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Research Article

Efficacy of Ozone in Combination with UV for Inactivation of Selective Foodborne Pathogens

Divya Kumari[1], R Naveen Kumar[1], V Bhaskar[2], DP Chakravarthy[3] and V Sudershan Rao[1]*

[1]*Food and Drug Toxicology Research Center, National Institute of Nutrition, Indian Council of Medical Research (ICMR), Jamai Osmania, Tarnaka, Hyderabad, India*

[2]*Division of Bio-Statistics, National Institute of Nutrition, Indian Council of Medical Research (ICMR), India*

[3]*Easavyasa technologies Pvt. Ltd, Hyderabad, India*

***Corresponding Author:** V Sudershan Rao, Scientist-E, Food Toxicology Research Center, National Institute of Nutrition, Indian Council of Medical Research (ICMR), Jamai-Osmania, Tarnaka, Hyderabad, India.

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Abstract

Ozone is a powerful antimicrobial agent that is suitable for application in food. In this study effect of gaseous ozone alone of different concentrations (0.2, 0.8 and 1.4 ppm/second) for four different time periods (5, 10, 15 and 20 minutes) and in combination with UV on strains of *Salmonella spp. Staphylococcus aureus* was studied and a reduction in the total bacterial log reduction were examined.

The results indicated that the combination of ozone at 0.2 ppm for 20 min and ultraviolet light ($p < 0.05$) was effective against *Salmonella spp.* whereas solely ozone at 0.8 ppm for 20 min ($p < 0.05$) was effective against *Staphylococcus aureus*. Exposure of ozone in combination with ultraviolet light has been applied directly to foodborne pathogens. Additional research is needed to elucidate the mechanisms of ozone in combination with UV to optimize its use in food application.

Keywords: Foodborne pathogens; Ozone; Salmonella spp; Staphylococcus aureus; Ultraviolet light

Introduction

Ozone is well known for its strong oxidizing capacity and has been recognized as a powerful antimicrobial agent, reacting with organic substances approximately 3000 times quicker than chlorine [1]. It can effectively kill viruses, bacteria, fungi and parasites, including those causing food spoilage or human diseases. Ozone can be generated directly from water, air and pure oxygen by several methods, the most efficient being corona discharge. It can be used in gaseous and aqueous state. Ozone has the potential to fill a substantial gap in today's technologies that are used to ensure food safety. The use of ozone in food processing has become increasingly important as a result of the affirmation of ozone as GRAS (Generally Recognized as Safe) chemical [2] and its subsequent approval by the United States Food and Drug Administration (US FDA) as an antimicrobial additive for direct contact with foods of all types [3].

Efficacy of ozone, however, depends on the target microorganism and the treatment conditions. Ozone can be applied to foods as a gas or as a dissolved form in water. Ozone was applied to reduce fungal deterioration of blackberries and grapes [4]. It also appeared to inhibit growth and caused the death of gram-negative and gram-positive tested bacteria, this study also supported the proposed mechanism of the bacterial inactivation by ozone that caused cell membrane destruction and finally lysis reaction [5].

Ozone has been used routinely for washing and storage of fruits and vegetables [6,7]. Rinsing with dissolved ozone effectively removes of residual pesticides from vegetables [8]. Short exposure of ozone could be successfully used for reducing the coli form and *Staphylococcus aureus* of date fruits, but longer exposure times are required for elimination of the total mesophilic bacteria as well as yeast/mold [9].

Ozone was able to inactivate bacteria in tomatoes after only 3 minutes [10].

The overall objective of this research was to determine the effect of ozone treatment in combination with UV on selective food borne pathogens (both gram positive and gram negative).

Materials and Method

Fumigator

Fumigator is the instrument developed by Eesavyasa Technologies Pvt. Ltd. for the generation of ozone and UV. Basically the fumigator is an airtight chamber receives the ozone and pulsed UV light. The dimensions of the fumigator are 8 mm in length, 600 mm height and 500 mm breadth. The fumigator connects to a small instrument called ozonator where ozone is released into the fumigator. The ozone output of the ozonator was ranged from 0.2 ppm to 1.4 ppm. The ozonator also consists of a

frequency knob where the frequency of pulsed ultraviolet light can be increased or decreased. Pulsed UV was measured by pulse per second and it varied from 300 Hz to 14.7 KHz.

Bacterial Strains

The pure culture of *Salmonella spp.* (MTCC-1162) was obtained from the Microbial Type Culture Centre (MTCC), Chandigarh, India and *Staphylococcus aureus* (ATCC-700699) was from the American Type Culture Collection, USA.

Preparation of Pure Culture

All the strains of pure culture *Salmonella spp*. were initially inoculated on to Nutrient broth procured from HIMEDIA and was kept for incubation at 37ºC for 24 hr. After the incubation, the bacterial culture was sub-cultured on Nutrient Agar slant and was maintained on Nutrient Agar slant till further experiment. While all strains of pure culture Staphylicoccus spp. was initially inoculated on to Brain Heart Infusion broth and was sub-cultured on Brain Heart Infusion media and was maintained on the same till further experiment.

Inoculation of Bacteria

For the experiment a loopful of individual bacterial culture was inoculated into Nutrient broth and incubated at 37ºC for 24 hours. After incubation 1ml of the exponential growth phase was taken for serial dilution. Serial dilution was done in 0.85% saline solution. The dilution 10-3 was taken for the further experiment which yielded 886 cfu/ml (2.9log) of respective bacteria. After serial dilution 0.1 ml of bacterial culture (10-3) was placed on Nutrient agar for *Salmonella spp*. and on Brain Heart Infusion media for *Staphylococcus aureus* by spread plate method. One set of Petri-plates were kept in the incubator as control and the other set was exposed to disinfection treatments. The plating of each culture was done in duplicates.

Disinfection Treatments

Ozone

The inoculated plates were exposed in the fumigator with ozone for about 5, 10, 15 and 20 min. The amount of ozone released during the exposure was 0.2, 0.8 and 1.4 ppm/second. After the exposure, the plates were kept back in the incubator at 37ºC for 24 hr.

Ozone and UV

It was carried out in fumigator having the input of ozone from 0.2 p.m. to1.4 p.m./sec and pulsed UV light 0.380 pulses/sec/103 (fixed). The inoculated plates were exposed to ozone and ozone and UV at three different concentrations, i.e. 0.2, 0.8 and 1.4 ppm/second for about 5, 10, 15 and 20 min.

Microbiological Analysis

After the incubation the colonies of control and test plates were counted and tabulated. The effect of different treatments was counted in terms of log reduction and interpreted in terms of percent reduction of the particular microorganism, for specific concentration and specific time.

Statistical Analysis

It was done using SPSS Software; p-value less than 0.05 were considered statistically significant. In case of ozone exposure and in combination with UV, repeated measure of ANOVA was used to check the mean difference at 95% confidence interval at five different points. One way ANOVA was used to check for percent reduction differences in different groups.

Results

The study on the effect of ozone in combination with UV in reducing selective foodborne pathogens demonstrated its effectiveness against *Salmonella* and *S. aureus*. Combination of ozone and UV caused lethality that was greater than the effect of ozone applied individually Similar kind of study [11] indicated that the combination of UV and ozone had significantly more effective in killing microorganisms on the calyx of blueberries than UV alone indicating a synergistic effect.

Effect of Ozone on Food Borne Pathogens

Effect of Ozone on Gram-Negative (Salmonella Spp.) Bacteria

Salmonella spp. was exposed to ozone at different concentration i.e. 0.2 ppm, 0.8 ppm and 1.4 ppm (mg/L/second) and at different time points i.e. 5, 10, 15 and 20 minutes. Among all the three concentrations, exposure to 1.4 ppm was very much effective and resulted in more than 1log reduction (> 90%) of the bacteria when exposed for 15 and 20 minutes (Table 1).

Ozone	Exposure time (min.)	Ozone concentration (ppm)					
		0.2		0.8		1.4	
		Mean ± SD	Log reduction	Mean ±SD	Log reduction	Mean ±SD	Log reduction
	0	3.17 ± 0.03	3.04	3.17 ± 0.03	3.04	3.17 ± 0.03	3.04
	5	3.06 ± 0.05	3.1	2.80 ± 0.22	2.80	2.91 ± 0.01	2.91
	10	2.46 ± 0.07	2.4	2.30 ± 0.29	2.30	2.52 ± 0.04	2.52
	15	1.84 ± 0.05	1.8	2.23 ± 0.02	2.23	2.03 ± 0.07	2.00
	20	1.52 ± 0.19	1.5	0.66 ± 0.57	1.00	1.76 ± 0.05	1.99

Table 1: Effect of exposure time and ozone concentration on gram-negative bacteria (log reduction).

Effect of Ozone on Gram-Positive (Staphylococcus Aureus) Bacteria

The effect of ozone on gram positive bacteria indicated that a significant reduction was observed in Staphylococcus aureus i.e. more than 2log reduction (> 95%) when exposed to 0.8 and 1.4 ppm concentration for 20 minutes (Table 2).

Ozone	Exposure time (min.)	Ozone concentration (ppm)					
		0.2		0.8		1.4	
		Mean ± SD	Log reduction	Mean ±SD	Log reduction	Mean ±SD	Log reduction
	0	2.58 ± 0.15	2.60	2.58 ± 0.15	2.60	2.58 ± 0.15	2.84
	5	2.53 ± 0.09	2.53	2.49 ± 0.0	2.49	2.80 ± 0.04	2.80
	10	2.41 ± 0.03	2.41	2.29 ± 0.35	2.59	2.51 ± 0.03	2.51
	15	2.36 ± 0.02	2.36	2.25 ± 0.02	2.25	2.26 ± 0.01	2.26
	20	2.09 ± 0.19	2.09	0.87 ± 0.04	0.87	1.12 ± 0.07	0.54

Table 2: Effect of exposure time and ozone concentration on gram-positive bacteria (log reduction).

Effect of Ozone and UV on Food Borne Pathogens

Effect of Ozone and UV on Gram-Negative Bacteria

The effect of ozone and UV on *Salmonella spp*. were almost equally effective i.e. 2log reduction (~ 99%) at all the three concentrations i.e. 0.2, 0.8 and 1.4 ppm with exposure for 15 and 20 minutes (Table 3).

Ozone + UV	Exposure time (min.)	Ozone concentration (ppm)					
		0.2		0.8		1.4	
		Mean ± SD	Log reduction	Mean ±SD	Log reduction	Mean ±SD	Log reduction
	0	3.17 ± 0.03	3.19	3.17 ± 0.03	3.02	3.17 ± 0.03	2.56
	5	2.91 ± 0.007	2.91	2.92 ± 0.21	2.92	2.33 ± 0.01	2.33
	10	1.69 ± 0.06	1.69	1.50 ± 0.09	1.50	1.39 ± 0.02	1.39
	15	1.34 ± 0.06	1.34	0.99 ± 0.55	0.99	0.345 ± 0.48	0.34
	20	0.0	0.00	1.00 ± 0.14	1.00	0 0	0.00

Table 3: Effect of Exposure Time and Ozone Concentration in Combination with UV on Gram-Negative Bacteria (Log Reduction).

Effect of Ozone and UV on Gram-Positive Bacteria

Combination of ozone and UV was not that much effective against Staphylococcus aureus. It was effective less than 1log reduction (~ 60%) for all the three concentrations i.e. 0.2, 0.8 and 1.4 ppm when exposed for 20 minutes. All the above three concentrations were negligibly effective when exposed for 5, 10 and 15 minutes (Table 4).

Ozone + UV	Exposure time (min.)	Ozone concentration (ppm)					
		0.2		0.8		1.4	
		Mean ± SD	Log reduction	Mean ±SD	Log reduction	Mean ±SD	Log reduction
	0	2.58 ± 0.15	2.88	2.58 ± 0.15	2.87	2.58 ± 0.15	2.86
	5	2.77 ± 0.02	2.77	2.84 ± 0.04	2.84	2.86 ± 0	2.86
	10	2.75 ± 0.02	2.75	2.77 ± 0.04	2.77	2.84 ± 0	2.84
	15	2.68 ± 0.04	2.68	2.74 ± 0	2.74	2.81± 0	2.81
	20	2.45 ± 0.08	2.45	2.47 ± 0	2.47	2.31 ± 0.01	2.37

Table 4: Effect of exposure time and ozone concentration in combination with UV on gram-positive bacteria (log reduction).

Discussion

We demonstrated that ozone in combination with UV is effective against *Salmonella* and *S. aureus.* The bactericidal effect of ozone has been studied on a variety of organisms, including gram-positive and gram-negative bacteria as well as spores and vegetative cells [12, 13]. In this present study effect of ozone in combination with UV was checked for both gram-positive (*Staphylococcus aureus*) and gram-negative (*Salmonella spp*.) bacteria and its bactericidal effect. In a similar study Moore., *et al* [14] have shown that ozone was more effective against Staphylococcus aureus and E. Cole when exposed at a concentration of 5 ppm compared to 2 ppm and 4 hour exposure of ozone was more bactericidal than 1 hour exposure, but in the present study ozone was effective in reducing more than 90% of the both gram-positive and gram-negative bacteria even at the concentration of 1.4 ppm and exposure time was only 20 minutes.

Inactivation by ozone is a complex process which involves ozone acting upon various cell-membrane and wall constituents (e.g. unsaturated fats) along with cell content constituents (e.g. enzymes and nucleic acids). Ozone is responsible for the oxidation of lipids in the cell; it acts on unsaturated lipids of the cell membrane, and in the lipopolysaccharides coat of gram-negative bacteria [15]. Ozone may affect membrane glycoprotein or glycolipids [16], membrane bound enzymes [11] and oxidation of double bonds by singlet oxygen found in the cell [17], and possibly may damage proteins and DNA [11]. In the current study ozone was found to be more detrimental against gram-negative bacteria than gram-positive bacteria and was supported by a study [12] to effect of ozone on food-related microorganisms and observed that gram-negative bacteria were substantially more sensitive to ozone in pure water than were the gram-positive ones including *L. monocytogenes.*

The efficacy of ozone may be increased by use in combination with other technologies. The production of UV photons of different wavelengths has been proposed to be involved in dimerizing the thymine bases of DNA including that of spores [18].

The efficacy of ozone and ultraviolet light, used in combination, to inactivate *Listeria monocytogenes* in fresh and spent chill brines was determined. Ozonation for sufficient time had considerable listericidal activity in fresh brines and spent brines and when combined with UV treatment, is effective reducing L. *monocytogenes* to undetectable levels in fresh brines [19]. In a study inactivation of E. coli and bacteriophage MS2 by UV, ozone, UV/ozone co-exposure, and sequential UV-ozone and ozone-UV exposures was investigated and compared. These results show that the combination of UV and low-dose ozone is a promising technology for securing the microbiological quality of water [20]. In a study, shell eggs externally contaminated with *Salmonella spp*. was treated with combinations including UV followed by ozone treatment resulted in synergistic inactivation of *Salmonella spp.* by 4.6 log units or more in about 2 min of total treatment time. Salmonella was effectively inactivated on shell eggs in a short time and at low temperature with the use of a combination of UV radiation and ozone [21]. In the present study combination of ozone and UV was more effective than ozone alone, the combination has yielded the similar percent reduction at lower concentration (0.2 ppm) and less time period exposure (15 min.) when compared to only ozone exposure.

In the present study, the combination of ozone and UV was more effective against gram-negative bacteria (~ 95%) when compared gram-positive bacteria (~ 60%) and was supported by a study carried out by Restaino., *et al.* [12] which has shown that gram-negative bacteria are more sensitive to UV light rather than gram-positive bacteria. The composition of the cell wall in gram-negative bacteria do not offer any protection to the cells when they are exposed to different sanitizers, which act directly on the cellular inner structure.

Conclusion

Combination of ozone and UV was (~ 100%) effective against *Salmonellaspp*. when exposed for 20 min at 0.2 ppm concentration of ozone. Ozone was ~ 100% effective against Staphylococcus aureus at 0.8 ppm concentration when exposed for 20 minutes. We also found that exposure of ozone alone has shown more than 90% reduction in bacterial count for both gram-positive and gram-negative bacteria while the combination of ozone and UV was more effective against gram-negative bacteria (~ 100%) than gram-positive bacteria (~ 65%). Exposure of ozone and in combination with UV has been applied directly to foodborne pathogens so before recommendation it should be checked with food sample. Further work has to be done to elucidate the mechanisms of ozone in combination with UV to optimize its use in food application.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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