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ORIGINAL PAPER

Extension of button mushroom storability by ultrasound treatment in combination with calcium lactate

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Abstract

Browning, weight loss and microbial infection are the main deteriorations which occur during the mushroom postharvest. In current research the impact of calcium lactate (CL) and ultrasound (US) treatments on the increase of mushroom shelf-life were investigated. Mushrooms were washed in 0, 0.25 and 0.5% CL in dipping and US methods for 3 and 6 min, then, were packed in poly-ethylene containers using cellophane film. Treated mushrooms, then, were stored at 4ºC with 80% RH and evaluated at 7- and 14-day storage times. The results indicated that a combination of CL and US could effectively control the browning of mushrooms. L* and total phenol were higher in the combination of CL and US than the other treatments, but B* was lower. CL led to the control of weight loss and reduced the bacterial population and firmness loss during the experiment; however, this effect was improved when applied as US method.

Keywords Bacterial infection · Browning · Color parameters · Edible mushroom · Shelf life

Introduction

Button mushroom (*Agaricus bisporus*) is a valuable edible mushrooms worldwide that is rich source of a variety of nutrients, and its regular consumption is useful in preventing from disease like cancer [17, 22], however, because of absence of cuticle layer on the periderm, harvested button mushrooms are very susceptible to decay, and could be only stored for 3–4 days at ambient temperature after harvest, that is due to microbial infections, high water loss, high respiration rate and susceptibility to enzymatic browning [4, 24, 40]. Thus, button mushroom loses its quality quickly after harvest and, thus, it is highly essential to find alternative methods for increasing the shelf life of button mushroom [10, 21, 28]. Various technologies such as washing with antimicrobial and/or anti-browning materials, edible coatings, irradiation, modified atmosphere packaging, chemical treatments and cold storage have been investigated to enhance the shelf life of mushrooms [10, 13, 16, 17, 27]. Although effective to some degree, these methods reduce the nutritional value, safety, texture, flavor and taste of mushrooms [13, 21]. Therefore, finding alternative methods to enhance the shelf life of button mushroom without adverse effects on quality is of great significance.

In recent years, ultrasound (US) waves as a safe, effective, nontoxic and environmentally friendly physical technology has attracted attentions in terms of food products preservation [12, 37, 43]. US is sound waves with a frequency above 16 kHz based on acoustic cavitation [15]. US treatment may affect the postharvest life of fresh products because acoustic cavitation can deteriorate microbes existing on surfaces and pores of fruits and vegetables [20]. US treatment can also increase the postharvest life of fresh products by inactivation of enzymes involved in the postharvest deterioration [35]. Moreover, US treatment, as a physical elicitor, can induce the biosynthesis and accumulation of some antioxidant and antimicrobial compounds inside the produce, thereby slowing down quality deterioration [35, 45]. Recently, researchers interested in the application of US in washing the fruits and vegetables. US waves could enhance storability of strawberries [1, 6] and litchi [8] during storage and fresh cut apple [15] and mushroom [25, 35] during shelf

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life. However, it has been shown that antimicrobial efficacy of US treatment alone is limited and it should be combined with other effective treatments in washing fresh produces to be more effective [39].

Calcium is the most important mineral element in retaining the quality of fruits and vegetables during the postharvest period [33] and plays a vital role in preserving the firmness and induced resistance against pathogens by deactivating polygalactronase enzyme, postponing the cell wall degradation, maintaining membrane selective permeability and membrane integrity and increasing cell wall strength [5, 19, 29]. Usually, calcium chloride were used as calcium source in the studies, since it has been proved as an effective one in preserving firmness and enhancing resistance against diseases in the treated products [21]. However, calcium chloride induced bitter taste, while calcium lactate (CL) did not have that negative effect [34]. In former study, the impact of different calcium sources in relation to the increased shelf life of button mushroom was compared and the results showed that CL was better than other calcium sources in preserving mushroom quality during the shelf life, that is probably due to its higher solubility and diffusion capacity to the texture [21]. Furthermore, US has the potential to increase the calcium absorption inside the plant tissues, thereby improving the calcium treatments efficacy [45]. On the other hand, when combined with other preservation methods, US is more efficient [35]. Therefore, in this experiment, the effect of CL or US treatments solely or in combination form was investigated on button mushroom shelf life. Furthermore, the physiological attributes and microbial load of samples were studied.

Materials and methods

Sample preparation and treatments

Button mushrooms in closed cap stage and 40 mm diameter were obtained from the Corporation of Green Melina Farm, located near to Nazarabad, Alborz province, Iran. The mushrooms were transferred within 2 h to the postharvest laboratory of Shahed University under refrigerated conditions (≈4 °C and 80% RH) by refrigerator vehicle. The L* and b*values of mushrooms at harvest time were 95 and 17.5 respectively, and the firmness value was 2.56 kg cm^{-2}.

Uniform and defect free mushrooms were selected and divided into 12 groups, each containing 60 mushrooms and subjected to the following treatments:

- Dipping with distilled water (control) for 3 or 6 min.
- Dipping with 0.25% CL for 3 or 6 min.
- Dipping with 0. 5% CL for 3 or 6 min.
- Ultrasound in distilled water (control) for 3 or 6 min.
- Ultrasound in 0.25% CL for 3 or 6 min.
- Ultrasound in 0. 5% CL for 3 or 6 min.

Ultrasound treatment was carried out at constant power of 400 W using an ultrasonic cleaning bath system (Falc Instruments, Treviglio, Italy) with a center frequency of 34.722 kHz (nominal frequency 40 kHz) and band width of 320 Hz as continuous mode of sonication. The adopted concentrations of CL solution were based on our previous studies on button mushroom [21]. All CL solutions in both dipping method (DM) and US method (UM) were carried out in 10 L volume at 25 °C.

After the treatments, mushrooms were dried at room temperature (23 ± 2 °C) and 60% RH for 1 h; then, the mushrooms of each group were randomly distributed in six polyethylene containers, each containing 10 mushrooms. The containers were packed with cellophane film and were stored at 4 °C and ≥ 80% RH for 14 days. At 7- and 14-day storage times, three packages of each treatment as 3 replica were removed from the storage and evaluated. The severity of browning, color parameters, weight loss percent, total phenol content, firmness, bacterial colony formation unit (CFU) and calcium content were measured in the present experiment.

Browning index, L* and b*

The severity of mushrooms browning was assessed visually on the surface of mushroom at four levels scaled from 0 (no browning) to 4 (the highest browning severity), and the browning index (BI) was calculated according to the following equation [13]:

BI = ∑ [(Browning scale) × (number of sample at each browning scale)] / (4 × total number of sample in the lot).

The color parameter of L* and b* was determined at three upper surface of each mushroom using a colorimeter (TEST-300, Taiwan). Decrease in L* value represents darkening, and increase in b* represents yellowing in the white mushroom [4].

Weight loss and firmness

Weight loss percent was calculated by weighing the packages before and after the storage and using the following equation:

[(weight of samples before the storage – weight of samples after the storage)/weight of samples before storage] ×100.

The firmness of mushrooms was determined using a hand penetrometer (model VBR80, Italy) with 4-mm tip at 3 points of cap, and the results were expressed as kg/cm^2.

Table 1 Statistical analysis of parameters studied: treatment methods, CL solution and storage time (ST) and their interaction for button mushrooms through analysis of variance

	Method	CL	ST	Method×CL	Method×ST	CL×ST	Method×CL×ST
Browning index	**	**	**	**	ns	**	**
L* value	*	**	**	ns	ns	ns	*
b* value	**	**	**	**	ns	ns	**
Weight loss	ns	ns	**	**	**	ns	**
Bacterial counts	**	**	*	**	**	**	**
Firmness	**	**	**	**	*	**	**
Phenol content	ns	**	**	**	ns	ns	*
Ca content	*	**	**	**	ns	ns	ns

*, ** and ns represent significance at the 0.05 and 0.01 levels and non-significance respectively

Bacterial population

To determine the bacterial population, 20 g of mushrooms of each lot was taken and homogenized with 200 mL of tryptone phosphate buffer using a blender device (Midea BL-F016ABS model) for 2 min at high speed. Then, serial dilutions were prepared using saline peptone solution for 5 times (10^{-1} – 10^{-5}). Then, 100 µL of each dilution was spread on the 8% nutrient agar medium (produced by QUELAB, Canada) inside a Petri-dish. The Petri-dishes were incubated at 30 °C for 48 h and then, number of the colonies was counted and expressed as $\log_{10}$CFU per g fresh weight [4, 10, 13].

Total phenol content

A slice from each mushrooms of each package was taken and grounded in the presences of liquid nitrogen and stored at -70ºC. Total phenol content was measured by Folin-Ciocalteu reagent method as described by Hassani and Khademi [13]. Briefly, 1 g of frozen mushroom tissue was homogenized with 10 mL of 80% methanol. Then, the homogenate was centrifuged at 9800 g for 10 min at 4 °C using a centrifuge device. 1 mL of supernatant added to 7 mL of distilled water and 1 mL of Follin-Ciocaltaeu reagent were added. After 5 min, 1 mL of 20% sodium carbonate solution was added and the absorbance of resultant was measured at 760 nm within 1 h using spectrophotometer (model: Lambda, 650, PerkinElmer, USA). The amount of phenol content was estimated against standard of tannic acid and expressed as mg of tannic acid equivalent per kg of the fresh weight sample.

Calcium content

The samples oven dried at 70 ºC and was burned in a muffle furnace to obtain the ash. The ash was digested in 4 mL HCl: distilled water (1:1 v/v) and then filtered through 'Whatman' paper. The calcium content of filtrate was determined using an atomic absorption spectrophotometer (AAS, Shimadzu Instruments, Inc., SpectrAA-220, Japan) and the content of calcium was expressed as dry weight% [18].

Statistical analysis

A randomized design with three replicates per treatment was used in this experiment. To determine the effects of CL solutions (0, 0.5 and 1%) and treatment method (DM for 3 and 6 min, UM for 3 and 6 min), and storage time (7 and 14 day) 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 Least Significant Difference test (LSD, $P = 0.05$).

Results

The ANOVA results related to treatments methods, CL solutions and time of storage factors and their interaction on button mushrooms are shown in Table 1.

BI, L* and b*

Treatment methods by itself had no effects on browning index, because no noticeable differences were observed among the treatment methods in the control solution during the study. On the other hand, the effect of CL on the BI depended on the treatment method, so that during the first 7 days of storage, the lowest BI was observed in the samples treated by UM method for 3 min in 0.25% CL solution and the samples treated by DM for 6 min in 0.25% CL (Fig. 1). No considrable differencess were observed among the other samples in terms of BI at this time. The rate of BI in all samples increased significantly when the duration of the experiment was increased. At 14-day storage time, in DM, no significant differences were obsrved among the CL solutions and the control in BI. While in UM, the mushrooms treated in 0.25 and 0.5% CL had a significantly lower BI than the control mushrooms.

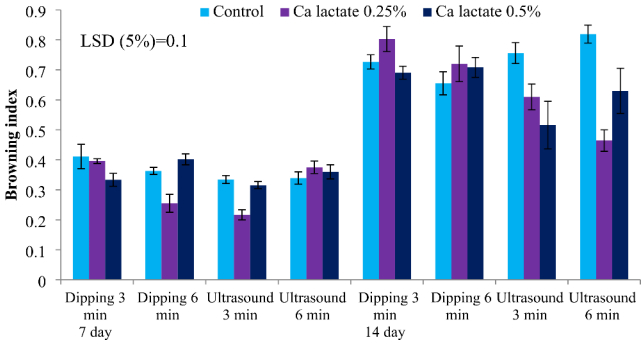

Fig. 1 Changes in browning index in button mushrooms treated by dipping or ultrasound methods in different concentrations of calcium lactate during 14 day storage at 4 °C. The data represent mean ± standard error

L* value of the mushrooms treated by UM was more than that of the mushrooms treated by DM. The effects of CL on preserving L* value of the mushrooms were more pronounced at 14-day storage time than the 7-day stotage time. At this time and in both DM and UM, the samples treated by CL solution had more L* value than the control samples. The highest L* value was observed in mushrooms treated by UM for 3 min in 0.5% CL solution or by UM for 6 min in 0.25 and 0.5% CL solutions (Fig. 2-A).

At 7-day storage time, no significant differences were observed among the CL solutions and treatment methods in terms of the b* value of the mushrooms. During the 14-day storage time, b* value increased significantly in more samples as compared to the 7-day one; however, both treatment method and CL solutions affected mushroom b* value at this time. The mushrooms treated by UM had lower b* value than those treated by DM. Regardless to the treatment method, the mushrooms washed in 0.25 and 0.5% CL solutions had lower b* value than the control mushrooms. The lowest b* value was observed in the mushrooms treated by UM in 0.5% CL solution (Fig. 2-B).

Weight loss and firmness

As shown in Fig. 3, the mushrooms treated by UM had a lower level of weight loss than those treated by DM at 7-day storage time. At this time, generally, the samples of CL solution had a lower weight loss than the control samples. Weight loss of all samples increased significantly when duration of the experiment was increased. At 14-day storage time, the lowest weight loss was observed in the mushrooms treated by UM in 0.25 and 0.5% CL solutions, while no significant difference was observed among the other samples with regard to the mushroom weight loss.

The effects of treatment methods and CL solutions on mushroom firmness during the cold storage are showed in Fig. 4. At first 7 days of storage time, among the mushrooms of the control solution, the mushrooms subjected to UM for 6 min had a higher firmness value than those subjected to DM for 3 and 6 min; however, no significant differences were observed among the UM for 3 min and DM in mushroom firmness values. The effect of CL solutions on mushrooms firmness was dependent upon the treatment method, so that in DM, the mushrooms subjected to CL solutions had a higher firmness value than the control mushrooms. However, in UM, CL solutions had no effect or had adverse effect on mushrooms firmness as compared to the control solution. The results of the 14-day storage time showed a pattern similar to that of the 7-day one.

Bacterial population

The results showed that bactrial count of more samples increased significantly when duration of the experiment was increased. At both 7- and 14- day storage times, CL solutions reduced bacterial counts significantly in mushrooms as compared to the control solution, and the effect of 0.5% CL solution was more pronounced than the effect of 0.25% CL solution in reducing bacterial counts. Mushrooms treated by UM for 6 min had lower bacterial counts than mushromms treated by other methods, so that the lowest bacterial count was recorded in the samples washed by UM for 6 min in CL sloutions (Fig. 5).

Total phenol content

Total phenol content of mushrooms showed a significant decrease at the 14 day storage time as compared to the 7-day one; however, no significant differences were observed among the methods in control solution at both study times, suggesting that the treatment methods by itself did not affect the mushrooms phenol content. Genreally, at this time, the samples treated by CL solutions had a higher phenol content than the control ones. At the 14-day storage time, in treatment by DM for 3 or 6 min, no significant differences were observed among the CL solutions in the mushrooms phenol content; in UM for 3 min, the mushrooms of 0.25% CL solution had a higher phenol content than those of the control solution, while there was no significant difference between the samples of 0.5% and the control CL solution in terms of the phenol content. In UM for 6 min, the samples of both 0.25 and 0.5% CL solutions had a higher phenol content than the control samples (Fig. 6).

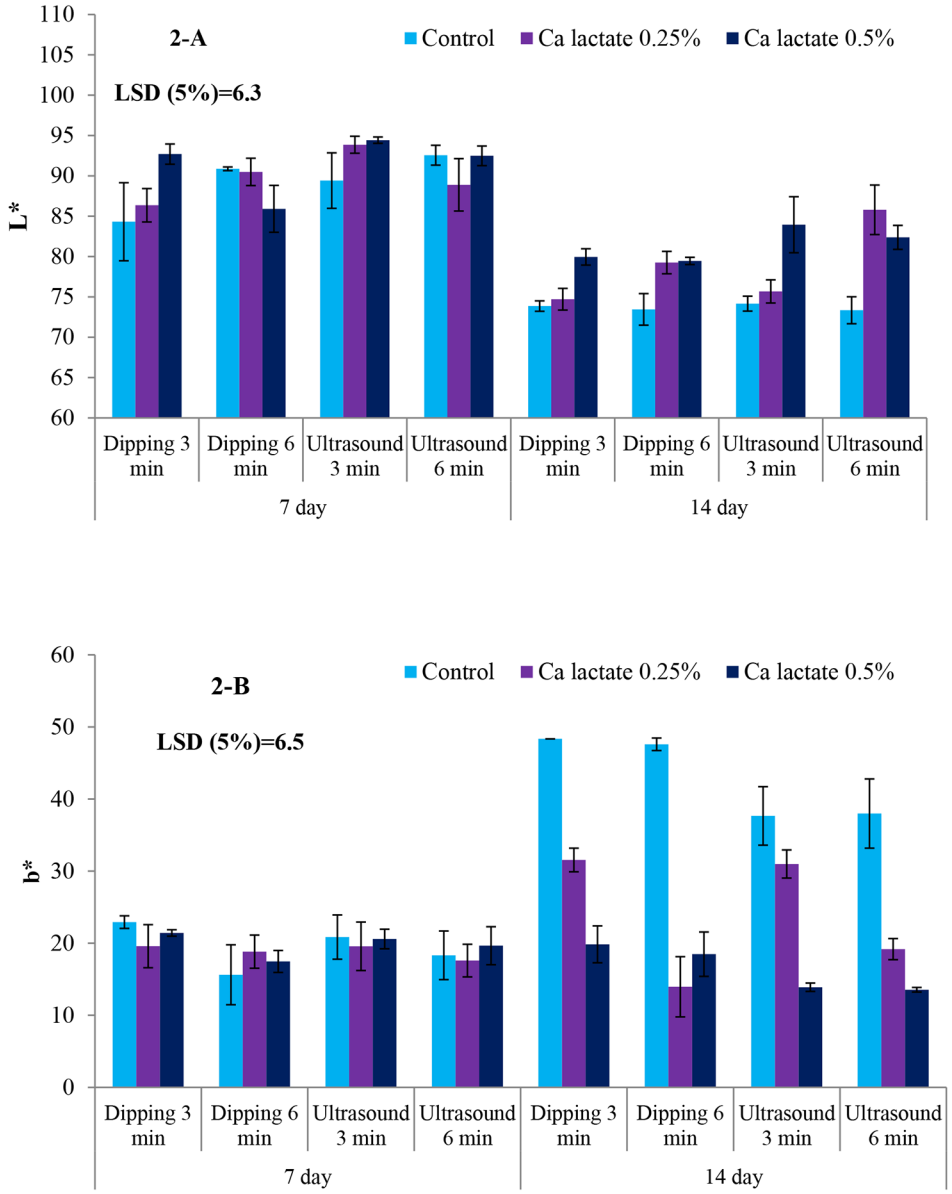

Fig. 2 Changes in L* (2-A) and b* (2-B) in button mushrooms treated by dipping or ultrasound methods in different concentrations of calcium lactate during 14 day storage at 4 °C. The data represent mean ± standard error

Calcium content

The content of calcium was affected more by CL treatment and was not significantly affected by treatment methods or experiment duration. In general, calcium content in the mushrooms treated with 0.25 and 0.5% CL solutions was significantly higher than the control mushrooms. Furthermore, no significant difference was observed between the samples of the two CL solutions in calcium content (Fig. 7).

Discussion

White colour is one of the main quality attribute in button mushrooms and a determining factor in purchase by the customer [27]. Browning is a serious problem for the mushroom postharvest, which reduces the white colour and the crop's marketability [25]. Browning in mushrooms is due to the senescence and oxidative stress; a close relationship have been shown between accumulation of reactive oxygen species (ROS) and browning in button mushroom. ROS disrupts the structure of cell membranes, which brings about interaction between substrate and enzyme that leads to

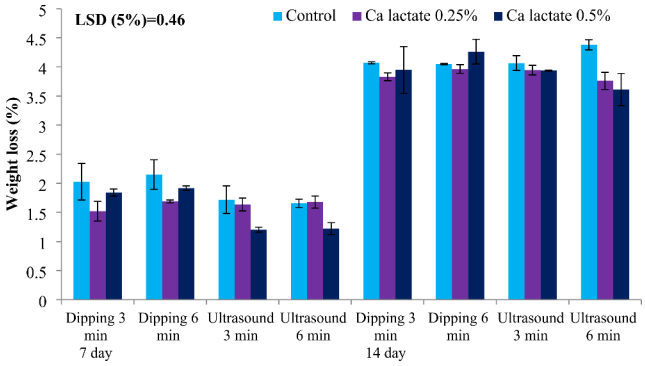

Fig. 3 Changes in weight loss of button mushrooms treated by dipping or ultrasound methods in different concentrations of calcium lactate during 14 day storage at 4 °C. The data represent mean ± standard error

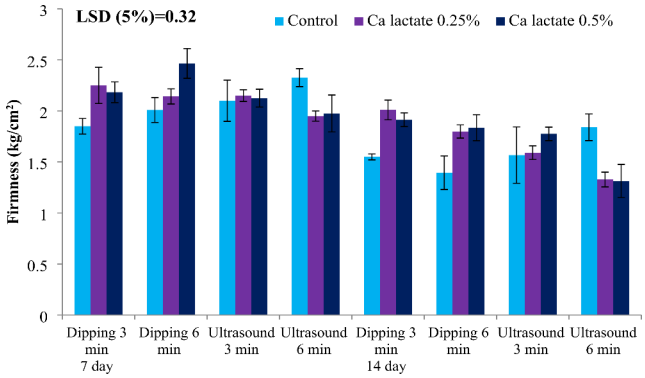

Fig. 4 Changes in firmness of button mushrooms treated by dipping or ultrasound methods in different concentrations of calcium lactate during 14 day storage at 4 °C. The data represent mean ± standard error

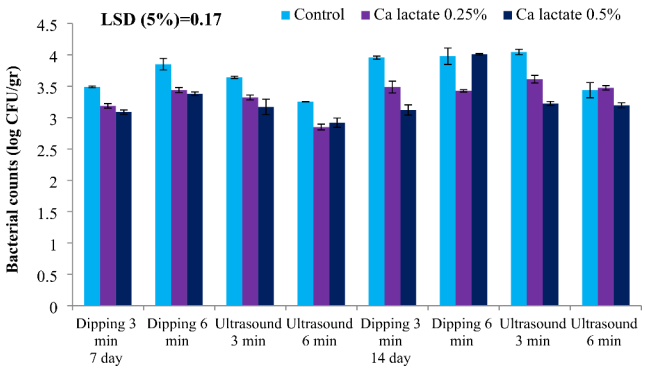

Fig. 5 Changes in bacterial counts of button mushrooms treated by dipping or ultrasound methods in different concentrations of calcium lactate during 14 day storage at 4 °C. The data represent mean ± standard error

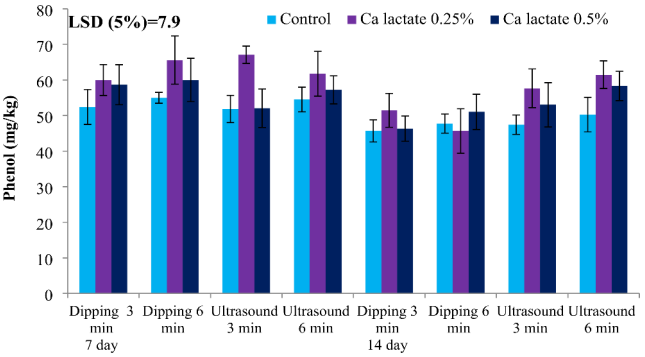

Fig. 6 Changes in total phenol content of button mushrooms treated by dipping or ultrasound methods in different concentrations of calcium lactate during 14 day storage at 4 °C. The data represent mean ± standard error

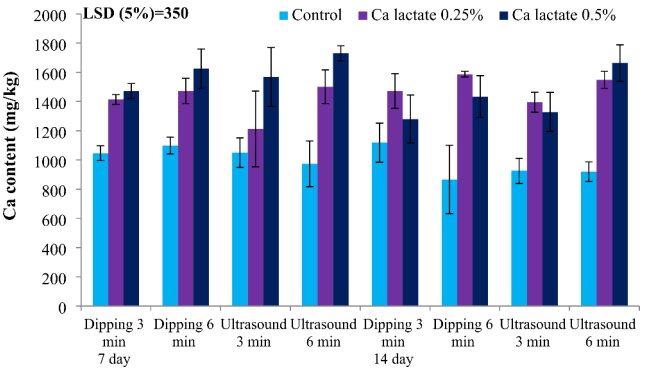

Fig. 7 Changes in Ca content of button mushrooms treated by dipping or ultrasound methods in different concentrations of calcium lactate during 14 day storage at 4 °C. The data represent mean ± standard error

browning reaction [31]. In most horticultural crops, activities of enzymes such as PPO at the presence of oxygen are responsible for browning raection [9]. Button mushroom browning occurs as a result of tyrosinase (belong to the PPO family) enzyme activity [10]. This enzyme catalyzes monophenol and di-phenol oxidation to poduce orthoquinones which finally polymerized and formed brown pigments of melanin [14, 25, 26]. In this experiment, the lowest browning severity (BI) in the desired mushrooms was in combination of UM and CL treatment. These results were also consistent with the L* value results, because only L* value of greater than 80 is acceptable for button mushrooms [10] and, accordingly, among the samples, only the mushrooms treated by combination of US and CL treatments were acceptable at the end of this study. Calcium hinders the exit of phenolic compounds through cellular organelles by increasing the membrane integrity and prevents the enzymatic browning by decreasing PPO enzyme activity [30]. It seems that the reasons for retaining white color of mushrooms in UM is the deactivation of tyrosinase enzyme, which has been reported previuosly [25]. US technique could deactivate the enzymes such as PPO by breaking the hydrogen and van der waals bonds in poly peptid chains and leades to changes in enzyme structure [3, 20].

It has been reported that UM has high potential to enhance the absorption of calcium within the tissue and accordingly, the calcium reatment impact is improved [44]. In this experiment, the useful effect of desired combined treatment on mushroom color preservation might be due to

more calcium infiltration under the imapc of UM. However, more investigating indicated that UM had no more effect on the calcium absorption than dipping method in this study. Similar result was reported by Zhi and Dong [45] in investigating cherry; they indicated that US treatment did not promote more Ca absorption inside the tissue. As a consequence, it seems that the reason for more effect of comination of CL and UM on the color preservation might be the cumulative effect of two treatments. In another mechanism, UM can improve the positive impact of calcium by increasing bonded calcium to the cell wall and membrane structues as compared to DM. Considering the calcium treatment application, calcium passed through cell epidermis, linked to the carboxyl group of pectins, which leds to cell wall stranges, followed by the membrane intigrity [31, 41, 44]. But all the calcium absorbed is not in form of banded and a large portion of it remains freely in the intercellular space or apoplast. It has been suggested that some treatments like hot water (and probably UM in this study) is effective in increasing the banded calcium to the cell wall or membrane, which incrases calcium impact [33].

Button mushrooms have high transpiration, respration rate and weight loss because of a thin epidermic layer [4, 16, 26]. Weight loss is an important physiological process affecting the mushrooms quality [24, 25]. According to the results, CL solution in the dippig form decreased the weight loss and the lowest weight loss was in CL combined with UM. Effects of calcium treatemnt in weight loss control of button mushrooms previously was reported [22]. Calcium might affected weight loss throght the improved membrane intigrity [21]. Calcium also entry to cytosol and acts as an intercellular signals, lead to delay of sencense process and decrease metabolism rate so that it hinders the weight loss in fresh products [38]. Increased effect of calcium in combination with UM might be due to the increased banded calcium on membrane or the increased cytosol calcium level. US treatment, influencing the cell membrane structure, facilitated the entry of different elements into the cell [41].

In button mushrooms, the texture is often one of the most important quality attributes, representing metabolic status and water content changes of the mushrooms, and rapid softening has been considered as a real problem limiting the marketability of the edible mushroom [10, 27]. Button mushroom has no pectin layer, and is well known that softening in mushrooms is directly linked to the degradation of cell walls compositions by microbial enzymes, which increases the endogenous autolysins activity as well as losses of turgor pressure caused by water loss [22–24]. In current study, the CL and UM had positive impact on the button mushroom firmness preservation. Calcium treatment effect on the mushroom firmness is more likely to be due to; creating a bond between carboxyl groups in cell wall or slowing down the degradation of pectin by microbial and internal enzymes, which cause the firmness preservation [8, 30]. As well, calcium retains the membrane structure, intercellular water and turgor pressure; thereby has a positive effect on tissue firmness [19, 30]. US mechanism has not been completely recognized concerning the prolongation of fruits and vegetables shelf life but it has been widely confirmed that US as non-thermal technology can delay the softening phenomenon [3, 7].

Bactria, especially *Pseudomonas tolaasii*, has been considered as the most abundant microorganism responsible for limiting button mushrooms shlef life. Bacteria along with the growth, degrade mushroom cell walls, leading to the mushroom softening [24]. The bacteria also secreted toxin called tolaasii to the mushroom tissue, which resulted in membrane degradation, and then enzymatic browning. Effective treatments in avoiding the mushroom browning were of prohibiting impact on bacterial growth [4]. In this experiment, CL and US treatments, especially at combination form, significantly reduced the bacteria population. Similarly, US treatment prevented microbial growth in apple juice and strawberry [6, 11]. US waves can induce the pressure inside the solution, which could damage various parts of the microbes including cell wall, cytoplasmic membrane and intercellular structure and inactivate them [24, 39]. Calcium ion enhanced the cell wall strength and resistance against the microbes [2], though direct anti-microbial effect of CL solution in button mushrooms has also been shown [21]. As an organic acid salt, CL can confer antibacterial properties to the solution [2]. The antibacterial mechanism of the organic acid is as follows: after penetrating the cell, the high intracellular pH environment stimulates molecular dissociation, and the dissociated molecules accumulated in the cell can attack DNA and RNA, inhibit energy metabolism, induce protein denaturation, block ion channels, and cause cell deformation [39].

Similar to our results, in other studies, US in combination with chemical compounds has been assumed as an appropriate option in order to decrease microbe population in fresh products and augment the impact of chemical compounds [32, 36]. A study on oyster mushrooms, investigated the effect of combination of organic acids and US treatments on the control of *Listeria monocytogenes* bacterium, and it was revealed that the combined treatments controlled compeletly the bacterium without any adverse effect on the quality of the mushrooms [42]. Investigating three cherries cultivars, Zhi and Dong [45] found that although US treatment did not promote more Ca absorption inside the tissue, the combination of US and $CaCl_2$ treatments improved resistance against pathogen infection. In fact the combination of US, as a surface disinfectant, and CL, as an organic

acid, accelerates the antibacterial effects of washing solution by inducing intracellular and cell membrane damage to the bacteria [39].

Phenols are important components of fresh products which have dual performance so that, phenols can be oxidated by PPO and induced browning of white products like mushrooms, or they can protect cell structures against the stresses, since they have antioxidant capacity [11, 24]. Phenols are introduced as the substrate of browning enzyme reaction and consequently, their amount are likely to be reduced after browning [7]. It has been reported that treatments, which affected the white color preservation in the mushrooms, could better preserve phenolic compounds [9, 14, 24]. It has been also shown that lower level of browning in button mushroom is correlated positively with higher level of total phenolic content [10]. Accordingly, in this study, the most phenol content and least browning intensity were observed in the samples treated with the combination of CL and UM. Also, the samples with more browning intensity had less phenol content. Ca, as an intercelluar signal, and US, as an abiotic elicitor, can induce phenylpropanoid pathway, involving in phenolic components bio-synthesis [33, 39], higher level of the phenolic components, as antioxidant can control browning in white mushrooms [10, 28]. Similar result was reported by Liu et al. [28], who studied the effect of gallic acid grafted chitosan film in preserving mushroom and reported that packaging greatly stimulated the accumulation of phenols, and accumulated phenols showed antioxidant activity, which alleviated browning in mushrooms through maintaining membrane intgreity.

In conclusion, CL was effective in controlling the browning, retaining the firmness, reducing the bactrial population, preserving phenol content and controling weight loss in the mushrooms and its effects were greater when combined with US. Generally, 0.25% CL solution was better than the 0.5% one. UM treatment by itself (in control solution) was effective in few indices such as browning control or bacterial population decrease, but its impact was more pronounced when combined with CL solution.

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