Postharvest Biology and Technology 85 (2013) 141–146

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

High carbon dioxide and low oxygen storage effects on reactive oxygen species metabolism in *Pleurotus eryngii*

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ARTICLE INFO

Article history:
Received 19 November 2012
Accepted 9 May 2013

Keywords:
Pleurotus eryngii
Antioxidant enzyme activity
Respiratory enzyme activity
Controlled atmosphere
Reactive oxygen species (ROS)

ABSTRACT

This study addressed the influence of high carbon dioxide and low oxygen levels on *Pleurotus eryngii* samples, stored at 20–25 °C and 90–95% RH for 5 d. Evaluations of sensory characteristics, malondialdehyde (MDA) content, superoxide anion ($O_2^{\bullet-}$) production rate and the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and cytochrome *c* oxidase (CCO) were made in the mushrooms in response to high carbon dioxide and low oxygen treatments. The results showed that 2% O_2 + 30% CO_2 significantly prolonged mushroom shelf-life when compared to the control. The 2% O_2 + 30% CO_2 mixture was better suited to maintaining mushroom sensory characteristics and delaying the MDA increase and $O_2^{\bullet-}$ production rate during storage. The activities of SOD, POD, and CAT in 2% O_2 + 30% CO_2-treated mushrooms were significantly higher than those of the control. However, the CCO activity was not affected by the atmospheric treatment (2% O_2 + 30% CO_2). These results indicated that the 2% O_2 + 30% CO_2 treatment could alleviate lipid peroxidation and enhance antioxidant enzyme activities, but it exerted little influence on the CCO activity of *Pleurotus eryngii*.

1. Introduction

Pleurotus eryngii (king oyster mushroom) is very nutritious and has biological activities such as antioxidation and antihypercholesterolemia (Oke and Aslim, 2011), but the shelf-life of these mushrooms is shorter than that of other supermarket products after harvest because the mushrooms lack a cuticle to protect them from physical damage, microbial attack, and water loss (Aguirre et al., 2008).

Reactive oxygen species (ROS), which are generated in the mitochondrion under normal respiratory conditions, are involved in the postharvest senescence of fresh products (Huang et al., 2007; You et al., 2012). ROS production can be enhanced in response to various biotic and abiotic stress conditions (Gill and Tuteja, 2010). Postharvest storage can be considered as a sort of abiotic stress for mushrooms because the storage conditions are quite different from the growing conditions, which can lead to electron transport inhibition in mitochondria as well as increased ROS production. Enzymatic antioxidant defense systems including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) can efficiently scavenge ROS (Duan et al., 2011). When the ROS content exceeds the antioxidant capacity of cells, oxidative stress occurs and mediates cell structure damage to lipids, membranes, proteins, and DNA (Prasad et al., 2010). As a result, the mitochondria, especially the enzymes localized in the mitochondrial membrane and related to mitochondrial respiratory metabolism such as CCO, would be damaged and suffer a more serious decline in function (Choksia et al., 2007; Sedlák et al., 2010). It has been reported that the ROS content increased rapidly in many products during storage (Sabban-Amin et al., 2011; Wu et al., 2012). However, to the best of our knowledge, little has been reported as to whether ROS metabolism would be disturbed, leading to accelerated mushroom senescence during postharvest storage.

Cold storage (Dama et al., 2010), UV-C treatment (Jiang et al., 2010), modified atmosphere packaging (MAP) (Guillaume et al., 2010), and coatings (Jiang et al., 2012) have all been applied to extend mushroom shelf-life. Recently, Akram et al. (2012) reported that *P. eryngii* mushrooms treated with 1 kGy of irradiation could maintain optimum quality characteristics for 4 weeks. In addition, controlled atmosphere (CA) is a very effective technique for maintaining the quality of fruits and vegetables during postharvest periods (Escalona et al., 2006; Singh and Pal, 2008; East et al., 2009). However, to our knowledge, few studies on the effects of a controlled atmospheres, with high carbon dioxide and low oxygen conditions, on the shelf-life of *P. eryngii* have been completed.

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http://dx.doi.org/10.1016/j.postharvbio.2013.05.006

The objective of this work was to investigate the effects of high carbon dioxide and low oxygen treatments on the sensory characteristics, MDA content, $O_2^{\bullet-}$ production rate, and enzyme activities of SOD, POD, CAT, and CCO in *P. eryngii.*

2. Materials and methods

2.1. Materials and treatments

P. eryngii mushrooms were harvested from Xinghua Edible Mushroom Company in Jiangsu, China, and transported to the laboratory within 2 h. The mushrooms were selected for uniformity of shape and color, and freedom from mechanical damage and disease.

Forty mushrooms were randomly selected and placed in a container (Lock & Lock). For each gas mixture (for which there were five groups as follows: 2% O_2, 2% O_2 + 10% CO_2, 2% O_2 + 30% CO_2, 2% O_2 + 50% CO_2, and ambient air), three containers were connected as a group in a series and flushed continuously at a flow rate of 10 L h^{-1} and controlled with a closed-loop test system by CNROtech (Cnrotech Company, Tianjin, China). The gas mixture was humidified with water before going into the containers. The containers were closed and stored at 20–25 °C and 90–95% RH for 5 d. The required gas mixtures (2% O_2, 2% O_2 + 10% CO_2, 2% O_2 + 30% CO_2, 2% O_2 + 50% CO_2, and air) were ready-made and stored in cylinders provided by the Nanjing Special Gas Company. Mushroom samples were collected every day from storage, placed in liquid N_2, and stored at −80 °C.

2.2. Sensory evaluation

Sensory evaluation was conducted by 10 skilled panelists. The samples were scored using a 5-point hedonic scale (5 = like very much; 1 = dislike very much in relation to mushroom color, odor and overall acceptability).

2.3. MDA content determination

The MDA content was assayed on the basis of a method by Hodges et al. (1999) with some modifications. Frozen tissue (5 g) was homogenized with a mortar and pestle in 10 mL of 5% (w/v) trichloroacetic acid (TCA), and then centrifuged for 20 min (9000 × *g*, 4 °C). The supernatant (2 mL) was mixed with 2 mL of 10% TCA (including 0.67% 2-thiobarbituric acid) and the mixture was heated at 95 °C for 10 min. It was cooled immediately in an ice bath and centrifuged for 10 min (10,000 × *g*, 4 °C). The supernatant absorbance was recorded at 532 nm, and then corrected for non-specific turbidity by subtracting the absorbance at 600 nm and the interference generated by TBA–sucrose complexes at 440 nm. The MDA concentration was calculated with an extinction coefficient of 155 mM^{-1} cm^{-1}. The MDA content was expressed in μmol g^{-1} FW (fresh weight).

2.4. $O_2^{\bullet-}$ production rate determination

Frozen tissue (5 g) was homogenized with 10 mL of 50 mmol L^{-1} K-phosphate buffer (pH 7.5). The homogenate was centrifuged for 20 min (9000 × *g*, 4 °C). The resulting supernatant was collected and used as a crude extract for assaying the $O_2^{\bullet-}$ production rate and CAT, SOD, and POD activities.

The $O_2^{\bullet-}$ production rate was determined as described by Jiang et al. (2010). The crude extract (0.5 mL) was mixed with 0.5 mL of 50 mmol L^{-1} K-phosphate buffer (including 1 mmol L^{-1} hydroxylamine hydrochloride) and incubated at 25 °C for 60 min. A quantity of 3-aminophenylsulfonic acid (58 mmol L^{-1}) including 1-naphthylamine (7 mmol L^{-1}) was added, and the resulting mixture was incubated at 25 °C for 30 min. The resulting optical density was recorded immediately at 530 nm. A KNO_2 solution was used for the standard curve. The specific $O_2^{\bullet-}$ production rate was expressed in nmol min^{-1} g^{-1} FW.

2.5. SOD activity determination

SOD activity was assayed according to Jiang et al. (2010). The reaction mixture (3 mL) contained crude extract (1 mL), 750 μmol L^{-1} nitro-blue tetrazolium (NBT), 130 mmol L^{-1} L-methionine, 0.1 mmol L^{-1} EDTA, and 20 mmol L^{-1} riboflavin in 0.05 mol L^{-1} K-phosphate buffer (pH 7.8). The reaction was started by adding riboflavin and placing the tubes of reaction mixture under 4000 lx irradiance at 25 °C for 60 min. The absorbance was recorded at 560 nm. One unit of SOD enzyme activity was defined as the enzyme quantity that inhibited NBT photoreduction by 50% under assay conditions. The specific SOD activity was expressed in U g^{-1} FW.

2.6. POD activity determination

POD activity was measured according to the method of Singh and Singh (2012). The reaction mixture (3 mL) contained 33 mmol L^{-1} K-phosphate buffer (pH 6.1), 16 mmol L^{-1} guaiacol, 2 mmol L^{-1} H_2O_2, and 0.2 mL of enzyme extract. The increase in absorbance at 470 nm was monitored for 3 min with and without added enzyme extract. The POD activity was expressed in U g^{-1} FW.

2.7. CAT activity determination

CAT activity was determined according to Candan and Tarhan (2003). It was assayed in the reaction mixture (3 mL) containing 50 mmol L^{-1} K-phosphate buffer (pH 7.0), 10 mmol L^{-1} H_2O_2 and 0.5 mL enzyme. The decomposition of H_2O_2 was observed at 240 nm. CAT activity was expressed in U g^{-1} FW.

2.8. CCO activity determination

Samples were homogenized in ice-cold 0.25 mol L^{-1} sucrose (Prasad et al., 2010). The homogenate was centrifuged (800 × *g* 4 °C, 10 min) and then the consequent supernatant was centrifuged for 30 min (9000 × *g*, 4 °C). The resulting pellet, which was made up of mitochondria, was resuspended in sucrose solution to assay the CCO activity.

The CCO activity was determined according to Ishikawa et al. (2011). The cytochrome *c* (63 mmol L^{-1}) in the buffer solution (10 mmol L^{-1} Tris, 0.2 mmol L^{-1} EDTA, and 0.05% *n*-dodecyl β-D maltoside, pH 7.6) was incubated in 12.5 mmol L^{-1} ascorbic acid for 30 min at 20 °C to convert ferric cytochrome *c* to ferrous cytochrome *c*. The mitochondrial suspension (0.5 mL, 1 mg mL^{-1}) was added to 2 mL of the ferrous cytochrome *c* solution at 37 °C. The reaction was spectrophotometrically monitored at 550 nm. CCO activity was expressed as U g^{-1} on a protein basis. Protein content was determined according to the method of Bradford (1976), with bovine serum albumin as a standard.

2.9. Statistical analysis

Experiments were arranged in a completely randomized design in triplicate. The data were processed by using an analysis of variance (ANOVA). Duncan's multiple range test (SPSS version 20.0) was used to compare treatments at a significance level of 5%.