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ORIGINAL PAPER

The Synergistic Effects of Low-Concentration Acidic Electrolyzed Water and Ultrasound on the Storage Quality of Fresh-Sliced Button Mushrooms

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Abstract The objective of this study was to determine the effects of low-concentration acidic electrolyzed water (LcEW) combined with ultrasound (US) on fresh-sliced button mushrooms (*Agaricus bisporus*), by evaluating the enzymatic browning, physio-biochemical changes, and microbial loads. The sliced button mushrooms were divided randomly into three groups (control, LcEW, and LcEW+US) and treated separately with tap water, LcEW, and LcEW+US for 3 min, and afterwards, they were stored at 5 °C for 8 days. The results indicated that LcEW delayed surface browning and maintained the flesh firmness of fresh-cut mushrooms compared with the control, and ultrasound enhanced these effects. Moreover, LcEW+US slowed down the electrolyte leakage (EL) rate and malondialdehyde (MDA) content, which were 5.29% and 0.5227 μmol/fresh weight less than those in control at day 8, respectively. The combined treatment of LcEW and US also significantly inhibited the activities of polyphenol oxidase (PPO) and peroxidase (POD) and controlled the counts of total bacteria (TBC) and yeast and mold counts (Y&M). Meanwhile, LcEW+US maintained a high level of total soluble protein and total phenolic contents, which were 1.49 and 1.24 times higher than those in control at the end of the storage time, respectively, and the combined treatment was more effective than LcEW treatment alone. These results demonstrated that the combination of LcEW and US could be an effective pretreatment technology in maintaining the product quality and prolonging the shelf life of fresh-sliced button mushrooms.

Keywords *Agaricus bisporus* · Low-concentration acidic electrolyzed water · Ultrasound · Enzymatic browning · Cell membrane damage · Microbial growth

Introduction

The button mushroom (*Agaricus bisporus*) is the most appreciated mushroom worldwide (Khan 2015). This fungus offers various nutrients; regular consumption of this mushroom is beneficial in preventing scurvy, cancer, and arteriosclerosis as well as improving neurological function (Cui 2010). However, previous studies indicate that harvested button mushrooms are susceptible to browning, softening, and microbial attack on account of their tender tissue, high water content, and cap without distinct protection structure, so they could be only preserved for 3–4 days at room temperature (Ge et al. 2009). Polyphenol oxidase (PPO) and peroxidase (POD) have important influences on mushroom browning (Sun et al. 2013; Wang et al. 2012), and the electrolyte leakage (EL) rate and malondialdehyde (MDA) content indicate the changes of the firmness of mushrooms indirectly (Cui 2010; Zhu 2016). Particularly, the consumption demand for fresh-cut vegetables has grown rapidly in the last few years, because of their

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convenience (Ma et al. 2017). However, the damage of surface cells and underlying tissues caused by the cutting process leads to faster deterioration of the quality of fresh-cut mushrooms compared with the original produce (López-Gálvez et al. 2010). The quick quality reduction of the button mushroom results in enormous economic loss. It is imperative to explore more effective methods of delaying the deterioration of the button mushrooms.

Various technologies that prolong the shelf life of button mushrooms have been investigated, including chemical coating, electron beam irradiation, modified atmosphere packaging, and low-temperature storage (Jiang 2013). However, these technologies have some disadvantages, such as off-flavor, residue of poison, expensive equipment, and the promotion difficulty (Zhang et al. 2013). Therefore, this research aims to find an effective and safe to human health strategy to preserve button mushrooms.

Washing is an important step for removing dirt and microorganisms attached to the surfaces of vegetables and fruits (Bilek and Turantas 2013). Recently, low-concentration acidic electrolyzed water (LcEW), a promising disinfectant, has been extensively applied in vegetable and fruit preservation (Koide et al. 2011). LcEW has a pH of 5.0–6.5, and its concentration of hypochlorous acid is up to approximately 95% (Issa-Zacharia et al. 2011). There are findings reported by Issa-Zacharia et al. (2011) that the antimicrobial activity of LcEW is equivalent to sodium hypochlorite solution and may be even more efficient than it. Meanwhile, its low available chlorine content reduces its corrosion on equipment surfaces, hazard to human beings, and security events from Cl_2 off-gassing (Issa-Zacharia et al. 2011). Its simplicity of production and application, efficient antimicrobial activity, and cost-effectiveness render its utilization a sustainable technology (Rahman et al. 2016). Most published data indicate that LcEW has effective disinfection ability to lessen the microbial biomass of romaine, iceberg lettuce, tomatoes, and oyster mushroom (Pang and Hung 2016).

Ultrasound has also been observed to be an effective approach in maintaining the freshness of vegetables. It is an acoustic wave whose frequency is higher than 16 kHz. The application of ultrasound is based on its acoustic cavitation (Ge 2014). Previous research reported by Lagnika et al. (2013) has implied that ultrasound pretreatment is a promising preservation method for mushrooms to retain freshness and extend shelf life during cold storage. Since the microbes and dust attached to the surfaces and stomata of fruits and vegetables will be stripped due to the acoustic cavitation, ultrasound-assisted chemical treatments have more significant effects on the quality of fresh produce (Kentish and Feng 2014). The application of ultrasound in the washing process of vegetables and fruits gained much attention recently, particularly its combination with LcEW (Alexandre et al. 2013). Ge et al. (2014) found that ultrasound-assisted LcEW treatment prolongs the shelf life of strawberry and cherry tomato; their results are consistent with the results of Luo et al. (2016) about potato.

To our knowledge, there are few available reports about the synergistic effect of LcEW and ultrasound on the storage quality of fresh-sliced button mushrooms so far. Thus, this study is conducted to investigate the effects of the combination of LcEW and ultrasound on the browning, softening, and microbial attack that are linked to quality changes of button mushrooms compared with the individual treatment of tap water and LcEW.

Materials and Methods

Chemicals

All chemicals were of analytical reagent-grade purity and purchased from Beijing Solarbio Science & Technology Co., Beijing, China.

Sample Preparation

The first flush of button mushrooms [*A. bisporus* (J.E. Lange) Imach stain A-15] was harvested at commercial-maturity stage (approximately 3–4 cm) from a planting base located in Daxing district, Beijing, China. The mushrooms were transported to the laboratory within 1 h. All samples were immediately stored in darkness at 5 °C and 80 to 90% relative humidity (RH) for 24 h before the experiment. Mushrooms that were uniform in color and free from mechanical damage were selected.

The selected mushrooms were sliced to a width of 0.5 cm by a slicer (Sunnex Metal Products (Shenzhen) Co., Ltd., Shenzhen, Guangdong Province, China) after cutting the stipes and then divided into three groups (control, LcEW, LcEW+US). The sliced mushrooms were dipped in the control (tap water), LcEW, and LcEW+US for 3 min at 25 °C, respectively. Each group of samples (200 pieces of sliced mushrooms) was taken out to drain the water and put onto a stainless steel tray (40 cm × 30 cm × 70 cm), then overwrapped with 0.03-mm-thick polyvinyl chloride (PVC) film and stored at 5 °C and 80–90% RH for 8 days. Each treatment was replicated three times. During the storage, a number of sliced mushrooms from each group were taken out at 0, 2, 4, 6, and 8 days. Twenty pieces of sliced mushrooms per replicate of each treatment were taken to measure the changes of surface color, firmness, and electrolyte leakage rate. Twelve pieces of sample were chopped and frozen immediately in liquid nitrogen and then stored at − 80 °C to assess the content of malondialdehyde (MDA), active compounds, and enzyme activity. Five pieces of samples were taken out for microbiological analysis at 0, 4, and 8 days.

Generation of Low-Concentration Electrolyzed Water

LcEW was generated by mixing 1% (*v*/*v*) hydrochloric acid solution and tap water using an LcEW generator (Fangxin water treatment equipment Co., Ltd., Yantai, Shandong Province, China), setting at 3 ± 0.1 A and maintaining the flow rate of tap water at 60.0 l/h, and 180 ml 1% (*v*/*v*) hydrochloric acid solution is consumed for 1 h. The physical and chemical properties of LcEW are in direct relation to the operating parameters of the generator. The pH and oxidation reduction potential (ORP) of LcEW were measured with a pH meter (Sartorius Co., Ltd., Germany) and a lab conductivity meter (Sanxin Co., Ltd., Shanghai, China), respectively.

The Concentration of Available Chlorine and Chlorine Species (HOCl and ClO^-)

Available chlorine concentration (ACC) was determined according to the iodometric method (American Public Health Association, 1975, pp. 316–317). Moreover, in order to evaluate the existing form of ACC, the chlorine concentrations of HClO and ClO^- were measured by a TU1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). Ultraviolet spectra was accessed at 25 °C with tap water used as the reference. The absorbances at 234 and 292 nm were obtained to calculate the separate chlorine concentration of HClO and ClO^- according to Lambert-Beer's law (Eq. (1)) and mass concentration (Eq. (2)) as follows (Zheng et al. 2012):

$$A = \varepsilon lc \tag{1}$$

$$\rho_{\mathrm{cl}} = 1000(\mathrm{mg/g})cM_{\mathrm{cl}} \tag{2}$$

where A is absorbance at 234 or 292 nm; ε is the molar absorptivity at 234 or 292 nm, which is 100 and 350 l/mol cm, respectively; l is the length of the path through the sample in cm; c is the concentration of the compound in the solution in mol/l; ρ_{Cl} is the chlorine concentration in HClO and ClO^- in mg/l; and M_{Cl} is the chlorine molar mass of 35.5 g/mol. All determinations were operated in triplicate.

Ultrasound Treatment

The sliced mushrooms were immersed in the ultrasonic chamber (Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, Jiangsu Province, China) with an ultrasonic vessel (300 cm × 240 cm × 150 cm) containing 9.5 l of LcEW and treated with 40-kHz frequency at a power of 200 W for 3 min.

Color Measurement

The cut surface color of the sliced mushrooms was measured by a CR-400 colorimeter (Konica Minolta Co., Ltd., Japan) in reflectance mode. A standard white plate accompanying the colorimeter was used for calibration. Then, the values of L^* (lightness), a^* (red/green), and b^* (yellow/blue) were determined at three equidistant points on the cut surface of the samples from the three groups. The lightness value, total color difference (ΔE), and browning index (BI) were measured during the storage at 0, 2, 4, 6, and 8 days according to the method of Lagnika et al. (2013) with minor modifications. Total color difference (ΔE) indicates the magnitude of total color difference and is expressed by the following (Eq. (3)), where L_0^*, a_0^*, and b_0^* are the values of control samples at day 0. The browning index (BI) represents the purity of the brown color and was calculated according to the following (Eq. (4)) (Borchert et al. 2014):

$$\Delta E = \left[\left(L^* - {L_0}^*\right)^2 + \left(a^* - {a_0}^*\right)^2 + \left(b^* - {b_0}^*\right)^2\right]^{1/2} \tag{3}$$

$$\mathrm{BI} = [100(x - 0.31)]/0.172 \tag{4}$$

where $x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*)$.

Firmness Measurement

A penetration test was performed on the sliced mushrooms by a TA-XT2i-plus texture analyzer (Stable Micro Systems Co., Ltd., UK) equipped with a flat-end cylindrical probe of 2-mm diameter. The test mode was compression. The sample was penetrated with a probe speed of 1 mm/s during the testing penetration and 2 mm/s during the pretest and the post-test. Firmness was defined as the average force when the probe penetrated samples during 3~4 s.

Electrolyte Leakage Rate

Electrolyte leakage rate was evaluated according to the method of Zhao et al. (2009) with minor modifications. Sliced mushrooms were cut into small cakes of 1 cm in diameter and 0.3 cm in depth by a puncher. Surface contamination was first removed from each of the six pieces of sample cakes by washing with distilled water, and afterwards, they were put into a 100-ml beaker containing 40 ml deionized water. The conductivity of the suspending solution was determined (P_0) by a MP513 conductivity meter (Sanxin Co., Ltd., Shanghai, China) immediately. P_1 was measured after shaking the solution at 100 cycles/min for 2 h. The solution including the samples was subsequently boiled for 10 min and cooled to room temperature, after which deionized water was added to the volume of 40 ml. A final conductivity measurement was taken (P_2). The relative electrolyte leakage rate was calculated by the following (Eq. (5)):

$$\textit{Electrolyte leakage rate} = 100\% \times (P_1 - P_0)/(P_2 - P_0) \tag{5}$$

MDA Content

The MDA content was determined with reference to the method described by Ding et al. (2007) with little modifications. Mushroom samples (1.5 g) were homogenized in 7 ml of 10% (*w*/*v*) trichloroacetic acid and then centrifuged at 10,000×*g* for 15 min at 4 °C. Two milliliters of the supernatant was mixed with 2 ml of 0.67% (*w*/*v*) thiobarbituric acid. The mixture was incubated at 95 °C for 20 min in a water bath and then centrifuged again at 10,000×*g* for 15 min at 4 °C after it was cooled to room temperature. The absorbance of the supernatant was measured at 450, 523, and 600 nm respectively. The MDA content was expressed in micromoles per gram fresh weight (FW).

PPO and POD Activities

The enzyme extracts used for the assays of polyphenol oxidase (PPO, EC 1.10.3.1) and peroxidase activities (POD, EC 1.11.1.7) were obtained according to the method described by Zhao et al. (2011) with modifications. Frozen mushrooms (5 g) were extracted with 5 ml of 0.1 M potassium-phosphate buffer (pH 7.0), which contained 1 mM ethylene diamine tetraacetic acid (EDTA), 5% (*w*/*v*) polyvinyl polypyrrolidone (PVPP), and 1% (*v*/v) Triton X-100 under ice-cold condition. The homogenate was centrifuged at 10,000×*g* for 20 min at 4 °C, and the supernatant was used as crude enzyme extract for the enzymatic activities assays. PPO activity was measured using the method of Luh and Phithakpol (1972). One unit of PPO was defined as the amount of enzyme that causes an increase of 0.1 absorbance per minute. PPO activity was expressed in unit per gram FW. POD activity was determined according to the method using guaiacol as the substrate described by Jiang et al. (2002). One unit of POD was defined as the amount of enzyme that causes a change of 0.1 absorbance per minute at 470 nm. PPO activity was expressed in unit per gram FW as well.

Total Phenolic Content and Total Soluble Protein Content

Quantification of the total phenolic content was done using the method established by Singleton and Rossi (1965) with little modifications. Ten grams of frozen samples was homogenized with 40 ml of distilled water. The mixture was put into a volumetric flask, and distilled water was added to bring the volume to 100 ml after boiling for 30 min. Four layers of cheesecloth were used to filter the homogenized mixture. Extra distilled water was added to 0.4 ml of the filtered liquid to bring the volume to 1 ml, and it was mixed with 5 ml of distilled water, 1 ml of Folin-Ciocalteu reagent, and 3 ml of 7.5% (*w*/*v*) sodium carbonate. The absorbance was then read at 765 nm after the mixture was left to settle for 1 h in the dark. A standard curve of gallic acid was used for quantification.

The total soluble protein was determined with BCA protein assay kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing). Three grams of frozen mushrooms was homogenized with 6 ml of PBS (50 mM, pH 7.8). The homogenized mixture was centrifuged at 10,000×g for 20 min at 4 °C. Then, the total soluble protein in the supernatant liquid was detected by applying the kit mentioned above.

Microbiological Analysis

After each treatment, all samples were analyzed for their total bacterium counts (TBCs) and yeast and mold count (Y&M). Twenty-five grams of each sample was put into a sterile lateral filter bag (Interscience Inc., France) containing 225 ml of aseptic physiological saline and homogenized for 2 min with a stomacher (Hanu Inc., Shanghai, China) (Khayankarn et al. 2013). Serial dilutions (10^{-1}~10^{-9}) were made in serial tubes by taking 1 ml of the sample with 9 ml of aseptic physiological saline. TBC and Y&M were determined by plate count agar (PCA) and potato dextrose agar (PDA), respectively, and the plates were incubated for 2 days at 37 °C and 7 days at 28 °C, respectively.

Statistical Analysis

All the experiments were conducted in triplicate with a randomized design. The data were analyzed statistically with one-way analysis of variance (ANOVA) using SPSS (version 19.0) statistical analysis software (IBM SPSS, Inc., Chicago, IL, USA). The differences between means were assessed by Duncan's multiple-range tests. Differences of $P < 0.05$ were considered significant.

Results and Discussion

Effect of US Treatment on LcEW

PH, ACC (mainly HOCl), and ORP are the main properties of electrolyzed water contributing to biocidal activity (Ding et al. 2015). As shown in Table 1, there was no significant difference in pH, ACC, ORP, HOCl, and ClO^- concentrations in US-treated LcEW compared to LcEW ($P > 0.05$). The results indicate that US treatment had no effect on the attributes of LcEW related to its bactericidal efficiency, which is consistent with the results reported by Ding et al. (2015).

Color

Color is one of the most important factors in determining the quality of fresh-sliced button mushrooms. When the lightness (L^*) values of button mushrooms were less than 80 and 69, they were considered unacceptable from a whiteness point of

Table 1 Effect of ultrasonic treatment on LcEW with respect to pH, ACC, ORP, HOCL, and CLO^-

Solutions	pH	ACC (mg/l)	ORP (mV)	HOCl ($\times 10^{-4}$ mol/l)	ClO^- ($\times 10^{-4}$ mol/l)
LcEW	5.50 ± 0.02a	16.86 ± 0.30a	514.67 ± 1.53a	3.20 ± 0.08a	0.43 ± 0.01a
LcEW+US	5.50 ± 0.02a	16.35 ± 0.34a	513.67 ± 2.31a	3.72 ± 0.04a	0.38 ± 0.01a

Values with the same lowercase letter in the same column showed no significant difference ($P > 0.05$). Data are presented as mean ± standard deviation, $n = 3$

LcEW low-concentration acidic electrolyzed water, *US* ultrasound, *ORP* oxidization reduction potential (mV), *ACC* available chlorine concentration (mg/l)

view at wholesale and consumer levels, respectively (Meng et al. 2012). LcEW+US treatment exhibited a pronounced effect with respect to delaying sliced button mushroom browning, followed by the LcEW treatment, compared to the control from day 4 to the last day of storage ($P < 0.05$). Visual inspection at day 8 reflected these results directly (Fig. 1).

The L^* values from sliced mushrooms in the control and LcEW remarkably decreased to 78.85 ± 0.25 at day 6 and 78.89 ± 0.10 at the end of the storage, respectively. This might be unacceptable for wholesale. However, the sliced button mushrooms treated with LcEW+US had a higher L^* value of 81.12 ± 0.06 until the last day of the storage (Table 2). Besides, the L^* value of the control samples at the end of the storage decreased by 10.38 compared to that at day 0, and those of LcEW and LcEW + US samples reduced by 6.45 and 3.7, respectively. As shown in Table 2, the browning of the control samples developed quickly during the storage, and the ΔE value dramatically increased from 0 at day 0 to 11.38 ± 0.07 at day 8. LcEW treatment effectively retained the color of sliced mushrooms, with the ΔE value of 6.97 ± 0.09 at day 8. Moreover, the combination was more functional than the application of LcEW alone; the ΔE value increased slowly from 0.15 ± 0.01 to 4.38 ± 0.03 during the storage. Similarly, the BI of the control samples was significantly higher than that treated with LcEW and LcEW+US during the storage ($P < 0.05$). The BI of the control mushrooms at day 8 increased by 10.91 compared to that at day 0, and those of the LcEW and LcEW+US mushrooms rose by 7.44 and 5.27, respectively. The overall result indicates that the combined treatment is better in maintaining the surface color of sliced button mushroom than LcEW treatment alone. This finding is consistent with the conclusion of Aday (2016).

Fig. 1 Browning appearance of sliced button mushrooms treated with control, low-concentration acidic electrolyzed water (LcEW), and LcEW+ultrasound (US) and stored at 5 °C on the 8th day

Firmness

The firmness of mushrooms is considered a key indicator contributing to consumer satisfaction. It depends on the cell

Table 2 The values of lightness (L^*), total color difference (ΔE), and Browning index (BI) from sliced button mushrooms treated with control, LcEW, and LcEW+US and stored at 5 °C for 8 days

Days at 5 °C	Control	LcEW	LcEW+US
L^*			
0	85.16 ± 0.20abA	85.34 ± 0.24aA	84.82 ± 0.14bA
2	83.78 ± 0.17bB	84.57 ± 0.21aB	84.71 ± 0.14aA
4	80.60 ± 0.21cC	82.72 ± 0.13bC	83.74 ± 0.09aB
6	78.85 ± 0.25cD	80.02 ± 0.10bD	82.67 ± 0.06aC
8	74.78 ± 0.14cE	78.89 ± 0.10bE	81.12 ± 0.06aD
ΔE			
0	0	0.17 ± 0.04aE	0.15 ± 0.01aE
2	1.67 ± 0.17aD	0.48 ± 0.20bD	0.55 ± 0.12bD
4	4.83 ± 0.07aC	2.62 ± 0.08bC	1.60 ± 0.03cC
6	6.97 ± 0.07aB	5.46 ± 0.03bB	2.64 ± 0.06cB
8	11.38 ± 0.07aA	6.97 ± 0.09bA	4.38 ± 0.03cA
BI			
0	16.49 ± 0.15aE	16.24 ± 0.10bE	15.23 ± 0.11cD
2	18.28 ± 0.23aD	16.47 ± 0.10bD	15.92 ± 0.08cD
4	20.54 ± 0.09aC	18.12 ± 0.12bC	17.11 ± 0.10cC
6	23.18 ± 0.31aB	20.03 ± 0.11bB	17.95 ± 0.08cB
8	27.40 ± 0.28aA	23.68 ± 0.14bA	20.50 ± 0.10cA

Values followed by different lowercase letters in the same row or followed by different capital letters in the same column are significantly different ($P < 0.05$). Data are presented as means ± standard deviation, $n = 3$

turgor pressure, the integrity of the cell wall, and the intercellular adhesion (Aday and Caner 2013; Khan 2015). Some data suggest that the cell wall is vulnerable to decomposition as a result of bacterial enzymes and endogenous autolysins (Zivanovic et al. 2000). Figure 2 shows that the firmness of mushroom slices in all three treatments suffered loss throughout the whole storage ($P < 0.05$), and there were no significant variations between LcEW and LcEW+US samples. Samples in the control showed the most severe softening rate, losing about 36% of their original firmness at day 8, compared with samples treated with LcEW and LcEW+US, which reduced by only 26 and 25%, respectively. This result exhibits that LcEW retarded the loss of firmness in sliced mushrooms, but US did not enhance the ability of LcEW in this regard. This concurred with the findings reported by Ding et al. (2015) that the firmness of strawberries treated with ultrasound did not change significantly.

Electrolyte Leakage Rate and MDA Content

EL rate is a key index reflecting the semipermeable properties of cell membranes and the damage of cell membrane integrity resulting from lipid peroxidation, which aggravates the membrane leakage and accelerates cell senescence (Aday and Caner 2013). Besides, MDA is the final product of lipid peroxidation. So the EL rate and MDA content play a key role in evaluating the reduction of cell membrane (Cui 2010; Zhu 2016).

In Fig. 3a, the EL rate of mushroom slices in all samples presented a rising trend along with the increasing time. The EL rate in LcEW- and LcEW+US-treated samples was significantly lower compared to that in the control ($P < 0.05$). Meanwhile, the EL rate of mushroom slices treated with LcEW+US was the lowest in all the three treatments ($P < 0.05$). The results suggest that LcEW is an effective

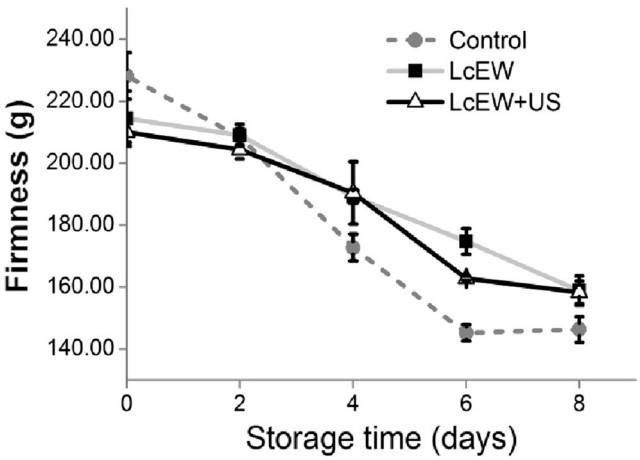

Fig. 2 Firmness of sliced button mushrooms treated with control, LcEW, and LcEW+US and stored at 5 °C for 8 days. Data represent the means ± SE, $n = 3$

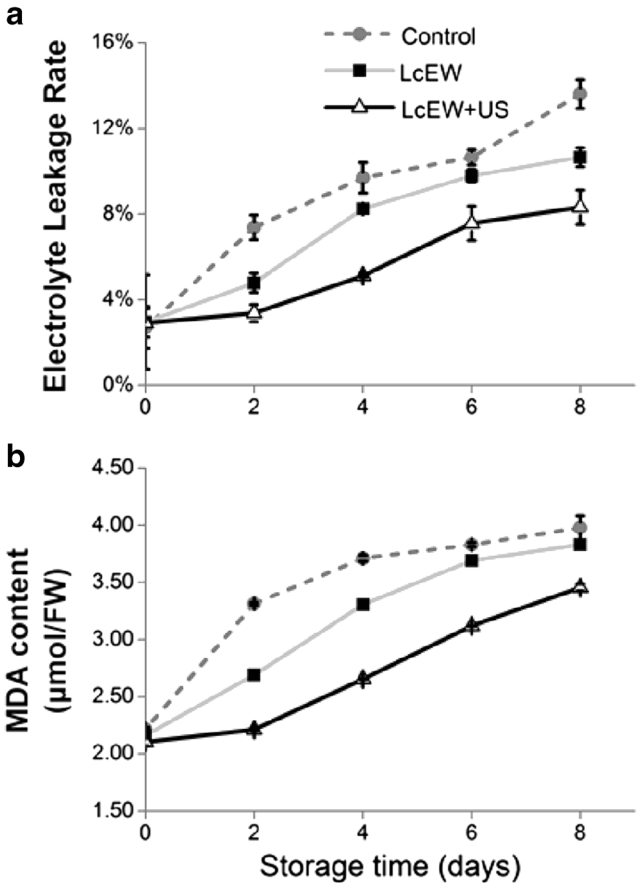

Fig. 3 The electrolyte leakage rate (**a**) and malondialdehyde (MDA) content (**b**) of sliced button mushrooms treated with control, LcEW, and LcEW+US and stored at 5 °C for 8 days. Data represent the means ± SE, $n = 3$

way to reduce the EL rate so as to protect the cell membrane of fresh-cut mushrooms. It is in conformity with the conclusions published by Aday (2016) about button mushroom. Besides, US-assisted treatment promoted the protective effects of LcEW. The variation of the MDA content has a similar trend to the changes in the EL rate in mushroom slices from all the three groups in the observation period (Fig. 3b). Compared to the control, LcEW treatment slowed down the accumulation of MDA content ($P < 0.05$). In addition, the combined treatment of LcEW and US was more functional in inhibiting the increase in MDA content than the individual LcEW treatment ($P < 0.05$).

The results imply that LcEW+US has better potential to maintain the integrity of cell membrane among the three groups when ultrasound treatment was set with 40-kHz frequency at a power of 200 W for 3 min. The reason might be that LcEW+US treatment prevented lipid peroxidation by reducing the related microbe enzyme on account of its disinfection. Previous studies had pointed out that the damage of cell membrane promoted the contact of phenolic compounds and PPO (Liu 2010). Liu et al. (2010) stated that the damage of the cell membrane causes mushroom browning. Besides, the

integrity of the cell membrane is associated with the flesh firmness (Aday and Caner 2013). The result of this part is consistent with the impacts of LcEW+US on the color and firmness of sliced mushrooms (Table 2 and Fig. 2).

PPO and POD Activities

PPO is capable of oxidizing phenolic compounds into reddish-brown quinones, which further react with amino acid to form melanins in aerobic condition. These result in the browning of button mushrooms ultimately (Sun et al. 2013). POD is also responsible for mushroom browning by catalyzing the oxidization and polymerization of phenolic compounds (Wang et al. 2012). Additionally, elevated POD activity is an important index reflecting the maturity and senescence of vegetables and fruits (Wang 2012).

The PPO activities of sliced mushrooms in the control exhibited a significantly higher level compared to the other two treatments in Fig. 4a ($P < 0.05$). The PPO activities of LcEW+US-treated mushrooms were remarkably lower than those of the mushrooms treated with LcEW alone from day 6 of the storage ($P < 0.05$). The variation of POD activities had a similar trend with PPO activities (Fig. 4b). The results indicate that the combination of LcEW and US inhibited the activities of PPO and POD more than the individual treatment of LcEW. It is a more effective approach to retard mushroom browning and senescence. It is consistent with the result shown in Table 2. The reason of enzyme inactivation might be that the collapse of microbubbles in the ultrasonic cavitation caused mechanical forces and localized energy accumulation with instantaneous high temperatures and pressures (Vercet et al. 2001). Consequently, the combination of LcEW and US was more efficient in inhibiting enzyme activity than the single treatment of LcEW and tap water.

Total Phenolic Content and Total Soluble Protein Content

Button mushroom browning is mainly attributed to enzymatic reaction with phenolic compounds as substrate. Therefore, the changes in total phenolic content are closely related to the browning degree of button mushrooms (Zhang et al. 2015). In Fig. 5a, compared to the control, total phenolic contents from the samples treated with LcEW+US showed significantly higher levels, followed by LcEW, throughout the storage. The total phenolic contents of LcEW and LcEW+US were 14 and 23% higher than that in the control mushroom slices at the last day of the storage, respectively ($P < 0.05$). This result is consistent with the inhibition of PPO activities and the delay in mushroom browning resulting from LcEW+US, as shown in Fig. 4a and Table 2, respectively.

Total soluble protein content exhibited a continuous decrease during the preservation of button mushrooms, and it is a key indicator of tissue senescence (Cui 2010). The free

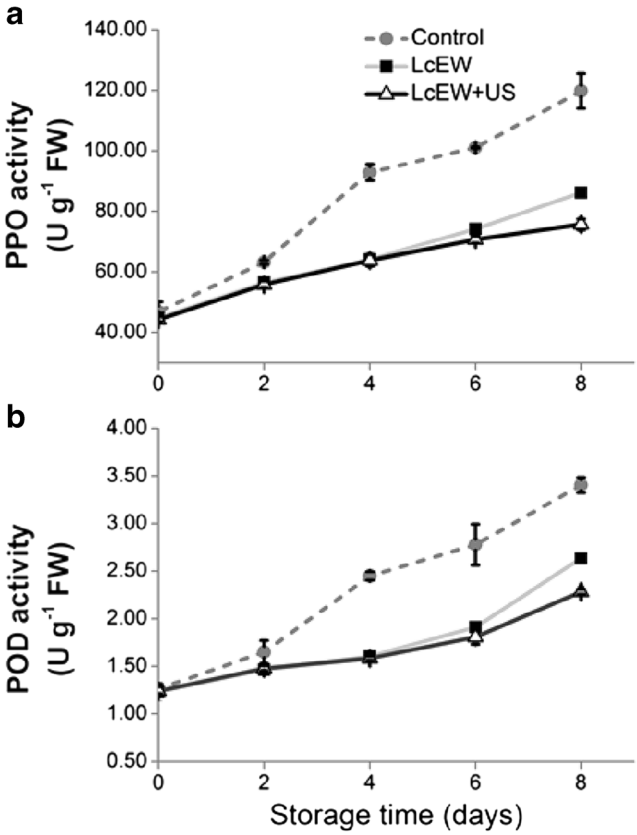

Fig. 4 Polyphenol oxidase (PPO) activity (**a**) and peroxidase (POD) activity (**b**) of sliced button mushrooms treated with control, LcEW, and LcEW+US and stored at 5 °C for 8 days. Data represent the means ± SE, $n = 3$

amino acids from the degradation of protein reacted with quinones to form melanins, which further aggravated the browning of mushrooms (Meng et al. 2010). As shown in Fig. 5b, total soluble protein contents from the samples treated with US-assisted LcEW presented a higher level, followed by LcEW treatment alone, compared to the control. The result indicates that US-assisted LcEW treatment was the most effective in delaying the senescence of mushroom slices among the three methods.

Microbiological Quality

Pathogenic microbial species accounting for the spoilage of button mushrooms might be pseudomonads, flavobacterium, and yeasts (Pan et al. 2015). They can contaminate mushrooms during the processing, transportation, distribution, storage, and handling (Xuan et al. 2017). Therefore, it was a requisite to investigate effective disinfection technologies to prolong the shelf life of button mushrooms. The effects of LcEW and LcEW+US treatments on TBC and Y&M are shown in Table 3. The TBC values of all samples exhibited a gradual increase during the storage ($P < 0.05$). At day 0, both the treatments of LcEW and LcEW+US reduced the TBC of

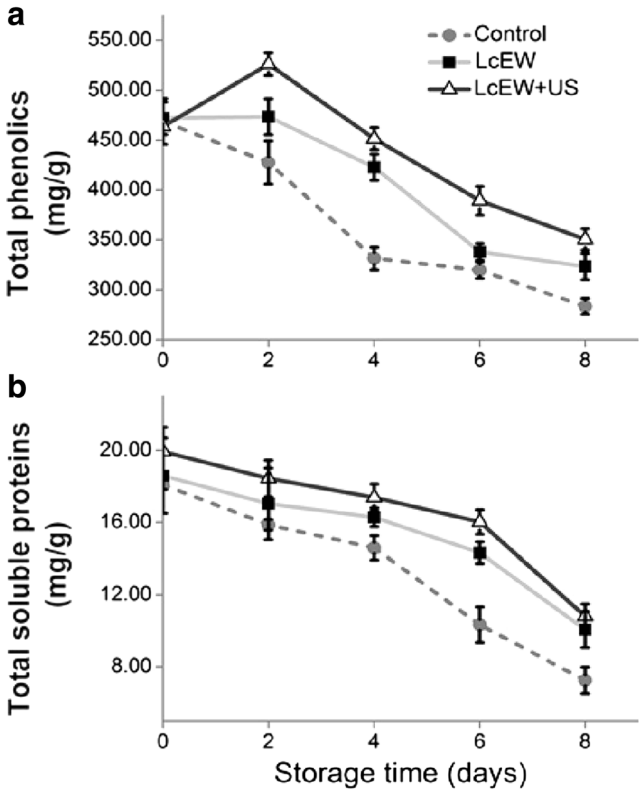

Fig. 5 Total phenolic content (**a**) and total soluble protein content (**b**) of sliced button mushrooms treated with control, LcEW, and LcEW+US and stored at 5 °C for 8 days. Data represent the means ± SE, $n = 3$

mushroom slices ($P < 0.05$). At the last day of the storage, the TBC value of the mushrooms treated with LcEW was 4.93 ± 0.02 log CFU/g, while that of LcEW+US treated mushrooms was lower with a value of 4.23 ± 0.04 log CFU/g. Both values are significantly lower than 5.33 ± 0.01 log CFU/g of the control samples ($P < 0.05$). Similarly, both the treatments of LcEW and LcEW+US were capable of reducing the initial Y&M compared to the control ($P < 0.05$). At the end of the storage, the Y&M of the control samples had an increase of 1.50 log CFU/g compared with its value at day 0. Also, the Y&M of the mushroom slices treated with LcEW was higher (0.86 log CFU/g) than that of the LcEW+US-treated mushrooms (0.70 log CFU/g) ($P < 0.05$).

The overall results revealed that the LcEW+US treatment is a more effective technology to maintain the quality of fresh-cut button mushrooms. All of these findings might be attributed to microorganism cell membrane damage caused by the cavitation efficacy of ultrasound. This led to effective chlorine compounds of LcEW getting inside the cells more easily (Khayankarn et al. 2013). Our findings concurred with the results published by Ge et al. (2014) that the combination of LcEW and US not only reduced the initial microbial loads of strawberries but also maintained the microbial biomass at a relatively lower level during storage. Similar conclusions were obtained about fresh-cut kale, kasha, and *Phaseolus vulgaris* L. reported by Mansur and Oh (2015), Forghani et al. (2015), and Zhang et al. (2016), respectively. Additionally, the studies of Forghani et al. (2013) about lettuce and Luo et al. (2016) about potato exhibited a consistent trend.

Table 3 Total bacterium counts (TBCs) and yeast and mold count (Y&M) (log 10 CFU/g) of sliced button mushrooms treated with control, LcEW, and LcEW+US and stored at 5 °C for 8 days

Days at 5 °C	Control	LcEW	LcEW+US
Total bacterium counts			
0	4.31 ± 0.03aC	2.94 ± 0.03bC	2.90 ± 0.04bC
4	4.6 ± 50.05aB	3.35 ± 0.01bB	3.20 ± 0.03cB
8	5.33 ± 0.01aA	4.93 ± 0.02bA	4.23 ± 0.04cA
Yeasts and molds			
0	3.40 ± 0.03aC	2.94 ± 0.03bC	2.33 ± 0.13cC
4	3.71 ± 0.02aB	3.31 ± 0.03bB	2.60 ± 0.14cB
8	4.90 ± 0.02aA	3.80 ± 0.03bA	3.03 ± 0.11cA

Values followed by different lowercase letters in the same row or followed by different capital letters in the same column are significantly different ($P < 0.05$). Data are presented as means ± standard deviation, $n = 3$

Conclusions

The present study demonstrated that the combination of LcEW and US treatment was more effective in delaying mushroom enzymatic browning resulting from the reduction of PPO and POD activities compared to the control and LcEW treatments. Meanwhile, LcEW+US treatment maintained flesh firmness of the sliced mushrooms. It might be related to the inhibition of the increase in the electrolyte leakage rate and the MDA accumulation. The treatment of LcEW+US slowed down the decrease of total phenolic content and total soluble protein contents as well. Furthermore, the combined treatment of LcEW and US had better potential to reduce the value of TBC and Y&M in contrast to the other two treatments. Thus, the LcEW combined with US pretreatment is a promising technology in extending the storage life and reducing the degradation of quality aspects of fresh-sliced button mushrooms.

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