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Evaluation of Rutability, Quality and Microbial Load in Hayani Date Palm Fruits during Cold Storage as Affected by Applying some Safe Postharvest Treatments

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ABSTRACT

This work was carried out during the two successive 2014 & 2015 seasons in the post-harvest lab., Agricultural Development System Project, Faculty of Agric. Cairo University, Giza, Egypt. The efficacy of using some safe substances i.e., chitosan (0.75 and 1.5 %) and sodium carbonate at 0.75 and 1.5 %, along with, control on storability, microbial load of Hayani date palm fruits and its quality under cold storage conditions (2 °C and 90 – 95 % relative humidity) was studied. It was observed that two conflicted trends were resulted with prolonging of cold storage period during both seasons. The percentage of weight loss, decay, rutab, soluble solids content and total sugars of fruits were increased; while fruit firmness, acidity and soluable tannins values were relatively reduced. Moreover, the different Hayani fruits measurements according to various tested treatments varied not only from one treatment to other but also, each characteristic reflected its own trend from the other side. Therefore, it could be concluded that all soaking treatments significantly reduced the fruit microbial load and decay percentage compared to control (water dipping). On the other hand, 1.5 % chitosan treatment was the superior. Chitosan treatments reduced the level of microorganisms load compared with control. At the same trend Sodium carbonate treatments reduced also, both bacterial and fungal count during the storage period of Hayani date. Four deferent fungi were isolated and identified from Hayani date as *Aspergillus niger, A. flavus, Rhizopus nigricans* and *Penicillium* sp. On the contrary of that , the total acidity was slightly affected by different treatments. The difference was more significant particularly with the two tested concentrations of chitosan during both seasons of study. Furthermore, the fruit firmness, total sugars and tannins content significantly affected by various treatments.

Keywords: Date palm, postharvest, safe substances, chitosan, sodium carbonate, physical and chemical properties, microbiological analysis and fungi identification.

INTRODUCTION

Date palms (*Pheonix dactylifera*, L) can grow from 12.7 to 27.5 °C average temperature, withstanding up to 50 °C and sustaining for a short period on the frost temperatures reach –5 °C (Chao and Krueger. 2011). Egypt is a subtropical country which lies between 22 ^{o} and 31^{o} North latitudes and between 25^{o} and 35^{o} East longitudes. Its climate, which comprises a mild and rainy winter from November to April and a hot and dry summer from May to October, is suitable for the production of several horticultural crops.

Several studies show that edible films and coatings can be used to help in fruits and vegetables preservation because they provide a partial barrier to moisture loss, O_2 and CO_2 permeability, (Olivas and Barbosa-Ca novas, 2005). Edible coatings have been known to protect food products from decay by suppressing respiration, decreasing dehydration. It also improve the textural quality, volatile flavor compounds and reducing microbial load (Özdemir *et al*., 2010).

On the last decade, more attention has been paid to natural polymers including polysaccharides and proteins in food processing applications. Most of these materials can be applied as films or processed as surface coatings to reduce catabolism . They also improve the handling and help maintain its structural integrity (Baldwin, 1994).

Chitosan application as pre- or postharvest treatment has been enhanced resistance against for fungal diseases (Reglinski *et al*., 2005). Chitosan also can be used as an antimicrobial and shown to interfere with the germination of most phytopathogenic fungi (Ben-Shalom *et al*., 2003). Furthermore, many researcher demonstrated that ,chitosan coating has the potential for inhibiting decay and prolong the storage life of citrus fruits (Chien *et al*., 2007).

Carbonic salts like sodium carbonate and sodium bicarbonate are widely used in the food industry as food additives (Lindsay 1985 and Multon 1988). The antimicrobial activity of these chemicals has been studied *in vitro* by (Marloth, 1931 and Corral *et al.,* 1990) . Sodium bicarbonate solution used as immersion of citrus fruits by (Barger 1928) he showed that the fruit green mold disorder had been reduced.

The objective of this research is study

1- The possibility of using natural safe alternatives postharvest treatments such as chitosan and sodium carbonate for improving the storability and quality of date palm fruits during cold storage, as well as. marketing period of the Hayani variety
2- Induce the ripening process of Bisr Hayani date fruits during cold storage by safe postharvest treatments for increasing the percentage of Rutab fruits.

MATERIALS AND METHDOS

This study was conducted to evaluate the response of cold stored mature fruits of Hayani date palm cv. to emulsifying with some safety substances during two successive seasons of 2014 and 2015. Mature fruits of Hayani date palm trees (*Pheonix dactylifera*, L.) grown in a private orchard at OM EL – Reda village, Damietta Governorate, Egypt were collected at full colored (Khalal stage), then directly transferred to the laboratory of the Agriculture Development Systems (ADS) Project in Faculty of Agric. Cairo University, Giza Governorate, Egypt.

Similar intact fruits as possible in their shape, colour and size and free from any disorders, defects and apparent pathogen infection were carefully selected, washed, air-dried and subjected to one of the following dipping treatments: Control (T1), Chitosan at 0.75% (T2), Chitosan at 1.5% (T3), Sodium carbonate at 0.75% (T4) and Sodium carbonate at 1.5% (T5).

Experimental layout

Fruits of each treatment were dipped for two min in the corresponding emulsion solution devoted for each of the above mentioned five treatments. Treated fruits were left to dry aerobically, then fruits of each treatment were subdivided into two sections (A&B). Section (A) devoted for estimating the periodical changes in some fruit physical characteristics include percentage of fresh weight loss, rutab fruits, decay, fruit firmness and microbial load. Other section (B) was used for determining the changes in chemical fruit characteristics i.e. fruit juice contents of total soluble solids, total sugars, total acidity and tannins, during cold storage. The complete randomized design with three replicates was applied for arranging the differential five postharvest treatments. Each replicate was represented by one perforated carton box contained 1.5 Kg Hayani date palm fruits. Cold storage at 2 ºC ± 2 and relative humidity (RH) of 90-95% was extended during each season up to 45 days.

Preparation of chitosan

The chitosan solutions were prepared by dissolving [l% or 2% (w/v)] chitosan in 0.25 N HCI. Then the solutions centrifuged to remove undissolved particles. The pH was adjusted to 5.6 with 0.1 N NaOH. At this pH, is positively charged and has maximal biological activity (Stössel and Leuba, 1984). Tween 80 [0.l% (v/v)] was added to the solutions to improve wettability. (El Ghaouth *et al*., 1992).

Measurements

The responses to the different investigated postharvest treatments were evaluated at the beginning of the storage periodically and at 15 days intervals by determining the changes exhibited in the following measurements of Hayani date palm fruits.

A) Fruit physical properties

1. **Weight Loss (%):** Fruits were weighted and the loss weight was recorded for each replicate as percentage of the initial weight according to the following formula.

$$\text{Fruit weight loss\%} = \frac{\text{Initial weight} - \text{Weight at specific interval}}{\text{Initial weight}} \times 100$$

2. **Decay Percentage (%):** Decayed fruits were determined according to skin appearance, shriveling, chilling injury, and pathogenic rots. In the inspection time , the decayed fruits per each replicate were weighted and discarded, then decay percentage was estimated according to the following formula:

$$\text{Decay Percentage (\%)} = \frac{\text{Weight of decay fruits}}{\text{Initial weight}} \times 100$$

3. **Fruit firmness (Ib/inch^2)**

The firmness of fruit was determined as Lb/inch^2 by using fruit pressure tester FT 327 (3-27 Lbs mod.

4. **Rutab percentage (%):** all fruits that showed visual change in color to dark brown and softening of about 20% of its surface were considered rutab. Percentage of rutab was calculated according to the following equation:

$$\text{Rutab \%} = \frac{\text{Weight of rutab fruits}}{\text{Initial weight}} \times 100$$

B) Fruit chemical properties

1. **Titratable Acidily (%)**: five ml sample of fruit juice was used to determine the titratable acidity by the titration aganist 0.1 N sodium hydroxide in the presence of phph indicator (Total titrable acidity was expressed as mg citric acid /100 ml Juice).
2. **Total sugars (g/100 g "dried weight"):** Determined in stored date fruits by method described by Dubois *et al*. (1956).
3. **Soluble solids content (SSC %):** Soluble solids content was measured by using a hand refractometer in fruit juice.
4. **Fruit tannins content:** Total tannins of Hayani date fruits were determined according to the method described by Schanderl (1970).

C) Microbiological studies

Total bacterial count of treated Hayani dates samples was determined according to APHA (1998) by plating suitable dilution in duplicates using nutrient agar medium. The plates were incubated for 3 days at 30°C. Also, plating technique method was used for counting total fungi on PDA medium (Oxoid, 2006).

Three plates incubated at 25°C for six days. After the incubation period, developed bacteria and fungi were counted in each plate. The mean count of plates was recorded to calculate the fungal and bacterial count as mentioned by (APHA, 1998). The fungal isolates were done by using single colonies of different morphologies developed fungi in PDA medium. which picked-up and it was transferred onto PDA plates for sub culturing to pure fungal isolates. Before use, the fungal isolates were sub-cultured on new slants and incubated at 25°C for 10 days. Identification of fungal was based on the visual observation of isolated fungal growth. Plates were identified according to morphological characteristics (color of the colonies and growth forms) (Ronald, 2006) and Czapek yeast extract agar with 20% sucrose (CY20S) medium (Ronald, 2006). In addition, the vegetative and reproduction strictness observed in microscope were also considered for the fungal isolates. Fungal isolates were identified by Agric. Microbiology Dept., Fac. of Agric., Damietta University, Damietta, Egypt. The identification based on the morphological characteristics such as color of the colonies and the growth on different cultivation media, also, the observations of vegetative and reproduction strictness using light microscope were considered. The taxonomic keys described by Frater *et al*. (2001), Balajee *et al*. (2007) and Buommin *et al.* (2009) were followed.

D) Statistical Analysis

The experimental layout is complete randomized design. Experimental data obtained was treated with two way Analysis of Variance (ANOVA) technique. The statistical analysis was performed using CoStat software program.

RESULTS AND DISCUSSION

1. Fruit weight loss percentage

Firstly, it is important to notice that storage life of cold stored Hayani date palm fruits (2 ± 2°C) extended up to 45 days in both seasons of study. Table 1 prove that fruit weight loss percentage increased as the storage period was prolonged in both seasons. Where the cold stored fruits for fifteen days scored the lowest weight loss percentage, however at 45 days the highest percentage of weight loss was recorded. The differences in fresh weight loss % of cold stored fruits due to the specific effect of prolonging storage duration were pronounced as compared each other during two seasons of study. Data of first season showed that, the weight loss of fruit did not significantly differed between treated and non-treated fruits (control) except dipping treatment in 0.75% chitosan emulsion which recorded the highest value of fruit weight loss (4.95%) at the end of storage period. On the other hand, the control (untreated fruits) exhibited the highest values of fruit weight loss at all intervals of cold storage in the second season.

The postharvest treatments by dipping in sodium carbonate solution at the concentration of 1.5% tended to be the most effective one for reducing the fruit weight loss. It recorded the lowest value of weight loss as the average of(0.89 and 0.65) and (1.35 and 1.02) during 15 and 30 days of cold storage of two seasons, respectively).

Weight loss is attributed to losing of water during metabolic processes. In addition, moisture loss and gaseous exchange from fruits is usually affected by the epidermal layers.

Table 1. Effect of postharvest treatments on weight loss of Hayani date fruits (khalal) under cold storage conditions during two seasons of study.

Treatments	Loss Weight (%)					
	2014			2015		
	Shelf life (days)			Shelf life (days)		
	15	30	45	15	30	45
Control	1.38bc	1.81bc	3.08b	1.40a	1.60a	2.61a
Chitosan at 0.75 %	1.79a	2.75a	4.95a	0.67b	1.18b	2.39ab
Chitosan at 1.5 %	1.29c	1.80bc	3.29b	0.61b	1.86a	2.09ab
Carbonate Sodium at 0.75 %	1.58ab	1.69c	2.75b	0.76b	1.28b	1.80b
Carbonate Sodium at 1.5 %	0.89d	1.35d	3.20b	0.65b	1.02b	1.96ab
LSD 5 %	0.28	0.32	0.77	0.55	0.43	0.65

2. Fruit decay percentage

Results presented in Table 2 show that investigated treatments significantly reduced decay % compared to control at the end of storage period. Cold storage for 45 days at 0.2°C ±2 reduced the storability of Hayani date palm fruits, hence it registered the highest fruit decay percentages when compared with the corresponding ones of fifteen and thirty days cold storage.

With regard to specific effect of tested postharvest treatments, Table 2 indicates that fruits treated with chitosan in both concentrations statistically recorded the lowest fruit decay percentages (ranged from 13.4% to 17.6%), followed by 1.5% sodium carbonate treatment which ranged from 41.7% to 46.4% in both seasons. On reverse, the highest fruit decay percentages (56.7 – 57.1) were coupled with control (tap water dipped fruits) followed by 0.75% sodium carbonate -treated fruits (50.4% & 49%) in both seasons.

The gas exchange between fruit and atmosphere could be modified by Chitosan. The internal gas composition by producing a film on the surface. The effect on decay by chitosan can be attributed to delaying the senescence. Chitosan, as a natural compound, reduced the fruit fungal decay by its antifungal activity (Bautista-Banos *et al*., 2006). Chitosan also maybe induction the host resistance to pathogens (Trotel-Aziz *et al*., 2006), (Gonzalez-Aguilar, 2009). The protection against deterioration by chitosan slowing decay and ripening and it has protective effect against infection (Reddy *et al*., 2000). For all above mentioned properties, chitosan has a potential to increase storage life and decrease decay of fruits.

Table 2. Effect of postharvest treatments on decay percentage of Hayani date fruits (khalal) under cold storage conditions during two seasons of study

Treatments	Decay %							
	2014				2015			
	Shelf life (days)				Shelf life (days)			
	0	15	30	45	0	15	30	45
Control	0.0	0.0	0.0	56.7a	0.0	0.0	0.0	57.1a
Chitosan at 0.75 %	0.0	0.0	0.0	15.1d	0.0	0.0	0.0	17.6d
Chitosan at 1.5 %	0.0	0.0	0.0	13.4d	0.0	0.0	0.0	16.0d
Carbonate Sodium at 0.75 %	0.0	0.0	0.0	50.4b	0.0	0.0	0.0	49.0b
Carbonate Sodium at 1.5 %	0.0	0.0	0.0	46.4c	0.0	0.0	0.0	41.7c
LSD 5 %	0.0	0.0	0.0	2.00	0.0	0.0	0.0	2.42

3. Fruit firmness (lb./in^2)

Firmness of fruit is one of the most important determinants of postharvest quality and fruit physiology. The data in Table 3 reveal that prolonging the storage period, increased generally softness of Hayani date fruits. All studied treatments recorded high firmness values than control fruits. At the end of the storage period (45 days), the firmness values of the tested treatments ranged from 11.4 to 12.3 lb./in^2 and from 10.3 to 11.5 lb./in^2 in the first and second seasons, respectively comparing to those of

control (10.4 and 9.7 lb./in^2, respectively). In both seasons, treatment of 1.5% chitosan showed the highest values of date's fruit firmness at each interval. It should be reported that all treatments had an effect in preserving firmness, whereas, at the end of the storage, the least firmness value resulted from the untreated fruits. With respect to specific effect of tested postharvest treatments, it is so clear to be noticed that response of fruit firmness to the different investigated postharvest dipping treatments was statistically significant.

The softening fruit after storage can be caused by breakdown of insoluble proto-pectin into soluble pectin (Matto *et al*., 1975). The first step in ripening process is the loss of pectic substances that leads to the loss of cell integrity and firmness (Solomes and Latics, 1973).

The application of chitosan onto fruits restraints of gas exchange CO2 and O2 inside the fruit thus depresses the metabolism process (Trenggono, 1992). So, the role of chitosan is able to blocks the activity of enzymes, which, may give better contribution in the storage (Li *et al*., 2006) and (Hanani *et al*., 2012).

Losses in firmness with the progress of storage period due to ripening of mango fruits as a result of an increase in activities of cell wall hydrolysis enzymes such as pectinesterase, polygalacturonase pectin methylesterase and pectatelyases during ripening and cold storage (Ali *et al*., 2004). The behavior of fruit firmness during the storage period of Hayani dates fruits as affected by post-harvest chitosan treatments are in harmony with those observed by (Brar *et al*., 1997 and Li and Yu, 2000) on peach fruits, they reported that chitosan treatments delayed the loss of firmness

Table 3. Effect of postharvest treatments on firmness of Hayani date fruits (khalal) under cold storage conditions during two seasons of study.

Treatments	Firmness (lb/inch^2)							
	2014				2015			
	Shelf life (days)				Shelf life (days)			
	0	15	30	45	0	15	30	45
Control	16.4bc	16.3c	13.6c	10.4d	16.0c	15.7c	12.9d	9.7d
Chitosan at 0.75 %	16.3bc	16.1b	14.6ab	11.9b	16.8ab	16.1b	14.2b	10.7b
Chitosan at 1.5 %	17.5a	17.3a	14.9a	12.3a	16.9a	16.7a	14.7a	11.5a
Carbonate Sodium at 0.75%	17.0ab	16.3c	13.9c	11.4c	16.2c	15.8bc	13.5c	10.3c
Carbonate Sodium at 1.5 %	17.0ab	16.5c	14.0c	11.5c	16.6b	15.9bc	13.9b	10.7b
LSD 5 %	0.78	0.32	0.44	0.29	0.29	0.38	0.33	0.31

4. Rutab percentage

At the Bisir stage, 'Hyani' fruit is considered physiologically mature and firm, reaches its maximum weight and size and the color changes from green to red. At the Rutab stage, the fruit starts ripening at the apex, changes in color to brown or black and becomes soft. The softening of the date fruit is mainly influenced by polygalacturonase, beta-galactosidase and cellulose enzymes (Glasner *et al*., 1999 and Serrano *et al*., 2001). During softening, the tannins which led under the skin are precipitated in an insoluble form, so that the fruit loses astringency with an increase in the reducing sugars and total sugars and total solids concentrations (Glasner *et al*., 1999 and Serrano *et al*., 2001).

Results presented in Table 4 clarify that all treatments significantly enhanced the transformation of fruits from the bisir stage to the rutab stage. This persisted throughout the whole storage period. The increase in rutab % was insignificant compared to control fruits after 45 days under cold storage. The treatment of dipping the fruits in sodium carbonate at a 1.5% concentration led to delay and decrease the rutability rate compared to control, especially in the second season of the study. It was also found that the higher chitosan concentration (1.5%) used, the more frequent fruits were transformed from the bisir to the rutab stage. Generally, all treatments increased the transformation from bisir to rutab stage and this effect was concentration dependent. Finally, it is worth mentioning that the insignificant effect of chitosan on rutab has been reported for dates fruits by Zahran *et al* . (2015).

Table 4. Effect of postharvest treatments on rutab percentage of Hayani date fruits (khalal) under cold storage conditions during two seasons of study

Treatments	Rutab (%)							
	2014				2015			
	Shelf life (days)				Shelf life (days)			
	0	15	30	45	0	15	30	45
Control	0.00	64.4a	94.4a	95.8a	0.00	54.0c	70.4d	91.9b
Chitosan at 0.75 %	0.00	64.9a	86.8b	94.7a	0.00	49.7d	78.2c	99.6a
Chitosan at 1.5 %	0.00	38.5d	83.6b	95.9a	0.00	57.8ab	81.3b	98.0a
Carbonate Sodium at 0.75 %	0.00	44.c	60.2c	87.6b	0.00	59.2a	87.0a	90.6b
Carbonate Sodium at 1.5 %	0.00	55.5b	82.3b	90.9ab	0.00	56.4b	64.9e	85.5c
LSD 5 %		2.4	5.3	7.4		2.0	3.5	4.9

5. Soluble solids content percentage

Table 5 demonstrates that prolonging cold storage period resulted in increasing total soluble solids percentage of fruit juice cv. In this concern, the initial time before cold storage at 2.0 ± 0.2°C scored the lowest TSS %, whereas prolonging cold storage period up to 45 days gave the

highest values in both seasons. Regarding the specific effect of post-harvest treatments, Table 4 shows that the differences in most cases were pronounced during two seasons. However, it could be generally observed that chitosan treated fruits at either 1.5 or 0.75% were statistically the richest in their TSS content as compared to those of other investigated treatments during two seasons.

The observed changes in TSS may be due to the hydrolytic conversion of polysaccharides into soluble sugar during the ripening process, which resulted in an increase in TSS % of the fruits (Abbasi *et al*., 2011). These results are also in agreement with those obtained by on Haden mango in Mexico. The abovementioned results are in agreement with those obtained by Attia (1995), EL-Badawy *et al*. (2012) and Baiea and EL-Badawy (2013) on orange, Pal (1998) and Carrillo *et al.* (2000) on Mango, Mpho *et al*., (2013) on avocado, Jiang and Li (2000) on longan fruit, Togrul and Arslan (2004) on peach, Rojas-Graü *et al.* (2007) and Raybaudi-massilia *et al*. (2008) on apple.

6. Total sugars content (g/100g dw)

Table 6 clear the effect of treatments on the total sugars. In all treatments, the sugars increased gradually as advancing the storage period. Treatment of 1.5 % sodium carbonate in 2014 and 2015 gave the highest values of total sugars 74.60 -77.27 (g/100 g dw) after 45 days of storage period in both seasons of the present study. The results in Table 6 indicate that all treatments of total sugars take nearly the same trend from harvest till end of storage. At the beginning or the end of storage period, all treatments resulted in higher total sugars than the control. Types of carbohydrates in dates are glucose, fructose, sucrose, mannose, maltose, small amounts of cellulose and starch (Al-Shahib and Marshall, 2003). The total sugars increase as the fruit mature. It is ranging from 32.99-38.20 (g/100 g dw) at the kimri stage and 77.97- 79.39 (g/100 g dw) at the tamr stage. The sucrose contents increased rapidly as the fruits grown from the kimri to the khalal stage then it decreased at the tamr stage to a non-detectable level.

Table 5. Effect of postharvest treatments on soluble solids content of Hayani date fruits (khalal) under cold storage conditions during two seasons of study.

Treatments	SSC (%)							
	2014				2015			
	Shelf life (days)				Shelf life (days)			
	0	15	30	45	0	15	30	45
Control	30.1d	37.8a	38.5b	40.0a	32.3c	39.5a	40.5c	43.8d
Chitosan at 0.75 %	30.4bc	35.1d	37.1d	37.1b	32.8a	38.5c	40.1d	45.8b
Chitosan at 1.5 %	30.8a	37.0b	39.1a	42.1a	32.9b	39.1b	42.5a	46.4a
Carbonate Sodium at 0.75 %	30.3cd	34.7d	36.1e	40.0a	32.7a	36.0e	39.2e	44.3c
Carbonate Sodium at 1.5 %	30.6ab	36.0c	38.2c	41.6a	32.7a	38.2d	41.3b	45.6b
LSD 5 %	0.24	0.60	0.22	2.52	0.20	0.11	0.12	0.49

The increase in total sugars values may be chiefly due to losses in water due to conversion of complex form, as carbohydrates like starch, to simple form of sugars with enzyme activities in date palm fruits as a- amylase (Wills *et al.,* 1980; Rohani *et al.,* 1997 and Wills and Rigney, 1979). These results are in accordance with those obtained by Hafez *et al.* (2012) who found that date palm fruits c.v Zaghlool, Samany, Amhat and Sewy recorded the highest significant content of total sugars obtained from fruits treated by soaking in 4% sodium carbonate.

Table 6. Effect of postharvest treatments on total sugars content of Hayani date fruits (khalal) under cold storage conditions during two seasons of study

Treatments	Total sugars (g/100 g dw)							
	2014				2015			
	Shelf life (days)				Shelf life (days)			
	0	15	30	45	0	15	30	45
Control	54.2a	57.5d	58.3d	65.4d	57.4bc	59.9d	60.1e	68.2d
Chitosan at 0.75 %	54.9a	64.7b	65.3b	69.8c	58.0a	67.4b	67.5d	72.8c
Chitosan at 1.5 %	54.9a	64.8b	65.5b	72.2b	57.6b	67.1b	68.3c	75.0b
Carbonate Sodium at 0.75 %	51.9b	60.5c	61.4c	72.1b	57.3c	63.6c	69.4b	75.1b
Carbonate Sodium at 1.5 %	54.6a	68.3a	69.9a	74.6a	57.2c	70.2a	71.4a	77.3a
LSD 5 %	0.79	0.10	0.65	0.14	0.28	0.32	0.10	0.21

7. Total acidity percentage (TA %) in fruit juice

As shown in the table 7, acidity reduced as cold storage periods preceded over 15 days. The titratable acidity decreased along with increased storage time in both treated fruits and the untreated one (control). These results agreed with those reported by El-Ghaouth *et al*. (1991) and Garcia *et al*. (1998) that the decrease of acidity during storage demonstrated fruit senescence.

Generally, it could be concluded that the lowest total acidity of fruit juice was always in concomitant to the control and fruits treated with 0.75% sodium carbonate after 45 days. A reduction of TA% during cold storage was previously reported for other fruits such as papaya and apricot (Ali *et al*., 2011). The authors attributed such decreases metabolic changes in fruits or due to the use of organic acids (citric and malic) as respiratory substrates. Moreover, chitosan proved to generally retain TA during cold storage of many fruits such as grapes and mango (Abd Elwahab *et al*., 2014 and Ampaichaichok, 2014). In this regard, El-Badawy and El-Salhy (2011) reported that a higher chitosan concentration was correlated with more TA control.

Table 7. Effect of postharvest treatments on titratable acidity of Hayani date fruits (khalal) under cold storage conditions during two seasons of study

Treatments	Acidity							
	2014				2015			
	Shelf life (days)				Shelf life (days)			
	0	15	30	45	0	15	30	45
Control	0.37e	0.35e	0.33d	0.30e	0.40d	0.40d	0.37d	0.35d
Chitosan at 0.75 %	0.52b	0.52b	0.50a	0.48a	0.59ab	0.58b	0.53b	0.51ab
Chitosan at 1.5 %	0.54a	0.51c	0.48c	0.42d	0.61a	0.60a	0.53b	0.50b
Carbonate Sodium at 0.75 %	0.50c	0.50d	0.49b	0.45c	0.56c	0.52c	0.50c	0.48c
Carbonate Sodium at 1.5 %	0.52b	0.53a	0.50a	0.47b	0.57bc	0.57b	0.55a	0.52a
LSD 5 %	0.006	0.004	0.002	0.004	0.026	0.013	0.019	0.019

8. Fruit tannins content (mg tannic acid mg/100 g fruit fresh weight)

It is obvious from Table 8 that the reduction in tannins content of Hayani date palm fruits is in proportionate with the advancement of storage period. Thus, forty five days cold stored fruits scored the lowest values of fruit tannins content. On contrary, the freshly harvested fruits (zero day storage) scored the highest values in this respect. Other values of cold storage periods occupied an intermediate position between the previously mentioned two categories. The differences between the studied storage periods were obvious to be significant. In regard to specific effect of post harvest treatments, statistical analysis of data in Tables 8 indicates that all studied treatments achieved to induce a remarkable effect on fruit tannins content. However the relative lower value of tannins content was generally coupled by sodium carbonate 0.75% - treated fruits in corresponding to relative higher content in sodium carbonate 1.5% in both seasons. In addition, other investigated treatments were in between the aforesaid two extends with a complete present of significance in the two seasons. Soluble tannins are responsible for the sensory astringency in dates (Serrano *et al*., 2001 and Pesis, 2005).

Table 8. Effect of postharvest treatments on soluble tannins content of Hayani date fruits (khalal) under cold storage conditions during two seasons of study

Treatments	Tannins content (mg/100g FW)							
	2014				2015			
	Shelf life (days)				Shelf life (days)			
	0	15	30	45	0	15	30	45
Control	201.1ab	195.5b	192.8a	182.2d	202.8a	198.2a	195.1a	185.3d
Chitosan at 0.75 %	202.1a	190.4d	187.2b	182.9c	202.8a	193.5b	190.8c	186.4c
Chitosan at 1.5 %	200.8b	196.7a	192.8a	190.2a	202.0a	198.5a	195.4a	193.6a
Carbonate Sodium at 0.75 %	200.9b	185.4e	182.0c	180.3e	202.7a	188.5c	187.1d	184.2e
Carbonate Sodium at 1.5 %	201.1ab	191.0c	187.4b	185.1b	203.0a	194.4b	193.8b	191.8b
LSD 5 %	1.04	0.13	0.39	0.30	2.59	1.14	0.31	0.50

9. Microbiological analysis

Table 9 shows that, the microbiological load of Hayani date after 0, 15, 30 and 45 days of cold storage at 2°C. Chitosan treatments significantly reduced the level of microorganisms load compared with control.

The mode of inhibition of chitosan on the growth of bacteria and fungi might be due to the interaction of chitosan with membranes or cell wall component, the mechanism underlying the inhibition of bacterial growth is thought to be that the cationic ally charged amino-group many combine with anionic components such as Nacetylmuramic acid, sialic acid and neuraminic acid on the cell surface, and may suppress bacterial growth by impairing the exchanges with the medium, chelating transition meal ions and inhibiting enzymes, due to the positive charge, resulting in increased permeability of the membranes and leakage of cell material from tissue, or due to water binding capacity and inhibition of various enzymes by chitosan (Jung *et al*., 1999 and Limam *et al*,. 2011). Chitosan also has bio absorption activity and can absorb nutrients of bacteria and may inhibit their growth (Knorr, 1991). Simillar results were obtained by Feliziani *et al*. (2013) who found application of 1% chitosan reduced postharvest diseases of sweet cherry. Moreover (Karabulut *et al*., 2003) found that baking soda (sodium bicarbonate) significantly reduced the total number of decayed grape berries caused by *Botrytis cinerea*, *Alternaria spp*., or *Aspergillus niger* after storage for 30 days at 1°C followed by 2 days at 20°C.

Table 9. Effect of chitosan and sodium carbonate dipping on the bacterial and fungal counts (CFU/g) of Hayani date fruits under cold storage.

Treatments	Microbiological analysis $\times 10^{-3}$ CFU/g date							
	Bacterial count				Fungal count			
	Storage period (days)				Storage period (days)			
	0	15	30	45	0	15	30	45
Control (water)	0.13b	0.33b	0.0b	0.0a	0.72b	6.60b	7.19b	9.24b
Chitosan at 0.75 %	0.06c	0.06d	0.0b	0.0a	0.0c	0.0d	0.12d	0.63d
Chitosan at 1.5 %	0.10b	0.0d	0.0b	0.0a	0.0c	0.05d	0.05e	0.30e
Sodium Carbonate at 0.75 %	2.63a	0.17c	2.92a	0.0a	4.38a	11.98a	11.68a	14.02a
Sodium Carbonate at 1.5 %	0.12b	0.55a	0.06b	0.06a	0.0c	0.93c	3.10c	3.10c
LSD 5 %	0.036	0.066	0.132	0.067	1.365	2.534	1.881	2.321

Four deferent fungal isolates were isolated from Hayani date and identified as *Aspergillus niger* (Figs. 1a and b), *A. flavus* (Figs. 2a and b), *Rhizopus nigricans* (Fig. 3a and b) and *Penicillium* sp. (Fig. 4a and b).

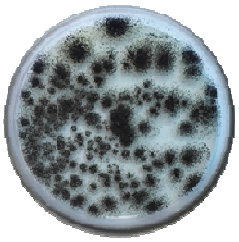

Fig. 1a. *Aspergillus niger* on PDA medium

Fig. 1b. *Aspergillus niger* under light microscope (400x)

Fig. 2a. *Aspergillus flavus* on PDA medium

Fig. 2b. *Aspergillus flavus* under light microscope (400x)

Fig. 3a. *Rhizopus nigricans* on PDA medium

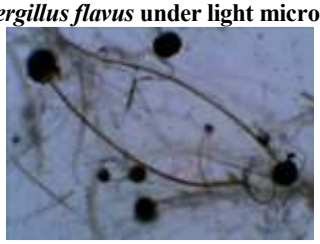

Fig .3b. *Rhizopus nigricans* under light microscope (400x)

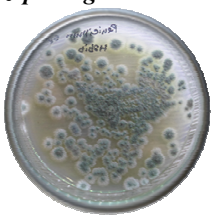

Fig. 4a. *Penicillium* sp. on PDA medium

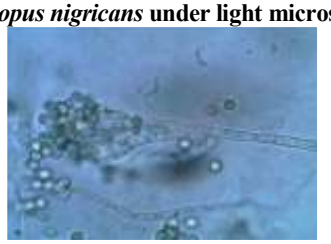

Fig. 4b. *Penicillium* sp. under light microscope (400x)

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تقييم الترطيب و الجودة و الحمل الميكروبى فى البلح الحيانى خلال التخزين البارد نتيجة تأثير إضافة بعض معاملات آمنة بعد الحصاد

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أجرى هذا البحث لدراسة تاثير الغمس بالشيتوزان وكربونات الصوديوم على ثمار البلح الحيانى كاملة التلوين فى مرحلة الخلال وذلك لدراسة معدل ترطيب الثمار وتحسين جودتها. وكذلك دراسة تاثير هذه المعاملات على الحمل الميكروبى للثمار و قدرتها التخزينية تحت ظروف التبريد على درجة حرارة ٢±٢ °م خلال عامى ٢٠١٤-٢٠١٥ . و كانت المعاملات المستخدمة كالاتى:١- غمس ثمار الكنترول فى ماء الصنبور فقط.٢- غمس الثمار فى محلول الشيتوزان بتركيز ٠.٧٥%.٣- غمس الثمار فى محلول الشيتوزان بتركيز ١.٥% .٤- غمس الثمار فى محلول كربونات الصوديوم بتركيز ٠.٧٥%.٥- غمس الثمار فى محلول كربونات الصوديوم بتركيز ١.٥ % .تم غمس ثمار البلح الحيانى السليمة فى محلول الشيتوزان وكربونات الصوديوم بتركيزى٠.٧٥ و ١.٥ % لمدة دقيقتين وتجفيفها هوائيا. ثم تعبئتها فى اكياس بلاستيك مثقبة ووضعها فى صناديق كرتونية مثقبة, وتم وضعها فى ظروف التخزين البارد على درجة حرارة ٢ م°±٢ و رطوبة نسبية ٩٠ – ٩٥ % لمدة ٤٥ يوم . وقد أوضحت النتائج المتحصل عليها أن الغمس فى الشيتوزان تركيز٠.٧٥ و ١.٥ % اعطى ثمار ذات جودة عالية من حيث الصلابة والمواد الصلبة الذائبة وأعلى نسبة ترطيب وأقل نسبة عفن وأقل محتوى ميكروبى. بالنسبة للفقد فى الوزن فوجد أن كربونات الصوديوم بتركيز ١.٥ % أعطت اقل فقد فى الوزن.أما بالنسبة للكنترول أعطت الثمار أقل نسبة حموضة لكن حدث انخفاض معنوى فى جودة الثمار وزيادة فى الفقد فى الوزن. كذلك يمكن إستنتاج ان جميع معاملات الغمس سواء بالشيتوزان أو بكربونات الصوديوم أدت إلى إنخفاض الحمل الميكروبى بشكل معنوى للبلح الحيانى، وخصوصا عند تركيز ١.٥٪ شيتوزان. كما أن المعاملة بكربونات الصوديوم قللت اعداد البكتيريا والفطريات خلال فترة تخزين البلح حيانى. كما تم عزل ٤ عزلات وتم تعرفها على انها أسبيرجيلس نيجر ، أسبيرجيلس فلافس ، ريزوبس نيجريكانس وبينيسليوم. ومن هذا البحث , يمكن التوصية بغمس ثمار البلح الحيانى فى محلول الشيتوزان أو كربونات الصوديوم بتركيز% ١.٥ لكل منهما حيث أنهما مادتان طبيعيتان , امنتان تماما , ولهما القدرة على خفض نسبة الثمار التالفة و كذلك نسبة الفقد الكلى فى الوزن خلال التخزين البارد. كما ساعدتا على المحافظة على صفات الجودة وحفظ صلابة الثمار.