Effect of ultrasound and chlorine dioxide on *Salmonella* Typhimurium and *Escherichia coli* inactivation in poultry chiller tank water

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ABSTRACT

This study evaluated the application of ultrasound alone or combined with chlorine dioxide (ClO_2) for *Salmonella* Typhimurium and *Escherichia coli* inactivation in poultry processing chiller tank water. A Full Factorial Design (FFD) 2^2 was conducted for each microorganism to evaluate the effect of ultrasound exposure time (x_1: 1 to 9 min; fixed: 37 kHz; 330 W; 25 °C) using a bath, and ClO_2 concentration (x_2: 1 to 17 mg L^{-1}) on microorganism count expressed in log CFU mL^{-1} in distilled water. Variable x_2 had a negative effect on *Salmonella* Typhimurium (-5.09) and *Escherichia coli* (-2.00) count, improving the inactivation; while a x_1 increase present no inactivation improvement, explaining the use of x_1 lower level (1 min) and x_2 higher level (17 mg L^{-1}). The best condition for microorganism inactivation based on FFD was evaluated in chiller tank water (with organic matter) at 25, 16, and 4 °C; x_1 was kept (1 min), however x_2 was adjusted to obtain the same residual free chlorine (2.38 mg L^{-1}) considering the ClO_2 consumption by organic matter, achieving the value of 30 mg L^{-1}. An inactivation of 49% and 31% were observed for *Salmonella* Typhimurium and *Escherichia coli*. When ultrasound was replaced by a simple agitation in the presence of ClO_2, there was no inactivation for both microorganisms. Moreover, at poultry carcass pre-chilling (16 °C) and chilling (4 °C) conditions, the synergism of ultrasound combined with ClO_2 was more pronounced, with microorganisms' reductions up to 100%.

1. Introduction

Considering the poultry meat productive chain complexity, poultry meat is susceptive to several pathogenic and deteriorating microorganisms. Therefore, the microbial load of poultry carcasses during processing is an ongoing concern and its control is a key factor in the quality and useful life of this product. The main bacterial contamination of chicken meat is focused on pathogenic bacteria such as *Salmonella, Campylobacter, Escherichia coli*, and *Listeria monocytogenes* [1,2].

Among the several steps in the poultry meat processing, the cooling carried out by continuous immersion of the carcasses in chilling water is

recognized as a source of cross contamination [3]. Studies suggest that the post-chill (final exit) poultry carcass microbiological population depends on: (1) upon pre-chill carcass contamination; and (2) chiller conditions, as the fresh water over-flow and the ratio carcasses/water [4]. In Brazil, according to federal legislation [5], it is determined that chillers water renewal occurs constantly in the opposite direction to the movement of the carcasses (countercurrent), in the minimum proportion of 1.5 L of water per carcass in the pre-cooling chiller and 1.0 L of water in the cooling chiller in order to minimize cross contamination.

Physical, chemical, and biological decontamination treatments may be applied after slaughtering, or even prior or during the chilling steps to

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reduce the prevalence of pathogenic and deteriorating microorganism in poultry carcass [6,7]. Physical treatments are based on hot water or steam, electrolyzed or ozonated, ionizing radiation, refrigeration, and freezing; chemicals ones include organic acids, phosphates, chlorine-based compounds, among others; the more limited is biological ones, which include bacteriophages and competitive microbial cultures [6,8]. Regardless of the chosen treatment, it is must be safe, viable, easy to apply, ecologically correct, and capable of keep the sensory properties of foods; however, keep all these principles is not always possible through the application of these methods.

Chlorine dioxide (ClO_2, CAS 10049–04-4) is a powerful chemical sanitizer that has broad and high biocidal activity, which offers many advantages compared to other chlorine-based sanitizers [9]. Chlorine dioxide is a chemical compound with oxidizing and disinfectants properties (2.5 times more efficient than liquid chlorine). Its use has been recommended by certain regulatory agencies to reduce contamination by *Salmonella* in fruits and vegetables, and poultry meat, without further indications of toxicity and adverse effects in the production process of meat products [10,11]. Studies indicated that foods subjected to high ClO_2 concentrations (200 mg L^{-1}) and for a long exposure time (24 h) had minimal chemical residues [12]. Therefore, chlorine dioxide in aqueous solution is rapidly reduced to chlorine and chlorate and if used, leaves no detectable residues of chlorine dioxide, chlorite, chlorate in fresh food as already observed in poultry carcasses [7].

On the other hand, emerging greener technologies as ultrasound have been developed to intensify industrial processes, resulting in reduction of water, chemicals, energy consumption, and wastewater generation [13]. The ultrasound stands out, which is a specialized and versatile technique that has applications in food processing, which is often used to extract, mix, emulsify, or sterilize [14,15]. Considering a liquid food (water, juice, milk or other), the ultrasound propagation generates compression and expansion cycles inside the liquid. These lead to the development of bubbles, which grow in size until the point when it is no longer sufficient to withstand the pressure from outside [16]. The bubbles collapse (implosion) causing a collision between liquid molecules and this process, called cavitation, along with the resulting shear and rise in temperature (approximately 5500 °C) and pressure (approximately 50 MPa) are responsible for disrupting the cell structure and subsequently the cell's death [17,18].

The cellular membrane is the first target for the lethal effects of cavitation, with at least 6 different injuries or modes of actions, generally referred to as cells disruption [19]. These effects would be caused by the different actions that the bubble formed during cavitation can perform on the microorganism. When the bubble increases during cavitation, its expansion can touch and push the membrane; it can implode near the membrane; pull the membrane during the compression/contraction phase, leading to its rupture; asymmetric bubble collapse can create a funnel; the flow of fluid around the oscillating bubble can create shear effects; and forces of high intensity ultrasonic radiation can diffuse across the membrane [20].

Several studies have demonstrated the synergistic effect of ultrasound and ClO_2 in microorganism inactivation in vegetables [21–23], and seaweed [24]. However, no studies have evaluated the effect of low frequency ultrasound alone or with the application together with ClO_2 on the microorganism inactivation in poultry processing chiller tank water. Therefore, the effect of ultrasound alone or combined with ClO_2 in the inactivation of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in poultry processing chiller tank water was studied. A Full Factorial Design (FFD) was conducted for each microorganism to evaluate the effect of ultrasound exposure time and ClO_2 concentration and consumption. Moreover, in order to prove the effect of ultrasound on the microorganism inactivation, a conventional mechanical agitation in the presence of ClO_2 to rule out the stirring effect on cells was compared.

2. Material and methods

2.1. Strains and materials

The strain *Salmonella* enterica sorovar Typhimurium ATCC 14028 and *Escherichia coli* serotype O157:H7 ATCC 43888 was activated in Brain Heart Infusion (BHI; Merck (Darmstadt, Germany) at 37 °C for 12 h up to obtain a cell count of 9 log CFU mL^{-1}.

The liquid ClO_2 ($\cong$ 25 °C) was obtained by the reaction of HCl and $NaClO_2$ using an acid generator (CDKc 3000, ProMaqua, Italy). In order to control the chlorine concentration, a N,N-diethyl-*p*-phenylenedi-amine colorimetric method was used; a pocket colorimeter (HI711, Hanna Instruments, Woonsocket, USA) with meter ranging from 0.00 to 2.50 mg L^{-1} and precision of $\pm$ 0.03 mg L^{-1} at 25 °C was employed.

Considering that water quality may affect the efficiency of chemical sanitizers [25], and in order to avoid chemical residue, especially chlorine, the chiller tank water was obtained by simulation procedure. Three plucked, gutted, and chilled chickens (7 to 9 °C), without foot and head, weighting 2.5 kg slaughtered following Animal Welfare Standards [26] 24 h prior, were individually and sequentially tumbled in distilled water (4.5 L) at $\approx$ 16 °C (kept by environment climatization) for 1 h each. The water obtained after tumbling was stored in 1 L boron silicate flasks, autoclaved at 121 °C for 15 min, characterized by lipid and protein content (AOAC, 2005), and kept at −18 °C.

2.2. Effect of ultrasound and ClO_2 on Salmonella Typhimurium and Escherichia coli inactivation in distilled water

Initially, the *Salmonella* Typhimurium and *Escherichia coli* inactivation were evaluated by a FFD 2^2 with triplicate at the central point (4 factorial points, 3 central points; total of 7 runs) in distilled water. The factors ultrasound exposure interval (x_1: 1 to 9 min) and ClO_2 concentration (x_2: 1 to 17 mg L^{-1}) were studied (Table 1); these variables were selected based on preliminary studies (data not shown).

The runs were conducted in ultrasound bath (Elmasonic P 120H, Elma, Singen, Germany), using a fixed frequency (80 and 37 kHz), nominal power (330 W), continuous and pulsed modes, frequency amplitude (40% and 100%), and temperature (25 °C). The 37 kHz fixed frequency, continuous mode, and 100% amplitude were selected for FFD considering the significantly high performance on cells inactivation (data not shown). The delivered power into US bath was estimated by calorimetry method according to previous works [27,28]. During run test conduction, the ultrasound bath water volume was controlled according to the equipment manufacturer's instructions, the sample layout was fixed (Fig. 1), and water temperature was controlled by ice addition, when necessary.

Table 1
FFD 2^2 matrix with coded and real factors and experimental responses for *Salmonella* Typhimuriumn and *Escherichia coli.*

Run	Coded (real) factors		*Salmonella* Typhimuriumn Cell count (Log CFU mL^{-1})	*Escherichia coli* Cell count (Log CFU mL^{-1})
	x_1	x_2		
1	− 1 (1)	−1 (1)	$7.09^{ab} \pm 0.03$	$6.99^a \pm 0.06$
2	1 (9)	−1 (1)	$7.10^{ab} \pm 0.02$	$6.88^{ab} \pm 0.05$
3	−1 (1)	1 (17)	$0.89^e \pm 0.16$	$5.38^d \pm 0.02$
4	1 (9)	1 (17)	$3.12^d \pm 0.05$	$4.50^e \pm 0.06$
5	0 (5)	0 (9)	$6.88^b \pm 0.05$	$6.58^c \pm 0.09$
6	0 (5)	0 (9)	$7.01^{ab} \pm 0.06$	$6.66^{bc} \pm 0.13$
7	0 (5)	0 (9)	$7.04^{ab} \pm 0.06$	$7.01^{ab} \pm 0.02$
C1	−	−	$7.16^a \pm 0.02$	$7.00^a \pm 0.06$
C2	0 (5)	−	$7.19^{ab} \pm 0.14$	$6.84^{abc} \pm 0.06$
C3	−	1 (17)	$5.13^c \pm 0.07$	$5.50^d \pm 0.08$

x_1: Ultrasound time (min); x_2: ClO_2 (mg L^{-1}); C1: without ultrasound and ClO_2 exposure; C2: only ultrasound exposure (5 min); C3 only ClO_2 exposure (17 mg L^{-1}); mean $\pm$ standard deviation (n = 2).
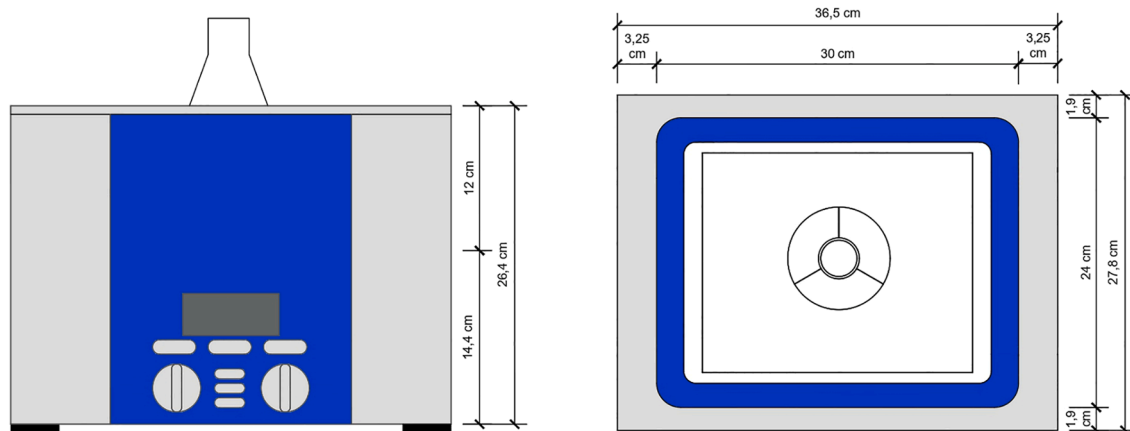
Fig. 1. Diagram of the position of the Erlenmeyer flask in the ultrasound bath for carrying out the runs.

Erlenmeyer flasks of 250 mL were filled with 100 mL of distilled water and autoclaved (121 °C for 15 min), followed by ClO_2 addition (according Table 1) and microorganism inoculation (1.0%; v v^{-1}). The samples were exposed to ultrasound one at a time and for pre-established intervals (Table 1). After the ultrasound application, the runs were kept at rest until completing 9 min of contact time with ClO_2 to maintain the same immersion conditions. Moreover, three control treatments were performed in parallel: (C1) without ultrasound and ClO_2 exposure; (C2) only ultrasound exposure (5 min); and (C3) only ClO_2 exposure (17 mg L^{-1}). The response variable was the microorganism count determined in duplicate by inoculating the water sample by pour plate in Plate Count Agar (PCA; Merck, Darmstadt, Germany) added of 2,3,5-triphenyltetrazolium chlorine (0.005%; m v^{-1}) and incubated at 37 °C by 24 h. The results were expressed by mean ± standard deviation in log CFU mL^{-1}.

The FFDs runs were randomly conducted, and the data were analyzed by experimental design procedure of Statistica 8.0 software (Statsoft Inc., Tulsa, USA). The adequacy of the linear models was expressed by the determination coefficient (R^2) and adjusted R^2 (R^2-adj), and statistical significance was determined by analysis of variance (ANOVA) ($p < 0.05$).

2.3. Inactivation of Salmonella Typhimurium and Escherichia coli in chiller tank water

The microorganism inactivation test was carried out in the chiller tank water in order to study conditions closer to observed in poultry slaughterhouse. Total chemical oxygen demand (TCOD) was determined according to the methodology 5220 D - Standard Methods for the Examination of Water and Wastewater (SMWW) [29]. After establishing the best condition for *Salmonella* Typhimurium and *Escherichia coli* inactivation according to FFD 2^2 ($x_1 = 1$ min; $x_2 = 17$ mg L^{-1}) in distilled water at 25 °C, the model was validated for both microorganisms replacing the distilled water by chiller tank water at temperatures of 4, 16, and 25 °C. The two first temperatures were based on pre-cooling and cooling immersion chiller tank systems, which should not exceed 16 °C and 4 °C, respectively [5]. Moreover, despite the lower reactivity of ClO_2 in relation to other chlorine-based disinfectant compounds [9], it was necessary to correct the ClO_2 concentration in order to supply the same free chlorine concentration provided by 17 mg L^{-1} of ClO_2 in distilled water. Thus, 30 mg L^{-1} of ClO_2 was added to chiller tank water to achieve the same free chlorine (2,38 mg L^{-1}) of distilled water.

Thus, the test in chiller tank water was performed in triplicate using an ultrasound exposure time of 1 min followed by 8 min of rest, 30 mg L^{-1} of ClO_2, at temperatures of 4, 16, and 25 °C; and the response was the microorganism count. Moreover, the controls C1, C2, and C3 were performed in the same context described for FFD. Furthermore, the test

in chiller tank water was also conducted replacing the ultrasound exposing by a simple manual stirring at different rotation speed (200, 400, 600, 800, and 1000 rpm), in order to evaluate the contact effect of 30 mg L^{-1} of ClO_2 with cells for 9 min at 4, 16, and 25 °C and to rule out the stirring effect. The data were analyzed by ANOVA and Tukey's test ($p < 0.05$) using the Statistica 8.0 software.

3. Results and discussion

3.1. Effect of ultrasound and ClO_2 on Salmonella Typhimurium inactivation in distilled water

Salmonella Typhimurium count from 7.10 to 0.89 log UFC mL^{-1} was observed for FFD runs (Table 1). The effects of curvature, ultrasound time (x_1), and interaction (x_1 by x_2) were positive ($p < 0.05$), indicating that an increase in these factors present no improvement on the *Salmonella* Typhimurium inactivation (Table 2). In contrast, the ClO_2 concentration (x_2) had a negative effect on microorganism count ($p < 0.05$), indicating that an increase in this factor improved the *Salmonella* Typhimurium inactivation (Table 2). In this context, run 3 with lower ultrasound time ($x_1 = 1$ min) and higher ClO_2 concentration ($x_2 = 17$ mg L^{-1}) was the best condition to inactivate the microorganism studied. Comparing run 3 and C1, a cell count reduction of 88% was observed. However, comparing C2 and C3 with C1, in the first case no reduction was observed, showing that the ultrasound alone had no effects on *Salmonella* Typhimurium inactivation understudied condition; moreover, comparing C3 with C1, only a reduction of 28% was observed, reinforcing that ClO_2 had a key role in inactivation, but acts

Table 2

FFDs effects on *Salmonella* Typhimurium and *Escherichia* coli inactivation.

Microorganism	Factor	Effect	Standard error	p-value
Salmonella Typhimurium	Mean	4.55	0.04	107.00	0.000
	Curvature	4.85	0.13	37.36	0.001
	Ultrasound time (x_1)	1.12	0.09	13.17	0.006
	ClO_2 concentration (x_2)	−5.09	0.09	−59.85	0.000
	x_1 by x_2	1.11	0.09	13.05	0.006
Escherichia coli	Mean	5.94	0.11	51.93	0.000
	Curvature	1.63	0.35	4.65	0.043
	Ultrasound time (x_1)	−0.50	0.23	−2.16	0.163
	ClO_2 concentration (x_2)	−2.00	0.23	−8.72	0.013
	x_1 by x_2	−0.38	0.23	−1.68	0.234

synergistically with short time ultrasound applications (run 3; $x_1 = 1$ min; $x_2 = 17$ mg L^{-1}).

A valid linear model (F calculated: 1330; F tabulated: 19; $p < 0.05$) was obtained for response *Salmonella* Typhimurium cells count. The adjusted linear model (with $R^2 \geq 0.99$ and adjusted $R^2 \geq 0.99$) is shown in Fig. 2 A. The model suggests that lower ultrasound time exposure and higher ClO_2 concentration in the studied range reduced the *Salmonella* Typhimurium cells count, improving the microorganism inactivation. The ultrasound exposure may has a lethal effect in microorganisms for increasing the permeability of the cell membrane due to the sonoporation effect [20,30], contributing to input ClO_2 into the cell. On the other hand, if long-term ultrasound application was performed, they cause ClO_2 degradation; it may be occur by ClO_2 evaporation caused by temperature increase due to cavitation, reducing ClO_2 antimicrobial effect as detailed by Huang et al. [22].

3.2. Effect of ultrasound and ClO_2 on *Escherichia coli* inactivation in distilled water

Escherichia coli count varied from 7.01 to 4.50 log UFC mL^{-1} for FFD runs (Table 1). The effect of curvature was positive ($p < 0.05$), indicating that an increase in this factor reduces the *Escherichia coli* inactivation. In contrast, the ClO_2 concentration (x_2) had a negative effect on microorganism count ($p < 0.05$), indicating that an increase in this factor improved the *Escherichia coli* inactivation (Table 2). The factors ultrasound time (x_1), and interaction (x_1 by x_2), although had negative effects, were no significant on the studied response ($p > 0.05$). In this context, run 4 and 3 with higher ClO_2 concentration ($x_2 = 17$ mg L^{-1}) were the best condition to inactivate the microorganism studied. Comparing run 4 and 3 with C1, a cell count reduction of 36% and 23% were achieved. However, comparing C2 and C3 with C1, in the first case a reduction of 2% was observed, showing that the ultrasound alone had practically no effects on *Escherichia coli* inactivation; moreover, in the second case, a reduction of 21% was observed, reinforcing that ClO_2 had a key role in *Escherichia coli* inactivation.

A valid linear model (F calculated: 26; F tabulated: 19; $p < 0.05$) was obtained for response *Escherichia coli* cells count. The adjusted linear model (with $R^2 \geq 0.98$ and adjusted $R^2 \geq 0.94$) is shown in Fig. 2 B. The model suggests that longer ultrasound exposure time and higher ClO_2 concentration in the studied range reduced the *Escherichia coli* cells count, improving the microorganism inactivation.

For *Escherichia coli* the combination of ultrasound exposure and ClO_2 action was less effective if compared with *Salmonella* Typhimurium. The

parameters of ultrasound exposure time had a negative effect for *Salmonella* and no effect for *Escherichia coli*, indicating an advantage of using 1 min; moreover, the ClO_2 use in the concentration of 17 mg L^{-1} was the best choose in the studied range for both microorganisms' inactivation.

Aqueous ClO_2, being a strong highly reactive oxidizing agent, is rapidly reduced to chlorite and chlorate in water [7]. The ultrasound exposure accelerates the reaction speed; therefore, longer ultrasound exposure times lead to a greater chemical compound degradation, reducing ClO_2 content available in the medium, reducing its microorganism inactivating efficiency [20]. Thus, the ultrasound treatment during 1 min would be enough to increase the bacteria cell permeability without causing significant ClO_2 degradation to the point of decreasing its efficiency. Thus, in the model validation using chiller tank water was performed using 1 min of ultrasound exposure.

3.3. Inactivation of *Salmonella* Typhimurium and *Escherichia coli* in chiller tank water

The chiller tank water used in the validation of the models obtained for *Salmonella* Typhimurium and *Escherichia coli* inactivation contained $0.196 \pm 0.008\%$ of proteins and $0.013 \pm 0.001\%$ of lipids. Due to the organic matter present, the ClO_2 was consumed, justifying a ClO_2 concentration adjustment in order to obtain the same residual free chlorine. In this aspect, the test conducted with chiller tank water had ClO_2 addition improved from 17 mg L^{-1} (distilled water) to 30 mg L^{-1}, ensuring a final free chlorine concentration of 2,38 mg L^{-1}, the same obtained by the addition of 17 mg L^{-1} in distilled water. The free chlorine concentration obtained both in distilled or chiller tank water was in accordance with the regulatory agencies. In Brazil, the legislation [5] establishes a free chlorine content up to 5 mg L^{-1} in poultry carcass chilling water. Moreover, the ClO_2 use as an antimicrobial in water for the meat (poultry) and fruit and vegetable industry was approved by the Food and Drug Administration (FDA) in 2001 [31]. The FSIS Directive 7120.1 (revision 45 of 19/01/18) of the USDA (United States Department of Agriculture) establishes the use of ClO_2 as the antimicrobial agent to be applied in several uses. Noteworthy, ClO_2 use is prevised in the water used in the poultry processing with a maximum limit of 3 mg L^{-1} residual chlorine dioxide [32,33].

Table 3 present the results obtained in FFD model validation for *Salmonella* Typhimurium and *Escherichia coli* inactivation in chiller tank water at 4, 16, and 25 °C. Comparing test 1, 2, and 3 for both microorganisms studied, it was observed that at lower temperatures the

(A) $y = 4.55 + 2.43C + 0.56x_1 - 2.55x_2 + 0.56x_1x_2$ **(B)** $y = 5.94 + 0.81C - 0.25x_1 - 1.00x_2 - 0.19x_1x_2$
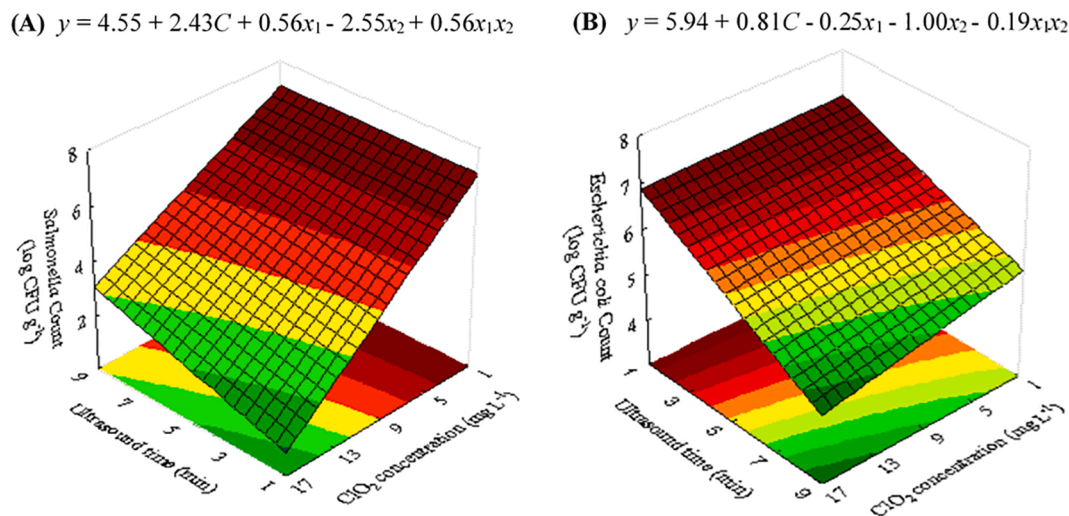

Fig. 2. Linear model surface response for (A) *Salmonella* Typhimurium and (B) *Escherichia coli*. C: curvature ($C = 1$ on central point, for x_1 and $x_2 = 0$; $C = 0$ on the other points); x_1: Ultrasound time (min); x_2: ClO_2 (mg L^{-1}).

Table 3
Results of FFD 2^2 model validation in chiller tank water for *Salmonella* Typhimurium and *Escherichia coli* inactivation.

Test	Test conditions			*Salmonella* Typhimurium Cells count (log CFU mL^{-1})	*Escherichia coli* Cells count (log CFU mL^{-1})
	x_1	x_2	Temperature (°C)		
1	1	30	4	$< 1.00^e \pm 0.00$	$2.58^f \pm 0.06$
2	1	30	16	$2.56^d \pm 0.06$	$3.83^d \pm 0.05$
3	1	30	25	$3.81^b \pm 0.03$	$5.27^c \pm 0.07$
C1	–	–	25	$7.50^a \pm 0.16$	$7.61^a \pm 0.08$
C2	1	–	25	$7.61^a \pm 0.06$	$6.94^b \pm 0.08$
C3	–	30	–	$3.07^c \pm 0.09$	$2.96^e \pm 0.03$

x_1: Ultrasound time (min); x_2: ClO$_2$ (mg L^{-1}); C1: without ultrasound and ClO$_2$ exposure; C2: only ultrasound exposure (1 min); C3 only ClO$_2$ exposure (30 mg L^{-1}); mean ± standard deviation (n = 3); different lowercase letters in the same column indicate significant differences by Tukey test (p < 0.05).

ultrasound exposure time and ClO$_2$ addition present a greater effect in microorganism inactivation. Considering test 3 and C1, it was observed a decrease of 49% and 31% for *Salmonella* Typhimurium and *Escherichia coli*, respectively. Furthermore, comparing C2 and C3 with C1, in the first case no reduction was observed for *Salmonella* Typhimurium and a slight reduction (9%) was observed for *Escherichia coli*, showing that the ultrasound alone had practically no effects for both microorganisms. In the second case, a reduction of 59% and 61% were observed for *Salmonella* Typhimurium and *Escherichia coli*, respectively. This reinforces the antimicrobial potential of ClO$_2$ on microorganism studied inactivation.

In contrast as observed in the FFDs preformed in distilled water, ultrasound exposure seems to not affect both microorganisms in chiller tank water at 25 °C. Some reasons should be associated with frequency value (37 kHz), delivered power (45 W L^{-1}), and amplitude (100%) employed. Another studied in the literature reported these parameters as the conditions to inactivate bacteria cells, generally in higher ultrasound time exposure than studied in the current article [34–37]. It is important to emphasize that in the presence of organic matter observed for chiller tank water, which increases the attenuation to ultrasound movement, an increase in amplitude would contribute to the ultrasound device obtaining the necessary mechanical vibrations for promoting greater cavitation and consequently greater efficiency in the microorganisms inactivation [38]. Although, the intensity may not be increased indefinitely, because as the pressure increases, the bubbles may become so thin that the time available for the collapse would be inadequate [39]. Furthermore, the FFDs results indicating that more aggressive conditions of ultrasound exposure may degraded the ClO$_2$. Even considering that interaction between ClO$_2$ and organic matter does not produce toxic by-products, as occurs with NaClO, ClO$_2$ is able to oxidize a large fraction of natural organic matter, reducing its availability in inactivating target microorganisms [40]; moreover, ultrasonication may quickly disperse the organic matter more, increasing its contact with ClO$_2$, reducing ClO$_2$ residue [22]. It was observed considering the free chlorine residual determined before (2.38 mg L^{-1}) and after (2.00 mg L^{-1}) ultrasound exposure during 1 min, without the presence of inoculum.

The model validation performed in 3 different temperatures indicated that at lower temperatures (4 and 16 °C) the results obtained using chiller tank water were satisfactory. Considering chiller conditions (4 °C) a 100% reduction of *Salmonella* Typhimurium and 66% reduction of *Escherichia coli* was achieved; for pre-chilling (16 °C), the reduction was 66% and 48%, respectively, improving the sanitary conditions of poultry slaughterhouse processing plants. According to Paniwnyk (2017), the temperature of the medium in which the ultrasound is applied influences the effect on microorganisms, with lower temperatures showing better results. At lower temperatures in the medium the cavitation has a better result, since the temperature of the solvent does not rise as its vapor pressure, while at high temperatures, more solvent

vapor fills the cavitation bubbles which then collapse less violently, causing less intense effects than expected [38]. Moreover, previous studies showed that residual ClO$_2$ in hot water was lower than in cold water, presumptively related to faster solubilization of ClO$_2$ [41].

The ultrasound exposure replacement by manual stirring demonstrated that no effect was added increasing the rotation speed from 200 to 1000 rpm in the presence 30 mg L^{-1} of ClO$_2$ both for *Salmonella* Typhimurium and *Escherichia coli* (p > 0.05 by Tukey test) (Fig. 3). Furthermore, the effect of stirring on cells inactivation was 2 and 1.5 times lower for *Salmonella* Typhimurium and *Escherichia coli* than ultrasound exposure. Comparing the mean data of Fig. 3 with data of Table 3 the combination of ultrasound exposure and 30 mg L^{-1} of ClO$_2$ at 4, 16, and 25 °C reduced the *Salmonella* Typhimurium count at least 4 cycles log more than stirring, and at least 2.5 cycles log more for *Escherichia coli*.

Despite the proven advantages of ClO$_2$ in the treatment of drinking water and in the sanitization of food, it has some limitations and cares about its use. Chlorine dioxide is unstable as a compressed gas and cannot be shipped and stored and it should be produced on-site. Furthermore, the gaseous ClO$_2$ applications in the food industry may be limited because the treatment must be conducted in a firmly and safely sealed chamber [42], high concentrations of the gas are potentially explosive [43], and numerous mechanical devices or steps are necessary to handle ClO$_2$ gas as well as to provide precise concentrations for sanitization [44]. Considering that gaseous ClO$_2$ and its principal precursors, sodium chlorite (NaClO$_2$), and sodium chlorate (NaClO$_3$) are strong oxidizers appropriate precautions must be taken in their handling and use. Thus, this study suggests the use of aqueous ClO$_2$ with several advantages: (1) a special chamber is not required for the sanitizing process; (2) handling is easier than with gaseous ClO$_2$; and (3) the liquid solution may be easily applied to the existing process without modifying subsequent steps [44].

Comparing the current study with the literature (Table 4), it was visible that, mainly, longer ultrasound times application was used in the microorganism's inactivation on wastewater; however, this did not correspond to better results. In the current study we indicated the absence of *Salmonella* in water previously contaminated with 7 log CFU mL^{-1}, and the reduction of 5 log cycle for *Escherichia coli*, also previously contaminated with 7 log CFU mL^{-1}. The present study proved an excellent result compared to the study of Drakopoulou et al. [45], which used probe and ultrasound exposure times of up to 60 min in order to treat municipal wastewater with TCOD of only 56 ± 14 mg L^{-1}. The cited authors achieved a 99% reduction for *Pseudomonas* spp. despite the addition of titanium dioxide (TiO$_2$) combined with ultrasound exposure. Moreover, when an ultrapure water was ultrasound treated using a probe and 70:30 Ar:O$_2$ mix, a 2.8 cycle log reduction was observed for *Escherichia coli* [46]; this result was 2 times inferior if compared with the present study that reach 5 cycle log reduction for the same microorganism.

Other studies were carried out to inhibit *Escherichia coli* using only ultrasound exposure, but they used times greater than 1 min. Dehghani et al. [47] obtained a 98% reduction for *Escherichia coli* applying 42 kHz during more than 45 min of ultrasound exposure. An ultrasound application using a probe and 20 kHz during 3 min reduced the *Escherichia coli* more than 90% using a pulsed mode; for a continuous mode, a 10 min of exposure was required for the same inactivation rate [48]. Moreover, Zhou et al. [49] reported lower inactivation for total coliforms in drinking water treatment sludge obtained with bath and probe, 25 and 40 kHz, 0.03 W mL^{-1}, over 30 min of exposure. After 5 min the bath and probe reduced the microorganism no more than 15% and 5%, and after 30 min reduced no more than 30% and 20% under the same conditions.

Furthermore, the results obtained in the current study indicate that it is not appropriate to just take a science-oriented approach to emerging technologies. However, the traditional food processes must consider and it is strongly recommended to improve or combine them with the
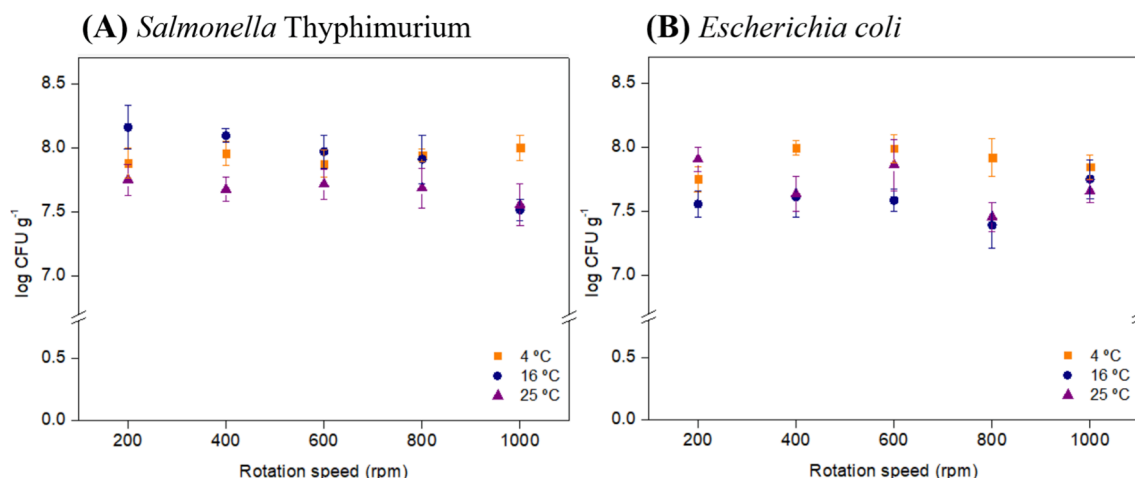
Fig. 3. Effect of stirring at different rotation speed in ultrasound exposure replacement combined with 30 mg L^{-1} of ClO$_2$ in chiller thank water for (A) *Salmonella* Typhimurium and (B) *Escherichia coli* at 4 °C (■), 16 °C (●), and 25 °C (▲). Data for comparison considering ultrasound exposure of 1 min and ClO$_2$ concentration of 17 mg L^{-1} in distilled water: 0.89 CFU mL^{-1} for *Salmonella* Typhimurium; and 5.38 CFU mL^{-1} for *Escherichia coli*.

Table 4
Comparison of studies reporting the ultrasound exposure on microorganism cells inactivation.

Sample	Reagent combined with US	Conditions used	Time	Energy density	Total chemical oxygen demand (TCOD)	Microorganism	Optimized conditions	Result	Reference
Poultry chiller tank water	ClO$_2$ (1 to 17 mg L^{-1})	Bath, 37 kHz; 330 W	1 to 9 min	–	2150 mg L^{-1}	*Salmonella* Typhimurium and *Escherichia coli*	4 °C, 30 mg L^{-1} ClO$_2$, 1 min	Reductions up to 100% to *Salmonella* Typhimurium, and reduction of 5 cycle log to *Escherichia coli*	Current study
Secondary treated municipal wastewater	TiO$_2$ g L^{-1}	Probe: 24 kHz, 300 W, in the absence and presence of TiO$_2$ particle	15, 30 and 60 min	1.5 W mL^{-1}	56 ± 14 mg L^{-1}	*Coliforms, Faecal coliforms, Pseudomonas* spp., *Faecal streptococci*, and *Clostridium perfringens*	60 min	Inactivation of 66% and 84% the gram-positive bacteria, and 5 g L^{-1} TiO$_2$ enhanced the destruction of gram-negative bacteria above 99%	[45]
Ultrapure water	Oxygen, argon, and an argon/oxygen mixture	Probe: 20 kHz, 205 a 1017 kHz; 80, 85, and 140 W; 74, 18.5, and 4.6 W cm^{-2}	0–60 min	0.27, 0.28, and 0.46 W mL^{-1}	–	*Escherichia coli*	70:30 Ar:O$_2$, 20 kHz, 80 W, 74 W cm^2, 0.27 W mL^{-1}, 60 min	2.8 cycle log reduction	[46]
Drinking water	–	Bath: 42 kHz, 155 W	1, 15, 30, 45, 60, 75, and 90 min	0.12 W mL^{-1}	–	*Escherichia coli*	42 kHz, 0.12 W mL^{-1}, above 45 min	microbial reduction was 98.0%	[47]
Purified wastewater	–	Probe: 20 kHz modes continuous and pulsed, and 40 kHz, mode continuous	0, 3, 5, 7, 10, 15, and 20 min	–	–	*Escherichia coli* bacilli	20 kHz, with the pulsation mode, 3 min	90% reduction	[48]
Drinking water treatment sludge	–	Bath: 25 kHz or 40 kHz	5, 10, 15, 20, 30 min	0.03 W mL^{-1}	298–331	Total bacteria, and Total coliforms	Probe, 5 W mL^{-1}, 5 min	Inactivation 81.25% of total bacteria, with 5 W ml^{-1} for 5 min, however low inactivation of total coliforms	[49]
		Probe: 25 kHz	5, 10, 15, 20, 30 min	0.03, 1, 3, 5 W mL^{-1}	298–331	Total Coliforms and Total bacteria			

technologies rising. Thus, the synergistic performance with pre-existing procedures may be viable. Future research should investigate the feasibility of chlorine dioxide application, given minimum effective concentrations of this study and higher ultrasound frequency ranges, as a chiller water disinfectant during pilot-scale processing.

4. Conclusions

The use of ClO$_2$ combined with ultrasound exposure (37 kHz; 330 W; 1 min) inactivated the microorganism *Salmonella* Typhimurium at 25 °C, in chiller tank water under pre-chilling and chilling temperatures (16 °C and 4 °C). For *Escherichia coli* the same effect was observed mainly at chilling conditions. The ClO$_2$ concentration required for microorganism

inactivation, both in distilled and chiller tank water, needs to ensure 2.38 mg L^{-1} of residual free chloride. Furthermore, the efficacy of ultrasound on microorganism's inactivation was confirmed by comparison with manual stirring in the presence of ClO_2.

5. Authors' contributions

Ana Paula Rossi: Development of methodology. Conducting of experiments. Application of statistical. Validation of results/experiments. Preparation, creation and writing of the published article. Daneysa Lahis Kalschne: Formulation of overarching research goals and aims. Oversight and leadership responsibility for the research activity planning and execution. Preparation, creation and writing of the published article. Ana Paula Iglikowski Byler: Development of methodology. Conducting of experiments. Application of statistical. Validation of results/experiments. Preparation, creation and writing of the published article. Eder Lisandro de Moraes Flores: Development of methodology. Conducting of experiments. Application of statistical. Validation of results/experiments. Preparation, creation and writing of the published article. Oldair Donizeti Leite: Development of methodology. Conducting of experiments. Application of statistical. Validation of results/experiments. Preparation, creation and writing of the published article. Daniel dos Santos: Development of methodology. Application of statistical. Validation of results/experiments. Preparation, creation and writing of the published article. Juliano Smanioto Barin: Development of methodology. Application of statistical. Validation of results/experiments. Preparation, creation and writing of the published article. Cristiane Canan: Formulation of overarching research goals and aims. Acquisition of the financial support for the project leading to this publication. Oversight and leadership responsibility for the research activity planning and execution. Preparation, creation and writing of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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