Postharvest Biology and Technology 139 (2018) 99–105

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

# Effects of UV-C treatment on browning and the expression of polyphenol oxidase (PPO) genes in different tissues of *Agaricus bisporus* during cold storage

Jing Lei[a], Bingjuan Li[a], Na Zhang[b], Ruixiang Yan[b], Wenqiang Guan[a,*], Charles S. Brennan[a,c], Haiyan Gao[d,*], Bo Peng[a]

[a] Tianjin Key Laboratory of Food Biotechnology, College of Biotechology and Food Sciences, Tianjin University of Commerce, Tianjin 300134, China
[b] Tianjin Key Laboratory for Postharvest Physiology and Storage of Agricultural Products, National Engineering and Technology Research Center for Preservation of Agricultural Products, Tianjin 300384, China
[c] Department of Wine, Food and Molecular Biosciences, Lincoln University, Lincoln, 7647, Canterbury, New Zealand
[d] School of Life Sciences, Shanghai University, Shanghai 200244, China

ARTICLE INFO

*Keywords:*
*Agaricus bisporus*
UV-C irradiation
Browning
PPO
Gene expression

ABSTRACT

Polyphenol oxidase (PPO) is the key enzyme leading to browning of *Agaricus bisporus* (*A. bisporus*). In order to determine the influence of UV-C treatment on polyphenol oxidase (PPO) of *A. bisporus* (Cv, 192), the visual quality and color, degree of browning, PPO activity and the expression of PPO genes in different tissues of *A. bisporus* during cold storage after 1.0 kJ/m$^2$ UV-C treatment were analysed. Results showed that the degree of browning and PPO activity of *A. bisporus* increased during 21 d of storage at 4 °C. UV-C treatment increased the PPO activity and exacerbated the browning of cap surface of *A. bisporus* during the beginning period of the storage. However, the degree of browning and the PPO activity in pileus, stipe and gill of *A. bisporus* were reduced by UV-C treatment. The expression of PPO genes (*Ab*PPO2, *Ab*PPO3 and *Ab*PPO4) in the peel of *A. bisporus* was stimulated by UV-C treatment during storage at 4 °C, while they were inhibited in pileus, stipe and gill. The patterns of *Ab*PPO2, *Ab*PPO3 and *Ab*PPO4 transcription level in different tissues of *A. bisporus* were similar to that of the degree of browning and PPO activity. *Ab*PPO1 gene transcription was not in accordance with the degree of browning and PPO activity. UV-C treatment inhibited browning of *A. bisporus* fruit body (pileus, stipe and gill) and the expression of *Ab*PPO genes.

## 1. Introduction

*A. bisporus* (button mushroom) is the most common edible cultivated mushroom in the world market (Mokochinski et al., 2015). The fruiting bodies of mushrooms have a very high respiration rate and a high water content, and they have no cuticle to protect them from physical or microbial attack or water loss. Therefore, mushrooms are prone to induced browning caused by enzymatic oxidation of phenolic compounds, bacterial disease occurance or mechanical damage during harvest, packaging, cold storage or transport (Guan et al., 2012; Brennan et al., 2000). The browning affects the quality of the mushrooms and leads to considerable economic losses in industry, and it has been a focus of research in recent years. The enzyme responsible for mushroom browning is polyphenol oxidase (PPO, EC 1.14.18.1) which is characterized with its chemical properties of cresolase and catecholase (Li et al., 2011). PPO activity and phenolic compounds content in different tissues of mushrooms are different before and after harvest (Bárbara et al., 2008). Gill of *A. bisporus* has the highest concentration of total phenolics, phenolic substrates (γ-glutaminyl-4-hydroxybenzene and γ-glutaminyl-3,4-dihydroxybenzene), followed by the stipe, while the lowest was present in the cap (Guan et al., 2016a,b). Two new PPO genes (*Ab*PPO3 and *Ab*PPO4, GenBank accession nos. GU936494 and GU936493) were identified to be main regulation gene for *A. bisporus* enzymatic browning (Li et al., 2011). PPO isozyme bands changed in cap, gill and stipe of *A. bisporus* as cold storage period prolonged according to our study (not published).

Ultraviolet (UV-C) irradiation has shown potential in the reduction of foodborne pathogens in different food matrices, and it is widely used as an alternative to chemical sterilization and microbial reduction in food products (Zhang et al., 2017; Lu et al., 2016; Ferrario et al., 2015; Falguera et al., 2012). UV-C can also have beneficial effect for antifungal enzymes induction and phytoalexin compounds formation in

* Corresponding authors.
*E-mail addresses:* gwq18@163.com (W. Guan), gaohy@shu.edu.cn (H. Gao).

https://doi.org/10.1016/j.postharvbio.2017.11.022
Received 17 August 2017; Received in revised form 23 November 2017; Accepted 28 November 2017
Available online 10 February 2018

fresh produce (Shama, 2007). Application of UV in the mushrooms as a postharvest treatment can inhibit microbial growth and browning lesion formation of mushrooms significantly (Guan et al., 2012, 2013), result in a significant increase in vitamin $D_2$ content in both caps and stipes without significantly affecting ergosterol content (Jasinghe and Perera, 2005; Guan et al., 2016a,b), inactivate PPO activity (Sampedro et al., 2014; Jiang et al., 2010), lead to the oxidation and conversion of phenolic compounds associated with browning of different tissues (Wu et al., 2016), and is proved to be suitable and safe for human (Simon et al., 2013). The mecanism for UV-C application on the browning of *A. bisporus* during cold storage has not been explored. To our knowledge, none of studies have reported the browning degree, PPO activity and gene expression of PPO isozymes in different tissues of UV-C irradiated *A. bisporus* during cold storage.

The objective of this study was to characterize the influence of UV-C radiation on the visual quality, browning degree, PPO activity and mRNA expression in different tissues of *A. bisporus*. For the first time, PPO activity and mRNA expression in different tissues of mushroom irradiated by UV-C were studied to explore the mechanism of mushroom browning during cold storage.

## 2. Materials and methods

### 2.1. Mushroom

Mushrooms (*A. bisporus*, Cv.192) were collected from a local mushroom production base in Tianjin. The ends of the stipes were removed according to the demand of commercial mushroom production. The mushrooms were then placed in foam container and quickly carried back to laboratory for UV-C irradiation.

### 2.2. UV-C irradiation

The UV-C irradiation treatment was applied using unfiltered General Electric 20 W germicidal lamps (G15T8). The lamp bank was horizontally suspended over the radiation vessel. Polystyrene trays were placed below the lamps at a distance of 0.3 m simulating a processing line. A wooden box covered with aluminium foil and supported by a metal framework enclosed the UV-C lamps, reflectors, and treatment area, providing UV protection for the operator. The mushrooms were placed in a single layer on the foam trays for treatment. The UV-C radiation dose was 1.0 kJ $m^{-2}$ (intensity 10 w $m^{-2}$, exposure time was 100 s). The intensity of the lamps was measured with a UVX × Radiometer Radiometer (Serial No. 2373, UVP INC., Upland, CA, USA). Mushrooms were exposed to UV-C with cap straight upwards for 100 s, and then turned over, to orientate the stipe straight to UV-C light for same time. Untreated mushrooms were used as the controls. After UV-C treatment, 30 mushrooms per sample were sealed in 0.2 m × 0.3 m bags of low density polyethylene (PE) ($3.0 \times 10^{-5}$ m thickness). They were then stored at 4 ± 0.5 °C, and the first day after cooling for 24 h was set as 1 d. Thirty replicate mushrooms were included in each treatment group. Three replicates from each treatment group were randomly selected and analyzed at 1, 7, 14 and 21 d. All the tissues of fruiting bodies of *A. bisporus* selected for analysis were immediately frozen in liquid nitrogen and stored at −80 °C until used.

### 2.3. Quality analysis and color

The sensory quality of whole mushrooms was evaluated using quality traits that included aroma, texture, color, and decay, and was examined in accordance with the standards in Table 1 (Caponigro et al., 2010). The coefficient of total visual quality was calculated based on the method described by Bernaś and Jaworska (2015).

Color (CIE L*) of mushroom cap peel was measured with a CR-400 Chroma Meter (Konica Minolta, Inc. Japan) using a $0.5 \times 10^{-2}$ m measuring aperture. The chroma meter was calibrated using a white tile. $D_{65}$ was the illuminant. L*(lightness), a*(reddish-greenish) and b* (yellowish–bluish) was analyzed based on the method described by Guan et al. (2012). One reading on the top surface of the cap was taken on each mushroom. Eight mushrooms for each replicate were measured and three replications for each treatment ateach time point.

**Table 1**
Evaluation standard for visually quality of mushrooms.

| Score | Aroma | Texture | Browning of surface | Decay |
|---|---|---|---|---|
| 9 | Full typical aroma | Very firm and turgid | None | No rotting |
| 7 | Moderately full | Firm | Slight browning | Rotting rate ≤ 1/5 |
| 5 | Moderate | Moderately firm | Moderate browning | 1/5 < Rotting rate ≤ 2/5 |
| 3 | Slight | Soft | Serious browning | 2/5 < Rotting rate ≤ 3/5 |
| 1 | None | Very soft | Poor | Rotting rate > 3/5 |

### 2.4. Browning degree assay

The browning degree of peel, pileus, stipe and gill was determined with the procedure described by Lee et al. (1990). Two grams of mushroom tissues was homogenized in 5 mL of ethanol solution (95%), and centrifuged at 8000 rpm for 10 min at 4 °C. The absorbance of the supernatant was measured at 410 nm on a spectrophotometer (Evolution 201, Thermo Fisher Scientific Inc., Madison, WI, USA). Browning degree was expressed as absorbance at 410 nm (OD 410 nm).

### 2.5. PPO activity assay

The extraction and assay of PPO activity were performed as described by Serradell et al. (2000). Tissue of mushroom peel, pileus, stipe and gill (1 g) was homogenized in 8 mL of phosphate buffer (0.1 mol/L, pH 6.5) and then was centrifuged at 8000 rpm for 15 min at 4 °C. The reaction mixture contained 1.0 mL 0.1% catechol (Sigma Aldrich, Shanghai, China), 1.0 mL PPO crude extract in 2.9 mL 0.1 mol/L phosphate buffer (pH 6.5). Changes in the absorbance at 420 nm were measured. One unit (U) of PPO activity was defined as a change of 0.01 at 420 nm per minute. PPO activity was expressed as U $kg^{-1}$ fresh weight.

### 2.6. Total RNA extraction, cDNA synthesis and quantitative real-time PCR (q-PCR)

After UV-C treatment, peel, pileus, stipe and gill of *A. bisporus* during storage were sampled and disrupted in liquid nitrogen using mortar and pestle stored at 4 °C. Total RNA was extracted and tested by RT-PCR using 18 s rRNA as a reference to analyze PPO gene mRNA expression in samples during storage. The four PPO genes in *A. bisporus* are *Ab*PPO1、 *Ab*PPO2、 *Ab*PPO3 and *Ab*PPO4 and specific nucleotide sequence information was retrieved from the GenBank databases.

Total RNA was isolated by the Trizol (Invitrogen, CA, USA) method (Shu et al., 2014). RNA concentration and purity were determined using a ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). Total RNA (2 μg) was reverse-transcribed into first-strand cDNA by using a TIANScript RT kit (Tiangen, Beijing, China).

Quantification of cDNA was performed on a step one plus real-time PCR system (Applied Biosystems, USA), and used into a TaqMan probe (Guan et al., 2016a,b). For PCR, all the listed primers and probes were designed using Premer3 software (version 0.4.0). Each reaction volume was 20 μL, comprised of 10 μL of real-time PCR Master Mix (Applied Biological Materials Inc., Canada), 10 μM of forward and reverse primers (primer pair mix, 1 μL) and 1 μL of appropriately diluted DNA or