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Modelling inactivation of *Staphylococcus* spp. on sliced Brazilian dry-cured loin with thermosonication and peracetic acid combined treatment

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Abstract: Ultrasound (US) has a high capacity to increase food safety. Although high and/or moderate temperature in combination with US has been studied, the knowledge about cooling/low temperatures as well as its combined effect with chemical preservation methods is scarce. Therefore, the aim of this study was to describe the inactivation of *Staphylococcus* spp. (SA) present in the natural microbiota of sliced Brazilian dry-cured loin (BDL) using US (40 kHz and 5.40 W/g) at 1.6-17.9 kJ/g, temperature (T) between 6.4-73.6 °C and peracetic acid (PA) between 5.5-274.5 mg/L employing the Central Composite Rotatable Design. The model fully describes how the combination of US, T, and PA affects SA inactivation. In BDL, an increase in US acoustic energy density (kJ/g) allows the reduction of T necessary to inactivate SA because of the occurrence of synergistic effect. However, US applied at low T was inefficient. On the other hand, PA was more efficient at low T, since high T degraded this compound at different rates according to the holding T. Therefore, the data indicates a relation between the technologies useds in the combined decontamination of sliced BDL improving dry-cured meat safety.

Keywords: Central composite rotatable design; Response surface methodology, Meat decontamination; Synergistic effect; Non-thermal technology; Mild processing.

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1. Introduction

Emerging technologies should be employed to reduce the risk to public health through the inactivation of pathogenic microorganisms that may be naturally present in food. Non-thermal food preservation technologies such as ultrasound (US) have been considered green technologies due to their ability to improve the food processing sparing energy (Awad et al. 2012). US has the ability to improve the antimicrobial effect of chemical compounds (Duarte et al., 2018; Rosário et al., 2017; Sarkinas et al., 2018). Microbial inactivation occurs mainly due to the formation, growth, and implosion of bubbles during the cavitation process (Chemat et al., 2011). The implosion process generates (i) micro-jets capable of breaking the cell wall, (ii) free radicals and (iii) hot-spots capable of inactivating microorganisms (Awad et al. 2012; Gogate and Kabadi, 2009). Recently, several studies have evaluated the effect of US on meat quality and processing (Barekat and Soltanizadeh, 2018; Inguglia et al., 2018; Ojha et al., 2016; Zou et al., 2018a; Zou et al., 2018b). In addition, high and moderate temperatures in combination with US (thermosonication) were extensively studied (Contreras et al., 2018; Evelyn et al. 2017; Evelyn and Silva, 2015a; Evelyn and Silva, 2018). However, studies on cooling/low temperatures, as well as its combined effect with chemical preservation methods, are scarce (Caraveo et al., 2015; Pedrós-Garrido et al., 2017).

Peracetic acid (PA) is among the chemical compounds with high inactivation efficiency against key foodborne pathogens (Srey et al., 2013). The Food and Drug Administration (FDA) regulates its use in the United States and concentrations of 80 mg/L for fruit and vegetables (21CFR173.315) and 220 mg/L on meat carcasses (21CFR173.370) are allowed on foods imported by the U.S. Moreover, the use of PA is environmentally friendly (Srey et al., 2013). Products of PA are: (i) acetic acid that reduces the intracellular pH causing perturbation of cellular enzymes and high consumption of ATP for resumption of the original intracellular pH (Eklund, 1985; Theron and Lues, 2007) and (ii) hydrogen peroxide that

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causes oxidation of substances, which are essential for the survival of microorganisms (Kitis, 2004; Srey et al., 2013; Yuan et al., 1997). On the other hand, one of the main methods applied in food processing and preservation is the use of heat which acts mainly on the inactivation of microbial enzymes and denaturation of proteins that constitute the microorganisms (Earnshaw et al., 1995), as well as denaturation of proteins and other constituents of food (Earnshaw et al., 1995; Tornberg, 2005). Recently, several studies used moderate heat combined with other technologies to inactivate pathogenic and spoilage microorganisms in meat and meat products (Condón-Abanto et al., 2016; Evelyn and Silva, 2015a; Wang et al., 2015).

Recent outbreaks of diseases involving several pathogenic bacteria have been reported worldwide mainly due to the consumption of contaminated meat and meat products (Agência Nacional de Vigilância Sanitária, 2017; Centers for Disease Control and Prevention, 2015; Centers for Disease Control and Prevention, 2018; European Food Safety Authority European, 2017). In addition, spoilage bacteria can cause high loss in meat quality and waste of the final product. Due to their low water activity and high and/or moderate NaCl content, dry-cured meat products have been considered microbiologically stable products (Menéndez et al., 2018). However, halotolerant pathogenic and spoilage microorganisms may be the main public health risk and cause losses during storage and commercialization due to the intrinsic characteristics of the matrix (Mutz et al., 2019; Menéndez et al., 2018; Ng et al., 1997). *Staphylococcus* spp. is a halotolerant bacterium found in dry-cured meat and ingredients for its production around the world (Ahhmed et al., 2017; García-Díez et al., 2016; Menéndez et al., 2018; Stavropoulou et al., 2018). Staphylococcal food poisoning is one of the most common in the world (Kadariya et al., 2014). Staphylococci are ubiquitous in nature, with humans and animals as their primary reservoirs. Consequently, the contamination of foods often happens via food handlers. Therefore, good personal hygiene and compliance with

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Good Manufacturing Practices is necessary, as well as an effective intervention/decontamination strategy. Among *Staphylococcus* spp. strains, *S. aureus* is the most notable bacterium associated with virulence factors such as Staphylococcal enterotoxins (SEs) that cause hypersalivation, nausea, vomiting, and abdominal cramping with or without diarrhea with a lethality rate of 0.03% (Kadariya et al., 2014). Moreover, *Staphylococcus* spp. are among the key microorganisms causing spoilage in processed meats, being fermentative organisms, producing both acid and gas from glucose. *Staphylococcus* spp. are also present in a significant percentage of raw, intact meat, and the predominant surface microbiota during curing (Sperber and Doyle, 2009).

High levels of reduction of spoilage and pathogenic microorganisms can be achieved using emerging technologies. Furthermore, the knowledge of the decontamination efficiency of combined methods is of great importance (Rajkovic et al., 2010). However, the effect of the combination of preservation methods such as US, T, and PA on microorganism inactivation has not been described yet. The concept of combining factors to preserve food was developed by Leistner (2000) who described that each factor involved in the microbial control process represents a hurdle to be overcome by the microorganism, making it difficult to survive. For several meat products, such as Brazilian dry-cured loin (BDL), natural hurdles are not sufficient to guarantee safety. Therefore, the aim of this study was to evaluate the effect of US, T, and PA on the inactivation of *Staphylococcus* spp. present in the natural microbiota of BDL using the Design of Experiments (DOE) approach to obtain a mathematical model of inactivation that describes the effects of combined preservation methods on reducing the target bacteria at various levels and with high confidence. To the best of our knowledge, this study is the first paper using DOE approach to evaluate US and PA on SA inactivation in meat products.

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2. Material and methods

2.1 Experimental design

A Central Composite Rotatable Design of Experiment (CCRD) was used in 2^3 experiment (Table 1), wherein nineteen experiments (Table 2) were performed in random order to evaluate the effects of ultrasound (US), temperature (T) and peracetic acid (PA) on *Staphylococcus* spp. (SA) inactivation in sliced BDL. The central point was performed with five replicates to evaluate experimental error and thus lack-of-fit of the model. Statistica 10® software was used. The whole experiment was performed with two independent replicates.

2.2 Sample preparation

A total of 2.0 kg of Brazilian dry-cured loin (Socol, BDL) was obtained from different batches in Venda Nova do Imigrante city (latitude 20°19'31.9"S and longitude 41°07'56.6"W), Brazil. Samples were produced during August-October 2017 (three months) with ripening conditions of room temperature (18.3 ± 2.3 °C and relative humidity of 80.7 ± 4.1%) according to the Capixaba Institute for Research, Technical Assistance and Rural Extension (2017). Immediately after their production, samples were vacuum packed and transported to the laboratory, also at room temperature, for analysis. Samples were sliced with a thickness of 0.15 ± 0.01 cm (approximate commercial thickness for dry-cured meat products) using a commercial slicer sanitized with alcohol 70% (Arbel® Ftd 178 MC/MC-X 3.0, São Paulo, Brazil). The average diameter obtained for the slices was 5.5 ± 0.1 cm. Therefore, each slice had 48.0 ± 1.7 cm^2 and 3.9 ± 0.1 g. Preservation methods were applied according to Section 2.3.

2.3 Preservation technologies

For each run, 10 g (122.0 ± 6.1 cm^2, approximately 3 slices) of BDL were placed in sterile polyethylene bags (20 × 35 cm and thickness of 0.05 mm) (Emba Freezer, Brazil)

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containing 500 mL (sufficient amount to avoid overlapping of the slices in US bath) of sterile distilled water. The dimensions of the immersed bag were 15.0 × 6.8 × 4.9 cm (length × width × height). The application conditions for each preservation technology are described in Table 2. A US bath equipment (Unique, USC-2800A, Brazil) with a power of 154 W, frequency of 40 kHz and capacity of 9 L was employed. The US power (P) dissipated into the liquid was calculated using Equation 1 (calorimetric method) and 54 W (5.40 ± 0.01 W/g) was obtained. To obtain these data the internal temperature (initial and final) of the bags was measured at different times. Acoustic Energy density (AED) was obtained using Equation 2 and expressed as kJ/g. For the application of the preservation technologies the bags containing water, sample, and PA (Proxitane®, Paraná, Brazil) were placed inside the US bath. The PA concentration of the commercial product was 15%, and the concentrations for each run (Table 2) were calculated in mg/L using the equation $C_i \cdot V_i = C_f \cdot V_f$ (initial (i) and final (f) concentrations (C) and volumes (V)). The treatment time (min) and the T (± 0.1 °C) of the water in the US bath were adjusted in the configuration of the equipment. For T lower than 25 °C the water bath was adjusted using ice cubes. During application, the temperature was monitored using a digital thermometer (Equitherm, TH439, Porto Alegre, Brazil).

$$P = m\mathrm{C_p}\left(\frac{\mathrm{d}T}{\mathrm{d}t}\right) \quad (1)$$

$$\mathrm{AED} = \frac{Power\ (W) \cdot process\ time\ (s)}{Sample\ weight\ (g)} \quad (2)$$

wherein Cp = water specific heat (4.18 J/g K), m = mass of water on US bath (g), dT = T increase (°C), dt = sonication time (s).

2.4 Microbiological analysis

The procedure for counting the *Staphylococcus* spp. naturally present in the matrix was performed according to the methodology of the American Public Health Association

described in the Compendium of Methods for the Microbiological Examination of Foods (Downes and Ito, 2001). In each experiment, 10 g of sample together with 90 mL of 0.1% peptone water were homogenized for 1 min in a Stomacher (Yka Tecnologia, Saparinga, Rio Grande do Sul, Brazil). The cultivation process was performed with Spiral Plater (Eddy Jet 2, IUL instruments, United States) using mode E50. Baird Parker agar (HiMedia®, Mumbai, India) containing egg yolk emulsion and potassium tellurite (Sigma-Aldrich®, Switzerland) was used as a selective and differential supplement and incubated at 35 ± 1 °C for 48 h. Characteristic colonies (grey-black shiny with an opaque zone around the colony) were considered *Staphylococcus* spp. according to Baird-Parker (1962). The colonies were counted in an electronic counter (Flash & Go, IUL instruments, United States). The data were expressed as $\log_{10}$ decimal reductions (DR) of colony forming units per gram of sample (cfu/g) calculated according to Equation 3. Initial count (control, CT) of *Staphylococcus* spp. in BDL was 5.8 ± 0.3 log cfu/g using Baird Parker agar as previously described.

$$\mathrm{DR} = \mathrm{Log}\,(N/N_0) \tag{3}$$

Wherein: N = count (cfu/g) after the treatment, N_0 = CT count (cfu/g).

2.5 Mathematical modelling

To obtain the polynomial model (Equation 4) (Baş et al. 2007) that describes the effect of the independent variables (US, T, and PA) on SA inactivation, multiple regression analysis was performed using Statistica 10® software. The presence of significant factors composing the model was determined respecting the level of significance of 0.05. To verify the normality of the residuals' data the Shapiro-Wilk's test was used. The graphical representation of the obtained model was presented using the response surface methodology. To describe the proportion of the variation explained by the independent variable level that the model

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represents the data the adjusted R^2_{adj} value was obtained. Mean square error (MSE) was obtained.

$$\text{Log}\,(\text{N}/\text{N}_0) = B_0 + \sum_{i=1}^{3} B_i X_i + \sum_{i=1}^{3} B_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j>i}^{3} B_{ij} X_i X_j + \varepsilon \quad (4)$$

Wherein: X is the variable (T, US, or PA), B is the regression coefficient and ε is the experimental error.

To evaluate the acceptable prediction zone of the model, the relative error (RE) was calculated according to Equation 5 (Delignette-Muller et al., 1995).

$$\text{RE} = \frac{DR_{observed} - DR_{fitted}}{DR_{fitted}} \quad (5)$$

2.6 Model validation

Validation of the model was performed with additional experimental conditions (random). The applied conditions were not used for the construction of the model (Table 3). The preservation technologies were applied according to Section 2.3. The experiments were performed with three independents replicates. The accuracy factor (A_f) and bias factor (B_f) were calculated according to Baranyi et al. (1999) (Equations 6 and 7), respectively. A_f indicates the spread of data around the prediction. B_f indicates the level of agreement between predicted and observed values. Ideally, A_f and B_f should have values equal to 1. However, the acceptable limit may increase 0.10-0.15 for each variable presented in the predictive model (Ross et al., 2000). Therefore, in this study, A_f value lower than 1.45 were accepted. According Ross et al. (1999) B_f value lower than 1.15 was accepted.

$$A_f = \exp\left(\sqrt{\frac{\sum_{k=1}^{m} (\text{Ln}\, f(x^{(k)}) - \text{Ln}\mu^{(k)})^2}{m}}\right) \quad (6)$$

$$B_f = \exp\left(\frac{\sum_{k=1}^{m} (\text{Ln}\, f(x^{(k)}) - \text{Ln}\mu^{(k)})}{m}\right) \quad (7)$$

Where Ln f(x) is the value predicted by the model, Ln μ the observed value and *m* the number of experiments.

The '*percent* discrepancy' between the model and observations was calculated according to Equation 8, and '*percent* bias' according to Equation 9 (Baranyi et al., 1999).

$$\%\mathrm{D}_f = \left(\mathrm{A}_f - 1\right) \cdot 100\% \quad (8)$$

$$\%\mathrm{B}_f = \mathrm{sgn}\left(\mathrm{Ln}B_f\right) \cdot \left(\exp\left|\mathrm{Ln}B_f\right| - 1\right) \cdot 100\% \quad (9)$$

3. Results and discussion

3.1 Mathematical modelling and model performance

The effect of US, T, and PA on SA inactivation is described by the model (Equation 10) obtained in the multiple regression analysis, wherein individual and interaction effects ($p < 0.05$) were presented. The residual values have significant normal distribution since in the Shapiro-Wilk's *P* value was 0.253 ($P > 0.05$) (Granato et al., 2014). The lack-of-fit value obtained non-significance ($P = 0.167$); indicating that the model adequately describes the functional relationship between dependent and independent variables. In addition, the MSE obtained was similar or lower than those found in other studies using response surface methodology (Table 4). The value of the adjusted coefficient of determination (R^2_{adj}), equal to one, indicates perfect fit of the model to the data. This study obtained adequate values close to 1 (Fig. 1A). Others studies using preservation technologies in meat and fish products found R^2_{adj} values between 0.83 and 0.99 (Table 4). In addition, the significance (*P* - value) for each regression coefficient is presented in Fig. 2. Concerning RE, models with pRE > 0.70 (pRE is the number of runs with RE in the acceptable prediction zone/total number of runs) offer acceptable values of bias and accuracy (Oscar, 2005). The prediction zone is the RE range between -0.3 (fail-safe) and 0.15 (fail-dangerous) (Oscar, 2005). In this study, the value of

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0.90 pRE was found, since only two runs were outside the prediction zone (Fig. 1B). Therefore, all model performance indices indicate goodness-of-fit.

$$\text{Log }(N/N_0) = 0.520 + 0.00253 \cdot T - 0.000925 \cdot T^2 + 0.0506 \cdot AED - 0.0120 \cdot PA - 0.00260 \cdot T \cdot AED + 0.000148 \cdot T \cdot PA \quad (10)$$

Wherein: Log (N/N_0) is the decimal reduction (cfu/g), T is the temperature (ºC), AED is the acoustic energy density (kJ/g) and PA is peracetic acid (mg/L).

In this study, the accuracy factor (A_f) and bias factor (B_f) lower than 1.45 and 1.15, respectively, were considered acceptable (Ross et al., 2000; Ross et al., 1999) and the value obtained is within this range. For the predicted and observed values, A_f values (*percent* discrepancy %D is 13%) were found close values to other predictive models (Baranyi et al., 1999) and smaller or similar to the models that used the design of experiments as described in Table 4. The (B_f) equal to 1 indicates that the model has a perfect agreement between predicted and observed values (Ross, 1996). Values above 1 indicate that the model is fail-dangerous due to predicted values higher than observed (Ross, 1996) as verified in Table 4. In addition, $\%B_f > 0$ indicates that the model predicts larger decimal reductions than those observed (Baranyi et al., 1999). Values below 1, in general, indicate that the model is fail-safe. However, excessively low values such as 0.5 indicate a conservative model that provides only half of the observed (Ross, 1996). In the present study, the model predicted values only 7.3% higher. Thus, the obtained model describes the effect of US, T, and PA on SA inactivation in BDL with high performance.

3.2 *Staphylococcus* spp. inactivation

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The effect between T and PA on SA inactivation in BDL obtained significant interaction ($p < 0.05$) as demonstrated in Fig. 3A. Level curves at low Ts (cooling) start with behaviour parallel to the T axis (Fig. 3A). This fact indicates that the reduction of SA is occurring due to the action of the factor described in the other axis (PA). However, the gradual increase of T causes a decrease in the level curves, inducing behaviour parallel to the PA axis (Fig. 3A). When a higher T is employed, the predominance of the behaviour parallel to the PA axis occurs, which shows that the inactivation of SA is occurring due to the effect of the high T. PA in the concentration of 200-250 mg/L maintains the integrity of the antimicrobial effect up to 15-20 °C. Concentrations between 100-199 mg/L maintain the integrity of the antimicrobial effect up to 7-10 °C. Concentrations < 100 mg/L gradually decrease effectiveness with increasing T and have low/no antimicrobial activity (Fig. 3A). Therefore, PA loses efficiency with increasing T. However, the loss of efficiency due to the effect of T is reduced with the increase of PA concentration. Although PA efficiency is reduced with increasing T, high PA concentrations require a longer time for total degradation (Chen et al., 2017). T between 20-30 °C causes the highest inactivation efficiency loss rates due to the declination of the levels of curve lines (Fig. 3A). T > 45 °C turns the efficacy of PA near null. The increase in T leads to increased decomposition (Chen et al., 2017) and reduced PA efficiency (Jorjani et al., 2004) since the spontaneous decomposition constant k is increased with the addition of T (Yuan et al., 1997). The spontaneous decomposition of this compound caused by the increase of T causes acetic acid and oxygen formation (Yuan et al., 1997). Acetic acid has low microorganism inactivation efficiency (Rosário et al., 2017). Broda (2007) used PA, heat, and US to inactivate *Clostridium esterthеticum* spores in vitro by applying preservation methods separately and reaffirming the efficiency in the inactivation of this pathogen. However, the understanding of the combined inactivation effect was not possible. Therefore, in this study, after evaluating an extensive range, concentrations within

the specific ranges for each situation are in agreement with the range described in the FDA recommendations and make its application promising for dry-cured meat products.

The effect between T and US on SA inactivation in BDL obtained significant interaction ($p < 0.05$) as demonstrated in Fig. 3B. Level curves with behaviour parallel to the US axis indicate the predominance of the effect of T on SA inactivation. However, the increase in T results in the formation of a decreasing level curve towards the US axis. For this reason, high and moderate T improved the effect of the US on SA inactivation. In addition, using the model to assess the isolated effects of US and T, reductions of -0.3 and -2.9 log cfu/g were obtained for US (17 kJ/g) and T (60 °C), respectively. Simulating the combined effects of the previous conditions a reduction of -4.5 log cfu/g was obtained. Therefore, in relation to high T's, there was a synergistic effect between US and T since the individual technologies presented smaller reduction than when applied in combination. All these effects can be explained by the microbial key inactivation mechanisms of US, one of which is associated with cavitation, i.e. formation and collapse of cavitating bubbles. This process causes the formation of zones of compression and expansion in the medium (Chemat et al., 2011). The expansion process results in the existence of negative pressure (Earnshaw et al., 1995). Obviously, the boiling point of liquids decreases with the decrease in pressure. Thus, at very low pressures the water liquid phase changes to water vapour at T less than usual (~100 °C, 1 atm) and outcomes in the formation of bubbles in the medium (Chemat et al., 2011; Earnshaw et al., 1995). For this reason, a T increase in the liquid favours the conversion of water to water vapour more easily due to the previous reduction of the local pressure (reduction of the boiling point). Therefore, the application of US at higher T increased the amount and/or rate of bubble formation. Finally, high T improved the ultrasonic reduction of SA in BDL and the physicochemical, nutritional and sensorial damage due to high T application could be reduced. Contreras et al. (2018) found that US accelerates mild heating

in sliced dry-cured ham. In accordance with the present study, thermosonication process also increased the reduction of *Bacillus cereus* spores (Evelyn and Silva, 2015a) and *Clostridium perfringens* spores (Evelyn and Silva, 2015b) in beef slurry. The application of ultrasonication treatment to microorganisms punctures their cell membranes and produces free radicals, and extrusion of the intracellular matrix which ultimately kills the microorganisms. Therefore, it is important to realize that factors such as shape (cocci or bacilli), diameter of microorganisms, cell size or surface area, gram positivity or negativity (cell wall thickness), and cell sensitivity or ability to recover from treatment greatly influence the treatment effectivity (Roobab et al., 2018).

In relation to low T's, the application of US during cooling increased SA survival (Fig. 3A, Table 3). Moreover, under these T conditions, the increase in AED caused an increase in SA count. During the cavitation process, the implosion of the bubbles is responsible for the formation of micro-jets that rupture the cell wall of the bacteria and consequently cause inactivation (Joyce et al., 2003). However, low T requires higher negative pressure for efficient bubble formation and collapse. Therefore, it is possible to suggest that low bubble formation and collapse were not sufficient to cause cell wall pores and lethal rupture in the SA population in BDL. The formation of pores by US facilitates the passive transport of nutrients from the extracellular medium into the cell (Ojha et al., 2017; Tizazu et al., 2018). On the other hand, it is possible to suggest that the mechanical effect of US in non-lethal conditions can propel bacteria into the meat. In others studies, US improves NaCl diffusion in pork meat curing due to the effect of sound waves that propel the salt of the aqueous solution into the matrix (Inguglia et al., 2018; McDonnell et al., 2018). Microjets cause the microinjection process of brine into the meat (Cárcel et al., 2007). During the cavitation process in a solid medium, a rapid series of alternative contractions and expansions cause an effect similar to squeeze and release repeatedly and for this reason it is called ''sponge effect''

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(Gallego-Juarez et al., 1999). This effect provides the creation of microchannels for water movement (Gallego-Juarez et al., 1999; Muralidhara et al., 1985) and increases the distance between muscle fibres (Siró et al., 2009). All these effects can be corroborated with bacteria mobility/internalization. In addition, other mechanical effects such as blade tenderization cause *E. coli* O157:H7 internalization in meat (Luchansky et al., 2008).

The effect of US and PA on SA inactivation in BDL obtained non-significant interaction ($p > 0.05$). According to the findings discussed earlier, T influenced otherwise the bactericidal effect of US and PA. US improves the antimicrobial effect of chemical compounds, since micro-jets damage the cell wall and propel the chemical into the cell (Gogate and Kabadi, 2009; Rosário et al., 2017). In the present study, at low Ts, PA showed action and US had low efficiency. On the other hand, at high Ts, US showed efficiency (micro-jets were formed). However, PA was degraded. Therefore, the effect of T caused no significant interaction between US and PA. In addition, this finding corroborates with other interactions observed in our study (T × US and T × PA).

4. Conclusion

From the analysis and application of the model we can state that the increase in T (i) improves the antibacterial effect of US with the occurrence of synergistic effect, however, (ii) it reduces the bactericidal effect of the PA. (iii) The use of US allows the reduction of the T level to inactivate SA. (iv) US (40 kHz, 5.40 W/g) during cooling is not suitable to inactivate SA in BDL. (v) PA sanitizing solution during cooling is more efficient than at higher Ts. (vi) T and PA threshold values for optimized SA inactivation were determined. Finally, the understanding of the combined inactivation effect of these three factors allows the improvement of the SA decontamination process in sliced BDL. In addition, this study demonstrated that a high amount of sanitizing solution is required for sliced meat products.

However, the high efficiency of the methods studied for bacteria inactivation encourages future studies to improve and optimize the employment of these preservation technologies in meat.

Declarations of interest

None.

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Figure Captions (Use color only online)

Fig. 1: Validation indexes. A: Fitted versus observed values and B: relative error (RE) (prediction zone of -0.3 (fail-safe) and 0.15 (fail-dangerous)) of the decimal reduction (DR) of *Staphylococcus* spp. after application of ultrasound (kJ/g), temperature (°C) and peracetic acid (mg/L) in Brazilian dry-cured loin.

Fig. 2: Pareto chart of standardized effects and *P*-value for inactivation of *Staphylococcus* spp. T: temperature (ºC), AED: acoustic energy density (kJ/g), PA: peracetic acid (mg/L). Linear term (L) and quadratic term (Q).

Fig. 3: Response surface graphs of *Staphylococcus* spp. inactivation (Log N/N_0) in Brazilian dry-cured loin. A: effect of peracetic acid (mg/L) and temperature (°C). B: effect of ultrasound (kJ/g) and temperature (°C).

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Table 1: Factors (independent variables) coded and not coded according to the central composite rotatable design.

Factors	Levels				
	-1.68	-1	0	+1	+1.68
T	6.4	20	40	60	73.6
AED (tm)	1.6 (4.8)	4.9 (15)	9.7 (30)	14.6 (45)	17.9 (55.2)
PA	5.5	60	140	220	274.5

T: Temperature (°C), AED: Acoustic energy density (kJ/g), tm: Application time (min), PA: Peracetic acid (mg/L)

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Table 2: Decimal reduction and standard deviation of *Staphylococcus* spp. after application of temperature, ultrasound, and peracetic acid in Brazilian dry-cured loin according to central composite rotatable design.

Run	T (ºC)	AED (kJ/g)	PA (mg/L)	DR (log cfu/g)
1	20.0	4.9	60.0	-0.4 ± 0.08
2	20.0	4.9	220.0	-1.8 ± 0.36
3	20.0	14.6	60.0	-0.2 ± 0.05
4	20.0	14.6	220.0	-1.5 ± 0.31
5	60.0	4.9	60.0	-3.6 ± 0.72
6	60.0	4.9	220.0	-4.1 ± 0.82
7	60.0	14.6	60.0	-4.5 ± 0.90
8	60.0	14.6	220.0	-4.8 ± 0.96
9	6.4	9.7	140.0	-0.9 ± 0.18
10	73.6	9.7	140.0	-5.5 ± 0.60
11	40.0	1.6	140.0	-1.4 ± 0.28
12	40.0	17.9	140.0	-2.8 ± 0.56
13	40.0	9.7	5.5	-1.1 ± 0.22
14	40.0	9.7	274.5	-2.9 ± 0.58
15	40.0	9.7	140.0	-2.3 ± 0.46
16	40.0	9.7	140.0	-2.5 ± 0.50
17	40.0	9.7	140.0	-2.0 ± 0.40
18	40.0	9.7	140.0	-2.3 ± 0.46
19	40.0	9.7	140.0	-2.3 ± 0.44

T: temperature, AED: acoustic energy density, PA: peracetic acid, DR: decimal reduction (means and standard deviation of two analytical replicates).

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Table 3: Additional experimental conditions for validation of the predictive model for inactivation of *Staphylococcus* spp. in Brazilian dry-cured loin using temperature, ultrasound and peracetic acid.

Test	Independent variables			Decimal reduction (log cfu/g)	
	T (ºC)	AED (kJ/g)	PA (mg/L)	Observed value	Predicted value
1	8.0	1.6	10.0	0.3 ± 0.02	0.4
2	10.0	2.3	150.0	-1.0 ± 0.06	-1.1
3	15.0	6.5	80.0	-0.2 ± 0.02	-0.3
4	35.0	11.3	200.0	-2.2 ± 0.11	-2.3
5	45.0	13.0	100.0	-2.6 ± 0.13	-2.6
6	65.0	3.2	180.0	-3.8 ± 0.19	-4.0
7	70.0	1.9	40.0	-4.1 ± 0.21	-4.2

T: temperature, AED: acoustic energy density, PA: peracetic acid. Initial count: 5.4 ± 0.31 log cfu/g.

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Table 4: Validation data of inactivation models (design of experiments) of foodborne pathogens in meat and fish products using preservation technologies.

Matrix	Bacteria	Preservation technology (factors)	R^2_{adj}	*P* value	A_f	B_f	MSE	Reference
Brazilian dry-cured loin	*Staphylococcus* spp.	US (acoustic energy density, peracetic acid and temperature)	0.970	< 0.0001	1.13	1.07	0.203	This study
Spanish dry-cured ham	*Salmonella* London	HPP (pressure, time and temperature)	0.834	< 0.0005	1.31	1.00	0.398	Bover-Cid et al. (2012)
Cooked shrimp	*Vibrio parahaemolyticus*	AEW (NaCl concentration, time and temperature)	0.950	< 0.0001	1.28	1.19	0.185	Wang et al. (2014)
Meat medium	*Listeria monocytogenes*	HPP (pressure, pH, NaCl, $NaNO_2$ and time)	0.910	< 0.0001	1.06	1.04	-	Possas et al. (2018)
Spanish chorizo sausage	*Listeria monocytogenes*	HPP (a_w, time and pressure)	0.880	< 0.0001	1.45	1.32	-	Rubio et al. (2018)
Shelled Fresh Shrimp	*Vibrio parahaemolyticus*	AEW and HPP (NaCl, pressure and time)	0.986	< 0.0001	1.03	1.01	0.036	Du et al. (2016)
Spanish dry-cured ham	*Listeria monocytogenes*	HPP (pressure, time and time)	0.9884	< 0.0001	1.36	1.06	0.062	Bover-Cid et al. (2011)

AEW: acidic electrolyzed water, HPP: high-pressure processing, US: ultrasound, MSE: mean-square error. Accuracy (A_f) factor lower than 1.45 (Ross et al., 2000) and bias (B_f) factor lower than 1.15 (Ross et al., 1999) are acceptable.

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Highlights

- The combined effect of US, PA, and T was quantified
- Heat improves ultrasonic reduction of *Staphylococcus* spp.
- Synergistic effect between US and T in SA inactivation
- Cold combined with US (40 kHz, 5.40 W/g) increased SA survival
- Effective T and PA thresholds for SA inactivation were determined

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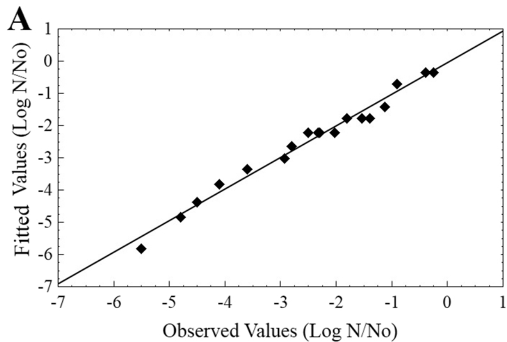


Figure 1

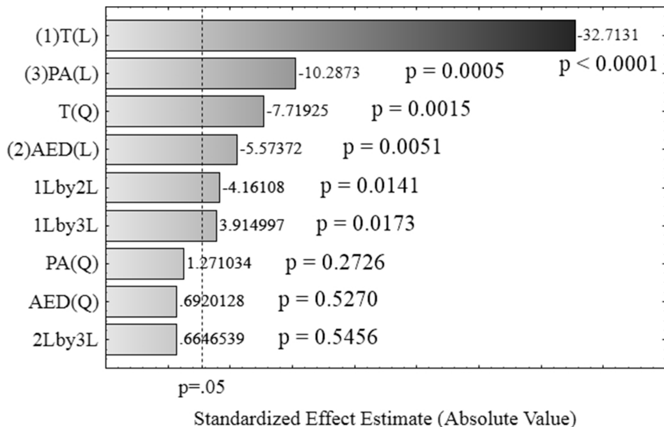
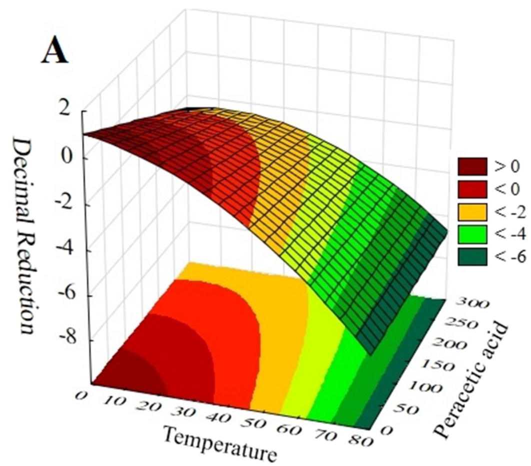
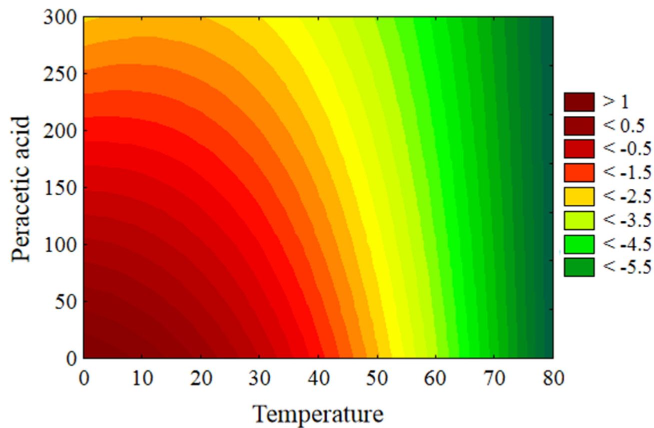
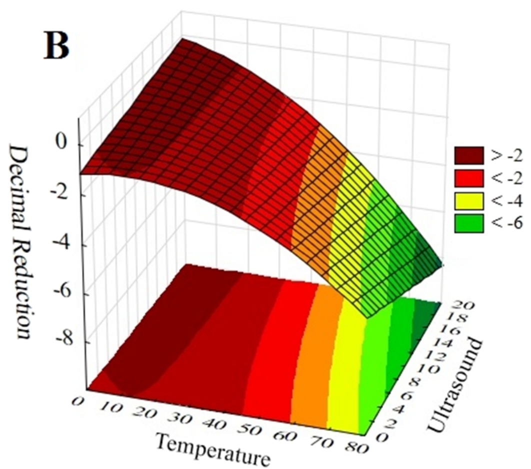


Figure 2







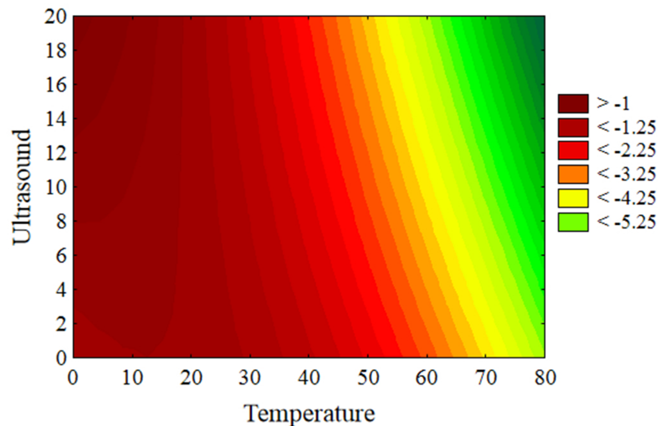


Figure 3