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Effects of Calcium Salts on Browning of Edible Mushroom Caps

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Abstract

Recently, various edible coatings have been employed to extend the shelf life of fruits and vegetables. This study investigated the effects of immersion of calcium salt solutions (calcium chloride and calcium nitrate) at concentrations of 5 and 10 millimolar on the post-harvest quality of edible mushrooms stored at refrigeration temperature (4 degrees Celsius) for 15 days. The experiment was conducted in triplicate. The results indicated that, compared to uncoated control samples, immersion of the mushrooms in a 5 millimolar calcium nitrate solution significantly outperformed the samples coated with a 5 millimolar calcium chloride solution. Visually, the mushrooms coated using the spraying method exhibited a longer shelf life than those treated by immersion. In terms of appearance and browning, both concentrations (5 and 10 millimolar) of calcium nitrate performed similarly in the immersion method, presenting an acceptable appearance. However, for the calcium chloride samples, it appeared that the 5 millimolar concentration was more effective than the 10 millimolar concentration, positively influencing the shelf life of the edible mushrooms. Similarly, in the sprayed samples, calcium nitrate at both concentrations demonstrated a better effect on the shelf life of the mushrooms.

Keywords:Calcium salts, Edible coatings, Mushroom, Post-harvest quality, Shelf life.

1. Introduction

Fresh and preserved mushrooms have been valued since ancient times due to their organoleptic, nutritional, and medicinal properties Malekzadeh, Kamrani, Abbasi, and Sadeghi (2025). Consumer demand for mushrooms, as well as their production, has significantly increased in recent years (Badalyan & Zambonelli, 2023). White button mushrooms are the most common and widely consumed edible mushrooms in the world due to their delicious taste and nutritional value (Ba et al., 2023). Although button mushrooms are low in calories, they are a good source of high-quality protein, amino acids, dietary fiber, vitamins (B2, folates, and niacin), as well as many healthy minerals (iron, calcium, phosphorus, potassium, selenium, zinc, and copper) and phenolic compounds. Button mushrooms also contain a wide range of therapeutic compounds such as triterpenoids, glycoproteins, natural antibiotics, enzymes, enzyme inhibitors, and soluble and insoluble fibers, which

have diverse and strong health properties (Brady, 2024; Gholami, Aghili Nategh, & Rabbani, 2023). Additionally, they contain bioactive phytochemicals responsible for preventing and treating many diseases such as cancer, hyperlipidemia, allergies, heart and liver diseases, and immune problems (Gukasyan, Griffiths, Yaden, Antoine, & Nayak, 2023; Guo et al., 2022). However, the high moisture content (over 90%), rapid metabolic activity, high respiration and transpiration rates, and lack of an epidermal structure make button mushrooms highly perishable (Guo et al., 2022; Hou et al., 2023). The increase in bacterial and fungal load, water loss, surface browning, and tissue softening are among the main phenomena that occur during the storage of mushrooms (Beetz & Greer, 2004) and lead to their spoilage and loss of commercial value (Nasri, Khademi, Saba, & Ebrahimi, 2023). Additionally, reactive oxygen species (ROS) increase lipid oxidation, which is indicated by the elevated content of malondialdehyde (MDA), damaging cell membrane integrity and accelerating aging (Xu, Gao, Meng, & Chang, 2022; Yang et al., 2022).

Regarding the role of mushrooms in human nutrition and health, they can be classified into four main categories based on Cheung's research (2010): edible (e.g., button mushroom *Agaricus bisporus*), medicinal (such as Ganoderma lucidum), toxic (like *Amanita muscaria*), and other species whose properties have not yet been fully identified. Notably, some edible mushrooms also possess bioactive compounds, placing them simultaneously in both edible and medicinal categories (Blasco, Esteve, Frígola, & Rodrigo, 2004).

A major challenge in the mushroom industry is the short post-harvest shelf life of button mushrooms, which lasts only 3–4 days at room temperature without packaging, creating significant distribution, sales, and consumption hurdles (Hou et al., 2023; Jebelli Javan et al., 2015). To address this limitation, developing advanced preservation methods (e.g., modified atmosphere packaging or edible coatings) and optimizing post-harvest processes appear essential.

Mushroom preservation methods can be divided into three main categories: thermal (including drying and freezing), chemical (such as edible coatings, protective films, and wash solutions), and physical (like packaging, irradiation, pulsed electric field processes, and ultrasound). These processes can affect the nutritional value and bioactive properties of mushrooms. Drying, especially at high temperatures, may lead to the degradation of polysaccharides, proteins, and aromatic compounds, while freezing, although it extends shelf life, is associated with a reduction in vitamin content. Edible coatings and films are effective in preserving total sugars, ascorbic acid, and bioactive compounds, but wash solutions may reduce amino acid content. On the other hand, gamma and electron beams reduce unsaturated fatty acids, while UV-B irradiation significantly increases vitamin D2 levels. However, the impact of methods like packaging, pulsed electric field, and ultrasound on the nutritional and bioactive properties of mushrooms is not yet fully understood and requires further research. This review provides strategies for mushroom processing industries to produce value-added food products by introducing innovative and cost-effective technologies.

In addition to storage temperature control, various methods have been proposed to preserve the quality and extend the shelf life of edible mushrooms, including washing with antimicrobial and anti-browning solutions, applying nanocomposite films and edible coatings, modified atmosphere packaging (MAP), controlled atmosphere storage, irradiation, pulsed electric field (PEF), and ultrasound [2, 12]. Among these, edible coatings have gained recent attention due to their advantages over alternative preservation methods—such as irradiation (which faces lower consumer acceptance) or MAP (which is less economically viable) (Brady, 2024; Lian et al., 2024).

During storage, mushrooms tend to develop brown spots due to the enzymatic conversion of phenolic compounds into quinones, leading to melanin (brown pigment) synthesis. The key enzymes involved in these reactions are polyphenol oxidase (PPO) and, to a lesser extent, peroxidase (POD). Typically, browning occurs when the cellular membrane integrity of mushrooms is disrupted. This structural compromise facilitates contact between phenolic compounds and enzymes—a critical prerequisite for the browning cascade (Malekzadeh et al., 2025).

Edible coatings can significantly enhance the postharvest appearance and preservation of mushrooms by maintaining their phytochemical

content and physicochemical properties over extended periods, thereby preserving their nutritional and health benefits while extending shelf life. These coatings form a semi-permeable layer on the product surface, providing additional protection by limiting microbial contamination, controlling moisture loss, suppressing respiration and ethylene production rates, and delaying senescence. Moreover, they impart a desirable gloss and sheen to coated products, increasing their visual appeal to consumers (Ziogas, 2024). Polysaccharides—such as pectin, chitosan, cellulose derivatives, starch derivatives, alginate, agar, carrageenan, and gums—are among the most widely used materials for edible coating production, often combined with proteins and lipids. Notably, these coatings pose no adverse effects on human health, are safely consumed as part of the product, and are environmentally friendly due to their biodegradable nature (Hammann & Vetter, 2016).

The objective of this research is to investigate the effects of two calcium salt solutions (calcium nitrate and calcium chloride) at concentrations of 5 mM and 10 mM on the postharvest quality of button mushrooms (*Agaricus bisporus*). Specifically, the study focuses on evaluating their impact on shelf-life extension, texture firmness, browning inhibition, and weight loss reduction during storage.

2. Material and methods

2.1. Mushroom treatment and storage

Button mushrooms were purchased from Tehran and promptly transferred to the Biotechnology and Plant Physiology Research Laboratory at Qom University. The selected mushrooms were of similar size, with completely closed caps, and showed no signs of browning or mechanical damage. Upon arrival at the laboratory, the immersion samples were washed and dried using absorbent paper, while the sprayed samples were tested without rinsing with distilled water. The mushrooms were coated using two methods: immersion and spraying. After being treated with the desired solutions, the samples were allowed to rest for 15 minutes at room temperature, followed by vacuum packaging. They were then stored in a refrigerator at 4 degrees Celsius with controlled humidity and evaluated at 5-day intervals. For the preparation of 5 and 10 millimolar calcium nitrate solutions, 0.82 grams and 1.64 grams of calcium nitrate were weighed using a digital balance and dissolved in 1 liter of distilled water to obtain a homogeneous and clear solution. Similarly, for the preparation of 5 and 10 millimolar calcium chloride solutions, 0.5 grams and 1.11 grams of the respective salt were weighed and dissolved in 1 liter of distilled water.

2.2. weight loss

Weight loss of the samples was quantified based on the methodology outlined by Malekzadeh et al. (2025). In this approach, the initial weight of the samples is accurately measured, and then after a specific storage or testing period, the final weight is measured again. The difference between the initial and final weights indicates the amount of weight loss, with measurements taken every four days to monitor changes over time.

2.3. cap browning

The surface color of the mushroom caps was assessed every five days during storage at 4 °C by measuring ten mushrooms per replicate using a Minolta spectrophotometer (CR-400). To determine the L* (lightness/darkness), a* (red/green), and b* (yellow/blue) values, each mushroom was evaluated at three evenly spaced points on the cap. The percentage of cap browning, which indicates the intensity of the brown color, was analyzed according to the method described by Malekzadeh et al. (2025).

Data analysis

The data analysis was performed using SPSS version 25. Two-way analysis of variance (ANOVA) was performed on the data, and subsequent post-hoc multiple comparisons were carried out. The threshold for determining statistical significance was established at a significance level of $P < 0.05$. The results were presented as the mean ± standard error of triplicate experiments, indicating the average value and the variability among the three replicates. Data manipulation was performed using Microsoft Excel, while GraphPad Prism 8 software was used for graphing the results.

3. Results

3.1. Effect of Ca salt treatment on Physiological Weight Loss (PWL%)

As shown in Figure 1, the results of the study on the impact of different calcium salts on the storage of edible mushrooms indicate that the use of these salts can effectively reduce the physiological weight loss of mushrooms during the storage period. In this study, various treatments were examined, including a control group (without salt addition), calcium chloride ($CaCl_2$) at concentrations of 0.05% and 0.1%, and calcium nitrate (Ca $(NO_3)_2$) at concentrations of 0.05% and 0.1%. The results demonstrated that over the 15-day storage period, the rate of physiological weight loss in the control group increased, with the most significant percentage of physiological weight loss observed in this group on day 15. The application of calcium treatments at concentrations of 0.05% and 0.1% revealed that the 0.1% calcium chloride treatment did not have a significant effect on reducing physiological weight loss, and no significant difference was found between the control group and the 0.1% calcium chloride treatment.

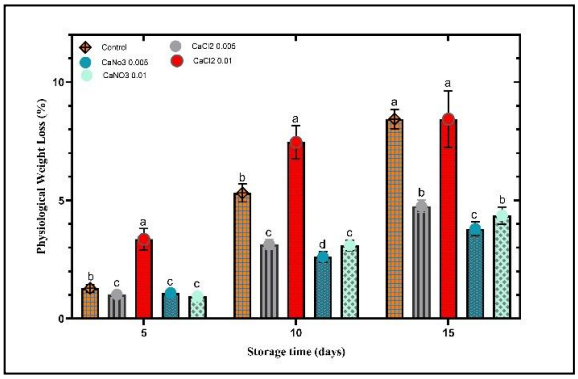

Figure 1.
Impact of Various Concentrations of Calcium Salts on the Postharvest Shelf Life PLW percentage of Mushrooms During a 15-Day Storage Period.

However, the use of the 0.05% calcium chloride treatment showed a significant effect in preventing physiological weight loss and slowed the rate of increase. Compared to the control group, the application of calcium nitrate at concentrations of 0.05% and 0.1% also slowed the rate of physiological weight loss, with the least amount of reduction observed at the 0.05% and 0.1% calcium nitrate concentrations on days 10 and 15 of storage. As the results indicated, the calcium nitrate treatment was more effective than the calcium chloride treatment in preventing physiological weight loss, with less than a 5% weight loss observed in the calcium nitrate treatment on day 15. In contrast, the control group and the group treated with calcium chloride experienced a weight loss of approximately 10% of the mushroom's weight.

3.2. Effect of Ca salt treatment on Cap Browning

In Figure (A), the results of the experiment examining the effect of various calcium treatments on the degree of browning in edible mushrooms over time (days 0, 5, 10, and 15) are presented. The treatments included a control, 5 mM calcium chloride, 10 mM calcium chloride, 5 mM calcium nitrate, and 10 mM calcium nitrate. The results indicate that the calcium treatments, particularly at the higher concentration (10 mM), were able to significantly inhibit the browning of the mushrooms during the 15-day storage period, compared to the control samples.

The effect of calcium salt treatments on the browning of the mushroom cap is shown in Figure 2B. In a study on the effect of calcium salts on the storage of edible mushrooms, it was found that the use of these treatments can be effective in reducing the browning of the mushroom cap during the storage period. Five different treatments were studied, including a control group, calcium chloride at concentrations of 0.05% and 0.1%, and calcium nitrate at concentrations of 0.05% and 0.1%. Over the course of 15 days of storage, the trend of cap browning increased in all treatments, but the use of calcium nitrate at both concentrations was able to more effectively reduce this trend, so that on the 15th day of storage, the browning in these treatments was the lowest compared to other treatments and the control group. The 0.05% calcium nitrate treatment showed the least amount of browning.

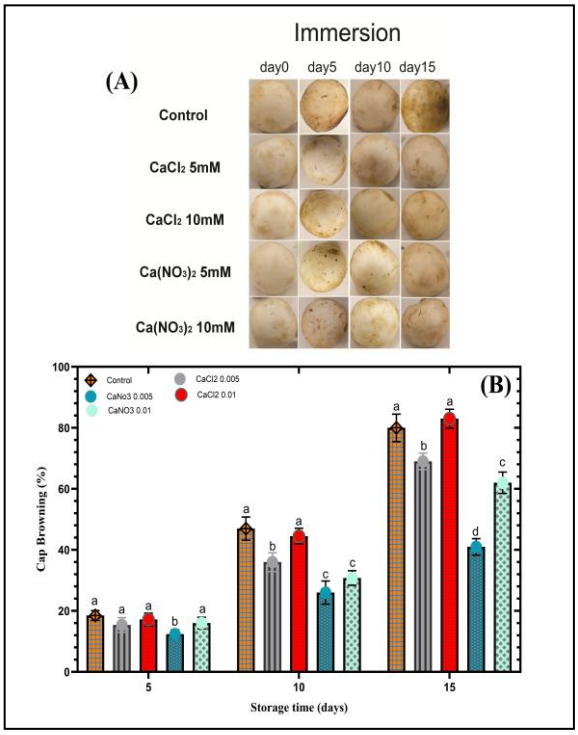

Figure 2.
Impact of Various Concentrations of Calcium Salts on the external appearance (A) and Cap Browning (B) of Mushrooms During a 15-Day Storage Period.

3.3. Effect of Ca salt treatment on H_2O_2 Content

As shown in Figure 3, the results of the study on the effect of different calcium salts on the storage life of edible mushrooms indicate that the use of these salts can be effective in reducing the hydrogen peroxide content in edible mushrooms during the storage period. In this study, the treatments under investigation included a control group (without added salt), calcium chloride ($CaCl_2$) at concentrations of 0.05 and 0.1%, and calcium nitrate (Ca $(NO_3)_2$) at concentrations of 0.05 and 0.1%. The results showed that during the 15-day storage period, the hydrogen peroxide content had an increasing trend in all control and treatment groups. However, on the first and fifth days of storage, no significant difference was observed in the hydrogen peroxide content among the treatment and control groups. On the tenth day of storage, the hydrogen peroxide content was the highest in the control group. In the other treatments, the hydrogen peroxide content also showed an increasing trend, but the values were lower compared to the control group. The lowest hydrogen peroxide content on the tenth day was observed in the 0.1% calcium nitrate treatment. On the 15th day of storage, the hydrogen peroxide content maintained its increasing trend compared to the tenth day, and a significant difference was observed between the control group and the other calcium treatments. On the 15th day of storage, the highest hydrogen peroxide content was observed in the control group and the 0.1% calcium chloride treatment. The lowest hydrogen peroxide content on the 15th day was observed in the 0.05% calcium nitrate treatment.

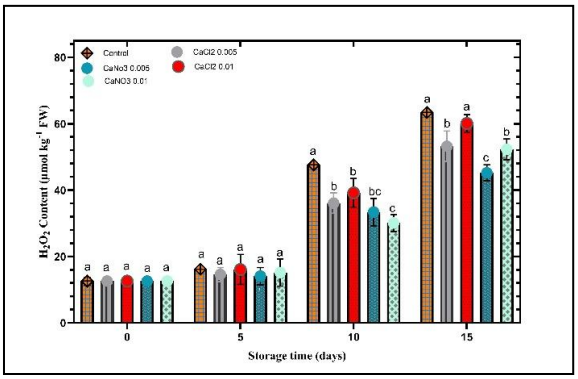

Figure 3.
Impact of Various Concentrations of Calcium Salts on H_2O_2 Content of Mushrooms During a 15-Day Storage Period.

3.4. Effect of Ca salt treatment on Catalase (CAT) activity

As shown in Figure 4, the results of the study on the effect of different calcium salts on the storage life of edible mushrooms indicate that the use of these salts can be effective in reducing the catalase activity in edible mushrooms during the storage period. In this study, the treatments under investigation included a control group (without added salt), calcium chloride

($CaCl_2$) at concentrations of 0.05 and 0.1%, and calcium nitrate (Ca $(NO_3)_2$) at concentrations of 0.05 and 0.1%. The results showed that during the 15-day storage period, the catalase activity had an increasing trend in all control and treatment groups. However, on the first day of storage, no significant difference was observed in the catalase activity among the treatment and control groups. On the fifth day of storage, the 0.1% calcium nitrate and calcium chloride treatments

showed a significant difference compared to the other treatments and the control group. On the tenth day of storage, the catalase activity was the lowest in the control group. In the other treatments, the catalase activity also showed an increasing trend, but the values were higher compared to the control group. The highest catalase activity on the tenth day was observed in the 0.1% calcium nitrate treatment. On the 15th day of storage, the catalase activity maintained its increasing trend compared to the tenth day, and a significant difference was observed between the control group and the other calcium treatments. On the 15th day of storage, the highest catalase activity was observed in the 0.05% calcium chloride treatment group. The lowest catalase activity on the 15th day was observed in the 0.05% calcium chloride treatment and the control group.

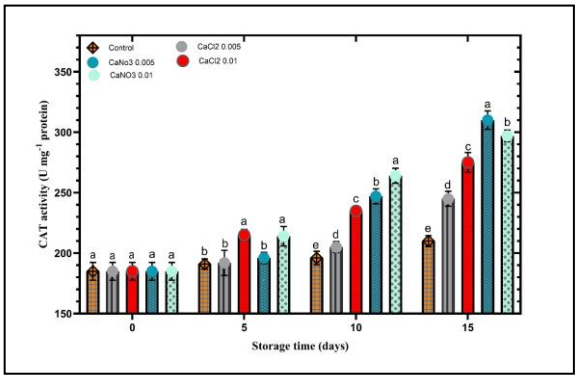

Figure ٤.
Impact of Various Concentrations of Calcium Salts on H2O2 Content of Mushrooms During a 15-Day Storage Period.

4. Discussion

In the present study, the effect of potassium salts on the shelf life of edible mushrooms was investigated. The results showed that the use of these salts, particularly calcium salts, had a significant impact on the quality and longevity of the mushrooms. In this study, the browning percentage of the mushrooms was examined as one of the quality indicators, and it was found that calcium salts significantly reduced this percentage. This reduction in browning is attributed to the positive influence of these salts on the metabolic processes of the mushrooms and their improved condition during storage. Additionally, a decrease in the weight of the mushrooms was observed as a result of using potassium salts, especially calcium, which indicates the preservation of moisture and prevention of water evaporation in the mushroom tissue.

The results presented in Figure 1 demonstrate the impact of adding various concentrations of calcium salts, including calcium chloride and calcium nitrate, on the physiological weight loss (%) of edible mushrooms during storage. The data indicates that the incorporation of calcium nitrate at a higher concentration of 0.01% was particularly effective in mitigating the browning process and preserving the overall quality of the mushrooms over the storage duration compared to the control and other treatments. While the calcium chloride treatment at 0.01% also exhibited a reduction in the browning of the mushrooms, its effect was less pronounced than that of the calcium nitrate. These findings suggest that the strategic use of calcium salts, especially calcium nitrate, can significantly extend the shelf life and maintain the desirable characteristics of fresh mushrooms during storage.

Browning in edible mushrooms significantly influences their nutritional quality. This phenomenon can result in the loss of vital nutrients, including vitamins and antioxidants, which are important for health (Malekzadeh et al., 2025; Ojeda, Sgroppo, Martín-Belloso, & Soliva-Fortuny, 2019). Moreover, browning impacts the sensory attributes of mushrooms, such as flavor and visual appeal, which may diminish their attractiveness in the market. Consequently, controlling browning is essential for preserving both the nutritional integrity and overall value of edible mushrooms (Guo et al., 2022). As observed in the results and Figure 1, the effect of adding calcium salts (calcium chloride and calcium nitrate) on the browning of edible mushrooms over storage time is evident. Calcium salts, particularly calcium nitrate at higher concentrations (0.01 percent), have been able to more effectively prevent the browning of mushrooms, indicating better quality preservation and shelf life of the mushrooms during storage. Additionally, the addition of calcium chloride has somewhat reduced the browning process of the mushrooms, although its effect has been less than that of calcium nitrate.

Antioxidant enzymes play a crucial role in mitigating cold stress in plants by scavenging reactive oxygen species (ROS) that accumulate during chilling

or freezing conditions. These enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidases, help maintain cellular integrity by preventing oxidative damage to proteins, lipids, and DNA. By enhancing the plant's ability to cope with oxidative stress, antioxidant enzymes contribute to improved survival and recovery from cold-induced injuries (Almas et al., 2021; Amini et al., 2021).

In Figure 3, the activity levels of the enzyme CAT in various samples over storage time are presented. It is observed that in samples containing $CaNO_3$ at different concentrations, the activity of the CAT enzyme increased over time. In contrast, samples containing $CaCl_2$ at various concentrations exhibited a relatively lower increase in CAT activity. These results indicate that the addition of $CaNO_3$ to the environment enhances catalase activity. In Figure 4, the content of H_2O_2 in different samples over storage time is depicted. It is noted that in samples with varying concentrations of $CaNO_3$, the H_2O_2 content decreased over time. Meanwhile, in samples containing $CaCl_2$ at different concentrations, the H_2O_2 content experienced an increase, although this increase was less pronounced compared to the control group. These findings suggest that the addition of $CaNO_3$ to the environment reduces H_2O_2 content by enhancing the activity of the catalase enzyme.

5. Conclusion

In this study, the effect of potassium salts on the shelf life of edible mushrooms was investigated, and the results showed that the use of these salts, particularly calcium salts, had a significant impact on the quality and longevity of the mushrooms. The reduction in the browning percentage of the mushrooms, as one of the quality indicators, indicated the positive influence of these salts on the metabolic processes and improved storage conditions of the mushrooms. Additionally, the decrease in the weight of the mushrooms due to the use of potassium salts, especially calcium, reflects the preservation of moisture and prevention of water evaporation in the mushroom tissue. The results demonstrated that the addition of calcium nitrate at a higher concentration (0.01%) effectively reduced the browning process and preserved the overall quality of the mushrooms during storage. Considering the importance of controlling browning to maintain the nutritional value and market appeal of mushrooms, these findings suggest that the strategic use of calcium salts, particularly calcium nitrate, can significantly extend the shelf life and desirable characteristics of fresh mushrooms during storage.

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