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# Reduction of date microbial load with ozone

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**Background:** Date is one of the foodstuffs that are produced in tropical areas and used worldwide. Conventionally, methyl bromide and phosphine are used for date disinfection. The toxic side effects of these usual disinfectants have led food scientists to consider safer agents such as ozone for disinfection, because food safety is a top priority. The present study was performed to investigate the possibility of replacing common conventional disinfectants with ozone for date disinfection and microbial load reduction. **Materials and Methods:** In this experimental study, date samples were ozonized for 3 and 5 hours with 5 and 10 g/h concentrations and packed. Ozonized samples were divided into two groups and kept in an incubator which was maintained at 25°C and 40°C for 9 months. During this period, every 3 month, microbial load (bacteria, mold, and yeast) were examined in ozonized and non-ozonized samples. **Results:** This study showed that ozonization with 5 g/h for 3 hours, 5 g/h for 5 hours, 10 g/h for 3 hours, and 10 g/h for 5 hours leads to about 25%, 25%, 53%, and 46% reduction in date mold and yeast load and about 6%, 9%, 76%, and 74.7% reduction in date bacterial load at baseline phase, respectively. Appropriate concentration and duration of ozonization for microbial load reduction were 10 g/h and 3 hours. **Conclusion:** Date ozonization is an appropriate method for microbial load reduction and leads to an increase in the shelf life of dates.

**Key words:** Date, food safety, methyl bromide, microbial load, ozone

## INTRODUCTION

Date is one of the nutritious foodstuffs used significantly worldwide, especially in tropical and Middle East areas. In Iran, date cultivation has a long history, and nowadays date is one of the most important agricultural productions of Iran. Shahani date is one of the best export dates worldwide.[1] Iran is one of the largest producers of dates with an average production of more than one million tons per year (about 13% of global production).[2] In fresh phase (Rotab) of Shahani date, the meat completely sticks to the skin and has high nectar and is considered to be of best quality. Due to high moisture level there is an increased risk of fermentation and spoilage and at normal temperature (about 20°C – 25°C); therefore, dates should be stored at low temperatures.[3] To increase the shelf life, dates should be protected from risk factors such as microorganisms and other environmental factors.[4] One of the most commonly used chemicals for decreasing date microbial load and storage pests is methyl bromide ($CH_3Br$). Although, methyl bromide is an effective pesticide, it is known to be harmful for the ozone layer. According to Montreal Protocol in 1992, methyl bromide was listed as an ozone depleting substance and was banned in all developing countries until 2015. Nowadays, researchers follow alternate methods for disinfection and insect control of date and other agricultural products.[1,5]

The main reason for the ban of methyl bromide is its damaging effect on the environment. Over 50% of methyl bromide eventually enters the atmosphere during or after the process of fumigation. Bromine ($Br_2$) released into the atmosphere then reacts with ozone molecules and destroys the protective ozone layer in the stratosphere.[5,6] Phosphine ($PH_3$) is another chemical compound used for date disinfection and insect control. Phosphine is more toxic than methyl bromide. According to the studies conducted by international assemblies in America and Europe phosphine may be included in the list of toxic chemicals used illegally.[1] Treatment of food products with active oxygen gas (ozone) has been considered by food product manufacturers as the alternate disinfection method.[6] In 1886, Dehmitenz discovered the capability of ozone for disinfecting contaminated water. In 1982, use of ozone for bottled water was approved by Food and Drug Administration of United Stated of America (US FDA) and in 1997, US FDA included it in the "generally recognized as safe" (GRAS) list. Also, in June 26, 2001 US FDA approved the use of active oxygen as an antibacterial agent in food industries.[7,8] In December 21, 2001 The Food Safety and Inspection Service of U.S. Department of Agriculture (USDA/FSIS) approved use of ozone for meat and poultry, from raw to fresh cooked and pre-packed products.[6] Use of active oxygen in food industries and drinking water was approved by Food and Drug deputy of Ministry of Health and Education of Islamic Republic of Iran

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in November 29, 2003.[1] American National Institute of Health (NIH) restricted exposure to ozone and determined 0.1 ppm and 0.3 ppm ozone concentration per day for long-term and short-term exposure, respectively. Definition of long-term and short-term is inhalation of ozone at work for 8 hours and 5 days per week and inhalation of ozone for 15 minutes, respectively.[1]

Ozone treatment can destroy spores, yeasts, and other pathogens on fruits and vegetables and their products.[1,9-30] Washing strawberry with ozonized water for 2 minutes decreases aerobic mesophile bacteria, mold and yeast up to 92.3% and 91%, respectively.[12] Strawberry ozonization for 2 days at 2°C, decreases mold and yeast growth level up to 15%. However, after transition of ozonized samples to 20°C, microbial load of molds and yeasts increased.[13] It was observed that in peaches and grapes that were ozonized at 0.3 ppm concentration at 5°C for 28 days, mold and yeast microbial load was less than the load in the non-ozonized samples, and ozone had no adverse effects on the fruits. However, after 14 days, mold and yeast level were equal in both samples.[14] In another study, ozonization at 5 ppm for 3 hours decreased fig mesophile bacteria, mold and yeast up to 72%.[17] Moreover, Emer *et al*., showed that ozonization of pepper for 120, 240, and 360 minutes decreased *Escherichia coli* colonies and bacterial activity without any change in the organoleptic properties of the pepper and without any significant difference in its bitterness percentage, taste, flavor, color and palatability.[11]

In their study, Liew and Prange found that ozonization of carrot at 60 ppm within 28 hours reduced *Sclerotinia sclerotorum* load by 50%. Storage of carrot at 15 ppm ozone concentration could reduce microbial load without any adverse effect on physical specifications.[15] On the basis of the results obtained in different studies, it can be summarized that controlled use of ozone could decrease food microbial load and help increase the shelf life of food products. The present study was performed to investigate the possibility of replacing common conventional disinfectants with ozone for date disinfection and microbial load reduction.

## MATERIALS AND METHODS

Shahani dates were used for the experimental study. Ozone generator machine (Mog-10 GH model) was from ARDA Green Technology Company. The oxygen delivery machine was from BNP Ozone Technology Co., Ltd. Produced oxygen gas pressure was 1-1.2 kg/cm$^2$ and its rate was 0.5-1 liter/minute. This machine was set up to produce ozone with 5 g/h and 10 g/h concentrations when oxygen entrance rate was 1 and 2 liters per minute, respectively. The ozone concentrations of 5 g/h and 10 g/h were equal to 2335 and 4670 ppm, respectively. Ninety 100-g packages of Shahani dates were used as samples. First, as a pilot study, 72 samples were divided into three groups (i.e., 0.2 g, 5 g, and 10 g) and 3 samples of each group were ozonized for eight intervals (i.e., 1, 2, 3, 4, 5, 6, 22, and 28 hours) to attain best concentration and time period of ozonization based on maximum microbial load reduction. Then, on the basis of results and experiments conducted by other researchers, considering the time period effect on microorganisms, and observing the microbial, mold and yeast load results of examined dates in previous studies, two time periods (3 and 5 hours) and two concentrations (5 and 10 g/h) were chosen for the main study.

Samples were ozonized for 3 and 5 hours with 5 g/h and 10 g/h concentrations and were packed under vacuum in two-layer polyethylene and polypropylene bags that were sterilized by microwave irradiation.[31] Ozonization and packaging processes were done at ARDA Green Technology Company. Then, samples were transferred to the laboratory of health faculty of Baqiyatallah University of Medical Sciences. The ozonized samples (i.e., samples ozonized with 5 g/h for 3 and 5 hours and samples ozonized with 10 g/h for 3 and 5 hours) and the non-ozonized samples were then placed in an incubator maintained at 25°C and 40°C for 9 months. Microbial load (total count), mold and yeast of ozonized and non-ozonized samples taken as control group were examined and compared at baseline and every 3 months based on national standard of Iran No. 9899,[32] standard No. 8923-1,[33] and standard No. 10899-2,[34] respectively. The data were analyzed using SPSS 17. The level of significance was set at $P < 0.05$ for all analyses. ANOVA test was used to compare five groups at the baseline phase of study. Repeated measurements were used for time trend analysis in each group. *Post hoc* tests were used to compare the two groups at baseline and follow-up examination.

## RESULTS

The present study showed that bacterial count in samples ozonized with 10 g/h concentration within 3 hours was less than the bacterial count in samples ozonized with 5 g/h concentration within 3 and 5 hours ($P < 0.05$). Ozonization with 10 g/h concentration within 3 hours reduced bacterial count up to 98.5%. There was no significant difference between bacterial total count in samples ozonized with 10 g/h concentration within 3 hours and bacterial total count in samples ozonized with 10 g/h concentration within 5 hours [Figure 1].

Ozonization at baseline with 10 g/h concentration for 3 hours was more effective than ozonization with 5 g/h concentration and reduced mold and yeast count up to 52%. There was no significant difference between mold and yeast

count of samples ozonized with 10 g/h concentration for 3 hours and samples ozonized with 10 g/h concentration for 5 hours [Figure 2]. Also, there was no significant difference between mold and yeast count of samples ozonized with 5 g/h concentration for 3 hours and samples ozonized with 5 g/h concentration for 5 hours.

Bacterial count in samples ozonized with 10 g/h concentration within 3 hours after 3 month reduced by about 50% ($P < 0.05$) compared with baseline examination.

Entirely, ozonization at baseline reduced bacterial count by 98.5% ($P < 0.05$) and this reduction continued until final examination (after 9 months) and finally ozonized samples had less microbial load than non-ozonized samples [Figure 3].

In ozonized samples with 10 g/h concentration, mold and yeast count after 3-month maintenance at 25°C reduced ($P < 0.05$) although mold and yeast count after 6 months increased, but nonsignificantly. Mold and yeast count of ozonized samples after 3, 6 and 9 months of maintenance at 25°C reduced by 75%, 74%, and 87%, respectively ($P < 0.05$). In non-ozonized samples, mold and yeast count after 3, 6 and 9 months of maintenance at 25°C reduced significantly ($P < 0.05$). However, at each phase, mold and yeast count was higher than ozonized samples ($P < 0.05$) [Figure 4].

Bacterial count after 3-month maintenance at 40°C reduced by 93% compared with baseline examination. However, bacterial count after 6-month maintenance at 40°C increased nonsignificantly compared with previous examination. Due to sever moisture level reduction and high temperature and damage of the samples after 6 months, examination after 9 month at 40°C was not performed.

Mold and yeast count after 3-month maintenance at 40°C reduced by 87%. However, mold and yeast count after 6-month maintenance at 40°C increased nonsignificantly compared with previous examination because of high temperature of incubation [Figure 5].

## DISCUSSION

The present study showed that ozonization reduced microbial load of date samples in various phases of examination. It means that after ozonization, mold and yeast count of samples reduced by 52.5%. After 3 and 9 months of maintenance at 25°C, mold and yeast count of samples reduced by 88% and about 94% ($1 \times 10^3$ CFU/g) compared with the mold and yeast count of non-ozonized samples ($1.6 \times 10^4$ CFU/g) at baseline examination. This decrease during maintenance may be due to anaerobic condition of date package, because mold can proliferate only in aerobic mode. This microbial load decrease occurred in bacterial count. Thus, ozonization could reduce date microbial load within acceptable limits. These findings are

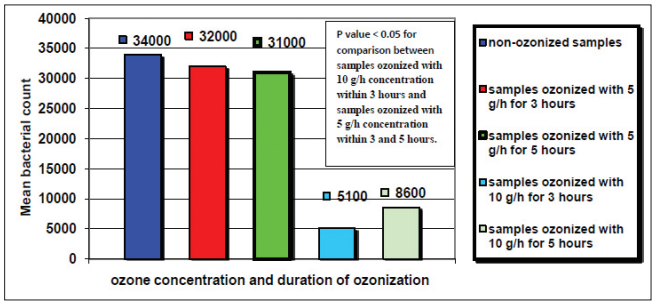


**Figure 1:** Effect of ozone concentration and duration of ozonization on bacterial count of samples at baseline examination

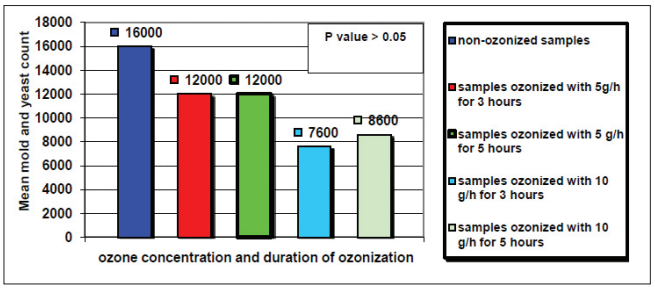


**Figure 2:** Effect of ozone concentration and duration of ozonization on mold and yeast count of samples at baseline examination

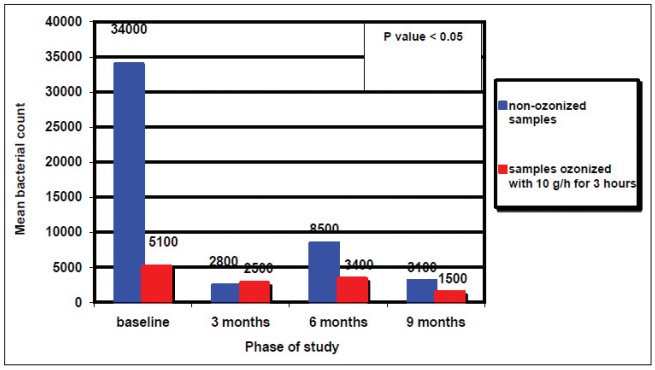


**Figure 3:** Bacterial count variations of samples maintained at 25°C for 9 months

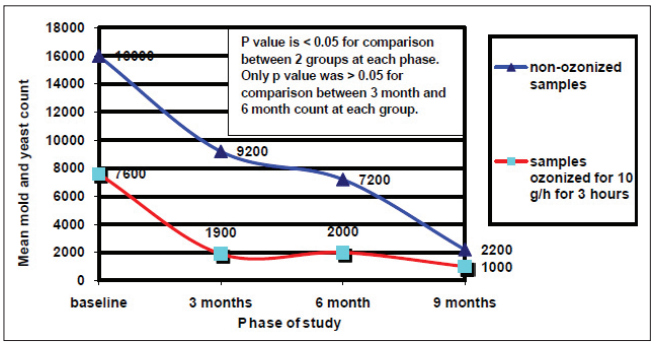


**Figure 4:** Mold and yeast count variations of samples maintained at 25°C for 9 months

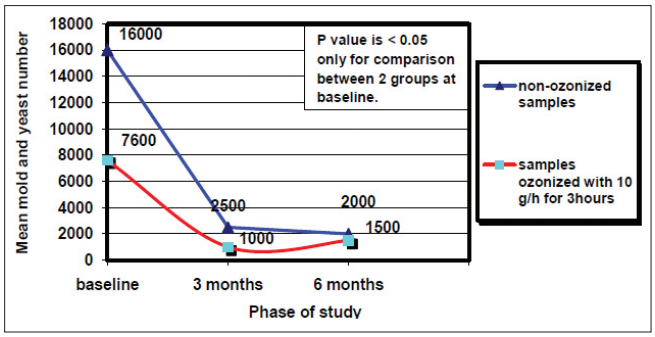


**Figure 5:** Mold and yeast count variations of samples maintained at 40°C for 6 months

consistent with results of the study on dates by Habibi Najafi and Haddad Khodaparast[16] and results of the study by Palou *et al*.,[14] in which they observed the ozonization effect on blue and green molds of fruits stored in cold storage. It was found that ozonization reduced not only the mold and yeast count of ozonized samples but also the bacterial count of the ozonized samples by 98.5% compared with the non-ozonized samples.

In addition to study of ozonization effect on reduction of microbial load, it is necessary to determine ozone concentration and duration of ozonization for maximum saving of time and consumption of ozone in industrial processes. This study showed that ozonization with 5 g/h for 5 hours and 10 g/h for 3 hours was more effective in reducing microbial load compared with other concentrations ($P < 0.05$). However, there was no significant difference between ozonization with 10 g/h and 5 g/h in reducing mold and yeast count, whereas there was significant difference between bacterial counts of samples ozonized with 10 g/h compared with 5 g/h ($P < 0.05$). Thus, ozone concentration is effective on bacterial count. There was no significant difference between ozonization with 10 g/h for 5 hours and 10 g/h for 3 hours in reducing microbial load. However, reduction of mold, yeast, and bacterial count in samples ozonized with 10 g/h for 3 hours was considerable. Thus, based on these results, the best and most appropriate ozone concentration and duration for date ozonization was found to be 10 g/h for 3 hours and ozonization more than 3 hours is not necessary and increased process costs. These results are consistent with results of the study by Zorlugenç *et al*. They found that ozonization reduced fig mesophile bacteria (i.e., *Escherichia coli and Bacillus cereus)* and mold and yeast count by 72%.[17] Our findings are consistent with results of the study on black pepper by Emer *et al*.,[11] in which they recorded the antibacterial effect of ozonization (for 120 minutes, 240 minutes and 360 minutes).

Microbial load of samples maintained at 40°C for 3 months decreased significantly ($P < 0.05$). However, after 6-month maintenance, despite the moisture level reduction, microbial load increased slightly, and finally samples dried and deteriorated and had to be excluded from the study. All types of dates should be kept in cold storage to save them from decomposing and spoilage. In this study, temperature higher than cold storage temperature is used to calculate the shelf life of dates at refrigerator temperature (0°C-5°C). Our experience showed that temperature higher than 25°C should not be used for shelf life determination because of the physicochemical characteristics of dates. The limitation of this study, as we mentioned above, was maintenance of dates at 40°C, which destroyed the date samples and the effect of ozonization could not be studied for longer period.

## CONCLUSION

The results of the present study showed that date ozonization decreased the microbial load in the samples. However, increasing duration of ozonization (more than 3 hours) had no incremental effect on microbial load reduction. According to results of microbial examination of samples maintained at 25°C for 9 months and $Q_{10}$ of chemical and biochemical reaction of food stuffs, ozonization of dates is an appropriate method for microbial load reduction and leads to increased shelf life of dates.

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