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1 **CORRECTION OF DEFECTIVE TEXTURES IN PACKAGED DRY-CURED PORK**
2 **HAM BY APPLYING CONVENTIONAL AND ULTRASONICALLY-ASSISTED MILD**
3 **THERMAL TREATMENTS**

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19

20 **Abstract**

21 Pastiness is a textural defect characterized by an excessive softness and loss of
22 elasticity which lacks corrective actions at industrial level. The objective of this study
23 was to evaluate the textural and microstructural changes of dry-cured pork ham, with
24 different pastiness levels, subjected to conventional and ultrasonically-assisted
25 corrective mild thermal treatments. Pastiness was assessed by an expert sensory
26 panel and hams were classified into three categories: high (HP), medium (MP) and no
27 (NP) pastiness. Ham samples (n=108) were heated (40 and 50 °C) with power
28 ultrasound (PuS) and without (CV) PuS application. After heating, all of the textural
29 parameters assessed were improved. Hardness increased by 102% and adhesiveness
30 decreased by 55% and the ham became less viscoelastic. The largest modifications
31 were found in the samples heated at 50 °C and no differences were found between CV
32 and PuS treatments. The microstructure of pasty samples revealed that the treatment
33 produced a shrinkage of the myofibrils, which could explain the increase in hardness
34 and the improvement in texture of defective ham.

35 **Keywords:** Dry-cured ham; texture; microstructure; heating; ultrasound.

36

37 **1. Introduction**

38 During dry-cured ham processing, there are many factors such as temperature, pH,
39 muscle type, water content and availability or salt content, among others, which affect
40 the development of the product's characteristic texture (Bermúdez, Franco, Carballo, &
41 Lorenzo, 2014). In this regard, low or high pH in the raw ham, low salt contents, high
42 temperatures and a short resting period may induce defective textures (Arnau et al.,
43 1998; Garcia-Garrido et al., 1999). The most relevant textural defects are softness and
44 pastiness, which influence negatively the consumer acceptance of dry-cured ham and
45 also promote technological problems, such as the adhesiveness. Recently, Contreras
46 et al. (2020) characterized the defect of pastiness using conventional techniques, such
47 as instrumental texture, chemical and microstructural analysis, and also non-
48 destructive ultrasonic testing. Diverse studies have reported that at the end of the ham
49 processing the extension of the aging stage and a slight temperature increase could
50 reduce the intensity of these textural defects. In this sense, Cilla, Martínez, Beltrán, &
51 Roncalés, (2005) stated that extending ham maturation time to 20 months (18 °C, 75%
52 RH) increased hardness and decreased adhesiveness. Similarly, Gou, Morales, Serra,
53 Guàrdia, & Arnau (2008) confirmed that including a final aging stage at 30 °C and low
54 relative humidity (40-45%) during the last 10 days of ham manufacturing could improve
55 its texture. While, Morales et al. (2008) tested in sections of dry-cured ham (4 cm thick)
56 a slight temperature increase (30 °C) during a short storage (30 days) that involved a
57 decrease in softness, pastiness and adhesiveness in BF muscle. The main drawback
58 of these approaches was the long time employed for texture correction. Additionally,
59 high hydrostatic pressure (HHP) treatments were also tested to improve the ham
60 texture. In this sense, Garcia-Gil et al. (2014) found that the HHP treated ham (500
61 MPa) was harder and presented more elastic behavior. Likewise, Coll-Brasas et al.
62 (2019) identified an increase in hardness and a decrease in pastiness in dry-cured
63 hams with different levels of pastiness after HHP treatment (600 MPa), which was
64 more intense as the treatment temperature rose. In this regard, the use of HHP at the

65 end of the processing of dry-cured ham could help to improve its texture in addition to
66 eliminating pathogenic microorganisms and extending its shelf-life. However, the
67 implementation of HHP at industrial level is constrained by its high cost compared to
68 other more affordable alternatives.

69 The feasibility of using mild temperatures, from 40 to 50 °C, and short treatment times
70 has recently been explored in order to bring about texture modifications in dry-cured
71 ham in both air (Contreras, Benedito, Bon, & Garcia-Perez, 2018a) and water medium
72 (Contreras, Benedito, Bon, & Garcia-Perez, 2018b). The mild thermal treatment
73 induced an increase in the sample hardness; thus, the higher the treatment
74 temperature, the harder the ham. Contreras et al. (2018a and 2018b) used a small
75 number of samples of commercial dry-cured hams without textural defects, since the
76 main aim of both studies was to test the feasibility of power ultrasound (PuS) to
77 accelerate the heating process (Lacivita et al., 2018; Sun et al., 2019). In addition,
78 samples were heated only until they reached a target temperature defined as 5 °C
79 below the temperature of the heating medium. Thus, very short treatment times were
80 applied, ranging from 16 to 24 min, depending on the sample size, air-water medium
81 and temperature used. In this way, it should be expected that an additional holding
82 phase at the heating medium temperature could promote a greater modification of the
83 texture than that found in the aforementioned studies. Other previous studies
84 addressing corrective actions for textural enhancement based on a slight temperature
85 increase have also tested a limited number of samples (Garcia-Gil et al., 2014; Morales
86 et al., 2008). In this sense, a more exhaustive experimentation, using a large number of
87 samples with a wide range of pastiness values is necessary to evaluate the
88 performance of the corrective action depending on the initial product properties, which
89 constitutes an approach never addressed before to our knowledge. Therefore, the aim
90 of this study was to assess the textural and microstructural modifications undergone by
91 dry-cured ham with different levels of pastiness subjected to mild thermal treatments
92 using conventional and PuS heating systems.

93 **2. Materials and methods**

94 *2.1. Raw material*

95 Dry-cured hams (n=108) from Large White and Landrace animal breed crosses were
96 used. Dry-cured ham manufacturing was modified as described in detail by Contreras
97 et al. (2020) to induce pastiness over a wide intensity range. At the end of the
98 processing, the cushion part of dry-cured hams was sliced into different formats. Thin
99 slices (thickness 1.5 mm) were used for sensory pastiness evaluation and
100 microstructural analyses, and packages of 4 slices were prepared for the adhesiveness
101 test. Thicker slices (thickness 20 mm), meanwhile, were used for hardness and
102 elasticity tests and ultrasonic analysis. Afterwards, all the samples were vacuum-
103 packed in individual plastic bags of polyamide/polyethylene (oxygen permeability of 50
104 cm³/m²/24h at 23 °C and water permeability of 2.6 g/m²/24h at 23 °C and 85% RH,
105 Sacoliva® S.L., Spain). From each ham piece, two thick slices (20 mm) and 2
106 packages of thin slices (1.5 mm) were used. Thereby, the destructive instrumental
107 textural tests could be performed in identical samples before (control) and after the
108 thermal treatment. Finally, packaged samples were stored in a chamber at 4±2 °C until
109 the experiments were performed.

110

111 *2.2. Sensory texture analysis*

112 A three-member expert panel, trained following the American Society for Testing and
113 Materials standards (ASTM, 1981), performed the sensory texture analysis on dry-
114 cured ham slices (thickness 1.5 mm). The textural attribute evaluated in BF muscle
115 was pastiness, which can be defined as a feeling similar to the mouth-coating
116 sensation produced by flour-water paste during the mastication process. Dry-cured
117 ham slices presented different levels of pastiness. The levels of ham pastiness were
118 ranked from 0 (absence) to 6 (maximum intensity). The pastiness level of the samples
119 was set as the average score of the three experts' scores. Thus, dry-cured hams were

120 classified according to the textural defect into samples with no pastiness (pastiness<1),
121 medium pastiness (pastiness between 1-2.5) and high pastiness (pastiness>2.5). For
122 every level of pastiness, 36 samples were selected.

123

124 *2.3. Mild thermal treatments*

125 Mild thermal treatments were carried out by placing packaged samples into a
126 temperature controlled water bath following the same methodology already described
127 by Contreras et al. (2018a). Two different temperatures were tested (40 and 50 °C) and
128 the treatment time was 5 h in both cases. In conventional thermal treatments (CV), a
129 mechanical stirrer was used to improve the liquid turbulence, while ultrasonically
130 assisted treatments (PuS) were carried out in an ultrasonic bath (600 W, 20 kHz)
131 supplied with a custom temperature control (Contreras et al., 2018a). Ultrasound was
132 only applied during the heating phase, which represents the time needed to reach a
133 temperature of 5 °C below the temperature of the heating medium in the center of the
134 slice. The duration of the heating phase was determined by the mathematical model
135 proposed by Contreras et al. (2018a) for dry-cured ham slices. Once the heating phase
136 finished, the ultrasound generator was switched off and samples were held at the pre-
137 set temperature until completing a total treatment time of 5 h. During this holding
138 phase, the same mechanical stirrer as in CV experiments was used.

139 Every experimental condition (40-50 °C, CV-PuS) was tested in dry-cured ham with
140 different pastiness levels (high, medium and no pastiness). For each pastiness level, 9
141 slices (20 mm thick) and 9 packages (containing 4 slices, 1.5 mm thick per package)
142 were thermal treated (CV and PuS assisted) at 40 and 50 °C, which makes 108
143 treatments for each sample thickness (slices 20 mm thick and sliced packages).

144 A preliminary test was conducted in order to choose the appropriate duration of the
145 heat treatments. The objective of this test was to obtain the largest textural
146 modifications without inducing a cooked flavor or appearance in the dry-cured ham. For

147 that purpose, 18 cylinders (diameter 2.52 ± 0.11 cm and height 1.9 ± 0.14 cm) from
148 commercial dry-cured hams were heated with (PuS) and without (CV) ultrasound
149 application at 50 °C modifying the total treatment time: 1, 3 and 5 h. Each experiment
150 was replicated at least three times. Finally, the initial hardness of the samples (F_i),
151 which was used as control, was compared to the final hardness (F_f) after the treatment
152 (Fig. 1). The experimental results from the preliminary test showed that the hardness
153 ratio (F_f/F_i) in CV and PuS treatments ranged from 2.3 ± 0.8 to 3.3 ± 0.8 when heating for
154 1 and 5 h, respectively (Fig. 1). A hardness ratio above one indicated that softness,
155 which is one of the main consequences of pastiness, was reduced. Although there was
156 a considerable dispersion in the hardness ratio, probably due to the heterogeneity of
157 the commercial dry-cured ham used, it could be observed that the hardness ratio
158 increased as the treatment time lengthened in the case of both CV and PuS
159 experiments. Longer treatment times (6, 7 and 10 h) were also evaluated in preliminary
160 tests but were discarded since they caused the appearance of cooking flavors. For that
161 reason, the treatment time chosen to analyze the improvement in the textural
162 properties of dry-cured ham brought about by mild thermal treatments was 5 h.

163

164 *2.4. Instrumental texture analysis*

165 In order to evaluate the changes caused by the heat treatment in dry-cured ham
166 texture, different properties (hardness, elasticity and adhesiveness) were measured
167 before (X_i) and after (X_f) heating. The ratio between the final and the initial textural
168 property (X_f/X_i) was computed in order to standardize and make reliable comparisons
169 between treatments with samples of different initial textural properties.

170

171 *2.4.1. Hardness and elasticity*

172 Hardness and elasticity were measured using a texturometer (TA-XT2, SMS,
173 Godalming, UK) provided with a load cell of 50 kg. From the slices of 20 ± 4 mm, 5

174 parallelepipeds of BF muscle were carved (20 mm length x 20 mm width x 15 mm
175 height). Stress-relaxation tests were carried out at a constant temperature (4 ± 1 °C)
176 using a flat 75 mm diameter aluminum probe (SMS P/75). The samples were
177 compressed to 25% of their original height perpendicular to the fiber bundle direction at
178 a crosshead speed of 1 mm/s and, afterwards, the probe was held for 90 s to monitor
179 relaxation. The experimental data were recorded and processed with Exponent Lite
180 6.1.4.0 software (SMS, Godalming, UK). Thus, hardness (F) was computed from the
181 force versus time profiles as the maximum force achieved during compression, while
182 elastic behavior was indirectly assessed by computing the force decay, Y_t , logged
183 during relaxation since its increase reflects a more viscoelastic behavior (Eq. 1):

$$184 \quad Y_t = \frac{F_{\max} - F_t}{F_{\max}}$$

185 (1)

186 where F_{\max} is the maximum force during compression (N) and F_t is the force recorded
187 after t seconds of relaxation. Y_t was calculated after 2 s of the relaxation period (Y_2)
188 and at the end of the stress-relaxation test (90 s, Y_{90}).

189

190 2.4.2. Adhesiveness

191 The adhesiveness was analyzed using a texturometer (TA-XT Plus, SMS, Godalming,
192 UK) provided with a load cell of 0.5 kg following the methodology proposed by López-
193 Pedrouso et al. (2018). From a dry-cured ham package containing 4 slices (1.5 mm
194 thick), these were separated one by one in order to measure adhesiveness. The probe
195 was placed at one end of the slice and displaced horizontally (100 mm) at a crosshead
196 speed of 5 mm/s, detaching both slices. The adhesiveness measurements were carried
197 out at a constant temperature (20 ± 2 °C). The experimental data were recorded and
198 processed with Exponent Lite 6.1.4.0 software (SMS, Godalming, UK). Thus,
199 adhesiveness was computed from the force versus time profiles as the maximum force

200 achieved during the separation test with a single-cycle. For each package, three
201 measurements were taken.

202

203 *2.5. Microstructure*

204 The dry-cured ham microstructure was analyzed using two microscopic techniques:
205 light microscopy (LM) and transmission electron microscopy (TEM). Between 4-5
206 different samples per level of pastiness were randomly chosen and analyzed. Thus,
207 from slices 1.5 mm thick, small sections (5 x 3 mm) from BF muscle were cut with a
208 disposable blade. In order to include the sections, samples were fixed with a 25 g/L
209 glutaraldehyde solution (0.025M phosphate buffer, pH 6.8, at 4 °C, 24 h), postfixed with
210 a 20 g/L OsO₄ solution (1.5 h), dehydrated using a graded acetone series (300, 500,
211 700 and 1000 g/kg), contrasted in 40 g/L uranyl acetate dissolved in acetone and
212 embedded in epoxy resin (Durcupan, Sigma–Aldrich, St. Louis, MO, USA). The
213 samples were cut using a Reichert Jung ultramicrotome (Leica Microsystems, Wetzlar,
214 Germany). Thin sections (1.5 µm) were stained with 2 g/L toluidine blue and examined
215 in a Nikon Eclipse E800 light microscope (Nikon, Tokyo, Japan). Ultrathin sections (0.5
216 µm) were stained with 40 g/L lead citrate and observed in a Philips EM400 (Philips,
217 Eindhoven, Holland) transmission electronic microscope at 80 kV. Dry-cured ham
218 samples with high, medium and no pastiness were observed before and after PuS heat
219 treatment at 50°C.

220

221 *2.6. Statistical analysis*

222 One-way analysis of variance (ANOVA) ($p < 0.05$) was performed to assess the
223 influence of the type of thermal treatment (CV-PuS) on the textural parameters of
224 treated samples. Likewise, multifactor ANOVA ($p < 0.05$) was performed in order to
225 evaluate the influence of the temperature (40-50 °C) and the level of pastiness intensity
226 (high, medium and no pastiness) and also whether their interactions had a significant

227 influence on every measured textural parameter. ANOVAs and least significant
228 difference (LSD) intervals were estimated using the statistical package Statgraphics
229 Centurion XVI (Statpoint Technologies Inc., Warrenton, VA, USA) considering a
230 significance level of 95%.

231

232 **3. Results and discussion**

233 *3.1. Effect of mild thermal treatment on dry-cured ham texture*

234 *3.1.1. Influence of PuS application*

235 The present study is exploring whether the previously reported kinetic improvement of
236 PuS during the heating phase (Contreras et al., 2018a) was coupled to an additional
237 textural modification by testing dry-cured hams over a wide range of pastiness
238 intensities. Table 1 shows the ratios of the different textural parameters analyzed
239 (hardness, elasticity and adhesiveness) before and after heat treatment for both CV
240 and PuS experiments. There were not any statistical difference ($p>0.05$) for the
241 analyzed parameters between CV and PuS. The negligible effect of PuS was already
242 anticipated by the preliminary test carried out to determine the duration of the thermal
243 treatment (Fig. 1). This fact could be explained by considering that the ultrasound
244 application was restricted only to the heating phase, which only represents a short time
245 (7.5-11 min) compared to the duration of the whole treatment (5 h). Therefore, although
246 the use of PuS during mild thermal treatments could be used to speed-up the heating
247 phase, allowing the desired temperature in the ham slice to be reached faster
248 (Contreras et al., 2018a), it does not induce additional textural changes to the one
249 caused by the heating itself. Previous studies reported similar results; thus, Lyng, Allen,
250 & McKenna (1997, 1998) confirmed that the texture of sonicated beef was not changed
251 by ultrasonic treatment. Notwithstanding this, different studies have also demonstrated
252 the feasibility of using PuS for improving meat tenderness in different products, such as
253 poultry (Xiong et al., 2012) or beef (Kang et al., 2017). This contrary effect could be

254 related to the energy applied, since long, high power treatments may cause a reduction
255 in hardness. Future research should be conducted in order to elucidate whether
256 extending ultrasonic application to the holding phase during the thermal treatment
257 could bring about some textural modifications in dry-cured ham.

258

259 *3.1.2. Influence of the thermal treatment on hardness*

260 The computed hardness ratio (F_f/F_i) constitutes a simple way of assessing if hardness
261 increased ($F_f/F_i > 1$) or decreased ($F_f/F_i < 1$) after the treatments. Fig. 2 shows the
262 relationship between the level of pastiness and its hardness ratio for each of the 108
263 samples under study; these were grouped according to the temperature applied (40 or
264 50 °C) since, as already mentioned, the effect of ultrasound was statistically negligible
265 ($p > 0.05$). It has to be remarked that 98% of the hardness ratios at 50 °C were over one,
266 indicating that heating caused an overall increase in hardness. However, when
267 samples were heated at 40 °C, the hardness ratio was scattered around one. Thus,
268 only 62% of the samples heated at 40 °C presented a ratio of more than one. Thereby,
269 an average hardness ratio of 1.22 ± 0.51 was found for the samples heated at 40 °C,
270 which was significantly ($p < 0.05$) smaller than that found at 50 °C, 2.72 ± 0.85 (Fig. 2).
271 Therefore, the temperature played a relevant role in the increase in hardness provoked
272 by the mild thermal treatment. These results agree with those previously reported by
273 Morales et al. (2008), who stored BF muscle parallelepipeds (20 x 20 x 15 mm)
274 wrapped in film for 24 h at temperatures from 4 to 46 °C. They found that the hardness
275 values increased from 17.3 to 26.9 N when the temperature rose from 36 to 46°C.

276 As observed in Fig. 2, level of pastiness did not have a statistically significant ($p > 0.05$)
277 effect on the hardness ratio; notwithstanding this, at 50 °C it was observed that the
278 highest ratios belonged to the sample group with medium pastiness (Table 1).
279 Therefore, for each temperature, ham samples experienced a similar relative variation
280 in hardness when subjected to the mild thermal treatment, regardless of their initial

281 pastiness. There have been no previous references to the impact caused by the mild
282 thermal treatments on the textural attributes of samples differing in pastiness intensity.

283

284 3.1.3. Influence of the thermal treatment on elastic behavior

285 The material relaxation when subjected to prior compression stress was analyzed as
286 an indicator of elastic behavior since an ideal elastic material would have a force decay
287 of 0. Thereby, the higher the force decay, the more relevant the viscoelasticity (Eq. 1).

288 Fig. 3 plots the $Y_{90,f}/Y_{90,i}$ ratio according to the pastiness of every dry-cured ham

289 slice, showing the same pattern as the one found for the $Y_{2,f}/Y_{2,i}$ ratio (data not

290 shown). At 50 °C, 100% of the treated samples showed a $Y_{90,f}/Y_{90,i}$ ratio of under one,

291 which points to the fact that elasticity increased after the treatment. Otherwise, 90% of

292 dry-cured ham samples heated at 40 °C presented a $Y_{90,f}/Y_{90,i}$ ratio of below one. The

293 average values of $Y_{90,f}/Y_{90,i}$ were 0.96 ± 0.04 and 0.86 ± 0.06 at 40 and 50 °C,

294 respectively. The lower value of $Y_{90,f}/Y_{90,i}$ at 50 °C reflects the fact that the treatment

295 at this temperature was more effective at improving elasticity than at 40 °C. The effect

296 of temperature is also shown in Table 1 since, for the three levels of pastiness and for

297 both CV and PuS, $Y_{90,f}/Y_{90,i}$ and $Y_{2,f}/Y_{2,i}$ ratios were always significantly ($p<0.05$)

298 lower at 50 than at 40 °C. In the aforementioned study published by Morales et al.

299 (2008), a reduction of Y_{90} in ham was found as the treatment temperature increased

300 from 36 (0.621) to 46 °C (0.575). Gou et al. (2008) also reported a reduction of Y_2 in

301 ham when the ageing temperature was increased from 18 (0.339) to 30 °C (0.318),

302 which again supports the experimental results shown in Fig. 3. Therefore, the 5 hour-

303 long mild thermal treatment at 50 °C emerges as a simple and reliable means of

304 correcting softness and elasticity loss of dry-cured ham. Thus, heating would improve

305 not only consumer perception during mastication but also industrial slicing.

306 The statistical analysis revealed that both the temperature and the level of pastiness
307 had a significant effect on $Y_{90,f}/Y_{90,i}$ ratio ($p<0.05$) (Table 1). When samples were
308 grouped into three levels of pastiness (Table 1), it was found that the $Y_{90,f}/Y_{90,i}$ ratio
309 was the lowest in the group with no pastiness and the highest in the group with high
310 pastiness, while intermediate values were found for the samples with medium
311 pastiness. As an example, at 50 °C in PuS experiments, the $Y_{90,f}/Y_{90,i}$ ratio ranged
312 from 0.81 ± 0.03 in the group with no pastiness to 0.92 ± 0.03 in the one with high
313 pastiness, 0.84 ± 0.03 being the ratio for samples with medium pastiness. The same
314 behavior was found for the $Y_{2,f}/Y_{2,i}$ ratio, as illustrated in Table 1. Therefore, the
315 capacity of the mild thermal treatment to improve elastic behavior was moderately
316 reduced as the pastiness increased. This could be due to the more intense effect of the
317 thermal treatment on the proteins of non-pasty samples, since they retain the native
318 structure (Coll-Brasas et al., 2019).

319

320 *3.1.4. Influence of the treatment on adhesiveness*

321 The modification of adhesiveness brought about by the thermal treatment was
322 computed by instrumental texture analysis. Mild thermal treatment led to relevant
323 modifications of adhesiveness; thus, its ratio (A_f/A_i) was below one for every sample
324 (Fig. 4). This indicates that slice adhesiveness was reduced after heating, regardless of
325 the conditions. Morales et al. (2008) also found a decrease in the adhesiveness of dry-
326 cured ham BF muscle after a 168 h thermal treatment at 30 °C. Similarly, Pérez-
327 Santaescolástica et al. (2018) also reported a decrease in adhesiveness between
328 control (0.84 N) and conventionally heated ham samples (0.38 N). Unlike the trend
329 observed in hardness and elasticity, a multifactor ANOVA showed that neither the
330 temperature nor the level of pastiness influenced the adhesiveness ratio significantly
331 ($p>0.05$). What should be highlighted is the great performance of the mild thermal

332 treatment in the reduction of slice adhesiveness, since the ratios were around 0.5 in
333 every case (Table 1). This confirms that adhesiveness was reduced by 50%, which
334 represents an excellent result since adhesiveness is one of the main issues related to
335 consumer rejection of pasty dry-cured ham. The adhesiveness decrease after the mild
336 thermal treatment could be explained by denaturation and other structural changes in
337 the proteins.

338

339 *3.2. Effect of mild thermal treatment at 50 °C on dry-cured ham microstructure*

340 As the most relevant textural modifications in dry-cured ham were observed for
341 treatments at 50 °C, microstructural analysis only focused on these samples. In general
342 terms, the muscle tissue of non-pasty dry-cured ham BF muscle (Fig. 5A) was formed
343 by cells that maintained their structural individuality despite manufacturing adopting a
344 compact appearance. However, in some areas, small gaps were observed due to
345 myofibrillar protein denaturation, which causes the loss of its three-dimensional
346 conformation, a typical consequence of the salt action (Mora et al., 2013). Z-disks were
347 visible, although they were not aligned (Fig. 6A) (Larrea et al., 2007). Practically the
348 whole length of the sarcomere seemed to be occupied by an A band. The treatment
349 carried out at 50 °C did not seem to affect the structural integrity of no pastiness dry-
350 cured ham negatively (Fig. 5B). In the heated samples, more empty intercellular
351 spaces could be observed (Fig. 5A) and some structural elements, such as I and A
352 bands, could be differentiated more clearly in the cellular inner (Fig. 6B) if compared to
353 the ham structure before the treatment (Fig. 6A). Z-disks still had the characteristic
354 discontinuity originated from the curing process (Picouet et al., 2012). In summary, for
355 non-pasty ham, the effect of the treatment on the microstructure was very light.
356 Likewise, Contreras et al. (2020) analyzed the micro and ultrastructure of medium (Fig.
357 5 C, D and Fig. 6 C, D) and high pastiness (Fig. 5 E, F and Fig. 6 E, F) dry-cured ham
358 and manifested that exists a large myofibrillar disintegration compared to the non-pasty

359 ham. As increase the level of pastiness, the muscle tissue is converted in an
360 unstructured protein matrix with many disintegrated areas and intercellular spaces
361 (Contreras et al., 2020). Similar results were obtained by Fulladosa, Rubio-Celorio,
362 Skytte, Muñoz, & Picouet (2017), who used LM to observe dry-cured ham with a high
363 proteolysis index (47%) but that had been induced artificially by a protease enzyme.

364 In medium pasty ham, the mild thermal treatment seemed to produce the structuring of
365 the muscle. Before treatment, muscle cells presented significant disintegration (Fig.
366 5C); however, after the heat treatment, the muscle cells were less disintegrated, with
367 better myofibrillar bundling (Fig. 5D). Furthermore, the endomysium tissue seemed to
368 be less shattered. Myofibril-sarcolemme unions were unattached in some areas,
369 leading to the myofibrils shrinking inside the muscle cell, giving rise to empty
370 intercellular spaces. This shrinkage, and thereby the tissue compaction (Tornberg,
371 2005), could somehow be responsible for the increase in the hardness and elasticity
372 provoked by the treatment. As regards the ultrastructure of the ham (Fig. 6, D), an
373 enhancement in the myofibrillar structure was found since the limits between myofibrils
374 were more easily distinguished and sarcomere structures such as I and A bands
375 seemed more organized.

376 The heat treatment of dry-cured ham with high pastiness provoked substantial changes
377 in the muscle structure. The tissue seemed to be more organized than before the
378 treatment, structured in individual cells surrounded by endomysium connective tissue
379 with a high enough degree of integrity (Fig. 5, F). However, some myofibril-sarcolemme
380 joints disappeared and the myofibrils seemed to be retracted into the cellular inner to a
381 greater extent than in ham with medium pastiness after treatment. As a consequence,
382 large empty intercellular spaces were created between myofibrils and endomysium
383 convective tissue. As mentioned previously, the increase in hardness and more elastic
384 behaviour in ham after heat treatment could be closely related with the myofibrillar
385 shrinkage. As for the ultrastructure of the ham with high pastiness after the treatment,

386 some sarcomeric structures could be appreciated; notwithstanding this, they were
387 highly distended (Fig. 6, F).

388

389 **4. Conclusions**

390 Mild thermal treatments in liquid medium emerge as a reliable, affordable and simple
391 strategy to modify the textural properties in dry-cured ham. The performance of the
392 thermal treatment was dependent on the temperature applied; thus, the higher the
393 temperature, the greater the effect on the texture. The application of PuS during
394 heating, which could be used to accelerate the process, did not involve any additional
395 textural change. Thermally-treated samples were harder, more elastic in their behavior
396 and less adhesive. Thereby, softness and adhesiveness, which are the typical
397 problems related to pastiness, were improved by the thermal treatment. In general
398 terms, the magnitude of the observed effects on the textural parameters was not linked
399 to the level of pastiness of the dry-cured ham. Micro and ultrastructural analyses
400 revealed that the thermal treatment caused substantial modifications in ham structure,
401 such as the shrinkage of myofibrils in pasty hams, which helped to explain the reported
402 textural effects. Future studies should address different aspects. Firstly, the impact of
403 the thermal treatments on sensory properties and the assessment of the inherent
404 microbial risks has to be necessarily analyzed. Secondly, the extension of the mild
405 thermal treatments to whole hams also has to be explored. Finally, although the
406 thermal treatment has to be limited to defective hams, it should be elucidated if the
407 increase in hardness caused in non-pasty hams may negatively affect the consumer
408 acceptance.

409

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416

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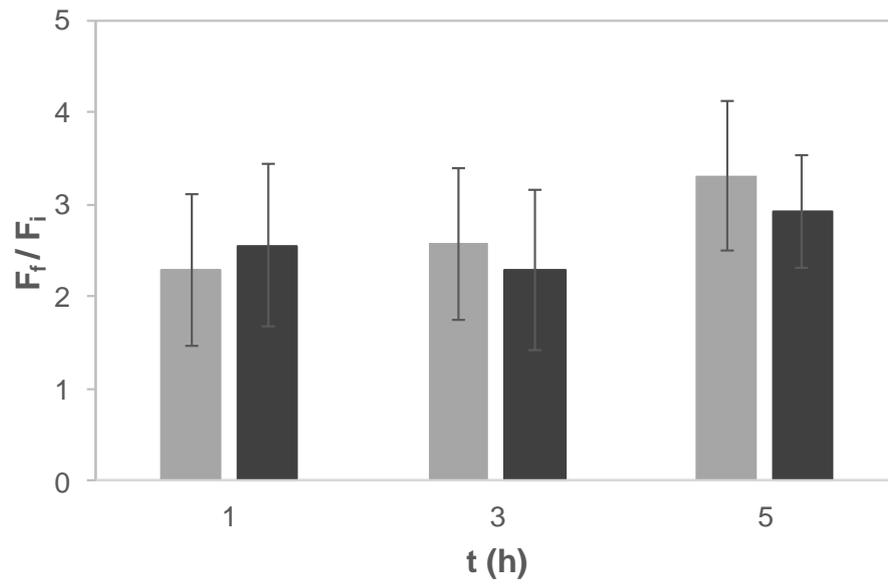


Figure 1. Influence of treatment time at 50 °C on the hardness ratio (F_f/F_i) of dry-cured ham. F_i and F_f are the initial and final sample hardness values, respectively. Average values \pm standard deviation values are plotted. Conventional (■CV) and ultrasonically-assisted (■PuS) mild thermal treatments are shown.

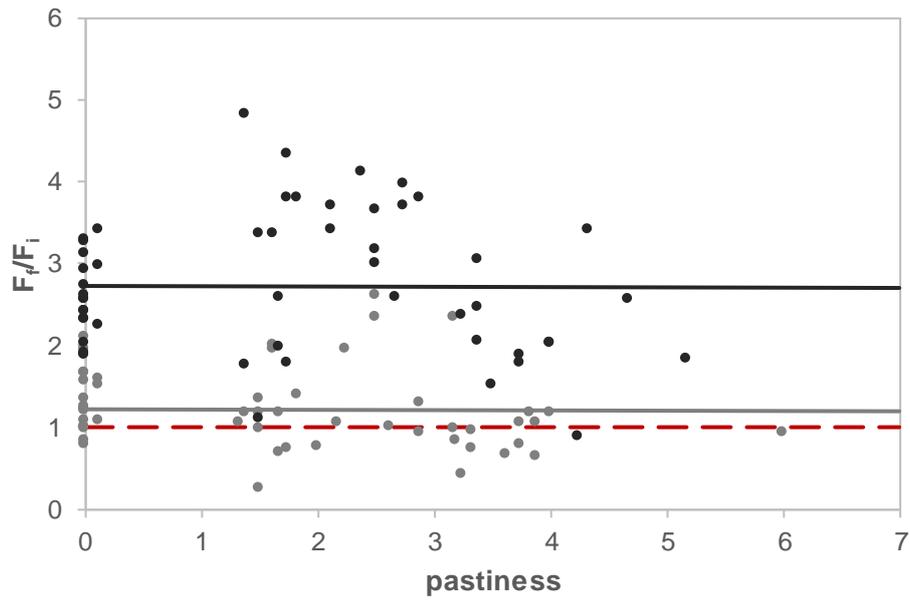


Figure 2. Relationship between pastiness and hardness ratio (F_f/F_i) of dry-cured ham heated at 40 (●) and 50 (●) °C. F_i and F_f are the initial and final sample hardness values, respectively. Points from both conventional (CV) and ultrasonically-assisted (PuS) experiments are shown together. Lower and upper continuous lines show average F_f/F_i for 40 and 50 °C treatments, respectively, and dashed line shows F_f/F_i equal to one.

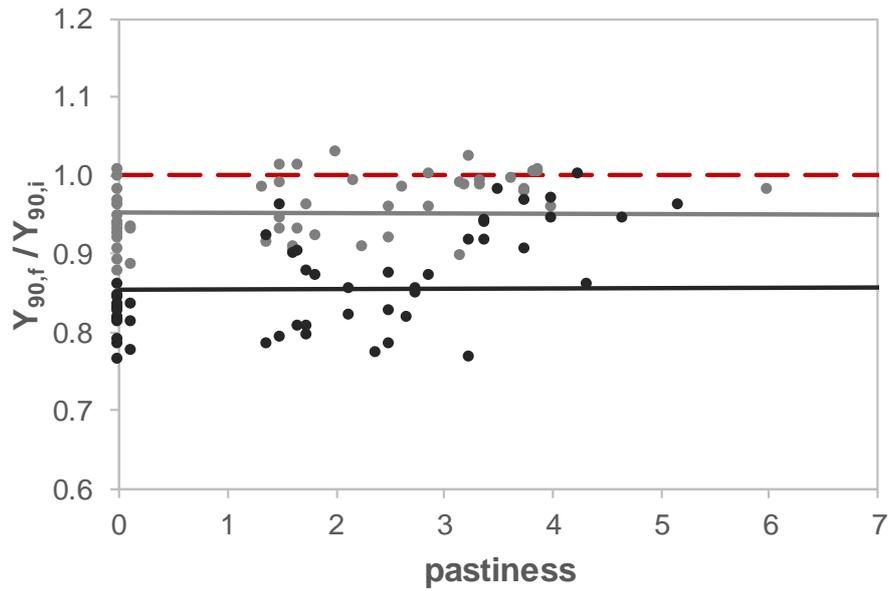


Figure 3. Relationship between pastiness and elasticity ratio ($Y_{90,f} / Y_{90,i}$) of dry-cured ham heated at 40 (●) and 50 (●) °C. $Y_{90,i}$ and $Y_{90,f}$ are the initial and final sample elasticity values, respectively, at the end of the compression test (90 s). Points from both conventional (CV) and ultrasonically-assisted (PuS) experiments are shown together. Upper and lower continuous lines show average $Y_{90,f} / Y_{90,i}$ for 40 and 50 °C treatments, respectively, and dashed line shows $Y_{90,f} / Y_{90,i}$ equal to one.

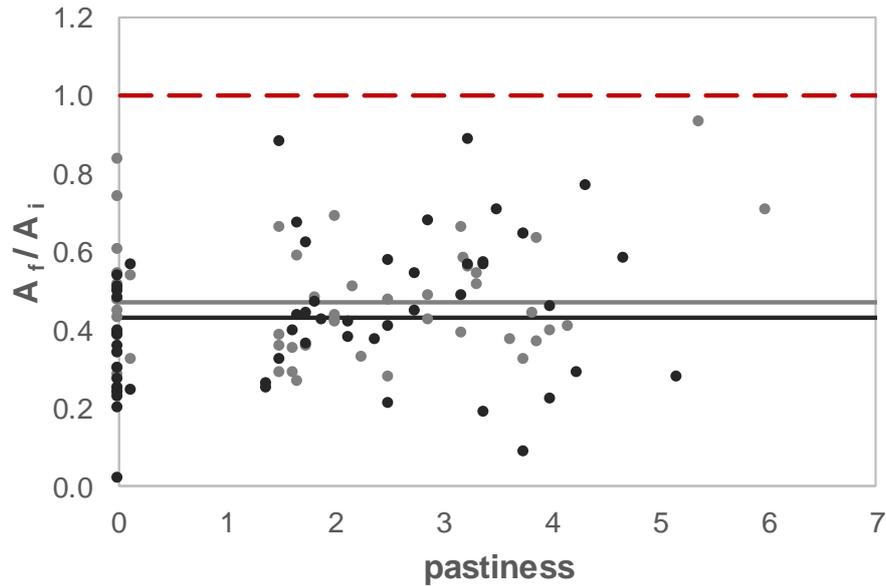


Figure 4. Relationship between pastiness and adhesiveness ratio (A_f/A_i) of dry-cured ham heated at 40 (●) and 50 (●) °C. A_i and A_f are the initial and final sample adhesiveness values, respectively. Points from both conventional (CV) and ultrasonically-assisted (PuS) experiments are shown together. Upper and lower continuous lines show average A_f/A_i for 40 and 50 °C treatments, respectively, and dashed line shows A_f/A_i equal to one.

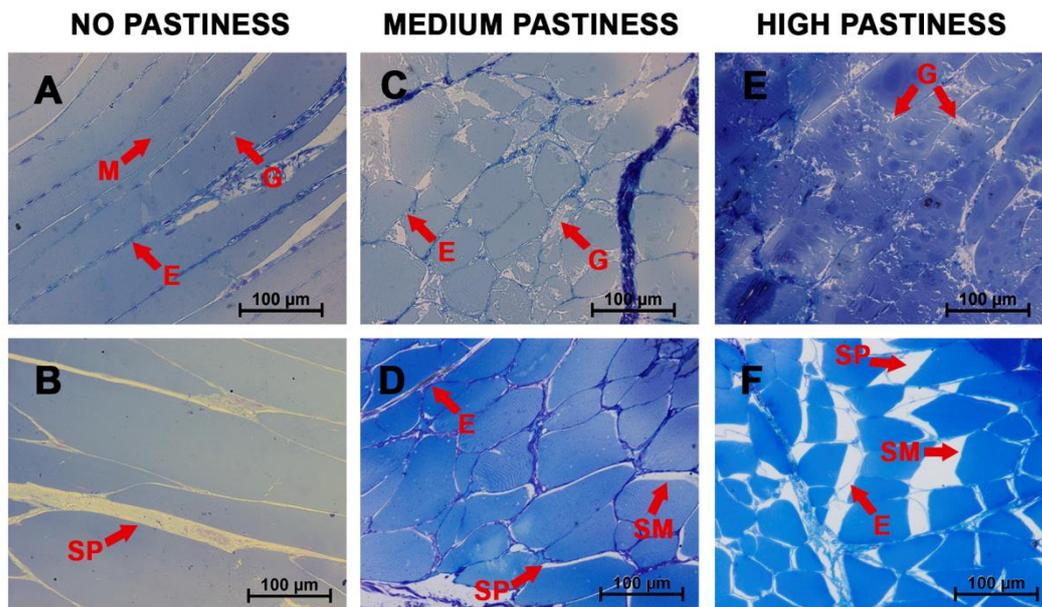


Figure 5. LM micrographs of muscle tissue, *Biceps femoris*, from dry-cured ham with different levels of pastiness before and after treatment carried out at 50 °C with PuS application (Before treatment: A, C, E; After treatment: B, D, F; Magnification: 20x). E: Endomysium; G: Gap; M: Myofibrill; SM: Shrunk Myofibrills; SP: Intercellular Space.

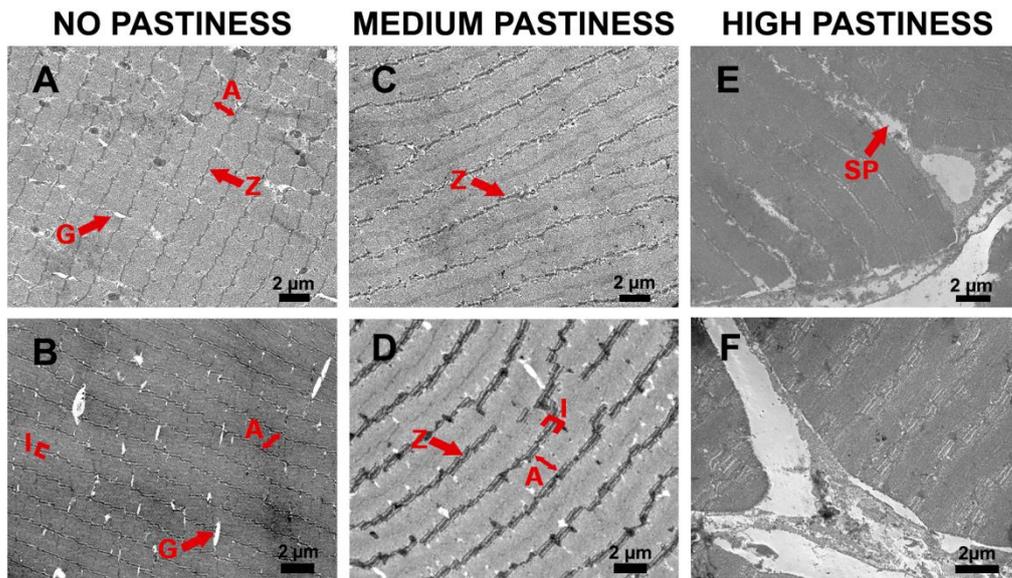


Figure 6. TEM micrographs of muscle tissue, *Biceps femoris*, from dry-cured ham with different levels of pastiness before and after treatment carried out at 50 °C with PuS application (Before treatment: A, C, E; After treatment: B, D, F; Magnification: 1200x).

A: A band; G: Gap; I: I band; SP: Intercellular Space; Z: Z disk.

Table 1. Ratios of hardness (F), relaxation capacity parameters (Y_2 , Y_{90}) and adhesiveness (A) of dry-cured ham heated at 40 and 50 °C without (CV) and with (PuS) ultrasound application of samples with high (HP), medium (MP) and no pastiness (NP). Ratios refer to the relationship between the final (f) and the initial (i) textural properties.

	CV			PuS		
	HP	MP	NP	HP	MP	NP
F_f/F_i , 40 °C	1.10±0.52 ^a	1.28±0.74 ^a	1.44±0.42 ^a	0.87±0.27 ^x	1.34±0.57 ^x	1.26±0.36 ^x
F_f/F_i , 50 °C	2.61±0.64 ^b	3.03±0.83 ^b	2.64±0.51 ^b	2.78±0.6 ^y	3.24±0.65 ^y	2.54±0.45 ^y
$Y_{2,f}/Y_{2,i}$, 40 °C	0.97±0.04 ^c	0.95±0.06 ^c	0.93±0.06 ^{bc}	0.99±0.02 ^z	0.93±0.07 ^{yz}	0.97±0.12 ^{yz}
$Y_{2,f}/Y_{2,i}$, 50 °C	0.87±0.04 ^b	0.81±0.05 ^a	0.80±0.04 ^a	0.89±0.06 ^y	0.79±0.09 ^x	0.78±0.05 ^x
$Y_{90,f}/Y_{90,i}$, 40 °C	0.98±0.04 ^c	0.96±0.04 ^{bc}	0.92±0.05 ^b	0.98±0.02 ^z	0.95±0.04 ^{yz}	0.94±0.04 ^{yz}
$Y_{90,f}/Y_{90,i}$, 50 °C	0.90±0.03 ^b	0.84±0.05 ^a	0.82±0.02 ^a	0.92±0.03 ^y	0.84±0.06 ^x	0.81±0.03 ^x
A_f/A_i , 40 °C	0.49±0.16 ^b	0.40±0.12 ^{ab}	0.47±0.18 ^b	0.54±0.13 ^{xy}	0.44±0.14 ^{xy}	0.44±0.15 ^{xy}
A_f/A_i , 50 °C	0.51±0.12 ^b	0.41±0.13 ^{ab}	0.30±0.14 ^a	0.49±0.19 ^{xy}	0.47±0.19 ^{xy}	0.39±0.14 ^x

Average values ± standard deviation are shown. Superscript letters (a, b, c) and (x, y, z) show homogeneous groups in both CV and PuS experiments, respectively, established from LSD (Least Significance Difference) intervals ($p < 0.05$) considering the influence of temperature and pastiness level on each textural parameter ratio.