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) (3.0×10^{-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 0.5×10^{-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

Table 2
Primers and probes for *Ab*PPO1、*Ab*PPO2、*Ab*PPO3 and *Ab*PPO4 genes.

Gene	Accession NO.	Forward primer	Reverse primer	Probe
*Ab*PPO1	X85113	CGACCATGTAGCCGAGGAA	GAGCACGCGCCACTGTAAA	ATTGCCGAGCACACGGGTTTACCTTC
*Ab*PPO2	X89382	AATGCTTTGTTGGATCAATTAACG	TCAAGGTCACGAGCAAGATAATG	CCGTGGGACTGCTCCCGAAACTT
*Ab*PPO3	GU936493	CTCCGACGAATTGCGCTAAT	TCCTTCAAGTAACGATGAACAGCTT	TTCAAGAAGGTTTCGTTCATCTCGATCGC
*Ab*PPO4	GU936494	CAGCATGCGGATCACGAA	CGACGACAAGAGACGTGAGTTC	ATGACGCTGTTCCGCTTTATGAGCCC

cDNA template, 0.4 μL probe and deionized water. No-template control (NTC) reactions were included in each plate to monitor potential formation of primer dimers. The amplification program was 50 °C for 2 min; 95 °C for 10 min; 40 cycles at 95 °C for 15 s, 60 °C for 1 min. All the samples, including no-RT and no-template controls, were analyzed in triplicate. The 18 s gene was used as the internal reference gene for normalizing the transcript profiles following the $2^{-\Delta\Delta Ct}$ method, relative to the calibrator sample (control, 1 d).

All primers and probes used in this research were synthesized by the Sangon DNA Technologies, Inc. (Shanghai, China) (Table 2).

2.7. Statistical analysis

The data was analysed by one way analysis of variance (ANOVA) and general linear model (GLM) followed by Newman–Keuls test. Statistical analysis was performed using SPSS 17.0 software.

3. Results and discussion

3.1. Effect of UV-C treatment on visual quality and color of mushrooms during storage

Mushroom quality is influenced by many parameters, including aroma, color, texture, microbial induced decay. Visual quality is the most intuitive factor when consumers purchase the mushrooms (Xu et al., 2016). The effect of UV-C treatment on visual quality of mushrooms is shown in Table 3.

The sensory quality of all the mushrooms decreased during storage. The assessment scores of the control samples showed the greatest degree of decrease (from 9.0 to 2.9 on a 9-point scale). While the scores decreased more slowly (from 8.8 to 4.5) in the UV-C treatment group. The peel of mushroom had slight browning after UV-C irradiation at 1 d of the storage. The sensory score of UV-C irradiated mushrooms was lower than those of the control group, but the difference was not significant ($P > 0.05$). During 7–14 d of the storage at 4 °C, the mushrooms began soften with slight odor of putrefaction emitted from a few fruiting bodies in the control group. Additionally several of them appeared to have brown spots. The sensory score of control had decreased to 4.7 by 14 d, which meant that the mushrooms had lost commercial value by 14 d. Meanwhile, the sensory score of UV-C treatment remained at 6.5, which was significantly higher than control ($P < 0.05$). At the end of storage, the sensory score of mushrooms in control group was 2.9 points, and the peel of mushroom had a lot of browning spots and cap opening. However the UV-C treatment reduced the degree of fruit softening and maintained color, with the sensory scores being significantly higher than control samples ($P < 0.05$).

Table 3
Changes of visual quality of mushrooms during 21 days at 4 °C.

Treatment	Storage time/d			
	1	7	14	21
CK	9.0 ± 0.0Aa	8.3 ± 0.2Aa	4.7 ± 0.3Ba	2.9 ± 0.3Ca
UV-C	8.8 ± 0.1Aa	8.5 ± 0.1Aa	6.5 ± 0.2Bb	4.5 ± 0.4Cb

Note: Each value represents mean ± standard deviation of three replicates. Different capital letters within the same line mean significant difference at $P < 0.05$, and lower case letters within the same column mean significant difference at $P < 0.05$ according to the Newman–Keuls' test. 9 = excellent; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible.

L*(lightness), depends on the reflectivity of the determined surface, and is an indicator of the luminosity of the sample surface (Du et al., 2009). The effect of UV-C treatment on L* values of mushroom cap surface during storage at 4 °C is shown in Fig. 1A. L* values of all the mushrooms decreased during storage, compared to the control mushrooms, those treated with UV-C had lower L* at initial treatment, but the L* values decreased more slowly in the UV-C treatment group than in the control group. The mushroom from the UV-C treatment group had a higher L* value than the control samples after 14 days storage. Smaller changes in a* and b* of mushrooms during cold storage were also observed for the UV-C treated samples compared to the control (Fig. 1B, C).

These results indicate that the use of UV-C treatment on mushrooms may cause slight damage to the mushroom cap surface tissue immediately after irradiation, while the protective effect of UV-C treatment was shown after a long storage term of 14 days at 4 °C. This finding is consistent with our previous findings (Guan et al., 2012). In addition, UV-C treatment could reduce the total bacteria including *pseudomonas tolaasii* that caused browning lesion of the mushrooms surface, which leading to the better retention of mushroom whiteness in later stage of cold storage (Brennan et al., 2000).

The changes of a* and b*values of the pileus surface are also seen in Fig. 1B and C, which increased by the UV-C treatment. Manzocco et al. (2009) also reported an increased browning in apple juice by UV-C light exposure. Despite the inactivation of PPO, direct photo-oxidation of its phenolic compounds browned the apple juice (Manzocco et al., 2009).

3.2. Effect of UV-C treatment on degree of browning in different tissues of mushrooms during storage

Browning affects consumers' acceptability and is one of the main causes of quality losses (Serradell et al., 2000). The browning degree in different mushroom tissues (peel, pileus, stipe, gill) is showed in Fig. 2.

During 21 d of storage at 4 °C, the degree of browning of pileus increased in all treatment groups, but the value of the UV-C treatment group was significantly lower than that of control ($P < 0.05$) (Fig. 2B).The browning degree in the stipe and the gill of *A. bisporus* (Fig. 2C, D) showed a similar pattern during storage at 4 °C and a lower value was observed than control by UV-C treatment stored for 7–21 d ($P < 0.05$). However, a profoundly higher browning degree was observed in the peel of UV-C exposed *A. bisporus* than that of the control during 14 d storage ($P < 0.05$). However, compared to the control, a reduction of browning degree of more than 0.02 OD_{410}/g was observed when stored for 21 d, which may suggest that UV-C treatment functioned to retard dark brown spots (lesions) of mushroom peel. A reduction of dark brown spots (lesions) by UV-C is probably attributed to the inactivation of *Pseudomonas tolaasii* (Guan et al., 2012), and inducement of phenolic/antioxidant compounds by UV-C treatment (Wu et al., 2016). These compounds also possess antimicrobial properties and are involved in disease resistance by contributing to the healing of wounds by lignification of cell walls around wound sites (Shahidi et al., 2011). These results are in agreement with those reported previously in different produce at a laboratory scale (Shahidi et al., 2011).

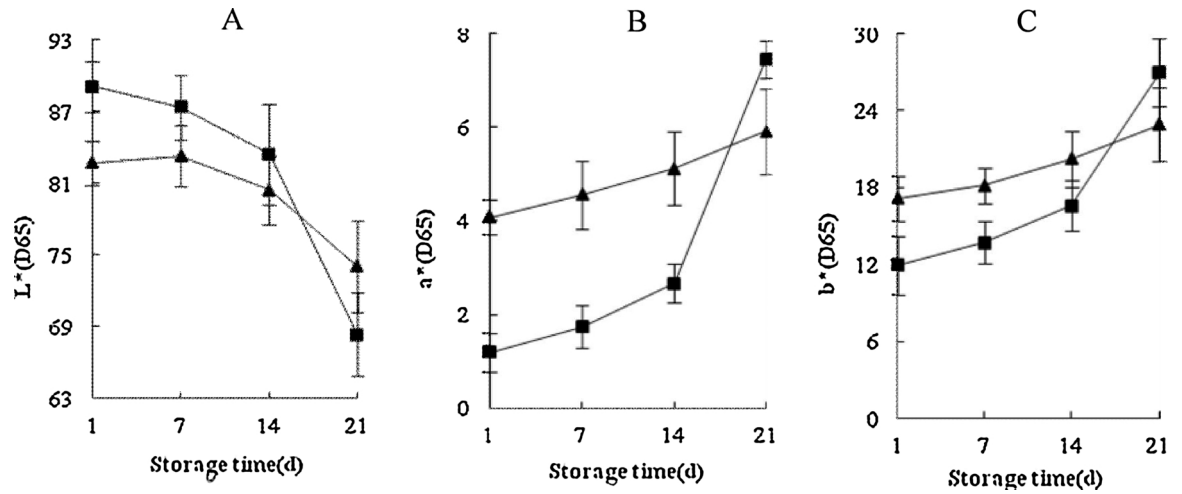

Fig. 1. Effect of UV-C dose on L*, a* and b*values of mushroom peel stored for 21 d at 4 °C. Vertical bars represent standard deviation (n = 10). ■ (CK) ▲ (UV-C).

3.3. Effect of UV-C treatment on PPO activity in different tissues of mushrooms during storage

The PPO enzymatic activities are depicted in Fig. 3. It shows that the differences of PPO activity in different mushroom tissue (the peel, the pileus, the stipe, the gill) were more distinct during storage.

The evolution of PPO activity in the peel of *A. bisporus* with UV-C treatment is shown in Fig. 3A, the PPO activity increased during storage (about 24 U g^{-1}) over 21 d at 4 °C, while PPO activity of the control group increased by more than 11 U when stored for 21 d. The PPO activity for UV-C treated sample was higher than control because UV-C light could cause damages on the outer tissues of mushrooms (Guan et al., 2012, 2013). The evolution of PPO activity of pileus with UV-C treatment is shown in Fig. 3B. The PPO activity increased during storage (about 50 U g^{-1}) at 4 °C. Compared to the control, a reduction of PPO activity of more than 17 U was observed in the treated samples stored for 14 d after UV-C treatment. Similar changes in PPO activity were observed in the stipe and the gill of *A. bisporus* (Fig. 3C, D). The PPO activity increased during storage for 21 d at 4 °C, and the changed magnitude of the PPO activity in the stipe (60 U g^{-1}) was higher than that in the pileus and the gill (10 U g^{-1}). Compared to the control, the PPO activity was shown to be lower in the stipe and gill after UV-C treatment. This may be due to inhibition expression of PPO gene after irradiation of UV-C. These results about PPO enzyme activity in pileus,

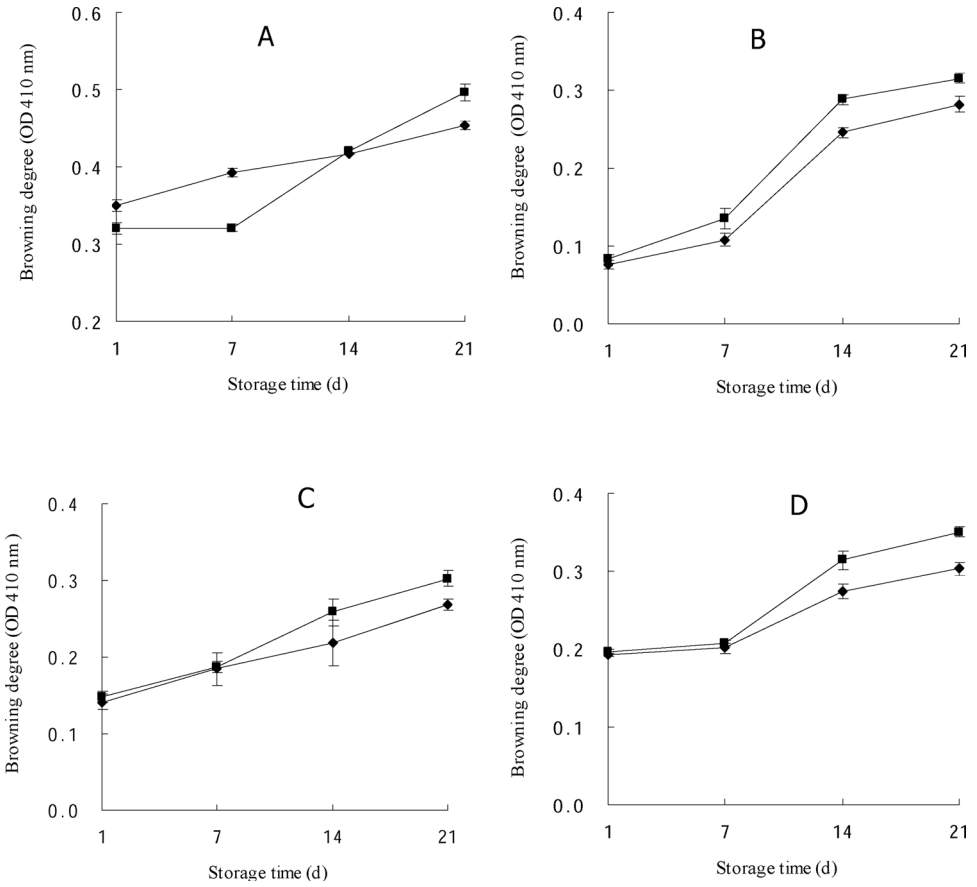

Fig. 2. Effect of UV-C treatment on browning degree of mushrooms stored for 21 d at 4 °C. A. peel, B. pileus, C. stipe, D. gill. Vertical bars represent standard deviation (n = 3). ■(CK)▲(UV-C).

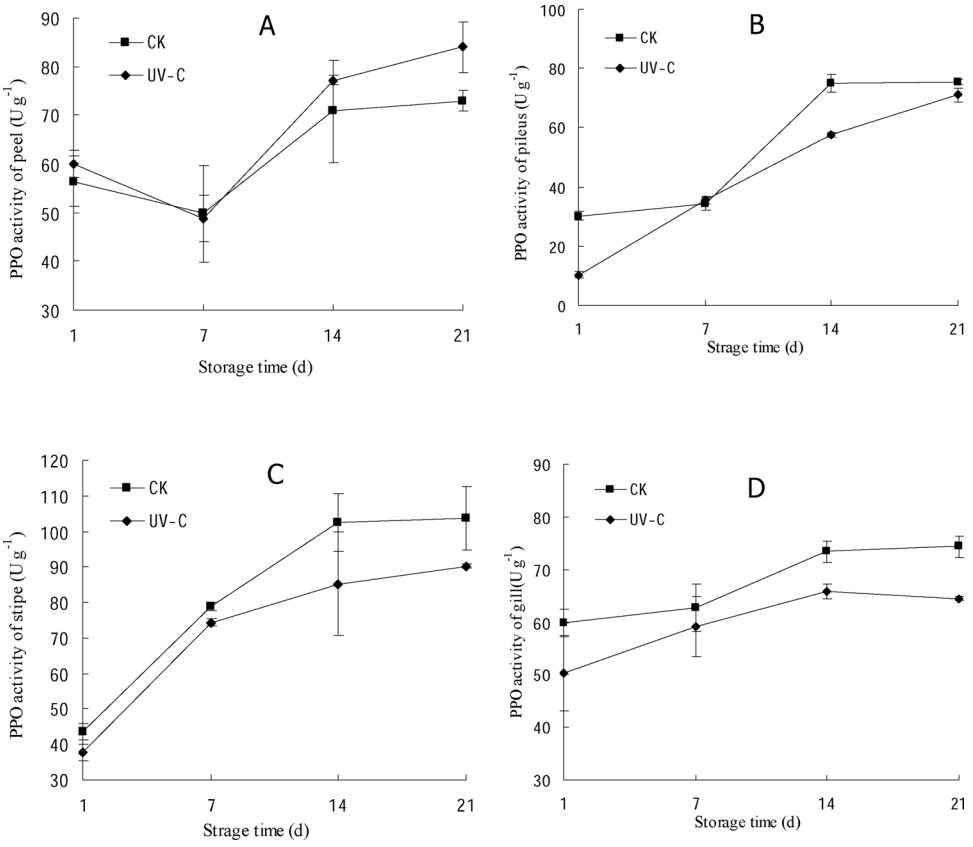

Fig. 3. Effect of UV-C treatment on PPO activity of mushrooms stored for 21 d at 4 °C. A. peel, B. pileus, C. stipe, D. gill. Vertical bars represent standard deviation (n = 3). ■ (CK)▲(UV-C).

gill and stipe of UV-C treated mushrooms are in agreement with those reported previously by Falguera et al. (2012) which suggested that a reduction of 58.7% in PPO enzyme activity after ultraviolet irradiation containing 12 U mL^{-1} in vitro of *A. bisporus* was achieved in the first 90 s, and the PPO enzyme activity was completely inactivated after 35 min of treatment. Our previous research found that the concentrations of phenolics (GHB, GDHB and Tyrosine) in *A. bisporus* tissues (including pileus, stipe and gill) were increased after UV-C treatment (Wu et al., 2016), which is probably due to the activating of some enzymes in phenypropanoid metabolism and inhibiting the acitivity of PPO by UV-C irradiation, which was consistent with our study.

It has been shown previously that enzyme activities increased following UV-C treatment (Sampedro et al., 2014; González-Aguilar et al., 2007). Researchers have suggested that the enzyme was either located in the thylakoid membrane-bound or soluble form of chloroplasts (Liu et al., 2015). It has been hypothesized that membrane-bound PPO can be easily activated, thereby contributing to browning and rotting (Orenes-Piñero et al., 2006). The enhancement in PPO activity has been shown to be related to the release of membrane-bound enzymes after suffering from UV-C irradiation damage or from separation of a part of the protein molecule and subsequent liberation of a second active site (Eisenmenger and Reyes-De-Corcuera, 2009).

3.4. Effect of UV-C treatment on expression of PPO genes in different tissues of mushrooms during storage

The mRNA levels of four PPO genes in the mushrooms (peel, pileus, stipe and gill) irradiated by UV-C stored for different times (1, 7, 14 and 21 d) at 4 °C (Fig. 4) were analyzed.

The transcription level of *Ab*PPO1 gene was higher under UV-C treatment than that of the control in the peel of mushrooms (Fig. 4A), and the same condition was found in other tissues (pileus, stipe and gill). However, the transcription level of *Ab*PPO1 gene was not in accordance with the browning degree (Fig. 2) and PPO activity (Fig. 3). This result indicated that *Ab*PPO1 might not be involved in mushroom browning.

The transcription level of *Ab*PPO2 gene increased in the peel of mushrooms during storage at 4 °C (Fig. 4A), and the level was significantly higher than control after treated with UV-C at the first 7 d ($P < 0.05$). This was probably due to the infection of *Pseudomonas tolaasii* (Soler-Rivas et al., 1999) which can induce the expression of *Ab*PPO2 gene (Soler-Rivas et al., 2001). However, during the storage of 14–21 d, the transcription level of *Ab*PPO2 gene significantly decreased in the UV-C treatment group compared to the control samples ($P < 0.05$). This was probably owing to the inactivation of *Pseudomonas tolaasii* (Guan et al., 2012). Similar conditions of *Ab*PPO2 transcription were obtained in other tissues (pileus, stipe and gill) of mushrooms (Fig. 4B–D). Thus, this result suggests that *Ab*PPO2 was involved in mushroom browning.

The transcription patterns of the *Ab*PPO3 and *Ab*PPO4 genes were similar (Fig. 4). The transcription levels of the two genes decreased until7d after storage at 4 °C in the pileus, stipe and gill of mushrooms. This observation is in agreement with previous reports by Li et al. (2011). The transcription of *Ab*PPO3 and *Ab*PPO4 genes were significantly inhibited in the stipe and pileus of mushrooms after UV-C irradiation ($P < 0.05$), which corresponds to the browning degree and PPO activity pattern. However, the transcription level of the two genes in the gill of mushrooms was higher in UV-C irradiation group than control ($P < 0.05$). It is known that the transcription of *Ab*PPO3 and *Ab*PPO4 genes can also be induced by inoculation with *Pseudomonas tolaasii* (Li et al., 2011). Thus, these results also indicate that *Ab*PPO3 and *Ab*PPO4 contributed to the browning of mushrooms during the storage at 4 °C.

4. Conclusions

UV-C irradiation has different effect on browning degree, PPO activity and the gene transcription level of PPO genes of different tissues

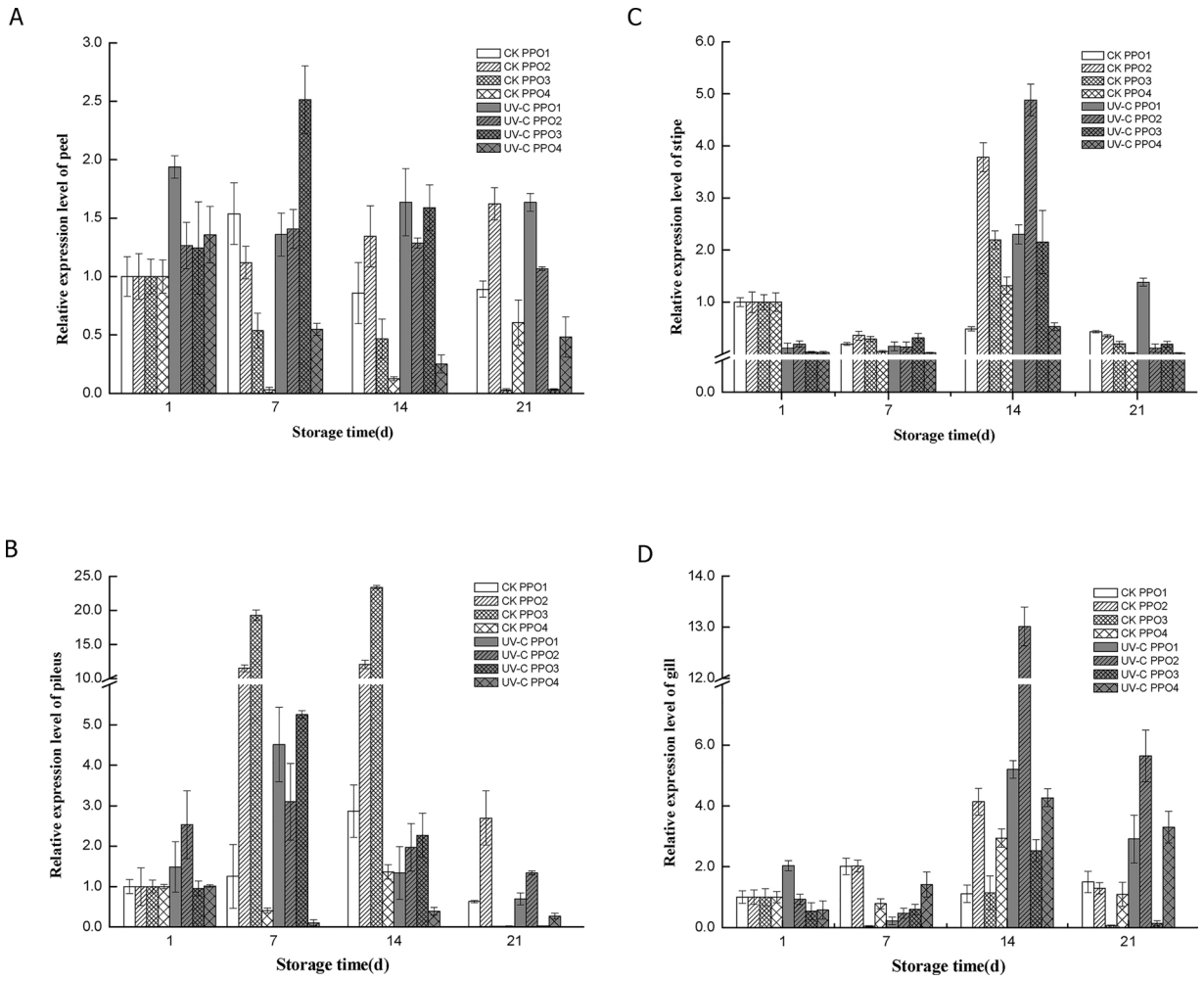

Fig. 4. Effect of UV-C treatment on expression PPO genes of mushrooms stored for 21 d at 4 °C. A.peel, B. pileus, C. stipe, D.gill. Vertical bars represent standard deviation (n = 3).

of mushrooms. The trend of browning degree, PPO activity, and the gene transcription level of PPO genes (*Ab*PPO2, *Ab*PPO3 and *Ab*PPO4, respectively) in mushroom tissues (pileus, stipe and gill) exposed to UV-C decreased during storage at 4 °C. UV-C effectively inhibited browning of mushrooms through a delayed peak in the appearance of PPO activity, and the inhibition of gene expression. Meanwhile, the expression pattern of *Ab*PPO2, *Ab*PPO3 and *Ab*PPO4 genes were associated with changes in browning degree and PPO activity, and this finding implies that these genes were the major contributors to browning of *A. bisporus*. The modeling browning and PPO gene mRNA expression by means of q-PCR need to be further studied to predict the storage life at the beginning of the storage.

Acknowledgments

This research was supported by Key Project of Tianjin Natural Science Foundation (Grant No. 16JCZDJC34000), the National Natural Science Foundation of China (Grant No. 31271949) and National Science and Technology Support Program (Grant No. 2015BAD16B02). We also would like to thank Dr. Brennan Margaret (Linclon University) for technical support.

References

Bárbara, R., Rosário, L., Paula, B.A., Rosa, M.S., Rui, F.G., Paula, B., Ines, Q., Patrícia, V., 2008. Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes. Food Chem. 110, 47–56.

Bernaś, E., Jaworska, G., 2015. Use of onion extract to prevent enzymatic browning of frozen *Agaricus bisporus* mushrooms. J. Int. J. Refrig. 57, 257–264.

Brennan, M., Port, G.L., Gormley, R., 2000. Post-harvest treatment with citric acid or hydrogen peroxide to extend the shelf life of fresh sliced mushrooms. LWT − Food Sci. Technol. 33, 285–289.

Caponigro, V., Ventura, M., Chiancone, I., Amato, L., Parente, E., Piro, F., 2010. Variation of microbial load and visual quality of ready-to-eat salads by vegetable type, season, processor and retailer. J. Food Microbiol. 27, 1071–1077.

Du, J.H., Fu, Y.C., Wang, N.Y., 2009. Effects of aqueous chlorine dioxide treatment on browning of fresh-cut lotus root. J. LWT-Food Sci. Technol. 42, 654–659.

Eisenmenger, M.J., Reyes-De-Corcuera, J.I., 2009. High pressure enhancement of enzymes: a review. J. Enzyme Microb. Technol. 45, 331–347.

Falguera, V., Pagán, J., Garza, S., Garvín, A., Ibarz, A., 2012. Inactivation of polyphenol oxidase by ultraviolet irradiation: protective effect of melanins. J. Food Eng. 110, 305–309.

Ferrario, M., Alzamora, S.M., Guerrero, S., 2015. Study of pulsed light inactivation and growth dynamics during storage of *Escherichia coli* ATCC 35218, *Listeria innocua* ATCC 33090, *Salmonella* Enteritidis MA44 and *Saccharomyces cerevisiae* KE162 and native flora in apple, orange and strawberry juices. J. Int. J. Food Sci. Technol. 50, 2498–2507.

González-Aguilar, G.A., Zavaleta-Gatica, R., Tiznado-Hernández, M.E., 2007. Improving postharvest quality of mango 'Haden' by UV-C treatment. J. Postharvest Biol. Technol. 45, 108–116.

Guan, W.Q., Fan, X.T., Yan, R.X., 2012. Effects of UV-C treatment on inactivation of *Escherichia coli* O157:H7, microbial loads, and quality of button mushrooms. J. Postharvest Biol. Technol. 64, 119–125.

Guan, W.Q., Fan, X.T., Yan, R.X., 2013. Effect of combination of ultraviolet light and hydrogen peroxide on inactivation of *Escherichia coli* O157:H7, native microbial loads, and quality of button mushrooms. Food Control. 12 (34), 554–559.

Guan, W.Q., Zhang, J., Yan, R.X., ShaoS.Q. Zhou, T., Lei, J., Wang, Z.D., 2016a. Effects of UV-C treatment and cold storage on ergosterol and vitamin D2 contents in different parts of white and brown button mushrooms. Food Chem. 210, 129–134.

Guan, X., Cai, Q., Zhang, W.J., Chen, Q., 2016b. Development of a real-time quantitative PCR assay using a TaqMan Minor groove binder probe for the detection of α-lactalbumin in food. J. Dairy Sci. 99, 1716–1724.

Jasinghe, V.J., Perera, C.O., 2005. Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D_2 by UV

irradiation. J. Food Chem. 92, 541–546.
Jiang, T., Jahangir, M.M., Jiang, Z., Lu, X., Ying, T., 2010. Influence of UV-C treatment on antioxidant capacity: antioxidant enzyme activity and texture of postharvest shiitake (*Lentinus edodes*) mushrooms during storage. Postharvest Biol. Technol. 56, 209–215.
Lee, C.Y., Kagan, V., Jaworski, A.W., Brown, S.K., 1990. Enzymatic browning in relation to phenolic compounds and polyphenoloxidase activity among various peach cultivars. J. Agric. Food Chem. 38, 99–101.
Li, N.Y., Cai, W.M., Jin, Q.L., Qin, Q.P., Ran, F.L., 2011. Molecular cloning and expression of polyphenoloxidase genes from the mushroom, *Agaricus bisporus*. J. Agric. Sci. China 10, 185–194.
Liu, F., Zhao, J.H., Wen, X., Ni, Y.Y., 2015. Purification and structural analysis of membrane-bound polyphenol oxidase from Fuji apple. J. Food Chem. 183, 72–77.
Lu, Y.Y., Zhang, J., Wang, X.T., Lin, Q., Liu, W., Xie, X.F., Wang, Z.D., Guan, W.Q., 2016. Effects of UV-C irradiation on the physiological and antioxidant responses of button mushrooms (*Agaricus bisporus*) during storage. J. Int. J. Food Sci. Technol. 51, 1502–1508.
Manzocco, L., Quarta, B., Dri, A., 2009. Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. J. Innov. Food Sci. Emerg. Technol. 10, 506–511.
Mokochinski, J.B., López, B.G.C., Sovrani, V., Dalla Santa, H.S., González-Borrero, P.P., Sawaya, A.C.H.F., Schmidt, E.M., Eberlin, M.N., Torres, Y.R., 2015. Production of *Agaricus brasiliensis* mycelium from food industry residues as a source of antioxidants and essential fatty acids. J. Int. J. Food Sci. Technol. 50, 2052–2058.
Orenes-Piñero, E., García-Carmona, F., Sánchez-Ferrer, A., 2006. Latent polyphenol oxidase from quince fruit pulp (*Cydonia oblonga*): purification, activation and some properties. J. Sci. Food Agric. 86, 2172–2178.
Sampedro, F., Phillips, J., Fan, X.T., 2014. Use of response surface methodology to study the combined effects of UV-C and thermal processing on vegetable oxidative enzymes. LWT − Food Sci. Technol. 55, 189–196.
Serradell, M.A., Rozenfeld, P.A., Martínez, G.A., Civello, P.M., Chaves, A.R., Añón, M.C., 2000. Polyphenoloxidase activity from strawberry fruit (*Fragaria ananassa*, Duch., cv Selva): characterisation and partial purification. J. Sci. Food Agric. 80, 1421–1427.
Shahidi, F., Chandrasekara, A., Zhong, Y., 2011. Bioactive phytochemicals in vegetables. In: Sinha, N.K. (Ed.), Handbook of Vegatables and Vegetable Processing. Blackwell-Publishing Ltd., Ames Iowa, pp. 125–158.
Shama, G., 2007. Process challenges in applying low doses of ultraviolet light to fresh produce for eliciting beneficial hormetic responses. J. Postharvest Biol. Technol. 44, 1–8.
Shu, C.W., Sun, S., Chen, J.L., Chen, J.Y., Zhou, E.X., 2014. Comparison of different methods for total RNA extraction from sclerotia of *Rhizoctonia solani*. J. Electron. J. Biotechnol. 17, 50–54.
Simon, R.R., Borzelleca, J.F., DeLuca, H.F., Weaver, C.M., 2013. Safety assessment of the post-harvest treatment ofbutton mushrooms (*Agaricus bisporus*) using ultraviolet light. Food Chem. Toxicol. 56, 278–289.
Soler-Rivas, C., Jolivet, S., Arpin, N., Olivier, J.M., Wichers, H.J., 1999. Biochemical and physiological aspects of brown blotch disease of *Agaricus bisporus*. J. FEMS Microbiol. Rev. 23, 591–614.
Soler-Rivas, C., Möller, A.C., Arpin, N., Olivier, J.M., Wichers, H.J., 2001. Induction of a tyrosinase mRNA in *Agaricus bisporus*, upon treatment with a tolaasin preparation from *Pseudomonas tolaasii*. J. Physiol. Mol. Plant Pathol. 58, 95–99.
Wu, X.L., Guan, W.Q., Yan, R.X., Lei, J., Xu, L.X., Wang, Z.D., 2016. Effects of UV-C on antioxidant activity, total phenolics and main phenolic compounds of the melanin biosynthesis pathway in different tissues of button mushroom. J. Postharvest Biol. Technol. 118, 51–58.
Xu, Y.Y., Tian, Y., Ma, R.N., Liu, Q.H., Zhang, J., 2016. Effect of plasma activated water on the postharvest quality of button mushrooms, *Agaricus bisporus*. J. Food Chem. 197, 436–444.
Zhang, J., Yuan, L., Liu, W., Lin, Q., Wang, Z.D., Guan, W.Q., 2017. Effects of UV-C on antioxidant capacity, antioxidant enzyme activity and colour of fresh-cut red cabbage during storage. J. Int. J. Food Sci. Technol. 52, 626–634.