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Effects of high CO_2 in-package treatment on flavor, quality and antioxidant activity of button mushroom (*Agaricus bisporus*) during postharvest storage

Qiong Lin[a], Yuanyuan Lu[a], Jie Zhang[a], Wei Liu[a], Wenqiang Guan[b], Zhidong Wang[a,*]

[a] Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences/Key Opening Laboratory of Agricultural Products Processing and Quality Control, Ministry of Agriculture, Beijing 100193, China
[b] Tianjin Key Laboratory of Food Biotechnology, College of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China

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ABSTRACT

Button mushroom (*Agaricus bisporus*) is marketed for its good flavor and health benefits. However, the shelf life of fresh button mushroom is limited and quality is lost rapidly during storage. In this study, button mushrooms were treated with high CO_2 (95%–100%) at the time of sealing of the packages and the packages were ventilated after 0, 12, 24 and 48 h by puncturing the film at four corners. Results showed that 12 h high CO_2 treatment had a significant effect in reducing browning index (BI) and maintaining flavor of button mushroom during storage. In addition, the malonaldehyde (MDA) content was significantly inhibited while catalase (CAT) and peroxidase (POD) activities were significantly promoted by high CO_2 treatment. High CO_2 treatment increased antioxidant ability of button mushroom, which in turn maintained the flavor, quality and consumer acceptance of button mushroom during postharvest storage.

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1. Introduction

Button mushroom (*Agaricus bisporus*) is a popular edible mushroom, which is considered not only as nutritional vegetable but also as functional food due to the free radical scavenging and antioxidant activities (Guan et al., 2013; Wu et al., 2016). Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. The smell of button mushroom is primarily ascribed to an abundance of flavor volatiles, particularly 8-carbon compounds, with 1-octen-3-ol being reported to be one of the major 8-carbon components responsible for the typical mushroom smell (Dong et al., 2012). The secondary compounds in mushrooms are of great interest to consumers and are possible protective agents for human health.

However, mushrooms lose their quality rapidly during postharvest storage at ambient temperature because of their high moisture content and overall structure (Oliveira et al., 2012). Loss of qualities for mushrooms include browning, softening, cap development, off-flavor and secondary mold growth (Kim et al., 2006). Different treatment have been reported to extend the shelf life of mushrooms such as modified atmosphere packaging, washing with hydrogen peroxide and ozone treatment (Kim et al., 2006; Yuk et al., 2006).

Modified atmospheres are created by altering normal air composition, in order to provide an appropriate atmosphere surrounding the product for decreasing its deterioration rate and increasing its shelf life (Ares et al., 2007). It has been reported that modified atmospheres rich in CO_2 can modify respiration rate, energy metabolism, ethylene reaction and physiological changes in postharvest storage or package of many fresh products (Blanch et al., 2015; Lumpkin et al., 2015; Yi et al., 2016). However, excessive accumulation of CO_2 in modified atmosphere packages can damage the cell membrane and cause physiological injuries to the product, such as enzymatic browning and loss of firmness (Briones et al., 1992; Burton et al., 1987). Thus, the exact concentration and exposure time should be determined for specific fresh produce during modified atmosphere packaging or storage.

In this study, button mushrooms were treated with high CO_2 (95%–100%) at the time of sealing of the packages and then the packages were ventilated at 0, 12, 24 and 48 h using a new packaging method. The sensory evaluation, browning index (BI), flavor compounds, total phenolics, total antioxidant activity and

* Corresponding author.
E-mail address: wangzhidong@caas.cn (Z. Wang).

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antioxidant enzyme activities in treated and untreated mushrooms were measured. The objective is to select appropriate high CO_2 in-package treatment for button mushroom during postharvest storage.

2. Materials and methods

2.1. Plant material and CO_2 package

Button mushrooms (*Agaricus bisporus*) were harvested from a local edible fungus cultivation base in Beijing, China. Intact, closed and uniform mushrooms with a fresh white color were selected and pre-cooled for 12 h at 4 °C in a cold room. Afterwards, three mushrooms were placed in a 28 cm × 20 cm × 4 cm box and were sealed with high CO_2 (95%–100%) using low density polyethylene (PE) of 0.04 mm thickness (He Yuan Hua Feng Plastic Co., Ltd., China). The packages were stored at 4 °C with 85% relative humidity (RH) in refrigerators, and were punched with 4 holes (r = 0.3 cm) at each corner of the package after 0, 12, 24 and 48 h storage. Analyses were carried out on the first day and subsequently at 4 d intervals until 16 d. Mushroom caps were frozen by liquid nitrogen and stored at −80 °C for analysis.

2.2. Sensory evaluation

Sensory evaluation was conducted according to the method from Huang et al. (2008). Five key attributes, color, off-odour, cap shape, texture and consumer acceptance, were selected for evaluation. A scale of 0 to 10 was used in sensory evaluation: Color (White: 10-8, Slight browning: 8-6, Mild browning: 6-4, Heavy browning <4); Off-odour (No: 10-8, Slight: 8-6, Obvious: 6-4, Severe <4); Cap shape (Closed: 10-8, Slightly open: 8-6, Half open: 6-4, Totally open <4); Texture (Stretchy: 10-8, Slight soft: 8-6, Mild soft: 6-4, Severe soft <4); Consumer acceptance: (Intense: 10-8, Acceptable: 8-6, Discount: 6-4, Unacceptable <4). The sensory evaluations were carried out by ten panelists. The sensory score for each sample was calculated as a mean value. Fresh mushrooms were used as the control each time (score = 10).

2.3. Color analysis

The surface color of mushroom caps was measured with a WSC-S Colorimeter (Shanghai precision instrument Co. Ltd., China). 'L^*' (light/dark), 'a^*' (red/green) and 'b^*' (yellow/blue) values were used to calculate the browning index (BI) according to the following equation (Gao et al., 2014):

$$BI = [100(x - 0.31)]/0.172, \quad \text{where} \quad x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*).$$

2.4. Flavor compounds analysis

Flavor volatiles were measured according to the method from Costa et al. (2013) with modifications. Exactly 1 g of each sample was placed in a 10 mL vial and homogenized with 5 mL buffer solution, containing 20% $CaCl_2$ and 200 mM ethylene diamine tetraacetic acid (EDTA). Solid-phase micro-extraction (SPME) was carried out in the headspace mode by means of an AOC-5000 autosampler (Shimadzu, Kyoto, Japan) connected with the GC–MS-QP2010 Plus system (Shimadzu, Kyoto, Japan). The polydimethyl-siloxane/divinylbenzene (65 μm, 1 cm) fiber was held in the headspace for 30 min at 50 °C under agitation for extraction.

Analytes were then desorbed for 2 min at 250 °C in the GC injector with a splitless mode fitted with DB-WAX column (30 m × 0.25 mm × 0.25 μm). The column temperature was held at 40 °C for 2 min, and then increased to 120 °C with a temperature gradient of 3 °C min^{-1}, before being increased to 200 °C at 5 °C min^{-1} and held for 5 min.

Identification of the metabolites was conducted using the NIST/EPA/NIH Mass Spectral Library (NIST-11) of the GC–MS data system. Relative contents of the identified compounds were normalized by an internal standard method.

2.5. Malondialdehyde (MDA) content analysis

MDA content was measured according to the method described by Heath and Packer (1968) with modifications. Absorbencies of the aqueous phase at 450 nm, 532 nm and 600 nm were measured. The MDA content in the aqueous phase was calculated according to the following formula:

$$MDA\ (mol\ L^{-1}) = [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times 10^{-6}.$$

2.6. Total phenolics content analysis

The total phenolics content analysis was carried out using the method described by Gao et al. (2014).

2.7. Antioxidant assay

The 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assay were used as described by Lu et al. (2016).

2.8. Enzyme activity analysis

One gram mushroom powder was homogenized with 8 mL of 0.05 mol L^{-1} PBS at pH 6.8, then centrifuged at 8000*g* at 4 °C for 10 min. The supernatant was used to measure the activity of polyphenol oxidase (PPO) and peroxidase (POD). The PPO and POD activity was measured according to the method described by Hu et al. (2015).

Catalase (CAT) activity was analyzed according to the method described by Kan et al. (2010) with modifications. One gram of mushroom powder was homogenized with 5 mL 0.1 mol L^{-1} PBS (pH 7.0) and then centrifuged for 20 min at 8000*g* at 4 °C. The supernatant was used as the crude extract. The reaction mixture contained 0.02 mol L^{-1} H_2O_2 and crude enzyme. Catalase activity was determined as the amount of enzyme that caused an absorbance decrease of 0.01 at 240 nm in 1 min. The protein concentrations for all the enzyme assays were determined with Bradford and Williams (1977) method.

2.9. Statistical analysis

The figures were drawn using Origin 8.6 software (Microcal Software Inc., Northampton, MA, USA). Least significant difference (LSD) or Duncan's test at the 0.05 level were analyzed by SPSS Statistics 22 Software.

3. Results and discussion

3.1. Sensory evaluation

The assessment of produce quality is one of the core aspects of applied postharvest biology and the sensory evaluation of both the control and high CO_2 treated button mushrooms was conducted using a sensory score. The sensory scores all declined in button mushroom in all the treatments during storage, but the decline was reduced by the CO_2 packaging treatment. Compared with high CO_2

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