

## Effect of high argon pressure and modified atmosphere packaging on the white mushroom (*Agaricus bisporus*) physico-chemical and microbiological properties

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### Summary

The effect of high argon pressure (HAP) combined with modified atmosphere packaging (MAP) processing on the physico-chemical and microbiological characteristics of white mushrooms were assessed during 15 days of storage at 4 °C. The comparisons between different samples showed, HAP samples exhibited the smaller rate of weight loss and higher increase in total flavonoid and protein contents. Weight losses after 15 days of storage were 2.3%, 3.8%, 4.8% and 7.6% for HAP, MAP, HAP-MAP and untreated mushrooms, respectively. Furthermore, MAP treatment showed smaller polyphenol oxidase (PPO, E.C. 1.14.18.1) activity during storage. PPO activity in white mushrooms increased during the 15 days of storage from initial activity of 1009 U·min<sup>-1</sup>·g<sup>-1</sup> to 2070, 2418, 2976 and 3174 U·min<sup>-1</sup>·g<sup>-1</sup> corresponding to MAP, HAP-MAP, HAP and control treatments, respectively. MAP-HAP treatment revealed beneficial effects on white mushroom colour, total phenol content and ascorbic acid content, and moderate effect on total flavonoid and polyphenol oxidase (PPO). All treatments were effective in retarding mushroom colour change. Thus, application of MAP-HAP could extend the storage life of white mushrooms to 15 days at 4 °C.

### Keywords

high argon pressure; modified atmospheres package; white mushroom; shelf life; storage

Edible mushrooms are widely consumed in many parts of the world owing to their characteristic taste, aroma and nutritional relevance. Edible mushrooms are valuable components of diet and their culinary and commercial values are mainly due to their organoleptic properties such as texture and flavour [1, 2]. They also are excellent source of several essential amino acids, vitamins (B<sub>2</sub>, niacin and folates) and minerals (potassium, phosphorus, zinc and copper). Mushroom protein is comparable to the high-quality animal protein and superior to vegetables and cereals proteins [3, 4].

Postharvest browning of *Agaricus bisporus* mushrooms is a severe problem that reduces the shelf life of harvested ones because of their continual respiration and biochemical activities. Mushrooms have a shelf life of 3 to 4 days, which is short compared to other vegetables at room

temperature, because they have no cuticles to protect them from physical or microbial attack or water loss [5, 6]. More importantly, the high tyrosinase and phenolic contents of mushrooms make them prone to rapid enzymatic browning [7]. 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. Gram-negative microorganisms, such as *Pseudomonas tolaasii* and *Pseudomonas fluorescens*, and yeasts, such as *Candida sake*, have been associated with mushroom spoilage [1, 6].

Storage of mushrooms affects their quality in the following ways: darkening of the tissue, elongation of the stems, opening of the caps and hardening of the flesh [8]. Therefore, one alternative to extend mushroom shelf life during post-harvest storage and commercialization is modified

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atmosphere packaging (MAP) and high argon pressure (HAP) at cold storage. MAP is a method of conservation and may be suitable to extend the shelf life of fresh mushrooms due to its unique technological characteristics involving the use of atmospheric gases, where O<sub>2</sub> concentration is reduced and CO<sub>2</sub> concentration is enriched. This does not only reduce respiration rate, but potentially can inhibit microbial growth and physiological disorders thereby preserving product's quality and increasing shelf life.

Recently, a great deal of interest has been shown in the potential benefits of argon in food preservation. Beyond its greater efficiency, argon is much more soluble in both water and oils than nitrogen, making it more efficient at displacing entrained and dissolved oxygen from food media [9]. Apart from its physical effects in media, argon is also effective in controlling the inhibition of respiratory enzymes, including oxidases [10]. Noble gases have been shown to have an effect on enzyme reaction rates and yields [11]. Additionally, argon has been shown to inhibit oxidases even when oxygen is present, whereas nitrogen does not.

Antimicrobial activity of argon alone has been shown to be particularly high for yeasts and moulds. The polarizability and a higher ionization potential of argon affect liganding of gases to active sites, penetration of gases through membranes, and biological reaction potentials [9]. Argon is reported to be biochemically active, probably due to its enhanced solubility in water compared with nitrogen and possible interference with enzymatic oxygen receptor sites [11].

When a noble gas is dissolved in water under appropriately selected temperature and pressure conditions, highly ordered "iceberg-like" structures form around solute molecules in aqueous solution due to hydrophobic hydration [12], called gas hydrate or clathrate hydrate. Noble gases such as argon (Ar), krypton (Kr) and xenon (Xe) can form clathrate hydrates in some vegetables and fruits more readily under higher pressure than the critical pressure point [13, 14]. By subjecting mushrooms to high pressure, microorganisms and enzymes can be inactivated without the degradation of flavour and nutrients. To obtain effective sterilization, the combination of pressure with other treatments is required [15].

To date there are no literature data available documenting the effect of combined application of high gas pressure and modified atmosphere on the shelf life of white mushrooms. The objective of this study was to investigate the potential application of high argon pressure and modified atmosphere

on physico-chemical and microbiological properties of white mushrooms.

## MATERIAL AND METHODS

### Sample preparation, treatment and storage

Freshly harvested white mushrooms (*Agaricus bisporus*) were purchased from Frostar Fruits and Vegetables (Nanjing, China) and submitted to the steps of minimal processing. The mushrooms were transported to the laboratory and stored at 4 °C. Mushrooms (52.5 g ± 2.5 g) were selected at random and treated. Treatments included four groups: control without treatment; high argon pressure treatment (HAP); modified atmosphere packaging (MAP) and high argon pressure treatment combined with modified atmosphere packaging (HAP-MAP). MAP experiments were conducted using a MAP-H360 machine (Senrise Fresh Care Equipment, Suzhou, China) to create a mixture consisting of 5% O<sub>2</sub>, 10% CO<sub>2</sub> and 85% N<sub>2</sub>. The permeability of films at 23 °C and 90% relative humidity for CO<sub>2</sub> and O<sub>2</sub> was 10.2 × 10<sup>-12</sup> and 3.2 × 10<sup>-12</sup> mol·s<sup>-1</sup>mm<sup>-2</sup>kPa<sup>-1</sup>, respectively. All samples without high pressure gases treatment were packaged in the low-density polyethylene (LDPE) bags. The mixture used was selected after performing a number of preliminary experiments (data not shown).

HAP treatments were performed with an apparatus HCYF-3 (Hunan Scientific Research Instrument, Jiangsu, China), which had an operating temperature range from 0 °C to 50 °C at a maximum working pressure of 30 MPa. The primary components of the apparatus consist of a 500 ml stainless steel treatment vessel, a plunger pump, a vacuum pump and a thermally controlled bath.

For HAP, the sample was subjected to a treatment of 20 MPa at (4 ± 1) °C for 60 min. After treatment, the mushrooms were immediately packaged (air: O<sub>2</sub> 21%, CO<sub>2</sub> 0%). For the combined treatment HAP-MAP, samples were pressurized at 20 MPa at (4 ± 1) °C for 60 min and flushed with gas 5% O<sub>2</sub>, 10% CO<sub>2</sub> and 85% N<sub>2</sub>. Mushrooms packed with air were used as control. Samples were then stored for 15 days at (4 ± 1) °C and 90% relative humidity. Every 3 days, three replicates from each treatment group were randomly selected and analysed for physico-chemical and microbiological qualities.

## PHYSICO-CHEMICAL ANALYSIS

### Respiration rate (Gas composition)

Carbon dioxide and oxygen concentrations in packages were evaluated using an O<sub>2</sub> and CO<sub>2</sub>

Analyzer Cyes-II (Jiading Federation Instrument, Shanghai, China). Gas samples were taken from the bags with a 20 ml syringe. Oxygen used or uptake ( $\Delta O_2$ ) and  $CO_2$  production ( $\Delta CO_2$ ) were calculated:

$$\Delta O_2 [\%] = O_{2i} - O_{2f} \quad (1)$$

$$\Delta CO_2 [\%] = CO_{2f} - CO_{2i} \quad (2)$$

where  $O_{2i}$ ;  $CO_{2i}$  are the gas concentrations on the first day and  $O_{2f}$ ;  $CO_{2f}$  gas concentrations on final storage day.

#### Weight loss

Weight loss was determined by weighing the contents of the packages before and after the storage period, which was expressed as the percentage of loss of weight with respect to the initial weight.

$$\text{Weight loss} [\%] = \frac{(W_0 - W_f)}{W_0} \times 100 \quad (3)$$

where  $W_0$  is the weight on the first day and  $W_f$  the weight on final storage day.

#### Polyphenoloxidase (PPO)

Polyphenoloxidase (PPO, E.C. 1.14.18.1) activity in mushroom extract, during the storage period was determined according to PIZZOCARO et al. [16] with slight modifications. 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  $\Delta A_{420}$ . One unit of PPO was defined as the amount of enzyme present in the extract that resulted in an absorbance increase of 0.001 U per minute. The activity was expressed in units of PPO per minute and gram ( $U \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ) of fresh mushrooms.

#### Total phenolics and flavonoids

The sample total phenolic contents were measured according to SINGLETON and ROSSI [17]. Extract (200  $\mu\text{l}$ , 0.1  $\text{g} \cdot \text{ml}^{-1}$ ) with 1.80 ml distilled water was diluted with 1 ml of Folin and Ciocalteu's phenol reagent. After 2 min, 2 ml of 20% sodium carbonate solution ( $\text{Na}_2\text{CO}_3$ ) was added. The reaction was kept in the dark for 90 min and then the absorbance was read at 750 nm using the spectrophotometer. Gallic acid was used to construct the standard curve and the results were expressed as milligrams of gallic acid equivalents (GAE) per

kilogram of extract fresh weight. Estimation of the phenolic compounds was carried out in triplicate.

Flavonoids were extracted and determined according to the methods of LILLIAN et al. [18] with slight modifications. The mushroom extract (1.8 ml, 0.1  $\text{g} \cdot \text{ml}^{-1}$ ) was diluted with 20  $\mu\text{l}$  distilled water and 75  $\mu\text{l}$  5% sodium nitrite ( $\text{NaNO}_2$ ). After 6 min, 150  $\mu\text{l}$  10% aluminium chloride ( $\text{AlCl}_3$ ) were added. After another 5 min, 2 ml of 1  $\text{mol} \cdot \text{l}^{-1}$  sodium hydroxide ( $\text{NaOH}$ ) were added to the mixture. Immediately, the absorbance of the mixture, pink in colour, was determined at 510 nm. Rutin was used to construct the standard curve and the total flavonoids of mushrooms were expressed on a fresh weight as milligrams of rutin equivalents (RUE) per kilogram.

#### Total ascorbic acid

Ascorbic acid (AA) was analysed according to the 2,6-dichlorophenol-indophenol titration method by JAMES [19]. Ten grams of fresh mushrooms were mixed with 10 ml (3%) metaphosphoric acid and homogenized. Ten millilitres of the filtrate were titrated with dye until the distinct rose pink colour persisted for 15–20 s. Results were expressed as  $\text{mg} \cdot \text{kg}^{-1}$  of the sample.

#### Soluble protein

Soluble protein was determined according to the method of BRADFORD [20] using bovine serum albumin as standard.

#### Microbiological analysis

All samples were analysed for mesophilic and psychrophilic bacteria, and for yeasts and moulds during 12 days of storage. 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). Aerobic counts were determined on plate count agar (PCA; Merck, Darmstadt, Germany) following incubation at 35 °C over 3 days for mesophilic bacteria, and at 4 °C over 7 days for psychrophilic bacteria. Yeasts and moulds were estimated on potato dextrose agar (PDA; Merck) with incubation at ( $28 \pm 1$ ) °C for 7 days.

#### Acceptance test

Sensory quality of mushrooms was evaluated using the following parameters such as colour and veil opening. Whiteness is an important indicator among many that determine the quality of mushrooms. Mushroom deterioration has been related

to the development of the sporophore, such as breaking of the veil, elongation of the stipe, opening of the pileus, expansion of gill tissue and spore formation [21, 22].

### Colour

The 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.  $\Delta E$  was described by the following equation [23]:

$$\Delta E = [(L_t^* - L_i^*)^2 + (a_t^* - a_i^*)^2 + (b_t^* - b_i^*)^2]^{1/2} \quad (4)$$

where  $\Delta E$  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.

### Veil opening rate

Veil opening rate can be used to characterize the aging of white mushrooms. The veil opening is the detachment of the head from the stem of the mushroom. The veil opening was evaluated manually by visual observation. It was defined as the ratio of the number of mushrooms with cap opening out to the total number of mushrooms.

### Statistical analysis

All experiments were conducted at least in triplicate. Analysis of variance (ANOVA) was per-

formed 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

### PHYSICO-CHEMICAL PROPERTIES

#### Respiration rate (Gas composition)

Fig. 1 shows the variation of gas composition (%) during storage at 4 °C for 15 days of fresh white mushrooms under different treatment. The four samples generated significantly ( $P < 0.05$ ) different atmosphere. Oxygen concentration decreased during storage with increasing CO<sub>2</sub> concentration in the package. After 6 days, the modified atmosphere packaged samples reached equilibrium. The HAP-MAP, HAP and MAP exhibited reduced respiration rate compared with that of the control. HAP treatment showed a higher respiration rate during the first 6 days of storage then decreased and increased after 12<sup>th</sup> day when compared with the other treatments.

Mushrooms treated with MAP and HAP-MAP showed lower respiration rate compared to HAP and control. However, HAP-MAP treatment revealed higher efficiency than MAP. The high argon pressure and the lower O<sub>2</sub> concentration coupled with higher CO<sub>2</sub> concentration than in air appeared to be reasons explaining the reduction of respiration rates. Similar work has been previously carried out and an increase in CO<sub>2</sub> concentration was reported to exhibit an inhibitory effect on respiration rate of mushrooms [1, 24, 25]. ZHANG et al. [26] reported a decreased respiration rate on *Asparagus* spears using argon and xenon treat-

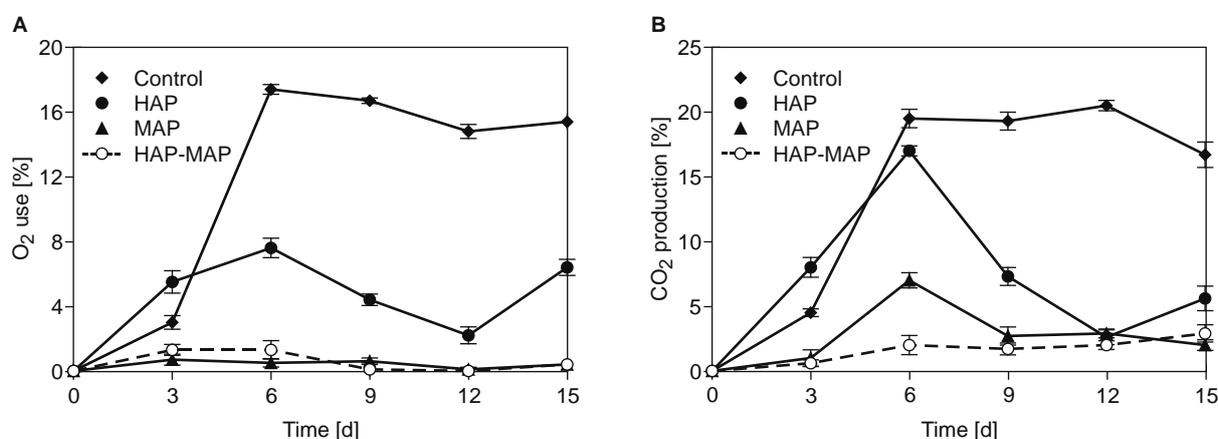


Fig. 1. Gas composition variation in white mushrooms packaged during storage at 4 °C for 15 days under different treatments.

**Tab. 1.** Physico-chemical characteristics of white mushrooms during storage at 4 °C for 15 days under different treatments.

Days	Control	HAP	MAP	HAP-MAP
	Weight loss [%]			
0	—	—	—	—
3	1.95 ± 0.01 <sup>a</sup>	0.23 ± 0.03 <sup>d</sup>	1.68 ± 0.10 <sup>b</sup>	1.63 ± 0.29 <sup>c</sup>
6	3.63 ± 0.99 <sup>a</sup>	1.85 ± 0.4 <sup>c</sup>	1.92 ± 0.91 <sup>b</sup>	3.63 ± 1.06 <sup>a</sup>
9	3.77 ± 1.72 <sup>b</sup>	1.88 ± 0.90 <sup>c</sup>	1.92 ± 0.51 <sup>c</sup>	3.92 ± 0.02 <sup>a</sup>
12	5.88 ± 1.00 <sup>a</sup>	1.92 ± 0.96 <sup>d</sup>	3.29 ± 0.67 <sup>c</sup>	4.66 ± 0.04 <sup>b</sup>
15	7.69 ± 1.50 <sup>a</sup>	2.32 ± 0.01 <sup>d</sup>	3.84 ± 1.55 <sup>c</sup>	4.88 ± 0.86 <sup>b</sup>
	PPO [U·min <sup>-1</sup> ·g <sup>-1</sup> ]			
0	1009 ± 8.54 <sup>a</sup>	1009 ± 8.54 <sup>a</sup>	1009 ± 8.54 <sup>a</sup>	1009 ± 8.54 <sup>a</sup>
3	2069 ± 23.64 <sup>a</sup>	1729 ± 21.07 <sup>b</sup>	1122 ± 24.02 <sup>d</sup>	1203 ± 1.73 <sup>c</sup>
6	2159 ± 15.71 <sup>a</sup>	1875 ± 28.51 <sup>c</sup>	1275 ± 11.13 <sup>d</sup>	1981 ± 6.24 <sup>b</sup>
9	2210 ± 9.16 <sup>a</sup>	2151 ± 36.37 <sup>b</sup>	1754 ± 10.53 <sup>d</sup>	2065 ± 3.60 <sup>c</sup>
12	2774 ± 16.70 <sup>a</sup>	2440 ± 15.62 <sup>b</sup>	1932 ± 16.52 <sup>d</sup>	2215 ± 2.64 <sup>c</sup>
15	3174 ± 24.75 <sup>a</sup>	2976 ± 7.93 <sup>b</sup>	2070 ± 14.29 <sup>d</sup>	2418 ± 9.84 <sup>c</sup>

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

ment. Similar results were reported by ZHAN and ZHANG [13] for cucumber with compressed xenon treatment.

#### Weight loss

Tab. 1 displays some physico-chemical characteristics of mushrooms packed with different methods of packaging during refrigerated storage. Dehydration is an important process in mushroom quality loss during post-harvest storage. That could be attributed to the fact that mushrooms have no cuticle and are only protected by a thin and porous epidermal structure, which does not prevent a quick superficial dehydration [6, 27, 28].

Evaluation of the three methods in this study demonstrated a significant reduction ( $P < 0.05$ ) in the weight loss. Weight losses after 15 days of storage for HAP, MAP, HAP-MAP and untreated mushrooms were 2.3%, 3.8%, 4.8% and 7.6% respectively. This observation is in good agreement with that reported by ANTMANN et al. [29] and ARES et al. [30], who observed that shiitake mushrooms showed a reduction in weight lower than 5% and 5.6% for 18 and 16 days of storage period, respectively. The lower weight loss at HAP treatment could be attributed to the formation of clathrate hydrate, due to the water molecules confined by hydrogen bonds, which decreased the loss of water.

#### Polyphenoloxidase (PPO)

It has been documented that PPO is respon-

sible for browning of damaged fruits and vegetables by catalysing hydroxylation of monophenols to *o*-diphenols and dehydrogenation of *o*-diphenols to *o*-quinones in the presence of oxygen. PPO activity in white mushrooms increased during the 15 days of storage from initial activity of 1009 U·min<sup>-1</sup>·g<sup>-1</sup> to 2070, 2418, 2976 and 3174 U·min<sup>-1</sup>·g<sup>-1</sup> corresponding to MAP, HAP-MAP, HAP and control treatments, respectively (Tab. 1). The lowest activity was observed with MAP followed by HAP-MAP treatment. This low activity in MAP and HAP-MAP could be due to low O<sub>2</sub> (5%) compared to HAP and control (21%). DEVI et al. [31] and SOMMER et al. [32] reported that low O<sub>2</sub> decreased tyrosinase activity reducing enzymatic browning. HAP, compared to control, had also low activity due to high pressure inert gases, which interfered with enzymatic oxygen receptor sites or could be due to argon clathrate hydrate formation that suppressed the activity. Behnke [33] demonstrated that high pressure inert gases inhibited tyrosinase systems by decreasing oxygen availability rather than by physically altering the enzyme. When noble gases dissolve in water, enzymatic reactions are inhibited, resulting in restrained vegetable metabolism [26]. ZHANG et al. [34] indicated that argon reduced the tyrosinase activity. According to XUAN et al. [35], the high pressure carbon dioxide reduced approximately by 95% the PPO activity in red beet extract. Otherwise, the respiration rate decreased with the reduction of the O<sub>2</sub> concentra-

tion. In our results, we observed at low O<sub>2</sub> a correlation between PPO activity and respiration rate. Therefore, the low O<sub>2</sub> reduced the respiration rate and PPO activity.

#### Total phenolics and flavonoids

The antioxidants contained in foods, in particular vegetables, are total phenolics, flavonoids, ascorbic acid, carotenoids and tocopherol, which are important protective agents for human. They are the most plentiful classes of constituents in the plant kingdom and have been reported to have multiple biological effects [36, 37, 38]. Similar to PPO, all the treatments showed a reduction in total phenolic compounds during storage (Tab.2). However, MAP and HAP-MAP treatment appeared to be significantly ( $P < 0.05$ ) efficient in delaying the reduction in phenolics in the white mushrooms as compared to HAP treatment. Whereas untreated white mushrooms presented a lower level of total phenolics, all treatments affected significantly ( $P < 0.05$ ) the flavonoid content in white mushrooms during the 15 days

storage period. Unlike with total phenolics, HAP and HAP-MAP treatments of the white mushrooms were more effective than MAP treatment in delaying the decrease in flavonoids.

#### Total ascorbic acid

All treatments influenced significantly the decrease in ascorbic acid content during the storage ( $P < 0.05$ ). Between day 0 and 9, HAP treatment lead to 29.25 mg·kg<sup>-1</sup>, HAP-MAP 29.52 mg·kg<sup>-1</sup> and MAP 24.58 mg·kg<sup>-1</sup> of ascorbic acid.

However, after day 9, MAP followed by HAP-MAP treatments showed moderate vitamin C reduction (Tab. 2). At the end of the storage, the contents of ascorbic acid in HAP and control treatment were not significantly ( $P < 0.05$ ) different. BIGNON [39] observed that vitamin C content of fruit and vegetable products was not significantly affected by pressure treatment in contrast to thermal treatment. ZHANG et al. [26] reported that compressed argon and xenon mix treatment on asparagus spears did not reduce significantly the loss of vitamin C.

**Tab. 2.** Changes in functional components in white mushrooms during storage at 4 °C for 15 days under different treatments.

Days	Control	HAP	MAP	HAP-MAP
	Total phenolics [mg·kg <sup>-1</sup> ]			
0	971 ± 0.06 <sup>a</sup>	971 ± 0.06 <sup>a</sup>	971 ± 0.06 <sup>a</sup>	971 ± 0.06 <sup>a</sup>
3	800 ± 0.04 <sup>c</sup>	864 ± 0.02 <sup>b</sup>	969 ± 0.09 <sup>a</sup>	970 ± 0.03 <sup>a</sup>
6	759 ± 0.04 <sup>d</sup>	773 ± 0.04 <sup>c</sup>	790 ± 0.02 <sup>b</sup>	904 ± 0.09 <sup>a</sup>
9	654 ± 0.10 <sup>c</sup>	703 ± 0.01 <sup>a</sup>	703 ± 0.09 <sup>a</sup>	675 ± 0.05 <sup>b</sup>
12	599 ± 0.02 <sup>c</sup>	603 ± 0.03 <sup>b</sup>	666 ± 0.08 <sup>a</sup>	669 ± 0.06 <sup>a</sup>
15	523 ± 0.08 <sup>c</sup>	528 ± 0.02 <sup>b</sup>	549 ± 0.04 <sup>a</sup>	532 ± 0.03 <sup>b</sup>
Total flavonoids [mg·kg <sup>-1</sup> ]				
0	687 ± 0.05 <sup>a</sup>	687 ± 0.05 <sup>a</sup>	687 ± 0.05 <sup>a</sup>	687 ± 0.05 <sup>a</sup>
3	561 ± 0.06 <sup>c</sup>	683 ± 0.10 <sup>a</sup>	642 ± 0.03 <sup>b</sup>	475 ± 0.09 <sup>d</sup>
6	439 ± 0.03 <sup>d</sup>	667 ± 0.05 <sup>a</sup>	543 ± 0.08 <sup>b</sup>	520 ± 0.02 <sup>c</sup>
9	312 ± 0.02 <sup>c</sup>	434 ± 0.04 <sup>a</sup>	316 ± 0.04 <sup>c</sup>	393 ± 0.01 <sup>b</sup>
12	250 ± 0.04 <sup>d</sup>	327 ± 0.09 <sup>a</sup>	304 ± 0.01 <sup>c</sup>	309 ± 0.03 <sup>b</sup>
15	220 ± 0.02 <sup>d</sup>	257 ± 0.08 <sup>a</sup>	238 ± 0.02 <sup>c</sup>	249 ± 0.04 <sup>b</sup>
Vitamin C [mg·kg <sup>-1</sup> ]				
0	39.64 ± 0.07 <sup>a</sup>	39.64 ± 0.07 <sup>a</sup>	39.64 ± 0.07 <sup>a</sup>	39.64 ± 0.07 <sup>a</sup>
3	39.28 ± 0.01 <sup>a</sup>	39.40 ± 0.02 <sup>a</sup>	29.52 ± 0.10 <sup>b</sup>	29.52 ± 0.04 <sup>b</sup>
6	27.05 ± 0.01 <sup>b</sup>	31.99 ± 0.19 <sup>a</sup>	29.40 ± 0.09 <sup>ab</sup>	29.52 ± 0.09 <sup>ab</sup>
9	19.64 ± 0.06 <sup>c</sup>	29.25 ± 0.01 <sup>a</sup>	24.58 ± 0.00 <sup>b</sup>	29.52 ± 0.01 <sup>a</sup>
12	14.70 ± 0.03 <sup>c</sup>	14.70 ± 0.06 <sup>c</sup>	19.64 ± 0.16 <sup>b</sup>	22.11 ± 0.02 <sup>a</sup>
15	11.02 ± 0.03 <sup>b</sup>	11.76 ± 0.09 <sup>b</sup>	14.92 ± 0.10 <sup>a</sup>	16.58 ± 0.08 <sup>a</sup>

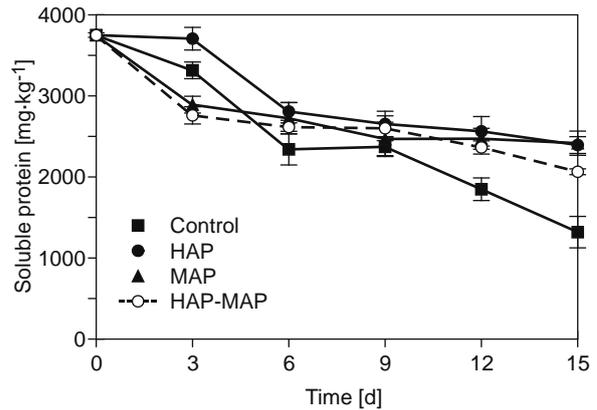
Values are mean ± standard deviation of triplicates. Data in same row with different letters are significantly different ( $P < 0.05$ ).

**Soluble protein**

Soluble protein levels decreased in all treatments during the storage (Fig. 2). However, HAP (1.1%) treatment caused a slight decrease in soluble protein levels during the first 3 days. The highest rate of decline was observed with HAP-MAP (25.3%) and MAP (21.9%) compared to control (11.1%). After day 3 until day 15 of storage, all treatments, i. e. MAP (34.1%), HAP (34.6%) and HAP-MAP (43.1%), exhibited ascending effect on delaying the decrease in protein in white mushrooms compared to control (62.1%; Fig. 2).

**Microbiological analysis**

The browning of mushrooms might also be caused by the action of bacteria and moulds on the mushroom tissues. Earlier report showed that cultivated mushrooms had high initial mesophiles counts ranging from 6.2 logCFU·g<sup>-1</sup> to 7.2 logCFU·g<sup>-1</sup> at harvest time. Tab. 3 shows mesophilic bacteria, psychrophilic bacteria, yeasts and moulds counts of white mushrooms during 12 days of storage. During the first 9 days of storage, the highest amount of mesophilic bacteria was observed in control (4.432 to 7.165 logCFU·g<sup>-1</sup>), followed by MAP (4.432 to 5.652 logCFU·g<sup>-1</sup>), but lower amounts were found in HAP-MAP (4.432 to 5.431 logCFU·g<sup>-1</sup>) and HAP (4.432 to



**Fig. 2.** Changes in soluble protein of fresh white mushrooms during storage at 4 °C for 15 days at different treatments.

5.372 logCFU·g<sup>-1</sup>). After this period, no significant ( $P < 0.05$ ) changes were observed in all samples.

For the psychrophilic bacteria, yeasts and moulds counts, no significant ( $P < 0.05$ ) difference was observed in all samples during the first 3 days of storage. After 3<sup>rd</sup> day, it is evident from this study that HAP-MAP followed by HAP and MAP treatments showed efficient effect in reduc-

**Tab. 3.** Microbial counts in white mushrooms packaged during storage at 4 °C for 12 days under different treatment.

Days	Control	HAP	MAP	HAP-MAP
	Mesophilic bacteria [log CFU·g <sup>-1</sup> ]			
0	4.432 ± 0.34 <sup>a</sup>	4.432 ± 0.34 <sup>a</sup>	4.432 ± 0.34 <sup>a</sup>	4.432 ± 0.34 <sup>a</sup>
3	5.314 ± 0.10 <sup>a</sup>	4.477 ± 0.21 <sup>b</sup>	4.633 ± 0.18 <sup>b</sup>	4.380 ± 0.16 <sup>b</sup>
6	5.732 ± 0.27 <sup>a</sup>	4.653 ± 0.03 <sup>b</sup>	5.097 ± 0.32 <sup>a</sup>	4.875 ± 0.43 <sup>b</sup>
9	7.165 ± 0.02 <sup>a</sup>	5.372 ± 0.09 <sup>b</sup>	5.652 ± 0.07 <sup>b</sup>	5.431 ± 0.28 <sup>b</sup>
12	7.189 ± 0.25 <sup>a</sup>	6.213 ± 0.88 <sup>a</sup>	6.803 ± 0.42 <sup>a</sup>	6.104 ± 0.07 <sup>a</sup>
Psychrophilic bacteria [log CFU·g <sup>-1</sup> ]				
0	4.637 ± 0.21 <sup>a</sup>	4.637 ± 0.21 <sup>a</sup>	4.637 ± 0.21 <sup>a</sup>	4.637 ± 0.21 <sup>a</sup>
3	5.262 ± 0.28 <sup>a</sup>	4.861 ± 0.20 <sup>a</sup>	5.013 ± 0.32 <sup>a</sup>	4.682 ± 0.60 <sup>a</sup>
6	6.429 ± 0.08 <sup>a</sup>	5.293 ± 0.03 <sup>bc</sup>	6.071 ± 0.44 <sup>ab</sup>	4.958 ± 0.39 <sup>c</sup>
9	7.861 ± 0.14 <sup>a</sup>	5.435 ± 0.07 <sup>c</sup>	6.412 ± 0.10 <sup>b</sup>	5.218 ± 0.34 <sup>c</sup>
12	7.839 ± 0.07 <sup>a</sup>	6.172 ± 0.52 <sup>b</sup>	6.764 ± 0.03 <sup>ab</sup>	5.932 ± 0.84 <sup>b</sup>
Yeasts and moulds [log CFU·g <sup>-1</sup> ]				
0	2.000 ± 0.06 <sup>a</sup>	2.000 ± 0.06 <sup>a</sup>	2.000 ± 0.06 <sup>a</sup>	2.000 ± 0.06 <sup>a</sup>
3	3.076 ± 0.30 <sup>a</sup>	2.207 ± 0.73 <sup>a</sup>	2.477 ± 0.54 <sup>a</sup>	2.007 ± 0.05 <sup>a</sup>
6	4.604 ± 0.40 <sup>a</sup>	3.569 ± 0.10 <sup>b</sup>	3.787 ± 0.17 <sup>ab</sup>	3.353 ± 0.45 <sup>b</sup>
9	5.477 ± 0.30 <sup>a</sup>	4.477 ± 0.43 <sup>ab</sup>	4.602 ± 0.55 <sup>ab</sup>	4.000 ± 0.16 <sup>b</sup>
12	6.158 ± 1.05 <sup>a</sup>	4.706 ± 0.46 <sup>ab</sup>	4.830 ± 0.16 <sup>ab</sup>	3.700 ± 0.26 <sup>b</sup>

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

**Tab. 4.** Colour changes of white mushrooms at different types of treatment during storage at 4 °C for 15 days.

Days	Control		HAP	
	$L^*$	$\Delta E$	$L^*$	$\Delta E$
0	92.92 ± 0.91 <sup>a</sup>	1.34 ± 0.52 <sup>a</sup>	92.92 ± 0.91 <sup>a</sup>	1.34 ± 0.52 <sup>a</sup>
3	84.60 ± 1.01 <sup>b</sup>	8.85 ± 1.21 <sup>a</sup>	88.56 ± 0.97 <sup>a</sup>	3.51 ± 0.42 <sup>b</sup>
6	83.87 ± 0.04 <sup>a</sup>	9.97 ± 0.47 <sup>a</sup>	84.26 ± 0.83 <sup>a</sup>	8.22 ± 0.93 <sup>a</sup>
9	81.10 ± 1.54 <sup>a</sup>	11.85 ± 1.64 <sup>a</sup>	81.93 ± 1.31 <sup>a</sup>	10.75 ± 1.62 <sup>a</sup>
12	80.19 ± 0.56 <sup>b</sup>	13.26 ± 1.01 <sup>a</sup>	81.11 ± 1.03 <sup>ab</sup>	11.82 ± 0.23 <sup>a</sup>
15	77.86 ± 0.45 <sup>b</sup>	15.21 ± 1.06 <sup>a</sup>	80.30 ± 1.12 <sup>a</sup>	12.45 ± 0.80 <sup>b</sup>

Days	MAP		HAP-MAP	
	$L^*$	$\Delta E$	$L^*$	$\Delta E$
0	92.92 ± 0.91 <sup>a</sup>	1.34 ± 0.52 <sup>a</sup>	92.92 ± 0.91 <sup>a</sup>	1.34 ± 0.52 <sup>a</sup>
3	85.56 ± 0.86 <sup>b</sup>	8.75 ± 2.72 <sup>a</sup>	85.67 ± 0.72 <sup>b</sup>	3.27 ± 0.66 <sup>b</sup>
6	84.60 ± 1.05 <sup>a</sup>	9.48 ± 1.48 <sup>a</sup>	84.69 ± 0.67 <sup>a</sup>	9.42 ± 1.14 <sup>a</sup>
9	82.81 ± 0.75 <sup>a</sup>	12.01 ± 1.43 <sup>a</sup>	83.10 ± 1.18 <sup>a</sup>	12.16 ± 1.05 <sup>a</sup>
12	81.02 ± 1.30 <sup>ab</sup>	12.82 ± 0.68 <sup>a</sup>	82.62 ± 0.33 <sup>a</sup>	12.14 ± 0.07 <sup>a</sup>
15	80.01 ± 0.40 <sup>a</sup>	14.80 ± 1.04 <sup>ab</sup>	80.03 ± 0.82 <sup>a</sup>	14.38 ± 1.06 <sup>ab</sup>

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

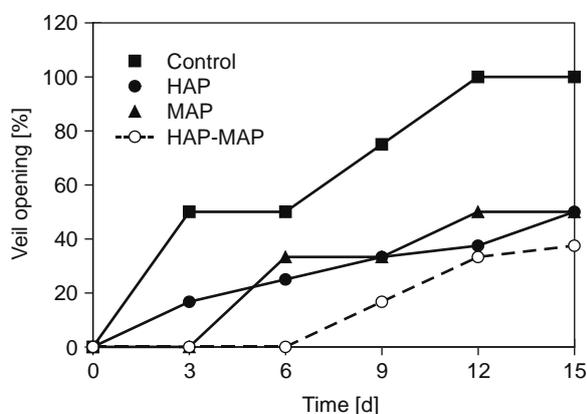
ing microbial counts during storage (Tab. 2). This was lower than the results obtained by GONZÁLEZ-FANDOS et al. [40]. The reduction of microbial contamination during storage was due to low O<sub>2</sub> and high CO<sub>2</sub> concentration level, and may be caused by starvation of microorganisms due to insufficient quantities of O<sub>2</sub> for respiratory activities. Thus, the extension of the shelf life of mushrooms due to modified atmosphere can be mainly attributed to the low O<sub>2</sub> and high CO<sub>2</sub> concentration in the system that surrounded the product, causing a decrease in respiration rate thereby inhibiting microbial growth. The inhibitory effect of HAP

treatment on microbial growth can be explained by presence of the argon, which reduced water activity and remained in the micropores to reduce the growth of microorganisms.

### Colour

All mushroom samples showed a decrease in luminosity ( $L^*$ ) and the colour difference ( $\Delta E$ ) during storage (Tab. 4). Previously reported relationship between different quality levels in mushrooms (*A. bisporus*) and Hunter  $L$ -value provided a criterion for classification [5, 22]. 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 [41]. This grading method is the most frequently used indicator of mushroom shelf-life both in the industry and research [22].

During the first 3 days of storage, there were significant differences ( $P < 0.05$ ) in colour  $L$ -values between HAP and the control, MAP and HAP-MAP mushrooms. From 3 to 9 days of storage, there were no significant differences ( $P < 0.05$ ) between control and other samples. From 10 to 15 days of storage, HAP-MAP mushrooms had higher  $L$ -value followed by HAP and MAP treatment. According to LÓPEZ-BRIONES et al. [41], the control samples had to be rejected. The behaviour in the colour difference ( $\Delta E$ ) during the first 3 days differed according to the treatment. HAP and HAP-MAP had lower col-



**Fig. 3.** Veil opening of fresh white mushrooms during storage at 4 °C for 15 days at different treatments.

our difference compared to MAP and control (Tab. 4). There was no significant difference in  $\Delta E$  for all the samples between 6 to 12 days of storage. At day 15, HAP treatment still showed lower  $\Delta E$  followed by HAP-MAP and MAP compared to control.

#### Veil opening rate

The veil opening of white mushrooms is an indicator of aging. Fig. 3 shows that all treatments reduced the veil opening rate of mushrooms during the 15 days of storage. We observed veil opening in samples treated with HAP-MAP and MAP after 6th and 3rd day. In the case of HAP and control, veil opening started on the 3rd day of storage. The veil opening rate of HAP was lower than in control. At day 15 of storage, HAP-MAP had 37.5% veil opening followed by HAP (50%) and MAP (50%). Therefore, these treatments were effective in delaying the decrease of veil opening rate compared to control (100%).

#### CONCLUSIONS

Mushrooms treated with MAP showed the lowest PPO activity and increased the phenolics contents. HAP treatment revealed significant reduction of weight loss, and increased protein and flavonoid contents. HAP-MAP treatment showed to be the most effective treatment in delaying decrease in antioxidants, reduction of respiration rate, colour change and microbial counts. The physico-chemical and microbiological properties of mushrooms stored at 4 °C under HAP-MAP, HAP and MAP had significant difference from those of untreated sample. Effect of HAP treatments could be due to clathrate hydrates, which are formed in mushrooms and are known to restrict the activity of enzymes.

Based on our study, application of HAP combined with MAP could extend the storage life of white mushrooms up to 15 days at 4 °C.

#### Acknowledgment

The authors are grateful to China National Natural Science Foundation for supporting this research under contract No. 30972058.

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Received 8 April 2011; revised 17 June 2011; accepted 5 September 2011.