

## The Effect of Peanut Coating with Chitosan and Chitosan Nanoparticles on its Taste Sensory Properties and Growth Inhibition of *Aspergillus flavus*

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### Abstract

Food contamination with fungi and their toxins is a serious public health issue. Peanuts can be exposed to microbial spoilage and adverse changes in their taste sensory properties. Chitosan is one of the biopolymers that is biodegradable and has antimicrobial activity. The purpose of this study was to investigate the effect of peanut coating on chitosan and chitosan nanoparticles on *Aspergillus flavus* and the taste sensory properties of peanut. Mold counting tests were performed by culture on Saburo dextrose agar medium, culture progress percentage by slide culture and microscopic observation and sensory properties (taste, color and general acceptance) by 5-point hedonic method by 15 evaluators. For this purpose, the experiments were performed on peanuts by covering the levels of 0.5, 1 and 1.5% of chitosan and chitosan nanoparticles and the control sample (7 treatments in total) at room temperature and darkness on days 0, 14, 28 and 42. The effect of type of treatments and storage time on the total count and the rate of progression of *Aspergillus flavus* and sensory characteristics was significant ( $P < 0.01$ ). But the interaction between treatments and the storage time of the samples were not significantly different. The highest and lowest antimicrobial effects were related to concentrations of 0.5% chitosan and 1.5% chitosan nanoparticles. But the highest sensory score was observed in the control and day zero samples and the lowest score was observed in the samples coated with 1.5% chitosan nanoparticles. The most suitable coating concentration can be 0.5% chitosan and 1% chitosan nanoparticles.

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### Introduction

Today's customers are always looking for healthy food and prefer their personal health to anything. In this regard, food science has made it a priority to provide a healthy food product with all the desired tissue, sensory, physiological and rheological characteristics (Mozaffari

Nejad, 2011). Peanut is one of the most useful products around the world due to the high sensitivity of this food, concerns about pollution to pathogens. In terms of cultivation, it is considered as the second legumes after soybean and can provide the most important nutritional needs of human nutrition (Jiao, Zhu, Deng, & Zhao, 2016).

The important dangers for peanuts can be referred to two destructive and pathogenic fungal species called *Aspergillus flavus* and *Aspergillus Parasiticu*, which are the producer of the aflatoxin (Mexis, Badeka, Riganakos, Karakostas, & Kontominas, 2009). Unfavorable storage conditions and storage period of this valuable food cause aflatoxin, moisture absorption, fat oxidation, taste of aging and, by nature, reduce the quality of the product. One way to prevent and reduce the incidence of this problem is to use food coatings that act as a barrier against the transfer of moisture, oxygen and solutes in food and are part of food products (Mozaffari Nejad, 2011).

Edible coatings prevent food migration and microorganisms and prevent the release of free radicals because most of them are made from very natural materials with anti-cancer and anti-radical properties (Badawy & Rabea, 2009). Among the food coatings used, chitosan and chitosan nanoparticle are widely used and are widely used as food coatings (Mohammadpour Dounighi *et al.*, 2012).

Chitosan is a cheap, non-toxic, biodegradable and friendly compound (Gholami, Ahmadi, & Ahmadi, 2020) and has antimicrobial activity (Rozman *et al.*, 2019; Skandari, *in press*). It was observed that chitosan films containing *Zataria multiflora* essential oil had a significant increase in the anti-listeria properties of chitosan films. Also, these films were significantly ( $P < 0.05$ ) more effective on gram-positive bacteria than gram-negative bacteria (Molaei Aghaei, Kamkar, Akhondzadeh Basti, Khanjari, & Kontominas, 2016). Apart from its direct microbial activity, especially its antifungal properties; other studies show that chitosan induces a series of defense reactions associated with enzymatic activity, increasing the production of glucohydrolases, phenolic compounds, and the synthesis of phytotoxins. Certain phytotoxins with antifungal activity and

also reduce the production of enzymes such as polygalacturonases, pectin methyl esterase, etc. In addition, due to its ability to create a semi-finished coating, it extends the shelf life of fruits and vegetables by minimizing respiration and reducing water loss (Bautista-Baños *et al.*, 2006).

Due to the importance of controlling spoilage caused by fungi and chemical spoilage in food products, this study was conducted to investigate the antifungal effects of chitosan and chitosan nanoparticles on peanuts, especially *Aspergillus flavus* and its sensory properties.

## Materials and methods

### Sampling

*Aspergillus flavus* PTCC 5004 was prepared from the collection of microorganisms of the Scientific-Industrial Research Organization of Iran. Peanut samples were prepared from Sanandaj market and immediately transferred to the refrigerator located in the microbiology laboratory.

### Preparation method

First, 1 kg of raw peanuts was purchased from the market and then peeled (manually). Peanuts were tested with chitosan (Sigma-Aldrich, USA) and chitosan nanoparticle (Sigma-Aldrich, USA) with a thickness. 27-240 nm were coated, 7 samples were prepared so that one treatment without coating as a control and other treatments with chitosan 0.5, 1 and 1.5 wt% in acetic acid (Kian Kaveh, Iran) 0.1 volumes and were coated with 0.5, 1 and 1.5 wt% chitosan nanoparticle in 0.1 vol. acetic acid. Treatments were performed to perform total count counts of mold, percentage of *Aspergillus* mold progression and sensory characteristics (taste, color, general acceptance) inside foam packages with cellophane coating at ambient temperature and darkness on days 0, 14, 28, 42 and at these intervals.

### Activation of *Aspergillus flavus*

Lyophilized ampoule containing *A. flavus* strain with a special razor from the marked part under sterile conditions and slightly cut next to the flame to thin and easily broken with a push. Using pasteurized pipette, some of the culture medium of sterile liquid chloramphenicol dextrose extract (Qlab, Canada) was diluted, poured into the vial itself, a suspension was prepared and some of it was transferred to the test tubes. Test tubes with screws containing 10 mL of culture medium were closed and discarded after transferring the suspension. Subsequent tubes were cultured in the same way. Finally, the tubes were placed in a greenhouse (Memmert, Germany) at a temperature of 22 to 25 °C for 7 to 14 days until the greenhouse was placed and sporulation took place (Iranian National Standardization Organization [ISIRI], 2015).

### *Aspergillus flavus* count

After preparation of physiological saline dilution up to 6-10 dilution with peanut samples, cultured them superficially on Saburo dextrose agar (Merck, Germany) and incubated (Memmert, Germany) at 25 °C for 7 days were placed (Trucksess & Pohland, 2002).

### Progression of *Aspergillus flavus*

From each treatment, 10 peanuts were placed in sterile glass-lined culture dishes containing wet filter paper contaminated with *A. flavus* in a 25 °C oven. After 5 days, the number of peanuts that had grown in each mold containing mold was counted and the rate of growth of this mold was cultured on slide culture. Observed under the microscope (Olympus, Japan) to ensure the growth of *Aspergillus species* (Muñoz, Moret, & Garcés, 2009).

### Evaluation of sensory characteristics (consumer opinion)

15 male and female panelists in the age

range of 20 to 30 years were selected using a degree or quality recognition test to examine the samples in terms of quality factors that can be perceived by the senses such as taste, color, general acceptance. Then graded them according to preference. 15 identical samples of each treatment were prepared and given to the judges along with a special form that had a 5-point hedonic scale to complete the forms according to their taste. For this purpose, 5 points were allocated for good quality and 1 point for bad quality. The distribution was such that people's opinions about each other had the least effect. The evaluation was performed within a specific hour (preferably at the time) of evaluation between 11 and 9 am. The completed forms including the overall consumer evaluation were converted into a numerical value and the results were analyzed by analysis of variance (Han, Lederer, McDaniel, & Zhao, 2005).

### Statistical test

Two-factor factorial experiment was performed in a completely randomized design (CRD) with three replications. The first factor was coverage treatment at 7 levels and the second factor was time (day) in 4 time periods. Test data were subjected to analysis of variance (ANOVA). Fisher's least significance difference (LSD) at ( $P < 0.01$ ) was used to compare the radial growth means. Minitab 16 software was used for statistical operations and Excel software 2010 was used to draw graphs.

### Results and discussion

The results of the analysis of variance for counting and progression of *Aspergillus flavus* Peanut samples showed that the effect of chitosan and nanochitosan coating on storage time and the interaction of time and treatments were significant ( $P < 0.01$ ) (Table 1).

**Table 1.** Results of analysis of variance of the effect of different coatings of chitosan and nanochitosan on antifungal properties in peanuts

Sources change	Degrees of freedom	P-Value	
		Mold count	Mold progress
Treatment	6	0.000**	0.000**
Day	3	0.000**	0.000**
Treatment×Day	18	0.000**	0.000**
Error	56	-	-

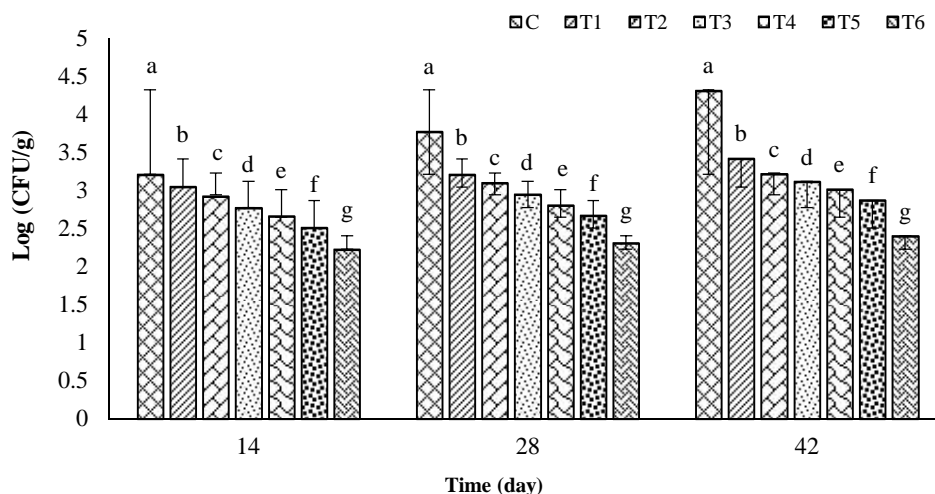
\*\* Significant at 1% level probability.

The highest mold count was related to the control sample and the lowest was related to the treatment of 1.5% chitosan nanoparticles. The number of molds also decreased with increasing time. In all treatments, the lowest and highest number of molds were related to days 14 and 42, respectively. All treatments had lower total mold count than the control and with increasing the coverage percentage, the total mold count of the product decreased, so that the highest total mold count in the control sample and day 42 of storage and the lowest total mold count in the coated samples it was stored with 1.5% nanochitosan on day 14 (Fig. 1).

The highest development of mold was observed in the control sample and then decreased in the treatments containing chitosan but no growth was

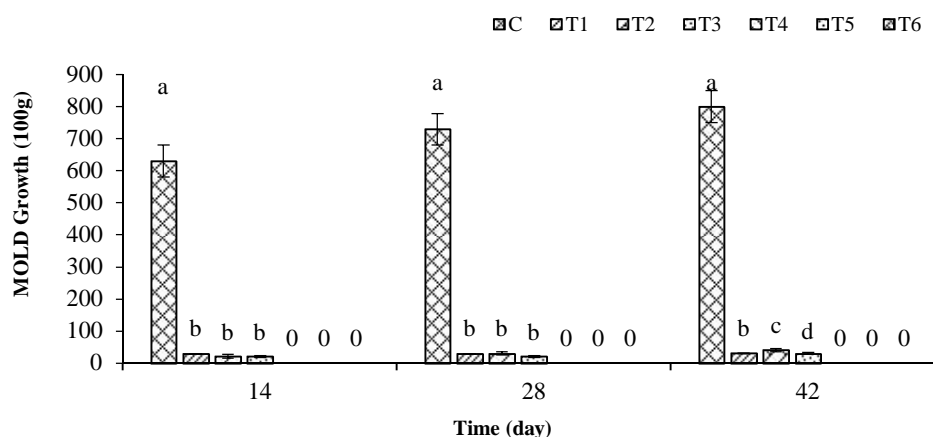
observed in the treatments containing chitosan nanoparticle. In control and chitosan-containing treatments, the progression of mold also increased with increasing time. Therefore, the highest and lowest mold progression was related to the control sample on day 42 of storage and chitosan 1.5% on day 14 of storage (Fig. 2).

The highest development of mold was observed in the control sample and then decreased in the treatments containing chitosan but no growth was observed in the treatments containing chitosan nanoparticle. In control and chitosan-containing treatments, the progression of mold also increased with increasing time. Therefore, the highest and lowest mold progression was related to the control sample on day 42 of storage and chitosan 1.5% on day 14 of storage (Fig. 2).



**Fig. 1.** Comparison of different coating treatments of chitosan and nanochitosan in terms of the mean count of *Aspergillus flavus* at the storage times of 14, 28 and 42 days

C: Sample without chitosan and chitosan nanoparticle, T1: Sample with 0.5% chitosan, T2: Samples with 1% chitosan, T3: Sample with 1.5% chitosan, T4: Sample with 0.5% nanochitosan, T5: Sample with 1% nanochitosan and T6: Sample with 1.5% nanochitosan. Dissimilar letters in each column indicate a significant difference ( $P < 0.01$ ).



**Fig. 2.** Comparison of different coating treatments of chitosan and nanochitosan in terms of the mean rate of progression of *Aspergillus flavus* at storage times of 14, 28 and 42 days

C: Sample without chitosan and nanochitosan, T1: Sample with 0.5% chitosan, T2: Samples with 1% chitosan, T3: Sample with 1.5% chitosan, T4: Sample with 0.5% nanochitosan, T5: Sample with 1% nanochitosan and T6: Sample with 1.5% nanochitosan. Dissimilar letters in each column indicate a significant difference ( $P < 0.01$ ).

**Table 2.** Results of analysis of variance of the effect of different coatings of chitosan and nanochitosan on sensory properties of peanut

Sources Change	Degrees of freedom	P-Value	
		Flavor acceptance	Color
Treatment	6	0.002**	0.000**
Day	3	0.000**	0.000**
Treatment × Day	18	0.695 <sup>ns</sup>	1.000 <sup>ns</sup>
Error	56	-	-

\*\* Significant at 1% level probability, ns not significant.

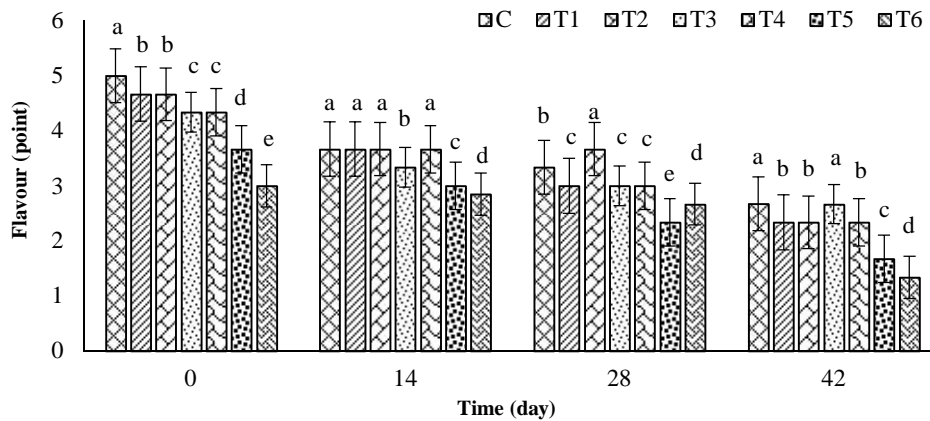
The results and comparison of means of sensory properties of peanut under the influence of chitosan and chitosan nanoparticle in Table (2), the results of analysis of variance based on the effect of type of treatments, storage time and interaction of treatments and time on the amount of taste, color and overall acceptance of peanuts were significant at the level of 1% probability.

The results related to sensory characteristics (taste, color, general acceptance) are shown (Figs. 3, 4 and 5). The least effect on peanut taste was related to the control sample and the most effect was related to the treatment of 1.5% chitosan nanoparticle. In all treatments, the effect of taste increased with increasing time. Therefore, the highest score of this feature was in the control sample and day zero and the lowest in the samples coated with 1.5% chitosan nanoparticle (Fig. 3).

The lowest effect on peanut color was related to the control sample and the highest effect was related to the treatment of 1.5% chitosan nanoparticle. So that in all treatments, the effect of color increased with increasing time. Therefore, the highest score of this feature was in the control sample and zero day of storage and the lowest in the samples coated with 1.5% chitosan nanoparticle (Fig. 4).

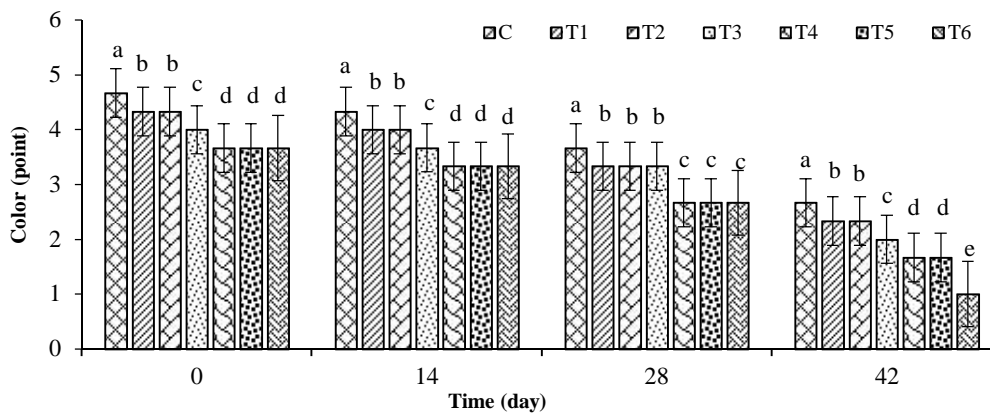
The lowest effect on the total acceptance of peanut was related to the control sample and the highest effect was related to the treatment of 1.5% chitosan nanoparticles. In all treatments, the effect on overall acceptance increased with increasing time. Therefore, the highest score of this feature was in the control sample and zero day of storage and the lowest in the samples coated with 1.5% chitosan nanoparticle (Fig. 5).





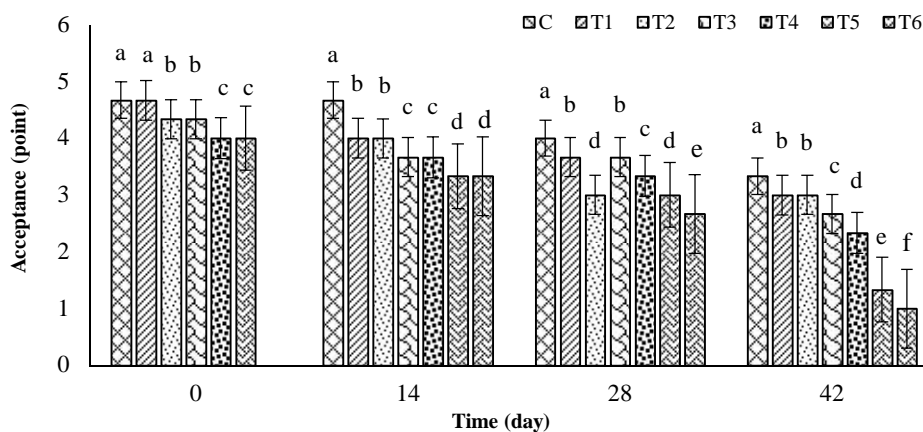
**Fig. 3-** Comparison of different coating treatments of chitosan and nanochitosan in terms of peanut flavor mean at the storage times of 14, 28 and 42 days

C: Sample without chitosan and nanochitosan, T1: Sample with 0.5% chitosan, T2: Samples with 1% chitosan, T3: Sample with 1.5% chitosan, T4: Sample with 0.5% nanochitosan, T5: Sample with 1% nanochitosan and T6: Sample with 1.5% nanochitosan. Dissimilar letters in each column indicate a significant difference ( $P < 0.01$ ).



**Fig. 4.** Comparison of the mean effect of treatments and storage time on peanut color with different coatings of chitosan and nanochitosan

C: Sample without chitosan and nanochitosan, T1: Sample with 0.5% chitosan, T2: Samples with 1% chitosan, T3: Sample with 1.5% chitosan, T4: Sample with 0.5% nanochitosan, T5: Sample with 1% nanochitosan and T6: Sample with 1.5% nanochitosan. Dissimilar letters in each column indicate a significant difference ( $P < 0.01$ ).



**Fig. 5.** Comparison of the average effect of treatments and storage time on the overall acceptance of peanuts with different coatings of chitosan and nanochitosan

C: Sample without chitosan and chitosan nanoparticle, T1: Sample with 0.5% chitosan, T2: Samples with 1% chitosan, T3: Sample with 1.5% chitosan, T4: Sample with 0.5% nanochitosan, T5: Sample with 1% nanochitosan and T6: Sample with 1.5% nanochitosan. Dissimilar letters in each column indicate a significant difference ( $P < 0.01$ ).

In the present study, the count and the rate of mold development, the control sample showed higher values than the treatments coated with chitosan and chitosan nanoparticle and even in the treatments containing nanochitosan no mold improvement was observed, so the effect of peanut coating with chitosan and chitosan nanoparticles is inevitable. Is (Figs. 1 and 2).

The reduction in the development of mold in chitosan samples may also be due to the antimicrobial effect of acetic acid dissolved with chitosan. Acetic acid is a weak organic acid that becomes molecular (non-ionized) at neutral pH, so the power to enter the cell has microorganisms and kills them. The zero rate of mold development in nanochitosan samples may be related to the interaction of nanochitosan polycationic molecules with the anionic components of the microorganism cell wall, which causes changes in cell wall permeability.

In one study, the results showed that in tomatoes treated with nanochitosan, the percentage of *Aspergillus* mold development was zero, which is consistent with the results of the present study (Li, Feng, Yang, Wang, & Su, 2008; Muñoz *et al.*, 2009). The taste of higher concentrations of chitosan can be detected due to the aftertaste similar to the taste of fish or other seafood. High concentrations of chitosan slightly altered the taste of peanuts (Devlieghere, Vermeulen, & Debevere, 2004).

Chitosan acts as a protector on products and prevents unwanted reactions and undesirable discoloration. Preventing the penetration of oxygen and moisture into the product tissue and trapping metal ions are important and effective properties of chitosan. These properties prevent adverse enzymatic and non-enzymatic reactions that lead to discoloration of the product (Cao, Xue, & Liu, 2009). In a recent study, a decrease in the score of sensory properties (taste, color, general acceptance) of chitosan-coated and chitosan

nanoparticles was observed compared to the control, which was consistent with the results of another study. These results showed that in tomatoes, the coatings treated with chitosan and chitosan nanoparticles had lower scores than the control (Cao *et al.*, 2009).

In a recent study, a decrease in the score of sensory properties (taste, color, general acceptance) of chitosan-coated and chitosan nanoparticles was observed compared to the control, which was consistent with the results of another study. These results showed that in tomatoes, the coatings treated with chitosan and chitosan nanoparticles had lower scores than the control (Cao *et al.*, 2009).

The effect of chitosan coating on pork during refrigerated storage for 7 days has been investigated. Based on the results of chitosan coating during the storage period, the natural color of meat did not change, which is consistent with the results of the present study (Chang, Chen, & Tan, 2011). One study found that chitosan-coated strawberries were not significantly different from fresh strawberries after one week compared to fresh strawberries, and their properties did not change (Han *et al.*, 2005).

By examining the effect of pure Aloe Vera gel coatings, 1% chitosan and 50:50 chitosan/Aloe Vera combination coating on grapes, it has been suggested that chitosan/Aloe Vera composite coating can delay the decrease of acidity and increase pH and increase the amount of soluble solids is effective. It was also observed that the coatings used prevented the growth of *Aspergillus niger* in grapes and had little effect on the sensory properties of grapes. The results of this study are consistent with the present study in inhibiting the growth of molds and also have less effect on sensory properties by covering 1.5% of chitosan (Iranian National Standardization Organization [ISIRI], 2015).

In one study it was shown that the properties of chitosan film are completely affected by the amount of extract used and

improve the antioxidant properties and phenol of the whole chitosan film. Such films are very suitable for use in food (Moradi *et al.*, 2012).

### Conclusions

Due to the fact that the greatest effect on inhibiting the growth and development of mold *Aspergillus flavus* is chitosan 0.5%

and nanochitosan 1.5% and on the other hand nanochitosan 1.5% has a greater effect on the sensory properties of peanut than other treatments. Therefore, the most suitable peanut coating concentration to inhibit the growth of *Aspergillus flavus*, which does not have a significant negative effect on its taste sensory properties, is 0.5% chitosan and 1% nanochitosan.

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## بررسی تأثیر پوشش دهی بادام زمینی با کیتوزان و نانوذره کیتوزان بر خواص حسی و مهار رشد آسپرژیلوس فلاووس

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### چکیده

آلودگی غذا به قارچ‌ها و سموم آنها مسئله جدی سلامت جامعه است. بادام زمینی می‌تواند در معرض فساد میکروبی و تغییرات نامطلوب در خصوصیات حسی چشایی آن قرار گیرد. کیتوزان یکی از پلیمرهای زیستی است که دارای قابلیت تجزیه‌پذیری و خاصیت ضد میکروبی است. هدف از انجام این تحقیق بررسی اثر پوشش دهی بادام زمینی توسط کیتوزان و نانوذره کیتوزان بر آسپرژیلوس فلاووس و خواص حسی بادام زمینی بود. آزمون‌های شمارش کپک با کشت روی محیط کشت سابورو دکستروز آگار، درصد پیشرفت کپک توسط اسلاید کالچر و مشاهده با میکروسکوپ و خواص حسی (طعم، رنگ و پذیرش کلی) با روش هدونیک 5 نقطه‌ای توسط 15 نفر ارزیاب آموزش دیده بود. بدین منظور آزمون‌های فوق روی بادام زمینی با پوشش دهی سطوح 0/5، 1 و 1/5 درصد کیتوزان و نانوذره کیتوزان و نمونه شاهد (در مجموع 7 تیمار) در دمای محیط و تاریکی در روزهای صفر، 14، 28 و 42 انجام گرفت. شمارش کلی کپک، میزان پیشرفت کپک، تأثیر نوع تیمارها و زمان نگهداری بر ویژگی‌های حسی در سطح احتمال 1 درصد معنی‌دار بود ( $P < 0/01$ ). اما اثر متقابل تیمارها و زمان نگهداری نمونه‌ها تفاوت معنی‌داری با هم نداشتند. بیشترین و کمترین اثر ضد میکروبی مربوطه به غلظت‌های 0/5 درصد کیتوزان و 1/5 درصد نانوذره کیتوزان بود. بیشترین امتیاز حسی چشایی در نمونه‌های شاهد و روز صفر نگهداری و کمترین امتیاز در نمونه‌های پوشش داده شده با 1/5 درصد نانوذره کیتوزان مشاهده شد. مناسب‌ترین غلظت پوشش دهی مربوط به 0/5 درصد کیتوزان و 1 درصد نانوذره کیتوزان بود.

واژه‌های کلیدی: ارزیابی حسی، بادام زمینی، ضد قارچی، کیتوزان، نانوذره کیتوزان