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Effect of different packaging films and pre washing on the shelf life of button mushrooms

Narges Hassani and Orang Khademi*

Department of Horticulture, Faculty of Agriculture, Shahed University, Tehran, Iran

ABSTRACT

Button mushroom quickly loses its quality after harvest. Weight loss, enzymatic browning and microbial contamination are the main factors limiting the shelf life of edible mushroom. In this study, the effects of pre-washing with 1% H_2O_2, pre-washing with distilled water, and no washing, as well as the effects of packaging by using nanosilica-polyethylene, conventional polyethylene and cellophane films on the maintenance of postharvest quality of button mushrooms were investigated. After being washed, the mushrooms were packaged by using these films, stored at 4 °C, and then studied at 7and 14 day after storage. The results showed that mushrooms packaged in nanosilica-polyethylene film compared to the two other packaging films had lower values of browning (23 and 16% less than cellophane and conventional polyethylene, respectively) and weight loss (66% less than cellophane and 48% less than conventional polyethylene), as well as higher value of L* (6.5 and 5% more than cellophane and conventional polyethylene, respectively) and phenol content (10 and 7.5% more than cellophane and conventional polyethylene, respectively). In addition, pre-washing with H_2O_2 before packaging was more effective treatment in preserving the quality and color of the mushroom compared to washing with distilled water and no washing treatments, due to the reduction of microbial load. Therefore, the combination of washing with H_2O_2 and packaging in nanosilica-polyethylene film can be effective treatment in increasing the shelllife of the mushrooms.

Keywords: *Agaricus bisporus*, bacterium, nano packaging, enzymatic browning, weight loss.

INTRODUCTION

Button mushroom (*Agaricus bisporus*) is the most common edible mushroom in the world due to having good taste and high nutritional value (Khan *et al.*, 8). The main limiting factor in the industrial development of mushroom is its short shelf life, which is due to the lack of thick cuticle protecting the product against physical and microbial injuries, water loss and high rate of metabolism (Donglu *et al.*, 4; Lagnika *et al.*, 11). For this reason, mushroom is more susceptible to spoilage, enzymatic browning and eventually aging. Extening postharvest life by preventing the enzymatic browning and controlling bacterial agents can be helpful for mushroom industry (Lagnika *et al.*, 11). To this end, various methods have been reported to be applied to maintain the storage quality of mushroom, such as; the use of packaging with nano films (Donglu *et al.*, 4), sodium metabisulfite (Brennan *et al.*, 3), organic acids (Brennan *et al.*, 3), ultrasound waves and high pressure of argon (Lagnika *et al.*, 11). All of the mentioned methods, in addition to increasing the shelf life of the mushroom, have some disanvantages, such as nutritional value diminution, texture changes, discoloration, contamination by pathogen microorganisms, elimination of flavor and aroma, high energy consumption, and not being usable at industrial scale. Therefore, searching for alternative methods is of high importance (Rico *et al.*, 14).

Hydrogen peroxide (H_2O_2) is known as a chemical compound used against the microorganisms existed on the outer surfaces of fruits and vegetables. By producing reactive oxygen species such as hydroxyl radicals, H_2O_2 shows a good antimicrobial effect. The remains of this compound are degradedin to water and oxygen by catalase enzyme, and its use in the disinfection of food materials is recognized to be safe (Kniel *et al.*, 9). In edible mushroom, washing with H_2O_2 before packaging has been reported to be an effective pre-treatments in reducing microbial load, maintaining proper color and increasing the shelflife (Andrawis and Kahn, 1; Brennan *et al.*, 3). In addition to the whole mushroom, the positive effect of H_2O_2 treatment on the reduction of bacterial populations and consequently the prevention of browning in fresh cut sliced mushroom was also observed (Bernnan *et al.*, 3).

Nanocomposite materials that are developed for use in food packaging industry include a polymer with an additive nano particle that changes the physical properties of the polymer (Douglas *et al.*, 5). The nano particles used in advanced nanopolymers (nanocomposites) cause an increase in flexibility,

*Corresponding author's Email: o.khademi@shahed.ac.ir

increase in the prevention of gas entry and exit, and increase in moisture and thermal stability of the polymers. These nanoparticles make the plastic cover light, firm and heat-resistant, and are an obstacle to the movement of gases. Douglas *et al.* (5) reported that the silica nanoparticle-rich plastic bag is lighter and stronger than other similar products, and exhibits high resistance to heat. These plastics can prevent the drying of materials and protect them against humidity and oxygen. The positive effect of nano packaging on the increase of storage life and improvement of jujube quality was reported by Li *et al.* (12). Therefore, films containing nanoparticles, due to changing physical properties, can be very useful in increasing the shelf life of edible mushroom. Donglu *et al.* (4) showed that use of nanocomposite packages containing nano-Ag and nano-TiO_2 reduced the aging development in edible mushroom by decreasing respiration rate.

In this study, the effect of polyethylene film containing silica nano particles on the preservation of postharvest quality of edible mushroom in comparison with polyethylene film without nanoparticles and cellophane (as a common film for packaging of edible mushroom) was investigated. In addition, the effect of washing with H_2O_2 before packaging compared with no washing and washing with water was studied.

MATERIALS AND METHODS

Button mushroom samples with closed cap and uniform diameter of 40 ml were provided from the commercially mushroom production unit in Mallard of Karaj and transferred quickly to the postharvest laboratory. The TSS value of mushrooms at harvest time was 5.58% and the firmness was 1.95 kg/cm^2.

Uniform and without defect mushrooms were selected and divided into three groups, each containing 126 mushrooms. The first group was treated with distilled water and the second group was treated with 1% H_2O_2 solution for 10 min, and they were then dried in the laboratory for one hour. The third group was not washed as a control. Then the mushrooms of each group were divided into three subgroups, each containing 42 mushrooms. 42 mushrooms of each subgroup were randomly distributed in six polyethylene containers special for mushroom, each containing 7 mushrooms. The first, second and third subgroups were packaged with cellophane film, nanosilica-polyethylene (NS-PE) and common polyethylene (PE), respectively. NS-PE film, which was prepared using a silicon nano-emulsion solution, was provided from Aitak Nano BisPar Corporation.

Packaged mushrooms were stored at 4 °C and relative humidity ≥ 80%, and three packages of each packaging film, as three replicates, were removed from the storage and evaluated at 7- and 14-day periods. The rate of browning, L* value, total phenol content, weight loss percentage, bacterial colony formation unit (CFU), firmness, and soluble solids content were measured in the present experiment. The severity of mushrooms browning was assessed visually on the surface of mushroom at five levels ranging from 0 (no browning) to 5 (maximum browning), and the browning index was calculated according to formula of:

Browning Index = $\sum$ [(Browning level) × (number of mushroom at each browning level)] / (5 × total number of mushroom in the packaging).

The color parameter of L* was determined using a colorimeter (TEST-300, Taiwan) at three points of each mushroom. Total phenol content of mushrooms was measured by Follin-Ciocaltaeu method. To do so, 1g of mushroom tissue was homogenized with 10 ml of 80% methanol using a mortar for 5 min. The obtained homogenate was centrifuged at 10000 rpm at 4°C for 10 min, and then 7 ml distilled water for each 1 ml of the extract was added in a test tube, followed by adding 1 ml of Folin-Denis reagent for color development. After 5 min, 1 ml saturated sodium carbonate solution was added and the absorbance was measured at 760 nm within 1 hours by using spectrophotometer. The amount of phenol content was estimated against standard tannic acid, expressed as mg of tannic acid equivalent per 100g of the fresh weight sample.

Weight loss (%) during the storage was calculated by weighing the mushrooms before and after the storage by using the formula of [(weight of fruits before the storage – weight of fruits after the storage)/weight of fruits before storage] ×100.

In order to determine the CFU, 20 g of mushroom was homogenized in 200 ml sterilized distilled water by a blender device (Midea BL-F016ABS model) at 600 w power and high speed for 2 min. Consecutive concentrations (10^{-1} - 10^{-5}) were prepared by mixing one ml of the extract with 9 ml of sterile distilled water. Then, one drop of each concentration was distributed homogeneously in petri dishes containing nutrient agar medium (2.8%). The petridishes were kept in incubator at 27°C for 27 hours, and finally the number of colonies per petri dishes was counted and reported as $\log_{10}$CFU.

Mushroom firmness was determined using a hand penetrometer (model VBR80) equipped with a 4-mm tip at 2 equatorial points, and the results were expressed as Newton (N). Total Soluble Solid (TSS) of the mushroom juice was determined by a hand refractometer (RF40).

A randomized design with three replicates per treatment was used in this experiment. To determine

the effects of pre-washing treatment, packaging film and storage time on each dependent variable, a three-way analysis of variance was carried out using SAS software (version 9.2). Mean values of the treatments were compared by using Least Significant Difference test (*LSD*, *P*=0.05).

RESULTS AND DISCUSSION

The ANOVA results related to treatments of pre washing, packaging film, time of storage factors and their interaction on button mushrooms were shown in Table 1. The mushrooms packaged with NS-PE film had significantly lower rate of browning (0.47) compared to the mushrooms packaged with PE and cellophane films (0.56 and 0.62, respectively), while there was no significant difference in the intensity of browning between the mushrooms packaged with PE and cellophane (Fig. 1). On the other hand, at both 7- and 14-day storage times, the lowest rate of browning (0.37) was observed in the samples washed with H_2O_2 before packaging. During the 7-day storage time, samples of water pre washing had higher rate of browning than non-washed samples, while at 14-day storage time, no significant difference was observed between the two treatments (Fig. 2).

L* value of mushroom samples showed significant decrease at 14-day storage time compared to the 7-day. Moreover, samples washed by H_2O_2 had higher value of L* (78.23) than those washed with water as well as non-washed samples (with L* values of 71.2 and 69.87, respectively), while there was no significant difference between samples of water washing treatment and non-washed samples. The highest value of L* (75.81) was observed in NS-PE packaging film. There was also no significant difference between PE packaging film and cellophane (with values of 72.48 and 71.02, respectively) in terms of L* (Fig. 3).

One of the main factors reducing the postharvest quality of button mushroom is rapid browning and discoloration. Browning of button mushroom occurs as a result of tyrosinase activity, an enzyme from polyphenol oxidase family, and the attack of *Pesudomonas tolasii* bacterium (Brennan *et al.* 3; Munsch *et al.*, 13). As a result of increase in time and the aging of mushroom, as well as the

Table 1. Statistical analysis of parameters studied: time of storage (T), washing treatment (WT) and packaging film type (PFT) and their interaction for mushroom through analysis of variance.

	T	WT	PFT	T×WT	T×PFT	WT×PFT	T×WT×PFT
Browning index	**	**	**	**	ns	ns	ns
L*	**	**	*	ns	ns	ns	ns
Weight loss	*	**	**	ns	ns	**	**
Log CFU	**	**	ns	**	**	*	**
Phenol	**	**	*	ns	ns	ns	ns
Firmness	*	**	ns	**	ns	ns	ns
TSS	*	**	ns	ns	ns	ns	ns

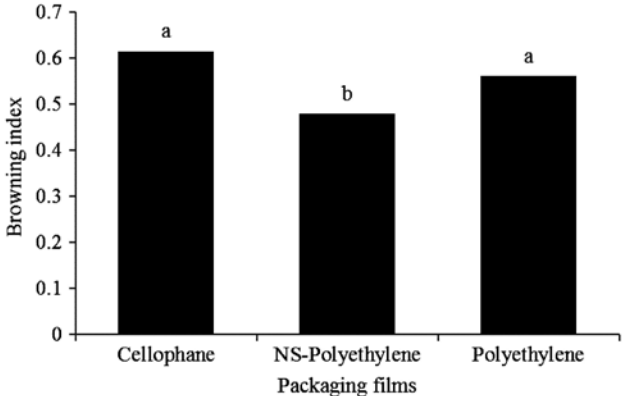


Fig. 1. Effect of different packaging films on brwoning index of button mushroom. Means with the same letter are not significantly different at 5% level of the *LSD* test.

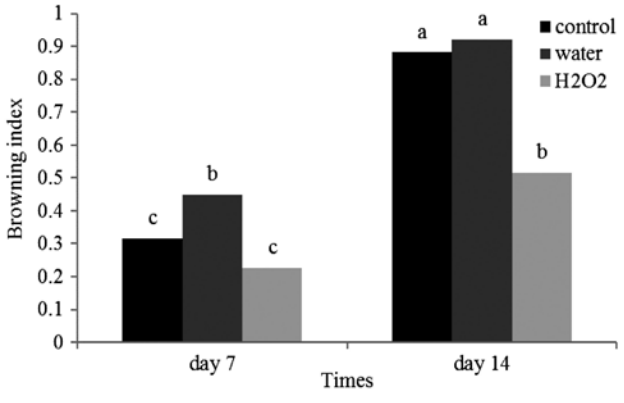


Fig. 2. Effect of prewashing treatemnts on brwoning index of button mushroom during storage at 4°C. Means with the same letter are not significantly different at 5% level of the *LSD* test.

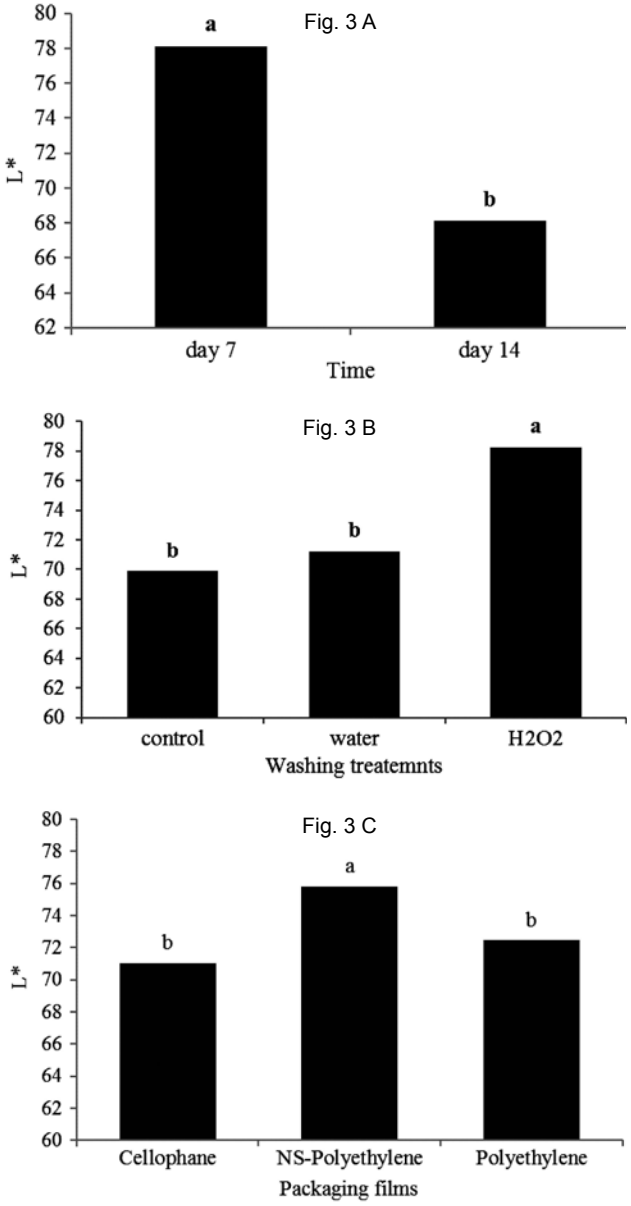


Fig. 3. Effect of storage times (3 A), prewashing treatments (3 B) and different packaging films (3 C) on L* value of button mushroom. Means with the same letter are not significantly different at 5% level of the *LSD* test.

intensification of the enzyme activity and the attack of microbial agents, color of the mushroom gradually gets darker and more brownish (Brennan *et al.,* 3). In this study, the results of browning and L* (whiteness) indices showed that H_2O_2 washing and NS-PE packaging film treatment was effective in maintaining the optimum color of button mushroom at 14-day storage time. H_2O_2 exhibits high antimicrobial effect by producing reactive oxygen species, such as hydroxyl radicals (Kniel *et al.*, 9). Besides having anti-bacterial properties, H_2O_2 also prevents enzymatic browning by inhibiting the tyrosinase enzyme activity, thereby increasing the shelf life of button mushroom (Andrawis and Kahn, 1). The effect of this treatment on the preservation of white color of edible mushroom has also been shown in other studies (Brennan *et al.,* 3; Lagnika *et al.*, 11).

The NS-PE package can be considered as a modified atmosphere packaging, which creates a favorable gas mixture around the product due to having relative impermeability to atmospheric gases and respiration of the product (Farber *et al.*, 6). According to the experiments, it was found that the NS-PE film permeability rates to water vapor, carbon dioxide and oxygen were 0.3g/m2.24h, 20 ml/m2.24h, and 37 ml/m2.24h, respectively, and the amount of distilled water movement in this film was also 0.4365 mg/dm^2, which was 14.29% lower than conventional polyethylene bags. These properties of NS-PE create a MAP that greatly reduces respiration rate and ethylene production (Li *et al.*, 12). When the amount of oxygen reduces, respiration rate of the mushroom and aging process are controlled, as well as the enzymatic browning is reduced since the enzymatic browning process is dependent on the concentration of oxygen (Rico *et al.*, 14).

At 7-day storage time, no significant difference was found between different treatments in terms of weight loss percentage, though the lowest rate of weight loss was observed in mushrooms packaged with NS-PE film which were washed with H_2O_2 and water (with less than 1% weight loss). Weight loss percentage of all the samples increased significantlywhen duration of the experiment increased. At 14-day storage time, the samples packaged with NS-PE film had lower weight loss percentage (less than 2%) compared to the samples packaged with PE (more than 3%) and cellophane (more than 4%). At this study time, samples packed with cellophane film had the highest weight loss precentage, and no significant difference was found in the samples of this packaging among the three washing treatments. However, in PE film, the mushrooms washed with H_2O_2 and water had lower weight loss percentage than non-washed samples (Fig. 4). In general, NS-PE packaging had the greatest effect on the reduction of weight loss precentage of mushrooms in the present expriment.

One of the most important attributes used to determine the quality and shelf life of fruits and vegetables is the amount of weight loss during the storage, occurring due to water loss and respiration process. Extra weight loss causes the crop to spoil and shrink in terms of appearance (Jiang *et al.*, 7). The results of this study showed that packaging with NS-PE film had higher potential for better

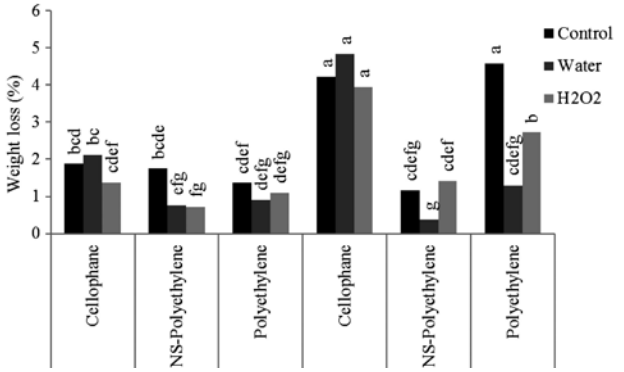


Fig. 4. Weight loss values of button mushroom prewashed with water and H_2O_2 and then packaged in different types of pakaging films during 4°C storage. Means with the same letter are not significantly different at 5% level of the *LSD* test.

maintenance of the initial weight of edible mushroom compared to polyethylene and cellophane films. Due to better control of the entry and exit of gases and thus creation of MAP conditions, the NS-PE film led to the reduction in the rate of respiration and exit of water vapor from the surroundings of mushrooms, thus reducing the mushrooms weight loss. In general, button mushroom has high respiration rate, and the film used for their packaging should maintain the oxygen around them at optimum level to prevent the creation of anaerobic respiration conditions (Koushki *et al.*, 10). Cellophane and polyethylene films have greater penetration and permeability to water vapor and oxygen, and faster exit of water vapor and high respiration rate accelerate weight loss in these films.

On the other hand, aging process in mushrooms is accompanied by continuous increase in oxidation, which leads to the accumulation of ROS and oxidative damage (Rogiers *et al.*, 15). The accumulation of ROS in the cell causes damage to the structure and function of membrane, and membrane damage, in turn, causes postharvest problems, such as weight loss, tissue softening, pathogens attack, and mushroom browning. Packaging with Nano-silica stimulates the activity of antioxidant enzymes by releasing Si, thus preventing the accumulation of ROS and damage to the membrane, and increasing the preservation of mushroom quality (Donglu *et al.,* 4).

The results showed that at 7-day storage time and in all the three types of NS-PE, PE and cellophane films, the H_2O_2-washed samples had lower CFU (3.92 log CFU/gr FW) than those water-washed and non-washed samples (5.2 and 5.1 log CFU/gr FW respectively). However, there was no significant difference between water washing and no washing in the three types of packages in terms of the CFU. The lowest number of bacteria was found in the treatment of cellophane film and H_2O_2 washing (3.2 log CFU/gr FW), and the number of bacteria in NS-PE packaging film and H_2O_2 washing (4.1 log CFU/gr FW) was lower than PE packing film and H_2O_2 washing (4.5 log CFU/gr FW). At 14-day storage time, there were no significant differences among the treatments in terms of CFU. Generally, H_2O_2 treatment had the greatest effect on the reduction of bacterial population in button mushroom in the current experiment (Fig. 5). Mushrooms after harvest show high susceptibility to spoilage (Wang *et al.*, 16). H_2O_2 exhibits high antimicrobial effect by producing reactive oxygen species, such as hydroxyl radicals (Kniel *et al.*, 9).

The results of total phenol content were similar to those of L* value, so that total phenol content during the 7-day storage time was significantly higher than that observed during the 14-day. Furthermore, H_2O_2-washed samples (with value of 83.87 mg/kg FW) had higher total phenol content compared to the samples washed with water and non-washed samples (with values of 78.22 and 78.89 mg/kg FW, respectively). There was no significant difference in terms of total phenol content between water-washed samples and non-washed samples. Total phenol content in the samples packaged with NS-PE film (with value of 84.8 mg/kg FW) was significantly higher than total phenol content in samples packaged with cellophane and PE films (with values of 77.1 and 79 mg/kg FW, respectively). No significant difference was observed between the samples of two cellophane and PE films regarding total phenol content (Fig. 6). Therefore, NS-PE packaging and H_2O_2 washing resulted in better preservation of total phenol content than other treatments.

Polyphenols have a high nutritional value for consumers, and their preservation in mushrooms is of high importance. Polyphenols are the substrate of

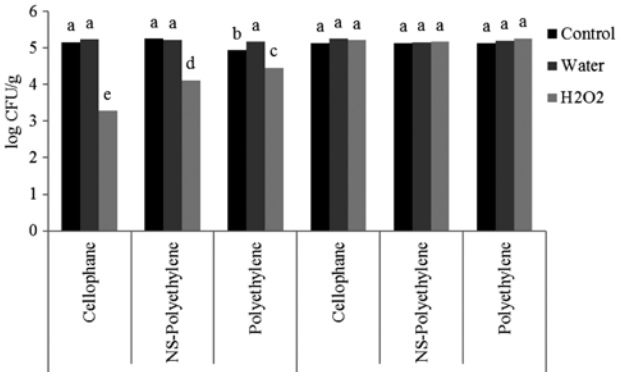
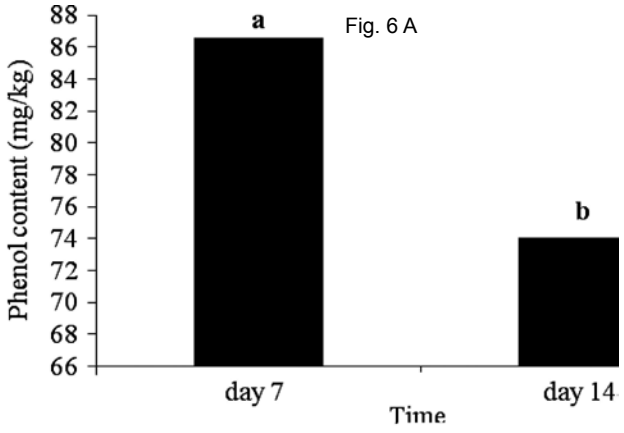
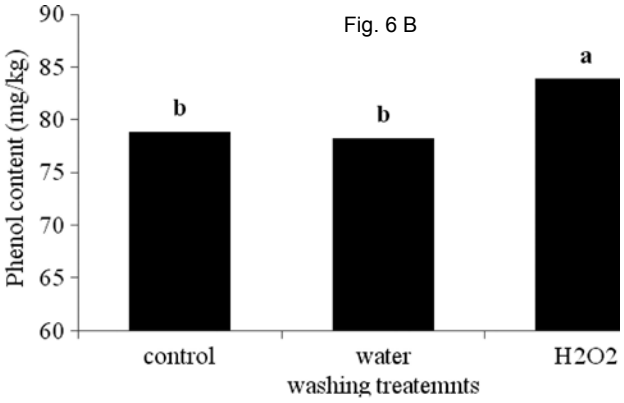


Fig. 5. CFU values of button mushroom prewashed by water and H_2O_2 and then packaged in different types of packaging films during 4°C storage. Means with the same letter are not significantly different at 5% level of the *LSD* test.





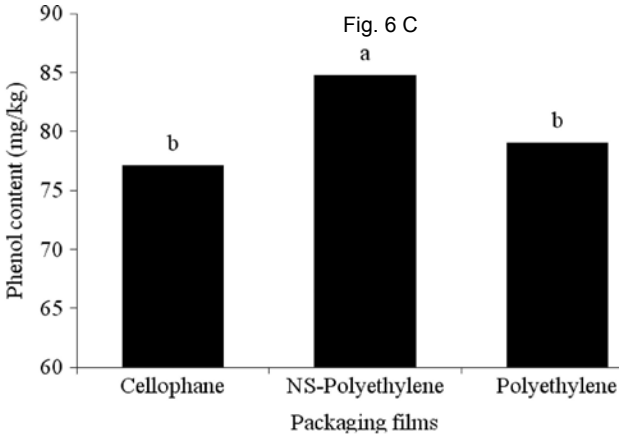


Fig. 6. Effect of storage times (6 A), prewashing treatments (6 B) and different packaging films (6 C) on total phenol content of button mushroom. Means with the same letter are not significantly different at 5% level of the *LSD* test.

polyphenol oxidase enzyme and hence, the process of enzymatic browning in mushroom is associated with a reduction in phenolic compounds (Lagnika *et al.*, 11). In the present experiment, the H_2O_2-washed samples packaged with NS-PE film had a higher phenol content, which was better preserved due to lower rate of browning of the phenolic compounds, while in other mushrooms with higher browning rate, the amount of phenol were lower.

Firmness of mushrooms was not affected by the type of film, but the treatment of washing before packaging had a significant effect on it. At the time of 7-day study, the highest rate of firmness was observed in the samples washed with H_2O_2 (14.5 N), while no significant difference was found between washed and non-washed samples regarding firmness. When the time of evaluation increased from 7 to 14 days, firmness of the samples decreased, though at 14-day examination, no significant difference was observed between any of the washing treatments in terms of this parameter (Fig. 7).

During the postharvest period, mushroom softening occurred due to the degradation of cell wall polysaccharide by microbial enzymes (Khan *et al.*, 8). In this study H_2O_2 preserved fruit firmness better than other pre washing treatments due to antimicrobial effects. The TSS in mushrooms decreased significantly when the time of evaluation increased. The TSS in non-washed mushrooms (4.2%) was significantly higher than that of mushrooms washed with water (3.66%) or H_2O_2 (3.82%), while no significant difference was observed between mushrooms washed with water or H_2O_2 in terms of TSS (Fig. 8).

The amount of TSS decreased over the time of the experiment, due to being consumed during the respiration process (Ayala-Zavala *et al.*, 2), but, on the other hand, TSS in non-washed samples, compared to those washed with water and H_2O_2, was higher. The study showed that, in general, the weight loss was higher in non-washed samples than in washed ones; therefore the concentration of cell sap in these samples was concentrated, thus increasing the amount of TSS (Khan *et al.*, 8).

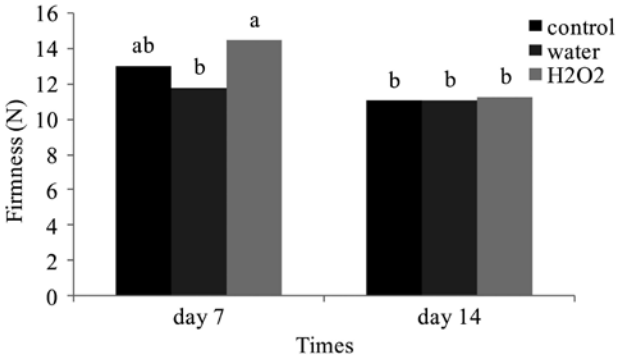


Fig. 7. Effect of prewashing treatments on the firmness of button mushroom during storage at 4°C. Means with the same letter are not significantly different at 5% level of the *LSD* test.

Fig. 8 A

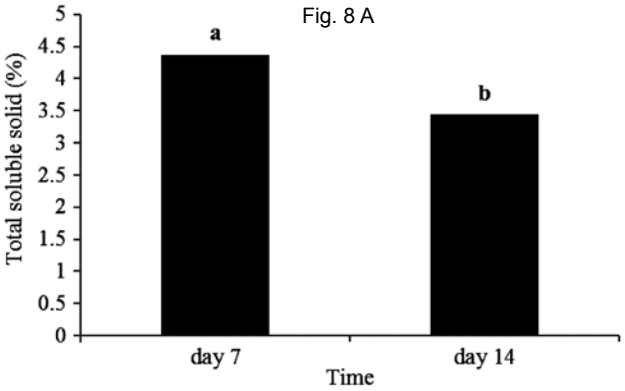


Fig. 8 B

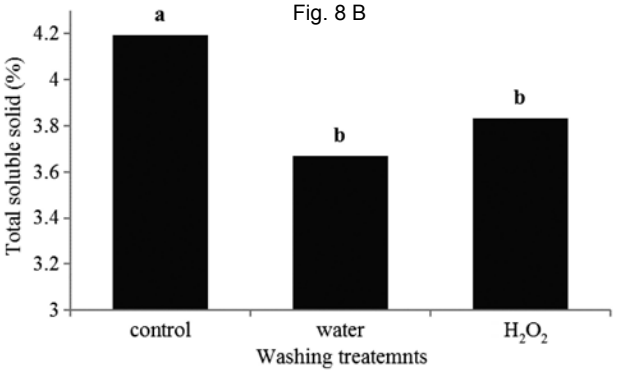


Fig. 8. Effect of storage times (8 A) and prewashing treatments (8 B) on total souble solids content of button mushroom. Means with the same letter are not significantly different at 5% level of the *LSD* test.

CONCLUSION

In conclusion, based on the decrease in browning rate, higher value of L*, preservation of total phenol content, and control of weight loss percentage, use of Nano silica polyethylene- film was useful in the packaging of edible mushroom. However, if mushrooms are washed with 1% H_2O_2 before packaging, the quality of mushrooms will be better preserved because of the reduced microbial load. Therefore, the combination of washing with H_2O_2before packaging and packaging with Nano silica polyethylene film is very effective in maintaining postharvest quality of button mushroom.

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