Postharvest Biology and Technology 118 (2016) 51–58

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Effects of UV-C on antioxidant activity, total phenolics and main phenolic compounds of the melanin biosynthesis pathway in different tissues of button mushroom

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ARTICLE INFO

Article history:
Received 6 December 2015
Received in revised form 6 March 2016
Accepted 19 March 2016
Available online 28 March 2016

Keywords:
Agaricus bisporus
UV-C irradiation
Antioxidant activity
Phenolic compounds

ABSTRACT

The effect of 1.0 kJ/m^2 ultraviolet-C (UV-C) light on antioxidant activity, total phenolics and main phenolic compounds associated with browning of button mushroom (*Agaricus bisporus*) tissues during 21 days storage at 4 °C was studied. The distribution of antioxidant activity and phenolic compounds varied significantly in different parts of button mushroom. The highest DPPH scavenging activity was investigated in the stipe, and the gill contained the highest concentration of total phenolics, γ-glutaminyl-4-hydroxybenzene (GHB) and γ-glutaminyl-3,4-dihydroxybenzene (GDHB). UV-C irradiation enhanced antioxidant activity, total phenolics and phenolic compounds in inner cap, stipe, gill and whole mushroom during storage compared to control. However, UV-C irradiated mushroom reduced the total phenolics, GHB, GDHB and tyrosine contents of the peel fraction compared to the control sample during the storage. Application of 1.0 kJ/m^2 UV-C treatment could effectively induce the increase of antioxidant capacity and compounds in whole mushroom during storage, and likely lead to the oxidation and conversion of phenolic compounds in peel tissue which participate in the formation of melanin associated with browning of mushrooms.

1. Introduction

Mushrooms have been widely used as a human food for centuries and have been appreciated for their healthy properties. They have proven to be effective as antiinflammatory, antitumor, antibacterial, antioxidant and antiviral agents (Barros et al., 2007). Among the biologically active substances present in mushrooms, phenolics have attracted much attention due to their high antioxidant activity (Palacios et al., 2011). For example, mushroom compounds have been linked to the prevention and treatment of more than one hundred diseases including several kinds of cancer and diabetes by mediating oxidative stress-induced damage (Ferreira et al., 2009). Hence, natural products with antioxidant activity, in particular mushrooms, are used with increasing interest in the antioxidative role of functional foods or nutraceutical products (Ferreira et al., 2009; Reis et al., 2011). Various genera of edible mushrooms could be a viable and economical source of antioxidants in the diet. *Agaricus bisporus*, specifically portobello and crimini mushrooms, shows high antioxidant potential relative to the other mushrooms tested (Dubost et al., 2007). It is well known that browning is a major cause of quality losses of mushrooms and decreases the commercial value of the products. Browning of button mushroom during picking, handling, and storage are considered to be mediated by polyphenol oxidase (PPO) enzyme, which catalysed oxidation of phenolic substrates such as tyrosine, γ-glutaminyl-4-hydroxybenzene (GHB) and γ-glutaminyl-3,4-dihydroxybenzene (GDHB) into quinones (Soulier et al., 1993). Quinones are then converted into a dark pigmented chemical substance, melanin (Mohapatra et al., 2008).

Ultraviolet (UV) irradiation is generally classified as UV-C (200–280 nm), UV-B (280–320 nm) and UV-A (320–400 nm). The shortest wavelengths of the UV spectrum are also the most energetic ones, and most of the information on the biological effects of UV-radiation is derived from experiments using artificial

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http://dx.doi.org/10.1016/j.postharvbio.2016.03.017
0925-5214/

UV-C, particularly 254 nm irradiation (Shama, 2007). As a postharvest treatment on fresh produce, UV irradiation has been proven beneficial to reduce respiration rates, control rot development, induce disease resistance and delay senescence and ripening in different fruits and vegetables (Jiang et al., 2010; Shama, 2007; Park and Kim, 2015). Several studies have correlated UV-C induced disease resistance to the biosynthesis of defensive secondary metabolites, mainly phenolics, in treated tissue (Charles et al., 2008; Park and Kim, 2015). Hormetic phenomena manifest themselves after exposure to UV-C at periods of time ranging from hours to days, and occur throughout the entire fruit significantly (Shama, 2007). At the same time, UV-C irradiation is an environmental-friendly technology used in food products to reduce microbial contamination and improve food safety, and have been approved for use as a disinfectant for surface treatment of food (US-FDA, 2002). Our previous research showed that UV-C radiation applied at proper doses (lower than 1.0 kJ/m^2) was effective in reducing *Escherichia coli* O157:H7 and microbial loads and inhibiting browning lesion development on button mushroom surface, and may potentially extend storage periods without causing deterioration of nutritional quality of button mushrooms (Guan et al., 2012, 2013). UV-C radiation also could induce higher vitamin D_2 concentration in mushrooms significantly (Viraj and Conrad, 2005; Simon et al., 2013). The chemical composition of different tissues of mushroom varies in relation to different structures and morphologies. To our knowledge, no reports have investigated the effect of UV-C illumination on phenolic substrates (GHB, GDHB and tyrosine) involved browning and antioxidant capacity of different mushroom tissues during cold storage.

The objective of this work was to evaluate the effects of UV-C irradiation on total phenolic content and DPPH scavenging activity in peel, inner cap, gill, stipe of mushroom during cold storage, which would help to elucidate the mechanism of UV-C induced antioxidant capacity. For the first time, the browning phenolic substrates in different tissues of mushroom irradiated by UV-C were studied in order to explore the mechanism of mushroom browning.

2. Materials and methods

2.1. Sample preparation

Button mushroom material was used in this study and obtained from Tianjin Minzong Edible Fungi Co, Ltd., China. The mushrooms were immediately transferred to the laboratory. Whole, white, closed cap mushrooms with caps of 4–5 cm in diameter were chosen for each trial and treated within 6 h after harvest.

A germicidal UV-C irradiator containing two 0.6 m UV-C emitting bulbs with a peak emission at 254 nm (Ruisente Ultraviolet CO., Tianjin, China) was used. The UV-C dose rate was determined by UVX Digital Radiometer (Serial No. 2373, UVP Inc, Upland, CA, USA), and the intensity of the UV-C lamp is 1 W m^{-2} at a distance of 0.2 m. Mushrooms were illuminated with the UV-C lamp from the caps and stem sides for 100 s, respectively. Each side of mushrooms was subjected to irradiation treatment at a dosage of 1.0 kJ/m^2. Irradiation experiments were carried out at ambient temperature (ca. 25 °C).

After UV-C irradiation, mushrooms were packed in a plastic HDPE film bags(30 cm × 20 cm × 0.03 mm)perforated with 2 holes (0.06 m in diameter). The packed mushrooms were then stored at 4 °C. Control mushrooms (CK) were handled similarly without UV-C exposure. At 0–3 weeks of storage, antioxidant activities, total phenolics, γ-glutaminyl-4-hydroxybenzene (GHB), GDHB and tyrosine of mushroom were measured. For analysis of nutritional properties, peel, inner cap, stipe, and gill were carefully separated with a sharp stainless steel blade, and these four parts were separately frozen in liquid nitrogen and stored at −76 °C. The samples were freeze-dried with a vacuum freeze dryer (FD-8, Boyikang Co., Beijing, China), and were ground into powder with a mortar and pestle before the extraction and analysis. Each experiment was conducted independently 3 times (n = 3).

2.2. Determination of antioxidant activities

The free radical scavenging activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (CAS17912-87-7, Sigma, USA) following the protocols of Bougatef et al. (2009) with little modification. One milliliter of each sample solution was added to 1 mL of 0.1 mM DPPH dissolved in ethanol. The mixture was shaken and left for 30 min at room temperature, and the absorbance of resulting solution was read at 517 nm. A lower absorbance represents a higher DPPH scavenging activity. The scavenging effect was expressed as shown in the following equation:

DPPH scavenging activity (%) = [(absorbance of control − absorbance of sample)/absorbance of control] × 100.

The control was conducted in the same manner, except that ethanol was used instead of sample. 70% ethanol was used as the blank.

2.3. Extraction and determination of total phenolics

Freeze-dried mushroom powder (5×10^{-5} kg) was mixed with 10 mL of 70% ethanol. After 20 min treatment with 45 kHz and 400 W ultrasonic treatment at 60 °C, the mixture was centrifuged for 15 min with a speed of 10000*g* at 4 °C. The pooled supernatant was harvested for total pheolics and antioxidant activities analysis.

Total phenolic content of the material was measured using the Folin–Ciocalteu colorimetric method with little modification. The extract (0.5 mL) used for the antioxidant activity assay was mixed with 1 mL of Folin–Ciocalteu reagent (Shanghai Herochem, Shanghai, China), and incubated for 5 min at 25 °C. Then 3 mL of 20% sodium carbonate was added. Absorbances (at 760 nm) were recorded for the mixtures after 120 min incubation at 25 °C. The results were expressed as g of gallic acid equivalents per kilogram of dried mushroom.

2.4. Extraction of phenolic compounds and HPLC analysis

Sample preparation for analysis of phenolic compounds was conducted according to Soler-Rivas et al. (1998). Freeze-dried mushroom powder 5×10^{-4} kg) from each sample was mixed with 15 mL of 0.5% (w/v) sodium bisulphite in 1% (v/v) acetic acid solution (Jolivet et al., 1999). The mixtures were homogenized with a Vortex (VORTEX1, IKA, DE) for 2 min and centrifuged (10000*g* for 10 min at 4 °C). The supernatant containing the phenols was collected and the pellet was used for a second extraction with 10 mL of the solution. After the second centrifugation, the supernatants were pooled, filtered through a 0.45 μm nylon membrane filter (Jinlong, Tianjin, China). The filtered sample (10 μL) was injected into a HPLC (SSIEVANS, USA) system equipped with a UV detector (Pharmacia) and eluted through a reverse phase HPLC column (Hypersil BDS C18 5 μm 250 × 4.6 mm, Thermo) using 1.8% (v/v) tri-fluoroacetic acid (TFA) (A) and 10% (v/v) acetonitrile in 1.8% (v/v) TFA (B) as the mobile phase following a linear gradient of 95/5 (A/B) to 54/46 (A/B) from 0 to 40 min. The UV detection was performed at 230 nm for tyrosine and 245 nm for GHB and GDHB. Tyrosine, GHB and GDHB were identified on the basis of their retention times compared to those of standards (tyrosine: CAS60-18-4, Sigma, USA; GHB: CAS30383-24-2, Extrasynthese, FR; GDHB: CAS58298-77-4, Extrasynthese, FR), and quantification was done by using a calibration curve. Obtained

values were the average of three replicates per data point. The results were expressed as gram per kilogram of dried mushroom.

2.5. Statistical analysis

Statistical analysis was performed using SPSS 17.0 software. The evaluation of statistical significance was determined by ANOVA, followed by Newman–Keuls test. The level of significance was set at $P < 0.05$ (95% statistical confidence level).

3. Results and discussion

3.1. Antioxidant activity, total phenolic and phenolic substrates in different tissues of button mushroom

Antioxidant molecules can quench DPPH free radicals by providing hydrogen atoms or by electron donation. DPPH scavenging activity test is a commonly employed assay in antioxidant studies (Ferreira et al., 2007). The nutritional components and antioxidant content in fruits and vegetables vary with tissue types, for example, the biological properties, phenolic compounds and antioxidant capacity were different in different tissues of the mushroom (Bárbara et al., 2008; Tim et al., 2001). DPPH scavenging activity, total phenolic, GHB, GDHB and tyrosine of different parts of button mushroom were illustrated in Table 1.

Significant differences ($P < 0.05$) in the distributions of DPPH scavenging activity, total phenolics content, GHB and GDHB were observed between mushroom tissues (Table 1). However tyrosine content did not show significant differences among the mushroom tissues. The highest DPPH scavenging activity was found in the stipe, followed by the peel, while the lowest was present in the gill. Other researchers have found that the cap of five species of Portugal wild mushrooms had greater antioxidant effect than the stipe, while one stipe methanolic extracts from wild mushroom (*Amanita rubescens*) exhibiting greater antioxidant effect than cap (Ferreira et al., 2007; Bárbara et al., 2008). Freshly harvested mushrooms contained the highest concentration of total phenolics, GHB and GDHB in the gill, followed by the stipe, while the lowest was present in the peel and inner cap respectively.

Previous research has illustrated that the antioxidant activity of plant materials is correlated with the content of phenolic compounds, and that the major contribution on the antioxidant activities was the amount of polyphenolic compounds in the foods (Choi et al., 2006). Barros et al. (2007) reported that Portuguese wild edible mushrooms *Leucopaxillus giganteus* contained the highest content of total phenols and antioxidant capability in all the antioxidant activity assays compared with that in other species. It was also reported that total phenolic content in the mushrooms was significantly correlated with ORAC values (Dubost et al., 2007). However, in this study, DPPH scavenging activity of different mushroom tissues correlated neither with the total phenoic amount nor with the GHB, GDHB or tyrosine content of mushroom tissues, which may indicate that each phenolic compound or a group of them possess different antioxidant activity (Palacios et al., 2011). Palacios et al. (2011) also investigated that inhibition extent for linoleic acid oxidation and observed that there was no correlation with the total phenolic amount nor with the flavonoid content. This may be attributed to variations in the radical or oxidant sources or variations in analytical methodology (Wang et al., 2015). More than one methods have been used to study the complex antioxidant activities (Reis et al., 2012). At the same time, it should be noted that antioxidants are not only phenolic based, but other compounds (e.g., phytic acid, tocopherol, etc.) might also contribute for the antioxidant activity (Erkan et al., 2008). It is possible that some compounds of low quantities may have greater antioxidative power than other phenolic compounds which are more abundant (Palacios et al., 2011). As such it can be hypothesized that some compounds in stipe of button mushroom may possess high levels of radical scavenging ability. Therefore, more methods need to be applied to detect antioxidant activity of mushrooms, and more compounds that contribute to the antioxidant activity need to be further identified and justified.

3.2. Effect of UV-C irradiation on antioxidant activity in different tissues of button mushroom during storage

All tissues of UV-C treated mushrooms had higher DPPH scavenging activity than the controls ($P < 0.05$) during storage (Fig. 1). Both control and UV-C treated inner cap, stipe and gill showed decreasing trend in DPPH scavenging activity during the initial 7 days. After 7 days, the DPPH scavenging activity increased, before decreasing just prior to the end of storage. UV-C treated peel showed increasing trend in DPPH scavenging activity during the initial 7 days, which may result from the direct irradiation of UV-C on the peel of mushroom.

Previous studies regarding the effects of UV-C irradiation on antioxidant activity of fruits and vegetables have reported an increase in antioxidant capacity. Increase in antioxidant capacity of inner cap and gill might be as part of the defense mechanism produced by mushroom tissues in reacting to stress induced by UV-C exposure despite the low penetrating ability of UV-C. Jiang et al. (2010) found that UV-C (4 kJ/m^2) irradiation reduced reactive oxygen species (ROS) production and induced higher activities of antioxidant enzymes in shiitake mushrooms during cold storage. The finding of this study is also in agreement with previous studies which reported that UV-C treatment increased antioxidant activity of fresh fruits and vegetables such as strawberry (Erkan et al., 2008), peeled garlic (Park and Kim, 2015) and fresh-cut mango (González-Aguilar et al., 2007). Therefore, we may suggest that UV-C can initiate a stress response in button mushroom, and the response would manifest throughout the entire mushroom and elicit the changes in different tissues observed in this study.

Table 1
DPPH scavenging activity, total phenolic, GHB, GDHB and tyrosine of different parts of button mushroom.

Mushroom tissues	DPPH scavenging activity (%)	total phenolic (g/kg)	GHB(g/kg)	GDHB (g/kg)	Tyrosine(g/kg)
peel	62.56 ± 0.9b	4.40 ± 0.11b	0.42 ± 0.03a	3.58 ± 0.26b	0.64 ± 0.01a
inner cap	59.14 ± 1.32ab	3.50 ± 0.04a	0.74 ± 0.03a	2.06 ± 0.14a	0.80 ± 0.30a
gill	57.22 ± 0.28a	6.87 ± 0.33d	3.50 ± 0.51c	4.19 ± 0.23c	0.48 ± 0.08a
stipe	69.52 ± 4.57c	5.86 ± 0.14c	2.48 ± 0.06b	4.17 ± 0.17c	0.50 ± 0.15a
Whole	61.57 ± 1.27	4.17 ± 0.08	1.04 ± 0.26	2.65 ± 0.19	0.52 ± 0.09

Note: Each value represents mean ± standard deviation of three replicates. Means followed by the same letters within same column are not significantly different ($P < 0.05$) according to the Newman–Keuls' test.

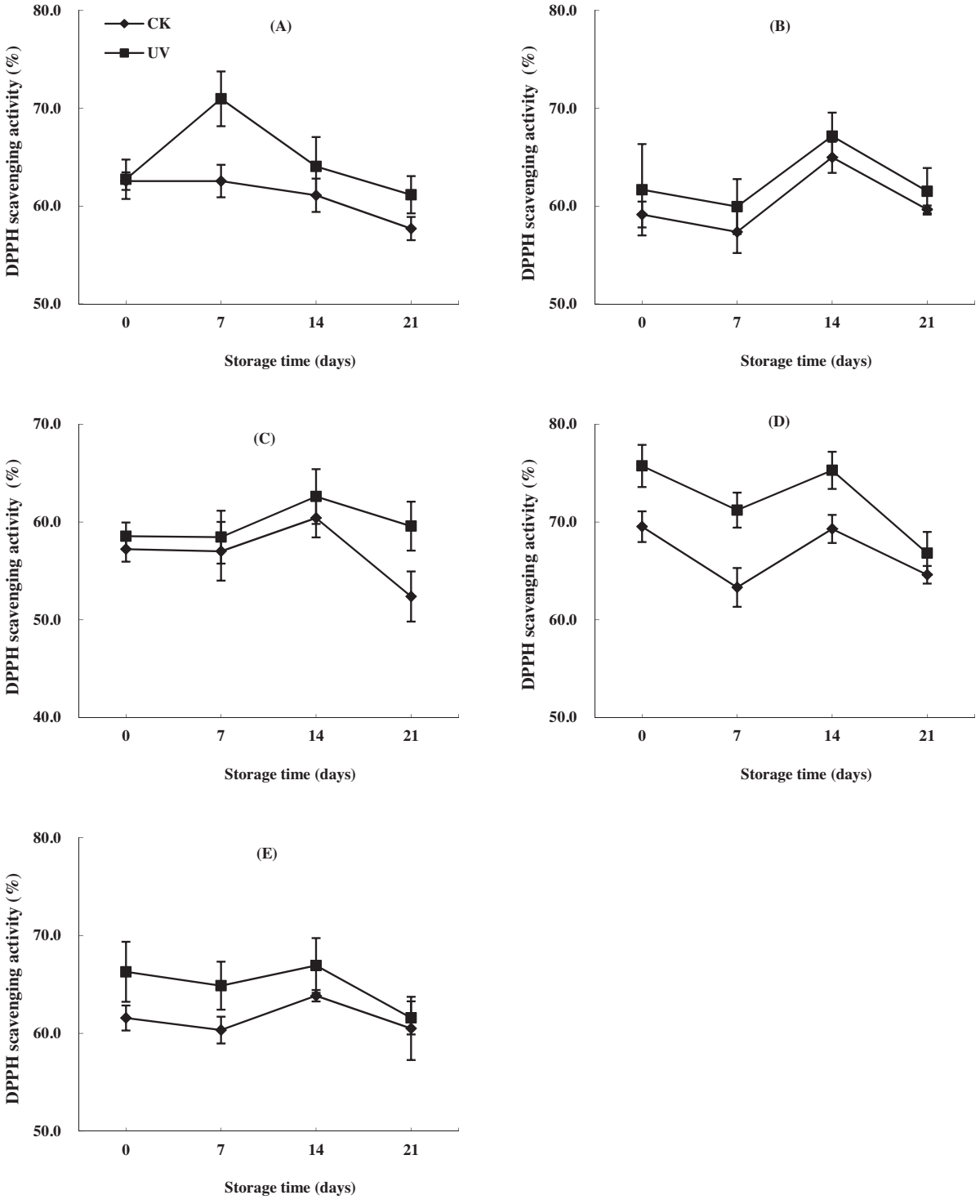

Fig. 1. Effect of UV-C treatment on DPPH scavenging activity of peel (A), inner cap (B), gill (C), stipe (D) and whole fruiting body (E) of button mushrooms stored at 4 °C. Values are means of standard errors (n = 3).Vertical bars indicate the standard errors of the means, where they exceed the size of the symbol used.

3.3. Effect of UV-C irradiation on total phenolic content in different tissues of button mushroom during storage

The changes in total phenolic content in different tissues of control and UV-C treated mushrooms are shown in Fig. 2.

Total phenolic contents in the UV-C treated whole mushroom and different tissues including inner cap, stipe and gill were higher than the control during storage ($P < 0.05$). Both the control and the UV-C treated inner cap, stipe and gill showed decreasing trend in total phenolic content during the initial 7 days, which might be caused by the stress reaction of phenylalanine ammonialyase (González-Aguilar et al., 2007). The total phenolic content slightly increased in the following 7 days, before decreasing by the end of storage. Total phenolic content reached the highest at 14th day and

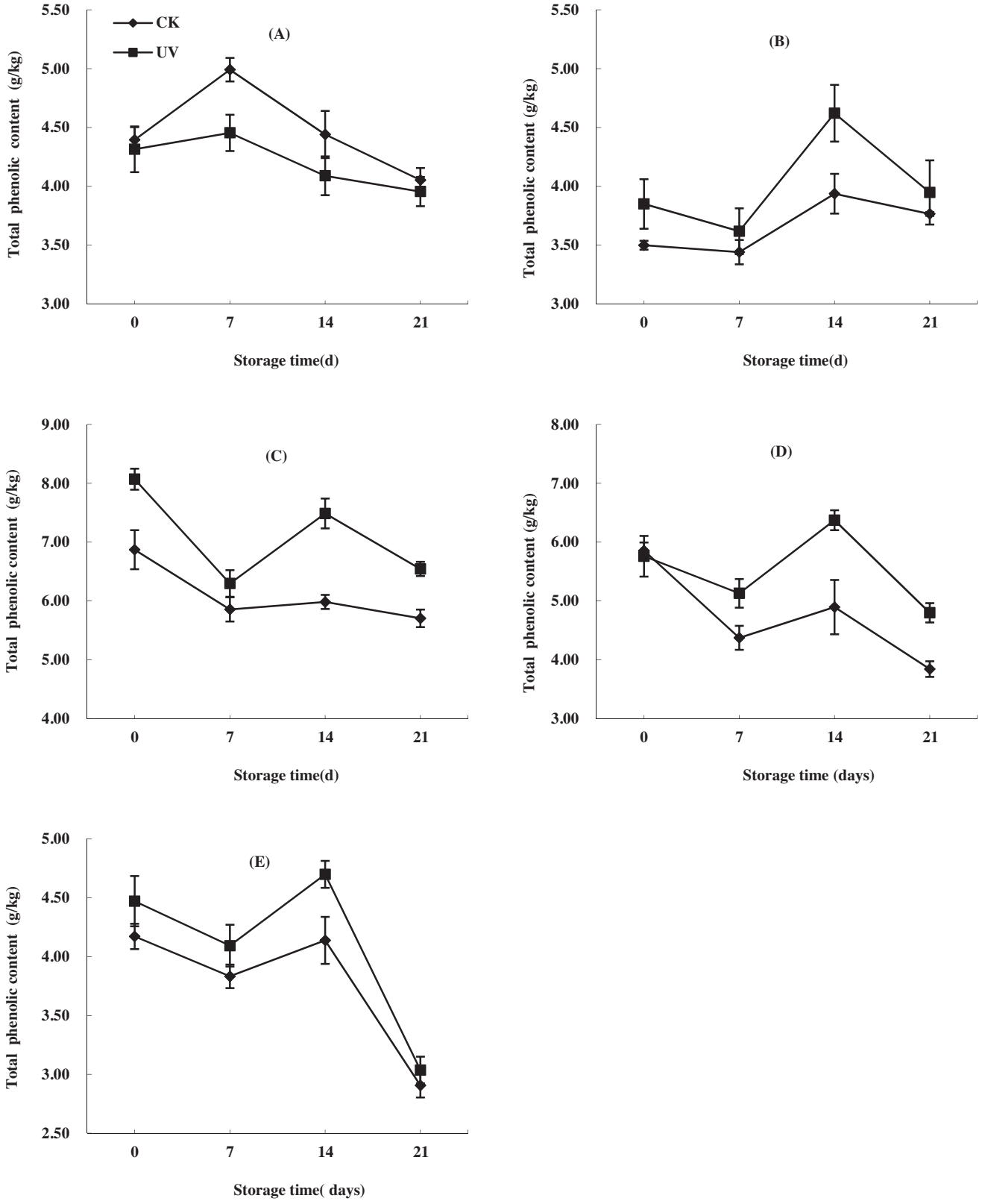

Fig. 2. Effect of UV-C treatment on total phenolic content of peel (A), inner cap (B), gill (C), stipe (D) and whole fruiting body (E) of button mushrooms stored at 4 °C. Values are means of standard errors (n = 3). Vertical bars indicate the standard errors of the means, where they exceed the size of the symbol used.

decreased with the increasing time of storage. However, the UV-C treated peel had significant lower total phenolic content than the controls during storage ($P < 0.05$). Total phenolic content in the peel showed increasing trend during the initial 7 days of storage, and then decreased by the end of 21 days storage.

Phenolic compounds in fruits and vegetables may produce the beneficial effects by scavenging free radicals and protecting cells

against the oxidative damage caused by free radicals (Erkan et al., 2008). UV-C irradiation could increase total phenolic content in fruits and vegetables by activating enzymes of phenylpropanoid metabolism (Jose et al., 2015). The results were consistent with studies that UV-C treatment was effective in increasing total phenolic content of fruit and vegetables (Erkan et al., 2008; Jiang et al., 2010; Park and Kim, 2015). However, the total phenolic of the mushroom peel was lower than that of control after UV-C irradiation, which is probably because higher UV-C exposure caused too much stress and resulted in injury of peel cell of mushroom. Similar results about injury of UV-C on fruits and vegetables were found by some other authors. It was reported that UV-C doses ($4.30 \sim 6.45\ kJ/m^2$) tended to suppress increases of total phenols and anthocyanins in blueberries (Baka et al., 1999). Doses of UV-C ($1.0\ kJ/m^2$) exposure resulted in strawberries injury as these fruits exhibited a higher level of conductivity (Baka et al., 1999). Liu et al. (2012) found that higher dose of $80\ kJ/m^2$ UV-C irradiation caused negative effects on antioxidants. Excessive doses of UV light produced membrane disorganization and progressive disruption of thylakoids, and facilitated enzymatic and/or non-enzymatic formation of pheophytin (Kovács and Keresztes, 2002). Overall, UV-C may induce different responses of different parts to UV-C stress and affect the increment of phenolics, which needs further study.

3.4. Effect of UV-C irradiation on GHB, GDHB and tyrosine in different tissues of button mushroom during storage

The phenolic substrates (GDHB, GHB and tyrosine) in UV-C irradiated button mushrooms during storage are shown in Table 2.

Note: Each value represents mean ± standard deviation of three replicates. Different lower case letters within the same column of same tissue mean significant difference at $P < 0.05$, and capital letters within the same line of same phenolic compound between the control and UV-C treatment mean significant difference at $P < 0.05$ according to the Newman–Keuls' test.

During the initial 7 days, GHB and GDHB contents decreased in both control and UV-C treated mushroom tissues ($P < 0.05$), and increased in the following storage period. At the end of storage, the contents of GHB in all tissues were significantly lower than the initial content (day 1), while the content of GDHB in inner cap and stipe was not significantly ($P < 0.05$) different from the initial content. The content of GDHB in gill was significantly higher than the initial content (day 1). The level of tyrosine in both control and UV-C treated mushroom tissues increased significantly between day 1 and day 14, whereas decreased significantly ($P < 0.05$) on day 21. The finding is in agreement with the results by Tseng and Mau (1999) who reported that an increase in L-tyrosine and L-phenylalanine contents in button mushrooms during storage. However, the obtained results contradicted those found by Soler-Rivas et al. (1998) who showed that concentration of GHB, GDHB and Tyrosine decreased during 50 h of storage, which may be due to the different storage time and temperature. At first day after treatment, the difference of GDB, GDHB and tyrosine between the control and UV-C treatment was not significant. However, the phenolic substrates varied significantly between UV-C treatment and control ($P < 0.05$) during days 7 and 21 of storage. Inner cap, stipe and gill of UV-C treated mushrooms had higher GHB, GDHB and Tyrosine concentration than the controls during days 7 and 21 of storage ($P < 0.05$), while GHB, GDHB and Tyrosine concentration in peels of UV-C treated mushrooms were significantly lower than the controls during storage ($P < 0.05$). This was similar to the change of total phenolic content in UV-C treated mushrooms. UV-C probably induced synthesis of GHB, GDHB and tyrosine in inner tissue of mushroom, but the injury of peel cell might lead to oxidation of phenolic compounds. The degree of decrease in the phenolic compounds of peel was completely different. The content of GHB in peel was, on average, more than 4 times higher in control than that in UV-C treatment, whereas GDHB and tyrosine were no more than 1 times higher.

Although optimal doses of UV-C irradiation could increase phenolic compounds and antioxidant capacity in fruits and

Table 2
Changes of phenolic substrates during 21 days of different parts of button mushroom (levels in g/kg).

Days	GHB		GDHB		Tyrosine	
	CK	UV-C	CK	UV-C	CK	UV-C
Peel						
1	0.42 ± 0.03Ac	0.34 ± 0.03Bb	3.58 ± 0.26Ab	3.59 ± 0.05Ab	0.64 ± 0.01Aa	0.62 ± 0.02Aa
7	0.21 ± 0.05Ba	0.04 ± 0.01Aa	2.45 ± 0.20Aa	2.40 ± 0.40Aa	2.05 ± 0.17Ac	1.61 ± 0.27Ac
14	0.27 ± 0.05Bab	0.05 ± 0.01Aa	4.61 ± 0.14Bc	2.93 ± 0.06Aa	1.85 ± 0.12Bc	1.13 ± 0.18Ab
21	0.37 ± 0.09Bbc	0.05 ± 0.01Aa	5.40 ± 0.37Bd	3.56 ± 0.36Ab	1.08 ± 0.05Bb	0.82 ± 0.09Aab
Inner cap						
1	0.74 ± 0.03Aa	0.75 ± 0.03Ac	2.06 ± 0.14Abc	2.62 ± 0.27Aa	0.80 ± 0.30Aa	0.77 ± 0.14Aa
7	0.12 ± 0.02Ab	0.23 ± 0.06Aa	1.94 ± 0.12Ab	2.09 ± 0.37Aa	1.03 ± 0.09Aa	1.27 ± 0.01Abc
14	0.08 ± 0.01Ab	0.33 ± 0.05Bb	1.85 ± 0.24Aa	4.71 ± 0.33Bc	1.11 ± 0.15Aa	1.41 ± 0.09Bc
21	0.10 ± 0.01Ab	0.15 ± 0.06Aa	2.32 ± 0.15Ac	3.35 ± 0.30Bb	0.81 ± 0.01Aa	1.04 ± 0.25Bab
Gill						
1	3.15 ± 0.51Ab	3.14 ± 0.68Ac	4.19 ± 0.23Aa	4.21 ± 0.09Aa	0.48 ± 0.08Aa	0.43 ± 0.02Aa
7	0.55 ± 0.04Aa	0.56 ± 0.03Aa	3.56 ± 0.52Aa	3.85 ± 0.55Aa	0.87 ± 0.12Ab	1.13 ± 0.17Bb
14	0.23 ± 0.01Aa	1.50 ± 0.13Bb	5.04 ± 0.37Aab	6.90 ± 0.35Bb	1.52 ± 0.02Ac	1.83 ± 0.08Ac
21	0.38 ± 0.03Aa	0.44 ± 0.05Aa	5.81 ± 0.98Ab	6.86 ± 0.16Ab	0.95 ± 0.07Ab	1.15 ± 0.01Ab
Stipe						
1	2.48 ± 0.06Ad	2.61 ± 0.17Ac	4.17 ± 0.17Ab	4.62 ± 0.38Ab	0.50 ± 0.50Aa	0.48 ± 0.16Aa
7	0.44 ± 0.02Aa	0.37 ± 0.07Aa	2.92 ± 0.43Aa	2.98 ± 0.16Aa	0.87 ± 0.03Ab	0.90 ± 0.03Ab
14	0.64 ± 0.03Ab	1.28 ± 0.03Bb	2.94 ± 0.20Aa	4.85 ± 0.37Bb	1.14 ± 0.09Ac	2.89 ± 0.27Bd
21	0.77 ± 0.04Ac	0.95 ± 0.07Bb	4.24 ± 0.03Ab	4.55 ± 0.32Ab	0.86 ± 0.07Ab	1.23 ± 0.08Bc

Note: Each value represents mean ± standard deviation of three replicates. Different lower case letters within the same column of same tissue mean significant difference at $P < 0.05$, and capital letters within the same line of same phenolic compound between the control and UV-C treatment mean significant difference at $P < 0.05$ according to the Newman–Keuls' test.

vegetables by hormesis (Baka et al., 1999), excessive doses of UV-C exposure caused injury and abnormal browning in some fruits and vegetables (Maharaj et al., 1999). For example, UV-C irradiation resulted a more severe browning with increasing dosage in *A. bisporus* (Guan et al., 2012). γ-irradiation also induced light browning of mushroom (Beaulieu et al., 1999). In this study, immediately after UV-C irradiation, a light browning effect of mushroom peel was observed. The mechanism for this observation appears to be that UV-C irradiation causes oxidation of phenolic compounds (GHB, GDHB and tyrosine) present in vacuoles, which induces brownish discoloration of mushroom (Beaulieu et al., 1999).

Numerous reports have described the phenolic compounds involved browning of mushrooms. GHB, GDHB and tyrosine have been identified as main phenolic substrates in mushrooms leading to browning (Weijn et al., 2013; Jolivet et al., 1998). In the presence of tyrosinase and oxygen, GHB is oxidized into GDHB and subsequently into quinine which polymerizes into GHB-melanins, and tyrosine is oxidized into DOPA and polymerizes into DOPA-melanins (Weijn et al., 2013; Beaulieu et al., 1999; Espin et al., 1999). More and more studies showed that the oxidation of GHB was crucial for the melanin formation of mushroom browning. GHB/GDHB pair is more important than L-tyrosine/L-DOPA pair to yield the enzymatic browning (Espin et al., 1999; Pierce and Rast, 1995 Pierce and Rast, 1995). It was also reported that GDHB and GHB were most abundant in the bruising-sensitive fungi strains, and were 3 and 2.5 times higher than that in the tolerant white strains, respectively, while L-tyrosine did not shown a correlation with bruising sensitivity (Weijn et al., 2013). Formation of brown GHB-melanin in skin tissue seemed to be the predominant pathway in bruising-related discoloration of button mushrooms (Weijn et al., 2013; Sylvie Jolivet et al., 1998). Our results showed that content of GHB in UV-C treated mushroom peel did not increase and was significantly lower compared to the control during storage, whereas GDHB and tyrosine increased during storage in UV-C treated mushrooms. GHB-melanins polymerization might be the main source for skin browning caused by UV-C irradiation, which is consistent with the results by above mentioned authors (Weijn et al., 2013; Espin et al., 1999; Pierce and Rast, 1995 Pierce and Rast, 1995). In present study, content of GDHB was higher than GHB, and did not show apparent decrease during storage. The explanation might be that GDHB were immediately replenished via de novo synthesis, indicating a dynamic flux of compounds from a reservoir of precursors, especially GHB (Weijn et al., 2013).

Previous studies have shown that UV-C could significantly increase antioxidant activity and total phenonic contents in most of mushroom tissues. Whereas it has been reported that the strongest responses of fruit to UV-C treatment occurred instantly after the irradiation and the effects diminished with time (Wang et al., 2009). There is also a lack of studies on the biochemical and molecular mechanisms by which UV irradiation increases or decreases the antioxidant status of fruits and vegetables (Jose et al., 2015). UV-C treatment has been demonstrated to inhibit microbial growth of mushrooms significantly (Guan et al., 2012) and it can be concluded that the application of UV light technology for mushrooms is suitable and safe (Simon et al., 2013). However, it is unclear whether the UV-C treatment will impact flavor of button mushroom during shelf life as a result of changes in phenolics. Further research may be conducted to evaluate possible changes in sensory properties of UV-C treated mushroom. The effect of UV-C on biochemical and molecular mechanism of enzymatic browning and potential health-promoting nutraceutical compounds in mushroom are also needed to be further explored.

4. Conclusion

The distribution of antioxidant activity and phenolics in different tissues of button mushroom were varied. The gill had the highest concentration of total phenolics, GHB and GDHB, while the stipe showed highest antioxidant capacity. Compared with control, UV-C irradiation could induce the defense mechanism by enhancing antioxidant activity, total phenolics, GHB, GDHB and tyrosine in inner cap, stipe and gill of mushroom during storage, while UV-C irradiation probably caused injury and browning of mushroom peel and led to the decrease of phenolic compounds. UV-C treatment likely leaded to the oxidation and conversion of phenolic compounds associated with browning of mushrooms. The interaction of phenolic compounds and browning among different tissues of button mushrooms in response to UV-C irradiation still need to be further studied.

Acknowledgments

The authors would like to thank Dr. Xuetong Fan for critical reviewing the manuscript. This research was supported by the National Natural Science Foundation of China (Grant no. 31271949), Tianjin Municipal Project of Science and Technology (Grant no. 13ZCZDNC01500 and TD12-5049) and the financial support of China Academy of Agricultural Sciences Innovation Project.

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