Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

The effect of edible fox gum coating containing Lactobacillus fermentum bacteria on the quality characteristics of button mushroom.

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ARTICLE INFO

Article History:
Received:2024/1/6
Accepted:2024/3/13

Keywords:

edible coating,
salep gum,
Lactobacillus fermentum bacteria,
button mushroom

DOI: **10.22034/FSCT.21.151.86.**

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ABSTRACT

In this research, the shelf life, physicochemical, and sensory properties of button mushrooms were investigated using salep gum edible coating containing *Lactobacillus fermentum* bacteria. For this purpose, the effect of salep gum at the levels of 0.25, 0.75, and 1.5 and the constant addition of *Lactobacillus fermentum* probiotic with the amount of 1.5 x 108 CFU/gr (0.5 McFarland half) for 15 days at a temperature of 4 Celsius was evaluated. The results showed that by increasing the coverage of salep gum, pH, acidity, soluble solids, total phenol, antioxidant, and parameters a* and b*, histologically, they maintained at a high level compared to the untreated fruit, and the total number of probiotic bacteria in The coating preserved better the probiotic bacteria suspension compared to the immersion treatment. But weight loss, L* parameter, and browning index decreased with the increase of salep gum. With increasing shelf life of dissolved solids, acidity, weight loss, a*, b*, and browning index increased, but pH, ascorbic acid, total phenol, antioxidant and L*, histology, total number of probiotics decreased The sensory evaluation of different treatments showed that the coating containing probiotics did not hurt the sensory properties of edible mushrooms; Rather, it improved the sensory and nutritional quality of the fruit over time and compared to the control sample. Therefore, the edible coating of salep gum containing Lactobacillus fermentum bacteria can be used as a suitable coating material to preserve the organoleptic, chemical, microbial properties and shelf life of button mushrooms.

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[DOI: 10.22034/FSCT.21.151.86]

1- Introduction

Edible coatings are matte, light or milky, and consumers prefer clear and invisible coatings. Food products are usually coated by dipping or spraying. Currently, immersion is the most common method for coating fruits and vegetables, but other methods such as brushing and spraying are also used according to the type of food product and the characteristics of the coating [1]. Foods (fruits, vegetables, dried fruits, etc.) have a natural protective layer in the form of skin, which acts as a controller of disturbing environmental factors. In most cases during processing and storage, this Natural coatings lose their effectiveness and this reduces the quality and shelf life of food products. Therefore, food needs to be protected from environmental factors [2] Biopolymer films and coatings may be edible or only biodegradable, this formulation depends on the production method and corrective treatments. Covers Edibles are thin layers that are prepared from natural resins and are cut by different mechanical methods for packaging They are placed on the surface of food and in this way they control biological, physical, chemical and microbial changes [3]. Edible coatings have been proposed as an alternative to synthetic food packaging to improve the quality and safety of food products. These coatings protect food from water loss and respiratory gas exchanges. In addition, edible coatings can carry active compounds such as antimicrobials, antioxidants, and tissue improvers [4]. According to statistics, button mushroom (*Agaricus bisporus*) has the highest amount of mushroom production in the world [5]. East Asian countries, especially China, are the largest producers of edible mushrooms in the world, the United States of America and Canada occupy the next ranks of edible mushroom production in the world [6]. Mushrooms are rich in protein in their dry matter and contain essential amino acids such as glutamic acid, aspartic acid and arginine, and are also an excellent source especially of the amino acid lysine. The short-term shelf life of edible mushrooms is a problem that has undermined its economic value and marketability. In the post-harvest stage, the process of quality degradation of edible mushrooms begins, such as moisture, color change, texture change, taste, and loss of nutrients [7]. The shelf life of button mushrooms that were kept in the refrigerator is reported to be about 8 days. This short shelf life has a primary structural reason: button mushrooms have no cuticle to act as a physical barrier against mechanical damage, water loss, or microbial attack. High respiration and high humidity contribute to rapid aging of button mushrooms, causing microbial attack and enzymatic browning [8]. Fresh mushrooms have a high moisture content of about 85%-95%. In the post-harvest period, the moisture content of the mushroom gradually decreases, resulting in a continuous weight loss. Various factors affect the quality of mushrooms in the post-harvest period. These factors can be divided into two categories: internal factors related to the fungus itself (water activity, respiration rate, and microbial activity) and external factors related to storage conditions (storage temperature, relative humidity, and mechanical damage). Therefore, the mushroom is likely to be contaminated by various microorganisms. The high water content and neutral pH in mushrooms provide an ideal environment for microbial growth [9]. salep is the ground flour of the tubers of a kind of wild orchid from the Orchidaceae family. Different species of salep grow in Iran, mostly in the north and northwest of the country. salep components are different in different species and include 54% glucomannan, 38% starch, 5% protein and 3% ash, and also glucomannan is a natural fiber soluble in water. The most important substance in salep gum polysaccharide is glucomannan, which gives it an important stabilizing capacity and is suitable for forming an edible film. Glucomannan, a polymer with a high molecular weight, which has given the salep an extraordinary hydrocolloid property [10]. Lactobacilli are non-sporulating, gram-positive, catalase-negative, and usually non-motile bacteria. Bifidobacteria are also Gram-positive and can grow at a pH between 4.5 and 8.5, but the most important thing about them is that they are obligate anaerobes [11]. Today, the consumption of food with probiotics is increasing due to the increasing desire of consumers to have healthy and safe nutrition. Amiri et al. (2021) investigated the effect of the composite coating of concentrated milk protein-

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[DOI: 10.22034/FSCT.21.151.86]

pectin reinforced with calcium chloride and black seed essence to increase the shelf life and quality characteristics of strawberry fruit after harvest. After optimizing the microbial quality, the physicochemical, antioxidant, anthocyanin content and texture hardness of the coated strawberries were compared with the control samples on days 5 and 10 after storage. All qualitative characteristics of the studied sample were significantly different from the control samples ($p<0.05$). They concluded that this edible coating preserved the quality of strawberries better than the control samples due to the reduction of decay [12]. In another study, Shahrampour et al. (2020), investigated the effect of using a multi-layer coating of calcium alginate containing native probiotic bacteria *L. plantarum KMC45* and suspension of this bacteria in distilled water on the quality of strawberry fruit during storage and shelf life. They observed that the treatment of fruit with calcium alginate coating containing probiotic bacteria was more effective in maintaining the quality characteristics of strawberry compared to the treatment of immersion in probiotic bacteria suspension. So that the weight loss, tissue softening and also the percentage of strawberry decay significantly decreased during the 14-day period compared to the control treatment. Also, in this study, the changes in color indices including *L, hue angle and chroma were less in the coated samples than the control treatment. In addition, the results of counting the population of probiotic bacteria *L. Plantarum* KMC45 in different treatments of strawberries during storage at 4 degrees Celsius showed that after two weeks, the rate of bacterial population decline in the immersion treatment in the bacterial suspension was 2.97 logarithmic cycles, while In the treatment of calcium alginate coating, only 0.95 logarithmic cycles were determined. Therefore, calcium alginate coating is recommended as a suitable carrier for the transfer of probiotic microorganisms in live form on the surface of fresh strawberries, which can increase its shelf life and provide the basis for the production and development of new probiotic products [13]. Also, Rod et al. (2019), investigating the effect of edible coating and increasing shelf life of button mushrooms with The use of enzyme removal and then coating with carboxymethyl cellulose and sodium metabisulfite were discussed on the organoleptic, chemical, and microbial characteristics. Their findings showed that the investigated coating significantly reduced the growth of microorganisms and the number of mold and yeast, also the least color changes, weight loss and decrease in tissue stiffness were observed in these coated samples. Compared to the control sample, it significantly increased the pH and the amount of dissolved solids [14]. The aim of the present study was to use edible salep gum coating containing Lactobacillus fermentum in order to increase the shelf life of button mushroom and to investigate the effect of coating on the physicochemical and quality characteristics of button mushroom during storage in the refrigerator.

2- materials and methods

1-2- preparation of button mushroom

Button mushrooms were obtained in bulk form from the famous Spaid Zarin greenhouse in Urmia, and for several hours after harvesting, they were transferred to the laboratory of Urmia University in a dark environment, and then the button mushrooms were uniform in terms of shape, size, and color. and being healthy were used to check the effect of coatings. *Lactobacillus fermentum strain (PTCC 1744)* was purchased from Iran Industrial Microorganism Collection Center in lyophilized form. salep ***(Orchis mascula)*** powder was purchased from a domestic company and agr MSR and MRS broth were purchased from Ibresco. Barium chloride, glycerol, sodium hydroxide, metaphosphoric acid, ethanol, methanol were obtained from Merck, Germany. 2,2-diphenyl-1-picrylhydrazyl, gallic acid and folinic acid were obtained from Sigma-Aldrich, USA.

2-2 Preparation of coating formulation

In order to prepare the coating solution of salep gum at the levels of 0.25%, 0.75%, and 1.5% (weight/volume), salep gum powder was first poured into 100 ml of distilled water on a heater with a temperature of 60 degrees Celsius and stirred. until it is a clear and clear solution. After

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cooling the solution, glycerol was added to the solution as a plasticizer in the amount of 1%. All formulations were further mixed by magnetic stirrer for another 10 minutes and then autoclaved to remove pathogenic microorganisms and then kept at 4 degrees Celsius for 24 hours [15]. Then, McFarland's 0.5 bacterial suspension was prepared with a concentration equal to the concentration of McFarland's half-standard solution by adding the mass of bacteria to sterile physiological serum (0.85%). The turbidity of the prepared suspension was measured in a spectrophotometer with a wavelength of 625 nm, 0.882 [16].

2-3- Coating and storage

Button mushrooms were uniformly separated in terms of shape, size, color and health, and were washed and dried with distilled water before testing. Button mushroom samples were immersed in the prepared solutions for 3 minutes. Then they were removed from the solution and transferred to sterile baskets to be drained. The samples were placed in the refrigerator at 4°C for one hour to dry the coating and were distributed in polyethylene containers with cellophane covers in dimensions of 10 x 10 square centimeters. Covered mushrooms were sampled at intervals (0-8-15) once every eight days for tests. In this test, the control treatment included button mushroom samples that were immersed only in sterile distilled water [13].

2-4- Physicochemical properties

2-4-1- pH

This factor shows the degree of acidity or alkalinity, which is based on the amount of hydrogen ion and depends on it. First, 5 grams of mushroom caps were homogenized in 10 ml of water. After filtration, the pH was measured by a digital pH meter (model PM12E, Fan Azmagaster Co., Iran) [17].

2-4-2- total acidity (TA)

To measure organic acids, first, 10 ml of mushroom extract was poured into 10 mm Erlenmeyer flask and 10 ml of distilled water was added to it, and then by placing the electrode of the digital pH meter, titration was performed with 0.1 normal hydroxide (4 g per liter). It was done until pH = 8.4. The amount of titratable acid was calculated based on the amount of sodium hydroxide consumed according to link (1) below [18]

$$A=\frac{V\times 0/0065\times 100}{m}$$

A is acidity in terms of citric acid (grams per milliliter), V is the volume of 0.1 normal sodium hydroxide in milliliters, m is the amount of the sample in milliliters. * One milliliter of 0.1 normal soda is equivalent to 0.0064 grams of citric acid.

2-4-3- total soluble solid (TSS)

For this purpose, a few drops of mushroom extract were placed on a manual refractometer (model R-5000, Atago, Japan) at room temperature, which was calibrated before starting the measurement, and then the refractometer was read and the data was recorded in Brix degrees [19].

2-4-4- weight loss

Treated and control button mushrooms were measured with an accuracy scale of 0.001 during 0, 8, 15 days intervals during storage in the refrigerator. Then, the percentage of their weight loss compared to the initial weight was calculated using equation (2) [20]

$$WL=\frac{M1-M2}{M1}\times 100$$

WL is the amount of weight loss (%), M1 is the initial weight of the mushroom package (g), M2 is the weight of the mushroom package (g) after a certain period of time.

2-4-5- ascorbic acid (AA)

One gram of the mushroom cap was mixed with 3 ml of 1% metaphosphoric acid (to extract the extract) and then the mushroom cap along with the meat and skin was pounded in a mortar and the sample was kept in the Falcon for 30 minutes in a refrigerator at a temperature of 4 degrees Celsius. After half an hour, the samples were centrifuged at a speed of 4000 rpm for 10 minutes (model CE-148, Shimi Fan Company, Iran). Then, 500 microliters of the extract was taken from the transparent supernatant solution and 2

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ml of sodium 2 and 6-dichlorophenol indophenol (DCPIP) was added to it until the pink color change (which remains for 10 to 50 seconds) and finally the absorption rate of the sample. were read at a wavelength of 520 nm by a spectrophotometer (model Shimadoz 4100, Termonicolt, Japan), the control sample had the above compounds except the mushroom extract. Ascorbic acid was also used as a control to draw the standard curve[21].

2-4-6- total antioxidant activity (TAA)

The antioxidant activity of the extracts was determined through DPPH free radical neutralization property. For this purpose, 2000 microliters of DPPH (0.1 mmol solution) was added to 100 microliters of methanolic extract. The resulting solution was stirred immediately and then kept for 60 minutes at room temperature and in the dark until it reached a uniform state. The decrease in absorbance was read by a spectrophotometer at a wavelength of 517 nm. Finally, the antioxidant capacity was calculated as the percentage of DPPH inhibition through the equation (3) below [22].

٪ $DPPH_{SC}= (A_{cont}-A_{samp})/A_{cont}\times100$

$DPPH_{SC}$ %: inhibition percentage (free radical scavenging activity), Asamp: absorption rate (sample+DPPH), Acont: DPPH absorption rate

2-4-7- total phenolic concentrations (TPC)

The amount of total phenol was measured using Folin-Ciocalchu (Folin) method. For this purpose, one gram of mushroom cap tissue was added to 10 ml of extraction solvent (80% methanol and 20% distilled water), then the extract obtained from the samples was kept in a refrigerator at 4 degrees Celsius for 24 hours. And finally, after being poured into a microtube, they were centrifuged at 4000 rpm for 10 minutes. First, 500 microliters of concentrated extract was poured into the test tube and 180 microliters of distilled water was added to it. In the next step, 1200 microliters of Folin 10% (diluted 10:1 with distilled water) was added to it. After 5 minutes of adding Folin, 960 microliters of 7.5% sodium carbonate was added. The samples were placed in dark conditions. After keeping for 1.5 hours in the dark and at room temperature, the absorbance of the extract was read by a spectrophotometer at a wavelength of 760 nm. Finally, the amount of total phenol was calculated based on the absorption rate of the sample and its comparison with the standard in terms of milligrams of acegallic acid per gram of fresh tissue [23].

2-4-8- color

The color of the samples was evaluated using a colorimeter (model CR-400, Tokyo company, Japan) based on Hunter lab parameters. The amount of color was reported through Hunter's parameters, in terms of brightness (L*), red-green (a*), and yellow-blue (b*). The L* index represents the brightness of the sample and its range is from 0 (black) to 100 (white). The a* index shows the closeness of the color of the sample to green and red colors and its range varies from -120 (pure green) to +120 (pure red). The b* index shows the closeness of the color of the sample to blue and yellow colors and its range varies from -120 (pure blue) to +120 (pure yellow) [24].

$$x = \frac{a+1.75\,L}{5.645\,L+a-3.012\,b}$$

$$BI = \frac{100\,(X-0.31)}{0.172}$$

9-4-2-Mechanical test of tissue measurement

A tissue tester (TA Plus XT model, Stable Microsystems, England) was used to determine the texture hardness of the samples. For this purpose, a cylindrical steel probe was used with a displacement speed of 1 mm / s and the penetration test was performed with a displacement of 6 mm. The penetration force values were recorded with an accuracy of 0.1 newton and the displacement of the probe with an accuracy of 0.001 seconds. Using force-time diagrams, the maximum penetration force was calculated according to gram [25].

2-4-10- viscosity of salep gum

Viscosity of coating solutions of %, 0.75% and 1.5% of salep gum using a viscometer (model LVDV-IP, Brookfield Company, USA) at 37

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degrees Celsius and spindle number 63 and 62 type LV_2, rotation speed 10 rpm for concentration 1.5%, rotation speed of 60 rpm for concentration of 0.75%, rotation speed of 10 rpm for concentration of 0.25% and after a period of 15 seconds was measured. Before measuring, salep gum samples were manually stirred for 1 minute. The average of 3 replicates at different times was reported as the final viscosity.

2-4-11- Investigating the viability of *Lactobacillus fermentum* bacteria enclosed in button mushroom cover

In order to perform the microbial test, 10 grams of the samples were mixed and homogenized in 90 ml of 85% physiological serum and 2 dilutions were prepared for each sample. 1 ml of each dilution was used for the number of *Lactobacillus fermentum* in MRS agr culture medium by mixed culture method.Then, to count the total number of bacteria, the plates were placed upside down in the incubator at 37°C for 48 hours. After 48 hours, the plates were removed from the incubator and the colonies were counted and the results were obtained as CFU/g [27].

2-7-12- Sensory analysis

The sensory evaluation of the samples was done by 10 students of Urmia University using the 5-point hedonic method for the parameters of color, smell, texture and overall acceptance. In order to perform this test, each member was compiled in questionnaire forms. Each factor was given a score from 1 to 5. The number 5 represents the highest score and the best state, and the number 1 represents the lowest score and the worst state [28].

8-2- Statistical analysis

In the present study, the number of samples included the mushroom control sample as well as **s**alep gum treatments containing *Lactobacillus fermentum* bacteria at levels (0, 0.25%, 0.75%, 1.5%) and the storage time was 0, 8, 15 days. A factorial design based on a completely randomized test was carried out in three replications and the physical and chemical properties of the mushroom by the software. Expert.Design 7 was studied.

3- Discussion and results

1-3- salep viscosity

Before carrying out different coating treatments by immersion method, in order to better understand the characteristics of the film or coating layer, the important properties of the polymer solution were measured, including the viscosity. Adhesion and uniformity of the coating layer are two important criteria in evaluating the formation of coating on fruits. These criteria are directly influenced by the viscosity and surface tension of the coating solution. The effect of different concentrations of gum on the viscosity of the coating was significant ($p>0.05$), as shown in table (1-1), with the increase in the concentration of gum, the viscosity of the coating also shows a significant increase, and the most The viscosity with a value of 5363 centipoise was related to the coating sample containing 1.5% gum; Therefore, it can be concluded that salep gum is a polysaccharide compound, it is hydrophilic and has a high molecular weight. With the increase in salep gum concentration, the number of interstrand connections increases, the entanglement of the chains increases, and as a result, the viscosity of the solution increases [29-30].

Table 3-1: Investigating the viscosity of different concentrations of salep gum

Viscosity (centipoise)	The concentration of salep gum (gram of salep/100 ml of distilled water)
٤٠/٤٥±٠/٢٥^{c}	٠/٢٥
٣٣٥/٩٠±٠/٢٥^{b}	٠/٧٥
٥٣٦٣/٠٠±٦٠^{a}	١/٥

2-3- Effect of coating on pH

The results of pH evaluation in uncoated and coated mushrooms during a period of 15 days are shown in Figure (1-3). The effect of salep gum concentration, storage time and the interaction of concentration and storage time on mushroom pH were significant ($p<0.05$). As can be seen in the

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figure, the pH of the treatments increased with the increase in the concentration of salep gum, but the pH of the treatments decreased with the increase of the storage time from day zero to the fifteenth day. Also, in the control sample that was not coated, the pH decreased sharply during the storage time, but when the samples were coated with different concentrations of gum, the decrease in pH during the storage time was less; Therefore, the most pH fluctuations are in the control sample that has not been coated. Also, although the presence of probiotics in the coating reduced the initial pH value, it did not change its increasing trend over time. However, it can be said that the presence of probiotics slowed down the increase in pH, which can be attributed to preventing or slowing down the activity of mold and spoilage yeast that cause an increase in pH [31]. The coating of other treatments with concentrations of 0.25%, 0.75% and 1.5% of salep gum decreased the pH during the storage time. Also, the pH balance can be related to the stability of salep gum in acidic environment. The pH value indicates the acidity or alkalinity of the horticultural products, but the pH value of the fruit is not always directly related to the amount of organic acids in the product. Organic acids are mainly weak acids and do not have much effect on the pH of the fruit, and strong acids cause a rapid change in pH. pH or the concentration of H^+ ions do not affect the taste and their importance is more due to the effect on enzyme reactions and the activity of microorganisms (yeasts and bacteria), so compared to titratable acidity, pH changes are important. It has less taste as a quality factor. In most fruits, the pH of the fruits increases during storage, and this is due to the reduction of organic acids, but this increase in the pH of most fruits is different, because in addition to acids, other substances in the fruit, such as sugars, can also affect the pH. [32]. Similar results were reported by Amiri et al (1400) that the increase in pH of the fruit is due to the biochemical changes of the fruit, such as the breakdown of organic acids into sugars and the participation in the respiration cycle, which is the composite edible coating of condensed milk protein, pectin, fortified with calcium chloride and black seed essence. It was able to reduce the rate of respiration and decomposition of organic acids, and as a result, kept the pH of strawberries low during the storage period [12].

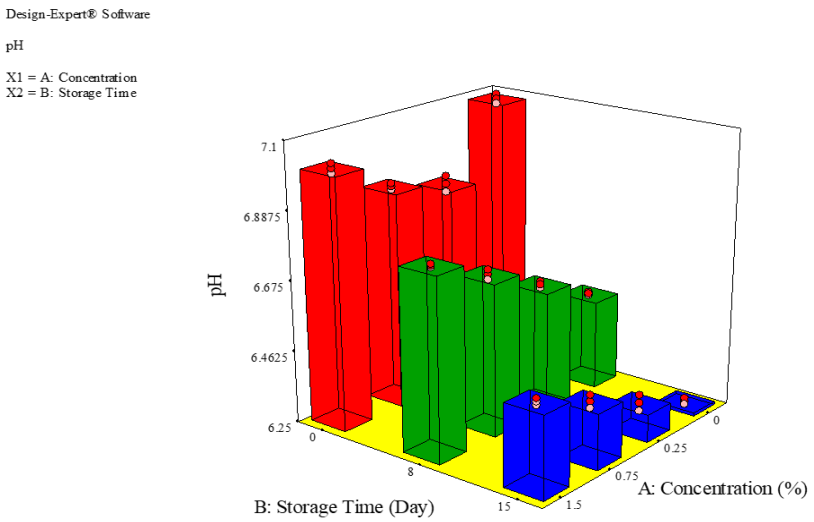

Figure 3-1: Effect of different levels of salep gum edible coating and storage time on button mushroom

3-3- effect of coating on acidity (TA)

The results of the assessment of acidity in coated and uncoated mushrooms during a period of 15 days are shown in Figure (2-3). The effect of salep gum concentration, storage time and the interaction of concentration and storage time on mushroom acidity was significant ($p<0.05$). As can be seen in the figure, with the increase in the concentration of salep gum, the acidity of the

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treatments has increased, which can be attributed to the decrease in respiration intensity of the fruits covered with salep, citric acid which is one of the main ingredients of respiration. By increasing the concentration of the coating, citric acid is protected against oxidation, so the concentration of citric acid increases with the increase of the coating concentration, and as a result, the acidity increases [31]. And with increasing storage time from day 0 to day 15, the acidity of the treatments increased. The control sample had a greater drop in acidity than other treatments during the storage period. Regarding the effect of increasing time, with the passage of time, the amount of moisture decreases, the amount of dry matter increases, and as a result, the concentration of acid in the specific weight of the mushroom sample increases, this increases acidity [33]. Verbken et al. (2003) reported that titratable acidity in fruits treated with basil essential oil in all concentrations used was higher than the control fruit, which is consistent with the results of this study [34]. The results obtained with the results of Emami et al.'s research (2019) showed that the combination of grape seed extract with **salep**,coating can prevent and control fruit ripening and improve acidity in coated samples compared to the control. To prevent the increase of soluble solids in strawberries by reducing their respiration and microbial infection during storage [15].

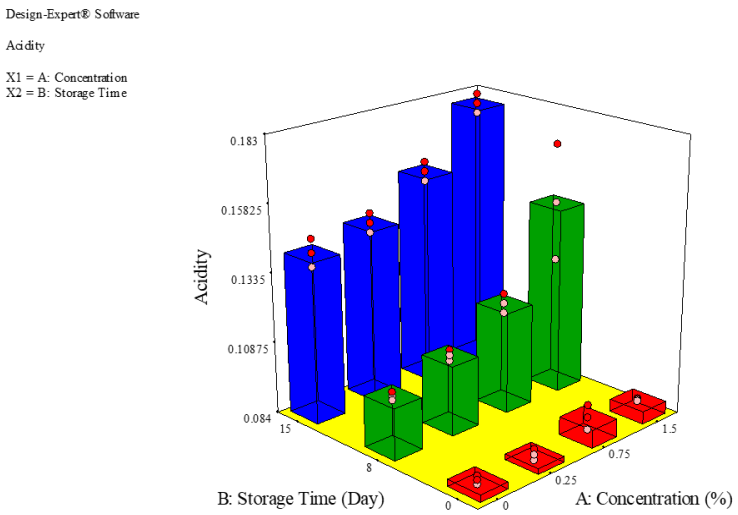

Figure 3-2: Effect of different levels of salep gum edible coating and storage time on button mushroom TA

4-3- effect of coating on total soluble solid

The results of evaluating the amount of soluble solids (TSS) in coated and uncoated mushrooms during a period of 15 days are shown in Figure (3-3). The effect of salep gum concentration, storage time and the interaction of concentration and storage time on mushroom TSS was significant ($p<0.05$). As can be seen in the figure, the TSS values of the treatments increased with the increase in the concentration of salep gum, and also with the increase of the storage period from day 0 to day 15, the TSS of all treatments also increased. Regarding the increase of TSS with the increase of gum concentration, probably during the test, the measurement of TSS from the dry matter of the gum was also included in the test, and this causes the increase of dry matter in the results of this test [15]. Ghorbani et al. (2015) the reason for the increase of soluble solids can be attributed to the criterion of maturity and ripening of the fruit and the destruction of carbohydrates and the beginning of fruit spoilage and on the other hand the breakdown of acid into sugar during fruit respiration, the gradual decrease in the amount of available water It is in mushroom that happens with the passage of time and during the storage period and causes its soluble solids to be less in the amount of water, as a result Brix becomes more concentrated [35]. Siahroudi et al. (2014) investigated the effect of aloe vera coating along with nettle plant extract

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on the shelf life of edible mushrooms and concluded that with the passage of time during the storage period in cold storage, the amount of soluble solids in mushrooms significantly has increased, which is consistent with the results of the present study [36].

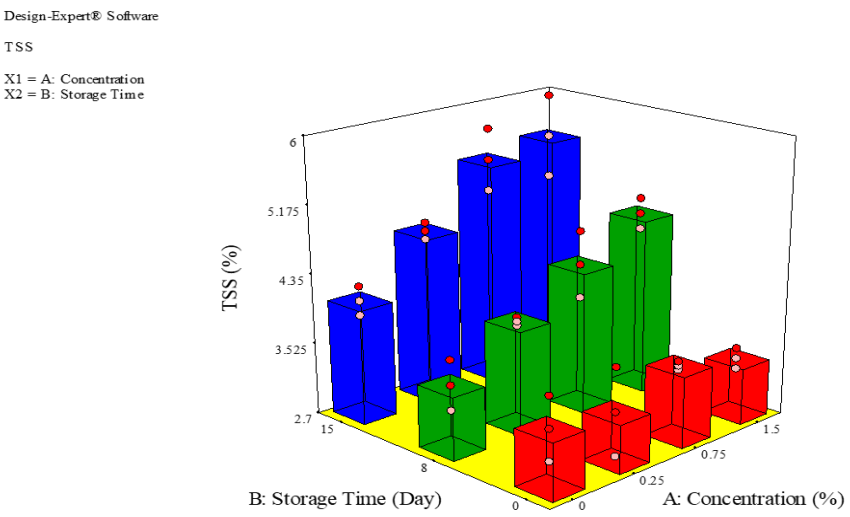

Figure 3-3: Effect of different levels of salep gum edible coating and storage time on button TSS mushroom

5-3 effect of coating on weight loss

The results of evaluating the amount of weight loss in coated and uncoated mushrooms during a period of 15 days are shown in Figure (3-4), the effect of salep gum concentration, storage time and the interaction of concentration and storage time on mushroom weight loss was significant. ($p > 0.05$). As can be seen in the figure, the weight loss is less with the increase in the concentration of salep gum, the results of the weight loss from days 0 to 15 were an increasing trend with the passage of time. Addition of probiotic strain as an active compound inside the coating was effective in reducing weight loss. The reason for this can be due to the antimicrobial properties of probiotics, by reducing the number of mold and yeast that cause spoilage on the surface of edible mushrooms, probiotics reduce the quality loss and naturally the weight loss caused by fungal spoilage. The causes of weight loss in fruits and vegetables are transpiration and the release of carbon dioxide during the breathing process, which is more severe in fruits with thin skin, and after the release of moisture, shriveling and spoilage of the fruit is accelerated. Edible coating delays moisture loss by creating a barrier and by limiting water transfer and protecting the fruit epidermis against the attacks of spoilage microorganisms or by covering possible injuries on the surface of the fruit [37]. Weight loss is an important physiological process and is one of the most important quality indicators of fresh mushrooms. Similar results of Hajbi et al. (2021) showed the combined coating of aloe vera gel, chitosan and calcium chloride on mango fruit, the coating treatments had less weight loss than the control during storage. Edible coatings form a smooth layer on the surface of the fruit that covers the stomata and guard cells and thus reduces water transfer and evaporation as well as gas exchange through the surface of the fruit and ultimately reduces respiration and transpiration [38].

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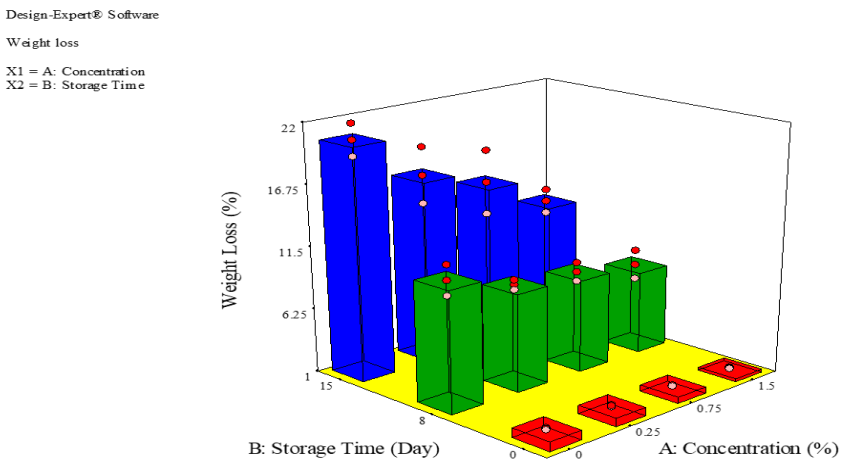

Figure 3-4: Effect of different levels of salep gum edible coating and storage time on button mushroom weight loss

6-3- Effect of coating on ascorbic acid

Ascorbic acid is an important nutrient in measuring product quality, and with oxidation, its amount decreases compared to other nutrients during storage. The highest amount of ascorbic acid is seen in fresh fruits and in the stage before ripening, and then its amount decreases due to the activity of ascorbic acid oxidase enzymes. The oxidation process of ascorbic acid is downward, and its reduction rate depends on the level of dissolved oxygen and storage temperature [39]. The results of evaluating the amount of ascorbic acid in coated and uncoated mushrooms (control) during a period of 15 days are shown in Figure (3-5). As can be seen, with increasing storage time, the amount of ascorbic acid in both control and treated samples decreased slowly. By increasing the concentration of salep gum, compared to the control sample, it significantly prevented the loss of ascorbic acid after 15 days of storage. In the first days between treatments, no significant changes were observed for ascorbic acid ($p<0.05$). And on the eighth day, the changes between the treatments for ascorbic acid were mildly reduced, and at the end of the storage period, the greatest reduction of ascorbic acid was related to the control sample (18.09 mg/100g) and the greatest effect of the coating was related to the concentration of 1.5% (mg/100g 36/28) and the reason is that since the loss of ascorbic acid is closely related to the presence of oxygen, the use of salep gum coating reduces the penetration of oxygen and the rate of respiration, leading to the slowing down of the wilting process, which results Better protection from ascorbic acid and delayed mushroom spoilage. While the control sample has more degradation of ascorbic acid due to the presence of oxygen. Ascorbic acid is a powerful antioxidant that may prevent or reduce damage caused by reactive oxygen species. Ascorbic acid content not only directly affects the antioxidant capacity, but is also an important indicator of the freshness of mushrooms [40]. Ascorbic acid plays a role in controlling browning in several ways. By decreasing tissue pH, it decreases the activity of polyphenol oxidase family enzymes and decreases the bacterial population [41]. The presence of the probiotic Lactobacillus fermentum in the edible coating of the fruit helps to preserve vitamins by reducing the number of pathogenic microorganisms and also by reducing moisture [31]. Amiri et al. (1400) by using a composite edible coating of concentrated milk protein-pectin reinforced with calcium chloride and black seed essential oil for strawberries, showed that the reduction of permeability to oxygen is an important factor in increasing the shelf life of ascorbic acid [12].

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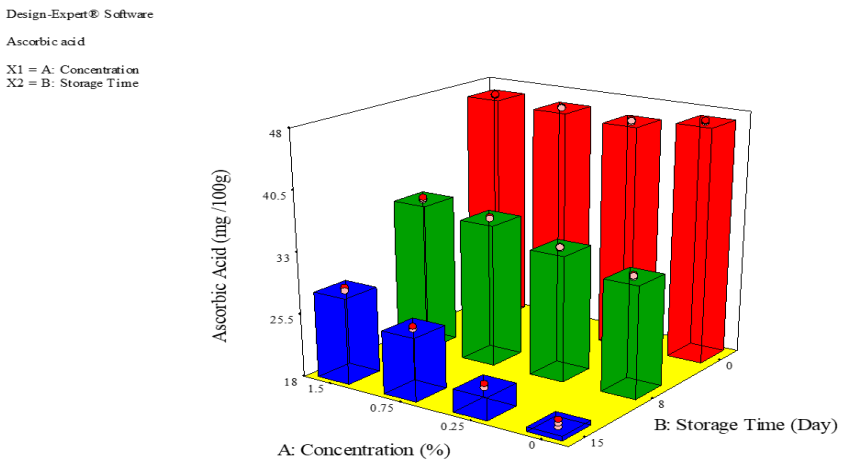

Figure 3-5: Effect of different levels of salep gum edible coating and storage time on button mushroom ascorbic acid

7-3- effect of coating on total antioxidant activity (TAA)

The results of evaluating the amount of antioxidants in coated and uncoated mushrooms (control) during a period of 15 days are shown in Figure (3-6). The effect of salep gum concentration, storage time and the interaction of concentration and storage time on the amount of mushroom antioxidant were significant ($p<0.05$). With the increase of salep gum, the antioxidant capacity increased, but with the increase of shelf life, its value decreased, which is a significant difference ($p<0.05$). By increasing the concentration of the coating due to the protection of phenolic compounds against oxygen, these phenolic compounds have antioxidant properties and show that the antioxidant activity increases. But with the increasing duration of the antioxidant, it decreases, which is a significant difference ($p < 0.05$). With the passage of time, because these compounds are decomposed, they are affected by the polyphenol oxidase enzyme, so the antioxidant activity due to the decrease Phenolic compounds are reduced. Total antioxidant activity changes are consistent with the phenolic content changes in the samples. During storage, the antioxidant activity in mushrooms decreases, which is due to the protection of cells against damage caused by free radicals [15]. More antioxidant activity was preserved in the coated button mushroom, which can be due to the preservation of more ascorbic acid compared to the control sample. The results were consistent with the results of Eshghi et al. (2013) who showed that in strawberries coated with nanoemulsion coating containing chitosan, antioxidant activity is preserved more, which can be due to the preservation of ascorbic acid and anthocyanin more than the control sample [42] . Similarly, in another study by Shahi et al. (2018), the results of comparing the average effect of edible coatings on the antioxidant activity of fresh jujube fruit showed that the antioxidant activity of treated jujubes was higher than the control sample [43].

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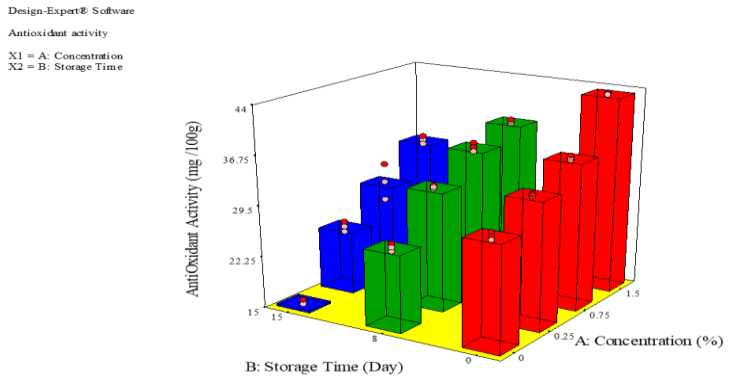

Figure 3-6: Effect of different levels of salep gum edible coating and storage time on button mushroom antioxidants

3-8- Effect of coating on total phenolic concentrations (TPC)

The results of evaluating total phenol content in coated and uncoated mushrooms (control) during a period of 15 days are shown in Figure (3-7). The effect of salep gum concentration, storage time and the interaction of concentration and storage time on the total phenolic compounds of the mushroom was significant ($p<0.05$). Total phenol content increases with increasing the concentration of salep gum and also with increasing storage time, total phenol content increased, phenolic compounds are secondary metabolites that are derived from pathways in plants. These compounds play an important role in the characteristics of color and sensitivity of fruits and vegetables. The phenolic compounds found in plants are an important part of the human diet and due to their antioxidant properties, considerable attention has been paid to them. Phenolic content can serve as an indicator of antioxidant capacity. In the presence of oxygen, the polyphenol oxidase enzyme causes the polymerization of phenolic compounds, reducing their amount and turning them into brown compounds [44]. When the concentration of the coating increases, due to the reduction of contact with oxygen, the protection of phenolic compounds occurs more, as a result, the total phenolic content increases with the increase of the concentration of the coating. The increase in total phenol content with the increase of time can be attributed to the decrease in moisture and increase in dry matter, and with the wilting and shriveling of mushroom samples, the phenol content per unit weight of the mushroom increases, like acidity, and this causes an increase in phenol compounds up to It decreases again on the eighth day and after that, it can be attributed to the starting activity of the polyphenol oxidase enzyme, which once again decomposes some of the phenolic compounds and the phenolic content decreases on the final day [45]. On the other hand, it can be said that the main role of probiotics in reducing the loss of phenolic compounds can be attributed to the property of competition with pathogenic microorganisms and the production of antimicrobial compounds that help to maintain the quality of the fruit and the intensity of the destructive reactions caused by these microorganisms. reduces [31]. The obtained results are in line with the results of Ghorbani et al. (2015), who investigated the effect of edible coating of royal jelly mucilage on the shelf life of button mushrooms and by analyzing the results, they showed that the edible coating had a significant effect in preventing the reduction of the phenolic compounds of the mushroom. corresponded[35].

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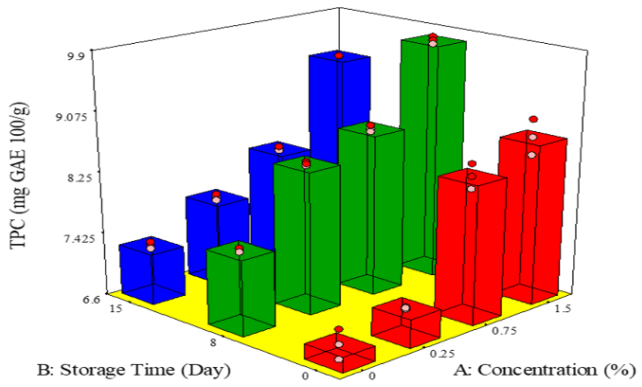

Figure 3-7: Effect of different levels of salep gum edible coating and storage time on button mushroom Total phenol

9-3- parameter L *

As can be seen in the results, the data obtained from the L* parameter generally showed a decreasing trend with the passage of time in most of the samples, the highest level of the L* index in the control sample was 76.34 on day zero and the lowest level was The same sample on the 15th day was 47/32. Because the surface coating by immersion method reduces the brightness of the mushroom surface due to the nature of the coating material. With the increase of salep gum, the brightness of mushrooms increased, but with the passage of time, the brightness decreased, which means that the difference is significant ($p < 0.05$). Esperanza et al. (2018) observed that the use of edible coatings with probiotics preserved the color characteristics of apple and cantaloupe slices better compared to the case where probiotics were used without coating [46].

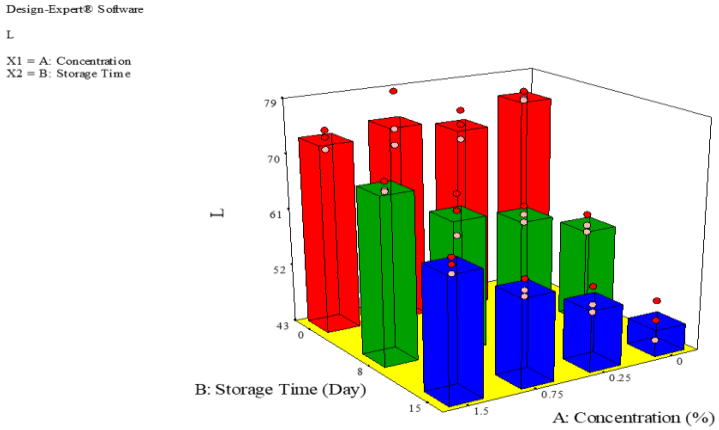

Figure 3-8: Effect of different levels of salep gum edible coating and storage time on button mushroom

10-3- parameter a*

A higher a* indicates an increase in browning reactions during the storage period. As it can be seen in Figure 3-9, in general, the increase in salep gum concentration and duration is significant on the a* parameter ($p>0.05$). On the zero day, two concentrations of salep gum,

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0.75% and 1.5%, respectively, on the a* index increased steadily and had the same effect, and on the same day, 0.25% and the control, respectively, decreased in the same way. On the 8th day of keeping mushrooms in the order of control and the concentration of salep gum, there was an increase of 0.75%, and on the 15th day of keeping the mushrooms in the order of control and different concentrations of salep gum, an increase in the a* parameter was observed

This index, which shows the changes in the redness of the samples during the storage time, increases gradually. Maturation and ripening and respiration of the samples led to an increase in redn. The higher respiration and enzyme activity of the control compared to the coated samples and the faster ripening process of the fruit leads to an increase in the redness of the samples [46].

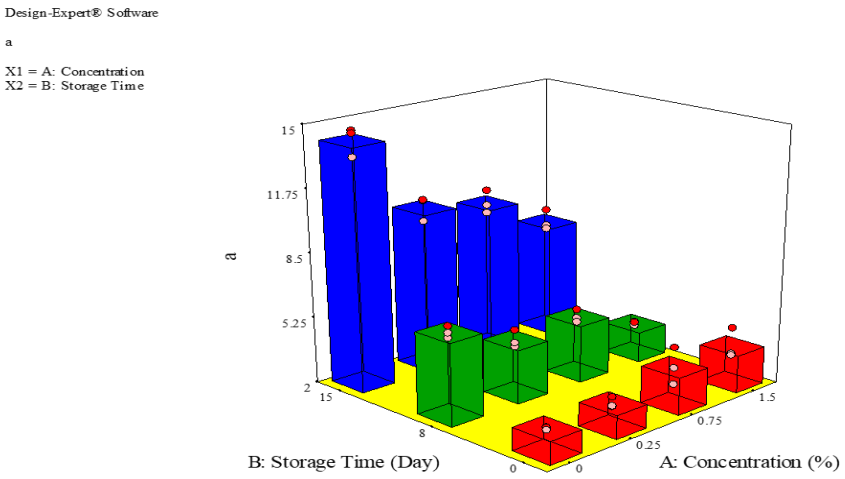

Figure 3-9: Effect of different levels of salep gum edible coating and storage time on button mushroom a*

11-3- parameter b*

As can be seen in the results, with the increase of salep gum, the b* parameter increased with a very slow process, but with the increase of the storage time, b* increased with a rapid process. The lowest value was on day zero for the control sample and the subsequent treatments had constant trends and there was no significant difference ($p>0.05$). On the 8th day, the highest value was 0.75% and the lowest value was 1.5%, and on the 15th day, the highest value was 1.5%, and the rest of the treatments followed a constant trend. The results of b* yellowness were the same as the results of a* redness. During the storage time, moisture leaving the product causes damage to the cell wall and the contact of the phenol oxidase enzyme with the phenolic substrates and eventually browning of the mushroom [44]; Therefore, with increasing storage time, the samples tend to turn yellow. The parameter b*, which is the indicator of color change from blue to yellow, is similar to the changes of parameter a*.

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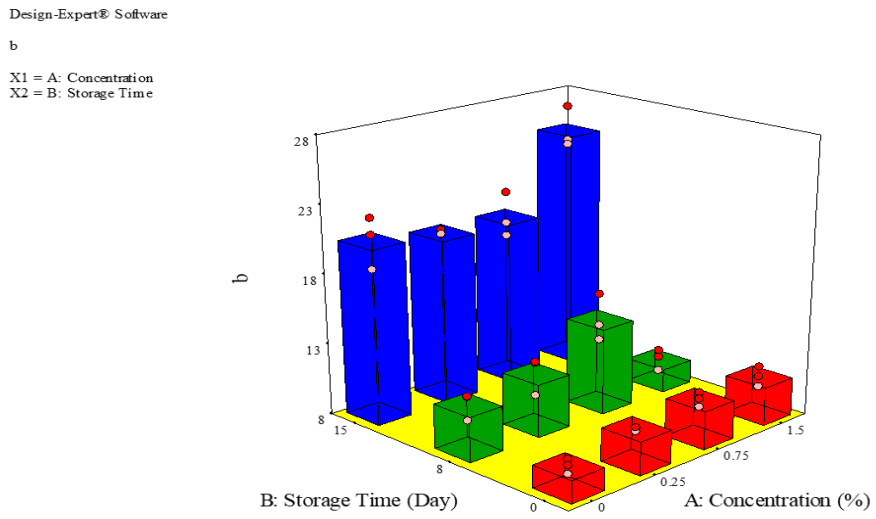

Figure 3-10: Effect of different levels of salep gum edible coating and storage time on button mushroom b*

3-12 browning index Bi

To better describe the color changes due to mushroom coating, the parameter Bi (browning index) can be used. The lower this index is, the lower the browning rate and, as a result, the better the color of the mushroom.[۲۷] . As can be seen in the results, the data obtained from the Bi parameter in all samples showed an increasing trend with the passage of time. The results of statistical analysis showed that there is a significant difference in all treatments ($p<0.05$). There were no significant changes with the increase of salep gum on day zero ($p<0.05$). The greatest effect on the browning color is on the fifteenth day in the control sample and the least effect is on the zero day in the same sample. Also, on the eighth day, the changes were constant, except for the treatment, which decreased by 1.5%, and it may be because of It is a strong antioxidant and the lowest amount is on the 15th day for gum with a concentration of 1.5%. One of the most important reasons for the brown color of mushrooms is related to dark pigments and melanins, which are the product of oxidation and polymerization of phenolic substrates [44]. In another study by Baldwin et al. (1991), edible coating delayed ripening in tomatoes and bananas and also prevented browning reactions, resulting in a better preservation of the color and appearance of the fruits [47]. Due to providing a thick barrier against the exchange of gas between the environment inside and outside the cover and reducing the oxygen level, it caused a delay in the color change and the wilting of the mushroom [48].

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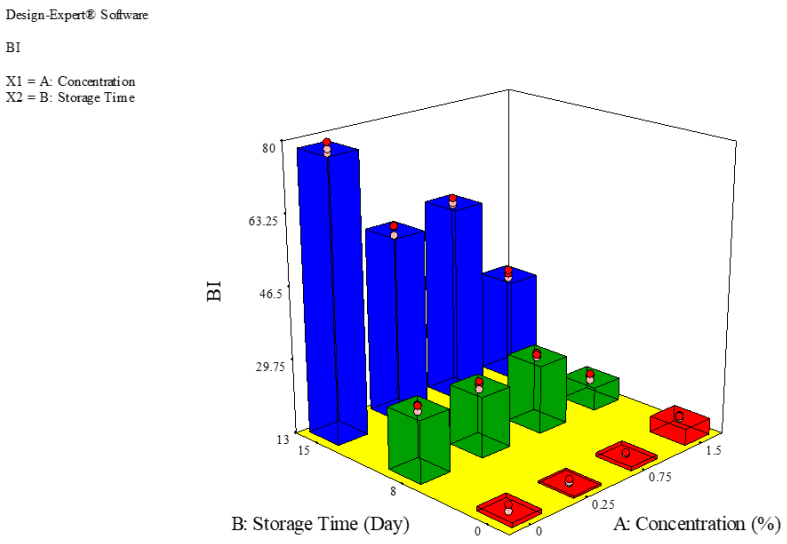

Figure 3-11: Effect of different levels of salep gum edible coating and storage time on button mushroom Bi

13-3- effect of coating on button mushroom tissue

The results of evaluating the degree of tissue stiffness in coated and uncoated mushrooms during a period of 15 days are shown in Figure (12-3). The firmness of button mushroom tissue decreased significantly in all treatments and with the passage of fifteen days of storage time ($p<0.05$). The sample containing 0.25 %salep gum on the 15th day has more coverage than other treatments. This treatment was able to keep the firmness of the mushroom until the 15th day. That is, the mentioned coating prevented the mushroom from breathing too much, and the other treatments, 0.75%, 1.5%, and the control, decreased over time, the texture became softer, and they had no effect on the firmness of the button mushroom. During mushroom storage, its tissue becomes soft and damaged, and the reason for the decrease in tissue strength may be enzyme activity and cell wall destruction, loss of parenchyma tissue, and dissolution of pectin in intracellular fluid [35]. Polysaccharide coatings, such as carboxymethyl cellulose, act like a membrane and as a result delay the dehydration of water and thereby lead to preservation of the texture and firmness of the mushroom sample during the storage period [49]. The reaction of the gum with the cell wall compounds leads to the stiffening of the tissue. It is also possible that excessive use of hydrocolloids causes the creation of resistant layers and covers pores, which results in increased stiffness. Increasing the amount of hydrocolloids creates frequent bonds between polysaccharides and increases tissue stiffness [2]. Lima et al. (2010) investigated the effect of guar gum as an edible coating to increase the shelf life and improve the quality of tomatoes. They concluded that the firmness of uncoated tomatoes is faster than that of coated tomatoes ($p<0.05$). Dropped. After the fourth day, the strength loss increases significantly and continuously and is more prominent in the control samples [50].

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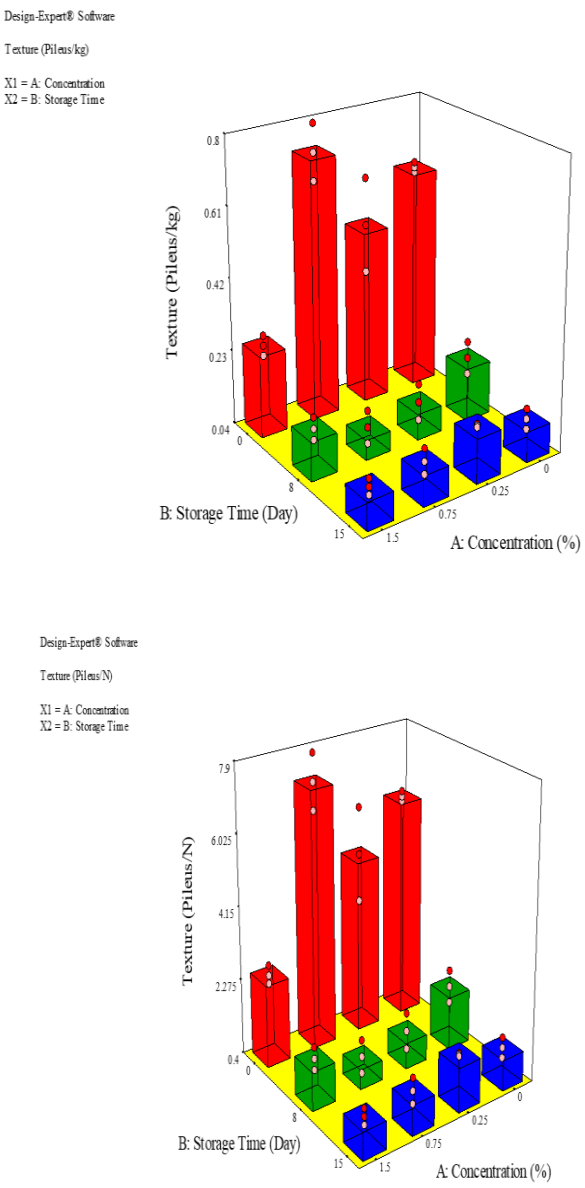

Figure 3-12: Effect of different levels of salep gum edible coating and storage time on button mushroom texture

14-3- effect of coating on the viability of probiotics

The results of the evaluation of the viability of probiotics in coated and uncoated mushrooms (control) during a period of 15 days are shown in Figure (13-3). The effect of salep gum concentration, storage time and concentration interaction on the viability of the probiotic mushroom was significant ($p<0.05$). The results showed that with the increase of time, the number of *Lactobacillus fermentum* bacteria's viability decreases, but with the increase of 0.25%, 0.75% and 1.5% of salep gum, the number of bacteria's viability increases, and in fact, the trapping of probiotic bacteria in The structure of the network, like salep gum, unlike distilled water, can help protect them against environmental stress [2]. Considering that the minimum amount of population of probiotic microorganisms suggested by many researchers when consuming probiotic products is 10^6 CFU/g for the occurrence of health-giving effects in the host [13]. In this study, according to Figure 13-3, the population of probiotic bacteria loaded on the button mushroom surface covered with salep gum at the end of the storage period (4.4×10^8 CFU/g) was more than the recommended amount

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for probiotic products; Therefore, the consumption of 100 g of coated button mushrooms can lead to the transfer of about 10^8 probiotic bacteria cells to the intestine after enduring the stresses of the digestive system. According to these results, in the last decade, similar studies of fresh fruits have been introduced as a suitable carrier for the transfer of probiotic bacteria to the human digestive system. For example, Tapia et al. (2007) used alginate and gelatin coatings containing antioxidants, sunflower oil and *Bifidobacterium lactis* bacteria to coat apples and mangoes. The research results of these researchers showed that after 10 days of storage at 2°C, the bacterial population in apples and mangoes coated with two types of alginate and gellan coatings remained almost constant, and this amount was estimated to be more than 10^6 CFU/g [13]. Shahrampour et al. (2019) reported that the effect of using calcium alginate multi-layer coating containing native probiotic bacteria *L. plantarum KMC45* increased the survival of bacteria during 14 days of cold storage on the surface of strawberry fruit [13]. In another study, Russo et al. (2015) found that the probiotic bacteria species inoculated by immersion method on the surface of sliced cantaloupe was effective in their survival during 12 days of storage at 4°C. So that the live population of B plantarum Lactobacillus was counted more than *PBCC fermentum* Lactobacillus at the end of the storage period[51]. In fact, keeping fruits at low temperature by reducing the metabolic activity of probiotic bacteria increases their survival in refrigerator conditions. Khodayi and Hamidi Esfahani (2019) reported that by increasing the concentration of probiotic bacteria in the carboxymethyl cellulose coating solution, the survival of bacteria increased during 15 days of cold storage on the surface of strawberry fruit [13].

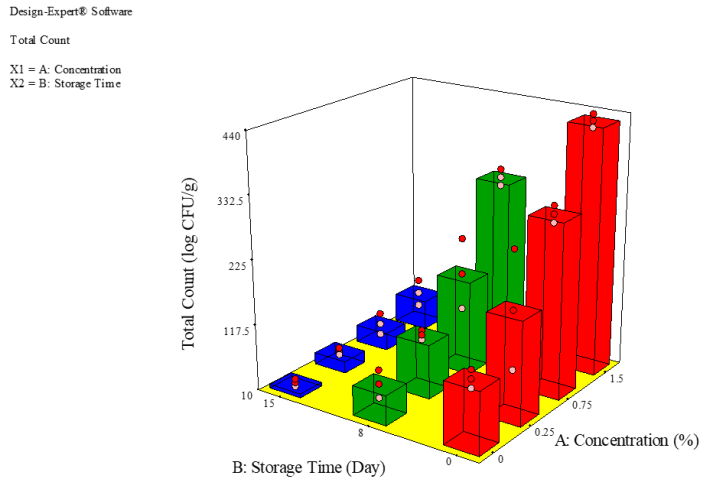

Figure 3-13: Effect of different levels of salep gum edible coating and storage time on probiotic survival on button mushroom

3-15- effect of coating on sensory properties

Descriptive and hedonic sensory evaluation for coated samples was done based on acceptance of color, smell, texture and overall acceptance. The effect of edible salep gum coating containing *Lactobacillus fermentum* and storage time and the interaction effect of coating and storage time had a significant effect on the overall acceptance of coated samples ($p<0.05$). On day zero, the evaluators made the same diagnosis for the color of the treatments and the control sample, and the highest score is related to the overall acceptance, texture and smell of the control sample, and the lowest score in the overall acceptance and texture belongs to the coverage of 0.75. On the eighth day, the highest score for texture, color and overall acceptance is related to treatment 1.5% and 0.75%. The lowest score of color, smell and overall acceptance is 0.25% and the control. On the 15th day, as on the 8th day, the highest score of color, texture, smell and overall acceptance is related to the treatments of 1.5% and 0.75% and the control, and the lowest score was 0.25% and the control. As a result, the mushrooms covered

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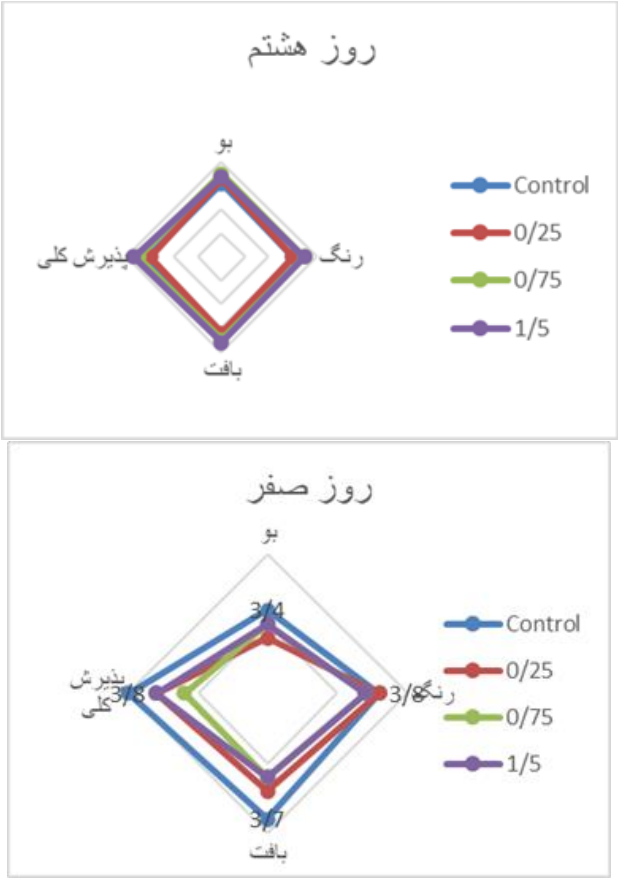
by 1.5% and 0.75% had a better appearance compared to all treatments. Also, no signs of decay or microbial accumulation and sliminess were observed in these treatments. According to the results of this research, Nowrozizadeh et al. (2020) reported the effect of carboxymethyl cellulose/pectin edible coating containing hop extract on orange fruit slices, the overall acceptance of the samples in terms of color, taste, aroma and texture during the storage period. It was preserved even until the 14th day. Also, the taste of the samples did not change significantly during the storage period. It can be said that the shelf life had the greatest impact on the overall acceptance of the samples. The overall acceptance of the samples decreased with the storage time, this decrease was due to the softening of the tissue of the samples [52]. In another study, Russo et al. (2015) stated that apart from smell (caused by the production of bacterial metabolites), other sensory characteristics of sliced cantaloupe changed after inoculation with probiotic bacteria Lactobacillus plantarum B2 and Lactobacillus fermentum during ten days of cold storage. did not [51].



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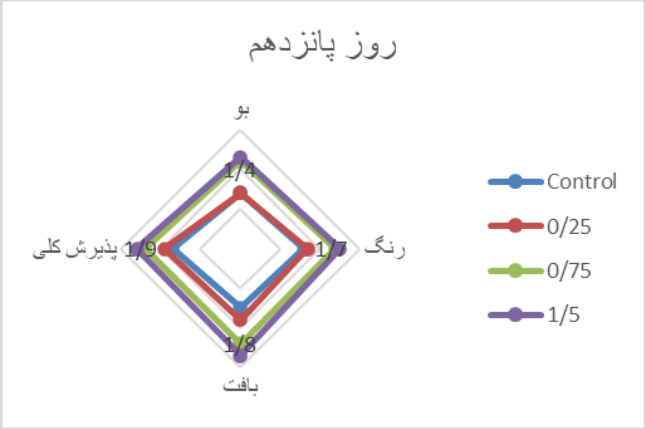

Figure 3-14: Effect of different levels of salep gum edible coating and storage time on sensory properties of button mushroom

4 - Conclusion:

The results of this study showed that coating edible mushrooms with edible salep gum coating containing probiotic bacteria while increasing shelf life can also improve its health value. It can also improve its health value. . Most of the proposed models of this research had high and significant 2R and 2R-adj. The neutral pH of salep gum as well as the lactic acid secreted by the *Lactobacillus fermentum* bacterium has a positive effect on the stability to maintain button mushroom pH, acidity and dissolved solids. The high score of 1.5% coating in reducing the weight loss and maintaining the nutritious compounds of ascorbic acid and total phenol, the antioxidant of the fruit during 15 days of storage. The treatment containing 0.25% salep gum was able to keep the firmness of the mushroom until the 15th day. The use of salep gum coating and probiotic improves and preserves the color evaluation indicators of mushrooms and delays browning. The aforementioned edible coatings contain the number of active cells necessary for the acceptable level of probiotic products. Based on the results, it can be introduced as a bioactive coating and probiotic product. The lack of effect of probiotics on the sensory properties of edible mushrooms even at high concentrations, although the coating along with the presence of probiotics caused a reduction in the amount of spoilage and improved the quality of the fruit, but it was not able to prevent the changes caused by ripening and aging of the fruit, and physical and chemical spoilage occurred. falls down.

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مقاله علمی_پژوهشی

تاثیر پوشش خوراکی صمغ ثعلب حاوی باکتری *لاکتوباسیلوس فرمنتوم* بر ویژگی‌های کیفی قارچ دکمه‌ای

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۱۰/۱۶

تاریخ پذیرش: ۱۴۰۲/۱۲/۲۳

کلمات کلیدی:

پوشش خوراکی،

صمغ ثعلب،

باکتری *لاکتوباسیلوس فرمنتوم*،

قارچ دکمه‌ای

DOI:10.22034/FSCT.21.151.86.

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چکیده

در این پژوهش به بررسی مدت زمان ماندگاری، خواص فیزیکو شیمیایی و حسی قارچ دکمه‌ای با استفاده از پوشش خوراکی صمغ ثعلب حاوی باکتری *Lactobacillus fermentum* پرداخته شد. بدین منظور اثر صمغ ثعلب در سطوح ۰/۲۵، ۰/۷۵، ۱/۵ و افزودن ثابت پروبیوتیک لاکتوباسیلوس فرمنتوم با میزان $1/5 \times 10^{8}$ CFU/gr (۰/۵ نیم‌مک فارلند) به مدت ۱۵ روز در دمای ٤ درجه سانتی‌گراد مورد ارزیابی قرار گرفت. نتایج نشان داد، با افزایش پوشش صمغ ثعلب، pH، اسیدیته، مواد جامد محلول، فنول کل، آنتی‌اکسیدان و پارامتر a* و b* ، بافت‌سنجی در سطح بالایی نسبت به میوه تیمار نشده در حفظ ویژگی‌های کیفی قارچ دکمه‌ای موثرتر بود و تعداد کل باکتری‌ها پروبیوتیک در پوشش در مقایسه با تیمار غوطه‌وری در سوسپانسیون باکتری پروبیوتیک بهتر حفظ شد. ولی افت وزنی، پارامتر L*، شاخص قهوه‌ای شدن با افزایش صمغ ثعلب کاهش یافتند($p<0.05$). و با افزایش مدت زمان ماندگاری مواد جامد محلول و اسیدیته و افت وزنی و a* و b* و شاخص قهوه‌ای شدن افزایش یافتند ولی pH و اسیدآسکوربیک، فنل کل، آنتی‌اکسیدان و L* ، بافت‌سنجی، تعداد کل پروبیوتیک کاهش یافتند. ارزیابی حسی تیمارهای مختلف نشان می‌داد که پوشش حاوی پروبیوتیک بر خواص حسی قارچ خوراکی تأثیر منفی نداشته؛ بلکه سبب بهبود کیفیت حسی و تغذیه‌ای میوه طی زمان و در مقایسه با نمونه شاهد شد؛ بنابراین پوشش خوراکی صمغ ثعلب حاوی باکتری *Lactobacillus fermentum* را می‌توان به‌عنوان یک ماده پوشش‌دهنده مناسب برای حفظ خصوصیات ارگانولپتیکی، شیمیایی، میکروبی و ماندگاری قارچ دکمه‌ای مورد استفاده قرار داد.

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