


## EFFECT OF EDIBLE COATINGS ON SOME PHYSICO-CHEMICAL PROPERTIES AND FUNGI GROWTH DURING DIFFERENT STORAGE PERIODS OF PISTACHIO NUTS

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Article info	Abstract
<p><b>Received:</b> 13-07-2019 <b>Accepted:</b> 08-12-2019 <b>Published:</b> 31-12-2020</p> <p><b>DOI -Crossref:</b> 10.32649/ajags.2022.170521</p> <p><b>Cite as:</b> Hamasalih, T. H., and Rasul, N. H. (2020). Effect of edible coatings on some physicochemical properties and fungi growth during different storage periods of pistachio nuts. <i>Anbar Journal of Agricultural Sciences</i>, 18(2): 154–166.</p> <p>©Authors, 2020, College of Agriculture, University of Anbar. This is an open-access article under the CC BY 4.0 license (<a href="http://creativecommons.org/licenses/by/4.0/">http://creativecommons.org/licenses/by/4.0/</a>).</p> 	<p>The aim of this study was to examine the effects of regular edible coating treatments on the local <i>Pistacia vera</i> Kaleghouchi to determine changes in composition, physicochemical parameters, and microbial analysis of the pistachio during different storage periods. Pistachio nuts were coated with chitosan at different concentrations of 1%, 1.5%, and 2%, with 50% and 60% of whey protein isolate by immersion method. Samples were stored at room temperature for 5 months. The obtained results showed that there was a significant difference (<math>p \leq 0.05</math>) between coated and uncoated samples. The highest acid value, peroxide value, viscosity and density were observed in the control samples (1.05 mg KOH/g oil, 9.33 mEq active O<sub>2</sub>/kg oil, 142.77 cP and 0.922 g/mL) respectively, while the lowest peroxide value was found in the treated nuts with chitosan 1.5% and whey protein isolate (50%), which both were 4 mEq of active oxygen kg<sup>-1</sup>. lowest acid value (0.73 mg KOH/g oil) and viscosity (80.21 cP) was observed in the chitosan 1.5% coated samples. As the lowest density was resulted in coated pistachios with both 1% and 2% chitosan and they both were 0.916 g/mL. Chitosan significantly (<math>p \leq 0.05</math>) reduced the mould count (<i>Aspergillus</i>), the highest mould count (<math>75 \times 10^1</math> CFU/g nuts) was noticed in control sample, while lowest (<math>5 \times 10^1</math> CFU/g nuts) was observed in the treated samples with 2% chitosan .</p>
<p><b>Keywords:</b> Coating, Pistachio, Physical, Chemical Analysis, Microbiological Analysis.</p>	

## تأثير الطلاءات الصالحة للأكل على بعض الخصائص الفيزيائية - الكيميائية والنمو الفطري خلال فترات تخزين مختلفة من الفستق

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### الملخص

الغرض من هذه الدراسة هو فحص تأثير معالجات الطلاء المنتظمة الصالحة للأكل على الفستق المحلي *Pistacia vera* Kaleghouchi لتقدير التغيرات في المكونات، الصفات الفيزيائية - الكيميائية والتحليل الميكروبي للفستق خلال فترات مختلفة من الخزن. الفستق المغطاة مع chitosan بتركيز مختلفة 1%، 1.5% و 2% مع 50% و 60% من whey protein isolate بطريقة الغمر. تم تخزين العينات في درجة حرارة الغرفة لمدة 5 أشهر. أوضحت النتائج يوجد الفروقات المعنوي بين العينات المغطاة وغير المغطاة. أعلى نسبة من قيمة الحموضة، قيمة البيروكسيد، الكثافة واللزوجة كانت في العينات غير المعاملة وبلغت (10.50 ملغم / KOH / غم زيت، 9.33 مكافئ / O<sub>2</sub> / كغم. أدنى قيمة لكل من الحموضة (0.37 ملغ / KOH / زيت) واللزوجة (80.21 سنتي بويز) للزيت المستخلص كانت في العينة المغطاة مع chitosan بتركيز 1.5%. أدنى نسبة للكثافة النسبية كانت للفستق المغطى مع chitosan 1% و 2% وكانت كلاهما (0.916 غم/مل). أظهرت الدراسة ان chitosan كانت لها تأثير معنوي ( $P \leq 0.05$ ) على خفض تعداد الأعفان (*Aspergillus*)، أعلى رقم لتعداد الأعفان (75  $\times 10^1$  وحدة فطرية/جرام فستق) لوحظت في العينات الغير المعاملة (غير المغطاة)، وفي نفس الوقت أقل قيمة ( $5 \times 10^1$  وحدة فطرية/جرام فستق) كانت للعينات المعاملة بـ 2% chitosan.

**كلمات مفتاحية:** الطلاء، مكونات الفستق، التحليل الفيزيائي لزيت، التحليل الكيميائي، التحليل الميكروبي.

### Introduction

Edible films and coatings are thin layers of edible substances applied to the product surface as a replacement of its natural protective, and to make a solute barrier, oxygen, and movements of moisture in a food product (1, 2 and 3). Generally edible coatings consist of three main components: polymers with high molecular weights [lipids, polysaccharides, and proteins] (4, 5 and 6). The most common polysaccharides available are extracted from marines, animals and agricultural plants. Films commercially used to protect meats, nuts, fruits, and vegetables. In addition, edible films have the possibility to reduce the packaging costs, complexities and wastes (7 and

8). Polysaccharide edible coating, such as chitosan, which is created from crab waste and shrimp, is utilized like edible coats and they have activities of anti-fungal (9). The increasing interests in high quality food products with an extended shelf-life and low packaging wastes have encouraged more studies and improvements of biodegradable packaging with edible polymer films and coatings (10). Edible coatings have showed the ability to improve the quality of fresh food products, by regulating of the transformations of oxygen, carbon dioxide, moisture, aroma, and taste compounds in a food system (11, 12 and 13). One of the most important storage factors that can affect lipid oxidations, is the oxygen concentration (14). By decreasing the concentration of oxygen around the nuts, oxidation of the lipid can be decreased dramatically, how better the barrier, the lipid oxidation will be slower (15). It is a linear polysaccharide made up of  $\beta$ —(1→4)—linked 2—amino—2—deoxy D - glucose remains, deriving from de-acetylated derived of chitin, which is the second most plenteous polysaccharide naturally next cellulose (16 and 17). In addition, solutes have a high possibility to hold active and useful compounds such as antimicrobials (18). Chitosan treatments reduced the activity of various fungi (19). Whereas the using of biopolymer coatings has increased in the recent years, due to the general concerns of the natural resource limitations (raw materials) and the environmental issues that caused by non-biodegradable packaging materials (20).

(15) found that WPI coatings can delay the development of rancidity of dry roasted peanuts. (9) investigated that chitosan coatings significantly inhibited the growth of the *Aspergillus* and its effect was increased with increased concentration.

The aim of this paper was to investigate the use of some edible coatings (chitosan and whey protein isolate), which they were environmentally friendly packagings for the physicochemical parameters, and microbial analysis.

## Materials and Methods

*Pistacia vera* Kaleghouchi nuts were harvested on September 2018. The sample mixture was obtained from a local orchard in Erbil /Kurdistan region-Iraq. Coating process: Chitosan solutions at three different concentrations (1%, 1.5%, and 2%) were prepared by dissolving (1, 1.5, and 2) grams of chitosan powder in acetic acid (1%) solution and then continued the addition to make the final volume to 1000 mL. Then they were filtered and autoclaved. The pistachios kernel samples were immersed in the previously prepared chitosan solutions for 30 to 40 seconds and dried in the oven at 45 °C for 3 hours (9).

Meanwhile the whey protein isolate (WPI) was dissolved in distilled water in two different concentrations (50% and 60%). Then the both solutions were heated to 90 °C for 30 minutes in a water bath. After they were degassed and cooled to ambient temperature, glycerol (Gly) added as plasticizer (50 mL and 40 mL) respectively, and then the solutions were again degassed. The coating procedure was performed by dipping the samples for 1 second (21), and then dried by a drier.

The coated samples, by the two different coatings and at the various concentrations, are kept at room temperature for 5 months. During the storage time, samples were examined in three different times (1<sup>st</sup> month, 3<sup>rd</sup> month and 5<sup>th</sup> month) for some physicochemical changes except for total mould count and moisture content of the samples, which both parameters were examined monthly during the experiment.

**Composition:** Some compositions of the control and treated pistachios kernel were examined to determine their amounts and changes. The moisture, Ash, Protein to nitrogen, Crude dietary fiber, Carbohydrate contents were measured according to AOAC Official Methods No.925.40, 950.49, 955.04, 4.06.01, 50 -1-16 respectively (22). As the oil in the controls and treated samples for the 1<sup>st</sup> month of storage were extracted by using a Soxhlet system according to AOAC official method NO.920.39C (23)

**Physical Analysis of Oil:** Crude oils were obtained by a cold pressing of the nuts (oil extraction machine/ LPP205). Density meter was used to measure the density of the oil samples according to the method described by (24 and 25). Brookfield viscometer was used to measure the viscosity of the crude oil samples (24).

**Chemical Analysis of Oil:** Peroxide value and acid value of the crude oil were determined according to AOAC Official Method No. 965.33 and 940.28 respectively (22). **Microbiological analysis:** Microbiological analysis was conducted to determine mould count for control and treated samples of different treatments of the coatings at different periods of storage (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> months). The total mould was determined as it was described in ISO international standard methods No.21527-2 (26).

**Statistical Analysis:** Statistical analysis was done by using the property program SAS software (27), to analysis data by the experiment of the factorial in a completely randomized design. The means were compared by using Duncan's multiple range tests at ( $P \leq 0.05$ ).

## Results and Discussion

**Compositions:** Table 1 indicates the compositions of the examined coated and uncoated pistachio nuts. The highest oil content (58.10%) was observed in the coated pistachio sample with 1.5% of chitosan, which is located within the range stated by (28). There was no significant difference among coated pistachios with chitosan and WPI samples (Table 1). The minimum oil content was extracted in the control sample (54.05%), these values agree with those reported by other authors (29 and 30).

The protein content of the coated and uncoated nuts varied from 17.84% to 20.54%, which is consistent with (28), their results were ranged from 15.0 to 21.2%. Coated pistachio kernels with 50% and 60% WPI have had the highest protein content (20.44% and 20.54% respectively), same results were reported by (31 and 32). As can be noticed, the protein contents in the two concentrations of the WPI were significantly different than the chitosan coatings and the non-coated nuts. There was a probability of being effect by coating material with WPI increased the protein of pistachios.

There were no significant variations ( $P \leq 0.05$ ) in the ash contents between the coated and non-coated samples, with an exceptional for the 2% chitosan coating, when compared with control samples (Table 1). The lowest ash content (2.86%) was recorded in the coated pistachios (2% chitosan), and the highest amount (3.10 %) was observed in the uncoated samples, which is agrees with (29 and 33). This could be due to being calculating the ash percent based on the total weight. Dietary fiber content of coated and non-coated pistachio samples (Table 1) was between (6.02 and 7.18), approximated results with (31) their result was 9%. And found that there were no considerable differences between coated pistachio with 1.5%, 2% chitosan and 60% WPI samples. On the other hand, there were noticeable differences between each of control, chitosan coating (1%) and 50% WPI. This might be due to the same reason as mentioned for the ash content.

**Table 1 Mean values of pistachio composition (dry weight basis)**

Compositions	Control	Chitosan 1%	Chitosan 1.5%	Chitosan 2%	WPI 50%	WPI 60%
<b>Oil content</b>	54.05 c	57.09 ab	58.10 a	56.79 ab	56.89 ab	56.82 ab
<b>Protein</b>	18.21 bc	18.77 b	18.19 bc	17.84 c	20.44 a	20.54 a
<b>Moisture</b>	4.00 a	3.00 b	2.78 bc	2.56 c	2.59 bc	3.01 b
<b>Ash</b>	3.10 a	2.98 ab	2.93 ab	2.86 b	2.94 ab	2.94 ab
<b>Fiber</b>	7.04 a	6.02 c	6.29 bc	6.39 b	7.18 a	6.45 b
<b>Carbohydrate</b>	13.60 a	12.13 b	11.70 b	13.55 a	9.86 c	10.24 c

\* Means with the same letter for each line are not significantly different at  $P \leq 0.05$

Carbohydrate contents in control and coated samples are presented in (Table 1). The majority of treatments showed lower percentages of carbohydrate than the control, this can be related to the increased percent of both of the oil and the protein contents in this study. There were no statistical variations between each of control with 2% chitosan, same result was reported by (34). Also 50% with 60% WPI, and 1% with 1.5% chitosan coatings.

**Table 2 Density of different pistachio – coated and non-coated samples during storage period.**

Months	Control	Chitosan 1%	Chitosan 1.5%	Chitosan 2%	WPI 50%	WPI 60%
<b>Month 1</b>	0.916 cd	0.915 d	0.915 d	0.915 d	0.915 d	0.916 cd
<b>Month 2</b>	0.917 bc	0.915 d	0.915 d	0.915 d	0.916 cd	0.916 cd
<b>Month 3</b>	0.922 a	0.916 cd	0.916 d	0.916 cd	0.918 b	0.917 bc

\* Means with the same letter for each line are not significantly different at  $P \leq 0.05$

Density provides information about the establishment, the oxidation state or polymerization (35). Table 2 shows different values of specific gravity that may be attributed to the different fatty acid composition, different total solid content and different degree of saturation (36). Furthermore, illustrates the densities of oil samples that varied between 0.915 and 0.916 g/mL in the first month of the storage, which is consistent with (37). And there were no significant differences between all samples in the first month of the storage. The examined densities for all the coating treatments were insignificant among themselves. While the same test for the control sample

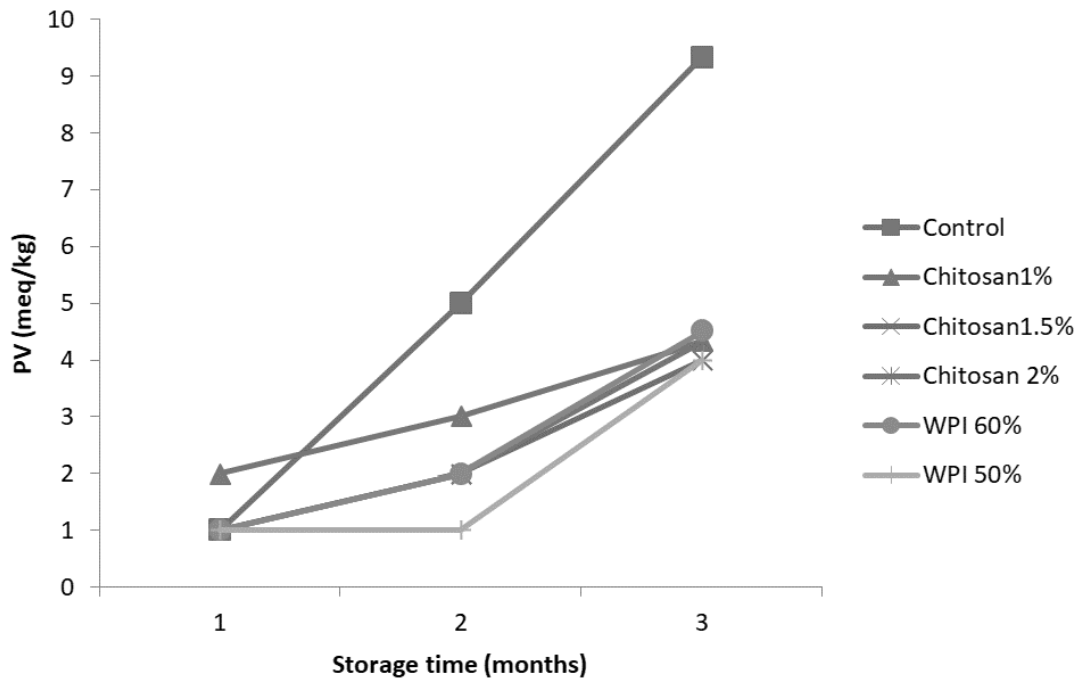
(uncoated pistachios) were significantly higher than all of the treated samples, in the last month of the storage. Density was increased during periods of storage, density of control samples were consistently higher than those of coated dried pistachio kernels. This it could be due to the effect of chitosan and WPI coatings. The highest density observed in control sample was 0.922 g/mL, and the lowest density noticed in coated dried pistachios was with 1%, 1.5% and 2% chitosan, and they have had the same values of density (0.916 g/mL), which is agreed with (25), they observed that density of pistachio oil was 0.916 g/mL. According to the (37) mean values of pistachio oil for specific gravity was  $0.9143 \pm 0.006$ . Density was higher for uncoated nuts at all times of the storage. There was significant difference between the specific gravity of the coated pistachios and control samples. In conclusion, it can be observed that the coating treatments have led to a kind of stability in the density parameter of the extracted crude oils (Fig. 3) over the storage.

**Table 3 Viscosity of different pistachio - coated samples during storage period.**

Months	Control	Chitosan 1%	Chitosan 1.5%	Chitosan 2%	WPI 50%	WPI 60%
Month 1	72.41 cd	68.84 cd	65.05	62.96 d	61.56 d	60.23 d
Month 2	110.72 b	71.37 cd	78.98 cd	77.89 cd	81.63 cd	84.12 cd
Month 3	142.77 a	80.21 cd	85.23 cd	83.07 cd	82.92 cd	90.18 bc

\* Means with the same letter for each line are not significantly different at  $P \leq 0.05$

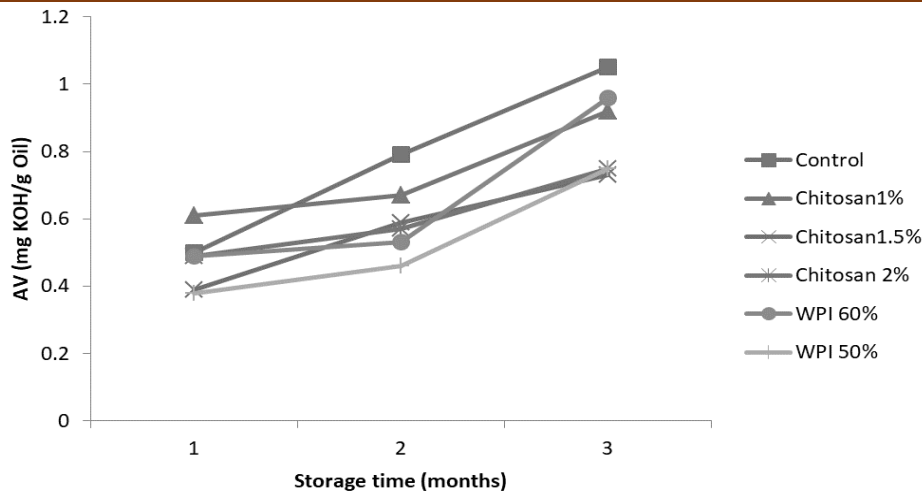
Viscosity is another important physical property of vegetable oil (38 and 39). This is attributed to the degree of saturation and chain length of free fatty acids. For edible fats and oils, viscosity decreases as both saturation level and chain lengths decrease (40). As shown in Table 3, viscosity of oil samples varied between 60.23 and 72.41 Centipoises (cP) of the pistachio coated with WPI 60% and control samples respectively in the first month of the storage. It was elevated dramatically throughout the storage time, which is consistent with (41). The highest viscosity was found in uncoated pistachio (control) samples (142.77 cP), while lowest was noticed in coated pistachios with 1% chitosan samples (80.21 cP) after 150 days of storage, and this is similar or approximated results with (42). Results showed insignificant changes in the viscosity of the crude oils in the coated samples over the time. Whereas, the control samples was significantly increasing in their viscosities over the same time, and they all were noticeably different than the studied treatments.



**Figure 1 Effect of time on the peroxide value of uncoated nuts and coated nuts with different formulations stored at room temperature.**

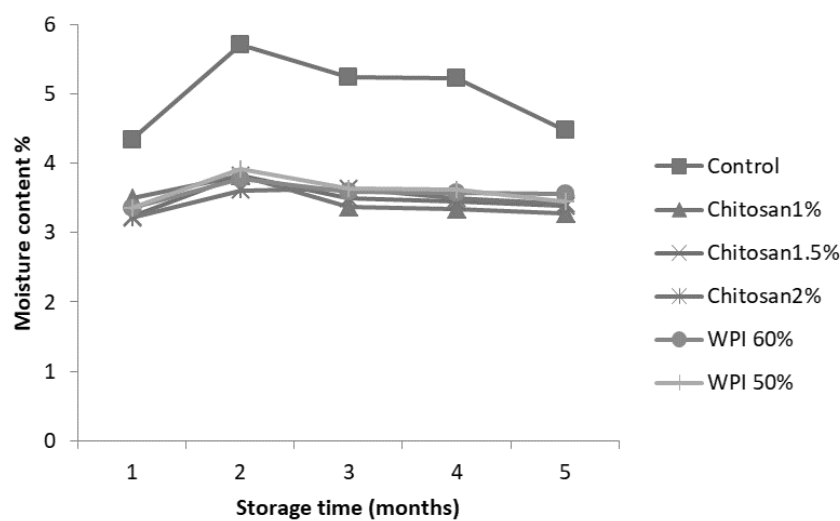
The peroxide value (PV) determines the formation of hydro peroxides (primary oxidation products) (5). It gives the initial evidence of rancidity in unsaturated oils (43). Elevated peroxide value indicates that lipid oxidation has taken place (40). Fig. 1 shows the peroxide values of crude oils extract from all treated and untreated pistachios during two main periods of storage. And as can be seen, the PVs in each examined sample have increased significantly over the time and this consistent with (42).

In the term of the different treatments in this experiment, there was no significant difference in the PV between all coated samples, and their PV were significantly lower than the control samples at the both examined storing times (Fig. 1). The lowest PV was recorded by the chitosan 1.5% and WPI 50% in the last month of the storage, both results was (4.0 mEq kg<sup>-1</sup>). For the same time, the highest level of PV was observed in the control (crude oil screwed from uncoated samples). According to (44), the PV for a cold pressed and virgin oils are (up to 15 mEq active O<sub>2</sub>/kg oil). In this study, all extracted oils have had PVs lower than the limit stated in the standard, which is coincided with (25).



**Figure 2 Effect of time on the acid value of uncoated nuts and coated nuts with different formulations stored at room temperature.**

Acid value (AV) is an indicative index for oil quality (45). When the oil quality is considered, the number of free fatty acids is shown to be a good indicator throughout production and storage (46). Oils, which have higher free fatty acid contents, possess poor quality (47). AVs for the different crude oils that extracted from the pistachios (coated and non-coated) were increased significantly over the time (Fig. 2). In the last month of the storage (the 5<sup>th</sup>), the AV in the uncoated pistachio's crude oil was considerably higher than the both coating treatments. The highest recorded numbers were (1.05 mg KOH/g) for the uncoated samples. The obtained results (AVs) from this study, were within the limits of acid value reported for a cold pressed and virgin oil in the Codex Standard for named vegetable oils (44), which it is (4 mg KOH/g oil). And the recorded range for the coated and non-coated samples were (0.73 – 1.05 mg KOH/g oil) respectively, in the final month of the storage.

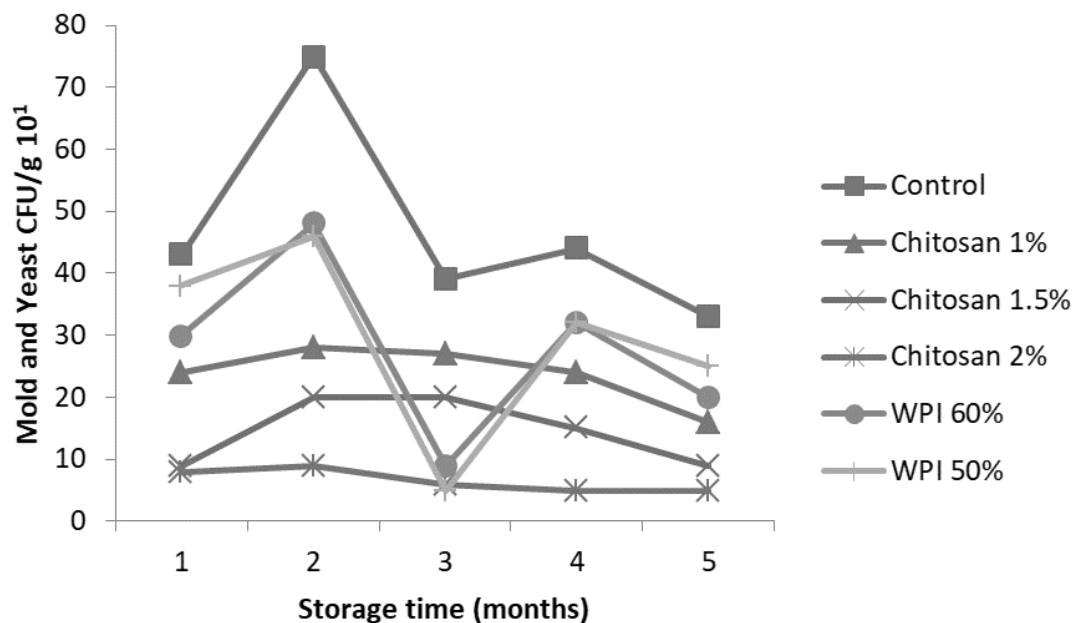


**Figure 3 Chart of changes in moisture content of pistachio nuts during 5-month storage.**



Moisture content: Other important parameter that has been studied in this paper was the moisture content of the different treated and untreated samples, stored in room temperature, at the different storage time (Fig. 3). Although the humidity trends in all samples (coated and non-coated) are slightly increased by the storing time, a significant difference occurred between the non-coated and coated samples. Whereas, the higher levels of humidity were recorded in the uncoated pistachios. Moisture transfer between a food and its surrounding atmosphere decreases as the food covered up with an edible coating (9). In conclude, the coating treatments (Chitosan and WPI), at the different concentrations have had a significant impact in reducing undesirable moisture gain by the coated pistachios.

Although the storage conditions, such as temperature, was controlled to some extent, in the 2<sup>nd</sup> month of the storage, there was a noticeable increase in the moisture content almost in all examined samples (coated and non-coated) (Fig. 3), as the relative humidity in this month was higher than other months during the experiment. This result is coincidence with the increased mould count in the same month of the storage. Hence, this result can confirm the fact that relative humidity can affect on mould counts in coated and uncoated nuts (48).



**Figure 4 Chart of changes in moulds growth in pistachio nuts during 5 month storage.**

Figure 4 illustrates the total mould count in the pistachios kernel samples for different coating treatments and different periods of storage at room temperature. Obviously, the total mould count decreased over the storage time. There was an exceptional increase in the second month of the storage, but then it falls again on the third month (which it coincides the March), particularly for both treatments of WPI, then the reduction continued during storage periods. As relative humidity and temperature both have a direct impact on the growth of moulds (48), this fluctuation is believed to be as a result of the effects of environment humidity in these months.

Generally, during the total storage time, the lowest colony count ( $5 \text{ colonies} \times 10^{-1}$ ) CFU/g was found in coated pistachio with 2% chitosan, while the highest count of mould noticed in non-coated samples and it was ( $75 \text{ colonies} * 10^{-1}$ ) CFU/g. Results showed that chitosan caused reduction in microbial population and its microbial-growth inhibition increased with high concentrations (49). In this study, the chitosan coated samples, showed low levels of mould count. It was noticed that the mold count in the chitosan treated nuts, at different concentrations, was lower than both, the WPI coated samples and uncoated samples with an exceptional for the WPI treatments in the third months of the storage (Fig. 4). The low microbial growth was almost during the entire periods of the storage, particularly treated pistachios with 2% chitosan, which it showed no significant growth of the mould. This it could be due to the antifungal effects of chitosan. Protein and polysaccharide-based films have a potential to fulfill customer demands and expectations of new packaging systems that are biodegradable and made from renewable sources. Edible films or coatings are promising alternative to conventional packaging systems that among their functions can be added maintenance of organoleptic and other quality attributes. Moreover, edible coatings can protect perishable products from deterioration by delaying dehydration, suppressing respiration, improving texture quality, helps in retaining volatile flavor compounds and reducing microbial contamination. Hence, it can extend the shelf life of foods by preventing chemical, physical, or biological damages. The oxidation of oils and fats can be controlled by application of edible coatings, such as chitosan and WPI. From results in this study, it was possible to conclude that the use of chitosan and WPI/Gly coatings increased the shelf life of pistachio kernels and preserved their fresh-like characteristics. These coatings universally are good oxygen barriers at low-to-intermediate relative humidity.

Chitosan solution at 2% concentration showed to be the most suitable coatings for a reduction in mould growth in pistachio nuts. Treated pistachios with 1.5% chitosan and 50% WPI/Gly showed the lowest peroxide value, meanwhile the best acid value was recorded in 1.5% chitosan. The lower and most proper density was noticed in coated pistachios by 1%, 1.5% and 2% chitosan. In comparison with the other samples, coated pistachios with 1% chitosan have had the low viscosity. It is reasonable to mention that the all the treatments did not significantly affected the ash content of the pistachio nuts overall coatings were effective in preventing the oxidation and hydrolytic rancidity of pistachio nuts. It is evident that all tested coatings have had significantly ( $P < 0.05$ ) lower peroxide and acid values, moisture contents, density and viscosity, and moreover decreased the growth of mould in stored pistachio nuts compared to those of uncoated nuts.

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