Influence of High Intensity Ultrasound Application on Mass Transport, Microstructure and Textural Properties of Pork Meat (*Longissimus dorsi*) Brined at Different NaCl Concentrations.

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ABSTRACT

The aim of this work was to evaluate the effect of high intensity ultrasound and NaCl concentration on the brining kinetics (5±1 ºC) of pork loin as well as its influence on the textural and microstructural changes. In order to identify the effect of both factors on NaCl and moisture transport, kinetics were analyzed by taking the diffusion theory into account. The textural and microstructural analysis of raw and brined meat both with and without ultrasound application was carried out. The experimental results showed that the brine NaCl concentration not only determined the final NaCl content in meat samples but also the direction of water transport. The NaCl and moisture effective diffusivities were improved by ultrasound application. The final NaCl and moisture content and the ultrasound application promoted changes in instrumentally measured meat texture, which were confirmed via microstructural observations.

Keywords: mass transfer, power ultrasound, modeling, diffusivity, texture, Cryo-SEM, SEM.

1. Introduction

Over 1.2 million tons of meat products produced per year makes Spain the fourth most important country in the European Union in this regard, being the dry-cured products the most valuable ones in the Spanish meat industry (AICE, 2011). One of the main stages during the processing of dry-cured products is salting. Actually, this operation is mainly carried out by salting the meat pieces with solid salts (NaCl), but it could also be performed by brining (Barat et al., 2006). Compared with other food processes, brining is a slow process and, for that reason, the food industry is searching for alternative technologies for improving the mass transfer kinetics (Rastogi et al., 2002), such as high intensity ultrasound application (Cárcel et al., 2007a).

In liquid medium, ultrasound induces cavitation (Leighton, 1998), temperature gradients within the material (Mason and Lorimer, 2002), mechanical phenomena, such as the "sponge effect", generation of microchannels and microstirring on interfaces (Muralidhara et al., 1985). These effects can not only increase the mass transport kinetics but also imply structural changes and, consequently, changes in textural properties. Thereby, textural changes induced by ultrasound have been observed in, among others, tomato juice (Vercet et al., 2002), yoghurt (Wu et al., 2000), bell peppers (Gabaldón-Leyva et al. 2007) or meat (Jayasooriya et al., 2007; Pohlman et al., 1997).

Meat has great biochemical and structural complexity. During meat brining, changes in NaCl and moisture content take place (Graiver et al., 2006), which extend meat's shelf life and modify organoleptic characteristics, such as juiciness, texture and flavor. Mass transfer driving forces between meat and brine are linked to chemical gradients and NaCl-induced changes in the water holding capacity (WHC) of meat proteins (Shi and Le Maguer, 2002; Vestergaard et al., 2005). The NaCl concentration in brine affects the direction of moisture transport as well as the equilibrium state (Cheng and Sun, 2008). A low NaCl content in the meat increases the water holding capacity (WHC) (Nguyen et al., 2010), a phenomenon known as "salting-in" which is linked to the meat protein net charge modification. However, a high NaCl content in meat could also bring about a decrease in WHC, probably due to the insolubilization of proteins (“salting-out”) (Graiver et al., 2009). Therefore, the NaCl concentration in the brine solution can not only affect the chemical gradients, but also the WHC, thus affecting the magnitude of mass transport.

Modeling is a fundamental tool to quantify the mass transport (Cárcel et al., 2007a), as well as to evaluate the effectiveness on that of new technologies, such as ultrasound. But in addition, modeling also provides relevant information to understand the changes undergone by foodstuffs during processing, which usefulness complements the information given by other techniques, such as textural and microstructural ones (Pérez-Munuera et al., 2008). Thereby, different electron microscopy techniques, Larrea et al. (2007) have been used to characterize the microstructure of *Biceps femoris* and *Semimebranousus* muscles during the processing of Teruel dry-cured ham. Ruiz-Ramírez et al. (2005) described the effect of NaCl and pH on the relationship between water content and textural parameters in dry-cured muscles.

The main aim of this work was to evaluate the influence of high intensity ultrasound application on the meat brining kinetics using different NaCl concentrations in the brine solution. Moreover, the ultrasonic effects in transport phenomena have been quantified by modeling and linked to the induced changes in microstructural and textural meat properties.

2. Materials and methods

2.1. Raw material and sample preparation

Fresh pork loins (*Longissimus dorsi)* were purchased at a local slaughterhouse (Valencia, Spain). The pieces selected had a pH of 5.3 ± 0.30, which was measured in-situ by means of a pH-meter (pH STAR, Matthäus, Germany) at three different points along the muscle avoiding fatty areas. Parallelepiped shaped samples (length 50 x width 30 x thickness 10 mm) were obtained from the central part of loin pieces using a sharp knife. Before brining, samples were wrapped in plastic waterproof film and kept frozen at -18 ± 0.5 ºC (maximum storage time 120 hours).

2.2. Brining treatments

Brining experiments with (US, 40 kHz; 37.5 W/dm^3) and without ultrasound application (CONTROL) were carried out using brine solutions of different NaCl concentrations (50, 100, 150, 200, 240 and 280 kg NaCl/m^3). The highest NaCl concentration used (280 kg NaCl/m^3) involved brine saturation at 5±1 ºC, which was pointed to by the presence of NaCl crystals in the solution.

Brining treatments were carried out in an ultrasonic cleaning bath (4 L, Selecta, Spain) where the temperature was held at 5±1 ºC by brine recirculation from a cooling reservoir. A peristaltic pump (302S Limited, Watson/Marlow, United Kingdom) drove the brine from the cooling reservoir equipped with a chiller (3000778, J.P. Selecta, Spain) and a mechanical stirrer (RZR 1, Heidolph Instruments, Germany).

Before each brining experiment, 6 meat samples were thawed at constant temperature (2±1 ºC) for 24 h. Then, the samples were blotted, weighed (PB3002-S/PH, J.P., Mettler Toledo, Spain) and their size was measured by using a Vernier caliper. Afterwards, they were placed in a hollow sample holder and simultaneously introduced in the brine. For a homogenous brining, the position of the meat samples was changed every 5 min. In the US experiments, ultrasound was continuously applied. Samples were taken out of the brine at preset times (15, 30, 45, 60, 90 and 120 minutes) and immersed in distilled water for 20 s to remove any adhered surface brine. Finally, samples were blotted, wrapped in plastic waterproof film and frozen (-18 ± 0.5 ºC) until moisture and NaCl measurements were taken.

After brining, moisture and NaCl content were measured from ground meat (at 300 r.p.m., Blixer® 2, Robot coupe, France). The moisture content was determined following AOAC standards (Method No. 950.46 AOAC, 1997). While in the case of NaCl, the procedure reported by Cárcel et al. (2007b) was used. Both measurements were carried out in triplicate at least.

2.3. Mass transfer modeling

A mathematical model based on Fick's 2nd law was used to separately describe the evolution of moisture and NaCl content in the sample during brining (Cranck 1975). Samples were considered to become slab geometry bodies due to the fact that they were not nearly as thick (10 mm) as they were high (50 mm) and wide (30 mm), thus, mass transfer was simplified as a one-dimensional problem. Constant effective diffusivities (D_s and D_w), negligible changes in temperature and sample volume, solid symmetry, homogeneous NaCl and moisture initial content and negligible external resistance (Gou et al., 2003) were assumed during processing. Eqs. 1 and 2 show the solution of the diffusion model in terms of average moisture and NaCl content.

$$W = W_{eq} + (W_0 - W_{eq})\left[2\sum_{n=0}^{\infty}\frac{1}{\lambda_n^2 L^2}e^{-D_W \lambda_n^2 t}\right] \qquad (1)$$

$$S = S_{eq} + \left(S_0 - S_{eq}\right)\left[2\sum_{n=0}^{\infty}\frac{1}{\lambda_n^2 L^2}e^{-D_S \lambda_n^2 t}\right] \qquad (2)$$

Where λ_n are the eigenvalues obtained from $\lambda_n L = (2n+1)\frac{\pi}{2}$. The equilibrium moisture and NaCl content values (S_e, W_e) were determined by immersing meat samples in the different brine solutions for at least 48 h. From previous experiments, this time was considered to be long enough to achieve the equilibrium.

Both effective diffusivity values, D_s and D_w, were identified by separately fitting Eqs. 1 and 2 to moisture and NaCl transport kinetics. The identification was performed by minimizing the squared differences between the experimental and calculated average sample moisture and NaCl content. For that purpose, the Generalized Reduced Gradient (GRG) optimization method, available in the Microsoft Excel spreadsheet from Microsoft Office XP Professional, was used.

2.4. Textural and microstructural analysis

Meat texture and microstructure were studied in raw material, US (40 kHz; 37.5 W/dm^3) and CONTROL brined samples for 120 minutes using low, intermediate and high NaCl concentrations (50, 200 and 280 kg NaCl/m^3, respectively). Samples were brined in triplicate at least.

2.4.1. Texture

Hardness, characterized as maximum penetration force, was evaluated in brined samples using a Texture Analyzer (TAX-T2®, Stable Micro System, United Kingdom). Penetration tests were conducted with a 2 mm flat cylinder probe (SMS P/2N), a crosshead speed of 1 mm/s and a strain of 60 % (penetration distance 6 mm). In each meat slice, penetration tests were carried out at 12 points at least.

2.4.2. Cryo-scanning electron microscopy (Cryo-SEM)

Cubic samples (side 3 mm) of raw and brined meat were immersed in slush Nitrogen (-210 ºC), and quickly transferred to a cryo-trans (CT 15000 C, Oxford Instruments, England) linked to a scanning electron microscope (JSM-5410, Jeol, Japan). Samples were cryo-fractured at -180 ºC, etched at -90 ºC and gold-coated, allowing cross-section visualization.

The microscopic observations were carried out at 10 kV, a working distance of 15 mm and a temperature below -130 ºC.

2.4.3. Scanning electron microscopy (SEM) with combined dispersion X-ray analysis (SEM-EDX).

Cubic samples (side 3 mm) from raw and brined meat were immersed in liquid N_2 and then freeze-dried at 1 Pa for 3 days (LIOALFA-6, Telstar, Spain). The fixed samples were vacuum sealed in vials in the same freeze-drier so that they would remain stable (Llorca et al., 2001). The fixed samples were individually placed on SEM slides with the aid of colloidal silver and then gold-coated with (SCD005, Baltec, Germany) at 10^{-2} Pa and an ionization current of 40 mA. The samples were observed in a scanning electron microscope (JSM-5410, Jeol, Japan) equipped with an X-ray detector and LINK data-processing system (INCA 4.09, Oxford Instruments, England) at an acceleration voltage of 10-20 kV which provides internal information about the standards of energy dispersive X-ray spectra of the elements analyzed (Na^+ and Cl^-). This technique is an analytical tool that allows the ions Cl^- and Na^+ inside the samples to be identified (Grote and George, 1984). For EDX (energy-dispersive X-ray) analysis, samples were carbon-coated (CEA035, Baltec, Germany). Mapping images of Cl^- and Na^+ distribution in meat samples were made using a voltage of 20 kV and at a working distance of 15 mm.

2.5. Fitting model evaluation and statistical analysis

In order to evaluate the ability of the models to fit the experimental data, the percentage of explained variance (%VAR) was computed (Eq. 3) (Cárcel et al., 2007a). Confidence intervals for the estimation of the effective diffusivities (D_s and D_w) were assessed in order to determine the reliability of the model prediction.

$$\%VAR = \left[1 - \frac{S^2_{tw}}{S^2_w}\right] x100 \qquad (3)$$

Where S^2_w and S^2_{tw} are the variance of the sample and the estimation, respectively.
Multifactor ANOVA and LSD (Least Significant Difference) intervals were estimated to perform a statistical evaluation of the influence of ultrasound application and NaCl concentration on the textural properties. The statistical analysis was carried out using the Statgraphics Centurion XVI software package (Statistical Graphics Corp., Herdorn, USA).

3. Results and discussion

3.1. NaCl and water transport

3.1.1. NaCl and water content

Fig. 1 shows the NaCl content of loin samples during brining, which is also considered the NaCl gain, due to the fact that the NaCl content in meat samples was negligible. NaCl concentration in the brine influenced significantly on NaCl content (Fig. 2A). Thus, when using a brine solution of 50 kg NaCl/m^3, the NaCl content in the meat after 120 min of brining was almost four times lower than when using 280 kg NaCl/m^3. The NaCl gain is mainly linked to osmotic mechanisms, thus the hydrodynamic flux increases as the pressure gradients between the meat and brine get higher (Schmidt et al., 2008). Other factors, such as temperature, pH and muscle microstructure, can also affect the NaCl gain (Barat et al., 2006).
Ultrasound also significantly ($p<0.05$) influenced the NaCl gain (Fig. 2 B); as an example, for a brining time of 90 min and using the highest NaCl concentration (280 kg NaCl/m^3) (Fig. 1), the NaCl content in the CONTROL samples was 0.315±0.020 kg NaCl/kg initial d.m. while the content in US samples reached 0.359±0.033 kg NaCl/kg initial d.m. Among other phenomena, the US application in liquid media induces cavitation, temperature gradients within the material, alternative compression and decompression of the material, the

generation of microchannels and microstirring on interfaces, which are responsible for the increased gain in NaCl. Cárcel et al. (2007a) and Gabaldón-Leyva et al. (2007) also found significant differences ($p<0.05$) in the net increase of dry matter content during the ultrasound assisted osmotic treatment of apple and red bell pepper.

Regarding the moisture content, pork loin showed average initial moisture content of 3.10±0.12 kg water/kg initial d.m. As can be observed in Fig. 3, the concentration of NaCl in the brine solution was a key parameter in moisture transport, since it determined the direction of water flux. The ANOVA carried out with samples brined for 120 min reflected that samples could be classified in three significantly different groups according to the moisture content (Fig. 2 C). The first group included samples brined using 50 and 100 kg NaCl/m^3, which showed a significant ($p<0.05$) water gain. Samples brined using 150 and 200 kg NaCl/m^3 (the second group) neither lost nor gained water, having similar moisture content to raw meat. This result coincided with what was reported by Graiver et al. (2009) and Nguyen et al. (2010), who did not find a clear moisture transport when using brines close to 200 kg NaCl/m^3, either. The water activity of meat samples and the 200 kg NaCl/m^3 brine were 0.980±0.007 and 0.868±0.001 respectively (Aqua Lab Serie 3, Decagon Devices, Inc., USA). Therefore, the lack of net moisture transport at this concentration may not to be explained from a water activity point of view. Finally, the third group included samples brined at 240 and 280 kg NaCl/m^3, which underwent dehydration. During brining however, hydration or dehydration are not only affected by chemical potential gradient (Shi and Le Maguer, 2002) but also by structural changes brought about in the meat by salt gain (Schmidt et al., 2008). On the one side, the low NaCl content increases the muscle's WHC by protein solubilization, which is known, as aforementioned, the "salting-in" phenomenon (Offer and Trinick, 1983). On the other side, the high NaCl content reduces the WHC and meat muscle shrinks, which is the "salting-out" phenomenon (Graiver et al., 2006).

Regarding ultrasound effect on moisture content, US and CONTROL brined samples for 120 min did not show significant differences. (Fig. 2 D). This fact has been also been observed in ultrasound assisted brining of beef muscles (Pohlman et al., 1997; Jayasooriya et al. 2007) and pork meat (Siró et al. 2009). The negligible effect of ultrasound on water content could be linked to the great variability in moisture content of samples (Fig. 3). In addition, it should be considered that the ultrasound intensity may be not enough to provoke significant differences in water transport due to a minim amount of ultrasonic energy is necessary in the medium, and that threshold could be different for water and NaCl content. Cárcel et al., (2007 b) reported that in high intensity ultrasound fields, brine could be microinjected into the meat leading to a direct increase of NaCl and water content.

3.2. Modeling transport kinetics

The analysis of the experimental results has been focused on the final salt and moisture content (samples brined for 120 min). Modeling the experimental transport kinetics (Figs. 1 and 3) will help to identify whether the brining conditions (US application and/or NaCl concentration) affect the process rate. The fit of the models to experimental kinetics achieved percentages of explained variance (Table 1) for NaCl transport ranging from 93 to 99%. These values were lower for moisture transport (81.1 to 95.6%), which could indicate a poor fit in this case. However, as can be observed in Fig. 4, there exists a similar trend between calculated and experimental moisture contents, which highlights that the proposed diffusion model was adequate to describe the brining process. The low explained variance provided by the diffusion model in the moisture transport should mostly linked to the great variability of the initial composition of raw meat. Therefore, diffusion could be considered the predominant mass transport mechanism during brining.

The D_s values were similar for all the different brine concentrations tested; a lower figure was only found for all NaCl concentration of 50 kg NaCl/m^3, probably due to the structural changes in meat samples brought about by low salt gain. The effective NaCl diffusivities identified for the CONTROL samples agree closely with the values reported in the literature (Graiver et al., 2006; Vestergaard et al., 2007), which actually range between 2–4×10^{-10} m^2/s. Regarding moisture transport, the D_w values identified in experiments where meat was hydrated (NaCl concentration lower than 200 kg NaCl/m^3) decreased as the NaCl content rose. Thus, in CONTROL experiments using a brining solution of 50 kg NaCl/m^3, the D_w was 0.76x10^{-10} m^2/s, while D_w decreased to 0.17x10^{-10} m^2/s for experiments at 150 kg NaCl/m^3, where hydration was almost negligible. The same fact was also observed in US experiments and has also been previously reported by Gou et al., (2003), who found that the D_w decreased when the NaCl content of the salting solutions increased from 20 to 80 kg NaCl/m^3. On the other hand, the D_w values were higher when meat was dehydrated (NaCl concentration higher than 200 kg NaCl/m^3) than when meat was hydrated (NaCl concentration lower than 200 kg NaCl/m^3) (Table 2). It must be clarified that the model was not fit to the experimental data of moisture content obtained for the 200 kg NaCl/m^3 brine due to no net moisture transport was observed. For this reason, the diffusivity value is not included in Table 2 at this experimental conditions. These differences could be ascribed to the different product structure induced and controlled by NaCl transport (Schmidt et al., 2008; Gou et al., 2003; Offer and Trinick, 1983). Therefore, the NaCl concentration in the brining solution is not only affecting the direction of water flux (hydration or dehydration) but also the water transport rate as a consequence of the structural changes brought about by the NaCl content in the meat.

Ultrasound application led to a significant ($p<0.05$) improvement in both D_s and D_w, which points to an acceleration of both the global bring process. The increase in D_s ranged from 23 to 45% and is in a similar range to other improvements reported for solid transport in the literature. Thus, Siró et al. (2009) found increases of 96% in D_s meat brining and Gabaldón-Leyva et al. (2007) stated an improvement of 190% in the total solid diffusion coefficients. In the case of D_w, the improvement was higher than in D_s for the lowest and highest NaCl concentrations used (50 and 280 kg NaCl/m^3) (Table 1), being in this case the improvement close to 100%. Gabaldón-Leyva et al., 2007 and Cárcel et al., 2007a reported increases in D_w of around 128 and 117% when ultrasound was applied in bell pepper brining and osmotic dehydration of apple. Smaller increases were observed in the D_w values for intermediate NaCl concentration brines tested, these being 76% and 41% for 100 and 150 kg NaCl/m^3, respectively. The different effectiveness of ultrasound application, depending on brine NaCl concentration, could be explained considering that the ultrasound effects on mass transport are largely dependent on product structure (Gabaldón-Leyva et al., 2007).

Finally, it should be remarked that, as stated before, there was not a significant ($p<0.05$) difference on the moisture content at the end of the brining process (120 min) for CONTROL and US samples, however, the analysis of transport kinetics showed an improvement on the moisture transport rate by ultrasound. The effective moisture diffusivities identified from experimental results are kinetic parameters that not only include diffusion mechanisms but also other existing phenomena not considered in the model, such as external mass transport. Ultrasound may affect both internal mass transport resistance, by alternating cycles of expansions and contractions ("sponge effect") and the generation of microchannels, and external by microstirring at the interfaces (Muralidhara et al., 1985, Cárcel et al., 2007b). These effects that US induce into the medium are responsible for the kinetic improvement.

3.3. Texture

In order to study the influence of ultrasound application and NaCl concentration on meat texture, instrumental penetration tests were carried out in raw material, US (40 kHz; 37.5

W/dm^3) and CONTROL samples brined for 120 min using NaCl concentrations of 50, 200 and 280 kg NaCl/m^3. At least 12 points were measured in each meat slice.

Raw material showed a hardness of 1.60 ± 0.49 N. With regard to brined samples, the hardness was dependent on the salting conditions used. Thus, CONTROL samples brined at 200 and 280 kg NaCl/m^3 were significantly ($p<0.05$) harder than those brined at 50 kg NaCl/m^3 (Table 3). As aforementioned, the higher the NaCl concentration in the brine, the greater the NaCl gain. In such a way, the NaCl gain promoted changes in meat texture, leading to harder samples, a fact already showed by Ruiz-Ramírez et al. (2005). Non-significant differences ($p<0.05$) in hardness were found in samples brined at 200 and 280 kg NaCl/m^3 (Table 3). This indicates that the level of NaCl gained by both samples was enough to produce the same change in meat texture. The effect of the NaCl concentration in US samples was similar to that reported in CONTROL ones.

On the other hand, the application of ultrasound during brining significantly increased ($p<0.05$) the meat hardness. As already explained, ultrasound application intensified NaCl transport during brining, increasing not only the NaCl diffusivity but also the final NaCl content (brining time 120 min, CONTROL 0.326±0.007 kg NaCl/kg initial d.m. and US 0.409.29±0.11 kg NaCl/kg initial d.m.). So, the effects of ultrasound on meat texture could be linked to the intensification of NaCl transport, which provoked structural changes in meat proteins. Lee and Feng (2011) reported that the texture of ultrasound-treated food is influenced by protein changes during sonication, as well as Siró et al., (2009), who linked the hardening of meat tissue to the high ultrasonic intensities applied. Sanchez et al., (2001) showed that US application in the brining of Mahon cheese increased the sample hardness due to the improvement of proteolysis and lipolysis reactions.

Raw and brined CONTROL and US pork loin previously characterized from instrumental texture were studied by Cryo-SEM and SEM techniques in order to contribute to a better understanding of the effects produced by ultrasound application, being this fact analyzed in the following section.

3.4. Microstructure

3.4.1. Cryo-scanning electron microscopy (Cryo-SEM)

First of all the microstructure of raw pork loin was characterized. Fig. 5A shows a cross section of the raw pork loin, where cells are surrounded by the membrane or sarcolemma (Fig. 5A, S). These cells are interconnected by endomysial connective tissue (Fig. 5A, EC), which keeps the muscle fibers tightly attached. In addition, the myofibrils inside the muscle cell can be observed, which are fundamental components of the cell's contractile apparatus (Fig. 5A, M).

Intercellular spaces appear full of typical eutectic artifacts in brined samples (Figs. 5B, C and D) due to solute aggregation after water sublimation provoked by Cryo-SEM technique (Pérez-Munuera et al., 2008). The accumulation of solutes in the intercellular spaces can be linked to both the penetration of the NaCl and the strong solubilization and dehydration in muscle tissue. The greater the brining NaCl concentration, the greater the solute accumulation and the more compact the eutectic artifacts (Larrea et al., 2007). The bundles of muscle fibers in CONTROL samples brined at 280 kg $NaCl/m^3$ (Fig. 5D) seem to be more compact, showing the more intense sample dehydration linked to the "salting-out" phenomenon. The denaturation and precipitation of proteins involves progressive structural shrinkage and less space for water (Vestergaard et al., 2005). These effects are widely related with the hardening observed in the textural analysis of meat brined at the highest NaCl concentration (280 kg $NaCl/m^3$) (Table 3). In samples brined at the lowest NaCl concentration (50 kg $NaCl/m^3$, Fig. 5B), however, may be observed an expansion of myofibrils coupled with the protein solubilization that is related with the "salting-in" phenomena (Graiver et al., 2006). Some authors have already reported that an increase in water binding and hydration in the muscle fibers of brined meat at low brine concentrations (< 50 kg NaCl/m^3) is ascribed to enhanced electrostatic repulsion between myofibril filaments causing the filament lattice to expand for water entrapment (Graiver et al., 2009; Cheng and Sun, 2008). These phenomena in meat proteins can explain the hydration of samples brined at low NaCl concentrations (Fig. 3) and the meat softening observed in the textural analysis (Table 3).

Samples brined with 200 kg NaCl/m^3 (Fig. 5C), showed a mixed effect. Some parts of the sample show structural dehydration due to a high NaCl concentration (Fig. 5C, 1), whereas in other parts of the sample, the opposite phenomena (hydration) can be observed in the myofibrillar structure (Fig. 5C, 2). As mentioned before (Fig. 3), no net transport of moisture was identified at 200 kg NaCl/m^3, which can be linked to the combined effect of hydration-dehydration observed in the meat microstructure (Fig. 5C).

From micrographs obtained with Cryo-SEM, no effects of ultrasound in the meat structure were found. Thus, the Cryo-SEM microstructural analysis was completed with SEM observations.

1.1.1. Scanning electron microscopy (SEM) with combined dispersion X-ray analysis (SEM-EDX).

CONTROL samples brined with the lowest NaCl concentration tested (50 kg NaCl/m^3, Fig. 6B) showed a swelling of muscle fibers (width 87.6±7.3 μm) compared to raw meat (width 72.5±9.1 μm) (Fig. 6A). The swelling of muscle fibers could be mainly ascribed to the "salting-in" phenomena. Samples brined at higher NaCl concentrations (280 kg NaCl/m^3 kg behaved in the opposite way and presented a dehydration of muscle fibers and an accumulation of NaCl; these effects may be observed in Figs. 7A, C, E.

The SEM technique also allowed the effect brought about by high intensity ultrasound in meat structure to be observed, this effect mainly focusing on myofibrils (Figs. 7B, D, F). The micrograph of the US sample brined at 50 kg NaCl/m^3 (Fig. 7B) shows the disruption and the dispersion of the connective tissue of the fibers caused by US application. In Fig. 7F the rupture of a myofibril provoked by the acoustic energy was identified. The aforementioned myofibrillar changes could be explained by the alternating compressions and decompressions induced by US in solid materials ("sponge effect"). Another important effect produced by high intensity ultrasound in liquid media is cavitation, which may be observed in Fig. 7D, where the erosion of meat fiber produced by cavitation is shown. The asymmetric implosion of bubbles near the solid surface could produce violent microjets that collide with the samples, which can improve mass transfer by disturbing the boundary layer and producing changes in the meat structure. These results coincide with those found by several authors that related the application of high power ultrasound with the physical disruption of cellular and sub-cellular components (Reynolds et al., 1978), the degradation of collagen macromolecules (Nishira and Doty, 1958), and the creation of micro channels (Muralidhara et al., 1985).

The obtained SEM-EDX mapping images confirmed the presence of NaCl in meat after brining, which is shown up by the red (Cl^-) and green (Na^+) dots in the micrographs. As can be observed in Fig. 8, the NaCl concentration in US samples (Figs. 8B, D and F) was higher than those observed in CONTROL samples, as manifested by a higher number of dots in the micrographs (Figs. 8A, C and E). In CONTROL samples (Figs. 8A, C and E), NaCl molecules are mainly located around the myofibrils. However, the US brined samples (Figs. 8B, D and F) showed a more homogeneous NaCl dispersion due to the collapse of myofibrillar structure caused by the effects of high power ultrasound, permitting a higher NaCl penetration in the meat. The obtained SEM-EDX images showed the intensification of

NaCl transport brought about by US application and the increase in NaCl content, which confirms the results provided by modeling and textural tools.

Therefore, ultrasound application could improve the brining process by reducing the brining time, involving a faster and more uniform distribution of sodium chloride. In this sense, this technology could be an interesting alternative to reduce NaCl levels in dry-cured meat foods.

4. Conclusions

The NaCl concentration in the brine solution significantly ($p<0.05$) affected moisture and NaCl transport during meat brining. At NaCl concentrations lower than 200 kg NaCl/m^3, the meat was hydrated while, dehydration took place at concentrations higher than 200 kg NaCl/m^3. As for the NaCl transport, the more concentrated the brine, the greater the NaCl sample gain. Ultrasound application intensified the brining kinetics, increasing both moisture effective and NaCl diffusivities. The NaCl gain promoted changes in meat texture, high NaCl contents leading to harder samples. Microstructural analyses showed that the application of high intensity ultrasound during brining brought about relevant effects on meat microstructure, such as a more homogeneous NaCl distribution in meat. Therefore, ultrasound could be considered a potential technology with which to accelerate the brining process.

Acknowledgments

This work is financed by project CARNISENUSA (CSD2007-00016) included in the CONSOLIDER-INGENIO-2010.

Nomenclature

W_{eq}	Equilibrium moisture content, kg water/kg initial d.m.
W_0	Initial moisture content, kg water/kg initial d.m.
S_{eq}	Equilibrium sodium chloride content, kg NaCl/kg initial d.m.
S_0	Initial sodium chloride content, kg NaCl/kg initial d.m.
D_S	Effective NaCl diffusivity (m^2/s)
D_W	Effective moisture diffusivity (m^2/s)
L	Half length, m

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Figure captions

Fig 1. Experimental NaCl transport kinetics in pork loin slices (thickness 10 mm) brined at different NaCl concentrations with (US, 40 kHz, 37.5 W/dm^3) and without ultrasound application (CONTROL). In this figure, each point represents the average of 9 measurements: three independently brined samples analyzed in triplicate. Average ± standard deviation being plotted.

Fig 2. Least Significant Difference intervals ($p<0.05$) for moisture and NaCl contents of pork loin slices (thickness 10 mm) brined at 5±1 ºC for 120 min with different brine concentrations with (US, 40 kHz, 37.5 W/dm^3) and without ultrasound application (CONTROL). Dotted line (---) represents the average initial moisture content of pork loin.

Fig 3. Experimental moisture transport kinetics in pork loin slices (thickness 10 mm) brined at different NaCl concentrations with (US, 40 kHz, 37.5 W/dm^3) and without ultrasound application (CONTROL). In this figure, each point represents the average of 9 measurements: three independently brined samples analyzed in triplicate. Average ± standard deviation being plotted.

Fig 4. Experimental vs calculated moisture content of pork loin slices (thickness 10 mm) brined with (US, 40 kHz, 37.5 W/dm^3) and without (CONTROL) ultrasound application.

Fig 5. Cross-section of *Longissimus dorsi* muscle of pork meat observed by Cryo-SEM (x 750). Raw meat (A), meat brined for 120 min in 50 kg NaCl/m^3 (B), 200 kg NaCl/m^3 (D) and 280 kg NaCl/m^3. S: sarcolemma, EC: endomysial connective tissue, M: myofibrills, 1: “salting-out effects”, 2: “salting-in effects”.

Fig 6. Longitudinal section of *Longissimus dorsi* muscle of pork meat observed by SEM (x500). Raw meat (A) and meat brined in 50 kg NaCl/m^3 for 120 min (B).

Fig 7. Longitudinal section observed by SEM of *Longissimus dorsi* muscle of pork meat brined for 120 min in 50 kg NaCl/m^3 (A,B: x1500), 200 kg NaCl/m^3 (C,D: x150) and 280 kg NaCl/m^3 solution (D, E: x750) without (CONTROL: A, C and E) and with (US: B, D and E) ultrasound application (40 kHz, 37.5 W/dm^3). CV: impact of cavitation buble implosion on myofibrill.

Fig 8. Effect of ultrasound application on NaCl dispersion. Longitudinal section observed by SEM-EDX of *Longissimus dorsi* of pork meat brined for 120 min in 50 kg NaCl/m^3 (A,B: x500), 200 kg NaCl/m^3 (C,D: x500) and 280 kg NaCl/m^3 (D,E: x150) without (CONTROL: A, C and E) and with (US: B, D and E) ultrasound application (40 kHz, 37.5 W/dm^3).

Figure 1

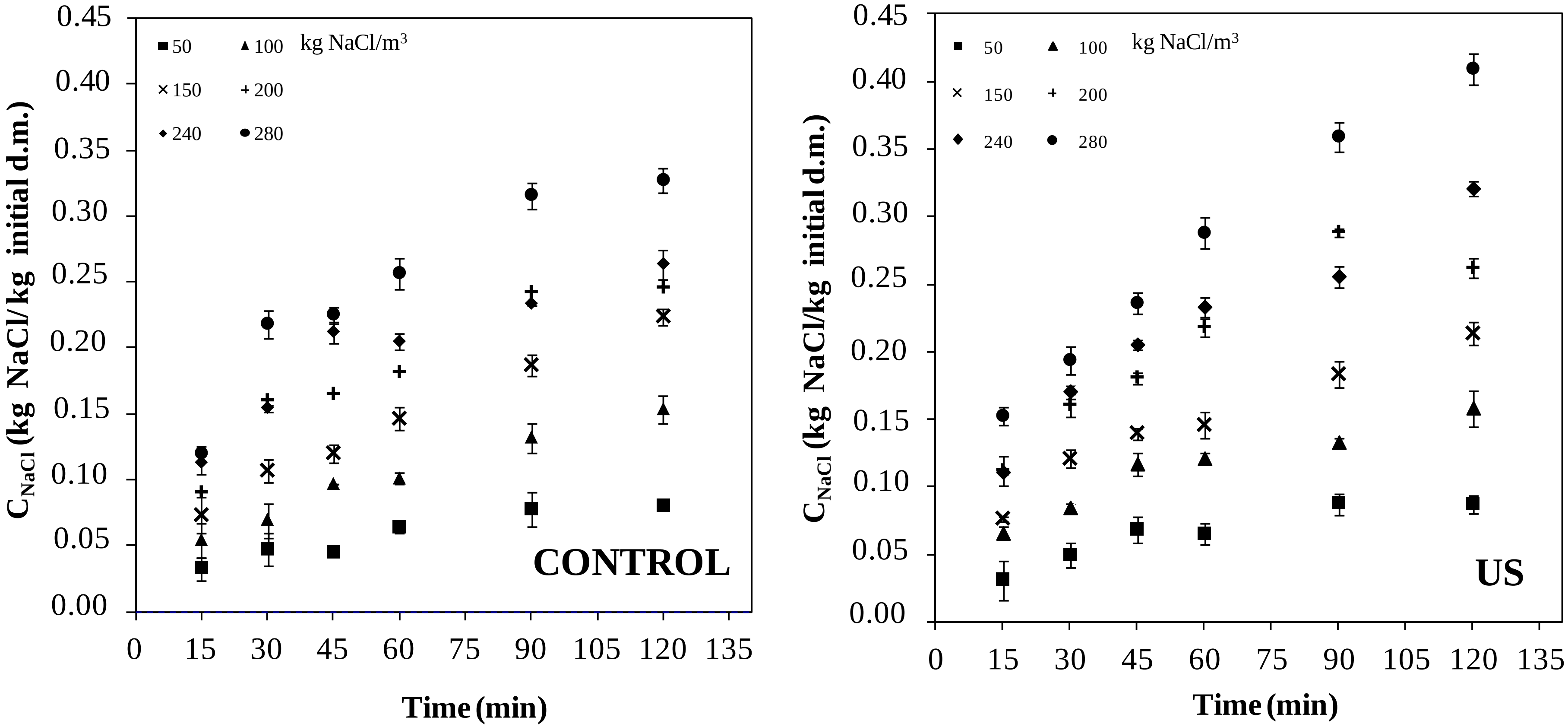

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Figure 2

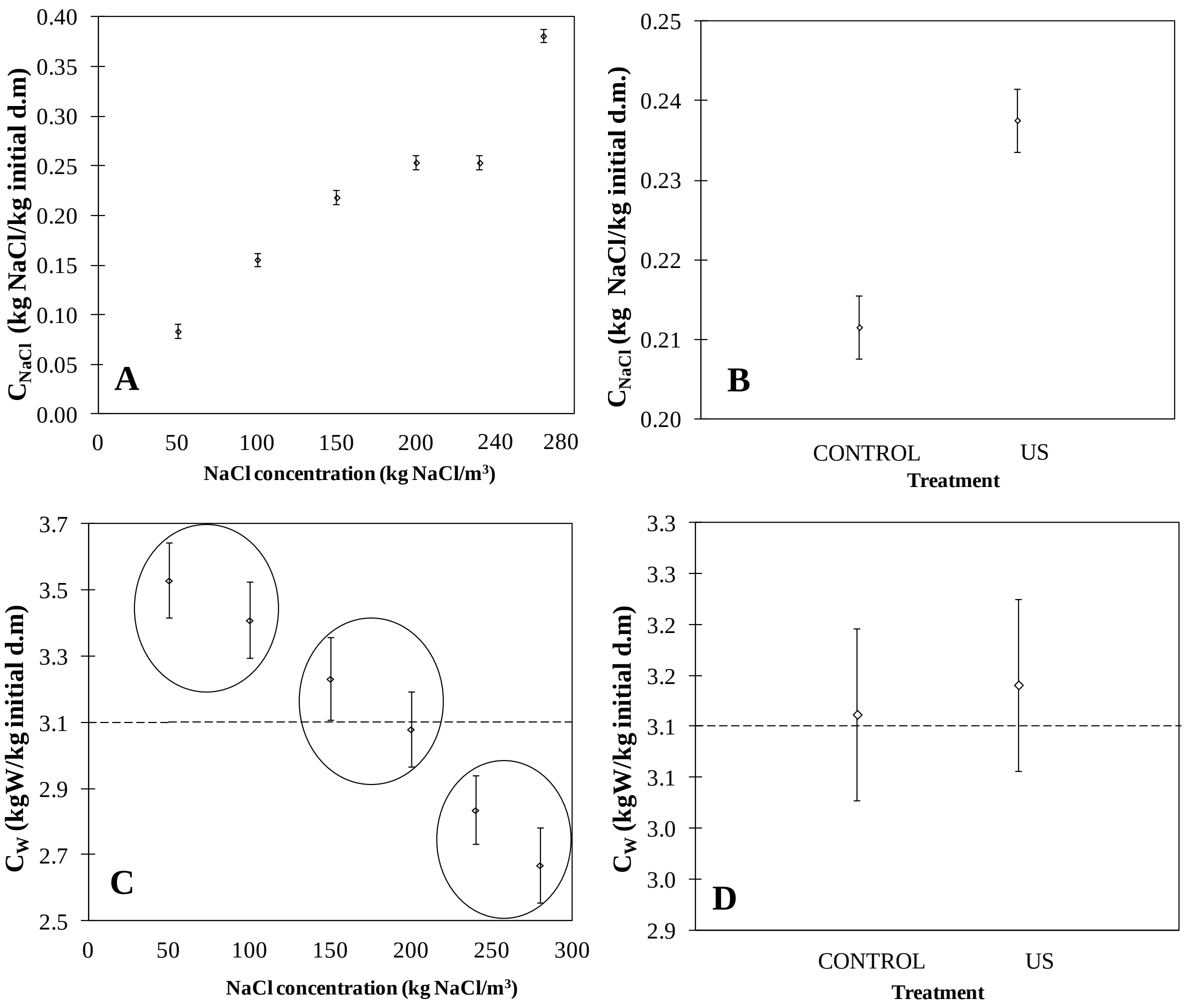

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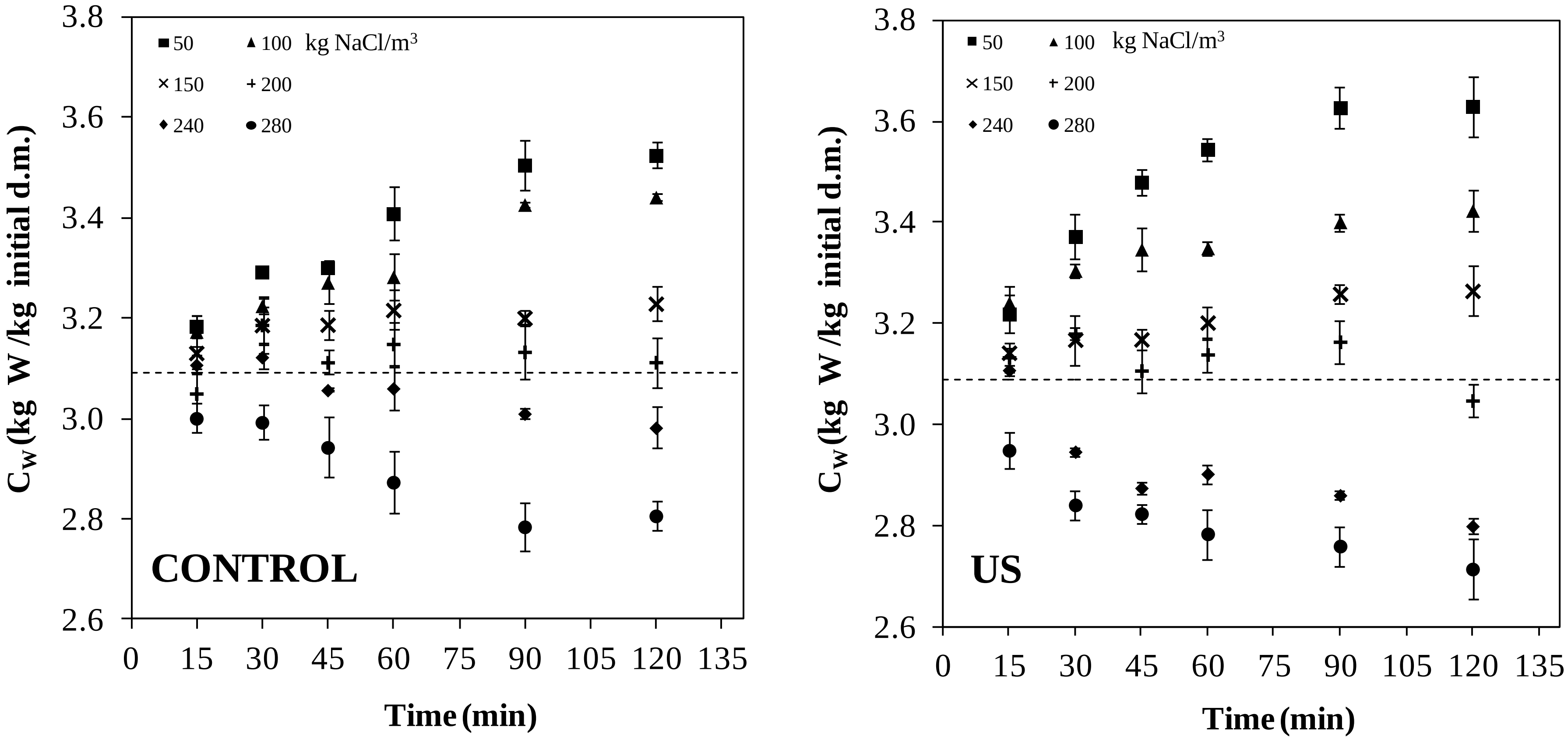
Figure 3



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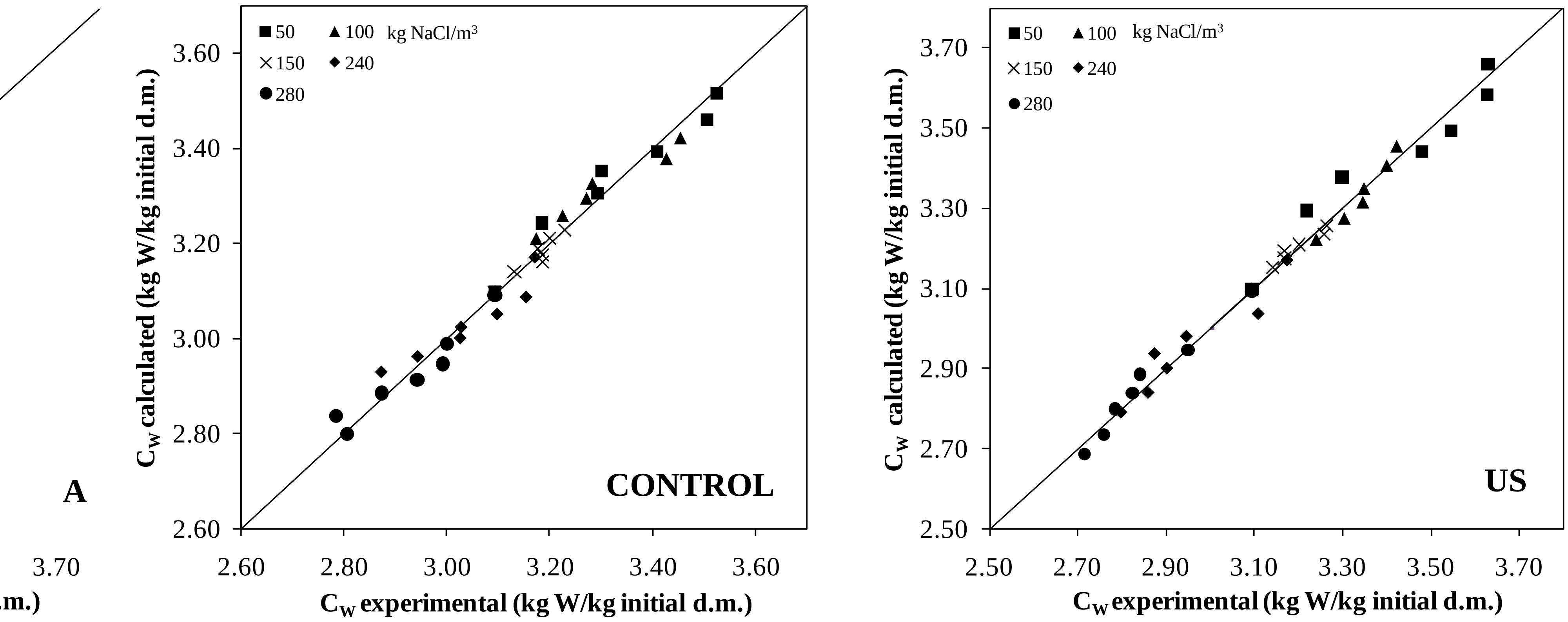

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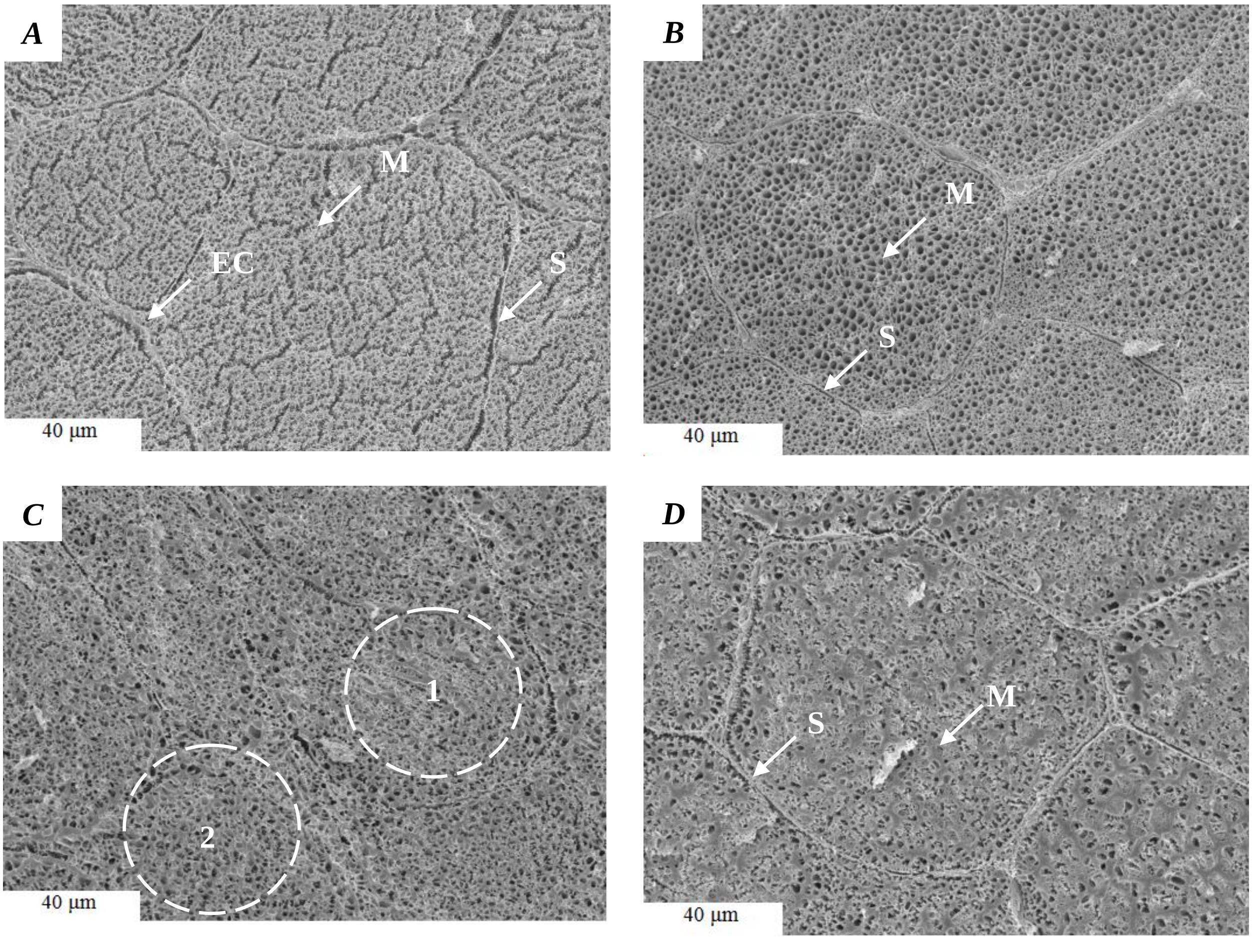
Figure 5



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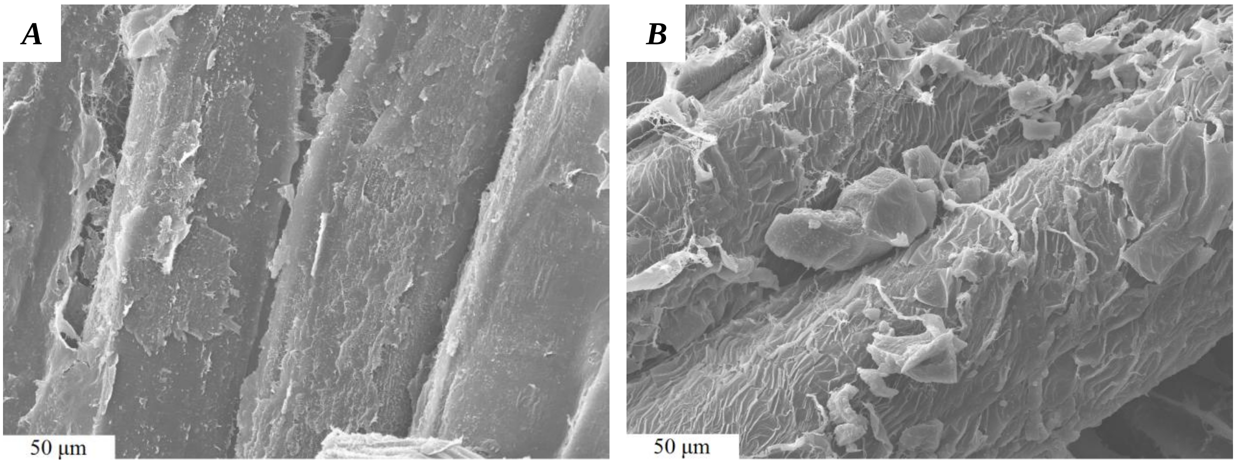

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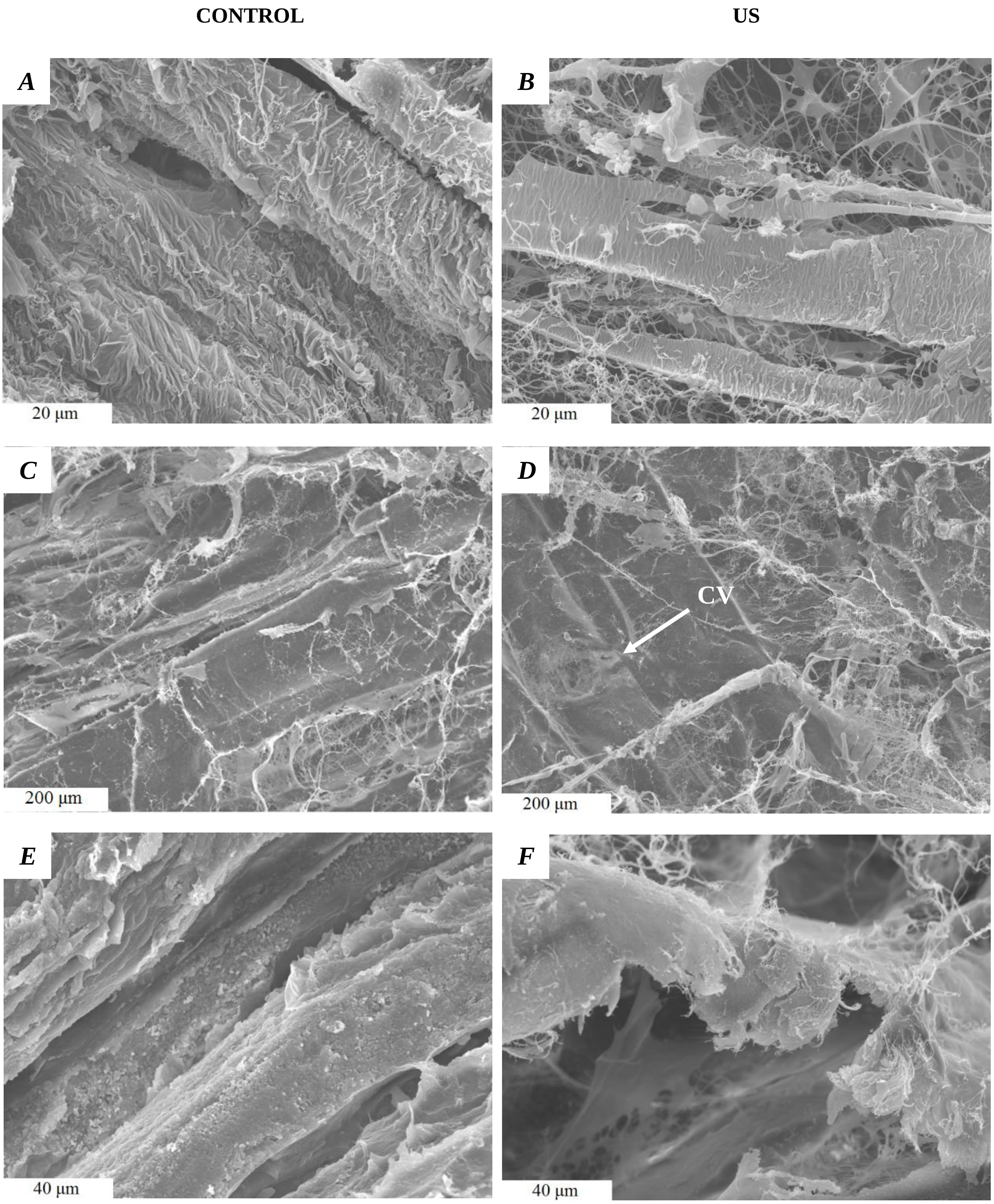

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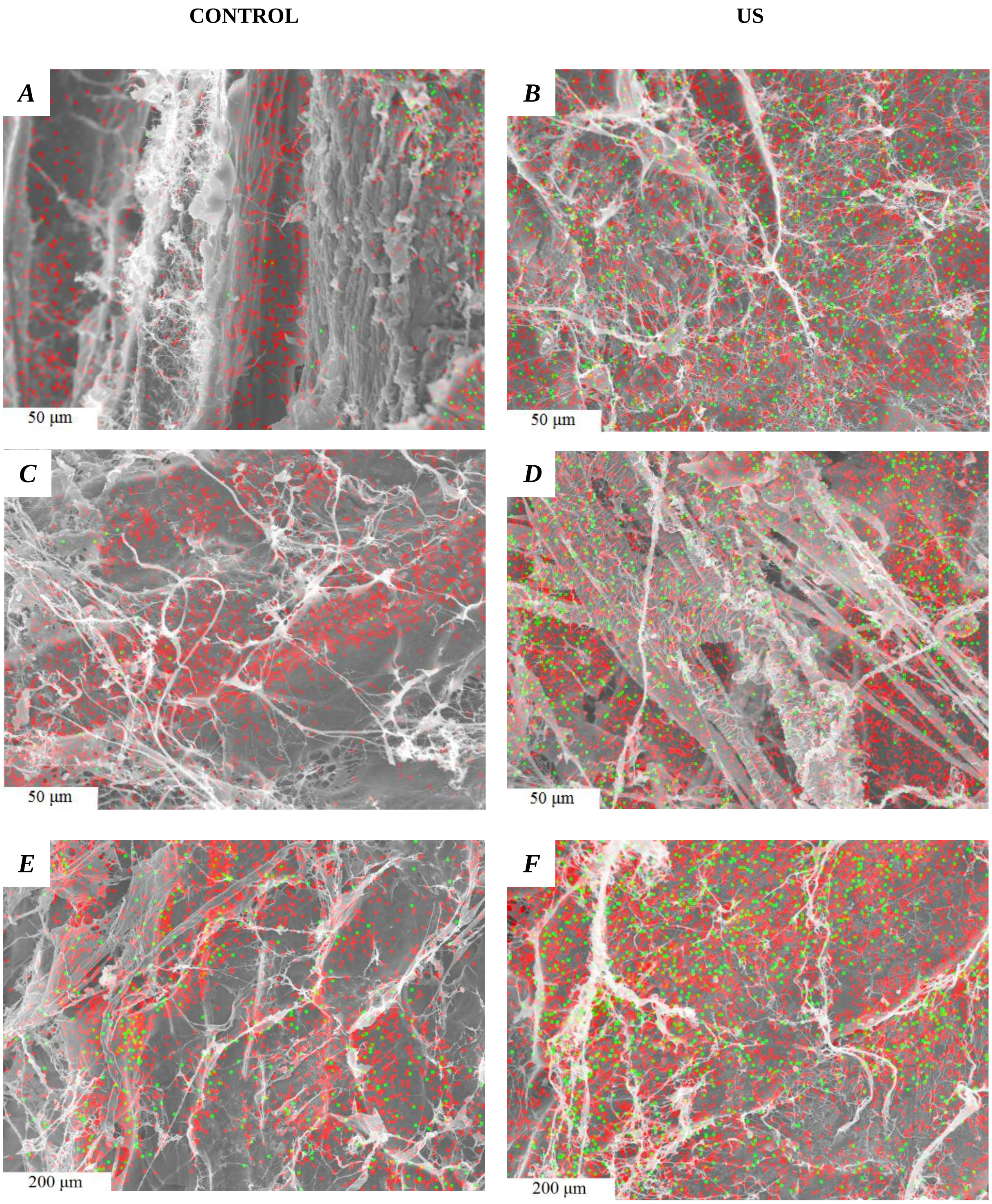

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Table captions

Table 1. Modeling NaCl transport in pork meat brining (5±1 ºC) at different NaCl concentrations with (US, 40 kHz, 37.5 W/dm^3) and without (CONTROL) ultrasound application. Effective diffusivity and percentage of explained variance. Average ± confidence intervals of the estimation (significance level 95%) are shown.

Table 2. Modeling moisture transport in pork meat brining (5±1 ºC) at different NaCl concentrations with (US, 40 kHz, 37.5 W/dm^3) and without (CONTROL) ultrasound application. Effective diffusivity and percentage of explained variance. Average ± confidence intervals of the estimation (significance level 95%) are shown.

Table 3. Hardness (N) of pork meat brined for 120 min in different NaCl concentrations with (US, 40 kHz, 37.5 W/dm^3) and without (CONTROL) ultrasound application. Average ± standard deviations are shown. Subscripts (a,b,c,d) show homogeneous group established from LSD intervals ($p<0.05$).

Tables

Table 1

NaCl concentration (kg/m^3)	CONTROL D_s (10^{-10} m^2/s)	CONTROL % VAR	US D_s (10^{-10} m^2/s)	US % VAR	Increment (%)
50	1.24±0.11	98.1	1.73±0.51	97.5	40
100	2.04±0.16	99.2	2.93±0.54	96.6	44
150	2.11±0.19	99.0	2.60±0.24	98.9	23
200	1.99±0.32	96.4	2.68±0.51	95.1	35
240	1.73±0.18	96.1	2.51±0.52	93.8	45
280	1.96±0.32	96.1	2.62±0.16	99.5	34

Table 2

NaCl concentration (kg/m^3)	CONTROL D_W (10^{-10} m^2/s)	CONTROL % VAR	US D_W (10^{-10} m^2/s)	US % VAR	Increment (%)
50	0.76±0.21	90.7	1.92±0.27	91.6	153
100	0.62±0.19	90.2	1.09±0.14	94.7	76
150	0.17±0.04	89.1	0.24±0.07	92.2	41
200	-	-	-	-	-
240	1.04±0.52	81.1	2.60±0.83	88.8	150
280	1.83±0.57	90.0	3.67±0.68	95.6	101

Table 3

Treatment	Hardness (N) 50 kg $NaCl/m^3$	Hardness (N) 200 kg $NaCl/m^3$	Hardness (N) 280 kg $NaCl/m^3$
CONTROL	$1.27±0.39_{a}$	$1.78±0.60_{b,c}$	$1.69±0.70_{b,c}$
US	$1.65±0.52_{b,c}$	$1.94±0.46_{b,c,d}$	$2.08±0.66_{d}$

ANÀLISI I SIMUL·LACIÓ
DE PROCESSOS AGROALIMENTARIS

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Dr. R. P. Singh
Editor in chief of Journal of Food Engineering

Valencia, October 30, 2012

Dear Editor,

We would like to submit our manuscript "INFLUENCE OF HIGH INTENSITY ULTRASOUND APPLICATION ON MASS TRANSPORT, MICROSTRUCTURE AND TEXTURAL PROPERTIES OF PORK MEAT (*Longissimus dorsi*) BRINED AT DIFFERENT NaCl CONCENTRATIONS", for your consideration for publication in Journal of Food Engineering, the corresponding author being:

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The manuscript is addressing the application of ultrasound at an important stage in industrial meat processing, the brining process. The influence of high intensity ultrasound application on water and salt transport during meat brining using brine solutions with different NaCl concentrations have been studied. The experimental work has been thoroughly designed and conducted and is appropriate to reach the objectives proposed. The ultrasound effects on water and salt transport kinetics are quantified using mechanistic-diffusion models. Moreover, the meat structure and microstructure changes brought about by ultrasound application have been observed from instrumental texture analysis and Cryo-SEM and SEM techniques. In addition, the structure study has been linked to the kinetic one in order to explain how ultrasound may affect the mass transport during brining. Therefore, we believe the manuscript is an interesting and relevant piece of work useful for authors that are going to deal with ultrasonic applications in mass transfer in food or chemical processing. For this reason, we believe the paper's relevance is well demonstrated and would like to submit it for your consideration. We thank you for your time and interest in our research and look forward to hearing from you.

Yours sincerely

César Ozuna, Ana Puig, Jose V. García-Pérez, Antonio Mulet and Juan A. Cárcel

Authors of the manuscript

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	NaCl concentration (kg/m^3) 50	NaCl concentration (kg/m^3) 200	NaCl concentration (kg/m^3) 280
CONTROL	$1.27\pm0.39_{a}$	$1.78\pm0.60_{b,c}$	$1.69\pm0.70_{b,c}$
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Influence of High Intensity Ultrasound Application on Mass Transport, Microstructure and Textural Properties of Pork Meat (*Longissimus dorsi*) Brined at Different NaCl Concentrations.

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ABSTRACT

The aim of this work was to evaluate the effect of high intensity ultrasound and NaCl concentration on the brining kinetics (5±1 ºC) of pork loin as well as its influence on the textural and microstructural changes. In order to identify the effect of both factors on NaCl and moisture transport, kinetics were analyzed by taking the diffusion theory into account. The textural and microstructural analysis of raw and brined meat both with and without ultrasound application was carried out. The experimental results showed that the brine NaCl concentration not only determined the final NaCl content in meat samples but also the direction of water transport. The NaCl and moisture effective diffusivities were improved by ultrasound application. The final NaCl and moisture content and the ultrasound application promoted changes in instrumentally measured meat texture, which were confirmed via microstructural observations.

Keywords: mass transfer, power ultrasound, modeling, diffusivity, texture, Cryo-SEM, SEM.

1. Introduction

Over 1.2 million tons of meat products produced per year makes Spain the fourth most important country in the European Union in this regard, being the dry-cured products the most valuable ones in the Spanish meat industry (AICE, 2011). One of the main stages during the processing of dry-cured products is salting. Actually, this operation is mainly carried out by salting the meat pieces with solid salts (NaCl), but it could also be performed by brining (Barat et al., 2006). Compared with other food processes, brining is a slow process and, for that reason, the food industry is searching for alternative technologies for improving the mass transfer kinetics (Rastogi et al., 2002), such as high intensity ultrasound application (Cárcel et al., 2007a).

In liquid medium, ultrasound induces cavitation (Leighton, 1998), temperature gradients within the material (Mason and Lorimer, 2002), mechanical phenomena, such as the "sponge effect", generation of microchannels and microstirring on interfaces (Muralidhara et al., 1985). These effects can not only increase the mass transport kinetics but also imply structural changes and, consequently, changes in textural properties. Thereby, textural changes induced by ultrasound have been observed in, among others, tomato juice (Vercet et al., 2002), yoghurt (Wu et al., 2000), bell peppers (Gabaldón-Leyva et al. 2007) or meat (Jayasooriya et al., 2007; Pohlman et al., 1997).

Meat has great biochemical and structural complexity. During meat brining, changes in NaCl and moisture content take place (Graiver et al., 2006), which extend meat's shelf life and modify organoleptic characteristics, such as juiciness, texture and flavor. Mass transfer driving forces between meat and brine are linked to chemical gradients and NaCl-induced changes in the water holding capacity (WHC) of meat proteins (Shi and Le Maguer, 2002; Vestergaard et al., 2005). The NaCl concentration in brine affects the direction of moisture transport as well as the equilibrium state (Cheng and Sun, 2008). A low NaCl content in the meat increases the water holding capacity (WHC) (Nguyen et al., 2010), a phenomenon known as "salting-in" which is linked to the meat protein net charge modification. However, a high NaCl content in meat could also bring about a decrease in WHC, probably due to the insolubilization of proteins (“salting-out”) (Graiver et al., 2009). Therefore, the NaCl concentration in the brine solution can not only affect the chemical gradients, but also the WHC, thus affecting the magnitude of mass transport.

Modeling is a fundamental tool to quantify the mass transport (Cárcel et al., 2007a), as well as to evaluate the effectiveness on that of new technologies, such as ultrasound. But in addition, modeling also provides relevant information to understand the changes undergone by foodstuffs during processing, which usefulness complements the information given by other techniques, such as textural and microstructural ones (Pérez-Munuera et al., 2008). Thereby, different electron microscopy techniques, Larrea et al. (2007) have been used to characterize the microstructure of *Biceps femoris* and *Semimebranousus* muscles during the processing of Teruel dry-cured ham. Ruiz-Ramírez et al. (2005) described the effect of NaCl and pH on the relationship between water content and textural parameters in dry-cured muscles.

The main aim of this work was to evaluate the influence of high intensity ultrasound application on the meat brining kinetics using different NaCl concentrations in the brine solution. Moreover, the ultrasonic effects in transport phenomena have been quantified by modeling and linked to the induced changes in microstructural and textural meat properties.

2. Materials and methods

2.1. Raw material and sample preparation

Fresh pork loins (*Longissimus dorsi)* were purchased at a local slaughterhouse (Valencia, Spain). The pieces selected had a pH of 5.3 ± 0.30, which was measured in-situ by means of a pH-meter (pH STAR, Matthäus, Germany) at three different points along the muscle avoiding fatty areas. Parallelepiped shaped samples (length 50 x width 30 x thickness 10 mm) were obtained from the central part of loin pieces using a sharp knife. Before brining, samples were wrapped in plastic waterproof film and kept frozen at -18 ± 0.5 ºC (maximum storage time 120 hours).

2.2. Brining treatments

Brining experiments with (US, 40 kHz; 37.5 W/dm^3) and without ultrasound application (CONTROL) were carried out using brine solutions of different NaCl concentrations (50, 100, 150, 200, 240 and 280 kg NaCl/m^3). The highest NaCl concentration used (280 kg/m^3) involved brine saturation at 5±1 ºC, which was pointed to by the presence of NaCl crystals in the solution.

Brining treatments were carried out in an ultrasonic cleaning bath (4 L, 40 kHz; 150 W, Selecta, Spain) where the temperature was held at 5±1 ºC by brine recirculation from a cooling reservoir. A peristaltic pump (302S Limited, Watson/Marlow, United Kingdom) drove the brine from the cooling reservoir equipped with a chiller (3000778, J.P. Selecta, Spain) and a mechanical stirrer (RZR 1, Heidolph Instruments, Germany).

Before each brining experiment, 6 meat samples were thawed at constant temperature (2±1 ºC) for 24 h. Then, the samples were blotted, weighed (PB3002-S/PH, J.P., Mettler Toledo, Spain) and their size was measured by using a Vernier caliper. Afterwards, they were placed in a hollow sample holder and simultaneously introduced in the brine. For a homogenous brining, the position of the meat samples was changed every 5 min. Samples were taken out of the brine at preset times (15, 30, 45, 60, 90 and 120 minutes) and immersed in distilled water for 20 s to remove any adhered surface brine. Finally, samples were blotted, wrapped in plastic waterproof film and frozen (-18 ± 0.5 ºC) until moisture and NaCl measurements were taken.

After brining, moisture and NaCl content were measured from ground meat (at 300 r.p.m., Blixer® 2, Robot coupe, France). The moisture content was determined following AOAC standards (Method No. 950.46 AOAC, 1997). While in the case of NaCl, the procedure reported by Cárcel et al. (2007b) was used. Both measurements were carried out in triplicate at least.

2.3.Mass transfer modeling

A mathematical model based on Fick's 2nd law was used to separately describe the evolution of moisture and NaCl content in the sample during brining (Cranck 1975). Samples were considered to become slab geometry bodies due to the fact that they were not nearly as thick (10 mm) as they were high (50 mm) and wide (30 mm), thus, mass transfer was simplified as a one-dimensional problem. Constant effective diffusivities (D_s and D_w), negligible changes in temperature and sample volume, solid symmetry, homogeneous NaCl and moisture initial content and negligible external resistance (Gou et al., 2003) were assumed during processing. Eqs. 1 and 2 show the solution of the diffusion model in terms of average moisture and NaCl content.

$$W = W_{eq} + (W_0 - W_{eq})\left[2\sum_{n=0}^{\infty}\frac{1}{\lambda_n^2 L^2}e^{-D_W \lambda_n^2 t}\right] \qquad \text{where, } \lambda_n L = (2n+1)\frac{\pi}{2} \qquad (1)$$

$$S = S_{eq} + \left(S_0 - S_{eq}\right)\left[2\sum_{n=0}^{\infty}\frac{1}{\lambda_n^2 L^2}e^{-D_S \lambda_n^2 t}\right] \qquad \text{where, } \lambda_n L = (2n+1)\frac{\pi}{2} \qquad (2)$$

Where the equilibrium moisture and NaCl content values (S_e, W_e) were determined by immersing meat samples in the different brine solutions for at least 48 h. From previous experiments, this time was considered to be long enough to achieve the equilibrium.

Both effective diffusivity values, D_s and D_w, were identified by separately fitting Eqs. 1 and 2 to moisture and NaCl transport kinetics. The identification was performed by minimizing the squared differences between the experimental and calculated average sample moisture and NaCl content. For that purpose, the Generalized Reduced Gradient (GRG) optimization method, available in the Microsoft Excel spreadsheet from Microsoft Office XP Professional, was used.

2.4. Textural and microstructural analysis

Meat texture and microstructure were studied in US and CONTROL brined samples for 120 minutes using low, intermediate and high NaCl concentrations (50, 200 and 280 kg NaCl/m^3, respectively). Samples were brined in triplicate at least.

2.4.1. Texture

Hardness, characterized as maximum penetration force, was evaluated in brined samples using a Texture Analyzer (TAX-T2®, Stable Micro System, United Kingdom). Penetration tests were conducted with a 2 mm flat cylinder probe (SMS P/2N), a crosshead speed of 1 mm/s and a strain of 60 % (penetration distance 6 mm). In each meat slice, penetration tests were carried out at 12 points at least.

2.4.2. Cryo-scanning electron microscopy (Cryo-SEM)

Cubic samples (side 3 mm) of raw and brined meat were immersed in slush Nitrogen (-210 ºC), and quickly transferred to a cryo-trans (CT 15000 C, Oxford Instruments, England) linked to a scanning electron microscope (JSM-5410, Jeol, Japan). Samples were cryo-fractured at -180 ºC, etched at -90 ºC and gold-coated, allowing cross-section visualization.

The microscopic observations were carried out at 10 kV, a working distance of 15 mm and a temperature below -130 ºC.

2.4.3. Scanning electron microscopy (SEM) with combined dispersion X-ray analysis (SEM-EDX).

Cubic samples (side 3 mm) from raw and brined meat were immersed in liquid N_2 and then freeze-dried at 1 Pa for 3 days (LIOALFA-6, Telstar, Spain). The fixed samples were vacuum sealed in vials in the same freeze-drier so that they would remain stable (Llorca et al., 2001). The fixed samples were individually placed on SEM slides with the aid of colloidal silver and then gold-coated with (SCD005, Baltec, Germany) at 10^{-2} Pa and an ionization current of 40 mA. The samples were observed in a scanning electron microscope (JSM-5410, Jeol, Japan) equipped with an X-ray detector and LINK data-processing system (INCA 4.09, Oxford Instruments, England) at an acceleration voltage of 10-20 kV which provides internal information about the standards of energy dispersive X-ray spectra of the elements analyzed (Na^+ and Cl^-). This technique is an analytical tool that allows the ions Cl^- and Na^+ inside the samples to be identified (Grote and George, 1984). For EDX (energy-dispersive X-ray) analysis, samples were carbon-coated (CEA035, Baltec, Germany). Mapping images of Cl^- and Na^+ distribution in meat samples were made using a voltage of 20 kV and at a working distance of 15 mm.

2.5. Fitting model evaluation and statistical analysis

In order to evaluate the ability of the models to fit the experimental data, the percentage of explained variance (%VAR) was computed (Eq. 3) (Cárcel et al., 2007a).

$$\%VAR = \left[1 - \frac{S_{tw}^2}{S_w^2}\right] x100 \qquad (3)$$

Where S^2_w and S^2_{tw} are the variance of the sample and the estimation, respectively.

Multifactor ANOVA and LSD (Least Significant Difference) intervals were estimated to perform a statistical evaluation of the influence of ultrasound application and NaCl concentration on the effective diffusivity and textural properties. The statistical analysis was carried out using the Statgraphics Centurion XVI software package (Statistical Graphics Corp., Herdorn, USA).

3. Results and discussion

3.1. NaCl and water transport

3.1.1. NaCl and water content

Fig. 1 shows the NaCl content of loin samples during brining, which is also considered the NaCl gain, due to the fact that the NaCl content in meat samples was negligible. NaCl concentration in the brine influenced significantly on NaCl content (Fig. 2A). Thus, when using a brine solution of 50 kg NaCl/m^3, the NaCl content in the meat (brining time 120 min) was almost four times lower than when using 280 kg NaCl/m^3. The NaCl gain is mainly linked to osmotic mechanisms, thus the hydrodynamic flux increases as the pressure gradients between the meat and brine get higher (Schmidt et al., 2008). Other factors, such as temperature, pH and muscle microstructure, can also affect the NaCl gain (Barat et al., 2006). Ultrasound also significantly ($p<0.05$) influenced the NaCl gain (Fig. 2 B); as an example, for a brining time of 90 min and using the highest NaCl concentration (280 kg NaCl/m^3), the NaCl content in the CONTROL samples was 0.315±0.020 kg NaCl/kg initial d.m. while the content in US samples reached 0.359±0.033 kg NaCl/kg initial d.m. (Fig. 1). Among other phenomena, the US application in liquid media induces cavitation, temperature gradients within the material, alternative compression and decompression of the material, the generation of microchannels and microstirring on interfaces, which are responsible for the increased gain in NaCl. Cárcel et al. (2007a) and Gabaldón-Leyva et al. (2007) also found significant differences ($p<0.05$) in the net increase of dry matter content during the ultrasound assisted osmotic treatment of apple and red bell pepper.

Regarding the moisture content, pork loin showed average initial moisture content of 3.10±0.12 kg water/kg initial d.m. As can be observed in Fig. 3, the concentration of NaCl in the brine solution is a key parameter in moisture transport, since it determines the direction of water flux. The ANOVA carried out with samples brined for 120 min reflected that samples could be classified in three significantly different groups according to the moisture content (Fig. 2 C). The first group included samples brined using 50 and 100 kg NaCl/m^3, which showed a significant ($p<0.05$) water gain. Samples brined using 150 and 200 kg NaCl/m^3 (the second group) neither lost nor gained water, having similar moisture content to raw meat. This result coincided with what was reported by Graiver et al. (2009) and Nguyen et al. (2010), who did not find a clear moisture transport when using brines close to 200 kg NaCl/m^3, either. Finally, the third group included samples brined at 240 and 280 kg NaCl/m^3, which underwent dehydration. During brining however, hydration or dehydration are not only affected by chemical potential gradient (Shi and Le Maguer, 2002) but also by structural changes brought about in the meat by salt gain (Schmidt et al., 2008). On the one side, the low NaCl content increases the muscle's WHC by protein solubilization, which is known, as aforementioned, the "salting-in" phenomenon (Offer and Trinick, 1983). On the other side, the high NaCl content reduces the WHC and meat muscle shrinks, which is the "salting-out" phenomenon (Graiver et al., 2006).

Regarding ultrasound effect on transport kinetics, the moisture content of US and CONTROL samples brined for 120 min was not significantly ($p<0.5$) different. (Fig. 2 D). This fact has been also been observed in ultrasound assisted brining of beef muscles (Pohlman et al., 1997; Jayasooriya et al. 2007) and pork meat (Siró et al. 2009). The negligible effect of ultrasound on water content could be linked to the great variability in moisture content of samples (Fig. 3) if it is compared to the NaCl content (Fig. 1). In addition, it should be considered that the ultrasound intensity may be not enough to provoke significant differences in water transport due to a minim amount of ultrasonic energy is necessary in the medium, and that threshold could be different for water and NaCl content. Cárcel et al., (2007 b) reported that in high intensity ultrasound fields, brine could be microinjected into the meat leading to a direct increase of NaCl and water content.

3.2. Modeling transport kinetics

The analysis of the experimental results has been focused on the final salt and moisture content (samples brined for 120 min). Modeling the experimental transport kinetics (Figs. 1 and 3) will help to identify whether the brining conditions (US application and/or NaCl concentration) affect the process rate. The fit of the models to experimental kinetics achieved percentages of explained variance (Table 1) ranging from 93 to 99% for NaCl transport, being the figures slightly lower for moisture transport (81.1 to 95.6%), which could indicate a poor fit in this case. However, as can be observed in Fig. 4, there exists a similar trend between calculated and experimental moisture contents in both cases, which highlights how suitable the proposed diffusion model is to describe the brining process. The low explained variance provided by the diffusion model in the moisture transport should mostly linked to the great variability of the initial composition of raw meat. Therefore, diffusion could be considered the predominant mass transport mechanism during brining.

The D_s values were similar for all the different brine concentrations tested; a lower figure was only found for all NaCl concentration of 50 kg NaCl/m^3, probably due to the structural changes in meat samples brought about by low salt gain. The effective NaCl diffusivities identified for the CONTROL samples agree closely with the values reported in the literature

(Graiver et al., 2006; Vestergaard et al., 2007), which actually range between 2–4×10^{-10} m^2/s. Regarding moisture transport, the D_w values identified in experiments where meat was hydrated (NaCl concentration lower than 200 kg/m^3) decreased as the NaCl content rose. Thus, in CONTROL experiments using a brining solution of 50 kg $NaCl/m^3$, the D_w was 0.76x10^{-10} m^2/s, while D_w decreased to 0.17x10^{-10} m^2/s for experiments at 150 kg/m^3, where hydration was almost negligible. The same fact was also observed in US experiments and has also been previously reported by Gou et al., (2003), who found that the D_w decreased when the NaCl content of the salting solutions increased from 20 to 80 kg $NaCl/m^3$. On the other hand, the D_w values were higher when meat was dehydrated (NaCl concentration higher than 200 kg $NaCl/m^3$) than when meat was hydrated (NaCl concentration lower than 200 kg $NaCl/m^3$) (Table 2). These differences could be ascribed to the different product structure induced and controlled by NaCl transport (Schmidt et al., 2008; Gou et al., 2003; Offer and Trinick, 1983). Therefore, the NaCl concentration in the brining solution is not only affecting the direction of water flux (hydration or dehydration) but also the water transport rate as a consequence of the structural changes brought about by the NaCl content in the meat.

Ultrasound application led to a significant ($p<0.05$) improvement in both D_s and D_w, which points to an acceleration of both the global bring process. The increase in D_s ranged from 23 to 45% and is in a similar range to other improvements reported for solid transport in the literature. Thus, Siró et al. (2009) found increases of 96% in D_s meat brining and Gabaldón-Leyva et al. (2007) stated an improvement of 190% in the total solid diffusion coefficients. In the case of D_w, the improvement was higher than in D_s for the lowest and highest NaCl concentrations used (50 and 280 kg/m^3) (Table 1), being in this case the improvement close to 100%. Gabaldón-Leyva et al., 2007 and Cárcel et al., 2007a reported increases in D_w of around 128 and 117% when ultrasound was applied in bell pepper brining and osmotic dehydration of apple. Smaller increases were observed in the D_w values for intermediate NaCl concentration brines tested, these being 76% and 41% for 100 and 150 kg/m^3, respectively. The different effectiveness of ultrasound application depending on brine NaCl concentration could be explained considering that the ultrasound effects on mass transport are largely dependent on product structure (Gabaldón-Leyva et al., 2007). Finally, it should be remarked that there was not a significant ($p<0.05$) difference on the moisture content at the end of the brining process (120 min) for CONTROL and US samples, however, the analysis of transport kinetics showed an improvement on the moisture transport rate by ultrasound, which resulted no significant at the end of the brining process.

The effective moisture diffusivities identified from experimental results are kinetic parameters that not only include diffusion mechanisms but also other existing phenomena not considered in the model, such as external mass transport. Ultrasound may affect both internal mass transport resistance, by alternating cycles of expansions and contractions (“sponge effect”) and the generation of microchannels, and external by microstirring at the interfaces (Muralidhara et al., 1985, Cárcel et al., 2007b). These effects that US induce into the medium are responsible for the kinetic improvement.

3.3.Texture

In order to study the influence of ultrasound application and NaCl concentration on meat texture, instrumental penetration tests were carried out in US and CONTROL samples brined for 120 min using NaCl concentrations of 50, 200 and 280 kg/m^3. At least 12 points were measured in each meat slice.

CONTROL samples brined at 200 and 280 kg NaCl/m^3 were significantly ($p<0.05$) harder than those brined at 50 kg NaCl/m^3 (Table 3). As aforementioned, the higher the NaCl concentration in the brine, the greater the NaCl gain. In such a way, the NaCl gain promoted changes in meat texture, leading to harder samples, a fact already showed by Ruiz-Ramírez et al. (2005). Non-significant differences ($p<0.05$) in hardness were found in samples brined at 200 and 280 kg NaCl/m^3 (Table 3). This indicates that the level of NaCl gained by both samples was enough to produce the same change in meat texture. The effect of the NaCl concentration in US samples was similar to that reported in CONTROL ones.

On the other hand, the application of ultrasound during brining significantly increased ($p<0.05$) the meat hardness. As already explained, ultrasound application intensified NaCl transport during brining, increasing not only the NaCl diffusivity but also the final NaCl content (brining time, 120 min, CONTROL 0.326±0.007 kg NaCl/kg initial d.m. and US 0.409.29±0.11 kg NaCl/kg initial d.m.). So, the effects of ultrasound on meat texture could be linked to the intensification of NaCl transport, which provoked structural changes in meat proteins. Lee and Feng (2011) reported that the texture of ultrasound-treated food is influenced by protein changes during sonication, as well as Siró et al., (2009), who linked the hardening of meat tissue to the high ultrasonic intensities applied. Sanchez et al., (2001) showed that US application in the brining of Mahon cheese increased the sample hardness due to the improvement of proteolysis and lipolysis reactions.

Raw and brined CONTROL and US pork loin previously characterized from instrumental texture were studied by Cryo-SEM and SEM techniques in order to contribute to a better understanding of the effects produced by ultrasound application, being this fact analyzed in the following section.

3.4. Microstructure

3.4.1. Cryo-scanning electron microscopy (Cryo-SEM)

First of all the microstructure of raw pork loin was characterized. Fig. 5A shows a cross section of the raw pork loin, where cells are surrounded by the membrane or sarcolemma (Fig. 5A, S). These cells are interconnected by endomysial connective tissue (Fig. 5A, EC), which keeps the muscle fibers tightly attached. In addition, the myofibrils inside the muscle cell can be observed, which are fundamental components of the cell's contractile apparatus (Fig. 5A, M).

Intercellular spaces appear full of typical eutectic artifacts in brined samples (Figs. 5B, C and D) due to solute aggregation after water sublimation provoked by Cryo-SEM technique (Pérez-Munuera et al., 2008). The accumulation of solutes in the intercellular spaces can be linked to both the penetration of the NaCl and the strong solubilization and dehydration in muscle tissue. The greater the brining NaCl concentration, the greater the solute accumulation and the more compact the eutectic artifacts (Larrea et al., 2007). The bundles of muscle fibers in CONTROL samples brined at 280 kg/m^3 (Fig. 5D) seem to be more compact, showing the more intense sample dehydration linked to the "salting-out" phenomenon. The denaturation and precipitation of proteins involves progressive structural shrinkage and less space for water (Vestergaard et al., 2005). These effects are widely related with the hardening observed in the textural analysis of meat brined at the highest NaCl concentration (280 kg NaCl/m^3) (Table 3). In samples brined at the lowest NaCl concentration (50 kg NaCl/m^3, Fig. 5B), however, may be observed an expansion of myofibrils coupled with the protein solubilization that is related with the "salting-in" phenomena (Graiver et al., 2006). Some authors have already reported that an increase in water binding and hydration in the muscle fibers of brined meat at low brine concentrations (< 50 kg NaCl/m^3) is ascribed to enhanced electrostatic repulsion between myofibril filaments causing the filament lattice to expand for water entrapment (Graiver et al., 2009; Cheng and Sun, 2008). These phenomena in meat proteins can explain the hydration of samples brined at low NaCl concentrations (Fig. 3) and the meat softening observed in the textural analysis (Table 3).

Samples brined with 200 kg NaCl/m^3 (Fig. 5C), showed a mixed effect. Some parts of the sample show structural dehydration due to a high NaCl concentration (Fig. 5C, 1), whereas in other parts of the sample, the opposite phenomena (hydration) can be observed in the myofibrillar structure (Fig. 5C, 2). As mentioned before (Fig. 3), no net transport of moisture was identified at 200 kg NaCl/m^3, which can be linked to the combined effect of hydration-dehydration observed in the meat microstructure (Fig. 5C).

From micrographs obtained with Cryo-SEM, no effects of ultrasound in the meat structure were found. Thus, the Cryo-SEM microstructural analysis was completed with SEM observations.

1.1.1. Scanning electron microscopy (SEM) with combined dispersion X-ray analysis (SEM-EDX).

CONTROL samples brined with the lowest NaCl concentration tested (50 kg NaCl/m^3, Fig. 6B) showed a swelling of muscle fibers (width 87.6±7.3 μm) compared to raw meat (width 72.5±9.1 μm) (Fig. 6A). The swelling of muscle fibers could be mainly ascribed to the "salting-in" phenomena. Samples brined at higher NaCl concentrations behaved in the opposite way and presented a dehydration of muscle fibers and an accumulation of NaCl; these effects may be observed in Figs. 7A, C, E.

The SEM technique also allowed the effect brought about by high intensity ultrasound in meat structure to be observed, this effect mainly focusing on myofibrils (Figs. 7B, D, F). The micrograph of the US sample brined at 50 kg NaCl/m^3 (Fig. 7B) shows the disruption and the dispersion of the connective tissue of the fibers caused by US application. In Fig. 7F the rupture of a myofibril provoked by the acoustic energy was identified. The aforementioned myofibrillar changes could be explained by the alternating compressions and decompressions induced by US in solid materials ("sponge effect"). Another important effect produced by high intensity ultrasound in liquid media is cavitation, which may be observed in Fig. 7D, where the erosion of meat fiber produced by cavitation is shown. The asymmetric implosion of bubbles near the solid surface could produce violent microjets that collide with the samples, which can improve mass transfer by disturbing the boundary layer and producing changes in the meat structure. These results coincide with those found by several authors that related the application of high power ultrasound with the physical disruption of cellular and sub-cellular components (Reynolds et al., 1978), the degradation of collagen macromolecules (Nishira and Doty, 1958), and the creation of micro channels (Muralidhara et al., 1985).

The obtained SEM-EDX mapping images confirmed the presence of NaCl in meat after brining, which is shown up by the red (Cl^-) and green (Na^+) dots in the micrographs. As can be observed in Fig. 8, the NaCl concentration in US samples (Figs. 8B, C and D) was higher than those observed in CONTROL samples, as manifested by a higher number of dots in the micrographs (Figs. 8A, C and E). In CONTROL samples (Figs. 8A, C and E), NaCl molecules are mainly located around the myofibrils. However, the US brined samples (Figs. 8A, C and E) showed a more homogeneous NaCl dispersion due to the collapse of myofibrillar structure caused by the effects of high power ultrasound, permitting a higher NaCl penetration in the meat. The obtained SEM-EDX images showed the intensification of NaCl transport brought about by US application and the increase in NaCl content, which confirms the results provided by modeling and textural tools.

4. Conclusions

The NaCl concentration in the brine solution significantly ($p<0.05$) affected moisture and NaCl transport during meat brining. At NaCl concentrations of under 200 kg/m^3, the meat was hydrated while, at concentrations of over 200 kg/m^3, it underwent dehydration. As for the NaCl transport, the more concentrated the brine, the greater the NaCl sample gain. Ultrasound application intensified the brining kinetics, increasing both moisture effective and NaCl diffusivities. The NaCl gain promoted changes in meat texture, high NaCl contents leading to harder samples. Microstructural analyses showed that the application of high intensity ultrasound during brining brought about relevant effects on meat microstructure, such as a more homogeneous NaCl distribution in meat. Therefore, ultrasound could be considered a potential technology with which to accelerate the brining process.

Acknowledgments

This work is financed by project CARNISENUSA (CSD2007-00016) included in the CONSOLIDER-INGENIO-2010.

Nomenclature

W_{eq}	Equilibrium moisture content, kg water/kg initial d.m.
W_0	Initial moisture content, kg water/kg initial d.m.
S_{eq}	Equilibrium sodium chloride content, kg NaCl/kg initial d.m.
S_0	Initial sodium chloride content, kg NaCl/kg initial d.m.
D_S	Effective NaCl diffusivity (m^2/s)
D_W	Effective moisture diffusivity (m^2/s)
L	Half length, m

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