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Effects of Pressurized Argon and Krypton Treatments on the Quality of Fresh White Mushroom (*Agaricus bisporus*)

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ABSTRACT

Effects of argon, krypton and their mixed pressure treatments on the quality of white mushrooms were studied during 9 days of storage at 4˚C. Among all treatments in this study, the minimum respiration rate, polyphenoloxidase activity, retained color change, antioxidants and delayed pseudomonas growth were observed with pressure argon (5 MPa) followed by mixing argon and krypton (2.5 MPa each) treatments. Respiration rates after 9 days of storage were 5.35%, 6.20%, 7.50%, 7.60%, 7.91% and 8.95% for HA5, HAK, HA2, HK5, HK2 and control, respectively. DPPH inhibition percentages of free radical for HA5, HAK, HK5, HA2, HK2 and control mushrooms were 28.03%, 25.24%, 24.96%, 21.87%, 20.56% and 19.06%, respectively, after 9 days of storage. The pressurized argon treatment was the most effective compared to pressurized krypton. Thus, application of pressurized argon and krypton treatments could extend the storage life of white mushrooms to 9 days at 4˚C.

Keywords: Pressurized Argon; Krypton; Clathrate Hydrates; White Mushroom; Storage

1. Introduction

Production and consumption of mushrooms have been gaining substantial ascendency in many parts of the globe due to their deliciousness, flavor and overall nutritional value. White mushroom (*Agaricus bisporus*) is rich in acidic polysaccharides, dietary fiber, and antioxidants including vitamins (C, B12, and D), folate, ergothioneine and polyphenol [1,2]. Due to these nutrients, consumption of white mushrooms may have potential anti-inflammatory, hypoglycemic and hypocholesterolemic consequences. Unfortunately, fresh mushrooms have very short shelf life (3 to 4 days) compared to most other vegetables at room temperature [3]. This might be due to the fact that mushrooms do not have cuticles to protect them from physical or microbial attack or water loss [4] also because of their high respiratory rate. Postharvest browning of *Agaricus bisporus* is a severe problem that reduces the shelf-life. The most important factors that determine the rate of enzymatic browning are the concentrations of active polyphenol oxidase (PPO) and phenolic compounds present [5].

Moulds, bacteria, enzymatic activity and biochemical changes can cause spoilage during storage. Gram-negative microorganisms, such as Pseudomonas bacteria, have been associated with mushroom spoilage.

Thus, since mushrooms are highly perishable, they need special care, especially during harvesting and storage to retain freshness and overall quality.

Parameters such as visual appearance, respiration rate, color, microbial growth and weight loss are usually used to determine the quality of mushrooms [6]. Moreover, the antioxidant status of fruits and vegetables is related to its shelf life and may provide a useful indicator of the quality during storage [7]. Various studies have demonstrated that shelf life of fruits and vegetables is modulated by antioxidants [8,9].

Recently, a great deal of interest has been shown in the potential benefits of using argon in food preservation. Many studies on the application of pressurized inert gases in preserving fresh fruits and vegetables have been

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published [10-16]. Use of argon, a major component of the atmosphere in modified atmosphere packaging (MAP) has been reported to reduce microbial growth and improve product overall quality retention [12,17]. Noble gases dissolved in water under appropriately selected temperature and pressure conditions, could result in the formation of highly ordered "iceberg-like" structures (called gas hydrate or clathrate) around solute molecules in aqueous solution due to hydrophobic hydration [18]. At 0˚C, argon and nitrogen clathrate hydrates can form and remain stable at more than 8.7 and 14.3 MPa respectively [19]. Zhan and Zhang, 2005 [11] observed clathrate hydrates (structure-type I) using a mixture of argon and xenon at a pressure range of 0.4 - 1.1 MPa in cucumber samples. Ando *et al.*, [20] examined the formation of the hydrate crystals of fresh-cut onions, which were preserved under Xe pressure up to 0.8 MPa at 5˚C for few hours. Purwanto *et al.*, [21] found the formation of gas hydrate in distilled water and coffee solutions at 8˚C and 0.70 MPa.

Fresh-cut vegetables and fruits pressurized in the presence of inert gases under appropriate conditions of pressure and temperature, cause the inert gases to form hydrate in these fresh-cut material's tissue and lower the activity of intracellular water and inhibit the enzymatic reactions. The combination of these two phenomena contributes to the reduction of metabolism of fruits and vegetables [10,12].

To the best of our knowledge, there are no reports of scientific research works on the effects of combined pressure argon and krypton treatments on the shelf life of white mushrooms. Therefore, the present research was designed to investigate the effects of pressurized argon and krypton, as well as, the mixture of the two on the physico-chemical, microbiological properties and sensory quality of mushrooms during cold storage.

2. Materials and Methods

High-pressure equipment HCYF-3 (HuaAn Scientific Instruments Co. Ltd., Jiangsu-China), commercially available argon and krypton of 99.7% purity (Wuxi Xinnan Gas Co. Jiangsu-China) were used. Freshly harvested, white mushrooms (*Agaricus bisporus*) were purchased from a local market at Wuxi, China. All other chemicals and solvents used were of analytical grade.

The freshly harvested mushrooms were transported to the laboratory and selected base on uniformity of shape and colour and free from mechanical damage. Mushrooms obtained were randomly divided into six groups, and each group was samples (65 ± 5 g) at least three times using glass jars. The different groups were subjected to the following treatments:

- Control (C): mushrooms washed with distilled water to remove soil then storage at 4˚C;
- pressurised argon (HA2): mushroom treated with Ar under pressure 2.5 MPa at 4˚C for 1 h;
- pressurised argon (HA5): mushroom treated with Ar under pressure 5 MPa at 4˚C for 1 h;
- pressurised krypton (HK2): mushroom treated with Kr under pressure 2.5 MPa at 4 ˚C for 1 h;
- pressurised krypton (HK5): mushroom treated with Kr under pressure 5 MPa at 4˚C for 1 h;
- pressurised mixed argon and krypton (HAK): mushroom treated with mixed Ar and Kr under pressure 2.5 MPa each gas at 4˚C for 1 h.

Fresh mushrooms were placed in a high pressure chamber, and then argon or/and krypton was passed into the chamber after the evacuation time. After the pressurized argon or/and krypton treatments, all samples were stored at 4˚C with 90% relative humidity for 9 days.

Measurements and analyses of the mushrooms were performed on the following days of storage period; 0, 3rd, 6th, and 9th day. Twelve replicates were included in each treatment group, and subsequently every 3 days, three replicates from each treatment group were analyzed. All measurements were done in triplicates.

2.1. CO_2 Production

Mushrooms (65 ± 5 g) were placed in 500 mL glass jars and sealed with high gas barrier film then stored at 4˚C for 9 days. Carbon dioxide production was measured on the 3rd, 6th and 9th day 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. Carbon dioxide production (ΔCO_2) was calculated as follows:

$$\Delta CO_2\,(\%) = CO_{2f} - CO_{2i} \quad (1)$$

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

2.2. Weight Loss

Weight losses were determined by weighing of all mushrooms contained in one package (initially 65 ± 5 g) before and after the storage period, which was expressed as weight loss percentage with respect to the initial weight.

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

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

2.3. Color

Surface color of mushrooms was measured with a Minolta spectrophotometer (CR-400, Konica Minolta Sensing, Tokyo, Japan) using CIE color parameters L^* (light/dark), a^* (red/green) and b^* (yellow/blue) values.

Three readings were taken at three equidistant points on each mushroom cap. Numerical values of L^* and color difference (ΔE) were considered for the evaluation of color modification of fresh mushroom. The value ΔE defines the magnitude of total color difference and is expressed by the equation [15]:

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

where ΔE indicates the degree of overall color change in comparison to color 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.

2.4. Polyphenoloxidase (PPO) Activity

Polyphenoloxidase (PPO, E.C. 1.14.18.1) activity in mushroom, during the storage period was determined according to the method proposed by Pizzocaro *et al.* [22] with slight modifications. Fresh mushroom (10 g) was ground in 10 mL of McIlvaine citric-phosphate buffer, pH 6.5. The homogenate was centrifuged at 3000 × g at 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. A 2 mL 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 ($U \cdot min^{-1} \cdot g^{-1}$) of fresh mushroom.

2.5. DPPH Free Radical-Scavenging Assay

The determination of free radical scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was carried out according to the method of Alothman *et al.*, [23] with slight modifications. Mushroom samples (2 g) were homogenised with a mortar and pestle in 10 mL of methanol and centrifuged at 6000 × g for 15 min at 4˚C and filtered through a Whatman No 1 paper. Aliquots of 0.05 mL of the supernatant were mixed with 1 mL of DPPH and 1.5 mL of Tris buffer. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured with the UV-visible spectrophotometer at 517 nm against methanol as blank. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\text{DPPH radical scavenging activity } (\%) = \left[\left(A_0 - A \right) / A_0 \right] \times 100 \quad (4)$$

where, A_0 is the absorbance of DPPH solution without extracts and A is the absorbance of the mushroom extract.

2.6. Total Phenolic and Flavonoids Contents

Total phenolic contents were measured according to Singleton and Rossi [24]. Mushroom samples (5 g) were crushed and homogenised in 50 mL methanol. The mixture was centrifuged at 3000 × g for 30 min at 4˚C, filtered with Whatman no. 4 filter paper and the mushroom extract was collected. Mushroom extract (200 µL) was mixed 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.

Flavonoids were extracted and determined according to the methods of Barros *et al.* [25] with slight modifications. Namely, 1.8 mL of mushroom extract was added to 20 µL distilled water and 75 µL of 5% sodium nitrite ($NaNO_2$) then allowed standing for 6 min. Thereafter, 150 µL of 10% aluminium chloride ($AlCl_3$) was added. After standing for another 5 min, 2 mL of 1 $mol \cdot L^{-1}$ sodium hydroxide (NaOH) was 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.

2.7. Microbiological Analysis

All samples were analyzed for the pseudomonas bacteria counts. Mushrooms samples (10 g) were removed aseptically from each pack and diluted with 90 mL of 0.1% sterile peptone water. The samples were homogenised 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 ($CFU \cdot g^{-1}$) of mushroom was determined.

2.8. Sensory Analysis

Sensory analysis of the mushroom was evaluated according to Abdallah *et al.* and Conesa *et al.* [26,27] with slight modifications on days 3, 6 and 9 by 10 semi-trained recruited among students of the Food Science and Technology, Jiangnan University. Sensory evaluation was performed based on four aspects (color, aroma, texture and overall acceptability). The aspects were evaluated on a scale of 9-1, where 9—excellent, 8—very good, 7—good, 6—fairly good, 5—satisfactory and limit of marketability, 4—fair and limit of usability, 3—bad, 2—very bad and 1—extremely bad and inedible.

2.9. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The Tukey's test and one-way analysis of variance (ANOVA) were used for multiple comparisons by the SPSS 17.0 (SPSS, Chicago, Illinois, USA). Difference was considered to be statistically significant if $P < 0.05$.

3. Results and Discussion

3.1. CO_2 Production and Weight Loss

Changes of the CO_2 production during storage under different treatments at 4˚C are shown in **Figure 1(A)**. As can be seen from the figure a progressive increase in CO_2 production during the entire storage period was observed with all samples. At the end of storage time, CO_2 productions were 5.35%, 6.20%, 7.50%, 7.60%, 7.91% and 8.95% for HA5, HAK, HA2, HK5, HK2 and control samples, respectively. The results also demonstrated that, argon or krypton treatment at 5 MPa showed lower CO_2 production compared to that at 2 MPa. However, argon treatments were significantly more efficient than krypton treatments ($P < 0.05$).

The weight loss of the control sample was the highest (1.04%) among the six treatments during the storage time (**Figure 1(b)**). Samples treated by HA5 had significantly lower weight loss than the other samples throughout the storage ($P < 0.05$). The weight loss of the HAK, HA2, HK5 and HK2 treatments increased progressively during storage to a maximum of 0.95% for HK2 sample after 9 days, without significant differences among the HAK and HA2 treatments. In our results, we observed a correlation between CO_2 production and weight loss. HAK treated ones showed reduced weight loss and respiration rate compared to samples with 2.5 MPa treatments and the control. Therefore, the lowest CO_2 production and water loss observed in argon treatment could be related to the highest solubility nature of argon which caused the highest capability of gas hydrate formation compared to krypton [28]. Argon hydrate is the most fundamental clathrate hydrate in the sense that argon is spherical and

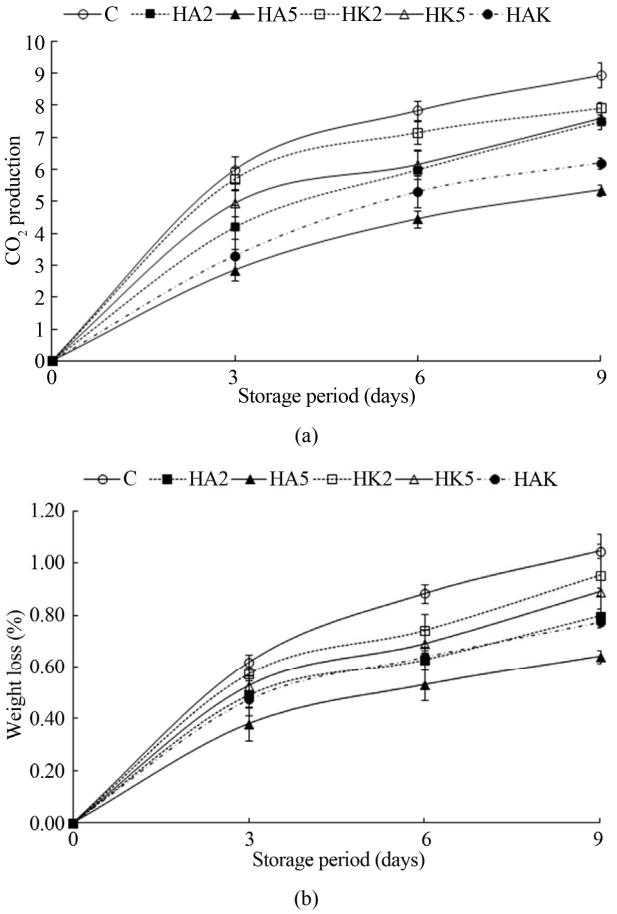

Figure 1. Changes in (a) CO_2 production and (b) Weight loss in white mushrooms during storage at 4˚C for 9 days under different treatments (n = 3).

the smallest of the molecules which can be accommodated in the clathrate cages and therefore, its interaction with the lattice is the weakest [29].

3.2. Color

Browning after harvest is a common and economically detrimental phenomenon in the mushroom industry, which may have negative effect not only on the appearance quality, but also on the flavor and nutrient composition.

All mushroom samples showed a decrease in whiteness (L^*), however, the color difference (ΔE) increased during storage (**Table 1**). Compared to the control mushrooms, those treated had a higher L^* ($P < 0.05$) and lower ΔE ($P < 0.05$) values. Mushroom with HA5 and HAK treatments followed by HK5, HA2 and HK2, respectively, had a higher L^* value than that of control during the 9 days of storage.

Color difference (ΔE) during storage differed among treated ones with HA5 and HAK samples recording lower color difference in contrast with HK5 followed by

Table 1. Colour changes of white mushrooms at different types of treatment during storage at 4˚C for 9 days.

Days	0	3	6	9
	L			
C	91.02 ± 1.66[a]	85.30 ± 0.66[a]	82.40 ± 0.33[a]	63.28 ± 1.43[a]
HA2	90.40 ± 0.69[a]	87.47 ± 1.66[b]	86.18 ± 0.84[c]	71.51 ± 1.03[b]
HA5	90.78 ± 1.69[a]	90.78 ± 1.43[e]	87.29 ± 1.06[d]	84.61 ± 1.21[e]
HK2	90.92 ± 0.27[a]	86.85 ± 0.33[b]	83.41 ± 1.16[b]	70.58 ± 0.16[b]
HK5	90.70 ± 0.97[a]	87.51 ± 1.04[c]	86.57 ± 1.34[c]	77.58 ± 1.36[c]
HAK	91.04 ± 0.52[a]	88.73 ± 1.31[d]	87.00 ± 0.96[d]	80.64 ± 2.01[d]
	ΔE			
C	3.89 ± 1.32[a]	9.52 ± 0.98[a]	13.08 ± 0.53[a]	30.09 ± 1.72[a]
HA2	4.20 ± 1.06[a]	8.42 ± 1.03[b]	8.62 ± 1.01[c]	25.30 ± 2.03[b]
HA5	4.35 ± 1.35[a]	4.73 ± 1.23[e]	7.49 ± 1.03[d]	11.03 ± 1.54[e]
HK2	4.44 ± 0.37[a]	8.46 ± 0.17[b]	12.07 ± 0.69[b]	25.62 ± 1.05[b]
HK5	4.44 ± 0.17[a]	7.40 ± 61.06[c]	8.30 ± 1.09[c]	19.13 ± 0.87[c]
HAK	4.33 ± 0.35[a]	6.54 ± 1.64[d]	7.94 ± 1.48[d]	15.80 ± 1.08[d]

Values are mean ± standard deviation of triplicates. Data in same column with different letters are significantly different ($P < 0.05$). Control (C): mushrooms washed with distilled water to remove soil then storage at 4˚C; (HA2): 2.5 MPa pressure argon treatment at 4˚C for 1 h; (HA5): 5 MPa pressure argon treatment at 4˚C for 1 h; (HK2): 2.5 MPa pressure krypton treatment at 4˚C for 1 h; (HK5): 5 MPa pressure krypton treatment at 4˚C for 1 h; (HAK): mixing argon and krypton treatment at 2.5 MPa pressure each at 4˚C for 1 h.

HA2 and HK2 and control. Previously reported relationship between different quality levels in mushrooms (*A. bisporus*) and Hunter *L*-value provided a criterion for classification [6,30]. Mushrooms with *L*-values greater than 93 were classified as excellent sample, however, that with L-values ranging between 90 to 93, 86 to 89, 80 to 85, and 69 to 79 were classified as very good, good, fair and poor sample, respectively.

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 [31]. This grading method is the most frequently used indicator of mushroom shelf-life both in the industry and research [30]. According to that criterion [31], except the HA5 and HAK samples, other samples had to be rejected.

3.3. Polyphenoloxidase (PPO) Activity

PPO plays an important role in the browning process of many fruits and vegetables. Browning reactions are generally assumed to be a direct consequence of PPO actions on polyphenols to form quinones, which ultimately polymerize to produce the browning appearance of fruit and vegetable [32]. PPO activity in white mushrooms increased on the first day of storage and reached maximum value on day 6 for all treatments and decreased during the latter period afterward (**Table 2**). The lowest activity was observed in HA5, HAK followed by HK5, HA2 and HK2 treatment as compared to control. Mobility of water is restricted by the formation of clathrate hydrates [33]. The lowest PPO activity observed with treated samples compared to control could be attributed to the formation of clathrate hydrate. This implied that, structured water contributed into low water mobility which consequently delayed the enzymatic browning. It's also might be due to the capacity of these gases to dissolve in the aqueous layer of the mushroom through the cells of the flesh. Therefore, they can inactivate some chemically-active sites on the enzymes and/or reduce the level of dissolved oxygen, whose presence is necessary for oxidative enzymes to catalyze metabolic reactions. Behnke [34] 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 [12].

3.4. DPPH Free Radical-Scavenging Assay

Changes during storage in the percentage of inhibition of DPPH radical by antioxidants present in white mushroom, are shown in **Table 2**. DPPH scavenging power of the mushroom generally showed a reduction trend over

Table 2. Changes in polyphenoloxidase (PPO) activity and free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) of white mushrooms at different types of treatment during storage at 4˚C for 9 days.

Days	0	3	6	9
		PPO activity ($U \cdot min^{-1} \cdot g^{-1}$)		
C	3132 ± 3.56[a]	3851 ± 8.02[a]	4496 ± 7.23[a]	4275 ± 9.20[a]
HA2	3132 ± 3.56[a]	3673 ± 3.24[b]	4229 ± 4.67[b]	3863 ± 2.65[c]
HA5	3132 ± 3.56[a]	3210 ± 1.45[e]	3809 ± 2.56[c]	3155 ± 5.63[f]
HK2	3132 ± 3.56[a]	3826 ± 2.34[a]	4449 ± 5.65[a]	4143 ± 4.65[b]
HK5	3132 ± 3.56[a]	3394 ± 6.12[c]	4200 ± 4.34[b]	3781 ± 2.64[d]
HAK	3132 ± 3.56[a]	3488 ± 5.32[d]	4037 ± 1.54[c]	3451 ± 6.34[e]
		DPPH (%)		
C	40.61 ± 0.64[a]	31.29 ± 0.81[a]	27.51 ± 0.76[a]	19.06 ± 0.53[a]
HA2	40.61 ± 0.64[a]	32.02 ± 0.68[b]	30.29 ± 0.81[c]	21.87 ± 0.44[d]
HA5	40.61 ± 0.64[a]	37.55 ± 0.37[d]	34.30 ± 0.27[f]	28.03 ± 0.76[f]
HK2	40.61 ± 0.64[a]	31.24 ± 0.63[a]	28.54 ± 0.57[b]	20.56 ± 0.16[b]
HK5	40.61 ± 0.64[a]	31.94 ± 0.78[b]	31.18 ± 0.14[d]	24.96 ± 0.84[c]
HAK	40.61 ± 0.64[a]	35.02 ± 0.68[c]	32.22 ± 0.68[e]	25.24 ± 0.59[e]

Values are mean ± standard deviation of triplicates. Data in same column with different letters are significantly different ($P < 0.05$). Control (C): mushrooms washed with distilled water to remove soil then storage at 4˚C; (HA2): 2.5 MPa pressure argon treatment at 4˚C for 1 h; (HA5): 5 MPa pressure argon treatment at 4˚C for 1 h; (HK2): 2.5 MPa pressure krypton treatment at 4˚C for 1 h; (HK5): 5 MPa pressure krypton treatment at 4˚C for 1 h; (HAK): mixing argon and krypton treatment at 2.5 MPa pressure each at 4˚C for 1 h.

the 9 days storage time in all samples but at different extent. However, all the treated samples delayed in the decrease but at different degrees, with significant differences ($P < 0.05$) in DPPH scavenging power between the treated samples and the control. After 9 days of storage, percentage of inhibition of DPPH radical for HA5, HAK, HK5, HA2, HK2 and untreated mushrooms were 28.03%, 25.24%, 24.96%, 21.87%, 20.56% and 19.06% respectively. It was observed that, high pressure argon could delay the reduction of antioxidant capacity of mushroom during the refrigerator storage, probably due to noble gas hydrate formation and residual gas in micropore of fruit tissue.

3.5. Total Phenolic and Flavonoids Contents

In this study, total phenolics levels declined in all treatments during the 9 days of storage (**Table 3**). However, pressurized argon (5 MPa) was more effective in delaying decrease of phenolics than other samples. Mushrooms treated samples presented a higher level of total phenolics, compared to control.

Similar to total phenolics, all the treatments showed a reduction in flavonoids contents during storage (**Table 3**). However, HA5 and HAK treatments appeared to be significantly ($P < 0.05$) efficient in delaying the reduction in flavonoids in the white mushrooms as compared to other treatment. Whereas, untreated white mushrooms presented a lower level of flavonoids, all treatments affected significantly ($P < 0.05$) the flavonoid content in white mushrooms during the 9 days.

It demonstrated that, HA5 and HAK treatments were significantly effective in maintaining the total phenolic and flavonoids compounds, which might be due to clathrate hydrate formation that inhibited the enzyme activity of phenolic and flavonoids compounds degradation. The pressurized argon treatment was the most effective in delaying the reduction in total phenolics and flavonoids content compared to pressurized krypton may be due to the high solubility of argon.

3.6. Microbiological Analysis

Figure 2 presents growth of pseudomonas bacteria (expressed as log $CFU \cdot g^{-1}$) of fresh mushroom during 9 days of storage at 4˚C. 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. Pressurized argon samples (5 Mpa) followed by combined of argon-krypton and HK5 samples, were found to be effective in delaying pseudo-

Table 3. Changes in functional components in white mushrooms during storage at 4˚C for 9 days under different treatments.

Days	0	3	6	9
		Total phenolics (min·g^{-1})		
C	1.03 ± 0.92^{a}	0.90 ± 0.63^{a}	0.62 ± 0.10^{a}	0.43 ± 0.18^{a}
HA2	1.03 ± 0.92^{a}	0.99 ± 0.17^{bc}	0.75 ± 0.25^{b}	0.63 ± 0.16^{d}
HA5	1.03 ± 0.92^{a}	1.02 ± 0.15^{c}	0.89 ± 0.34^{e}	0.76 ± 0.32^{f}
HK2	1.03 ± 0.92^{a}	0.97 ± 0.23^{b}	0.77 ± 0.32^{c}	0.50 ± 0.71^{b}
HK5	1.03 ± 0.92^{a}	0.98 ± 0.43^{bc}	0.77 ± 0.45^{c}	0.58 ± 0.58^{c}
HAK	1.03 ± 0.92^{a}	1.01 ± 0.68^{c}	0.85 ± 0.30^{d}	0.74 ± 0.63^{e}
		Total flavonoids (min·g^{-1})		
C	0.71 ± 0.44^{a}	0.50 ± 0.36^{a}	0.38 ± 0.46^{a}	0.25 ± 0.36^{a}
HA2	0.71 ± 0.44^{a}	0.57 ± 0.56^{c}	0.46 ± 0.34^{c}	0.33 ± 0.32^{c}
HA5	0.71 ± 0.44^{a}	0.66 ± 0.14^{d}	0.54 ± 0.17^{e}	0.40 ± 0.37^{d}
HK2	0.71 ± 0.44^{a}	0.51 ± 0.44^{ab}	0.41 ± 0.32^{b}	0.29 ± 0.43^{b}
HK5	0.71 ± 0.44^{a}	0.53 ± 0.65^{b}	0.43 ± 0.45^{bc}	0.30 ± 0.48^{b}
HAK	0.71 ± 0.44^{a}	0.61 ± 0.35^{cd}	0.50 ± 0.33^{d}	0.34 ± 0.23^{c}

Values are mean ± standard deviation of triplicates. Data in same column with different letters are significantly different ($P < 0.05$). Control (C): mushrooms washed with distilled water to remove soil then storage at 4˚C; (HA2): 2.5 MPa pressure argon treatment at 4˚C for 1 h; (HA5): 5 MPa pressure argon treatment at 4˚C for 1 h; (HK2): 2.5 MPa pressure krypton treatment at 4˚C for 1 h; (HK5): 5 MPa pressure krypton treatment at 4˚C for 1 h; (HAK): mixing argon and krypton treatment at 2.5 MPa pressure each at 4˚C for 1 h.

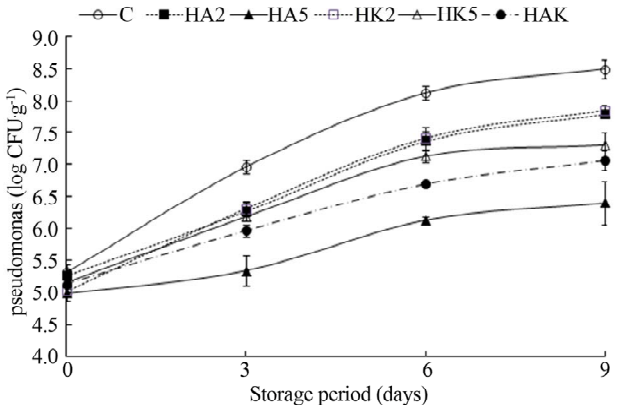

Figure 2. Growth of pseudomonas bacteria of fresh mushroom during 9 days of storage at 4˚C under different treatments (n = 3).

monas bacteria growth in mushroom during the 9 days of cold storage. For HA2 and HK2 treatments, no significant ($P < 0.05$) difference was observed during the storage. In food, microbial growth is closely related to the water activity of those products and can be delayed by pressurized inert gases. The inhibitory effect of pressurized gases treatment on microbial growth in white mushroom might be owned to clathrate hydrates formation, which reduced water activity and remained gas in the micropore mushroom to reduce the growth of microorganism.

3.7. Sensory Analysis

Figure 3 shows the sensory evaluation including color, aroma, texture and overall preference of the six treatments for the three typical storage days. On day 3, all the treatments showed a moderate decrease in the overall quality. As expected, color, aroma, texture and overall acceptability significantly changed ($P < 0.05$) with storage time, supporting the validity of using these parameters as indicators of mushroom deterioration. As the storage time progressed to day 6, there was a continued decrease in sensory quality. On day 9 of storage, considering the development of the evaluated sensory attributes, HA_5 mushrooms showed the lowest deterioration rate, followed by HAK, HK5 and HA2. On the other hand, control and HK_2 samples reached a score lower than 5 a value that is below the borderline of acceptability and marketability.

4. Conclusion

Compared to the untreated (control) samples, treated samples had significantly ($P < 0.05$) longer shelf-life. The argon treatment delayed quality deterioration, reduced the loss of water, exhibited the smallest respiration rate, retained mushrooms color change, showed smaller

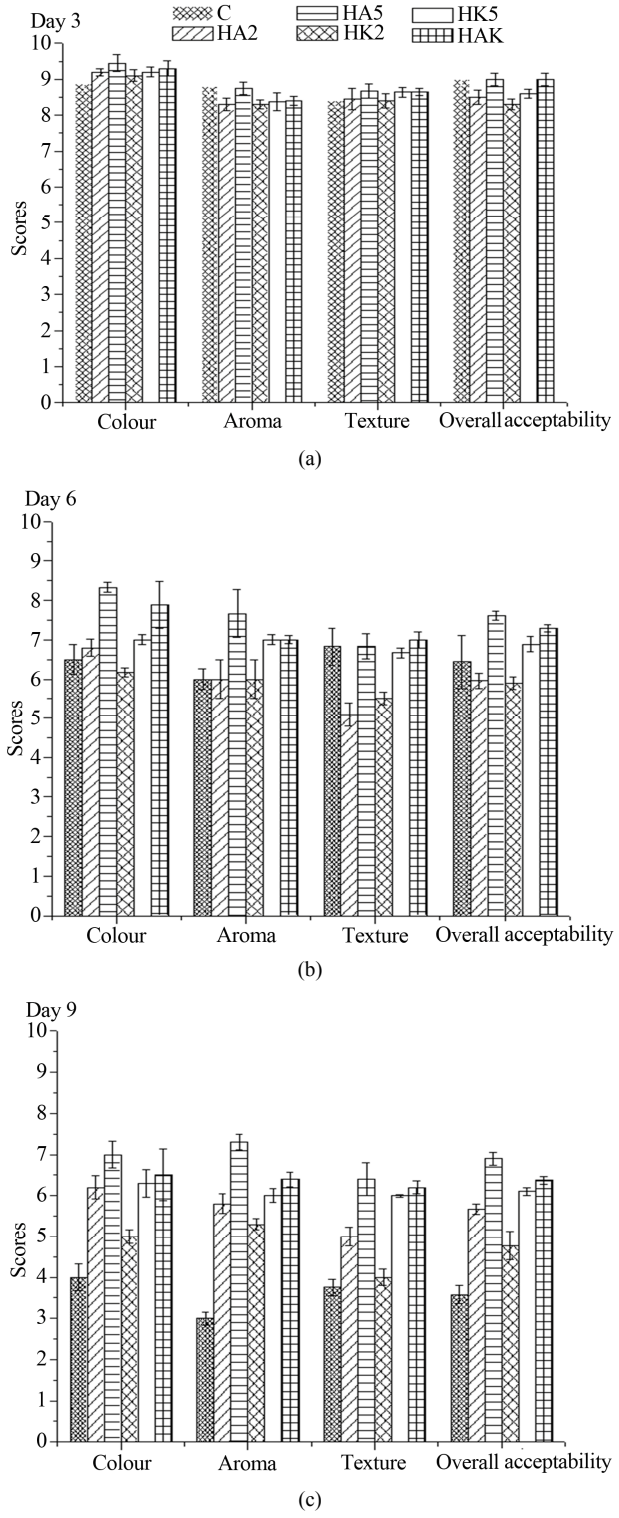

Figure 3. Sensory characteristics of fresh mushroom of six treatments stored for 3, 6 and 9 days at 4˚C. Values represent the means of the replicates and error bars represent the standard error of the means (n = 3).

polyphenoloxidase activity, retained antioxidants, delayed pseudomonas growth and maintained sensory quality compared to krypton treatment. Our research showed that pressurized argon and mixed argon-krypton may be a useful way of maintaining quality and extending the shelf-life of white mushroom.

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