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Determination the best source of calcium for button mushroom conservation

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ABSTRACT

Purpose: Button mushroom has short postharvest life due to high respiration rate, fast browning and high transpiration rate. Calcium is regarded as a suitable material in order to preserve the quality and increase the postharvest life of button mushroom, but, in the research conducted on the edible mushrooms, usually calcium chloride has been used as a calcium source. However, calcium chloride is of a negative effect on the taste of products and finding a suitable alternative is highly significant. In this study, the impact of other calcium sources has been studied in relation to the increased shelf-life of button mushroom in comparison to calcium chloride. **Research method:** Mushrooms were treated in calcium chloride, calcium lactate and calcium nitrate (Ca concentration equivalent to 0.5% calcium chloride) for 5 min and then were dried at room temperature and packed in polyethylene containers using cellophane film. The packed mushrooms were stored at 4°C and estimated during the cold storage up to the 14 days. **Findings:** Results indicated that all three calcium treatments led to the reduction of browning in mushrooms by reducing polyphenol oxidase enzyme activity as compared to the control, and also, treated mushrooms had higher firmness value and calcium content but lower phenol content. In addition, desired indices, the calcium lactate treatment was better than two other treatments in maintaining mushrooms quality during the cold storage. **Research limitations:** No limitations were encountered. **Originality/Value:** calcium lactate can be an appropriate alternative for calcium chloride treatment in terms of increased shelf-life of button mushrooms.

INTRODUCTION

Button mushroom (*Agaricus bisporus*), belonging to the family Agaricaceae, is one of the most important edible mushrooms, which is accounted for 40% of total mushroom consumed worldwide (Soler-Rivas et al., 1999). Button mushroom has short postharvest life as compared to a variety of vegetables and fruits (Brennan et al., 2000), so that it has only 2-4 days' shelf life at the ambient temperature (Jafri et al., 2012). High respiration rate, weight loss, enzymatic browning and microbial attacks are considered as the main reasons of limited shelf life of button mushroom (Brennan et al., 2000). With respect to more production of button mushroom, introducing methods to control the postharvest limitations and increase the shelf life is of high importance.

Calcium is one of the effective treatments in the fresh products after harvest. The products with low calcium content are sensitive to more physiological disorders and diseases and have short postharvest life (Chen et al., 2006). The presence of calcium along with the increased rigidity of cell wall and preserved integrity of protoplast membrane led to the control of cellular water exchanges and avoided the products weight loss during postharvest period (Jafri et al., 2012).

Browning of white mushrooms is done by the activity of Tyrosinase enzyme belonging to the poly phenol oxidase family (PPO); consequently, phenol compounds are oxidized and converted to brown compounds of melanin (Nerya et al., 2006). Calcium ion penetrates to vacuole membrane, enhances the membrane integrity and prevents the exit of phenolic compounds from vacuole, thereby postponing the browning (Kukur et al., 1998). Positive effect of calcium ion has been shown in the reduced cellular respiration in different products (Aguayo et al., 2008; Chen et al., 2006; Manganaris et al., 2005; Silveira et al., 2011). Aguayo et al. (2008) studied the melon fresh cuts and indicated that various calcium salt caused the reduced growth of microbial elements especially bacteria; it was stated that Ca treatments increase bond Ca in the texture, which increases cellular wall strength against microbial attacks and colonization. In another mechanism, it seems that Ca treatments induce the synthesis of phytoalexins components inside the cells. Therefore, calcium treatment can effectively remove the postharvest limitations of button mushroom and increase its shelf life. Of course, in some studies, it has been shown that adding calcium chloride to irrigation water or the treatment of calcium chloride at postharvest stage is effective in improving the color and decreasing the browning of mushrooms (Barden et al., 1990; Khan et al., 2017; Philippoussis et al., 2001; Srivastava et al., 2020). Finally, in most of the researches, calcium chloride was used as the calcium source.

Among Ca salts, calcium chloride has been widely used to increase the shelf life of fresh products in postharvest research; however, it has been shown that this salt imparted an undesirable bitter taste in the products, especially at higher concentrations. Therefore, finding an alternative calcium source to calcium chloride is important (Naser et al., 2018). Calcium lactate, calcium nitrate, calcium propionate, calcium carbonate, calcium citrate, calcium ascorbate, calcium tartrate, calcium silicate, calcium acetate have been used in different researches as calcium sources (Aguayo et al., 2008; Naser et al., 2018; Silveira et al., 2011; Zhi et al., 2017).

To the best of our knowledge, little information is available about the effects of different Ca salts on the increased shelf life of button mushrooms. The objective of this research was to study the effects of different Ca salts (using the same Ca concentration) on the increased button mushrooms shelf life. Thus, the main aim of this study was to find a suitable alternative Ca salt for calcium chloride.

MATERIALS AND METHODS

Samples and treatments

White Button mushrooms with closed cap and around 40 mm diameter were obtained from a commercial mushroom producer, located near Shahriar city, Tehran province, Iran. At harvest time, the average TSS value of mushrooms was 4 % and the average firmness value was 20 Newton (N).

The mushrooms were transferred immediately to the postharvest biology and technology laboratory in Shahed University of Tehran, where the intact and defect free mushrooms were selected and divided into 4 homologues groups, each group containing 42 mushrooms. Mushrooms were dipped in Ca solutions including water (as control), calcium chloride ($CaCl_2$), calcium lactate (CaLac), and calcium nitrate ($Ca(NO_3)_2$) for 5 min at 20°C. The concentrations of the calcium salts were adopted ensuring that they provide the same Ca concentration equivalent to 0.5% $CaCl_2$ (Sarlak et al., 2017). All chemicals were of analytical reagent-grade purity and purchased from Merck Company, Germany.

After the treatments, mushrooms were dried at ambient condition and then were packed in polyethylene containers using cellophane film. Each treatment included 6 homologues packages, each package containing 7 treated mushrooms. Then, the containers were stored at 4°C with RH ≥ 90% up to 14 days, and at 7th and 14th day after storage, three packages of each treatment as 3 replicates were removed from the storage and were evaluated.

Physicochemical analysis

The severity of 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 severity was calculated according to the following equation (1) (Hassani & Khademi 2018):

$$\text{Browning Severity} = \sum [(\text{Browning scale}) \times (\text{number of samples at each browning scale})] / (4 \times \text{total number of samples in the lot}) \quad (1)$$

The color parameter of L^*, a^* and b^* was determined at five upper surfaces of each mushroom using a colorimeter (model TES-300, Taiwan) and browning index (BI) was calculated by the below equation (2): (Gómez et al., 2012).

$$BI=\frac{100(X-0.31)}{0.17} \quad (2)$$

$$X=\frac{(1.75\times L^*)+a^*}{(5.645\times L^*)+a^*-(0.3012\times b^*)}$$

To determine the weight loss of mushrooms, the packages were weighted before and after the storage and weight loss percent was calculated using the following equation (3):

$$\text{Weight Loss} = [(\text{weight of package before the storage} - \text{weight of package after the storage}) / \text{weight of package before storage}] \times 100 \quad (3)$$

The firmness (penetration test) of the samples was evaluated on the mushroom cap using a hand penetrometer (model VBR80, Italy) with 4-mm plunger and the result was expressed as N (Sarlak et al., 2017). Measurements were performed in each replicate on 5 mushrooms and the mean was calculated.

Total soluble solid (TSS) of mushrooms was measured by a hand Refractometer. A drop of juice obtained from ground mushroom was placed on the refractometer glass prism and the percentage of TSS was recorded (Lagnika et al., 2014). TSS Measurements were performed in each replicate on 5 mushrooms and the mean was calculated.

Total phenol content was measured by Follin-Ciocaltaeu reagent method as described by Hassani and Khademi (2018). 1g of mushroom texture was homogenized in 10 mL of 80% methanol and the homogenate was centrifuged at 9800 g for 10 min at 4°C. Inside a test tube, 7 mL of distilled water, 1 mL of supernatant and 1 mL of Follin-Ciocaltaeu reagent were added. After 5 min, 1 mL of 20% sodium carbonate solution was added to the mixture, and the absorbance of resultant was measured at 760 nm after 1h using spectrophotometer (model: Lambda, 650, PerkinElmer, USA). The amount of total phenol content was calculated according to a standard curve obtained from different concentrations of tannin acid solution, and the result was expressed as mg of tannic acid equivalent per kg of the fresh weight sample.

PPO enzyme activity was determined as a method described by Khademi et al. (2019). For that, 2 g of frozen mushrooms was homogenized in 12 ml of potassium phosphate buffer (50 mM, pH 6.8) containing 0.5 M KCl and 10% PVPP using a mortar and pestle. The homogenate was centrifuged at 9800 g for 20 min at 4°C and then, the supernatant was collected. Reaction mixture contained 1800 µl potassium phosphate buffer (50mM, pH=7), 500 µl pyro-catechol solution (0.5 M) and 700 µl enzyme extract that the increase in absorbance of the reaction was recorded using the spectrophotometer at 420 nm for 2 min. One unit of enzyme activity was defined as an increase in absorbance per g FW per min.

To determine calcium content, randomly selected mushrooms per replicate were dried at 65°C and then were grinded and passed from a 40 mesh sieve. Two gr of the grinded samples were put at 550°C until obtaining ashes, the ashes were dissolved in 2 M hot HCL, then filtered, and brought to a final volume of 50 mL with distilled water. Ca concentration was determined using an atomic absorption spectrophotometer (AAS, Shimadzu Instruments, Inc., SpectrAA-220, Japan) and the content of Ca was expressed as dry weight percent (Kukur et al., 1998).

Statistical analysis

A randomized design with three replicates per treatment was used in this experiment. To determine the effects of calcium solutions and storage time on each dependent variable, a two-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, $P \leq 0.05$).

Table 1. Statistical analysis of parameters studied: Ca salts treatments and storage time (ST) and their interaction for Button mushrooms through analysis of variance

	Browning index	Browning Score	Weight loss	TSS	Firmness	Phenol content	PPO	Ca content
Ca salts	**	**	ns	**	**	**	**	**
ST	**	**	*	*	ns	ns	ns	ns
Ca×ST	ns	ns	ns	ns	ns	ns	ns	ns
CV%	8.53	17.44	23.5	11.5	17.68	12.2	36.57	25.9

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

Table 2. Effect of different calcium salts on physicochemical properties of button mushroom, stored at 4°C for 14 days

Traits / Treatment	Browning index	Browning Score	TSS (%)	Firmness (N)	Phenol content (mg.100g^{-1}FW)	PPO (U/grFW/min)	Ca content (% DW)
Control	11.01±0.46 a	0.76±0.05a	2.49±2.4b	11.08±1.47c	27.89±1.76a	6.16±0.61a	4.375±0.92d
Ca Lactat	7.44±0.5 c	0.40±0.046c	2.990±2.9a	18.85±1.09a	23.84±1.49bc	3.26±0.44bc	26.333±2.2a
Ca Cl_2	8.45±0.49 b	0.44±0.058bc	3.08±3.08a	16.22±0.94ab	20.86±1.3c	2.56±0.51c	17.517±1.41b
Ca $(NO_3)_2$	8.79±0.4 b	0.54±0.048b	2.64±2.6ab	14.70±1.07b	26.35±1.63ab	4.41±0.96b	6.35±0.96c

Means with the same letter in each column are not significantly different at 5% level of the LSD test. Values represent the means ± standard error of two studied times and three replicates (n = 6).

Table 3. Effect of storage time on physicochemical properties of button mushroom, treated with different calcium salts and stored at 4°C for 14 days

Traits / Time	Browning index	Browning Score	Weight loss (%)	TSS (%)
7 day	8.09±0.46b	0.46±0.06b	4.29±0.46b	3.22±0.09a
14 day	9.76±0.4a	0.61±0.05a	6.92±0.88a	2.38±0.14b

Means with the same letter in each column are not significantly different at 5% level of the LSD test. Values represent the means ± standard error of four calcium treatemnts and three replicates (n=12).

RESULTS

The ANOVA results related to Ca salts treatments and storage time factors and their interaction on Button mushrooms have been shown in Table 1.

Results showed that both Ca treatment and storage time affected mushrooms BI. All calcium solutions including CaLac, $CaCl_2$ and $Ca(NO_3)_2$ reduced BI in button mushrooms as compared to control, among Ca treatments; CaLac was more effective than $CaCl_2$ and $Ca(NO_3)_2$ in control of BI in mushroom, while no significant differences were observed between $CaCl_2$ and $Ca(NO_3)_2$ treated mushrooms in BI. Results for browning severity (visually assessment) were similar to results of BI, confirming browning disorder attenuate in button mushroom by Ca treatments. The most effective treatment was CaLac (Table 2). The rate of browning (as presented by BI and browning severity) in all samples increased significantly when the duration of the experiment was increased (Table 3).

The weight loss of mushrooms was affected more by the experiment duration and was not significantly affected by Ca treatments. Weight loss of mushrooms indicated a significant increase at 14th day storage time as compared to the 7th day one; however, at both study times; no significant differences were detected among the treatments regarding weight loss (Table 3). The reason has been the appropriate primary quality of mushrooms and suitable preservation conditions during the experiment.

Results showed that the TSS value of the mushrooms was affected by both Ca treatments and storage time. The TSS in all mushrooms decreased significantly when the time of evaluation increased (Table 3). The TSS value in CaLac and $CaCl_2$ treated mushrooms was significantly higher than that of control mushroom, while no significant difference was observed between $Ca(NO_3)_2$ treated mushrooms and control mushrooms in terms of TSS (Table 2).

In similar trends, the firmness value, phenol content, PPO activity and Ca content of mushrooms were affected by Ca treatments and were not affected by study times.

The lowest firmness value was detected in control samples in this study. Samples treated by Ca solutions had higher firmness value than the control samples. The highest firmness value was observed in CaLac treatment; even though no significant difference was observed between CaLac and $CaCl_2$ solutions regarding mushroom firmness (Table 2).

CaLac and $CaCl_2$ treated mushrooms had lower phenol content than control one; however, $Ca(NO_3)_2$ treated mushrooms showed no significant differences with control mushrooms regarding phenol content. No significant differences were observed between CaLac, $CaCl_2$ mushrooms in phenol content (Table 2).

The highest PPO activity was related to control mushrooms during this study. CaLac, $CaCl_2$ and $Ca(NO_3)_2$ treatments reduced PPO activity in treated mushrooms as compared to the control. The effect of $CaCl_2$ was more pronounced than $Ca(NO_3)_2$ in control of mushroom PPO activity; however, no significant differences were detected CaLac and $CaCl_2$ samples in PPO activity (Table 2).

All applied Ca solutions including CaLac, $CaCl_2$ and $Ca(NO_3)_2$ increased Ca content as compared to the control. Among them, the highest Ca content was detected in CaLac treatment; on the other hand, $CaCl_2$ treatment increased Ca content in mushrooms more than that of $Ca(NO_3)_2$ treatment (Table 2).

DISCUSSION

Absence of cuticle, high respiration rate and high water content make button mushrooms very susceptible to microbial attacks and enzymatic browning (Brennan et al., 2000). The white colour is one of the main quality features in button mushrooms and a determining factor in purchase by the customer; the discoloration caused by the enzymatic browning leads to the decreased acceptance by the customers (Li et al., 2019). In this experiment, calcium treatments showed a significant impact on the reduction of browning; the highest impact was seen in CaLac treatment. In browning process, phenolic compounds as the substrate and PPO enzyme are usually located in separate cellular locations, but the breakdown of the cellular membranes leads to the mixing of substrate and enzyme and finally, the browning reaction take place (Zhang et al., 2018). In button mushrooms, membrane breakdown usually occurs under the impact of microbial attacks and or senescence (Li et al., 2019). Ca ion can avoid the enzymatic browning (Silveira et al., 2011), because Ca ions by maintaining the functionality of the cell membranes, can slow down the senescence process, and by stacking to the cell wall components, they can increase the cell wall resistance against bacterial infection, thereby, it prevents the enzymatic browning (Tarlak et al., 2020). Impact of CaLac treatment on the decreased browning intensity in button mushrooms can be due to more penetration of Ca ion under the effect of this treatment on the mushrooms tissue as compared to other treatments ($CaCl_2$ and $Ca(NO_3)_2$), because, diffusion capacity and solubility of Ca salts vary depending on the source (Silveira et al., 2011). Also, the effect can be resulted from the direct anti-microbial effects of CaLac treatment. Although all Ca treatments indirectly increase the cell wall rigidity and the resistance against microbial agents as well, the direct antimicrobial properties of CaLac as an organic acid salt is dependent on its ability to form acids in solutions which are able to decouple microorganism transport through the membrane and oxidative phosphorylation influencing the electron transport system (Aguayo et al., 2008).

In this study no significant differences were observed among the treatments regarding weight loss. The reason could be the appropriate primary quality of mushrooms and suitable preservation conditions during the experiment.

According to the results during the experiment, TSS amount was reduced; and generally, TSS amount was higher in $CaCl_2$ and CaLac treatments than control. Since the soluble sugars in edible mushrooms have most effects on TSS amounts, and since the one of the important impacts of Ca ion presence on plant cells is the reduction of respiration rate leading to the decreased consumption of soluble sugars (Chen et al., 2006; Manganaris et al., 2005),

therefore more preservation of TSS amounts in the Ca treated mushrooms is due to the decreased respiration rate and soluble sugar consumption. The decreased TSS over the time is due to consumption of soluble sugars during respiration process (Hassani & Khademi, 2018).

In button mushrooms, the texture is often one of the most important quality attributes assessed by the consumers; therefore, rapid softening has been considered as a real problem limiting the marketability of the edible mushroom (Li et al., 2019; Ni et al., 2017). Textural changes during postharvest stage on button mushrooms are related to the water loss and changes in cell wall structure (Cheng et al., 2020); in this study, we found no significant differences among the mushrooms in terms of weight loss, so that the main reason for the difference between the treatments in terms of mushrooms firmness has been resulted from the changes in cell wall structure. The button mushroom has no pectin layer and its softening is directly linked to the degradation of cell wall compositions, which have occurred by bacterial enzymes and/or endogenous enzymes (Khan et al., 2017). ß-1,3 glucan, ß-1,6 glucan and chitin are the main constituents of the fungal cell wall that may play main roles in mushroom textural properties. Glucanase, cellulase and chitinase are the main enzymes involved in degradation of cell wall matrix resulting in softening in button mushrooms during the postharvest stage (Khan et al., 2017; Ni et al., 2017). In this study, the higher firmness values were found in CaLac, $CaCl_2$ and $Ca(NO_3)_2$ treatments, respectively. As well, the most Ca contents were seen in the CaLac, $CaCl_2$ and $Ca(NO_3)_2$ treatments, respectively. Thus, there was a definite relationship between texture Ca content and mushrooms firmness. The relationship between Ca content and firmness value in button mushroom has been also reported by Khan et al. (2017). Preservation of firmness by the addition of Ca ions can be related to stabilization of membrane system, increasing rigidity of cell wall and retarding of cell wall degradation enzymes (Khan et al., 2017; Zhi et al., 2017). Similar to our result, Aguayo et al. (2008) in an investigation on the fresh cut melon showed that CaLac and $CaCl_2$ improved and preserved firmness of fresh cut melon better than other Ca sources during the cold storage; they explained that the differential effects of Ca solutions on firmness maintenance might be due to the differences among the Ca salts in solubility and diffusion capacity to the texture.

It has been documented that there was a normal relationship between phenylalanine ammonia lyase (PAL) activity and firmness value in button mushrooms, and any treatment which can maintain the firmness in button mushrooms has also inhibitory effect on the PAL activity (Khan et al., 2017). PAL is a key enzyme in the phenyl propanoid pathway and is involved in the biosynthesis of the phenolic compounds. Although PAL enzyme activity was not measured in the research, total phenol content in Ca treatments was less than control, indicating the decreased activity of PAL enzyme with the presence of Ca ion. Since in this study, the decrease in total phenol content was consistent with a decrease in browning degree in mushrooms, the control of PAL enzyme activity and reduction of phenol content can be regarded as one of the major reasons for the decreased browning in Ca treatments.

Phenolic compounds oxidation by the activity of PPO enzyme and melanin pigment formation is the basic element of browning in button mushrooms (Nerya et al., 2006). Since the Ca treatments lead to the decreased activity of PPO enzyme in the experiment as compared to the control, the main factor of decreased browning in the mushrooms by the desired treatments has been the decreased activity of PPO enzyme. Direct impact of Ca ion in the reduction of PPO enzyme activity has been reported (Li et al., 2019).

CONCLUSION

Button mushrooms are of short postharvest life and browning and softening are the main limiting factors in terms of mushrooms postharvest life. In this experiment, Ca treatments could preserve mushrooms firmness and decrease the enzymatic browning, however, the impact of CaLac treatment was more than $CaCl_2$ and $Ca(NO_3)_2$ treatments. Since, this treatment has no undesirable impact on the products taste; it can be a suitable alternative for calcium chloride in the increased postharvest life of button mushrooms.

Conflict of interest

The authors have no conflict of interest to report.

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