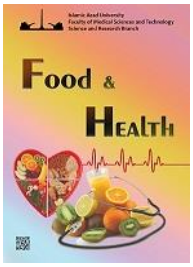

Food & Health Journal

Journal homepage: fh.srbiau.ac.ir

Evaluation of the effect of application of ascorbic acid and chitosan on quality, nutritional value, and storage of *Agaricus bisporus*

Kimia Eyalati [1], Elham Danaee [1*]

[1] Department of Horticulture, Garmsar Branch, Islamic Azad University, Garmsar, Iran

ARTICLE INFO

Original Article

Article history:
Received 23 April 2021
Revised 07 June 2021
Accepted 10 August 2021
Available online 20 September 2021

Keywords:
Button mushroom
Chitosan
Ascorbic acid
Antioxidant activity
Nutritional value

ABSTRACT

To investigate the effect of ascorbic acid and chitosan on the postharvest life of button mushrooms (*Agaricus bisporus*), an experiment was conducted based on a completely randomized design with three replications. The treatments were grouped in ascorbic acid (0, 1.5, and 3 mM) and chitosan (0, 0.25 and 0.5%) levels and their interaction. At the end of the experiment, the nutritional value including Vitamin C, total soluble solids, total acidity, Phenol, and antioxidant activity in button mushrooms were recorded. The results stated that the maximum levels of all traits were obtained in response to use '1.5 mM ascorbic acid + 0.5% chitosan. The minimum percentage of weight loss was observed at 3 mM ascorbic acid + 0.25% chitosan at 12th days and the maximum value belongs to control treatment. Delaying of senescence and increasing of the nutritional value of button mushrooms in response to the association of these materials with molecules and prevention of their breakdown. The highest shelf life equivalent to 14.6 days was in 5'1.5 mM ascorbic acid + 0.25% chitosan' treatment that caused an increase of 8 days in comparison with control. It can be said that the application of treatments in the post-harvest stage has more influence on the nutritional value and durability of a button mushroom.

1. Introduction

Agaricus bisporus is an edible basidiomycete mushroom native to grasslands in Europe and North America. It is one of the most common commercial mushrooms in the world and Iran has 85% of edible mushrooms growing production. This is one of the valuable agricultural and important protein products that have been part of the human diet for many centuries (1). Dried mushrooms are very aromatic and some chefs prefer to use them for cooking instead of fresh mushrooms. Indeed, mushrooms contain on average from 80% to 90% water. Drying them removes the water, resulting in the concentration of the mushroom carbohydrates, proteins, and fats, and the rest is composed of various substances from metabolism, including vitamins and aromatic substances. Carbohydrates include simple sugars as well as polysaccharides make up the largest amount of dry matter. Fungal cell walls contained higher amounts of neutral amino acids and proteins, pigments, sugars, phosphates, and various minerals. Mushrooms are edible fungus that can provide several important nutrients. The many kinds of mushrooms have varying compositions and nutritional profiles. It also contains a small amount of vitamin D. They are rich in B vitamins, such as riboflavin, folate, thiamine, pantothenic acid, and niacin which can have various health benefits. Free radicals are toxic byproducts of metabolism and other bodily processes. They can accumulate in the body, and if too many collect, oxidative stress can result. The antioxidant content in mushrooms may help prevent lung, prostate, breast, and other types of cancer, according to the National Cancer Institute. This amount of energy (250 Kcal/kg fresh weight) shows that the consumption of mushrooms is completely in line with the demand for a low-energy diet in rich and industrialized countries and is very valuable and attractive for diets. In addition, fungi can partially compensate for the lack of valuable food, despite all the conditions that arise from

*Corresponding author: Department of Horticulture, Garmsar Branch, Islamic Azad University, Garmsar, Iran

E-mail address: elhamdanaeii@gmail.com (Elham Danaee).

different perspectives on nutritional value (2). Mushrooms are highly perishable and tend to lose quality immediately after harvest. Their shelf life is short when compared to most vegetables at room temperature. Many postharvest quality losses are as a result of various pre-harvest factors. The short shelf life is due to high water content and post-harvest changes such as browning, cap opening, cap diameter increase, weight loss, tissue changes, high respiration rate, and lack of physical protection to prevent dehydration and microbial attack. In recent years bio-preservation is one of the alternative food preservation technologies that improve shelf life and improving food safety through the use of natural antimicrobial or antimicrobial compounds. In post-harvest technology, biopreservation is done by using plant products such as ascorbic acid and chitosan to increase the shelf life or storage of fruits and vegetables (3). Ascorbic acid (vitamin C) is one of the natural compounds not only because of its maintaining the quality and storage of products but also due to the high content of various antioxidant compounds. The role of vitamin C is as a cofactor or cosubstrate for many enzymes. In the presence of ascorbic acid, the activity of the glutathione-ascorbate and catalase cycles is increased and the defense system against oxidative stress is strengthened. Ascorbic acid serves a variety of functions critical to inhibiting the enzyme polyphenol oxidase at any given inhibitor concentration, it does not have adverse effects on the taste of the product. Adding ascorbic or other acids leads to lowers the pH and prevents enzymatic browning (4). Chitosan is the second abundant natural polysaccharide after cellulose, which is insoluble in most organic solvents and soluble in dilute acidic solutions below pH 6. It has many unique physiological and biological properties and has great potential for use in the food industry as a coating, preservative, antioxidant and antimicrobial. Chitosan coating is beneficial to maintaining the storage quality and prolonging the shelf life of postharvest fruits and vegetables, which is always used as the carrier film for the antimicrobial agents for fresh products (5). Therefore, the one aim of this article was to highlight the potential of chitosan and ascorbic acid as the active biobased coating material for antimicrobial agents. Moreover, another aim was to review their activity on some quantitative and qualitative traits, improving shelf life and biochemical features and activity of antioxidant enzymes mushrooms. It is expected to provide insights for researchers working on the postharvest preservation of agricultural products.

2. Materials and methods

The present study was performed as a factorial experiment in a completely randomized statistical design with 9 treatments, 3 replications, and each replication containing 5 button mushrooms. Treatments included ascorbic acid with (0, 1.5, and 3 mM) and chitosan with (0, 0.25, and 0.5%) levels and their interaction. First, healthy white button mushrooms were prepared with approximately the same size. After applying the treatments (immersion for 2 minutes in ascorbic acid, 5 minutes in chitosan, or both treatments), they were left in the laboratory for 20 minutes to dry. Then, 5 mushrooms were placed in each dish and the treated mushrooms were transferred to the cold storage with a temperature of 4±1 ℃ and relative humidity of 80±5 %. Traits were evaluated on days (start of experiment: 0), 4, 8, and 12. Distilled water is used as a solvent to 1.5- and 3-mM ascorbic acid. The chitosan solution was prepared with 2% (w/v) in 1% (v/v) acetic acid using adjustable volume micropipettes pH. Weight loss was calculated using the difference in weight of the samples before and after storage. Measurement of cell membrane stability evaluated by electrolyte leakage using the EC-meter Singh et al. (6) method by the following formula:

$$Electrolyte\ leakage\ (\%) = EC_1 / EC2 \times 100$$

The amount of soluble solids was measured by a digital refractometer in the first, 4, 8, and 12 days (7). The acidity was recorded by titration method with 0.1 normal NaOH, in the first 4, 8, and 12 days and was expressed in terms of percentage of citric acid (7). The content of vitamin C was calculated by the method of Mashayekhi and Atashi (8) as a two-stage oxidation-reduction titration. The phenol content of samples was determined according to (9) with some modifications. Briefly, 300 µL of appropriately diluted bitter melon extract or standard solution was mixed and incubated with 300 µL of FC solution for 2 min before 2400 µL of a 50 g/L sodium carbonate solution was added. The solutions were mixed well and placed in the dark at room temperature for 2 h before the absorption was measured at 765 nm using a spectrophotometer. Gallic acid was used as the standard and the phenol content was expressed as mg gallic acid equivalents per g of dry powder weight (mg GAE/g). Enzyme activity was measured according to Aebi (10) method based on the reduction of hydrogen peroxide adsorption at a wavelength of 240 nm. The enzyme extract was prepared by homogenizing fresh samples (2 g). The antioxidant enzyme activities of superoxide dismutase were assayed spectrophotometrically at 560 nm by spectrophotometer (11). The enzymatic extract was prepared the same as SOD enzyme. Enzyme activity values are calculated by spectrophotometer at 530 nm (12). The mushrooms were stored in the refrigerator at 4±1°C and relative humidity of 80±5 %, and their shelf life was recorded in terms of symptoms such as dehydration, cap opening, browning, and mold contamination by day (13). The information was entered into Excel software after measurement. Then, the data were analyzed by SPSS 19 statistical software and the comparison of means was evaluated using Duncan's Multiple Range Test at the level of 1% and 5%. Excel software was also used to draw the graphs.

3. Results and discussion

As shown in Table 1, the effect of treatment, time, and their interaction on the weight loss, cell ion leakage, acidity, vitamin C, phenol, soluble solids, superoxide dismutase, peroxidase enzymes, catalase activity was significant at the 1%and 5% level. 3 mM ascorbic acid + 0.25% chitosan treatment had the

lowest weight loss with 2.42% and the control treatment with 7.41% had the highest weight loss on the 12th day. Also, the percentage of weight loss increases in all treatments during storage (Fig. 1). The 12 Days of storage ion leakage under ascorbic acid and chitosan treatment illustrated the highest amount in '1.5 mM ascorbic acid + 0.5% chitosan' (27.43%), and the lowest in control (57.98%) (Fig. 2). Water loss due to damage to the cell membrane wall leads to increased ion leakage of cells and increased weight loss, which, even in the absence of wilting and changes in appearance, causes significant changes in cell composition and metabolism. Ascorbic acid due to its high antioxidant properties reduce

Table 1. Analysis of variance of the trait.

	DF	TSS	Total acidity	Loss weight	Ion leakage	Vitamin C	Peroxides	Catalase	Superoxide dismutase	Shelf life
Time	8	1.611**	1.975**	22.641**	13.618**	4.822**	8.951**	5.920*	6.383**	-
Treatments	3	7.867**	6.778*	49.197**	16.793**	17.279*	17.780**	16.87**	23.038*	13.287
Time × Treatments	24	1.59**	0.455**	3.428**	7.974**	3.733**	3.936**	2.314*	0.823**	-
Error	288	0.031	0.008	0.016	0.070	0.10	0.041	0.025	0.013	0.043

NS, *, and ** indicate non-significant, significant at p≤ 0.05 and p≤ 0.01, respectively.

oxidative stress damage to the vacuole membrane and reduces the oxidation of lipids in cell membranes and maintains cellular turbulence, which prevents ion leakage and subsequent weight loss. Chitosan coating also reduces respiration and cell damage by creating hydrophilic layers around the mushrooms and preventing gas exchange. In this way, reducing ion leakage and water loss through transpiration prevents the reduction of surface moisture, and as a result, decreases ion leakage and weight loss of button mushrooms. Therefore, our results agree with the findings of Zhu et al. (14).

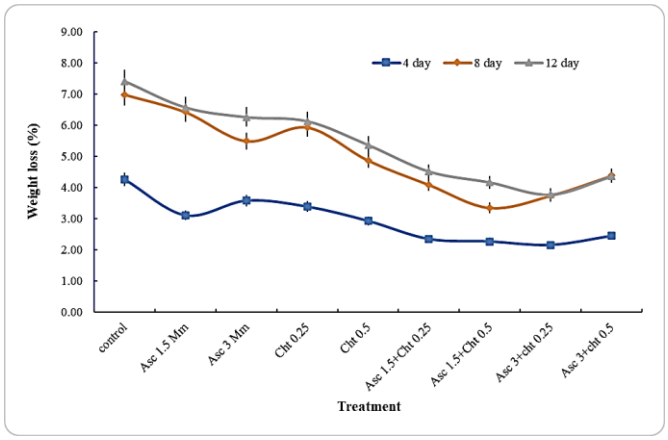

Fig.1. Changes of weight loss in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan].

In terms of total soluble solids, as seen in Fig. 3, a wide difference was declared among treatments during times from '1.5 mM ascorbic acid + 0.5% chitosan' (6.28° Brix) to 3.25 ° Brix at the 12th day (Fig. 3). The total acidity differed in mushroom grading from 1.03 to 2.37 mg/100 g FW. The maximum and the minimum values of manganese were appeared in '1.5 mM ascorbic acid + 0.5% chitosan' and 'control', respectively (Fig. 4). Post-harvest weight loss with cell material concentration plays an important role in increasing the amount of soluble solids. On the other hand, cellular oxidation reactions with depolymerization and degradation of polysaccharides increase the amount of soluble solids. However, degradation of cell wall polysaccharides can also be effective in increasing soluble solids.

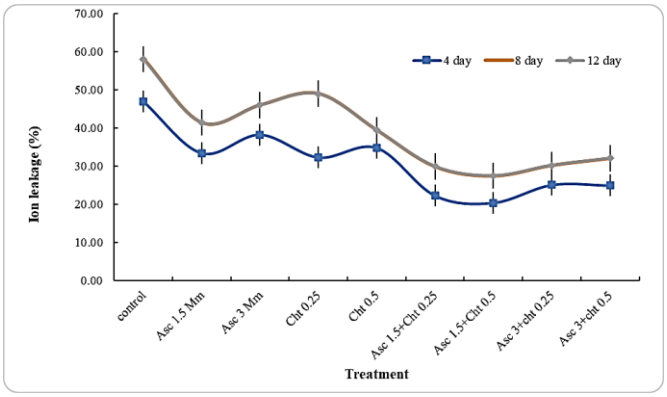

Fig. 2. Changes ion leakage in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan]

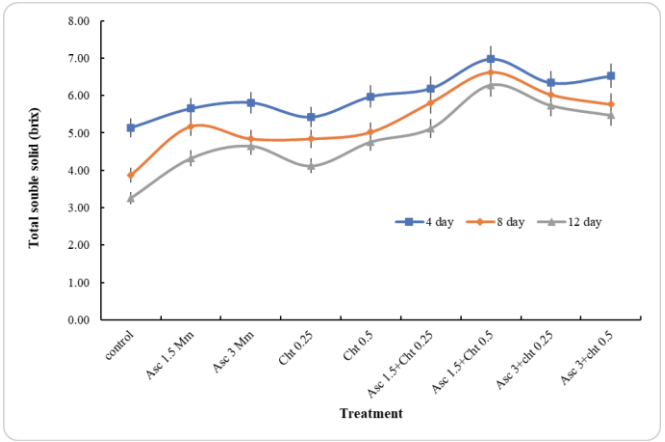

Fig. 3. Changes of total soluble solid in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan].

Also, the acidity decreases over time due to respiration and the use of organic acid in enzymatic reactions. Ascorbic acid due to its strong antioxidant role by reducing respiration and

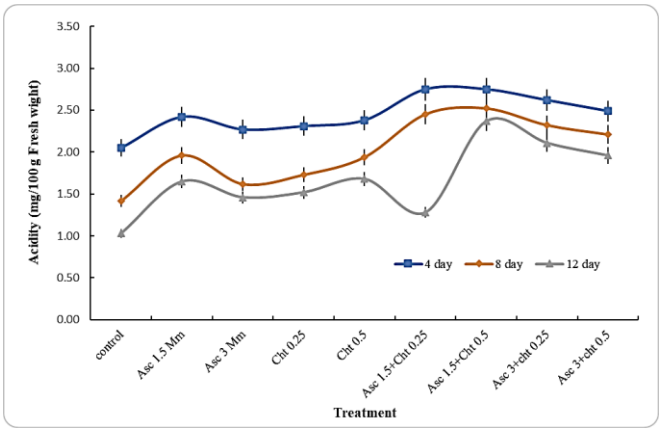

Fig. 4. Changes of total acidity in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan].

chitosan coating by inhibiting gas exchange can slow down the metabolic activities and aging process in edible mushroom, thus increasing the process of increasing soluble solids, reducing acidification and acidity. Therefore, the findings of this study were according with Eshghi et al. (15).

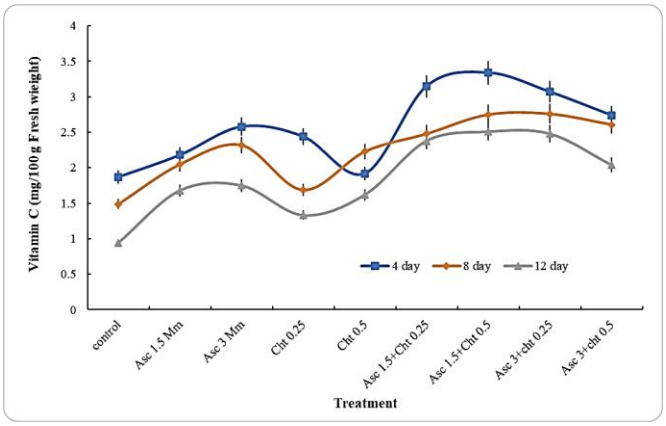

Fig. 5. Changes of vitamin C in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan].

Fig. 5 depicts the effect of the treatments on the vitamin C during 12 days of storage. The initial count was 0.94 mg/100 g FW, which increased during storage in all the samples, especially in the '1.5 mM ascorbic acid + 0.5% chitosan' samples (2.51 mg/100 g FW). The internal ascorbic acid of horticultural products is sensitive to oxidation reactions and decomposition during the storage process. The possible reason for this is the reduction of ascorbic acid during storage due to its auto-oxidation, which occurs spontaneously in the presence of oxygen in the air. By using ascorbic acid and edible chitosan, the amount of oxygen penetration into the fungal tissue is reduced and as a result, the activity of ascorbic acid oxidizing enzymes is decreased. This finding is Similar to Ghaisarbigi et al. (16) in lemon, cucumber, and mushroom.

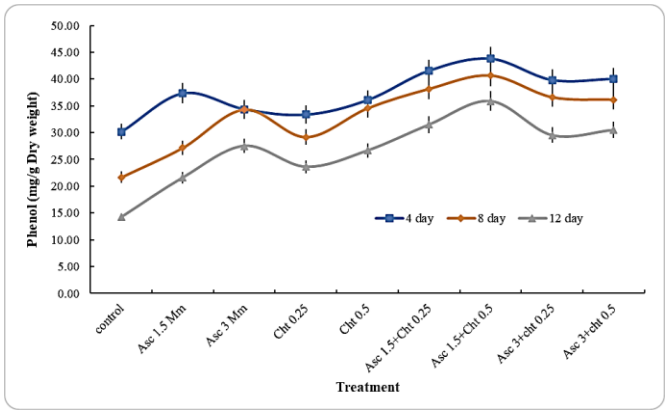

Fig. 6. Changes of phenol in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan].

During the 12 days in the current research (Fig. 6), the phenol content in the samples in the control group (27.83 mg/g DW) enhanced to (41.4 mg/g DW) in the '1.5 mM ascorbic acid + 0.5% chitosan'. Phenolic secondary metabolites play an important role in plant yields. The decomposition of phenolic compounds is the result of the activity of polyphenol oxidase and peroxidase enzymes. Preservation of phenolic compounds improves the quality and storage of products by affecting quality characteristics such as appearance and taste. Coating the mushrooms with chitosan and using ascorbic acid helps reduce the activity of polyphenol oxidase and peroxidase enzymes and delays the aging process. These are close to the study of strawberry and apple by Mighani et al (17).

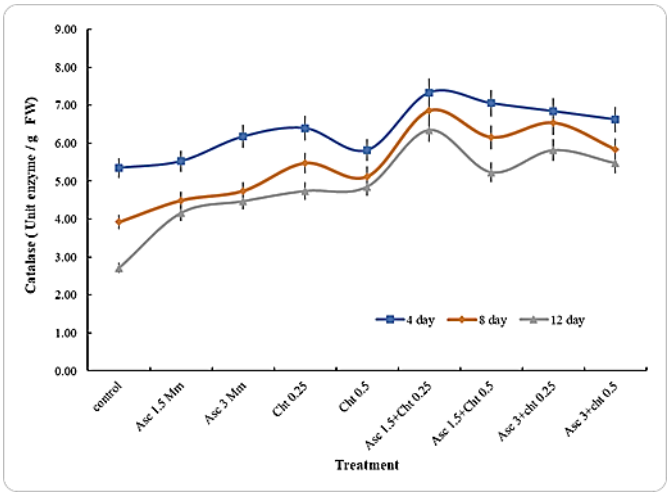

Fig. 7. Changes of catalase enzyme activity in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan].

As can be seen in Fig. 7, the highest enhancement rate of Catalase enzyme activity (6.34 Unit g/FW) was observed in the '1.5 mM ascorbic acid + 0.25% chitosan' samples compared to the control samples. Comparison of the treatment used in the present study (Fig. 8), indicated that ' 1.5 mM

ascorbic acid + 0.25% chitosan' (4.27 Unit g/FW) exerted significant effects on Superoxide dismutase while the control had the lowest amount.

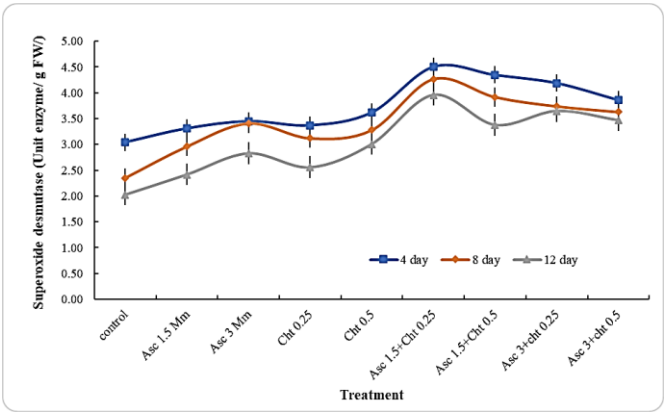

Fig.8. Changes of superoxide dismutase enzyme activity in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan].

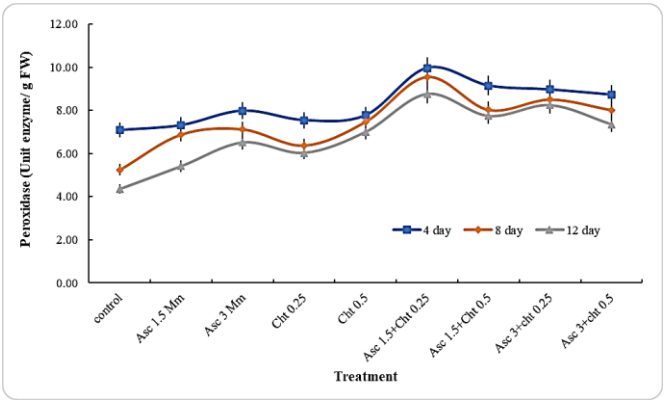

Fig. 9. Changes of peroxides' enzyme activity in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan].

According to the results in Fig. 9, the peroxides' activity in control was (4.36 Unit g/FW) and the use of '1.5 mM ascorbic acid + 0.25% chitosan' caused a significant increase of enzyme activity (8.25 Unit g/FW). Free radicals are the products of normal cellular metabolism that can disintegrate cell membranes, but the intracellular antioxidant system can scavenge free radicals and thus delay aging. During storage, the activity of internal natural antioxidant systems decreases over time, and due to increased oxidative stress and the production of free radicals, the aging process is accelerated. The use of ascorbic acid as an antioxidant and chitosan coatings with the ability to reduce the penetration of oxygen, alone or in combination with each other leads to delay the aging process. This finding is consistent with the study of Rostamzadeh et al. (18). Maximum of shelf life equivalent to 14.6 days was in 5'1.5 mM ascorbic acid + 0.25% chitosan' treatment that caused an increase of 8 days in comparison with control (Fig. 10). The present finding on the length of the shelf life is in agreement with a study on (mushrooms) by Sarlak et al. (13) and Khavarpour et al. (19).

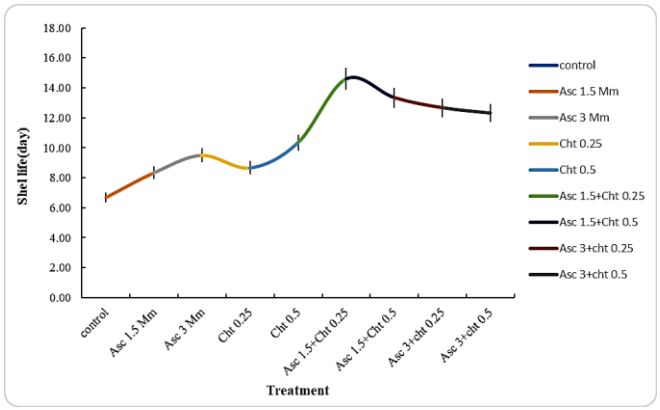

Fig. 10. Changes shelf life in mushroom treated with ascorbic acid (0, 1.5 and 3 mM) and chitosan (0, 0.25 and 0.5%); [Asc: ascorbic acid; Cht: chitosan]

4. Conclusion

Due to the results of this finding, it seems that the treatment of button mushrooms with chitosan along with ascorbic acid resulted in the promotion of TSS, TA, Phenol, Vitamin C, and antioxidant activity. Delaying of senescence and increasing of the nutritional value of button mushrooms in response to the association of these materials with molecules and prevention of their breakdown. However, for increasing nutritional factors of button mushrooms 1.5 mM ascorbic acid + 0.25% chitosan' treatment is the most effective factor. Totally, post-harvest treatment button mushroom with the above-mentioned treatment could preserve the quality and the shelf life of button mushrooms.

References

1. Masson Y. Growth of Pseudomonas fluorescens and Candida sake in homogenized mushrooms under modified atmosphere. *Journal of Food Engineering*. 2002;54,125-31.
2. Riahi, H. Cultivation of edible mushrooms (button). Arad Publications. 2015. (In Persian)
3. Rai RD, Arumuganathan T. Post Harvest technology of Mushrooms. National Research Centre for Mushroom, 2008.
4. Ahmadi Baghi M, ErfaniMoqaddam J, Khademi A .Maintaining the quality of sliced persimmon pieces using organic acids and 1-MCP. *Journal of Food Industry Research*. 2015;27,72-61. (In Persian)
5. Devlieghere F, Vermeulen A, Debevere J. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology*. 2004; 21:703-14.
6. Singh A, Kumar J, Kumar P. Effect of plant growth regulators and sucrose on post harvest physiology, membrane stability and vase life of cut spikes of Gladiolus. *Journal of Plant Growth Regulator*. 2008;55, 221-9.
7. Mostofi Y, Najafi F. Analytical laboratory methods in horticultural sciences. University of Tehran Press. 2005. (In Persian)
8. Mashayekhi K, Atashi P. 2016. Guide to Plant Physiology Experiments. Agricultural Education Research Publications. (In Persian)
9. Cicco N, Lanorte MT, Paraggio M, Viggiano M, Lattanzio V. A reproducible, rapid and inexpensive folin-ciocalteu micro-method in determining phenolics of plant methanol extracts. *Microchemistry Journal*. 2009; 91,107-10.

10. Aebi H. Catalase in vitro. *Meth Enzymologists*. 1984;105,121-6.
11. Bayer WF, Fridovich I. Assaying for superoxide dismutase activity: some large consequences of minor changes in condition. *Annals Biochemistry*.1987;161,559–66.
12. Putter J. In: Methods of enzymatic analysis (2Ed Bergemeyer, A.) Academic press. New York. 1974.
13. Sarlak F, Khademi A, Erfanimoghadam J. The effect of ascorbic acid, calcium chloride and hydrogen peroxide on the viability of button mushrooms. *Journal of Production and Processing of Crops and Horticulture*. 2016;25,1146-35. (In Persian)
14. Zhu X., Wang Q., Cao J, Jiand, W. Effect of chitosan coating on postharvest quality of Mango (*Mangifera indica* L. CV. Tainong). *Fruits*. 2008; 32,770-784.
15. Eshghi S, Hashemi Mohammadi AR., Badiei F, Ahmadisome K, Ghantai K. The effect of chitosan-containing nanoemulsion coating on increasing the shelf life and quality characteristics of post-harvest strawberry fruit. *Iranian Journal of Nutrition Sciences and Food Industry*. 2011;2,19-9.
16. Ghaisarbigi Sh, Ramin A. Amini. F. The effect of chitosan oral coating on maintaining the quality and shelf life of sweet lemon fruit. *Journal of production and processing of agricultural and horticultural products*. Fifth year. Issue 18. 2015.(In Persian)
17. Mighani H, Boroumand N, Moghbeli A. The effect of chitosan and calcium chloride on post-harvest quality and antioxidant compounds of strawberry fruit. *Food Science and Industry*. 2016;76-15. (In Persian)
18. Rostamzadeh B, Ramin AA, Amini F, Pirmoradian M. The effect of chitosan coating on increasing postharvest life and maintaining fruit quality of Soltani cultivar. Journal of Production and Processing of Agricultural and Horticultural Products. 2015; Issue 17. (In Persian)
19. Khavarpour M, Ariaei P. Investigation of the effect of chitosan coating and lavender essential oil on the persistence of button fungus. *The First International Congress and Exhibition of New Sciences and Technologies*. 2015. (In Persian).