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Ultrasound for improving the preservation of chicken meat

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Abstract

The objective of this study was to evaluate the effect of power ultrasound on the microbiota of chicken meat. Samples were treated under the following conditions of frequency and power: 20 kHz and 27.6 W/cm^2; 40 kHz and 10.3 W/cm^2; 850 kHz and 24.1 W/cm^2. Microbial counts were done before the ultrasound treatment, immediately after and following 7 days of aerobic storage at 4 °C. The results indicate that high intensity ultrasound helps inhibit the growth of lactic acid, mesophilic and psychrophilic bacteria present in chicken meat at the ultrasound frequency levels used in this study. The number of mesophilic bacteria decreased with the ultrasound probe at 20 kHz and 27.6 W/cm^2 in relation to the treatment with higher frequency and less intensity. In conclusion, high-intensity ultrasound has a bactericidal effect. Therefore, it can be useful in the preservation of meat products and thus play an important role in the food industry.

Keywords: bacterial growth; bacterial count; chicken meat; deterioration; storage.

Practical Application: The high intensity ultrasound has a bactericidal effect which can be considered one option for controlling the growth of bacteria in chicken meat stored at 4 °C. Therefore may assist in the preservation of meat products and potential role in the food industry.

1 Introduction

Most studies of high-power ultrasound have been conducted in milk and dairy products, as well as eggs, rice, soy, peas, and meat. However, application in meat has been delimited due to the structural complexity of the skeletal muscle (Higuera-Barraza et al., 2016). The intrinsic (nutrients, water availability and pH) and extrinsic (collection, processing and storage) characteristics of chicken meat, make it highly susceptible to the development of pathogens and decomposing microorganisms, and are inconvenient in the different stages of slaughter (Alonso et al., 2014).

In order to inactivate the microorganisms present in the meat, different methods are commonly used: a) antimicrobial wash (using chlorine or organic acids); b) thermal processing (lowering the nutritional value), dehydration and/or addition of preservatives. However, these latter procedures modify the physical (Jayasooriya et al., 2007) and chemical characteristics of the meat and in some cases they result in undesirable changes in color, flavor and texture (Ercan & Soysal, 2013). Power ultrasound offers an alternative to the traditional methods (Feng et al., 2013; Turantas et al., 2015) and it is regarded as a green, versatile and promising technology (Majid et al., 2015) with a dynamic development in applied research and in the food industry respect to processing, preservation, transformation and extraction procedures to increase the efficiency of production and contribute to the preservation of the environment. Ultrasound used in the "Green Food Processing" is based on the discovery and design of technical processes to: a) reduce energy and water consumption; b) allow the recycling of products through bio-refinery; c) ensure high quality and safe food (Chemat et al., 2017).

The antimicrobial effectiveness of high-intensity ultrasound depends on many factors, including the frequency, intensity and duration of the ultrasound waves, the characteristics of the food being treated and the type of microorganism (Joyce et al., 2011). It has been reported that high intensity ultrasound has effective antimicrobial activity against *Escherichia coli* (Lee et al., 2009; Luna et al., 2015; Patil et al., 2009; Zhou et al., 2009) and *Listeria monocytogenes* (Birk & Knøchel, 2009; Cameron et al., 2009), highlighting the importance of ultrasound in the microbiological quality of food (Sango et al., 2014).

Although the effectiveness of high-intensity ultrasound has been demonstrated, there are still inconsistent results. Therefore, the aim of this study was to determine the effect of power ultrasound on bacteria commonly found in chicken meat.

2 Materials and methods

2.1 Experiment site

The study was conducted in the laboratory of Microbiology and Sonochemistry of the Faculty of Health and Life Sciences at the University of Coventry, located in the City of Coventry, England.

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2.2 Nature of the sample, experimental design and treatment

Forty portions (150 g per portion) of chicken breasts were used. An experimental unit was defined as 150 g of the chicken meat, each was individually packed in polyethylene bags, sealed and stored during 48 h (4 °C) to allow bacteria to adapt to the packaging and storage conditions. Samples were assigned randomly to each ultrasonic treatment (ten servings per treatment). The design was completely randomized and the ultrasonification was performed under the following conditions of frequency and power: T1: 20 kHz and 27.6 W/cm^2; T2: 40 kHz and 10.3 W/cm^2; T3: 850 kHz and 24.1 W/cm^2 and the control.

2.3 Application of ultrasound

For T1 the meat and diluent were poured into a glass beaker (previously disinfected) and sonicated with a 20 kHz probe and 27.6 W/cm^2 ultrasonic probe (Jencons® model VC-505 (USA)). Similarly, for T2 the glass beaker containing the meat in diluent was placed into a 40 kHz probe and 10.3 W/cm^2 ultrasonic bath (Langford® model Sonomatic-575 (England)) and, finally, for T3 (850 kHz and 24.1 W/cm^2) the meat samples and 200 ml of MRD were placed directly into the device after disinfected it ultrasound bath (Meinhardt Ultraschalltechnik® Model 5 / 1575 (Germany)). Temperature was kept constant at 4 °C during the ultrasound application.

2.4 Power measurement of three ultrasound systems

The effective ultrasound power introduced in the system was determined using the calorimetric technique described by Margulis & Margulis (2003). The method was applied to estimate the power of the acoustic (for the different ultrasound amplitudes) wave transmitted to a solution of distilled water and dissipated as heat through the following steps. US was applied to an established volume of the solution while the temperature change of the sonicated fluid was recorded at short time intervals for 180 s. The value of dT/dt was estimated from the graph of temperature as a function of time. The power of the US transmitted to the fluid was determined from the Equation 1:

$$P = m \times Cp \times dT \,/\, dt \quad (1)$$

where P is the ultrasonic power (W); m is the mass of the sonicated liquid (kg); Cp is the specific heat at constant pressure (J/g K); dT is the increment in temperature; and dt is the increment in time. The effective ultrasound power is expressed in watts per unit area of the emitting surface (W/cm^2) (Jambrak et al., 2014).

The ultrasound system T1 (20 kHz potency probe) had a value of 0.0248, which was substituted into the US potency equation given above. In the equation, water was considered to have a caloric capacity of 4.186 J/kg °C and a dissolvent mass (M) of 200 g resulting in a US output power of the system of 27.6 W/cm^2. Similarly, ultrasound system T2 (40 kHz) had a value 0.0142 with an ultrasonic power of 10.3 W/cm^2, and the ultrasound system T3 (850 kHz), same procedure is performed whit a value 0.043 with an ultrasonic output power of 24.1 W/cm^2.

2.5 Collection of microbiological samples

After 48 hour of storage, each sample was unpacked and 200 mL of MRD [Maximum recovery diluent (peptone saline diluent: peptone 1.0 g/L, sodium chloride 8.5 g/L, pH 7.0 ± 0.2)] was added to the meat, from this, 1 mL of exudate was taken for microbiological analyses (Haughton et al., 2012).

Subsequently, the ultrasound treatment was applied for 5 minutes (as it corresponded) and immediately, a second collection (1 mL of exudate) was taken. The samples were repacked in polyethylene packages and was stored at 4 °C. After 7 days the bags were opened and the breast meat was immersed in 200 mL of MRD sterile solution and a third sample of exudate was taken for analysis (sample of 1 mL).

The control breast meat samples were immersed in MRD sterile solution for 5 minutes and samples were taken before and after this time and after 7 days storage at 4 °C.

2.6 Microbiological analysis

Microbial counts were performed using decimal serial dilutions. A series of dilutions from 1:10 to 1:100 in MRD were prepared with the collected exudate sample solution of the chicken breasts before and after treatment. With the samples taken after 7 days of storage dilutions were prepared as follows from 1:10 to 1:1000000 in MRD. 100 µL of each dilution was inoculated by the spread plate technique on standard agar plates (Plate Count Agar (PCA), CM0325, Oxoid, Basingstoke, UK) (Luna et al., 2015). The plates were incubated for mesophilic microorganisms at 25 °C for 3 d and for psychrophilic organisms at 4 °C for 6 d.

Moreover, for the lactic acid bacteria, the dilutions were inoculated into De Man agar, Rogosa, Sharpe (MRS, CM0361, Oxoid, Basingstoke, UK) and incubated for 6 d at 35 °C in carboxyphilic conditions (Vera et al., 2009). Finally the microbial counts were performed for each petri dish, the number of colony forming units per milliliter (CFU/mL) were calculated and transformed into a logarithmic scale (Log_{10} CFU/g). Control samples were not subjected to ultrasound treatment.

2.7 Statistical analysis

This study had a completely randomized design with two factors: ultrasound application time and atmosphere. The application time factor had three levels (0, 30, and 50 min), and the packaging atmosphere had two levels (aerobic and vacuum packaging). Each 150 g portion was considered an experimental unit. The transformed data were analyzed using PROC GLM of SAS Institute (2002) at $\alpha = 0.05$. The model included the level of ultrasound as a fixed effect and the initial bacteria count as a covariate. Sampling time as an indicator variable for trend analysis was also included.

3 Results and discussion

3.1 Mesophilic bacteria

According to Dolatowski & Stasiak (2002), aerobic bacteria can be controlled based on the treatment of power ultrasound, which may explain the effect thereof on the mesophilic bacteria (Figure 1a). It has been reported that in fresh chicken mesophilic

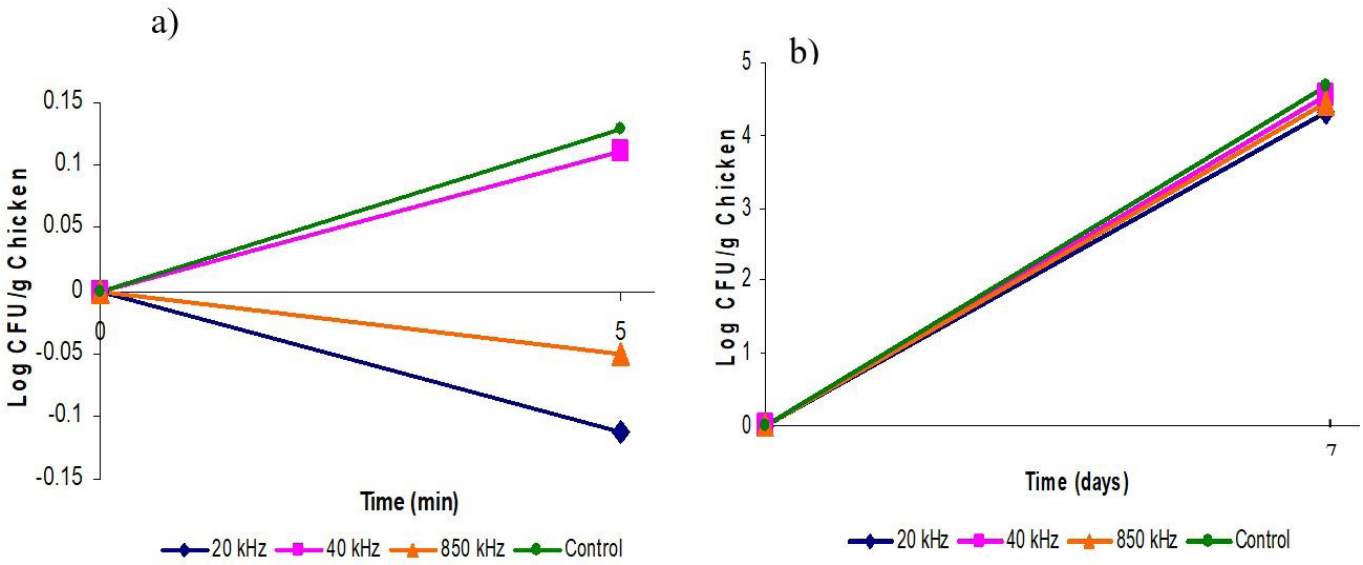

Figure 1. Adjusted plots of mesophilic bacteria in chicken breast. (a) grown during sonication; (b) grown during refrigeration (Storage time).

total counts decrease when subjected to power ultrasound (Haughton et al., 2012). However Figure 2b shows that the effect during storage is not notorious or permanent.

A cubic type trend (P = 0.004) was observed among the four treatments when analyzing the average mesophilic bacteria count after 5 min of the application of ultrasound and after 7 d of storage at 4 °C (Figure 3). Significant differences between the control and treatment 20 kHz and 27.6 W (P = 0.003) were discovered. The 40 kHz and 850 kHz treatments (10.3 and 24.1 W respectively) were statistically equal (P = 0.45).

In the results for mesophilic bacteria it is noteworthy that the high intensity ultrasound helped control the growth of the treated samples, as the microbial content of samples subjected to the treatment of 20 kHz (3.7 ± 0.4 and 8 ± 0.6 Log_{10} CFU/g) was lower than those of the control samples and of those treated with 40 and 850 kHz. These results coincide with those mentioned by Dolatowski & Stasiak (2002) and Haughton et al. (2012), who found a significant decrease in the total number of mesophilic colonies after applying high intensity ultrasound to pieces of fresh chicken meat.

Joyce et al. (2011) and Hoover (2000) indicate that the effect of ultrasound depends on the intensity and frequency of the treatment. The greater intensity of ultrasound used in this study was 27.6 W (20 kHz), which explains why this treatment presented lower counts of mesophilic bacteria (P = 0.02).

The main effect of high intensity ultrasound (40 kHz) is the agglomeration of microbial cells (Joyce et al., 2011; Mason & Lorimer, 2002) and not their inactivation. Thus, this explains that the decline of mesophilic bacteria was statistically equal (P = 0.45). In another study it was suggested that frequencies above 740 kHz can produce overheating (due to cavitation) in the treated surface, which leads to less penetration of ultrasound waves (Miles et al., 1999). The type of microorganisms should also be considered, as the sensitivity to the ultrasonic waves

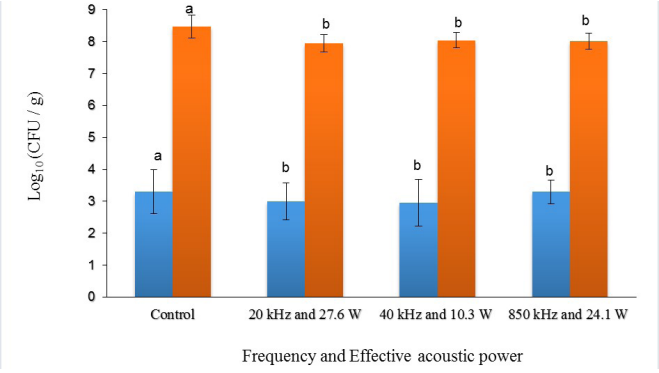

Figure 2. Psychrophilic bacteria count (Log_{10} CFU/g) in chicken meat, after application of ultrasound (▪) and after 7 d of storage (aerobic) at 4 °C (▪). Different letters show significant differences between treatments.

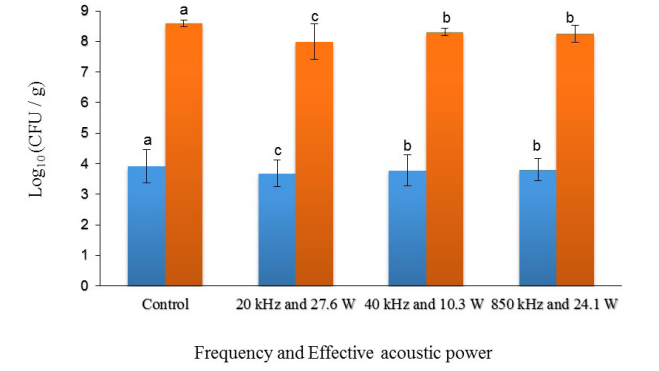

Figure 3. Mesophilic bacteria count (Log_{10} CFU/g) in chicken meat, after application of ultrasound (▪) and after 7 d of storage (aerobic) at 4 °C (▪). Different letters show significant differences between treatments.

is related to structural differences between microorganisms. Gram-negative bacteria have a thinner cell wall with an outer lipid bilayer whereas the cell wall of Gram-positive bacteria

has a thick peptidoglycan layer surrounding the cytoplasmatic membrane (Piyasena et al., 2003).

Gram-positive bacteria are more resistant to ultrasonicacion than the gram-negative ones; also it should be considered that aerobic bacteria are more resistant than the anaerobic bacteria. The non-significant reduction, in general, suggests the possibility that there are micro-organisms susceptible and resistant to ultrasound treatments. Therefore, it can be inferred that the efficiency of the utrasonicacion is related to the heterogeneity of the microbiota present in a food.

Recently, Sienkiewicz et al. (2017) proposes the destruction of pathogenic bacteria with the use of ultrasound waves. They demonstrated the impact of ultrasound of 20, 40 and 100 kHz frequencies and the power of 10.5 W/cm^2 on the growth of the strain of *Salmonella enterica* subs. *typhimurium*. Likewise, Kang et al. (2017) confirmed the effects of ultrasound on the inactivation of microorganisms during the curing processing. The particle size distribution of bacterial and cell fluorescence staining analysis showed that ultrasound could result in the formation of cell fragments through destroying the integrality of the membrane of *E. coli* O157:H7 and *Bacillus cereus.*

3.2 Psychrophilic bacteria

The psychrophilic bacteria show a biggest decrease after 5 minutes of sonication with the 20 kHz probe, plot were adjusted to clearly see the effect of each device (Figure 4). The initial amount of bacteria is not an important factor, in the samples treated in the 20 and 850 kHz device the microbial content were higher and yet there was a decrease in microbial count during the 5 minutes of sonication. Unlikely those samples treated with 40 kHz showed an increase in bacteria counts compared to the control samples.

In the growth of psychrophilic bacteria (Figure 2) there was a quadratic trend (P = 0.02) for the effect of the frequency of high-intensity ultrasound on samples of chicken meat. Microbial content was lower in the treatments with ultrasound (20, 40 and 850 kHz) compared with the control (P = 0.008), again indicating that the high intensity ultrasound has a bactericidal effect. The effect of ultrasound was found to persist throughout the experimental time period, even after storage at 4 °C. Conditions which are known to be optimal for the growth of psychrophilic bacteria (Stanier et al., 2005). These results are in accord with others which claim that ultrasonication helps slow the growth of psychrophilic bacteria in different meat (Dolatowski & Stasiak, 2002) and milk products (Bermúdez-Aguirre & Barbosa-Cánovas, 2010).

Although the samples subjected to ultrasonication had lower microbial contents than the control samples, there were no significant differences between the different treatments (P = 0.32). That is, the effect of ultrasound on psychrophilic bacteria did not depend, on the frequency or intensity used in each treatment.

The bactericidal effect of ultrasound is due to the acoustic cavitation phenomenon, where the changes in pressure allow the formation and collapse of microbubbles in milliseconds, which burst into structural and functional components in the cells resulting in cell lysis. In addition, breakdown and thinning of cell membranes, and the damage to the DNA due to free radical production (Joyce et al., 2003).

Prior research has found that some microbial cells are resistant to high pressure and the presence of free radicals (Foladori et al., 2010; Pitt et al., 1994), which are the main effects of high-intensity ultrasound and may be associated with the lack of difference in the effectiveness of the treatments presented in this investigation. However, the microbial content was slightly lower in the treatments of 20 and 850 kHz, where the highest intensity (27.6 and 24.1 W respectively) was used (Figure 2).

Mainly changes observed of ultrasound are due to the cavitation effect on biological structures. Marchesini et al. (2015) evaluated the effect of ultrasound against *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and

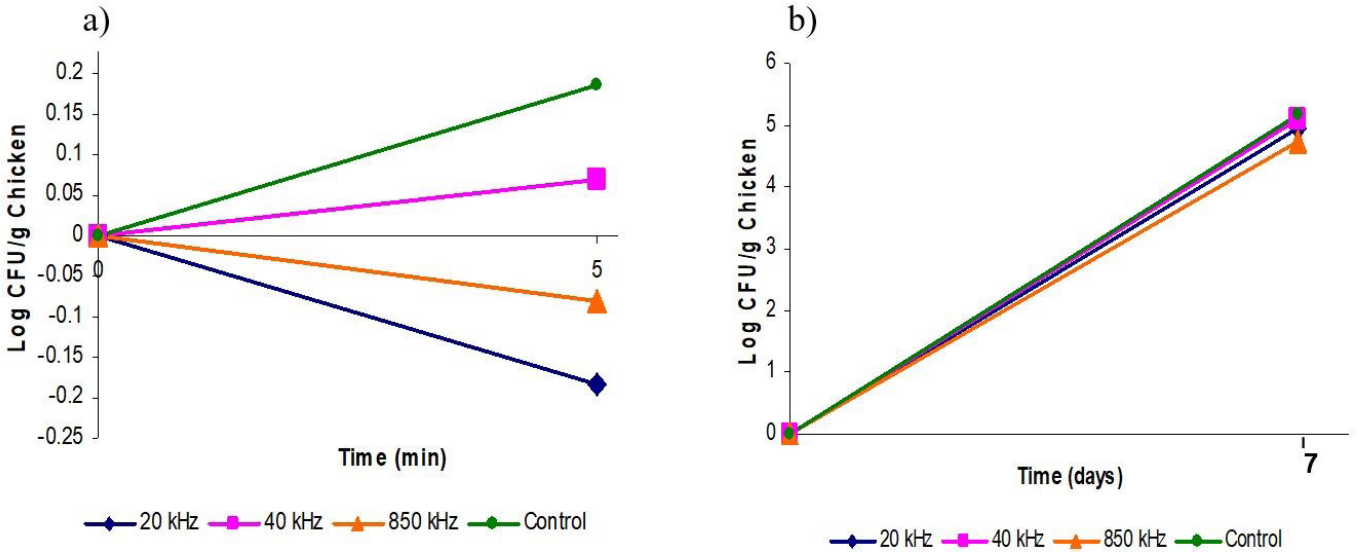

Figure 4. Adjusted plots of psychrophilic bacteria in chicken breast. (a) grown during sonication; (b) grown during refrigeration (Storage time).

Debaryomyces hansenii and reported that the strongest treatment (100% Å~ 300 s) led to a population reduction but caused milk sensorial deterioration. However, Johansson et al. (2016) showed that ultrasound treatment does not significantly influence the oxidative changes in milk and they concluded that the lipid oxidation derived volatiles produced are below the human sensory detection level. The study of Saeeduddin et al. (2017) indicated that ultrasound-pasteurization is a promising pear juice processing technology at low temperature, retaining bioactive compounds, meeting safety standards, and increasing shelf life.

3.3 Lactic acid bacteria

The lactic acid bacteria did not decrease during the sonication; however the untreated samples had a higher growth than the treated ones (Figure 5a). As it can see in Figure 4b, the ultrasonic effect is slightly evident with the 20 kHz and 850 kHz devices but the highest growth was observed in samples treated with 40 kHz bath, being this values higher than those of control samples.

Samples subjected to 20 kHz probe had lower mesophile bacteria growth until the end of the experiment.

As in the case of psychrophilic bacteria, ultrasound treatment had a significant effect on the lactic acid bacteria content of the treated samples (P = 0.05). A quadratic trend between the treatments (P = 0.04) was observed, with no significant differences (P = 0.26) between the samples subjected to different ultrasound wave frequencies (20, 40 and 850 kHz). Dolatowski & Stasiak (2002) reported that high-intensity ultrasound is an effective method for controlling the growth of lactic acid bacteria, which coincides with the findings of the present study (Figure 6).

However, their study did not mention whether such bacteria are equally vulnerable to different methods of ultrasonication. In addition, Cameron et al. (2009) suggests that some lactic acid bacteria are resistant to various methods and parameters of high intensity ultrasound, its resistance can also be a major cause of non-decrease effect in the samples subject at 40 kHz treatment.

The content of lactic acid bacteria after application of 5 min ultrasound was lower (2.0 ± 0.2 Log_{10} CFU/g) in the samples assigned to treatment of 40 kHz. In this treatment, a low intensity ultrasound (10.3 W) was used. However, at the end of storage (7 d at 4 °C) the content of lactic acid bacteria in the samples subjected to the treatment of 40 kHz was higher (4.0 ± 0.2 Log_{10} CFU/g) than that of the other two treatments. In conclusion, the effect of 40 kHz did not inhibit growth of lactic acid bacteria under refrigeration as reported previously (Pohlman et al., 1997). It was observed that the effect of the initial content of microorganisms, used as a covariate to adjust the quantification of bacteria, was highly significant (P <0.0001) for mesophilic, psychrophilic, and lactic acid bacteria in the samples after ultrasound treatment.

Worth mentioning that the total count of microorganisms is reduced by applying steam and ultrasound immediately after slaughter. However, there is still a need for more thorough research in the previous fields of meat processing. Other novel techniques such thermosonization, manipulation and manothermostation, can be an alternative energy processing relevant to the food industry. In meat processing, power ultrasound can modify cell membranes that can help heal, marinate and soften tissue and control the growth of bacteria in meat.

Even though high-power ultrasound improves microbiological properties of meat, also has thermal and mechanical effects on structure. Application of ultrasound led to irreversible changes in structure (growth of porosity, loss of tissue coherence, formation of microchannels, etc.) and cell composition (destruction of cell components, e.g., the nucleus) (Rajewska & Mierzwa, 2017). Another degradation effect of ultrasound is on permeability, solubility and diffusion coefficients of oxygen through the biaxially oriented polypropylene films (Ščetar et al., 2017).

Regarding mass transfer in meat is accelerated by ultrasound mechanisms, such as cavitation, independent of temperature effects (McDonnell et al., 2018) and this effect represents an advantage in the curing process. Another effect induced by

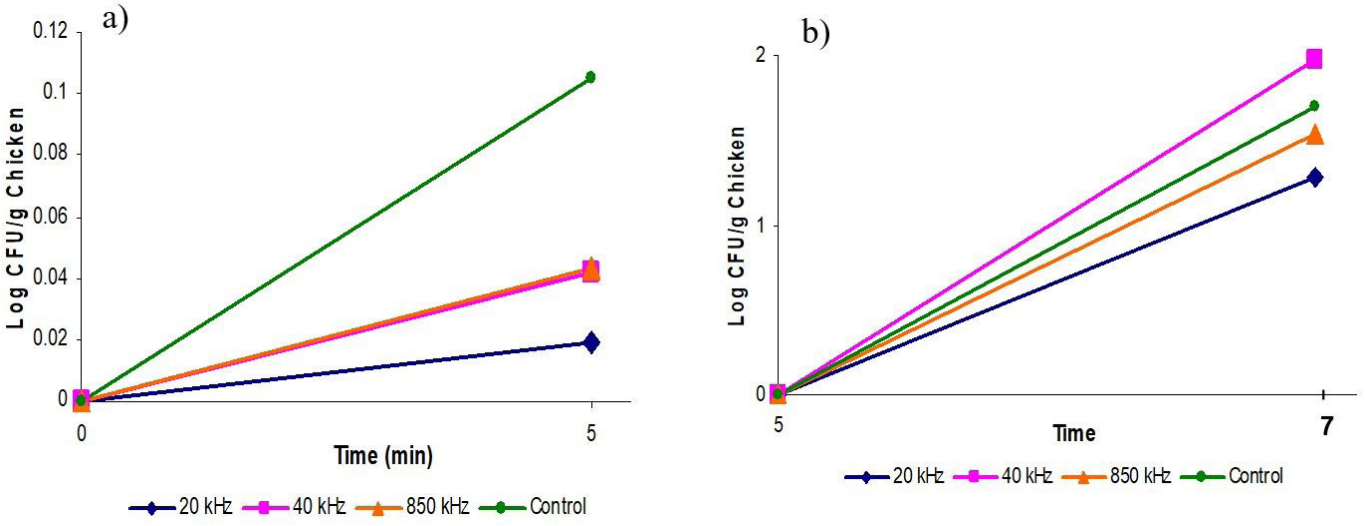

Figure 5. Adjusted plots of lactic acid bacteria in chicken breast. (a) grown during sonication; (b) grown during refrigeration.

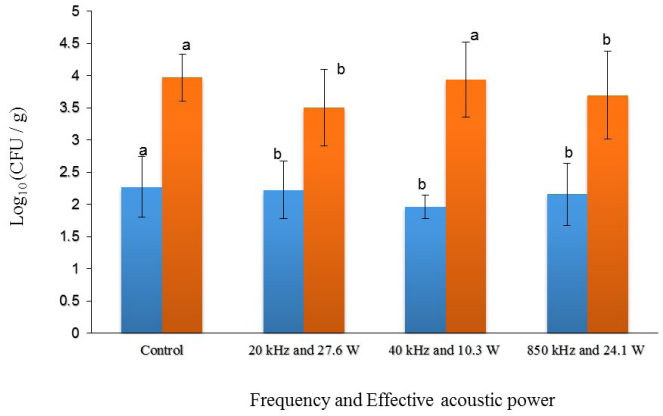

Figure 6. Lactic acid bacteria count (Log_{10} CFU/g) in chicken meat, after application of ultrasound (▪) and after 7 d of storage (aerobic) at 4 °C (▪). Different letters show significant differences between treatments.

ultrasound on meat is the tenderizing effect which is due to proteolysis during the postmortem storage and it is reflected by an increased degradation of desmin and troponin-T (Barekat & Soltanizadeh, 2017). Power ultrasound leads to changes in structures and oxidation of beef proteins caused by mechanical effects of cavitation and the resultant generation of free radicals (Kang et al., 2016).

Finally, the food industry requires the use processes such as high power ultrasound to reduce carbon food printing, this meets the future trend concept of "Green Food Processing" and consumer demand of greener products. However, these processes must be further developed before they can be implemented at a full industrial level. Some ultrasonic innovations are already close to being used on a large scale; but research to date has not been consistent enough to establish the effect of ultrasound on the structure and properties of meat. Therefore, additional research is still required until the ultrasound technology is widely applied in the meat industry.

4 Conclusion

High intensity ultrasound at 20 kHz, 40 kHz and 850 kHz frequencies immediately controls the development of mesophilic, psychrophilic and lactic acid bacteria in chicken meat. Based on our work the ultrasonic probe system (20 kHz) can be considered the best option for controlling the growth of such bacteria in chicken meat stored at 4 °C. The duration of treatment and the frequency used are vital factors that determine the effect of treatment on microorganisms.

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