EFFECT OF CHITOSAN COATING AND STORAGE PERIODS ON SOME PHYSICOCHEMICAL PROPERTIES AND FUNGAL GROWTH OF GRAPE ”CV. “RASHMIRI

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Abstract

Postharvest deterioration of table grapes have economic implications for the producer countries every year which need to control by some techniques, among them, applying of natural edible coatings such as chitosan as an alternative, to mitigate the negative impact of chemical coatings on human health. This study was carried out to evaluate the effects of chitosan coating (0.5% & 1%) on some physicochemical properties of grapes CV. “Rashmiri including shriveling, shattering and berry abscission, Firmness, springiness, cohesiveness, weight loss percentage, soluble solid content (TSS), total acidity (TA %), and Maturity index MI (TSS/TA) as well as fungal counts of table grape during storing at 4oC temperatures over a period of 60 days. The Results shows a significant effect of chitosan coating on berries' quality properties. It prevent their shattering, beery abscission and reduce shriveling percent. Chitosan coatings grapes recorded significant improvement in weight loss, firmness, and cohesiveness, however springiness increased during 60 day of cold storage in all grapes treatment and higher springiness value recorded in samples coated with 1.0% chitosan. Moreover grapes coated with Chitosan recorded slight declines in total soluble solids (TSS) and titratable acidity (TA %). Less variation recorded between the treatments in Maturity index MI (TSS/TA) during 60 day of cold storage. The main property of chitosan that achieve in this study its ability to preserve table grape from spoilage by fungi, since no fungal growth were recorded in these samples during 60 days of storage time, indicating that it can be a valid alternative to fungicides in controlling postharvest decay of table grapes. However, the use of biopolymers can be improved by the addition of cross linking, antioxidant and antimicrobial agents

Keywords: Grape, Chitosan, storage period, quality properties

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Introduction

Grape is the most produced fruits in the world, due to their nutritional value, it exhibits antioxidant, cardio protective and anticancer activities, hence their annual world production increase to approximately 75 million tons (1). The production of grapes also increased In Iraq and reached to about 421868 tons in 2023 of which high present of these were produced in the Kurdistan Region of Iraq (KRG-Iraq) (2). However about one third of this product is wasted annually, because of, non-operating in ideal post-harvest condition. As a perishable fruits, grapes are exposed to berry drop, rachis browning, accelerated softening, microbial rot and high incidence of berry decay that leads to shelf-life reduction (3). In order to mitigate

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grapes deterioration and contamination, as well as the associated financial losses and health hazards, the food industry is currently concentrating on creating innovative solutions that prolong food shelf life and prevent microbiological contamination (4). Among the strategies that implemented for this purpose, are using some agrochemical compounds (SO2) with cold storage after harvest (5). However, high SO2 cause potential hazards to consumers environment during grape storage (6), hence it was replaced by natural polymers like polysaccharides, lipids, proteins, and some plants extracts that rich in bioactive compounds. They are used as edible coatings and can be processed as fruit surface coatings to protect perishable fruits like grape from deterioration by reduce respiration, retarding dehydration, improving mechanical handling properties of the fruits and helping retain volatile flavor compounds, as well as reducing microbial growth (7). One of the most used edible coatings is Chitosan, deacetylated β-(1-4)- linked D-glucosamine polymer, due to its bioactivity and biodegradability. It could be obtained from the polysaccharide, chitins which is typically derived from the shell of crustaceans (shrimp and crabs) and fungal cell wall, as well as some plants (Luffa spp) (8). Chitosan can produce a semipermeable film on fruit and vegetable surfaces, which reduces respiration rate by adjusting the permeability of O2 consumption and CO2 production and has the potential to enhance resistance against pathogenic fungi and hence prolong the shelf life of deferent kinds of fruit including citrus and plum fruits (9), moreover it has antibacterial action. The positive effect of chitosan is based on their hygroscopic properties which induce formation of a water barrier and consequently reduce external water transfer and reduce the weight loss caused by pathogenic fungi (10). It has many applications in preservation of fresh vegetables and fruits. Two chitosan formulations were approved by the European Union (Reg. EU 2014/563) and (Reg. EU 2022/456) as main substances for plant protection [11 .[

A wide range of studies demonstrated the effectiveness of chitosan applied alone or combined with other compounds such as essential oils to maintain the physical properties of some fruits and vegetables recently (12, 13). Since 30% of the total output of grapes was lost in our region because of poor management of post-harvest condition , which lead to economic implications, this study was conducted to improve storage life of grapes by investigate the effect of chitosan coating treatments of some physicochemical traits of table grapes CV. “Rashmiri” .This grape cultivar grows in KRG-Iraq, it have sweet black berries with heavy cluster and distinctive taste and flavor nurtured by the area’s unique climate and soil, they have the potential to become a global cultivar when given the support it deserve.

.2Materials and Methods

.2.1Sampling

A local table grape CV. Rashmiri (Black-colored) from vineyards of Sitak (KRG-Iraq) were used in this study. Grapes samples was taken to the lab of Food Science and Quality Control Department in University of Sulaimani for analysis. The Grapes berries were chosen for the current study harvested on October-2022 based on their size, color, and form, after excluding the grapes with obvious signs of mechanical damage or fungal infection. After washing with distill water (DW), grapes samples were disinfected using 200 ppm of Prodein (8 mg codeine phosphate/500 mg paracetamol) in order to get

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rid of the microbiological load from farming and handling. Subsequently, they submerged again in DW water to eliminate any remaining disinfectant .

.2.2Preparation of chitosan coating solution: As described by Ngo et.al., (14) 2g of chitosan was dispersed in 98ml of acetic acid %2 concentration to obtain 2% (w/w) of chitosan with stirring at 45oC for 2 h. The grapes samples were coated with 0.5 and 1% (v/v) chitosan then the grapes were allowed to dry at room temperature. Ultimately, they divided into clear, disposable plastic hinged containers with around 200±20 grams for each container, with three replicate. Following that, kept in accordance with storage periods at 4 °C and 65-70% RH. In order to ensure of the results, these treatments were repeated at the Hamsa lab for quality control of fruit and vegetable in Sulaymaniyah City.

.2.3Qualitative traits analysis

.2.3.1Analysis of physicochemical properties of grapes CV. "Rashmiri

The visual properties of grape fruit include evaluation of berry shriveling, Incidence of cracked or shattered berries and berry abscission percentages (15). Each character in every cluster was measured by weight of character and dividing it by the overall weight of the bunch and computed on a % weight basis.

The Physical analysis of grape fruit include: Weight loss percentages (%) which were calculated by first weighing the fresh fruit on harvest day (which is regarded as day 0), and subsequently recording the fresh weight every 20 days till 60 days (0, 20, 40, and 60). The calculation that follows was used to get the weight loss percentage.

Weight loss percentage (%)=(fresh weight at 0 day-fresh weight at X day)/(fresh weight at 0 day)*100

After choosing at random 10 berries per cluster for each treatment, the fruits' firmness (gm/cm2), cohesiveness and springiness (mm) were evaluated using compression twice. Firmness is the force required to compress a sample, cohesion is the degree to which the material adheres to one another and springiness is the rate of return former shape and recovered quickly. The berries textures were then examined at room temperature using a CT3-Brookfield texture analyzer (The Laboratory Store, Inverness, United Kingdom). The 500 g trigger load and 5.0 mm deformation at a speed of 3 mm/second were the settings for the instrument. The TA-OC (Ottawa Cell; 43*40 mm) piercing probe was used to finish each test.

The chemical analysis of grape fruit include: The percentage of soluble solids content (TSS %) which determined using a manual press, the juice from the berries was extracted, filtered through cheesecloth, and the supernatants were saved for juice analysis. The TSS of the juice was determined using a digital refractometer (HANNA model HI96801, Bedfordshire, UK) with a range of 0-85%. When calibrating the device with DW, the Brix refractometer displayed, when a few drops of fruit juice solution were added to the glass plate. Titratable acidity percentage (TA%) was determine by titrating 25 mL of diluted filtered juice and adding 0.1 N NaOH solution to it, in order to attain the neutral point as a pH indicator (5). Titratable acidity percentage (TA %) was measured .

TA as tartaric acid %= (V * N* 0.0075)*100/ v

V = volume (mL) of sodium hydroxide solution used for titration

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N = normality of sodium hydroxide solution

v = must/wine sample volume (ml.(

Regarding to Maturity index (MI), it was determined by dividing soluble solid content on acid content as the next equation MI: TSS/TA.

.2.3.2Fungal Analysis: For the determination of mold counts during the storage period, samples of 25 g from each group were taken and homogenized in 225 mL of sterile peptone water. Serial dilutions were carried out, and 0.1 mL from each solution was spread to the potato dextrose agar (PDA) for 5 days at 25 ℃. Samples were prepared in triplicate, and the counts between 30 and 300 colony-forming units (CFU/gm) were only considered [16]. Calculate the colony-forming unit for fungi by using the following formula :

CFU/gm= the colonies number*dilution factor / volume of culture plate (ml.(

.2.4Statistical Analysis: A 3×3 factorial experiment (two factors at three levels) was conducted according to Complete Randomized Design (CRD) with three replicates, the first factor was chitosan treatments at three concentrations (0. %, 0.5% and 1.0%), and the second factor was storage periods of three times (20, 40, and 60 days). Data were analyzed in Excel 2011 and SPSS 24 software. Analysis of variance (ANOVA) and significant differences were performed using Duncan's multiple comparison procedure ($p < 0.05$.(

.3Results and Discussion

.3.1Visual quality properties

The results of Table 1 shows that the visual quality properties of studied treatments in general declined after 60 days of storage. Regardless of chitosan coating treatments with both concentrations (1.0% and 0.50%), they were able to preserve the visual appearance of grapes fruits during storage time, since no shattering recorded in both treatment as well as the percent of berry abscission were zero in both treatments, however the fruit that treated with 1.0% chitosan was the best treatment, as less percent of shriveling was recorded in this concentration of chitosan (10.6) compared to control (43.2). Chitosan coating combined with lower storage temperature were also used to increase the shelf life of pears, tomatoes and mandarin (17-18) .When it comes minimally treated horticultural products (mainly fruit), their visual appearance is crucial (19) According to earlier research the degree of shriveling is important variable that affect how fresh product looks. Even a little decrease in water content can have a significant impact on the berry visual quality of table grapes, such as wilting, shriveling, weight loss, color changes and rachis browning which lead to loss of grape quality traits and reduce their shelf-life (20). The greatest probability of shattering (cracking) occurs when water on the surface of the berry is absorbed by the berry cells resulting un increase in their water contents, especially when combined with High relative humidity, this generates turgor pressure resulting berry cracking (21). The storage-life extension because of chitosan coating might be due to the formation of a semipermeable that induce the formation of a water barrier and consequently reduce water loss from the coated surfaces

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Table 1. Visual quality properties (Shriveling, Shattering and berry abscission) of local table grape (Rashmiri) at 4oC

Time (Day)	Defect	Blank	0.50%	1.00%
20	Shriveling	29.9	5.9	1.5
	Berry Abscission	0.0	0.0	0.0
	Shattering	5.9	0.0	0.0
40	Shriveling	29.0	36.1	19.4
	Berry Abscission	2.9	0.0	0.0
	Shattering	29.0	0.0	0.0
60	Shriveling	43.2	35.1	10.6
	Berry Abscission	5.7	0.0	0.0
	Shattering	35.1	0.0	0.0

.3.2Effect of chitosan coating on some physical properties of grape

.3.2.1Wight loss

Figure 1 displays variations in weight loss of grape samples CV "Rashmiri" during the cold storage at 4°C for 60 days. Throughout storage, every fruit exhibited a steady loss of weight. The longer the storage period, the highest the weight loss recorded. Significant variation recorded between the chitosan coating treatments, grapes coated with concentrations of 1% chitosan, recorded less percent of weight loss (6.2%) after 60 days of cold storage compared to the berries (without chitosan) which recorded weight reduction value that was noticeably greater (10.7%). Humidity ratio of the storage environment have an effect on the %weight losses. Water loss is excessive when there is a greater disparity between the relative humidity of the surroundings and the water activity of the fruit (22). Relative humidity of stored environment was about 70% while moisture content in grapes berries is at least 80%.this explain the lower water loss during stored coated chitosan with high relative humidity (85%) (23). Besides, the stage of ripening considers as remarkable factors that cannot ignored. It directly connected to physiological characters of fruits during postharvest that may affect the results (24.(

The biggest alteration in horticulture products during storage is water loss, which eventually results in weight loss. Since fresh fruits, like grapes, typically have moisture contents of more than 80%, water content loss occurs during storage. As a result, avoiding product from losing water is a crucial concern that directly impacts how well the storage works (25). It is worth to mention that the majority of the water in grape dehydration evaporates via the rachis in its initial stages, while the skin serves as the primary pathway for water loss in the latter stages. The positive effect of chitosan coating is based on their hygroscopic properties that induce the formation of a water barrier and consequently reduce external water transfer. The metabolic activity of fruits that linked to tissue senescence throughout extended storage periods, reduces down after coating application, which explains why the weight loss in the uncoated samples increased throughout storage. The usefulness of polysaccharide coatings as a water barrier in fruit has been documented in other studies, and the addition of lipids improves this barrier (26, 27.(

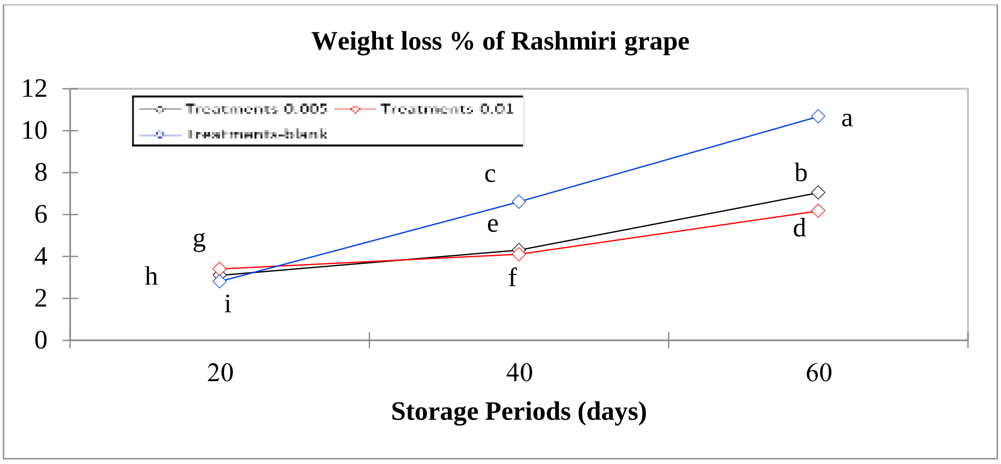

Figure 1. Means weight loss% of Rashmiri grape during storage for 60 days at 4 oC.

.3.2.2Changes in mechanical properties of grapes samples during storage

Figure 2 display the firmness, springiness and cohesiveness averages for several coated and uncoated grape samples across 60 days of cold storage. In general, the berries' skin rupture force progressively dropped during time of storage, and the mechanical response varied depending on the storage duration, however the uncoated samples exhibited a significantly higher loss of firmness, from 1221.73 to 763.33 g/cm2 with 41.2% loss, in comparison to the remaining coated samples, in which grapes coated with 1.0% demonstrated the highest levels of firmness values. This mean that chitosan coatings have positive effect on their firmness, with a concentration-dependent impact. Similar results were obtained by other studies (28, 29). The fruit firmness values obtained in this study decreases with increasing water loss. In this way, chitosan coatings have the ability to block water vapor and helps to preserve the texture of the fruits and increase their firmness values. Moreover, chitosan coatings consistently prevented berries from bruises and breaking of berry skin (30.(

There was fluctuation found of springiness (figure 2) of the grape's treatments. In contrast to firmness and cohesiveness, springiness increased during 60 days of cold storage in all chitosan coating however higher springiness value recorded in grapes samples coated with 1.0% chitosan. Similar results obtained by Gao et.al. (31), since chitosan–coating effectively decreased grapes respiration rate and maintained fruit texture, which have an effect on berry springiness .

Regarding to cohesiveness, there were no significant changes found between the treatments till 40 days of storage, after that, at end of storage (after 60 days), cohesiveness value decrease in all of the samples, however chitosan coated samples had relatively slight declines, its loss about 27.7% of their cohesiveness value) compared to the control treatment which loss 40.0% of their cohesiveness value (31). Overall, chitosan coatings have been found to contribute to improved preservation of fruit texture.

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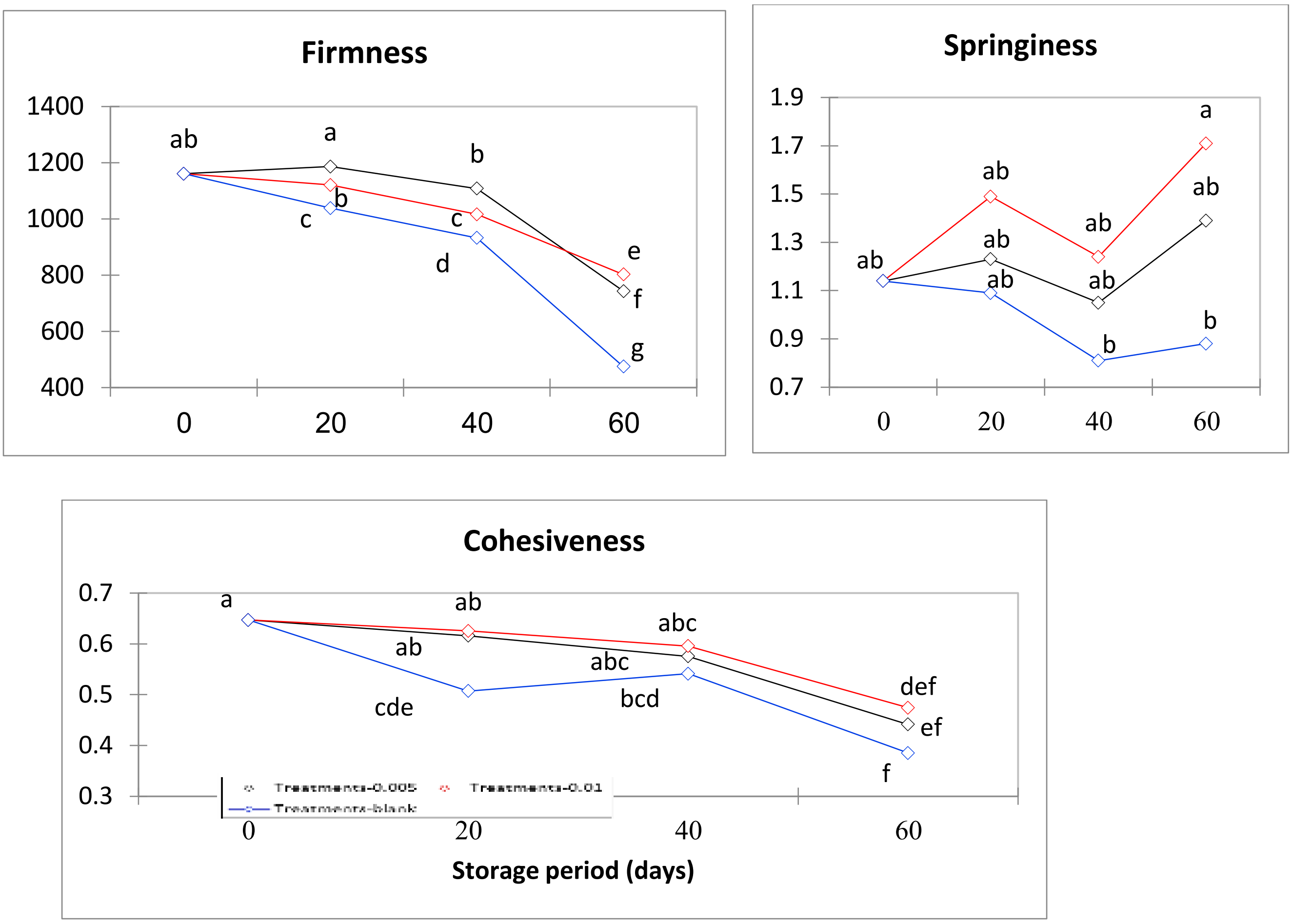

Figure 2. Mean change of firmness, springiness and cohesiveness during storage of Rashmiri grape at 4 oC. Data are given as the average (n=10), and error bars indicate standard deviations. Different letters indicate a statistical difference ($p \leq 0.05$) between treatments according to Duncan's multiple ranges.

.3.3Effect of chitosan coating on some chemical attributes

Figure 3 displays the effect of chitosan coating of grape on total soluble solids (TSS), titratable acidity (TA %) and Maturity index MI (TSS/TA) values during 60 days of cold storage. In general, the concentrations of TSS and TA reduced with time, however slight declines recorded in the chitosan-coated grapes, similar results obtained in other studies (32,33), suggesting that the coating process may delayed the physiological senescence of grape berries and ultimately the catabolism of organic acids throughout the extended duration of storage and consequently TA% reduction. Less variation recorded in MI during 60 days of cold storage in which lower MI recorded in 1% chitosan-coated grapes. however higher MI values obtained in the study of Shiri (21) due to using deferent cultivar of grapes in their study with deferent level of TSS and TA values. Hence grapes must be harvested at high TSS (more than 17 Brix) and lower TA in order to get MI values within the maturity requirements (34), since higher MI ratio had better organoleptic quality at harvest (35

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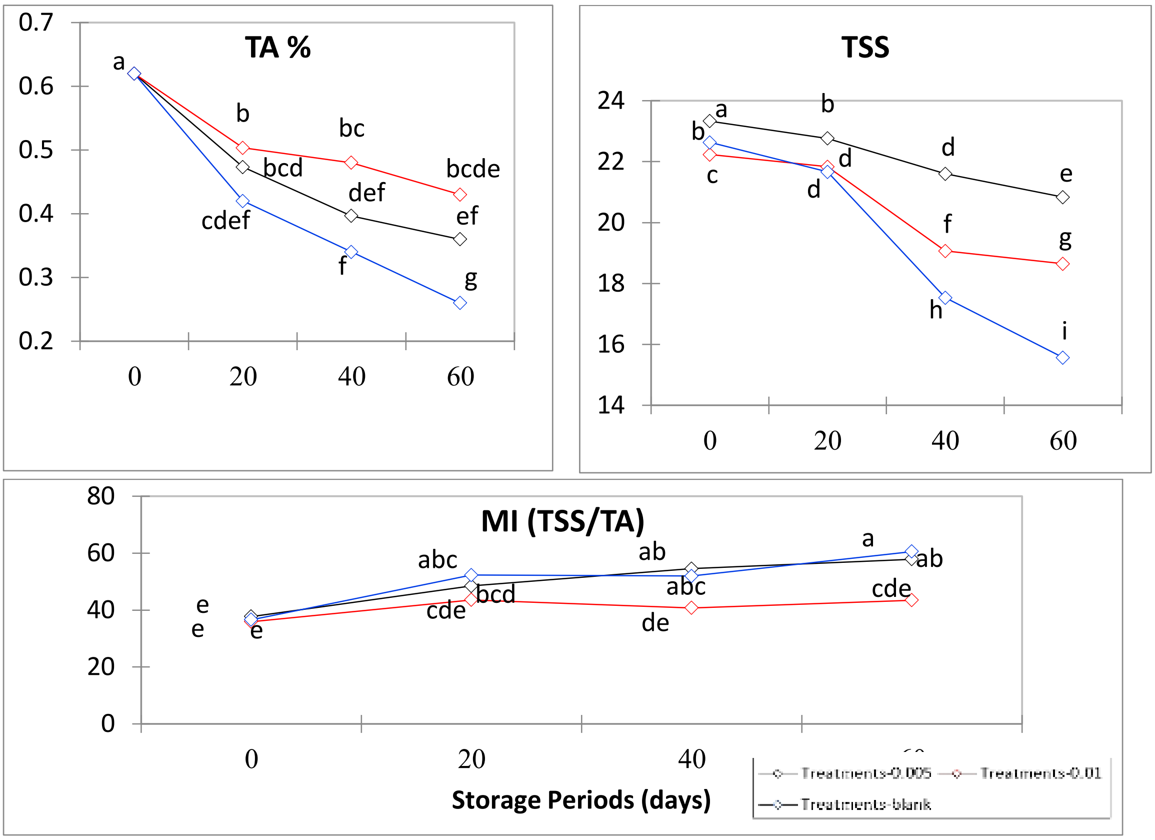

Figure 3. Mean change of TSS, TA% and MI (TSS/TA) and during storage of Rashmiri grape at 4 ℃. Data are given as the average (n=3), and error bars indicate standard deviations. Different letters indicate a statistical difference ($p \leq 0.05$) between treatments according to Duncan's multiple ranges.

.3.4 Effect of chitosan coating on Fungal spoilage of grapes .

As shown in figure 4, chitosan with concentration of 0.5 and 1 % have positive effect on preserve table grape from spoilage by fungi for 60 days, since no fungal growth were recorded in these samples during storage time compared to control, similar results obtained in other studies (33, 36), this is due to the inhibitor activity of chitosan against mold. In fact, chitosan inhibits the in vitro growth of many fungi, as well as a wide variety of foodborne pathogens (37). The antifungal activity of Chitosan might be related to its forming a physical barrier against infection, forming film matrix or gel, besides that, it is attributed to its specific physicochemical characteristics that enable it to interact with fungal cells and disrupt their normal functions (38). Chitosan is a linear poly amino saccharide obtained by N-deacetylation of the polysaccharide chitin. The mechanism of antimicrobial action of this polymer based on the electrostatic interactions between the positively charged (NH_3^+) groups of chitosan with the negatively charged of fungi cell walls , that leads to a change in permeability of cell wall, preventing cell metabolism and lead to cell death (39). The present study indicates that chitosan can be a valid alternative to fungicides in controlling postharvest decay of table grapes, it could be applied, to avoiding Sulphur dioxide applications, which was harmful to humans. However, other factors also affect this interaction such as molecular

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weight of chitosan, the pH of the solvent and others. Hence , chitosan is become one of the most widely used polymers as natural preservatives for food. Moreover it gained significant interest due to its non-toxicity, and biodegradability
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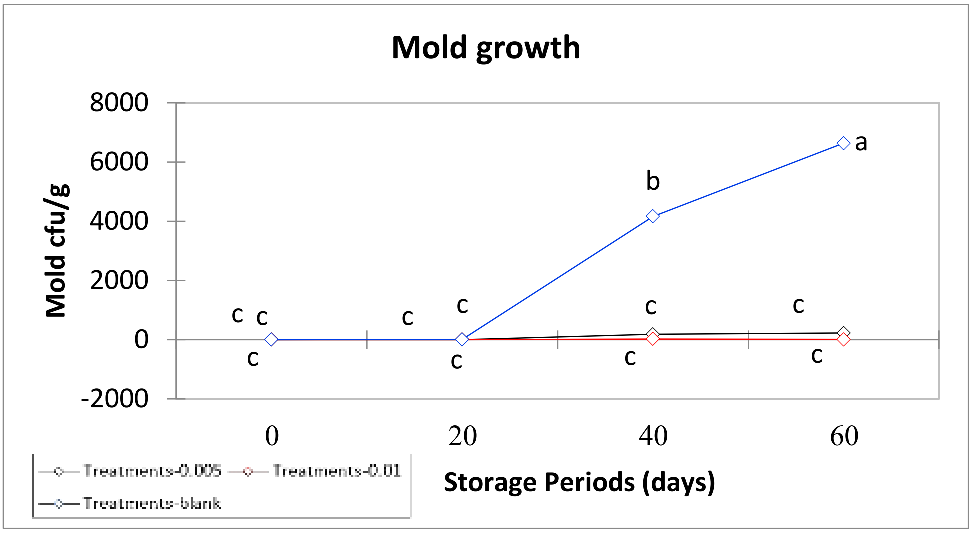

Figure 4. Mean change of mold growth during storage on Rashmiri grape at 4 oC. Data are given as the average (n=3), and error bars indicate standard deviations. Different letters indicate a statistical difference ($p \leq 0.05$) between treatments according to Duncan's multiple ranges.

Conclusion

Grape is a perishable fruit with rich nutritional contents; however, its post-harvest shelf-life is limited, which lead to economic loss. In order to solve the issues related to grape deterioration during storage chitosan with deferent concentrations were used as edible coating for table grapes CV. "Rashmiri" cultivated in Sulaimani /Iraq during storing at 4oC temperatures and 70% relative humidity over a period of 60 days. It was concluded from this study the positive effect of chitosan to extend the shelf life of grapes, since coating grape with chitosan prevent grape shattering, berry abscission and reduce shriveling percent. A significant improvement in weight loss (reduced by 50%), total soluble solids (TSS) and titratable acidity (TA %) were also recorded in this study. Moreover, chitosan exerts its inhibitory activity against fungi since no fungal growth were recorded in chitosan coated samples during 60 days of storage time, indicating that it can be a valid alternative to fungicides for prolonging postharvest shelf-life of table grapes. Further studies are needed to extend the shelf-life of table grapes by the addition of some antioxidant and antimicrobial agents to chitosan and study their effect on the sensory quality of grape
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