Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Evaluation of extending the shelf life of button mushroom *(Agaricus Bisporous)* using chitosan gum and *Ferulago angulate* essential oil

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ARTICLE INFO

Article History:
Received:2024/1/2
Accepted:2024/2/5

Keywords:

Agaricus bisporous,

Ferulago angulate,

Essential oil,

Chitosan

DOI: 10.22034/FSCT.21.151.45.

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ABSTRACT

The purpose of this study was to investigate the effect of chitosan coating and *Ferulago angulate* essential oil on button mushroom (*Agaricus Bisporous*) shelf life. Therefore, *Ferulago angulate* essential oil was extracted by clevenger method and whit different concentrations of chitosan (control sample without chitosan and essential oil, with 0.5% chitosan, chitosan with two different concentrations of 100 and 150 ppm) were used to coating the mushroom samples. After packing, the samples were stored in the refrigerator at 4°C for 15 days and samples were evaluated for physicochemical, structural and sensory tests every 3 days. With increasing shelf life, the hardness of all coated samples decreased and addition of chitosan to samples could have a positive effect on increase the hardness of the samples during storage, which was significantly increased by adding *Ferulago angulate* essential oil. All color parameters were significantly retained ($P<0.05$) By adding chitosan which the addition of essential oils exacerbated these conditions. Using the chitosan significantly ($p<0.05$) reduced the weight loss of samples, but the addition of essential oil had no significant effect on reducing weight loss changes. The use of chitosan significantly ($p <0.05$) prevented the growth of total count of microorganism and molds and yeasts, and the addition of essential oil to chitosan as a preservative compound also exacerbated the inhibition of microorganism growth. Similar results were also true for color and appearance evaluation. Chitosan structure, antimicrobial properties and phenolic compounds in essential oils were the main reason for maintaining the quality of mushroom samples during storage.

1- Introduction

Production and consumption of mushrooms have increased significantly in recent years due to their high protein content, bioactive compounds, and minimal carbohydrates, making them valuable as a nutritional and medicinal substance. Among them, button mushrooms are the most well-known edible mushrooms worldwide, dominating both production and consumption markets in Iran and globally [1]. It is evident that living tissues such as mushrooms, when exposed to atmospheric oxygen, insect infestation, airborne microbes, and chemical effects of oxygen, undergo changes in color, taste, and texture, leading to a decrease in product quality. This results in a useful shelf life of mushrooms limited to 3 to 4 days at ambient temperature; in other words, high moisture content and respiration rate in mushrooms, lack of cuticle, moisture loss, and microbial attacks contribute to their perishability. Additionally, temperature fluctuations during storage periods increase oxidative activity and physiological activity [2]. Therefore, only 45% of produced mushrooms are consumed fresh, while the rest undergo various processing operations for subsequent uses. Methods such as cold storage, freezing, heat processes, drying, and chemical additives exist to reduce or prevent these losses, which on one hand incur high costs and energy consumption, and on the other hand, reduce the nutritional value of these products [3].

The use of edible coatings for environmentally sensitive foodstuffs is a technique that has been utilized for many years. Edible coatings are thin layers deposited on the surface of a product that are consumable and capable of preserving the desired properties of the product to a considerable extent [4].

Films derived from polysaccharides often have low gas permeability due to their polymer chain arrangement, but their weak mechanical properties and hydrophilic nature result in poor water vapor permeability [5]. One way to improve this property is to combine them with water-repellent materials such as fatty acids. Various types of polysaccharides and their derivatives have been investigated as films and edible coatings, often including high-methoxyl pectin, Arabic gums, alginates, carrageenans, starch and its derivatives, cellulose and its derivatives, gelatin, guar gum, agar, and chitosan [6].

Chitosan, as a natural amino polysaccharide with unique structure and multifunctional properties, finds wide applications in medicine and industry. Among its prominent characteristics are high biocompatibility, acceptable biodegradability alongside low toxicity, as well as its antibacterial and anti-allergic properties [7].

To enhance the efficiency of edible coatings, antimicrobial, antioxidant, and other active substances can be added to them, which in this case are referred to as active coatings. In fact, edible coatings containing plant extracts, in addition to the advantages shared by all edible coatings, possess antimicrobial and antioxidant effects, making them considered for active packaging and increasingly used [8].

Chuvil plant, known as a natural source of monoterpenes and sesquiterpenes with antimicrobial properties, has been studied by some researchers in recent years. For instance, in one study, 33 compounds comprising 89.7% of the components were identified, of which 1.77% were monoterpenes and 6.12% were sesquiterpenes. The main identified compounds were alpha-pinene (3.17%), bornyl acetate (45.14%), and cis-asimene (4.14%) [9]. Additionally, the antioxidant properties and antimicrobial effects of this plant have been investigated. The yield of essence in dry chuvil plant was 5.0%, and out of the total 24 identified compounds, beta-cis-ocimene (35.41%), alpha-pinene (12.18%), gamma-terpinene (15.61%),

myrcene (3.83%), and para-cymene (3.24%) were the main components of its essence [10].

The use of edible coatings for the preservation of edible mushrooms leads to an increase in shelf life and quality retention, thereby reducing the amount of waste in mushrooms. In this study, chitosan was used as an edible coating along with chuvil plant essence as a synergistic agent for enhancing preservative factors, aiming to increase the shelf life of button mushrooms.

2-Materials and Methods

Extraction of Essence from Chuvil Plant

In this study, the Angulata Ferulago was obtained from a reputable source and received approval from the Plant Pathology Department of Shiraz University for further stages. The essence extraction was performed using the Kloenjor method with distilled water as the solvent. After drying, the extract was preserved using sodium sulfate at an appropriate temperature of 4 degrees Celsius for subsequent steps [11].

Mushrooms coating

To achieve an appropriate chitosan coating, a mixture of 5.0% chitosan powder along with 1.0% glacial acetic acid in distilled water was prepared. Chuvil plant essence was also prepared at two levels, 100 and 150 ppm, and then brought to volume with distilled water in various treatment formulations. The final mixture of different treatments was homogenized using a magnetic stirrer for 2 hours to ensure uniformity. Subsequently, it was sterilized at 121 degrees Celsius for 15 minutes and stored in suitable containers for further use [4].

Edible mushrooms were obtained from a reputable greenhouse and transferred to the laboratory within two hours after harvesting in a dark environment. Then, mushroom samples were coated with appropriate concentrations of the prepared edible coating along with the essence and dried in the laboratory environment for 15 to 20 minutes. After being placed in suitable packaging, they were stored in the refrigerator at 4 degrees Celsius. Samples were taken on days 0, 3, 6, 9, 12, and 15 for the desired tests [4]. The samples were coded as follows: the control mushroom sample without the use of chitosan and essence with the control code, the coated mushroom sample with 5.0% chitosan with the code K, the coated mushroom sample with 5.0% chitosan along with 100 ppm essence with the code KE100, and the coated mushroom sample with 5.0% chitosan along with 150 ppm essence with the code KE150.

Texture analysis

The tissue firmness of the mushrooms, as an indicator of changes in mushroom tissue, was measured using a texture analyzer. To measure the tissue firmness of the mushroom samples with different coating conditions, a texture analyzer (CNF Farnell) was employed. For the texture analyzer, a probe diameter of 3 millimeters, a penetration speed of 10 millimeters per second, and a penetration depth into the mushroom cap of 5 millimeters were considered. The maximum force required to create a hole and penetrate into the mushroom cap to a depth of 5 millimeters was recorded as the firmness (in Newtons) [12].

Color analysis

To measure and analyze the colors, XE, Reston VA Hunter, Lab Scan was employed. In this case, three parameters, a^*, b^*, and L^* were measured at specified intervals [13].

weight loss

The weight of the mushroom samples was measured and recorded before and after transfer to the refrigerator and on days 3, 6, 9, 12, and 15 using a digital scale. Then, the

percentage weight loss of the mushroom samples under different conditions was calculated using Equation 1.

$$weight\ loss\ percentage = \frac{W_0 - W_1}{W_0} \times 100 \qquad \text{Equation 1}$$

W_0 is the weight of the mushroom before coating.
W^1 is the weight of the mushroom sample after the specified time period (days 3, 6, 9, 12, or 15).

Total count of microorganisms

To examine and enumerate the total microorganisms grown in the mushroom samples, a quantity of 25 grams from all mushroom samples at different times was diluted with 225 milliliters of 1.0% peptone water. After a few minutes, the samples were thoroughly homogenized and then serially diluted from 10^-1 to 10^-9 in tubes using 9.0 milliliters of 1.0% peptone water. To estimate the total microbial count, the samples were cultured in PCA (Plate Count Agar) medium manufactured by Merck Germany and incubated at 32 degrees Celsius for 48 hours. After colony growth, they were counted [14].

mold and yeasts

To examine and enumerate the total molds and yeast grown in the mushroom samples, a quantity of 25 grams from all mushroom samples at different times was diluted with 225 milliliters of 1.0% peptone water to investigate the level of molds and yeast. The samples were thoroughly homogenized for a few minutes. Serially diluted samples were prepared in tubes from 10^-1 to 10^-9 using 9.0 milliliters of 1.0% peptone water. For calculating and estimating the amount of molds and yeast, the samples were cultured in PDA (Potato Dextrose Agar) medium manufactured by Merck Germany and incubated at 28 degrees Celsius for 5-7 days [13].

Sensory Evaluation of Color and Appearance of Mushroom Samples

Sensory evaluation for the coated samples was conducted using the 5-point Hedonic method based on color and appearance. For this test, 10 trained assessors were utilized, where 1 represented the lowest score and 5 represented the highest score. Mushroom samples, adequately coded, were provided to the assessors to evaluate and record their scores accordingly.

statistical analysis

A statistical analysis was conducted using a completely randomized design. One-sided analysis of variance (ANOVA) was performed, and the comparison between means was done using the Duncan multiple range test at a confidence level of 95%. Statistical analysis was carried out using SPSS software version 16. All experiments were replicated three times.

3-Result and discution

Texture analysis

The evaluation of tissue firmness of different mushroom samples with varying concentrations of chitosan and clove essential oil is presented in Table 1. As the results clearly demonstrate, during the 15-day storage period, the tissue firmness of all examined samples significantly decreased (p-value < 0.05). Examination of the results revealed that adding chitosan for mushroom coating effectively preserved the mushroom tissue during storage, and adding essential oil to chitosan reduced tissue destruction, with the greatest effect on preserving mushroom tissue observed with chitosan coating combined with 150 ppm essential oil. On the other hand, the control sample or mushrooms without coating deteriorated faster than the other samples. Until the third day of storage, the difference in compound percentages

compared to the control sample was not significant (p-value < 0.05), but from the third day onwards, the compounds and percentages used had a significant effect on preserving the firmness of mushroom tissue.

The main reason for the deterioration of mushroom tissue during storage is that over time, under the influence of pectolytic enzymes and microbial enzymes, cell walls are degraded, and the mushroom tissue is severely damaged and softened [15]. The destruction of the mushroom tissue structure can be attributed to the consumption of nutrients as respiratory substrates and the loss of surface moisture, resulting in the impact on cells and their destruction [16].

During fruit ripening, the polymerization of pectin and other pectic substances occurs with increased activity of pectinesterase and polygalacturonase [17]. Low oxygen concentration and high carbon dioxide concentration in the environment potentially reduce the activity of these enzymes and help maintain the strength of fruits and vegetables during storage [18]. It is expected that fruit coating can alter the internal gas composition of fruits, especially by reducing oxygen concentration and increasing carbon dioxide concentration, leading to slower tissue changes in coated fruits [19].

Table 1 - The effect of treatment and storage time on hardness of mushroom samples

	0	3	6	9	12	15
Control	33.8±0.32^{b}	30.5±0.32^{b}	25.7±0.27^{d}	22.6±0.32^{d}	16.8±0.26^{d}	12.6±0.41^{d}
K	35.6±0.42^{a}	33.4±0.42^{a}	30.2±0.52^{c}	26.3±0.48^{c}	21.3±0.37^{c}	15.7±0.63^{c}
K.E100	35.6±0.49^{a}	33.6±0.46^{a}	30.8±0.47^{b}	28.2±0.52^{b}	23.9±0.31^{b}	19.6±0.27^{b}
K.E150	35.6±0.61^{a}	33.7±0.61^{a}	31.6±0.55^{a}	29.6±0.71^{a}	26.4±0.59^{a}	22.1±0.33^{a}

*The same letters indicate insignificance at the 5% level in each column

Color analysis (a*, b* and L*)

The a* color index during storage has increased for all samples (Figure 1). Using chitosan coating significantly reduced the changes in color index ($p<0.05$) and adding essence to this combination could also have a significant effect ($p<0.05$) on maintaining and stabilizing this color index in mushroom samples. The least color changes in this index were related to mushroom samples coated with chitosan and 150 ppm essence. As clearly shown in Figure 1, the control sample or the sample without coating had the highest color changes during storage. Until the third day, there were no significant changes in the a* color index, but from the third day onwards, the compounds used had a significant effect on maintaining the a* index.

The increase in the a* color index during the storage period indicates the effect of physiological and microbial processes on the changes in this index, which varied depending on the type of compounds used for coating. Using chitosan significantly reduced the color changes in this index during storage ($p<0.05$), and adding essence to this compound further reduced the color changes significantly ($p<0.05$). It seems that from the sixth day onwards, the use of clove essence significantly reduced the change in the b* color index and contributed to maintaining color stability.

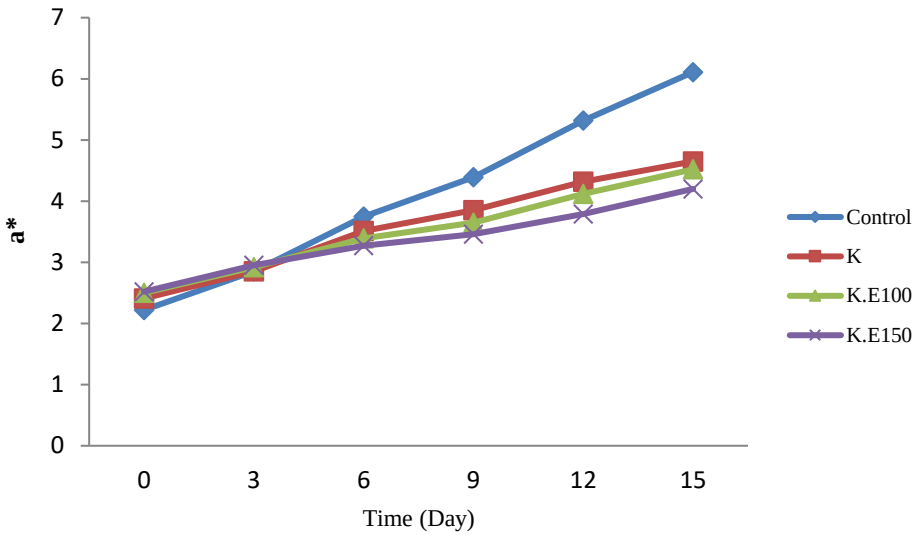

Figure 1- The effect of treatment and storage time on a* index in mushroom samples

The results of measuring the b* index in this study were also almost similar to the results of the a* index. With the increase in storage time, the b* color index of all mushroom samples increased, and this increase in the color index and its changes for the control sample was significantly higher than other samples (Figure 2). The use of chitosan significantly reduced the color changes in this index during storage ($p<0.05$), and the more essence was used, the more significant reduction in color changes occurred. It seems that from the sixth day onwards, the use of clove essence significantly reduced the change in the b* color index and contributed to maintaining color stability.

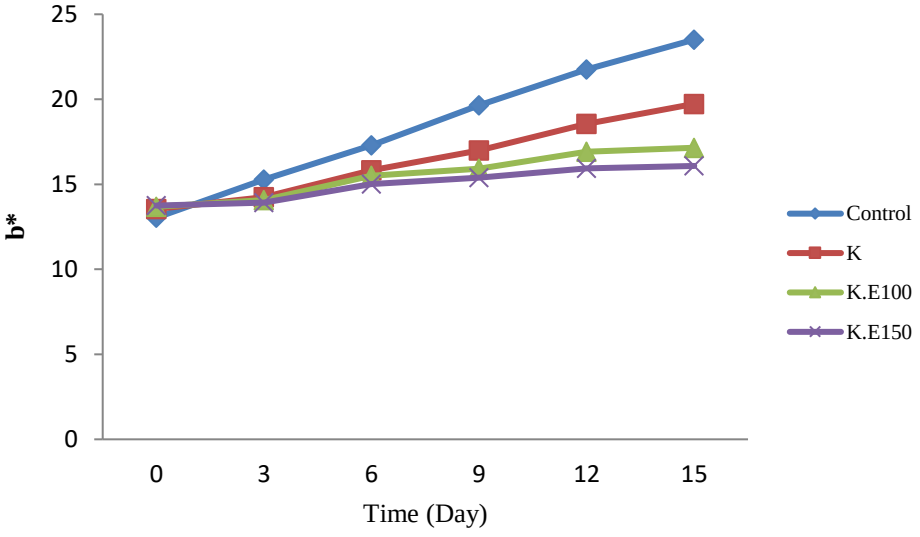

Figure 2- The effect of treatment and storage time on b* index in mushroom samples

The results of measuring the L* color index during the storage of mushroom samples for 15 days are shown in Figure 3. As it is clear, the L* color index value decreased with increasing storage time for all samples, resulting in a decrease in brightness and transparency of the mushrooms. It is also evident in Figure 3 that the color changes in all samples decreased, and this decrease was much

more significant ($p<0.05$) in the control sample, which lacked any coating. Like the other two color indices, the least color changes in the L* index were related to samples containing chitosan and clove essence, which seems that clove essence, especially in the final stages of storage, significantly ($p<0.05$) maintained the L* index and contributed to preserving the brightness and appearance of mushrooms.

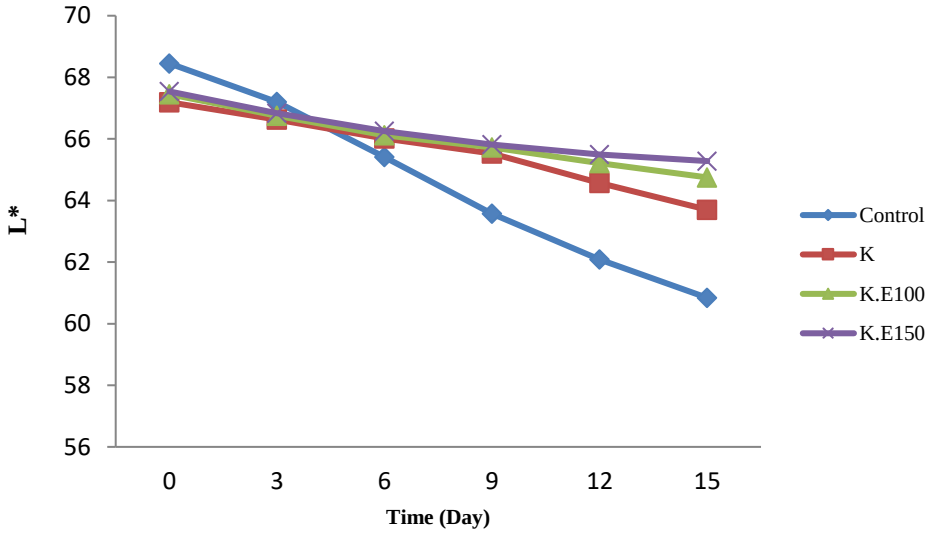

Figure 3- The effect of treatment and storage time on L* index in mushroom samples

The L* color index indicates the brightness and transparency of the product, and considering the white and bright nature of mushrooms, this index is very important, as the higher the L* color index of mushrooms, the more consumers will be inclined to buy them (Sapers et al., 1994).

Mahbobi et al. [21] investigated the effect of coatings of aloe vera and katira on mushrooms and the results showed that the combination of aloe vera and katira coating was the best sample in terms of the L* component, which is consistent with the present results.

In general, over time, the color of the mushroom cap changes from white to brown. Factors affecting the color of mushrooms during storage include: an increase in microbial population, enzyme activity, the physiological conditions of the mushrooms themselves, the quality of

Hashem et al. [20] reported that an increase in the value of a* indicates an increase in browning reactions, which here, chitosan coating on edible mushrooms controlled the a* value and improved color properties in coated samples, consistent with the present findings.

washing operations, cutting, transportation, and packaging [22].

During storage, moisture loss from the product causes damage to the cell walls and the activation of polyphenol oxidase enzymes, with phenolic substrates, ultimately leading to browning of the mushrooms. Explaining the positive performance of chitosan coating in preserving and stabilizing color changes over time compared to the control sample can be stated as follows: this combination preserves the structure and pores, prevents oxygen entry, thereby reducing respiration and physiological activities, as well as reducing microbial growth, all of which result in reducing physiological cell activities and consequently reducing enzymatic activities (thus delaying browning), thereby reducing color changes in mushroom samples. On the other hand, one of the main reasons for reducing color changes in using this polysaccharide

combination can be attributed to maintaining the water-holding capacity by cells during storage [23].

Eissa [24] investigated the use of chitosan coating on the shelf life and quality of mushroom slices, and the results showed that chitosan coating could affect the preservation of slice color. Chang et al. [25] examined the effect of chitosan coating on the quality of pork during storage for 7 days. Chitosan coating had no significant effect on the color of meat pieces, and there was no change in the natural color of the meat during storage. Preventing oxygen and moisture from penetrating into the product tissue and trapping metal ions are important and effective properties of chitosan. These properties prevent undesirable enzymatic and non-enzymatic reactions that lead to color changes in the product.

Furthermore, based on comparisons of a*, b*, and L*, clove essence has been recognized to effectively preserve the color of mushrooms and intensify the positive performance of chitosan, which can be attributed to the phenolic compounds present in clove essence that have very high antimicrobial properties and consequently reduce microbial growth and physiological activities [26].

The results of this study are consistent with other studies that have evaluated the shelf life of mushrooms based on imaging techniques, showing that mushroom storage negatively affects all color parameters over time [27].

Weight Loss

The results of weight loss during the 15-day storage of mushroom samples are presented in Table 2, and it is evident that the use of chitosan coating significantly (p-value < 0.05) reduced the weight loss of mushroom samples during storage. This significant effect (p-value < 0.05) compared to the control sample is clearly apparent. However, adding clove essential oil to chitosan for coating did not result in a significant (p-value < 0.05) change in weight loss during storage, and there was no significant difference in weight loss between samples coated with chitosan alone and those containing essential oil.

The primary mechanism for the reduction in weight loss during storage is attributed to the polysaccharide structure of chitosan, which leads to pore blockage on the surface of mushrooms [28, 29], reducing oxygen penetration and moisture loss, thereby inhibiting cellular respiration and water loss, ultimately resulting in decreased weight loss of the samples [13]. This reduction in weight loss can significantly impact other parameters such as microbial growth, color, and texture.

Li et al. in 2011 used nano-sized particles of zinc for coating apple slices, and their results showed that the use of these compounds for coating, by blocking pores, reduced the weight loss of samples and significantly increased the shelf life of apple slices [30].

Preserving fruit mass indicates the integrity of the cell wall. This is associated with reduced respiratory activity and demonstrates the protective effect of the coating. As water evaporates from the liquid to the gas phase, moisture is lost. When the product is harvested, it loses its water source, so rehydration of lost water is not possible. Additionally, when water causes metabolic changes, enzyme activity may change, accelerating aging. The results of this research are consistent with the results of other researchers' studies, where a decrease in fruit weight during aging was observed, and during aging, the metabolism of the cell wall is disrupted, reducing water retention during this period. Coating reduces weight loss, indicating a delay in aging [28].

Jiang et al. in 2011 stated that the main reason for the weight loss of mushrooms is due to water transpiration and carbon dioxide loss during respiration. It is a fact that mushrooms are only covered by a thin epidermis layer that cannot effectively protect them against water loss, and coating can prevent weight loss [14].

Nosrati and Kamp used alginate polysaccharide coating in 1993 to increase the shelf life of mushrooms, reporting that alginate coating significantly reduced the weight loss of mushrooms during storage [31].

Respiration is an inevitable biological process. This process is one of the prominent characteristics after harvest, during which stored materials such as carbohydrates, proteins, etc., are gradually consumed as respiratory substrates, leading to slow weight loss and irreversible changes in the product. With increased respiratory intensity, aging occurs more quickly, and any factor that delays respiration contributes to increased product shelf life. Coating is effective because it restricts oxygen entry with its barrier property, thereby slowing down respiration. As a result, weight loss of the product decreases [32].

Table 2 - The effect of treatment and storage time on weight loss of mushroom samples

Treatment/Time	3	6	9	12	15
Control	10.35±0.16[a]	19.55±0.19[a]	31.85±0.19[a]	44.15±0.24[a]	53.25±0.28[a]
K	8.6±0.08[b]	17.15 ± 0.15[b]	24.6 ±0.15[b]	31.3 ±0.17[b]	37.80 ±0.18[b]
K.E100	8.6±0.07[b]	17.15 ±0.08 [b]	24.4 ± 0.13[b]	31.00 ±0.15[b]	37.60 ±0.21 [b]
K.E150	8.6±0.05[b]	17.12±0.03[b]	24.2 ±0.12[b]	29.90 ±0.15[b]	37.70 ±0.23 [b]

*The same letters indicate insignificance at the 5% level in each column

Microbiological assessment (growth of indicator microorganisms)

The overall growth of microorganisms on mushroom samples during the 15-day storage under different coating conditions was measured, and the results are presented in Table 3. With the increase in storage time, the overall growth of microorganisms significantly increased (p-value < 0.05). Upon careful examination of the results, it was found that the use of chitosan significantly (p-value < 0.05) inhibited the overall growth of microorganisms compared to the control sample, with these differences becoming particularly evident from the sixth day of storage onwards. On the other hand, the addition of essential oil to the samples as part of the coating significantly (p-value < 0.05) inhibited the growth of microorganisms, and the higher the concentration of the essential oil used, the greater its effectiveness against microorganisms.

Table 3 - The effect of treatment and storage time on General growth of microorganisms in mushroom samples

Treatment/Time	0	3	6	9	12	15
Control	4.43 ±0.07[a]	4.95±0.02 [a]	5.47±0.03[a]	6.15±0.07[a]	6.8± 0.08[a]	7.23±0.11[a]
K	4.43± 0.07[a]	4.85±0.04[a]	5.20±0.07[ab]	5.36 ±0.08[b]	5.87±0.04[b]	6.46 ±0.09[b]
K.E100	4.43 ± 0.07 [a]	4.76 ±0.02[a]	5.04±0.05[b]	5.15±0.04[c]	5.38±0.08[c]	5.51±0.08[c]
K.E150	4.43 ± 0.07[a]	4.60±0.01[a]	4.95 ±0.01[b]	5.01 ±0.03[c]	5.17±0.06[cd]	5.28±0.07[cd]

*The same letters indicate insignificance at the 5% level in each column

In this study, the effect of coating under different conditions using chitosan and essential oil on the growth of mold and yeast was also evaluated and investigated, with the results presented in Table 4. As the examination of the results indicates, similar to the overall growth of microorganisms, the amount of mold and yeast significantly increased (p-value < 0.05) from the first day to the fifteenth day of storage. The highest increase was observed in the control sample, while the lowest increase in mold and yeast was observed in the sample

coated with chitosan and 150 ppm of essential oil. The results of this study clearly showed that the use of chitosan for coating edible mushrooms had a significant effect (p-value < 0.05) on reducing the amount of mold and yeast on mushrooms. Furthermore, the addition of clove essential oil was able to intensify the antifungal effect of the chitosan combination, with this effect being enhanced with increasing concentrations of the essential oil up to approximately 150 ppm.

Table 4 - The effect of treatment and storage time on mold and yeast growth in mushroom samples

Treatment/Time	0	3	6	9	12	15
Control	3.04±0.08^{a}	3.95±0.02^{b}	4.81±0.06^{a}	5.52±0.06^{a}	6.00±0.08^{a}	6.73±0.11^{a}
K	3.04±0.08^{a}	3.92±0.05^{b}	4.31±0.05^{b}	4.63±0.09^{b}	5.01±0.08^{b}	5.54±0.08^{b}
K.E100	3.04±0.08^{a}	3.76±0.03^{ab}	4.01±0.04^{bc}	4.29±0.07^{bc}	4.44±0.05^{c}	4.64±0.09^{bc}
K.E150	3.04±0.08^{a}	3.60±0.04^{ab}	3.84±0.04^{bc}	4.07±0.04^{bc}	4.30±0.06^{c}	4.41±0.08^{bc}

*The same letters indicate insignificance at the 5% level in each column

It seems that the surface pore coverage by chitosan, and consequently the reduction of available oxygen and moisture, preserves the higher activity of protective enzymes and cellular membrane integrity, enhancing the ability of fruits to withstand microbial attack and preventing excessive growth and proliferation of microorganisms compared to the control sample lacking coating. Additionally, the amino groups of chitosan have bacteriostatic effects and can reduce the number of microbes [32].

With increasing chitosan concentration, the positive charge resulting from the presence of amino groups increases, leading to stronger electrostatic interactions. This creates stronger reactions between chitosan and the cell walls of microorganisms, thereby increasing the antimicrobial effect of chitosan [33].

Khaleghi and colleagues investigated the effect of chitosan coating on protecting strawberries in 2010, and their results showed that all chitosan treatments reduced the activity of various fungi, and likewise, Jia et al. in 2010 studied the antifungal effect of chitosan coating on pear fruit, and their results indicated a significant effect of chitosan in preventing fungal growth [34,35]. All these studies are consistent with the results of this research.

Furthermore, the addition of clove essential oil has further enhanced this effect, with the main reason for this enhancement being attributed to the phenolic compounds present in the essential oil, including alpha-pinene, bornyl acetate, and cis-asarone, whose antimicrobial properties have been previously demonstrated [36].

Considering the number of chemical compounds in clove, a unified mechanism for its antibacterial activity cannot be considered. However, an important feature is its hydrophobic nature, which allows these substances to penetrate the lipid components of bacterial and mitochondrial membranes, disrupting bacterial structure and increasing their permeability. This problem leads to the leakage of ions and other cellular contents. Although the release of limited amounts of these substances by bacteria is tolerable, it affects their viability and, in the case of significant release of cellular contents or ions, leads to cell death [37].

Sensory evaluation

In this study, a sensory evaluation based on color and appearance was designed, the results of which are clearly shown in Figure 4. The sensory evaluation results of the color and appearance of edible mushrooms showed that the quality of the mushrooms in all examined coated samples and controls decreased significantly ($p< 0.05$) during the 15-day storage period. The results indicated that the use of chitosan had a significant

effect on the appearance and color of mushroom samples, reducing color changes during storage. Additionally, the use of clove essential oil was also able to significantly ($p< 0.05$) reduce color changes during the 15-day storage period. The lowest scores given for the appearance and color of mushroom samples belonged to the control sample, while the highest scores were given to the mushroom sample coated with chitosan and 150 ppm of clove essential oil.

The reason for this color stability in the coated samples containing essential oil can be attributed to the factors mentioned in previous sections, including inhibition of microbial growth, reduction of physiological activities, weight loss reduction, cellular respiration reduction, etc. This evaluation of color and appearance indices is consistent with the findings of Mahboubi et al. (2012), who investigated the effect of aloe vera with tragacanth gum on edible mushrooms [21]. In general, it can be stated that all factors influencing tissue preservation, weight loss reduction, microbial load reduction, and color indices of the product directly play a role in preserving sensory evaluation parameters. The significant effect of using essential oil and chitosan on preserving sensory evaluation indices was notable.

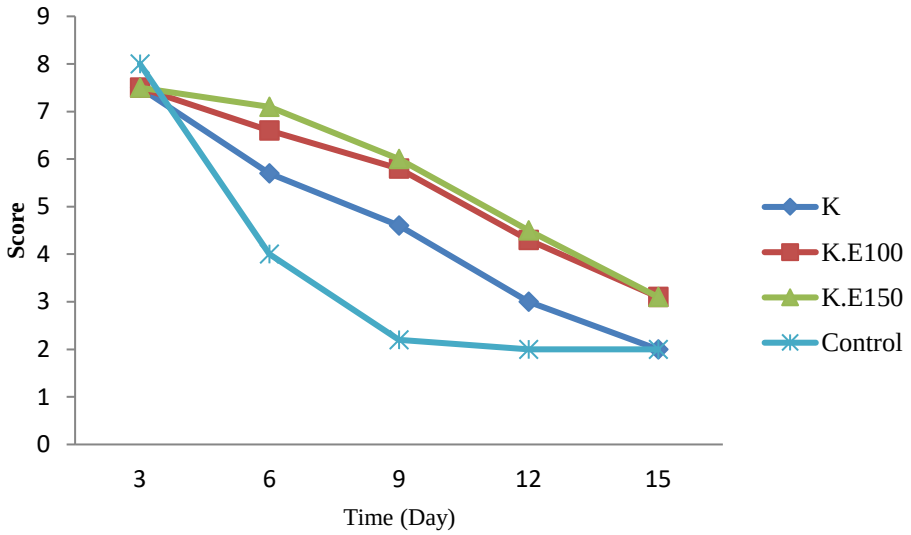

Figure 4– The effect of treatment and storage time on sensory evaluation in mushroom samples

4-Conclusion

The greatest effect on preserving the quality of mushroom samples was observed with chitosan along with 150 ppm of clove essential oil. On the other hand, the control samples or mushrooms without coating deteriorated earlier than the other samples. The use of the chitosan blend for coating significantly reduced the color changes of the color indices a^* and b^*, ($p < 0.05$), and adding essential oil to this blend was able to significantly maintain the stability of these color indices in mushroom samples ($p < 0.05$). The L* value, indicating the brightness of the mushroom samples, decreased with increasing storage time in all samples. However, the addition of chitosan resulted in less decrease in the L* color index, and adding clove essential oil was able to maintain the brightness of the mushrooms significantly more ($p < 0.05$).

The use of chitosan coating significantly reduced the weight loss of mushroom samples during storage, and adding essential oil to chitosan for coating did not create a significant change in weight loss during storage. Generally, with the increase in storage time for 15 days, the overall growth of microorganisms, molds, and

yeasts increased. The use of chitosan was significantly effective ($p < 0.05$) in preventing the overall growth of microorganisms, molds, and yeasts compared to the control samples. Additionally, adding essential oil to chitosan as a preservative compound also had an intensifying effect on inhibiting microbial growth. The higher the concentration of essential oil used, the greater the impact on microorganisms.

The color and appearance of the coated mushroom samples decreased during the 15-day storage period, which chitosan was able to significantly reduce the changes in appearance and color of the mushroom samples from the panelists' point of view ($p < 0.05$). On the other hand, adding clove essential oil to chitosan was also able to significantly reduce the intensity of color and appearance changes during storage ($p < 0.05$).

It seems that the carbohydrate structure of chitosan leads to blockage of surface pores of the mushrooms, reducing oxygen penetration into the tissue, and ultimately hindering cell respiration, which in turn reduces weight loss of the samples and decreases respiratory intensity. This reduction in weight loss and respiratory intensity can in turn significantly affect other parameters such as microbial growth, color, and tissue firmness. The main reason for the greater effectiveness of chitosan along with essential oil can also be attributed to the phenolic compounds present in the essential oil, whose antimicrobial properties have been previously proven, and these properties complement the lipid properties of chitosan, collectively impacting the preservation of sample quality.

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بررسی افزایش عمر ماندگاری قارچ خوراکی دکمه‌ای (آگاریکوس بیسپوروس) با استفاده از صمغ گیاهی کیتوزان و اسانس گیاه چویل

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۱۰/۱۲

تاریخ پذیرش: ۱۴۰۲/۱۱/۱۶

کلمات کلیدی:

قارچ دکمه‌ای،

اسانس چویل،

کیتوزان،

پوشش‌دهی

DOI:10.22034/FSCT.21.151.45.

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چکیده

هدف از انجام این پژوهش بررسی تأثیر پوشش‌دهی با کیتوزان و اسانس گیاه چویل بر روی ماندگاری قارچ دکمه‌ای بود؛ اسانس گیاه چویل به روش کلونجر استخراج شده و به همراه کیتوزان در غلظت‌های مختلف (نمونه کنترل بدون کیتوزان و اسانس، با ۰/۵ درصد کیتوزان، کیتوزان همراه با دو غلظت ۱۰۰ و ۱۵۰ پی‌پی‌ام اسانس) برای پوشش‌دهی قارچ استفاده گردید. پس از بسته‌بندی، قارچ‌ها به مدت ۱۵ روز در دمای ٤ درجه سانتی‌گراد در یخچال نگهداری شده و هر ۳ روز یک‌بار نمونه‌ها از نظر آزمون‌های فیزیکوشیمیایی، بافتی و حسی مورد ارزیابی قرار گرفتند. با افزایش زمان نگهداری، سفتی بافت همه نمونه‌های پوشش داده شده کاهش یافت و افزودن کیتوزان برای پوشش‌دهی قارچ توانست به‌طور مؤثری بر روی حفظ سفتی بافت قارچ در طی نگهداری تأثیر مثبت بگذارد که این تأثیرگذاری مثبت با افزودن اسانس چویل به‌طور معنی‌داری بیشتر گردید. با افزودن کیتوزان مورد استفاده، مقدار همه شاخص‌های رنگی به‌طور معنی‌داری ($p<۰/۰۵$) حفظ گردیدند که افزودن اسانس این تأثیرگذاری را تشدید کرد. استفاده از کیتوزان توانست افت وزنی نمونه‌های قارچ را به‌طور معنی‌داری ($p<۰/۰۵$) کاهش دهد، اما افزودن اسانس تأثیر چندانی بر روی کاهش تغییرات افت وزنی نداشت. استفاده از کیتوزان توانست به طور معنی‌داری ($p<۰/۰۵$) نسبت به نمونه شاهد مانع رشد کلی میکرواورگانیسم‌ها و کپک و مخمرها گردد و افزودن اسانس به کیتوزان به عنوان یک ترکیب نگهدارنده نیز نقش تشدیدکننده‌ای بر ممانعت از رشد میکرواورگانیسم اعمال کرد که این نتایج مشابه در مورد ارزیابی رنگ و ظاهر نیز کاملا صدق می‌کرد. ساختار کیتوزان و خاصیت ضد میکروبی و ترکیبات فنولی موجود در اسانس علت اصلی حفظ کیفیت نمونه‌های قارچ در طی نگهداری بود.