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The influence of gaseous ozone and ozonated water on microbial flora and degradation of aflatoxin B_1 in dried figs

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ABSTRACT

In this study, the effectiveness of gaseous ozone and ozonated water on microbial flora and aflatoxin B_1 content of dried figs were investigated. After dried figs were exposed to13.8 mg L^{-1} ozone gas and 1.7 mg L^{-1} ozonated water for 7.5, 15 and 30 min, variation of aerobic mesophilic bacteria (AMB), *E. coli*, coliform, yeast and mold counts were determined. Before and after ozone treatments molds on dried figs were also isolated and identified. In both ozone treatments, AMB was not exactly inactivated whereas *E. coli* was completely destroyed at 7.5 min. Coliform, and yeast were also destroyed at 7.5 and 15 min in ozonated water, respectively. Ozone applications at 15 min were sufficient for inactivation of all molds. *Aspergillus flavus* and *Aspergillus parasiticus* which cause aflatoxin formation were isolated from dried figs. Artificially contaminated with aflatoxin B_1 samples were also treated with gaseous ozone and ozonated water for 30, 60 and 180 min, respectively. In both of treatments, degradation of aflatoxin B_1 was increased due to increasing of ozonation time. Results indicated that gaseous ozone was more effective than ozonated water for reduction of aflatoxin B_1, whereas ozonated water was affected for decreasing microbial counts.

1. Introduction

Dried fig is one of the most important agricultural export commodities of Turkey. In 2004 45,867 tons of dried figs were exported from Turkey with a total value of US $83 Million that covers about 25% of world dry fig production (Anonymous, 2004). The conventional dried fig production method leads considerable quality and quantity losses caused by microbial pathogens, Aflatoxin B_1 and insect infestations, mainly *Ephestia* spp. (Öztekin et al., 2001). Insect and microbial count of sun dried figs could be higher than permitted levels due to natural growing conditions and poor agricultural practices in cultivation areas. The processing plants provide the dried figs which have wide range of quality from growers. Size and visible appearance of figs under UV light are two important criteria to accept the dry fruit. As even low level of insect or fungal infestations could spread very rapidly in improper storage conditions, suitable precautions should be taken to reduce spoilage risks.

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 ozone in distilled water at 20 °C is about 20–30 min. Thus, it does not accumulate substantially without continual ozone generator (Peleg, 1976; Miller et al., 1978). Ozone has a pungent, characteristic odor described as similar to "fresh air after a thunderstorm" (Coke, 1993). It has a longer half-life in the gaseous state than in aqueous solution (Rice, 1986). Ozone in pure water rather quickly degrades to oxygen, and even more rapidly in impure solution (Hill and Rice, 1982). Ozone is a blue gas at ordinary temperature but at concentrations at which it is normally produced the color is not noticeable. Ozone can be generated by electrical charges in air and is currently used in the medical industry as disinfectant against microorganisms and viruses, as a means of reducing odor, and for removing taste, color, and environmental pollutants in industrial applications (Kim et al., 1999).

Ozone eliminates the handling, storage, and disposal problems of conventionally used post-harvest pesticides. Attractive aspect of ozone is that it decomposes rapidly to molecular oxygen without leaving a residue. These attributes make ozone an attractive candidate for controlling insects and fungi in stored products. At low concentrations ozone protected clean surfaces from subsequent fungal contamination and growth, although higher doses were required to kill fungi on contaminated surfaces (Rice et al., 1982). Five mg kg^{-1} ozone inhibited surface growth, sporulation, and mycotoxin production by cultures of *Aspergillus flavus* link: Fr and *Fusarium moniliforme* Sheldon (Mason et al., 1997).

One of the important usages of ozone in agriculture is the post-harvest treatment of harvested crops. Ozone can be applied to foods as a gas or dissolved form in water. Main purposes of ozone application at post-harvest stage are given below: Inactivation of bacterial growth (Sharma et al., 2002; Achen and Yousef, 2001; Kim and Yousef, 2000; Xu, 1999), prevention of fungal decay (Palou et al., 2002; Perez et al., 1999), destruction of pesticides and

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chemical residues (Hwang et al., 2001; Ong et al., 1996) controlling of storage pests (Mendez et al., 2003; Kells et al., 2001), degradation of aflatoxin from agricultural products (McKenzie et al., 1998).

Developing strategies to control aflatoxin formation and to reduce level of aflatoxin from agricultural commodities is quite significant for about fifteen years. Techniques for the inactivation of aflatoxins include physical (Das and Mishra, 2000), chemical (Moerck et al., 1980; Rodriguez and Mahoney, 1994; Wheeler and Bhatnagar, 1995; Netke et al., 1997) and biological methods (Karunaratne et al., 1990; Brown et al., 1991; Luchese et al., 1992; El-Nezami et al., 1998; Haskard et al., 2000; Horn et al., 2000). Any degradation process must be technically and economically feasible. However, no universally applicable, effective and practical methods are currently available (Peltonen et al., 2000). However, limited research has been performed on reduction of aflatoxins by ozone treatment in dried fruits.

The objective of this study was to evaluate the effectiveness of ozone gas and ozonated water on natural microbial flora and degradation of aflatoxin B_1 in dried figs.

2. Material and method

2.1. Material

Dried Sarılop (Calimyrna) figs were obtained from commercial company (Selcuk Gıda) in Aydın, Turkey. Dried fruits were stored at 4 °C until use.

2.2. Ozon generator

Ozone gas was generated using a laboratory corona discharge ozone generator (Model OZO-1VTT, Ozomax Inc., Canada) from purified extra dry oxygen. The output of generator was 5 grams per hour.

2.3. Chemicals

Aflatoxin (Sigma) standard solution (12.7 mg kg^{-1}) was obtained from Ministry of Agriculture and Rural Affairs Control Laboratory Ankara, Turkey. Methanol, phosphoric acid, *n*-hexane, sodium chloride, dichloromethane, anhydrous sodium sulphate, chloroform, acetonitrile, potassium bromide, nitric acid and all culture media used in this study were purchased from Merck (Darmstadt, Germany). All chemicals were in HPLC or analytical grade. Ultra pure water (Milli-Q system Millipore, Bedford, MA) was used for analytical purposes.

2.4. Method

2.4.1. Ozone application

The experimental set up was consisted of ozone generator, oxygen cylinder, check valve, mass flow-meter, small aquarium stone and 3-L exposure jar. Ozone gas was generated dry oxygen feed gas. The generator is capable of producing 13.8 mg L^{-1} ozone at an oxygen flow rate of 6 L min^{-1} at room temperature. The concentration of ozone solubilizing in water was monitored during ozone treatments by ATI Model Q45H/64 dissolved ozone analyzer.

Ozonation was applied in ozone chamber that consisted of 3 L glass jar equipped with metal lid that consist of inlet and outlet tube connections. For each jar, 200 g of dried fig sample was load. Ozonation was carried out by gaseous ozone and ozonated water. Ozone gas was continuously given to chamber during both treatments. Aqueous ozone was produced by bubbling ozone gas into 3 liter sterile deionized water using small aquarium stone. For microbial study, dried figs were exposed 13.8 mg L^{-1} ozone gas concentration and 1.7 ± 0.17 mg L^{-1} of average concentration of dissolved ozone in water for 7.5, 15 and 30 min of ozonation period.

In order to determine the effect of ozone treatments on reduction of aflatoxin levels in dried figs, 30 g of dried figs were artificially contaminated with AFB_1 (50 μl) at a level of 21 μg kg^{-1} using a standard of AFB_1. Aflatoxin contaminated figs were treated with gaseous ozone (13.8 mg L^{-1} ozone gas concentration) and ozonated water (1.71 ± 0.17 mg L^{-1} of average dissolved ozone concentration) for 30, 60 and 180 min.

Each test was replicated at three times. From each replicated two dried fig samples were randomly selected ($n = 6$). All experimental work with ozone gas and ozonated water was done in a chemical hood. Protective clothes, gloves and mask were worn during the experiments. Microbial and aflatoxin analysis have been carried out immediately after each trial.

2.4.2. Microbial analyses

Microbial analyses were carried out aseptically by mixing 10.0 g dry fig with 90.0 ml sterile 0.1% peptone water using a blender. Serial dilutions from 10^{-1} to 10^{-5} levels were made. 0.5 ml of 10^{-1} dilution and 0.1 ml of the other dilutions was spread onto the surface of selective media. AMB (Nutrient agar), coliform (Violet red bile agar) and yeast/mold (Potato dextrose agar) were enumerated at 30 °C for 48 h, 37 °C for 24 h and 25 °C for 3–5 days, respectively (Anonymous, 2006; Messley and Norrell, 2003). *E. coli* was determined by MPN method on Florocoult lauryl sulfat broth at 37 °C for 24 h (Anonymous, 2006; Williams and Busta, 2000). Molds were identified by examination of colony and cell morphology. For macroscopic examination, isolates were cultured on Malt extract agar, Potato dextrose agar and Dichloran rose bengal chloramphenicol agar. Plates were incubated at 25 °C for 7 days (Samson et al., 2004). Microbial counts were expressed as log CFU g^{-1}.

2.4.3. Extraction of aflatoxin B_1

Fig. (30 g) were extracted according to the BF method with modification. Samples were blended with methanol (75 ml), ultra pure water (7.5 ml) and phosphoric acid (0.75 ml) for 3 min in a Waring blender. Then ultra pure water (22.5 ml) was added into the mixture and blended for 3 min. The eluate was filtered through Whatman No. 4 filter paper. 26.25 ml of filtrate was poured into separating funnel. After *n*-hexane (18.75 ml), ultra pure water (1.87 ml) and sodium chloride (1.87 g) were added; separating funnel was shaken for 3 min. After partition of the phases, methanol–water phase was kept and upper layer was removed. Dichloromethane (33.75 ml) and ultra pure water (7.5 ml) were added into methanol–water phase and shaken for 5 min. Dichloromethane phase was filtered through filter paper (Whatman No. 4) with anhydrous sodium sulphate (3.75 g) (Çoksöyler, 1997; Duman, 2001).

Extracts were concentrated in rotary evaporator (Buchi) at 45 °C until dichloromethane removed. The residue was transferred with chloroform (2 ml) to a coloured vial and evaporated to dryness in a vacuumed oven (Nüve) at 45 °C. The vial with the residue was stored at −18 °C until analysis (Çoksöyler, 1997; Duman, 2001).

2.4.4. Determination of aflatoxin B_1

The residue in vials was redissolved in 1 ml of methanol and 1 ml of ultra pure water before injection. 100 μl of the sample was injected into HPLC. Determinations of AFB_1 levels were carried out by HPLC using the following equipment: A Hewlett Packard HPLC system (Hewlett Packard, Agilent 1100, Palo Alto, USA) equipped with an auto sampler Agilent 1100 Series and a HP Agilent 1100 fluorescence detector; excitation and emission wavelengths were 365 and 435 nm, respectively. The stationery phase was Ace 5 C18 (25 cm × 4.6 mm i.d.) column (Advanced Chromatography Technologies, Aberdeen, Scotland). The mobile phase was the mixture of acetonitrile–methanol–water (2:3:5, v/v/v) with the addition of 120 mg l^{-1} potassium bromide and 350 μl L^{-1} nitric acid. The flow rate was 1 ml min^{-1}. For the post column derivatization a Kobra cell (Rhone Diagnostics, Glasgow, UK) was used to increase the quantification of aflatoxin B_1.

Recovery experiments were performed with the samples non-contaminated with aflatoxins. Dried figs (30 g) were spiked at a level of 21 μg kg^{-1} using a standard AFB_1. Level of AFB_1 was determined with HPLC. Then recovery ratio was calculated.

2.5. Statistical analyses

The data was evaluated by analysis of variance (ANOVA) using SPSS 11.0 (SPSS Inc, Chicago, IL, USA). Duncan's multiple range (DMR) test was used at a significance level of 0.05 (Watts et al., 1989).

3. Results and discussion

3.1. AMB microorganisms

The effectiveness of ozone gas and ozonated water on count of AMB is shown in Fig. 1. AMB microorganism count was reduced by 0.81, 1.0 and 1.42 log CFU g^{-1} at 7.5, 15 and 30 min, respectively. Application of gaseous ozone at 7.5 min had a significant effect on reduction of AMB count ($P < 0.05$), whereas reduction of AMB count at 15 and 30 min were statistically insignificant. In treatment of ozonated water, AMB microorganism count was degreased by 1.49, 2.13 and 2.42 log CFU g^{-1} at 7.5, 15 and 30 min, respectively. Statistical analyses showed that reduction of AMB count was significant in all ozonated water treatments ($P < 0.05$). Comparing with gaseous ozone, treatment of ozonated water was more effective on reduction of AMB count. The differences between two ozone treatments were significant ($P < 0.05$).

Kim et al. (1999) reported that bubbling ozone gas (49 mg L^{-1}, 0.5 L min^{-1}) in a lettuce-water mixture decreased total plate counts by 2 log in 5 min. Kondo et al. (1989) obtained greater than

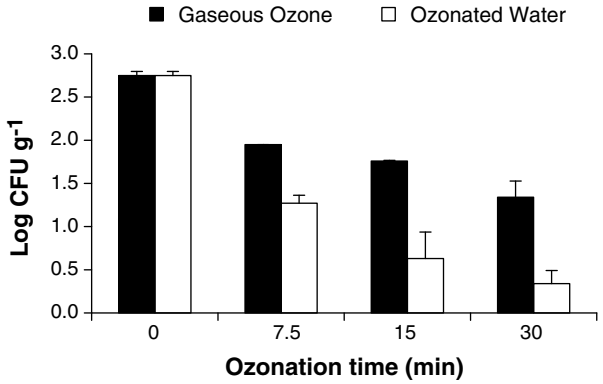

Fig. 1. Effect of gaseous ozone and ozonated water on the growth of AMB.

90% reduction in total bacterial counts for Chinese cabbage by the same washing method. Beltran et al. (2005) also indicated that ozonated water reduced the total mesophilic population by 1.6 log.

3.2. E. coli and coliform

Coliforms, which were used as indicator microorganism of fecal contamination in water and foods, include potentially pathogenic species such as *E. coli*. Therefore, it was necessary to control this microbial group by an effective treatment/process. As shown in Fig. 2, *E. coli* was completely destroyed at 7.5 min after gaseous ozone and ozonated water treatment. Similarly Öztekin et al. (2006) reported that *E. coli* was not detected in ozonated dried fig samples.

The reduction of *E. coli* achieved with gaseous ozone and ozonated water treatments were same at the end of the 7.5 min. This result is contrary to that reported by Singh et al. (2002), who inoculated *Escherichia coli* in lettuce and carrot. They concluded that in comparison to aqueous washing higher log reductions were observed in gaseous ozone treatment. It was supposed that initial load affected the survival rate of microorganisms. Daş et al. (2006) similarly demonstrated that in high microbial load, *S. enteritidis* survived with a decrease but in the case of low microbial load the death was observed by ozonation.

In application of gaseous ozone, reduction of 0.46, 0.84 and 1.84 log CFU g^{-1} were observed in coliform count after 7.5, 15 and 30 min, respectively (Fig. 3). However, coliform were destroyed at 7.5 min in treatment of ozonated water. In this study, exact inactivation of *E. coli* and coliform was provided by ozonated water treatment. Except for *E. coli*, the results were in agreement with Rice et al. (1982). They indicated that gaseous ozone had a lower bacterial effect than ozonated water.

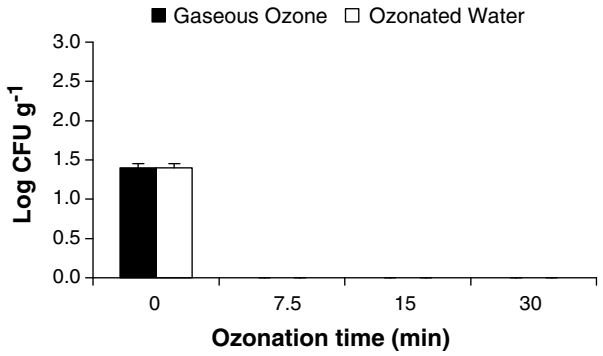

Fig. 2. Effect of gaseous ozone and ozonated water on the growth of *E. coli*.

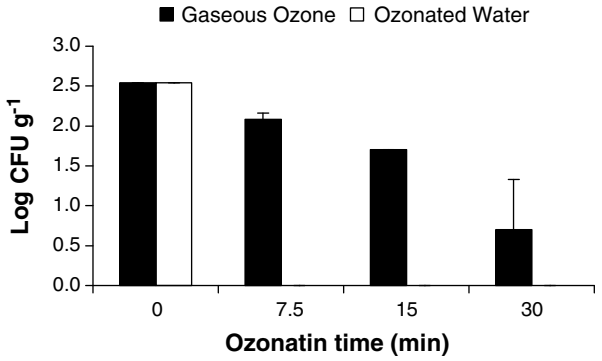

Fig. 3. Effect of gaseous ozone and ozonated water on the growth of coliform.

3.3. Yeasts

In treatment of gaseous ozone, total yeast count was reduced by 0.16, 1.57 and 2.09 log CFU g^{-1} at 7.5, 15 and 30 min, respectively (Fig. 4). Reduction in yeast count was found statistically significant ($P < 0.05$). Ozonated water caused 1.33 log reductions at 7.5 min and none of yeast was detected after 15 min. Comparing with gaseous ozone, treatment of ozonated water was more effective on reduction of yeast count. The differences between two ozone treatments were significant ($P < 0.05$).

3.4. Molds

Total mold count was degreased by 0.59 log CFU g^{-1} at 7.5 min in gaseous ozone, whereas a reduction of 1.73 log CFU g^{-1} was observed in ozonated water at the same period (Fig. 5). In both ozonation process, molds were completely inactivated at 15 min and

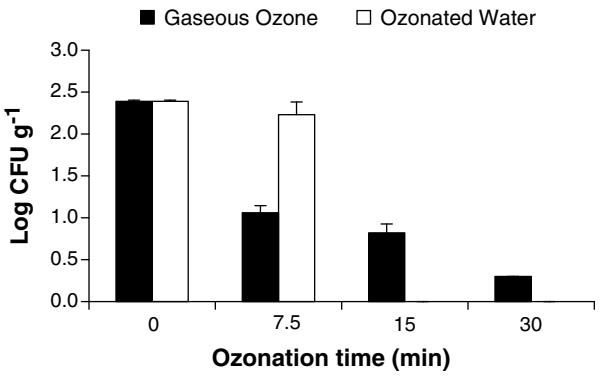

Fig. 4. Effect of gaseous ozone and ozonated water on the growth of yeast.

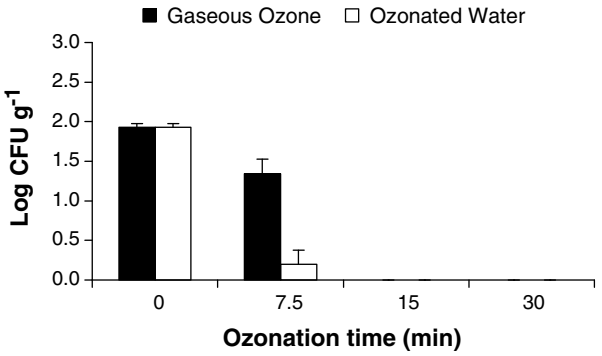

Fig. 5. Effect of gaseous ozone and ozonated water on the growth of molds.

reduction in mold count was found statistically significant ($P < 0.05$).

Molds isolated from dried figs prior to ozone treatments were identified as *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus, Byssochlamyys fulva, Cladosporium clodosporiodes, Mucor hiemalis, Mucor plumbeus Bon. Mucor racemosus Fres, Scopulariopsis bain.* Molds were identified to genera or species by their macro-micromorphology features using appropriate identification keys (Samson et al., 2004).

After 7.5 min gaseous ozone treatment, all molds except *A. parasiticus* and *M. hiemalis* were inactivated, whereas only *A. niger* and *Mucor plumbeus Bon*. were found at 7.5 min in ozonated water. In treatment of neither gaseous ozone nor ozonated water, there were no molds to identify at 15 and 30 min. Gaseous ozone treatment was completely inhibited growth of *Cladosporium* sp. and effectively reduced growth of *Lasiodiplodia* sp. in longan fruit (Whangchai et al., 2006). It has been also indicated that the effect of ozone gas on *Botrytis cinerea* was fungustatic but not fungicidal in carrot (Aguayo et al., 2006). Perez et al. (1999) found 0.35 μl L^{-1} O_3 ineffective for preventing fungal decay in strawberries. However, Palou et al. (2002) pointed out that 0.3 μl L^{-1} O_3 did not prevent decay in peaches although grey mold in table grapes was completely inhibited.

Ozone was thought to kill microorganisms by oxidation of cellular components such as sulphydryl groups and amino acids of enzymes, peptides, proteins and polyunsaturated fatty acids, and oxidation of the cell membrane (Victorin, 1992; Xu, 1999; Young and Setlow, 2004; Daş et al., 2006). Degradation of unsaturated lipids of the cell membrane by ozone was resulted in cell disruption and subsequent leakage of cellular contents. In gram-negative bacteria, destruction of lipoprotein and lipopolysaccharide layers was resulted in increasing cell permeability and eventually cell lysis. Due to potent destruction and damage of nucleic acids, cellular death could be occurred (Escriche et al., 2001; Daş et al., 2006).

Sensitivity of microorganisms to ozone was affected by several factors including the method of applied ozone, strain of the microorganism, physiological state of cells, growth level, pH of the medium, temperature, humidity and presence of other chemicals such as acids, surfactants and sugars. In pure suspensions of bacteria, yeasts, molds, viruses and parasites, low concentrations of ozone and short contact times were sufficient to inactivation. However, the ozone concentration of water was rapidly reduced in presence of organic material in foods (Sharma et al., 2002). The disagreement of most studies on the effectiveness of ozone treatments might be attributed to these findings.

3.5. Variation of aflatoxin B_1 level

Average recovery of aflatoxin B_1 was found to be 82.44% ($n = 6$). According to recovery, aflatoxin levels of the samples were calculated again. The effects of ozone gas and ozonated water on level of AFB_1 were shown in Fig. 6. Gaseous ozone treatment was resulted in a 48.77, 72.39 and 95.21% reduction at 30, 60 and 180 min, respectively. At 30 min, rapid decrease was observed in AFB_1 content whereas a little variation was occurred in ozonated water treatment. Statistical analysis showed that treating time of ozone gas was significant on degradation of aflatoxin B_1 ($P < 0.05$). At the end of 180 min, level of AFB_1 was decreased to 1.01 μg kg^{-1} from 21 μg kg^{-1}.

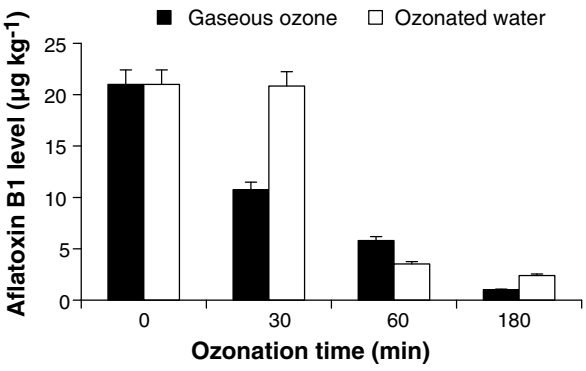

Fig. 6. Effect of gaseous ozone and ozonated water on degradation of aflatoxin B_1 in artificially contaminated dried figs.

McKenzie et al. (1998) achieved reduction of greater than 95% of AFB_1 by treating contaminated corn (30 kg batches) for 92 h with O_3 at 200 mg/min. Besides, storage of cotton seed and peanut meals in ozonized air was resulted in complete destruction of Aflatoxin B_1 within 1 h (Dwarakanath et al., 1968).

In application of ozonated water, reduction of 0.76%, 83.25% and 88.62% were observed in AFB_1 content at 30, 60 and 180 min, respectively. According to initial value, reduction of AFB_1 at 30 min was statistically insignificant. Reduction ratio of AFB_1 was increased at 60 min and this difference was found to be significant ($P < 0.05$). However, there were no significant differences between ozone treatments at 60 and 180 min. AFB_1 level was decreased to 2.39 μg kg^{-1} from 21 μg kg^{-1} after 180 min. Comparing with ozonated water, treatment of ozone gas was more effective on AFB_1 reduction. The differences between two ozone treatments were significant ($P < 0.05$). McKenzie et al. (1997) indicated that aflatoxin B_1 and aflatoxin G_1 were rapidly degraded using 2% O_3, while aflatoxin B_2 and aflatoxin G_2 were more resistant to oxidation and required higher levels of O_3.

4. Conclusion

During the ozonated water process, *E. coli*, coliform, yeast and molds were completely inactivated whereas approximately an 88% reduction was observed in AMB count at the end of the 30 min. In gaseous ozone treatment, *E. coli* and molds population were exactly destroyed. However, substantial reduction in AMB, coliform and yeast counts were determined. *A. flavus and A. parasiticus* which cause aflatoxin formation were isolated from non-ozonated dried figs. Due to inactivation of all molds in dried figs, aflatoxin formation potential was decreased after ozonation process.

The best way to control contamination with aflatoxins is to reduce infestation of agricultural commodities with aflatoxigenic molds. Therefore, new methods to control aflatoxin formation and reduce level of aflatoxin are needed to limit economic losses and health hazards. The experimental results of this research indicated that application of gaseous ozone and ozonated water had significant effect on degradation of AFB_1 in contaminated dried figs.

In this research, organoleptic properties of dried figs were not examined before and after the ozonation process. Barely, initial visual appearance of dried figs was maintained during ozonation. Further investigations will be necessary to determine the effects of ozone on organoleptic properties and nutritional values of dried products and also degradation products of aflatoxins. Consequently, this study suggests that ozonated water can be used in washing treatment or giving a shape before packaging at the fig processing plant. Besides, gaseous ozone can be use in storage rooms to preserve dried figs from undesirable microbial growth, aflatoxin formation and pest damage. In addition, contradictory results previously reported on effects of ozone suggest that the efficacy of ozone must be assessed individually for each commodity.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Achen, M., Yousef, A.E., 2001. Efficacy of ozone against *Escherichia coli* O157:H7 on apples. Journal of Food Science 66, 1380–1384.

Aguayo, E., Escalona, V.H., Artés, F., 2006. Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. Postharvest Biology and Technology 39, 169–177.

Anonymous, 2004. Dried Fig. – Export Statistics of Export Promotion Center of Turkey, IGEME, Available from: http://www.igeme.org.tr.

Anonymous, 2006. Microbiology Manuel. 12th ed. Merck, Germany, 688 p.

Beltran, D., Selma, M.V., Marin, A., Gil, M.I., 2005. Ozonated water extends the shelf life of fresh-cut lettuce. Journal of Agricultural Food Chemistry 53, 5654–5663.

Brown, R.L., Cotty, P.J., Clevel, T.E., 1991. Reduction in aflatoxin content of maize by atoxigenic strains of *Aspergillus flavus*. Journal of Food Protection 54, 623–626.

Coke, A.L., 1993. Mother nature's best remedy: Ozone. Water Conditioning and Purification, 48–51.

Çoksöyler, N., 1997. Extraction ve Extract Cleaning. Mersin University, Engineering Faculty, Food Engineering Department, Seminar Notes, Mersin, 41 p. (in Turkish).

Das, C., Mishra, H.N., 2000. Effect of aflatoxin B_1 detoxification on the physicochemical properties and quality of ground nut meal. Food Chemistry 70, 483–487.

Daş, E., Candan Gürakan, G., Bayındırlı, A., 2006. Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella enteritidis* on cherry tomatoes. Food Microbiology 23, 430–438.

Duman, A.D., 2001. Effects of processing techniques on mould growth and aflatoxin production in Kahramanmaraş and Şanlıurfa types red pepper flakes. Çukurova University Institute of Natural and Applied Sciences, Food Engineering Department, PhD Thesis, 136 p. (in Turkish).

Dwarakanath, C.T., Rayner, E.T., Mann, G.E., Dollear, F.G., 1968. Reduction of aflatoxin levels in cottonseed and peanut meals by ozonization. Journal of the American Oil Chemists' Society 45, 93–95.

El-Nezami, H., Kankaapaa, P., Salminen, S., Ahokas, J., 1998. Physicochemical alterations enhance the ability of dairy of strains of lactic acid bacteria to remove aflatoxin from contaminated media. Journal of Food Protection 61, 466–468.

Escriche, I., Serra, J.A., Gomez, M., Galotto, M.J., 2001. Effect of ozone treatment and storage temperature on physicochemical properties of mushrooms (*Agaris bisporus*). Food Science and Technology International 7, 251–258.

Haskard, C., Binnion, C., Ahokas, J., 2000. Factors affecting the sequestration of aflatoxin by *Lactobacillus rhamnosus* strain GG. Chemico–Biological Interactions 128, 39–49.

Hill, A.G., Rice, R.G., 1982. Historical background properties and applications. In: Rice, R.G. (Ed.), Ozone Treatment of Water for Cooling Application. Ann Arbor Science Publishers, Ann Arbor, MI, pp. 1–37.

Horn, B.W., Greene, R.L., Dorner, J.W., 2000. Inhibition of aflatoxin B_1 production by *Aspergillus parasitucus* using nonaflatoxigenic strains: role of vegetative compatibility. Biological Control 17, 147–154.

Hwang, E.S., Cash, J.N., Zabik, M.J., 2001. Postharvest treatments for the reduction of mancozeb in fresh apples. Journal of Agricultural Food Chemistry 49, 3127–3132.

Karunaratne, A., Wezenberg, E., Bullerman, L.B., 1990. Inhibition of mould growth and aflatoxin production by *Lactobacillus* spp. Journal of Food Protection 53, 230–236.

Kells, S.A., Mason, L.J., Maier, D.E., Woloshuk, C.P., 2001. Efficacy and fumigation characteristics of ozone in stored maize. Journal of Stored Products Research 37, 371–382.

Kim, J.G., Yousef, A.E., 2000. Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone. Journal of Food Science 65, 521–528.

Kim, J.G., Yousef, A.E., Chism, G.M., 1999. Use of ozone to inactivate microorganisms on lettuce [Cited by: Xu, L., 1999. Use of ozone to improve the safety of fresh fruits and vegetables]. Food Technology 53, 58–61.

Kondo, F., Utoh, K., Rostamibashman, M., 1989. Sterilizing effect of ozone water and ozone ice on various microorganisms [Cited by: Xu, L., 1999. Use of ozone to improve the safety of fresh fruits and vegetables]. Food Technology 53, 58–61.

Luchese, R.H., Martins, J.F.P., Harrigan, W.F., 1992. Aflatoxin production in a meat mix model system in the presence of *pediococcus* and *lactobacillus*. Journal of Food Protection 55, 583–587.

Mason, L.J., Woloshuk, C.P., Maier, D.E., 1997. Efficacy of ozone to control insects, moulds and mycotoxins. In: Donahaye, E.J., Navarro, S., Varnava, A. (Eds.), Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products. Nicosia, Cyprus Printer Ltd., Nicosia, pp. 665–670.

McKenzie, K.S., Sarr, A.B., Mayura, K., Bailey, R.H., Miller, D.R., Rogers, T.D., Norred, W.P., Voss, K.A., Plattener, R.D., Kubena, L.F., Phillps, T.D., 1997. Oxidative degradation and detoxification of mycotoxins using a novel source of ozone. Food and Chemical Toxicology 35, 807–820.

Mckenzie, K.S., Kubena, L.F., Denvir, A.J., Rogers, T.D., Hitchens, G.D., Bailey, R.H., Harvey, R.B., Phillips, S.A., Buckley, T.D., 1998. Aflatoxicosis in turkey poultry is prevented by treatment of naturally contaminated corn with ozone generated by electrolysis. Poultry Science 77, 1094–1102.

Mendez, F., Maier, D.E., Mason, L.J., Woloshuk, C.P., 2003. Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. Journal of Stored Products Research 39, 33–44.

Messley, K., Norrell, S.A., 2003. Microbiology Lab Manual, second ed. Benjamin Cummings. p. 288.

Miller, G.W., Rice, R.G., Robson, C.M., Scullin, R.L., Kuhn, W., Wolf, H., 1978. An assessment of ozone and chlorine dioxide technologies for treatment of municipal water supplies. US Environmental Protection Agency Report No. EPA-600/2-78-147. Washington DC: US Government Printing Office.

Moerck, K.E., McElfresh, P., Wohlman, A., Hilton, B.W., 1980. Aflatoxin destruction in corn using sodium bisulfite, sodium hydroxide and aqueous ammonia. Journal of Food Protection 43, 571–574.

Netke, S.P., Roomi, M.W., Tsao, C., Niedwiecki, A., 1997. Ascorbic acid protects Guinea pigs from acute aflatoxin toxicity. Toxicology and Applied Pharmacology 143, 429–435.

Ong, K.C., Cash, J.N., Zabik, M.J., Siddiq, M., Jones, A.L., 1996. Chlorine and ozone washes for pesticide removal from apples and processed apple sauce. Food Chemistry 55, 153–160.

Öztekin, S., Başçetinçelik, A., Soysal, Y., 2001. Processing of Some Agricultural Crops having Export Potential. İstanbul Trade Chamber, Turkish. No: 2001-38. 142 p.

Öztekin, S., Zorlugenç, B., Kıroğlu Zorlugenç, F., 2006. Effects of ozone treatment on microflora of dried figs. Journal of Food Engineering 75, 396–399.

Palou, L., Crisosto, C.H., Smilanick, J.L., Adaskaveg, J.E., Zoffoli, J.P., 2002. Effect of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. Postharvest Biology and Technology 24, 39–48.

Peleg, M., 1976. The chemistry of ozone in the treatment of water. Water Research 10, 361–365.

Peltonen, K.D., El-Nezami, H.S., Salminen, S.J., Ahokas, J.T., 2000. Binding of aflatoxin B_1 by probiotic bacteria. Journal of the Science of Food and Agriculture 80, 1942–1945.

Perez, A.G., Sanz, C., Rios, J.J., Olias, R., Olias, J.M., 1999. Effects of ozone treatment on postharvest strawberry quality. Journal of Agricultural Food Chemistry 42, 1652–1656.

Rice, R.G., Farquhar, J.W., Bollyky, L.J., 1982. Review of the applications of ozone for increasing storage times of perishable foods. Ozone Science and Engineering 4, 147–163.

Rice, R.G., 1986. Application of ozone in water and waste water treatment. In: Rice, R.G., Browning, M.J. (Eds.), Analytical aspects of ozone treatment of water and waste water. The Institute, Syracuse, NY, pp. 7–26.

Rodriguez, S.B., Mahoney, N.E., 1994. Inhibition of aflatoxin production by surfactants. Applied and Environmental Microbiology 60, 106–110.

Samson, R.A., Hokestra, E.S., Frisuad, J.C., 2004. Introduction to Food and Airbone Fungi, seventh ed. ASM Press. 389 p.

Sharma, R.R., Demirci, A., Beuchat, L.R., Fett, W.F., 2002. In activation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment. Journal of Food Protection 65, 447–451.

Singh, N., Bhunia, A.K., Stroshine, R.L., 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequence washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. Lebensm-Wiss. u.-Technol. 35, 720–729.

Victorin, K., 1992. Review of genotoxicity of ozone. Mutation Research 227, 221–238.

Watts, B.M., Ylimaki, G.L., Jeffery, L.E., Elias, L.G., 1989. Basic sensory methods for food evaluation. The International development research center Ottowa, Canada, (160 p).

Whangchai, K., Saengnil, K., Uthaibutra, J., 2006. Effect of ozone combination with some organic acids on the control of postharvest decay and pericarp browning of longan fruit. Crop Protection 25, 821–825.

Wheeler, M.H., Bhatnagar, D., 1995. Inhibition of aflatoxin production by *Aspergillus flavus* with pentachlorobenzyl alcohol, phthalide and pyroquilon. Pesticide Biochemistry and Physiology 52, 109–115.

Williams, M.G., Busta, F.F., 2000. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Most probable number (MPN) encyclopedia of food microbiology, vol. 3. Academic Press, pp. 2166–2168.

Xu, L., 1999. Use of ozone to improve the safety of fresh fruits and vegetables. Food Technology 53, 58–63. 0-12-227070-3.

Young, S.B., Setlow, P., 2004. Mechanism of *Bacillus subtilis* spore resistance to and killing by aqueous ozone. Journal of Applied Microbiology 96, 1133–1142.