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EFFICACY OF OZONE TO REDUCE TOTAL VIABLE COUNT, YEAST AND MOULD COUNT, COLIFORM COUNT AND ENTEROBACTERIACEAE COUNT IN RAW ONION AND DEHYDRATED ONION PRODUCTS

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Abbreviations

Total Viable Count: TVC, Yeast and Mould Count: YMC, Coliform Count: CC, Enterobacteriaceae Count: EC, Before treatment: initial, (before treatment Log CFU count – after treatment Log CFU count) = Log CFU reduction

Dehydrated Onion: DHO, Kibbled Onion: KO, Minced Onion: MN, Granulated onion: GR, Onion Standard powder: SP. Standard Error of Mean: SEM.

Abstract

Microbial food safety and quality issues are of great importance in food processing industry. Various antimicrobial agents including disinfectant used in food processing industry should ensure the quality and microbiological safety of food, also they should not endanger the health through toxic intermediate products. Ozone molecule is a strong oxidant and effective agent to destroy microorganisms. The objective of this study is associated with treatment and effect of ozone to reduce microbial population in raw and dehydrated onion products. Current research focused on determination of the ozone concentration and contact time to reduce microbial load on raw onion and dehydrated onion products. Microbial load in terms of TVC, YMC, CC and EC of raw and dehydrated onion products was monitored initially and after ozone treatment. The average maximum count before and after treatment in terms of Log CFU/g reduction was calculated. Effect of ozone experimentally carried out at three different concentrations of (0.3, 0.5 and 1 ppm) with respect to three different exposure times (1, 3 and 5 minutes), for raw onion, DHO, KO, MN, GR and SP independently. For raw onion 1 ppm ozone for 3 minutes was collectively effective to reduce TVC, YMC, CC, and EC Log CFU/g. For DHO 1ppm ozone for 3 minutes reduced TVC and CC whereas 0.5 ppm for 5 minute reduced, YMC and EC. For GR, SP, KO and MN, 1 ppm for 3 and 5 minute treatment was found to be effective for total microbial load reduction.

Key words: Ozone, total microbial load, toxic intermediate, raw onion, dehydrated onion products

Introduction

To reduce the risk of infection associated with raw fruits and vegetables, washing is one of the most important steps during processing of food products; it removes soil and surface microorganisms. However all washing methods are not equally effective. Chemical sanitizers have been widely used in food processing industry to reduce microbial load; still the effect of common sanitizers on the surface of fruits and vegetables may be limited or volatile when a large amount of organic matter is present in the wash water and it is also important they should not leave any harmful residues (Danyluk and Schaffner, 2011; Zhuang *et al.*, 1995).

Fruits and vegetables are contaminated by microorganisms on their surfaces from sources, such as soil, water, wild animals, birds and insects during the growing stage. Process involving harvesting, washing, cutting, packaging and shipping may possibly create

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additional contamination. In addition a large area of cut surfaces can provide ideal conditions for growth of microorganisms including food borne pathogens and spoilage microorganisms (Zhang and Farber, 1996). Several types of treatment are known to be partially effective in removing pathogenic organisms from the surface of whole raw fruits and vegetables or from contact surfaces during handling (Danyluk and Schaffner, 2011; Zhuang *et al., 1995;* Brackett, 1988; Zhang *et al.*, 2009). Each type of disinfectant has its own efficacy to kill microbial cells; effectiveness depends on the nature of the cell as well as the characteristics of fruit and vegetable tissues (Beuchat *et al.*, 2004; Selma *et al.*, 2008).

Ozone was discovered by the European researcher C.F. Schonbein in 1839. Ozone is the second powerful common oxidizing agent. Common methods for ozone generation by ultraviolet radiation (188 nm wave length) and corona discharge method for commercial levels are used (Manley and Niegowski, 1967).

Ozone is used in the food processing industry to reduce the microbial load from the surface of food product and extension of its shelf life. Ozone is the most effective disinfectant to inactivate even a lot of resistant infective microorganisms like protozoa (e.g. *Cryptosporidium parvum oocysts*) wherever standard disinfectants (chlorine, chemical element dioxides) fail. Treatment of ozone increases the shelf life of apples and oranges. Fungal deterioration of black berries and grapes was decreased by ozonation of the fruits. At the concentration of 0.03mg/m^3 for 120 minutes to deactivate the *Salmonella enteritidis* and *Staphylococcus aureus,* although *Escherichia coli* was inactivated after 80 minutes (Uradzinski *et al.*, 2005). Studies have shown the effective action of ozone towards microorganisms inducing food decay (*P. aeruginosa* and *Z. bailii*), Faecal contaminants (*E. coli* and *E. faecalis*) and pathogens causing food poisoning, e.g. *L. monocytogenes, B. cereus, S. Typhimurium* (Restaino *et al.*, 1995). Application of ozone in apple and orange juice as alternative method for thermal pasteurization to control the population of *E. coli* O157:H7 and *Salmonella* sp. considerably reduced by heat and ozone (Williams *et al.*, 2005).

Ozone is a very pungent gas with strong (highly reactive) oxidizing properties, it dissolves in water ten times better than oxygen and its solubility decreases with respect to water temperature (Gordon, 1995; Holcman and Domoradzki, 2003).

Ozone is highly unstable in water and decomposes to oxygen in a very short time. Ozone disinfectant activity is only marginally affected at a water pH from 6.0 to 8.5. Ozone is extremely corrosive to equipment and is dangerous to humans with prolonged exposure at concentration above 4ppm. Ozone is readily detectable by human smell at 0.01 to 0.04 ppm. Federal occupational safety and health administration (OSHA) limits of exposures specify a 0.1 ppm threshold for continuous exposure throughout an eight hour period and 0.3 ppm for a 15 minute period.

The property of ozone to split of third atom of oxygen makes it very effective in destroying microorganisms. (It has been proven that ozone destroys viruses inducing hepatitis *A. influenza, A. vesicular stomatitis*, and infectious bovine rhinotracheitis). Ozone damages the protein and peptidoglycan molecules in cell wall of bacteria. It has also same effect on destroying various strains of bacteriophages (Rice *et al.*, 1981).

Bacteriocidal properties of ozone have also been described in the case of Gram-positive (*Listeria monocytogenes, Staphylococcus, Enterococcus faecalis*) and Gram-negative microorganisms (*Yersinia enterocolitica, Pseudomonas aeruqinosa, Salmonella typhimurium*); in both spores and vegetative cells (Rice *et al.*, 1981; Guzel-Seydim *et al.*, 2004).

Two major mechanisms have been identified through which the bactericidal effect of ozone is exerted, (a) oxidation of sulfhydryl groups and amino acid of enzymes, peptides and proteins. (b) Oxidizing Poly unsaturated fatty acids (PUFA$_S$) (Victorin, 1992).

In Gram-negative bacteria, the lipoprotein and lipopolysaccharide layers are the main sites where ozone acts, which contribute to increased cell permeability and results in application its lysis. Ozone acts as an intracellular oxidant (Khadre *et al.*, 2001; Kim *et al.*, 1999). Though, ozone is used for disinfection of range of food products; its application for raw onion and processed onion products are not yet thoroughly studied in literature. The study suggests, optimized condition effectively reduces total microbial load in raw onion and onion products.

Materials and methods

1. **Collection of Samples:** Samples of raw onion and processed onion products such as DHO, KO, MN, GR and SP were collected from onion dehydration unit of Jain Irrigation Systems Ltd. Jalgaon, India. These products were studied for their microbial profile (TVC, YMC, CC and EC) before and after treatment of ozone.

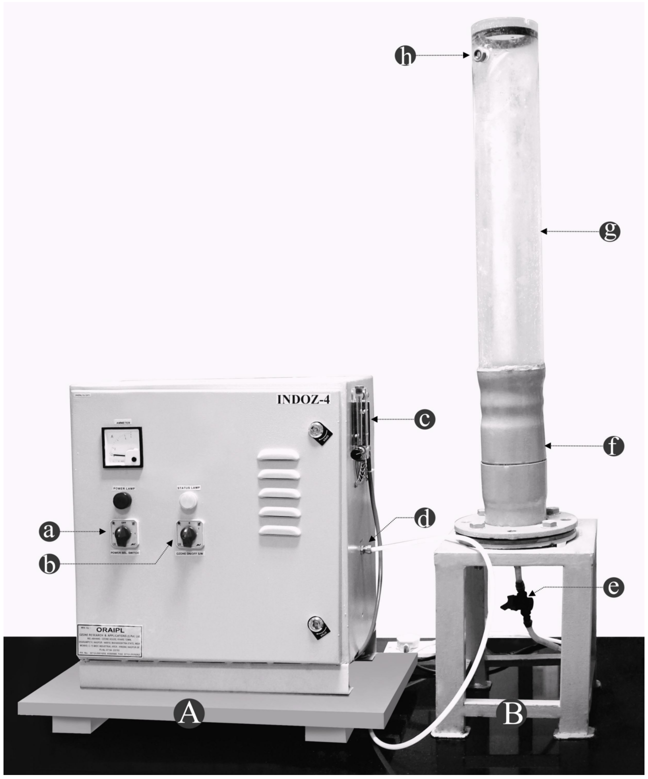

Fig.1: (A) Ozone generator machine, (B) Ozone treatment column, (a) Ozone flow regulator knob, (b) ON/OFF switch, (c) Flow meter, (d) Pipe carrying generated ozone towards treatment column, (e) Inlet of ozone gas in column, (f) Area for sample loading, (g) Passage of ozone gas after treatment, (h) Outlet of ozone gas from column.

2. **Preparation of ozone gas for treatment:** Ozone gas was generated by ozone generator machine (Ozone Research and Application Pvt. Ltd., Nagpur, India) 0.3, 0.5, and 1.0 ppm ozone gas was dissolved in RO water and this water samples was used to determine the final ozone concentration with help of ozone test kit (Prerna Lab., Pune, India) and the same ozone concentration was supplied to treatment column. (Fig.1)
3. **Determination of ozone concentration:** Ozone concentration was determined by ozone test kit as per manufacturer's instructions.
4. **Sample preparation:** 10 kg of total sample weight was taken out of which 1 kg was used for initial analysis before treatment for TVC, YMC, CC and EC. The same sample was loaded in sample holding (d) part of ozone treatment column (B) as shown in fig.1.
5. **Treatment of samples:** Different ozone concentrations (0.3, 0.5, 1.0 ppm) was provided for different time period (1, 3, 5 minutes) and regulated by flow regulator in ozone generator, for each sample loaded in treatment column (table 1).
6. **Microbial analysis of samples after ozone treatment:** Treated sample was collected and analyzed for TVC, YMC, CC and EC according to ISO 4833-1:2003, IS 5403:1999, ISO 4832:1991, ISO 21528-2:2004 respectively.

Statistical Analysis

All experiments were performed ten times in triplicate sets, the standard error of mean (SEM) for all experiments were determined. Graph preparation, and log reduction calculation was performed in excel 2010. Analysis of variance (ANOVA) was applied to know the level of significance (p-value) before and after ozone treatment in different ozone concentration with different times by Tukey-Kramer multiple comparison test by GraphPad software (Tukey 1949).

Total TVC, YMC, CC and EC values were expressed in log CFU/g for graphical representation.

Results

Raw onion and its products (DHO, KO, MN, GR and SP) were analyzed in triplicate sets for microbial load, before and after ozone treatment. Three different ozone concentrations (0.3, 0.5 and 1 ppm) were selected for varying exposure time (1, 3 and 5 minutes). TVC, YMC, CC and EC were expressed in log CFU/g count. Maximum log CFU/g count along with its reduction it was decided to select optimum concentration of ozone. Maximum log CFU/g count was used for further description and comparison at different ozone concentration for various time limits then from maximum log CFU/g values total log reduction at various concentrations and times were detected, which shown in table 2 to 7 and fig. 2 to 7.

Effect of ozone on raw onion

Table 2 summarized the effect of ozone on raw onion. The initial maximum log CFU/g count for TVC was found to be 8.982 that was reduced to 2.323 log CFU/g at 1 ppm ozone for 3 minutes. In case of CC, the maximum log CFU/g reduction of 1.724 was obtained with initial maximum of 6.724 log CFU/g count, after application of 1 ppm of ozone for 3 minutes. For YMC, the initial maximum log CFU/g count 5.681 was reduced to 1.505 log units and for EC, it is reduced to 2.646 log CFU/g from initial maximum 6.643 log CFU/g counts at 1 ppm of ozone for 3 minutes.

So 1 ppm ozone concentration was found to produce a significant ($P<0.001$) reduction in total microbial load if applied for 3 minutes to raw onion. Fig. 2.

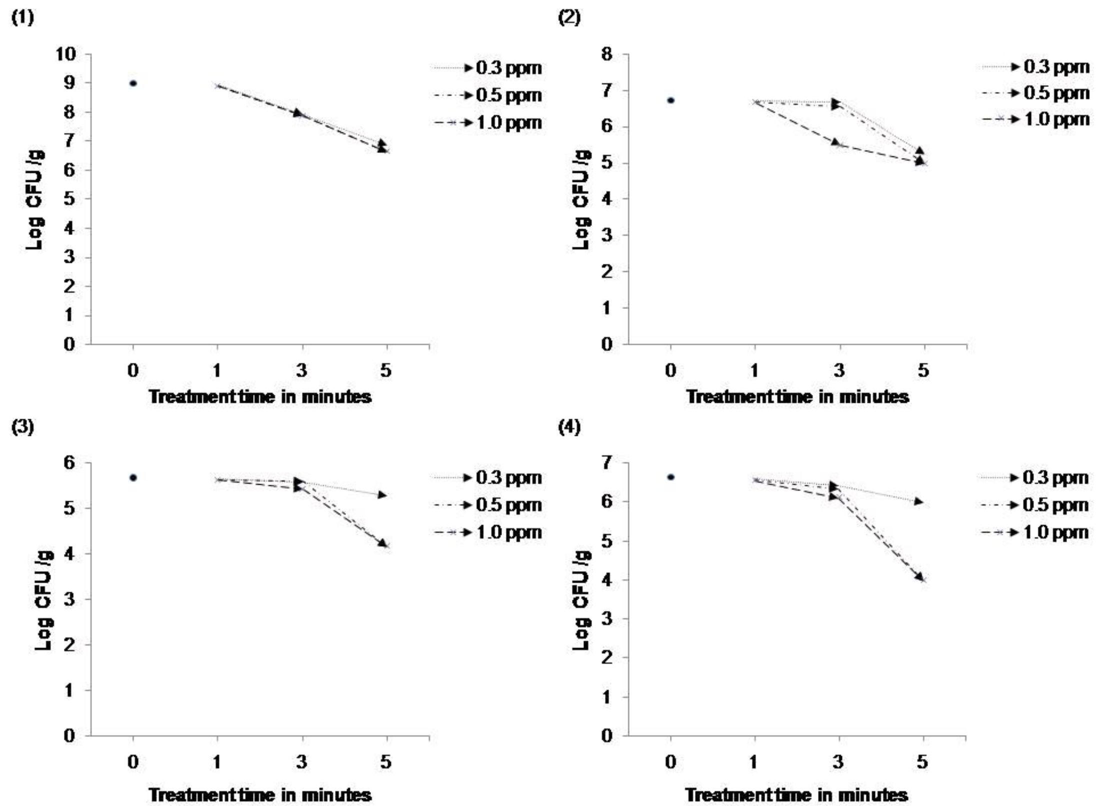

Fig. 2: Raw onion before and after ozone treatment maximum Log CFU/g count. (1) Raw onion TVC before and after ozone treatment, (2) Raw onion CC before and after ozone treatment, (3) Raw onion YMC before and after ozone treatment, (4) Raw onion EC before and after ozone treatment.

Table 1: Experimental Design for Ozone treatment of raw and processed onion products inside treatment column.

Sr. no.	Onion Product	Ozone Concentration (ppm)	Time (min)		
1	Raw Onion	0.3	1	3	5
		0.5	1	3	5
		1	1	3	5
2	DHO	0.3	1	3	5
		0.5	1	3	5
		1	1	3	5
3	KO	0.3	1	3	5
		0.5	1	3	5
		1	1	3	5
4	MN	0.3	1	3	5
		0.5	1	3	5
		1	1	3	5
5	GR	0.3	1	3	5
		0.5	1	3	5
		1	1	3	5
6	SP	0.3	1	3	5
		0.5	1	3	5
		1	1	3	5

Table 2: Microbial count of raw onion initial and after ozone treatment reduction.

		logCFU/ g count	SEM
Maximum initial count (before treatment)	TVC	8.982	0.012
Maximum log reduction, after 1 ppm ozone treatment for 3min.		2.323	0.030
Maximum initial count (before treatment)	CC	6.724	0.040
Maximum log reduction, after 1 ppm ozone treatment for 3min.		1.724	0.053
Maximum initial count (before treatment)	YMC	5.681	0.040
Maximum log reduction, after 1 ppm ozone treatment for 3min		1.505	0.114
Maximum initial count (before treatment)	EC	6.643	0.047
Maximum log reduction, after 1 ppm ozone treatment for 3min.		2.646	0.046

Effect of ozone on dehydrated onion (DHO)

Table 3 summarized effect of ozone on dehydrated onion (DHO). DHO exposed to 1ppm of ozone for 3 minutes resulted in final log CFU/g count 1.6021 from

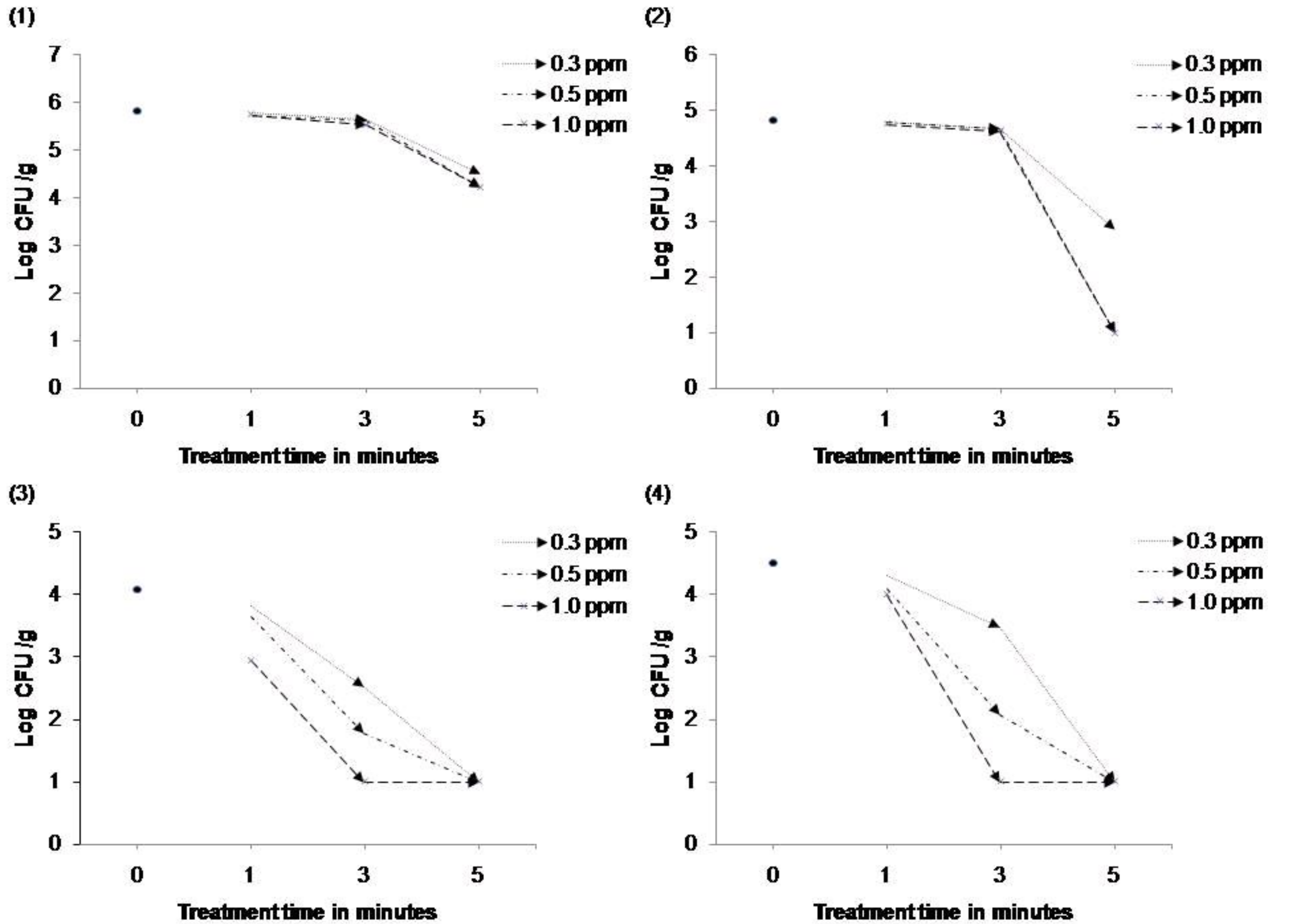

Fig. 3: DHO before and after ozone treatment maximum Log CFU/g count. (1) DHO TVC before and after ozone treatment, (2) DHO CC before and after ozone treatment, (3) DHO YMC before and after ozone treatment, (4) DHO EC before and after ozone treatment.

Table 3: Microbial count of DHO initial and after ozone treatment reduction.

		logCFU/ g count	SEM
Maximum initial count (before treatment)	TVC	5.820	0.054
Maximum log reduction, after 1 ppm ozone treatment for 3min.		1.602	0.056
Maximum initial count (before treatment)	CC	4.826	0.021
Maximum log reduction, after 1 ppm ozone treatment for 3min.		3.826	0.021
Maximum initial count (before treatment)	YMC	4.079	0.030
Maximum log reduction, after 0.5 ppm ozone treatment for 5min		3.079	0.030
Maximum initial count (before treatment)	EC	4.505	0.042
Maximum log reduction, after 5 ppm ozone treatment for 5min.		3.505	0.042

Table 4 : Microbial count of KO initial and after ozone treatment reduction.

		logCFU/ g count	SEM
Maximum initial count (before treatment)	TVC	5.959	0.011
Maximum log reduction, after 1 ppm ozone treatment for 5min.		0.767	0.042
Maximum initial count (before treatment)	CC	3.806	0.023
Maximum log reduction, after 1 ppm ozone treatment for 3min.		2.806	0.023
Maximum initial count (before treatment)	YMC	3.908	0.017
Maximum log reduction, after 1 ppm ozone treatment for 3min		2.898	0.141
Maximum initial count (before treatment)	EC	3.756	0.024
Maximum log reduction, after 1 ppm ozone treatment for 3min.		2.756	0.024

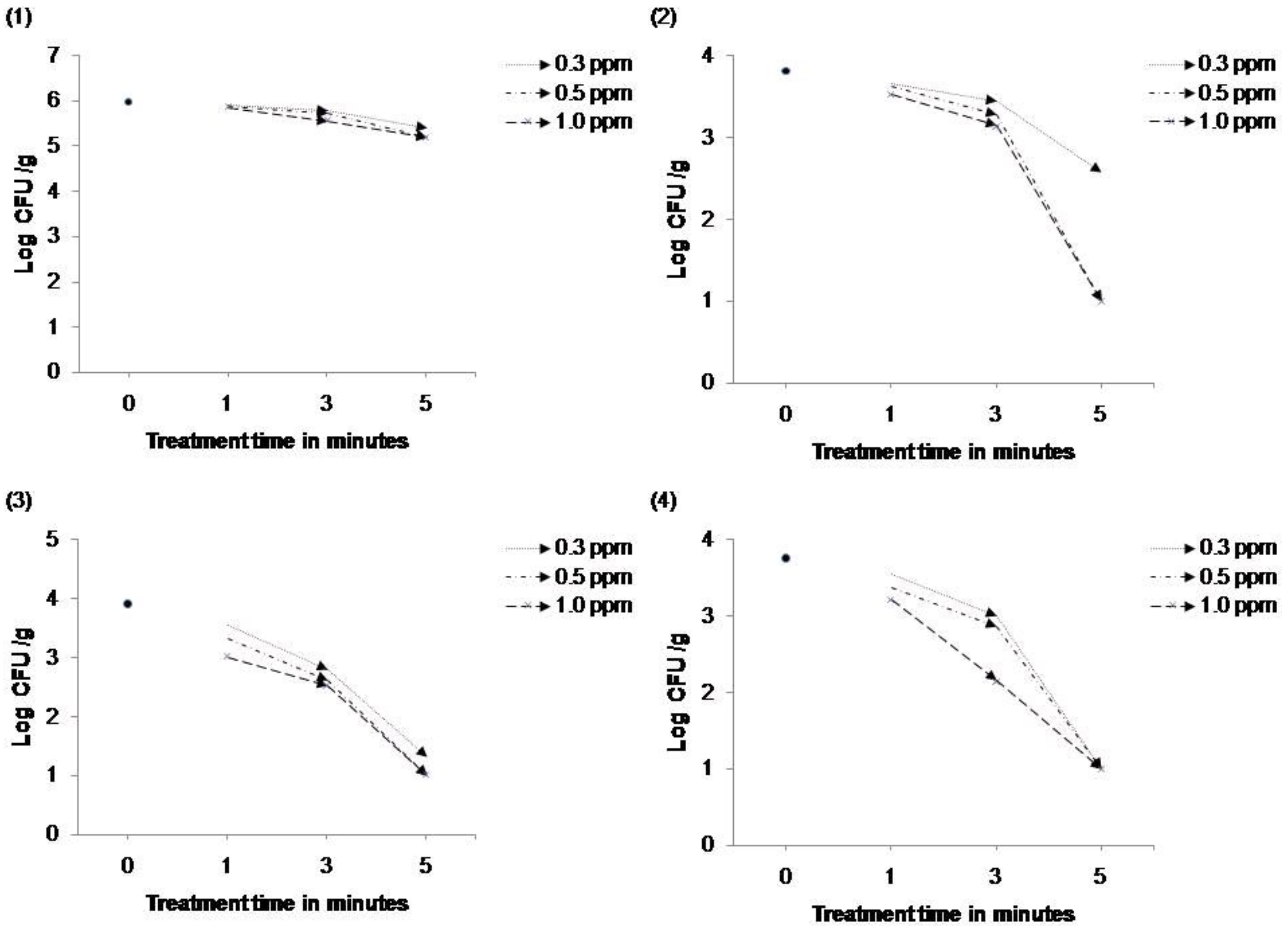

Fig. 4: KO before and after ozone treatment maximum Log CFU/g count. (1) KO TVC before and after ozone treatment, (2) KO CC before and after ozone treatment, (3) KO YMC before and after ozone treatment, (4) KO EC before and after ozone treatment.

Table 5: Microbial count of MN initial and after ozone treatment reduction.

		logCFU/ g count	SEM
Maximum initial count (before treatment)	TVC	6.255	0.034
Maximum log reduction, after 1 ppm ozone treatment for 3min.		1.058	0.450
Maximum initial count (before treatment)	CC	3.908	0.024
Maximum log reduction, after 0.5 ppm ozone treatment for 3min.		2.908	0.024
Maximum initial count (before treatment)	YMC	3.845	0.015
Maximum log reduction, after 1 ppm ozone treatment for 3min		2.000	0.039
Maximum initial count (before treatment)	EC	3.681	0.030
Maximum log reduction, after 1 ppm ozone treatment for 1min		2.681	0.030

Table 6 : Microbial count of GR initial and after ozone treatment reduction.

		logCFU/ g count	SEM
Maximum initial count (before treatment)	TVC	6.279	0.020
Maximum log reduction, after 1 ppm ozone treatment for 3min.		0.952	0.037
Maximum initial count (before treatment)	CC	4.041	0.022
Maximum log reduction, after 1 ppm ozone treatment for 3min.		1.467	0.018
Maximum initial count (before treatment)	YMC	3.964	0.014
Maximum log reduction, after 1 ppm ozone treatment for 5min		1.256	0.031
Maximum initial count (before treatment)	EC	3.940	0.040
Maximum log reduction, after 1 ppm ozone treatment for 5min		1.207	0.030

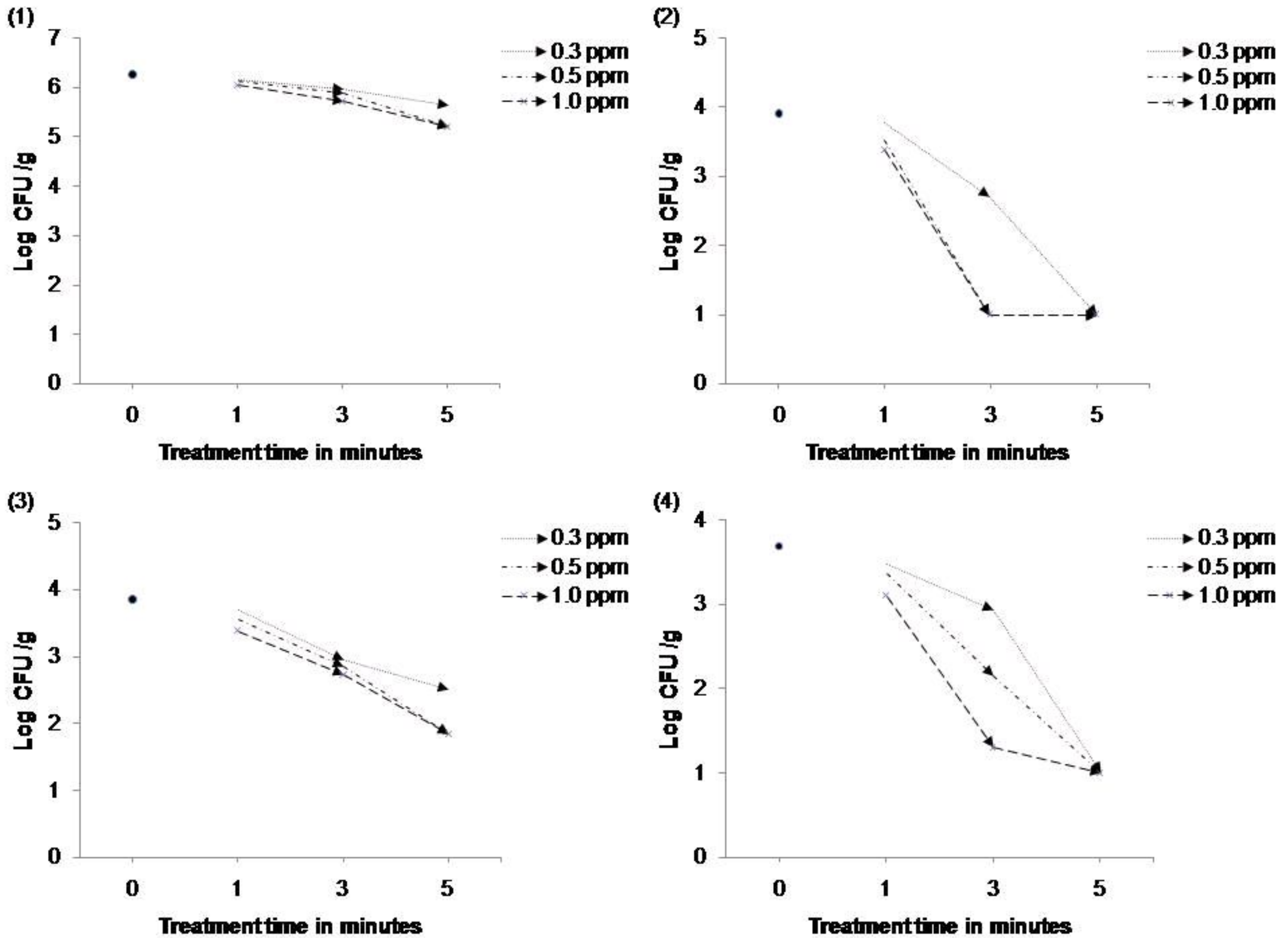

Fig. 5: MN before and after ozone treatment maximum Log CFU/g count. (1) MN TVC before and after ozone treatment, (2) MN CC before and after ozone treatment, (3) MN YMC before and after ozone treatment, (4) MN EC before and after ozone treatment.

Table 7: Microbial count of SP initial and after ozone treatment reduction.

		logCFU/ g count	SEM
Maximum initial count (before treatment)	TVC	6.556	0.025
Maximum log reduction, after 1 ppm ozone treatment for 3min.		0.645	0.019
Maximum initial count (before treatment)	CC	4.940	0.040
Maximum log reduction, after 1 ppm ozone treatment for 5min.		1.966	0.054
Maximum initial count (before treatment)	YMC	3.881	0.033
Maximum log reduction, after 1 ppm ozone treatment for 3min		0.818	0.039
Maximum initial count (before treatment)	EC	3.914	0.026
Maximum log reduction, after 1 ppm ozone treatment for 1min		1.552	0.051

initial maximum 5.820 log CFU/g in case of TVC. Also the same 1 ppm for 3 minutes found to produce significant ($P<0.001$) reduction in CC initial maximum 4.826 log CFU/g to log reduction of 3.826.

YMC in dehydrated onion product reduced to 3.079 log CFU/g starting from initial maximum log count 4.079 and for EC maximum log CFU/g reduction 3.505 where the initial maximum log CFU/g count was 4.505. The ozone concentration that was effective for reducing YMC and EC was 0.5 ppm for 5 minutes. Fig. 3.

Effect of ozone on kibbled onion (KO)

For KO 1 ppm ozone concentration for 5 minutes was significantly ($P<0.001$) reducing TVC, while 1 ppm concentration of ozone applied for 3 minutes was more effective to CC, YMC and EC. table 4 and Fig. 4.

Effect of ozone on Minced dehydrated onion (MN)

Table 5 corresponds to effect of ozone on MN. Ozone concentration of 1 ppm for 3 minutes was responsible for maximum log CFU/g reduction in MN on TVC and YMC to 1.058 and 2.000 respectively. Whereas

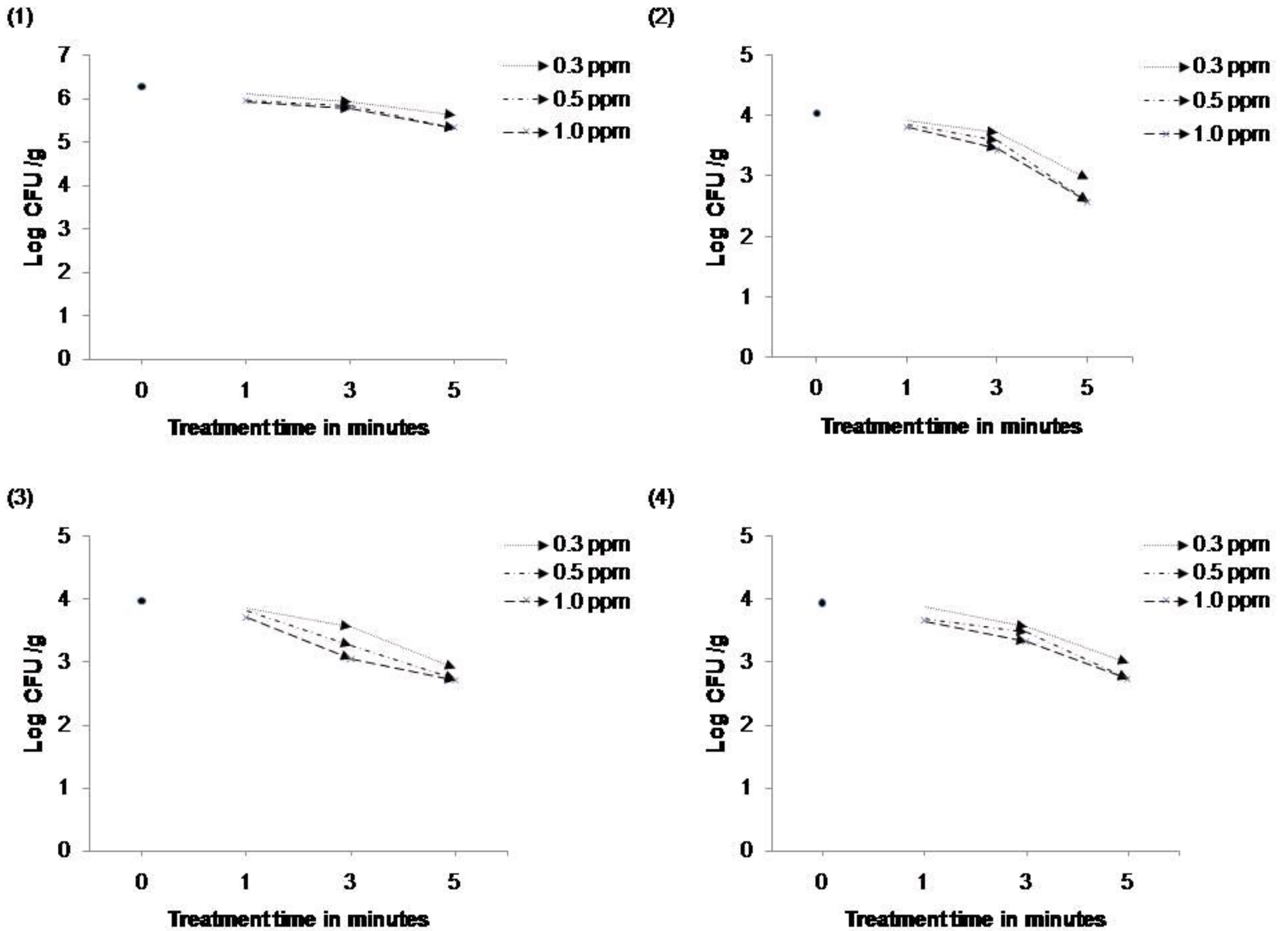

Fig. 6: GR before and after ozone treatment maximum Log CFU/g count. (1) GR TVC before and after ozone treatment, (2) GR CC before and after ozone treatment, (3) GR YMC before and after ozone treatment, (4) GR EC before and after ozone treatment.

for CC 0.5 ppm ozone for 3 minutes was sufficient for maximum log CFU/g reduction to 2.908. However 1 ppm for 1 minutes ozone concentration significantly (P<0.001) reduced EC. Fig. 5.

Granulated dehydrated onion (GR)

According to table 6 for results of effect of ozone on granulated dehydrated onion, 0.952 log CFU/g count of TVC and 1.467 log CFU/g count of coliform were obtained after treatment of 1 ppm ozone for 3 minutes. Significant (P<0.001) reduction was found. Also YMC and EC 1 ppm ozone for 5 minutes, significantly (P<0.001) reduced maximum log CFU/g count 3.964 and 3.940 to 1.256 and 1.207 log CFU/g respectively. Fig. 6.

Dehydrated Standard powder (SP).

According to table 7 the effect of ozone on SP, 0.645 log CFU/g count of TVC and 0.818 log CFU/g count of YMC were obtained after treatment of 1 ppm ozone for 3 minutes, significant (P<0.001) reduction was found. For CC 1 ppm ozone treatment for 5 minutes and for EC1 ppm ozone treatment for 1 minute, significantly (P<0.001) reduced maximum log CFU/g count 1.966 and 1.552 log CFU/g respectively. Fig. 7.

Discussion

The results obtained for each product of raw onion and its dehydrated products were gathered and summarized in table and figure 2 to 7. The effect of ozone to reduce microbial load of TVC, YMC, CC and EC was determined.

Effect of ozone on TVC of raw onion and dehydrated onion products:

The 1 ppm ozone for 3 minute was found most effective for reduction of TVC in raw onion with maximum log reduction of 2.323, whereas the same concentration responsible for moderately reducing TVC of DHO, MN, and GR by 1.602, 1.058 and 0.952 log reduction respectively. 1 ppm for 3 minutes is not sufficentely reducing TVC of KO so 5 minutes exposure of 1 ppm ozone is required for 0.767 log reduction.

Very few studies have been carried out treatment of ozone on onion and related produce, but various other

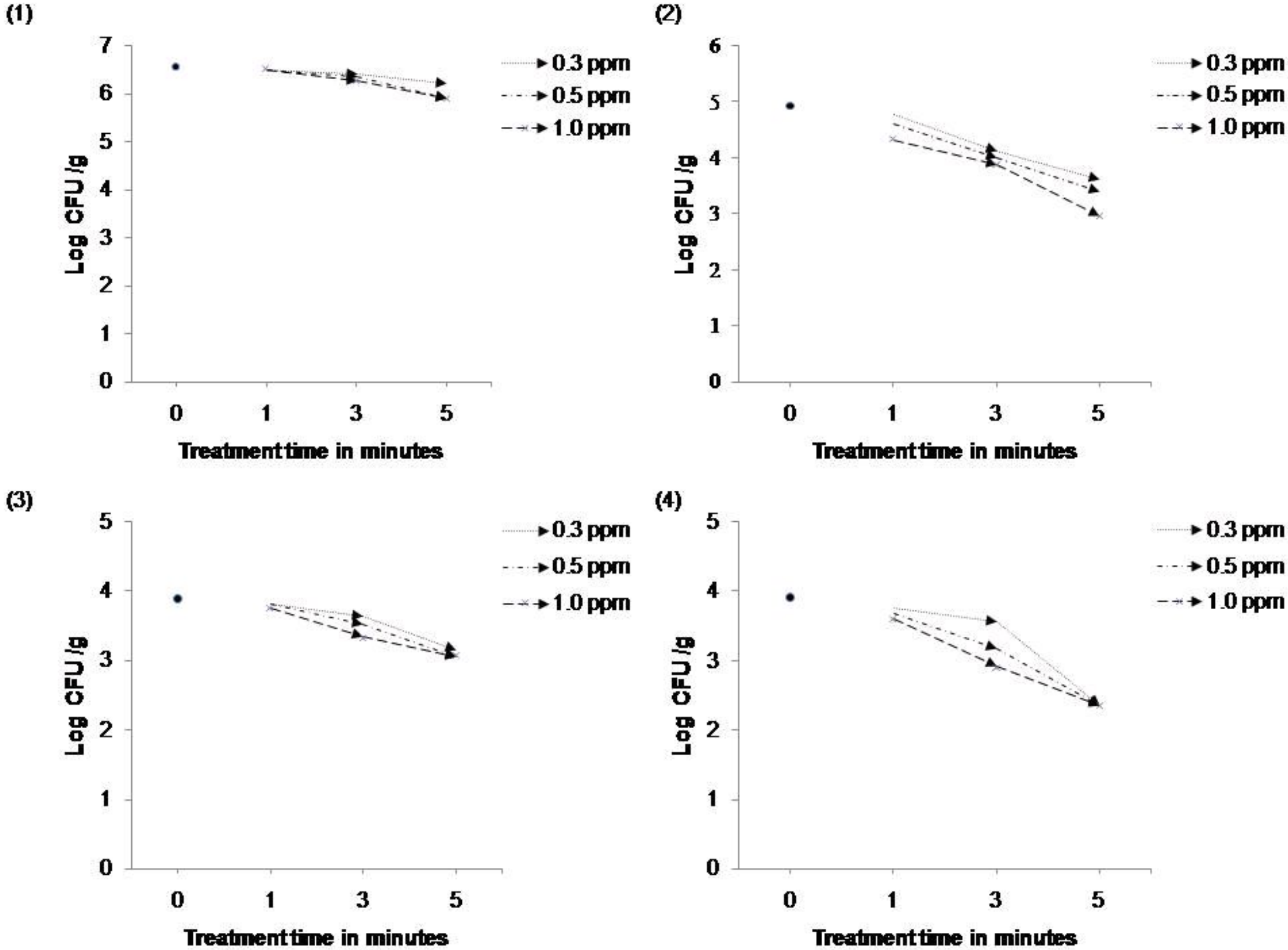

Fig. 7: SP before and after ozone treatment maximum Log CFU/g count. (1) SP TVC before and after ozone treatment, (2) SP CC before and after ozone treatment, (3) SP YMC before and after ozone treatment, (4) SP EC before and after ozone treatment.

food products were studied by number of researchers.

A study carried out by Najafi and Khodaparast, 2009 to check efficacy of ozone to reduce total microbial population on date fruits; 5 ppm ozone for different time period (15 to 60 minutes) successfully reduced after longer exposure time (Najafi and Khodaparast, 2009). Another study on dried figs by Oztekin *et al.*, 2006 minimum 3 to 5 hours of treatment of 5 ppm ozone was required for effective reduction of TVC (Oztekin *et al.*, 2006). Even one hour treatment of 40 ppm ozone exposure exerted minimal effect on TVC of asparagus, cucumber, green bean and pepper showing just 0.75, 1.04, 0.38 and 1 log reduction (Qiang *et al.*, 2005). Alegria *et al.* (2009) carried out studies on alternative decontamination procedures for reduction of total microbial load of carrot and ozone was effectively reduced total aerobic mesophile bacteria by 0.41 log reductions. The inconsistent results are obtained probably due to different kind of produce in each study.

Effect of ozone on CC of raw onion and dehydrated onion products

Maximum log reduction of 1.724, 3.826, 2.806 and 1.467 in CC of raw onion, DHO, KO and GR are observed after treatment of 1 ppm ozone for 3 minutes. CC of DHO and KO are strongly inactivated. MN CC is inhibited to 2.908 log reduction after successful exposure of 0.5 ppm ozone for 3 minutes. Slight increase in exposure time (1 ppm ozone for 5 minutes) is required to reduce CC of SP.

A study by Najafi *et al.* (2009) proposed that 1 hr. ozone treatment at 5 ppm could be used for reducing coliform of date fruits. Another study was conducted by Zorlugenc *et al.* (2008) on Influence of gaseous ozone with ozonated water on microbial flora in dried figs. 13.8 ppm of gaseous ozone for 7.5 to 30 minutes found effectively decreasing coliforms by 0.46 to 1.84 log. While Oztekin *et al.* (2006) successfully inactivated all coliforms from dried figs after 3 hr. treatment of 5 ppm ozone. Studies on shelf life extension of fresh cut lettuce by

Beltran *et al.* (2005) using efficient treatment of ozone at 10 ppm 3.2 log reduction of coliform count were achieved. Two separate studies on watercress to check impact of ozone in foods using 0.3 ppm ozone containing water for 3 minutes resulted in 0.4 log cycles (Alexandre *et al.*, 2011) and 1.7 log cycles reduction of total coliforms (Alexandre *et al.*, 2011).

Effect of ozone on Yeast and Mold count (YMC) of raw onion and dehydrated onion products

Ozone is significantly effective against YMC for all onion products. 1 ppm ozone treatment for 3 minutes is most effective in achieving maximum log reduction of 1.505, 2.898, 2.000 and 0.818 for Raw Onion, KO, MN and SP. Whereas 0.5 ppm for 5 minutes is sufficient to reduce YMC by 3.079 in DHO. GR only required 5 minutes exposure of 1 ppm ozone.

Patil *et al.* (Patil *et al.*, 2011) reported the effectiveness of ozone (33 to 40 ppm for 8 minutes) for the extension of shelf life of apple juice restricting the growth of *Saccharomyces cerevisiae*. Exposure of 1, 3 and 5 ppm of ozone for 15, 30, 45 and 60 minutes for dates fruit successfully reduced YMC (Najafi and Khodaparast, 2009). Zorlugenc *et al.* (2008) found decrease in YMC by 0.16-2.09 and 0.59 log respectively using 13.8 ppm ozone for 7.5 to 30 minutes (Alegria *et al.*, 2009).

YMC on dried figs were efficiently reduced by 1.33 and 1.73 log respectively after washing with 1.7 ppm ozone containing water (Zorlugenc *et al.*, 2008). About 1 to 10 ppm ozone for 5 to 10 minutes in ozone reactor inactivated all YMC from strawberries and lettuce with 0.78 log and 0.99 log respectively (Wei *et al.*, 2007). Alegria *et al.* (2009) carried out studies on alternative decontamination procedures for reduction of total microbial load of carrot and ozone was effectively reduced YMC by 0.6 to 0.7 log reductions.

Effect of ozone on EC of raw onion and dehydrated onion products

EC was reduced significantly to 2.646, 2.756 in raw onion and KO respectively, after 1 ppm ozone treatment for 3 minutes. The same concentration (1 ppm) of ozone is effective against EC of MN (2.681 log reduction in 1 minute), GR (1.207 log reduction in 5 minutes) and SP (1.552 log reduction in 1 minute). EC of DHO is reduced by 3.505 log for the treatment of 0.5 ppm ozone for 5 minutes.

Olmez *et al.* (2009) conducted experiments on effect of 0.5-4.5 ppm ozone for 0.5-3.5 minutes and concluded that water containing ozone at 2 ppm for 2 minutes was found to be optimum processing condition for lettuce disinfection. EC decreased to 1.5 log. Whereas Wang *et al.* (2004) found 5 minutes washing time required to reduce EC with ozonated water.

Conclusion

The use of gaseous ozone decreased the total microbial load of raw onion and its dehydrated products that is essential step to meet the safety aspects of processed food products. Therefore, ozone causes effective control agent for raw and processed onion foods.

The ozone treatment was effective in restricting growth of microbes in terms of TVC, CC, YMC and EC. Maximally, 1 ppm ozone exposure for 3 minutes was overall treatment of choice for most of products. On an average ozone concentration of 0.5 ppm-1 ppm was effectively controlling microbes within the time period of 1-5 minutes. This study concludes that very low ozone concentration and less exposure time as compared to preveous studies, is sufficient to treat various onion products.

Our results confirm the hypothesis that ozone in gaseous condition performs best disinfectant by restricting microbial population and thereby improving the product quality.

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Author's contributions

Dr S.S. Bhardwaj, V.S. Subramaniam conceived of the presented idea and supervised the findings of this work. V.A. Agnihotri, H.K. Bhandarkar, K.P. Borse writing of the manuscript.

Conflict of Interests

The authors declare no conflict of interests.

References

Alegria, C., J. Pinheiro, E.M. Goncalves, I. Fernandes, M. Moldao and M. Abreu (2009). Quality attributes of shredded carrot (Daucuscarota L. cv. Nantes) as affected by alternative decontamination processes to chlorine. *Innov. Food Sci. Emerg. Technol.,* **10(1)**: 61–69. doi: 10.1016/ j.ifset.2008.08.006.

Alexandre, E.M.C., D.M. Santos-Pedro, T.R.S. Branda˜o, and C.L.M. Silva (2011). Influence of aqueous ozone, blanching and combined treatments on microbial load of red bell peppers, strawberries and watercress. *J. Food Eng.,* **105(2)**: 277–282. doi: 10.1016/j.jfoodeng.2011.02.032.

Alexandre, E.M.C., T.R.S. Branda˜o, and C.L.M. Silva (2011). Modelling microbial load reduction in foods due to ozone impact. *Procedia Food Sci.,* **1**: 836–841. doi: 10.1016/ j.profoo.2011.09.126.

Beltran, D., M.V. Selma, A. Marin, and M.I. Gil (2005). Ozonated water extends the shelf life of fresh-cut lettuce. *J. Agric.*

Food Chem., **53(14)**: 5654–5663. doi: 10.1021/jf050359c.

Beuchat, L.R., B.B. Adler, M.M. Lang (2004). Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. *J. Food Prot.,* **67**: 1238-1242.

Brackett, R.E. (1988). Fruits, vegetables, and grains. In Doyle, M.P., L.R. Beuchat and T.J. Montville (Eds.). *Food Microbiol*: Fundamentals and Frontiers, ASM Press, Washington, DC, 117-126.

Danyluk, M.D and D.W. Schaffner (2011). Quantitative microbial assessment for *E. coli* O157:H7 in leafy greens from farm to consumption using published data. *J. Food Prot.,* **74**: 700-708.

Gordon, G. (1995). The chemistry and reactions of ozone in our environment. *Prog. Nucl. Energy*, **29**(Supl.): 89-96.

Guzel-Seydim, Z., P.I. Bever, and A.K. Greene (2004). Efficacy of ozone to reduce bacterial populations in the presence of food components. *Food Microbiol,* **21**: 475-479.

Holcman, J., and M. Domoradzki (2003). Fundamental reactions of ozone in the water environment. *Ekologia i Technika* **11**:16–19 (in Polish).

IS 5403:1999 (First revision) Method for yeast and Mold count of food stuffs and animal feeds.

ISO 21528-2 (First edition 2004-08-15) Microbiology of food and animal feeding stuffs- Horizontal methods for the detection and enumeration of Enterobacteriaceae. Part – 2 colony count method. Reference number ISO 21528-2:2004(E).

ISO 4832 (Third edition 2006-02-15) Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coliforms. Colony count Technique. Reference number ISO 4832:2006(E).

ISO 4833-1, Microbiology of the food chain-Horizontal method for the enumeration of microorganisms. Part-1: Colony count at 30 °C by the pour plate T Technique. Reference number ISO 4833-1:2013(E).

Khadre, M.A., A.E. Yousef, and J.G. Kim (2001). Microbiological aspects of ozone applications in food: A review. *J. Food Sci.,* **66**: 1242-1251.

Kim, J.G., A.E. Yousef, and S. Dave (1999). Application of ozone for enhancing the microbiological safety and quality of foods: A review. *J. Food Prot.,* **62**: 1071-1087.

Manley, T.C. and S.J. Niegowski (1967). Ozone. In Encyclopedia of chemical Technology (Vol. 14, 2nd ed.), Wiley: New York, 410–432.

Najafi, M.B. and M.H. Khodaparast (2009). Efficacy of ozone to reduce microbial populations in date fruits. *Food Control,* **20(1)**: 27–30. doi: 10.1016/j.foodcont.2008.01.010

Olmez, H. and M.Y. Akbas (2009). Optimization of ozone treatment of fresh-cut green leaf lettuce. *J. Food Eng.,* **90(4)**: 487-494. doi: 10.1016/j.jfoodeng.2008.07.026.

Oztekin, S., B. Zorlugenc, and F.K. Zorlugenc (2006). Effects of ozone treatment on microflora of dried figs. *J. Food Eng.,* **75(3)**: 396–399. doi: 10.1016/j.jfoodeng.2005.04.024.

Patil, S., V.P. Valdramidis, B.K. Tiwari, P.J. Cullen, and P. Bourke (2011). Quantitative assessment of the shelf life of ozonated apple juice. *Eur. Food Res. Technol.,* **232(3)**: 469-477. doi: 10.1007/s00217-010-1416-2.

Qiang, Z.M., O. Demirkol, N. Ercal, and C. Adams (2005). Impact of food disinfection on beneficial biothiol contents in vegetables. *J. Agric. Food Chem.,* **53(25)**: 9830-9840. doi: 10.1021/jf051359f

Restaino, L., E.W. Erampton and J.B. Hemphill (1995). Efficacy of ozonated water against various food-related microorganisms. *Appl. Environ. Microbiol.,* **61**: 3471-3475.

Rice, R.G., C.M. Robson, G.W. Miller and A.G. Hill (1981). Uses of ozone in drinking water treatment. *J. Am. Water Works Assoc.,* **73**: 44–57.

Selma, M.V., A.M. Ibañez, A. Allende, M. Cantwell and T. Suslow (2008). Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *Escherichia coli* O157:H7 during cutting. *Food Microbiol,* **25**: 162–168.

Tukey, John (1949). Comparing Individual Means in the Analysis of Variance. *Biometrics*, **5(2)**: 99–114.

Uradzinski, J., B. Wysok, Z. Bielicki and M. Gomólka-Pawlicka-Pawlicka (2005). Ozonation as an alternative method of disinfecting knives for use in meat processing. *Bull. Vet. Inst. Pulawy,* **49**: 399-402.

Victorin, K. (1992). Review of the genotoxicity of ozone. *Mutat. Res.,* **277**: 221-238.

Wang, H., H. Feng and Y.G. Luo (2004). Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. *Food Res. Int.,* **37(10)**: 949-956. doi: 10.1016/j.foodres.2004.06.004

Wei, K.J., H.D. Zhou, T. Zhou and J.H. Gong (2007). Comparison of aqueous ozone and chlorine as sanitizers in the food processing industry: impact on fresh agricultural produce quality. *Ozone Sci. Eng.,* **29(2)**: 113–120. doi: 10.1080/01919510601186592.

Williams, R.C., S.S Sumner and D.A. Golden (2005). Inactivation of Escherichia coli O157:H7 and Salmonella in apple cider and orange juice treated with combinations of ozone, dimethyl dicarbonate, and hydrogen peroxide. *J. Food Sci.,* **70(4)**: 197-201.

Zhang, G., L. Ma, L.R. Beuchat, M.C. Erickson, V.H. Phelan, and M.P. Doyle (2009). Evaluation of treatments for elimination of food borne pathogens on the surface of leaves and roots of lettuce (*Lactuca sativa* L.). *J Food Prot* **72(2)**: 228–234.

Zhang, S. and J.M. Farber (1996). The effects of various disinfectants against Listeria monocytogenes on fresh-cut vegetables. *Food Microbiol,* **13**: 311-321.

Zhuang, R.Y., L.R. Beuchat and F.J. Angulo (1995). Fate of Salmonella Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Appl. Environ. Microbiol,* **61**: 2127-2131.

Zorlugenc, B., F.K. Zorlugenc, S. Oztekin and I.B. Evliya (2008). The influence of gaseous ozone and ozonated water on microbial flora and degradation of aflatoxin B1 in dried figs. *Food and Chemical Toxicology,* **46(12)**: 3593-3597. doi: 10.1016/j.fct.2008.09.003.