Scientia Horticulturae 225 (2017) 380–385

Contents lists available at ScienceDirect

Scientia Horticulturae

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

UV-C Treatment maintains quality and delays senescence of oyster mushroom (*Pleurotus ostreatus*)

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ARTICLE INFO

Keywords:
Oyster mushrooms
UV-C treatment
Quality
Shelf-life

ABSTRACT

The feasibility of UV-C treatment was studied for extending the shelf-life of oyster mushrooms. Oyster mushrooms were treated with 4.0 kJ/m^2 ultraviolet-C (UV-C) radiation before packaging with LDPE pouches, stored at 4 °C for 15 days. During storage, color, tissue electrolyte leakage, soluble solid content, soluble protein, methane dicarboxylic aldehyde (MDA), phenylalanine ammonia lyase (PAL), catalase (CAT), total viable counts, lactobacillus, yeast & mold counts and overall quality of oyster mushrooms were measured. Oyster mushrooms treated with UV-C resulted in slower changes in color, soluble solid content, soluble protein, exhibited higher CAT,PAL activities and overall quality, lower tissue electrolyte leakage and MDA content compared to untreated oyster mushrooms. Besides, oyster mushrooms treated with UV-C also show a high potential ability for surface decontamination of oyster mushrooms. Results show that UV-C treatment could be useful to extend the shelf life of oyster mushrooms.

1. Introduction

Oyster mushroom (*Pleurotus ostreatus*) is one of the most popular health foods across the world because of special flavor, pleasant texture, abundant nutrient and medicinal value (Ren et al., 2012). A large number of researches have shown that oyster mushrooms have been valued as edible and medicinal resources. Oyster mushrooms contain various nutrients required by humans, such as proteins, vitamins, fibers, minerals. Among nutrients, protein content is higher than other foods and contains all nine essential amino acids required by humans, enabling their use as a substitute for meat diet (Kakon et al., 2012). However, oyster mushrooms are easy to deteriorate and browning because of high metabolic activity, respiration rate and dehydration (Ares et al., 2007) after harvesting. They can only keep commercial quality of the 1–3 days at ambient temperature, and the 4–7 days at 4 °C. Therefore, some measures should be taken to extend the shelf life of oyster mushrooms.

In the past studies, many postharvest treatments have been used to extend the shelf life of oyster mushrooms. Methods mainly include modified atmosphere packaging (MAP) (Guillaume et al., 2010), coating (Krochta and De Mulder-Johnson, 1997), cooling (Tao et al., 2006), chemical treatment (Jafri et al., 2013) and ozone treatment (Watanabe et al., 1994). Although aforementioned measures can prolong the shelf period, it still exists some imperfections. MAP has a preservative effect on the color by slowing down respiration, but it leads to water accumulation at the product surface, promoting microbial growth and sliminess (Singh et al., 2010). Method of coating is relatively complex, because processing effect of coating is related to not only coating solutions, but also coated objects. Regarding to cooling, there are some strict demands for storage conditions, for example, temperature and humidity. During ozone and chemical treatment, higher expenditures of apparatus and reagents will happen and weight losses are incurred. So a powerful treatment being fast, economic and environmental friendly will be needed.

UV-C irradiation (200–280 nm) belonging to a small part of the electromagnetic spectrum is a non-thermally disinfection method used in fruits and vegetables. It is also characterized by favorable costs of equipments, energy and maintenance (Keyser et al., 2008). According to predecessor's researches, UV-C treatment has many of benefits, and can make up for the defects of thermally technology. Compared with thermal treatment, UV-C treatment by minimal processing can enhance commercial and nutritional value, reduce destructive impact on the sensory quality, and remove microbial contamination and spoilage of fruits and vegetables (Islam et al., 2016). The theory of sterilization is that UV-C exposure can damage microbial DNA and cause cross-linking between neighboring thymine and cytosine. DNA transcription and replication will be blocked, finally, losing cellular functions and leading to cell death (Rame et al., 1997; Sastry et al., 2000). UV-C treatment also is safer for workers and processed foods. Some simple measures can be taken to defend workers from light exposure. Moreover, the Food

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http://dx.doi.org/10.1016/j.scienta.2017.07.019
Received 1 March 2017; Received in revised form 4 June 2017; Accepted 13 July 2017
Available online 27 July 2017

and Drug Administration (FDA) approved UV-C light as a disinfectant technology for surface treatment of food (FDA, 2003). This technology has been widely applied to fresh-cut fruits (Beaulieu, 2007; Gómez et al., 2010) and vegetables (Escalona et al., 2010; Lu et al., 2016a, 2016b), fruit juice (Caminiti et al., 2012; Gouma et al., 2015) with mushrooms (Guan et al., 2012; Simon et al., 2013), but few studies related to oyster mushroom are reported on the literature. Therefore, the main objectives of this study were to evaluate the effect of 4.0 kJ/m^2 UV-C treatment on quality maintenance, microbial growth, and enzyme activities.

2. Materials and methods

2.1. Sample preparation and treatments

Oyster mushrooms were purchased from a local farmers market and immediately transported to the laboratory. Mushrooms that have a good commercial quality (diameter of cap: 5 cm; length of stipe: 6 cm; color of gills: white; without slimy feeling; free of mechanical trauma) were selected to study. Selected oyster mushrooms were randomly divided into two groups. Each group included 3 replicates, and each replication had 10 mushrooms.

UV-C radiation intensity varied with distances between UV-C lamps and samples. In this study, distance used for this study was 42 cm, and the ultraviolet intensity of irradiation was 230 μW/cm^2. Intensity was measured by a portable UV-C intensity meter (LVU254, heng oda instrument, Beijing, China). Before placed on low-density polyethylene (LDPE) pouches (18 cm × 15 cm × 0.04 mm) and sealed, one group of oyster mushrooms was treated with doses of 4.0 kJ/m^2 and another group (control samples) were handled similarly without any further UV-C processing. Mushrooms (60 ± 5 g per bag) were stored in a refrigerator at 4 °C for 15 days. Subsequently every 2 days, qualities, enzymes activity and microbial growth were analyzed.

2.2. Quality attributes

2.2.1. Color

Cap color of oyster mushrooms were analyzed using a colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-300 measuring head(Liu and Wang, 2012). Color was measured on three equidistant points of the cap (Lu et al., 2016a, 2016b). Prior to color measurement, the colorimeters were calibrated to a standard black glass and standard white tile. Color of cap was expressed in L* (lightness). L* was expressed as an average of 3 replicates.

2.2.2. Tissue electrolyte leakage

Fresh mushrooms (3 g) were submerged into 30 mL distilled water at 5 °C for 30 min. The electrical conductivity of the solution was measured using a conductivity meter (DDSJ-308A, LeiCi Instrument Co. Ltd., Shanghai, China) by inserting a probe into the sample solutions. Total electrolyte of the tissues was determined on the same samples after freezing at −20 °C for 24 h and thawing at room temperature (Kim et al., 2005). Tissue electrolyte leakage was expressed as a percentage of total electrolytes. Result was expressed as an average of 3 replicates.

2.2.3. Soluble solid content

Soluble solid content of oyster mushrooms was measured by a digital refractometer (REF121, Atago, China). Oyster mushrooms (20 g) were homogenized in a grinder and then centrifuged (Sigma 3-3OK, Germany) for 20 min at 4000 r/min. The supernatant phase was collected for determination of soluble solid content (Eissa, 2010). Soluble solid content was expressed as percentage. Result was expressed as an average of 3 replicates.

2.2.4. Soluble protein

Soluble protein content of mushroom was measured according to a modified method of Bradford (1976) using Coomassie brilliant blue G250. Fresh oyster mushrooms (2 g) were ground in 8 mL distilled water. Extracting the solution thoroughly and letting it standing for 30 min before centrifuging (Sigma 3-3OK, Germany) for 15 min at 4000 r/min. Pipette the supernatant liquid (2 mL) and dilute with water to 10 mL, mixing to get the sample extract liquid.

0.1 mL sample extract liquid was added to 5 mL Coomassie brilliant blue G250, mixed well and incubated at room temperature. After 5 min, absorbance was recorded at 595 nm by Uv–vis spectrophotometer (Analytik Jena UV–vis L 40, Germany). The blank was distilled water. Soluble protein was expressed as mg of protein equivalent per 1 g of oyster mushroom. The result was determined from the following formula:

$$X\ (mg/g) = (C \times V/A)/W$$

X: Soluble protein content; mg/g

C: Value of the standard curve; mg

V: The total volume of extract; mL

A: Measuring volume of extract; mL

W: weight of sample; g

2.2.5. Methane dicarboxylic aldehyde

Malondialdehyde (MDA) was measured by the procedure described by Shah et al. (2001) with a slight modification. Fresh oyster mushrooms (1 g) were ground using a mortar with 10 mL 10% trichloroacetic acid (TCA). The homogenate was centrifuged at 4000 r/min for 10 min at 4 °C. The supernatant liquid (2 mL) was mixed with 2 mL of 1% 2-thio-barbituricacid (TBA) to the mixture. After heating at 100 °C for 15 min, the mixture was rapidly cooled in an ice bath and centrifuged at 3000 r/min for 10 min. The absorbance of the supernatant was measured at 450, 532, and 600 nm. The blank was 10% TCA. MDA was expressed as μ mol/g of oyster mushroom

MDA level was calculated with following formula:

$$\mathrm{MDA}(\mu\ \mathrm{mol/g\ FW}) = \frac{[(OD_{532} - OD_{600}) \times 6.452 - 0.559 \times OD_{450}] \times V_T}{W}$$

V_T: volume of extract; mL

W: Weight of sample; g

2.2.6. Overall quality

A Five-member trained panel evaluated overall quality according to a modified procedure from Luo et al. (2004). Overall quality was evaluated with a 9-point hedonic scale, where 9 = like extremely, 5 = neither like nor dislike and 1 = dislike extremely (Meilgaard et al., 1991).

2.3. Enzyme activities

Two important enzymes that were responsible for scavenging free radicals were selected for analysis: phenylalanine ammonia lyase (PAL; EC 4.3.1.5), catalase (CAT; EC 1.11.1.6)

Tissues (5 g) of oyster mushrooms were homogenized with 50 mL of 50 mM phosphate buffer (pH 7.8) containing 1.33 mM EDTA, 1% PVPP. The mixture was centrifuged at 12,000 r/min for 15 min at 4 °C, the supernatant liquid was collected as the extraction of crude enzyme.

2.3.1. PAL

PAL activity was measured with the method of Assis et al. (2001). The reaction mixture contained 3 mL sodium borate buffer (50 mM, pH 8.8), 0.5 mL L-phenylalanine (20 mM), and 0.5 mL extract. After continuous shaking at 37 °C for 60 min, the reaction mixture was immediately measured at 290 nm. One unit of PAL activity was defined as the quantity of enzyme that increased the absorbance at 290 nm by 0.01/min per g fresh weight in comparison with tubes lacking enzymes. The results were expressed as U/g of oyster mushroom.

2.3.2. CAT

CAT activity was measured according to the method of Havir and Mchale (1987) with slight modifications. CAT was assayed in a reaction mixture comprised by 2.9 mL 20 mM H_2O_2 and 100 μL enzyme extract. The absorbance at 240 nm was recorded one time every 30 s for 3 min. One unit of enzymatic activity was defined as the amount of the enzyme that caused a change of 0.01 in absorbance per minute and causes changes in the substrate. The specific CAT activity was expressed as U/g on a protein basis.

2.4. Microbial counts

Microbiological determinations were measured as followed: Under sterile conditions, 25 g of tissues per replicate were macerated in 250 mL saline water and homogenized for 1 min. Serial dilutions of each suspension were made in saline water and analyzed for microbial counts. Appropriate aliquots (0.1 mL or 1 mL) were spread on agar plates.

Total viable counts (TVC) were measured by plating appropriately aliquots on a Plate Count Agar (PCA, Difco) and incubated at 30 °C for 24–48 h. Lactobacillus (LAB) were plated on man ragosa sharpe (MRS, Difco) and incubated at 30 °C for 72 h. Potato dextrose ager medium (PDA, Difco) was used for enumeration of yeast & mold for at 28 °C for 48 h. All microbial counts are reported as $\log_{10}$ colony forming units per gram of sample ($\log_{10}$ CFU/g).

2.5. Statistical analysis

All experiments were implemented in triplicate and the results were expressed as mean values ± SD (standard errors). Results were analyzed using SPSS v19.0. The evaluation of statistical significance was determined by ANOVA followed by Duncan's multiple-range test. Differences at $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Effect of UV-C on quality attributes

3.1.1. Color

The color of oyster mushrooms is the first impression of quality attributes judged by the consumers. The surface color was measured in terms of L* value (brightness) only, the reason is that oyster mushrooms have no obvious yellowness and redness (Dhalsamant et al., 2015). The changes in L* value of oyster mushrooms during storage for the untreated and treated samples are shown in Figs. 1A. Before day 6 of storage, there was no significant difference in L* value ($P > 0.05$) between untreated and treated groups. L* value of untreated samples significantly lower ($P < 0.05$) when compared with UV-C treatment after day 6. The reason was most likely that oxidase activities were decreased by UV-C radiation, preventing browning of oyster mushrooms surface. The result was in agreement with previous studies which reported that UV-C treatment could reduce the browning of button mushrooms (Lu et al., 2016a, 2016b).

3.1.2. Tissue electrolyte leakage

Tissue electrolyte leakage was an important indicator for tissues and membrane integrity (Rolny et al., 2011). As shown in Fig. 1B, the trends of rapid increases in tissue electrolyte leakage were noticed in untreated and treated samples during storage period. Samples treated with UV-C had relatively low electrolyte leakage until after 12 day in storage whereas significant increase was observed in untreated samples. Similar change of increase in tissue electrolyte leakage was reported by earlier researchers reported about shiitake mushrooms (Ye et al., 2012). However, UV-C exposure had a significant effect on tissue electrolyte leakage during whole storage period. It could be found that tissue electrolyte leakage of treated samples be significantly lower ($P < 0.05$) in untreated samples. The result corroborated earlier findings that UV-C irradiation could reduce electrolyte leakage of vegetables such as fresh-cut bell peper (Rodoni et al., 2012) and broccoli (Lemoine et al., 2007). So, UV-C treatment will make a difference in decreasing tissue electrolyte leakage to protect the membrane stability at some level.

3.1.3. Soluble solid content

Soluble solid content (SSC) of samples is an index of maturity and closely related to respiration rate of mushrooms (Pal and Roy, 1988). The changes of SSC were shown in Fig. 1C. The trends of change were similar between the treated and untreated samples. SSC of each group was 1.20% on day 0. The untreated samples declined quickly and reached the lowest (1.06%) on day 6, the treated samples decreased gradually and SSC reached lowest (1.19%) on day 9, the major reason of decrease of content was that stored SSC was converted into energy under aerobic conditions (Nourian et al., 2003). In the rest of storage, SSC of treated showed a continuous increase. However, there were no significant differences ($P > 0.05$) for SSC of treated samples and nearly maintained at a steady level (1.20%) during entire storage period. For untreated samples, changes of SSC were more pronounced ($P < 0.05$). The result demonstrates that UV-C radiation is a potential means to retain soluble solid content of oyster mushrooms for the prevention of higher metabolic rate.

3.1.4. Soluble protein

Soluble protein is one of the nutritional and functional components in mushrooms and related to texture, flavor of oyster mushrooms which directly determined commercial value. The relevant study also shows reducing content of protein will lead to softening of mushrooms and losing of nutrition (Zivanovic et al., 2000). Fig. 1D showed that soluble protein contents of oyster mushrooms in treated and untreated groups quickly declined during the storage, such a decreased was consistent with previous research about button mushroom (Jahangir et al., 2011; Khan et al., 2014), which might be attributed to the proteolytic enzyme that was active and still maintained a function of decomposing protein. Although there was similar change between untreated and treated samples, soluble proteins of treated samples were significantly higher ($P < 0.05$) than untreated samples during entire storage period… A similar result had been reported for leek and cabbage (Liao et al., 2016). On day 9, soluble protein of the untreated samples was 1.8007 mg/g, while on day 15, treated samples still kept 1.9568 mg/g. Thus, the UV-C irradiation treatment could retain soluble protein of oyster mushrooms.

3.1.5. MDA

MDA is an index of lipid peroxidation that can result in bad flavors and will produce potentially toxic substances. Excess MDA undoubtedly contribute to an increase in membrane leakage and damage membrane system (Jiang et al., 2012). Fig. 1E showed the changes of MDA during the storage period. Similar trends of rapidly increasing in MDA content was found in untreated and treated groups, and this was consistent with the previous study findings (Tao et al., 2007; Jafri et al., 2013). The treatments with UV-C irradiation dramatically delayed the increase in MDA content of oyster mushrooms. MDA of untreated samples was significantly ($P < 0.05$) higher than treated samples at any time of

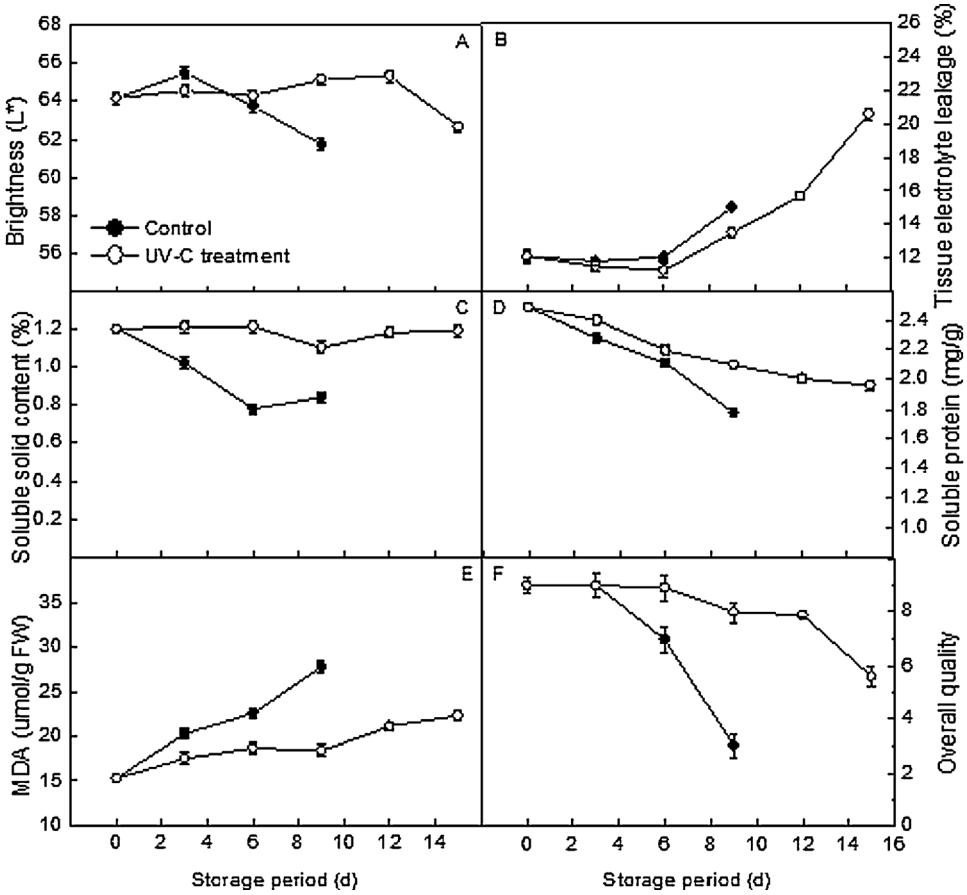

Fig. 1. Effect of UV-C treatment on L* (A), Tissue electrolyte leakage (B),Soluble solid content (C), Soluble protein (D), MDA(E) and Overall quality (F) of oyster mushrooms stored at 4 °C for 15 days. Data presented are the means of three replications, Vertical bars indicate the standard errors of the means. —○— UV-C treatment —●— Contorl.

storage. At the day 9 of storage, MDA of untreated samples increased to 27.80 μmol/g FW, while on day 15, MDA of treated samples was 22.31 μmol/g FW. This study corroborated earlier findings that UV-C irradiation could reduce MDA content of fruits such as blueberries (Xu et al., 2016) and banana peel (Pongprasert et al., 2011). Therefore, UV-C treatment could reduce MDA content to prevent lipid peroxidation of oyster mushrooms.

3.1.6. Effect of UV-C on overall quality

Mushrooms treated UV-C exhibited significantly higher overall quality ($P < 0.0001$) during storage than untreated mushrooms (Fig. 1F). On day 3, both treatments samples maintained the same sensory scores from day 0. Three days after storage, untreated samples showed signs of quality deterioration, while samples from UV-C treatment maintained the same sensory scores from day 0. On day 6, quality deterioration was noted for untreated samples, while UV-C treated samples scored high in overall quality. On day 9, untreated samples had a sharp decline (score is 3.0) in overall quality and developed a strong off-odor, significant decay, and were thus removed from storage. From day 6 to day 12, UV-C treated samples remained in acceptable the range. On day 15, UV-C treated samples had positive quality attributes rated at 5.6 (out of 9).

3.2. Effect of UV-C on enzyme activity

PAL takes a significant role in plants of fungi. When pathogenic bacteria violate the plant tissue, it can resist disease by involving in the synthesis of secondary metabolite, leading to the formation of lignin and flavonoid (Barron et al., 2017). Changes of PAL were observed in Fig. 2A, PAL activities of untreated and treated samples showed similar change trends, which showed an increasing tendency during the storage period from day 0 to day 9. However, due to UV-C exposure, the PAL activity of oyster mushrooms compared with untreated group was different during the storage period, and treated samples were significantly higher than untreated samples ($P < 0.05$) at any time without day 0. At the end of the storage, the PAL activities of treated and untreated sample were 78.9 U/mL on day 15 and 62.5 U/mL on day 9, respectively. The result of this study is similar with a report for blueberries (Xu et al., 2016).

CAT is one of the important antioxidant enzymes which play a key role in antioxidant defense systems, and destroy H_2O_2 by degrading into water and oxygen. Some relevant reports have shown that higher activities of CAT could delay the occurrence of browning of mushrooms (Lacan and Baccou, 1998). The changes of CAT enzyme activities were shown in Fig. 2B. The increases in CAT activity of untreated and treated samples were found before day 9 of storage, which were followed by its decrease. This trend was similar with earlier research (Tao et al., 2007). UV-C treated group of CAT activity was significantly higher than untreated groups during entire storage period.

3.3. Effect of UV-C on microbiological quality

Edible fungi are easily contaminated by spoilage microorganisms and food-borne pathogens because of its abundant nutrients including high moisture and sugar. As a consequence, it is necessary to analyze microbiological quality during storage. The changes about microbiological quality of oyster mushrooms were shown in Fig. 3.

Fig. 3A and C showed growth trends of TVC and yeast & mold counts during the storage, respectively. Apparently, TVC and yeast & mold counts were successfully reduced by UV-C treatment during the first 3 days of storage, but rapidly increased over the rest of storage days. For untreated samples, it was found that counts of TVC and yeast & mold still showed a rapid increasing trend. Moreover, UV-C treatment significantly ($P < 0.05$) delayed increase in TVC and yeast & mold of

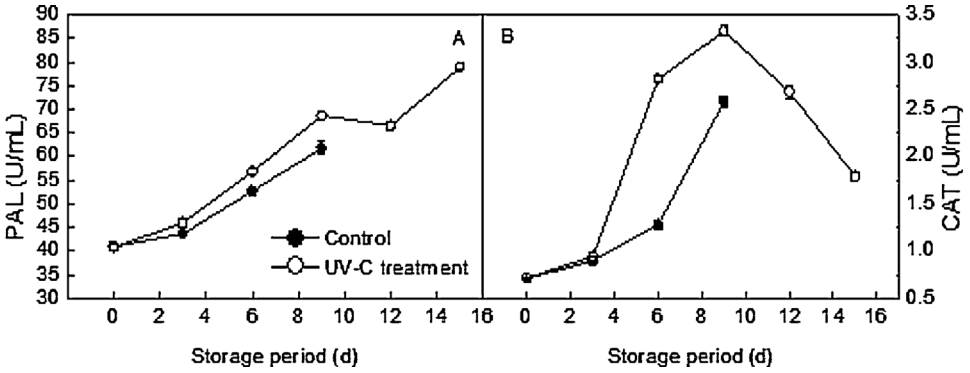

Fig. 2. Effect of UV-C treatment on PAL (A), CAT (B) of oyster mushrooms stored at 4 °C for 15 days. Data presented are the means of three replications, Vertical bars indicate the standard errors of the means. —○— UV-C treatment —●— Control.

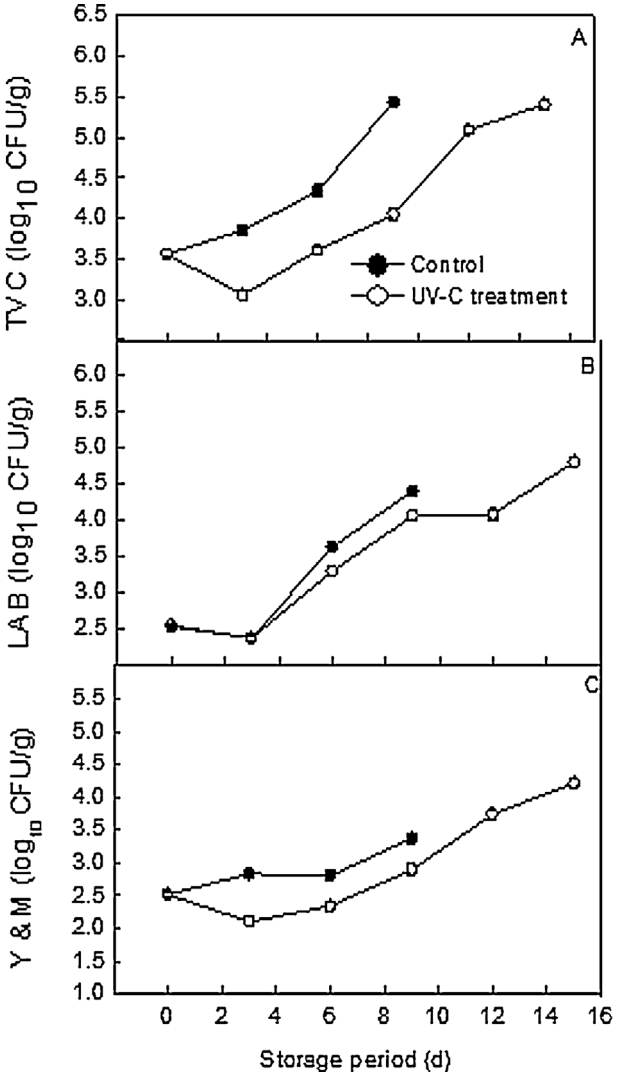

Fig. 3. Effect of UV-C treatment on Total viable count (A), Lactobacillus (B),Yeasts & Mold (C) of oyster mushrooms stored at 4 °C for 15 days. Data presented are the means of three replications, Vertical bars indicate the standard errors of the means. —○—UV-C treatment —●—Control.

oyster mushrooms from day 0 to day 15. This finding was in agreement with following UV-C treatments of foods such as grapefruit juice (La Cava and Sgroppo, 2015) and fresh-cut watermelon (Fonseca and Rushing, 2006).

The changes of LAB of oyster mushrooms were showed in Fig. 3B. The count of LAB decreased and there are no differences ($P > 0.05$) between untreated and treated samples during the first 3 days of storage. This change can be explained that other microorganisms rapidly grew and reproduced, consuming a lot of nutrients and inhibiting the growth of LAB. Treated samples and untreated samples showed a rapid increase in LAB from day 3 to day 9 and increased to 4.0 and 4.4 $\log_{10}$ CFU/g at day 9, respectively. UV-C treatment significantly ($P < 0.05$) delayed increase in LAB of oyster mushrooms from day 0 to day 15.This result was similar to early research about kumquat and orange fruit (Rodov et al., 1992).

It is worth to mention that criteria of microbiological populations in edible foods have been adopted in some countries. European countries (Spain, France and Germany) established 7.0 Log_{10} CFU/g as a maximum limit for total viable counts and LAB (Francis et al., 1999). In our study, three kinds of microorganisms reached the limit of 7.0 Log_{10} CFU/g, respectively. These results show that UV-C radiation could delay the growth of microbiological population of oyster mushrooms during the storage period.

4. Conclusions

The results presented in this study demonstrate that UV-C treatment is a useful non-thermally technology maintaining the quality in term of color, tissue electrolyte leakage, SSC, soluble protein, overall quality and MDA, delaying the decrease of enzyme activities for PAL and CAT, decontaminating the surface of oyster mushrooms. Therefore, the UV-C treatment has a good promise in maintaining quality and delaying senescence of oyster mushroom when stored at 4 °C.

Acknowledgement

The authors gratefully acknowledge the financial support provided by the Shaanxi Program for Science and Technology Development (Grant No. 2016NY-150).

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