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Research Article

Power Ultrasound Coupled with Modified Atmosphere Packaging: Synergistic Effects on the Shelf-Life of White Button Mushroom (*Agaricus bisporus*)

Kiana Vazirianjafari, Mahmoud Omid, and Mahmoud Soltani Firouz

Department of Agricultural Machinery Engineering, Faculty of Agriculture, University of Tehran, Karaj, Iran

Correspondence should be addressed to Mahmoud Omid; omid@ut.ac.ir and Mahmoud Soltani Firouz; mahsoltani@ut.ac.ir

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This study investigated the application of ultrasound (US) and modified atmosphere packaging (MAP), alone and in combination, on the mechanical and physicochemical properties and appearance of button mushrooms to increase their shelf-life. The mushrooms were treated with US (40 kHz, 100 W) for 5, 10, and 15 min; packaged in modified atmosphere of 10% O_2, 5% CO_2, and 85% N_2; and stored at ambient temperature of 4°C and 30 ± 5% relative humidity for 12 days. The synergistic effect of 10 min US in combination with MAP had the best protective role on the quality characteristics of mushrooms such as total soluble solids, pH, lightness, total color changes, browning index, and weight loss. Also, the treatment recorded fairly appropriate effect on preserving the firmness of mushrooms. The result proved that US is effective to enhance storage quality and is a promising tool to improve storage quality and the preservative effect of MAP for mushrooms.

1. Introduction

Edible mushrooms are very popular all over the world due to its unique organoleptic properties, texture, and nutritional values [1]. Their high nutrient level is related to protein and amino acids (tryptophan, lysine, proline, arginine, alanine, and leucine) [1, 2], minerals (potassium, iron, zinc, phosphorus, and magnesium) [1, 3], valuable dietary fiber (chitin, hemicellulose, chitosan, and glucans) [1], and vitamins (vitamin C and vitamin B complex, especially niacin, riboflavin, pantothenic acid and biotin [2, 3], folic acid, cobalamin, and ascorbic acid) [1]. White button mushroom (*Agaricus bisporus*) is known as the most popular and most commonly consumed edible mushroom in the world [4]. However, mushrooms are one of the most perishable agricultural products during the postharvest period [2]. The *Agaricus bisporus* mushrooms can keep their quality at an acceptable level for 1-3 days after harvesting when stored at ambient temperature (20-25°C) and 5-7 days when stored at 0-2°C [5]. In principle, the lack of a cuticle layer on the surface of mushrooms makes them very sensitive to moisture loss, physical damage, and microbial attack [2]. On the other hand, the respiration rate of mushrooms is relatively high due to their thin and porous skin structure [1]. The enzymatic activities are another important factor that contribute to the short life of button mushroom [4]. The disadvantage of the short shelf-life of mushroom limits its supply chain. Several methods have been proposed to control one or some of quality factors and to increase the shelf-life of edible mushroom [5]. Drying, cooling, modified atmosphere packaging (MAP), irradiation, pulsed electric field, washing with antimicrobial agents, ozone, electrolyzed water treatments, controlled atmosphere (CA), moisture absorbers, coatings, nanocomposite films, etc. are the preservation methods that effectively reduce postharvest quality deterioration and shelf-life extension of mushrooms [3–7]. The MAP technology is a simple, economical, ecofriendly, nonchemical, and commercially suitable method [8, 9]. Additionally, it has succeeded to get public acceptance because of the absence of some toxic residues [8]. The MAP method reduces the respiration rate of the product through the changing gas concentration within the package [10]. Thus,

it can potentially decrease physiological and biochemical processes contributing to deterioration of produce quality [9]. Numerous studies have been performed on the application of the MAP technology or its combination with other method to increase the shelf-life of agricultural products [4, 8, 11–17]. While other assistant technologies may be useful to promote the MAP technology for shelf-life extension of mushrooms, the power ultrasound (US) in combination with MAP seems to be a good approach to extend the shelf-life of mushrooms. Jiang et al. [18] reported that the combination of US as an auxiliary preservation technology with the traditional methods (e.g., refrigeration and MAP) would be needed since US demonstrates a synergistic effect on the shelf-life of the product. However, very limited research has been conducted on the application of US and MAP [19–23]. The US method is usually simple to use, economic, environment-friendly, highly efficient, and energy-saving compared with the traditional treatments. In addition, US does not need external chemical reagents and additives during the treatment [18]. Some studies have been performed on the application of US or its combination with other techniques to increase the shelf-life of fruits and vegetables [24] such as pomegranate [25], bok choy [26], white button mushrooms [27], fresh-sliced button mushrooms [28], straw mushroom [29], strawberries [30], and fresh-cut lettuce [31]. Lagnika et al. [27] studied on high-pressure argon and power ultrasound effects on the quality of white mushrooms. However, they stored the treated mushrooms in impermeable glass jars which were sealed by an impermeable film. In other words, they did not apply the MAP technology to prolong the shelf-life of the mushrooms. Li et al. [29] used power US to improve the quality of straw mushroom. They stored the sonicated mushrooms at relative humidity of 75% or 95% for four days at 15°C. Wei et al. [32] studied on different MAP types to store pine mushrooms. They used polyvinyl chloride and polyethylene packaging films as well as polyvinyl chloride packaging with silicon windows. However, no adjusted gas was flush into the packages before sealing. To the best of our knowledge, there is no research to study synergistic effects of MAP and US on white button mushroom to extend its shelf-life during the cold storage. This research is aimed at studying the impact of US in combination with the MAP technology on the quality factors of white button mushrooms during the cold storage. The studied quality factors are TSS, pH, firmness, and color properties including lightness (L^*), browning index (BI), and total color difference (ΔE).

2. Materials and Methods

2.1. Sample Preparation and Treatments. Freshly harvested white button mushrooms (*Agaricus bisporus*) were cautiously handpicked from the Malard Mushroom Cultivation Industry Company (Tehran, Iran) in January 2023 and immediately transported to the laboratory. Before packaging, the mushrooms were stored in a constant temperature and relative humidity (4°C with 30 ± 5%) for 24 h until the core temperature was the same as that of the environment. To perform the experiments, 236 mushrooms were selected at their maturity stage, with a completely closed cap, almost similar in shape and weight and free from scars and blemishes. Some of them (12 mushrooms) were randomly selected for initial experiments (the freshly harvested or day 0 category) to measure firmness, pH, TSS, and color properties of the fresh ones. The remaining samples were randomly divided into 8 categories (28 samples for each category) for the corresponding treatments. The samples in each category were divided into 4 groups (7 samples in each group) to be studied on the 3rd, 6th, 9th, and 12th days. The 8 treatments were as follows:

Control: untreated samples

MAP: the samples were washed with distilled water and then packaged in the modified atmosphere condition

US_5: 5 min ultrasonication in distilled water

US_{10}: 10 min ultrasonication in distilled water

US_{15}: 15 min ultrasonication in distilled water

US_5+MAP: 5 min ultrasonication in distilled water combined with MAP

US_{10}+MAP: 10 min ultrasonication in distilled water combined with MAP

US_{15}+MAP: 15 min ultrasonication in distilled water combined with MAP

All groups of each category were packaged in the packing films (each package contained 7 mushrooms, and each package was representative of each treatment). The packing films used were $30 \times 20\,cm^2$ in size made of polyamide 20 μm-polyethylene 70 μm with ethylene vinyl alcohol sealant layer. Carbon dioxide and oxygen permeability of the packaging films were 1.7×10^{-8} and $5.8 \times 10^{-9}\,mL\,m^{-2}\,s^{-1}\,Pa^{-1}$ (both at 23°C and 0% relative humidity), respectively, and the water vapor transmission rate was equal to $3.2 \times 10^{-5}\,g\,m^{-2}\,s^{-1}$ (at 23°C and 85% relative humidity) [33]. Finally, all packages were stored in a refrigerator, in dark conditions, at temperature and relative humidity of 4°C and 30 ± 5% for 12 days while the experiments were implemented at an interval of 3 days (four groups).

2.2. Ultrasonication. Samples were immersed in distilled water in an ultrasonic bath (vCLEAN1–L4, Backer, Iran). The volume of the ultrasonic bath was 4 L which was filled with distilled water as the medium liquid. The device was equipped with a temperature control system, which made it possible to adjust the liquid temperature. The temperature of the medium was set on 30°C throughout the treatment process. The ultrasonication process was separately applied to each of the treatment categories (US_5, US_{10}, US_{15}, US_5+MAP, US_{10}+MAP, and US_{15}+MAP). In each time of ultrasonication, 7 mushroom samples were simultaneously processed. Sonication was performed on the immersing mushrooms at a fixed frequency of 40 kHz, acoustic intensity of $2.1\,kW/m^2$, and power of 100 W for 5, 10, and 15 min based on the mentioned treatments (Figure 1). Immediately after treated by US, the mushrooms were placed on a clean towel for 10 min to remove excess water on their surface and instantly were packaged.

2.3. Modified Atmosphere Packaging. To prepare the MA packages, a desktop food packing machine (200A,

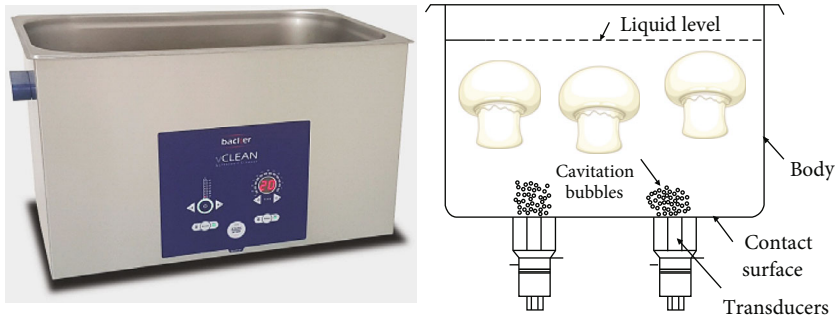


Figure 1: Schematic diagram of the ultrasound system.

Henkelman, Germany) was used, and the headspace of the packages was filled with the gas mixture of 85% N_2, 10% O_2, and 5% CO_2.

2.4. Weight Loss. The samples in each package were weighed before and during the storage at specified time intervals using a digital scale (SKY-600, Jadever, China) with an accuracy rate of 0.01 g. The percentage of weight loss of packages according to their initial weight was calculated by [4]

$$\Delta W = \frac{W_0 - W_1}{W_0} \times 100, \quad (1)$$

where ΔW is the weight loss (%), W_1 is the weight of each package at the given day (g), and W_0 is the initial weight of each package (g).

2.5. Firmness. In order to evaluate firmness of the samples, the penetration test was carried out using an Instron Universal Testing Machine (STM-5, Santam, Iran) controlled by a PC-based data acquisition card on a personal computer. For the test, a standard flat-end cylindrical steel probe of 5 mm was used. The probe penetrated perpendicularly into the cap of the mushroom up to the depth of 10 mm with the constant speed of 10 mm min^{-1} [4], while the force-displacement diagram was drawn. The maximum penetration force was recorded as firmness [4, 34, 35]. Three samples for each treatment were performed, while the mean values were calculated.

2.6. TSS and pH. For the TSS and pH measurements, each sample was crushed using a minishredder (Moulinex, China) and then the juices of mushrooms were extracted by filter paper and hand squeezing. The filter paper was 124 mm in diameter and 40 μm in mesh size with a circular shape (Whatman, Germany). A digital pH meter (ATC, China) with an accuracy of 0.01 was used to measure pH of mushroom juices.

A hand-held refractometer (ATC, China) with accuracy rate of 0.5% was used to measure TSS of the juices. Three samples for each treatment were performed at 20°C, and the mean values were calculated.

2.7. Color Properties. Digital image processing was applied to extract the color features of the mushroom samples. Image feature extraction using image processing can be divided into two general steps. The first step is detecting the edges and objects that are in the image, and the second step is extracting the features of these areas [36].

2.7.1. Image Acquisition. The image acquisition system was composed of an optically insulated portable chamber with dimensions of $15 \times 15 \times 40\,cm^3$. The samples were illuminated using a circular white light LED (12 V) with a constant brightness which was positioned beneath the chamber ceiling, perpendicular to the samples. The images were captured using a mobile phone with the camera of 16 MP (LG-H818P, Korea). The camera lens was located at a fixed position on the ceiling aperture, vertically over the samples at a distance of 40 cm with an angle of 0° to the light source. The images of seven samples were captured for each treatment. All images were in sRGB color space with the JPG format, resolution of 2988×5312 pixels, and 24-bit pixel depth.

2.7.2. Object Description. To extract the features of each image, an image processing algorithm was written in Google's Colaboratory framework on https://colab.research.google.com/, in Python. According to the algorithm, median filter was applied to remove blurs around the mushroom image (Figure 2(a)). Each RGB image was converted into a binary one by the threshold technique used by Otsu [37] (Figure 2(b)). In order to eliminate noises within the object of interest, morphological opening-closing operators were carried out. Dilation was applied to smooth the edge of mushroom (Figure 2(c)). The binary image was masked with the original color image (Figure 2(d)), and finally, the nonlinear RGB color space was converted to the CIELAB color space (Figure 2(e)).

2.7.3. Color Feature Extraction. The average values of each component L^*, a^*, and b^* were computed for all pixels in each image. Total color changes (ΔE) and browning index (BI) were calculated according to the following [4]:

$$\Delta E = \left[(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2\right]^{1/2},$$
$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*},$$
$$\mathrm{BI} = \frac{x - 0.31}{0.172} \times 100, \quad (2)$$

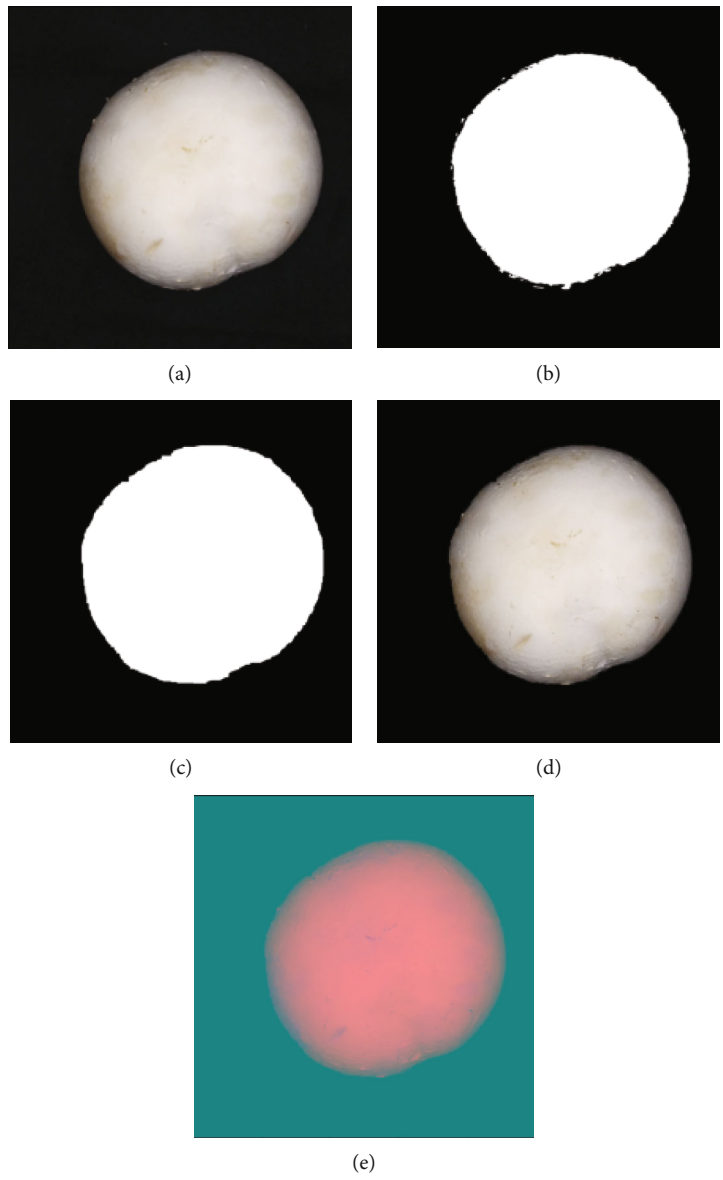


Figure 2: (a) A median filter applied on color image; (b) the binary image obtained after applying the Otsu thresholding; (c) the image after morphological opening-closing operators and dilation; (d) the binary image masked with the original color image; (e) final image in the CIELAB color space.

where ΔE is the total color changes and ΔL^*, Δa^*, and Δb^* are the difference between the values of L^*, a^*, and b^* before and after a certain period of storage, respectively.

Finally, the mean values of L^*, ΔE, and BI for each treatment were calculated.

2.8. Statistical Analysis. A simple random design for analysis of variance (ANOVA) and Duncan's method for comparing the means of treatments was implemented using the SPSS software version 26 (IBM, USA). The properties of mushrooms were expressed as the mean ± standard deviation for each treatment. Also, significant difference between the treatments was expressed at the level of 5% probability ($p < 0.05$). The graphs were also drawn using the Microsoft Excel 2016 software (Microsoft, USA).

3. Results and Discussion

3.1. Weight Loss. The weight loss during 12 days of storage has gradually increased in each treatment with respect to the samples of 0^{th} day (Figure 3). The untreated (control) and US_{10}+MAP treatments exhibited the highest and lowest level of weight loss at the end of the storage time, respectively. The results confirmed that the combined treatments (US +MAP) effectively reduced the weight loss of mushrooms, followed by US alone. So, ultrasonication at different times (5, 10, and 15 min) seems to be helpful for inhibiting weight loss of the MA-packaged mushrooms. Especially, 10 min ultrasonication was more effective than 5 and 15 min ones.

Weight loss of fresh mushrooms is one of the major challenges during postharvest which causes to shrivel and

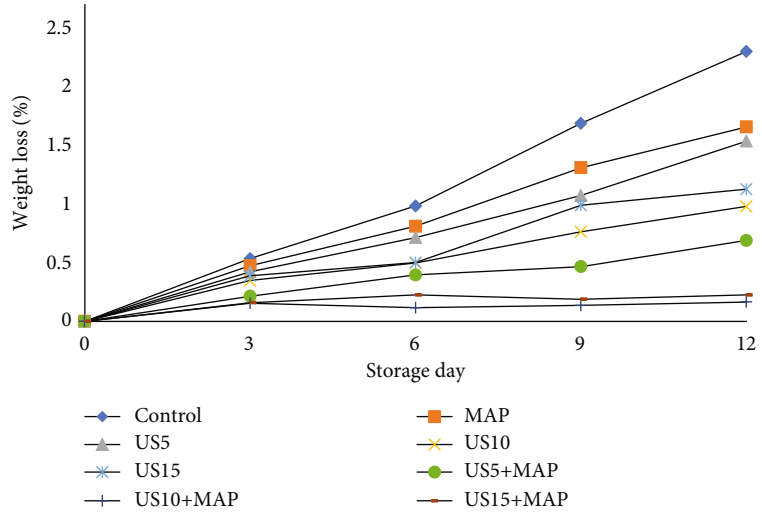


Figure 3: Changes in weight loss of white mushroom packages at 4°C for 12 days. Vertical lines represent standard error.

deteriorate when it becomes excessive. This physiological change is mainly related to transpiration and respiration rates, as these two factors result in losing water/dehydration (transpiration) and losing dry matter of product (respiration) [17, 27, 29]. On the other hand, button mushrooms contain 90% moisture content [17]. However, due to the fact that they are only covered by a thin and porous layer of epidermal structure, they are not properly able to prevent superficial dehydration [17, 38]. Therefore, depending on the relative humidity of the environment, mushrooms seem to be very susceptible to dehydrate leading to the increased weight loss. According to Singh et al. [39], "when the harvested produce loses 5-10% of its fresh weight, it begins to wilt and becomes unusable" (p. 1393). In this study, the greatest weight loss was equal to 2.3% with respect to 0^{th} day, after 12 days (related to the untreated samples). Therefore, from the point of view of weight loss, all types of packages were acceptable. The low weight loss of samples may be attributed to the film used for packaging. As Antmann et al. [38] reported, the slight water vapor transmission rate of film in combination with the high transpiration rate of mushrooms produces a saturated condition inside the packages, which prevents further transpiration and slows down weight loss. The good control of weight loss observed in the ultrasonicated mushrooms may be due to the fact that the hydrogen bonding between water molecules and macromolecules in the mushrooms could be better preserved due to the ultrasonication which could reduce the water loss and metabolic rate [40]. Also, as MAP is aimed at reducing the respiration rate by providing a desirable atmosphere in packages [9], it could show beneficial performance to reduce weight loss. Thus, the lowest weight loss was observed in the combined treated mushrooms, which could be a result of synergetic effects of US and MAP on weight loss of mushrooms. Among all combined treatments, the US_{10}+MAP recorded the lowest weight loss during the storage. Ultrasonication over 10 min created more cavitation bubbles, and the prolonged exposure of mushroom samples to the cavitation bubbles may lead to cell damages and reduced cell wall stability, resulting in an increased water loss [29]. The same results are reported by Li et al. [29] that the 10 min US-treated straw mushrooms showed lower weight loss than 5 and 15 min US.

There is a considerable issue of water absorption by samples during the ultrasonication process followed by weight gain. Simón et al. [12] reported 5-6% weight gain because of water absorption of *Agaricus bisporus* after washing for 5 min in citric acid solution (with gentle shaking) and then immersion for 3 min in distilled water. Sapers et al. [41] also recorded 2.5-3% weight gain after dipping time of 1-2 min in browning inhibitor solution and up to 8% after adding agitation to the process. Furthermore, sliced mushrooms, due to their larger surface area, showed 7.5-10 times more weight gain than a whole mushroom [41]. According to Sapers et al. [41], dipping time causes nearly little effects on water absorption of mushrooms, because the main absorption occurs within the first 5-10 s of immersion.

3.2. Firmness. Figure 4 shows the effect of different treatments on the firmness of button mushrooms. According to the results, the firmness of mushrooms has decreased compared to the 0^{th} day within the storage period. The untreated samples showed the lowest level of firmness in the total storage time. On the 12^{th} day, US_5+MAP with the firmness of 34.46 N demonstrated the greatest value significantly ($p < 0.05$), followed by US_5 and US_{10}+MAP with firmness of 31.11 N and 30.09 N, respectively.

Hence, 5 min ultrasonication can more appropriately maintain the firmness of samples compared to the other ultrasonication time (10 and 15 min). Also, the MA-packaged mushrooms showed lower softness compared to US_{10} and US_{15} ones in the total storage period.

The firmness of mushrooms is considered as a key indicator with regard to consumer preference and reflects the cell wall integrity and the intercellular adhesion [28]. The firmness of mushrooms gradually declines over the storage period. As some researchers have reported, the loss of firmness in fresh fruit and vegetables can be directly attributed to

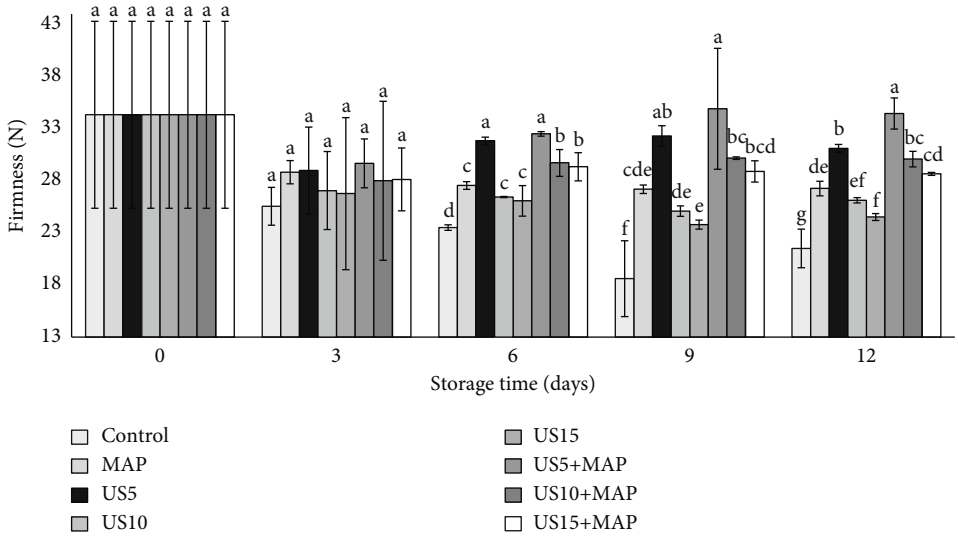


FIGURE 4: Changes in firmness values of white mushrooms at 4°C for 12 days ($n = 3$). Vertical bars represent Duncan's significant difference for 5% significance level.

the reduction of water content and weight, the growth of bacteria, and enzymatic reactions [4, 20]. According to Lagnika et al. [20], extensive dehydration of mushrooms leads to texture changes and reduction in firmness. The activity of proteases which are secreted by bacteria degrades protein to liberated amino acids [42]. Also, *Pseudomonas*, a kind of microorganisms, breaks down the intracellular matrix and disrupts the activity of central vacuole, resulting in a loss of turgor pressure and collapsed cells. During postharvest storage, to perform metabolic activities of mushroom, polysaccharides of cell wall are utilized as a source of carbohydrates [42] and made transformations by enzymatic reactions [20]. The two most important polysaccharides are glucan and chitin that contain the main chemical composition of cell wall of mushrooms. The main function of chitin is to improve strength of cell wall, and glucan acts to assemble chitin, bond the cells, and form the cell wall scaffold. Therefore, changing the structure of these cell wall components seems to be important reason of firmness loss [43].

In this study, US_5+MAP recorded the highest firmness value followed by US_5. It may be due to the potential of the US treatment to restrain respiratory and metabolic activity in mushrooms during storage. The decrease in the metabolic activity rate results in slowing the consumption of chitin, glucan, and other cell wall components [43]. Also, according to Lagnika et al. [20], US can control the loss of mushroom firmness which is related to polyphenol oxidase (PPO) activity and microorganism's growth such as *Pseudomonas*. The MAP richer in CO_2 and poorer in O_2 than air can inhibit biochemical reactions which are responsible for mushroom tissue degradation [38]. Therefore, US especially in combination with MAP can effectively show helpful synergistic effects on firmness of mushrooms.

As the results showed, US for 5 min was observed in higher firmness value than those treated for 10 and 15 min, either alone or combined treatments. It may be related to the prolonged exposure of US followed by production of more cavitation bubbles which leads to make negative effects on cell wall stability and firmness. The observation is in agreement with that of Li et al. [29] that 3 min US-treated straw mushrooms showed higher firmness than other times of US (10 and 30 min). Also, Fan et al. [21] reported that the treated fresh-cut cucumbers by US for 5 min have shown better result on firmness than the 10 and 15 min ultrasonication processes.

3.3. pH. According to Figure 5, the pH of all treatments increased within the storage period. The most changes were observed in the untreated samples with an increase of 11.93%, and the least changes were observed in the US_{10} +MAP treatments with an increase of 2.39%, with respect to 0^{th} day. In general, the combined treatments showed better performance to preserve the original conditions and freshness of mushrooms than US ones. The US treatment in different times especially for 10 min revealed an improved synergistic effect with MAP on pH retaining of mushrooms. Furthermore, the assessment of mean values exhibited that there is no significant difference ($p > 0.05$) between 10 and 15 min ultrasonication, either in combined or alone treatments.

The pH value refers to the hydrogen ion concentration of product which indicates the acidity level [44]. Keeping pH values constant can help to retain the initial conditions and maintain the quality of product after harvesting [4]. Variation in pH is attributed to the growth of microorganisms followed by production of organic acids [33]. The pH value of all mushrooms gradually increased during the 12-day storage, in the same way with Sami et al. [45]. The lowest changes are related to the US+MAP-treated mushrooms. MAP can decrease respiration rate of mushrooms by using a different gas composition than air [9]. According to Gholami et al. [4], the reduction of respiration rate results in retarding the aging and helps to inhibit the changes in pH.

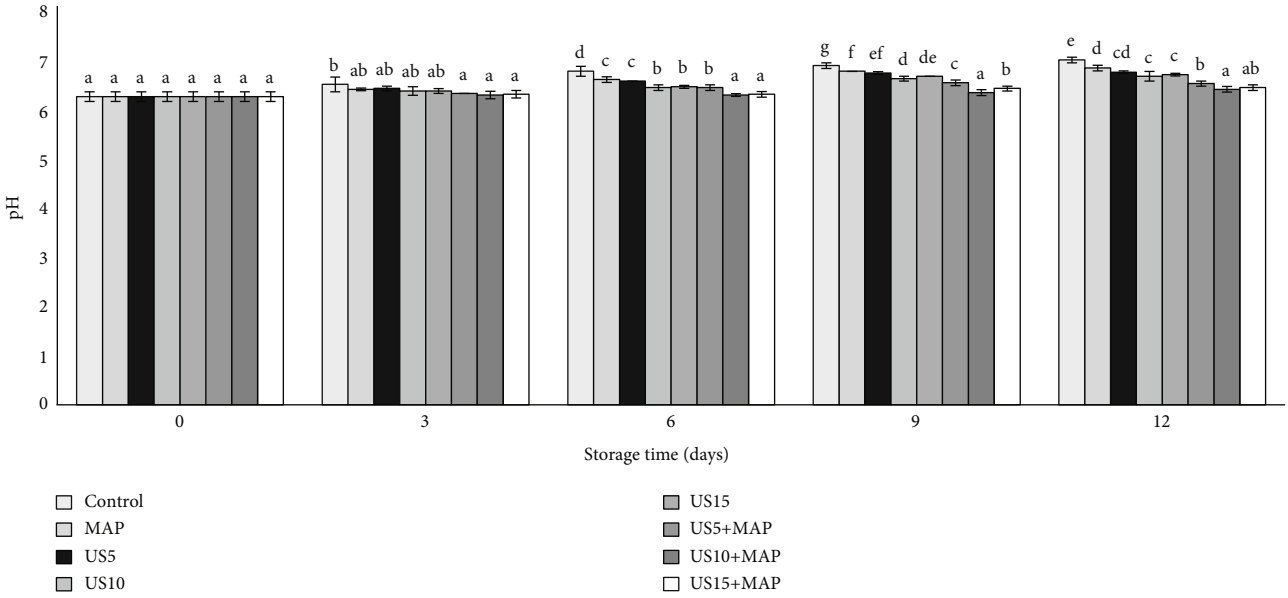


FIGURE 5: Changes in pH of white mushrooms at 4°C for 12 days ($n = 3$). Vertical bars represent Duncan's significant difference for 5% significance level.

On the other side, the cavitation bubbles produced by US make a rapid rise of localized pressure which leads to the damage of microbial cell walls, resulting in lethal effects [21, 46]. Thus, the obtained results proved that US+MAP is an effective method for controlling pH level of mushrooms. According to the results, US for 10 min was observed as the most helpful to delay pH changes than other time of US (5 and 15 min). This result can be due to the ability of 10 min US to reduce microbial number on products during storage, as Cao et al. [47] and Fan et al. [21] have reported. According to Cao et al. [47], the US treatment for 10 min can inhibit the bacteria, yeast, and mold growth on strawberry fruit. Also, Fan et al. [21] found that 10 min US was effective on inhibiting microbial growth and reduction of total number of colonies, mold, and yeast in the MA packages of fresh-cut cucumbers.

3.4. TSS. The effect of different treatments on TSS of mushrooms is shown in Figure 6. The TSS values decreased during the storage time for 12 days. The untreated samples demonstrated the greatest decrease of 57.35%, while US_{10} +MAP remained with the slightest decrease of 6.71% during the storage period. After US_{10}+MAP, the lowest decrease is related to US_{15}+MAP with 9.43% changes in TSS of samples. According to the trends of US_{10}+MAP and US_{15} +MAP, it seems that these treatments retain TSS of the samples at relatively high and constant values. The obtained results prove the potential of US for maintaining TSS of mushrooms in acceptable levels. Moreover, 10 min ultrasonication showed better preservation of TSS level than the 5 and 15 min treatments.

TSS is known as one of the indicators of mushroom postharvest decay [29]. It can be formed by breaking of long-chain carbohydrate compounds into soluble sugar compounds [44]. TSS in products acts as a temporary energy store and mainly involved in carbohydrate metabolism in cells [29]. Because of the high respiration rate in the fresh produce, the stored soluble solids convert into energy during the storage time. The conversion leads to a decrease in nutritional properties as well as shelf-life of produce [14]. After a downward trend in TSS values in this study, on the 12th days, the lowest and highest TSS was observed in control and US+MAP-treated samples, respectively. Li et al. [29] proved that MAP controls TSS of mushrooms by decreasing their respiration rate by creating a respiration-inhibiting atmosphere within the package. They reported that US inhibits respirations via inactivating respiration-related enzymes such as succinic dehydrogenase (SDH), phosphohexose isomerase (PGI), cytochrome C oxidase (CCO), glucose-6-phosphate dehydrogenase (G-6-PDH), and 6-phosphogluconate dehydrogenase (6-PGDH) in mushrooms, which confirms our finding on the synergistic effect of MAP and US for shelf-life extension of mushrooms. Among the combined treatments, US_{10}+MAP revealed higher TSS than US_{15}+MAP and US_5+MAP at the end of the storage. The same results have been represented by Li et al. [29] that 10 min US resulted in minimum CO_2 production rate within 72 h storage and keeps TSS in a relatively higher level than 3 and 30 min US in straw mushrooms.

3.5. Color. As shown in Figure 7(a), ΔE presented a growth during the storage time. The most color change, equals to 163.52, is related to the untreated samples after 12-day storage. The MAP treatment effectively controlled ΔE of mushrooms compared to the control, while the combination of MAP and US severely decreased the total color changes of mushrooms during the storage time. It confirms that US can improve effectiveness of MAP in maintaining the appearance of mushrooms. The lowest ΔE, equal to 23.04 and 30.03, are related to US_{10}+MAP and US_{10}, respectively.

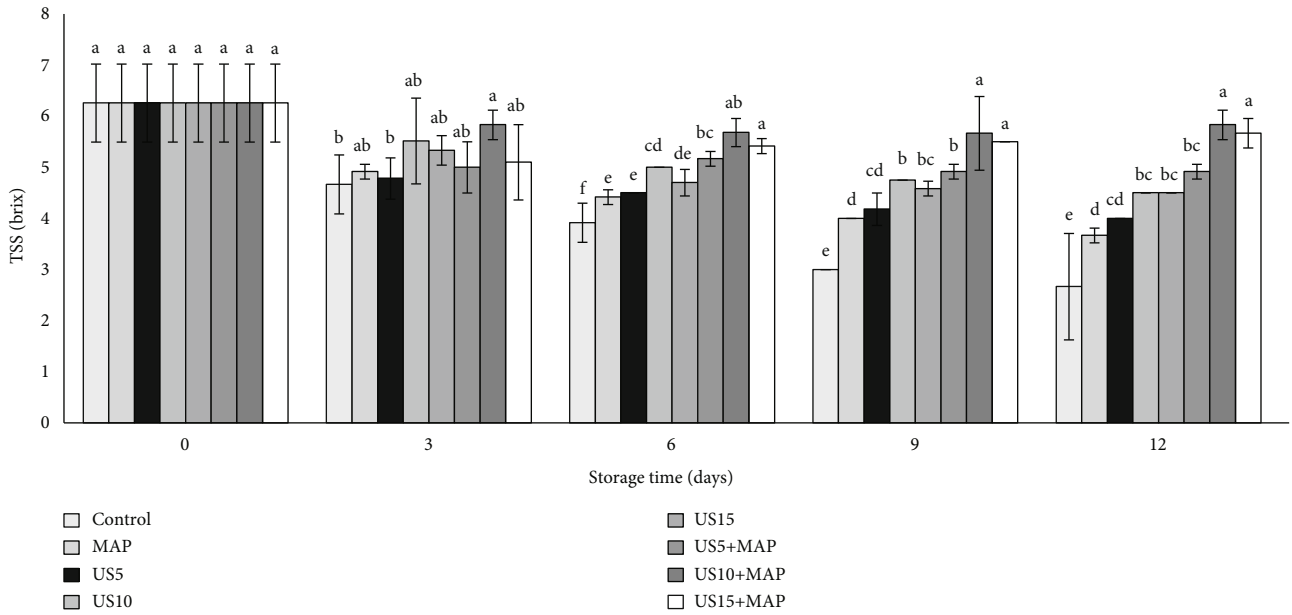


FIGURE 6: Changes in TSS of white mushrooms at 4°C for 12 days ($n = 3$). Vertical bars represent Duncan's significant difference for 5% significance level.

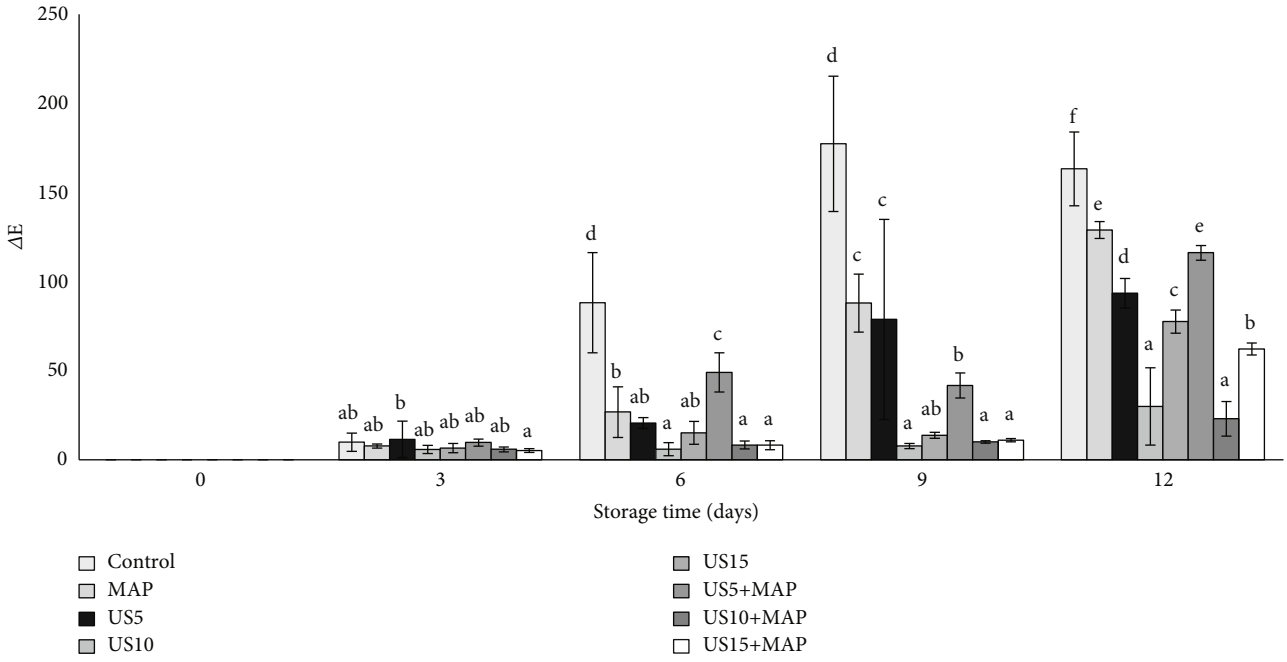
Therefore, 10 min ultrasonication is more susceptible to inhibit variations in color compared to the other US treatments.

According to Figure 7(b), US_{10}+MAP with L^* of 48.73 exhibited the most ability in preserving the whiteness of mushrooms on the 12th day. Also, US_{15}+MAP resulted a good control on L^* followed by the US treatments (US_{15}, US_{10}, and US_5).

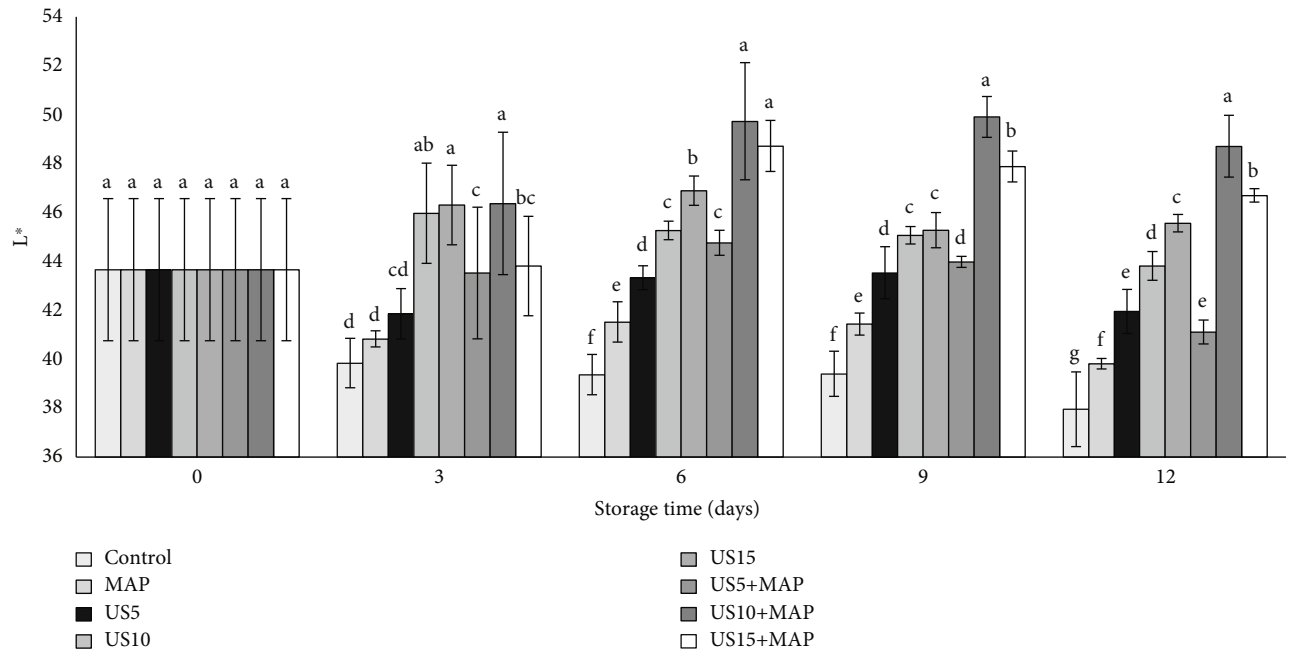
Figure 7(c) shows BI changes of mushrooms during storage. At the end of the storage, BI of untreated and US_{10}+MAP samples demonstrated the highest and lowest increase with respect to 0th day, respectively. Followed by US_{10}+MAP, US_{10} showed fewer browning degree than the other treatments. It can be found that 10 min US, especially in combination with MAP, is very impressive to prevent the surface of samples to turn yellow.

Consumers consider the appearance of the product as the main criterion for acceptance [27]; color is known as the most important visual indicator that reflects the aesthetic value and marketability of the products [21]. Thus, ΔE, L^*, and BI were measured and assessed during the storage period of mushrooms. ΔE represents the degree of overall color change in comparison with color values of an ideal mushroom [27]. L^* and BI indicate whiteness and the purity of brown color of samples, respectively [14, 17]. The color of mushrooms gradually turns from white to brown during storage in the postharvest period [14]. The browning trend of mushroom surface can be due to water loss, microbial contamination, and enzyme activities [5]. Microbial spoilage of mushrooms is generally because of the growth of *Pseudomonas* bacteria. As they grow, they break down the mushroom fibers which cause surface browning [20]. Also, enzymatic reactions usually occur on the surface in the presence of oxygen and present browning as a serious processing problem during the postharvest period [5, 20]. Enzymatic browning is closely related to the PPO activity [4]. Generally, PPO catalyzes the hydroxylation of monophenols to *o*-diphenols and dehydrogenation of *o*-diphenols to *o*-quinones which further react with the amino acid to form melanins in an aerobic condition [20, 28]. Therefore, as a result of such enzyme-catalyzed reactions, brown spots appear on the mushrooms. Among the PPO families, tyrosinase has the highest content in mushrooms, so it is known as the responsible enzyme for *Agaricus bisporus* browning [5]. In this study, US_{10}+MAP treatment recorded the highest L^* values and lowest ΔE and BI during storage for 12 days. It may be related to the collapse of cavitation microbubbles which results in an extreme increase in localized temperature and formation of free radical which help to destruct microbial agents [20, 22]. Also, the hydrogen peroxide (H_2O_2) activity which is formed in distilled water during the ultrasonication process causes oxidizing effects on bacteria such as *Pseudomonas* and thereby exerts an inhibitory effect on the browning phenomenon [20, 27]. On the other hand, US directly acted in effectively inhibiting enzymatic activities of lipoxygenase, heat-resistant lipase, protease, and browning-related enzymes such as peroxidase (POD) and PPO by producing the free radicals and localized energy accumulation with instantaneous high temperatures and pressures [27–29]. Moreover, mechanical forces created by cavitation, such as shock waves, caused strong shear forces which mainly affect the structures and activities of the involved PPO and POD [22, 28, 29].

According to Wu et al. [28], the damage of cell membrane of product promotes the contact rate of PPO and phenolic compounds which can accelerate browning of the surface. As mentioned earlier, there is a possibility of cell wall damaging of the mushrooms during ultrasonication. However, the results showed that ultrasonication for 10 and 15 min seems to be appropriate to inhibit the negative

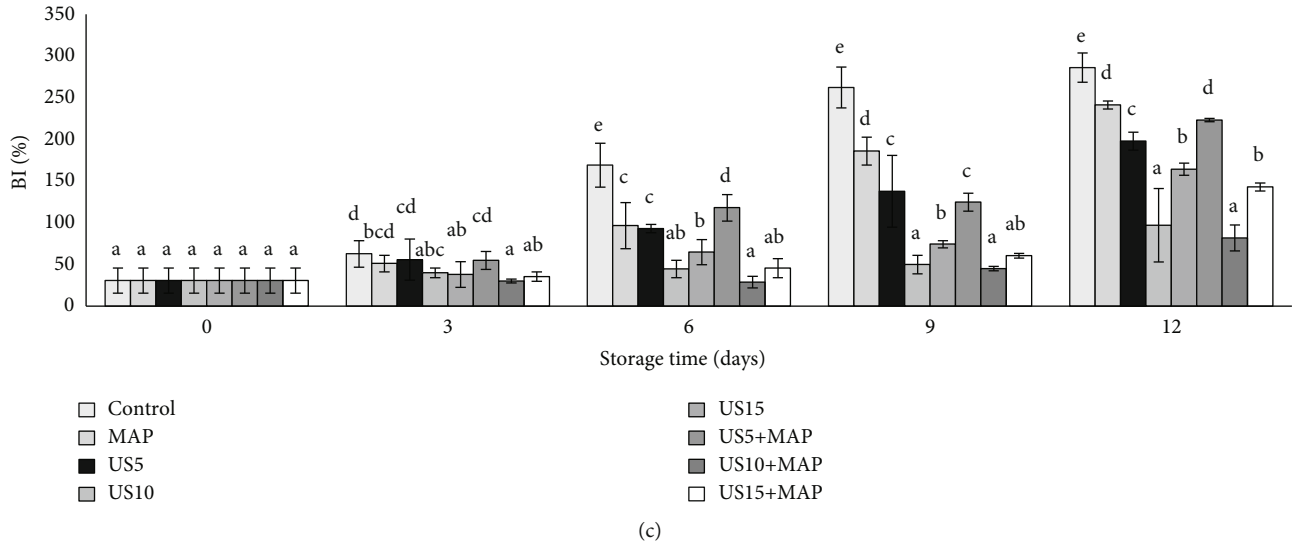


(a)



(b)

Figure 7: Continued.



(c)

Figure 7: Changes in (a) ΔE, (b) L^*, and (c) BI of white mushrooms at 4°C for 12 days ($n = 7$). Vertical bars represent Duncan's significant difference for 5% significance level.

effect of cell wall damage and effectively keep the appearance color by controlling enzymatic and microbial activities.

The MAP treatment recorded higher L^* and lower ΔE and BI than the control significantly ($p < 0.05$). The good control of MAP on color may be due to the increased CO_2 concentration that reduces O_2 availability in the packages and eventually suppresses POD and PPO activities followed by oxidation of phenolics [8]. Also, Ali et al. [8] found that the MAP-stored fruit recorded much lower POD and PPO activities than the unbagged group. Therefore, the application of US in combination with MAP can significantly have a synergistic effect which promote the color preservation of mushrooms during storage.

As the US_{10}+MAP and US_{10} treatments were very effective in retarding the color changes of samples, it can be found that 10 min US keep samples in better appearance than the other times of US (5 and 15 min). Zhang et al. [22] reported that the pakchoi treated by 10 min ultrasonication+MAP exhibited the lowest PPO activity and the highest L^* value at the end of the storage period. Also, according to the research of Birmpa et al. [48] on fresh-cut lettuce and strawberry, the US-treated samples for 10 min effectively preserved the ΔE and L^* values of the products.

4. Conclusion

In this research, the effects of US for different durations (5, 10, and 15 min) in combination with MAP were studied on increasing the shelf-life of white button mushrooms. After storage for 12 days at 4°C, the results showed that US can effectively enhance the preservative effect of MAP technique. The results showed that US_{10}+MAP is the most effective treatment in preventing TSS reduction and pH changes and especially controlling the color and weight loss of mushrooms. Also, the treatment had quite beneficial effect in preventing the decrease of firmness of the mushrooms during storage. Therefore, the application of US for 10 min prior to MAP is a promising tool to extend the shelf-life of white button mushrooms during the cold storage. The results of this research provided useful information for synergistic effects of applying US coupled with MAP for prolongation of shelf-life and reduction of the quality deterioration of mushroom. However, our research showed that US+MAP can be preferred over MAP or US alone; further investigation is needed to study some microbiological and sensory tests, absorbance of water by mushrooms within the ultrasonication process, and the effect of indirect US by placing mushrooms in polymer bags prior to immersing in water.

Data Availability

The data used to support the findings of this study are available from the corresponding authors (Mahmoud Omid and Mahmoud Soltani Firouz) upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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