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Application of Ozone to Control Dried Fig Pests—*Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and *Ephestia kuehniella* (Lepidoptera: Pyralidae)—and Its Organoleptic Properties

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Abstract

Ozone is a powerful oxidant which can be used for killing insects and microorganisms. In this study, ozone was applied in the gaseous form to control two species of pests in stored dried figs. The samples of figs (50 g each) were infested with adults of *Oryzaephilus surinamensis* L. and larvae of *Ephestia kuehniella* Zeller and were subjected to different combinations of ozone concentrations (2, 3, and 5 ppm) and exposure times (15, 30, 45, 60, and 90 min). Changes in organoleptic properties (color, sweetness, firmness, aroma, and overall acceptability) during ozonation were studied. The results showed that the mortality rate increased with an increase in ozone concentration and exposure time. The total mortality of both pests was achieved at an ozone concentration of 5 ppm and exposure time of 90 min. Sensory evaluation showed that ozone only had a negligible effect on aroma. Therefore, the usage of ozone is recommended during the postharvest process instead of other chemical fumigants, such as methyl bromide and phosphine.

Key words: ozone, dried fig, organoleptic property

Fig is a fruit of *Ficus carica* L., a member of the Moraceae family, and is known as a healthy fruit owing to its high dietary fiber and mineral content. Iran is the fifth-largest producer of figs in the world, and the annual market value is >46.5 million dollars (Food and Agriculture Organization [FAO] 2011). Fresh figs are sensitive to mechanical damage in the postharvest process. They are also susceptible to decay; therefore, the postharvest life of fresh figs is extremely short (Kong et al. 2013). For this reason, figs are usually consumed as dried fruit. Dried figs are one of the most important agricultural exports of Iran (Javanmard 2010). The conventional dried fig storage system leads to considerable quality and quantity losses caused by pests, mainly adults of *Oryzaephilus surinamensis* L. and larvae of *Ephestia kuehniella* Zeller (Oztekin et al. 2001).

Fumigation helps in controlling the insect population and helps preserve the stored product in the warehouse. However, registered fumigants, such as phosphine and methyl bromide, are few, and their use must be restricted owing to the development of pest resistance, health hazards, and risk of environmental contamination (Shadia and Abd El-Aziz 2011). Moreover, chemical pesticides have an irreversible effect on humans and the environment (Na and Ryoo 2000, Sauer and Shelton 2002). An alternative method is to use ozone which decomposes rapidly into molecular oxygen without leaving a residue (Mahapatra et al. 2005, Sousa et al. 2008, Bonjour et al. 2011, Hansen et al. 2012, Miller et al. 2013).

Ozone (O_3) is a triatomic form of oxygen and is referred to as activated oxygen, allotropic oxygen, or pure air. It is an unstable gas, and the half-life of ozone in the air is between 36 and 72 depending on the temperature. Thus, it does not accumulate substantially without a continuous ozone generator (Acton 2013).

Also, ozone can be used for destruction of pesticides and chemical residues (Ong et al. 1996, Hwang et al. 2001). Recently, ozone was used to control storage pests (Sousa et al. 2008, Bonjour et al. 2011, Hansen et al. 2012, Keivanloo et al. 2014). Bonjour et al. (2011) employed ozone against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.), *Cryptolestes ferrugineus* (Stephens), and *O. surinamensis*, they achieved 100% mortality for *S. oryzae* and *T. castaneum* after 2 and 4 d, respectively. The impact of different ozone treatments on the mortality rate of *O. surinamensis* and *E. kuehniella* and their effects on the organoleptic properties of dried figs has not been studied. Therefore, this study aims to 1) determine the effect of ozone concentrations and exposure times on mortality rate of *O. surinamensis* and *E. kuehniella* and 2) investigate the changes in sensory properties (organoleptic properties) of dried figs affected by the ozone treatments.

Materials and Methods

Dried figs were purchased from a local supplier in Mashhad (main city in Khorasan Razavi Province, Northeast of Iran). The pests used in this study, *O. surinamensis* and *E. kuehniella*, were collected from an infested food warehouse in Mashhad. The insects were reared in a laboratory on an artificial diet consisting of 99% wheat flour and 1% yeast for *O. surinamensis* and 90% wheat flour and 10% yeast for *E. kuehniella* (Finkelman et al. 2006). The rearing conditions were 27 ± 1 °C, 65 ± 5% relative humidity (RH), and photoperiod of 13:11 (L:D) h in a germinator (Razi Company, Iran). Adults of *O. surinamensis* and larvae of *E. kuehniella* were used for ozonation experiments.

Ozone was applied with the assistance of a laboratory corona discharge ozone generator (AS-1200 M, Ozoneab Company Inc., Iran), including a control panel to set the generated ozone rate. The system consisted of a 50-liter ozonation tank. The concentration of ozone in the ozonation tank was determined by ozone detector (model LFY-I-5A-W, Logfel Company, China) in the range between 0 and 10 ppm with an accuracy of 0.01.

Cylindrical transparent plastic containers that were covered with mesh on both ends were used for the bioassays. Each container (volume 0.5 liter) was filled with 50 g of figs and 10 individuals of the species being evaluated for 48 h before experiments. For each treatment, 20 such containers were placed in the ozonation tank in the same direction of ozone flow using special fixtures. The ozonation system was run with 15 combinations of 2, 3, and 5 ppm concentration and 15-, 30-, 45-, 60-, and 90-min time periods. For the control treatment, the above containers were exposed to the room air (25 ± 2 °C, 55 ± 5% RH). The tests were arranged in a factorial, complete randomized design with four replications. The experimental runs were performed separately for each species. The mortality rates of the insects were obtained by counting the dead insects 24 h after the ozonation process. The insects were considered dead if they failed to respond to gentle rubbing with a small brush (Vadivambal et al. 2007). The mortality data were corrected for control mortality using Abbott's formula, as described by Rosenheim and Hoy (1989).

Changes in sensory attributes, such as aroma, color, sweetness, firmness, and the overall acceptability of dried figs following ozone treatment were evaluated by the method recommended by Ranganna (1994). A trained 10-member panel (five men and five women), formed from the university community, was selected for sensory evaluation. The dried fig samples were coded and presented randomly in identical containers. The panelists were asked to evaluate their degree of liking or disliking on a seven-point hedonic scale using descriptive categories, ranging from 1–7 (1: extremely dislike, 2: very dislike, 3: dislike, 4: no difference, 5: like, 6: very like, and 7: extremely like). During testing, panel members washed their mouths with water between evaluations.

The effect of insect species, ozone, and exposure time on mortality of insects was statistically analyzed using the SPSS software (version 19.0) by analysis of variance (ANOVA) using 3 factorial design models (2 insect species [adults of *O. surinamensis* and larvae of *E. kuehniella*] × 3 ozone concentrations [2, 3, and 5 ppm] and 5 exposure times [15, 30, 45, 60, and 90 min]. The means comparison was performed using Tukey's HSD test ($\alpha = 0.05$; SPSS 2010).

Results and Discussion

The analysis of variance for the effects of time and ozone concentration on the mortality of *O. surinamensis* adults and *E. kuehniella* larvae are shown in Table 1. The results showed that ozone concentrations and time had separate significant effects on the mortality of adult *O. surinamensis* and larvae *E. kuehniella*, but the interaction of these two factors only affected adult *O. surinamensis* and had no significant effect on larvae of *E. kuehniella*.

The mortality of *O. surinamensis* and *E. kuehniella* at three ozone concentrations and five exposure times is summarized in Table 2. The mortality was in the range of 15.33–100% and 14–100% for *E. kuehniella* and *O. surinamensis*, respectively.

The mortality of both *E. kuehniella* and *O. surinamensis* at ozone concentrations of 2 and 5 ppm for exposure times of 15 and 90 min are statistically significant ($P < 0.05$). No significant differences were observed in the mortality of *E. kuehniella* between exposure times of 15 and 90 min at ozone concentration of 3 ppm. For each exposure time, an increase in ozone concentration from 2 ppm to 3 ppm led to a significant increase in *E. kuehniella*'s mortality.

The mortality rates of *E. kuehniella* for different combinations of ozone concentration and exposure time are shown in Table 2. The minimum level of mortality (15.33%) was at an ozone concentration of 2 ppm and exposure time of 15 min. Complete mortality (100%) was achieved at 5 ppm and 90 min. For the ozone concentration of 5 ppm, the mortality increased from 59.67% to 64.00%,

Table 1. Analysis of variance for the effects of time and ozone concentration on mortality percent of adults of *O. surinamensis* and larvae of *E. kuehniella*

Insect	Source of variation	df	Mean square	F
Adults of *O. surinamensis*	Concentrations	2	5612.356	163.673**
	Time	4	4306.467	125.594**
	Time × concentrations	8	394.717	11.512^{ns}
	Error	30	34.289	
	Total	45		
Larvae of *E. kuehniella*	Concentrations	2	10376.822	330.238**
	Time	4	998.856	31.788**
	Time × concentrations	8	93.739	2.983^{ns}
	Error	30	31.422	
	Total	45		

** Significant ($P < 0.01$).
^{ns} Not significant ($P > 0.01$).

Table 2. Mortality percent of *O. surinamensis* and *E. kuehniella* (mean ± SD) at different combinations of ozone concentration and exposure time

Insect	Time (min)	Ozone concn.		
		2 ppm	3 ppm	5 ppm
Adults of *O. surinamensis*	15	14.00 ± 3.60a	18.67 ± 2.88ab	28.00 ± 5.56abc
	30	18.67 ± 3.51ab	29.67 ± 7.09abc	37.00 ± 7.00c
	45	27.67 ± 9.45abc	39.00 ± 2.64c	74.67 ± 7.57de
	60	36.00 ± 7.93bc	61.00 ± 6.24d	88.67 ± 3.05ef
	90	38.67 ± 2.08c	78.00 ± 9.00de	100.00 ± 0.00f
Larvae of *E. kuehniella*	15	15.33 ± 3.78a	46.33 ± 6.50cd	59.67 ± 4.04cd
	30	23.00 ± 4.00ab	52.33 ± 6.11cd	64.00 ± 5.00de
	45	25.33 ± 6.50ab	54.67 ± 9.45cd	77.67 ± 4.04ef
	60	29.00 ± 7.00ab	56.67 ± 6.50de	86.33 ± 5.13f
	90	34.00 ± 6.55bc	66.33 ± 3.21de	100.00 ± 0.00f

Values with the common letters for each insect are not significantly different ($P > 0.05$).

Table 3. Sensory evaluation of dried fig (mean±SD)

Ozone concn. (ppm)	Time (min)	Color	Aroma	Sweetness	Firmness	Overall acceptability
Control		6.57 ± 1.13a	5.98 ± 0.25a	3.62 ± 0.89a	2.93 ± 1.93a	6.87 ± 0.83a
2	15	6.33 ± 1.21a	5.82 ± 1.47a	3.5 ± 1.51a	2.67 ± 0.63a	6.50 ± 1.22a
	30	6.00 ± 1.21a	5.33 ± 1.16ab	3.17 ± 1.51a	3.17 ± 1.75a	6.45 ± 0.19a
	45	6.17 ± 0.81a	4.17 ± 1.67bc	3.83 ± 1.83a	3.17 ± 1.82a	6.33 ± 1.09ab
	60	5.67 ± 1.63a	3.83 ± 0.89bc	3.69 ± 1.9a	3.00 ± 1.41a	6 ± 0.16ab
	90	5.83 ± 1.15a	3.17 ± 1.68bc	3.33 ± 1.02a	2.89 ± 0.04a	5.83 ± 1.81b
3	15	6.33 ± 0.16a	4.20 ± 1.25ab	3.00 ± 1.46a	3.20 ± 1.09a	6.33 ± 0.47ab
	30	5.67 ± 1.33a	4.17 ± 1.26b	3.63 ± 1.04a	3.17 ± 1.47a	6.17 ± 0.75ab
	45	5.83 ± 1.16a	3.17 ± 1.86bc	3.45 ± 1.21a	2.95 ± 1.21a	6.50 ± 0.91a
	60	5.50 ± 1.65a	2.63 ± 1.05bc	3.33 ± 1.97a	3.67 ± 1.63a	6.17 ± 1.63ab
	90	5.67 ± 1.13a	2.40 ± 1.04c	3.82 ± 1.60a	2.83 ± 1.95a	6.00 ± 1.80ab
5	15	6.33 ± 0.41a	3.00 ± 1.09bc	3.45 ± 1.50a	3.33 ± 1.36a	6.50 ± 1.36a
	30	6.00 ± 1.21a	2.83 ± 0.50bc	3.17 ± 0.94a	3.00 ± 1.67a	6.33 ± 0.09ab
	45	5.83 ± 0.15a	2.50 ± 0.75bc	3.00 ± 1.16a	3.17 ± 1.16a	6.00 ± 1.98ab
	60	5.50 ± 0.83a	2.33 ± 1.08c	3.83 ± 0.83a	2.83 ± 0.98a	6.17 ± 0.83ab
	90	5.45 ± 1.16a	2.17 ± 0.12c	3.87 ± 0.12a	3.00 ± 1.26a	5.83 ± 0.16b

Values with the common letters in the columns are not significantly different ($P > 0.05$).

77.67%, 86.33%, and 100% along with increasing the exposure times from 15 to 30, 45, 60, and 90 min. Therefore, it is proposed to apply 90 min of ozonation to achieve complete mortality.

Table 2 shows the mortality rates of *O. surinamensis* for different ozonation conditions. Similar to the above results, the minimum level of mortality (14%) was at the lowest ozone concentration (2 ppm) and exposure time. Complete mortality (100%) was at the highest ozone concentration (5 ppm) and exposure time, respectively. Actually, the two parameters affect mortality in different ways. These results are depicted in Table 2, which show the effects of ozone concentration and exposure time on the mortality of *E. kuehniella* and *O. surinamensis*.

Our findings are comparable with the results of other researchers. Bonjour et al. (2011) studied ozone fumigation of hard red winter wheat and found that increasing the exposure time may decrease the survival ratios of egg, larvae, and pupa of *Plodia interpunctella* (Hubner). The mortality of Indian meal moth, *P. interpunctella* (17-d-old larvae) at 5 ppm ozone concentration at exposure times of 30, 60, 90, and 120 min were reported to result in mortality rates of 71.66%, 68.33%, 78.33%, and 85%, respectively (Keivanloo et al. 2014). Also, Kells et al. (2001) reported that increasing the exposure time from 3 to 5 d leads to an increase in the mortality rate from 77% to 94.5% in stored maize. For *Tribolium confusum* du Val, mortality rate increased from 50% to 95% as a result of increasing the exposure time from 71.4 h to 151.8 h (Pereira et al. 2008). This study demonstrated that for each level of ozone concentration, the mortality rate increases with an increase in exposure time. Işikber and Öztekin (2009) reported that *T. confusum* larvae with a mortality rate of 86.31%, was more susceptible to ozone than at the pupa stage with a mortality rate of 44.1%. Işikber and Öztekin (2009) found a higher mortality rate at the larval and adult stages compared with the pupa and egg stages of *E. kuehniella* exposed to 13.88 mg/liter ozone for 2 h. Also, the larvae of *E. kuehniella* were more resistant to ozone compared with the adult (Hansen et al. 2012).

Results of sensory evaluation in terms of sensory attributes, such as color, aroma, sweetness, firmness, and overall acceptability are presented in Table 3. The statistical analyses were performed separately for each sensory parameter. No statistically ($P > 0.05$) significant differences could be found for color, sweetness, and firmness between ozonated and nonozonated dried fig samples. These results indicate that dried figs retained their original color, sweetness, and firmness during ozonation. However, ozone treatments had the greatest effect on product aroma as the panelists scored it 2.17 (the dislike category based on the scores explained in the material and methods) for ozone concentration of 5 ppm at an exposure time of 90 min. The sensory evaluation was performed 24 h after the ozone treatment.

In conclusion, the effect of ozone on the mortality of two major pests in dried figs (*O. surinamensis* and *E. kuehniella)* was studied. It was statistically proven that ozone concentration and exposure time had significant effects on mortality. The lowest mortality (15.33% for *E. kuehniella* and 14% for *O. surinamensis*) was observed at an ozone concentration of 2 ppm and exposure time of 15 min. Complete mortality was observed for 90 min at 5 ppm ozone concentration for both insects. The sensory evaluations of ozonated dried figs showed that ozone has no significant effect on color, sweetness, and firmness of dried figs, but its aroma could be reduced in some cases. Considering our results, ozone can thus be a potential fumigant for the disinfestation of stored dried figs. The results of this study are expected to have a significant benefit for dried fig processors.

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