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Extension of mushroom shelf-life by ultrasound treatment combined with high pressure argon**

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A b s t r a c t. Effects of ultrasound, high pressure argon, and treatments comprising their combinations on physicochemical and microbiological characteristics of white mushrooms were studied during 9 days of storage at 4°C. High pressure argon treatments were relatively effective in retaining firmness and were found to maintain the cell integrity. White mushrooms firmness after 9 days of storage was increased from 2.79 N for untreated mushrooms up to 3.01, 3.24, 3.58 N for ultrasound, treatments comprising ultrasound and high pressure argon, high pressure argon, respectively. Similarly, the loss of water, ascorbic acid and total soluble solid in fresh mushroom was also greatly reduced by the high pressure argon treatment. The ultrasound treatment followed by treatments comprising ultrasound and high pressure argon and high pressure argon, respectively exhibited a pronounced effect on retarding browning and in delaying mesophilic and psychrotrophic bacteria, yeasts and moulds growth in white mushroom, compared to the control during 9 days of cold storage. Treatments comprising ultrasound and high pressure argon treatment delayed pseudomonas growth, implying that it could extend shelf life of white mushrooms to 9 days at 4°C.

K e y w o r d s: high pressure argon, ultrasound, white mushroom, shelf life, storage

INTRODUCTION

Production and consumption of mushrooms have been growing substantially in many parts of the globe due to their delicious taste, flavour and overall nutritional value. White mushroom (*Agaricus bisporus*) is rich in acidic polysaccharides, dietary fibre, and antioxidants including vitamins (C, B12, and D), folate, ergothioneine and polyphenol (Mattila *et al*., 2001). Due to these nutrients, white mushrooms may have potential anti-inflammatory, hypoglycaemic, and hypocholesterolaemic effects when consumed. Unfortunately, they have very short shelf life (3 to 4 days), particularly in fresh form, compared to most other vegetables at room temperature (Preeti *et al*., 2010). This might be due to the fact that they lack cuticles to protect them from physical or microbial attack or water loss (Martine *et al*., 2000). Freshly harvested mushrooms have bright white colour, closed caps, and firm texture. Moreover, they can be easily sheared and chewed in fresh state. However, during storage these characteristics undergo a rapid change within a short time, leading to a deterioration of the mushrooms during a relatively shorter period.

This deterioration is manifested by:
– colour darkening,
– caps opening, exposing gills,
– stipes elongation,
– and texture becoming spongy and difficult to chew and shear.

Although texture is an important quality, understanding of what causes textural changes of mushroom tissue during storage remains largely unknown. In this light, mushrooms need special care, especially during storage, to retain freshness and extended shelf life.

There are many methods used to extend the shelf life of mushrooms. Conventionally, preservation under low temperature storage is often employed, but it has detrimental effects on product quality in the event of prolonged storage periods. Recently, a great deal of interest has been shown in the potential benefits of argon in food preservation. Spencer and Humphreys (2003) reported that the effects of argon in the inhibition of respiratory enzymes, including oxidases, could be due to high solubility and interference with

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enzymatic oxygen receptor sites in fruits and vegetables. When a noble gas is dissolved in water under appropriately selected temperatures and pressure conditions, highly ordered 'iceberg-like' structures known as gas hydrate or clathrate hydrate form around the solute molecules in aqueous solution due to hydrophobic hydration (Davidson, 1973). Noble gases such as argon (Ar), krypton (Kr) and xenon (Xe) can form clathrate hydrates in some vegetables and fruits more readily under pressure higher than the critical pressure point (Purwanto *et al*., 2001; Zhan and Zhang, 2005).

Ultrasound treatment has been reported to have attracted attention in food science and technology because of its promising effects in food processing and preservation. Knorr *et al*. (2004) reviewed direct food processing improvements such as cleaning surfaces, enhancement of dewatering, drying and filtration, inactivation of microorganisms and enzymes, extraction (enzymes, proteins and antioxidant compounds), degassing of liquid food and acceleration of heat transfer. The potential use of this novel technology to produce permanent changes in food in liquid systems is due the generation of intense cavitation. This cavitation could lead to the inactivation of microorganisms and enzymes. Again, treatment by ultrasound demonstrates its bene?ts (alone or combined with heat and/or high-pressure techniques) as a food preservation tool (Vercet *et al*., 1997; Villamiel and Jong, 2000).

In considering various techniques that are able to complement and strengthen low temperature storage in improving products quality, ultrasound (U) and high pressure argon (H) may be ideal to employ prior to cold storage. Though a lot of work has 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 literature references available on the effects of combined application of ultrasound and high gas pressure on the shelf life of white mushrooms. The present research was designed to investigate the combination of ultrasound and high gas pressure effects on the physicochemical and microbiological properties and on the ultrastructure of mushroom during cold storage.

MATERIALS AND METHODS

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 were eliminated, after which the wholesome samples were submitted to minimal treatment. Briefly, the soil on the mushroom was washed away with distilled water, then the mushrooms were placed on absorbent paper to remove excess surface water. They were then kept in cold storage at 4°C for 24 h to reduce the respiration rate.

Mushrooms (60±5 g) were selected at random and subjected to the following treatments:
– control (C) – not treated,
– ultrasound with distilled water (U),
– high pressure argon (H),
– ultrasound combined with high pressure argon treatment (UH).

Ultrasound treatment experiments were carried out at constant power of 400 W and frequency of 20 kHz for 10 min. Mushroom weight to distilled water ratio was maintained at 1:4 (w/w) (Fernandes *et al*., 2008). Temperature of distilled water was maintained at 4°C to prevent rapid temperature rise due to ultrasound. Ultrasonic treatments were carried out with sonotrodes of 20 mm in diameter, model JY98-3D, Ningbo Scientz Biotechnology Co., Ltd, China. For the combined treatment (UH), samples were immediately treated with high pressure argon after ultrasound treatment.

For high pressure argon processing, the samples were subjected to a treatment of 10 MPa at 4 ± 1°C for 60 min. High pressure machine (HCYF-3, HuaAn Scientific Research Instrument, Jiangsu, China) was working within the temperature range 0 to 50°C and maximum pressure of 30 MPa. Measurements and analyses of the mushrooms were performed on the following days of storage period; 0, 3rd, 6th, and 9th days. All measurements were done in triplicates from each treatment group. The samples were randomly selected and analysed for physicochemical and microbiological properties and ultrastructure.

Surface colour of mushrooms was measured with a spectrophotometer (CR-400, Konica Minolta Sensing, Tokyo, Japan) using CIE colour parameters L^* (light/dark), a^* (red/green) and b^*(yellow/blue). 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*., 2012a):

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

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^* represents the reading of fresh mushroom without any treatments, and L_t^*, a_t^* and b_t^* refers to the instantaneous individual readings during storage time after the mushrooms were treated.

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. The speed of the probe was 5.0 mm s^{-1} during the pre-test and penetration. Measurements were performed in triplicate on four mushroom caps for each sample and the mean was calculated.

Water loss of fresh mushroom was determined by difference in weight of the sample before and after storage, and expressed on a wet weight basis (%).

Total soluble solids (TSS) were determined at 20°C using a refractometer (AB-1, Mecsens Asia Co., Ltd. Taipei, Taiwan). A drop of juice obtained from ground mushroom was placed on the refractometer glass prism and the percentage total soluble solids were obtained (Tao *et al*., 2005).

Ascorbic acid (AA) was analysed according to the 2,6-dichlorophenol-indophenol titration method by James (1995). 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 mg kg^{-1} of the sample.

Samples for scanning electron microscopy (SEM) were carefully cut into cubes of 5 mm per side and fixed with 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The fixative was then dehydrated in successive series of acetone-water mixtures. After dehydration, the samples were dried with liquid CO_2, adhered to stubs, coated with a gold-palladium layer, and observed under SEM Quanta-200 (Fei Company, Shanghai, China).

All samples were analyzed for mesophilic and psychrophilic bacteria, yeasts, moulds and pseudomonas. Ten grams of mushrooms were removed aseptically from each pack and homogenised in 90 ml of 0.1% sterile peptone water using stomacher at high speed for 2 min. Serial dilutions (10^{-1}-10^{-8}) were made in tubes (1.0 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 ± 1 °C for 7 days. Pseudomonas was counted on cephaloridin fucidin cetrimide agar (CFC, Difco), with selective supplement SR 103 (Oxoid). Incubation temperature was 25°C and plates were examined after 48 h.

Sensory analysis of the mushroom was evaluated according to Jia *et al*. (2009) with slight modifications on days 0, 3, 6 and 9 by ten semi-trained persons recruited among students of the School of Food Science and Technology, Jiangnan University. Sensory evaluation was performed based on four aspects (colour, aroma, texture and overall acceptability). The aspects were evaluated on a scale of 9-1 points, where 9-excellent, 7-very good, 5-good and limit of marketability, 3-fair and limit of usability, and 1-poor and inedible.

All experiments were conducted at least in triplicate. Analysis of variance (one-way ANOVA) was performed and significant differences in mean values were evaluated by Tukey test at ($p<0.05$) using SPSS version 18.0 (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Consumers consider product appearance to be the primary criterion for acceptance. Colour has been considered to play a key role in food choice, preference and overall acceptance. It may even influence taste thresholds, sweetness perception and pleasantness. In this regard, it is important to bear in mind that for a given treatment, colour maintenance should be given consideration to ensure that the outcome of the final product meets consumer choice and acceptability.

Colour change data under the given treatments (Table 1) showed that all four treatments did not influence the colour of the samples since the same values of colour parameter for treated mushrooms and controls were noted on day 0 of storage. When the storage progressed to day 3, no significant difference was observed among samples as well as control. However, as storage time advanced, untreated mushrooms became darker, evidenced by higher L^* value and lower colour difference (ΔE) values (Table 1) compared to those of the treated ones. The effect of the treatments on colour became more pronounced when the storage was continued for

T a b l e 1. Colour change of white mushrooms at different types of treatment during storage at 4°C

Treatment	Time (day)			
	0	3	6	9
		L^*		
Control	90.38±0.62a	85.42±1.19a	80.58±1.44a	74.60±0.56a
U		85.89±1.31a	83.92±0.54b	82.20±0.23b
H		87.14±0.98a	84.66±1.73b	78.54±1.55ab
UH		87.90±1.35a	86.11±1.56b	82.86±0.76b
		ΔE		
Control	2.08±0.35a	8.13±0.68a	8.45±0.14a	15.64±0.20a
U		2.59±0.40b	5.23±0.45c	10.30±1.73c
H		6.98±0.12a	8.51±0.10a	12.75±0.99b
UH		3.94±0.95b	6.47±0.76b	9.81±0.83c

Values are mean ± standard deviation of triplicates. Data in same column with different letters are significantly different ($p<0.05$). U – ultrasound, H – high pressure argon, UH – ultrasound combined with high pressure argon.

6 days. There was a significant difference ($p<0.05$) between control and treated samples on day 6. From the 6th to the last day of storage, U and UH treatments exhibited a pronounced effect on retarding white mushroom browning, followed by H treatment. Colour difference (ΔE) during storage differed among treated ones with U and UH samples recording lower colour difference ($p<0.05$) in contrast with H. This observation might be due to cavitation caused by sonication. Thus the mechanical effects and/or hydrogen peroxide (H_2O_2) formation in distilled water during sonication are responsible for the retardation of mushroom browning. Hydrogen peroxide (H_2O_2) has been demonstrated to exert an inhibitory effect on the browning to maintain whiteness of mushrooms (Sapers *et al*., 2001). High pressure treatment, on the other hand, has been reported to largely preserve fresh colour of fruits and vegetables (Kadam *et al*., 2012). Low colour change in high pressure treatment could also be due to argon clathrate hydrate formation which could suppress the activity of enzyme responsible for browning.

Texture is one of the main attributes that governs the acceptability of food by consumers. The textural firmness kinetics of white mushrooms under different treatments is shown in Fig. 1. The mushrooms were not affected by the treatments as far as their firmness was concerned. Thus the treated materials exhibited the same firmness as that of the control. The untreated (control) mushrooms gradually became more softened than the treated ones under low temperature storage with increase in storage duration. These results were in agreement with the previous reports of Jiang *et al*. (2010).

Ultrasound and high pressure argon treated mushrooms showed significantly higher flesh firmness than untreated ones ($p<0.05$) during 9 days of storage. Mushrooms treated with U, H and UH exhibited no significant differences ($p>0.05$) in firmness during the first three days of storage compared to the control. During the last 6 days of storage, the levels of reduction in textural firmness were in the order of H (4.21 N to 3.58 N) > UH (4.28 N to 3.24 N) > U (4.20 N to 3.01 N) and were all greater than that of untreated sample (4.04 N to 2.79 N). High pressure treatments were relatively more effective in retaining firmness than ultrasound treatments. Texture changes in fruits and vegetables can be related to transformations in cell wall polymers due to enzymatic reactions (Sila *et al*., 2008).

High pressure inert gases which interfered with enzymatic oxygen receptor sites could have suppressed the enzyme activity due to argon clathrate hydrate formation. Behnke *et al*. (1969) also demonstrated that high pressure inert gases inhibited tyrosinase systems by decreasing oxygen availability rather than by physical alteration of the enzyme. When noble gases dissolve in water, enzymatic reactions are inhibited, resulting in restrained vegetable metabolism (Zhang *et al*., 2008). High pressure argon could reduce the activity of cell wall degradation by enzymes and consequently delay softening which affects the cell wall disassembling rate. This might be the explanation of higher degrees of firmness in H and UH treated mushrooms.

The freshness and weight of a plant material are characterized by the amount of water it retains as well as the total soluble mass that it constitutes. Accordingly, water loss (WL), total soluble solids (TSS) and ascorbic acid (AA) are among the key factors in determining the money value as well as the overall quality of white mushroom.

In the present study, it was realized that WL of the control sample was greater than those of the treated materials (Table 2). It therefore appeared that the four treatments exerted an influence that curtailed water of the mushrooms caused by either respiration, evaporation or

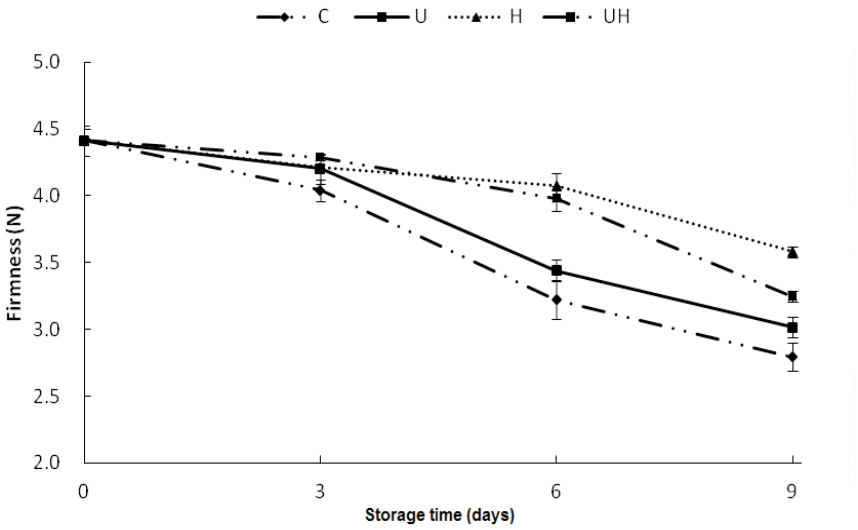

Fig. 1. Firmness changes in fresh white mushroom under different treatments during 9 days of storage at 4°C. Values are the means with standard error (n=3). Explanations as in Table 1.

T a b l e 2. Changes in water loss, total soluble solids and ascorbic acid of white mushrooms at different types of treatment during storage at 4°C

Treatment	Time (day)			
	0	3	6	9
		Water loss (%)		
Control	0	2.74±0.73a	3.75±0.19a	6.16±0.83a
U		0.39±0.39d	2.72±0.53b	4.63±0.42b
H		1.52±0.21b	2.03±0.16d	3.13±0.61d
UH		1.08±0.58c	2.42±0.42c	3.95±0.33c
		Total soluble solids (%)		
Control	8.03±0.13a	7.65±0.06c	6.03±0.23a	4.32±0.02a
U		7.03±0.10a	6.63±0.25b	6.12±0.14b
H		7.98±0.43d	7.42±0.18d	7.04±0.04d
UH		7.34±0.29b	6.93±0.02c	6.86±0.31c
		Ascorbic acid (mg kg^{-1})		
Control	34.30±0.13a	28.20±0.26a	19.10±0.37a	15.70±0.19a
U		30.20±0.12b	24.90±0.21b	21.40±0.09b
H		33.10±0.23c	29.40±0.17d	25.30±0.31c
UH		32.80±0.32c	27.10±0.16c	23.80±0.23bc

Explanations as in Table 1.

leaching. Comparatively, the effect of the argon treatment in controlling water loss was better than the other treatments. Water loss values at the end of storage for H, UH, U and untreated mushrooms were 3.13, 3.95, 4.63, and 6.16%, respectively.

Table 2 displays changes in TSS of mushrooms under the four treatments. As the storage time progressed, the TSS of the samples assumed a decreasing trend. The observed reduction in TSS content of the samples is a manifestation of high respiration rate and water loss resulting in quality deterioration with the onset of senescence. Like all living tissues, harvested mushroom continues to respire throughout its postharvest life. During the process of respiration, carbohydrates are broken down to their constituent parts to produce energy to run cellular processes, thus keeping the cells and organism alive. Throughout this process, oxygen is consumed, and water, carbon dioxide, and energy are released. The carbohydrates stored in the harvested mushroom are continually 'burned' as energy to keep the vegetable alive; as respiration continues, compounds that affect plant flavour, sweetness, weight, turgor (water content), and nutritional value are lost.

During the first 3 days of storage, the U and UH treatments, carried out with distilled water, showed a rapid decrease of TSS and low water loss compared to other treatments. This could be due to the transfer of soluble solids from the mushroom to the distilled water under the action of ultrasound treatment. Moreover, H treatment showed a slight decrease during 9 days of storage. High pressure treatment could maintain significantly ($p<0.05$) the TSS and water of fresh mushroom. This might be attributed to argon clathrate and residual argon in the micropores of mushroom which restrain the activity of intracellular water and enzyme in fruits, and slow down the respiration metabolism (Purwanto *et al*., 2001; Wu *et al*., 2012b; Zhan and Zhang, 2005; Zhang *et al*., 2008).

As shown in Table 2, the AA content decreased with increasing storage time for all samples, which is due to ascorbic acid degradation through oxidative processes (Davey *et al*., 2000). During 9 days at 4°C, there was a high reduction of ascorbic acid in the control samples (54%), a moderate reduction in U (38%) followed by UH (30%), and a low reduction in H (26%), thus demonstrating that high pressure treatments were effective in reducing the loss of ascorbic acid in fresh mushroom. This is in agreement with Wu *et al*. (2012a) who reported that high pressure argon treatments were effective in reducing AA in pineapples.

SEM study of white mushroom was conducted to understand the microstructural changes during the ultrasound, compressed argon and combined treatments. Figure 2 shows mushroom cap tissue with different treatments. All samples showed no hyphae orientation. Similar results were observed in fresh cap tissue of *Boletus edulis* (Hernando *et al*., 2008). Hyphae appeared to be shrunken, with large

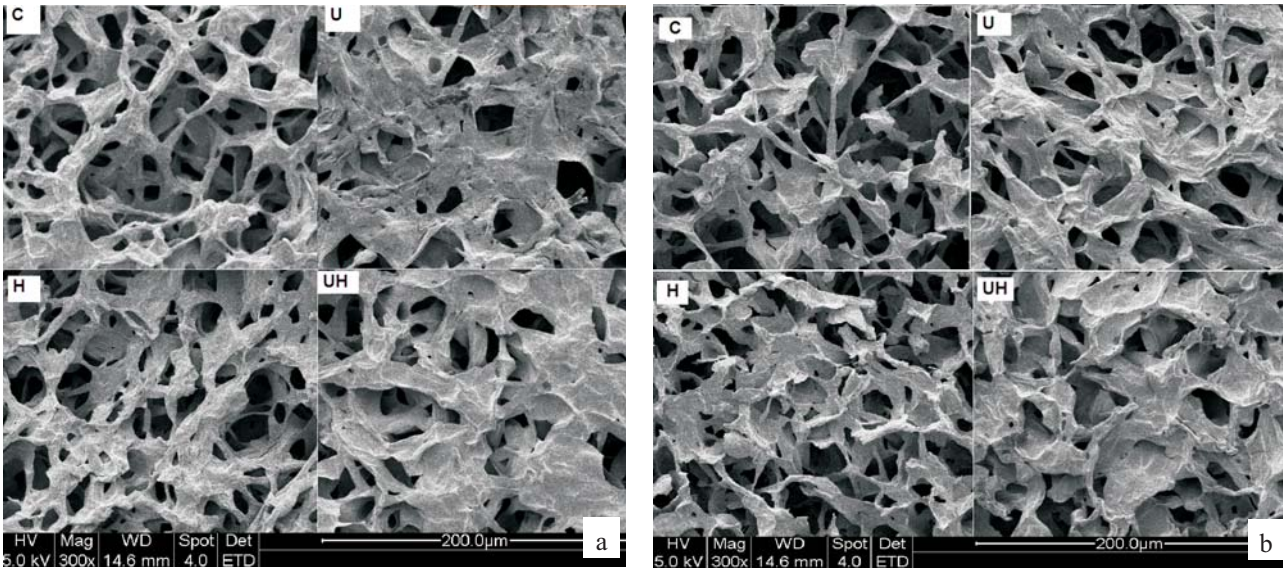

Fig. 2. Scanning electron micrographs demonstrating changes in mushrooms cells: a – immediately after different treatments, b – on the 6th day of storage at 4°C under different treatments. Explanations as in Table 1.

T a b l e 3. Microbial counts in white mushrooms during storage at 4°C under different treatments

Treatment	Time (day)			
	0	3	6	9
	Mesophilic (log CFU g^{-1})			
Control	4.27±0.06a	4.93±0.50a	5.42±0.12a	7.08±0.08a
U	3.97±0.92a	4.45±0.11c	4.54±0.03c	4.76±0.12c
H	4.02±0.11a	4.62±0,28ab	4.93±0.35b	5.74±0.14b
UH	3.83±0.31a	4.33±0.27c	4.72±0.12bc	5.03±0.39c
	Psychrophilic (log CFU g^{-1})			
Control	2.71±0.18a	4.11±0.12a	4.65±0.26a	6.57±0.20a
U	1.95±0.70bc	2.01±0.40c	3.92±0.07c	4.25±0.16b
H	2.15±0.15b	2.43±0.19b	4.15±0.81b	4.43±0.08b
UH	1.78±0.90c	2.26±0.09bc	3.25±0.52d	4.28±0.05b
	Yeasts and Molds (log CFU g^{-1})			
Control	4.13±0.75a	4.54±0.52a	5.34±0.19a	6.27±0.32a
U	2.02±0.08d	2.36±0.14d	2.97±0.26d	3.43±0.41c
H	2.48±0.15b	3.27±0.26b	3.68±0.14b	4.68±0.43b
UH	2.26±0.40c	2.68±0.45c	3.24±0.24c	3.52±0.36c
	Pseudomonas (log CFU g^{-1})			
Control	5.75±0.07a	6.43±0.06a	8.07±0.17a	8.97±0.13a
U	4.34±0.05c	5.92±0.13b	7.06±0.07bc	8.36±0.3bc
H	4.86±0.06b	5.83±0.56b	7.23±0.11b	8.65±0.71b
UH	4.23±0.17c	5.56±0.07c	6.80±0.50c	8.20±0.09c

Explanations as in Table 1.

inter-cellular spaces in untreated samples (C), compared to treated samples. Immediately after treatment (Fig. 2a), *Agaricus bisporus* showed different microstructure. U sample became flattened, and the cells closely joined, thereby resulting in a reduction of the intercellular spaces. The same observations could be seen with UH treatment, but less pronounced than in U treatment. These observations in U and UH treatments might be due to the effect of cavitations which might have removed water from intracellular environment into the extra-cellular space. The cavitation phenomenon is also believed to be responsible for the creation of microscopic channels where the intracellular water of the cells accumulates. Similar observations have been made on melon and pineapple (Fernandes *et al.*, 2008). Those authors argued that when using distilled water, ultrasound induced disruption of cells and formation of microscopic channels in the fruit structure but did not induce breakdown of the cells which likely might have occurred in this study as well. For the H treatment, hyphae cells appeared slightly thicker than in the control.

After the sixth day of storage (Fig. 2b), intercellular space between hyphae cells enlarged during storage. The inter-cellular spaces became more pronounced and were in the order of the control> U > UH > and H treatments. This was so probably due to the loss of hyphae turgidity caused by water loss.

The control hyphae showed a pronounced collapsing and flattening, with large intercellular spaces. The observed results with UH and U samples might be due to the presence of free water. For the H treatment, the result might be attributed to argon clathrate where water molecules form variously sized clathrates. That phenomenon is due to the reduction of water mobility.

Organisms which are usually responsible for spoilage of mushrooms include Gram-negative, psychrotrophic bacteria, particularly those belonging to the *Pseudomonasae* family. These organisms contaminate the product from compost. Table 3 shows mesophilic, psychrophilic, Pseudomonas, yeasts and moulds counts of white mushrooms treated with U, H, UH and control stored under low temperature for a period of 9 days.

Gradual growth of all microorganisms was seen during storage in all samples. However, some treatments retarded the microbial growth more than others. Generally, yeasts and moulds were present in relatively lower numbers during

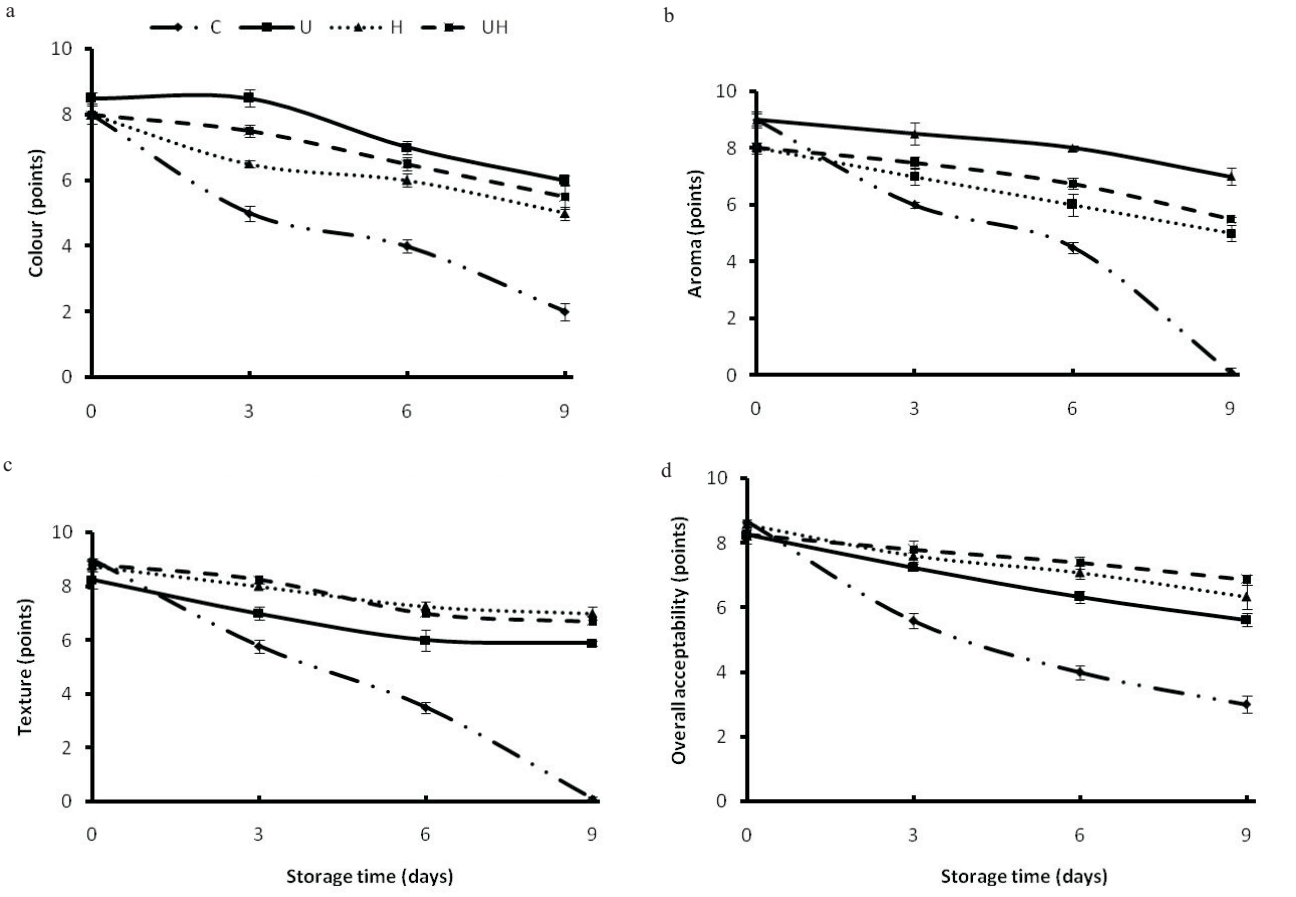

Fig. 3. Changes in sensory quality of fresh mushroom under treatments: a – clolour, b – aroma, c – texture, d – overall acceptability points; during 9 days of storage at 4°C. Values represent the means of the replicates and error bars represent the standard error of the means (n=3). Explanations as in Table 1.

the storage period. The highest amount of microorganisms was observed in control samples. Ultrasound treatment, followed by UH and H in increasing order, were found to be effective in delaying mesophilic bacteria, aerobic psychrotrophic bacteria, yeasts and moulds growth in mushroom during the 9 days of cold storage.

Moreover, during the storage, the pseudomonas bacteria in the UH and U, followed by H samples, were maintained at lower levels than those in the control ($p<0.05$). 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 research carried out by Dolatowski and Stasiak (2002) proved that ultrasound processing has a significant influence on microbiological contamination of meat. The lowest amounts of microorganisms found in U treated samples might be due to ultrasound process that creates the cavitation caused by 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). Moreover, the inhibitory effect of H treatment on microbial growth can be explained by the presence of argon which reduced water activity and remained in the micropores to reduce the growth of microorganisms.

All the selected sensory attributes assessed by the panelists were significantly ($p<0.05$) affected by storage time (Fig. 3). According to the physical appearance, the cap of mushrooms gradually became less firm, with uneven surfaces which showed dark zones that became intense with time, for all the evaluated conditions.

As the storage time progressed, the sensory attributes investigated generally recorded lower scores given by the panelists. However, the degree of the overall acceptance based on the panel members assessment of the aroma, colour and firmness varied among the samples. After 9 days of storage, the control mushrooms reached in all sensory attributes a score lower than 5, while the other treatments maintained mushrooms acceptability. It was apparent that H followed by UH and U treatment had higher texture and aroma scores than the untreated sample. The combined treatment (UH) had higher overall acceptability scores. Ultrasound treatment slightly affected the sensory attribution in terms of aroma, but had higher scores in the colour than the other treatments. The colour changes were different than the changes in other parameters. That result could be related to the sonication phenomenon (Sapers *et al*., 2001). The results of sensory analysis were in agreement with instrument analysis data (Table 1 and Fig. 1). These results were not in good agreement with those reported by Wu *et al*. (2012b), who observed that high pressure argon did not reap a better score on pineapples firmness.

CONCLUSIONS

1. Ultrasound treatment in white mushroom was able to delay the browning process and inhibited the growth of microorganisms within 9 days at cold storage conditions.

2. Mushrooms treated with high pressure argon revealed a significant effect in retaining firmness and reduction of microbial counts compared to control.

3. Application of ultrasound combined with high pressure argon and ultrasound appeared to be the most effective treatment in decreasing pseudomonas counts.

4. The positive effects of high pressure argon treatment on the quality of fresh white mushroom might be due to the occurrence of Argon clathrate hydrates.

5. Ultrasonic pre-treatment is a promising technique to decrease the deterioration and to extend shelf-life during cold storage. However, more research is needed to give deeper understanding of ultrasound and high pressure argon mechanism on fruit and vegetables to promote the application of those techniques.

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