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Inhibition the growth of *Aspergillus flavus* in fresh pistachios by edible coating based on carboxymethyl cellulose/aloe vera gel containing thyme and savory essential oils

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ARTICLE INFO	ABSTRACT
<p>Article History:</p> <p>Received:2024/08/26</p> <p>Accepted:2024/12/30</p>	<p>Iran is one of the largest exporters of pistachios in the world. Pistachios are one of the valuable export products that are very susceptible to <i>Aspergillus flavus</i> contamination. In this regard, studies revealed that edible coatings are highly effective in preventing pistachio fungal contamination, aflatoxin production and thus increasing its shelf life. This study aimed to investigate the effect of edible coating based on carboxymethyl cellulose and aloe vera containing savory (<i>Satureja hortensis</i>) and Shirazi thyme (<i>Zataria multiflora</i>) essential oil on the production of aflatoxin by <i>Aspergillus flavus</i>, weight loss, fat and protein content of the samples during the storage period. Also, the antifungal properties of different concentrations of savory essential oil, thyme, and aloe vera and their synergistic effect on <i>Aspergillus flavus</i> were investigated. The results showed that savory and thyme essential oils at a concentration of 1000 ppm and aloe vera gel at a concentration of 20% significantly inhibited the growth of <i>Aspergillus flavus</i>. The highest prevention of weight loss, reduction of fat content, and reduction of the protein content of the samples during 30 days of storage were observed by coating with aloe vera and thyme, coating with carboxymethyl cellulose and thyme, and coating with aloe vera and carboxymethyl cellulose, respectively. Coating the samples using 1% carboxymethyl cellulose and 20% aloe vera gel containing the essential oil of savory and thyme with a concentration of 1000 ppm significantly prevented the contamination of samples with aflatoxins B₁ and B₂ until the 40th day of storage ($p < 0.05$). As a result, using edible coating based on carboxymethyl cellulose and aloe vera containing savory essential oil and thyme is a suitable solution to increase the shelf life of pistachio and prevent its fungal contamination and aflatoxin production.</p>
<p>Keywords:</p> <p>aloe vera,</p> <p>thyme,</p> <p>edible coating,</p> <p>pistachio,</p> <p>savory.</p>	
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1- Introduction

The pistachio tree is a plant of the Anacardiaceae family that grows in subtropical regions and its cultivation and cultivation in Iran has a historical background. Currently, Iran is one of the largest pistachio exporters in the world. Studies have shown that unfavorable environmental conditions during storage cause mold growth in pistachios, production of toxins, especially aflatoxin, moisture absorption and the appearance of stale flavors, and ultimately a decrease in the quality and marketability of the product [1]. Aflatoxins are metabolites produced by toxic strains of molds, mainly *Aspergillus parasiticus* and *Aspergillus flavus*. Due to their high lipid and carbohydrate content, *Aspergillus* fungi have a strong tendency to grow on seeds and oilseeds [2]. The growth and development of these fungi on pistachios and oilseeds has caused serious problems in humans and animals and is considered a major cause of liver cancer in developing countries. *Aspergillus flavus* and *Aspergillus parasiticus* are two important aflatoxin-producing species among the various *Aspergillus* species [3]. One way to prevent the growth of microorganisms and solve storage problems is to use edible coatings/films. In various studies, proteins, carbohydrates and lipids have been used to produce edible coatings. In the production of edible coatings based on carbohydrates, various polysaccharides such as starch, cellulose, carboxymethyl cellulose, alginate and chitin are used. Carboxymethyl cellulose (CMC) is produced by reacting cellulose with sodium hydroxide and chloroacetic acid. This compound is soluble in water and forms flexible and strong films on its own. Carboxymethyl cellulose is also one of the

cheapest biopolymers produced industrially [4]. Aloe vera gel is widely used as a coating for fresh fruits and vegetables due to its antimicrobial properties and reduced weight and moisture loss [5]. Edible coatings can contain bioactive compounds such as antimicrobial, antioxidant, and antibiotic compounds, the release of which from the coating on the food increases the shelf life of the product and prevents the increase in its microbial load [6]. Among the bioactive compounds that can be used in edible coatings, the savory plant with the scientific name *Satureja hortensis* belongs to the mint family. Sage mainly contains phenolic compounds thymol and carvacrol, which have antifungal activity [7]. On the other hand, thyme (*Thymus vulgaris* L.) is another plant of the mint family that has a variety of uses in the food, pharmaceutical, health and cosmetic industries. Thyme has long been widely used in traditional medicine and has been shown to have properties such as antispasmodic, carminative, antifungal, antiseptic, anthelmintic, antirheumatic and expectorant. Thyme essential oil has a special place in global trade and is used as a preservative in food products due to its antibacterial, antifungal and antioxidant properties [8]. In various studies, different edible coatings such as chitosan [1], aloe vera gel [9], carboxymethyl cellulose [10] and alginate [11] have been used to increase the shelf life of pistachios. Therefore, considering the above, the aim of this study was to investigate the effect of edible coating based on CMC/Aloe vera gel containing savory or thyme essential oil on the physicochemical and antifungal properties of coated pistachios during storage.

2- Materials and Methods

2-1- Chemicals

Chemicals used in this study, including Tween 80, carboxymethyl cellulose, and PDA culture medium, were purchased from Merck (Germany). The fresh pistachio used in this study was the Akbari variety, which was purchased from Kermanshah city under the supervision of a horticultural expert from Jihad Keshavarzi and stored at -20°C until the start of the experiment.

2-2- Extraction of essential oil

Extraction of essential oil was carried out using a cologne machine. In this way, 50 grams of dried plant was ground and poured into a 500 ml flask, about 300 ml of distilled water was added to it, and the extraction and extraction of essential oil were carried out. The time required for extraction was about 3 hours. The resulting essential oil was collected in dark containers and stored at 4°C [12].

2-3- Investigation of the antifungal properties of plant essential oils and aloe vera gel

PDA culture medium was used to prepare *Aspergillus flavus* suspension. Incubation was carried out at 30°C for 7 days. The suspension was prepared by adding 10 ml of distilled water and suspending the contents of the 7-day fungal culture in the culture medium. After being removed from the culture medium, the conidia were placed in sterile distilled water containing 0.5% Tween 80. Then, they were mixed for 15 seconds and allowed to settle for 5 minutes. The spore concentration was adjusted to 1×10^6 spores per ml using a hemocytometer slide. The concentrations used to investigate the antifungal

properties of savory and thyme essential oils were 200, 400, 600, 800 and 1000 ppm, respectively, and the concentrations considered for Aloe vera gel were 10, 20 and 30%. The desired stocks were obtained by dissolving the essential oils and Aloe vera gel in a 10% DMSO solution. In order to investigate the inhibitory effect of the coatings against *Aspergillus flavus* and to select a coating with an appropriate formulation at the moment of pouring the culture medium into the plates, the prepared essential oil and Aloe vera gel stocks were mixed with the culture medium. After closing the culture medium, 10 µl of *Aspergillus* spore suspension was added to the center of the plate. The plates were placed in an incubator at 28°C for 7 days, and at the end of 7 days, the diameter of the fungal colony growth (mycelium growth diameter) was measured using a caliper [13].

2-4- Preparation of coating solutions

The base of the desired edible coating was distilled water and carboxymethyl cellulose at a concentration of 1%. Sage and thyme essential oils were used at a concentration of 1000 ppm, as well as Aloe vera gel at a concentration of 20%. In order to ensure uniformity of the essential oil and Aloe vera gel compositions in the edible coating, distilled water was used with Tween 80 at a concentration of 0.5%. Considering the use of plant essential oils, 1% propylene glycol was used to create a stabilizing effect in the coating solution. Briefly, to prepare the coating solution, 1 g of carboxymethyl cellulose powder was added to 100 ml of sterile distilled water and stirred on a magnetic stirrer for 30 minutes to completely dissolve. Then, 20 ml of aloe vera gel was added to it and

stirred again for 10 minutes. Then, Tween 80 and 4 ml of a mixture of savory and thyme essential oils were added to the resulting solution and stirred using a magnetic stirrer for 30 minutes to obtain a uniform solution [14].

2-5- Application of edible coating on fresh pistachios

For coating, fresh pistachios were immersed in the edible coating prepared in section 2-3. Then, the samples were placed on filter paper under a laminar hood (UV lamp and fan were on) for two hours to completely dry.

2-6- Contamination of fresh pistachios using fungal suspension:

In this stage, two treatment groups were investigated: Treatment 1: Pistachios without edible coating and contaminated with *Aspergillus flavus* fungus Treatment 2: Pistachios with edible coating and contaminated with *Aspergillus flavus* fungus. The treatments were subjected to fungal contamination using a prepared fungal suspension. The fungal suspension was applied to the pistachios using a spray. Then, the pistachios were placed in zip-top plastic bags whose bottom surface was covered with paper. The bags containing pistachios were stored in clean cabinets at room temperature for 4 weeks [15].

2-7- Investigating the level of aflatoxin production in pistachio samples:

In order to investigate the level of aflatoxin contamination in pistachio samples with and without edible coating and contaminated with *Aspergillus flavus* fungus, sampling was carried out in three stages at intervals of 10 days. In such a way that 10 grams of pistachio kernels were separated from the shell from each

sample and sent to the laboratory for HPLC analysis. In this study, contamination with aflatoxins of type B1 and B2 was considered. HPLC analysis was carried out according to the Iranian National Standard No. 6872 entitled Human and Animal Feed - Measurement of Group B Aflatoxin by high-performance liquid chromatography and purification with an immunoaffinity column [16].

2-8- Investigation of the effect of coating on the shelf life of fresh pistachios during storage

2-8-1- Measurement of weight loss

To measure weight loss, fresh pistachios were weighed at the beginning and end of a specified storage period and the weight loss was measured using equation 1 [17].

$$(1) \quad \text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Secondary weight}}{\text{Initial weight}} \times 100$$

2-8-2-Fat Content

Fresh pistachio samples were dried in an oven at 70°C after peeling. After removing the skin, the pistachio kernels were ground. Oil extraction from pistachio powder was performed by Soxhlet extraction using hexane at 69°C, and the fat percentage was calculated based on the gravimetric method [18].

2-8-3- protein content

The nitrogen percentage of the samples was measured using a Kjeldahl apparatus based on the AOAC 2003 method, and finally the protein percentage was determined using Formula 2 [19].

درصد ازت × فاکتور پروتئین = درصد پروتئین

2-9- Statistical analysis

In this study, the test results were evaluated using a completely randomized design with one-way analysis of variance and using SPSS version 16 software. All tests were performed in 3 repetitions and means were compared using Duncan's multiple range test to check the significance of the variable at a 95% confidence level. Graphs were also drawn using Excel version 2007 software.

3-Results and Discussion

3-1-Investigation of the antifungal properties of thyme, savory and aloe vera essential oils

The diameter of the growth halo of *Aspergillus flavus* is listed in Table 1. According to the results, the antifungal activity of savory and savory essential oils was concentration-dependent, such that increasing the concentration from 200 to 1000 ppm significantly inhibited the growth of *Aspergillus flavus* ($p < 0.05$). In line with these results, in the case of savory essential oil, the highest (2.54 cm) and lowest (2.16 cm) diameter of mycelium growth was related to essential oil concentrations of 200 and 1000 ppm, respectively. In the case of thyme essential oil, the lowest (1.51 cm) and highest (2.16 cm) mycelium growth was related to essential oil concentrations of 1000 and 200 ppm. Comparison of the antifungal properties of savory and thyme essential oils showed that thyme had a greater antifungal property at the same concentrations because at the same concentrations, the diameter of mycelium growth in samples treated with thyme was

significantly less than that in samples treated with savory. In the case of Aloe vera gel, the results also showed that its antifungal property was concentration-dependent, but increasing the concentration by more than 20% did not have a significant difference ($p < 0.05$). Studies have shown that Aloe vera gel has significant antifungal properties due to its saponin and salicylic acid compounds [20]. In this regard, Sitara et al. (2011) evaluated the antifungal effect of Aloe vera gel and reported that a concentration of 0.15% of Aloe vera gel showed moderate antifungal activity against *Aspergillus flavus* and *Penicillium digitatum* fungi and significant antifungal properties against *Alternaria alternata* fungus [21]. Regarding the antifungal properties of thyme essential oil, it has been reported that thymol and carvacrol present in Shirazi thyme pass through the cell membrane of microorganisms and disrupt the action of enzymes by destroying the cell membrane, ultimately leading to a delay in the germination of fungal spores [22]. Similar to these results, Tavakolipour et al. (2016) also reported the inhibitory effect of Shirazi thyme essential oil on the growth and toxin production of *Aspergillus flavus* [23]. In accordance with these findings, Afshari et al. (2013) evaluated the antifungal properties of thyme and savory essential oils against *Aspergillus flavus* and stated that the antifungal activity of the evaluated essential oils was concentration-dependent, and savory essential oil had less inhibitory ability than thyme [24].

Table 1. The growth diameter of *A. flavus* under the effect of different concentrations of thyme, savory essential oil and aloe vera gel

	Aloe vera concentration (%)			Thyme essential oil concentration (ppm)					Savory essential oil concentration (ppm)				
	10	20	30	200	400	600	800	1000	200	400	600	800	1000
Growth diameter zone	2.06±0.12a	1.55±0.21b	1.55±0.11b	2.16±0.12a	1.89±0.25b	1.77±0.15c	1.63±.17c	1.51±0.13d	2.54±0.14a	2.48±0.13a	2.3±0.11b	2.21±0.16b	2.16±0.12c

*Different letter shows significant differences at $p<0.05$

3-2-Investigating the synergistic properties of thyme, savory and aloe vera gel essential oils on the growth of *Aspergillus flavus*

According to the results of section 2-2, 1000 ppm of savory and thyme essential oils and 20% of aloe vera gel were selected to investigate their synergistic properties on the growth of *Aspergillus flavus*. The results are listed in Table 2. According to the results, in all treatments, the diameter of the *Aspergillus flavus* mycelium growth was significantly less than the control sample (without any essential oil or aloe vera gel). This finding confirms the antifungal properties of thyme, savory and aloe vera gel essential oils. The diameter of *Aspergillus flavus* mycelium growth in the treatment with 1000 ppm thyme and savory essential oils was 1.4 mm, while in the treatments containing 1000 ppm thyme essential oils or 1000 ppm savory essential oils it was 2.16 and 1.51 (mm), which was significantly greater than the treatment containing thyme and savory essential oils simultaneously ($p<0.05$). These results confirm the synergistic effect of thyme and savory. On the other hand, in the case of Aloe vera, the diameter of mycelium

growth in the treatment containing 20% aloe vera was 1.55 mm, while in the treatments containing Aloe vera gel with thyme or savory essential oils, the diameter of the mycelium growth halo was greater and was 2.06 and 2.16 mm, respectively. This observation indicates that the simultaneous application of Aloe Vera gel with thyme or savory essential oil did not have a significant synergistic effect. In the case of the treatment containing 1000 ppm thyme and savory essential oil and 20% aloe vera gel, the growth diameter of *Aspergillus flavus* mycelium was 1.36 mm, which was significantly less than the growth diameter of the treatments containing each of the mentioned compounds alone. This finding indicates a very significant synergistic effect of thyme, savory and Aloe Vera gel, which significantly inhibited the growth of *Aspergillus flavus* mycelium ($p<0.05$). In general, the synergistic effect of essential oils and plant extracts has been reported as an efficient strategy to inhibit a wide range of microorganisms, including fungi. Essential oils are mixtures of various compounds, usually dominated by one or two components (phenolic terpenes,

phenylpropanoids or alcohols) that are responsible for their antifungal activity. However, minor components (mainly hydrocarbons) present in essential oils are also of particular importance because they enhance the antifungal activity of essential oils through interactions with the major components present in the essential oil. Therefore, synergistic activity between essential oils with strong and weak antifungal properties is also important, as it can effectively enhance the biological activities of weaker oils [25]. In general, it has been reported that the increased antifungal activity of plant essential oils when used simultaneously (synergistic

effect) is due to the simultaneous activity of two or more components of the compounds present in the essential oils [26]. In this regard, various studies have evaluated the synergistic effect of plant essential oils, including Hlebova et al. (2021) who reported that thyme and lemongrass essential oils showed a significant synergistic effect against *Aspergillus* [27]. Also, the synergistic effect of oregano and lavender essential oils against *Fusarium* fungi and the synergistic effect of thyme and oregano essential oils against *Aspergillus flavus* has been reported [28].

Table 2. The synergistic effect of thyme, savory essential oil, and aloe vera gel on *A. flavus*

Treatments	Growth zone of (mm) <i>A. flavus</i>
Thyme essential oil (1000 ppm)	2.16±0.21 ^b
Savory essential oil (1000 ppm)	1.51±0.11 ^c
Aloe vera gel (20 %)	1.55±0.23 ^c
Thyme and Savory essential oil (1000 ppm)	1.4±0.11 ^d
Thyme essential oil (1000 ppm) and Aloe vera gel (20 %)	2.06±0.13 ^b
Savory essential oil (1000 ppm) and Aloe vera gel (20 %)	2.16±0.14 ^b
Thyme and Savory essential oil (1000 ppm) and Aloe vera gel (20 %)	1.36±0.12 ^d
control	3.23±0.11 ^a

*Different letter shows significant differences at $p < 0.05$

3-3-weight loss

The weight loss of coated and control (uncoated) pistachio samples after 30 days of storage is shown in Figure 1. In general, the weight loss in all coated samples was significantly lower than in the control (uncoated) sample; this finding indicates the positive effect of coating on preventing moisture loss and, consequently, weight

loss. According to the results, the highest weight loss (0.54%) was for the control sample and the lowest weight loss (0.20%) was for the sample coated with aloe vera and thyme. In general, it has been reported that the weight loss of fresh fruits during the storage period is due to increased respiration and moisture loss from the samples, which is prevented by coating by

creating a protective layer. In general, it has been reported that the main mechanism of weight loss in fresh fruits and vegetables is due to various reasons, including: the presence of vapor pressure in different places and also respiration. The positive effect of coating on preventing weight loss is due to the fact that coatings act as a semi-permeable barrier against oxygen, carbon dioxide, moisture, and the movement of soluble particles, thereby reducing respiration, moisture loss, and oxidation [29]. Similar

to these results, Ahmadi et al. (2013) reported that coating fresh pistachios with Aloe vera gel significantly prevented weight loss of samples stored for 40 days, and the highest weight loss was in the control sample (without coating) [30]. Valverde et al. (2005) also reported that the use of Aloe vera coating material increased the shelf life of grapes and their eating quality during and after storage, while the control sample showed greater respiration and weight loss [31].

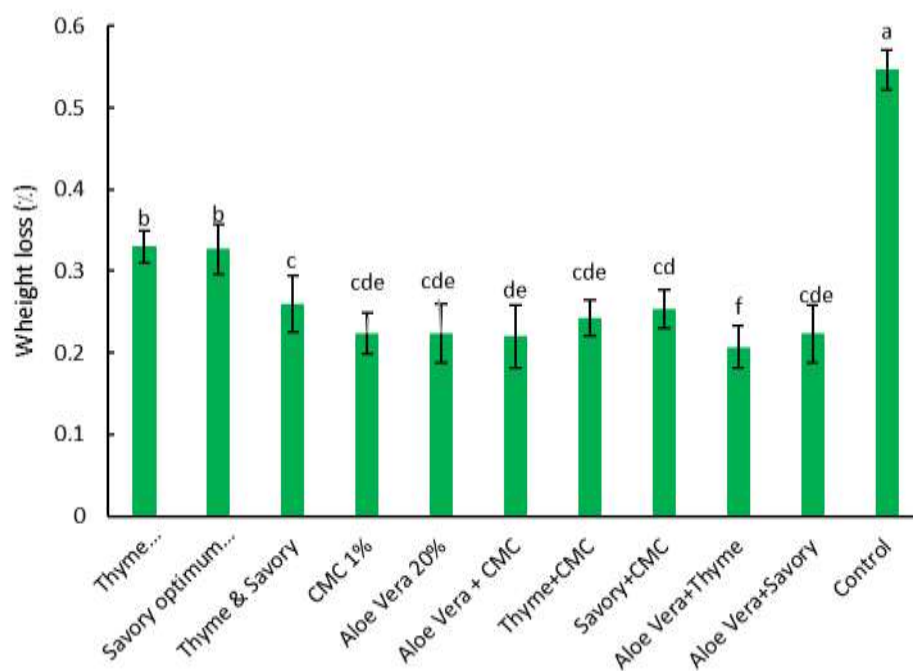


Figure 1- Weight loss of coated pistachio samples after 30 days of storage

3-4-Fat content

The fat content of pistachio samples after 30 days of storage is shown in Figure 2. In general, considering that the average fat content of the samples on the first day of storage was 59.2%, the results showed that the fat content of all samples decreased during the storage period. According to the results, the fat content of the control

treatment (without coating) was significantly lower than that of the other coated samples ($p < 0.05$). The highest (55.71%) and lowest (48.76%) fat content of the samples were related to the sample coated with thyme + CMC and the control sample, respectively. After the samples coated with thyme + CMC, coating the samples with a combination of aloe vera

and thyme, aloe vera and CMC, or savory and CMC also appropriately reduced the fat loss of the samples. These results indicate the synergistic effect of combining thyme with CMC or aloe vera and also combining aloe vera with CMC or thyme on preventing respiration and oxidation of pistachio samples during storage. In this regard, it has been reported that the decrease in fat content of pistachio samples during storage can be due to fermentation and anaerobic respiration of the samples, because it has been reported that during the respiration process in fresh pistachios, storage compounds in pistachios such as fats and carbohydrates are consumed. Using appropriate coating by creating a barrier on the surface of pistachios can prevent or delay the penetration of oxygen and oxidation, which in turn prevents the reduction of fat content during storage [30]. In this regard, Maghsoudloo et al. (2013) reported that coated samples had a lower peroxide index compared to the control sample by coating

pistachio kernels using chitosan. They reported that coating prevents moisture, oxygen, and peroxides from penetrating into pistachios by creating a protective layer, thereby preventing oxidation [1]. The positive effect of coating with aloe vera gel on preventing oxidation and reducing the fat content of the samples was also reported by Ahmadi et al. (2013). They stated that coating pistachios using aloe vera gel at concentrations of 25 and 33% significantly prevented the loss of fat in the samples during a 40-day storage period at refrigerated temperature, but coating with aloe vera gel at a concentration of 50% had a negative effect [30]. Also, Babapour et al. (2022) reported that coating based on potato starch containing zinc oxide and fennel nanoparticles on the aflatoxin content of pistachio samples during 15 days of storage at refrigerated temperature significantly prevented the loss of fat in the samples [32].

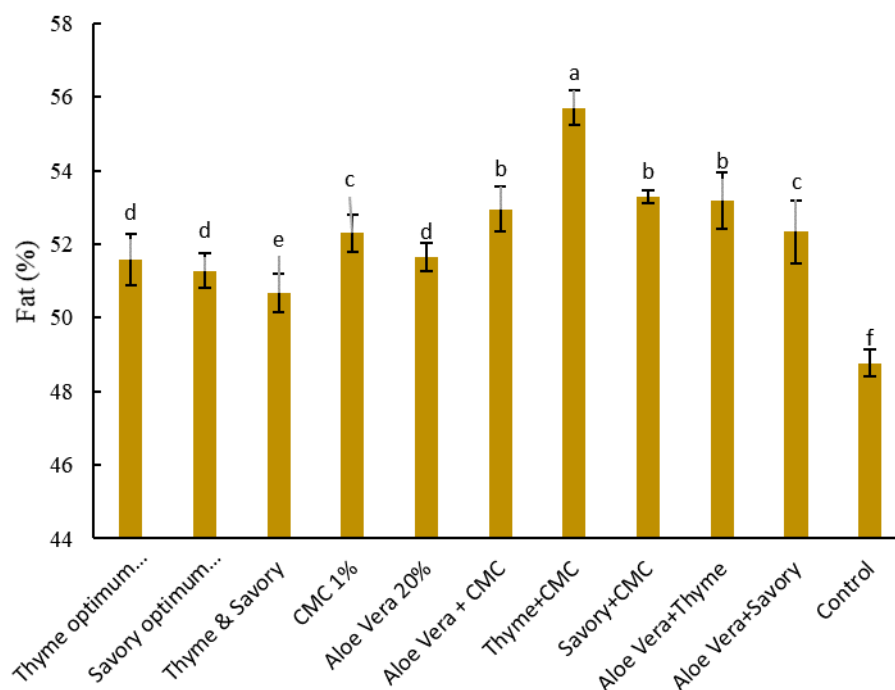


Figure 2- Fat content of coated pistachio samples after 30 days of storage

3-5-protein content

The protein content of pistachio samples after 30 days of storage is shown in Figure 3. In general, considering that the average protein content of the samples on the first day of storage was 23.35%, the results showed that the protein content of all samples decreased during the storage period. According to the results at the end of the storage period, the protein content of the control treatment (without coating) was significantly lower than that of the other coated samples ($p < 0.05$). In general, the highest (22.85%) and lowest (19.89%) protein content of the samples were related to the sample coated with Aloe Vera + CMC and the control sample, respectively. It is worth mentioning that coating pistachio samples with aloe vera and thyme or savory, as well as coating with

CMC and thyme and savory, or coating with aloe vera or CMC alone, significantly prevented the decrease in protein content of the samples during storage. However, as mentioned earlier, the highest protective effect was observed for the sample coated with aloe vera + CMC. These results indicate the positive effect of appropriate coating on preventing the quality loss of pistachio samples during storage due to respiration or oxidation, which significantly preserved nutritional factors such as protein [32]. Similar to these results, Taghipour et al. (2024) reported the positive effect of coating on preventing the decrease in protein content by investigating the effect of zinc oxide and chitosan nanocomposite coating on the physicochemical properties of fresh pistachios during 75 days of storage, while

the protein content of the control sample (without coating) decreased [33].

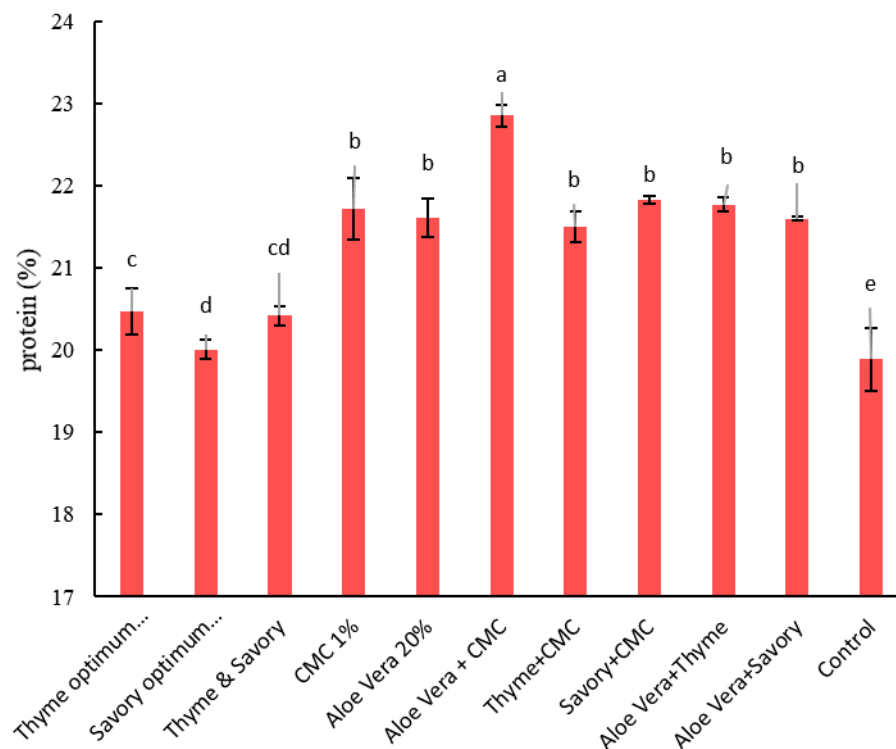


Figure 3- Protein content of coated pistachio samples after 30 days of storage

3-6-Investigation of aflatoxin production in pistachio samples:

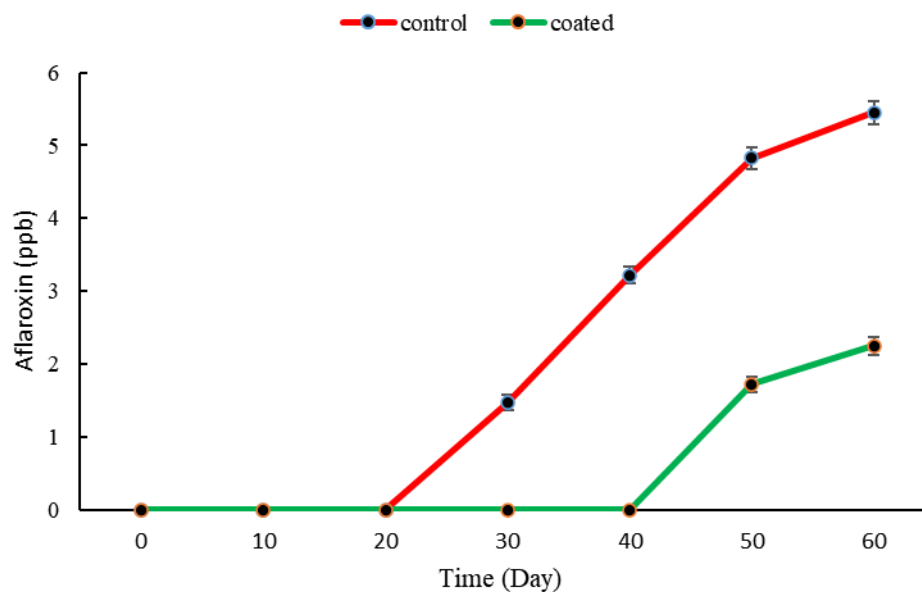
The levels of aflatoxin B1 and aflatoxin B2 in uncoated (control) and coated pistachio samples during a 60-day storage period are shown in Figure 4, parts A and B, respectively. According to the results of aflatoxin B1 production, no aflatoxin contamination was detected in the uncoated sample until 20 days of storage. Then, on the 30th day of storage, aflatoxin was detected in the samples at a level of 1.47 ppb, which increased until the end of the storage period and reached 5.45 ppb. In the case of the sample coated with aloe vera, CMC, thyme and savory, no contamination was detected until the 40th

day of storage, but after that, the aflatoxin level increased and reached 2.25 ppb at the end of the storage day. In the case of aflatoxin B2, no contamination was detected in the control sample until day 30 and the coated sample until day 40, but with increasing storage time, contamination with aflatoxin B2 was detected, which reached 3.24 ppb in the control sample and 2.12 ppb in the coated sample at the end of the storage period. In general, these results indicate the positive effect of coating pistachios with aloe vera, CMC, thyme, and savory in controlling the growth of *Aspergillus flavus* and consequently the production of aflatoxins B1 and B2. In this regard, it has been

reported that humidity and temperature are two important factors in the growth of *Aspergillus* and consequently the production of aflatoxins; in this study, the storage temperature of the samples was constant, but the prevention of aflatoxin production could be due to the positive effect of coating on the transfer of humidity between the storage atmosphere and the samples [34]. On the other hand, the antifungal effect of the applied coating due to the presence of thyme, savory and aloe vera is another reason for preventing and delaying aflatoxin contamination. In other words, the antifungal compounds present in the applied coating prevented the growth and production of aflatoxin by *Aspergillus* fungi, which resulted in the

production of aflatoxin B1 and B2 in the samples until day 40 of storage [35]. Similar to these results, Moslehi et al. (2022) reported that coating pistachios with methyl cellulose up to a concentration of 0.1% significantly prevented the contamination of the samples with aflatoxin B1 and B2 during a 4-month storage period [36]. Also, Taghipour et al. (2024) investigated the effect of zinc oxide and chitosan nanocomposite coating on aflatoxin production by *Aspergillus* fungi and reported that coating significantly prevented aflatoxin contamination of Akbari and Ahmad Aghaei pistachios stored until day 25 [33].

(A)



(B)

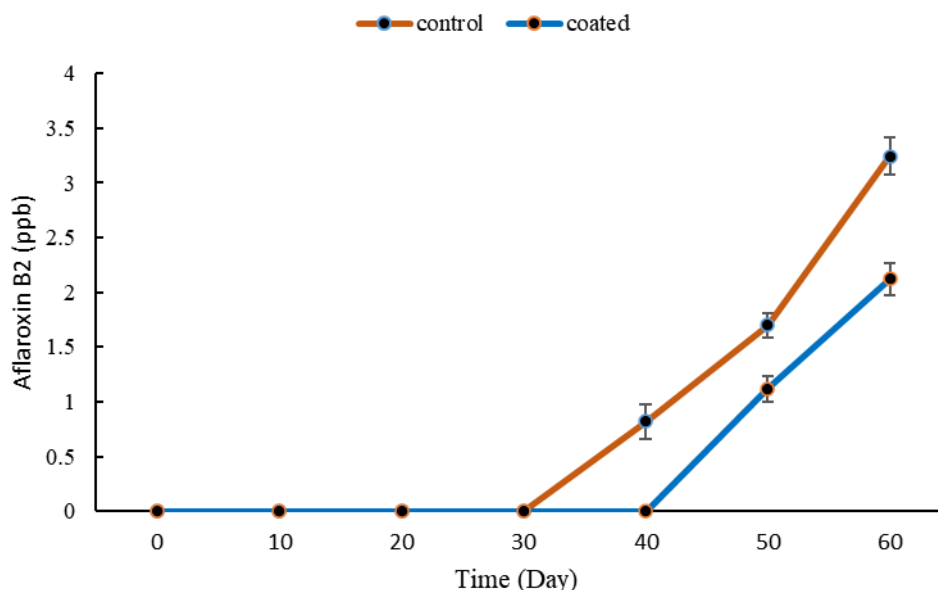


Figure 4- A: Aflatoxin B1 and B: Aflatoxin B2 content of control (uncoated) and coated pistachio samples during 60 days of storage

4-Conclusion

Growth and development of fungi such as *Aspergillus flavus* on pistachios, in addition to the risks of causing dangerous diseases to humans, reduces shelf life and results in heavy economic losses to producers. In this regard, studies have shown that the use of edible coatings is a very useful solution to prevent fungal contamination of pistachios and also reduce environmental pollution. The results of this study showed that thyme and savory essential oils as well as aloe vera gel have a significant antifungal effect against *Aspergillus flavus*. Examination of the synergistic effect of the compounds showed that thyme and savory essential oils and thyme and savory essential oils and aloe vera gel had a synergistic effect and were able to significantly prevent the growth of *Aspergillus flavus*. In general, coating

pistachio samples increased their shelf life by preventing weight loss and maintaining the fat and protein content of the samples. Coating pistachio samples with 1% carboxymethyl cellulose and 20% aloe vera gel containing thyme and savory essential oils at a concentration of 1000 ppm significantly prevented contamination of the samples with aflatoxin B1 and B2 until day 40 of storage. Therefore, it can be concluded that the use of the edible coating studied in this study is a useful solution to preserve the physicochemical and nutritional properties of pistachios and prevent their contamination with *Aspergillus flavus*. As a result, the above coating can be used to coat pistachio samples for export, which is a valuable solution to maintain product quality and prevent economic losses for pistachio producers and exporters. On the other hand, the use of biodegradable edible coatings in food storage is a

valuable step towards environmental protection due to the reduction in the use of plastic coatings that pollute the environment.

5-References

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Aflatoxin, microbial contamination, sensory attributes, and morphological analysis of pistachio nut coated with methylcellulose. *Food Science & Nutrition*, 9(5), 2576-2584.



جلوگیری از رشد قارچ *آسپرژیلوس فلاووس* در پسته تازه با استفاده از پوشش خوراکی بر پایه کربوکسی متیل سلولز/ژل آلوه‌ورا حاوی اسانس گیاه آویشن و مرزه

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چکیده

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ایران از بزرگترین صادرکننده‌های پسته در جهان است. پسته یکی از محصولات با ارزش صادراتی است که بسیار مستعد به آلودگی به قارچ *آسپرژیلوس فلاووس* می‌باشد. در این راستا مطالعات نشان داده است که پوشش‌های خوراکی کارایی بسیار زیادی جهت جلوگیری از آلودگی قارچی پسته، تولید آفاتوکسین و در نتیجه افزایش عمر انبارمانی آن دارند. هدف از این پژوهش بررسی تاثیر پوشش خوراکی بر پایه کربوکسی متیل سلولز و آلوه‌ورا حاوی اسانس مرزه و آویشن شیرازی بر تولید آفاتوکسین توسط قارچ *آسپرژیلوس فلاووس*، افت وزن، میزان چربی و پروتئین نمونه‌ها طی دوره نگهداری بود. همچنین خاصیت ضد قارچی غلظت‌های مختلف اسانس مرزه، آویشن و آلوه‌ورا و همچنین تاثیر سینرژیستی آنها بر *آسپرژیلوس فلاووس* بررسی شد. نتایج نشان داد که اسانس‌های مرزه و آویشن در غلظت ۱۰۰۰ ppm و ژل آلوه‌ورا در غلظت ۲۰ درصد به‌طور معنی‌داری مانع از رشد قارچ *آسپرژیلوس فلاووس* شدند. بیشترین میزان جلوگیری از افت وزن، کاهش میزان چربی، کاهش میزان پروتئین نمونه‌ها طی دوره ۳۰ روز نگهداری به ترتیب با پوشش‌دهی با آلوه‌ورا و آویشن، پوشش‌دهی با کربوکسی متیل سلولز و آویشن و پوشش‌دهی با آلوه‌ورا و کربوکسی متیل سلولز مشاهده شد. پوشش‌دهی نمونه‌ها با استفاده از کربوکسی متیل سلولز ۱٪ و ژل گیاه آلوه‌ورا با غلظت ۲۰٪ حاوی اسانس گیاهان مرزه و آویشن با غلظت ۱۰۰۰ ppm به‌طور معنی‌داری مانع از آلودگی نمونه‌ها به آفاتوکسین‌های B₁ و B₂ تا روز ۴۰ نگهداری شد. با توجه به نتایج استفاده از پوشش خوراکی بر پایه کربوکسی متیل سلولز و آلوه‌ورا حاوی اسانس مرزه و آویشن راهکاری مناسب جهت افزایش عمر ماندگاری پسته و جلوگیری از آلودگی قارچی آن و تولید آفاتوکسین می‌باشد.