



Effect of Physical Methods on Date Fruits Insects and Microbes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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: In the first method, the date fruits were frozen at -18°C at different times (15, 30, 60, 120, 180, 240, 300, and 360 min). In the second method, the date fruits were exposed to gamma rays in different doses as 25, 50, 75, 100, 200, 300, 400, 500, 600, and 700 grays. In the third method, the date fruits were exposed to different ozone concentrations of 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 a dose of 700 grays, 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. The 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 processing [1]. 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 appropriate harvesting, handling, processing, packaging, storage, transport, distribution, and retail sale operations [2]. 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 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 that is stored after harvesting for processing and marketing. Also, Coleoptera and Lepidoptera are the 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 increases with the increase of water content, therefore, the temperature of storage and water content are the major factors that affect the shelf life of dates [5]. The traditional method of eliminating insects and their stages in date is fumigation with phosphine before storage. Yahia and Kader [6] showed that freezing at -18°C or lower for at least 48 hrs. (from the time when the fruit temperature reaches -18°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°C the *Ectomyelois ceratoniae* Zeller larvae die within 2 hrs., and the eggs and larvae – within 24 hrs., respectively. They were recommended for storage dates at -18°C to prevent pests and improve the quality. It is apparently advantageous for the 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 the 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 foods [10]. 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. Zinhoum and El-Shafei [12] 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. Khalil [13] found that the Zaghloul date fruit at khalal stage were exposed to 150 ppm ozone (O_3) 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 the 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 preservative methods were used to study their 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 of 200,400,600 and 800 ppm at different exposure periods (1, 2, 3 and 4 hrs).

2.1 Mortality Insects

A stereo microscope 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:-

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

2.2 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* (25×10^7 cfu). After grinding the well, the samples were exposed to carbon dioxide and 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 Association [15].

Total bacterial and yeast & molds count: Total bacterial count (TBC), and yeast and molds count (Y and M) were determined using the plate count method according to American Public Health Association [15].

3. RESULTS AND DISCUSSION

3.1 Effect of Freezing Temperatures at -18°C at Different Time on Mortality Percentage (Eggs and Larvae of *E. cautella* and Adults of *O. surinamensis* of Siwi Date Fruits

The results in table (1) indicated that increasing the time of treatment at -18°C increased mortality percentages to 300 min (5hr) regardless of insect type or its stages. The result in the same Table 1 showed that the time to control the larvae of *E. cautella* is faster than the eggs of the same insect or the adult stage of *O. surinamensis*. These results are in agreement with [16] 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*.

3.2 Effect of Freezing Temperatures (-18°C) at a 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* were 25×10^7 , respectively. It is clear from Table (2) the effect of freezing at -18°C on microbial growth is 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, Table (2) showed that *E. coli* was reduced by more than 50% after 5 hr of treatment. On the other hand, the counts of *E. coli* were high of the allowed limit of date fruit standard. The initial microbial load of *E. coli* is due to its development in the laboratory. Therefore, 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, respectively [20]. The counts of total and yeast & mold were within the G.S.O. [21].

3.3 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 grays. 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 Gy [22].

3.4 Effect of Radiation Treatments on Microbial Load of Siwi Date Fruits

The results indicated that increasing radiation dose was combined with reducing the microbial load to 300 grays of the total count, *E. coli*, and yeast & molds (Table 4). Also, 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 [23,24].

3.5 Effect of Ozone Treatments on Mortality Percentage of (Eggs and Larvae of *E. cautella* 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. The result in Table (5) illustrated that ozone concentration of 200ppm caused mortality up to 30, 23.33 and 20% for eggs and larvae of *E. cautella* and adults of *O. surinamensis* after 4 hrs., respectively. While ozone concentration 400 ppm was recorded for mortality up to 70, 26.67, and 40% for the same insects' stage after 4 hrs respectively. On the other hand ozone concentration 800 ppm resulted in mortality up to 100 % for eggs of *E. cautella* 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 ($82 \pm 3\%$) than in control samples ($8 \pm 3\%$). The low mortality rate of larvae at low concentrations or short exposure time could be due to the low penetration capability of gaseous ozone. The mortality of the 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 one [14]. Shaghaghian et al. [26] exposed kab kab 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 1. Effect of freezing temperatures (-18°C) at a different time on mortality percentage (eggs and larvae of *E. cautella* and adults of *O. surinamensis* of Siwi date fruits

Insects % Mortality Time treatment (min)	<i>E. cutella</i>		<i>O. surinamensis</i>
	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

Table 2. Effect of freezing temperatures (-18°C) at a different time on microbial load of Siwi date fruits

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

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 Con.(gray)	<i>E. cutella</i>		<i>O. surinamensis</i>
	Egg	Larva	Adults
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		

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

Microbes Con.(gray)	TC(cfu/g)	<i>E. coli</i> (cfu/g)	Yeasts and molds(cfu/g)
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

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 being treated with 400 ppm of ozone gas for 4 hrs [25]. 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 cultivar [27]. Another reason for this low mortality rate was the fact that ozone needs a much longer exposure time to enter the respiratory system of larvae and/or react with the larvae cell system [28].

3.6 Effect of Ozone Treatments on Microbial Load of Siwi Date Fruits

The result in 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 x10¹ to 112 x10¹, 90 x10¹, 0 and 0 at 200, 400, 600 and 800ppm for 1hr, respectively. It also was

noted that the concentrations of ozone between 200 and 400 ppm were not eliminated *E. coli* and yeast and molds until 4 hours while total counts were complete inhibition at 400ppm for 3 hr. Also, the same result in the efficacy of ozone at 600ppm was complete inhibiting of the total count and *E. coli* for 1 hr only. The results were in agreement with [29] 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 release, 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 of ozone at 800ppm for 1 hr was the best treatment to complete inhibiting yeast and mold and other microbial. 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 concentrations or conversely for short periods with higher concentrations [30].

3.7 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 affect the color of the dates fruits. Therefore, the methods of freezing or ozone is suitable than radiation.

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

Ozone concentration 200 ppm					
Microbial counts (cfu/g)	Initial counts	1hr	2hr	3hr	4hr
Total count (TC)	150×10^1	112×10^1	75×10^1	80	70
<i>E. coli</i>	25×10^7	65×10^5	115×10^4	192×10^2	73×10^2
Yeast and Molds(Y&M)	70×10^1	36×10^1	44×10^1	17×10^1	100
Ozone concentration 400 ppm					
Microbial counts (cfu/g)	Initial counts	1hr	2hr	3hr	4hr
Total count (TC)	150×10^1	90×10^1	78	0	0
<i>E. coli</i>	25×10^7	226×10^2	113×10^1	98	22
Yeast and Molds(Y&M)	70×10^1	30×10^1	33×10^1	86	55
Ozone concentration 600 ppm					
Microbial counts (cfu/g)	Initial counts	1hr	2hr	3hr	4hr
Total count (TC)	150×10^1	0	0	0	0
<i>E. coli</i>	25×10^7	0	0	0	0
Yeast and Molds(Y&M)	70×10^1	21×10^1	106	63	38
Ozone concentration 800 ppm					
Microbial counts (cfu/g)	Initial counts	1hr			
Total count (TC)	150×10^1	0			
<i>E. coli</i>	25×10^7	0			
Yeast and Molds(Y&M)	70×10^1	0			

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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