Inactivation of *Escherichia coli* O157:H7 by ozone in different substrates

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Abstract

Ozone has a broad antimicrobial spectrum and each microorganism species has inherent sensitivity to the gas. The objective of this study was to evaluate the effect of ozone gas on *Escherichia coli* O157:H7 inoculated on an organic substrate, and the efficacy of ozonated water in controlling the pathogen. For the first experiment, *E. coli* O157:H7 (ATCC® 43890™) was inoculated in milk with different compositions and in water, which was ozonated at concentrations of 35 and 45 mg L⁻¹ for 0, 5, 15, and 25 min. In the second experiment, water was ozonated at 45 mg L⁻¹ for 15 min. *E. coli* O157:H7 was exposed for 5 min to the ozonated water immediately after ozonation, and after storage for 0.5, 1.0, 1.5, 3.0, and 24 h at 8 °C. The results showed that the composition of the organic substrate interfered with the action of ozone on *E. coli* O157:H7. In lactose-free homogenized skim milk, reductions of 1.5 log cycles were obtained for ozonation periods of 25 min at the concentrations tested. Ozonated water was effective in inactivating of *E. coli* O157:H7 in all treatments. The efficiency of ozone on *E. coli* O157:H7 is influenced by the composition of the organic substrates, reinforcing the need for adequate removal of organic matter before sanitization. Furthermore, refrigerated ozonated water stored for up to 24 h is effective in the control of *E. coli* O157:H7.

Keywords Milk · Ozone gas · Ozonated water · Inactivation of microorganisms

Introduction

The ability of microorganisms to survive or multiply depends on a number of factors, including those related to the characteristics of the substrates on which the microorganisms are inserted, called intrinsic factors, such as water activity, and those related to the environment which are called extrinsic factors, such as ambient temperature [1]. One of the major concerns of the food industry is guarantee of the microbiological quality of its products, seeking to reduce losses resulting from deterioration and reducing risks to consumer health [2].

The genus *Escherichia* belongs to the family Enterobacteriaceae and includes *E. coli*, *E. blattae*, *E. fergusonii*, *E. hermannii*, *E. vulneris*, *E. albertii*, and

E. senegalensis [3]. It is believed that most *E. coli* serotypes are devoid of any virulence factor; however, during the evolutionary process, some strains acquired different sets of genes that gave them the ability to cause diseases, a fact that determines the great pathogenic versatility of the species [4]. Shiga toxin-producing *E. coli* (STEC) are also called verotoxigenic or verotoxin-producing *E. coli* (VTEC) and make up an important group of emerging foodborne pathogens [5, 6]. According to the Centers for Disease Control and Prevention (CDC), most outbreaks caused by *E. coli* O157:H7 have mainly been related to the consumption of undercooked ground beef [7]. However, currently, outbreaks involving non-meat foods such as milk and its unpasteurized derivatives, unpasteurized fruit juices, lettuce, spinach, raw vegetables, and seed sprouts have also been reported [8–10].

Among the sanitizers used in the food industry, chlorine and chlorinated compounds predominate [11, 12]. The ease of use, low cost, antimicrobial activity, and complete dissolution in water makes chlorinated agents frequently used as sanitizers in the food industry [13, 14]. However, chlorine is a corrosive substance that can cause skin and respiratory tract irritation [15]. Furthermore, another concern with the use of chlorine is associated with the possibility of hyperchlorination

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of residual water, which together with the high organic carbon content may result in high concentrations of trihalomethanes and other disinfection byproducts [13, 16].

Considering the importance of the sanitization step, the study of alternative methods and/or agents should receive special attention and ozone is an option that can be used with different objectives. This technology associated with sanitizers allows for a greater reduction of microbial contamination of foods [17, 18]. In 2001, FDA (US Food and Drug Administration) approved the use of ozone as an antimicrobial agent in food, both in the gas phase and dissolved in water [19]. As a sanitizer, ozone first acts on the cell membrane, reacting with glycoproteins, glycolipids, and nucleic acids. Microorganisms are inactivated by disruption of the cell as a result of the action of molecular ozone or free radicals during decomposition of the gas [20–22]. Although ozone inactivates several microorganisms, studies are still needed to elucidate its efficacy on the elimination of *E. coli* O157:H7, in addition to determination of the most appropriate concentration and form of application. Thus, the aims of this study were (i) to assess the effects of direct application of ozone gas on *E. coli* O157:H7 inoculated in organic substrates and (ii) to evaluate the efficacy of ozonated water in controlling the pathogen.

Material and methods

Experiment I

The strain used was *E. coli* O157:H7 (ATCC® 43890™). Prior to each inoculation, the strain that was stored in MRS broth (Oxoid) added to glycerol at 30% at $-20\text{ }^{\circ}\text{C}$ was recovered in Tryptone Soy Broth with 0.6% of Yeast Extract (TSB-YE) (Bacto™) with incubation at $35\text{ }^{\circ}\text{C}$ for 24 h. The strain was plated on Tryptone Soy Agar supplemented with 0.6% of Yeast Extract (TSA-YE) (Kasvi®) where it was again incubated at $35\text{ }^{\circ}\text{C}$ for 24 h.

The organic substrates used were as follows: unhomogenized whole milk (UHWM), homogenized whole milk (HWM), skim milk (SM), lactose-free skim milk (LFSM), and lactose-free whole milk (LFWM), obtained from commercial establishments, which were sterilized by boiling in the laboratory to ensure the elimination of any other microorganisms and cooled before inoculation. Milk was used only as a substrate, considering the differences in fat and lactose contents. All procedures were also conducted in distilled water for comparison.

Colonies developed on TSA-YE were inoculated in 0.85% saline solution until obtaining the degree of turbidity corresponding to tube 1 of the McFarland nephelometric scale (Nefelobac®, Probac do Brasil), containing approximately $3.0 \times 10^8\text{ CFU mL}^{-1}$. From this inoculum, 9.0 mL was transferred to a volumetric flask containing 91 mL of each

substrate, obtaining approximately 10^6 CFU mL^{-1} ; then, 3.0 mL was inoculated in 297 mL of the substrate in order to obtain approximately 10^4 CFU mL^{-1} (parent inoculum).

For gas generation, the ozone generator Model O&L 3.0-O2-RM (Ozone & Life, Sao Jose dos Campos, São Paulo, Brazil) was used, with a flow rate of 0.5 L min^{-1} at the temperature of $20\text{ }^{\circ}\text{C}$. The ozone concentration was determined as recommended by Clescerl, Greenberg, and Eaton [23]. For ozonation, each sample was initially transferred from the substrates into a glass column, with a 500-mL capacity. Before the ozonation of each sample, the glass column was sanitized with bubbling of the ozone gas itself in water for 5 min. Based on results obtained by Couto et al. [24], the concentrations of ozone gas of 35 and 45 mg L^{-1} were applied at 0, 5, 15, and 25 min with three repetitions each for the microorganism. The residual ozone concentrations were determined after passage through the glass column containing the different substrates.

Experiment II

E. coli O157:H7 colonies grown on TSA-YE were inoculated in a 0.85% saline solution to obtain the degree of turbidity corresponding to tube 1 of the McFarland nephelometric scale (Nefelobac®, Probac do Brasil), containing approximately $3.0 \times 10^8\text{ CFU mL}^{-1}$. From this inoculum, 1.0 mL was withdrawn and serial decimal dilutions were performed in the 0.85% saline solution until obtaining the inoculum with approximately 10^4 CFU mL^{-1} (parent inoculum).

Ozone gas was obtained as described in the “Experiment I” section. To obtain ozonated water, 2 L of autoclaved distilled water was used. The water was transferred to a glass column and ozonated at a concentration of ozone gas of 45 mg L^{-1} for 15 min. After ozonation, the water was maintained under refrigeration at $8 \pm 1\text{ }^{\circ}\text{C}$. The storage periods of the ozonated water were 0, 0.5, 1.0, 1.5, 3.0, and 24 h. After each storage period, 9.0 mL of ozonated water was pipetted into sterile test tubes. Then, 1.0 mL of the inoculum (10^4 CFU mL^{-1}) was added. The contact time of the inoculum with the ozonated distilled water was 5 min. It is emphasized that by adding 1 mL of 0.85% saline solution to 9.0 mL of ozonated distilled water, the electrical conductivity of the solution was 1.1 mS cm^{-1} .

Quantification of ozone dissolved in water was performed in a photometer, Model I-2019 (CHEMetrics, Inc., Midland, VA, USA), with a measurement range of 0.2 to 5.0 mg L^{-1} .

Microbiological analyses

The counts of *E. coli* O157:H7 were performed by plating 0.1 mL on the surface of Violet Red Bile agar (VRB) (Acumedia®), with incubation at $35\text{ }^{\circ}\text{C}$ for 24 h. Results of the counts were converted to $\log_{10}$.

Experimental design

In the “**Experiment I**” section, a completely randomized design was used in a $2 \times 6 \times 4$ factorial scheme, consisting of two ozone concentrations (35 and 45 mg L⁻¹), six substrates (UHWM, HWM, SM, LFSM, LFWM, and water), and four gas exposure periods (0, 5, 15, and 25 min), where three replicates were conducted for each assay. An analysis of variance was performed at 5% probability using the ASSISTAT 7.7 BET software (Federal University of Campina Grande, Brazil). Subsequently, a mean test was performed using the Tukey test at 5% probability or regression analysis. The regression analysis was adopted when the effect of the ozonation period was evaluated. The SigmaPlot v.10 (Systat Software Inc., Germany) was used to obtain the regression equations and to plot the graphs. In the “**Experiment II**” section, in which the efficacy of ozonated water on the control of *E. coli* O157:H7 was evaluated, only the ozonated water storage period was varied (0, 0.5, 1.0, 1.5, 3.0, and 24 h). Descriptive statistics were used in the presentation and analysis of the data.

Results

There was a significant variation ($p < 0.01$) in the reduction of *E. coli* O157:H7 counts as a result of the triple interaction between ozone concentration, substrate, and gas exposure period. Table 1 shows the mean values for the reduction in *E. coli* O157:H7 counts in log cycles, when different combinations of substrates and ozone exposure periods were evaluated as a function of gas concentration. There was no significant increase ($p > 0.05$) in the reduction of *E. coli* O157:H7

counts with the increase in ozone concentration, except in the LFWM substrate combined with the 5-min ozonation period.

Table 2 presents the mean values of log cycle reductions in the *E. coli* O157:H7 count for different combinations of ozone concentration and gas exposure periods on different substrates. There was a significant difference ($p < 0.05$) in all combinations of ozone concentration and gas exposure period as a function of the substrate used. Greater reductions in *E. coli* O157:H7 counts were obtained when using distilled water and LFSM as substrates, with mean values between 3.90 and 3.98 log cycles and between 0.66 and 1.54 log cycles, respectively. In the HWM and SM substrates, reductions in *E. coli* O157:H7 counts remained below 0.40 log cycles in all combinations of ozone concentration and gas exposure period.

Table 3 and Fig. 1 show the regression curves referring to the reduction in *E. coli* O157:H7 as a result of ozonation, in the different substrates and at the concentrations of 35 and 45 mg L⁻¹, as a function of the ozonation period. The reduction was more pronounced as the ozone exposure period increased when water was used as the substrate. It is important to note that when the ozonated substrate was water (DW), independent of the ozone concentration, a maximum detectable reduction was obtained in the *E. coli* O157:H7 count with 5 min of gas exposure, considering the initial inoculum. In the substrates containing organic matter, this behavior was more accentuated in the LFSM substrate. In the HWM and SM substrates, the reductions were less than 0.4 log cycles.

Also observed was a variation in the residual concentration of ozone gas after passage through the column containing the different substrates. The presence of organic matter caused a reduction in the residual ozone gas concentration. Highlighted were the results obtained when the substrates were unhomogenized whole milk (UHWM), homogenized whole milk

Table 1 Mean values for the reduction in *Escherichia coli* O157:H7 counts (log N_0/N) in different combinations of substrate (S) and exposure period (P) at ozone concentrations (C) of 35 and 45 mg L⁻¹

S × P (min)	C (mg L ⁻¹)		S × P (min)	C (mg L ⁻¹)	
	35	45		35	45
UHWM/5	0.24 ± 0.04a	0.26 ± 0.06a	LFSM/5	0.66 ± 0.12a	0.78 ± 0.09a
UHWM/15	0.38 ± 0.10a	0.41 ± 0.11a	LFSM/15	0.82 ± 0.23a	0.86 ± 0.07a
UHWM/25	0.89 ± 0.17a	0.96 ± 0.31a	LFSM/25	1.53 ± 0.34a	1.54 ± 0.23a
HWM/5	0.23 ± 0.02a	0.23 ± 0.04a	LFWM/5	0.27 ± 0.02b	0.46 ± 0.09a
HWM/15	0.20 ± 0.04a	0.30 ± 0.05a	LFWM/15	0.44 ± 0.07a	0.56 ± 0.02a
HWM/25	0.29 ± 0.06a	0.38 ± 0.09a	LFWM/25	0.65 ± 0.17a	0.73 ± 0.06a
SM/5	0.02 ± 0.07a	0.01 ± 0.01a	DW/5	3.98 ± 0.01a	3.95 ± 0.04a
SM/15	0.03 ± 0.04a	0.03 ± 0.02a	DW/15	3.98 ± 0.01a	3.94 ± 0.04a
SM/25	0.10 ± 0.04a	0.11 ± 0.05a	DW/25	3.97 ± 0.02a	3.90 ± 0.02a

Means followed by the lower case letters (a–b) on the line do not statistically differ by the Tukey test at 5% probability

UHWM, unhomogenized whole milk; HWM, homogenized whole milk; SM, skim milk; LFSM, lactose-free skim milk; LFWM, lactose-free whole milk; DW, distilled water.

n = 3 sample replicates

Table 2 Mean values for the reduction in *Escherichia coli* O157:H7 counts ($\log N_0/N$) in different combinations of gas concentration (C) and exposure period (P) on different substrates (S)

C(mg L ⁻¹)/ P(min)	S					
	UHW	HWM	SM	LFSM	LFWM	DW
35/5	0.24 ± 0.04 cd	0.23 ± 0.02 cd	0.02 ± 0.07d	0.66 ± 0.12b	0.27 ± 0.02c	3.98 ± 0.01a
35/15	0.38 ± 0.10 cd	0.20 ± 0.04de	0.03 ± 0.04e	0.82 ± 0.23b	0.44 ± 0.07c	3.98 ± 0.01a
35/25	0.89 ± 0.17c	0.29 ± 0.06d	0.10 ± 0.04d	1.53 ± 0.34b	0.65 ± 0.17c	3.97 ± 0.02a
45/5	0.26 ± 0.06c	0.28 ± 0.04c	0.01 ± 0.01d	0.78 ± 0.07b	0.46 ± 0.09c	3.95 ± 0.04a
45/15	0.41 ± 0.11 cd	0.30 ± 0.05d	0.03 ± 0.02e	0.86 ± 0.23b	0.56 ± 0.02c	3.94 ± 0.04a
45/25	0.96 ± 0.31c	0.38 ± 0.09d	0.11 ± 0.05e	1.54 ± 0.09b	0.73 ± 0.06c	3.90 ± 0.02a

Means followed by the lower case letters (a–e) on the line do not statistically differ by the Tukey test at 5% probability

UHW, unhomogenized whole milk; HWM, homogenized whole milk; SM, skim milk; LFSM, lactose-free skim milk; LFWM, lactose-free whole milk; DW, distilled water

n = 3 sample replicates

(HWM), and lactose-free skim milk (LFSM). Residual concentrations of gaseous ozone at the initial concentration of 45 mg L⁻¹ after 25 min were equivalent to approximately 36.0, 40.5, and 31.50 mg L⁻¹ when the substrates used were UHW, LFSM, and HWM, respectively.

Table 4 shows the values of ozone concentration dissolved in water stored at 8 °C for up to 24 h. Also presented are the water temperatures at the time of each ozone concentration measurement. The residual ozone concentration remained in the range of 1.97 to 0.82 mg L⁻¹ during the ozonated water storage period at 8 °C. It should be noted that there was a change in temperature of the ozonated water during storage. This variation can be justified by the fact that water ozonation was carried out in an environment with a temperature of approximately 20 °C. Therefore, during this process, the water temperature increased to 14 °C. It should be noted that the temperature of the water prior to ozonation was 8 °C.

The count of *E. coli* O157:H7 prior to ozonation was equivalent to 4.50 log CFU mL⁻¹. On the other hand, the presence of the pathogen was not detected after exposure to ozonated water. This result was obtained for all ozonated water storage periods at a temperature of 8 ± 1 °C, with concentrations of dissolved ozone in the range of 0.82 to 1.97 mg L⁻¹ (Table 4).

Discussion

Ozone has a broad antimicrobial spectrum [25] and each microorganism species has inherent sensitivity to the gas [21]. Ozonation has been tested for the control of fungi, Gram-negative and Gram-positive bacteria, and viruses [26–28]. The inactivation of microorganisms by ozone is a process that occurs due to rupture of the cellular envelope and subsequent dispersion of the cytoplasmic constituents, since this gas has a

Table 3 Adjusted regression equations and respective coefficients of determination (*R*²) to the reduction in *E. coli* O157:H7 ($\log N_0/N$) count as a function of the ozone exposure period (x) in different substrates (S) and in the concentrations (C) of 35 and 45 mg L⁻¹

C (mg L ⁻¹)	S	Adjusted equation	<i>R</i> ²	SEE
35	UHW	$y = 0.007 + 0.033x$	0.95	0.10
	HWM	$y = 0.077 + 0.009x$	0.64	0.09
	SM	$y = 0.005 + 0.004x$	0.88	0.02
	LFSM	$y = 0.144 + 0.054x$	0.91	0.23
	LFWM	$y = 0.069 + 0.024x$	0.95	0.08
	DW	$y = 3.978(1 - e^{(-0.808x)})$	0.99	0.01
45	UHW	$y = 0.007 + 0.036x$	0.95	0.11
	HWM	$y = 0.102 + 0.012x$	0.68	0.11
	SM	$y = -0.012 + 0.004x$	0.89	0.02
	LFSM	$y = 0.201 + 0.053x$	0.87	0.28
	LFWM	$y = 0.157 + 0.025x$	0.79	0.17
	DW	$y = 3.941(1 - e^{(-0.945x)})$	0.99	0.01

UHW, unhomogenized whole milk; HWM, homogenized whole milk; SM, skim milk; LFSM, lactose-free skim milk; LFWM, lactose-free whole milk; DW, distilled water; SEE, standard error of estimate

n = 3 sample replicates

Fig. 1 Reduction in the *Escherichia coli* O157:H7 count ($\log N_0/N$) on different substrates in the concentrations of 35 mg L⁻¹ (a) and 45 mg L⁻¹ (b) in function of the ozone exposure period. LFSM, lactose-free skim milk; UHWM, unhomogenized whole milk; LFWM, lactose-free whole milk; HWM, homogenized whole milk; SM, skim milk

high oxidative potential. Ozone is able to react with cellular constituents including proteins, unsaturated fatty acids, cell wall peptidoglycans, enzymes, and nucleic acids [25]. In aqueous media, the inactivation of microorganisms is attributed to molecular ozone and the hydroxyl, hydroperoxyl, and superoxide radicals, generated from their decomposition, according to Manousaridis et al. [29]

Among the factors that affect the action of ozone on microorganisms, highlighted are pH, temperature, and composition of the medium. In addition, organic compounds can compete with microorganisms for ozone [25]. This justifies the greater efficiency of microorganism inactivation by ozone in substrates with less organic matter. In organic substrates, the greatest *E. coli* O157:H7 inactivation efficiency was obtained in the substrate LFSM, characterized by its reduced fat and lactose contents. In this substrate, the maximum reductions in *E. coli* O157:H7 counts were 1.53 and 1.54 log cycles when adopting the concentrations of 35 and 45 mg L⁻¹ for 25 min, respectively. These results are in agreement with those obtained by Restaino et al. [30], who evaluated the efficacy of ozone for inactivating Gram-positive and Gram-negative bacteria, yeasts, and fungi in water with or without starch and bovine serum albumin. Güzel-Seydim et al. [31] studied the effect of the presence of fat, proteins, and carbohydrates on the inactivation of *Bacillus stearothermophilus*, *E. coli*, and *S. aureus* by ozone. The constituents presenting the greatest protective effect on the microorganisms were cream of milk and caseinate. Similar results were obtained by Choi et al. [32] for apple juice with different soluble solids contents. Ozone was able to inactivate *E. coli* O157:H7, *Salmonella typhimurium*, and

Listeria monocytogenes, but the efficacy of ozonation was dependent on the soluble solids content in apple juice.

Another factor that affects the efficiency of ozone on microorganisms is the effect of organic compounds present on decomposition of the gas. Ozone is noted for being unstable both in the gas phase and when dissolved in water [29]. In distilled water, the half-life of ozone at 20 °C is in the range of 20 to 30 min [25]. In media rich in organic matter, faster decomposition of the ozone and consequently reduced effectiveness of microorganism inactivation are expected. Beltrán [33] affirmed that initially in an aqueous medium containing organic matter, there is a rapid consumption of ozone. As degradation of organic compounds occurs, ozone decomposition becomes slower. In the present work, the rapid decomposition of ozone in substrates rich in organic matter justifies the reduced efficacy of the gas for inactivation of microorganisms.

A significant difference was observed in inactivation of *E. coli* O157:H7 when comparing the effect of ozone on the UHWM and HWM substrates, at concentrations of 35 and 45 mg L⁻¹ and 25 min exposure period. In the UHWM substrate, reductions equivalent to 0.86 and 0.96 log cycle were obtained, for concentrations of 35 and 45 mg L⁻¹, respectively, while in the HWM substrate, the reductions were 0.29 and 0.39 log cycle for concentrations of 35 and 45 mg L⁻¹, respectively. These differences may be attributed to the size difference of fat globules in the substrates. The HWM substrate is equivalent to homogenized milk, which results in the reduction of fat globule size and consequently an increase in the surface area [34]. The UHWM substrate that was not homogenized presents larger fat globules [35]. It is therefore possible that the greater surface area of the fat globules in the HWM substrate caused a more rapid degradation of the dissolved ozone, which justifies the less effective inactivation of *E. coli* O157:H7. Highlighted are the observed results regarding ozone concentration after passage through the column containing the substrates. When HWM was used, a lower residual concentration was obtained when compared to UHWM. A lower residual concentration is directly related to greater reactivity of the medium.

Table 4 Mean values of dissolved ozone concentration and temperature in water stored at 8 °C for 24 h

Variables	Stored period (h)					
	0	0.5	1.0	1.5	3.0	24
Concentration (mg L ⁻¹)	1.45	1.97	1.71	1.78	1.67	0.82
Temperature (°C)	14	12.0	11.0	11.0	9.5	8.0

The ozonated water used in the control of *E. coli* O157:H7 demonstrated a high efficiency of microorganism inactivation, even after storage for 24 h at a temperature of $8\text{ }^{\circ}\text{C} \pm 1$. Several studies have been found in literature that indicate the effectiveness of ozonated water for control of important microorganisms with regard to food conservation and safety. Mari et al. [36] observed in their study with pears that spore germination of the deteriorating fungi *Penicillium expansum*, *Botrytis cinerea*, and *Mucor piriformis* was inhibited by treatment with ozonated water at the concentrations of 0.99 to 0.4 mg L^{-1} for 5 min at $20\text{ }^{\circ}\text{C}$. Beltrán et al. [37] evaluated the effect of ozonated water on maintaining lettuce quality and indicated that ozone may be an alternative to chlorine for product conservation. Inatsu et al. [38] tested ozonated water as an antimicrobial agent for the in vitro control of several bacterial species, including *E. coli* O157:H7, *S. aureus*, *L. monocytogenes*, and *Pseudomonas aeruginosa*. The authors obtained a reduction of 7.4 log cycles in the counts of *E. coli* O157:H7 for an exposure time of 3.0 min and a concentration of dissolved ozone in water of 5.44 mg L^{-1} , at a temperature of $25\text{ }^{\circ}\text{C}$. Cavalcante et al. [19] tested ozonated water for the control of *E. coli* O157:H7 and *B. subtilis*, and observed reductions of 6.6 and 5.3 log cycles, respectively, for the ozone concentration of 1.0 mg L^{-1} and 1.0-min contact time. A study carried out by Tachikawa et al. [39] in which ozonated water was tested with concentrations between 0.9 and 3.2 mg L^{-1} and contact time between 1 and 20 min, for the disinfection and removal of biofilm from *P. fluorescence* and *P. aeruginosa*, reported that the survival rate of biofilms was 1.0% for ozone concentrations in water between 0.9 and 1.4 mg L^{-1} and exposure period of 5 min; however, when the dissolved ozone concentration in water increased to 3.2 mg L^{-1} , the survival rates were 0.01 and 0.00002% for exposure periods of 5 and 20 min, respectively.

Conclusions

The direct and indirect application of ozone produced significant reductions in the counts of *Escherichia coli* O157:H7 (ATCC® 43890™), proving its efficiency in controlling this pathogen. The effectiveness of ozonated water confirmed in this study, where the maximum reduction was obtained under the conditions evaluated, allows for recommending its use in the cleaning processes of equipment and utensils in the food industry, as an interesting alternative to traditional sanitizers. On the other hand, the efficiency of gaseous ozone in reducing and eliminating *E. coli* O157:H7 is influenced by the presence of organic substrates. This behavior reinforces the need for efficient removal of organic matter in cleaning processes, prior to the use of ozone.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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