

Effect of physical methods on date fruits insects and microbes

ABSTRACT

Aims: Siwi dates fruits are infected with many pests, which leads to a high deficiency in the quality and safety of dates. The study aimed to investigate the possibility of three physical methods (freezing, gamma radiation and ozone) as suitable methods for pest control.

Study Design: Original research.

Place and Duration Study: The study was conducted in Giza, Egypt in season 2021.

Methodology: The first method, the date fruits were frozen at -18°C at different times (15, 30, 60, 120, 180, 240, 300 and 360 min). The second method the date fruits were exposed to gamma rays with different doses as 25, 50, 75, 100, 200, 300, 400, 500, 600 and 700 gray. The third method, the date fruits were exposed to different ozone concentrations 200, 400, 600 and 800 ppm at different exposure periods (1, 2, 3 and 4 hrs.).

Results: The data showed that freezing at -18°C for 5 hrs, radiation at dose 700 gray and ozone at 800ppm for 4 hrs controlled insect stages and the microbial load of the sample date fruits, respectively.

Conclusion: The obtained data revealed that freezing or ozone treatments were the suitable methods followed by radiation.

Keywords: Date fruits, physical methods, insects, microbes, freezing and ozone

1. INTRODUCTION

Dates are one of the main ancient fruits in the Arab and Islamic world. Then dates were cultivated in many countries. Egypt produces 1.710.600 tons of date fruits. Only 16.5% of total date fruit production was directed for food processing¹. The achievement of an optimal shelf-life of date palm (*Phoenix*

dactylifera L.) starts by using high quality raw material, free of insect attack, and continues with appropriated harvesting, handling, processing, packaging, storage, transport and distribution and retail sale operations². Insect especially (*Ephestiacautella*) causes substantial damage to dates held in storage with an average infestation rate of 16.8%. However, the fruit losses may reach to 100%³. Date palms are attacked by many pests and diseases and their nature and severity vary with cultivar, location, weather and cultural practices. *Ephestia cautella* is a major pest of stored food products including dates, a highly valued annual fruit which is stored after harvesting for processing and marketing. Also, Coleoptera and Lepidoptera are main two orders that contain 23 species of insect pests inflicting damage on date fruits during harvest and storage^{3,4}. The microbial spoilage of date can be caused by yeasts, molds and bacteria, mainly yeast species of *Zygosaccharomyces* that are more tolerant of high sugar content. The deterioration of dates by fermentation and molds increase with increase of water content, therefore, the temperature of storage and water content are the major factors which affect the shelf life of dates⁵. The traditional method of eliminating insects and their stages in dates is fumigation with phosphine before storage. Yahia and Kader⁶ showed that freezing at $-18\text{ }^{\circ}\text{C}$ or lower for at least 48 hrs. (from the time when the fruit temperature reaches $-18\text{ }^{\circ}\text{C}$ or lower) is enough to kill all life stages of stored products insects. Lallouche *et al.*⁷ indicated that date fruits were stored at $-18\text{ }^{\circ}\text{C}$ the *Ectomyelois ceratoniae* Zeller larvae dies within 2 hrs., the eggs and larvae – within 24 hrs., respectively. They were recommended for storage dates at $-18\text{ }^{\circ}\text{C}$ to prevent pests and improve the quality. It is apparently advantageous for environment and climate if compared with other anti-pest treatment variants. Frag *et al.*,⁸ reported that the irradiation did not cause significant changes in semi dry dates quality, except the color; only more darkening in color during long storage, especially at room temperature where best color resulted with frozen fruits. γ -rays controlled the insects completely and decreased the microbiological contamination in irradiated samples. Ramadan *et al.*,⁹ showed that total bacterial counts were reduced immediately after irradiation to a greater extent, compared to the reduction in molds and yeasts of Sakkoty date fruits. In 2001, gaseous and aqueous ozone was approved by the U.S. Food and Drug Administration for application as an antimicrobial agent to foods¹⁰. Niakousari *et al.*,¹¹ exposed contaminated dates with all life stages (adults, larvae and eggs) of Indian meal moth (*Plodia interpunctella*) and sawtooth grain beetle (*Oryzaephilus surinamensis*) to gaseous ozone (600, 1200, 2000 and 4000 ppm) for 1–2 hours. Exposing samples to ozone concentrations of >2000 ppm for 2 hours resulted in complete mortality of larvae and adults. Zinhom and El-Shafei¹² reported that mortality of Indian meal moth, *P. interpunctella* (eggs, larvae, and pupae) infesting stored date increased by increasing the exposure time in each ozone concentration. They showed that the egg was the most

tolerant stage to the ozone gas while the 2nd larval instar was the most susceptible one. Khalil¹³ found that the Zaghloul date fruit at khalal stage were exposed to 150 ppm ozone (O₃) by using ozone generator (biofresh OZ80, UK) up to 30 days. The results indicated that ozone application reduced the yeasts and molds and mesophilic aerobic bacteria compared to control. Dates are preserved during storage by various methods, including the use of fumigation, thermal, or non-heat methods. Each method of preservation has advantages and disadvantages on the quality and safety of dates, so this research aims to compare some physical preservation methods and their effect on mortality percentages of date fruits insects and decreasing the microbial content.

2. METHODOLOGY

Date fruits (Siwi date semi-dry variety) were obtained from Al Bahreia Oasis, Giza, Egypt in season 2021.

Collection and rearing of *E. cautella* and *O. surinamensis* insects were prepared in Central Laboratory for Research and Development of Date Palm, Agricultural Research Center, Egypt to use of experiment according to Assous *et al.*,⁴.

Escherichia coli was obtained from the Egyptian Microbial Culture Collection, (EMCC), Cairo Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. All microbiological media used were obtained from Oxoid Division of Oxoid Ltd., London.

Irradiation process was carried out in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt using Co60 facility "Indian Gamma cell" type Ge-4000A.

Ozone gas was produced from the air using an ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada (OZO Max Ltd, Shefford, Quebec, Canada) from purified extra dry oxygen feed gas at the laboratory of Food Toxicology and Contaminants, National Research Center. The amount of ozone output was controlled by a monitor- controller having a plug-in sensor onboard which is changed for different ranges of ozone concentration and a belt pan in the monitor-controller allows controlling the concentration in a selected range.

Experimental procedure: The three preservatives methods were used to study its efficiency on insects' mortality percentage and microbial load of Siwi semi dry date fruits. The treatments were performed in three replicates for one kilogram of dates per experiment as follows:-

- 1- The date fruits were treated at -18 °C for different time (15, 30, 60,120,180, 240,300 and 360 min).
- 2- The date fruits were exposed to gamma rays with different doses as 25, 50, 75, 100,200,300,400,500,600 and 700 gray at doses rates (0.815k Gy / hrs) at room temperature 25 °C.
- 3- The date fruits were exposed to ozone concentrations 200,400,600 and 800 ppm at different exposure periods (1, 2, 3 and 4 hrs).

Mortality insects

A stereomicroscope with binocular head (Leica EZ4 HD, Leica Microsystems ,Stereo & Macroscopic Systems Switzerland Ltd) was used to count non-hatched and hatched larvae, eggs and adults of insects after 7 days for larvae of *E. cautella* and adults of *O. surinamensis* and calculated the insects corrected mortality percentages according to the formula of Shaghaghian *et al.*¹⁴, as follows :-

$$\text{Corrected mortality \%} = \frac{\% \text{Mortality in treatment} - \% \text{Mortality in control}}{100 - \% \text{Mortality in control}} \times 100$$

Microbial counts determination

***E. coli* count:** - *E. coli* was refreshed by suspending in nutrient broth for 24 hrs. at 37°C. Ten grams of treated date palm fruit samples were weighed and crushed in a sterile mortar pestle with 1 ml of *E. coli* (25x10⁷ cfu). After grinding well, the samples were exposed to carbon dioxide aluminum phosphide gas. Samples were added to 90 ml of saline solution (0.85%) and serial dilutions of samples were prepared in test tubes containing 9 ml saline solution up to the sixth dilution. Then 1 ml of each dilution is poured into petri plates followed by pouring of McConkey agar medium .Then plates were incubated at 37°C for 24-48 hr according to American Public Health Association¹⁵.

Total bacterial and yeast & molds count: Total bacterial count (TBC), yeast and molds count (Y and M) were determined using plate count method according to American Public Health Association¹⁵.

3.RESULTS AND DISCUSSION

Effect of freezing temperatures at -18°Cat different time on mortality percentage (eggs and larvae of *E. cautella* and adults of *O. surinamensis* of Siwi date fruits

The results in the table (1) indicated that increasing time of treatment at -18°C was increased mortality percentages till 300 min (5hr) regardless insect type or its stages. Result in the same Table 1 showed that the time to control of the larvae of *E. cautella* is faster than the eggs of the same insect or the adult

stage of *O. surinamensis*. These results are agreement with¹⁶ who evaluated three degrees of freezing temperatures (-5,-10 and -15°C) as a control method against various stages of the *E. cautella*, infesting date for exposure periods of: 15, 30, 60, 90,180 and 240 minute. The results showed that the mortality percentages of *E. cautella* stages increased by decreasing of freezing temperatures and or increasing of exposer period. The mortality of insects was probably related mainly to protein denaturation and membrane lipid phase transitions^{17, 18}. Also, Ben-Amor *et al.*,¹⁹ showed that the effect of different freezing treatments at -18°C (50, 77 and 125 hrs.) on the different stages of *E. ceratoniae* (young instars, old instars, and pupae) in Deglet Noor palm date and found that all freezing treatments used in this experiment resulted in 100% mortality of all the development stages of *E. ceratoniae*.

Table 1: Effect of freezing temperatures (-18 °C) at different time on mortality percentage (eggs and larvae of *E. cautella* and adults of *O. surinamensis* of Siwi date fruits.

Insects	<i>E. cautella</i>		<i>O. surinamensis</i>
% Mortality			
Time treatment (min)	Egg	larva	Adults
15	30.00	40.00	23.33
30	40.00	50.00	30.00
60	53.33	56.67	43.33
120	70.00	83.33	56.67
180	83.33	90.00	63.33
240	90.00	100.00	70.00
300	100.00	---	100.00

Effect of freezing temperatures (-18°C) at different time on microbial load of Siwi date fruits.

The Initial controls of total count (TC) were 150×10^1 yeasts and molds were 70×10^1 and *E. coli* was 25×10^7 , respectively. It is clear from the Table (2) the effect of freezing at -18°C on microbial growth inhibited with increased time of treatment under freezing, regardless of the type of microbes. It was also observed that the total count of bacteria was lower than that of yeasts and mold. Also, from Table (2) showed that *E. coli* was reduced more than 50% after 5 hr of treatment. On the other hand the counts of *E. coli* were high of allowed limit of date fruit standard. The initial of the microbial load of *E. coli* is due to its development in the laboratory. Therefore, the good agricultural and hygiene practices must be conducted, especially postharvest, handling and storage. The temperatures below -10°C inhibit bacterial growth, whereas yeasts and molds cannot multiply below -12°C and -18°C, respectively²⁰. The counts of total and yeast & mold were within the G.S.O.²¹.

Table2: Effect of freezing temperatures (-18 °C) at different time on microbial load of Siwi date fruits.

Microbes	TC(cfu/g)	<i>E. coli</i> (cfu/g)	Yeasts and molds (cfu/g)
Time of treatment (min)			

Initial counts	150 x10¹	25x10⁷	70x10¹
15	140 x10 ¹	227 x10 ⁶	68 x10 ¹
30	123 x10 ¹	172 x10 ⁶	70 x10 ¹
60	112 x10 ¹	129 x10 ⁶	68 x10 ¹
120	84 x10 ¹	97 x10 ⁶	60 x10 ¹
180	115	124 x10 ⁵	57 x10 ¹
240	74	250 x10 ⁴	55 x10 ¹
300	55	183x10 ⁴	50x10 ¹
360	40	133 x10 ⁴	44 x10 ¹

Effect of radiation treatments on mortality percentage (eggs and larvae of *E. cautella* and adults of *O. surinamensis* of Siwi date fruits.

The exposed date fruit to gamma rays with different doses as 25, 50, 75, 100, 200, 300, 400, 500, 600 and 700 gray and its efficiency on mortality percentage (eggs and larvae of *E. cautella* and adults of *O. surinamensis*). It is clear from Table(3)that the effect of radiation on mortality of insects was increased with increasing dose up to 700 gray. Also, the most resistant stage to radiation was the *E. cautella* eggs, followed the *O. surinamensis* adults then the *E. cautella* larval stage. Gamma radiation treatment with doses of 0 (control), 100, 200, 300 and 400 Gy in controlling two damaging and harmful mites attack date fruits during storage, *Tyrophagus putrescentiae* (Schrank) (Astigmata: Acaridae) and *Rhizoglyphus robini* Claparede (Astigmata: Acaridae) were studied and the results concluded that the mites' mortality percentage increases by increasing irradiation doses and the dose for controlling 100% of the two tested mites' species was 400 Gy²².

Table 3: Effect of radiation treatments on mortality percentage (egg and larva of *E. cautella* and adult of *O. surinamensis* of Siwi date fruits.

Insects % Mortality	<i>E. cutella</i>		<i>O. surinamensis</i>
	Egg	Larva	Adults
Con.(gray)			
25	10.00	16.67	6.67
50	16.67	26.67	26.67
75	26.67	46.67	46.67
100	43.33	56.67	63.33
150	53.33	76.67	76.67
200	56.67	93.33	86.67
300	63.33	100.00	93.33
400	73.33		100.00
500	76.67		
600	86.67		
700	100.00		

Effect of radiation treatments on microbial load of Siwi date fruits.

The results indicated that increasing radiation dose were combined reducing the microbial load till 300 gray of total count, *E. coli* and yeast & molds (Table 4). Also in the same table illustrated that the bacterial counts were higher sensitive than yeast and mold. Total bacterial counts of Siwi date Sakkoty fruits were reduced immediately after irradiation and or drying to a greater extent, compared to the reduction in molds and yeasts^{23, 23,24}.

Table 4: Effect of radiation treatments on microbial load of Siwi date fruits.

Microbes	TC(cfu/g)	E.coli(cfu/g)	Yeasts and molds(cfu/g)
Con.(gray)			
Initial counts	150 x10¹	25x10⁷	70x10¹
25	65 x10 ¹	33x 10 ⁵	70x10 ¹
50	56x 10 ¹	47x 10 ⁴	60x10 ¹
75	11x 10 ¹	122x 10 ²	53x10 ¹
100	66	84x 10 ¹	44x10 ¹
150	37	N*	37x10 ¹
200	N*		20x10 ¹
300			17x10 ¹
400			N*

*Not detected

Effect of ozone treatments on mortality percentage of (eggs and larvae of *E. cutella* and adults of *O. surinamensis*) of Siwi date fruits.

The effect of ozone concentration (200,400, 600 and 800ppm) at different times (1, 2, 3 and 4 hrs.) on the mortality percentages of some insects were studied. From result in Table (5) illustrated that ozone concentration 200ppm was caused mortality up to 30, 23.33 and 20% for eggs and larvae of *E. cutella* and adults of *O. surinamensis* after 4 hrs., respectively. While, ozone concentration 400ppm was recorded mortality up to 70, 26.67 and 40% for the same insects stage after 4 hrs respectively. On the other hand ozone concentration 800ppm was resulted in mortality up to100 % for eggs of *E. cutella* and adults of *O. surinamensis* after 4hrs. The larvae were completely dead at 800ppm ozone concentration after 4 hrs. Jemni *et al.*,²⁵ illustrated that the mortality of *E. ceratoniae* depended on the ozone level and the exposure time. In fact, with 12.2 mg L-1 for 80 min the carob moth mortality was ten-fold higher (82 ± 3%) than in control samples (8 ± 3%). Low mortality rate of larvae at low concentration or short exposure time could be due to low penetration capability of gaseous ozone. The mortality of Indian meal moth, *Plodia interpunctella* (eggs, larvae, and pupae) infesting stored date of life stages increased by increasing the exposure time in each ozone concentration and showed that the egg was the most tolerant stage to the ozone gas while the 2nd larval instar was the most susceptible one¹⁴. Shaghaghian *et*

al.,²⁶ exposed kabkab date fruits with four ozone concentrations (300 ± 10 , 1050 ± 40 , 2000 ± 40 , and 4000 ± 50 ppm) during four periods (2, 4, 6, and 8 h) and found that 2000 ppm of ozone concentration within 8 h resulted in complete mortality of larvae and adult insects and over 90% mortality of eggs.

Table 5: Effect of ozone treatments on mortality percentage (egg and larva of *E. cutella* and adult of *O. surinamensis*) of Siwi date fruits.

Insects	<i>E. cutella</i>		<i>O. surinamensis</i>
% Mortality	Egg	Larva	Adults
Exposure times (hr)	Concentration of ozone (ppm) 200		
1	0.00	10.00	0.00
2	0.00	10.00	0.00
3	20.00	16.67	0.00
4	30.00	23.33	20.00
	Concentration of ozone (ppm) 400		
1	0.00	20.00	0.00
2	40.00	23.33	0.00
3	60.00	23.33	30.00
4	70.00	26.67	40.00
	Concentration of ozone (ppm) 600		
1	40.00	36.67	20.00
2	50.00	36.67	50.00
3	90.00	43.33	80.00
4	100.00	63.33	100.00
	Concentration of ozone (ppm) 800		
1	90.00	76.67	60.00
2	100.00	83.33	80.00
3		90.00	100.00
4		100.00	

Four concentrations of 0 (control), 100, 200, 300 and 400ppm of ozone gas treatment in controlling two damaging and harmful mites attack date fruits during storage, *Tyrophagus putrescentiae* (Schrank) (Astigmata: Acaridae) and *Rhizoglyphus robini* Claparede (Astigmata: Acaridae). Results showed that the mites mortality percentage increases by increasing ozone concentrations and/or exposure period. The

results indicated that ozone has the potential to control the tested mites. The mortality percentages reached 100% after treated with 400 ppm of ozone gas for 4 hrs²⁵. The elimination of insect pests requires higher ozone doses than fungi. The efficacy of ozone in eliminating fungal and insect pests depends on its applied form, contact period, infesting species and fruit cultivar²⁷. Another reason for this low mortality rate was due to the fact that ozone needs much longer exposure time to enter the respiratory system of larvae and/or react with the larvae cell system²⁸.

Effect of ozone treatments on microbial load of Siwi date fruits.

Result in the Table (6) indicated that increasing ozone concentration reduced the microbial load, regardless of the time of exposure to ozone. Total counts were reduced gradually from 150×10^1 to 112×10^1 , 90×10^1 , 0 and 0 at 200, 400, 600 and 800ppm for 1hr, respectively. It also, was noted that the concentrations of ozone 200 and 400 ppm were not eliminate *E. coli* and yeast and molds until 4 hours while, total counts were complete inhibition at 400ppm for 3 hr. Also, in the same result in efficacy of ozone at 600ppm was complete inhibiting of total count and *E. coli* for 1 hr only. The results were agreement with²⁹ who reported that the insecticidal effect of ozone is due to a combination of its high oxidation potential and its ability to diffuse through biological cell membranes. Upon released, ozone is very efficient in destroying microorganisms and avoiding their growth by the progressive oxidation of vital cell components. From the same result the yeast and molds were more resistant at low concentrations of ozone up to 600 for 4 hr. But, the concentration ozone at 800ppm for 1 hr was the best treatment to complete inhibiting yeast and mold and other microbials. It may be due to the sensitivity of contaminating fungi to ozone may be affected by several factors including the method of application, strain of the microorganism, growth level, nature and water content of date tissue and quantitative amount of sugars, *Escherichia coli* and *S. aureus* were not found on cultured plates inoculated with the treated samples after treatment. Also, to reduce yeast/mold activity, ozone should be applied either for longer periods at low concentration, or conversely for short period with higher concentrations³⁰.

Table 6: Effect of ozone treatments on microbial counts (TC, *E. Coli* and y &M) of Siwi date fruits.

Microbial counts (cfu/g)	Ozone concentration 200 ppm				
	Initial counts	1hr	2hr	3hr	4hr
Total count (TC)	150 x10 ¹	112 x10 ¹	75 x10 ¹	80	70
<i>E.coli</i>	25x10 ⁷	65 x10 ⁵	115 x10 ⁴	192 x10 ²	73 x10 ²
Yeast and Molds(Y&M)	70x10 ¹	36 x10 ¹	44 x10 ¹	17x10 ¹	100
Microbial counts (cfu/g)	Ozone concentration 400 ppm				
	Initial counts	1hr	2hr	3hr	4hr
Total count (TC)	150 x10 ¹	90 x10 ¹	78	0	0
<i>E.coli</i>	25x10 ⁷	226 x10 ²	113 x10 ¹	98	22
Yeast and Molds(Y&M)	70x10 ¹	30 x10 ¹	33 x10 ¹	86	55
Microbial counts (cfu/g)	Ozone concentration 600 ppm				
	Initial counts	1hr	2hr	3hr	4hr
Total count (TC)	150 x10 ¹	0	0	0	0
<i>E.coli</i>	25x10 ⁷	0	0	0	0
Yeast and Molds(Y&M)	70x10 ¹	21 x10 ¹	106	63	38
Microbial counts (cfu/g)	Ozone concentration 800 ppm				
	Initial counts	1hr			
Total count (TC)	150 x10 ¹	0			
<i>E.coli</i>	25x10 ⁷	0			
Yeast and Molds(Y&M)	70x10 ¹	0			

Optimum condition of freezing, irradiation and ozone methods on preservative of siwi date fruits.

The efficiency of some methods of preserving dates before date storage in order to produce dates free from insects or one of their stages and control the microbial load (Table 7). The table shows the conditions necessary to reach product, free of insects and microbial load within the permissible limits in the standard specifications. The conditions were time, concentration, temperature and requirements during treatment. From data in Table (7) the method of preserving dates by freezing or ozone is done in the date's production areas, while, the method of preserving dates by radiation requires transferring the dates to the commercial radiation unit outside the production areas. From the same table it was observed that the three methods lead to the main purposes, which are the final disposal of insects and their instars and the reduction of the microbial load to the allowance limits. During the procedure, the temperature of freezing and ozone methods was -18°C, and room temperature, respectively while, radiation method was high of room temperature, which may be affect the color of the dates fruits. Therefore, the methods of freezing or ozone is suitable than radiation.

Table 7: Optimum condition of freezing, irradiation and ozone methods on preservative of siwi date fruits.

Methods	Freezing	Irradiation	Ozone
Time	5hr	5hr	4hr
Concentration or dose	--	700 gray	800ppm
Temperature during treatment	-18°C	> Room temperature	Room temperature
Mortality insect (%)	100%	100%	100%
Reduction of microbial count (%)	<100%	100%	100%
Requirement during treatment	In date fruit production area	In radiation center	In date fruit production area

4. CONCLUSION

It is clear from the results that we can recommend the use of freezing and ozone to preserve the dates and followed radiation to obtain dates of high quality and safety dates from pests and microbes.

SIGNIFICANCE STATEMENT

There are many ways for pest control, such as fumigation, heat and modified atmosphere before storage of dates. The physical methods, freezing, ozone and radiation were treated of date fruits to study the efficiency on the pest control. It is recommended that freezing or ozone more suitable for controlling insect or microbial loads.

References

1. Ministry of Agriculture and Land Reclamation (2017). Economic Affairs Sector (EAS), Agriculture Planning Central Administration, General Administration of Agric. Economic Resources, National Agricultural Income, pp. 74-79.
2. Jemni M., P.A. Gomez , M.Souza, , N. Chaira, A. Ferchichi, M. Oton, and F. Artes, 2014. Combined effect of UV-C, ozone and electrolyzed water for keeping overall quality of date palm. LWT - Food Science and Techno. 59 649-655.

3. Aldawood A.S, K.G.Rasool, A.H. Alrukban, A. Soffan, M. Husain, K.W. Sutanto and M.Tufail, 2013. Effects of temperature on the development of *Ephestia cautella* (Walker) (Pyralidae: Lepidoptera): A case study for its possible control under storage conditions. Pakistan J. Zool., vol. 45(6), 1573-1578.
4. Assous, M.T.M., W.K.M. El-Shafei, L.M. Lewaa, and E. M. E. Rehab Salem,2022. Efficiency of carbone dioxide and aluminum phosphide gasses on *Ephestia cautella* and *Oryzaephilus surinamensis* insects and microbial load on stored date fruits. Egypt. Acad. J. Biolog. Sci., 15(1):81-89.
5. Al Jasser,M.S. 2010. Effect of storage temperatures on microbial load of some dates palm fruit sold in Saudi Arabia market. African Journal of Food Science Vol. 4(6) pp. 359 – 363,
6. Yahia, E.M. and A.A. Kader, 2011. Date (*Phoenix dactylifera* L.). In: Yahia EM, editor. Postharvest biology and technology of tropical and subtropical fruits. Cambridge, UK: Woodhead Publishing Ltd. pp 41–79.
7. Lallouche, A., V. Kolodyaznaya , M.S. BOulkrane and D. Baranenko, 2017. Low temperature refrigeration as an alternative anti-pest treatment of dates. Environmental and Climate Techno.. vol. 20, 24–35.
8. Frag,S., A. Shaloot, M.Emam, M. El Nawawey and E.Asmaa, 2013. Physicochemical-microbiological studies on irradiated date fruits with studying migration monomers of packages materials. J. Microb. Biochem. Technol. Vol. 5(1): 006-012.
9. Ramadan, B.R., M.N.A. El-Rify, , A.A. Abd El-Hamiedand, M.H.Abd EIMajeed, 2017. Effect of gamma irradiation on quality and composition of Sakkoty date fruits (*Phoenix dactylifera* L) during storage. Assiut J. Agric. Sci., (48) No. (1-1) 80-97.
10. F.D.A. 2001. Food and Drug Administration. Secondary direct food additives permitted in food for human consumption. Fed Reg 66(123):33829–30.
11. Niakousari, M., Z. Erjaee, and S. Javadian, 2010. Fumigation characteristics of ozone in postharvest treatment of Kabkab dates (*Phoenix dactylifera* L.) against selected insect infestation. J Food Protect. 73: 763–8.
12. Zinhoum ,R.A. and W. K. M. El-Shafei, 2019. Control of one of the vital stored date insects, *Plodia interpunctella* (hübner) (lepidoptera: pyralidae), by using ozone gas. Egypt. Acad. J. Biolog. Sci. Vol. 11(3) 149- 156.
13. Khalil,H.A., 2016. Effect of ozone application on postharvest quality and microbiological state of “zaghloul” date palm fruits. J. Plant Production, Mansoura Univ., Vol. 7 (1): 43- 51.

14. Shaghaghian, S. M. Niakousari and S. Javadian, 2014. Application of ozone post-harvest treatment on kabkab date fruits: Effect on mortality rate of indian meal moth and nutrition components. *Ozone: Science and Engineering*, 36: 269–275.
15. American Public Health Association, 2012. Compendium of the methods for the microbiological examination of foods. (3rd edn), Washington, DC, USA, <https://ajph.aphapublications.org/doi/10.2105/9780875530024ch06>
16. El-Shafei, W. K. M., 2015. Studies on efficiency of certain methyl bromide alternatives against *Ephestia cautella* (Walker) Lepidoptera: Pyralidae (Doctoral dissertation, Ph. D. thesis, Benha Univ., Egypt, pp: 195.
17. Darwish, A.A.; F. A. M. El-Lakwah ; A. El-Hosary, Rasha ;A. A. EL-Banna and W.K.M. El-Shafei (2014). Effect of microwave on *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) as alternative to methyl bromide. *J. Biol. Chem. Environ. Sci.*, 2014, (9(4),247-263.
18. Kostal, V., J. Korbelova, J. Rozsypal, H. Zahradničková, J. Cimlova, A. Tomcala, P. and Simek, 2011. Long-term cold acclimation extends survival time at 0°C and modifies the metabolomic profiles of the larvae of the fruit fly *Drosophila melanogaster*. *PLoS ONE*, Vol. 6 | Issue 9 | e25025.
19. Ben-Amor, R., M.D. Miguel-Gómez, D.M. Habib, H. Nouha, and E. Aguayo, 2019. Freezing treatments for *Ectomyelois ceratoniae* mortality and maintenance of Deglet Noor palm date quality. *J. of Food Quality* Vol.2019, Article ID 8941407, 9 pages.
20. Zaritzky, N., 2008. Frozen storage. In *Frozen food science and technology*, ed. J.A. Evans, 224–247. Oxford: Blackwell Publishing.
21. G. S.O., 2014. Standardization Organization for G.C.C GSO /FDS 1016, https://members.wto.org/crnattachments/2014/sps/OMN/14_5658_00_e.pdf
22. Mahmoud, R. H., A. R. Abdel-Khalik, and W. K. M. El-Shafei, 2022. Comparison between two physical methods to control the stored dates fruit mites, *Tyrophagus putrescentiae* (Schrank) and *Rhizoglyphus robini* Claparede (Astigmata: Acaridae). *Egyptian Academic J. of Biol. Sciences B. Zoology*, 14(1), 149-158.
23. Selim, K. M. Abdel-Bary and O. Ismaael, 2012. Effect of irradiation and heat treatments on the quality characteristics of Siwi date fruit (*Phoenix dactylifera* L.). *AgroLife Scientific J.*, Vol. 1, 103-111.
24. Ramadan, B.R., M.N.A. El-Rify, A.A. Abd El-Hamied and, M.H. Abd El Majeed, 2016. Effect of some treatments on chemical composition and quality properties of Saidy date fruit (*Phoenix dactylifera* L.) during storage. *Asset J. Agric. Sci.*, (47) No. (5) 107-124.
25. Jemni, M., M. Oton, M. Souza, M.H. Dhouibi, A. Ferchichi and F. Artés, 2015. Ozone gas greatly reduced the survival of carob moth larvae in stored date palm fruit. *J. of New Science*, Vol. 16 (4);567-573.

26. Shaghaghian, S. M. Niakousari and S. Javadian, 2014. Application of ozone post-harvest treatment on kabkab date fruits: Effect on mortality rate of Indian meal moth and nutrition components. *Ozone: Science and Engineering*, 36: 269–275.
27. Al-Ahmadi, S.S., R.A. Ibrahim, and S.A. Ouf, 2009. Application of ozone to control insect pests and moulds of date fruits. *Biosciences, Biotechnol. Research Asia*. Vol. 6(2), 435-446.
28. Isikber, A.A., and S. Oztekin, 2009. Comparison of susceptibility of two stored-product insects, *Ephestia kuehniella* Zeller and *Tribolium confusum* du Val to gaseous ozone." *J. Stored Prod. Res.* 45: 159–164.
29. Abo-El-Saad, M.M., H.A. Elshafie, A.M. Al Ajlan and I.A. Bou-Khowh, 2011. Non-chemical alternatives to methyl bromide against *Ephestia cautella* (Lepidoptera: Pyralidae): microwave and ozone. *ABJNA* 2: 1222-1231.
30. Najafi, M.P.H. and M.H.H. Khodaparast, 2009. Efficacy of ozone to reduce microbial populations in date fruits. *Food Control*. 20, 27–30.

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