Journal of Horticultural Science & Ornamental Plants 7 (2): 71-79, 2015
ISSN 2079-2158

DOI: 10.5829/idosi.jhsop.2015.7.2.1158

Quality Characteristics of 'Zaghloul' Date Fruits During Cold Storage as Affected by Postharvest Irradiated Chitosan Treatments

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Abstract: The potential of chitosan (irradiated and unirradiated) in preserving bisir Zaghloul date fruits during cold storage was investigated. Solid chitosan powder was gamma irradiated with 25 and 50 kGy doses, chitosan solutions were prepared and fruits were treated prior to cold storage. Initial fruit quality characteristics were assessed before refrigeration at 0 ± 1°C and RH 85- 90 % for up to 42 days, during which physiochemical characteristics were determined periodically, every 14 days. Different treatments were applied by immersion in one of the following for two minutes with temperatures adjusted to 45°C: Control (water); 0.5%, unirradiated chitosan; 0.5 %, 25 kGy irradiated chitosan; 0.5%, 50 kGy irradiated chitosan; 1.0 %, unirradiated chitosan; 1.0 %, 25 kGy irradiated chitosan; 1.0 %, 50 kGy irradiated chitosan, solutions. Chitosan generally decreased weight loss and decay and increased rutab percentages and controlled increases in total soluble solids and total sugars and decreases in fruit firmness and titratable acidity. As for the effect of radiation applied to chitosan, it generally reduced rutab % compared to control and limited chitosan's efficiency in controlling respiration rate, weight loss, firmness loss and acidity decrements.

Key words: Date palm · Zaghloul · Chitosan · Irradiation · Postharvest

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most important trees grown in the Middle East region. The antioxidant properties its fruits possess is partly due to its high polyphenolic compounds which contributes to its nutritional importance along with other important essential dietary nutrients [1, 2]. Among different cultivars grown in Egypt, Zaghloul is of great commercial importance because it's highly demanded locally and for the export market. Increasing needs for alternative methods to extend storage period and maintain fruit quality evolved because of the proliferation of resistant pathogen strains due to the widespread usage of chemicals and because of concerns about public health and environmental issues [3, 4].

After harvesting at maturity, fruits ripen, then start to deteriorate specially under room temperature. Thus, it is important to slow down ripening and extend shelf life in order to market the fruits with minimal physical and physiological disorders. One of the potential approaches to extend storability is to apply edible coatings, followed by cold storage [5]. Among the natural biopolymers that play an important role in this regard is chitosan, which has been reported to likely modify the internal atmosphere without causing anaerobic respiration [6]. Quality maintenance during extended storage periods also assists in releasing the commodity according to market requirements without jeopardizing consumer's acceptability. Such a goal can be achieved through enhancing fruit resistance to deterioration, controlling fruit rots, reducing losses and maintaining fruit quality during cold storage. This study aims at evaluating the

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effects imposed on Zaghloul dates during refrigerated storage in response to postharvest chitosan (irradiated and unirradiated) treatments. Our goal is to determine the efficiency of such treatments in maintaining quality attributes in order to avoid commercial value losses and present it to the local and export markets, with high market value.

MATERIALS AND METHODS

Plant Material: Cultivar Zaghloul, date palm (*Phoenix dactylifera* L.) fruit strands bearing mature fruits were obtained from a local wholesale market in 6th of October City, Giza, Egypt in the two successive seasons of 2012 and 2013. The fruits where then transferred to the laboratory at the Agricultural Development System (ADS) project, Faculty of Agriculture, Cairo University, Egypt. Intact, undamaged, uniform in shape and size, colored, mature 4200 fruits, free from apparent pathogenic infections were selected each season for this study.

Treatments and Storage Conditions: Fruits were washed, air dried and divided into 7 groups (treatments), 600 fruit, each. Each group, was immersed for 2 minutes in one of the following:

- Control: water (45°C).
- Treatment 1: 0.5%, unirradiated chitosan solution at 45°C.
- Treatment 2: 0.5%, 25 kGy irradiated chitosan solution at 45°C.
- Treatment 3: 0.5%, 50 kGy irradiated chitosan solution at 45°C.
- Treatment 4: 1.0 %, unirradiated chitosan solution at 45°C.
- Treatment 5: 1.0 %, 25 kGy irradiated chitosan solution at 45°C.
- Treatment 6: 1.0 %, 50 kGy irradiated chitosan solution at 45°C.

High molecular weight solid chitosan power (>75% deacetylated) obtained from Sigma- Aldrich was subjected to radiation doses of 0, 25 and 50 kGy at the National Center for Radiation Research and Technology (NCRRT) using a self- contained dry- storage gamma irradiator (Indian gamma cell GE 4000A) that uses ^{60}Co as a radiation source. The radiation dose rate was 2.08 kGy/ h at the time of the experiment. Afterwards, chitosan solutions were prepared according to the procedure as described by Jiang *et al.* [7].

After drying, each group was divided into 6 groups (100 fruits each) and placed into perforated plastic boxes and refrigerated at 0 ± 1°C and relative humidity (RH) 85- 90 % for up to 42 days, during which physiochemical characteristics were determined after 14, 28 and 42 days, respectively. The initial fruit quality characteristics were also assessed before storage (zero time). For each treatment, (3 replicates * 100 fruit) were used to determine decay, weight loss and rutab percentage and (3 replicates * 100 fruit) were used to determine respiration rate, firmness, total soluble solids (TSS), total sugars (TS) and titratable acidity (TA).

Physiochemical Characteristics:

- Respiration rate: was determined according to AOAC [8] and expressed as [mg $CO_2 \cdot kg^{-1}h^{-1}$].
- Decay percentage: all unmarketable fruits where considered as decayed and decay percentage was calculated as follows:

$$\text{Decay \%} = \frac{a \text{ X } 100}{b}$$

where:
a = number of decayed fruits at time of sampling;
b = initial fruit number (100).

- Weight Loss [%]: Was measured by the difference between the initial and final weight of each replication. It was expressed as a percent [%] as follows:

$$\text{Weight loss \%} = \frac{(\text{Initial weight of fruits} - \text{weight of fruits at inspection date})}{\text{Initial weight of fruits}} \times 100$$

- Rutab [%] all fruits that showed visual softening of about 10% of its surface were considered rutab and its % was calculated in the same manner as decay.
- Fruit firmness was measured using a pressure tester mod. FT 327. Readings were taken in two positions in each tested fruit, averaged and recorded in [$lb/inch^2$].
- TSS [%] was determined at 22°C, with a hand refractometer using 2 to 3 drops of well mixed fruit juice obtained by squeezing the fruits.
- TS was determined by using the method described by Dubois *et al.* [9] and the concentration was calculated as glucose [g·100 g] fresh weight.
- TA [%] was calculated as malic acid (dominant organic acid in fruits), where 1 ml. of fruit juice was titrated with 0.1 $mol \cdot l^{-1}$ NaOH using phenolphthalein as an indicator and the percentage was calculated as follows:

$$TA\ [\%] = \frac{\text{ml of NaOH} \times \text{Normality} \times 0.067}{\text{ml juice used}} \times 100$$

Statistical Analysis: The experiment was laid out using a completely randomized block design (CRBD). Three replicates per treatment were evaluated for physiochemical fruit quality attributes. Experimental data obtained was treated with one way Analysis of Variance (ANOVA) at confidence level of 95 %, which is a procedure used for testing the differences among means of two or more treatments and the differences between means were detected using least significant difference (L.S.D.) at $P< 0.05$ according to Gomez and Gomez [10]. All data was analyzed using statistical software (MSTATC 2.10, Russell D. Freed).

RESULTS AND DISCUSSION

Respiration Rate: Data presented in Figure (1) show that respiration rates decreased with progress of storage time. It also reveal that all investigated chitosan postharvest treatments significantly reduced respiration after 14 and 42 days of cold storage. On the other hand, after 28 days of cold storage, insignificant reductions compared to control were recorded for all 0.5 % chitosan treatments in season 2012 and 0.5 % chitosan irradiated with a dose of 50 kGy in season 2013. As shown generally, the higher chitosan concentration applied and the lower irradiation dose used, the lower respiration rate recorded, though statistical significance was not always detected in-between.

Reduced respiration in response to chitosan postharvest treatments have been reported earlier for several fruits such as raspberry and guava [11, 12]. This is most probably attributed to selective permeability of chitosan to respiratory gases which makes it act as a barrier to oxygen [13]. Such control of gas exchange between fruits and its surrounding environment reduces respiration and reduces the action of 1-aminocyclopropane-1-carboxylate oxidase and synthase enzymes that are key enzymes of ethylene biosynthesis which are influenced by the lack of oxygen [12]. On the other hand, the inverse relation between chitosan irradiation dose and respiration inhibition found in this trial is in harmony with results reported for grapes treated with high and low molecular weight chitosan oligomers [14]. Here, it is worth mentioning that reduced respiration is directly correlated to fruit quality maintenance during cold storage.

Decay: Results presented in Table (1) show that all investigated treatments significantly reduced decay % compared to control at the end of storage period. Differences recorded for fruits treated with the same chitosan concentration showed insignificant differences in- between in response to irradiating chitosan with different doses. Generally, the higher chitosan concentration and the lower irradiation dose, the more effective the treatment was in controlling decay in both seasons. This is in accordance with the results of Hafez *et al*. [15] who reported increased decay % with cold storage progress and in line with the findings of Raymond *et al*. [16] who found that greater reduction of fungal incidence on green pepper was observed with increased chitosan concentration. Our results are also in harmony with results of Choi [17] and Ampaichaichok *et al.* [18] who found an inverse relation between chitosan molecular weight and disease incidence in grapes and mango, respectively. Our results are also confirmed by results of Lauzardo *et al.* [19] who stated that higher MW of chitosan could reduce germination rate of the pathogen *Rhizopus stolonifer* more effectively than lower MW chitosan. Contrarily, Chien *et al.* [20] reported that lower MW (15kDa) chitosan was more effective than higher MW (357kDa) chitosan in controlling citrus fruit fungal decay. In this regard, Meng *et al.* [21] results suggest that chitosan and oligo- chitosan trigger different mechanisms for pathogenicity inhibition and disease control.

The semipermeable thin layer ascribed to chitosan treatments, besides being reported to reduce pathogenic disorders, reduces physiological disorders in many fruits other than date fruits [15] such as grapes, peach, Japanese pear and kiwi fruit [22, 23]. Such reduced decay incidence in chitosan treated fruits may be due to its extensively reported antifungal activity. Such activity might be attributed to its chemical structure which inhibits the growth of fungi and bacteria through electrostatic forces between the protonated amino groups in chitosan and the negative charges of phosphoryl groups on the cell surface of microorganisms [13]. Decay reduction might also be attributed to reduced fungi polygalactronase production which results in limited ability to colonize on fruit tissue [24]. Moreover, chitosan has a potential of inducing defense- related enzymes and phenolic contents in plants which play an important role in controlling pathogens [25-27].

Weight Loss: As shown in Figure (2), weight loss increased as cold storage proceeded. Significant weight loss reductions compared to control fruits were recorded

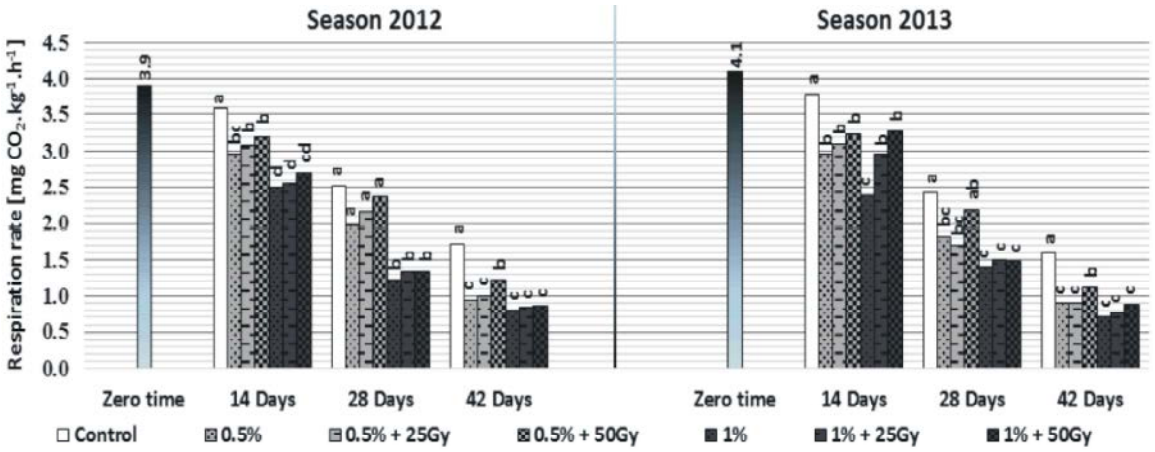

Fig. 1: Effect of irradiated and unirradiated chitosan on respiration rate [mg CO_2. kg^{-1} h^{-1}] during cold storage (Columns in the same group bearing a common letter are insignificantly different at P<0.05).

Table 1: Effect of irradiated and unirradiated chitosan on Zaghloul dates decay [%] during cold storage.

	Storage period (Season 2012)				Storage period (Season 2013)			
Treatments	0 days	14 days	28 days	42 days	0 days	14 days	28 days	42 days
Control	0.0	0.0	4.0 a	42.3 a	0.0	0.0	0.0	44.3 a
Chitosan 0.5 % + 0 kGy		0.0	0.0 b	23.3 bc		0.0	0.0	20.7 bc
Chitosan 0.5 % + 25 kGy		0.0	2.0 ab	26.7 b		0.0	0.0	23.0 b
Chitosan 0.5 % + 50 kGy		0.0	3.3 a	28.7 b		0.0	0.0	24.0 b
Chitosan 1.0 % + 0 kGy		0.0	0.0 b	14.7 d		0.0	0.0	12.7 c
Chitosan 1.0 % + 25 kGy		0.0	0.0 b	15.0 d		0.0	0.0	13.3 c
Chitosan 1.0 % + 50 kGy		0.0	0.0 b	15.3 cd		0.0	0.0	15.7 bc

Means in the same column bearing common letters/ no letters are insignificantly different at P<0.05.

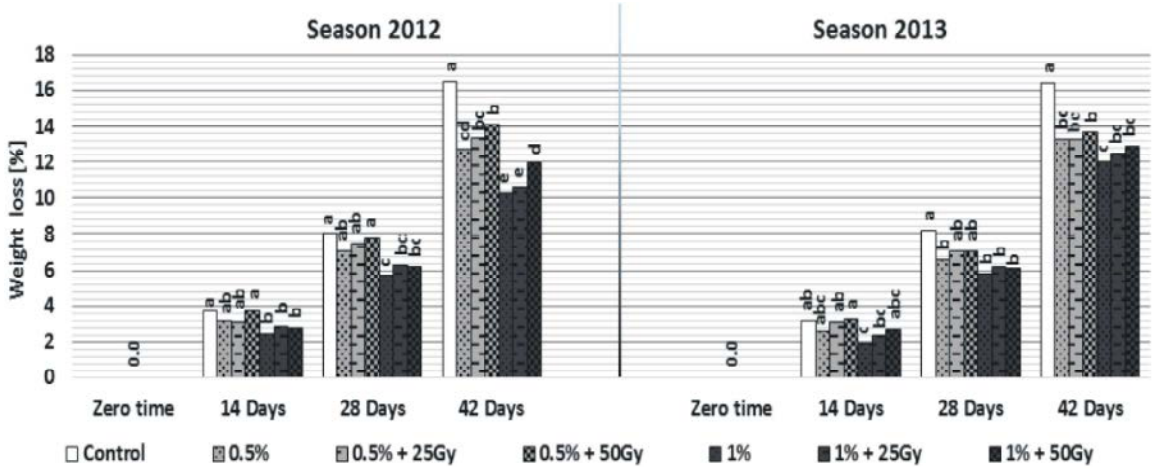

Fig. 2: Effect of irradiated and unirradiated chitosan on weight loss [%] during cold storage (Columns in the same group bearing a common letter are insignificantly different at P<0.05).

for all treatments at the end of storage period. Such significant effect was detected throughout the whole trial in all fruits treated with 1% chitosan in both investigated seasons. On the other hand, irradiating chitosan seemed to limit chitosan's efficiency in controlling weight loss, though statistical significance was not always detected.

Such results are in accordance with results reported by Hafez *et al*. [15], among many others, who recorded that weight loss % increased as cold storage proceeded. Chitosan coating has been reported to be effective in controlling water loss from other commodities such as strawberries, grapes and guava [28, 17, 12].

Results are also in agreement with previously reported effects of low and high molecular weight chitosan on weight loss of grapes and mango during cold storage [17, 19]. Such effect can be understood when putting into consideration that chitosan forms a selective barrier around fruits which controls moister loss through transpiration process (the main reason for weight loss from harvested horticultural crops) and respiration which the main metabolic process during which water loss occurs [29, 30]. Besides acting as a barrier that controls water transfer, chitosan seals small wounds and protects fruit skin from mechanical injuries and thus delays dehydration [28].

Rutab: Results presented in Table (2) 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, with only one exception in season 2013 when fruits were treated with 0.5 % chitosan irradiated with 50 kGy. The increase in rutab % compared to control fruits after 28 days under cold storage, was insignificant. It was also found that the higher chitosan concentration used, the more frequent fruits were transformed from the bisir to the rutab stage. Contrarily, most treatments incorporating irradiation reduced the transformation from bisir to rutab stage and this effect was concentration- dependent.

Fruit Firmness: As shown in Figure (3), fruit firmness dropped with storage advancement, which is compatible with results reported by Hafez *et al*. [15]. Such decrease in fruit firmness with the progress of storage period is mainly due to decompositions by enzymatic degradation of insoluble protopectins to simpler soluble pectins and the increase of pectin esterase activity which causes solubilization of cell and cell wall contents [31]. In this regard, Ruoyi *et al.* [32] reported that chitosan associated with other treatments reduced the increase of soluble pectinefic substances in peach fruits. Results also show that among all the treatments studied, only fruits treated with 1 % unirradiated chitosan showed significantly higher firmness compared to control fruits throughout the whole trial in both seasons. Moreover, although irradiating chitosan reduced its efficiency in maintaining fruit firmness, but fruits treated with 1% chitosan irradiated with 25 kGy showed relatively high firmness compared to control fruits at the end of the storage period in both seasons. Similarly, the 0.5 % unirradiated chitosan treatment resulted in maintaining fruit firmness after 42 days of cold storage.

Zaghloul fruits firmness maintenance in response to chitosan treatments is in harmony with results recorded for mango, grape and apricot [18, 22, 27]. This might be due to previously reported low polygalacturonase activity and pectin methylesterase content, which are key enzymes that reduce plant cell wall strength during fruit ripening [33].

TSS %: As shown in Table (3), TSS generally increased as time elapsed in the cold storage. Results also reveal that all treatments recorded lower TSS % compared to control fruits, though statistical significance was not detected. On the other hand, trivial increases in TSS % were recorded for fruits treated with the same chitosan concentration in response to higher irradiation doses. TSS increases as storage proceeded could be due to increased activity of enzymes responsible for starch hydrolysis or might be due to water loss or because of degradation of complex insoluble compounds to simple soluble compounds, particularly sugars that accumulate in fruits [34, 35]. As for the reduced TSS records for chitosan treated fruits, it might be an indirect outcome for the barrier effect imposed by chitosan, which in turn limited moisture loss and consequently reduced TSS relative to moisture content.

TSS increases associated with irradiating chitosan is in accordance with what Ampaichaichok *et al.* [18] reported regarding slowed down metabolism and respiration and delayed ripening and senescence of mango in response to high MW chitosan compared to medium MW chitosan. They attributed that to more rapid senescence in the latter treatment that caused more sugar consumption in metabolism [36]. Finally, it is worth mentioning that the insignificant effect of chitosan on TSS has been reported for other fruits such as apricot and mango [27, 37].

TS %: Results presented in Table (4) show that all chitosan treatments insignificantly affected TS in date fruits during cold storage in season 2013. On the other hand, fruits treated with 1% chitosan (regardless of irradiation doses), recorded significantly lower TS compared to control fruits in season 2012. Moreover, fruits treated with unirradiated and 25 kGy irradiated 0.5 % chitosan showed significantly low total sugar content at the end of storage period in that same season. Such results might be attributed to chitosan's barrier effect that resulted in limited water loss and consequently lower sugar content. It may also be because of the inhibited metabolism and respiration and delayed senescence discussed earlier in this study.

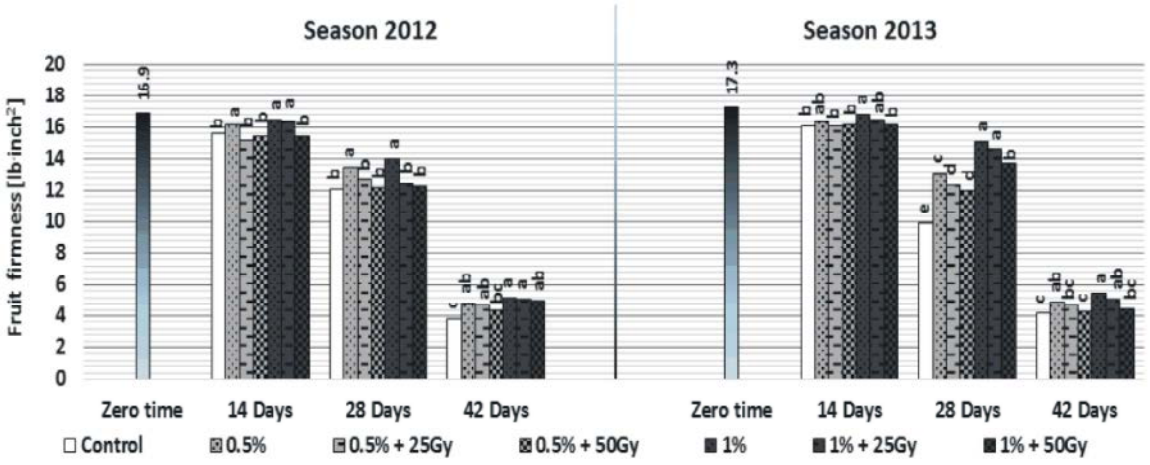

Fig. 3: Effect of irradiated and unirradiated chitosan on Zaghloul dates firmness [lb·inch^{-}]^{2}during cold storage (Columns in the same group bearing a common letter are insignificantly different at P<0.05).

Table 2: Effect of irradiated and unirradiated chitosan on Zaghloul dates rutab [%] during cold storage.

	Storage period (Season 2012)				Storage period (Season 2013)			
Treatments	0 days	14 days	28 days	42 days	0 days	14 days	28 days	42 days
Control	0.0	4.3 f	12.7 d	44.3 d	0.0	8.7 c	24.3 f	48.7 e
Chitosan 0.5 % + 0 kGy		27.7 c	41.0 b	88.7 a		29.0 ab	38.7 cd	84.0 bc
Chitosan 0.5 % + 25 kGy		20.0 d	43.7 b	76.0 b		27.3 ab	36.0 de	80.0 c
Chitosan 0.5 % + 50 kGy		16.0 e	20.3 c	56.3 c		25.3 b	30.0 ef	68.7 d
Chitosan 1.0 % + 0 kGy		36.3 a	51.3 a	91.7 a		34.3 a	53.3 a	94.3 a
Chitosan 1.0 % + 25 kGy		32.3 b	46.3 ab	92.0 a		29.3 ab	46.0 b	88.3 ab
Chitosan 1.0 % + 50 kGy		30.0 bc	45.7 ab	88.3 a		25.7 b	44.0 bc	82.3 bc

Means in the same column bearing a common letter are insignificantly different at P<0.05.

TA %: As shown in Figure (4), acidity dropped as storage proceeded. Although, all chitosan treatments limited TA decreases, but the only fruits that recorded significantly high values compared to controls throughout the whole storage period in both seasons, where those treated with unirradiated 1% chitosan. In the latter season, at the end of storage period, also fruits treated with 1% chitosan irradiated with a 25 kGy dose showed significantly high TA compared to control fruits. Here it is worth mentioning that irradiating chitosan limited its ability to control acidity decreases. This is in harmony with previously reported reduced decrease of TA in grape in response to high molecular weight chitosan treatment compared to low molecular weight chitosan [17].

TA% reductions during cold storage was previously reported for other fruits such as papaya and apricot [36, 27]. The authors attributed such decreases to 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 [22, 18]. In this regard, El-Badawy and El-Salhy [27] reported that a higher chitosan concentration was correlated with more TA control. This could be due to reduction in metabolic conversion of organic acids to carbon dioxide and water as a result of inhibited respiration rate and therefore maintenance of higher acid contents. Contrarily, it was reported that chitosan treatments had no significant effect on TA in guava fruits during storage [12]. On the other hand, Ampaichaichok *et al.* [18] reported that high MW chitosan treated fruits maintained very high TA compared to lower MW chitosan, which suggested that high MW chitosan interfered with acid metabolism in fruits.

Table 3: Effect of irradiated and unirradiated chitosan on TSS [%] in Zaghloul dates during cold storage.

	Storage period (Season 2012)				Storage period (Season 2013)			
Treatments	0 days	14 days	28 days	42 days	0 days	14 days	28 days	42 days
Control	22.3	25.8	27.7	28.4	23.0	25.2	27.3	29.9
Chitosan 0.5 % + 0 kGy		23.6	24.7	26.6		23.9	26.1	26.7
Chitosan 0.5 % + 25 kGy		24.5	27.6	27.7		23.9	26.6	26.9
Chitosan 0.5 % + 50 kGy		25.2	27.9	28.1		24.9	26.7	27.6
Chitosan 1.0 % + 0 kGy		23.5	24.0	25.2		23.5	25.2	25.7
Chitosan 1.0 % + 25 kGy		23.9	25.0	26.2		23.4	25.2	26.3
Chitosan 1.0 % + 50 kGy		25.0	26.6	27.9		24.9	27.6	28.0

Means in the same column bearing no letters are insignificantly different at P<0.05.

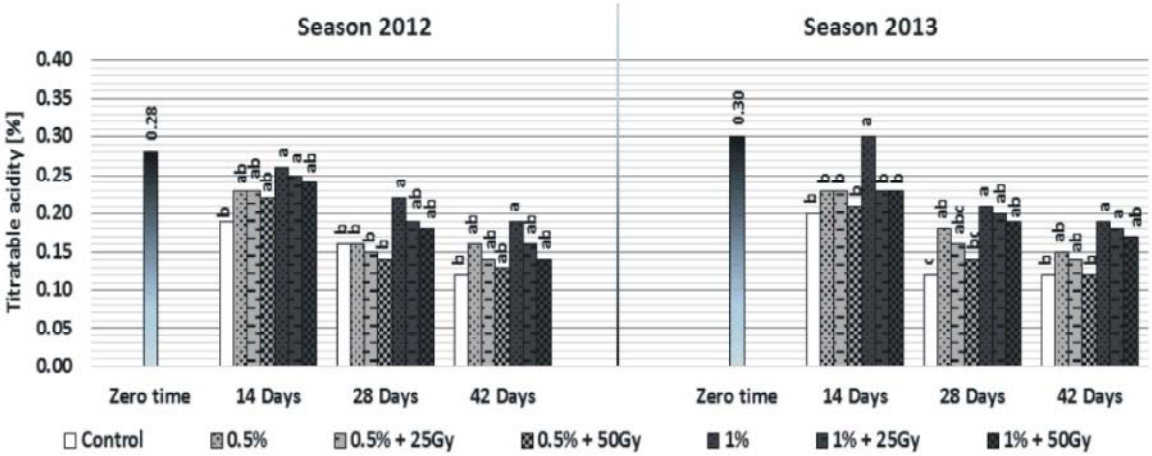

Fig. 4: Effect of irradiated and unirradiated chitosan on TA [%] in Zaghloul dates during cold storage (Columns in the same group bearing a common letter are insignificantly different at P<0.05).

Table 4: Effect of irradiated and unirradiated chitosan on TS [%] in Zaghloul dates during cold storage.

	Storage period (Season 2012)				Storage period (Season 2013)			
Treatments	0 days	14 days	28 days	42 days	0 days	14 days	28 days	42 days
Control	20.4	28.8 a	31.3 a	32.2 a	21.0	28.1	30.2	31.1
Chitosan 0.5 % + 0 kGy		28.2 a	29.5 ab	30.6 b		27.7	28.9	29.4
Chitosan 0.5 % + 25 kGy		28.4 a	30.4 ab	30.9 b		28.2	29.3	29.6
Chitosan 0.5 % + 50 kGy		28.5 a	30.8 a	32.2 a		28.6	29.8	30.0
Chitosan 1.0 % + 0 kGy		26.8 b	28.4 b	29.5 c		28.3	28.9	29.9
Chitosan 1.0 % + 25 kGy		27.1 b	28.6 b	29.8 c		28.6	29.0	29.9
Chitosan 1.0 % + 50 kGy		27.0 b	28.6 b	29.7 c		29.1	29.2	30.5

Means in the same column bearing common letters/ no letters are insignificantly different at P<0.05

CONCLUSION

Chitosan application to bisir Zaghloul dates prior to cold storage failed to retain fruits in this developmental stage and resulted in high transition to rutab stage. On the other hand, it proved effective in reducing respiration rate and minimizing decay incidence and maintaining other quality attributes. That's why further studies are needed to develop protocols incorporating chitosan treatments with the aim of extending storage time of date fruits for late season release during scarcity of commodity and the prevalence of maximum market value.

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