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Effects of L-Phenylalanine Pretreatment on Reducing Frost Damage and Browning in Edible Mushrooms (*Agaricus bisporus*)

Yareb Kasad Madloul[1], Ali Asghar Hatamnia[1]*, Parviz Malekzadeh[2*]

[1]*Department of Biology, Faculty of Sciences, Ilam University, Ilam, Iran.*

[2]*Department of Biology, Faculty of Basic Sciences, University of Qom, Qom, Iran.*

[1*]*Corresponding author: a.hatamnia@ilam.ac.ir Pajuhesh Blvd, Ilam University, Ilam province, Iran. Tel: +98-9188440569*

[2*]*Corresponding author: p.malekzadeh@Qom.ac.ir.*

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Abstract

One of the challenges in storing edible mushrooms is the browning of the caps during storage. The use of edible compounds such as potassium can affect the shelf-life of the fruit. In this study, the effects of pretreatment with exogenous L-phenylalanine at concentrations of 0, 1, 2, 5, 7- and 10-mM during storage at 4°C for 15 days were investigated. Compared with the control treatment, the L-phenylalanine treatment delayed the browning of the mushroom caps. A concentration of 5 mM L-phenylalanine was selected for measuring physiological parameters and biochemical enzyme activities. Compared with the control treatment, the 5 mM potassium treatment improved the physiological weight loss, fruit firmness, electrolyte leakage, and malondialdehyde content in the treated group. Additionally, potassium treatment increased the activities of antioxidant enzymes and PAL while reducing polyphenol oxidase (PPO) activity, leading to increased ascorbic acid and total phenol contents compared with those in the control group. Overall, these findings suggest that the reduction in frost damage in edible mushrooms during low-temperature storage may be partially related to improvements in membrane systems and antioxidant systems, including antioxidant compounds and enzyme activities.

Keywords: Storage, L-phenylalanine, polyphenol oxidase, phenylalanine ammonia-lyase, edible mushrooms.

1. Introduction

The white button mushroom, a globally popular edible fungus, is a rich source of essential nutrients, including polysaccharides, minerals, essential amino acids, dietary fiber, vitamins (D, B12, C), and polyphenols. Its nutritional profile, coupled with low cholesterol content and the presence of various bioactive compounds, positions it not only as a valuable food source but also as a promising ingredient in the pharmaceutical industry (Ba et al., 2023; Blasco et al., 2004). However, a major challenge in the mushroom industry is its short shelf life, which typically ranges from 3--4 days at room

temperature. This limited shelf-life is attributed primarily to the absence of a protective cuticle, rendering the mushroom highly susceptible to physical damage, microbial spoilage, and moisture loss. Moreover, the high-water content and respiration rate of mushrooms significantly contribute to tissue softening, moisture loss, and the development of undesirable browning (Brennan et al., 2000; Guo et al., 2022; Jebelli Javan et al., 2015).

Browning, a significant aesthetic and quality concern for consumers, is driven primarily by a cascade of events: increased activity of polyphenol oxidase enzymes, accumulation of phenolic compounds, lipid peroxidation, and disruption of the integrity of the cellular membrane. To address these challenges and extend the shelf-life of mushrooms, edible coatings have emerged as a promising strategy (Ojeda et al., 2019; Shekari et al., 2023). These coatings create a semipermeable barrier that regulates gas and moisture exchange, thereby reducing respiration rates, minimizing the loss of volatile aroma compounds, and preventing excessive moisture loss. This multifaceted approach can significantly increase the shelf-life and maintain the quality of mushrooms during storage and distribution (Brady, 2024; Hu et al., 2015).

Phenylalanine, an essential amino acid, significantly contributes to preserving fruit quality and extending shelf-life after harvest. This amino acid serves as a precursor for the biosynthesis of aromatic compounds, enhancing the flavor and sensory appeal of fruits for consumers. Moreover, phenylalanine stimulates the production of antioxidants, such as flavonoids, which effectively combat oxidative stress, a major contributor to postharvest browning and spoilage (Abdalla et al., 2022; Almas et al., 2021; Osman and El-Naggar, 2022). By mitigating oxidative damage, phenylalanine helps maintain fruit appearance and quality. Furthermore, phenylalanine acts as a signaling molecule, influencing key metabolic processes and growth patterns in fruits. In summary, the utilization of phenylalanine-based strategies holds promise for improving the postharvest lifespan and overall quality of fruits (Nasr et al., 2021; Patel et al., 2023; Wang et al., 2023).

Mushrooms have a short shelf life, significantly limiting their distribution and market reach. While low-temperature storage is crucial for initial quality preservation, prolonged cold storage can negatively impact mushroom quality. To address this, strategies aimed at enhancing the capacity of mushrooms to counteract oxidative stress offer promising solutions. By bolstering the activity of reactive oxygen species (ROS) scavenging systems, we can effectively protect cellular integrity, minimize cap browning, and maintain sensory and nutritional quality during extended storage (Jebelli Javan et al., 2015; Lian et al., 2024; Shekari et al., 2023). Various approaches, including the application of exogenous antioxidants, the use of edible coatings, and the implementation of physical treatments such as irradiation and modified atmosphere packaging, show potential for enhancing the shelf life and market value of mushrooms (Wang et al., 2015; Brennan et al., 2000; Faraj and Nouri, 2024; Gholami et al., 2017).

In this study, a comprehensive assessment of mushroom health and quality was conducted by evaluating various key parameters. Firmness and color, critical determinants of market acceptance, were measured to assess sensory quality. To evaluate cellular integrity and oxidative stress, electrolyte leakage and malondialdehyde (MDA) levels were determined. The activity of antioxidant enzymes, including guaiacol peroxidase and ascorbate peroxidase, was assessed to understand the ability of mushrooms to mitigate oxidative damage. Furthermore, the activities of polyphenol oxidase and phenylalanine ammonia-lyase, enzymes involved in the synthesis of phenolic compounds, were measured to evaluate mushroom defense mechanisms against environmental stresses. Finally, the levels of hydrogen peroxide and ascorbic acid were determined to assess the overall oxidative status and antioxidant capacity of the mushrooms.

2. Material and methods

White button mushrooms were procured from the Mushroom Cultivation and Industry Company in Parand, Tehran, and subsequently transported to the physiology laboratory at Qom University. Following a thorough surface wash and the removal of any defective samples, the mushrooms were immersed in L-phenylalanine solutions at concentrations of 0 mM (control), 1 mM, 2 mM, 5 mM, 7 mM, and 10 mM for 10 minutes. After immersion, the mushrooms

were allowed to air dry at room temperature. The samples were subsequently stored at 4°C for a period of 15 days. Measurements of weight, browning intensity, and selected biochemical parameters were conducted on days 1, 5, 10, and 15, with three biological replicates. A subset of samples was additionally stored at -25°C for subsequent analyses.

2.1. 2.1. Measurement of Physiological Weight Loss (WL)

The weight loss of the button mushrooms was determined by weighing 5 fruits during the 5-, 10-, and 15-day storage periods. The percentage of physiological weight loss was measured via the method described by Malekzadeh et al., (2017). The percentage of weight loss was calculated via a four-decimal Ohaus scale, with weight changes compared with those on day one.

2.2. Measurement of Cap Browning and Firmness

The color of the mushroom caps was evaluated every 5 days during storage at 4°C on 10 mushrooms from each group. The percentage of cap browning was assessed as a browning index according to the method of Xu et al, (2022). The firmness of the mushrooms was evaluated via a fruit firmness tester (Lutron, Germany) according to the method of Shekari et al. (2023).

2.3. Measurement of electrolyte leakage (EL) and malondialdehyde (MDA) content

Electrolyte leakage was measured via a conductivity meter in an aqueous solution containing mushroom pieces. Malondialdehyde (MDA) accumulation was assessed via the thiobarbituric acid (TBA) method Shahbazi et al., (2023). One gram of frozen powder was homogenized in 25 mL of 5% trichloroacetic acid (w/v).

2.4. Measurement of Antioxidant Enzyme Activities

To prepare the enzyme extract, 5 grams of mushroom were ground in 50 mL of potassium phosphate buffer. Then, 10 mL of the extract was centrifuged at 6000 RPM for 10 minutes.

Measurement of Catalase (CAT) Activity

The activity of catalase was measured via the method of Aebi (1984). The mushroom sample was mixed with guaiacol, hydrogen peroxide, and buffer. The enzyme activity resulted in the production of a colored product, and the concentration changes were measured at 240 nm over 60 s via a spectrophotometer.

Measurement of superoxide dismutase (SOD) activity:

The activity of superoxide dismutase was measured according to the methods of Wang et al., (2014). To measure SOD activity in edible mushrooms, samples were mixed with phosphate buffer and stored in a refrigerator. The samples were then centrifuged, and the supernatant was collected. Finally, the supernatant was mixed with ascorbate and hydrogen peroxide, and optical absorbance changes were measured at 560 nm.

2.5. Measurement of polyphenol oxidase (PPO) activity

To measure PPO activity, 10 grams of powdered and frozen mushrooms were prepared according to the method of Xu et al., (2022) and then centrifuged at 15000 RPM for 30 minutes. The resulting supernatant was used as the enzyme extract for PPO activity. The PPO activity was measured in the obtained solution, and the absorbance was assessed at 420 nm via a spectrophotometer.

2.6. Measurement of Phenylalanine Ammonia-Lyase (PAL) Activity

Four grams of powdered sample was combined with 0.4 grams of polyvinylpyrrolidone and 16 mL of borate buffer (pH 8.5) and stirred for 30 minutes. The mixture was subsequently centrifuged at 32000 RPM for 15 minutes, and the resulting supernatant was used as the enzyme extract. The enzyme extract was incubated at 40°C for 5 minutes. Two sets of samples were prepared: one containing the extract and distilled water (control) and the other containing the extract and 100 mM L-phenylalanine. The absorbance of the samples was measured at time zero and after 1 hour at 40°C and 290 nm (Arabia et al., 2023).

2.7. Measurement of Hydrogen Peroxide and Ascorbic Acid Contents

To measure ascorbic acid, 5 grams of mushroom powder were mixed with 15 mL of 5% metaphosphoric acid (Mahmoudi et al., 2022).

2.8. Statistical analysis

All the statistical analyses were performed via SPSS version 25. The data for each analysis were subjected to one-way ANOVA, and the means were separated via the least significant difference (LSD)

test at a significance level of $p < 0.05$. The presented data represent the mean ± standard deviation.

3. Results

3.1. Effect of L-Phenylalanine Treatment on Browning in Edible Mushrooms

Figure 1 shows the changes in cap browning of mushrooms stored at 4°C for 15 days with different concentrations of phenylalanine (Phe). The control group (0 mM Phe) presented a significant increase in cap browning over time, reaching nearly 100% by day 15. In contrast, all the Phe-treated groups presented a significant reduction in cap browning compared with the control group, especially at higher concentrations (5, 7, and 10 mM). On day 5 (week 1), the control group presented approximately 25% cap browning. In contrast, all the Phe-treated groups presented significantly lower levels, with the 10 mM concentration resulting in the least browning. On day 10 (week 2), the difference between the control and Phe-treated groups became more pronounced; the control group reached nearly 60% browning, while the Phe-treated groups remained below 40%, with the 10 mM group still showing the lowest level. By day 15 (week 3), the fruits in the control group had nearly 100% browning, while those in the Phe-treated groups were significantly lower, and those in the 10 mM group remained below 20%. Furthermore, analysis of browning progression over time revealed that the control group presented a continuous increase in cap browning, with a significant difference between each week ($p < 0.05$). Compared with the control group, the Phe-treated groups presented a slower increase in cap browning, with smaller differences between each week. The 10 mM group maintained the most stable browning levels over the 15 days. These results indicate that phenylalanine can effectively inhibit cap browning in mushrooms during cold storage and potentially prolong their shelf-life. The higher the concentration of phenylalanine is, the more effective it is in inhibiting cap browning.

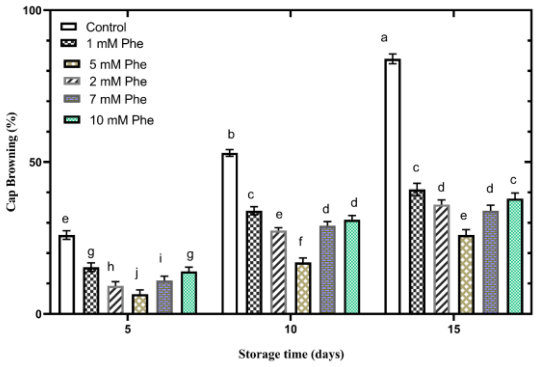

Figure 1- Effects of postharvest Phe treatment (0, 1, 2, 5. 7, and 10 mM) on cap browning of mushrooms during storage at 4°C for 15 d. Each value is expressed as the mean ± SE (n = 3). Different letters indicate significant differences ($P < 0.05$).

3.2. Effect of L-Phenylalanine Treatment on Physiological Weight Loss (WL%) in Edible Mushrooms

Figure 2 shows the changes in mushroom weight loss across different phenylalanine (Phe) concentrations during storage. The control group (0 mM Phe) experienced a substantial increase in weight loss, reaching approximately 7% by day 15. Conversely, the 5 mM Phe treatment significantly mitigated weight loss throughout the 15-day period. On day 5 (week 1), the control group exhibited approximately 1% weight loss, whereas the Phe-treated group presented a lesser degree of weight reduction. This difference became more apparent by day 10 (week 2), with the control group reaching approximately 4% weight loss compared with approximately 2.5% in the Phe-treated group. By day 15 (week 3), weight loss had increased to approximately 7% in the control group and approximately 3% in the Phe-treated group. Analysis of weight loss progression over time revealed a consistent increase in the control group, with statistically significant differences between weeks ($p < 0.05$). The Phe-treated group, however, demonstrated a much smaller increase in weight loss over time, with less pronounced week-to-week variations.

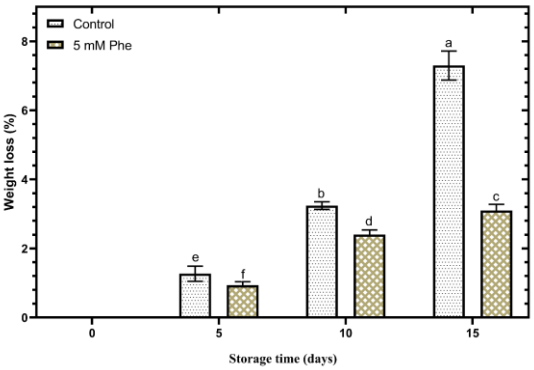

Figure 2- Weight loss of button mushrooms treated with 0- and 5-mM phenylalanine for 15 days at 4 ± 0.5°C. The data correspond to the means ± standard errors of three independent replicates. Different small letters in the same line indicate significant differences ($P < 0.05$) within the same storage period.

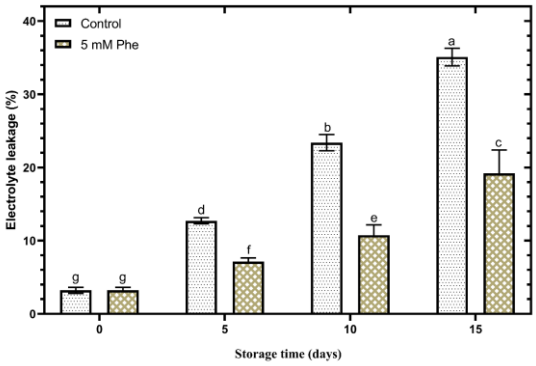

Figure 3- Leakage of button mushrooms treated with 0- and 5-mM phenylalanine for 15 days at 4 ± 0.5°C. The data correspond to the means ± standard errors of three independent replicates. Different small letters in the same line indicate significant differences ($P < 0.05$) within the same storage period.

3.3. Effect of L-phenylalanine treatment on electrolyte leakage (EL%) in edible mushrooms

concentrations of phenylalanine (Phe). The control group (0 mM Phe) presented a significant increase in electrolyte leakage over time, reaching approximately 35% by day 15. In contrast, the Phe-treated group (5 mM) exhibited much lower electrolyte leakage throughout the study period. On day 5 (week 1), the control group presented approximately 5% electrolyte leakage, whereas the Phe-treated group presented less electrolyte leakage. On day 10 (week 2), the difference between the control group and the Phe-treated group became more pronounced; the control group reached approximately 23% electrolyte leakage, whereas the Phe-treated group showed approximately 11% electrolyte leakage. By day 15 (week 3), the control group reached approximately 35% electrolyte leakage, whereas the Phe-treated group showed approximately 19% electrolyte leakage. When comparing weeks for each concentration, the control group displayed a continuous increase in electrolyte leakage over time, with significant differences between each week ($p < 0.05$). The Phe-treated group presented less electrolyte leakage over time and smaller differences each week.

3.4. Effect of L-Phenylalanine Treatment on Malondialdehyde (MDA) Content in Edible Mushrooms

Figure 4 shows the changes in the MDA content (µmol g-1 FW) of mushrooms stored for 15 days in the presence or absence of phenylalanine (Phe). The control group (without Phe) presented a significant increase in the MDA content over time, reaching approximately 16 units by the end of the period. In contrast, the Phe-treated group (5 mM) presented a much smaller increase in MDA, with the MDA levels in this group reaching approximately 9 units on day 15. Statistical analysis revealed that the difference between the control and treated groups was significant at all time points ($p < 0.05$). Additionally, the increase in MDA in the control group was significant between weeks ($p < 0.05$), whereas the increase in the treated group was smaller and likely not significant. These results indicate that Phe can effectively prevent an increase in MDA and, consequently, oxidative damage in mushrooms.

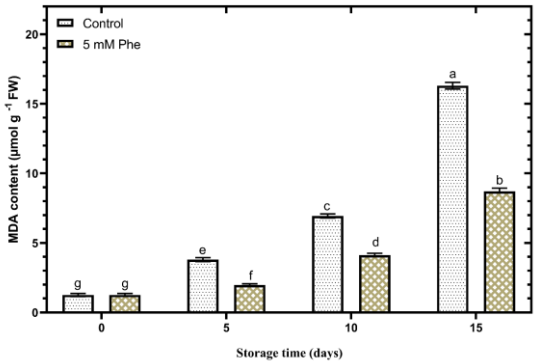

Figure 4- MDA content of button mushrooms treated with 0- and 5-mM phenylalanine for 15 days at 4 ± 0.5°C. The data correspond to the means ± standard errors of three independent replicates. Different small letters in the same line indicate significant differences ($P < 0.05$) within the same storage period.

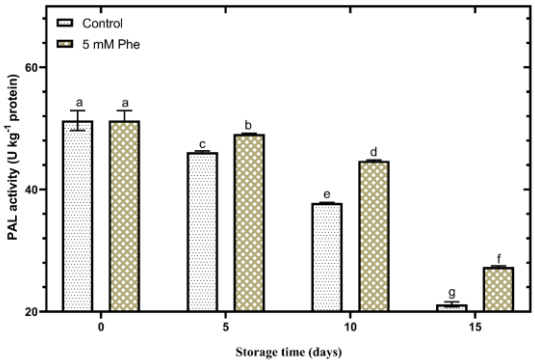

Figure 5- PAL activity of button mushrooms treated with 0- and 5-mM phenylalanine for 15 days at 4 ± 0.5°C. The data correspond to the means ± standard errors of three independent replicates. Different small letters in the same line indicate significant differences ($P < 0.05$) within the same storage period.

3.5. Effect of L-Phenylalanine Treatment on PAL Activity in Edible Mushrooms

Figure 5 presents the activity of polyphenol oxidase (PPO), an enzyme involved in browning, in mushrooms over a 15-day storage period. Two groups were compared: a control group (without phenylalanine) and a group treated with 5 mM phenylalanine. The control group maintained relatively low and stable PPO activity throughout the 15 days. In contrast, the phenylalanine-treated group presented a more pronounced increase in PPO activity, peaking at days 5 and 10. These results suggest that phenylalanine may stimulate PPO production in mushrooms, potentially contributing to increased browning.

3.6. Effect of L-Phenylalanine Treatment on PPO Activity in Mushrooms

Figure 6 shows the changes in the activity of the polyphenol oxidase (PPO) enzyme in mushrooms stored for 15 days in the presence or absence of phenylalanine (Phe). The control group (without Phe) presented relatively low PPO activity throughout the experiment. In contrast, the Phe-treated group (5 mM) presented a significant increase in PPO activity, particularly on days 5 and 10. However, from day 10 onward, the enzyme activity slightly decreased. Statistical analysis revealed that the mean PPO activity in the treated group was significantly greater than that in the control group ($p < 0.05$). These findings suggest that Phe can act as a stimulating agent for the production of the PPO enzyme in mushrooms and may lead to increased browning. The decrease in PPO activity from day 10 may indicate the regulation of enzyme levels after the initial response to stimulation.

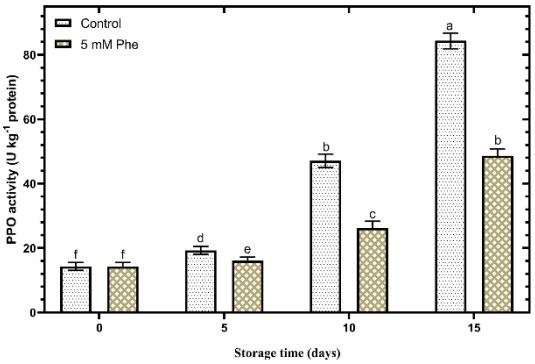

Figure 6- PPO activity of button mushrooms treated with 0- and 5-mM phenylalanine for 15 days of storage at 4 ± 0.5°C. The data correspond to the means ± standard errors of three independent replicates. Different small letters in the same line indicate significant differences ($P < 0.05$) within the same storage period.

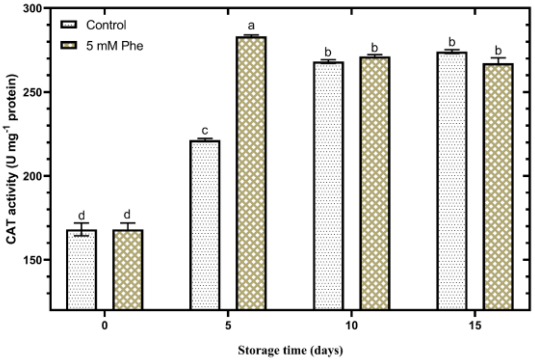

Figure 7- CAT activity of button mushrooms treated with 0- and 5-mM phenylalanine for 15 days of storage at 4 ± 0.5°C. The data correspond to the means ± standard errors of three independent replicates. Different small letters in the same line indicate significant differences ($P < 0.05$) within the same storage period.

3.7. Effect of L-Phenylalanine Treatment on CAT Activity in Edible Mushrooms

Figure 7 shows the changes in the activity of the enzyme catalase (CAT) in mushrooms stored for 15 days in the presence and absence of phenylalanine (Phe). The control group (without Phe) presented relatively low CAT activity throughout the experiment. In contrast, the Phe-treated group (5 mM) presented a significant increase in CAT activity, particularly on days 5, 10, and 15. Statistical analysis revealed that the mean CAT activity in the treated group was significantly greater than that in the control group ($p < 0.05$). These findings suggest that Phe can act as an inducing agent for the production of the CAT enzyme in mushrooms and may increase their resistance to oxidative stress.

3.8. Effect of L-Phenylalanine Treatment on SOD Activity in Edible Mushrooms

Figure 8 shows the changes in the activity of the enzyme superoxide dismutase (SOD) in mushrooms stored for 15 days in the presence or absence of phenylalanine (Phe). The control group (without Phe) presented relatively low SOD activity throughout the experiment. In contrast, the Phe-treated group (5 mM) presented a significant increase in SOD activity, particularly on days 5, 10, and 15. Statistical analysis revealed that the mean SOD activity in the treated group was significantly greater than that in the control group ($p < 0.05$). These findings suggest that Phe can act as an inducing agent for the production of the SOD enzyme in mushrooms and may increase their resistance to oxidative stress.

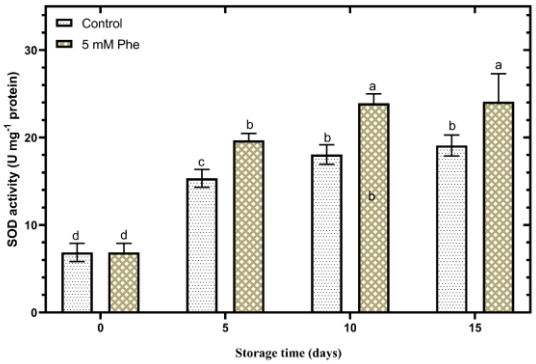

Figure 8- SOD activity of button mushrooms treated with 0- and 5-mM phenylalanine for 15 days at 4 ± 0.5°C. The data correspond to the means ± standard errors of three independent replicates. Different small letters in the same line indicate significant differences (P < 0.05) within the same storage period.

3.9. Effect of L-Phenylalanine Treatment on the Ascorbic Acid Content in Edible Mushrooms

The figure shows the changes in the level of ascorbic acid (AsA) in mushrooms stored for 15 days in the presence and absence of phenylalanine (Phe). The AsA levels in the control group (without Phe) gradually decreased over time. In contrast, the Phe-treated group (5 mM) maintained higher AsA levels throughout the storage period, particularly on days 5, 10, and 15. Statistical analysis revealed that the mean AsA level in the treated group was significantly greater than that in the control group ($p < 0.05$). These findings suggest that Phe can help maintain AsA levels in mushrooms and protect them against oxidative stress.

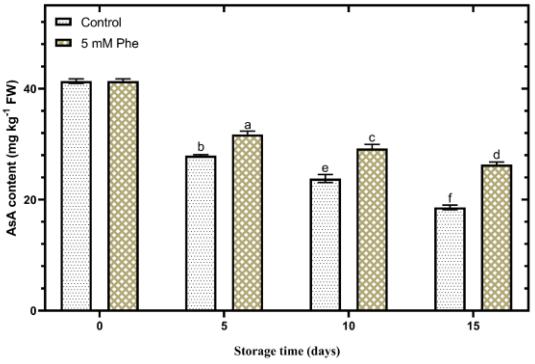

Figure 9- AsA content of button mushrooms treated with 0- and 5-mM phenylalanine for 15 days of storage.

3.10. Effect of L-Phenylalanine Treatment on Hydrogen Peroxide Content

The figure shows the changes in the level of hydrogen peroxide (H_2O_2) in mushrooms stored for 15 days in the presence and absence of phenylalanine (Phe). The control group (without Phe) showed a gradual increase in H_2O_2 levels over time. In contrast, the Phe-treated group (5 mM) showed lower H_2O_2 levels throughout the storage period, particularly on days 10 and 15. Statistical analysis showed that the mean H_2O_2 level in the treated group was significantly lower than the control group ($p < 0.05$). These findings suggest that Phe can help reduce H_2O_2 levels in mushrooms and protect them against oxidative stress.

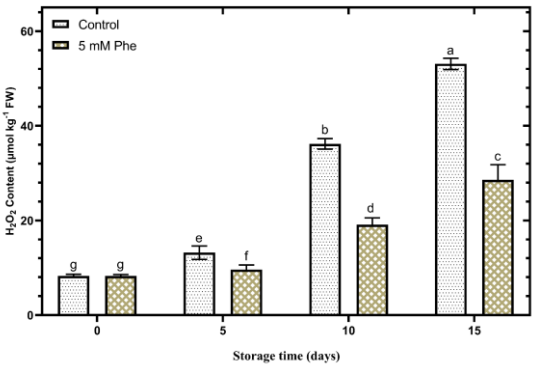

Figure 10- H2O2 content of button mushroom treated with 0- and 5-mM phenylalanine during 15 days of storage at 4 ± 0.5 °C. Data corresponds to the means ± standard error of three independent replicates. Different small letters in the same line show significant differences (P < 0.05) within the same storage period.

3.11. Total Phenolic Compounds in Edible Mushrooms

The figure shows the changes in the level of phenolic compounds in mushrooms stored for 15 days in the presence and absence of phenylalanine (Phe). The control group (without Phe) showed a gradual decrease in the level of phenolic compounds over time. In contrast, the Phe-treated group (5 mM) showed a higher level of phenolic compounds throughout the storage period, particularly on days 5 and 10. Statistical analysis showed that the mean level of phenolic compounds in the treated group was

significantly higher than the control group ($p < 0.05$). These findings suggest that Phe can help maintain the level of phenolic compounds in mushrooms and protect them against oxidative stress.

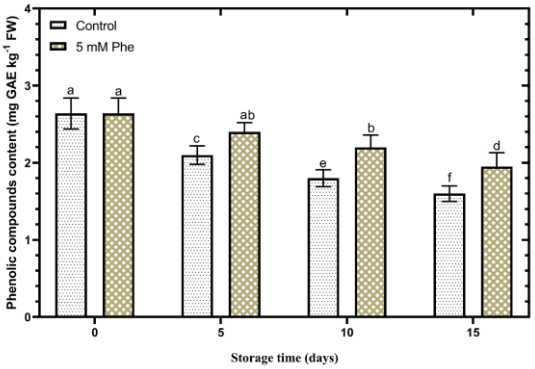

Figure 11- Phenolic compounds content of button mushroom treated with 0- and 5-mM phenylalanine during 15 days of storage at 4 ± 0.5 °C. Data correspond to the means ± standard error of three independent replicates. Different small letters in the same line show significant differences ($P < 0.05$) within the same storage period.

3. Discussion

The results of this study showed that treating edible mushrooms with phenylalanine (Phe) during storage at 4°C effectively protects their quality. Figure 1 clearly shows that Phe treatment, especially at higher concentrations (5, 7 and 10 mM), significantly prevents cap browning of mushrooms compared to the control group (without Phe). While the control group was almost completely browned by day 15, the Phe-treated groups, especially at 10 mM, showed a significant reduction in browning. This protective effect became more apparent over time, specifically in the second and third weeks. Statistical analysis also confirmed that the difference between the control group and the Phe-treated groups was significant over time ($p < 0.05$). These findings suggest that Phe can act as an anti-browning compound in mushrooms and potentially extend their shelf life. These findings are consistent with previous studies that have shown that Phe can prevent enzymatic browning in some fruits and vegetables (Chen, He et al. 2008, Hattori, Chen et al. 2019, Nasr, Pateiro et al. 2021, Abdalla, Sadak et al. 2022).

Physiological weight loss is also an important factor in mushroom quality. Figure 2 shows that Phe treatment (5 mM) effectively prevents weight loss of mushrooms during storage. While the control group experienced about 7% weight loss by day 15, the Phe-treated group had only about 3% weight loss. This significant difference ($p < 0.05$) suggests that Phe can help maintain the moisture and weight of mushrooms and prevent their shrinkage and quality loss. These results are consistent with studies that have shown that Phe can help maintain the weight and firmness of fruits and vegetables during storage (Leyva, Jarillo et al. 1995, Kumar, Elazari et al. 2021, Osman and El-Naggar 2022, Peng, Wang et al. 2022).

Electrolyte leakage is another indicator of membrane damage and cell degradation. Figure 3 shows that Phe treatment (5 mM) significantly prevents electrolyte leakage compared to the control group. This finding suggests that Phe can help maintain the integrity of the cell membrane of mushrooms and protect them against stressors. These findings are consistent with previous studies that have shown that Phe can prevent membrane damage in plant cells under oxidative stress conditions (Roubelakis-Angelakis and Kliewer 1986, Nasr, Pateiro et al. 2021, Xie, Yang et al. 2022, Wang, Li et al. 2023).

Oxidative stress plays an important role in reducing the quality and shelf life of mushrooms. Figure 4 shows that the level of MDA, as an indicator of oxidative stress, increases dramatically in the control group over time. In contrast, Phe treatment (5 mM) effectively prevents the increase in MDA. This finding suggests that Phe can act as an antioxidant compound and protect mushrooms against damage from free radicals. These results are consistent with studies that have shown that Phe can act as an antioxidant in various biological systems (Riov, Monselise et al. 1969, Zhang, Zhang et al. 2008, Qian, Lynch et al. 2019).

Antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) play an important role in combating oxidative stress. Figures 7 and 8 show that Phe treatment (5 mM) significantly increases the activity of these enzymes compared to the control group. This increased activity of antioxidant enzymes suggests that Phe can strengthen the antioxidant defense system of mushrooms and protect them against oxidative stress. These findings are consistent with the results of previous studies that have shown that Phe can increase the activity of antioxidant enzymes in plants and animals (Chen, He et al. 2008,

Aghdam, Moradi et al. 2019, Sogvar, Rabiei et al. 2020, Almas, -un-Nisa et al. 2021, Kumar, Elazari et al. 2021, Abdalla, Sadak et al. 2022, Peng, Wang et al. 2022).

In addition, Phe treatment showed a positive effect on the levels of ascorbic acid (AsA) and phenolic compounds (Figures 9 and 11). These compounds are also known as natural antioxidants and play an important role in maintaining the quality and nutritional value of mushrooms. Maintaining higher levels of these compounds in Phe-treated mushrooms suggests that this compound can help maintain the antioxidant properties and nutritional value of mushrooms during storage. These results are consistent with studies that have shown that Phe can help increase the levels of phenolic compounds and ascorbic acid in some plants (Kumar, Elazari et al. 2021, Osman and El-Naggar 2022, Xie, Yang et al. 2022, Wang, Li et al. 2023).

In contrast, the activity of the enzyme polyphenol oxidase (PPO), which plays a role in the browning of mushrooms, increased in the Phe-treated group (Figures 5 and 6). This finding may seem contradictory at first glance to the results related to the reduction of browning (Figure 1). However, the increase in PPO may be due to the induction of defense mechanisms in mushrooms in response to Phe treatment. In other words, the increase in PPO may be part of the complex response of the mushroom to Phe and does not necessarily mean an increase in browning. In fact, the results show that despite the increase in PPO, Phe prevents browning, which is probably due to its antioxidant and protective effects on other aspects of mushroom quality. Similarly, some studies have shown that increasing PPO in some plants can help increase their resistance to pathogens and environmental stresses (Xie, Yang et al. 2022, Wang, Li et al. 2023).

Finally, Figure 10 shows that Phe treatment can help reduce the level of hydrogen peroxide (H2O2) in mushrooms. Reducing H2O2 can also help reduce oxidative stress and improve the quality of mushrooms. These findings are consistent with the results of previous studies that have shown that Phe can help reduce H2O2 production in plant cells under oxidative stress conditions (Machado, Felizardo et al. 2013, Almas, -un-Nisa et al. 2021).

In general, the results of this study indicate that phenylalanine can be used as an effective compound to maintain the quality and increase the shelf life of edible mushrooms during storage. By affecting various quality indicators, including browning, weight loss, electrolyte leakage, oxidative stress and the activity of antioxidant enzymes, this compound can help maintain the freshness, nutritional value and marketability of mushrooms.

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