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

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Abstract

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

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

1. Introduction

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

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

2. Materials and methods

2.1. Raw material

Dry-cured hams (n=108) from Large White and Landrace animal breed crosses were used. Dry-cured ham manufacturing was modified as described in detail by Contreras et al. (2020) to induce pastiness over a wide intensity range. At the end of the processing, the cushion part of dry-cured hams was sliced into different formats. Thin slices (thickness 1.5 mm) were used for sensory pastiness evaluation and microstructural analyses, and packages of 4 slices were prepared for the adhesiveness test. Thicker slices (thickness 20 mm), meanwhile, were used for hardness and elasticity tests and ultrasonic analysis. Afterwards, all the samples were vacuum-packed in individual plastic bags of polyamide/polyethylene (oxygen permeability of 50 $cm^3/m^2/24h$ at 23 ºC and water permeability of 2.6 $g/m^2/24h$ at 23 ºC and 85% RH, Sacoliva® S.L., Spain). From each ham piece, two thick slices (20 mm) and 2 packages of thin slices (1.5 mm) were used. Thereby, the destructive instrumental textural tests could be performed in identical samples before (control) and after the thermal treatment. Finally, packaged samples were stored in a chamber at 4±2 ºC until the experiments were performed.

2.2. Sensory texture analysis

A three-member expert panel, trained following the American Society for Testing and Materials standards (ASTM, 1981), performed the sensory texture analysis on dry-cured ham slices (thickness 1.5 mm). The textural attribute evaluated in BF muscle was pastiness, which can be defined as a feeling similar to the mouth-coating sensation produced by flour-water paste during the mastication process. Dry-cured ham slices presented different levels of pastiness. The levels of ham pastiness were ranked from 0 (absence) to 6 (maximum intensity). The pastiness level of the samples was set as the average score of the three experts' scores. Thus, dry-cured hams were classified according to the textural defect into samples with no pastiness (pastiness<1), medium pastiness (pastiness between 1-2.5) and high pastiness (pastiness>2.5). For every level of pastiness, 36 samples were selected.

2.3. Mild thermal treatments

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

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

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

2.4. Instrumental texture analysis

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

2.4.1. Hardness and elasticity

Hardness and elasticity were measured using a texturometer (TA-XT2, SMS, Godalming, UK) provided with a load cell of 50 kg. From the slices of 20±4 mm, 5 parallelepipeds of BF muscle were carved (20 mm length x 20 mm width x 15 mm height). Stress-relaxation tests were carried out at a constant temperature (4±1 ºC) using a flat 75 mm diameter aluminum probe (SMS P/75). The samples were compressed to 25% of their original height perpendicular to the fiber bundle direction at a crosshead speed of 1 mm/s and, afterwards, the probe was held for 90 s to monitor relaxation. The experimental data were recorded and processed with Exponent Lite 6.1.4.0 software (SMS, Godalming, UK). Thus, hardness (F) was computed from the force versus time profiles as the maximum force achieved during compression, while elastic behavior was indirectly assessed by computing the force decay, Y_t , logged during relaxation since its increase reflects a more viscoelastic behavior (Eq. 1):

$$Y_t = \frac{F_{max} - F_t}{F_{max}} \qquad (1)$$

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

2.4.2. Adhesiveness

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

2.5. Microstructure

The dry-cured ham microstructure was analyzed using two microscopic techniques: light microscopy (LM) and transmission electron microscopy (TEM). Between 4-5 different samples per level of pastiness were randomly chosen and analyzed. Thus, from slices 1.5 mm thick, small sections (5 x 3 mm) from BF muscle were cut with a disposable blade. In order to include the sections, samples were fixed with a 25 g/L glutaraldehyde solution (0.025M phosphate buffer, pH 6.8, at 4 ºC, 24 h), postfixed with a 20 g/L OsO_4 solution (1.5 h), dehydrated using a graded acetone series (300, 500, 700 and 1000 g/kg), contrasted in 40 g/L uranyl acetate dissolved in acetone and embedded in epoxy resin (Durcupan, Sigma–Aldrich, St. Louis, MO, USA). The samples were cut using a Reichert Jung ultramicrotome (Leica Mycrosystems, Wetzlar, Germany). Thin sections (1.5 µm) were stained with 2 g/L toluidine blue and examined in a Nikon Eclipse E800 light microscope (Nikon, Tokyo, Japan). Ultrathin sections (0.5 µm) were stained with 40 g/L lead citrate and observed in a Philips EM400 (Philips, Eindhoven, Holland) transmission electronic microscope at 80 kV. Dry-cured ham samples with high, medium and no pastiness were observed before and after PuS heat treatment at 50ºC.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) ($p<0.05$) was performed to assess the influence of the type of thermal treatment (CV-PuS) on the textural parameters of treated samples. Likewise, multifactor ANOVA ($p<0.05$) was performed in order to evaluate the influence of the temperature (40-50 ºC) and the level of pastiness intensity (high, medium and no pastiness) and also whether their interactions had a significant influence on every measured textural parameter. ANOVAs and least significant difference (LSD) intervals were estimated using the statistical package Statgraphics Centurion XVI (Statpoint Technologies Inc., Warrenton, VA, USA) considering a significance level of 95%.

3. Results and discussion

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

3.1.1. Influence of PuS application

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

3.1.2. Influence of the thermal treatment on hardness

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

As observed in Fig. 2, level of pastiness did not have a statistically significant ($p>0.05$) effect on the hardness ratio; notwithstanding this, at 50 ºC it was observed that the highest ratios belonged to the sample group with medium pastiness (Table 1). Therefore, for each temperature, ham samples experienced a similar relative variation in hardness when subjected to the mild thermal treatment, regardless of their initial pastiness. There have been no previous references to the impact caused by the mild thermal treatments on the textural attributes of samples differing in pastiness intensity.

3.1.3. Influence of the thermal treatment on elastic behavior

The material relaxation when subjected to prior compression stress was analyzed as an indicator of elastic behavior since an ideal elastic material would have a force decay of 0. Thereby, the higher the force decay, the more relevant the viscoelasticity (Eq. 1). Fig. 3 plots the $Y_{90,f}/Y_{90,i}$ ratio according to the pastiness of every dry-cured ham slice, showing the same pattern as the one found for the $Y_{2,f}/Y_{2,i}$ ratio (data not shown). At 50 ºC, 100% of the treated samples showed a $Y_{90,f}/Y_{90,i}$ ratio of under one, which points to the fact that elasticity increased after the treatment. Otherwise, 90% of dry-cured ham samples heated at 40 ºC presented a $Y_{90,f}/Y_{90,i}$ ratio of below one. The average values of $Y_{90,f}/Y_{90,i}$ were 0.96±0.04 and 0.86±0.06 at 40 and 50 ºC, respectively. The lower value of $Y_{90,f}/Y_{90,i}$ at 50 ºC reflects the fact that the treatment at this temperature was more effective at improving elasticity than at 40 ºC. The effect of temperature is also shown in Table 1 since, for the three levels of pastiness and for both CV and PuS, $Y_{90,f}/Y_{90,i}$ and $Y_{2,f}/Y_{2,i}$ ratios were always significantly ($p<0.05$) lower at 50 than at 40 ºC. In the aforementioned study published by Morales et al. (2008), a reduction of Y_{90} in ham was found as the treatment temperature increased from 36 (0.621) to 46 ºC (0.575). Gou et al. (2008) also reported a reduction of Y_2 in ham when the ageing temperature was increased from 18 (0.339) to 30 ºC (0.318), which again supports the experimental results shown in Fig. 3. Therefore, the 5 hour-long mild thermal treatment at 50 ºC emerges as a simple and reliable means of correcting softness and elasticity loss of dry-cured ham. Thus, heating would improve not only consumer perception during mastication but also industrial slicing.

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

3.1.4. Influence of the treatment on adhesiveness

The modification of adhesiveness brought about by the thermal treatment was computed by instrumental texture analysis. Mild thermal treatment led to relevant modifications of adhesiveness; thus, its ratio (A_f/A_i) was below one for every sample (Fig. 4). This indicates that slice adhesiveness was reduced after heating, regardless of the conditions. Morales et al. (2008) also found a decrease in the adhesiveness of dry-cured ham BF muscle after a 168 h thermal treatment at 30 ºC. Similarly, Pérez-Santaescolástica et al. (2018) also reported a decrease in adhesiveness between control (0.84 N) and conventionally heated ham samples (0.38 N). Unlike the trend observed in hardness and elasticity, a multifactor ANOVA showed that neither the temperature nor the level of pastiness influenced the adhesiveness ratio significantly (p>0.05). What should be highlighted is the great performance of the mild thermal treatment in the reduction of slice adhesiveness, since the ratios were around 0.5 in every case (Table 1). This confirms that adhesiveness was reduced by 50%, which represents an excellent result since adhesiveness is one of the main issues related to consumer rejection of pasty dry-cured ham. The adhesiveness decrease after the mild thermal treatment could be explained by denaturation and other structural changes in the proteins.

3.2. Effect of mild thermal treatment at 50 ºC on dry-cured ham microstructure

As the most relevant textural modifications in dry-cured ham were observed for treatments at 50 ºC, microstructural analysis only focused on these samples. In general terms, the muscle tissue of non-pasty dry-cured ham BF muscle (Fig. 5A) was formed by cells that maintained their structural individuality despite manufacturing adopting a compact appearance. However, in some areas, small gaps were observed due to myofibrillar protein denaturation, which causes the loss of its three-dimensional conformation, a typical consequence of the salt action (Mora et al., 2013). Z-disks were visible, although they were not aligned (Fig. 6A) (Larrea et al., 2007). Practically the whole length of the sarcomere seemed to be occupied by an A band. The treatment carried out at 50 ºC did not seem to affect the structural integrity of no pastiness dry-cured ham negatively (Fig. 5B). In the heated samples, more empty intercellular spaces could be observed (Fig. 5A) and some structural elements, such as I and A bands, could be differentiated more clearly in the cellular inner (Fig. 6B) if compared to the ham structure before the treatment (Fig. 6A). Z-disks still had the characteristic discontinuity originated from the curing process (Picouet et al., 2012). In summary, for non-pasty ham, the effect of the treatment on the microstructure was very light. Likewise, Contreras et al. (2020) analyzed the micro and ultrastructure of medium (Fig. 5 C, D and Fig. 6 C, D) and high pastiness (Fig. 5 E, F and Fig. 6 E, F) dry-cured ham and manifested that exists a large myofribillar disintegration compared to the non-pasty ham. As increase the level of pastiness, the muscle tissue is converted in an unstructured protein matrix with many disintegrated areas and intercellular spaces (Contreras et al., 2020). Similar results were obtained by Fulladosa, Rubio-Celorio, Skytte, Muñoz, & Picouet (2017), who used LM to observe dry-cured ham with a high proteolysis index (47%) but that had been induced artificially by a protease enzyme.

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

The heat treatment of dry-cured ham with high pastiness provoked substantial changes in the muscle structure. The tissue seemed to be more organized than before the treatment, structured in individual cells surrounded by endomysium connective tissue with a high enough degree of integrity (Fig. 5, F). However, some myofibril-sarcolemme joints disappeared and the myofibrils seemed to be retracted into the cellular inner to a greater extent than in ham with medium pastiness after treatment. As a consequence, large empty intercellular spaces were created between myofibrils and endomysium convective tissue. As mentioned previously, the increase in hardness and more elastic behaviour in ham after heat treatment could be closely related with the myofibrillar shrinkage. As for the ultrastructure of the ham with high pastiness after the treatment, some sarcomeric structures could be appreciated; notwithstanding this, they were highly distended (Fig. 6, F).

4. Conclusions

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

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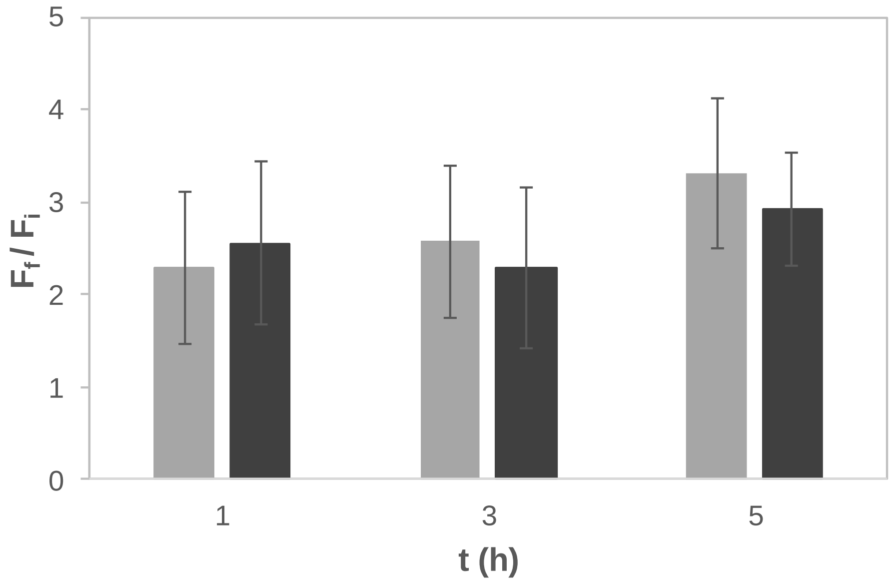

Figure 1. Influence of treatment time at 50 ºC on the hardness ratio (F_f/F_i) of dry-cured ham. F_i and F_f are the initial and final sample hardness values, respectively. Average values ± standard deviation values are plotted. Conventional (■CV) and ultrasonically-assisted (■PuS) mild thermal treatments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

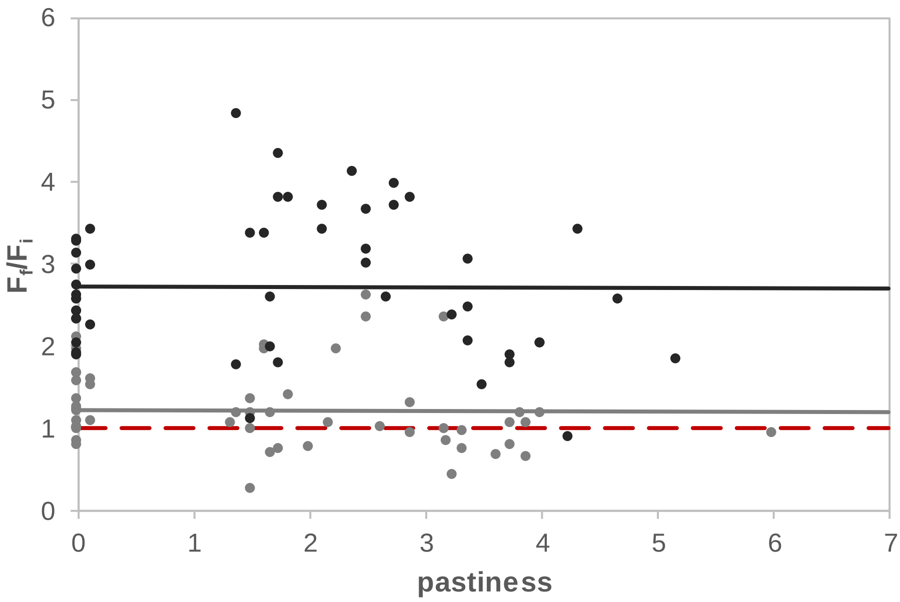

Figure 2. Relationship between pastiness and hardness ratio (F_f/F_i) of dry-cured ham heated at 40 (●) and 50 (●) ºC. F_i and F_f are the initial and final sample hardness values, respectively. Points from both conventional (CV) and ultrasonically-assisted (PuS) experiments are shown together. Lower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

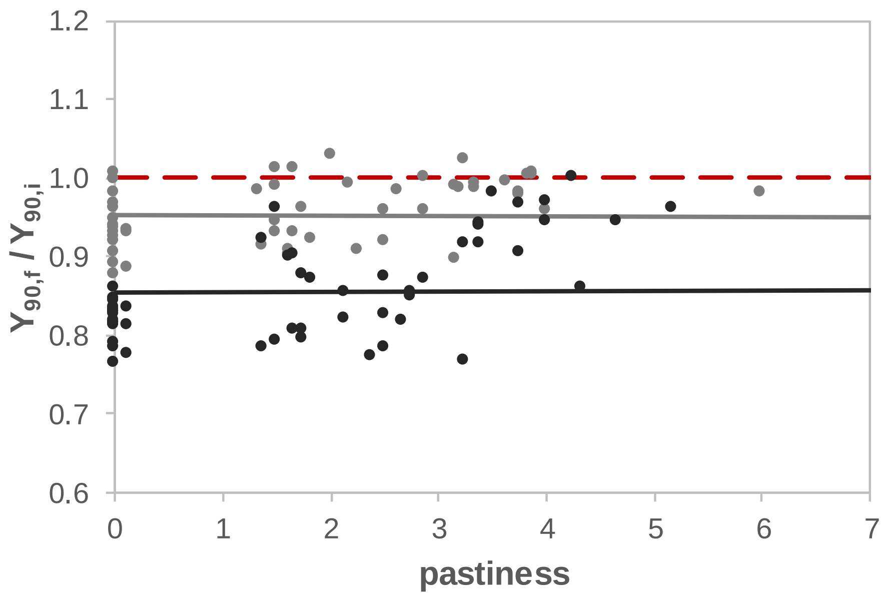

Figure 3. Relationship between pastiness and elasticity ratio ($Y_{90,f}/ Y_{90,i}$) of dry-cured ham heated at 40 (●) and 50 (●) ºC. $Y_{90,i}$ and $Y_{90,f}$ are the initial and final sample elasticity values, respectively, at the end of the compression test (90 s). Points from both conventional (CV) 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 ºC treatments, respectively, and dashed line shows $Y_{90,f}/ Y_{90,i}$ equal to one.

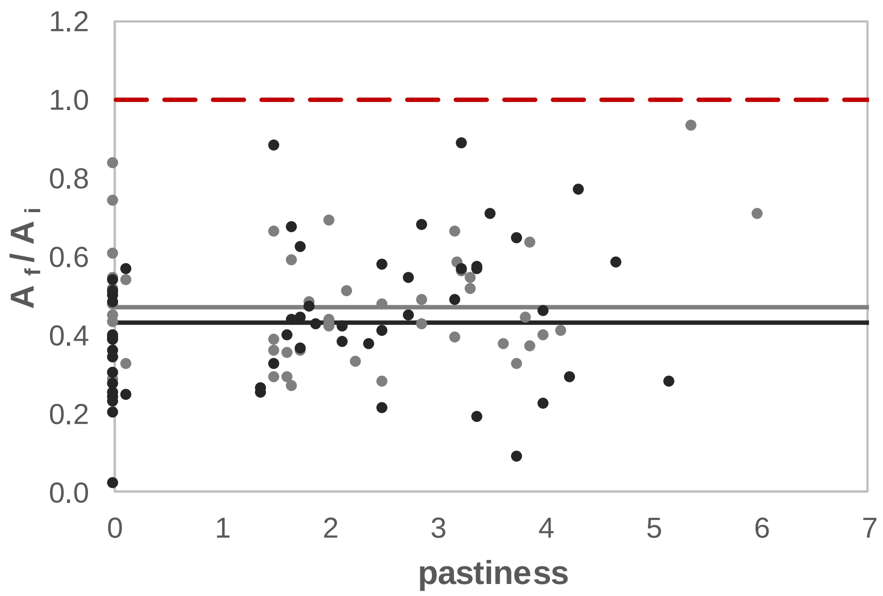

Figure 4. Relationship between pastiness and adhesiveness ratio (A_f/A_i) of dry-cured ham heated at 40 (●) and 50 (●) ºC. A_i and A_f are the initial and final sample adhesiveness values, respectively. Points from both conventional (CV) and ultrasonically-assisted (PuS) experiments are shown together.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

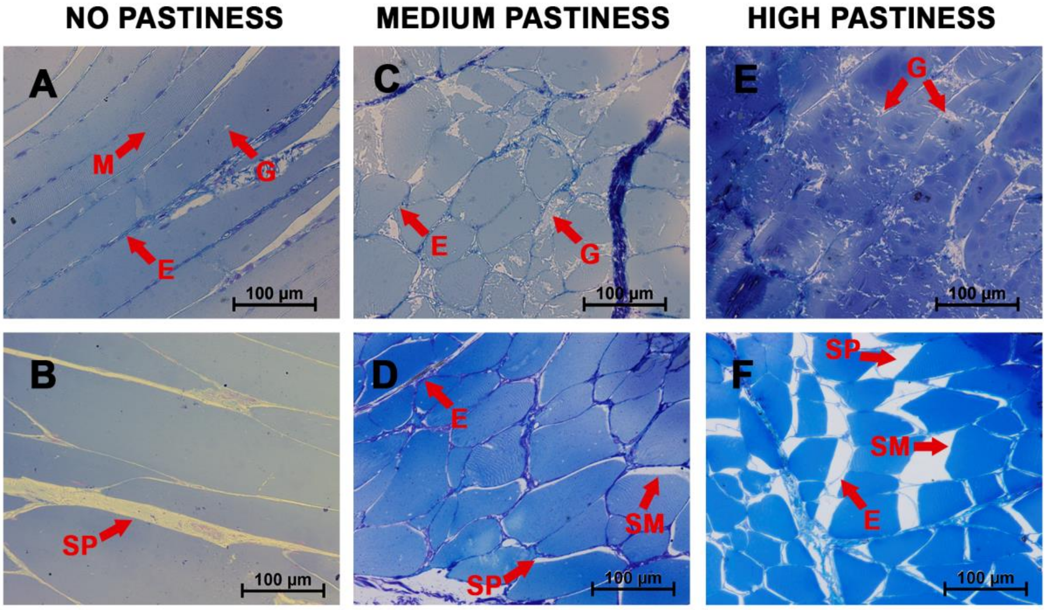

Figure 5. LM micrographs of muscle tissue, *Biceps femoris,* from dry-cured ham with different levels of pastiness before and after treatment carried out at 50 ºC with PuS application (Before treatment: A, C, E; After treatment: B, D, F; Magnification: 20x). E: Endomysium; G: Gap; M: Myofibrill; SM: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

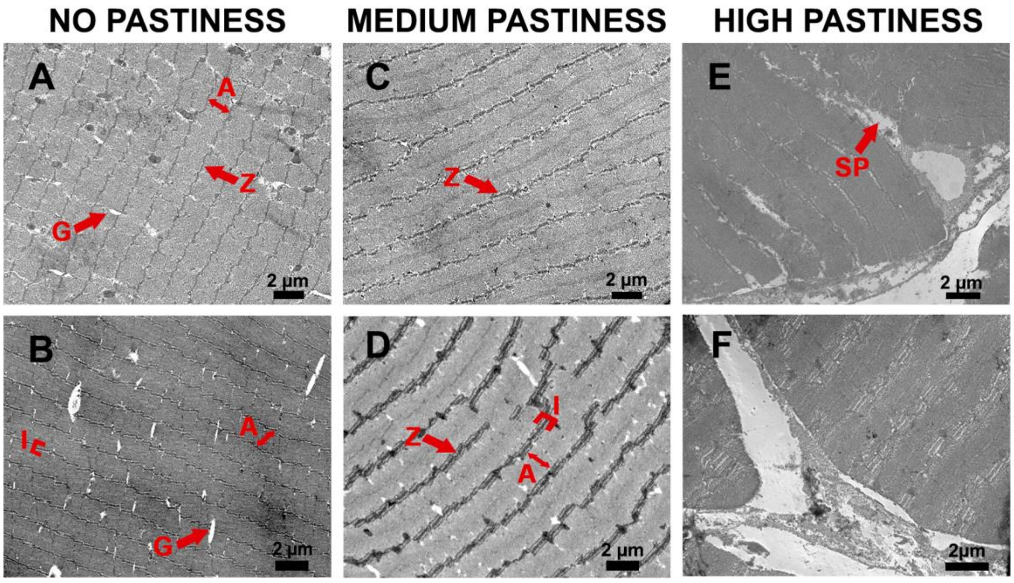

Figure 6. TEM micrographs of muscle tissue, *Biceps femoris,* from dry-cured ham with different levels of pastiness before and after treatment carried out at 50 ºC with PuS application (Before treatment: A, C, E; After treatment: B, D, F; Magnification: 1200x). A: A band; G: Gap; I: I band; SP: Intercellular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

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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 ºC 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**
$F_f/F_{i,\ 40\ ºC}$	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}
$F_f/F_{i,\ 50\ ºC}$	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}
$Y_{2,f}/Y_{2,i\ ,\ 40\ ºC}$	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}
$Y_{2,f}/Y_{2,i\ ,\ 50\ ºC}$	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}
$Y_{90,f}/Y_{90,i\ ,\ 40\ ºC}$	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}
$Y_{90,f}/Y_{90,i\ ,\ 50\ ºC}$	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}
$A_f/A_{i,\ 40\ ºC}$	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}
$A_f/A_{i,\ 50\ ºC}$	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.