Use of ozonated water for controlling microbial contamination of date palm fruits

Salah M. Aleid[1] and Siddig H. Hamad[2]
[1]Date Palm Research Center of Excellence, King Faisal University, P.O. Box 400, Alahsa, 31982, Saudi Arabia, Email: seid@kfu.edu.sa
[2]College of Agric. & Food Sciences, King Faisal University, P.O. Box 400, Alahsa, 31982, Saudi Arabia, Email. siddighamad@yahoo.com

ABSTRACT

Effects of treating date palm fruits with ozonated water (5, 15 and 30 minutes) on the general microbial contamination and on the added contamination with Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Aspegillus fumigates was investigated. Soaking date fruits for 15 minutes was effective to reduce general contamination with mesophillic aerobic bacteria, coliforms,molds and yeasts to levels that meet the requirements of Saudi Standards. The treatment was also found as the most effective for the reduction of added contamination with S. aureus, P. aeruginosa, E. coli and A. fumigates.

Key words: *Date fruits, preservation, microbial contamination, ozonated water.*

INTRODUCTION

Dates, like any other agricultural products, are subject to microbial contamination in the field and during handling processes. Studies conducted so far showed that date fruits in the rutab and Tamr stages are contaminated with many types of microorganisms (Abu-Zinada and Ali, 1982,Aidooetal.,1996,El-Sherbeenyetal.,1985, Nussinovitchetal.,1989, Hamad, 2008, Atia *et al.,* 2009 and Atia *et al.,* 2011). Potential spoilage microorganisms that contaminate date fruits include molds, yeasts and lactic acid bacteria, whereas potential pathogens include bacteria such as *Staphylococcus aureus* and yeasts such as *Candida pelliculosa*. Microbial contamination can lead to considerable losses in the crop especially at the *rutab* stage while contamination of the fruit at the tamr stage limits export chances, especially to the markets of the industrial countries. Treatments designed to control microbial contamination in date fruit processing factories in Saudi Arabia depend on washing with pure or chlorinated water. A survey conducted by the authors in a factory for date fruit processing that depend on washing with pure and chlorinated water showed that this treatment was not quite effective. In some cases the processed dates had higher microbial loads than the raw fruits (unpublished data).

The aim of this study was to investigate the effect of treatment with ozonated water on the microbial contamination of date fruits. Collected samples were treated with ozonated water by soaking for 5, 15 and 30 minutes. The effect of these treatments on the microbial load of the fruits was determined.

MATERIAL AND METHODS

Samples Collection

Date fruit samples of Khalas variety were purchased from one shop in Hofuf City, Saudi Arabia. Samples were collected in sterile containers and transferred to the laboratory for analysis and treatment.

Ozone Disinfection System

The Ozonated Water System FS-7200 (Biotek Ozone's Light Industrial Series FP-7200 Biotek Environmental Science Ltd.) was used. The system provides output flow rates of 600, 300 and 150 L/h, with ozone concentrations in the output of 1, 2 and 4 ppm, respectively. The 2 ppm ozone concentration was used in this study as recommended by the manufacturer.

Sample treatment

Treatment with ozonated water was performed by soaking 10 g date fruits sample in the ozonated water for 5, 15 and 30 minutes and then determining the microbial load. In a similar way 10 g date samples were soaked in sterile tap water to determine the effect of washing.

Microbiological analysis

Date fruit samples (10g) were weighed into sterile stomacher bags, 90 ml sterile peptone water (Oxoid, CM0009) added, homogenized in a stomacher (Lab-Blender 400, Seward Medical, England) for 45 seconds and aliquots (1.0 or 0.1 ml) plated out in duplicate as 10-fold dilutions in peptone water. Aerobic mesophillic bacteria were counted on plate count agar medium (PCA Oxoid, CM0325) incubated at 30˚C for 2 to 3 days, coliforms on violet red bile agar medium (VRBA Oxoid, CM0107) at 37˚C for 24 hours, yeasts and molds on PDA plates at 20 -30˚C for 3 to 7 days. *Escherichia coli* was cultivated on nutrient agar (CM0003, Oxoid), *Staphylococcus aureus* on baird-parker agar base (CM0961,Oxoid), *Pseudomonas aeruginosa* on pseudomonas agar base (CM0559) and *Aspergillus fumigatus* on potato dextrose agar (CM0139, Oxoid).

Contamination of fruits with selected microorganisms was performed as follows: the microorganisms were grown 2 days in Petri dishes containing appropriate media. Then suspensions of the individual microorganisms were made in bottles containing 50ml sterile peptone medium by transferring three lapfuls from the Petri dishes containing the individual microorganism into each bottle. Ten grams of each Date fruit samples were then contaminated with the selected microorganisms by immersing them in each suspensions alone for few seconds. The resulted contamination was about 106to 108cfu/g. For each date fruit sample three 10 gram portions were contaminated with the selected microorganism. One portion was treated with electrolyzed water by soaking for 5, 15 or 30 minutes, a second portion treated by soaking in sterile tap water for the same period of time, and a third portion analyzed as untreated sample. The microbial loads of the treated and untreated portions were then determined. The selected microorganisms were *S. aureus* ATCC 25923 as representative of potential pathogens, *P. aeruginosa* ATCC 27853 as representative of potential spoilage bacteria, *E. coli ATCC* 25922 as representative of coliforms, and *A. fumigatus* ATCC 204305 as a representative of potential spoilage molds.

Statistical Analysis

Analysis of variance was performed to detect differences between the microbial load of the applied treatments on samples. Duncan multiple ranges at 5% level of significance was used to compare between means.

The analysis was carried out using the PROC ANOVA procedure of Statistical Analysis System (SAS, 1996).

RESULTS AND DISCUSSION

Use of ozonated water to control general contamination with mesophillic aerobic bacteria, molds and yeasts

1. Treatment for 5 minutes

Contamination with mesophillic aerobic bacteria in the samples before treatment was in the range $10^2$to 10^4 cfu/g (Table 1). Soaking in sterile water for 5 minutes reduced this load by an average of 0.98 log cycles, which represent a reduction of about 88.6% of the bacterial population. Soaking in ozonated water reduced loads in the range 10^2 cfu/g to non-detectable level. Reductions in loads in the range 10^3 to 10^4 cfu/g were on average about 1.82 log cycles, i.e. an average of about 97.4% of the population was removed or an average of 0.84 log cycles over the washing effect of water. Since mesophillic aerobic bacteria are not considered an important potential spoilage agent of date fruits, their presence is only regarded as an index for the hygienic status of the fruits.

All 5 samples tested were found contaminated with molds and yeasts at loads in the range $10^2$to 10^4 cfu/g (Table 1) about 90% of which was molds. Soaking in sterile water reduced these levels of contamination by an average of 0.86 log cycles, i.e. about 83.9% of the population was removed. Soaking in ozonated water reduced contaminations in the range 10^2 cfu/g to non-detectable levels. Contaminations of the order 10^3-10^4 cfu/g, were reduced by an average of 1.69 log cycles or by 96.8%, and no yeasts were detected in the treated samples (result not shown). Molds and yeasts are considered important spoilage agents of date fruits. The Saudi standard for microbiological criteria of foods requires that the loads of yeasts in date fruits should not exceed 10 cfu/g in 3 out of 5 replicates of tested sample and that of molds not to exceed 10^2 cfu/g in 3 of 5 replicates of tested sample (SASO, 1998). This treatment reduced contamination to levels that meet Saudi standard for both molds and yeasts.

Coliforms contamination was detected in 2 of the 5 samples tested, with loads in the range $10^2$to 10^3 cfu/g. Soaking in water reduced these loads by an average of 0.84 log cycles, while soaking in ozonated water reduced contamination of the sample with 10^2 cfu/g to non-detectable level and that of the sample with 10^3 cfu/g by 1.8 log cycles (98.4%). It can therefore be concluded that contamination of date fruits with coliforms at normal levels could be controlled by this treatment.

2. Treatment for 15 minutes

The results of the 5 samples soaked in ozonated water for 15 minutes are presented in Table 2. Contamination

of the samples with mesophillic aerobic bacteria was in the normal range of 10^2 to 10^5 cfu/g. Soaking in sterile water for 15 minutes reduced these loads by an average of about 0.95 log cycles or by91.6%. Soaking in ozonated water reduced loads in the order 10^2 and 10^3 cfu/g to non-detectable levels. The loads in the order 10^4 and 10^5 cfu/g were reduced by an average of 2.29 log cycles or 99.5%, which was clearly higher than the effect of soaking in sterile water and in ozonated water for 5 minutes. This means that contamination levels of up to 10^3 cfu/g of mesophillic aerobic bacteria can be reduced to non-detectable levels by soaking in ozonated water for 15 minutes.

Contamination levels with molds and yeasts in the 5 samples tested were in the order 10^2 to 10^3 cfu/g (Table 2), again about 90% was molds. Soaking in sterile water reduced the contamination of $2.1x10^2$ cfu/g in one sample to non-detectable level, while contaminations in the other 4 samples were reduced by an average of 0.97 log cycles. Soaking in ozonated water reduced the contamination in all of the 5 samples to non-detectable levels. This treatment is therefore enough for reducing loads of molds and yeasts of up to 10^3 cfu/g to levels that make date fruits meet the Saudi standards for contamination with molds and yeasts.

Coliforms were detected in 2 of the 5 samples tested at loads of $1.4x10^2$ and $9.2x10^3$ cfu/g. Soaking in sterile water and in ozonated water reduced the load of $1.4x10^2$ cfu/g to non-detectable level, while soaking in sterile water and in ozonated water reduced the load of $9.2x10^3$ cfu/g by 1.11 and 2.07 log cycles, respectively. These results are comparable to results obtained for the other treatments discussed above and confirm that soaking in ozonated water for up to 5 minutes is quite enough to control normal levels of contamination of date fruits with coliforms.

3. Treatment for 30 minutes

The results of soaking date fruit samples in ozonated water for 30 minutes are shown in Table 3. The 5 samples were found contaminated with mesophillic aerobic bacteria at loads in the order $10^2$to 10^5 cfu/g. Soaking in sterile water for 30 minutes reduced a load of $3.0x10^2$ cfu/g in one sample to non-detectable level. The loads in the other 4 samples were reduced after soaking in water for 30 minutes by the usual values ranging between 0.84 and 1.11 log cycles. Loads of 10^2 and 10^3 cfu/g in 4 samples were reduced to non-detectable levels by soaking in the ozonated water for 30 minutes, while the load of the sample with $2.2x10^5$ cfu/g was reduced by 2.14 log cycles or about 99.3%. This effect is comparable to that reached by soaking in ozonated water for 15 minutes (2.29 log cycles for the 15 minutes soaking) which indicate that soaking for 30 minutes didn't bring about an increase in the killing effect of ozonated water. It can therefore be concluded that treatment with ozonated water for 15 minutes is the optimum treatment for the control of mesophillic aerobic bacteria in date fruits.

Molds and yeasts were found contaminating all of the 5 samples at loads in the order 10^2 to 10^4 cfu/g (Table 3). Soaking in sterile water reduced these contamination levels by an average of 0.96 log cycles or by about 88.8%, which was the usual value observed in the other treatments presented above. Soaking in the ozonated water for 30 minutes reduced the contamination in 4 samples with loads in the order 10^2 and 10^3 cfu/g to non-detectable levels. The load of the order 10^4 cfu/g was reduced by this treatment by 2.38 log cycles or by about 99.6%, with no yeasts detected in the treated samples (result not shown). This amount of reduction is enough to satisfy the Saudi standard for molds and yeasts. Anyway, soaking for 30 minutes doesn't seem to bring much more effect over soaking for 15 minutes. Since the normal level of contamination of date fruits with molds and yeasts doesn't exceed the order 10^3 cfu/g, treatment of date fruits by soaking in ozonated water for 15 minutes seem to be enough for the control of this group of microorganisms.

The picture for contamination with coliforms was as usual; only one of the 5 samples was found contaminated with this group of bacteria at a load of $4.2x10^3$ cfu/g (Table 3). Soaking in water reduced this load by 0.99 log cycles and soaking in the ozonated water reduced it to non-detectable level. It can be stressed again that contamination with coliforms is not a great problem for date fruits. Soaking in ozonated water for 15 minutes, which was found enough for the control of molds, yeasts and mesophillic aerobic bacteria, can be considered as enough for the control of contamination with coliforms.

Use of ozonated water to control contamination of date fruits with selected potential pathogenic and potential spoilage microorganisms

Table 4 contains the results of soaking 5 date fruit samples in ozonated water for 5 minutes. The levels of contamination of the untreated samples were 10^7 to 10^8 cfu/g for the bacteria and 10^6 to 10^7 cfu/g for the mold. Soaking in sterile tap water reduced the level of contamination in all tested samples by an average near one log cycle, which was the normal average value obtained for all other treatments discussed above. Soaking in ozonated water reduced the level of contamination with *S. aureus* by 1.59 to 2.11 log cycles with an average of 1.85 log cycles. In case of *P. aeruginosa*, this treatment reduced contamination by 1.22 to 1.37 log cycles with an average of 1.28 log cycles. *E. coli* was found more sensitive to treatment with ozonated water than *P. aeruginosa* but apparently less sensitive than *S. aureus*. On soaking in ozonated water for 5 minutes, contamination with this bacterium was reduced by 1.41 to

1.56 log cycles, with an average reduction of 1.49 log cycles. These results indicate that *S. aureus* was the most sensitive bacterium to treatment with ozonated water for 5 minutes, followed in sensitivity by *E. coli* and the least sensitive was *P. aeruginosa*. Treatment with ozonated water for 5 minutes reduced contamination with *A. fumigatus* by 1.83 to 2.19 log cycles with an average of about 2.0 log cycles, hence this fungus was the most sensitive to this treatment.

Results of treatments of the 4 microorganisms with ozonated water for 15 minutes are shown in Table 5. The effects of soaking in sterile tap water on the levels of contamination were similar to effects registered and discussed before. The average reduction in contamination with *S. aureus* after soaking in the ozonated water was 2.21 log cycles i.e. about 99.2%, which was more than that reached for treatment with ozonated water for 5 minutes (1.85 log cycles). Contamination of *P. aeruginosa* and *E. coli* was reduced as a result of this treatment by averages of 1.69 and 2.04 log cycles, respectively. Again the effect of this treatment on both bacteria was higher than that of treatment with ozonated water for 5 minutes. With respect to *A. fumigatus*, treatment with ozonated water for 15 minutes reduced the level of contamination by an average of 2.40 log cycles, i.e. about 99.4%. As expected the effect of this treatment was higher than that of the treatment with this water for 5 minutes.

Results of treatments with ozonated water for 30 minutes are presented in Table 6. The effect of treatment of the 4 microorganisms with ozonated water for 30 minutes was slightly higher than that of the treatment for 15 minutes (Tables 4 and 5). It was 2.30, 1.76, 2.09 and 2.53 log cycles for the 30 minutes treatment compared to 2.21, 1.69, 2.04 and 2.40 log cycles for the 15 minutes treatment for *S. aureus, P. aeruginosa, E. coli* and *A. fumigatus*, respectively.

Soaking date fruits in ozonated water for 15 minutes seems to be the most effective treatment for the control of contamination with *S. aureus,P. aeruginosa, E. coli* and *A. fumigatus*(Table 7). The effect of this treatment on the level of contamination with these microorganisms was significantly higher than that of treatment for 5 minutes but not significantly different from that of treatment for 30 minutes.

No work on treatment of date fruits with ozonated water was found in the literature cited. Xu (1999) reported >90% reduction in total bacterial count of cabbage after treatment with ozonated water for 3 minutes. According to the food Safety Network Canada (2008), *E. coli* contamination in water can be reduced by 2 log cycles after injection of 0.02 mg ozone per minute per liter. Manousaridis et al (2005) reported that ozonation (1 ppm for 60 to 90 minutes) reduced contamination of shucked mussels with aerobic mesophillic bacteria by 0.7 to 2.1 log cycles, *Pseudomonas* sp. by 0.5 to 1.1 log cycles, *Brochothrix thermosphacta* by 0.3 to 1.4 log cycles, and *Enterobacteriaceae* by 0.5 to 1.5 log cycles. Treatment with 0.12 to 3.8 ppm ozone inactivated gram-positive bacteria by 1.0 to 7.0 log cfu/ml and treatment with 0.004 to 6.5 ppm ozone reduced population of gram-negative bacteria by 0.5 to 6.5 log cfu/ml (Khadre et al. 2001). Treatment of lettuce in ozonated water (1.3 ppm) for 3 minutes reduced the load of mesophillic and psychrotrophic microorganisms by 1.2 and 1.8 log cycles, respectively (Rivera, 2005). Atia et al, (2011) found that hot air and hot water treatments of Deglate and Elak varieties date fruits reduced the number of the associated fungi (1.17, 1.17, 3.00, and 1.17 $x10^4$ colonies/g dates, respectively) compared to untreated control (12.5 and 7.84 $x10^4$ colonies/g dates). Washing date fruits with sterile water decreased numbers of fungal load (6.00 and 2.33 $x10^4$ colonies/g dates). Carnation oil treatment reduced fungal load on dates.

Conclusion

Contamination of date fruits with mesophillic aerobic bacteria was mostly in the range 10^2 to 10^4, with molds and yeasts in the range 10^2 to 10^3 and with coliforms less than 10^2 cfu/g. Treatment with ozonated water for 15 minutes reduced these levels of contamination by more than 2 log cycles which was enough to meet Saudi standard requirement. Hence normal microbial contamination of date fruits can be controlled by treatment with ozonated water.

Acknowledgments

The authors would like to thank the Ministry of Higher Education, Saudi Arabia for funding the project No. PR-1, through a generous financial support to the Date Palm Research Center of Excellence, King Faisal University, Alahsa, Saudi Arabia.

Literature Cited

Abu-Zinada AH, Ali MI. 1982. Fungi associated with dates in Saudi Arabia. *Journal of food Protection* 45(9):842-844.

Aidoo KE, Tester RF, Morrison JE, MacFarlane D. 1996. The composition and microbial quality of pre-packed dates purchased in Greater Glasgow. *International Journal of food Science and Technology* 31:433-438.

Atia M. M.M. 2011. Efficiency of Physical Treatments and Essential Oils in Controlling Fungi Associated with Some Stored Date Palm Fruits. Australian Journal of Basic and Applied Sciences, 5 (6): 1572-1580, 2011, ISSN 1991-8178.

Atia, M.M.M.; El Mahmodi, A.A.M. and El-Shili Manal. 2009. Survey of fungal contamination of some stored date palm fruits. The 3rd National Conference on Basic Science, Under the Title: Basic Sciences are the Main Source of Creativity, Gharian, Libya, BI 18, from 25-27 April 2009.

El-Sherbeeny MR, Saddik MF, Bryan FL. 1985. Microbial profiles of foods served by street vendors in Egypt. *International Journal of Food Microbiology* 2:355-364.

Food Safety Network, University of Guelph. www.foodsafetynetwork.ca

Hamad, S. H. 2008. Microbial spoilage of date *rutab* collected from the markets of Al-Hofuf City in the Kingdom of Saudi Arabia. *J. of Food Prot.*, 71 (7):1406-1411.

Khadre M. A., A. E. Yousef and J. G. Kim. 2001. Microbiological aspects of ozone applications in food: a review. *Journal of Food Science*, 66 (9):1242-1252

Manousaridis G., A. Nerantzaki, E. K. Paleologos, A. Tsiotsias, I. N. Savvaidis and M. G. Kontominas 2005. Effect of ozone on microbial, chemical and sensory attributes of shucked mussels. *Food Microbiology* 22:1-9.

Nussinovitch, A., Rosen, B., Salik, H. and Kopelman, I. J. 1989. Effect of heating media on the microbiology and shelf life of heat pasteurized soft dates. *Lebensmittel-Wissenschaft und Technologie,* 22, 245-247.

Rivera E. V. 2005. A review of chemical disinfection for minimally processed leafy vegetables. M. Sc. Thesis, College of Agriculture, Kansas State University, Kansas, USA.

SAS Program. 1996. SAS/STAT user's guide, release 6.12. Cary, NC, USA. SAS Inst. Inc

Saudi Standards, Metrology and Quality Organization (SASO). 1998. Microbial limits for food stuffs (in Arabic), part 1.

Xu, Liangji 1999. Use of ozone to improve the safety of fresh fruits and vegetables. *Food Technology*, 53 (10):58-62.

Tables

Table 1: Effect of soaking in tap water and ozonated water for 5 minutes on the contamination of date fruits with mesophillic aerobic bacteria (MAB), molds and yeasts (M & Y) and coliforms

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
1 (U)	$3.2x10^4$		$4.9x10^2$		n.d.	
1 (T)	$4.0x10^3$	0.91	79	0.79	n.d.	-
1 (O)	$5.2x10^2$	1.79	n.d.	-	n.d.	-
2 (U)	$7.7x10^2$		$2.7x10^4$		$5.3x10^3$	
2 (T)	$1.2x10^2$	0.81	$3.6x10^3$	0.87	$7.4x10^2$	0.85
2 (O)	n.d.	-	$2.8x10^2$	1.98	84	1.83
3 (U)	$7.0x10^3$		$8.1x10^2$		n.d.	
3 (T)	$8.5x10^2$	0.92	$1.4x10^2$	0.76	n.d.	-
3 (O)	$1.1x10^2$	1.81	n.d.	-	n.d.	-
4 (U)	$1.5x10^4$		$6.5x10^3$		$6.8x10^2$	
4 (T)	$9.7x10^2$	1.19	$4.6x10^2$	1.15	$1.0x10^2$	0.83
4 (O)	$2.5x10^2$	1.78	75	1.93	n.d.	-
5 (U)	$6.2x10^3$		$1.4x10^3$		n.d.	
5 (T)	$5.0x10^2$	1.09	$2.6x10^2$	0.74	n.d.	-

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
5 (O)	80	1.89	$1.0x10^2$	1.15	n.d.	-

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 2: Effect of soaking in tap water and ozonated water for 15 minutes on the contamination of date fruits with mesophillic aerobic bacteria (MAB), molds and yeasts (M & Y) and coliforms

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
1 (U)	$4.4x10^5$		$2.1x10^2$		$9.2x10^3$	
1 (T)	$3.7x10^4$	1.07	n.d.	2.32	$7.0x10^2$	1.11
1 (O)	$2.6x10^3$	2.23	n.d.	2.32	77	2.07
2 (U)	$5.1x10^3$		$8.4x10^2$		n.d.	
2 (T)	$6.6x10^2$	0.89	83	1.00	n.d.	-
2 (O)	n.d.	-	n.d.	2.92	n.d.	-
3 (U)	$4.1x10^2$		$1.9x10^3$		n.d.	
3 (T)	67	0.78	$2.4x10^2$	0.90	n.d.	-
3 (O)	n.d.	-	n.d.	3.28	n.d.	-
4 (U)	$5.5x10^3$		$7.0x10^2$		$1.4x10^2$	
4 (T)	$6.0x10^2$	0.96	64	1.04	n.d.	2.15
4 (O)	n.d.	-	n.d.	2.85	n.d.	2.15
5 (U)	$2.2x10^4$		$4.7x10^3$		n.d.	
5 (T)	$2.0x10^3$	1.04	$5.3x10^2$	0.95	n.d.	-
5 (O)	$1.0x10^2$	2.34	n.d.	3.67	n.d.	-

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 3: Effect of soaking in tap water and ozonated water for 30 minutes on the contamination of date fruits with mesophillic aerobic bacteria (MAB), molds and yeasts (M & Y) and coliforms

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
1 (U)	$3.0x10^2$		$4.0x10^3$		n.d.	
1 (T)	n.d.	-	$5.8x10^2$	0.84	n.d.	-
1 (O)	n.d.	-	n.d.	3.60	n.d.	-

Sample	MAB (cfu/g)	Reduction (log cycles)	M & Y (cfu/g)	Reduction (log cycles)	Coliforms (cfu/g)	Reduction (log cycles)
2 (U)	$4.8x10^3$		$5.5x10^4$		n.d.	
2 (T)	$3.7x10^2$	1.11	$4.1x10^3$	1.13	n.d.	-
2 (O)	n.d.	-	$2.3x10^2$	2.38	n.d.	-
3 (U)	$2.2x10^5$		$7.4x10^2$		n.d.	
3 (T)	$1.8x10^4$	1.08	90	0.92	n.d.	-
3 (O)	$1.6x10^3$	2.14	n.d.	2.87	n.d.	-
4 (U)	$5.3x10^3$		$6.3x10^2$		$4.2x10^3$	
4 (T)	$7.5x10^2$	0.84	66	0.98	$4.3x10^2$	0.99
4 (O)	n.d.	-	n.d.	2.80	n.d.	3.62
5 (U)	$7.1x10^2$		$8.0x10^3$		n.d.	
5 (T)	81	0.94	$9.1x10^2$	0.94	n.d.	-
5 (O)	n.d.	-	n.d.	3.90	n.d.	-

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 4: Effect of soaking in tap water and ozonated water for 5 minutes on the microbial load of date fruits contaminated with *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *A. fumigatus* ATCC 204305

Sample	*S. aureus* (cfu/g)	Reduction (log cycles)	*P. aeruginosa* (cfu/g)	Reduction (log cycles)	*E. coli* (cfu/g)	Reduction (log cycles)	*A. fumigatus* (cfu/g)	Reduction (log cycles)
1 (U)	$3.5x10^8$		$1.5x10^8$		$1.6x10^7$		$5.5x10^6$	
1 (T)	$4.4x10^7$	0.90	$3.1x10^7$	0.69	$2.4x10^6$	0.82	$3.0x10^5$	1.26
1 (O)	$5.3x10^6$	1.82	$8.7x10^6$	1.24	$4.4x10^5$	1.56	$6.5x10^4$	1.83
2 (U)	$6.5x10^7$		$7.5x10^7$		$2.1x10^8$		$6.9x10^6$	
2 (T)	$7.2x10^6$	0.95	$8.1x10^6$	0.97	$3.5x10^7$	0.78	$7.6x10^5$	0.96
2 (O)	$8.2x10^5$	1.90	$3.2x10^6$	1.37	$7.0x10^6$	1.47	$8.0x10^4$	1.94
3 (U)	$2.2x10^8$		$2.0x10^8$		$1.3x10^8$		$3.0x10^7$	
3 (T)	$2.8x10^7$	0.89	$3.5x10^7$	0.76	$3.0x10^7$	0.63	$4.2x10^6$	0.86
3 (O)	$5.6x10^6$	1.59	$1.2x10^7$	1.22	$5.0x10^6$	1.41	$3.4x10^5$	1.95

Sample	*S. aureus* (cfu/g)	Reduction (log cycles)	*P. aeruginosa* (cfu/g)	Reduction (log cycles)	*E. coli* (cfu/g)	Reduction (log cycles)	*A. fumigatus* (cfu/g)	Reduction (log cycles)
4 (U)	$5.8x10^7$		$6.3x10^7$		$4.2x10^7$		$3.2x10^7$	
4 (T)	$7.5x10^6$	0.88	$7.4x10^6$	0.93	$5.3x10^6$	0.90	$4.3x10^6$	0.88
4 (O)	$8.2x10^5$	1.85	$3.2x10^6$	1.29	$1.3x10^6$	1.51	$2.1x10^5$	2.19
5 (U)	$7.1x10^7$		$8.0x10^7$		$2.2x10^8$		$8.1x10^6$	
5 (T)	$8.4x10^6$	0.93	$9.1x10^6$	0.94	$3.3x10^7$	0.82	$9.0x10^5$	0.96
5 (O)	5.5x`0^5	2.11	$4.3x10^6$	1.27	$7.0x10^6$	1.49	$8.3x10^4$	1.99

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 5: Effect of soaking in tap water and ozonated water for 15 minutes on the microbial load of date fruits contaminated with *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *A. fumigatus* ATCC 204305

Sample	*S. aureus* (cfu/g)	Reduction (log cycles)	*P. aeruginosa* (cfu/g)	Reduction (log cycles)	*E. coli* (cfu/g)	Reduction (log cycles)	*A. fumigatus* cfu/g)	Reduction (log cycles)
1 (U)	$7.4x10^7$		$3.0x10^8$		$3.1x10^7$		$7.5x10^6$	
1 (T)	$6.8x10^6$	1.04	$4.5x10^7$	0.83	$4.2x10^6$	0.87	$6.7x10^5$	1.05
1 (O)	$5.0x10^5$	2.14	$4.6x10^6$	1.82	$2.3x10^5$	2.13	$4.5x10^4$	2.23
2 (U)	$2.4x10^8$		$8.8x10^7$		$8.2x10^7$		$1.8x10^7$	
2 (T)	$3.2x10^7$	0.87	$1.1x10^7$	0.90	$8.5x10^6$	0.98	$9.0x10^5$	1.31
2 (O)	$9.7x10^5$	2.39	$9.2x10^5$	1.98	$6.6x10^5$	2.09	$7.4x10^4$	2.39
3 (U)	$2.6x10^8$		$3.3x10^8$		$4.0x10^8$		$5.1x10^7$	
3 (T)	$1.7x10^7$	1.18	$4.0x10^7$	0.92	$5.9x10^7$	0.83	$4.6x10^6$	1.05
3 (O)	$1.5x10^6$	2.23	$4.7x10^6$	1.85	$3.0x10^6$	2.12	$2.0x10^5$	2.41
4 (U)	$7.2x10^7$		$1.4x10^8$		$6.3x10^7$		$6.2x10^6$	
4 (T)	$7.5x10^6$	0.94	$8.7x10^6$	1.21	$5.6x10^6$	1.05	$7.3x10^5$	0.93
4 (O)	$4.0x10^5$	2.22	$3.8x10^6$	1.57	$7.2x10^5$	1.94	$4.0x10^4$	2.19
5 (U)	$3.1x10^8$		$8.6x10^7$		$3.2x10^8$		$3.0x10^7$	
5 (T)	$4.4x10^7$	0.85	$7.7x10^6$	1.04	$4.0x10^7$	0.91	$4.2x10^6$	0.86
5 (O)	2.5x`0^6	2.09	$5.2x10^6$	1.21	$4.1x10^6$	1.90	$5.1x10^4$	2.77

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 6: Effect of soaking in tap water and ozonated water for 30 minutes on the microbial load of date fruits contaminated with *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *A. fumigatus* ATCC 204305

Sample	*S. aureus* (cfu/g)	Reduction (log cycles)	*P. aeruginosa* cfu/g)	Reduction (log cycles)	*E. coli* (cfu/g)	Reduction (log cycles)	*A. fumigatus* (cfu/g)	Reduction (log cycles)
1 (U)	$2.4x10^8$		$1.2x10^8$		$4.0x10^8$		$1.7x10^7$	
1 (T)	$3.0x10^7$	0.90	$3.0x10^7$	0.63	$5.2x10^7$	0.88	$2.1x10^6$	0.91
1 (O)	$1.6x10^6$	2.18	$2.2x10^6$	1.74	$3.3x10^6$	2.08	$6.4x10^4$	2.43
2 (U)	$4.1x10^8$		$7.9x10^7$		$7.6x10^7$		$5.9x10^6$	
2 (T)	$3.8x10^7$	1.03	$8.0x10^6$	1.00	$6.7x10^6$	1.05	$5.2x10^5$	1.05
2 (O)	$2.5x10^6$	2.21	$2.3x10^6$	1.54	$5.5x10^5$	2.14	$1.3x10^4$	2.66
3 (U)	$7.8x10^7$		$6.8x10^7$		$3.4x10^8$		$8.0x10^6$	
3 (T)	$8.2x10^6$	0.98	$5.7x10^6$	1.07	$2.2x10^7$	1.19	$6.8x10^5$	1.07
3 (O)	$2.1x10^5$	2.57	$8.7x10^5$	1.89	$2.7x10^6$	2.10	$5.3x10^4$	2.18
4 (U)	$9.0x10^7$		$7.0x10^7$		$2.9x10^8$		$7.5x10^6$	
4 (T)	$8.8x10^6$	1.01	$7.6x10^6$	0.97	$3.2x10^7$	0.95	$6.7x10^5$	1.05
4 (O)	$5.3x10^5$	2.22	$8.0x10^5$	1.95	$4.0x10^6$	1.86	$2.3x10^4$	2.52
5 (U)	$2.6x10^8$		$2.6x10^8$		$6.0x10^7$		$2.5x10^7$	
5 (T)	$3.0x10^7$	0.93	$3.5x10^7$	0.87	$5.8x10^6$	1.02	$3.2x10^6$	0.89
5 (O)	$1.2x10^6$	2.33	$5.3x10^6$	1.69	$3.1x10^5$	2.29	$3.6x10^4$	2.84

U=untreated, T= treated with tap water, O=treated with ozonated water, n.d.=non-detectable

Table 7: Effect of soaking in ozonated water for 5, 15 and 30 minutes on contamination of date fruit samples with some microorganisms

Time (minutes)	Amount of reduction (log cycles)			
	S. aureus	*P. aeruginosa*	*E. coli*	*A. fumigatus*
5	1.85^b	1.27^b	1.48^b	2.00^b
15	2.24^a	1.68^a	2.03^a	2.38^a
30	2.32^a	1.76^a	2.09^a	2.52^a

Means within columns having same letter are not significantly different ($P \leq 0.05$)