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The effect of cultivar, chitosan and storage period on qualitative characteristics of date palm fruits (*Phoenix dactylifera* L.) And their infection with the saw-toothed grain beetle

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Abstract. A storage experiment was carried out to study the the role of chitosan in controlling the infection of three date palm cultivars (*Phoenix dactylifera* L.) which were Bream , Khalas and Habssi with saw-toothed grain beetle (*Oryzaephilus surinamensis* L.). As samples were prepared from dates infected with the beetle from one of the dates stores in the province of Basrah and were identified in the department of plant protection at the faculty of Agriculture University of Basra. Beetles were bred under the laboratory conditions .Three cultivars of dates (Bream , Khalas and Habssi)were prepared and dipped in three concentrations of chitosan (0% , 1% , 2%) then put in cork packages. Fruits were polluted by the beetle from the colony which was prepared beforehand and then stored at room temperature starting from 5/1/2017 for four months. A factorial experiment was designed from three factors: cultivars, chitosan treatments and storage periods using Complete Randomized Design(CRD) with three replicates. The most important results showed the superiority of Habbsi in the controlling of the beetle, the loss of weight of fruits and the high content of the fruits of the total soluble solids. The highest percentage of weight loss was in the fruits of the Bream cultivar. The results indicated that the treatment with 2% chitosan was the best in reducing the percentage of beetle infection and the percentage of weight loss of fruits. The results showed that the loss of weight and total soluble solids increased with the increment of storage periods. The results of the statistical analysis indicate that the highest percentage of total soluble solids was recorded in the non-chitosan treated fruits of Habbsi date palm cultivar after four months of storage.

Keyword: Date palm cultivars, saw-toothed grain beetle , chitosan, weight loss

1. Introduction:

The date palm tree belongs to the palm family Areaceae cultivated in Iraq and some parts of the Middle East (Barreveld, 1993). Dates are sugary fruits, giving a kilogram of about 3000 calories in addition to supplying the body with vitamins and minerals (Bakr, 1972; Shabana *et al.*, 2006).

Oryzaephilus surinamensis L. considered as the most important economic beetle , which belongs to the order "Coleoptera " and to the family "Silvanidae" infected the most stored foods such as grain, dates and other fruits. The larva and whole beetle feed on mature dates with low humidity when beetle digs in the skin and the flesh of the fruit several small holes and feeds on the contents of the dates.. Severe infection occurs when the dates were shipped with long distance. The infection of the beetle



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appears on the stored dates after 1-2 months from the harvesting date and arises with the increment of the storage period to reach to 64% after six months of storage (Abdul Hussein, 1985).

In view of the significant losses of stored foods caused by this beetle, studies on the storage ability of fruits that included new ways of controlling the beetle have been developed and expanded in recent years. As a result of the total reliance on insecticides during the past decades and the result of these materials in the imbalance of the environment and the emergence of many cases of poisoning and environmental pollution led to the search for alternative and safe methods of controlling.

Chitosan is a vital polymer, the second largest biomaterial after cellulose, which is found in the outer structure of crustaceans, insects and fungal cell walls. It is also characterized by no toxicity and biological decay and has no local effects in living tissue. It is a compound with vital functions (Rudrapatnam and Farooqahmed 2003), which has attracted the interest of researchers in the last few years for its commercial uses. Chitosan is composed of glucosamine units, which are associated with each other with a type of beta-type (1-4) cyclic bonds. It possesses many free hydroxyl and amino acids that enable it to form ionic, hydrogen and hydrofluidic bonds with other molecules such as fats and proteins (Shahidi *et al.* 1999; Dutta *et al.*, 2004).

The aim of the experiment is to test the role of chitosan in controlling the infection of date palm fruits cultivars: Bream, Khalas and Habbasi stored at room storage temperature with the saw-toothed grain beetle (*Oryzaephilus surinamensis* L.).

2. Materials and methods:

1 - Preparation of the colony of the beetle: attended samples of dates infected with the saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) from a store dates in the province of Basrah. The beetle was identified in the department of Plant Protection, college of agriculture, University of Basrah, and the breeding of the beetle was under laboratory conditions.

2- The process of extracting the chitosan: The shrimps were obtained from the local fish market in Basrah and the crusts were washed with ordinary water and dried by leaving them exposed to the sun. The method mentioned in Salman and Abadi (2009) was followed to the extraction of chitin from the shrimp. The shrimp crust was crushed into small pieces using an electric mill then process of removal of the proteins (deprotenization) by treating the crusts with sodium hydroxide solution at a concentration of 3.5% for two hours at a temperature of 65 °C by 1:10 (weight / volume). After washing the crust with distilled water, the mineral elements removed in the process called Demineralization by using a solution of hydrochloric acid at the concentration of 1N for a period of 1/2 hour at room temperature by 1:15 (weight / volume). Crusts washing well with water several times, then the pigment was removed by acetone and then by sodium hypochlorite solution at 0.315% for 5 minutes at room temperature by 1:10 (weight / volume). Finally, the white product was washed with distilled water and dry in an oven at 60 °C for 24 hours to obtain the Chitin.

Chitosan was prepared according to the method mentioned by Kamil *et al.* (2002) by removing the acetyl groups (Deacetylation) by treating with 50% sodium hydroxide at 1: 10 (weight / volume) at 100° C for 20 hours to obtain chitosan with low molecular weight, and then dried at 110 °C for 6 hours. The resulting chitosan is white powder.

The viscosity was determined by the use of the Ostwald viscometer. After the preparation of the solution, the amount of time required to flow it at a certain distance at 25 °C (Hassan, 1987). Molecular weight was determined depending on the viscosity of the solution according to No *et al.* (2000). The degree of removal of acetyl groups was determined according to Khan *et al.* (2002) by mixing 40 mg chitosan with 120 mg potassium bromide and then pressed and dried, then determined by using the Fourier Transform Infrared Spectroscopy (FTIR) instrument.

The parameters of the product were measured as follows: Viscosity = 64.16 Centi Boyz, molecular weight 720 Kdalton, and degree of removal of acetyl groups 87.6%)

Three concentrations of chitosan were prepared:

- 1- 0% Chitosan (0.5 ml acetic acid and supplemented to 100 ml distilled water).
- 2 - 1% Chitosan (1 g chitosan + 0.5 ml acetic acid and supplemented to 100 ml distilled water).
- 3 - 2% Chitosan (2 g chitosan + 0.5 ml acetic acid and supplemented to 100 ml distilled water).
- 3- The storage experiment:

Three cultivars of date palm (Bream, Khalas and Habbasi) were prepared, soaked in the three concentrations of chitosan (0%,1% and 2%) and packed with cork package . Fruits were polluted by the beetle from the colony which was prepared in advance and thus stored at room temperature starting from 5/1/2017 for four months.

- 1- The Percentage of infection with the beetle: calculated monthly for four months as follows:

$$\text{Percentage of infection with the beetle} = \frac{\text{The weight of the infected fruits per package}}{\text{Weigh of total fruits per package}} \times 100$$

- 2 - Weight loss (%): was measured via the changes in fresh weight of fruits during storage.

3. Total soluble solids (T.S.S.) were measured by hand refractometer and the results were corrected to 20 °C according to Shirokov (1968).

The experiment included 36 factorial treatments came from the interaction among three cultivars(Bream, Khalas and Habbasi) post harvest soaking treatments with chitosan at the concentrations of (0, 2, 4 g .L⁻¹),four storage periods. Complete Randomized Design(CRD) was used with three replicates . The results were analyzed by the analysis of variance and mean values were compared using the Revised Least Significant Difference Test at 0.05 probability level(AI-Rawi and Khalf Allah,1980).

3. Results and discussion

3.1. Percentage of infection of the beetle

Table (1) showed the effect of chitosan treatment on date palm fruit cultivars Bream, Khalas and Habbasi on controlling the infection of saw-toothed grain beetle. It was noticed that the lowest percentage of infection was in Habbasi fruits with significant difference from the rest two cultivars, which amounted to 7.00%, while the highest percentage of infection was in Bream fruits, reached to 11.44% with no significant difference to Khalas cultivar. This may be due to the fact that the insect preferred the fruits of the Bream and Khalas cultivars more than the Habbasi cultivar, perhaps because of the low firmness of the two cultivars Bream and Khalas compared to the Habbasi.

The table also showed that the lowest percentage of the infection of saw-toothed grain beetle was in the fruits treated with 2% chitosan, which recorded 1.56%, whereas the highest percentage of infection was in untreated fruits, which amounted to 17.11%. The reason may be due to the role of chitosan in making layer around Fruit, which led to non-penetration and prevented the laying of beetle eggs inside.

The results indicated that there was no infection during the first and second months of storage and may be due to the lack of activity of the beetle due to the low temperature of the atmosphere of the store while starting to increase in the third and fourth months due to increase the activity of the beetle .The interaction between the cultivars and chitosan treatment showed that the lowest percentage of infection was in the fruits of Habbasi cultivar with the treatment of 2% chitosan, which was 1%, while the highest percentage of the infection was in the fruit of the non-treated chitosan that reached to 20.67% with no significant difference to untreated fruits of Khalas cultivar. As for the interaction between the cultivars and the duration of the storage, the results showed that the infection occurred in

the third month of storage and for all the cultivars. The highest percentage of infection was in the fruit of the Bream cv. after four months of storage with a significant difference to the Khalas fruits after four months of storage. The results also indicated to the significant interaction between the treatment and storage period. The results indicated that the highest percentage of infection was in untreated chitosan fruits after four months of storage. Regarding to triple interaction, the highest percentage of infection was in the untreated fruits of Bream cultivar after four months of storage with significant difference to the rest cultivars.

Table 1. The effect of Cultivar, Chitosan and storage period and the interaction among them on the percentage of infection of *Oryzaephilus surinamensis* L. on date palm fruits stored at room storage temperature.

Cultivars	Chitosan%	Pre-storage	Storage periods (month)				Cultivars x Chitosan
			1	2	3	4	
Bream	0	0.00	0.00	0.00	25.00	78.33	20.67
	1	0.00	0.00	0.00	11.67	45.00	11.33
	2	0.00	0.00	0.00	1.67	10.00	2.33
Khalas	0	0.00	0.00	0.00	25.00	68.33	18.67
	1	0.00	0.00	0.00	11.67	50.00	12.33
	2	0.00	0.00	0.00	0.00	6.67	1.33
Habbsi	0	0.00	0.00	0.00	18.33	41.67	12.00
	1	0.00	0.00	0.00	10.00	30.00	8.00
	2	0.00	0.00	0.00	0.00	5.00	1.00
Cultivars x storage periods							Means of Cultivars
Bream	0	0.00	0.00	0.00	12.78	44.44	11.44
Khalas	1	0.00	0.00	0.00	12.22	41.67	10.78
Habbsi	2	0.00	0.00	0.00	9.44	25.56	7.00
							Means of chitosan
Chitosan x storage periods	0	0.00	0.00	0.00	22.78	62.78	17.11
	1	0.00	0.00	0.00	11.11	41.67	10.56
	2	0.00	0.00	0.00	0.56	7.22	1.56
Means of storage periods		0.00	0.00	0.00	11.48	37.22	
RLSD 0.05							
Cultivars	Chitosan	Storage period	Cultivars x Chitosan	Cultivars x storage period	Chitosan x storage period	Cultivars x Chitosan x storage periods	
1.41	1.41	1.83	2.45	3.17	3.17	5.49	

3.2. Percentage of weight loss

Table (2) showed that the lowest percentage of weight loss was in the Habbsi fruits which was (0.140%), whereas the fruit of Bream cultivar gave the highest percentage of weight loss (0.280%). This may be due to the reduction of the percentage of infection of Habbsi fruits (Table 1), and therefore the loss of food content resulting from the feeding of the beetle beetle.

The results of the same table indicated that the lowest percentage of weight loss was in fruits treated with 2% ketosan (0.197%) while, the highest percentage of weight loss (0.297%) was in untreated fruits. The loss of weight in 2% kitosan-treated fruits may be due to the fact that the spray treatment of ketosan resulted in the formation of an insulating layer on the fruit surfaces, thus reducing the evaporation of water from the surface of the fruit, in addition to the reduction of respiration rate of fruits (Ribeiro *et al.*, 2007). This result is consistent with Jassim *et al.* (2016) on fruits of date palm cv. Burhi. It is noted from the table that the percentage of weight loss was relatively increased by increasing the storage period until it reached to (0.538%).

In the study of the interaction between cultivar and ketosan treatment, it is clear that the lowest percentage of weight loss was in the fruits of Habbsi cultivar treated with 2% ketosan, which was (0.094%) while , The highest percentage of weight loss was in untreated fruit of Bream cultivar that reached to (0.408%). The lowest percentage of weight loss was in Habbsi fruits after a month of storage (0.036%) whereas , the highest percentage of weight loss was in Bream fruits after four months of storage.

interaction between the treatment of ketosan and the storage period was significant as it was the lowest percentage of weight loss was in 2% ketosan after a months of storage (0.038), while, the highest percentage of weight loss was in untreated fruits after four months of storage(0.771%) . the triple interaction was also significant .The lowest percentage of weight loss was in Habbsi fruits treated with 2% ketosan after one month of storage (0.023%) while,the highest percentage of weight loss was in untreated Bream fruits after four months of storage, which recorded 1.076%)

Table 2. The effect of Cultivar, Chitosan and storage period and the interaction among them on the percentage of weight loss on date palm fruits stored at room storage temperature.

Cultivars	Chitosan%	Pre-storage	Storage periods (month)				Cultivars x Chitosan
			1	2	3	4	
Bream	0	0.00	0.186	0.220	0.556	1.076	0.408
	1	0.00	0.136	0.196	0.363	0.633	0.266
	2	0.00	0.060	0.083	0.266	0.420	0.166
Khalas	0	0.00	0.073	0.140	0.316	0.743	0.254
	1	0.00	0.043	0.096	0.193	0.413	0.149
	2	0.00	0.033	0.066	0.130	0.410	0.128
Habbsi	0	0.00	0.056	0.383	0.213	0.493	0.229
	1	0.00	0.030	0.073	0.133	0.356	0.118
	2	0.00	0.023	0.050	0.093	0.303	0.094
Cultivars x storage periods							Means of Cultivars
Bream	0	0.00	0.127	0.166	0.395	0.710	0.280
Khalas	1	0.00	0.050	0.101	0.213	0.522	0.177
Habbsi	2	0.00	0.036	0.168	0.146	0.384	0.147
							Means of chitosan
Chitosan x storage periods	0	0.00	0.105	0.247	0.362	0.771	0.297
	1	0.00	0.070	0.122	0.230	0.467	0.178
	2	0.00	0.038	0.066	0.163	0.377	0.129
Means of storage periods		0.00	0.071	0.145	0.251	0.538	
RLSD 0.05							
Cultivars	Chitosan	Storage period	Cultivars x Chitosan	Cultivars x storage period	Chitosan x storage period	Cultivars x Chitosan x storage periods	
0.019	0.019	0.025	0.033	0.043	0.043	0.075	

3.3. Percentage of total soluble solids :

Table (3) showed that Habbsi cultivar recorded the highest percentage of total soluble solids which was 75.96 % with no significant difference to Khalas cultivar , while the lowest percentage of total soluble was in Bream fruits which amounted 75.53%.

The results showed that the untreated chitosan fruits showed the highest percentage of total soluble solids (75.89 %), with no significant difference of 1% chitosan ,whereas the lowest percentage of total soluble was in fruits treated with 2% chitosan (75.62%).

The percentage of soluble solids increased with the increment of storage period reached to 76.32% at the end of the fourth month of storage. This result is consistent with Taain (2005) on Barhi date palm fruits .

In the same table as for the interaction between cultivars and chitosan treatment , the highest percentage of total soluble solids was in the untreated Habbsi cultivar fruits, which was 76.10 % .,whereas the lowest percentage of total soluble was in Bream fruits with 2% chitosan which gave 75.38%. As for the interaction between cultivars and storage period , the highest percentage of total soluble solids was in fruits of the Habbsi cultivar after four months of storage, which was 76.55%,whereas the lowest percentage of total soluble solids was in the Bream fruits after a month of storage, which was 75.32 % . The interaction between chitosan and storage period was significant, the highest percentage of total soluble solids was in untreated chitosan fruits after 4 months of storage, which recorded 76.46% . The fruits treated with 2%chitosan recorded the lowest percentage of total soluble solids after one month of storage which amounted to 75.42%. The triple interaction was also significant as the untreated with chitosan fruits of Habbsi cultivar recorded the highest percentage of total soluble solids after four months of storage, which was 76.63%, while the fruits of the Bream cultivar were treated 2% chitosan after one month of storage which amounted to 75.13%.

Table 3.The effect of Cultivar, Chitosan and storage period and the interaction among them on the percentage of total soluble solids on date palm fruits stored at room storage temperature.

Cultivars	Chitosan%	Pre-storage	Storage periods (month)				Cultivars x Chitosan
			1	2	3	4	
Bream	0	75.00	75.50	75.70	75.93	76.26	75.68
	1	75.00	75.33	75.53	75.73	76.06	75.53
	2	75.00	75.13	75.36	75.60	75.80	75.38
Khalas	0	75.00	75.80	76.00	76.20	76.50	75.90
	1	75.00	75.63	75.83	76.03	76.36	75.77
	2	75.00	75.43	75.66	75.90	76.26	75.65
Habbsi	0	75.00	76.16	76.30	76.43	76.63	76.10
	1	75.00	75.86	76.10	76.23	76.56	75.95
	2	75.00	75.70	75.90	76.13	76.46	75.83
Cultivars x storage periods							Means of Cultivars
Bream	0	75.00	75.32	75.53	75.75	76.04	75.53
Khalas	1	75.00	75.62	75.83	76.04	76.37	75.77
Habbsi	2	75.00	75.91	76.10	76.26	76.55	75.96
Chitosan x storage periods							Means of chitosan
Chitosan x storage periods	0	75.00	75.82	76.00	76.18	76.46	75.89
	1	75.00	75.61	75.82	76.00	76.33	75.75
	2	75.00	75.42	75.64	75.87	76.17	75.62
Means of storage periods		75.00	75.61	75.82	76.02	76.32	
RLSD 0.05							
Cultivars	Chitosan	Storage	Cultivars x	Cultivars x	Chitosan x	Cultivars x	
0.40	0.30	period	Chitosan	storage	storage	Chitosan	
		0.52	0.70	period	period	x storage	
				0.90	0.90	periods	
							1.57

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