Effects of ultrasound and chemical treatments on white mushroom (*Agaricus bisporus*) prior to modified atmosphere packaging in extending shelf-life

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Abstract Effects of pre-treatments of white mushrooms prior to modified atmosphere packaging on their physico-chemical and microbiological properties were studied during 12 days of storage at 4 °C. Physico-chemical and microbiological properties of differently treated mushrooms stored at 4 °C were significantly different ($P < 0.05$) from untreated ones. Washed samples exhibited the smallest respiration rate compared to all other samples. Hydrogen peroxide washing was effective in retaining mushrooms colour change. Furthermore, the diminishments in weight of ultrasound treated samples during storage were significantly ($P < 0.05$) low in comparison with the other four treatments. The weight loss for ultrasound treated samples were 3.52 %, 4.07 % and 4.59 % for U_{ca} , $U_{H_2O_2}$ and U_{H_2O} respectively. The lowest PPO activity was observed in U_{ca} , $U_{H_2O_2}$ followed by W_{ca} , $W_{H_2O_2}$, U_{H_2O} and W_{H_2O} treatments respectfully. Combined treatments showed lower polyphenol oxidase activity, retained antioxidants, delayed pseudomonas growth and did not cause any decline in tissue firmness during storage time implying that it could extend shelf life of white mushrooms up to 12 days at 4 °C.

Keywords Citric acid · Hydrogen peroxide · Ultrasound · Modified atmosphere packaging · White mushroom · Shelf life · Storage

Introduction

In recent years, production and consumption of fresh mushrooms have been increasing substantially in the world due to their deliciousness, flavor and high nutritious value. According to the Food and Agriculture Organization (2012) data, the world production of mushrooms and truffles in 2010 exceeded 5.9 million tonnes. Apart from nutritional and general organoleptic point of view, mushrooms are also consumed by humans due to their medicinal value as comestibles.

White mushroom (*Agaricus bisporus*) is rich in acidic polysaccharides, dietary fiber, and antioxidants including vitamins C, B12, and D; folate; ergothioneine; and polyphenol (Fukushima et al. 2000; Mattila et al. 2001). This nutrient rich vegetable may have potential anti-inflammatory, hypoglycemic, and hypocholesterolemic effects. Unfortunately, it has very short shelf life (3 to 4 days) in the fresh form compared to most other vegetables at room temperature (Singh et al. 2010). This might be due to the fact that they cuticles to protect them from physical or microbial attack or water loss (Lee 1999; Martine et al. 2000; Mahajan et al. 2008). More importantly, the high tyrosinase and phenolic contents of mushrooms make them prone to rapid enzymatic browning (Brennan et al. 2000). This phenomenon is the major cause of overall quality loss of mushrooms accounting for reduction in market value. Moulds, bacteria, enzymatic activity and biochemical changes can cause spoilage during storage. In this light, mushrooms need special care, especially during storage to retain freshness.

To extend the shelf life of fresh-cut products without compromising the nutritional quality, many methods have been proposed. Among them, modified atmosphere package (MAP) stands out, which replaces in-package atmosphere with an appropriate gas mixture that protects the products from oxidation-caused alterations, microbiological attack and colour and aroma variation (Briones et al. 1992; Nussinovitch and

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Kampf 1993). Coating (Nussinovitch and Kampf 1993; Jiang et al. 2012), refrigeration (Gormley 1975; Mau et al. 1993), cultivation with CaCl_2 solution (Miklus and Beelman 1996), ozone treatment (Escriche et al. 2001), washing into anti-microbial and anti-browning solutions (Simón et al. 2010; Cliffe-Byrnes and O'beirne 2008) and use of sorbitol (Roy et al. 1995) are some of the preservation methods. Use of modified atmosphere packaging as an adjunct to low temperature storage has been extensively reported to extend the shelf-life of mushrooms (Kim et al. 2006; Ares et al. 2006; Jiang et al. 2010).

Washing mushrooms with anti-microbial and anti-browning agents has recently gained commercial popularity as a means of removing casing soil particles and for the application of browning and microbial inhibitors (Singh et al. 2010). Several studies have been done on the efficiency of wash additives for mushrooms, among which included sodium metabisulfite, hydrogen peroxide, potassium sorbate, sodium salts of benzoate, ethylenediminetetraacetic acid (EDTA) and phosphoric acids. If effective washing processes with anti-microbial and anti-browning agents could be found, this combination with ultrasound prior to proper packaging techniques could further increase the market value.

Recently, a great deal of interest has been shown in the potential benefits of Ultrasound, which has attracted considerable interest in food science and technology due to its promising effects in food processing and preservation (Knorr et al. 2004). Though lots of works have been done on the application of ultrasound during food processing and preservation, little information is known about the effects of ultrasound treatment on decay incidence and quality of vegetables and fruit after harvest.

To date there are no literatures available on the effects of combined application of MAP with ultrasound and chemical treatments on the shelf life of white mushrooms. The present research was designed to investigate on the effects of ultrasound and chemical treatments prior to modified atmosphere packaging on the physico-chemical and microbiological properties of mushrooms during cold storage.

Material and methods

Sample preparation, treatment and storage

Freshly harvested, white mushrooms (*Agaricus bisporus*) were purchased from Frostar Fruits and Vegetables Co., Ltd. (Nanjing, China). The mushrooms were transported to the laboratory and unwholesome ones eliminated after which the wholesome samples were submitted to minimal treatment. Briefly, the soil on the mushroom was washed away with distilled water then placed on absorbent paper to remove excess surface water. They then kept in cold storage at 4 °C for 24 h to reduce the respiration rate.

Mushrooms (100 ± 5 g) were selected at random and subjected to the following treatments:

- Control (C): mushrooms washed with distilled water to remove soil then packaged in MAP;
- Washing with distilled water combined with MAP ($\text{W}_{\text{H}_2\text{O}}$);
- Washing with citric acid combined with MAP (W_{ca});
- Washing with hydrogen peroxide combined with MAP ($\text{W}_{\text{H}_2\text{O}_2}$);
- Ultrasound in distilled water combined with MAP ($\text{U}_{\text{H}_2\text{O}}$);
- Ultrasound in citric acid combined with MAP (U_{ca});
- Ultrasound in hydrogen peroxide combined with MAP ($\text{U}_{\text{H}_2\text{O}_2}$).

The mushrooms were soaked for 10 min in a chilled aqueous solution 4 °C of 10 gL^{-1} citric acid or 10 mL^{-1} hydrogen peroxide (H_2O_2) or distilled water. Following treatment, the mushrooms were placed on absorbent paper to remove excess moisture.

Ultrasound treatment experiments were carried out at constant power 400 W and frequency of 20 kHz for 10 min. Mushroom weight to solution ratio was also maintained at 1:4 (w/w) (Fernandes et al. 2006). Ultrasonic treatments were carried out with sonotrodes of diameter 20 mm (model JY98-3D, Ningbo Scientz Biotechnology Co., Ltd, China). After each treatment, the samples were immediately packed.

MAP experiments were conducted using a MAP-H360 machine (Senrui Fresh Care Equipment, Suzhou, China) creating gas mixture consisting of 5 % O_2 , 10 % CO_2 and 85 % N_2 . The CO_2 and O_2 permeation of films at 23 °C and 90 % relative humidity were 10.2×10^{-12} and $3.2 \times 10^{-12} \text{ mol s}^{-1} \text{ mm}^{-2} \text{ kPa}^{-1}$, respectively.

Measurements and analyses of the mushrooms were performed on the following days of storage period; 0, 4th, 8th, and 12th days. All measurements were done in triplicates from each treatment group. The samples were randomly selected and physico-chemical and microbiological analysis have been carried out during storage.

CO_2 production

Mushrooms (100 ± 5 g) were placed into polypropylene trays ($180 \times 120 \times 30$ mm) and the packages subsequently sealed with high gas barrier film then storage at 4 °C for 12 days. The initial gas concentration was 5 % O_2 and 10 % CO_2 . Carbon dioxide concentration was measured at 4, 8 and 12th days of storage period using an O_2 and CO_2 Analyzer (Cyes-II, Jiading federation Instrument, Shanghai, China). Gas samples were taken from the polypropylene trays packaging with a 20 mL syringe. Carbon dioxide production (ΔCO_2) was calculated as follows:

$$\Delta\text{CO}_2(\%) = \text{CO}_{2f} - \text{CO}_{2i} \quad (1)$$

Where, CO_{2i} is the gas concentration on the first day and CO_{2f} is gas concentration on the final day of storage.

Weight loss

Weight losses were determined by weighing of all mushrooms contained in one package (initially 100 ± 5 g) before and after the storage period, which was expressed as weight loss percentage with respect to the initial weight (Zhang et al. 2006).

$$\text{Weight loss(\%)} = (W_0 - W_f)/W_0 \times 100 \quad (2)$$

Where, W_0 is the weight on the first day and W_f the weight on final storage day.

Colour

Surface colour of mushrooms was measured with a Minolta spectrophotometer (CR-400, Konica Minolta Sensing, Tokyo, Japan) using CIE colour parameters L^* (light/dark), a^* (red/green) and b^* (yellow/blue) values. The mean of six readings at different locations was randomly taken on the cap and compared to an ideal mushroom. ΔE was described by the following equation (Wu et al. 2012).

$$\Delta E = \left[(L_t^* - L_i^*)^2 + (a_t^* - a_i^*)^2 + (b_t^* - b_i^*)^2 \right]^{1/2} \quad (3)$$

where ΔE indicates the degree of overall colour change in comparison to colour values of an ideal mushroom. L_i^* , a_i^* and b_i^* represented the reading of fresh mushroom without any treatments, and L_t^* , a_t^* and b_t^* referred to the instantaneous individual readings during storage time after the mushrooms were treated.

Texture

Firmness was evaluated on the mushroom cap using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Godalming, UK) with a 2 mm diameter cylindrical probe according to Jiang et al. (2010) with slight modifications. Samples were penetrated to a depth of 10 mm. To obtain a sample with the same tissue orientation and dimensions, cylindrical sample of 10 mm diameter was bored out from the mushroom cap using a steel borer and cut to 20 mm length using a sharp knife. The speed of the probe was 5.0 mm s^{-1} during the pretest and penetration. Measurements were performed in triplicate on four mushroom caps for each sample and the mean was calculated.

Polyphenoloxidase (PPO) activity

Polyphenoloxidase (PPO, E.C. 1.14.18.1) activity in mushroom extract, during the storage period was determined according to Pizzocaro et al. (1993) with slight modifications.

Ten grams of fresh mushroom was ground in 10 mL of McIlvaine citric-phosphate buffer, pH 6.5. The homogenate was centrifuged at 3,000 g and 4 °C for 30 min. The supernatant obtained was filtered with Whatman no. 4 filter paper and analyzed for PPO activity at 25 °C afterward. Two millilitres of catechol solution (0.1 %) and 2 mL of McIlvaine buffer pH 6.5 were added to 0.1 mL of PPO extract. PPO activity was assayed in triplicate using a spectrophotometer (UV-visible 2600, Precision Science Instrument, Shanghai, China) at 420 nm and calculated on the basis of the slope from the linear portion of the curve plotted with ΔA_{420} . One unit of PPO was defined as the amount of enzyme present in the extract that resulted in an absorbance increase of 0.001 units per minute. The activity was expressed in units of PPO per minute and gram ($\text{U min}^{-1} \text{ g}^{-1}$) of fresh mushroom.

Total phenolic and flavonoids contents

Total phenolic contents were measured according to Singleton and Rossi (1965). Five grams of fresh mushroom was ground in 50 mL of methanol. The homogenate was centrifuged at 3,000 g and 4 °C for 30 min. The supernatant obtained was filtered with Whatman no. 4 filter paper. Briefly, 200 μL of the extract (0.1 g mL^{-1}) was diluted with 1.80 mL distilled water then 1 mL of Folin and Ciocalteu's phenol reagent was added. After 2 min, 2 mL of 20 % sodium carbonate solution (Na_2CO_3) was added. Thereafter, the reaction was allowed to proceed in the dark for 90 min and absorbance was then read at 750 nm using the spectrophotometer. Gallic acid was used to calculate the standard curve and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract fresh weight. Estimation of the phenolic compounds was carried out in triplicate.

Flavonoids were extracted and determined according to the methods of Barros et al. (2008) with slight modifications. Namely, 1.8 mL of mushroom extract (0.1 g mL^{-1}) was added to 20 μL distilled water and 75 μL of 5 % sodium nitrite (NaNO_2) then allowed to stand for 6 min. Thereafter, 150 μL of 10 % aluminium chloride (AlCl_3) was added. After standing for another 5 min, 2 mL of 1 mol L^{-1} sodium hydroxide (NaOH) were added to the mixtures and immediately their absorbance (pink in colour) was determined at 510 nm. Rutin was used to establish the standard curve and the total flavonoids of mushroom were calculated and expressed on a fresh weight as mg Rutin equivalents (RUE) per g.

Microbiological analysis

All samples were analysed for the pseudomonas bacteria counts. Ten grams of mushrooms were removed aseptically from each pack and diluted with 90 mL of 0.1 % sterile peptone water. The samples were homogenized by a stomacher at high speed for 2 min. Serial dilutions (10^{-1} – 10^{-8}) were made in

tubes (1.0 mL with 9.0 mL of 0.1 % peptone water). *Pseudomonas* bacteria were counted on cephaloridin fucidin cetrimide agar (CFC; Difco), with selective supplement SR 103 (Oxoid). The plates were incubated for 48 h at 25 °C and the number of colony forming units per gram (CFU g⁻¹) of mushroom was determined.

Statistical analysis

All experiments were conducted at least in triplicate. Analysis of variance (ANOVA) was performed and significant differences in mean values were evaluated by Tukey's test at ($P < 0.05$) using SPSS version 17.0 (SPSS, Chicago, Illinois, USA).

Results and discussion

CO₂ production

Respiration rate is an important parameter in determining deterioration rate and onset of senescence in mushroom. It is proportional to product deterioration rate and inversely proportional to its shelf life (Farber et al. 2003). The effects of storage time at 4 °C on the CO₂ production of white mushrooms treated with different methods are shown in Fig. 1a. During the first 4 days of storage, except the control, the CO₂ production was lower in washed samples followed by ultrasound treated samples. All samples showed significantly ($P < 0.05$) high and strong CO₂ production when the storage duration was allowed beyond 4 days with control being the most affected and W_{ca} the least. The results demonstrated that W_{ca} and W_{H₂O₂} followed by U_{ca}, U_{H₂O₂}, U_{H₂O} and W_{H₂O} could lower the respiration rate of mushroom during the refrigerator storage compare to control. The citric acid and hydrogen peroxide treated samples were significantly more efficient than others treatments ($P < 0.05$). The lower respiration rate with W_{ca} and W_{H₂O₂} treatment could be attributed to the antimicrobial effect of those chemicals. Ultrasound effect on respiration rate could be due to hydrogen peroxide formation in distilled water during sonication (Mead et al. 1976).

Weight loss

Water loss is an important physiological process that affects the main quality attributes of fresh mushrooms. In mushrooms, the phenomenon causes shrinkage when it becomes excessive. The weight reduction of white mushroom during storage is shown in Fig. 1b. Generally speaking, there was reduction in weight in all samples as the time (days) increased. However, the diminishments in weight of ultrasound treated samples were significantly ($P < 0.05$) low, 3.52 %, 4.07 % and 4.59 % respectively for U_{ca}, U_{H₂O₂} and U_{H₂O}, in comparison with the other four treatments during storage. The citric acid

and hydrogen peroxide treated samples also presented a lower weight loss compared to water treated samples and control. The weight losses of the samples increased up to 12 days of storage by 5.14 %, 5.65 %, 6.24 % and 10.53 % for W_{ca}, W_{H₂O₂}, W_{H₂O} and Control, respectively. Antmann et al. (2008) observed that the weight loss in shiitake mushrooms was 5 % at 18 days; however, Ares et al. (2006) observed 5.6 % of weight loss at 16 days of storage.

The lowest weight loss observed in treated samples as compared to control could be attributed to the high relative humidity in MAP associated to the effects of ultrasound and chemical on the mushroom.

Singh et al. (2010) reported that, when the harvested mushroom loses 5–10 % of its fresh weight, it begins to wilt and soon becomes unusable. According to this criterion, the control and W_{H₂O} samples had to be rejected.

Colour

Colour is the most obvious indicator of quality of fruits and vegetables because it is the first parameter that consumers

Fig. 1 Changes in (a) CO₂ production and (b) Weight loss in white mushrooms treated by washing (W) or Ultrasound (U) in water (H₂O), citric acid (CA), or hydrogen peroxide (H₂O₂) and then packed under modified atmosphere (5 % O₂ and 10 % CO₂) at 4 °C for 12 days ($n=3$)

come into contact with. In mushrooms, colour relates to the age, degree of handling and microbial spoilage.

All mushroom samples showed a decrease in luminosity (L^*) and the colour difference (ΔE) increased during storage (Table 1). Compared to the control mushrooms, those treated had higher L^* ($P<0.05$) and lower ΔE ($P<0.05$) values.

Mushroom with $U_{H_2O_2}$ and U_{ca} treatments followed by $W_{H_2O_2}$, W_{ca} , U_{H_2O} and W_{H_2O} , respectively, had higher L^* value than that of control during the 12 days of storage. Ultrasound and washing with chemical treatments were effective in retaining on whiteness of mushroom at 4 °C, with ultrasound treatment showing a greater effect than washing treatment.

The behaviour of colour difference (ΔE) during the storage differed according to the treatment. Colour difference (ΔE) with citric acid was slightly higher than ($P<0.05$) the hydrogen peroxide treatment. This difference between the treatments might be due to a bleaching effect from the hydrogen peroxide and/or because citric acid can induce a slight yellowness to mushrooms. Fortunately it is only the surface which becomes slightly yellow and so when the mushrooms are sliced it is hardly noticeable. Previously reported relationship between different quality levels in mushrooms (*A. bisporus*) and Hunter L -value provided a criterion for classification (Aguirre et al. 2008; Taghizadeh et al. 2010). Mushrooms with an L -value greater than 93 were classified as excellent,

an L -value between 90 and 93 as very good, an L -value between 86 and 89 as good, an L -value between 80 and 85 as fair and L -value between 69 and 79 as poor.

This criterion can be used as an indicator of mushroom shelf life; for example mushrooms with an L -value less than 80 would not be acceptable at wholesale level (López-Briones et al. 1993). This grading method is the most frequently used indicator of mushroom shelf-life both in the industry and research (Aguirre et al. 2008). According to that criterion López-Briones et al. (1993), the control and W_{H_2O} samples had to be rejected.

Texture

One of the most important criteria in evaluating the quality of food products is texture. The evaluation of texture makes use of instrumental as well as sensory methods. Instrumental methods are easier and quicker to carry out, enable standardization and repeatable measurements, and require a smaller number of qualified staff than sensory methods (Green et al. 1985).

Figure 2 shows changes in the firmness of mushroom with different treatments during 12 days of storage. The untreated (control) mushrooms gradually became more softened than the treated ones when stored at low temperature with increase in number of days of storage confirming a

Table 1 Colour changes of white mushrooms treated by washing (W) or Ultrasound (U) in water (H_2O), citric acid (CA), or hydrogen peroxide (H_2O_2) and then packed under modified atmosphere (5 % O_2 and 10 % CO_2) at 4 °C for 12 days

Days at 4 °C	0	4	8	12
L^*				
C	88.2±0.12a	84.3±0.90a	81.7±1.15a	71.5±1.26a
W_{H_2O}	88.2±0.50a	85.6±1.53ab	81.9±2.16a	77.8±1.74b
W_{ca}	88.5±0.32a	87.3±2.47bc	85.1±2.56bc	82.5±1.00c
$W_{H_2O_2}$	90.8±0.68ba	88.6±1.62c	86.9±3.13de	83.6±2.00c
U_{H_2O}	88.4±0.34a	87.1±1.38bc	83.7±2.00b	80.8±0.13cb
U_{ca}	89.6±0.41a	85.3±1.47c	86.0±2.26 cd	82.9±1.06c
$U_{H_2O_2}$	92.8±0.24b	88.8±1.90c	88.0±3.23e	83.9±0.78c
ΔE				
C	8.1±0.21a	9.9±0.42a	12.1±0.16a	20.3±0.18a
W_{H_2O}	6.0±0.28b	7.0±0.86b	10.9±0.28b	16.8±0.80b
W_{ca}	5.4±0.38b	6.3±0.77b	9.9±0.43bc	12.8±0.93c
$W_{H_2O_2}$	3.1±0.65c	3.6±0.14c	6.2±0.97d	8.7±0.47d
U_{H_2O}	3.1±0.54c	3.8±0.52c	6.5±1.19d	9.7±0.65d
U_{ca}	3.2±0.52c	4.0±0.26c	9.3±0.35c	12.1±0.27c
$U_{H_2O_2}$	1.3±0.25d	1.5±0.49d	5.6±0.31d	8.6±0.13d

Values are mean ± standard deviation of triplicates. Data in same column with different letters are significantly different ($P<0.05$)

C Control; W_{H_2O} Washing with distilled water combined with MAP; W_{ca} Washing with citric acid combined with MAP; $W_{H_2O_2}$ Washing with hydrogen peroxide combined with MAP; U_{H_2O} Ultrasound in distilled water combined with MAP; U_{ca} Ultrasound in citric acid combined with MAP; $U_{H_2O_2}$ Ultrasound in hydrogen peroxide combined with MAP

Fig. 2 Firmness changes in fresh white mushrooms treated by washing (W) or Ultrasound (U) in water (H₂O), citric acid (CA), or hydrogen peroxide (H₂O₂) and then packed under modified atmosphere (5 % O₂ and 10 % CO₂) at 4 °C for 12 days (*n*=3)

gradual reduction in mushroom firmness under cold storage. This could be attributed to extensive dehydration with control sample compared to those samples during the storage. On the other hand, a significant decrease in the firmness of treated mushrooms was found with storage time. Reduction in textural firmness were in the order of U_{ca} (6.32 N to 4.12 N) > $U_{H_2O_2}$ (6.31 N to 3.84 N) > U_{H_2O} (6.31 N to 3.68 N) > W_{ca} (6.34 N to 3.46 N) > $W_{H_2O_2}$ (6.35 N to 3.28 N) > W_{H_2O} (6.30 N to 2.95 N) and were all greater than of untreated samples (6.33 N to 2.18 N). Texture changes in fruits and vegetables can be related to bacteria growth or to transformations in cell wall polymers due to enzymatic reactions (Sila et al. 2007). Loss of firmness in fresh mushroom had a direct relationship to PPO activity and bacteria spoilage, which could be correlated with the visual degree of mushroom browning. Microorganisms such as *Pseudomonas* degrade mushrooms by breaking down the intracellular matrix and reducing the central vacuole, resulting in partially collapsed cells and a loss of turgor. This kind of bacterial-induced softening was observed in control samples but was inhibited by ultrasonic and chemical washing in treated samples. Therefore, it is possible that the maintaining of mushroom firmness by ultrasonic and chemical washing treatment was related to PPO activity and microorganism's growth inhibition.

Polyphenoloxidase (PPO) activity

It has been documented that PPO is responsible for browning of damaged fruits and vegetables by catalysing hydroxylation of monophenols to *o*-di phenols and dehydrogenation of *o*-diphenols to *o*-quinones in the presence of oxygen (Espin et al. 1998). All treated materials showed significant ($P<0.05$) increase in enzyme activity increase during the 12 days of storage (Table 2). PPO activity in white mushrooms increased on the initial day of storage with ultrasound treatment probably due to the stress caused by ultrasound. After this time to

the last day of storage, the lowest activity was observed in U_{ca} , $U_{H_2O_2}$ followed by W_{ca} , $W_{H_2O_2}$, U_{H_2O} and W_{H_2O} treatment but were all lower than that of the control. Enzymatic reactions present a serious processing problem during postharvest period of mushroom and this usually occur on the surface in the presence of oxygen. Cavitation and heat generated by ultrasound might have created radicals that led to inactivation of enzymes which affected the surface of the mushrooms leading to low enzyme activity with ultrasound treated samples. This observation is in good agreement with that reported by O'donnell et al. (2010), who observed that ultrasound in combination with high pressure, has shown potential for enzyme inactivation. Metal ions are necessary for enzymatic browning reactions but citric acid as a metal chelator traps the metal ions and makes them unavailable for enzymatic browning to proceed.

Total phenolics and flavonoids contents

Polyphenolic compounds are very important fruit constituents because of their antioxidant activity in chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversion into reactive oxyradicals (Oliveir et al. 2009). Total phenol contents of mushrooms from different treatments at 4 °C during 12 days of storage are presented in Table 2. Apparently, all the treated samples and the control showed the same phenolic content at day zero of storage indicating that the treatments had no effect on total phenolic content of mushroom. After day zero, total phenolic content decreased with the oxidation of PPO in the presence of oxygen until the last day of storage. However, at the end of storage time, the total phenol content in U_{ca} , $U_{H_2O_2}$ and U_{H_2O} samples was 0.92, 0.85 and 0.80 mg g⁻¹ respectively, higher than W_{ca} (0.65 mg g⁻¹), $W_{H_2O_2}$ (0.62 mg g⁻¹), W_{H_2O} (0.61 mg g⁻¹) and Control (0.51 mg g⁻¹) samples. There was no difference in the total phenol content of control and W_{H_2O} samples throughout the storage time, except for the last day. Ultrasound and chemical washing treatments were effective in retaining the total phenolic content of mushroom at 4 °C, with ultrasound treatment showing a greater effect than washing treatment.

Similar to total phenolic, all the treatments showed a reduction in flavonoids compounds during storage (Table 2). In this study, like with total phenolics, ultrasound coupled with using chemical, synergistically caused a higher retention of flavonoids content during 12 days of storage. Kazunori (2010) demonstrated that, potatoes treated with ultrasound and electricity for 5–30 min retained high amount of antioxidants including phenols.

Microbiological analysis

Microbial spoilage of mushrooms is usually due to the growth of *pseudomonas* bacteria. As these bacteria grow,

Table 2 Changes in polyphenoloxidase (PPO) activity and functional components in white mushrooms treated by washing (W) or Ultrasound (U) in water (H₂O), citric acid (CA), or hydrogen peroxide (H₂O₂) and then packed under modified atmosphere (5 % O₂ and 10 % CO₂) at 4 °C for 12 days

Values are mean ± standard deviation of triplicates. Data in same column with different letters are significantly different ($P<0.05$)

C Control; W_{H_2O} Washing with distilled water combined with MAP; W_{ca} Washing with citric acid combined with MAP; $W_{H_2O_2}$ Washing with hydrogen peroxide combined with MAP; U_{H_2O} Ultrasound in distilled water combined with MAP; U_{ca} Ultrasound in citric acid combined with MAP; $U_{H_2O_2}$ Ultrasound in hydrogen peroxide combined with MAP

Days at 4 °C	0	4	8	12
PPO (U min ⁻¹ g ⁻¹)				
C	1653±3.41e	2399±2.66a	3665±5.73a	4176±7.56a
W_{H_2O}	1644±9.63f	2352±8.54b	3283±5.93b	3638±12.51b
W_{ca}	1607±3.67 g	2173±7.52e	2926±8.57e	3085±13.20e
$W_{H_2O_2}$	1677±7.80d	2275±4.68d	3026±3.63d	3329±2.44d
U_{H_2O}	1773±7.32a	2292±9.53c	3135±6.38c	3458±1.97c
U_{ca}	1756±2.75b	2016±7.60 g	2325±14.31 g	2421±3.62 g
$U_{H_2O_2}$	1734±3.50c	2148±4.92f	2391±11.26f	2573±4.84f
Total phenolics (mg g ⁻¹)				
C	1.44±0.40a	0.80±0.10a	0.65±0.03a	0.51±0.06a
W_{H_2O}	1.46±0.09a	0.89±0.07a	0.71±0.04a	0.61±0.21ab
W_{ca}	1.41±0.04a	0.97±0.02ab	0.78±0.07ab	0.62±0.12ab
$W_{H_2O_2}$	1.39±0.05a	0.93±0.05ab	0.76±0.14ab	0.65±0.06ab
U_{H_2O}	1.43±0.13a	1.10±0.09bc	0.91±0.14bc	0.80±0.09bc
U_{ca}	1.48±0.14a	1.28±0.11c	1.01±0.02c	0.92±0.03c
$U_{H_2O_2}$	1.40±0.07a	1.21±0.23c	0.95±0.05bc	0.85±0.04c
Total flavonoids (mg g ⁻¹)				
C	0.94±0.09a	0.68±0.03a	0.49±0.05a	0.26±0.10a
W_{H_2O}	0.94±0.09a	0.73±0.04ab	0.55±0.07ab	0.31±0.04a
W_{ca}	0.94±0.09a	0.80±0.06ab	0.61±0.01ab	0.39±0.02ab
$W_{H_2O_2}$	0.94±0.09a	0.78±0.08ab	0.62±0.02abc	0.36±0.10ab
U_{H_2O}	0.94±0.09a	0.87±0.12b	0.66±0.08abc	0.51±0.03bc
U_{ca}	0.94±0.09a	0.89±0.02b	0.80±0.01c	0.60±0.06c
$U_{H_2O_2}$	0.94±0.09a	0.88±0.07b	0.72±0.11bc	0.53±0.05bc

they break down the mushroom fibers which soften the mushroom and leads to enzymatic browning (Brennan and Gormley 1998). Table 3 presents growth of pseudomonas bacteria (expressed as log CFU g⁻¹) of fresh mushroom during 12 days of storage. Gradual growth of microorganisms was seen during storage in all samples. However, some treatments

retarded the microbial growth more than others. The highest amount of microorganisms was observed in control samples.

Samples combined ultrasound with chemical treatment followed by washing with chemical treatment, were found to be effective in delaying pseudomonas bacteria in mushroom during the 12 days of cold storage. The citric acid and the

Table 3 Pseudomonas counts in white mushrooms treated by washing (W) or Ultrasound (U) in water (H₂O), citric acid (CA), or hydrogen peroxide (H₂O₂) and then packed under modified atmosphere (5 % O₂ and 10 % CO₂) at 4 °C for 12 days

Days at 4 °C	0	4	8	12
C	5.6±0.54a	6.9±0.12a	8.0±1.03a	8.9±0.23a
W_{H_2O}	5.4±0.13b	6.6±0.43b	7.6±0.74b	8.2±0.32b
W_{ca}	5.3±0.64bc	6.4±1.01c	7.2±0.96c	7.9±0.23c
$W_{H_2O_2}$	5.3±0.28bcd	6.2±0.75d	7.1±0.65 cd	7.7±0.54d
U_{H_2O}	5.2±0.43 cd	6.0±0.92e	6.9±0.23d	7.6±1.43d
U_{ca}	4.9±0.76f	5.5±0.34f	6.5±0.56f	7.2±0.74e
$U_{H_2O_2}$	5.1±0.45e	5.7±1.32f	6.7±0.84e	7.4±0.38e

Values are mean ± standard deviation of triplicates. Data in same column with different letters are significantly different ($P<0.05$)

C Control; W_{H_2O} Washing with distilled water combined with MAP; W_{ca} Washing with citric acid combined with MAP; $W_{H_2O_2}$ Washing with hydrogen peroxide combined with MAP; U_{H_2O} Ultrasound in distilled water combined with MAP; U_{ca} Ultrasound in citric acid combined with MAP; $U_{H_2O_2}$ Ultrasound in hydrogen peroxide combined with MAP

hydrogen peroxide treatments both reduced pseudomonas bacteria number, but citric acid treatment had slightly more antibacterial effect than that of hydrogen peroxide treatment which agrees with Brennan et al. (2000) and Simón et al. (2010).

During the storage, the pseudomonas bacteria in the U_{ac} (4.91 to 7.28 log CFU g⁻¹) and $U_{H_2O_2}$ (5.14 to 7.43 log CFU g⁻¹) followed by W_{ac} (5.31 to 7.79 log CFU g⁻¹), $W_{H_2O_2}$ (5.36 to 7.97 log CFU g⁻¹), U_{H_2O} (5.21 to 7.63 log CFU g⁻¹) and W_{H_2O} (5.46 to 8.21 log CFU g⁻¹) samples, respectively, were maintained at a lower levels than those in the control ($P < 0.05$). The lowest amounts of microorganisms found in U treated samples might be due to ultrasound process that creates the cavitation caused by the changes in pressure responsible for the destruction of bacteria. The mechanism of microbial destruction is mainly due to thinning of cell membranes, localized heating and production of free radicals (Butz and Tauscher 2002; Fellows 2000). These observations are in agreement with those reported by Cao et al. (2010), where ultrasonic treatment was observed to inhibit microbial population on strawberry fruit. The results of a research carried out by Dolatowski and Stasiak (2002) proved that ultrasound processing is having a significant influence on microbiological contamination of meat. Moreover, the inhibitory effect of citric acid treatment on microbial growth can be explained by presence of its low pH and also by dissociation of the acid molecule within bacterial cells. Metal ions are necessary for bacterial growth but citric acid traps the metal ions and makes them unavailable. Hydrogen peroxide activity could be due to its oxidising effects on bacteria.

Conclusions

Overall, mushrooms washed with citric acid or hydrogen peroxide and packing had significant beneficial effects than those washed in water and untreated ones. Application of ultrasound and chemical washing combination on mushrooms prior to MAP was shown to be the most effective treatment in delaying antioxidants degradation, PPO activity, weight loss and microbial growth. Citric acid showed slightly antibacterial potential than the hydrogen peroxide but induced a slight yellowness to mushrooms. The combined treatment of MAP, ultrasound and washing with citric acid or hydrogen peroxide best to extend the shelf-life of fresh mushroom during cold storage and could be a better alternative to the other preservation method. However, more researches are needed to prove the effects of ultrasound on fruit and vegetables to promote the application of this technique.

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