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Ultrasound and slightly acid electrolyzed water application: An efficient combination to reduce the bacterial counts of chicken breast during pre-chilling

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ABSTRACT

Pre-chilling leads to a temperature decline of the pre-rigor muscle of poultry carcasses, and a reduction of the initial bacterial load may occur. Both ultrasound (US) and slightly acidic electrolyzed water (SAEW) have been used alone in the meat industry for the manufacture of emulsions, pasteurization, and prevention of bacteria growth. However, the impact of the combination of these technologies during the pre-chilling of chicken carcasses has not been evaluated. In this study, breast chicken cylinders (CBCs) were pre-chilled for 10 min at 10 °C using SAEW and different US frequencies (25 and 130 kHz). The microbiological characteristics, lipid and protein oxidation, shear force, and anaerobic glycolysis were evaluated. The US + SAEW combination led to an effective reduction ($P < 0.05$) of enterobacteria, mesophilic bacteria, lactic acid bacteria, and psychrotrophic bacteria, while the lipid and protein oxidation, shear force, anaerobic glycolysis, and muscle structure were not affected ($P > 0.05$). Therefore, the combination of these technologies may be promising in the pre-chilling stage of chicken carcasses.

1. Introduction

The world population should reach about 8.6 billion people by 2030 (United Nations Population Division, 2017). Poultry meat has a great impact on the diet of the world's population, thus the poultry industry must look for innovative process technologies that allow quality, and are cheap, quick and easy to use. Water immersion is one of the most common methods used for chilling poultry carcasses. However, this process is time-consuming and has the disadvantage of consuming a large amount of water and energy. Besides, poultry carcasses may present high microbiological contamination at the end of the process, if the water used in the process does not present good microbiological quality, and the renewal is not done efficiently (USDA, 2008).

The intestinal tract of birds, especially chickens and turkeys, is one of the main natural reservoirs of pathogenic microorganisms, such as Salmonella spp. and Campylobacter spp. In addition, other mesophilic bacteria responsible for food-borne infections, such as enterohemorrhagic *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*, can also be isolated from poultry meat (Barbut, 2001). For these reasons, procedures aimed at the decontamination of these carcasses are required. To reduce the carcass microbial load, the poultry industry uses hot water in the scalding stage, and chilled water to immerse the carcasses in the chiller in the pre-chilling and cooling stages (James et al., 2006). Consequently, new technologies can be used in these stages, aiming at improving the quality of the carcasses and immersion water.

Ultrasound (US) acts on the bacteria through direct and indirect effects of cavitation, and the generation of reactive oxygen species (Mason and Lorimer, 2002). Cavitation generates mechanical effects, which can produce micro-cracks in the bacterial cell membranes, allowing some substances to pass from the medium into the cells (Gao et al., 2014). The US can also modify the texture of meat, which is also related to the cavitation phenomenon, which leads to a weakening of muscle structure, with rupture of myofibrils (Stadnik and Dolatowski,

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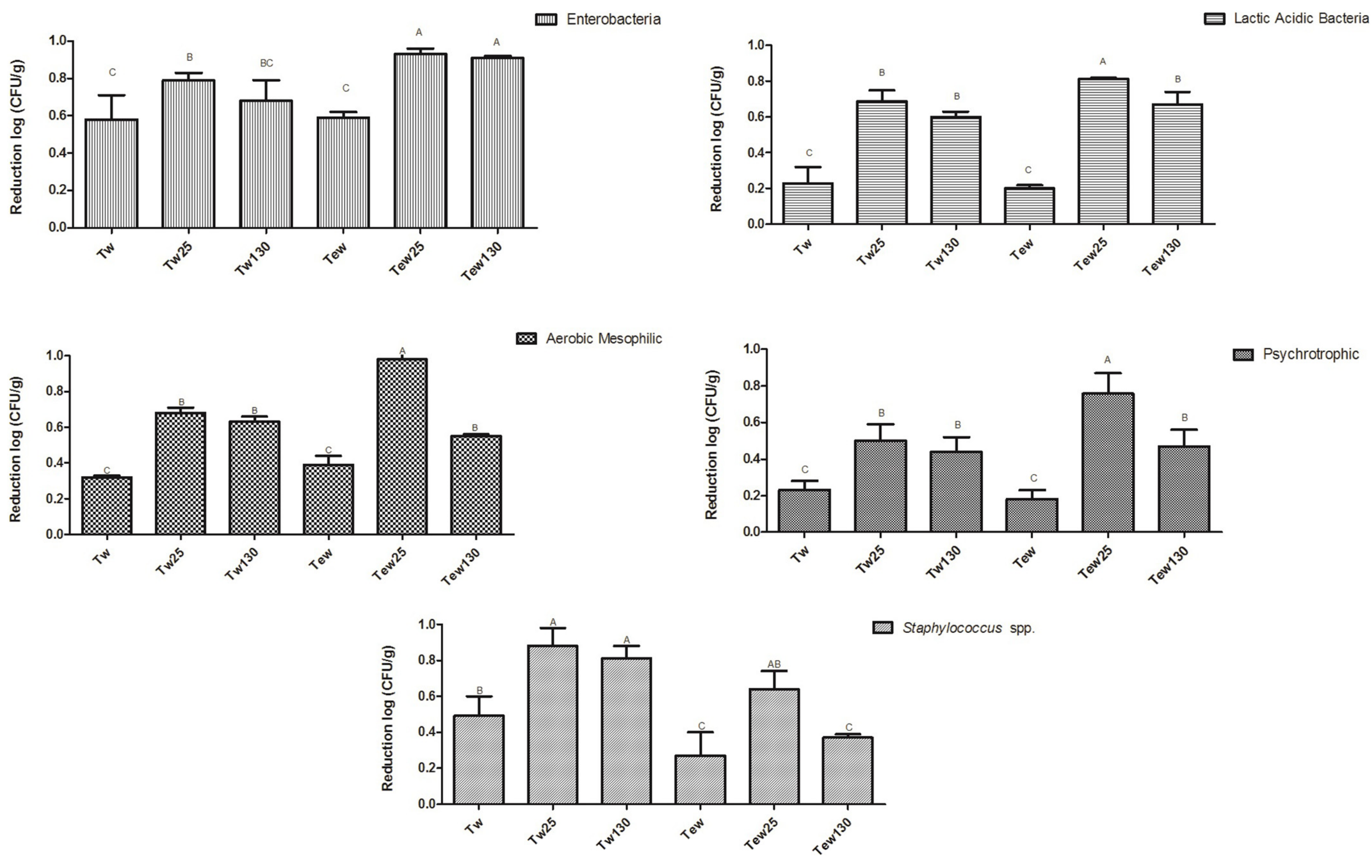

Fig. 1. Reduction of bacteria counts of the breast chicken cylinders (CBCs) pre-chilled for 10 min at 10 °C using SAEW and different US frequencies (25 and 130 kHz). Note: microbiological counts of the Control (CBCs without immersion): 3.47 log CFU/g psychrotrophic; 3.25 log CFU/g mesophilic; 3.22 log CFU/g lactic bacteria; 2.10 log log CFU/g enterobacteria and 2.25 log CFU/g *Staphylococcus* spp.
Different letters (A, B, C, D) indicate significant difference ($P < 0.05$) by Duncan test. Values expressed as mean ± standard deviation. Tw (CBCs immersed in water without the application of US), Tw25 (CBCs immersed in water with the application of US at 25 kHz), Tw130 (CBCs immersed in water with the application of US at 130 kHz), Tew (CBCs immersed in SAEW without the application of US), Tew25 (CBCs immersed in SAEW with the application of US at 25 kHz) and Tew130 (CBCs immersed in SAEW with the application of US at 130 kHz).

Table 1
Number of colonies of mesophilic and psychrotrophic bacteria (log CFU/mL) present in water and SAEW before and after immersion of CBCs for 10 min at 10 °C.

	Mesophilic		Psychrotrophic	
	Before immersion	After immersion	Before immersion	After immersion
Tw	2.84^b ± 0.14	3.59^a ± 0.12	2.12^b ± 0.07	3.32^a ± 0.13
Tw25	3.39^b ± 0.22	3.53^a ± 0.30	3.32^a ± 0.15	3.37^a ± 0.05
Tw130	3.42^a ± 0.10	3.45^a ± 0.06	2.91^b ± 0.20	3.30^a ± 0.06
Tew	< 1.00*	< 1.00*	< 1.00*	< 1.00*
Tew25	< 1.00*	< 1.00*	< 1.00*	< 1.00*
Tew130	< 1,00*	< 1.00*	< 1.00*	< 1.00*

Values expressed as mean ± standard deviation. Different letters in the same row (a, b, c) for each group of bacteria represent a significant statistical difference ($P < 0.05$) by the Duncan test. Batches: Tw (CBCs immersed in water without the application of US), Tw25 (CBCs immersed in water with the application of US at 25 kHz), Tw130 (CBCs immersed in water with the application of US at 130 kHz), Tew (CBCs immersed in SAEW without the application of US), Tew25 (CBCs immersed in SAEW with the application of US at 25 kHz) and Tew130 (CBCs immersed in SAEW with the application of US at 130 kHz).

2011). In addition, cavitation may cause damage to the structure of cellular organelles, promoting the release of the lysosome cathepsin enzymes and calcium from the sarcoplasmic reticulum, which activates the calpain enzyme system, thus accelerating proteolysis (Chandrapala et al., 2012). In turn, water sonication by the US can generate disulfide bonds and free radicals, which may favor the protein and lipid oxidation (Liao et al., 2007).

The slightly acidic electrolyzed water (SAEW) is a new technology with great potential to improve the microbiological quality of the cooling process of poultry carcasses. However, the application of SAEW in the cooling process of poultry carcasses is not yet regulated in Brazil and EU. On the other hand, in the USA the SAEW is allowed as a pH control agent of the water used in poultry and red meat processing (USDA-FSIS, 2014, Directive 7120.1). The SAEW contains hypochlorous acid (HClO) that can reduce microbial growth since it promotes the oxidation of protein compounds involved in metabolism, cellular rearrangements, and DNA damage (Huang et al., 2008). In addition, it may cause a modification in the electron transfer mechanism (redox potential) of microorganisms (Liao et al., 2007). However, SAEW may also accelerate the lipid and protein oxidation, once it can damage biological structures, as also observed for the US (Shirahata et al., 1997).

Table 2
Lipid (conjugated dienes, peroxides and TBARS) and protein (carbonyl and thiol) oxidation of the CBCs before (control) and after immersion in water and SAEW with and without application of US at 10 °C for 10 min.

	Control	Tw	Tw25	Tw130	Tew	Tew25	Tew130
Conjugated diene	$2.58^{A} \pm 1.71$	$1.13^{A} \pm 0.18$	$2.15^{A} \pm 0.18$	$2.57^{A} \pm 0.20$	$1.10^{A} \pm 0.08$	$1.42^{A} \pm 0.25$	$1.46^{A} \pm 0.12$
Peroxide	$0.67^{AB} \pm 0.37$	$0.23^{B} \pm 0.06$	$0.03^{C} \pm 0.01$	$0.04^{C} \pm 0.007$	$0.95^{A} \pm 0.04$	$0.20^{B} \pm 0.07$	$0.74^{A} \pm 0.05$
TBARS	$0.14^{A} \pm 0.11$	$0.07^{B} \pm 0.005$	$0.06^{B} \pm 0.01$	$0.06^{B} \pm 0.004$	$0.13^{A} \pm 0.001$	$0.14^{A} \pm 0.05$	$0.26^{A} \pm 0.02$
Carbonyl	$10.72^{A} \pm 6.45$	$2.21^{B} \pm 0.10$	$1.88^{B} \pm 0.39$	$1.49^{B} \pm 0.10$	$7.90^{AB} \pm 0.81$	$6.73^{AB} \pm 1.08$	$7.27^{AB} \pm 1.66$
Thiol	$109.6^{A} \pm 11.83$	$98.7^{A} \pm 4.74$	$100.22^{A} \pm 22.21$	$106.38^{A} \pm 13.38$	$123.52^{A} \pm 9.27$	$117.78^{A} \pm 11.71$	$115.54^{A} \pm 11.25$

Different letters on the same line indicate a significant difference ($P < 0.05$) by Duncan's test. Values expressed as mean ± standard deviation. Conjugated dienes expressed in milligrams of lipid per ml of cyclohexane; Peroxides expressed as milliequivalents of peroxide per kilogram of sample; TBARS expressed in milligrams of malonaldehyde (MDA) per kilogram of sample; Carbonyl expressed as dinitrophenylhydrazine (DNPH) nanomol per milligram of sample and Thiol expressed as nanophosphate of sulphhydryl (SH) per milligram of sample. Batches: Control (CBCs without immersion), Tw (CBCs immersed in water without the application of US), Tw25 (CBCs immersed in water with the application of US at 25 kHz), Tw130 (CBCs immersed in water with the application of US at 130 kHz), Tew (CBCs immersed in SAEW without the application of US), Tew25 (CBCs immersed in SAEW with the application of US at 25 kHz) and Tew130 (CBCs immersed in SAEW with the application of US at 130 kHz).

Table 3
Values of shear force, pH, glycogen and lactate of the CBCs before (control) and after immersion in water and SAEW with and without application of US at 10 °C for 10 min.

	Control	Tw	Tw25	Tw130	Tew	Tew25	Tew130
Shear force (N)	$13.60^{A} \pm 1.64$	$13.78^{A} \pm 0.19$	$14.47^{A} \pm 1.05$	$14.39^{A} \pm 1.66$	$12.47^{A} \pm 1.17$	$13.77^{A} \pm 0.65$	$13.97^{A} \pm 0.51$
pH	$6.10^{A} \pm 0.02$	$5.97^{A} \pm 0.02$	$6.03^{A} \pm 0.13$	$5.98^{A} \pm 0.14$	$6.16^{A} \pm 0.08$	$6.18^{A} \pm 0.09$	$6.14^{A} \pm 0.14$
Glycogen	$13.37^{A} \pm 2.08$	$16.37^{A} \pm 1.92$	$13.41^{A} \pm 1.90$	$13.70^{A} \pm 5.15$	$14.40^{A} \pm 1.47$	$15.14^{A} \pm 1.36$	$16.85^{A} \pm 3.78$
Lactate	$43.68^{A} \pm 4.71$	$40.12^{A} \pm 2.01$	$32.11^{A} \pm 5.75$	$33.23^{A} \pm 6.85$	$39.18^{A} \pm 12.11$	$38.25^{A} \pm 8.97$	$39.30^{A} \pm 1.47$

Different letters on the same line indicate a significant difference ($P < 0.05$) by Duncan's test. Values expressed as mean ± standard deviation. Glycogen values expressed in micromol of glucose per gram of tissue and Lactate values expressed in micromol of lactate per gram of tissue. Batches: Control (CBCs without immersion), Tw (CBCs immersed in water without the application of US), Tw25 (CBCs immersed in water with the application of US at 25 kHz), Tw130 (CBCs immersed in water with the application of US at 130 kHz), Tew (CBCs immersed in SAEW without the application of US), Tew25 (CBCs immersed in SAEW with the application of US at 25 kHz) and Tew130 (CBCs immersed in SAEW with the application of US at 130 kHz).

The use of new technologies during the cooling process of poultry carcasses can be useful to make the process more sustainable and efficient for the meat industry. In our previous study, we demonstrated that the application of ultrasonic bath in the chicken breast during chilling by immersion promoted a fast and uniform cooling (Flores et al., 2018). In addition, we also showed that the electrolyzed water might be a viable alternative to reduce the volume of water used at slaughter and to improve the microbiological quality of pork meat (Athayde et al., 2017).

To date, the combined application of US and SAEW during carcass chilling has not been studied. However, for these technologies to be applied industrially, studies that assess their effects on the microbiological quality of the immersion water and carcasses are necessary, as well as the biochemical and oxidative characteristics of chicken carcasses during cooling. Thus, in this study, breast chicken cylinders (CBCs) were immersed in water or SAEW and sonicated for 10 min at 10 °C in a US bath at frequencies of 25 and 130 kHz. The microbiological quality of water and SAEW used in the immersion, and the shear force, the anaerobic glycolysis, the oxidation profile (lipid and protein), and the microbiological characteristics of the CBCs before and after the immersion were evaluated.

2. Materials and methods

2.1. Obtaining the breast chicken cylinders (CBCs) and the US and SAEW application

This study was conducted in accordance with the European recommendations for the protection of animals used for scientific purposes (European Commission, 2012). The study consisted of 7 treatments, in triplicate on 3 different days. Poultry carcasses at 37 ± 1 °C were collected before reaching the pre-chiller in a slaughterhouse under federal inspection and placed in thermal boxes that were immediately transported to the laboratory. The breast (*M. pectoralis* major) was cut into cylinders with 5 cm in diameter and 3 cm in height and named as breast chicken cylinders (CBCs).

The CBCs (45 ± 5 g) were treated in two ultrasonic baths (Elma Schmidbauer GmbH, models TI-H-10 MF2 and TI-H-10 MF3, 8.2 L, Singen, Germany, nominal power 1000 W), at frequencies of 25 or 130 kHz, with acoustic power of 230 W in both frequencies, for 10 min at 10 °C. The volumetric power was determined by calorimetric method (Koda et al., 2003), thus obtaining the values of 28 W/L in both frequencies. The tank of the ultrasound baths had inner dimensions of approximately 300 × 240 × 150 mm (W × D × H). Water or SAEW was added until 10.5 cm of the height of the tank. During the US application, cold water or SAEW (10 °C) was supplied at a flow rate of 500 mL/min. These conditions gave rise to the following treatments: Control (CBCs without immersion), Tw (CBCs immersed in water without the application of US), Tw25 (CBCs immersed in water with the application of US at 25 kHz), Tw130 (CBCs immersed in water with the application of US at 130 kHz), Tew (CBCs immersed in SAEW without the application of US), Tew25 (CBCs immersed in SAEW with the application of US at 25 kHz) and Tew130 (CBCs immersed in SAEW with the application of US at 130 kHz).

The time and temperature used in the pre-chiller were based on the Technical Regulation for Technological, Hygienic, and Sanitary Inspection of Poultry Meat (Brazilian Ministry of Agriculture, Ordinance No.210 of 10/11/1998), which has established an immersion time of 30 min and water temperature up to 16 °C. The SAEW was obtained from a 0.02% sodium chloride (Dinâmica, Brazil) using a portable electrolyser (Envirolyte, Estonia). The characteristics of SAEW were pH 6.0 ± 0.1, 5 ppm chlorine, and oxidation-reduction potential (ORP) of 800–850 mV. The potable water used in the process exhibited pH 7.0 ± 0.1, and ORP of 130–140 mV.

2.2. Microbiological characterization

The microbiological parameters of CBCs, water, and SAEW were

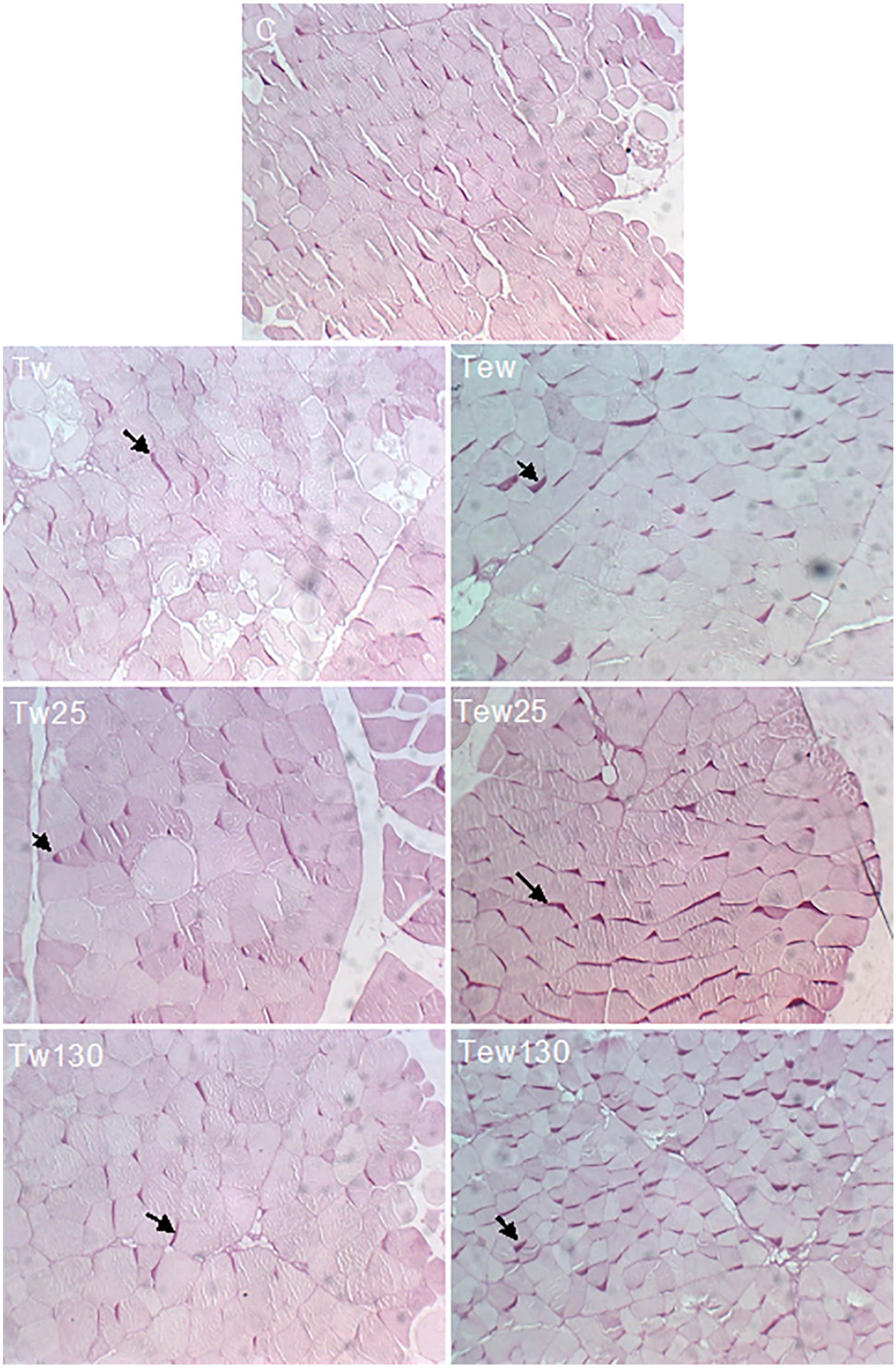

Fig. 2. Histological characterization of the CBCs, where the arrows indicate the presence of glycogen. Control (CBCs without immersion), Tw (CBCs immersed in water without the application of US), Tw25 (CBCs immersed in water with the application of US at 25 kHz), Tw130 (CBCs immersed in water with the application of US at 130 kHz), Tew (CBCs immersed in SAEW without the application of US), Tew25 (CBCs immersed in SAEW with the application of US at 25 kHz) and Tew130 (CBCs immersed in SAEW with the application of US at 130 kHz). Images increased by 100×.

determined before and after immersion of the CBCs. The lactic acid bacteria were plated on de Man Rogosa Sharpe agar (Oxoid, Basingstoke, United Kingdom) by the pour-plate method and incubated at 36 °C/48 h (Silva et al., 2007). *Staphylococcus* spp. was plated on Baird-Parker agar by the spread-plate method and incubated at 36 °C/48 h (Silva et al., 2007). Enterobacteria were inoculated by pour plating in double layer Violet Red Bile Glucose Agar (Oxoid) and incubated at 41.5 °C/24 h (Purnell et al., 2014). The aerobic mesophilic microorganisms were inoculated by the pour-plate method using Plate Count Agar (PCA) (Oxoid) and incubation at 36 °C/48 h (Brasil, 2003). The psychrotrophic bacteria were inoculated by the spread-plate method using PCA (Oxoid) and incubation at 7 °C/7 days (Silva et al., 2007).

2.3. Lipid and protein oxidation

The lipid oxidation of CBCs was evaluated by the determination of thiobarbituric acid reactive species (TBARS) (Raharjo and Sofos, 1993), conjugated diene (Recknagel and Glende, 1984) and peroxide value (Shanta and Decker, 1997). The protein oxidation was evaluated by determining the content of carbonyl groups (Levine et al., 1990) and thiol groups (Ellman, 1959). All analyses were performed before immersion and after immersion of the samples.

2.4. pH

The pH of CBCs was determined by potentiometry in apparatus DM-23 DC (Digimed®).

2.5. Shear force

The shear force (Warner Bratzler Shear-WBS) of the CBCs was evaluated shortly after the pre-chilling in a TA-XT plus texture meter (Texture Technologies Corp.). The samples were cooked in an electric grill (NKS) until reaching the temperature of 70 °C in the center of the meat piece. Six cuts were sampled perpendicular to the direction of the muscle fibers in each CBC and sheared at a speed of 5 cm/min (AMSA, 1995).

2.6. Biochemical characterization

Glycogen was determined by the method of Dubois et al. (1956) and lactate was determined according to methodologies of Montgomery (1957) and Harrower and Brown (1972). Both analyses were performed before and after the pre-chilling of CBCs.

2.7. Histological characterization

Muscle portions were collected from the surface of the CBCs in a transverse direction of the oblique muscle before and after the pre-chilling process. The Bauer-Feulgen method through the Periodic Acid Schiff staining (PAS) was used (Tolosa et al., 2003). To confirm the positive PAS reaction, the stained sections were submitted to the diastase with salivary amylase (Suvarna et al., 2012), and viewed under a microscope at 100× magnification (Olympus BX-40). Mouse liver was used as a positive control, and all slides subjected to the US and immersion process were also characterized for the diastase with salivary amylase.

2.8. Statistical analysis

The analyses were performed in triplicate. The experiments were modeled using a completely randomized design, at a significance level of 5%. The Duncan test was performed for analysis of the significant differences between means. The statistical program Statistica 8.0 (StatSoft) was used.

3. Results and discussion

3.1. Microbiological characterization

No significant ($P > 0.05$) effect of the SAEW alone were observed on the microbiological quality of the CBCs when compared to the conventional process since the treatment Tew presented a similar reduction in bacterial count with the treatment Tw for psychrotrophic bacteria, lactic acid bacteria, enterobacteria, and mesophilic bacteria. In addition, Tw showed a greater reduction of *Staphylococcus* spp. counts when compared to the treatment Tew (Fig. 1). A similar trend was reported by AlHoly and Rasco (2015) when applying electrolyzed water (EW) in chicken meat. According to these authors, the organic matter of chicken meat can react with free available chlorine and reduces EW bactericidal properties.

However, a great improvement in the microbiological quality of CBCs was observed in the treatment that combined the application of US 25 kHz and SAEW (Tew25), which presented the highest reduction ($P < 0.05$) of psychrotrophic bacteria, lactic acid bacteria, and mesophilic bacteria, with values of 0.76; 0.81; and 0.98 log (Fig. 1). A similar trend was noticed by Miks-Krajnik et al. (2017), which studied the combined application of US and acidified electrolyzed water in raw salmon fillets. This result may be due to the size of the bubbles produced by the cavitation at this frequency, once at 25 kHz the cavitation phenomenon leads to the formation of larger bubbles with low penetration power in the cell. When imploded on the surface of bacterial cells, a large amount of energy is generated, thus rupturing the cytoplasmic membrane. This event may have facilitated the SAEW entry into the bacterial cell. Similar behavior was not observed at 130 kHz, once the smaller bubbles were formed, with a high penetration power in the bacterial cells, thus generating a small amount of energy, which promotes the formation of small temporary channels in the cytoplasmic membrane (Chemat et al., 2011).

The action of the US on the different types of bacteria can vary according to the shape (cocci or bacilli), cell wall composition, ideal growth temperature, and pH (Pagan et al., 1999). Yokoyama et al. (2007) and Zacharia et al. (2010) reported that the differences in cell structure between gram-positive and negative bacteria may prevent HOCl from penetrating the microbial cell. The thick peptidoglycan layer of gram-positive bacteria would provide a greater resistance to the mechanical stress produced by the cavitation in US (Joyce et al., 2011). This fact may have hampered the penetration of SAEW into the Staphylococcus spp. cells. For this reason, SAEW was not efficient in reducing this microorganism (Fig. 1).

3.2. Microbiological characterization of SAEW and water before and after immersion of CBCs

In addition to cooling the chicken carcasses, the immersion liquid used in the pre-chilling stage contributes to the removal of bacteria from the surfaces. However, an opposite effect can take place if the liquid has a high microbiological load, including the cross-contamination of the carcasses and the environment (Barbut, 2001). The SAEW before and after immersion of CBCs of the treatments Tew, Tew25 and Tew130 did not present colonies of mesophilic and psychrotrophic bacteria (Table 1). This result suggest that SAEW could be reused in the cooling process. On the other hand, water from the Tw, Tw25 and Tw130 treatments presented before immersion of the CBCs a mesophilic and psychrotrophic counts close to 3 log CFU/mL. An increase in 0.7 and 1.25 log CFU/g in the number of mesophilic and psychrotrophic bacteria, respectively, was observed in the water after immersion of CBCs without US treatment (Tw).

3.3. Oxidation profile

All treatments presented low levels of lipid and protein oxidation (Table 2). The different immersion media (water and SAEW) and the application of US in CBCs has no effect ($P > 0.05$) on the formation of conjugated dienes and thiol. A similar trend was reported by Shimamura et al. (2016), when evaluated the effect of a combination treatment with alkaline electrolyzed water and strong acidic electrolyzed water on fresh chicken breasts. The treatments Tw25 and Tw130 had the lowest peroxide values when compared to the other treatments ($P < 0.05$). However, the treatments immersed in SAEW (Tew, Tew25, and Tew130) did not differ ($P > 0.05$) from the control. The TBARS values of the treatments Tw, Tw25, and Tw130 were lower ($P < 0.05$) in relation to the control. This lower lipid oxidation of the samples immersed in water suggests that a small fraction of peroxides and malonaldehyde may have been removed by water. The samples immersed in SAEW (Tew, Tew25, and Tew130) presented a higher ($P < 0.05$) TBARS values than samples immersed in water (Tw, Tw25, and Tw130). This increase in lipid oxidation of the SAEW treatments is probably due to the oxidizing properties of HClO (Winterbourn et al., 1992). The lipid oxidation should be prevented, since the final oxidation product leads to the development of oxidative rancidity, which affects the quality of meat *in natura* (Damodaran et al., 2010).

The protein oxidation leads to decreased solubility, decrease the water retention capacity and the gel formation ability (Zhang et al., 2013), besides to produce carcinogenic compounds (Damodaran et al., 2010). Chlorine and HClO in aqueous solution are recognized by their protein oxidation potential, due to the oxidation reaction of the hydroxyl groups leading to the formation of carbonyl derivatives (Fukayama et al., 1986; Estevez, 2015). In this study, the use of SAEW and US did not influence the protein oxidation of the CBCs, since the carbonyl and thiol values of the Tew, Tew25 and Tew130 treatments did not differ ($P > 0.05$) from Tw.

3.4. Texture, pH, glycogen, lactate levels, and histological sections

In pre-chilling, the chicken carcasses are in the pre-rigor stage, characterized by pH decline, the presence of glycogen and lactate, and maximum muscle flexibility and extensibility (Prandl et al., 1994). The breast chicken muscle consists of white fibers. These fibers perform anaerobic glycolysis more efficiently than red fibers, so the onset of rigor mortis occurs 1 h after slaughter (Olivo et al., 2006). The temperature of the pre-chilling and cooling stages directly influences the rate of anaerobic glycolysis. The rapid cooling of the carcasses at temperatures below 14 °C, associated with pH values higher than 6.2, tends to shorten the muscles, leading to an increase in meat hardness (Varnam and Sutherland, 1998). Studies have shown that the exposure time, US frequency and intensity, can promote structural modifications in muscle fibers (Turienzo et al., 2012). In the present study, the shear force values were similar ($P > 0.05$) between the control and the US treatments (Tw25, Tw130, Tew25, and Tew130), suggesting that the 10 min exposure of the CBCs to 25 and 130 kHz and 10 °C was not sufficient to promote muscle fiber modifications (Table 3).

Glycogen is the main reservoir of muscle energy, and its level can remain practically constant up to 2 h after slaughter, as well as lactate values, without affecting pH and glycolysis rate (Warris et al., 1993). The pH values of CBCs decreased shortly after the end of the immersion process for all treatments, in relation to the physiological pH of chicken (pH 7.0), indicating the onset of the anaerobic glycolysis (Savenije et al., 2002). No differences ($P > 0.05$) were observed for pH, glycogen, and lactate levels of the CBCs between the treatments (Table 3), thus demonstrating that the US and SAEW did not interfere in the anaerobic glycolysis process. Fig. 2 shows the presence of glycogen in the histological sections of each treatment.

4. Conclusions

Pre-chilling of the CBCs subjected to US at 25 kHz and SAEW led to a reduction in bacteria counts of the samples. In addition, the use of US at both frequencies, associated with SAEW did not accelerate the lipid and protein oxidation and anaerobic glycolysis, with no modifications in the muscle fiber structure. The present results demonstrated that the combination of US and SAEW has a great potential to improve the pre-chilling of poultry carcasses. In addition, lower microbial counts were detected in SAEW, even after immersion of the CBCs, thus further studies are required on the reuse of SAEW in the pre-chilling step, aiming at reducing the water consumption in the poultry industry.

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