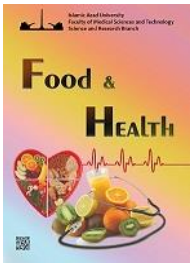

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Evaluation of the effect of polyamines and organic acids treatment on the nutritional value of button mushroom (*Agaricus bisporus*)

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ABSTRACT

To investigate the effect of polyamines and some organic acids' application on postharvest life of button mushroom (*Agaricus bisporus*), an experiment was conducted in 2016. The treatments included putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) and the control. Treatments were used in two stages, before and after harvest, in two separate experiments based on a completely randomized design with three replications. At the end of the experiment, the nutritional value including, K, Ca, Fe, Vitamin C, TSS, and Protein in button mushroom were measured. The results indicated that in post-harvest stages, the maximum level of protein and Vitamin C were obtained in response to use of spermidine 1.5 mM. The maximum value of protein was observed at 3 mM putrescine and 30 mM Vitamin C at post-harvest stages. The maximum level of TSS was shown in spermidine 1 mM in both pre- and post-harvest. The application of treatments affect on micro and macro elements significantly. It can be said that application of treatments in the post-harvest stage has more influence on the nutritional value and durability of button mushroom.

1. Introduction

Agaricus bisporus is an edible basidiomycete mushroom is cultivated in more than seventy countries. It belongs to the *Agaricaceae* family native to grasslands in Europe and North America which has two color states while immature –white and brown– both of which have various names, with additional names for the mature state (1). In a 100-gram serving, raw white mushrooms provide 93 kilojoules (22 kilocalories) of food energy and are an excellent source (>19% of the Daily Value, DV) of the B vitamins, riboflavin, niacin, and pantothenic acid. Fresh mushrooms are also a good source (10–19% DV) of the dietary mineral phosphorus. While fresh *A. bisporus* only contains 0.2 micrograms (8 IU) of vitamin D as ergocalciferol (vitamin D2), the ergocalciferol content increases substantially after exposure to UV light (2). Mushrooms include some of the most intense natural medicines on the earth. About 100 species of mushrooms are being investigated for their health-boosting profits. Of those hundred, about a half dozen stand out for their potency to release a massive promotion to the human immune system. It is important to eat only organically grown mushrooms because they absorb and concentrate whatever they cultivate in good or bad beds. Mushrooms are famous to accumulate heavy metals, as well as air and water pollutants, so healthy growing conditions is a critical factor. Edible mushrooms contain high amounts of ash, 80–120 g/kg of dry matter (mainly potassium, phosphorus, magnesium, calcium, copper, iron, and zinc). Carbohydrates are found in high proportions in edible mushrooms, including chitin, glycogen, trehalose, and mannitol; besides, they contain fiber, β-glucans, hemicelluloses, and pectic substances. Mushrooms contain just as high an antioxidant capacity as carrots, tomatoes, green and red peppers, pumpkins, green beans, and zucchini. Selenium is a mineral that does not exist in most fruits and vegetables but can be found in mushrooms. It plays a role in liver enzyme function and helps detoxify some cancer-causing compounds in the body. Many nutraceutical properties are described in mushrooms, such as the prevention or treatment of Parkinson, Alzheimer's, hypertension, and high risk of

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stroke. Additionally, selenium inhibits inflammation and reduces tumor growth levels (3). The fiber, potassium, and vitamin C content in mushrooms all provide cardiovascular health. Potassium and sodium work together in the body to help regulate blood pressure. Using mushrooms, which are high in potassium and low in sodium helps to lower blood pressure and decrease the risk of high blood pressure and cardiovascular diseases (4). It is important to remark that the growth characteristics, stage, and postharvest condition may influence the chemical composition and the nutritional value of edible mushrooms. However, this product is very spoilable and loses its marketability and nutritional value after harvest quickly. Internal and external browning is related to oxidation of phenolic compounds by the polyphenol oxidase enzyme (5). Organic acids have also been used in many studies to prolong the storage life and longevity of mushrooms. Ascorbic acid chemically is a derivative of sugars and is considered as a strong reluctant. It balances the activity of polyphenol oxidase through the reduction of A-quinones to their phenolic structure (6). Citric acid is an organic acid and probably causes complicated effects in plant metabolism, which increase post-harvest life is one of their effects. The effect of polyamines on longevity and post-harvest life of fungi has less been investigated (7). Therefore, this study was conducted the effect of some polyamines and organic acids on post-harvest life and nutritional value of button mushroom (*Agaricus bisporus*).

2. Material and methods

This research in contrast to a simple experimental design, which contains two levels: For this purpose, the mushroom spawn was purchased from Sepahan Yekta Mushroom Company. After the preparation of compost and cultivation rows, the mushroom mycelia were placed inside the bed, and operation was done during the growth of mushroom pins including management and controlling the temperature of compost air and hall air, uniform circulation of air, and level of carbon dioxide accurately. Treatments included putrescine (1, 2, and 3 mM), spermine (0.75, 1, and 1.5 Mm), spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) and control (distilled water). The experiments were carried out based on a completely randomized design with three replications. In the first stage (pre-harvest treatment), the treatment solutions were sprayed on the surface of mushrooms with irrigation water 24 hours before mushrooms harvesting. Then, they were packed in disposable cellophane products and were kept in a refrigerator at 4-5°C and humidity of 80-85% for 15 days. In the second experiment (post-harvest treatment), the mushrooms were placed at the refrigerator and transferred to the laboratory immediately after the harvest. In the laboratory, samples were immersed in treatment solutions, which were already prepared, and after drying of the surface; they were packaged and kept in the refrigerator with a similar temperature and humidity as with the first stage. Total soluble solids concentration (TSS) of the extracts were determined by refractor meter. Vitamin C was measured by titration with PotassiumIodide and was calculated with the formula of Shafiee et al. (8). K, Ca, Fe was determined by Atomic absorption spectrometers (Varian- SpectrAA.200). Protein was studied according to Sood et al (9).

3. Results

3.1. The effect of different treatments on the extent of TSS

The application of different pre-harvest treatments indicated significant effects on TSS (Table 1). Spermidine 1 mM treatment created the maximum level of TSS (5.8%), whereas the lowest level (3.7 %) was observed in control. TSS was also significantly influenced by different post-harvest treatments (Table 2). Utilized spermidine 1.5 mM obtained the maximum rate (5.9 %), which did not show any significant difference with other treatments including spermine 1 Mm. The lowest level of TSS (3.9%) was gain in response to the use of ascorbic acid 10 mM (Fig. 1, 2).

Table 1. Analyze variance pre-harvest.

SOV	DF	Fe	K	Ca	TSS	Protein	Vitamin C
Rep	2	0.08	0.19	0.67	0.9	0.11	0.09
Treatment	4	0.73	18.28	12.20	12.4	23.60	11.02
CV	-	8.1	8.11	12	9.8	15.1	15.2

Table 2. Analyze variance post-harvest.

SOV	DF	Fe	K	Ca	TSS	Protein	Vitamin C
Rep	2	0.05	0.17	0.66	0.7	0.19	0.20
Treatment	4	0.65	17.08	11.20	19.4	33.60	12.4
CV	-	7.1	4.11	11	8.1	9.3	6.7

3.2. The effect of different treatments on protein content

Pre-harvest treatment applications significantly affected protein content (Table 1). The maximum level (3.76 100g-1F.W) was measured in spermidine 1.5 mM treatment. Also, the lowest ones was obtained in 10Mm ascorbic acid (1.45 100g-1F.W). However, there was no significant difference with spermine 0.75 mM. Protein content significantly was impacted by different treatments at the post-harvest stage (Table 2). In this stage, the maximum protein rates were

obtained at (3.64 100g-1F.W) was observed in putrescine 3 mM while the lowest level (1.68 100g-1F.W) was shown in the control treatment (Fig. 3, 4).

3.3. The effect of different treatments on the level of Vitamin C

In the pre-harvest and post-harvest stage, Vitamin C levels were significantly affected by the use of different treatments (Table 1, 2). The highest amount of vitamin C (2.53 mg /100 g F.W) was obtained as a result of using 1.5 mM spermidine. Control treatment produced the lowest amount of vitamin C (1.1 mg /100 g F.W). Vitamin C levels were significantly affected by the use of different treatments. The highest amount of vitamin C (2.60 mg /100 g F.W) was illustrated as a result of 30 mM ascorbic acid. Control treatment had the lowest amount of vitamin C (1.07 mg /100 g F.W). (Fig. 5, 6).

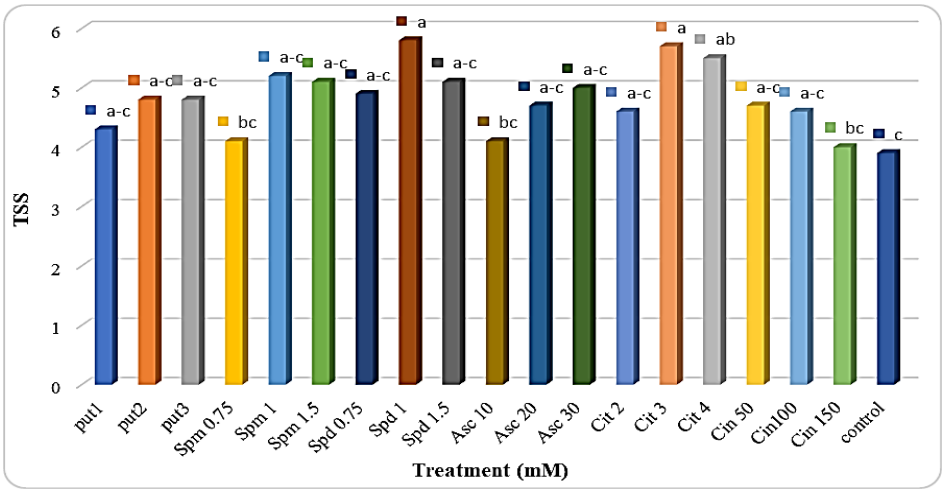

Fig. 1. Changes TSS to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [PRE- HARVEST).

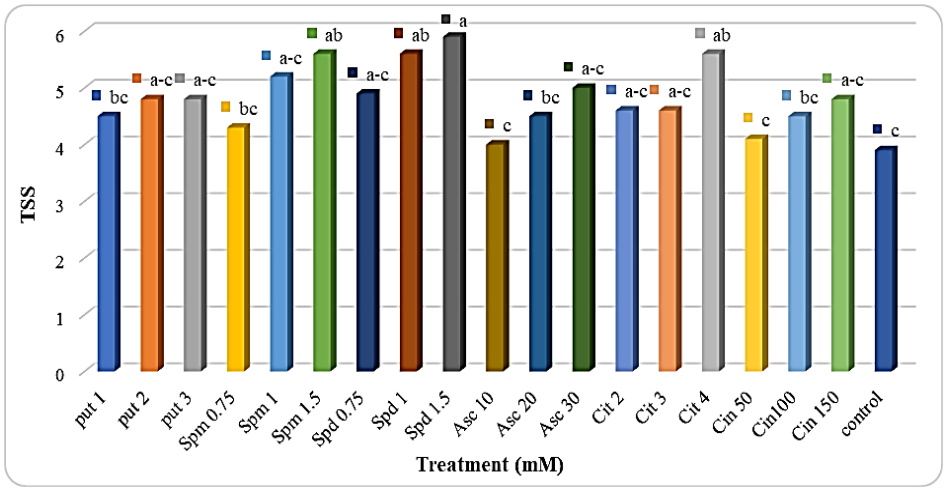

Fig. 2. Changes TSS to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [POST- HARVEST).

3.4. The effect of different treatments on Calcium

In the pre-harvest stage, the amount of calcium was affected by the use of different treatments (Table 1), so that the use of 30 mM ascorbic acid gives rise to the highest amount of calcium (272 mg/kg D.W). The lowest amount of calcium (191 mg/kg D.W) was induced because of applying spermine treatment of 0.75 mM. Calcium was influenced by the application of different treatments at post-harvest (Table 2), spermine 1.5 mM promoted the highest rate of calcium (258.3 mg/kg D.W). The lowest level of calcium (200 mg/kg D.W) was shown from the application of spermidine treatment of 0.75 mM (Fig.7, 8).

3.5. The effect of different treatments on the level of Iron

Using different treatments at pre- and post-harvest displays a significant effect on iron content (Table 1, 2). The highest

amount of Fe (78.3 mg/kg D.W) was obtained as a result of the application of 150 Mm cinnamic acid, while the lowest (55 mg/kg D.W) was obtained at control treatment. Similar results were achieved at post-harvest. The maximum dosage of iron (80 mg/kg D.W) was presented by the application of 30 mM ascorbic acid; while the lowest deal of iron, (63.3 mg/kg D.W) was measured using 0.75 mM spermine and 1 mmol spermine treatments (Fig. 9, 10).

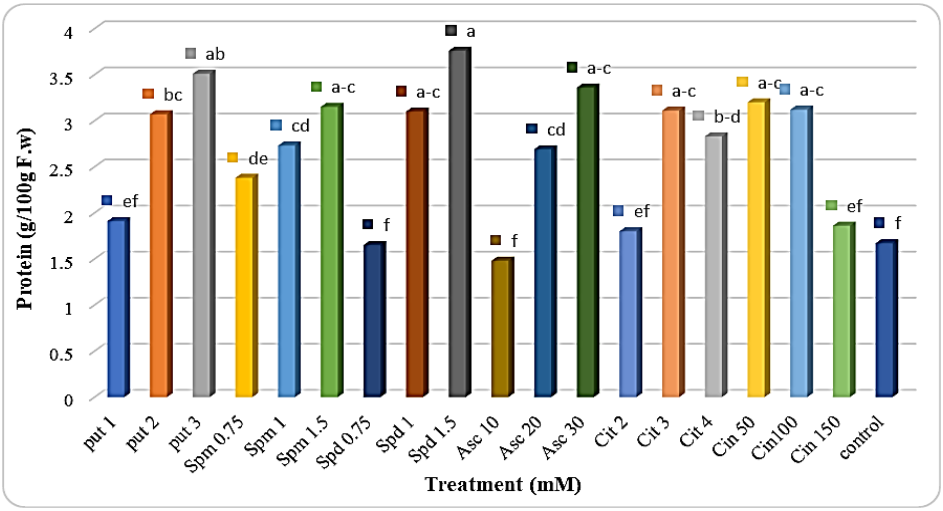

Fig. 3. Changes protein to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [PRE- HARVEST).

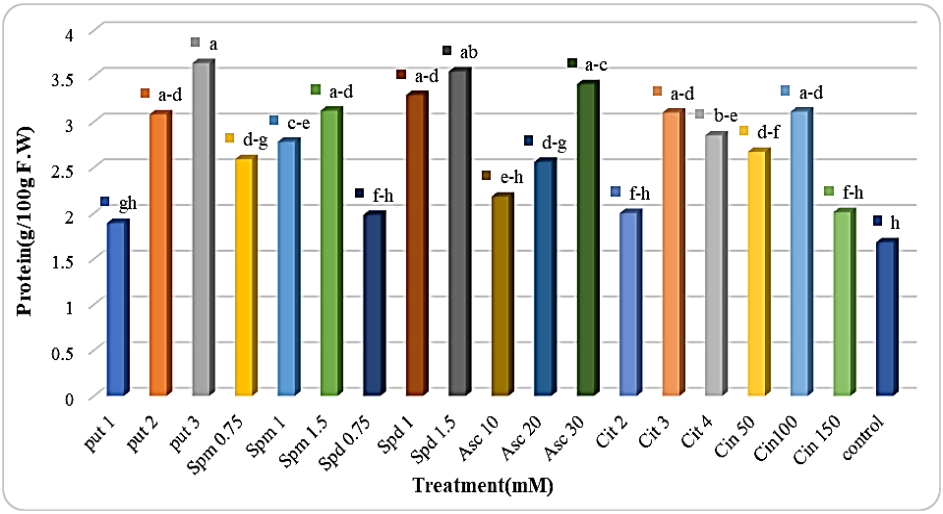

Fig. 4. Changes protein to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [POST HARVEST).

3.6. The effect of different treatments on the Potassium

In the pre-harvest stage, potassium content was simulated by the different treatments (Table 1). Application of 1 mM spermidine produced the highest level of potassium (3033.33 mg/kg D.W) and control treatment created the lowest amount of potassium (2296.67 mg/kg D.W). Similar results were illustrated in the post-harvest stage (Table 2). 1.5 mM spermidine produced the extreme dosage of potassium (3083.33 mg/kg. W) and 10 mM ascorbic acid made the lowest amount of potassium (2626.67 mg / kg D.W) (Fig. 11, 12).

4. Discussion

Because polyamines play important roles in diverse plant growth and developmental processes and environmental stress responses, they are considered as a new kind of plant biostimulant. In general, numerous studies have shown that the external application of polyamines affects fruit quality and

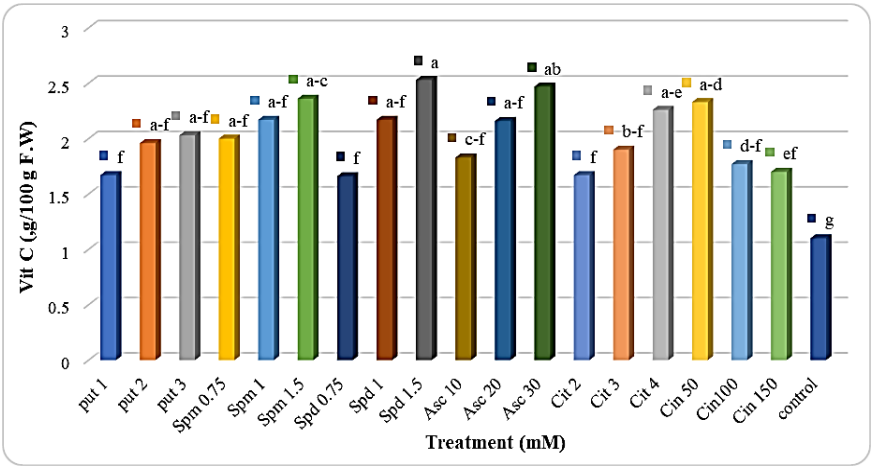

Fig. 5. Changes Vit C to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [PRE- HARVEST).

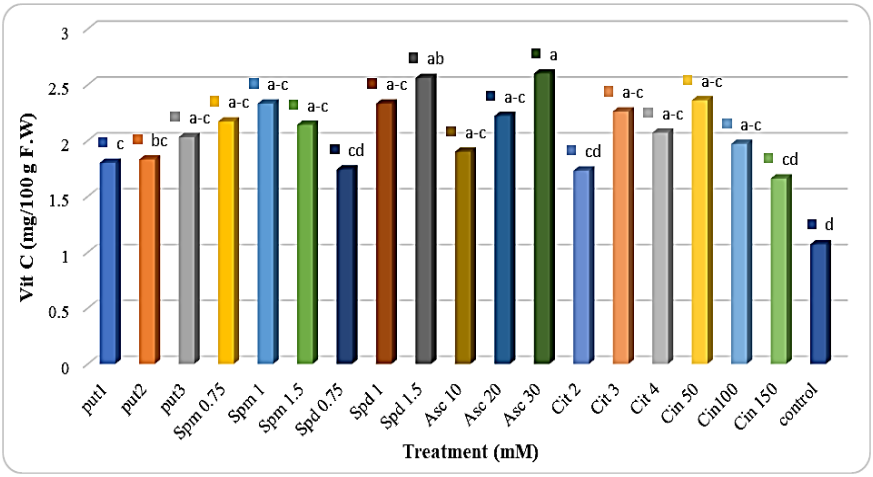

Fig. 6. Changes Vit C to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [POST- HARVEST).

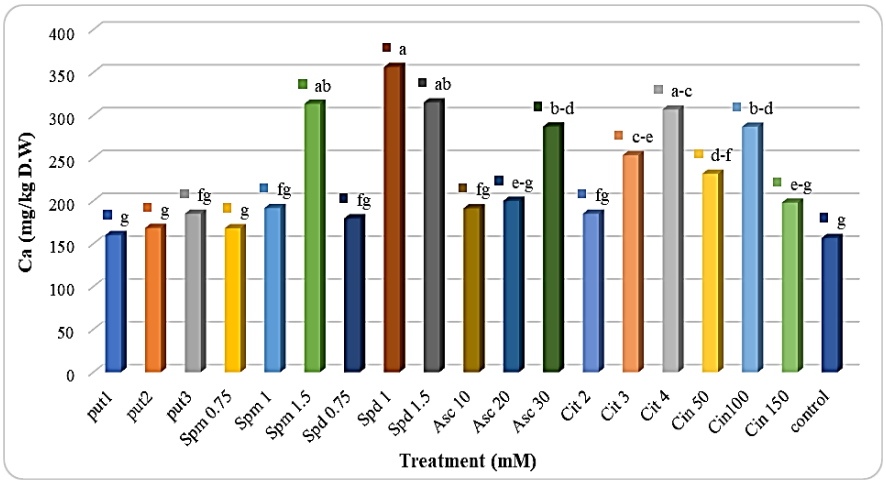

Fig. 7. Changes Ca to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [PRE- HARVEST).

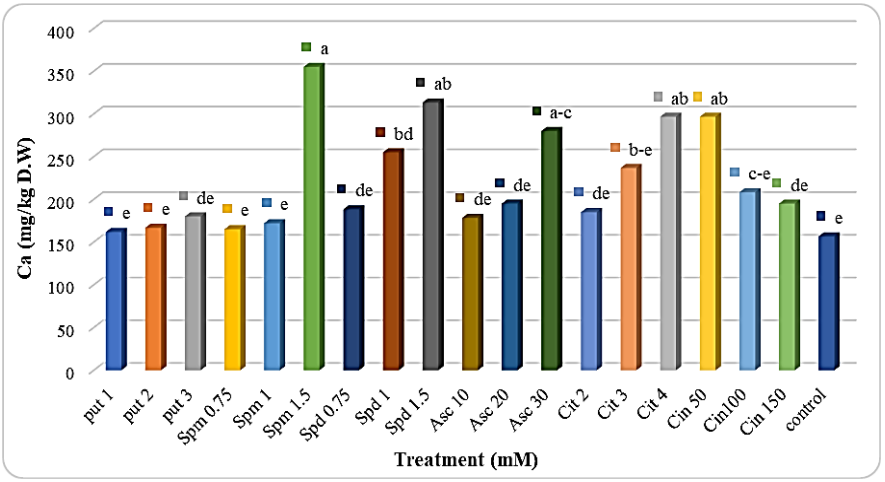

Fig. 8. Changes Ca to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [POST- HARVEST).

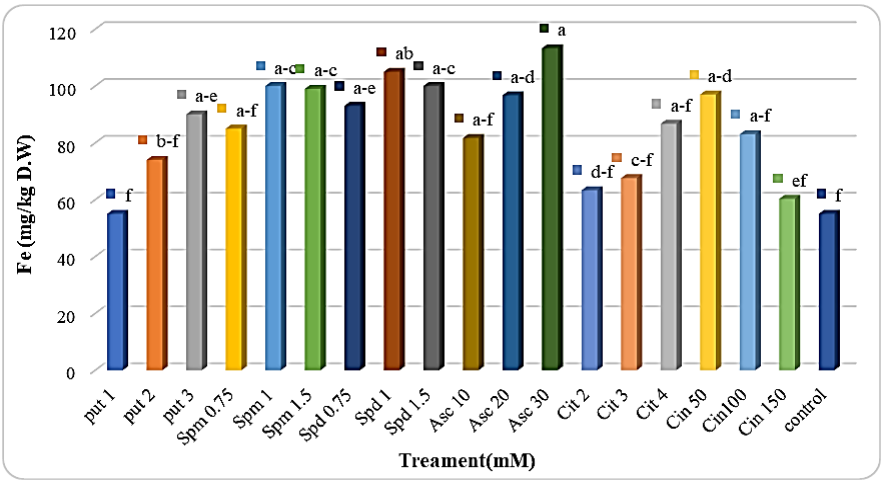

Fig. 9. Changes F to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [PRE- HARVEST).

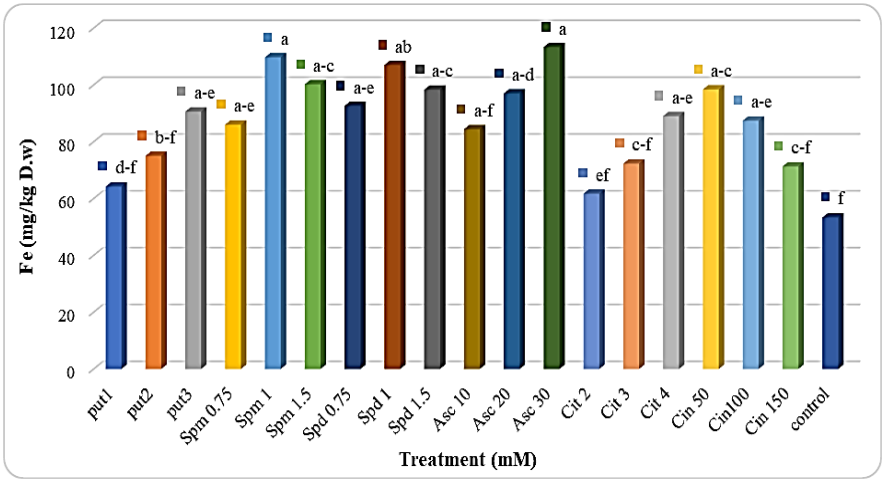

Fig. 10. Changes Fe to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [POST- HARVEST).

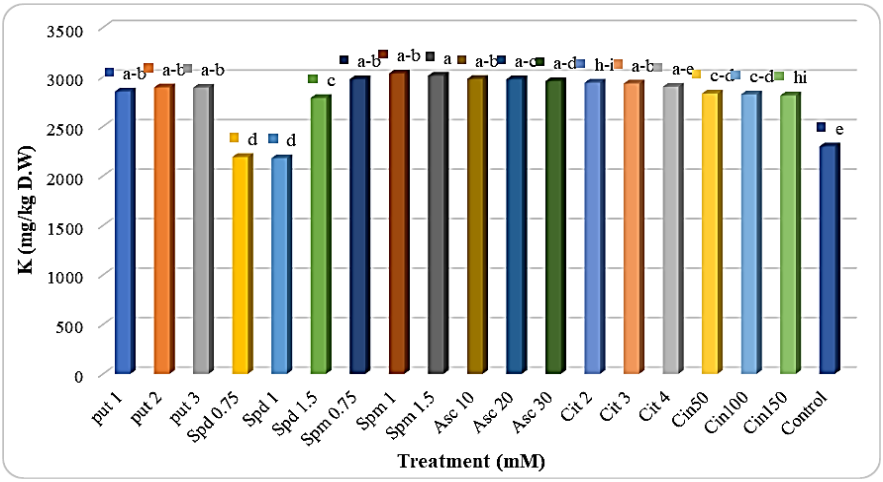

Fig. 11. Changes K to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [PRE- HARVEST).

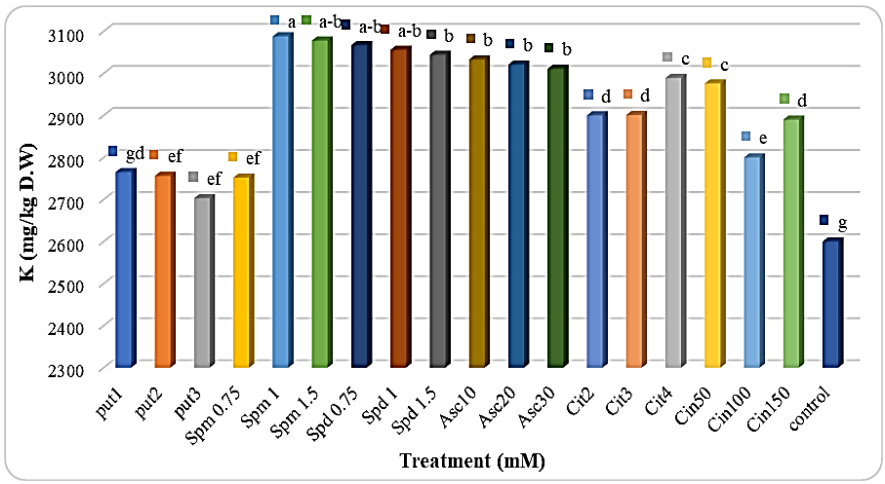

Fig. 12. Changes K to polyamines supplemented with ascorbic acid and cinnamic acid; putrescine (1, 2, and 3 mM), spermine and spermidine (0.75, 1, and 1.5 mM), ascorbic acid (10, 20, and 30 mM), citric acid (2, 3, and 4 mM), cinnamic acid (50, 100, and 150 mM) [POST- HARVEST).

soluble solids content. The presence of polyamines in all plant organs indicates their key role in plant growth regulation and maintaining fruit quality (10). Jafarpour et al. (11) also reported that the use of polyamines has a significant effect on the amount of soluble solids. An increasing soluble solid was observed under control treatment in strawberry. However, in the study of Assar et al. the percentage of soluble solids in some fruit juices related to polyamine treatments was significantly lower than the control. These contradict may be due to differences in product physiology and laboratory conditions (12). Endogenous polyamines are linked strongly to RNA and DNA and proteins (13), they also inhibit RNA-as enzyme activity and protease (14). Therefore, the reason for maintaining the highest protein level in the 1.5 mM spermidine treatment in this experiment may be due to the polyamine linking to the protein and the prevention of degradation. It is possible that spermidine treatments, by maintain the acidic condition of the fruit, preserve the total acidity of the fruit extract, and are effective on the stability and durability of vitamin C during the storage period. It is also possible that spermine and spermidine are effective in preventing severe vitamin C degradation during storage due to their competitive effect with ethylene hormone and due to the delay in the maturation process (15). The application of treatments influenced micro and macro elements significantly. In line with our study, the application of polyamines leads to increasing the concentration of calcium and iron in shoots and roots Strawberries, so that the highest amount of calcium in the shoots and roots of strawberries was observed in plants treated with spermidine 500 mg/L. (16). Enhancement of the biosynthesis of polyamines can protect plants from stress by eliminating ROS, stabilizing membranes and cell structures, balancing cations and anions, regulating ion channels, and increasing cell energy by stimulating ATP production (17). Increased uptake of nitrogen, phosphorus, and potassium minerals by polyamine treatment and increased growth and

productivity may be due to the effects of polyamines on numerous biochemical and physiological processes. The use of exogenous polyamines has been reported to reduce the effect of salinity on photosynthetic efficiency by preventing the degradation of DNA molecules by oxygen free radicals produced under salinity stress, but this effect strongly depends on the concentration of polyamines or the type and level of stress. An Arabidopsis double knockout (acl5/spms) compromised for tSpm and Spm biosynthesis showed hypersensitivity to NaCl and KCl but not to MgCl2. Altered tSpm and Spm levels in the mutant were shown to impair Ca^{++} homeostasis thereby affecting their overall monovalent: bivalent charge ratio leading to a differential response to stimulations (18, 19).

5.Conclusion

Based on the results of this research, it seems that the treatment of button mushrooms with spermidine and putrescine, in both pre- and post-harvest stages, resulted in the elevation of TSS and delay of senescence and increasing of the nutritional value of button mushrooms in response to the conjugation of these polyamines with protein molecules and prevention of their breakdown. However, according to this study, to enhance Vitamin C of button mushrooms, 1.5 mM putrescine treatment is the most effective factor. Post-harvest treatment button mushrooms with 1.5 mM spermidine could preserve the quality and increased protein and the shelf life of button mushrooms. Polyamine treatments are influenced by micro and macro elements significantly.

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