as the amount
164 of enzyme required to cause a change in absorbance of 0.01 per minute under the assay
165 conditions.
166 Polyphenol oxidase (PPO) was extracted and determined according to the methods of Li *et al*.
167 (2006).Weighed 2g of shredded and mixed mushrooms, added a little quartz sand and 7mL of
168 pre-cooled 0.05mol/L phosphate buffer (PBS, pH5.0), grinded, then centrifuged (4°C, 10000 r /
169 min, 15min) and collected the supernatant. The enzyme reaction system includes: 2.5mL
170 0.05mol/L PBS + 0.4mL 0.1mol/L catechol solution (present) + 0.5mL PPO crude enzyme
171 solution. PPO active unit (U) was set at 0.001 change per minute at 410nm absorbance at 20°C.
172 **Analysis of cell wall metabolic enzyme activity**
173 The activity of Chitinase and β-1,3-glucanase were extracted and determined according to the
174 methods of Ni *et al*. (2017). Enzyme extraction: 2g of each sample was added 7mL of 0.1mol/L
175 acetic acid-sodium acetate buffer (pH5.0) and grinded, then centrifuged at 10000 r/min for
176 20min at 4°C. The supernatants were kept at 4°C as enzyme extractions.
177 Chitinase: 0.5mL of enzyme extract was added to 0.5mL of 50mmol/L pH5.2 acetic acid-sodium
178 acetate buffer and 0.5mL of 10g/L colloidal chitin suspension. The mixture was incubated in a
179 water bath at 37°C for 1h, then, 0.1mL of 30g/L helicase was added and the incubation was
180 continued at 37°C for 1h. After the heat preservation, immediately added 0.2mL of 0.6mol/L
181 potassium tetraborate solution, 2ml of 3,5-dinitrosalicylic acid (DNS) reagent, then boiled for 5
182 min. After cooling, the volume was adjusted to 25mL. The absorbance was detected at 520nm
183 and the boiled enzyme extraction was used as the control.

184 $$\text{Chitinase activity} = \frac{\text{n} - \text{acetyl glucosamine weight} \times \text{total extraction volum} \times 1000}{\text{sample volum} \times \text{reaction time} \times \text{sample weight}}$$

185 β-1,3-glucanase: Two tubes were taken and 1mL of 4g/L of laminarin solution was added. Then,
186 1mL of the enzyme solution was added to one tube, and 1mL of boiled enzyme solution was
187 added to the other tube as a control. After mixing, the reaction tube was placed in a 37°C water
188 bath for 40min. After the incubation, 1.8mL of distilled water and 2mL of DNS reagent were
189 added to the reaction tube, and boiled for 5min, then diluted the reaction solution to 25mL with
190 distilled water. The absorbance of the mixture at 540nm was measured and repeated 3 times.

191 $$\beta - 1,3 - \text{glucanase activity} = \frac{\text{glucose weight} \times \text{total extraction volum} \times 1000}{\text{sample volum} \times \text{reaction time} \times \text{sample weight}}$$

192 **Observation of cell structure by transmission electron microscope (TEM)**
193 According to the methods of Yan (Yan*, Wang & Li*, 2002). The tissues of *C. comatus* with length
194 and width of about 2mm×1mm was cut out, fixed in 3.5% pentanediol, washed with 0.1mol/L
195 phosphate buffer of pH7.2, fixed with 1% azelaic acid, and flushed with 0.1mol/L phosphate
196 buffer. Then, 35%, 45%, 60%, 70%, 80%, 95%, 100% ethanol gradient dehydration, propylene

197   oxide transition and Spurr resin impregnation embedded. After polymerizing at 20°C for 8h,
198   sliced, double stained with uranyl acetate-lead citrate, and observed by TEM.

Statistical analysis

200   Data were subjected to analysis of variance (One-Way ANOVA) and least significant differences
201   (LSD) at the 5% level ($P \leq 0.05$) were used for comparing means using the software package
202   SPSS v24 (SPSS Inc., Chicago, USA). Graphs were created using Origin 2018 (Origin 2018.,
203   San Diego, USA).

204

Results

Appearance and browning evaluation

207   The appearance quality of *C. comatus* during the postharvest storage was shown in Fig.1. *C.*
208   *comatus* exhibited some degree of browning, cap opening and wilting of the whole fruit body
209   after 5 days of storage, and this situation was becoming more and more serious as the storage
210   days went by, since store mushrooms at day 20 were the worst. After 10 days of storage,
211   mushrooms appeared acutely browning, cap opening, stipe extending and flowing black juice,
212   rendering the mushrooms commercially unacceptable.

213   The fruit body of *C. comatus* suffered from serious browning during postharvest storage either
214   the stipe or the cap. The color difference value of cap sharply increased from 3.15 at day 0 to
215   27.67 at day 10 and to 57.91 at day 20, and the color difference value of stipe followed a similar
216   trend (Fig.2). The color difference value of the cap is basically larger than that of the stipe during
217   storage, indicated that the cap of *C. comatus* were more susceptible to browning than stipe.
218   Between day 5 to day 15 of postharvest storage, the ΔE of mushrooms cap increased significantly
219   ($p<0.05$) by 76.98%, while mushrooms stipe increased significantly ($p<0.05$) by 91.79%
220   between day 0 to day 5.

Weight loss analysis and Membrane permeability

222   The ratio of weight loss with respect to storage time and treatments in *C. comatus* was calculated
223   and the results showed on Figure 3. From the Fig. 3, it is overt that on the whole there was a
224   remarkable increase in the weight loss throughout storage, especially after 10 days weight losses
225   were particularly excessive, thus rendering the *C. comatus* commercially unacceptable. Similar
226   tendency in *L. edodes* was also reported by Jiang *et al*. (2010). The weight of *C. comatus*
227   decreased speedy, this may be due to only a thin epidermal structure on the surface of the
228   mushroom, which does not prevent a quick superficial dehydration. Roy (Roy, Anantheswaran &
229   Beelman, 2006) suggested that water transpiration and CO_2 loss during respiration are the main
230   causes of mushroom weight loss. While in our studies, respiration and evaporation might be the
231   main reasons of physiological weight loss rather than transpiration because the relative humidity
232   inside the storage chambers was near saturation.

233   Whether a membrane system is integrated could be reflected by membrane permeability, and
234   the adversity stress of postharvest plants cell, which could be expressed as electrolyte leakage
235   rate (Ran *et al*., 2010). From Fig. 3, we can visually see that the electrolyte leakage rates of *C.*
236   *comatus* rapidly increased during the whole storage (Fig. 3), reaching levels about 149.48%

237 higher than the initial values in the end. The results indicated that *C. comatus'* membrane
238 systems became more vulnerable to leakage during postharvest storage.

Analysis of chemical properties

240 Soluble protein, as a nutrient source to support sustaining metabolic activity after picking. In our
241 study, *C. comatus* contain approximately 3.78 mg/g FW soluble protein. A sharp decrease in
242 soluble protein content was observed, and only 60.05% of the initial levels was retained on the
243 twentieth day (Fig.4). Similar soluble protein level in *Agaricus bisporus* was also reported by
244 Meng *et al*. (2012). It is considered as an important indicator of tissue senescence that decline in
245 soluble protein concentration (Burton *et al*., 1997). This decline may due to the loss of energy
246 sources after picking.

247 MDA is one of the main outcomes of membrane lipid peroxidation, and its content is currently
248 used as the indicator of membrane lipid peroxidation. It can also make large molecules such as
249 proteins and nucleic acids become useless by altering their configuration or cross-linking (Duan
250 *et al*., 2011). Therefore, the accumulation of MDA can damage the cytoplasm and cell membrane
251 of mushrooms. The MDA content exhibited a gradual upward trend over the whole storage
252 (Fig.5), especially increased significantly($p<0.01$) by 95.82% between 10 and 15 days after
253 harvest. It seemed that severely damage had happened on cell membrane after day 10 and severe
254 membrane lipid peroxidation occurred in day 15 during the storage. MDA content had an
255 obvious decreasing in 5-10d. This may be related to the increase of antioxidant enzymes activity

256 Reactive oxygen species (ROS) refer to several metabolites of oxygen with high chemical
257 reactivity, such as $O_2^{\cdot-}$ and H_2O_2. They are considered as toxic by-products in plant metabolism,
258 which can cause damage to macromolecular substances such as lipids (Desikan *et al*., 2004). The
259 $O_2^{\cdot-}$ production rate sharply augmented throughout the postharvest period from 8.13umol/L to
260 28.19umol/L (Fig. 5), which increased significantly($p<0.01$) by 246.74%. The growth of $O_2^{\cdot-}$
261 production rate was obvious in the first five days, slowed down in the after 15 days during
262 storage.

Analysis of functional components

264 SOD, CAT and POD are the main antioxidant enzymes in edible fungi. During the mushroom
265 postharvest ripening process, they played a vital role in antioxidant defense and were thought to
266 extend food shelf life by protecting the integrity of membranes (Gill & Tuteja, 2010; Xing *et al*.,
267 2007). Time courses of SOD activity is shown in Fig. 6. SOD activity increased slowly at the
268 beginning of postharvest storage, afterward increased rapidly. peaked at the day 10 (31.62 U·g⁻
269 ¹·Fw), and then exhibited a sharply downward trend. A CAT activity increase was watched
270 during the first 15 days of storage, and then exhibited a slight drop over the next 5 days (Fig.6).
271 A relatively large increase of CAT activity was recorded between day 5 to day 15, with an
272 increase of 30 folds. POD could help plants get rid of reactive oxygen species (ROS) and was
273 one of the membrane lipid peroxidation defense systems of fruit and vegetable (Terefe, Buckow &
274 Versteeg, 2014). The changes of POD activity during the postharvest storage are also displayed in
275 Fig. 6. A rapid increase was observed during the first 5 days, then slowly up to a higher peak
276 (0.33 U/g FW) at day 10, afterwards sharply dropped to raw value.

277   A sharply increase was observed in 10 days after harvest, with a higher peak of total phenolics

278   ($0.89mg \cdot g^{-1} \cdot FW$). Then total phenolics level showed a gradual downward trend over the next 10

279   days (Fig.7). The oxidation of phenolic substances was the main factor of browning in

280   mushroom tissue, which is one of the key factors that influence the shelf-life and product quality

281   of the postharvest mushrooms. In our case, the content of total phenols increased significantly

282   ($p<0.01$) by 13.39% between 5 and 10 days after harvest, it may be caused by the reduction of

283   PPO activity, and the occurrence and timing of peak roughly coincides with the cap browning of

284   *C. comatus*.

285   The change of PPO activity of *C. comatus* during postharvest storage is given in Fig. 7. It

286   increased rapidly from the initial value, then decreased, afterwards increased to a peak, and

287   finally decrease, nonetheless on the whole there was an uplifted tendency. Similar tendency in

288   *Agrocybe aegirit* was also reported by Lo & Pck (2005). PPO participation in enzymatic

289   browning was thought to be the major factor of discoloration of many food (Vámos, Lilly &

290   Haard, 1981). It catalyzed the oxidation of polyphenols into quinones, which turned into melanin

291   by polymerization, thus severely affecting the mushrooms' nutritional value, flavor and

292   appearance quality. Hence, in order to preventing the synthesis of melanin in the browning of

293   mushrooms, control of PPO activity is indispensable. Increased PPO activity was associated with

294   mushroom browning, suggesting that the browning of *C. comatus* after postharvest was possibly

295   attributed to the action of PPO.

296   **Cell wall metabolism-related enzyme activity**

297   The change of cell wall metabolizing enzyme activity of *C. comatus* are given in Figure 8.

298   Studies have shown that the content of chitin in edible fungi decreases due to the effect of

299   chitinase in the storage process. Chitinase hydrolyzes the beta-1, 4-glycoside bond in chitinol to

300   produce n-acetylglucosamine oligomer or monomer (Buitimea *et al*., 2013). Lim & Choi (2009)

301   found that the *Agaricus bisporus* could produce black juice in the process of autolysis at 25°C

302   for 15h. By PCR confirmed that the amount of chitinase synthesis in the process of autolysis of

303   *A. bisporus mushroom* was significantly higher than that in the normal *A. bisporus mushroom*. In

304   this study, the activity of Chitinase increased rapidly from the initial low value to a peak during

305   the first 10 days, and finally declined over the next 20 days. β-Glucanase is a cell-wall-degrading

306   enzyme mainly effective on glucans. β-1,3-glucanase activity of *C. comatus* increased up to day

307   10 of storage and decreased thereafter. Thus, the higher degrees of cell wall polysaccharide

308   degradation observed during storage might be due to their high activities.

309   **Changes in stipe ultrastructure**

310   At 5,000 times magnification, we observed the cell of fresh *C. comatus* maintained complete

311   structure as shown in Fig.9-A. Cells arranged in neat rows and the cell wall structure was

312   complete. There are many nucleus and mitochondria in the cytoplasm. Cell of *C. comatus* stored

313   for 10d was shown in Fig. 9-B, the cells no longer line up. The distance between cells increased

314   and the thickness of the cell wall decreased, at the same time, the number of nucleus and

315   mitochondria decreased. Ultrastructure of *C. comatus* stored for 20d was given in Fig. 9-C,

316   which showed that cells had lost integrity already. Cell wall cracked and cell contents permeated
317   outside. Hence, *C. comatus* value had been lost.
318   At 10,000 times magnification, an integrated cell was observed in Fig. 9-D, the nucleus and
319   organelles were clearly visible. A relatively integrated cell was observed in Fig. 9-E, the nucleus
320   and the organelles become blurred. A dissolved cell was observed as shown in Fig. 9–F, the
321   nucleus and organelles were completely degraded into a dark mass.
322   At 15,000 times magnification, the structure of mitochondria was clear, and mitochondrial
323   cristae could be observed in fresh *C. comatus* (Fig. 9.G). Cell of *C. comatus* stored for 10d was
324   shown in Fig. 9.H, mitochondria still existed and mitochondrial membrane was visible. While,
325   the mitochondria had almost degraded when stored for 20d (Fig. 9.I), which just reserved the
326   structure of mitochondrial was disabled.

Discussion

328   In this study, we created a *C. comatus* softening model to evaluate changes of sensory
329   characteristics, chemical constituents, antioxidant activities and secondary metabolism of the cell
330   wall during a 20-day storage. *C. comatus* exhibited some degree of browning, wilting of the
331   whole fruit body and cap opening after 5 days of storage, and this situation was becoming more
332   and more serious as the storage days went by. The cap of *C. comatus* were more susceptible to
333   browning than stipe. Due to removing waters and nutrients after harvest, weight loss and soluble
334   protein declined. The continuous production of MDA and $O_2^{\bullet-}$ leads to the increasing
335   permeability of cell membrane, further leads to membrane lipid peroxidation. While, the
336   increased activity of SOD, CAT and POD can beneficial in protecting cells from ROS-induced
337   injuries, alleviating lipid peroxidation and stabilizing membrane integrity.
338   Color and textural changes are the two main factors limiting mushroom quality and shelf life
339   (Soler *et al*., 2010). In our study, relatively high positive correlation (r≥0.789) was found
340   between browning and polyphenol oxidase (PPO) enzyme. Thus, the browning of *C. comatus*
341   after postharvest was possibly attributed to the action of PPO. May be by inhibiting the activity
342   of PPO, the color quality of *C. comatus* can be effectively improved. Firmness decrease during
343   storage is generally attributed to changes in cell wall composition (Manolopoulou *et al*., 2007;
344   Singh *et al*., 2010). Ripening-related modifications in cell wall composition and structure are
345   commonly attributed to the finely tuned, coordinated action of a number of specific enzymatic
346   and non-enzymatic proteins on different cell wall polysaccharides, which eventually lead to cell
347   wall disassembly (Belge *et al*. 2015). Ni *et al*. (2017) studied secondary metabolism associated
348   with softening of *Lentinula edodes* at room temperature, and found that glucanase activity
349   reached the highest level at day 3, chitinase activity and cellulase activity increased since day 3
350   still day 6 during storage. Karakurt & Toka (2016) studied the effects of $CaCl_2$ and hot water
351   dips on the change hydrolase activities of *Agaricus bisporus*, and found xyloglucanase and beta
352   glucanase activities increased significantly during storage. In our study, the cell wall
353   metabolizing enzymes, including Chitinase, β-1,3-glucanase, they all increased firstly and
354   reached the highest level at day 10 during postharvest storage, then decreased. Thus, it is
355   possible that the higher degrees of cell wall degradation observed during storage might be due to

356   their high activities. Liu *et al*. (2015) suggested that β-1,3-glucanases plays a major role in the
357   autolysis of *Coprinopsis cinerea*. In our experiment, the activity of β-1,3-glucanases was the
358   strongest among cell wall metabolizing enzymes. It is also known that β-1,3-glucan with β-1,6-
359   glucan braches are the backbone of the fungal cell wall network (Aimanianda *et al*., 2017).
360   Based on the above, we speculate β-glucanases could destroy the cytoskeleton of *C. comatus*,
361   while the chitinases may degrade cell walls in synergy with β-1,3-glucanases. Surely, the activity
362   of enzymes is ultimately regulated by genes expression, so more experiments are needed to
363   verify this speculation in the next time. The ultrastructure changes of the postharvest *C. comatus*
364   were observed for the first time. The nucleus, mitochondria had disappeared and thinner cell wall
365   were detected with the storage duration. The lower integrity of cell walls may result in greater
366   loss of protein and polysaccharide.
367   ROS accumulates and leads to lipid peroxidation when plants suffer from biotic and abiotic
368   stresses (Ren *et al*., 2012). MDA is a lipid peroxidized product, and it can reflect the extent of
369   ROS-induced membrane lipid peroxidation (You *et al*., 2012). In this experiment, the MDA
370   content exhibited a gradual upward trend over the whole storage, especially increased
371   significantly(p<0.01) by 95.82% between 10 and 15 days after harvest. The result seems to
372   indicate that severely damage had happened on cell membrane after day 10 and severe
373   membrane lipid peroxidation occurred in day 15 during the storage. To protect cells from ROS-
374   induced injuries, plant tissues generated SOD, CAT and POD to scavenge ROS (Duan *et al*.,
375   2011). In our experiment, showed high activity and the $O_2^{\bullet-}$ production rate increased slowly
376   between 5 and 15 days after harvest. It validates that high SOD, CAT, POD activity could be
377   beneficial in protecting cells from ROS-induced injuries, alleviating lipid peroxidation and
378   stabilizing membrane integrity. Although the activity of SOD, CAT, POD of *C. comatus* peaked
379   up to 31.62 $U{\cdot}g^{-1}{\cdot}Fw$, 16.51 $U{\cdot}g^{-1}{\cdot}Fw$, 0.33 $U{\cdot}g^{-1}$ FW, respectively, they were lower than that of
380   *Agaricus bisporus* (Li *et al*., 2019) at the similar storage conditions. This indicated that
381   enzymatic antioxidant defense of *C. comatus* was inferior to that of *Agaricus bisporus*. It also
382   means *C. comatus* were more likely to spoil after harvest. Ding *et al*. (2016) studied the effects
383   of postharvest brassinolide (BL) treatment on the metabolism of *Agaricus bisporus*, and found
384   BL treatment significantly decreased the accumulation of ROS and induces the antioxidant
385   enzyme system. Li *et al*. (2019) also studied storage quality of *Agaricus bisporus* and the result
386   showed that 10mM L-arginine treatment inhibited PPO activities, while inducing SOD and POD
387   activities. Lia *et al*. (2013) explored low oxygen and high carbon dioxide storage effects on
388   reactive oxygen species metabolism in *Pleurotus eryngii*. They found the activities of SOD,
389   POD, and CAT in 2% O_2 + 30% CO_2 treated mushrooms were significantly higher than those of
390   the control (ambient air). In a study by Shi *et al*. (2017), the nanocomposite packaging material
391   could effectively delay the postharvest senescence of *Flammulina velutipes*. Jiang *et al*. (2010)
392   reported four macroholes treatment was a useful way of maintaining *Lentinula edodes* texture
393   during storage at 4°C. Above all, it is possible to apply these methods to the storage of *C*.
394   *comatus*, hence prolonged its shelf life. Furthermore, in the future, the expression of genes which

395 associated with antioxidant activity and cell wall metabolism could be artificially regulated
396 through gene editing, so as to extend the life of *C. comatus* and increased its preservation quality.
397

Conclusions

399 To sum up, these results provide insights into the quality deterioration of *C. comatus* after
400 postharvest. Identifying and reducing the quality deterioration is necessary to provide the edible
401 fungus industry more appropriate recommendations for postharvest management, which would
402 ultimately maximize both sensory and nutritional quality maintenance of fresh mushrooms.
403

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409

References

411 **Aimanianda V, Simenel C, Garnaud C, Clavaud C, Tada R, Barbin L. 2017.** The dual activity responsible
412 for the elongation and branching of β-(1,3)-glucan in the fungal cell wall. mBio 8(3): e00619-17 DOI
413 10.1128/mbio.00619-17
414 **Bailey C J, Turner S L, Jakeman K J. Hayes W A. 1985.** Effect of *Coprinus comatus* on plasma glucose
415 concentrations in mice. Planta Medica 50:525–526 DOI 10.1055/s-2007-969791.
416 **Belge B, Comabella E, Graell J, Lara I. 2015.** Post-storage cell wall metabolism in two sweet cherry
417 (*Prunus avium L.*) cultivars displaying different postharvest performance. Food Science and Technology
418 International 21(6):416-427 DOI 10.1177/1082013214541863.
419 **Bi X, Wu J, Zhang Y, Xu Z, Liao X. 2011.** High pressure carbon dioxide treatment for fresh-cut carrot slices.
420 Innovative Food Science & Emerging Technologies 12(3):0-304 DOI 10.1016/j.ifset.2011.04.001.
421 **Bo L, Fei L, Xiaomin S, Haijuan N, Bin L. 2010.** Antioxidant Properties of Cap and Stipe from *Coprinus*
422 *comatus*. Molecules 15(3):1473-1486 DOI 10.3390/molecules15031473.
423 **Bradford MM. 1976.** A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein
424 Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry 72(1-2):248-254 DOI 10.1016/0003-
425 2697(76)90527-3.
426 **Buitimea C G V, Rosas B E C, Cinco M F J, Burgos H A, Plascencia J, Cortez R M O, Gálvez R J C.**
427 **2013.** In Vitro Effect of Antifungal Fractions from the Plants Baccharis glutinosa and Jacquinia macrocarpa on
428 Chitin and β-1,3-Glucan Hydrolysis of Maize Phytopathogenic Fungi and on the Fungal β-1,3-Glucanase and
429 Chitinase Activities. J Food Safe 33(4):526−535 DOI 10.1111/jfs.12085.
430 **Burton K S, Partis M D, Wood D A, Thurston C F. 1997.** Accumulation of serine proteinase in senescent
431 sporophores of the cultivated mushroom (*Agaricus bisporus*). Mycological Research 101(2):146−152 DOI
432 10.1017/s095375 6296002316.
433 **Candan N, Tarhan L. 2003.** Relationship among chlorophyll–carotenoid content, antioxidant enzyme
434 activities and lipid peroxidation levels by Mg^{2+} deficiency in the Mentha pulegium leaves. Plant Physiology
435 and Biochemistry (Paris) 41:35-40 DOI 10.1016/s0981-9428(02)00006-2.
436 **Desikan R, Cheung M K, Clarke A, Golding S, Sagi M, Fluhr R, Rock C, Hancock J, Neill S. 2004.**
437 Hydrogen peroxide is a common signal for darkness- and ABA-induced stomatal closure in Pisum sativum.

438 Functional Plant Biology 31(9):913-920 DOI 10.1071/FP04035.

439 **Ding Y, Zhu Z, Zhao J, Nie Y, Zhang Y, Sheng J, Meng D, Mao H, Tang X. 2016.** Effects of Postharvest
440 Brassinolide Treatment on the Metabolism of White Button Mushroom (*Agaricus bisporus*) in Relation to
441 Development of Browning During Storage. Food and Bioprocess Technology 9(8):1327-1334 DOI 10.1007/
442 s11947-016-1722-1.

443 **Duan X, Liu T, Zhang D, Su X, Lin H, Jiang Y. 2011.** Effect of pure oxygen atmosphere on antioxidant
444 enzyme and antioxidant activity of harvested litchi fruit during storage. Food Research International 44(7):0-
445 1911 DOI 10.1016/j.foodres.2010.10.027.

446 **Fan J M, Zhang J S, Tang Q J, Liu Y F, Zhang A Q, Pan Y J. 2006.** Structural elucidation of a neutral
447 fucogalactan from the mycelium of *Coprinus comatus*. Carbohydrate Research 341(9):1130-1134 DOI
448 10.1016/j.carres.2006.03.039.

449 **Gill S S, Tuteja N. 2010.** Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in
450 crop plants. Plant Physiology and Biochemistry 48(12):909-930 DOI 10.1016/j.plaphy.2010.08.016.

451 **Huang R, Xia R, Hu L, Lu Y, Wang M. 2007.** Antioxidant activity and oxygen-scavenging system in orange
452 pulp during fruit ripening and maturation. Scientia Horticulturae 113(2):0-172 DOI 10.1016/j.scienta.2007.03.
453 010.

454 **Jayakumar T, Ramesh E, Geraldine P. 2006.** Antioxidant activity of the oyster mushroom, *Pleurotus
455 ostreatus*, on CCl4-induced liver injury in rats. Food and Chemical Toxicology 44(12): 1989-1996 DOI
456 10.1016/j.fct.2006.06.025.

457 **Jiang T, Feng L, Li J. 2012.** Changes in microbial and postharvest quality of shiitake mushroom (*Lentinus
458 edodes*) treated with chitosan–glucose complex coating under cold storage. Food Chemistry 131(3):780-786
459 DOI 10.1016/j.foodchem.2011.08.087.

460 **Jiang T, Jahangir M M, Jiang Z, Lu X, Ying T. 2010.** Influence of UV-C treatment on antioxidant capacity,
461 antioxidant enzyme activity and texture of postharvest shiitake (*Lentinus edodes*) mushrooms during storage.
462 Postharvest Biology & Technology 56(3):209-215 DOI 10.1016/j.postharvbio.2010.01.011.

463 **Jiang T, Wang Q, Xu S, Jahangir MM, Ying T. 2010.** Structure and composition changes in the cell wall in
464 relation to texture of *shiitake mushrooms* (*Lentinula edodes*) stored in modified atmosphere packaging. Journal
465 of the Science of Food & Agriculture 90(5):742−749 DOI 10.1002/jsfa.3876.

466 **Jiang X, Lian M, Han Y, Lv S. 2013.** RETRACTED: Antitumor and immunomodul -atory activity of a
467 polysaccharide from fungus *Coprinus comatus* (Mull.:Fr) Gray. International Journal of Biological
468 Macromolecules 58(26):349-353 DOI 10.1016/j.ijbiomac.2013.04.033.

469 **Karakurt Y, Toka D. 2016.** The Influence of Hot Water and Calcium Chloride on the Changes in Cell Wall
470 Composition and the Activities of Cell Wall Hydrolases during Storage in *Agaricus bisporus*. Journal of Food
471 Biochemistry 40(2):220-226 DOI 10.1111/jfbc.12219.

472 **Li B, Ding Y, Tang X, Wang G, Wu S, Li X. 2019.** Effect of l-arginine on maintaining storage quality of the
473 white button mushroom (*agaricus bisporus*). Food and Bioprocess Technology DOI 10.1007/s11947-018-
474 2232-0.

475 **Li H S. 2006.** The Experiment Principle and Technique on Plant Physiology and Biochemistry. Beijing:
476 Higher Education Press.

477 **Lia P, Zhang X, Hua H, Sun Y, Wang Y, Zhao Y. 2013.** High carbon dioxide and low oxygen storage
478 effects on reactive oxygen species metabolism in *Pleurotus eryngii*. Postharvest Biology and Technolog
479 85(Complete):141-146 DOI 10.1016/j.postharvbio.2013.05.006.

480 **Lim H, Choi H T. 2009.** Enhanced expression of chitinase during the autolysis of mushroom in *Coprinellus
481 congregatus*. Journal of Microbiology 47(2): 225−228 DOI 10.1007/s12275-008-0247-3.

482 **Liu Z, Niu X, Wang J, Zhang W, Yang M, Liu C, Xiong Y, Zhao Y, Pei S, Qin Q, Zhang Y, Yu Y, Yuan
483 S. 2015.** Comparative study of non-autolytic mutant and wild-type strains of *Coprinopsis cinerea* supports an

484 important role of glucanases in fruiting body autolysis. Journal of Agricultural & Food Chemistry 63:9609-
485 9614 DOI 10.1021/acs.jafc.5b03962.
486 **Liu Z, Wang X, Zhu J, Wang J. 2010.** Effect of high oxygen modified atmosphere on post-harvest
487 physiology and sensorial qualities of mushroom. International Journal of Food Science & Technology
488 45(6):1097-1103 DOI 10.1111/j.1365-2621.2010.02199.x.
489 **Lo K M, Cheung P C K. 2005.** Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita*
490 var. alba. Food Chemistry 89(4): 533-539 DOI 10.1016/j.foodchem.2004.03.006.
491 **Manolopoulou E, Philippoussis A, Lambrinos G, Diamantopoulou P. 2007.** EVALUATION OF
492 PRODUCTIVITY AND POSTHARVEST QUALITY DURING STORAGE OF FIVE AGARICUS
493 BISPORUS STRAINS. Journal of Food Quality 30(5):18 DOI 10.1111/j.1745-4557. 2007. 0014 8.x.
494 **Meng D, Song T, Shen L, Zhang X, Sheng J. 2012.** Postharvest Applicationof Methyl Jasmonate for
495 Improving Quality Retention of *Agaricus bisporus* Fruit Bodies. Journal of Agricultural and Food Chemistry
496 60(23):6056-6062 DOI 10.1021/jf3006454.
497 **Ni Z, Xu S, Bu J, Ying T. 2017.** Secondary metabolism associated with softening of shiitake mushroom
498 (*Lentinula edodes*) induced by O_2 depletion and CO_2 accumulation. International Journal of Food Science &
499 Technology 52(10):2303-2310 DOI 10.1111/ijfs.13512.
500 **Prasad S B, Rosangkima G, Kharbangar A. 2009.** Structural and biochemical changes in mitochondria after
501 cisplatin treatment of Dalton's lymphoma-bearing mice. Mitochondrion 10(1):38-45 DOI 10.1016 /j.mito.
502 2009. 09.002.
503 **Qi X D, Wei J M, Gao H S, Jia Y R, Zhang H. 2015.** Pectin Polysaccharide Degradation in Relation to the
504 Texture Softening in Pear Fruit. Scientia Agricultura Sinica.
505 **Ran Z, Li-ping Y, Jing X, Yun-fei L, Heng-he F. 2010.** Effects of edible coatings on quality of Shanghai
506 sand pear (*Pyrus pyrifolia*) during cold storage. Acta Agriculturae Shanghai 26(4):53-56 DOI 10.1080/ 00949
507 651003724790.
508 **Ren Y, Wang Y, Bi Y, Ge Y, Wang Y, Fan C, Li D, Deng H. 2012.** Postharvest BTH treatment induced
509 disease resistance and enhanced reactive oxygen species metabolism in muskmelon (*Cucumis melo L.*) fruit.
510 European Food Research and Technology 234(6) DOI 10.1007/s00217-012-1715-x.
511 **Roy S, Anantheswaran R C, Beelman R B. 2006.** Fresh Mushroom Quality as Affected by Modified
512 Atmosphere Packaging. Journal of Food Science 60(2):7 DOI 10.1111/j.1365-2621.1995.tb05667.x.
513 **Rui J, Feng-Lan L. 2006.** Research current situation and developing prospect of *Coprinus comatus*. Food
514 Science & Technology 27(12):890-889 DOI 10.1016/S1872-2040(06)60045-5.
515 **Shi C, Wu Y, Fang D, Pei F, Hu Q. 2017.** Effect of nanocomposite packaging on postharvest senescence of
516 *Flammulina velutipes*. Food Chemistry S0308814617317417 DOI 10.1016/j.foodchem.2017.10.103.
517 **Singh P, Langowski H C, Wani A A, Saengerlaub S. 2010.** Recent advances in extending the shelf life of
518 fresh *Agaricus mushrooms*: A review. J Sci Food Agric 90(9):1393-1402 DOI 10.1002/jsfa.3971.
519 **Singleton V, Rossi J A. 1964.** Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid
520 Reagents. American Journal of Enology and Viticulture 16(3) DOI doi:http://dx.doi.org/.
521 **Soler-Rivas C, Arpin N, Olivier J M, Wichers H J. 2010.** WLIP, a lipodepsipeptide of Pseudomonas
522 'reactans', as inhibitor of the brown blotch disease of *Agaricus bisporus*. Journal of Applied Microbiology
523 86(4):635-641 DOI 10.1046/j.1365-2672.1999.00709.x.
524 **Terefe N S, Buckow R, Versteeg C. 2014.** Quality-related enzymes in fruit and vegetable products: effects of
525 novel food processing technologies, part 1: high-pressure processing. Critical Reviews in Food Science and
526 Nutrition 54(1):24-63 DOI 10.1080/10408398.2011.566946.
527 **Tsai S Y, Tsai H L, Mau J L. 2009.** Antioxidant properties of *Coprinus comatus*. Journal of Food
528 Biochemistry 33:368–389 DOI 10.1111/j.1745-4514.2009.00224. x.
529 **Vámos-Vigyázó, Lilly, Haard N F. 1981.** Polyphenol oxidase and peroxidase in fruits and vegetables. Critical

530   Reviews in Food Science and Nutrition 15(1):49−127 DOI 10.1080/10408398109527312.

531   **Xing Z, Wang Y, Feng Z, Zhao Z, Liu X. 2007.** Effect of 60 Co irradiation on postharvest quality and
532   selected enzyme activities of Hypsizygus marmoreus fruit bodies. Journal of Agricultural and Food Chemistry
533   55(20):8126−8132 DOI 10.1021/jf070941w.

534   **Xue H. 2012.** Effect of postharvest calcium treatment on lentinan content and cell stability of *Lentinula*
535   *edodes*. Food Science 33(10):298−300.

536   **Yan C L, Wang J B, Li R Q. 2002.** Effect of heat stress on calcium ultrastructural distribution in pepper
537   anther. Environmental and Experimental Botany 48(2):161-168 DOI 10.1016/s0098-8472(02)00021-7.

538   **You Y, Jiang Y, Sun J, Liu H, Song L, Duan X. 2012.** Effects of short-term anoxia treatment on browning of
539   fresh-cut Chinese water chestnut in relation to antioxidant activity. Food Chemistry 132(3):1191-1196 DOI
540   10.1016/j.foodchem.2011.11.073.

541   **Zivanovic S, Buescher R, Kim S K. 2003.** Mushroom Texture, Cell Wall Composition, Color, and
542   Ultrastructure as Affected by pH and Temperature. Journal of Food Science 68(5):1860-1865 DOI 10.1111/
543   j.1365-2621.2003. Tb 12343.x.

Figure 1

Fig.1. The appearance quality of *Coprinus comatus* stored at 4 °C and 90% RH.

a: stored for 0d. **b**: stored for 5d. **c**: stored for 10d. **d**: stored for 15d. **e**: stored for 20d.

Figure 2

Fig.2. The color difference value of *Coprinus comatus* stored at 4 °C and 90% RH.
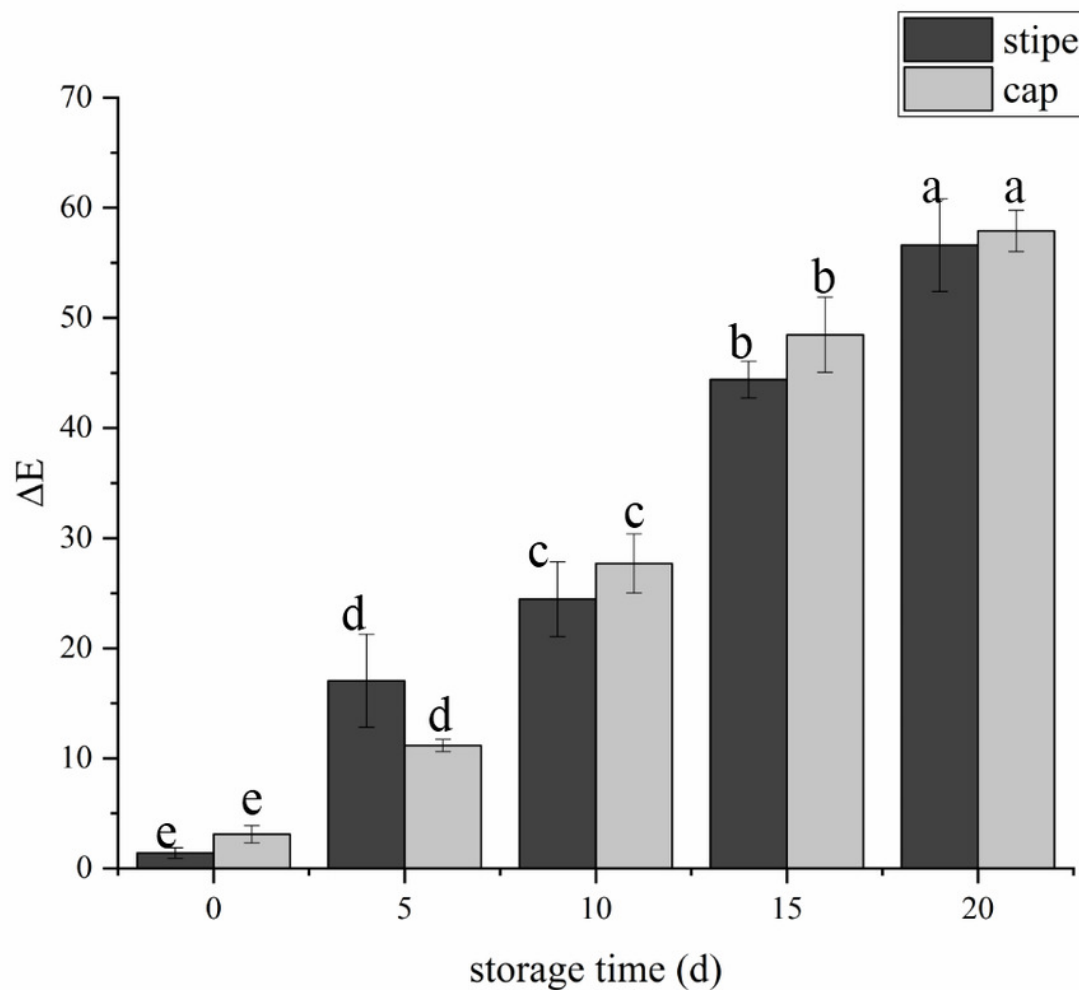
Figure 3

Fig.3. The weight loss ratio and membrane permeability of *Coprinus comatus* stored at 4 °C and 90% RH.
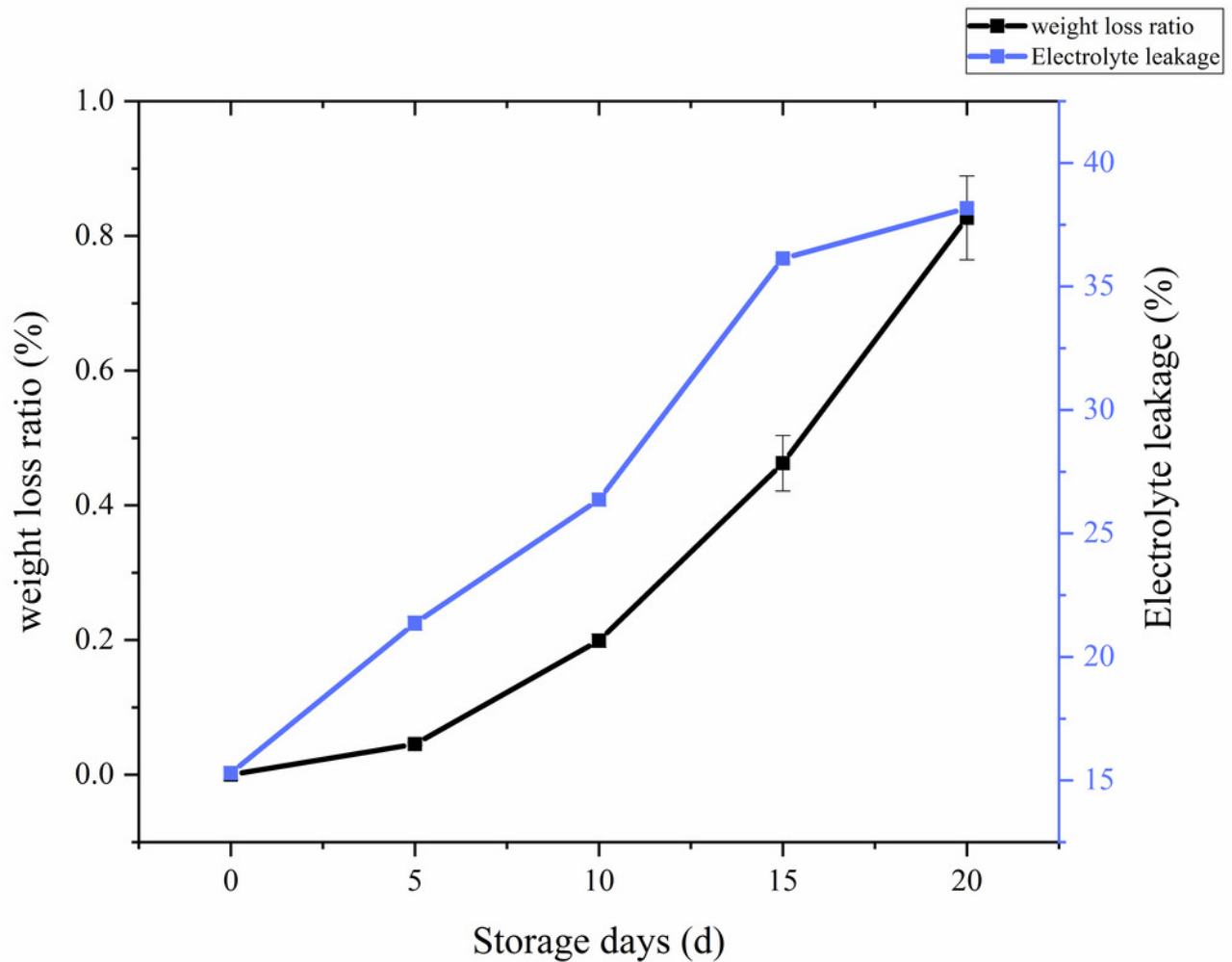
Figure 4

Fig.4. The soluble protein of *Coprinus comatus* stored at 4 °C and 90% RH
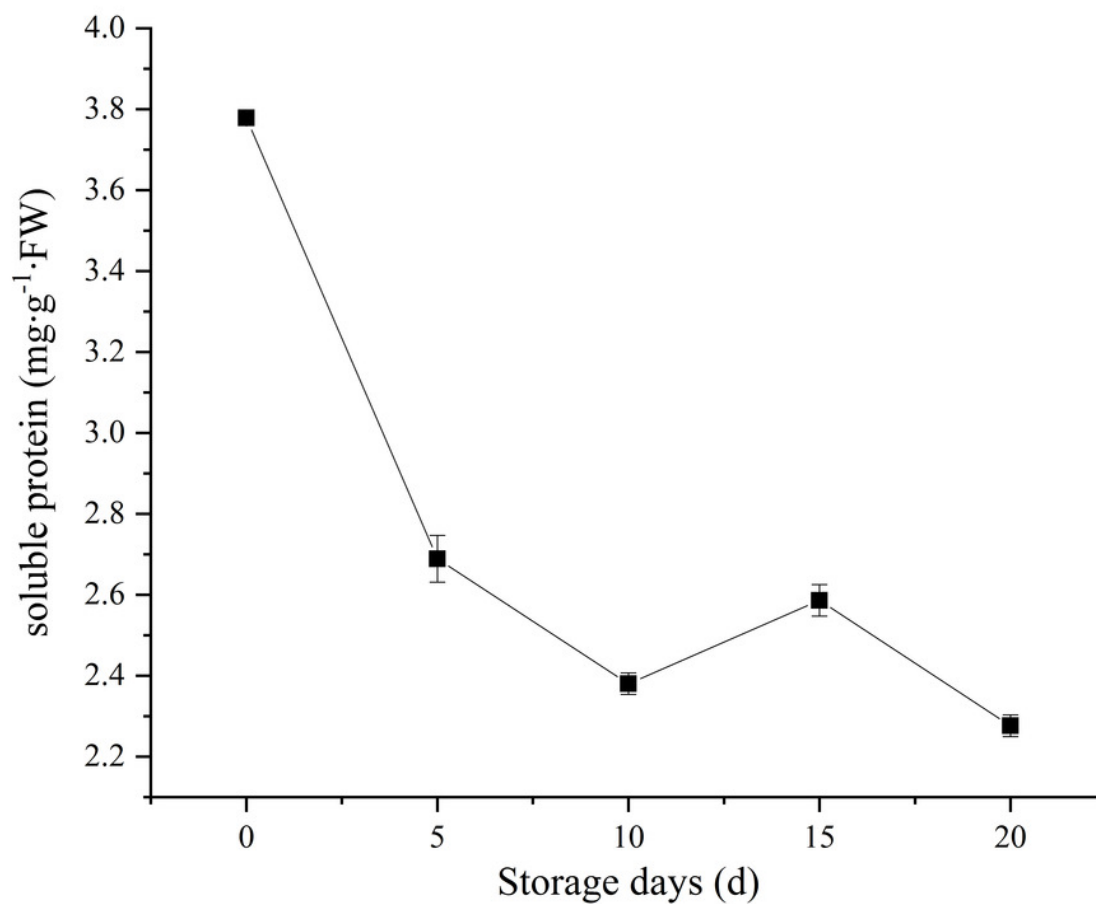
Figure 5

Fig.5. The MDA content and $O_2^{\bullet-}$ production rate of *Coprinus comatus* stored at 4 °C and 90% RH.
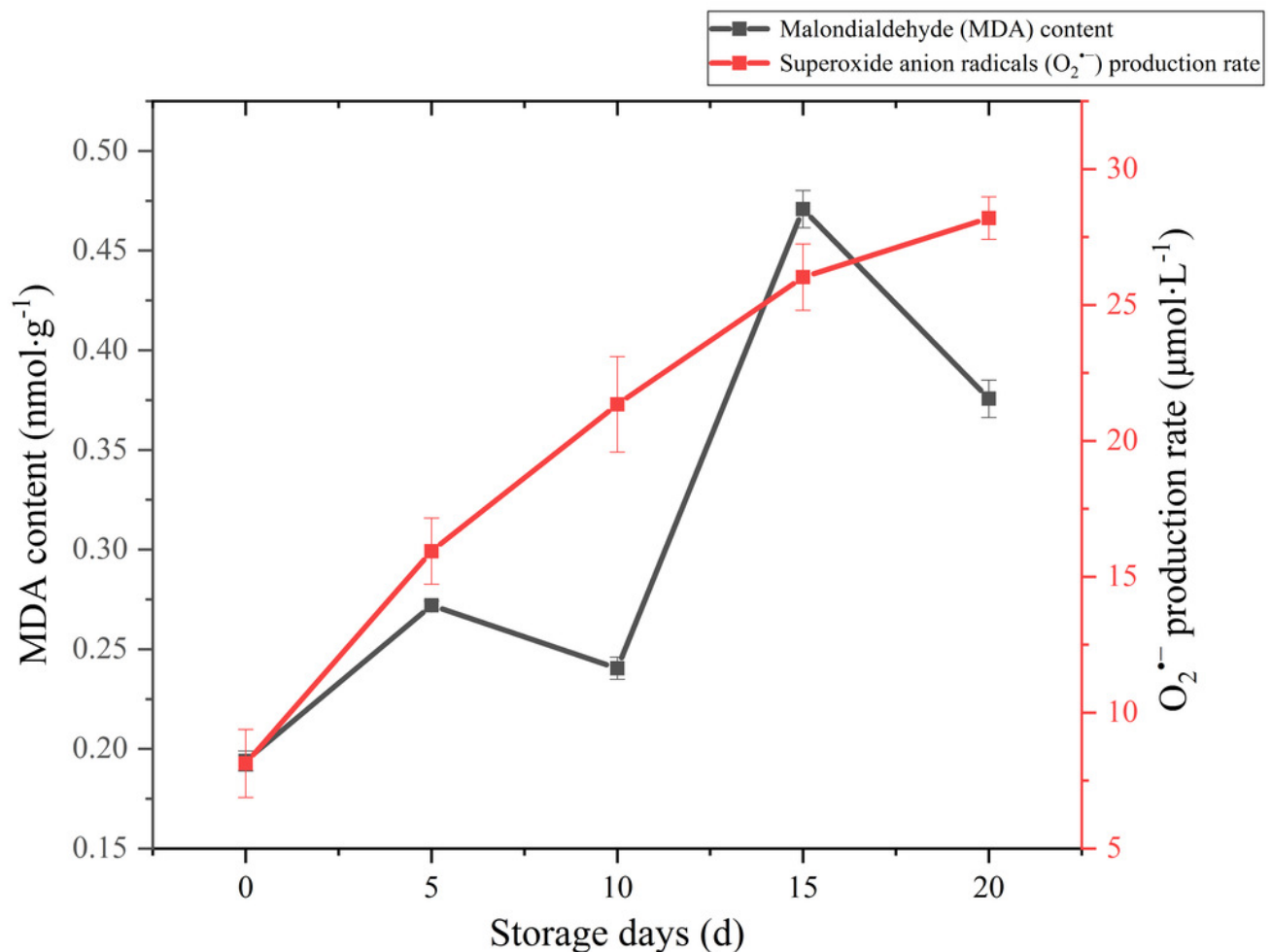
Figure 6

Fig.6. The SOD, CAT, POD activity of *Coprinus comatus* stored at 4 °C and 90% RH.
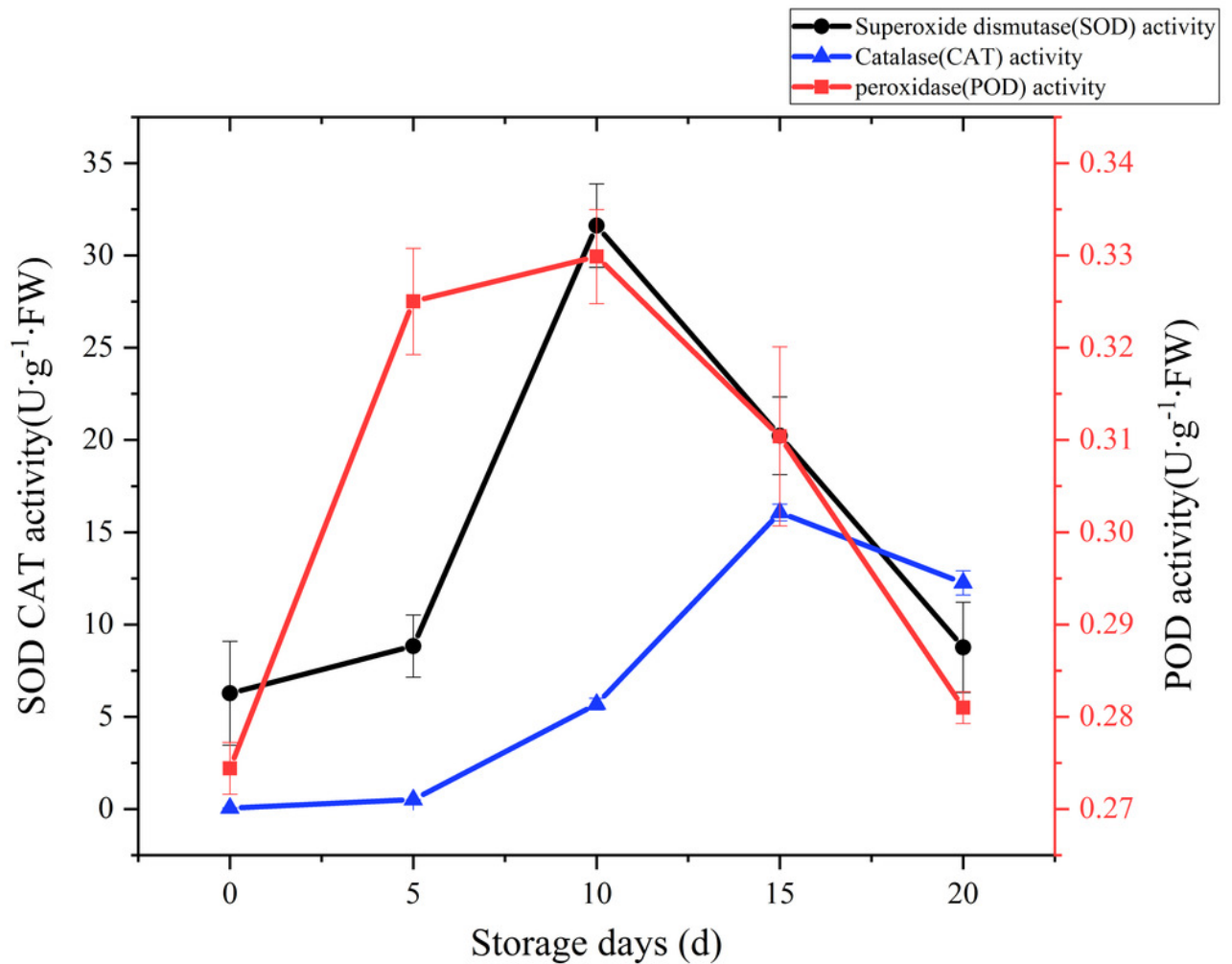
Figure 7

Fig.7. The total phenolics and PPO activity of *Coprinus comatus* stored at 4 °C and 90% RH.
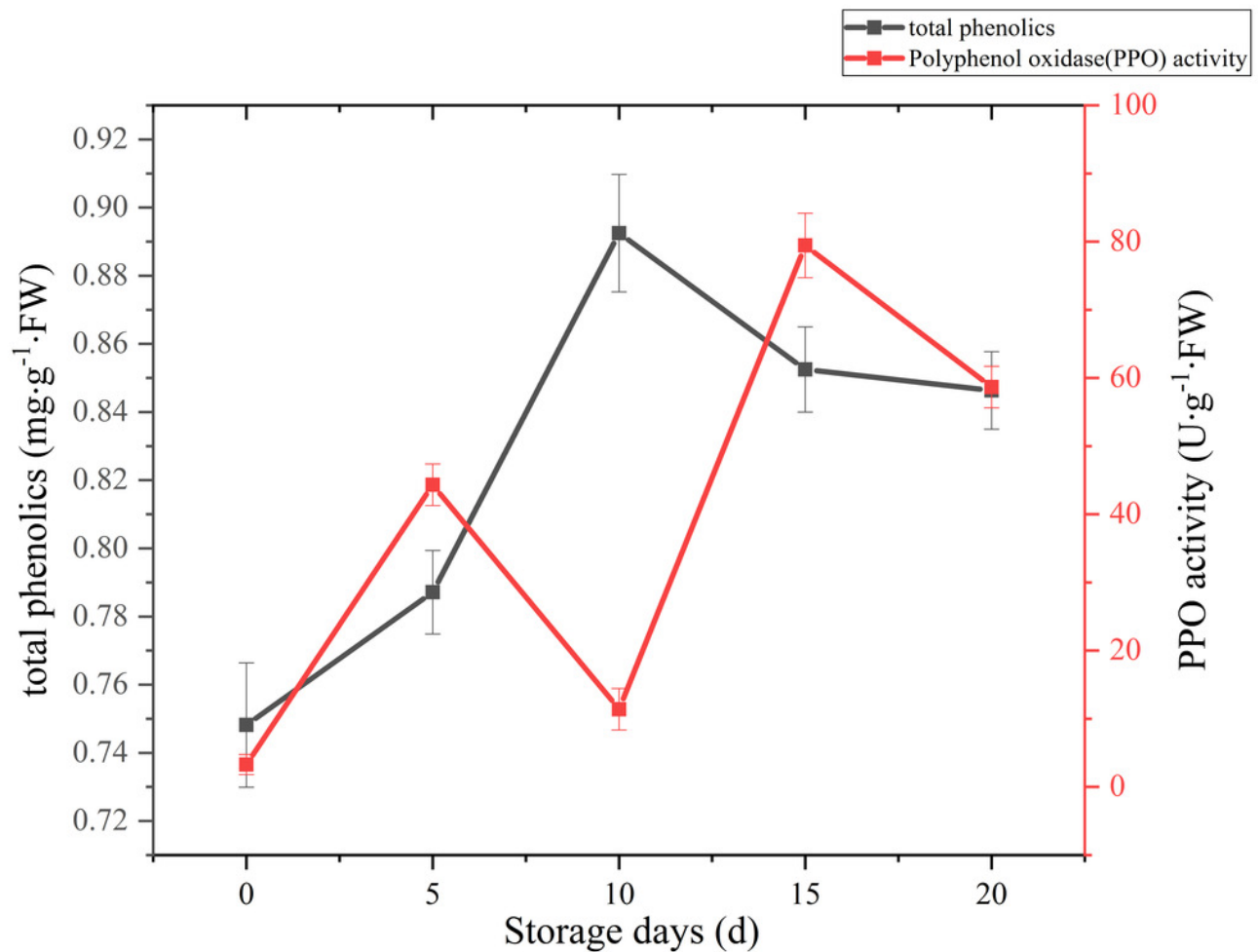
Figure 8

Fig.8 . The cell wall metabolizing enzyme activity of *Coprinus comatus* stored at 4 °C and 90% RH.
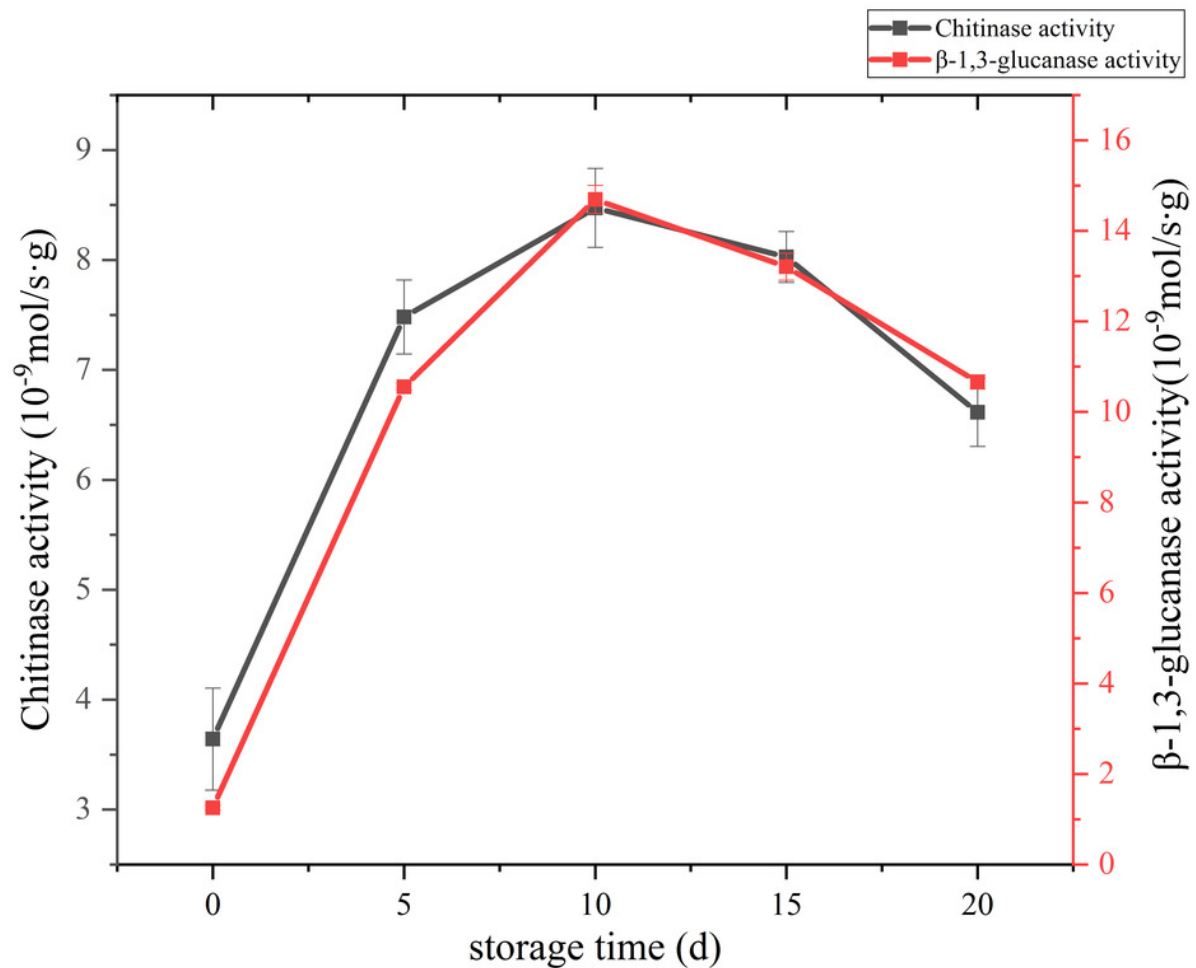
Figure 9

Fig.9 Changes in stipe ultrastructure of *Coprinus comatu s* though TEM stored at 4°C and 90% RH .

A: storage for 0d, **B**: storage for 10d, **C**: storage for 20d. amplification: 5×1000. **D**: storage for 0d, **E**: storage for 10d, **F**: storage for 20d. amplification:10×1000. **G**: storage for 0d, **H**: storage for 10d, **I**: storage for 20d. amplification:15×1000.

CW: cell wall, CY: cytoplasm, M: mitochondria, N: nucleus, V: vacuole, IM: mitochondrial inner membrane, OM: mitochondrial outer membrane.