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ORIGINAL ARTICLE

Examination of the Effect of Methylcellulose on the Reduction of Aflatoxin Production during Pistachio Storage

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A B S T R A C T

Pistachio is one of the valuable export products very susceptible to infection by *A. flavus*. The present study aimed to investigate the effect of coating fresh pistachio with hydrocolloid methylcellulose on aflatoxin amount during storage. In this study, pistachio seeds from the Abbasali cultivar were used. Methylcellulose solution with different concentrations (0.1%, 0.5%, 1%, and 2%) was prepared and a coating of this hydrochloride was created by immersing methylcellulose on pistachio samples. The coated samples and the control sample (uncoated) were kept in an incubator at a temperature of 25°C for 3 months. In the investigation of the impact of various methylcellulose concentrations in the composition of the pistachio kernel coating, on the inhibition rate of this micro colloid on the production of total aflatoxin and B1, B2, G1, and G2 aflatoxin which was measured by HPLC, showed that concentrations of 0.1% and 0.5% reduced total aflatoxin production significantly, it increased about 3.5 times after three months of storage. Also after 3 months of storage, aflatoxin B1, B2, G1, G2 in the samples coated by 0.5% and 0.1% methylcellulose was decreased to undetectable toxin production. Also, evaluation of moisture content revealed that the pistachio seed coated with methylcellulose had the highest moisture content at 2% and the lowest at 0.1% and 0.5% of the control samples.

Introduction

Aflatoxins are a great group of mycotoxins categorized as secondary metabolites of fungi produced by some species including *Aspergillus flavus, A. tamari, A. bombycis* Hs, and *A. minus*. Aflatoxins are regarded as the major group of mycotoxins due to their high prevalence in nature, toxicity, and carcinogenicity (Mahbobinejhad *et al.*, 2019). Until now several species of aflatoxins have been identified among which aflatoxins B1, B2, G1, and G2 are the most important ones (Ortega-Beltran *et al*., 2019).

Today, one of the most important health problems of the world community is the contamination of agricultural products with aflatoxins, and due to the serious dangers of mycotoxins, special laws and regulations have been set for the production, consumption, and import of food in different countries. (Holakouie Naieni *et al*., 2020). In USA purchasing, importing, and exporting food and pharmaceutical products containing total aflatoxin at more than 20 ppb and aflatoxin B1 at 15 ppb are forbidden (Catalán *et al*., 2017).

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Aflatoxin was discovered immediately after the occurrence of a Tukey disease of a known cause in England in 1960. This disease was called X turkey disease and it has been believed that Aspergillus flavus was its cause. In general, these toxic substances created by terrestrial microorganisms were called aflatoxin (Ortega-Beltran *et al*., 2019) .

Aspergillus flavus has been known as the most common fungus contaminant in food products since the detection of aflatoxins in 1960. It is a widespread infection, regarded as omnipresent microflora of soil and the air, and found in live and dead plants or animals the said species shows a certain tendency to infect nut and oil seeds as well as grains. These products are the most susceptible products to infection by fungus which favor fungus growth. Peanuts, corn, wheat, rice, pistachio, almond, and fig are the major hosts of fungal (Hadavi *et al*., 2017).

These fungi can produce spores that grow under the least conditions of life. Pistachio infection by Aspergillus species may occur during the ripening of products on farms. Obtaining healthy and fungus-free products requires that aflatoxin production conditions and prevention methods be understood.

Pistachio nuts are regarded as a nutritious snack since they are rich in protein, vitamin A, vital fatty acids, phenolics, and important minerals. The pistachio's edible dried shelled kernel or nut is widely sold across the world, but fresh pistachio consumption is also rising because of the fruit's distinctive flavor, high concentration of phytochemical antioxidants, and nutritional value. Postharvest loss is a factor in all production phases, from harvest and marketing through customer purchase. In underdeveloped nations, this loss occurs at a rate of 20–50%. These percentages, however, are significantly influenced by the kind of product, plant cultivar, environmental factors, and postharvest conditions. Fresh pistachios rapidly senesce after being harvested, browning and decaying, which lowers their market value. Hull browning occurs as endogenous polyphenols are oxidized. The conversion of ortho-diphenols into semiquinones and quinones is the process behind hull browning. Fruit cells contain the PPO enzyme, which causes fruits to become brown over time. Fresh fruits and vegetables frequently experience postharvest browning, a color response that is typically brought on by the unregulated interaction of polyphenols, oxygen, and the polyphenol oxidase (PPO) enzyme. Fresh items frequently experience browning reactions that degrade aesthetic appeal, cause nutrients and taste ingredients to be lost, and decrease customer acceptance. In fact, postharvest browning of the fresh pistachio's outer hull during processing and storage is a crucial quality criterion. Pistachios in their fresh state cannot be exported, and little study has been done on the subject. (Sharifkhah *et al*., 2020; Hashemi *et al*., 2021; Nazoori *e al*., 2022a). In Iran pistachio orchards are under cultivation at 440000 h. This country accounts for 57% of world production and is the greatest exporter of pistachio among countries producing this crop (Iran, U.S.A, Turkey, Syria, Greece, etc), (Shamshiri and Hasani, 2015; Catalán *et al*., 2017; Alipour, 2018; Norozi *et al*., 2019; Sharifkhah *et al*., 2020; Nazoori *e al*., 2022b). Economic obtained from Iranian pistachio export to 66 countries in the world and this crop is the second most important source of exchange income after oil, even though many of the orchards are suffering from drought and salinity stress (Behzadi Rad *et al.*, 2021).

The high nutritional value of pistachios renders them vulnerable to microbial deterioration and lipolytic oxidation in addition to their high lipid content, particularly unsaturated fatty acids (Roozban et al. 2006). In order to increase the shelf life of pistachio, antioxidant and antibacterial preservatives can be employed. Despite being used for many years to preserve food goods, synthetic antioxidants and preservatives have major negative consequences on the environment and human health. (Khajeh-Ali *et al*., 2022). Which requires higher optimization of world trade of this crop. Regarding import the ance of pistachio for Iran’s economy and increasing competition in the global market, improvement of quality during production and storage as well as the packaging of this product is needed. Therefore, mass export of pistachio as packaged in gunny or other undesirable packages should be avoided.

Nowadays research on food packaging is based mostly on biocompatible films, including films prepared from edible proteins derived from plants and animals (such as casein and whey proteins, gelatin, albumin, wheat gluten, soy, and peanuts). An initial package comprised of edible components, mostly biopolymers and GRAS (Generally Recognized as Safe) food additives, is referred to as an edible coating or film. Coatings are a unique kind of film that are placed directly to the material's surface and are regarded as a component of the finished item. (Hosseini *et al*., 2020) and act as a protector, thus preventing adverse changes in the taste, texture, and appearance of food. (Chong *et al*., 2019; Esfahani *et al*., 2020; Jafarzadeh & Jafari, 2020; Mei *et al*., 2020; Mohammadi Nafchi *et al*., 2017). The use of edible coating can be an alternative to packaging materials, increase shelf- life and acceptability of the product and solve the environmental problem due to packaging. Edible films can be prepared from protein, polysaccharide, and lipid materials (Ekramian *et al*., 2021; Kazemi *et al*., 2020; Mousavian *et al*., 2021).

Food films and coatings can be divided into four categories: polysaccharide, protein, lipid, and composite. Polysaccharide films include alginate, pectin, carrageenan, starch and cellulose derivatives, etc. Cellulose is a natural polymer and forms the principal structure of the cellular walls of green plants. Cellulose is the most abundant and least costly plant resource that can be recycled, broken down by microorganisms, and converted to carbon, hydrogen, and oxygen. The most important cellulose derivatives are cellulose ether, methylcellulose, carboxymethylcellulose, hydroxyl propylcellulose, and hydroxylpropylmethylcellulose, which are suitable for film production. These coatings are edible and have average strength, are transparent, and are also resistant to fats and oils. Their only weakness lies in their sensitivity to water.

The use of hydrocolloid coatings, especially the fat-resistant coating, creates a high ability to be impervious to oxygen and microorganisms. Methyl cellulose is a chemical compound that does not exist in nature. About 30% of the hydroxyl groups in cellulose, which is dissolved in cold water and turns into a transparent solution, are methylated to generate it. However, boiling water does not dissolve it, and with increasing temperature, it turns into a saturated solution of cellulose and leaves the soln as sediment. (Ahmad *et al*., 2016; Garavand *et al*., 2017, 2020; Moslehi *et al*., 2021. The most prevalent renewable and biodegradable polymer on earth is cellulose, which also offers a number of benefits including cheap cost, low density, non-toxicity, adaptability, and excellent mechanical qualities. (Zhao *et al*., 2022). It seems that due to its hydrophobic nature, methylcellulose high potential to prevent the penetration of water and oxygen and increase chemical and microbial stability compared to other cellulose derivatives. As different concentrations of methyl cellulose when coating pistachio show different oxygen barrier abilities (Hermawan *et al*., 2019).

A sort of active packaging that lengthens food's shelf life is antimicrobial packaging. and ensures their health from a microbial point of view. (KazemianBazkiaee *et al*., 2020). To control undesired microorganisms at the surface of the food products volatile and non-volatile antimicrobials can be incorporated into the formulation of packaging polymers.

Materials and Methods

Preparation of methylcellulose solution

Pistachio kernels have been randomly distributed into 1kg packages to be used as control samples and test samples should be covered using methylcellulose solution in various concentrations of 0.1, 0.5, 1, and 2%. It was used for coating (Delvarianzadeh *et al*., 2020; Molehill *et al*., 2021). In this test so certain amounts of powder were added to distilled water and allowed to solve by a mixer for 30-45 min at room temperature. The obtained solution was full of air bubbles therefore it was allowed to rest so that bubbles exited the solution. At this point, the solution is ready for coating the pistachio kern (Moslehi *et al*., 2015).

Coating

To perform the coating process at each concentration, one kilogram of pistachio nuts is cooled and degassed in methylcellulose solution. After 5 minutes; the samples are placed in an oven at a temperature of 25 degrees Celsius for 3 days to dry well. Then, one-kilogram samples are packed in zippered plastic bags made of heavy polyethylene (Figure 1) and stored in an incubator with a constant temperature of 25 degrees Celsius for 3 months. (Moslehi *et al*., 2015).

Sample assay

Aflatoxin contamination and humidity levels were measured at the beginning of the storage period, just after covering and drying, in the middle of the time, after two months, and at the conclusion of the period, after three months. It is connected to. All experiments were run three times, and using IBM SPSS version 19 software, 5% statistical analysis was used to determine the findings aflatoxin was measured using chromatography 2475 of capillary water column c18 (Novapack) outfitted with several detectors and fluorescence. Before centrifuging, a 50-gram sample of pistachios was first crushed with 5 grams of salt, 100 ml of hexane, and 200 ml of methanol. After obtaining the desired liquid (20 ml), 130 ml of water was added, and the mixture was homogenized for 5 minutes. Glass fibers were used to filter the homogeneous slurry through filter paper, and 70 ml of the filtrate was added to the Aflestest column. 15 ml of phosphate buffer and 15 ml of distilled water were used to wash the column. Aflatoxin was finally eliminated by running methanol down the column. Also included were 1.5 ml of distilled water (Kermi Esbo and Mirab al-Fathi, 2016). The selection produced by high-performance chromatography for aflatoxin B1, aflatoxin G1, B2, liquid, and aflatoxin G2 is shown in the Figure.

To measure moisture, a digital hydrometer (Sartorius, Germany) was used, to do so special plates of this system were washed with distilled water, dried with cotton, and placed inside the machine. After zeroing the weight of the empty plate, it was exited from the machine and the plate exited the machine and the plate containing the pistachio without kernels was placed inside the machine. The time needed for measuring pistachio moisture was 20 min. the value shown by the monitor was reported as the moisture content of samples.

Statistical analysis

Researching the result of several methylcellulose solutions coating on pistachio storage life at the beginning, middle, and end of the storage period, Tukey's test was utilized to establish the several during treatments p0.05 in the Duncan multi-range test. Software called IBM-SPSS version 19 was used for the statistical analysis.

Results

As can be seen in Fig. 1, the humidity in the studied concentrations had a significant difference over time ($p < 0.05$). Because the amount of moisture of food products decreases over because of the exchange of moisture using the surrounding environment, according to the type of environment (either free space or the environment within a container), of course, the reduction of moisture content in open packaged food products, according to It will be less based on the kind and type of packaging, as well as additional elements such ambient temperature, sunshine exposure, and the type of food are also involved in this.

The findings revealed that the loss of moisture due to the type of packaging (heavy polyethylene is impermeable to moisture), the unique characteristics of the methylcellulose coating, and the specific storage conditions are reduced (Fig. 1).

So that the highest moisture content of the pistachio kernel was covered the lowest concentration of 2% methylcellulose solution moisture content of the control sample was 0.1% and 0.5%. This disparity is due to the fact that the pistachio kernel absorbs parts of the solution when immersed in the methylcellulose solution through the scattering phenomenon, and since the pistachio kernels covered with the 2% solution are more impermeable, when dry being in the oven and also lose less moisture. During the storage period (Fig. 1).

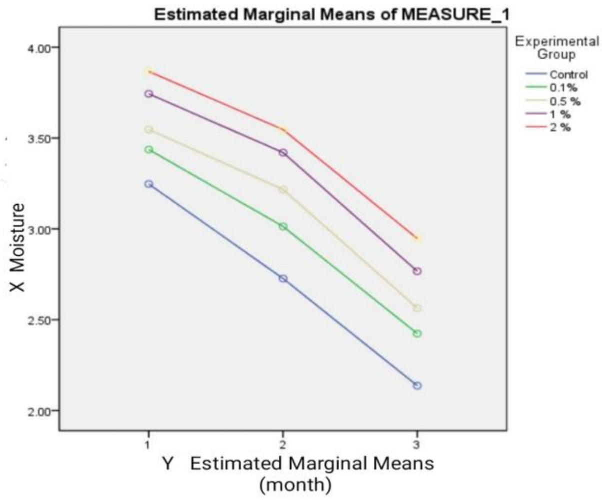

Fig.1. Results of moisture test during the time for different groups.

In the investigations, it was found that there are many aflatoxin B1 in the pistachio kernel without coating increased about 3 times during 3 months of storage (from 0.47 to 1.85 m m^{-1}) (Fig. 2). But No discernible change was seen in the amount of aflatoxin, in pistachio kernels coated with methyl cellulose (it reached from 0.47 to 1.85 m m^{-1}) (Fig. 2). However, no discernible variation in the aflatoxin amount was observed between samples coated with methyl cellulose and control sample (except the concentration of 2%). Also, after 3 months of storage, aflatoxin B1 was undetectable in the pistachio kerns with 0.5% and 0.1% methylcellulose.

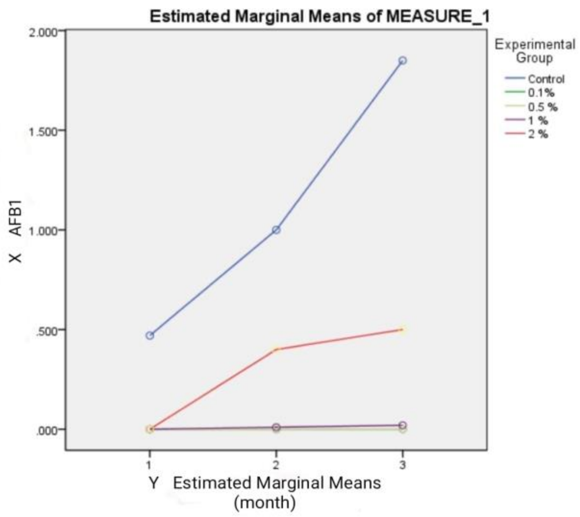

Fig.2. Aflatoxin B1 test results as repeated measures over time for different groups.

The results showed that the amount of aflatoxin B2 in uncovered pistachio kernels was stable during three months of storage, while the amount of aflatoxin increased at a concentration of 2% (Fig. 3). Aflatoxin samples were not significantly different from methylcellulose and the uncovered pistachio kernels (except for the concentration of 2%). Also, after three months of storage, the aflatoxin B2 sample was reduced by 0.5%, 0.1%, and 1% methylcellulose, and the production of poison became undetectable.

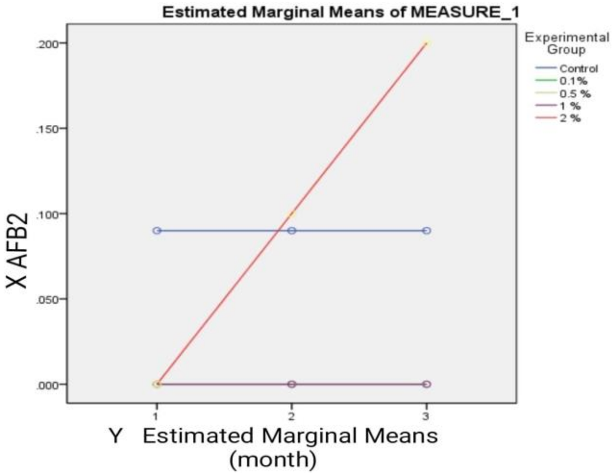

Fig.3. Aflatoxin B2 test results as repeated measures over time for different groups.

According to the results, the number of GI aflatoxin in the uncovered pistachio kernels increased after 3 months of storage about 4.5 times (from 0.4 to 1.72 m m^{-1}) in the concentrations of 1 and 2% (Fig. 4), but between the samples that were coated and the quantity of aflatoxin, there was no significant difference between methylcellulose and the control sample (except for the concentration of 2%). Also after 3 months of storage, test B1 in the pistachio kernels with 0.5% and 0.1% methyl cellulose was reduced until undetectable toxin production.

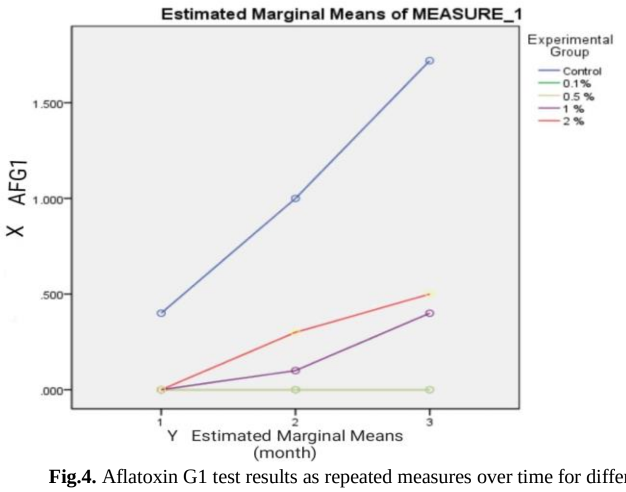

Fig.4. Aflatoxin G1 test results as repeated measures over time for different groups.

The results showed that the number of test G2 in the uncoated pistachio kernels was stable after 3 months, while the amount of aflatoxin increased the concentration by 1 by 2% (Fig. 5). However, a difference in the number of aflatoxins was observed between the samples with methylcellulose and the control sample (except for the concentration of 2%). Also, after 3 months of storage, the test G2 sample was reduced to undetectable toxin production by 0.5%, 0.1%, and 1% methyl cellulose.

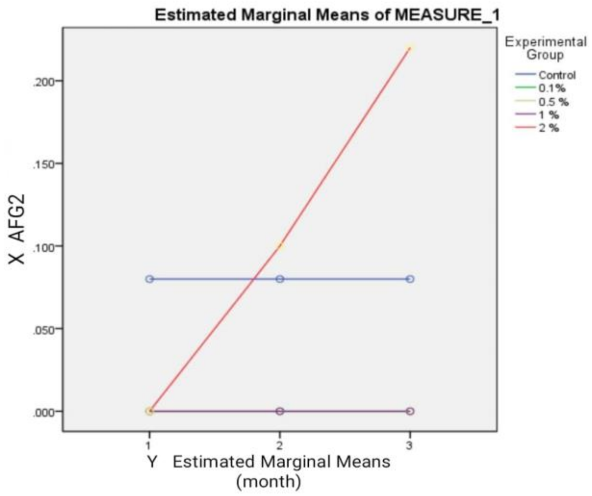

Fig.5. Aflatoxin G2 test results as repeated measures over time for different groups.

On the other hand, as one can see from the Results there is a significant difference between treatments so the total aflatoxin amount of uncoated pistachio kernels was measured from 1.04 m m^{-1} to 3.74 m m^{-1} (3.5 times as much) after 3 months of storage. At a concentration of 2% of methyl cellulose aflatoxin amount was increased to 1.5 times as much. Also, no A notable difference was found between pistachio kernels with 0.5% and 0.1% of methylcellulose concentrations. Results also revealed total aflatoxin amounts of pistachio kernels with 0.5 and 0.1% of methylcellulose concentrations. They were reduced to a large extent so that they could not be detected after three months of storage (Fig.6).

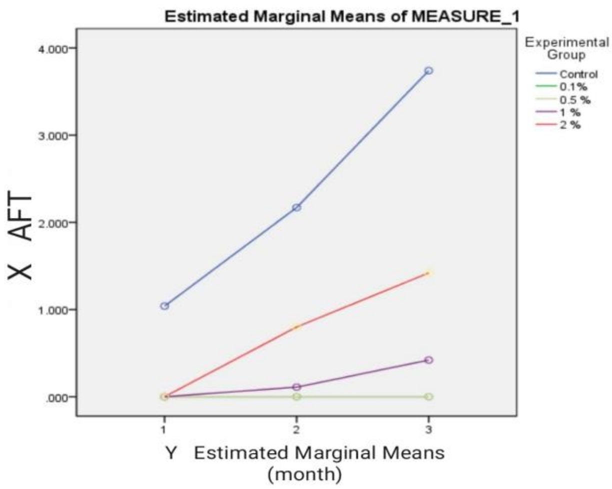

Fig.6. Total aflatoxin test results as repeated measures over time for different groups

Discussion

Because humidity and temperature are two factors in the production of aflatoxin, however in this research, the temperature was the same for all the samples, but the humidity vary with various concentrations. The uncoated had the most moisture loss pistachio kernels and the least was owned by the sample covered in methylcellulose solution at a concentration of 2%. It can be ascribed to the consequences of the type of packing, Storage in an incubator at a constant temperature, and a dense cover that acts as a good moisture barrier resulting in minimal moisture loss during storage. Also, the hydrophobic particles in the methylcellulose coating reduce surface vapor, limit water movement, and delay moisture loss (Tawaklipour *et al*., 2020). Contrarily, the control sample increased the amount of total aflatoxin due to a lack of proper coverage compared to other treatments (Table 1).

Table 1. Comparison between results during storage.

Concentration methyl cellulose	Start						Two month						Three month					
	Aflatoxin B1 (μg kg^{-1})	Aflatoxin B2 (μg kg^{-1})	Aflatoxin G1 (μg kg^{-1})	Aflatoxin G2 (μg kg^{-1})	Aflatoxin Total (μg kg^{-1})	Moisture (%)	Aflatoxin B1 (μg kg^{-1})	Aflatoxin B2 (μg kg^{-1})	Aflatoxin G1 (μg kg^{-1})	Aflatoxin G2 (μg kg^{-1})	Aflatoxin Total (μg kg^{-1}))	Moisture (%)	Aflatoxin B1 (μg kg^{-1})	Aflatoxin B2 (μg kg^{-1})	AflatoxinG1 (μg kg^{-1})	AflatoxinG2 (μg kg^{-1})	Aflatxin Total (μg kg^{-1})	Moisture (%)
Control sample	0.47	0.09	0.4	0.08	1.04	3.25	1	0.09	1.1	0.08	2.17	2.73	1.85	0.09	1.72	0.08	3.74	2.12
Coated with 0.1% CMC	n.d	n.d	0.01	n.d	n.d	3.44	n.d	n.d	1.01	n.d	n.d	3	n.d	n.d	2.01	n.d	n.d	2.42
Coated with 0.5% CMC	n.d	n.d	n.d	n.d	n.d	3.55	n.d	n.d	n.d	n.d	n.d	3.2	n.d	n.d	n.d	n.d	n.d	2.55
Coated with 1% CMC	n.d	n.d	0.74	n.d	n.d	3.75	0.01	n.d	1.74	n.d	0.11	3.4	0.02	n.d	2.74	n.d	0.42	2.75
Coated with 2% CMC	n.d	n.d	0.5	n.d	n.d	3.86	0.4	0.1	1.1	0.1	0.8	3.55	0.5	0.16	1.51	0.22	1.42	2.95

Abbreviations: nd, not detected.

By comparing pistachio kernels investigated by National Standard of Iran no. 218- kernel, the technique, and properties of test-regarding aflatoxin, it could be found that the amount of this factor in all samples was at the permitted level which means accuracy and validity of tests according to standard and high quality of Iran pistachio, and at a concentration of 2% methylcellulose moisture loss was noted.

Conclusions

Regarding the important effect of moisture on packaged product concentration of 2% methylcellulose is recommended when the moisture content of pistachio is in priority and a concentration of 1% is recommended when the objective of packaging is to control aflatoxin. It can be concluded that the appropriate coating of valuable products such as pistachio is effective level in lower aflatoxin amount and enhancing storage life as well as acceptability of these products.

Conflict of interests

No Conflict

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