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Improved postharvest quality and respiratory activity of straw mushroom (*Volvariella volvacea*) with ultrasound treatment and controlled relative humidity

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ABSTRACT

The present study proposed a synergistic method to extend the postharvest quality of straw mushroom (*Volvariella volvacea*) by controlling the conditions of ultrasound treatment and relative humidity (RH). Results showed that 10-min ultrasound treatment combined with storage at 95% RH prolonged the postharvest quality of straw mushroom from 24 h or 48 h to 72 h. The appearance of straw mushroom was maximally kept with original odor, minimum weight loss of 28.83%, malonaldehyde (MDA) content of 5.06 nmol g^{-1}, and higher total soluble sugar (TSS) of 28.32 g kg^{-1} and total soluble protein (TSP) of 30.27 g kg^{-1}. The ultrasound treatment also inhibited the browning and respiratory rates *via* inactivating the browning-related PPO activity to 236.52 U g^{-1}, and PGI to 0.038 U g^{-1}, SDH to 3.62 U mg^{-1} Pro, CCO to 9.29 U mg^{-1} Pro and G-6-PDH/6-PGDH to 69.87 μmol NADP g^{-1} min^{-1} activities of involved in respiratory pathways after 72 h of storage. Our results suggest that ultrasonic treatment with the control of relative humidity could potentially reduce the quality deterioration of straw mushroom.

1. Introduction

The straw mushroom (*Volvariella volvacea*) is a typical tropical/subtropical species of edible mushrooms presenting highly tasty and nutritional values with an estimated annual production of 330,000 tons in China occupying over 80% of global production (Cai et al., 1999; Bao et al., 2013). The straw mushroom grows at the relatively high temperatures (28–35 °C) and high moisture contents (80–90% relative humidity) for 4–5 week of vegetative growth and fruiting (Yen, 1992), and is harvested at the button or egg phase (Chang and Yau, 1971). However, fresh straw mushroom button rapidly loses its general quality and marketability within 48 h due to the chilling damage and fruit body autolysis at below 10 °C or high levels of respiration rate, metabolic activity and water content at over 25 °C (Chang and William, 2013). Hence, increase of straw mushroom shelf-life to benefit its long-distance distribution is still an urgent problem and extremely difficult issue to be dissolved. However, only few methods, such as combination of $CaCl_2$ (0.5%) treatment with 40-perforation packages, have been proposed to extend the shelf life of paddy straw mushroom up to 6 d with acceptable contents of total phenolic, antioxidants and protein, and total bacterial counts (Dhalsamant et al., 2015).

Mushroom shelf-life is closely related to its respiration rate during postharvest period, which metabolizing the nutrients such as carbohydrates, proteins and fats in the fruiting bodies with O_2 to simple end products such as organic acid or CO_2, which results in the mushroom ripening and senescence (Cliffe-Byrnes and O'Beirne, 2007). Generally, the maturity stage at harvest, ageing (time), temperature, gas composition, and cutting (mechanical damages) are the main internal and external factors affecting the respiration rate of fresh products. Therefore, methods such as modified atmosphere packaging (MAP) that reduce product respiration by creating a suitable atmosphere within a package can help in extending quality and shelf-life of mushrooms (Villaescusa and Gil, 2003). Some enzymes including succinic dehydrogenase (SDH), cytochrome C oxidase (CCO), glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenas (6-PGDH) are closely related to the respiration pathways. For example, respiration rate of peach fruit treated with ultraviolet-C could be inhibited *via* reducing SDH and CCO activity (Yang et al., 2014).

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Treatment with 50% O_2 + 50% CO_2 led to an obvious decrease in respiration rate of broccoli by inhibiting activities of SDH, CCO (cytochrome C oxidase), and activating the combined activities of G-6-PDH and 6-PGDH (Li et al., 2016). Straw mushroom has the significantly higher respiration during growing or after harvesting than majority of mushrooms. Industrial practice and our primary experiments have proved that the closed packaging accelerated the undesirable appearance changes of straw mushroom possibly due to its higher respiration rate and water release than other commercially cultivated mushrooms such as *lentinus edodes* and *Pleurotus eryngi*. Hence, inhibiting the respiration-related enzymes in the straw mushroom is a potential method to pro-long its shelf life.

Ultrasound has been increasingly applied for food processing and preservation due to its promising advantages of improved quality, reduced chemical damage and physical risks (Mizrach, 2008; Lagnika et al., 2013). For example, 30–40 min ultrasound (33 kHz, 60 W) treatment of strawberry gave the stable shelf quality during all the storage days (Gani et al., 2016). Ultrasound also has been applied to the preserve the physicochemical characteristics of white mushrooms (*Agaricus bisporus*) by slowing respiration rate and lowering polyphenol oxidase (PPO) activity (Lagnika et at., 2013). To date, there are few published references available to improve the quality retention of straw mushroom (*Volvariella volvacea*) by applying ultrasound and controlling storage humidity. Therefore, the objectives of present study were: 1) to maximize the postharvest quality of straw mushroom by using ultrasound treatment at 75% and 95% of relative humidity conditions, and 2) to elucidate the activity changes of enzymes involved in the respiration and deterioration of straw mushroom.

2. Materials and methods

2.1. Sample preparation, ultrasonic treatment and storage

Straw mushroom (*Volvariella volvacea*) was cultivated at 32 ± 2 °C and 90% of relative humidity in Jiangnan Biotech Co., Ltd (Zhenjiang, China). The fruiting body at egg stage was harvested after 4-week cultivation, transported to our laboratory within 1 h, selected for uniformity without any damage, and then randomly divided into 8 experimental groups.

Ultrasound equipment consisted of an ultrasonic reaction chamber (internal dimensions: 240 mm × 207 mm × 215 mm (width × length × height), Shangjia Biotechnology Co., Wuxi, Jiangsu, China) equipped with 3 alternate dual-frequency plates (Fig. 1), water bath for balancing the treatment temperature, and PLC control panel. The ultrasound generators were installed at three sides of bath reactor. The maximum output acoustic power of each plate is 600 W.

About 250 g mushroom samples were kept in a valve bag (20 × 30 × 0.12 mm), placed in the ultrasonic reactor chamber, and treated with 40 kHz frequency and 300 w power for 0, 3, 10 and 30 min, respectively. The experimental groups were set as follows,

(1) Control-75%: Mushrooms conditioned at relative humidity of 75%;
(2) Control-95%: Mushrooms conditioned at relative humidity of 95%;
(3) US-3-75%: Mushrooms treated by ultrasound for 3 min at relative humidity of 75%;
(4) US-3-95%: Mushrooms treated by ultrasound for 3 min at relative humidity of 95%;
(5) US-10-75%: Mushrooms treated by ultrasound for 10 min at relative humidity of 75%;
(6) US-10-95%: Mushrooms treated by ultrasound for 10 min at relative humidity of 95%;
(7) US-30-75%: Mushrooms treated by ultrasound for 30 min at relative humidity of 75%;
(8) US-30-95%: Mushrooms treated by ultrasound for 30 min at relative humidity of 95%.

Each experimental group had 15 replicates containing the untreated or ultrasonic-treated mushroom samples, and was stored at 75% or 95% of relative humidity and 15 °C for 96 h. Samples were taken on at every 12 or 24 h for collecting data including appearance, respiration rate, nutrients contents, and activities of enzymes involved in respiration and deterioration.

The crude extraction of straw mushrooms was prepared by homogenizing with 1:2 (w/v) of 50 mM sodium phosphate buffer (pH 7.8) and obtaining the supernatant fraction with centrifugation of 6800*g* for 20 min at 4 °C for subsequent analysis.

2.2. Respiration rate

Respiration rate was measured in accordance with Wang et al. (2015). The untreated or ultrasonic-treated mushrooms (100 ± 5 g) were weighed and placed in 1 L glass jars at 15 °C for 96 h. The glass jars were sealed by an impermeable film. Carbon dioxide concentration was measured at the 0 h, 12 h, 24 h, 48 h, 72 h and 96 h of storage period using an O_2 and CO_2 analyser (Cyes-II, Jiading federation Instrument, Shanghai, China). Gas samples were taken from the jars with a 20 mL syringe. CO_2 production was calculated as follows:

$$\Delta CO_2\ (mg\ kg^{-1}\ h^{-1}) = CO_{2f} - CO_{2i}$$

Where CO_{2i} represents the gas concentration on the first hour and CO_{2f}

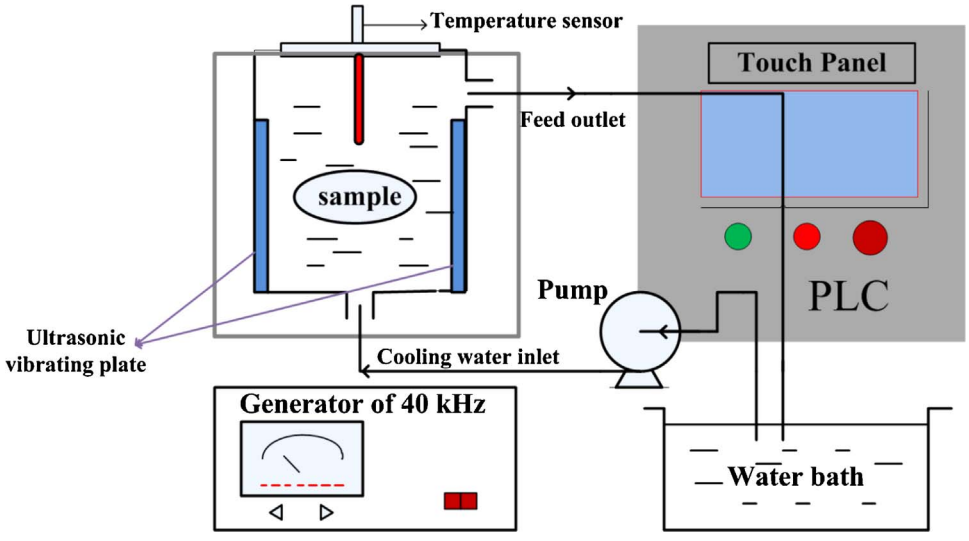

Fig. 1. Schematic diagrams of the Dual-frequency ultrasound equipment.

represents the gas concentration on the final hour of storage.

2.3. Firmness and weight loss

Straw mushrooms were placed on a XT2i Texture analyzer (Stable Micro Systems, Godalming, UK) fitted with a cylinder plunger SMS-P/10 CYL Delrin probe (2 mm diameter) and penetrated to a 10 mm depth at a speed of 2.0 mm s^{-1}. Firmness was recorded with the maximum force among 10 times test during penetration. Weight loss was determined by weighting the sample sat the 0 h, 12 h, 24 h, 48 h, 72 h and 96 h of storage period. Results were expressed as the percentage of loss of weight with respect to the initial weight.

2.4. Malondialdehyde (MDA) content

MDA content was measured according to the previously reported method (Zhou and Leul, 1998) with slight modification. 2 mL of crude extract of straw mushrooms was mixed with 2 mL of 0.6% 2-hiobarbituric acid (TBA), boiled for 15 min, immediately cooled, and centrifuged at 2000*g* for 10 min at 25 °C. The absorbance of supernatant using trichloroacetic acid (TCA) solution as blank was measured at 532, 600 and 645 nm. The MDA content was calculated through the formula:

$$\text{MDA (nmol g}^{-1}) = [6.45 \times (D_{532} - D_{600}) - 0.56 \times D_{450}] \times V/W$$

Where V represents the supernatant volume and W represents the fresh weight.

2.5. Total soluble protein and sugar contents

The concentrations of total soluble protein and sugar contents in the straw mushroom samples were determined with the method reported by Bradford (1976) and Dubois et al. (1956) using bovine serum albumin and glucose as a standard, respectively.

2.6. Browning degree and polyphenoloxidase (PPO) activity

Browning degree was performed by a UV spectrophotometer (UV-1801, Beijing Beifen-Ruili Analytical Instrument, China). The absorbance of straw mushrooms crude extract was measured at 410 nm using deionized water as blank. Browning degree was expressed as $A_{410nm} \times 10$.

Spectrophotometric measurement of PPO activity was followed the method by Pizzocaro et al. (1993). Briefly, sodium acetate buffer 3.9 mL (pH 4.8, 50 mmol L^{-1}), 1 mL of 0.1 M catechol were used as substrate and 0.8 mL of the enzyme solution were added and mixed thoroughly. The absorbance of the crude enzyme solution was measured at 420 nm and was recorded the increase in absorbance at 420 nm for 3 min. One unit (U) of PPO activity was defined as the amount of enzyme which increased the absorbance by 0.001 per minute under the assay condition. The specific PPO activity was expressed as U g^{-1}.

2.7. Activity determination of enzymes involved in respiration

2.7.1. Phosphohexoseisomerase (PGI)

The PGI activity was assayed using the procedure describing by Brown and Wary. (1968). Briefly, crude enzyme solution was mixed with 1.0 mL of 15 mM glucose-6-phosphate (dissolved with 50 mM sodium phosphate buffer, pH 7.4), following incubation at 30 °C for 5 min. Thereafter, 2 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 1872*g* for 10 min. One milliliter of supernatant, 3 mL of 3% HCl and1 mL of 0.1% resorcinol were mixed and maintained at 80 °C for 8 min. The absorbance of the reaction was recorded at 520 nm. Fructose was used as a standard and one unit (U) of PGI activity was defined as the amount of enzyme which liberated reducing sugars equivalent to 1 mg of fructose per minute.

2.7.2. Succinic dehydrogenase (SDH)

The measurement of SDH activity was carried out according to the method of Ackrell et al. (1984) with some modifications. The reaction was carried out at 30 °C for 5 min in a reaction mixture containing 1.5 mL of sodium phosphate buffer (pH 7.4, 50 mmol L^{-1}), 1 mL of 0.12 M sodium succinate, 0.1 mL of 0.9 mM 2,6-dichlorophenolindophenl (2,6-DCPIP) and 0.2 mL of distilled water. The absorbance at 600 nm was recorded after adding 0.1 mL of extract and 9 g L^{-1} methyl sulfenyl phenazine. One unit of SDH activity was defined as an increase of 0.01 in absorbance per minute at 600 nm under the assay conditions.

2.7.3. Combined glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) activities

The activity of G-6-PDH and 6-PGDH was carried out following the method of Brown and Wary (1968). Briefly, 0.9 mL reaction solution (containing 5 mmol L^{-1} 6-P-G, 5 mmol L^{-1} $MgCl_2$, 5 mmol L^{-1}, pH 7.4 Tris–HCl) were added to 2 mL of sodium phosphate buffer (50 mM, pH 7.4). After addition 0.1 mL of extract, the change of absorbance was recorded at 340 nm for 3 min and the combined activities of G-6-PDH + 6-PGDH were expressed as μmol NADP g^{-1} min^{-1}.

2.7.4. Cytochrome oxidase (CCO) activity analysis

The activity of CCO was measured using the procedure described by Errede et al. (1978). 0.1 mL of filtered supernatant, 0.2 mL of 0.04% cytochrome C and 1 mL of distilled water were mixed and incubated at 37 °C for 2 min. Thereafter, 0.1 mL of 0.4% *N*,*N*-dimethyl-*p*-phenylenediamine was added and maintained 37 °C for 3 min until red color appeared. 0.1 mol L^{-1} HCl was used for adjusting pH to 5.6–6.0. The reaction mixture was diluted 3 folds using sodium phosphate buffer. The absorbance was measured at 510 nm using sodium phosphate buffer as blank. One unit of cytochrome oxidase activity was defined as the change of absorbance per g fresh weight.

2.8. Statistical analysis

Data analyses were performed by Excel software, version 2010. The results were expressed as means ± standard deviations. Statistical comparisons were made by one-way analysis of variance (SPSS software, version 16.0), followed by Duncan's multiple-comparison test. Differences were considered significant when the *p*-values were < 0.05.

3. Results and discussion

3.1. Effect of ultrasound treatment and relative humidity on appearance of straw mushrooms

Generally, appearance of mushrooms directly influences the consumers' purchase intention. Fig. 2 presented the appearance of untreated or ultrasonic-treated straw mushrooms under 75% and 95% RH during 96-h storage at 15 °C. The brown rot and strong ammonia odor were observed in straw mushrooms without ultrasonic treatment at 48 h, and got worse with the following storage period up to 96 h. Similar results were also found in the industrial straw mushroom producer of Jiangnan Biotech, Co., Ltd with the shelf time of less 48 h. Too long (30 min) or short ultrasonic treatment time (3 min) could not significantly reduce the brown rot appearance of straw mushrooms. The 10-min ultrasonic treatment significantly improved the storage life to 72 h keeping straw mushrooms with stable color and original odor without spoilage. Lagnika et al. also found that the ultrasonic treatment with power of 400 W and frequency of 20 kHz for 10 min significanly retarded the white mushroom (*Agaricus bisporus*) browning (Lagnika et al., 2012; Lagnika et al., 2013). Interestingly, the relative humidity also affected the appearance of straw mushrooms. Lower RH of 75% resulted in the visibly shrinkage of untreated or ultrasonic-treated samples while samples in 95% RH had the acceptable appearance even

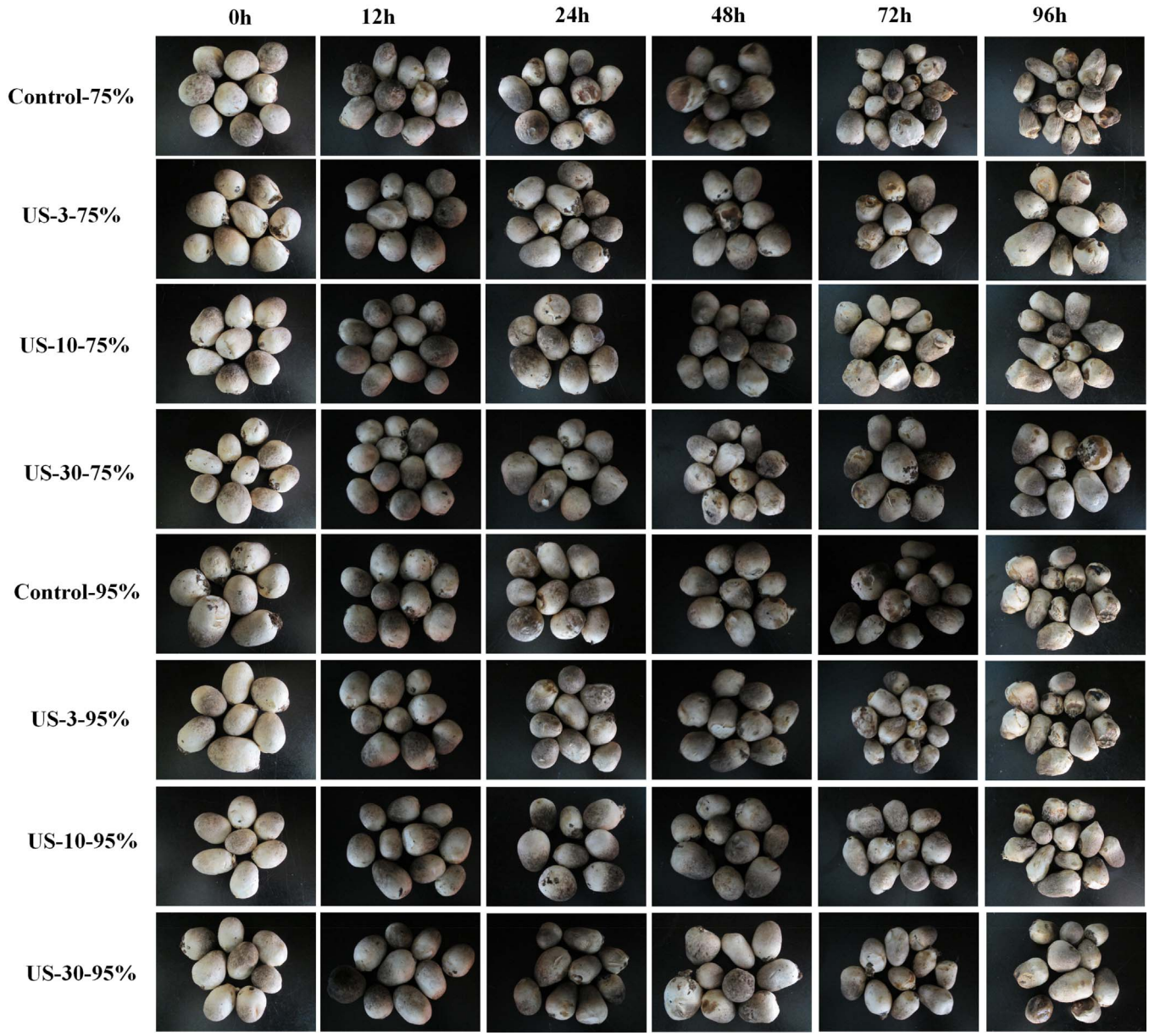

Fig. 2. Appearance of straw mushrooms treated by ultrasound (40 kHz, 300 W) for different time (0, 3, 10, 30 min) and relative humidity (75% RH or 95% RH).

after 72 h of storage. However, with the increase of storage time to 96 h, all ultrasonic-treated groups rapidly enter the deteriorated stages with brown rot appearance. Hence, it could be concluded that the ultrasonic treatment with storage in 95% RH maximally kept the appearance and original odor of straw mushrooms and possibly prolonged its marketability for 72 h.

3.2. Effect of ultrasound treatment and relative humidity on respiration rate

Respiration is an important parameter in determining deterioration rate and onset of senescence in mushroom and reflects the dissipative speed of tissue inclusion (Farber et al., 2003). Table 1 gave the change profile of CO_2 production rate of straw mushrooms with and without ultrasonic treatment at 75% and 95% RH, respectively. The CO_2 production rates increases to the maximum levels after 12 h or 24 h postharvest and decreased gradually with the storage period prolonged to 96 h. Compared to control group, ultrasonic treatment significantly inhibited the respiration of straw mushroom ($P < 0.05$), and 10-min ultrasonic treatment resulted in the minimum CO_2 production rate of 149.8 mg CO_2 kg^{-1} h^{-1} within 72-h storage. The respiration rates at 96 h were lower than those at 72 h which possibly was due to the deteriorated stages with lower respiratory activity and respiration rates.

Relative humidity also could affect the CO_2 production of straw mushrooms. From Table 1, 95% of relative humidity delayed the respiration peaks from 12 h to 24 h compared with those of 75%, which might attribute to the water lose in straw mushrooms at lower moisture conditions. Thus, a synergistic control of ultrasound time and humidity could effectively inhibit the respiration rate by slowing the metabolism of straw mushroom.

3.3. Effect of ultrasound treatment and relative humidity on firmness and weight loss

Firmness is an important food quality attribute and a parameter, related to the cell wall strength and intercellular adhesion (Toivonen and Brummell, 2008). Table 2 showed the influence of ultrasound treatment and relative humidity on firmness and weight loss changes during 96-h storage at 15 °C. The untreated (control) samples gradually softened with the firmness of 1.80 N and 1.57 N (at 75% RH and 95% RH) at the end of storage, respectively. Ultrasound treatment

Table 1
Effect of ultrasonic treatment and relative humidity on respiration rate in straw mushroom during stored at 75% RH and 95% RH, respectively (mg kg^{-1} h^{-1}). All mushrooms stored at 15 °C for 96 h.

Relative Humidity	Ultrasonic treatment time (min)	0 h	12 h	24 h	48 h	72 h	96 h
75%RH	0	223.8 ± 4.7a	250.9 ± 8.2a	212.3 ± 8.2ab	199.3 ± 7.3a	176.2 ± 5.9a	85.2 ± 9.6b
	3	220.9 ± 6.4ab	243.6 ± 5.9a	222.6 ± 7.7a	170.5 ± 2.8bc	165.4 ± 7.9ab	88.7 ± 7.7b
	10	220.0 ± 7.2ab	231.7 ± 3.9b	198.9 ± 6.5b	166.8 ± 5.1c	160.9 ± 6.8b	108.3 ± 2.3a
	30	210.3 ± 5.7b	242.2 ± 4.6ab	208.9 ± 8.9ab	179.9 ± 6.6b	168.8 ± 6.2ab	96.6 ± 3.8ab
95%RH	0	228.9 ± 10.3a	220.9 ± 11.4a	250.7 ± 6.7a	219.3 ± 9.8a	170.6 ± 11.2a	98.7 ± 5.9b
	3	223.7 ± 6.7a	212.2 ± 15.4ab	230.9 ± 3.8b	200.7 ± 7.5bc	165.1 ± 6.7ab	100.4 ± 5.2b
	10	224.5 ± 3.2a	196.7 ± 6.8b	214.7 ± 3.9c	192.6 ± 1.3c	149.8 ± 8.4b	130.4 ± 7.7a
	30	221.8 ± 12.8a	230.7 ± 8.4a	236.8 ± 7.8b	210.8 ± 8.4ab	157.7 ± 5.6ab	109.9 ± 6.8b

Data are means ± SD of three replicates.
Different following letters in the same column indicate significant differences ($P < 0.05$).

significantly retained the straw mushrooms firmness, while 3-min ultrasound treatment at 95% RH led to the maximum firmness retention of 1.90 N. Over ultrasound treatment (> 10 min) possibly produced more cavitation bubbles and prolonged exposure of ultrasound on stability of cell wall which results in cell injury and loss of firmness. These results were mainly consistent to that ultrasound power between 30 W and 60 W had improved quality and can be used to extend shelf life of strawberry (Aday et al., 2013).

Fresh mushrooms easily loss weight, shrivel, deteriorate and finally loss economic value. In most cases, mushroom weight loss is mainly related to the rapid water transpiration rate and CO_2 production during respiration process (Jiang, 2013). As shown in Table 2, weight loss increased as the storage period in all test groups. Compared to control, ultrasound treatment delayed the weight loss of straw mushrooms significantly. Ten-min ultrasound-treated mushrooms had the lowest weight losses of 31.22% and 28.83% at 75% RH and 95% RH, respectively. The lowest weight loss observed in ultrasound-treated mushrooms could be due to the water molecules were confined by hydrogen bonds, which decreased the loss of water. As predicted, higher relative humidity of 95% retained the weight of fruiting bodies than those at 75% RH by preventing the water release.

3.4. Effect of ultrasound treatment and relative humidity on browning degree and MDA content

Browning degree of mushrooms relates to their shelf life, handling degree and microbial spoilage. Table 3 showed the changes in the browning degree of straw mushrooms treated with various ultrasound treatments and relative humidity. After 72 h of storage, 10-min ultrasound treated group had the significant retention of color with the browning degrees of 4.25 and 3.42 at 75% RH and 95% RH, respectively ($P < 0.05$). Increase of ultrasound treatment time to 30 min showed the negative effect on the browning which possibly was related to the changes of microstructure and biochemical reactions in straw mushroom. Generally, ultrasound treatment could directly inactivate the enzymes activities in the plant or microbial cells (Piyasena et al., 2003). Herein, the ultrasound might affect the browning related PPO and POD activities in straw mushrooms and maximally kept the appearance color.

MDA, an index of lipid peroxidation, can reduce the membrane integrity and increase the membrane permeability (Long et al., 2006). As shown in Table 3, the MDA contents in 2 control groups at 75% RH and 95% RH kept the increase from 4.24 nmol g^{-1} to 6.52 nmol g^{-1}, and 3.19 nmol g^{-1} to 5.57 nmol g^{-1}, respectively. Ultrasound treatments for 10 min at 95% RH slowed the increase trend of MDA contents to 5.06 nmol g^{-1} ($p < 0.05$), while lower humidity or over-ultrasound conditions (30 min treatments) also contributed to the higher MDA (over 5.65 nmol g^{-1}). These results demonstrated that membrane permeability, as an indicator of membrane integrity, gradually increased during storage and showed that the treatments of 10 min ultrasound with 95% RH effectively could repress the increase of MDA content in mushrooms during storage, indicating that higher membrane integrity was maintained.

Table 2
Effect of ultrasonic treatment and relative humidity on firmness and weight loss in straw mushrooms during stored at 75% RH and 95% RH, respectively. All mushrooms were stored at 15 °C for 96 h (Mean ± SD; n = 3) ($p < 0.05$).

Relative Humidity	Items	Ultrasonic treatment time (min)	0h	12h	24h	48 h	72 h	96 h
75%RH	Firmness (N)	0	2.71 ± 0.02c	2.52 ± 0.02b	2.24 ± 0.04c	2.06 ± 0.04b	1.68 ± 0.06b	1.53 ± 0.01d
		3	2.83 ± 0.01a	2.73 ± 0.05a	2.39 ± 0.03a	2.22 ± 0.02a	1.83 ± 0.04a	1.86 ± 0.05a
		10	2.75 ± 0.04b	2.67 ± 0.04a	2.33 ± 0.02b	2.19 ± 0.01a	1.69 ± 0.05b	1.79 ± 0.04b
		30	2.73 ± 0.02bc	2.51 ± 0.05b	2.18 ± 0.03d	1.98 ± 0.05c	1.53 ± 0.03c	1.62 ± 0.03c
95%RH		0	2.73 ± 0.03c	2.56 ± 0.03b	2.35 ± 0.03b	2.07 ± 0.05d	1.80 ± 0.06c	1.57 ± 0.04b
		3	2.85 ± 0.02b	2.64 ± 0.02a	2.53 ± 0.06a	2.38 ± 0.03a	2.14 ± 0.03a	1.90 ± 0.02a
		10	2.91 ± 0.02a	2.52 ± 0.03b	2.37 ± 0.03b	2.28 ± 0.06b	2.02 ± 0.05b	1.51 ± 0.03b
		30	2.89 ± 0.03ab	2.46 ± 0.03c	2.29 ± 0.04b	2.18 ± 0.02c	1.72 ± 0.02d	1.42 ± 0.02 c
	Weight loss (100%)	0	0	5.82 ± 0.19b	12.26 ± 0.34bc	23.17 ± 0.28b	27.47 ± 0.39a	39.15 ± 0.31b
		3	0	5.41 ± 0.10b	12.85 ± 0.05b	22.42 ± 0.09c	24.51 ± 0.18b	37.86 ± 0.96c
		10	0	5.55 ± 0.24b	11.59 ± 0.34c	21.51 ± 0.07d	23.41 ± 0.24c	31.22 ± 0.15d
		30	0	6.37 ± 0.44a	15.61 ± 0.62a	26.66 ± 0.16a	27.32 ± 0.08a	42.17 ± 0.73a
95%RH		0	0	5.92 ± 0.12a	18.63 ± 0.11a	25.54 ± 0.14a	28.31 ± 0.23a	32.59 ± 0.38b
		3	0	6.23 ± 0.28a	18.21 ± 0.23a	22.42 ± 0.14b	24.35 ± 0.51c	31.52 ± 0.17c
		10	0	6.07 ± 0.06a	12.06 ± 0.16c	17.19 ± 0.18d	23.19 ± 0.39d	28.83 ± 0.27d
		30	0	6.18 ± 0.23a	16.55 ± 0.27b	20.60 ± 0.04c	26.93 ± 0.23b	40.12 ± 0.33a

Data are means ± SD of three replicates.
Different following letters in the same column indicate significant differences ($P < 0.05$).

Table 3
Changes in browning and MDA of straw mushrooms treated by ultrasound stored at 75% RH and 95% RH, respectively. All mushrooms were stored at 15 °C for 96 h.

Relative Humidity	Items	Ultrasonic treatment time (min)	0 h	12 h	24 h	48 h	72 h	96 h
75%RH	Browning (OD_{410nm})	0	2.13 ± 0.21a	3.22 ± 0.39a	3.58 ± 0.25ab	3.74 ± 0.15a	4.65 ± 0.24ab	5.13 ± 0.32a
		3	2.12 ± 0.19a	3.26 ± 0.31a	3.51 ± 0.14ab	3.23 ± 0.16b	4.39 ± 0.36b	4.96 ± 0.28a
		10	2.14 ± 0.15a	3.21 ± 0.23a	3.20 ± 0.19b	3.16 ± 0.22b	4.25 ± 0.19b	4.42 ± 0.17b
		30	2.16 ± 0.36a	3.65 ± 0.15a	3.68 ± 0.26a	3.96 ± 0.12a	5.11 ± 0.33a	5.36 ± 0.31a
95%RH		0	2.19 ± 0.12a	2.37 ± 0.28bc	2.64 ± 0.26ab	3.05 ± 0.17a	3.74 ± 0.15a	4.87 ± 0.18a
		3	2.22 ± 0.31a	2.66 ± 0.16ab	2.46 ± 0.35ab	2.96 ± 0.04a	3.66 ± 0.04a	4.65 ± 0.22a
		10	2.29 ± 0.16a	2.18 ± 0.13c	2.33 ± 0.17b	2.62 ± 0.12b	3.42 ± 0.16b	4.06 ± 0.11b
		30	2.34 ± 0.23a	2.69 ± 0.15a	2.91 ± 0.31a	3.22 ± 0.18a	3.82 ± 0.11a	4.92 ± 0.23a
75%RH	MDA (nmol/g)	0	4.24 ± 0.17a	4.95 ± 0.24a	5.12 ± 0.21a	5.23 ± 0.21a	5.75 ± 0.26a	6.52 ± 0.11a
		3	4.04 ± 0.06a	4.52 ± 0.15ab	4.84 ± 0.03b	4.96 ± 0.13a	5.26 ± 0.08b	5.82 ± 0.17b
		10	3.92 ± 0.08a	4.36 ± 0.16b	4.43 ± 0.05c	4.63 ± 0.18b	4.79 ± 0.14c	5.31 ± 0.15c
		30	4.16 ± 0.19a	4.75 ± 0.12ab	4.93 ± 0.12ab	5.16 ± 0.07a	5.97 ± 0.11a	6.03 ± 0.07b
95%RH		0	3.19 ± 0.22a	3.32 ± 0.13b	3.64 ± 0.16ab	3.95 ± 0.12a	4.74 ± 0.15a	5.57 ± 0.08b
		3	3.22 ± 0.11a	3.66 ± 0.06a	3.46 ± 0.25ab	3.96 ± 0.06a	4.66 ± 0.04a	5.65 ± 0.12b
		10	3.29 ± 0.16a	3.18 ± 0.09b	3.33 ± 0.17b	3.72 ± 0.05b	4.42 ± 0.06b	5.06 ± 0.11c
		30	3.34 ± 0.23a	3.69 ± 0.15a	3.91 ± 0.41a	3.22 ± 0.08c	4.82 ± 0.11a	5.92 ± 0.03a

Data are means ± SD of three replicates.
Different following letters in the same column indicate significant differences ($P < 0.05$).

3.5. Effect of ultrasound treatment and relative humidity on total soluble sugar (TSS) and total soluble protein (TSP) content

Total soluble sugars (TSS) are temporary energy storage mainly involved in the carbohydrate metabolism in cells, and regarded as an indicator of mushroom postharvest deterioration (Jiang et al., 2013). As shown in Table 4, TSS in all tested groups increased at first 12 h period due to the after-ripening, and began to decrease in the following storage. In 10-min ultrasound treated group at 95% RH, TSS kept a relatively higher level of 28.32 g kg^{-1} in straw mushrooms. The delaying effect on the process and senescence was more pronounced, which is attributed to the synergistic effect of ultrasound and high humidity storage. The ultrasound treat efficiency closely depends on the frequency, applied sound wave power, and treatment time. In most cases, treatment time could be shortened when applied high frequency and sound wave power. Similar results also could be found in plum fruit by hindering the process of decomposition of organic acids to sugars with combined effects of 100 W ultrasonic treatments for 10 min and aqueous chlorine dioxide (Chen and Zhu, 2011).

Total soluble protein (TSP) is considered as a sensitive indicator of tissue destruction and serves as a nutrient source to support continuing metabolic activity. From Table 4, TSP in the control groups under 75% RH and 95% RH decreased to 21.08 and 22.13 g kg^{-1} after 96 h of storage, respectively. Three or ten-min ultrasonic treatment could prevent the TSP utilization with the highest TSP residual value of 30.27 g kg^{-1}. Lower relative humidity (75% RH) and over-time ultrasonic treatment (30 min) had the negative effect on the TSP residual value (near to the control level).

3.6. Effect of ultrasound treatment and relative humidity on polyphenoloxidase (PPO) enzyme activity

As indicated previously, PPO promotes browning by catalyzing the oxidation of mono-and di-phenols to o-quinones, which are polymerized to produce brown pigments (Islam et al., 2014). Fig. 3 illustrated the influence of ultrasound treatment and relative humidity on PPO activities during 96-h storage at 75% RH (Fig. 3A) and 95% RH (Fig. 3B) respectively. The ultrasound treatments were found to inhibit the activity of PPO in straw mushrooms and also showed lower activity in comparison to control group. Minimum increases in PPO activity over initial levels ranged from 98.26 to 286.11 U g^{-1} in 10-min ultrasonic-treated samples stored at 95% RH ($P < 0.05$). Meanwhile, the lowest PPO activity was maintained to 236.52 U g^{-1} compared to other treatments at the 72nd h of storage, which coincided with the previous

Table 4
Changes in total soluble sugar and soluble of straw mushrooms treated by ultrasound stored at 75% RH and 95% RH, respectively. All mushrooms were stored at 15 °C for 96 h.

Relative Humidity	Items	Ultrasonic treatment time (min)	0h	12h	24h	48 h	72 h	96 h
75%RH	TSS (mg/g)	0	33.28 ± 0.94a	35.85 ± 0.09ab	32.14 ± 0.49b	27.69 ± 0.17b	26.99 ± 0.14b	23.38 ± 0.12c
		3	32.54 ± 1.23a	36.20 ± 1.12ab	32.66 ± 1.02b	31.20 ± 0.97a	27.51 ± 1.46b	25.71 ± 0.06b
		10	34.17 ± 1.09a	36.55 ± 1.05a	35.18 ± 1.54a	32.55 ± 0.92a	30.82 ± 1.11a	27.84 ± 0.13a
		30	32.94 ± 0.11a	34.71 ± 0.57b	32.38 ± 0.39b	28.71 ± 0.63b	27.38 ± 0.67b	25.92 ± 0.17b
95%RH		0	34.94 ± 0.14a	36.90 ± 0.18c	34.33 ± 0.24c	30.41 ± 0.43b	26.51 ± 0.16c	22.17 ± 0.28c
		3	33.82 ± 0.16b	38.98 ± 0.50b	36.01 ± 0.39b	33.44 ± 0.36a	29.02 ± 0.72b	24.63 ± 0.18b
		10	34.66 ± 0.18a	40.26 ± 0.33a	37.22 ± 0.57a	34.06 ± 0.32a	31.28 ± 1.39a	28.32 ± 0.14a
		30	32.12 ± 0.24c	36.56 ± 0.55c	33.80 ± 0.28c	31.67 ± 1.60b	28.11 ± 0.76bc	24.71 ± 0.08b
75%RH	TSP (mg/g)	0	34.57 ± 1.24a	30.81 ± 0.19c	28.44 ± 0.13c	27.34 ± 0.24c	25.02 ± 0.55b	21.08 ± 0.74c
		3	33.71 ± 0.74a	31.29 ± 0.14b	29.52 ± 0.39a	28.61 ± 0.20b	25.35 ± 0.23b	23.87 ± 0.58b
		10	33.92 ± 0.13a	32.44 ± 0.22a	30.07 ± 0.25b	29.34 ± 0.14a	28.61 ± 0.38a	27.88 ± 0.98a
		30	34.11 ± 0.26a	30.35 ± 0.37c	28.55 ± 0.32c	27.27 ± 0.07c	25.44 ± 1.17b	24.30 ± 1.22b
95%RH		0	33.57 ± 1.06a	30.27 ± 1.15c	28.08 ± 0.49c	26.33 ± 0.26d	24.02 ± 0.21b	22.13 ± 0.44b
		3	34.29 ± 0.91a	31.59 ± 0.16b	29.16 ± 0.43bc	27.26 ± 0.14c	25.35 ± 0.31b	25.40 ± 0.58ab
		10	34.97 ± 1.25a	33.17 ± 0.53a	32.43 ± 0.41a	31.93 ± 0.33a	31.61 ± 0.58a	30.27 ± 0.47a
		30	33.13 ± 0.76a	32.09 ± 0.15ab	30.19 ± 0.86b	28.72 ± 0.16b	24.78 ± 0.25c	23.78 ± 0.83ab

Data are means ± SD of three replicates.
Different following letters in the same column indicate significant differences ($P < 0.05$).

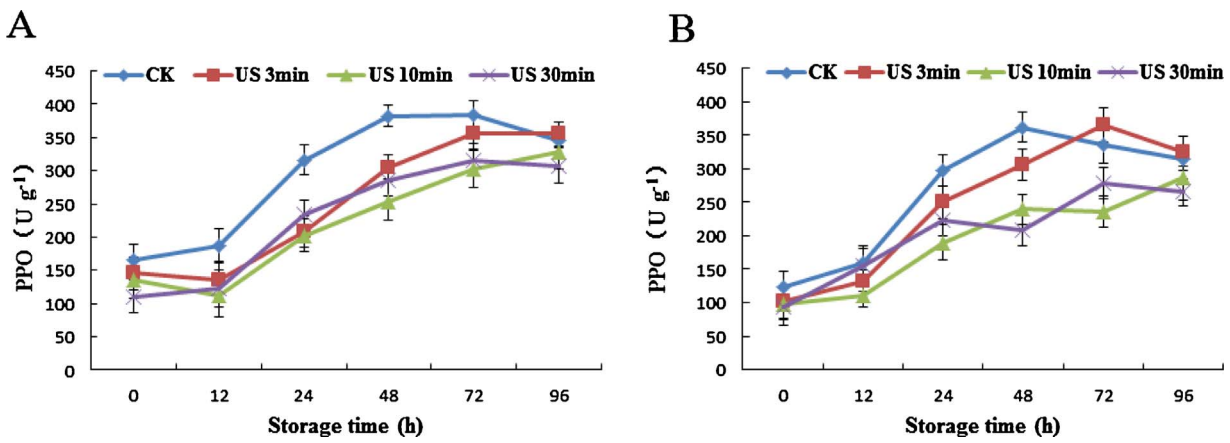

Fig. 3. Changes in PPO activity of straw mushrooms stored at 75% RH (A) and 95% RH (B) under ultrasonic pretreatment.

changes of browning degree. Ultrasound creates the shock waves which mainly contributed to the mechanical and chemical effects affecting the structures and activities of involved PPOs. Similarly, ultrasound treatment (400 W, 20 Hz) combined with citric acid inhibited the PPO activity to 3085 U g^{-1} in white mushroom of (*Agaricus bisporus*) mushroom stored at 4 °C for 12 d (Lagnika et al., 2012).

3.7. Effect of ultrasound pretreatment and relative humidity on activity of respiratory enzymes

3.7.1. Phosphohexoseisomerase (PGI) and Succinic dehydrogenase(SDH) activities

Respiration is a major factor contributing to senescence in postharvest mushrooms, involving a series of oxidation reduction reactions. Therefore, in the present study, it is crucial to maintain the respiration rate at a minimum level, as far as possible, to prolong the storage life of mushrooms. Phosphohexoisomerase (PGI) and Succinic dehydrogenase (SDH) are the important intracellular enzyme involved in glycolytic pathway and TCA pathway in respiration and play a key role in cellular energy metabolisms. The change profiles of the PGI activity of straw mushrooms during 96-h postharvest storage were shown in Fig. 4A. Compared to the control groups, PGI activities in the ultrasonic treated straw mushrooms showed a trend of sharp decrease. Among those treated groups, 10-min ultrasonic treated samples decreased the PGI activity to 0.038 U g^{-1} after 72 h of storage, which was significantly lower than those observed in 0, 3, 30 min treated samples at 95% RH, respectively. Hence, it could be concluded that the treatment with 10 min ultrasound and storage at 95% RH could effectively lower the PGI activities.

As shown in Fig. 4B, SDH activity first increased to the peak value of 10.21 U mg^{-1} Pro in control at 75% RH (Fig. 4B-1), while the maximum values of 9.68 U mg^{-1} Pro lagged to 24 h at 95% RH (Fig. 4B-2). The ultrasound treated mushrooms showed lower SDH activities than that in control sample during 72 h of storage. The lowest SDH activity of 3.62 U mg^{-1} Pro was observed in the 10-min ultrasound treated straw mushrooms storing at 95% RH ($P < 0.05$), which had the similar trend with the changes of respiratory rates. The obtained results indicated that 10-min of ultrasound treatment with 95% RH possibly inhibited the respiratory activity to delay mushrooms senescence *via* inactivating the SDH activity.

3.7.2. G-6-PDH and 6-PGDH activity

G-6-PDH and 6-PGDH are the key regulator enzymes of HMP pathway, which directly affect the activation of HMP pathway (Li et al., 2016). As shown in Fig. 4C, activities of G-6-PDH and 6-PGDH showed an increasing trend at first 12 h and then declined rapidly to approximate 2.98 μmol NADP g^{-1} min^{-1}. Meanwhile, straw mushrooms treated by ultrasound for 10 and 30-min maintained lower levels of less than 70.34 μmol NADP g^{-1} min^{-1} and 69.87 μmol NADP g^{-1} min^{-1} stored at 75% RH and 95% RH, respectively. Whereas, there is no obvious difference between G-6-PDH and 6-PGDH activities in straw mushrooms stored at 75% RH and 95% RH ($P > 0.05$).

3.7.3. Cytochrome oxidase (CCO) activity

CCO is the terminal enzyme in the respiratory electron transport chain of mitochondria, which catalyzes the transfer of electrons from Ferro cytochrome c to molecular oxygen to produce water (Soto et al., 2012). As presented in Fig. 4D, increase of ultrasound treatment time from 3 min to 30 min significantly decreased CCO activities during 96-h storage at 75% RH and 95% RH, respectively ($P < 0.05$). At the 72nd h of storage, 10 and 30-min treatment resulted in the 9.29 and 12.08 U mg^{-1} Pro of CCO activities at 95% RH, respectively (Fig. 4D-2).

4. Conclusion

A synergistic treatment of ultrasound waves and relative humidity control was proposed to extend the postharvest quality of straw mushrooms. The obtained results indicated that 10 min ultrasound treatment combined with control of relative humidity to 95% RH could prolong the storage of straw mushroom to 72 h with the beneficial appearance and physicochemical parameters like weight loss, firmness, malonaldehyde (MDA), and total soluble protein and sugar contents. Further investigations also proved that ultrasound treatment prolonged the commercial quality of straw mushrooms by inhibiting the browning and respiratory rates *via* inactivating the browning-related PPO activity, and SDH, PGI, CCO and G-6-PDH/6-PGDH activities involved in respiratory pathways. The results of this study provided useful information and reference for applying ultrasonic waves to prolong the shelf-life and reduce the quality deterioration of straw mushroom and other mushrooms. The genes expression of enzymes involved the respiratory pathways during the storage are ongoing in our lab to elucidating the mechanism of ultrasonic treatments on straw mushrooms.

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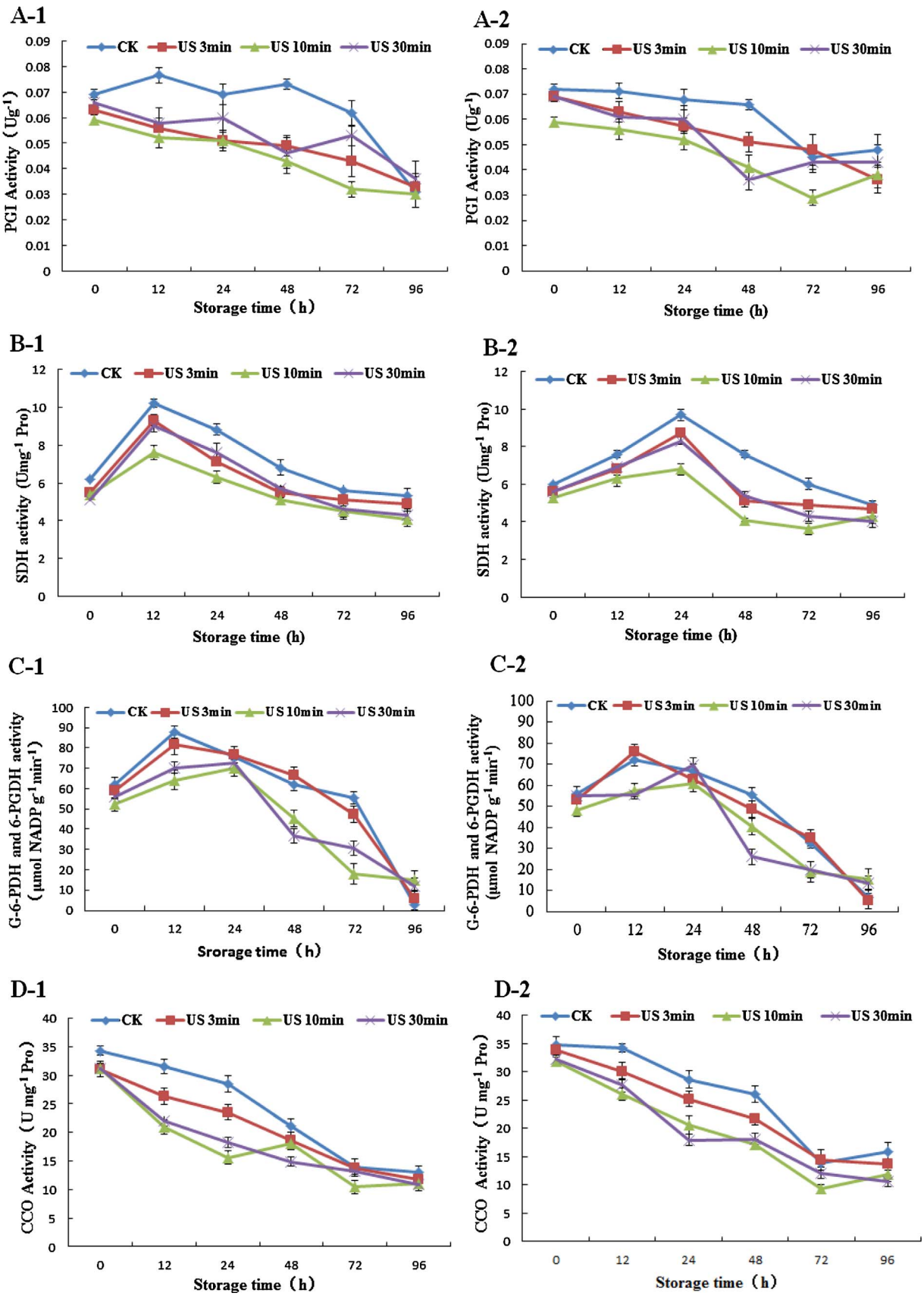

Fig. 4. Changes in PGI (A), SDH (B), G-6-PDH and 6-PGDH (C) and CCO (D) activities in straw mushrooms treated by ultrasound for different time (0, 3, 10, 30 min) and relative humidity (75% RH or 95% RH).

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