

Document downloaded from:

<http://hdl.handle.net/10251/72942>

This paper must be cited as:

Ozuna López, C.; Puig Gómez, CA.; García Pérez, JV.; Mulet Pons, A.; Carcel Carrión, JA. (2013). Influence of high intensity ultrasound application on mass transport, microstructure and textural properties of pork meat (*Longissimus dorsi*) brined at different NaCl concentrations. *Journal of Food Engineering*. 119(1):84-93.
doi:10.1016/j.foodeng.2013.05.016.



The final publication is available at

<https://dx.doi.org/10.1016/j.jfoodeng.2013.05.016>

Copyright Elsevier

Additional Information

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

4 **César Ozuna ^a, Ana Puig ^b, Jose V. García-Pérez ^a, Antonio Mulet ^a and Juan A.**
5 **Cárcel^{a,*}**

6 ^aGrupo de Análisis y Simulación de Procesos Agroalimentarios. Departamento de Tecnología
7 de Alimentos. Universitat Politècnica de València.

8 Camino de Vera. s/n. E46022. Valencia, Spain

9 ^bGrupo de Microestructura y Química de Alimentos. Departamento de Tecnología de
10 Alimentos. Universitat Politècnica de València.

11 Camino de Vera. s/n. E46022. Valencia, Spain

12
13
14
15
16
17
18
19 *Corresponding author.

20 Juan A. Cárcel.

21 Grupo de Análisis y Simulación de Procesos Agroalimentarios. Departamento de Tecnología
22 de Alimentos. Universitat Politècnica de València.

23 Camino de Vera. s/n. E46022. Valencia, Spain

24 Tel.: +34 96 387 93 65; Fax: +34 96 387 98 39

25 E-mail address: jcarcel@tal.upv.es.

26

27 **ABSTRACT**

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

39

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

41

42

43 **1. Introduction**

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

53 In liquid medium, ultrasound induces cavitation (Leighton, 1998), temperature gradients
54 within the material (Mason and Lorimer, 2002), mechanical phenomena, such as the “sponge
55 effect”, generation of microchannels and microstirring on interfaces (Muralidhara et al.,
56 1985). These effects can not only increase the mass transport kinetics but also imply structural
57 changes and, consequently, changes in textural properties. Thereby, textural changes induced
58 by ultrasound have been observed in, among others, tomato juice (Vercet et al., 2002),
59 yoghurt (Wu et al., 2000), bell peppers (Gabaldón-Leyva et al. 2007) or meat (Jayasooriya et
60 al., 2007; Pohlman et al., 1997).

61 Meat has great biochemical and structural complexity. During meat brining, changes in NaCl
62 and moisture content take place (Graiver et al., 2006), which extend meat’s shelf life and
63 modify organoleptic characteristics, such as juiciness, texture and flavor. Mass transfer
64 driving forces between meat and brine are linked to chemical gradients and NaCl-induced
65 changes in the water holding capacity (WHC) of meat proteins (Shi and Le Maguer, 2002;
66 Vestergaard et al., 2005). The NaCl concentration in brine affects the direction of moisture
67 transport as well as the equilibrium state (Cheng and Sun, 2008). A low NaCl content in the

68 meat increases the water holding capacity (WHC) (Nguyen et al., 2010), a phenomenon
69 known as "salting-in" which is linked to the meat protein net charge modification. However, a
70 high NaCl content in meat could also bring about a decrease in WHC, probably due to the
71 insolubilization of proteins ("salting-out") (Graiver et al., 2009). Therefore, the NaCl
72 concentration in the brine solution can not only affect the chemical gradients, but also the
73 WHC, thus affecting the magnitude of mass transport.

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

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

87

88 **2. Materials and methods**

89 2.1. Raw material and sample preparation

90 Fresh pork loins (*Longissimus dorsi*) were purchased at a local slaughterhouse (Valencia,
91 Spain). The pieces selected had a pH of 5.3 ± 0.30 , which was measured in-situ by means of a
92 pH-meter (pH STAR, Matthäus, Germany) at three different points along the muscle avoiding

93 fatty areas. Parallelepiped shaped samples (length 50 x width 30 x thickness 10 mm) were
94 obtained from the central part of loin pieces using a sharp knife. Before brining, samples were
95 wrapped in plastic waterproof film and kept frozen at -18 ± 0.5 °C (maximum storage time
96 120 hours).

97

98 2.2. Brining treatments

99 Brining experiments with (US, 40 kHz; 37.5 W/dm^3) and without ultrasound application
100 (CONTROL) were carried out using brine solutions of different NaCl concentrations (50, 100,
101 150, 200, 240 and 280 kg NaCl/m^3). The highest NaCl concentration used (280 kg NaCl/m^3)
102 involved brine saturation at 5 ± 1 °C, which was pointed to by the presence of NaCl crystals in
103 the solution.

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

109 Before each brining experiment, 6 meat samples were thawed at constant temperature (2 ± 1
110 °C) for 24 h. Then, the samples were blotted, weighed (PB3002-S/PH, J.P., Mettler Toledo,
111 Spain) and their size was measured by using a Vernier caliper. Afterwards, they were placed
112 in a hollow sample holder and simultaneously introduced in the brine. For a homogenous
113 brining, the position of the meat samples was changed every 5 min. In the US experiments,
114 ultrasound was continuously applied. Samples were taken out of the brine at preset times (15,
115 30, 45, 60, 90 and 120 minutes) and immersed in distilled water for 20 s to remove any
116 adhered surface brine. Finally, samples were blotted, wrapped in plastic waterproof film and
117 frozen (-18 ± 0.5 °C) until moisture and NaCl measurements were taken.

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

123

124 2.3. Mass transfer modeling

125 A mathematical model based on Fick's 2nd law was used to separately describe the evolution
 126 of moisture and NaCl content in the sample during brining (Cranck 1975). Samples were
 127 considered to become slab geometry bodies due to the fact that they were not nearly as thick
 128 (10 mm) as they were high (50 mm) and wide (30 mm), thus, mass transfer was simplified as
 129 a one-dimensional problem. Constant effective diffusivities (D_s and D_w), negligible changes
 130 in temperature and sample volume, solid symmetry, homogeneous NaCl and moisture initial
 131 content and negligible external resistance (Gou et al., 2003) were assumed during processing.
 132 Eqs. 1 and 2 show the solution of the diffusion model in terms of average moisture and NaCl
 133 content.

$$134 \quad W = W_{eq} + (W_0 - W_{eq}) \left[2 \sum_{n=0}^{\infty} \frac{1}{\lambda_n^2 L^2} e^{-D_w \lambda_n^2 t} \right] \quad (1)$$

$$135 \quad S = S_{eq} + (S_0 - S_{eq}) \left[2 \sum_{n=0}^{\infty} \frac{1}{\lambda_n^2 L^2} e^{-D_s \lambda_n^2 t} \right] \quad (2)$$

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

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

146

147 2.4. Textural and microstructural analysis

148 Meat texture and microstructure were studied in raw material, US (40 kHz; 37.5 W/dm³) and
149 CONTROL brined samples for 120 minutes using low, intermediate and high NaCl
150 concentrations (50, 200 and 280 kg NaCl/m³, respectively). Samples were brined in triplicate
151 at least.

152

153 2.4.1. Texture

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

159

160 2.4.2. Cryo-scanning electron microscopy (Cryo-SEM)

161 Cubic samples (side 3 mm) of raw and brined meat were immersed in slush Nitrogen (-210
162 °C), and quickly transferred to a cryo-trans (CT 15000 C, Oxford Instruments, England)
163 linked to a scanning electron microscope (JSM-5410, Jeol, Japan). Samples were cryo-
164 fractured at -180 °C, etched at -90 °C and gold-coated, allowing cross-section visualization.

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

167

168 2.4.3. Scanning electron microscopy (SEM) with combined dispersion X-ray analysis
169 (SEM-EDX).

170 Cubic samples (side 3 mm) from raw and brined meat were immersed in liquid N₂ and then
171 freeze-dried at 1 Pa for 3 days (LIOALFA-6, Telstar, Spain). The fixed samples were vacuum
172 sealed in vials in the same freeze-drier so that they would remain stable (Llorca et al., 2001).
173 The fixed samples were individually placed on SEM slides with the aid of colloidal silver and
174 then gold-coated with (SCD005, Baltec, Germany) at 10⁻² Pa and an ionization current of 40
175 mA. The samples were observed in a scanning electron microscope (JSM-5410, Jeol, Japan)
176 equipped with an X-ray detector and LINK data-processing system (INCA 4.09, Oxford
177 Instruments, England) at an acceleration voltage of 10-20 kV which provides internal
178 information about the standards of energy dispersive X-ray spectra of the elements analyzed
179 (Na⁺ and Cl⁻). This technique is an analytical tool that allows the ions Cl⁻ and Na⁺ inside the
180 samples to be identified (Grote and George, 1984). For EDX (energy-dispersive X-ray)
181 analysis, samples were carbon-coated (CEA035, Baltec, Germany). Mapping images of Cl⁻
182 and Na⁺ distribution in meat samples were made using a voltage of 20 kV and at a working
183 distance of 15 mm.

184

185 2.5. Fitting model evaluation and statistical analysis

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

$$190 \quad \% \text{VAR} = \left[1 - \frac{S_{tw}^2}{S_w^2} \right] \times 100 \quad (3)$$

191 Where S_w^2 and S_{tw}^2 are the variance of the sample and the estimation, respectively.

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

196

197 **3. Results and discussion**

198 3.1. NaCl and water transport

199 3.1.1. NaCl and water content

200 Fig. 1 shows the NaCl content of loin samples during brining, which is also considered the
 201 NaCl gain, due to the fact that the NaCl content in meat samples was negligible. NaCl
 202 concentration in the brine influenced significantly on NaCl content (Fig. 2A). Thus, when
 203 using a brine solution of 50 kg NaCl/m³, the NaCl content in the meat after 120 min of
 204 brining was almost four times lower than when using 280 kg NaCl/m³. The NaCl gain is
 205 mainly linked to osmotic mechanisms, thus the hydrodynamic flux increases as the pressure
 206 gradients between the meat and brine get higher (Schmidt et al., 2008). Other factors, such as
 207 temperature, pH and muscle microstructure, can also affect the NaCl gain (Barat et al., 2006).
 208 Ultrasound also significantly ($p < 0.05$) influenced the NaCl gain (Fig. 2 B); as an example, for
 209 a brining time of 90 min and using the highest NaCl concentration (280 kg NaCl/m³) (Fig. 1),
 210 the NaCl content in the CONTROL samples was 0.315 ± 0.020 kg NaCl/kg initial d.m. while
 211 the content in US samples reached 0.359 ± 0.033 kg NaCl/kg initial d.m. Among other
 212 phenomena, the US application in liquid media induces cavitation, temperature gradients
 213 within the material, alternative compression and decompression of the material, the

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

218 Regarding the moisture content, pork loin showed average initial moisture content of
219 3.10 ± 0.12 kg water/kg initial d.m. As can be observed in Fig. 3, the concentration of NaCl in
220 the brine solution was a key parameter in moisture transport, since it determined the direction
221 of water flux. The ANOVA carried out with samples brined for 120 min reflected that
222 samples could be classified in three significantly different groups according to the moisture
223 content (Fig. 2 C). The first group included samples brined using 50 and 100 kg NaCl/m³,
224 which showed a significant ($p < 0.05$) water gain. Samples brined using 150 and 200 kg
225 NaCl/m³ (the second group) neither lost nor gained water, having similar moisture content to
226 raw meat. This result coincided with what was reported by Graiver et al. (2009) and Nguyen
227 et al. (2010), who did not find a clear moisture transport when using brines close to 200 kg
228 NaCl/m³, either. The water activity of meat samples and the 200 kg NaCl/m³ brine were
229 0.980 ± 0.007 and 0.868 ± 0.001 respectively (Aqua Lab Serie 3, Decagon Devices, Inc., USA).
230 Therefore, the lack of net moisture transport at this concentration may not to be explained
231 from a water activity point of view. Finally, the third group included samples brined at 240
232 and 280 kg NaCl/m³, which underwent dehydration. During brining however, hydration or
233 dehydration are not only affected by chemical potential gradient (Shi and Le Maguer, 2002)
234 but also by structural changes brought about in the meat by salt gain (Schmidt et al., 2008).
235 On the one side, the low NaCl content increases the muscle's WHC by protein solubilization,
236 which is known, as aforementioned, the "salting-in" phenomenon (Offer and Trinick, 1983).
237 On the other side, the high NaCl content reduces the WHC and meat muscle shrinks, which is
238 the "salting-out" phenomenon (Graiver et al., 2006).

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

249

250 3.2. Modeling transport kinetics

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

263 The D_s values were similar for all the different brine concentrations tested; a lower figure was
264 only found for all NaCl concentration of 50 kg NaCl/m³, probably due to the structural
265 changes in meat samples brought about by low salt gain. The effective NaCl diffusivities
266 identified for the CONTROL samples agree closely with the values reported in the literature
267 (Graiver et al., 2006; Vestergaard et al., 2007), which actually range between $2-4 \times 10^{-10}$ m²/s.
268 Regarding moisture transport, the D_w values identified in experiments where meat was
269 hydrated (NaCl concentration lower than 200 kg NaCl/m³) decreased as the NaCl content rose.
270 Thus, in CONTROL experiments using a brining solution of 50 kg NaCl/m³, the D_w was
271 0.76×10^{-10} m²/s, while D_w decreased to 0.17×10^{-10} m²/s for experiments at 150 kg NaCl/m³,
272 where hydration was almost negligible. The same fact was also observed in US experiments
273 and has also been previously reported by Gou et al., (2003), who found that the D_w decreased
274 when the NaCl content of the salting solutions increased from 20 to 80 kg NaCl/m³. On the
275 other hand, the D_w values were higher when meat was dehydrated (NaCl concentration higher
276 than 200 kg NaCl/m³) than when meat was hydrated (NaCl concentration lower than 200 kg
277 NaCl/m³) (Table 2). It must be clarified that the model was not fit to the experimental data of
278 moisture content obtained for the 200 kg NaCl/m³ brine due to no net moisture transport was
279 observed. For this reason, the diffusivity value is not included in Table 2 at this experimental
280 conditions. These differences could be ascribed to the different product structure induced and
281 controlled by NaCl transport (Schmidt et al., 2008; Gou et al., 2003; Offer and Trinick, 1983).
282 Therefore, the NaCl concentration in the brining solution is not only affecting the direction of
283 water flux (hydration or dehydration) but also the water transport rate as a consequence of the
284 structural changes brought about by the NaCl content in the meat.

285 Ultrasound application led to a significant ($p < 0.05$) improvement in both D_s and D_w , which
286 points to an acceleration of both the global brine process. The increase in D_s ranged from 23
287 to 45% and is in a similar range to other improvements reported for solid transport in the

288 literature. Thus, Siró et al. (2009) found increases of 96% in D_s meat brining and Gabaldón-
289 Leyva et al. (2007) stated an improvement of 190% in the total solid diffusion coefficients. In
290 the case of D_w , the improvement was higher than in D_s for the lowest and highest NaCl
291 concentrations used (50 and 280 kg NaCl/m³) (Table 1), being in this case the improvement
292 close to 100%. Gabaldón-Leyva et al., 2007 and Cárcel et al., 2007a reported increases in D_w
293 of around 128 and 117% when ultrasound was applied in bell pepper brining and osmotic
294 dehydration of apple. Smaller increases were observed in the D_w values for intermediate NaCl
295 concentration brines tested, these being 76% and 41% for 100 and 150 kg NaCl/m³,
296 respectively. The different effectiveness of ultrasound application, depending on brine NaCl
297 concentration, could be explained considering that the ultrasound effects on mass transport
298 are largely dependent on product structure (Gabaldón-Leyva et al., 2007).

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

309

310 3.3.Texture

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

313 W/dm³) and CONTROL samples brined for 120 min using NaCl concentrations of 50, 200
314 and 280 kg NaCl/m³. At least 12 points were measured in each meat slice.

315 Raw material showed a hardness of 1.60 ± 0.49 N. With regard to brined samples, the
316 hardness was dependent on the salting conditions used. Thus, CONTROL samples brined at
317 200 and 280 kg NaCl/m³ were significantly ($p < 0.05$) harder than those brined at 50 kg
318 NaCl/m³ (Table 3). As aforementioned, the higher the NaCl concentration in the brine, the
319 greater the NaCl gain. In such a way, the NaCl gain promoted changes in meat texture,
320 leading to harder samples, a fact already showed by Ruiz-Ramírez et al. (2005). Non-
321 significant differences ($p < 0.05$) in hardness were found in samples brined at 200 and 280 kg
322 NaCl/m³ (Table 3). This indicates that the level of NaCl gained by both samples was enough
323 to produce the same change in meat texture. The effect of the NaCl concentration in US
324 samples was similar to that reported in CONTROL ones.

325 On the other hand, the application of ultrasound during brining significantly increased
326 ($p < 0.05$) the meat hardness. As already explained, ultrasound application intensified NaCl
327 transport during brining, increasing not only the NaCl diffusivity but also the final NaCl
328 content (brining time 120 min, CONTROL 0.326 ± 0.007 kg NaCl/kg initial d.m. and US
329 $0.409.29 \pm 0.11$ kg NaCl/kg initial d.m.). So, the effects of ultrasound on meat texture could be
330 linked to the intensification of NaCl transport, which provoked structural changes in meat
331 proteins. Lee and Feng (2011) reported that the texture of ultrasound-treated food is
332 influenced by protein changes during sonication, as well as Siró et al., (2009), who linked the
333 hardening of meat tissue to the high ultrasonic intensities applied. Sanchez et al., (2001)
334 showed that US application in the brining of Mahon cheese increased the sample hardness due
335 to the improvement of proteolysis and lipolysis reactions.

336 Raw and brined CONTROL and US pork loin previously characterized from instrumental
337 texture were studied by Cryo-SEM and SEM techniques in order to contribute to a better

338 understanding of the effects produced by ultrasound application, being this fact analyzed in
339 the following section.

340

341 3.4. Microstructure

342 3.4.1. Cryo-scanning electron microscopy (Cryo-SEM)

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

349 Intercellular spaces appear full of typical eutectic artifacts in brined samples (Figs. 5B, C and
350 D) due to solute aggregation after water sublimation provoked by Cryo-SEM technique
351 (Pérez-Munuera et al., 2008). The accumulation of solutes in the intercellular spaces can be
352 linked to both the penetration of the NaCl and the strong solubilization and dehydration in
353 muscle tissue. The greater the brining NaCl concentration, the greater the solute accumulation
354 and the more compact the eutectic artifacts (Larrea et al., 2007). The bundles of muscle fibers
355 in CONTROL samples brined at 280 kg NaCl/m³ (Fig. 5D) seem to be more compact,
356 showing the more intense sample dehydration linked to the “salting-out” phenomenon. The
357 denaturation and precipitation of proteins involves progressive structural shrinkage and less
358 space for water (Vestergaard et al., 2005). These effects are widely related with the hardening
359 observed in the textural analysis of meat brined at the highest NaCl concentration (280 kg
360 NaCl/m³) (Table 3). In samples brined at the lowest NaCl concentration (50 kg NaCl/m³, Fig.
361 5B), however, may be observed an expansion of myofibrils coupled with the protein
362 solubilization that is related with the “salting-in” phenomena (Graiver et al., 2006). Some

363 authors have already reported that an increase in water binding and hydration in the muscle
364 fibers of brined meat at low brine concentrations ($< 50 \text{ kg NaCl/m}^3$) is ascribed to enhanced
365 electrostatic repulsion between myofibril filaments causing the filament lattice to expand for
366 water entrapment (Graiver et al., 2009; Cheng and Sun, 2008). These phenomena in meat
367 proteins can explain the hydration of samples brined at low NaCl concentrations (Fig. 3) and
368 the meat softening observed in the textural analysis (Table 3).

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

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

378

379 1.1.1. Scanning electron microscopy (SEM) with combined dispersion X-ray analysis
380 (SEM-EDX).

381 CONTROL samples brined with the lowest NaCl concentration tested (50 kg NaCl/m^3 , Fig.
382 6B) showed a swelling of muscle fibers (width $87.6 \pm 7.3 \mu\text{m}$) compared to raw meat (width
383 $72.5 \pm 9.1 \mu\text{m}$) (Fig. 6A). The swelling of muscle fibers could be mainly ascribed to the “salting-
384 in” phenomena. Samples brined at higher NaCl concentrations (280 kg NaCl/m^3)
385 behaved in the opposite way and presented a dehydration of muscle fibers and an
386 accumulation of NaCl; these effects may be observed in Figs. 7A, C, E.

387 The SEM technique also allowed the effect brought about by high intensity ultrasound in meat
388 structure to be observed, this effect mainly focusing on myofibrils (Figs. 7B, D, F). The
389 micrograph of the US sample brined at 50 kg NaCl/m³ (Fig. 7B) shows the disruption and the
390 dispersion of the connective tissue of the fibers caused by US application. In Fig. 7F the
391 rupture of a myofibril provoked by the acoustic energy was identified. The aforementioned
392 myofibrillar changes could be explained by the alternating compressions and decompressions
393 induced by US in solid materials (“sponge effect”). Another important effect produced by
394 high intensity ultrasound in liquid media is cavitation, which may be observed in Fig. 7D,
395 where the erosion of meat fiber produced by cavitation is shown. The asymmetric implosion
396 of bubbles near the solid surface could produce violent microjets that collide with the
397 samples, which can improve mass transfer by disturbing the boundary layer and producing
398 changes in the meat structure. These results coincide with those found by several authors that
399 related the application of high power ultrasound with the physical disruption of cellular and
400 sub-cellular components (Reynolds et al., 1978), the degradation of collagen macromolecules
401 (Nishira and Doty, 1958), and the creation of micro channels (Muralidhara et al., 1985).

402 The obtained SEM-EDX mapping images confirmed the presence of NaCl in meat after
403 brining, which is shown up by the red (Cl⁻) and green (Na⁺) dots in the micrographs. As can
404 be observed in Fig. 8, the NaCl concentration in US samples (Figs. 8B, D and F) was higher
405 than those observed in CONTROL samples, as manifested by a higher number of dots in the
406 micrographs (Figs. 8A, C and E). In CONTROL samples (Figs. 8A, C and E), NaCl
407 molecules are mainly located around the myofibrils. However, the US brined samples (Figs.
408 8B, D and F) showed a more homogeneous NaCl dispersion due to the collapse of
409 myofibrillar structure caused by the effects of high power ultrasound, permitting a higher
410 NaCl penetration in the meat. The obtained SEM-EDX images showed the intensification of

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

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

416

417 **4. Conclusions**

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

428

429 **Acknowledgments**

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

432

433

434

435

436 **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

437

438

439 **References**

- 440 Asociación de industrias de la carne de España (AICE), available online <http://www.aice.es>.
441 Accessed on November 01, 2011.
- 442 Association of Official Analytical Chemists. Official Methods of Analysis; AOAC:
443 Washington, DC, 1997.
- 444 Barat, J.M., Grau, R., Ibáñez, J.B., Pagán, M.J., Flores M., Toldrá F., & Fito P. (2006).
445 Accelerated processing of dry-cured ham. Part I. Viability of the use of brine
446 thawing/salting operation. *Meat Science*, 72 (4), 757-765.
- 447 Cárcel, J.A., Benedito, J., Roselló, C., & Mulet, A. (2007a). Influence of ultrasound intensity
448 on mass transfer in apple immersed in a sucrose solution. *Journal of Food*
449 *Engineering*, 78 (2), 472-479.
- 450 Cárcel, J.A., Benedito, J., Bon, J., & Mulet A. (2007b). High intensity ultrasound effects on
451 meat brining. *Meat Science*, 76 (4), 611-619.
- 452 Cheng, Q., & Sun, D.-W. (2008). Factors affecting the water holding capacity of red meat
453 products: a review of recent research advances. *Critical Reviews in Food Science and*
454 *Nutrition*, 48 (2), 137-159.
- 455 Crank, J. (1975). *The Mathematics of Diffusion*. London: Oxford University Press.
- 456 Gabaldón-Leyva, C. A., Quintero-Ramos, A., Barnard, J., Balandrán-Quintana, R. R.,
457 Talamás-Abbud, R. T., & Jiménez-Castro, J. (2007). Effect of ultrasound on the mass
458 transfer and physical changes in brine bell pepper at different temperature. *Journal of*
459 *Food Engineering*, 81(2), 374–379.
- 460 Gou, P., Comaposada, J., & Arnau, J. (2003). NaCl content and temperature effects on
461 moisture diffusivity in the *Gluteus medius* muscle of pork ham. *Meat Science*, 63 (1),
462 29-34.

- 463 Graiver, N., Pinotti, A., Califano, A., & Zaritzky, N. (2006). Diffusion of sodium chloride in
464 pork tissue. *Journal of Food Engineering*, 77 (4), 910–918.
- 465 Graiver, N., Pinotti, A., Califano, A., & Zaritzky, N. (2009). Mathematical modeling of the
466 uptake of curing salts in pork meat. *Journal of Food Engineering*, 95 (4), 533-540.
- 467 Grote, M. & Georg, H. (1984) Determination of element concentrations in fresh and
468 processed vegetables by quantitative X-ray microanalysis. *Food Microstructure*, 3 (1),
469 49–54.
- 470 Jayasooriya S.D., Torley P.J., D’Arcy B.R. & Bhandari B.R. (2007). Effect of high power
471 ultrasound and ageing on the physical properties of bovine *Semitendinous* and
472 *Longissimus* muscles. *Meat Science*, 75 (4), 628-639.
- 473 Larrea, V., Pérez-Munuera, I., Hernando, I., Quiles, A., Llorca, E., & Lluch, M.A. (2007).
474 Microstructural changes in Teruel dry-cured ham during processing. *Meat Science*, 76
475 (3), 574-582.
- 476 Lee, H. & Feng, H. (2011). Effect of power ultrasound on food quality. In Feng, H., Barbosa-
477 Cánovas, G.M. & Weiss, J. (Eds.), *Ultrasound Technologies for Food and*
478 *Bioprocessing* (pp. 559–582). London: Springer.
- 479 Leighton, T. G. (1998). The principles of cavitation. In M. J. W. Povey & T. J. Mason (Eds.),
480 *Ultrasound in Food Processing* (pp. 151–182). London: Chapman & Hall.
- 481 Llorca, E., Puig, A., Hernando, I., Salvador, A., Fiszman S., & Lluch, M.A. (2001). Effect of
482 fermentation time on texture and microstructure of pickled carrots. *Journal of the*
483 *Science of Food and Agriculture*, 81 (15), 1553–1560.
- 484 Mason, T. J., & Lorimer, J. P. (2002). Applied sonochemistry. *The uses of power ultrasound*
485 *in chemistry and processing*. Weinheim: Wiley-VCH.
- 486 Muralidhara, H. S., Ensminger, D., & Putnam, A. (1985). Acoustic dewatering and drying
487 (low and high frequency): State of the art review. *Drying Technology*, 3(4), 529–566.

- 488 Nguyen, M.V., Arason, A., Thorarinsdottir, K.A., Thorkelsson, G. & Gudmundsdóttir, A.
489 (2010). Influence of salt concentration on the salting kinetics of cod loin (*Gadus*
490 *morhua*) during brine salting. *Journal of Food Engineering*, 100 (2), 225-231.
- 491 Nishira, T., & Doty, P. (1958). The sonic fragmentation of collagen macromolecules.
492 *Proceedings of the National Academy of Sciences of the United States of America*,
493 44(5), 411-417.
- 494 Offer, G. & Trinick, J. (1983). On the mechanism of water holding in meat: The swelling and
495 shrinking of myofibrils. *Meat Science*, 8 (4), 245-281.
- 496 Pérez-Munuera, I., Larrea, V., Quiles, A., & Lluch, M.A. (2008). Microstructure of muscle
497 foods. In L.M.L., Nollet & F. Toldrá (Eds.), *Handbook of muscle food analysis* (pp.
498 335-352). Boca Raton FL: CRC Press Taylor & Francis Group.
- 499 Pohlman, F.W, Dikeman M.E., & Kropf D.H. (1997). Effects of high intensity ultrasound
500 treatment, storage time and cooking method on shear, sensory, instrumental color and
501 cooking properties of packaged and unpackaged beef pectoralis muscle, *Meat Science*,
502 46 (1), 89–100.
- 503 Rastogi, N.K., Raghavarao, K.S.M.S., Niranjana, K., & Knorr, D. (2002). Recent
504 developments in osmotic dehydration: methods to enhance mass transfer. *Trends in*
505 *Food Science & Technology*, 13 (2), 48-59.
- 506 Reynolds, J.B., Anderson D.B., Schmidt, G.R., Theno, D.M., & Siegel D.G. (1978). Effects
507 of ultrasonic treatment on binding strength in cured ham rolls. *Journal of Food*
508 *Science*, 43 (3), 866-869.
- 509 Ruiz-Ramírez, J., Arnau, J., Serra, X., & Gou, P. (2005). Relationship between water content,
510 NaCl content, pH and texture parameters in dry-cured muscles. *Meat Science*, 70 (4),
511 579-587.

- 512 Sanchez, E. S., Simal, S., Femenía, A., Benedito, J. & Roselló, C. (2001). Effect of acoustic
513 brining on lipolysis and on sensory characteristics of Mahon cheese. *Journal of Food*
514 *Science*, 66(6), 892-896.
- 515 Schmidt, F.C., Carciofi, B.A.M. & Laurindo, J. B. (2008). Salting operational diagrams for
516 chicken breast cuts: Hydration-dehydration. *Journal of Food Engineering*, 88 (1), 36-
517 44.
- 518 Shi J. & Le Maguer M. (2002). Osmotic dehydration of foods: mass transfer and modelling
519 aspects. *Food Reviews International*, 18(4), 305-335.
- 520 Siró, I., Vén, C., Balla, C., Jónás, G., Zeke, I., & Friedrich, L. (2009). Application of an
521 ultrasonic assisted curing technique for improving the diffusion of sodium chloride in
522 porcine meat. *Journal of Food Science*, 91 (2), 363-362.
- 523 Vercet, A., Sánchez, C., Burgos, J., Montañés, L., & Buesa, P. L. (2002). The effects of
524 manothermosonication on tomato pectic enzymes and tomato paste rheological
525 properties. *Journal of Food Engineering*, 53 (3), 273–278.
- 526 Vestergaard, C., Lohmann Andersen, B., & Adler-Nissen, J. (2007). Sodium diffusion in
527 cured pork determined by ^{22}Na radiology. *Meat Science*, 76 (2), 258–265.
- 528 Vestergaard, C., Risum, J., & Adler-Nissen, J. (2005). ^{23}Na -MRI quantification of sodium and
529 water mobility in pork during brine curing. *Meat Science*, 69 (4), 663-672.
- 530 Wu, H., Hulbert, G. J., & Mount, J. R. (2000). Effects of ultrasound on milk homogenization
531 and fermentation with yogurt starter. *Innovative Food Science and Emerging*
532 *Technologies*, 1 (13), 211–218.
- 533
- 534

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/dm³) and without ultrasound application (CONTROL). In this figure, each point represents the average of 9 measurements: three independently brined samples analyzed in triplicate. Average \pm 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 °C for 120 min with different brine concentrations with (US, 40 kHz, 37.5 W/dm³) 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/dm³) and without ultrasound application (CONTROL). In this figure, each point represents the average of 9 measurements: three independently brined samples analyzed in triplicate. Average \pm 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/dm³) and without (CONTROL) ultrasound application.

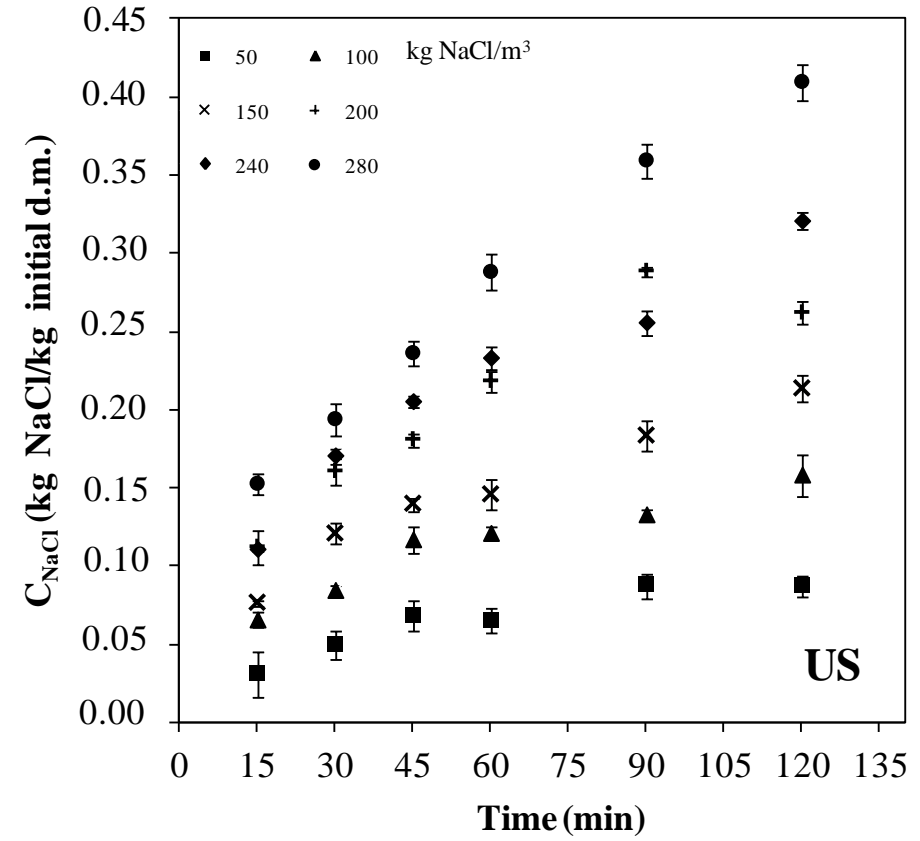
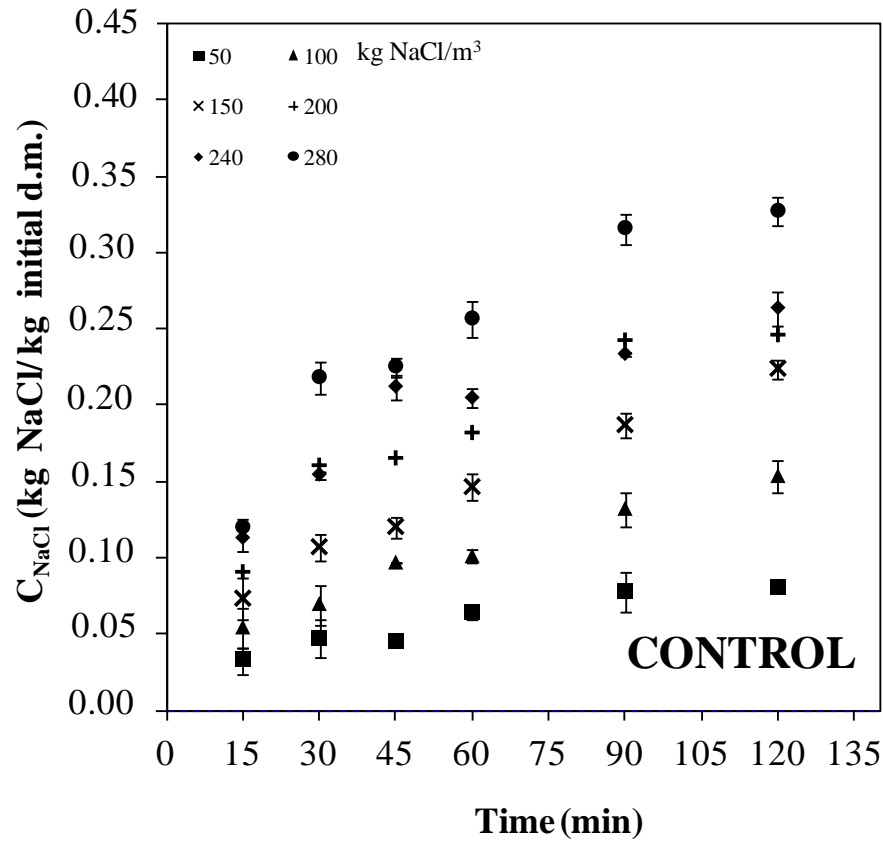
Fig 5. Cross-section of *Longissimus dorsi* muscle of pork meat observed by Cryo-SEM (x 750). Raw meat (A), meat brined for 120 min in 50 kg NaCl/m³ (B), 200 kg NaCl/m³ (D) and 280 kg NaCl/m³. S: sarcolemma, EC: endomysial connective tissue, M: myofibrills, 1: “salting-out effects”, 2: “salting-in effects”.

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/m³ 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/m³ (A,B: x1500), 200 kg NaCl/m³ (C,D: x150) and 280 kg NaCl/m³ solution (D, E: x750) without (CONTROL: A, C and E) and with (US: B, D and E) ultrasound application (40 kHz, 37.5 W/dm³). CV: impact of cavitation bubble 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/m³ (A,B: x500), 200 kg NaCl/m³ (C,D: x500) and 280 kg NaCl/m³ (D,E: x150) without (CONTROL: A, C and E) and with (US: B, D and E) ultrasound application (40 kHz, 37.5 W/dm³).

1

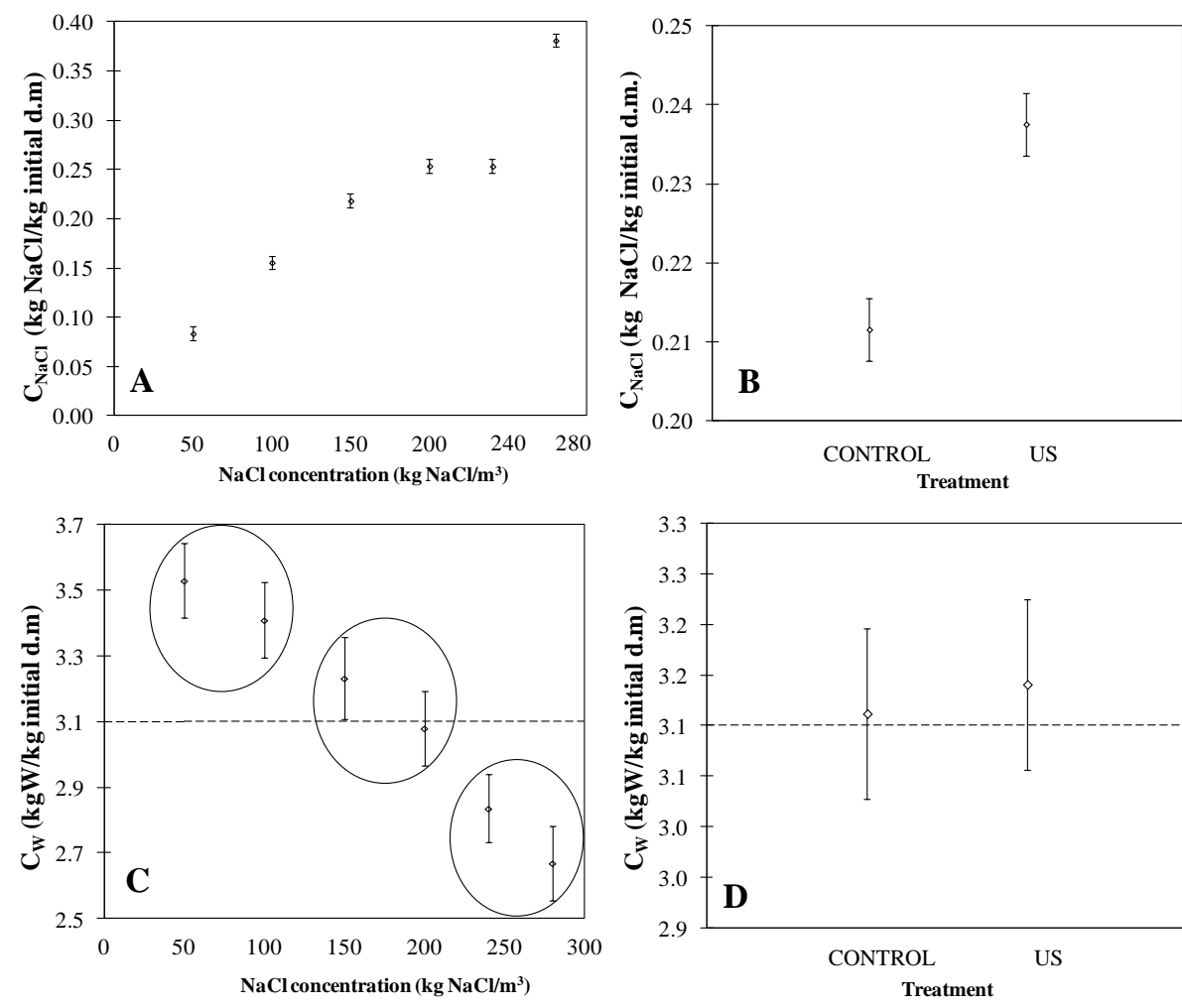


2

3

4 Fig 1. Experimental NaCl transport kinetics in pork loin slices (thickness 10 mm) brined at different NaCl concentrations with (US, 40 kHz, 37.5
 5 W/dm³) and without ultrasound application (CONTROL). In this figure, each point represents the average of 9 measurements: three
 6 independently brined samples analyzed in triplicate. Average ± standard deviation being plotted

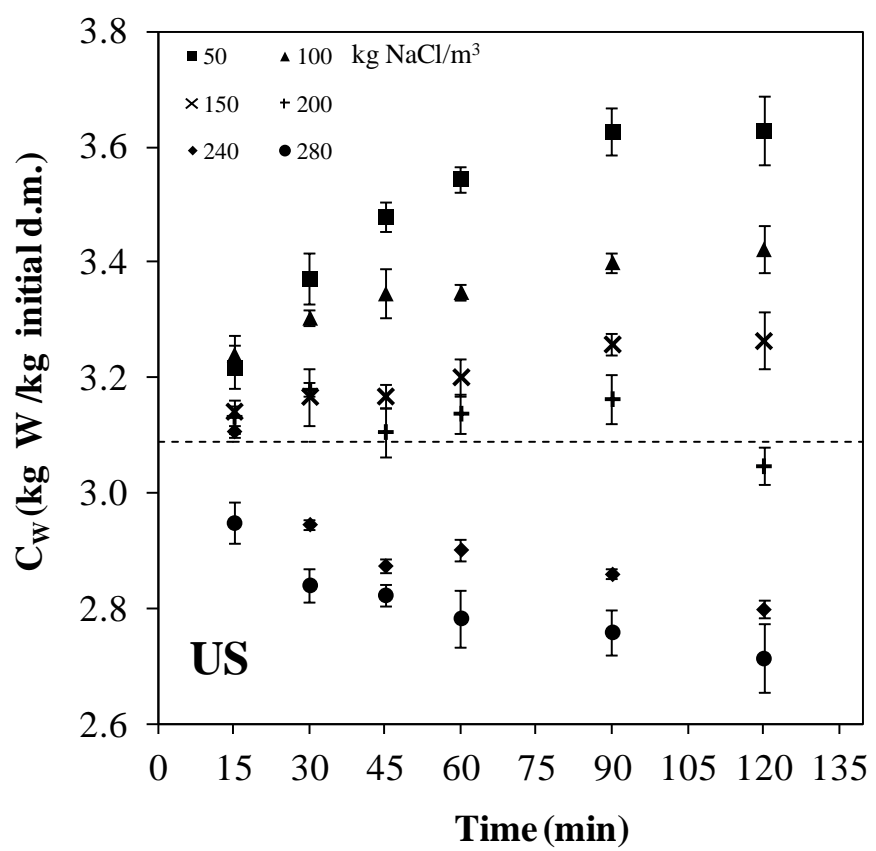
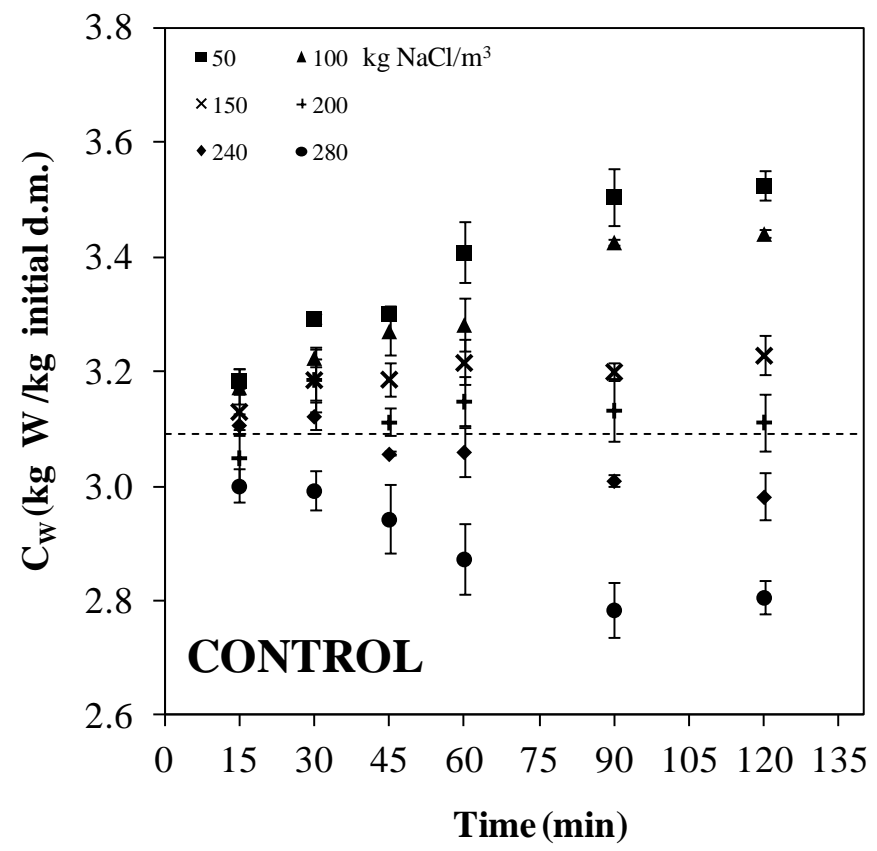
7



1

2 Fig 2. LSD intervals ($p < 0.05$) for moisture and NaCl contents of pork loin slices (thickness 10 mm) brined at 5 ± 1 °C for 120 min with different
 3 brine concentrations with (US, 40 kHz, 37.5 W/dm³) and without ultrasound application (CONTROL). Dotted line (---) represents the average
 4 initial moisture content of pork loin

1



2

3

4 Fig 3. Experimental moisture transport kinetics in pork loin slices (thickness 10 mm) brined at different NaCl concentrations with (US, 40 kHz,
5 37.5 W/dm³) and without ultrasound application (CONTROL). In this figure, each point represents the average of 9 measurements: three
6 independently brined samples analyzed in triplicate. Average ± standard deviation being plotted

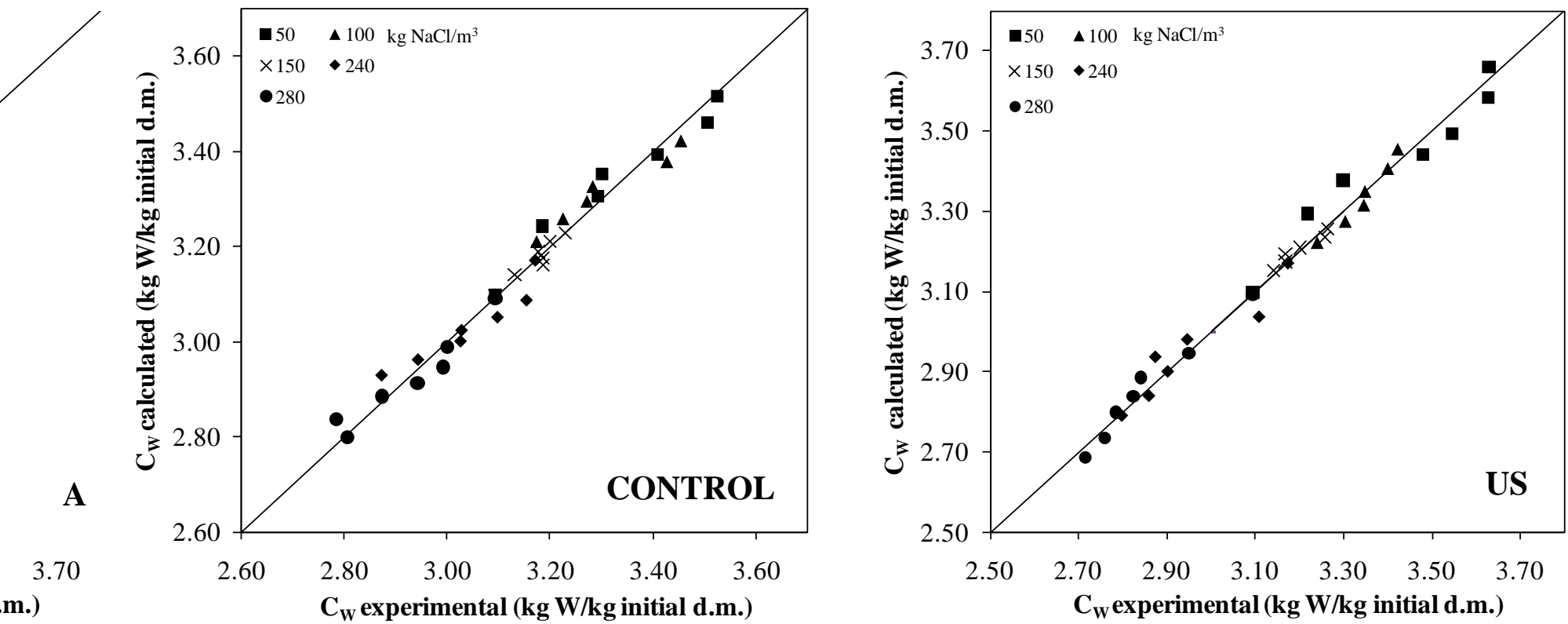


Fig 4. Experimental vs calculated moisture content of pork loin slices (thickness 10 mm) brined with (A) and without (B) ultrasound application

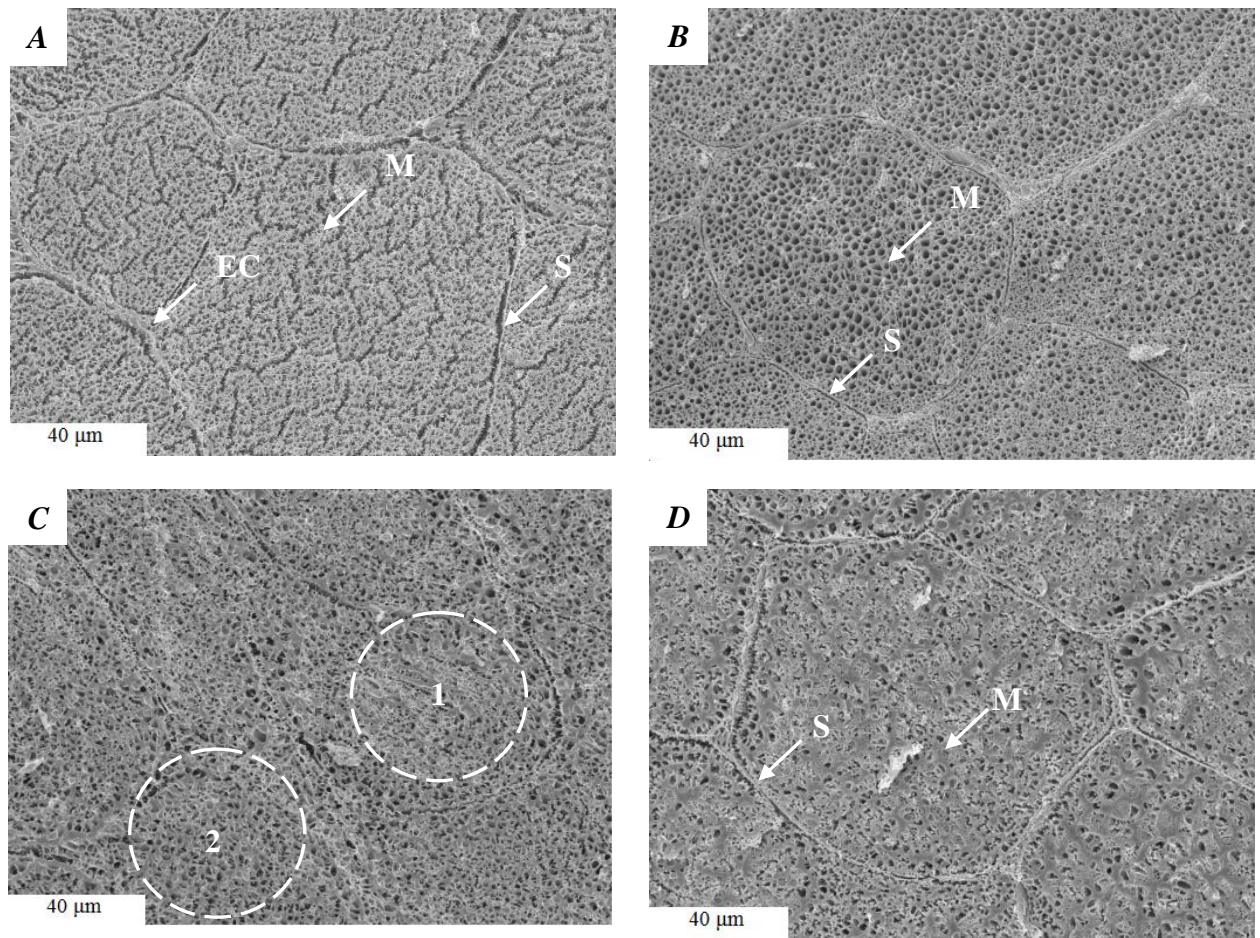


Fig 5. Cross-section of *Longissimus dorsi* muscle of pork meat observed by Cryo-SEM (x 750). Raw meat (A), meat brined for 120 min in 50 kg NaCl/m³ (B), 200 kg NaCl/m³ (D) and 280 kg NaCl/m³. S: sarcolemma, EC: endomysial connective tissue, M: myofibrils, 1: “salting-out effects”, 2: “salting-in effects”

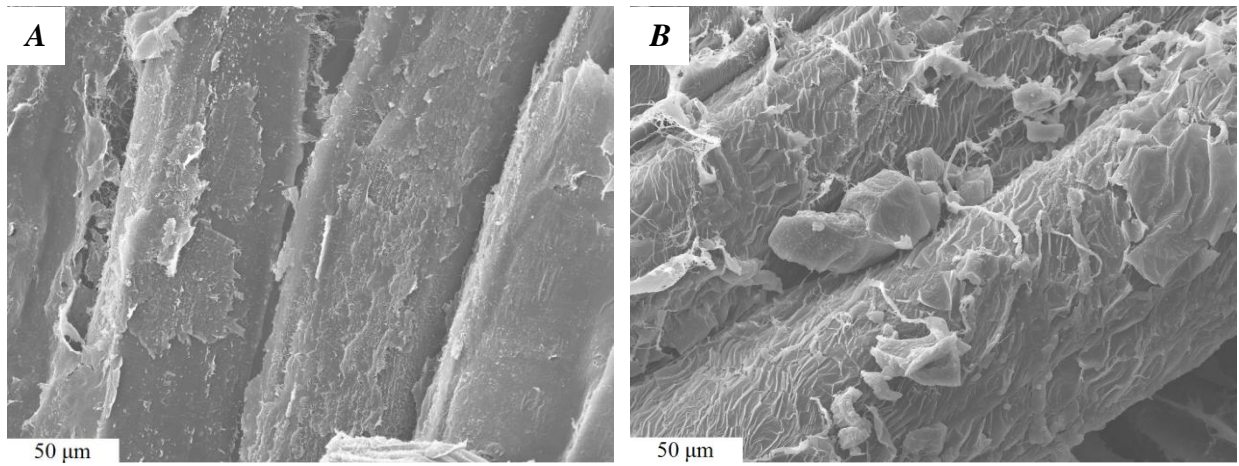


Fig 6. Longitudinal section of *Longissimus dorsi* muscle of pork meat observed by SEM (x500). Raw meat (A) and meat brined in 50 kg NaCl/m³ for 120 min (B).

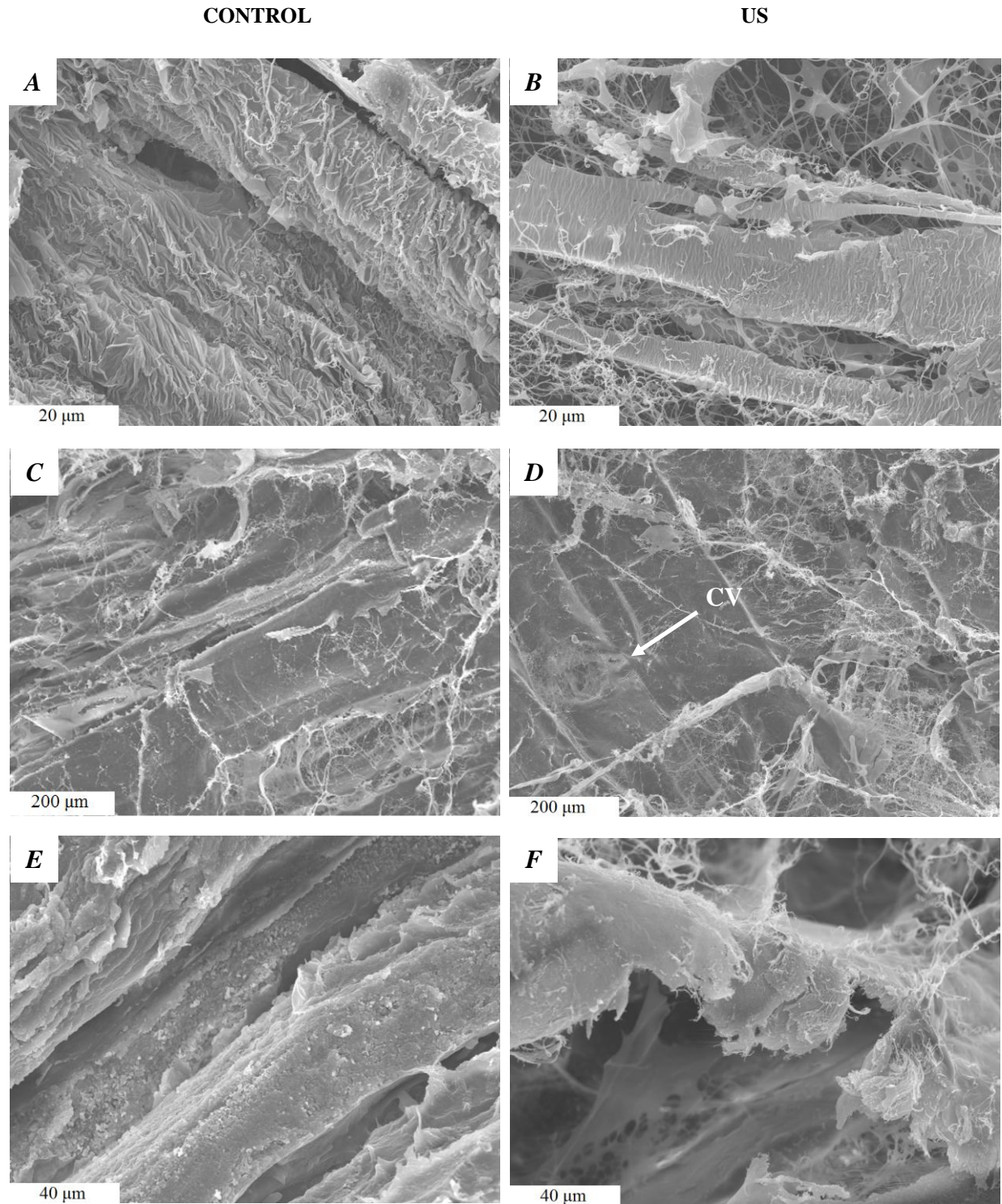


Fig 7. Longitudinal section observed by SEM of *Longissimus dorsi* muscle of pork meat brined for 120 min in 50 kg NaCl/m³ (A,B: x1500), 200 kg NaCl/m³ (C,D: x150) and 280 kg NaCl/m³ solution (D, E: x750). CV: impact of cavitation bubble implosion on myofibril

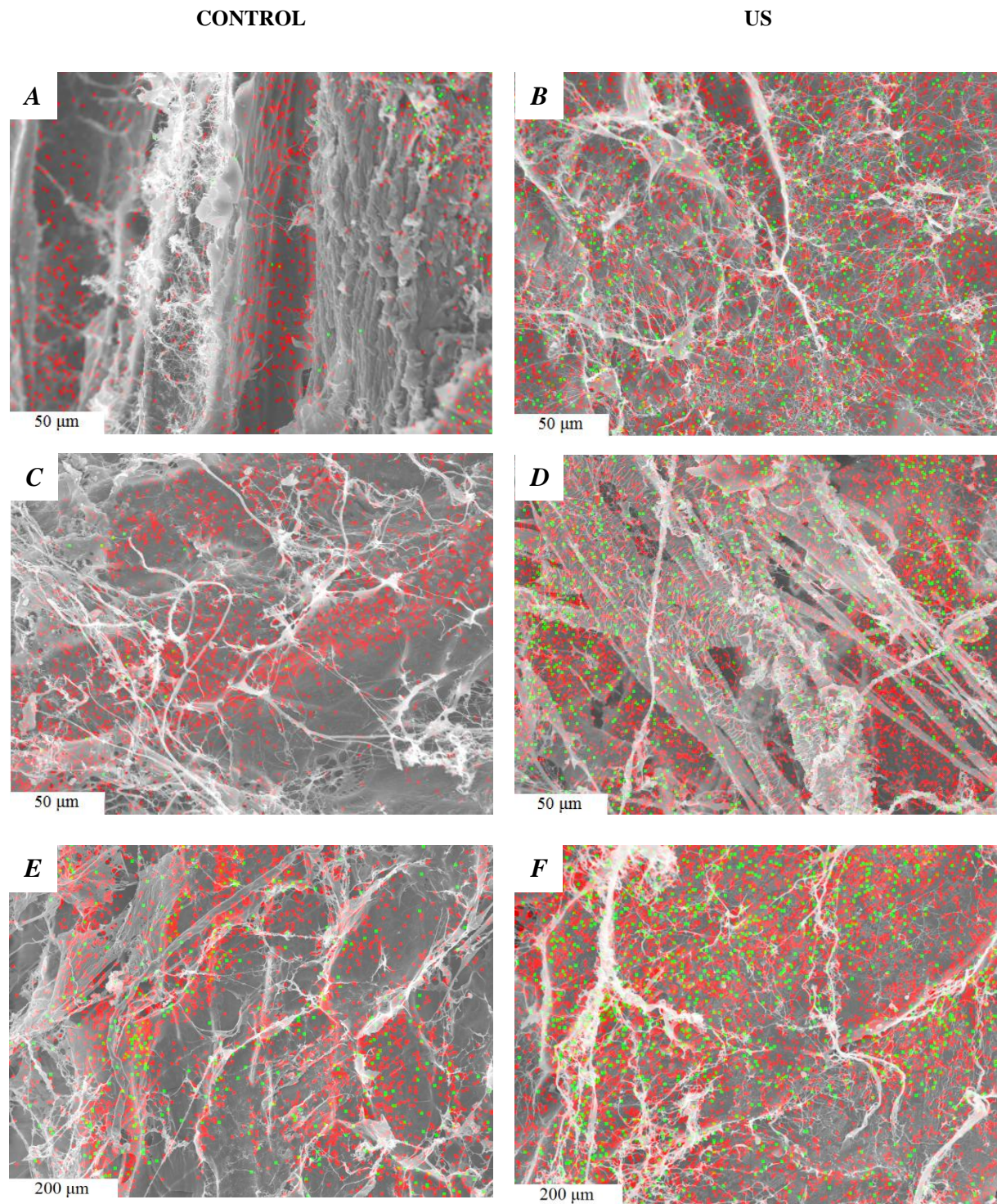


Fig 8. Effect of ultrasound application on NaCl dispersion. Longitudinal section observed by SEM-EDX of *Longissimus dorsi* of pork meat brined for 120 min in 50 kg NaCl/m³ (A,B: x500), 200 kg NaCl/m³ (C,D: x500) and 280 kg NaCl/m³ (D,E: x150)

1 **Table captions**

2

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

7

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

12

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

17

18

19 **Tables**

20

21 **Table 1**

NaCl concentration (kg/m ³)	CONTROL		US		Increment (%)
	D _s (10 ⁻¹⁰ m ² /s)	% VAR	D _s (10 ⁻¹⁰ m ² /s)	% VAR	
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

22

23 **Table 2**

NaCl concentration (kg/m ³)	CONTROL		US		Increment (%)
	D _w (10 ⁻¹⁰ m ² /s)	% VAR	D _w (10 ⁻¹⁰ m ² /s)	% VAR	
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

24

25 **Table 3**

Treatment	Hardness (N)		
	50 kg NaCl/m ³	200 kg NaCl/m ³	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

26

27



Universidad Politécnica de Valencia
Departamento de Tecnología de Alimentos
Grupo de Análisis y Simulación de procesos
C/ Camino de Vera, s/n
46022, Valencia, SPAIN

Dr. Juan A. Cárcel
Profesor Titular de Universidad
Tel: +34 96 387 93 65
Fax: +34 96 387 98 39
E-mail: jcarcel@tal.upv.es

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:

Juan A. Cárcel,
Food Technology Department
Universitat Politecnica de Valencia
C/ Camí de Vera s/n,
46022, Valencia, Spain.
Tel: +34963879365
Fax: +34963877369
E-mail: jcarcel@tal.upv.es

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

1 **Table captions**

2

3 **Table 1.** Modeling NaCl transport in pork meat brining (5 ± 1 °C) at different NaCl
4 concentrations with (US, 40 kHz, 37.5 W/dm³) and without (CONTROL) ultrasound
5 application. Effective diffusivity and percentage of explained variance.

6

7 **Table 2.** Modeling moisture transport in pork meat brining (5 ± 1 °C) at different NaCl
8 concentrations with (US, 40 kHz, 37.5 W/dm³) and without (CONTROL) ultrasound
9 application. Effective diffusivity and percentage of explained variance.

10

11 **Table 3.** Hardness (N) of pork meat brined for 120 min in different NaCl concentrations with
12 (US, 40 kHz, 37.5 W/dm³) and without (CONTROL) ultrasound application. Average \pm
13 standard deviations are shown. Subscripts (a,b,c,d) show homogeneous group established
14 from LSD intervals ($p<0.05$).

15

16

17 **Tables**18 **Table 1**

NaCl concentration (kg/m ³)	CONTROL		US		Increase (%)
	D _s (10 ⁻¹⁰ m ² /s)	% VAR	D _s (10 ⁻¹⁰ m ² /s)	% VAR	
50	1.24	98.1	1.73	97.5	40
100	2.04	99.2	2.93	96.6	44
150	2.11	99.0	2.60	98.9	23
200	1.99	96.4	2.68	95.1	35
240	1.73	96.1	2.51	93.8	45
280	1.96	96.1	2.62	99.5	34

19

20 **Table 2**

NaCl concentration (kg/m ³)	CONTROL		US		Increase (%)
	D _w (10 ⁻¹⁰ m ² /s)	% VAR	D _w (10 ⁻¹⁰ m ² /s)	% VAR	
50	0.76	90.7	1.92	91.6	153
100	0.62	90.2	1.09	94.7	76
150	0.17	89.1	0.24	92.2	41
200	-	-	-	-	-
240	1.04	81.1	2.60	88.8	150
280	1.83	90.0	3.67	95.6	101

21

22 **Table 3**

	NaCl concentration (kg/m ³)		
	50	200	280
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

23

24

25

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

4 **César Ozuna ^a, Ana Puig ^b, Jose V. García-Pérez ^a, Antonio Mulet ^a and Juan A.**
5 **Cárcel^{a,*}**

6 ^a Grupo de Análisis y Simulación de Procesos Agroalimentarios. Departamento de Tecnología
7 de Alimentos. Universitat Politècnica de València.

8 Camino de Vera. s/n. E46022. Valencia, Spain

9 ^b Grupo de Microestructura y Química de Alimentos. Departamento de Tecnología de
10 Alimentos. Universitat Politècnica de València.

11 Camino de Vera. s/n. E46022. Valencia, Spain

12

13

14

15

16

17

18

19 *Corresponding author.

20 Juan A. Cárcel.

21 Grupo de Análisis y Simulación de Procesos Agroalimentarios. Departamento de Tecnología
22 de Alimentos. Universitat Politècnica de València.

23 Camino de Vera. s/n. E46022. Valencia, Spain

24 Tel.: +34 96 387 93 65; Fax: +34 96 387 98 39

25 E-mail address: jcarcel@tal.upv.es.

26

27 **ABSTRACT**

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

39

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

41

42

43 **1. Introduction**

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

53 In liquid medium, ultrasound induces cavitation (Leighton, 1998), temperature gradients
54 within the material (Mason and Lorimer, 2002), mechanical phenomena, such as the “sponge
55 effect”, generation of microchannels and microstirring on interfaces (Muralidhara et al.,
56 1985). These effects can not only increase the mass transport kinetics but also imply structural
57 changes and, consequently, changes in textural properties. Thereby, textural changes induced
58 by ultrasound have been observed in, among others, tomato juice (Vercet et al., 2002),
59 yoghurt (Wu et al., 2000), bell peppers (Gabaldón-Leyva et al. 2007) or meat (Jayasooriya et
60 al., 2007; Pohlman et al., 1997).

61 Meat has great biochemical and structural complexity. During meat brining, changes in NaCl
62 and moisture content take place (Graiver et al., 2006), which extend meat’s shelf life and
63 modify organoleptic characteristics, such as juiciness, texture and flavor. Mass transfer
64 driving forces between meat and brine are linked to chemical gradients and NaCl-induced
65 changes in the water holding capacity (WHC) of meat proteins (Shi and Le Maguer, 2002;
66 Vestergaard et al., 2005). The NaCl concentration in brine affects the direction of moisture
67 transport as well as the equilibrium state (Cheng and Sun, 2008). A low NaCl content in the

68 meat increases the water holding capacity (WHC) (Nguyen et al., 2010), a phenomenon
69 known as "salting-in" which is linked to the meat protein net charge modification. However, a
70 high NaCl content in meat could also bring about a decrease in WHC, probably due to the
71 insolubilization of proteins ("salting-out") (Graiver et al., 2009). Therefore, the NaCl
72 concentration in the brine solution can not only affect the chemical gradients, but also the
73 WHC, thus affecting the magnitude of mass transport.

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

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

87

88 **2. Materials and methods**

89 2.1. Raw material and sample preparation

90 Fresh pork loins (*Longissimus dorsi*) were purchased at a local slaughterhouse (Valencia,
91 Spain). The pieces selected had a pH of 5.3 ± 0.30 , which was measured in-situ by means of a
92 pH-meter (pH STAR, Matthäus, Germany) at three different points along the muscle avoiding

93 fatty areas. Parallelepiped shaped samples (length 50 x width 30 x thickness 10 mm) were
94 obtained from the central part of loin pieces using a sharp knife. Before brining, samples were
95 wrapped in plastic waterproof film and kept frozen at -18 ± 0.5 °C (maximum storage time
96 120 hours).

97

98 2.2.Brining treatments

99 Brining experiments with (US, 40 kHz; 37.5 W/dm^3) and without ultrasound application
100 (CONTROL) were carried out using brine solutions of different NaCl concentrations (50, 100,
101 150, 200, 240 and 280 kg NaCl/m^3). The highest NaCl concentration used (280 kg/m^3)
102 involved brine saturation at 5 ± 1 °C, which was pointed to by the presence of NaCl crystals in
103 the solution.

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

109 Before each brining experiment, 6 meat samples were thawed at constant temperature (2 ± 1
110 °C) for 24 h. Then, the samples were blotted, weighed (PB3002-S/PH, J.P., Mettler Toledo,
111 Spain) and their size was measured by using a Vernier caliper. Afterwards, they were placed
112 in a hollow sample holder and simultaneously introduced in the brine. For a homogenous
113 brining, the position of the meat samples was changed every 5 min. Samples were taken out
114 of the brine at preset times (15, 30, 45, 60, 90 and 120 minutes) and immersed in distilled
115 water for 20 s to remove any adhered surface brine. Finally, samples were blotted, wrapped in
116 plastic waterproof film and frozen (-18 ± 0.5 °C) until moisture and NaCl measurements were
117 taken.

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

123

124 2.3. Mass transfer modeling

125 A mathematical model based on Fick's 2nd law was used to separately describe the evolution
 126 of moisture and NaCl content in the sample during brining (Cranck 1975). Samples were
 127 considered to become slab geometry bodies due to the fact that they were not nearly as thick
 128 (10 mm) as they were high (50 mm) and wide (30 mm), thus, mass transfer was simplified as
 129 a one-dimensional problem. Constant effective diffusivities (D_s and D_w), negligible changes
 130 in temperature and sample volume, solid symmetry, homogeneous NaCl and moisture initial
 131 content and negligible external resistance (Gou et al., 2003) were assumed during processing.
 132 Eqs. 1 and 2 show the solution of the diffusion model in terms of average moisture and NaCl
 133 content.

134

$$135 \quad W = W_{eq} + (W_0 - W_{eq}) \left[2 \sum_{n=0}^{\infty} \frac{1}{\lambda_n^2 L^2} e^{-D_w \lambda_n^2 t} \right] \quad \text{where, } \lambda_n L = (2n+1) \frac{\pi}{2} \quad (1)$$

$$136 \quad S = S_{eq} + (S_0 - S_{eq}) \left[2 \sum_{n=0}^{\infty} \frac{1}{\lambda_n^2 L^2} e^{-D_s \lambda_n^2 t} \right] \quad \text{where, } \lambda_n L = (2n+1) \frac{\pi}{2} \quad (2)$$

137

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

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

147

148 2.4. Textural and microstructural analysis

149 Meat texture and microstructure were studied in US and CONTROL brined samples for 120
150 minutes using low, intermediate and high NaCl concentrations (50, 200 and 280 kg NaCl/m³,
151 respectively). Samples were brined in triplicate at least.

152

153 2.4.1. Texture

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

159

160 2.4.2. Cryo-scanning electron microscopy (Cryo-SEM)

161 Cubic samples (side 3 mm) of raw and brined meat were immersed in slush Nitrogen (-210
162 °C), and quickly transferred to a cryo-trans (CT 15000 C, Oxford Instruments, England)
163 linked to a scanning electron microscope (JSM-5410, Jeol, Japan). Samples were cryo-
164 fractured at -180 °C, etched at -90 °C and gold-coated, allowing cross-section visualization.

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

167

168 2.4.3. Scanning electron microscopy (SEM) with combined dispersion X-ray analysis
169 (SEM-EDX).

170 Cubic samples (side 3 mm) from raw and brined meat were immersed in liquid N₂ and then
171 freeze-dried at 1 Pa for 3 days (LIOALFA-6, Telstar, Spain). The fixed samples were vacuum
172 sealed in vials in the same freeze-drier so that they would remain stable (Llorca et al., 2001).
173 The fixed samples were individually placed on SEM slides with the aid of colloidal silver and
174 then gold-coated with (SCD005, Baltec, Germany) at 10⁻² Pa and an ionization current of 40
175 mA. The samples were observed in a scanning electron microscope (JSM-5410, Jeol, Japan)
176 equipped with an X-ray detector and LINK data-processing system (INCA 4.09, Oxford
177 Instruments, England) at an acceleration voltage of 10-20 kV which provides internal
178 information about the standards of energy dispersive X-ray spectra of the elements analyzed
179 (Na⁺ and Cl⁻). This technique is an analytical tool that allows the ions Cl⁻ and Na⁺ inside the
180 samples to be identified (Grote and George, 1984). For EDX (energy-dispersive X-ray)
181 analysis, samples were carbon-coated (CEA035, Baltec, Germany). Mapping images of Cl⁻
182 and Na⁺ distribution in meat samples were made using a voltage of 20 kV and at a working
183 distance of 15 mm.

184

185 2.5. Fitting model evaluation and statistical analysis

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

188
$$\% \text{VAR} = \left[1 - \frac{S_{tw}^2}{S_w^2} \right] \times 100 \quad (3)$$

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

195

196 **3. Results and discussion**

197 3.1. NaCl and water transport

198 3.1.1. NaCl and water content

199 Fig. 1 shows the NaCl content of loin samples during brining, which is also considered the
200 NaCl gain, due to the fact that the NaCl content in meat samples was negligible. NaCl
201 concentration in the brine influenced significantly on NaCl content (Fig. 2A). Thus, when
202 using a brine solution of 50 kg NaCl/m³, the NaCl content in the meat (brining time 120 min)
203 was almost four times lower than when using 280 kg NaCl/m³. The NaCl gain is mainly
204 linked to osmotic mechanisms, thus the hydrodynamic flux increases as the pressure gradients
205 between the meat and brine get higher (Schmidt et al., 2008). Other factors, such as
206 temperature, pH and muscle microstructure, can also affect the NaCl gain (Barat et al., 2006).
207 Ultrasound also significantly ($p < 0.05$) influenced the NaCl gain (Fig. 2 B); as an example, for
208 a brining time of 90 min and using the highest NaCl concentration (280 kg NaCl/m³), the
209 NaCl content in the CONTROL samples was 0.315 ± 0.020 kg NaCl/kg initial d.m. while the
210 content in US samples reached 0.359 ± 0.033 kg NaCl/kg initial d.m. (Fig. 1). Among other
211 phenomena, the US application in liquid media induces cavitation, temperature gradients
212 within the material, alternative compression and decompression of the material, the
213 generation of microchannels and microstirring on interfaces, which are responsible for the

214 increased gain in NaCl. Cárcel et al. (2007a) and Gabaldón-Leyva et al. (2007) also found
215 significant differences ($p < 0.05$) in the net increase of dry matter content during the ultrasound
216 assisted osmotic treatment of apple and red bell pepper.

217 Regarding the moisture content, pork loin showed average initial moisture content of
218 3.10 ± 0.12 kg water/kg initial d.m. As can be observed in Fig. 3, the concentration of NaCl in
219 the brine solution is a key parameter in moisture transport, since it determines the direction of
220 water flux. The ANOVA carried out with samples brined for 120 min reflected that samples
221 could be classified in three significantly different groups according to the moisture content
222 (Fig. 2 C). The first group included samples brined using 50 and 100 kg NaCl/m³, which
223 showed a significant ($p < 0.05$) water gain. Samples brined using 150 and 200 kg NaCl/m³ (the
224 second group) neither lost nor gained water, having similar moisture content to raw meat.
225 This result coincided with what was reported by Graiver et al. (2009) and Nguyen et al.
226 (2010), who did not find a clear moisture transport when using brines close to 200 kg
227 NaCl/m³, either. Finally, the third group included samples brined at 240 and 280 kg NaCl/m³,
228 which underwent dehydration. During brining however, hydration or dehydration are not only
229 affected by chemical potential gradient (Shi and Le Maguer, 2002) but also by structural
230 changes brought about in the meat by salt gain (Schmidt et al., 2008). On the one side, the low
231 NaCl content increases the muscle's WHC by protein solubilization, which is known, as
232 aforementioned, the "salting-in" phenomenon (Offer and Trinick, 1983). On the other side,
233 the high NaCl content reduces the WHC and meat muscle shrinks, which is the "salting-out"
234 phenomenon (Graiver et al., 2006).

235 Regarding ultrasound effect on transport kinetics, the moisture content of US and CONTROL
236 samples brined for 120 min was not significantly ($p < 0.5$) different. (Fig. 2 D). This fact has
237 been also been observed in ultrasound assisted brining of beef muscles (Pohlman et al., 1997;
238 Jayasooriya et al. 2007) and pork meat (Siró et al. 2009). The negligible effect of ultrasound

239 on water content could be linked to the great variability in moisture content of samples (Fig.
240 3) if it is compared to the NaCl content (Fig. 1). In addition, it should be considered that the
241 ultrasound intensity may be not enough to provoke significant differences in water transport
242 due to a minim amount of ultrasonic energy is necessary in the medium, and that threshold
243 could be different for water and NaCl content. Cárcel et al., (2007 b) reported that in high
244 intensity ultrasound fields, brine could be microinjected into the meat leading to a direct
245 increase of NaCl and water content.

246

247 3.2. Modeling transport kinetics

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

260 The D_s values were similar for all the different brine concentrations tested; a lower figure was
261 only found for all NaCl concentration of 50 kg NaCl/m³, probably due to the structural
262 changes in meat samples brought about by low salt gain. The effective NaCl diffusivities
263 identified for the CONTROL samples agree closely with the values reported in the literature

264 (Graiver et al., 2006; Vestergaard et al., 2007), which actually range between $2-4 \times 10^{-10} \text{ m}^2/\text{s}$.
265 Regarding moisture transport, the D_w values identified in experiments where meat was
266 hydrated (NaCl concentration lower than $200 \text{ kg}/\text{m}^3$) decreased as the NaCl content rose.
267 Thus, in CONTROL experiments using a brining solution of $50 \text{ kg NaCl}/\text{m}^3$, the D_w was
268 $0.76 \times 10^{-10} \text{ m}^2/\text{s}$, while D_w decreased to $0.17 \times 10^{-10} \text{ m}^2/\text{s}$ for experiments at $150 \text{ kg}/\text{m}^3$, where
269 hydration was almost negligible. The same fact was also observed in US experiments and has
270 also been previously reported by Gou et al., (2003), who found that the D_w decreased when
271 the NaCl content of the salting solutions increased from 20 to $80 \text{ kg NaCl}/\text{m}^3$. On the other
272 hand, the D_w values were higher when meat was dehydrated (NaCl concentration higher than
273 $200 \text{ kg NaCl}/\text{m}^3$) than when meat was hydrated (NaCl concentration lower than 200 kg
274 NaCl/m^3) (Table 2). These differences could be ascribed to the different product structure
275 induced and controlled by NaCl transport (Schmidt et al., 2008; Gou et al., 2003; Offer and
276 Trinick, 1983). Therefore, the NaCl concentration in the brining solution is not only affecting
277 the direction of water flux (hydration or dehydration) but also the water transport rate as a
278 consequence of the structural changes brought about by the NaCl content in the meat.

279 Ultrasound application led to a significant ($p < 0.05$) improvement in both D_s and D_w , which
280 points to an acceleration of both the global brine process. The increase in D_s ranged from 23
281 to 45% and is in a similar range to other improvements reported for solid transport in the
282 literature. Thus, Siró et al. (2009) found increases of 96% in D_s meat brining and Gabaldón-
283 Leyva et al. (2007) stated an improvement of 190% in the total solid diffusion coefficients. In
284 the case of D_w , the improvement was higher than in D_s for the lowest and highest NaCl
285 concentrations used (50 and $280 \text{ kg}/\text{m}^3$) (Table 1), being in this case the improvement close to
286 100%. Gabaldón-Leyva et al., 2007 and Cárcel et al., 2007a reported increases in D_w of
287 around 128 and 117% when ultrasound was applied in bell pepper brining and osmotic
288 dehydration of apple. Smaller increases were observed in the D_w values for intermediate NaCl

289 concentration brines tested, these being 76% and 41% for 100 and 150 kg/m³, respectively.
290 The different effectiveness of ultrasound application depending on brine NaCl concentration
291 could be explained considering that the ultrasound effects on mass transport are largely
292 dependent on product structure (Gabaldón-Leyva et al., 2007). Finally, it should be remarked
293 that there was not a significant ($p < 0.05$) difference on the moisture content at the end of the
294 brining process (120 min) for CONTROL and US samples, however, the analysis of transport
295 kinetics showed an improvement on the moisture transport rate by ultrasound, which resulted
296 no significant at the end of the brining process.

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

304

305 3.3.Texture

306 In order to study the influence of ultrasound application and NaCl concentration on meat
307 texture, instrumental penetration tests were carried out in US and CONTROL samples brined
308 for 120 min using NaCl concentrations of 50, 200 and 280 kg/m³. At least 12 points were
309 measured in each meat slice.

310 CONTROL samples brined at 200 and 280 kg NaCl/m³ were significantly ($p < 0.05$) harder
311 than those brined at 50 kg NaCl/m³ (Table 3). As aforementioned, the higher the NaCl
312 concentration in the brine, the greater the NaCl gain. In such a way, the NaCl gain promoted
313 changes in meat texture, leading to harder samples, a fact already showed by Ruiz-Ramírez et

314 al. (2005). Non-significant differences ($p < 0.05$) in hardness were found in samples brined at
315 200 and 280 kg NaCl/m³ (Table 3). This indicates that the level of NaCl gained by both
316 samples was enough to produce the same change in meat texture. The effect of the NaCl
317 concentration in US samples was similar to that reported in CONTROL ones.

318 On the other hand, the application of ultrasound during brining significantly increased
319 ($p < 0.05$) the meat hardness. As already explained, ultrasound application intensified NaCl
320 transport during brining, increasing not only the NaCl diffusivity but also the final NaCl
321 content (brining time, 120 min, CONTROL 0.326 ± 0.007 kg NaCl/kg initial d.m. and US
322 $0.409.29 \pm 0.11$ kg NaCl/kg initial d.m.). So, the effects of ultrasound on meat texture could be
323 linked to the intensification of NaCl transport, which provoked structural changes in meat
324 proteins. Lee and Feng (2011) reported that the texture of ultrasound-treated food is
325 influenced by protein changes during sonication, as well as Siró et al., (2009), who linked the
326 hardening of meat tissue to the high ultrasonic intensities applied. Sanchez et al., (2001)
327 showed that US application in the brining of Mahon cheese increased the sample hardness due
328 to the improvement of proteolysis and lipolysis reactions.

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

333

334 3.4. Microstructure

335 3.4.1. Cryo-scanning electron microscopy (Cryo-SEM)

336 First of all the microstructure of raw pork loin was characterized. Fig. 5A shows a cross
337 section of the raw pork loin, where cells are surrounded by the membrane or sarcolemma
338 (Fig. 5A, S). These cells are interconnected by endomysial connective tissue (Fig. 5A, EC),

339 which keeps the muscle fibers tightly attached. In addition, the myofibrils inside the muscle
340 cell can be observed, which are fundamental components of the cell's contractile apparatus
341 (Fig. 5A, M).

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

362 Samples brined with 200 kg NaCl/m^3 (Fig. 5C), showed a mixed effect. Some parts of the
363 sample show structural dehydration due to a high NaCl concentration (Fig. 5C, 1), whereas in

364 other parts of the sample, the opposite phenomena (hydration) can be observed in the
365 myofibrillar structure (Fig. 5C, 2). As mentioned before (Fig. 3), no net transport of moisture
366 was identified at 200 kg NaCl/m³, which can be linked to the combined effect of hydration-
367 dehydration observed in the meat microstructure (Fig. 5C).

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

371

372 1.1.1. Scanning electron microscopy (SEM) with combined dispersion X-ray analysis
373 (SEM-EDX).

374 CONTROL samples brined with the lowest NaCl concentration tested (50 kg NaCl/m³, Fig.
375 6B) showed a swelling of muscle fibers (width 87.6±7.3 µm) compared to raw meat (width
376 72.5±9.1 µm) (Fig. 6A). The swelling of muscle fibers could be mainly ascribed to the
377 “salting-in” phenomena. Samples brined at higher NaCl concentrations behaved in the
378 opposite way and presented a dehydration of muscle fibers and an accumulation of NaCl;
379 these effects may be observed in Figs. 7A, C, E.

380 The SEM technique also allowed the effect brought about by high intensity ultrasound in meat
381 structure to be observed, this effect mainly focusing on myofibrils (Figs. 7B, D, F). The
382 micrograph of the US sample brined at 50 kg NaCl/m³ (Fig. 7B) shows the disruption and the
383 dispersion of the connective tissue of the fibers caused by US application. In Fig. 7F the
384 rupture of a myofibril provoked by the acoustic energy was identified. The aforementioned
385 myofibrillar changes could be explained by the alternating compressions and decompressions
386 induced by US in solid materials (“sponge effect”). Another important effect produced by
387 high intensity ultrasound in liquid media is cavitation, which may be observed in Fig. 7D,
388 where the erosion of meat fiber produced by cavitation is shown. The asymmetric implosion

389 of bubbles near the solid surface could produce violent microjets that collide with the
390 samples, which can improve mass transfer by disturbing the boundary layer and producing
391 changes in the meat structure. These results coincide with those found by several authors that
392 related the application of high power ultrasound with the physical disruption of cellular and
393 sub-cellular components (Reynolds et al., 1978), the degradation of collagen macromolecules
394 (Nishira and Doty, 1958), and the creation of micro channels (Muralidhara et al., 1985).

395 The obtained SEM-EDX mapping images confirmed the presence of NaCl in meat after
396 brining, which is shown up by the red (Cl⁻) and green (Na⁺) dots in the micrographs. As can
397 be observed in Fig. 8, the NaCl concentration in US samples (Figs. 8B, C and D) was higher
398 than those observed in CONTROL samples, as manifested by a higher number of dots in the
399 micrographs (Figs. 8A, C and E). In CONTROL samples (Figs. 8A, C and E), NaCl
400 molecules are mainly located around the myofibrils. However, the US brined samples (Figs.
401 8A, C and E) showed a more homogeneous NaCl dispersion due to the collapse of
402 myofibrillar structure caused by the effects of high power ultrasound, permitting a higher
403 NaCl penetration in the meat. The obtained SEM-EDX images showed the intensification of
404 NaCl transport brought about by US application and the increase in NaCl content, which
405 confirms the results provided by modeling and textural tools.

406

407 **4. Conclusions**

408 The NaCl concentration in the brine solution significantly ($p < 0.05$) affected moisture and
409 NaCl transport during meat brining. At NaCl concentrations of under 200 kg/m^3 , the meat was
410 hydrated while, at concentrations of over 200 kg/m^3 , it underwent dehydration. As for the
411 NaCl transport, the more concentrated the brine, the greater the NaCl sample gain. Ultrasound
412 application intensified the brining kinetics, increasing both moisture effective and NaCl
413 diffusivities. The NaCl gain promoted changes in meat texture, high NaCl contents leading to

414 harder samples. Microstructural analyses showed that the application of high intensity
415 ultrasound during brining brought about relevant effects on meat microstructure, such as a
416 more homogeneous NaCl distribution in meat. Therefore, ultrasound could be considered a
417 potential technology with which to accelerate the brining process.

418

419 **Acknowledgments**

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

422

423 **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

424

425

426 **References**

- 427 Asociación de industrias de la carne de España (AICE), available online <http://www.aice.es>.
428 Accessed on November 01, 2011.
- 429 Association of Official Analytical Chemists. Official Methods of Analysis; AOAC:
430 Washington, DC, 1997.
- 431 Barat, J.M., Grau, R., Ibáñez, J.B., Pagán, M.J., Flores M., Toldrá F., & Fito P. (2006).
432 Accelerated processing of dry-cured ham. Part I. Viability of the use of brine
433 thawing/salting operation. *Meat Science*, 72 (4), 757-765.
- 434 Cárcel, J.A., Benedito, J., Roselló, C., & Mulet, A. (2007a). Influence of ultrasound intensity
435 on mass transfer in apple immersed in a sucrose solution. *Journal of Food*
436 *Engineering*, 78 (2), 472-479.
- 437 Cárcel, J.A., Benedito, J., Bon, J., & Mulet A. (2007b). High intensity ultrasound effects on
438 meat brining. *Meat Science*, 76 (4), 611-619.
- 439 Cheng, Q., & Sun, D.-W. (2008). Factors affecting the water holding capacity of red meat
440 products: a review of recent research advances. *Critical Reviews in Food Science and*
441 *Nutrition*, 48 (2), 137-159.
- 442 Crank, J. (1975). *The Mathematics of Diffusion*. London: Oxford University Press.
- 443 Gabaldón-Leyva, C. A., Quintero-Ramos, A., Barnard, J., Balandrán-Quintana, R. R.,
444 Talamás-Abbud, R. T., & Jiménez-Castro, J. (2007). Effect of ultrasound on the mass
445 transfer and physical changes in brine bell pepper at different temperature. *Journal of*
446 *Food Engineering*, 81(2), 374–379.
- 447 Gou, P., Comaposada, J., & Arnau, J. (2003). NaCl content and temperature effects on
448 moisture diffusivity in the *Gluteus medius* muscle of pork ham. *Meat Science*, 63 (1),
449 29-34.

- 450 Graiver, N., Pinotti, A., Califano, A., & Zaritzky, N. (2006). Diffusion of sodium chloride in
451 pork tissue. *Journal of Food Engineering*, 77 (4), 910–918.
- 452 Graiver, N., Pinotti, A., Califano, A., & Zaritzky, N. (2009). Mathematical modeling of the
453 uptake of curing salts in pork meat. *Journal of Food Engineering*, 95 (4), 533-540.
- 454 Grote, M. & Georg, H. (1984) Determination of element concentrations in fresh and
455 processed vegetables by quantitative X-ray microanalysis. *Food Microstructure*, 3 (1),
456 49–54.
- 457 Jayasooriya S.D., Torley P.J., D’Arcy B.R. & Bhandari B.R. (2007). Effect of high power
458 ultrasound and ageing on the physical properties of bovine *Semitendinous* and
459 *Longissimus* muscles. *Meat Science*, 75 (4), 628-639.
- 460 Larrea, V., Pérez-Munuera, I., Hernando, I., Quiles, A., Llorca, E., & Lluch, M.A. (2007).
461 Microstructural changes in Teruel dry-cured ham during processing. *Meat Science*, 76
462 (3), 574-582.
- 463 Lee, H. & Feng, H. (2011). Effect of power ultrasound on food quality. In Feng, H., Barbosa-
464 Cánovas, G.M. & Weiss, J. (Eds.), *Ultrasound Technologies for Food and*
465 *Bioprocessing* (pp. 559–582). London: Springer.
- 466 Leighton, T. G. (1998). The principles of cavitation. In M. J. W. Povey & T. J. Mason (Eds.),
467 *Ultrasound in Food Processing* (pp. 151–182). London: Chapman & Hall.
- 468 Llorca, E., Puig, A., Hernando, I., Salvador, A., Fiszman S., & Lluch, M.A. (2001). Effect of
469 fermentation time on texture and microstructure of pickled carrots. *Journal of the*
470 *Science of Food and Agriculture*, 81 (15), 1553–1560.
- 471 Mason, T. J., & Lorimer, J. P. (2002). Applied sonochemistry. *The uses of power ultrasound*
472 *in chemistry and processing*. Weinheim: Wiley-VCH.
- 473 Muralidhara, H. S., Ensminger, D., & Putnam, A. (1985). Acoustic dewatering and drying
474 (low and high frequency): State of the art review. *Drying Technology*, 3(4), 529–566.

- 475 Nguyen, M.V., Arason, A., Thorarinsdottir, K.A., Thorkelsson, G. & Gudmundsdóttir, A.
476 (2010). Influence of salt concentration on the salting kinetics of cod loin (*Gadus*
477 *morhua*) during brine salting. *Journal of Food Engineering*, 100 (2), 225-231.
- 478 Nishira, T., & Doty, P. (1958). The sonic fragmentation of collagen macromolecules.
479 *Proceedings of the National Academy of Sciences of the United States of America*,
480 44(5), 411-417.
- 481 Offer, G. & Trinick, J. (1983). On the mechanism of water holding in meat: The swelling and
482 shrinking of myofibrils. *Meat Science*, 8 (4), 245-281.
- 483 Pérez-Munuera, I., Larrea, V., Quiles, A., & Lluch, M.A. (2008). Microstructure of muscle
484 foods. In L.M.L., Nollet & F. Toldrá (Eds.), *Handbook of muscle food analysis* (pp.
485 335-352). Boca Raton FL: CRC Press Taylor & Francis Group.
- 486 Pohlman, F.W, Dikeman M.E., & Kropf D.H. (1997). Effects of high intensity ultrasound
487 treatment, storage time and cooking method on shear, sensory, instrumental color and
488 cooking properties of packaged and unpackaged beef pectoralis muscle, *Meat Science*,
489 46 (1), 89–100.
- 490 Rastogi, N.K., Raghavarao, K.S.M.S., Niranjana, K., & Knorr, D. (2002). Recent
491 developments in osmotic dehydration: methods to enhance mass transfer. *Trends in*
492 *Food Science & Technology*, 13 (2), 48-59.
- 493 Reynolds, J.B., Anderson D.B., Schmidt, G.R., Theno, D.M., & Siegel D.G. (1978). Effects
494 of ultrasonic treatment on binding strength in cured ham rolls. *Journal of Food*
495 *Science*, 43 (3), 866-869.
- 496 Ruiz-Ramírez, J., Arnau, J., Serra, X., & Gou, P. (2005). Relationship between water content,
497 NaCl content, pH and texture parameters in dry-cured muscles. *Meat Science*, 70 (4),
498 579-587.

- 499 Sanchez, E. S., Simal, S., Femenía, A., Benedito, J. & Roselló, C. (2001). Effect of acoustic
500 brining on lipolysis and on sensory characteristics of Mahon cheese. *Journal of Food*
501 *Science*, 66(6), 892-896.
- 502 Schmidt, F.C., Carciofi, B.A.M. & Laurindo, J. B. (2008). Salting operational diagrams for
503 chicken breast cuts: Hydration-dehydration. *Journal of Food Engineering*, 88 (1), 36-
504 44.
- 505 Shi J. & Le Maguer M. (2002). Osmotic dehydration of foods: mass transfer and modelling
506 aspects. *Food Reviews International*, 18(4), 305-335.
- 507 Siró, I., Vén, C., Balla, C., Jónás, G., Zeke, I., & Friedrich, L. (2009). Application of an
508 ultrasonic assisted curing technique for improving the diffusion of sodium chloride in
509 porcine meat. *Journal of Food Science*, 91 (2), 363-362.
- 510 Vercet, A., Sánchez, C., Burgos, J., Montañés, L., & Buesa, P. L. (2002). The effects of
511 manothermosonication on tomato pectic enzymes and tomato paste rheological
512 properties. *Journal of Food Engineering*, 53 (3), 273–278.
- 513 Vestergaard, C., Lohmann Andersen, B., & Adler-Nissen, J. (2007). Sodium diffusion in
514 cured pork determined by ²²Na radiology. *Meat Science*, 76 (2), 258–265.
- 515 Vestergaard, C., Risum, J., & Adler-Nissen, J. (2005). ²³Na-MRI quantification of sodium and
516 water mobility in pork during brine curing. *Meat Science*, 69 (4), 663-672.
- 517 Wu, H., Hulbert, G. J., & Mount, J. R. (2000). Effects of ultrasound on milk homogenization
518 and fermentation with yogurt starter. *Innovative Food Science and Emerging*
519 *Technologies*, 1 (13), 211–218.
- 520
- 521