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ULTRASONIC CHARACTERIZATION AND ONLINE MONITORING OF PORK MEAT DRY SALTING PROCESS

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ABSTRACT

Bearing in mind the highly variable salt content in dry-cured meat products with anatomical integrity, such as pork loin or ham, non-destructive salt content characterization and the online monitoring of dry salting are highly relevant for industrial purposes. This study explores the ability of low-intensity ultrasound to monitor the dry salting of pork *Biceps femoris* (BF) and *Longissimus dorsi* (LD) online, as well as to estimate the salt content, both in these muscles and in hams. For this purpose, meat samples were dry salted for up to 16 d at 2ºC. During the salting of the muscles, the ultrasonic velocity was continuously measured at time intervals of 5min, while in the hams it was measured before and after salting. The ultrasonic velocity increased progressively during the salting due to salt gain and water loss, reaching a velocity variation (ΔV) of 46.8m/s after 16 d of dry salting for hams and 59.5 and 30.6m/s after 48h of dry salting for LD and BF, respectively. Accurate correlations between salt gain and ΔV were obtained (R^2 = 0.903 in LD-BF muscles and R^2 = 0.758 in hams), which allowed the assessment of the salt content with an average estimation error of 0.4% w.b. for both muscles and hams. Further research should investigate the use of the time of flight obtained through the pulse-echo mode, instead of the ultrasonic velocity, in order to improve the industrial applicability.

Keywords: Ultrasound; Pork meat; Dry salting; Online monitoring; Quality control; Salt content.

1. INTRODUCTION

Salting is one of the most ancient preservation methods used on meat products, such as ham, loin, bacon and sausages (Binkerd & Kolari, 1975). In the salting process, the fresh meat is stabilized due to a combined effect of the salt gain and the water loss. Salt is a multifunctional ingredient that affects both the food safety and quality. Meat products without anatomical integrity, such as dry-cured sausages, are formulated and a known quantity of salt is added to the minced meat. However, in meat products with anatomical integrity, salting is a complex and critical process due to the fact that it is affected by many factors, some of which cannot be controlled.

In the meat industry, dry salting is the most commonly-used salting process for the whole anatomical piece meat products and consists of covering the meat with coarse salt (Barat, Grau, Pagan-Moreno, & Fito, 2004). Usually, several salt/product layers are superimposed (Ventanas, 2001) and a particular salting time, temperature and relative humidity conditions are established for an entire batch (Jurado, Carrapiso, García, & Timón, 2002; Bello, 2008). Consequently, the salt content of meat pieces in the same batch varies greatly, not only due to the salting process itself but also to the heterogeneity in the weight, shape, composition and structure of the fresh meat (Gou, Composada, & Arnau, 2004; Ramírez & Cava, 2007; Castro-Giráldez, Fito, & Fito, 2010; Čandek-Potokar & Škrlep, 2012; Reig, Aristoy, & Toldrá, 2013). The variability linked to the dry salting process arises from the non-homogeneous ambient conditions of the salting chamber, the different position in the salting layers, the formation of brine between the sample surface and the dry salt and the size of the salt crystals, among other factors (Barat, Grau, Pagan-Moreno, & Fito, 2004; Van Nguyen, Arason, Thorarinsdottir, Thorkelsson, & Gudmundsdottir, 2010; Albarracín, Sánchez, Grau, & Barat, 2011). As a consequence of the variable salt absorption in meat pieces from the same batch, the behavior of each salted piece is different in the subsequent stages of the product manufacturing process, which gives rise to heterogeneous sensory and nutritional characteristics of the final batch (Garcia-Gil, Muñoz, Santos-Garcés, Arnau, & Gou, 2014). In addition, due to the above-mentioned variability in the salting process, meat products are commonly over-salted to ensure the product's safety, which increases the energy consumption, lengthens the process time and has a great impact on the product quality (Garcia-Gil et al., 2012). Thus, the online monitoring of the salt content of meat products during salting could be a useful tool in the meat industry with which to describe the salt evolution and to determine the optimal salting time, according to the salt content targeted for each particular piece.

The online monitoring of the salting process, as well as the salt content characterization, should be addressed through non-destructive and non-invasive techniques, such as low-intensity ultrasound technology. Ultrasonic velocity, acoustic impedance and the attenuation coefficient have been used to assess the physicochemical properties, such as the composition, structure and physical state, of many foods (Mulet, Benedito, Bon, & Sanjuan, 1999; Hæggström & Luukkala, 2001; Damez & Clerjon, 2008; Schöck & Becker, 2010). In the meat industry, ultrasound velocity has been used to estimate the intramuscular fat content in beef samples (Whittaker, Park, Thane, Miller, & Savell, 1992), to classify fresh hams according to the fat level (De Prados et al., 2015a) and to characterize formulated dry-cured meat products according to the breed and diet of the pigs (Niñoles, Clemente, Ventanas, & Benedito, 2007; Niñoles, Sanjuan, Ventanas, & Benedito, 2008) and the fat content (Corona, García-Pérez, Ventanas, & Benedito, 2014). Additionally, a recent study has demonstrated the relationship between the ultrasonic velocity measured in dry-cured hams and their salt content (Fulladosa et al., 2015a). De Prados, García-Pérez, and Benedito (2015b) studied the feasibility of using low intensity ultrasound to predict the salt content in pork meat samples (*Biceps femoris* and *Longissimus dorsi*) by measuring the ultrasonic velocity before and after salting by the through-transmission method. However, to our knowledge, the ultrasonic through-transmission method has not been applied either to predict the salt gain in meat products with great structural complexity, such as whole hams, or to perform the online evaluation of the salt gain evolution in meat muscles during dry salting.

Therefore, the aim of the present study was to investigate the ability of low intensity ultrasound to perform the online monitoring of pork meat (*Biceps femoris* and *Longissimus dorsi*) dry salting. The capacity of the ultrasonic models to estimate the salt content in both muscles and in dry salted whole hams was also assessed.

2. MATERIALS AND METHODS

2.1. MEAT SAMPLING

Fifteen fresh *Longissimus dorsi* (LD) and *Biceps femoris* (BF) pork muscles from *Large White* breed pigs were obtained from a local market. Muscles were selected with a pH ranging between 6.4 and 5.5. In both muscles, the subcutaneous fat and external connective tissue were removed. Samples of 20±2cm in length (L) and 1.0±0.1kg were obtained from each muscle, keeping the original width (Z) and thickness (T) of the

muscle (Fig. 1). Meat muscles were used for the online monitoring of dry salting and the salt gain estimation using ultrasound.

Additionally, thirty hams from the *Large White* breed, with an average weight of 11.2±0.5kg, were purchased in a slaughterhouse. The hams were used to estimate salt gain by measuring the ultrasonic properties before and after the salting process.

2.2. DRY SALTING EXPERIMENTS

Dry salting experiments were carried out on LD and BF muscles by covering the sample with 6kg of coarse salt (NaCl moisturized at 10% w/w) at 2±1ºC in a cold chamber (AEC330r, Infrico, Spain) (Fig. 2). Fresh samples and salt were previously stored for 24h at 2ºC for the purposes of tempering. Three replicates were carried out for each salting time (6, 12, 24, 36 and 48h) for both LD and BF muscles.

In the case of hams, all of them were salted following the standard dry-cured ham elaboration process. Thus, the hams were pile-salted with a layer of coarse salt (NaCl moisturized at 10% w/w) at least 10cm thick and kept for 2, 4, 7, 11 and 16 d at 2±2ºC and 85±5% relative humidity, in order to obtain a wide salt content range. Six hams were considered for each salting time.

2.3. ULTRASOUND MEASUREMENTS

The experimental set-up consisted of a pair of narrow-band ultrasonic transducers (1MHz, 0.5" crystal diameter, A303S model, Panametrics, Waltham, MA, USA, for the ultrasonic measurements in LD and BF muscles and 1 MHz, 0.75″ crystal diameter, A314S-SU model, Panametrics, Waltham, MA, USA, for the ultrasonic measurements in hams), a digital storage oscilloscope (Tektronix TDS5034, Digital phosphor oscilloscope. Tektronix Inc. Bearverton, OR, USA) and a pulser-receiver (Model 5058PR, Panametrics, Waltham, MA, USA). A custom designed digital height gage, linked to the computer by an RS232 interface, was used to measure the sample's thickness with a precision of ±0.01mm.

Fig. 2 shows the experimental set-up used for the measurement of the ultrasonic velocity during the dry salting experiments on LD and BF muscles. For the purposes of carrying out the ultrasonic measurements while the LD and BF meat was being salted, the sample was placed on 2kg of salt inside a plastic container (30x25x15cm) (Fig. 2) and the transducers were coupled to the sample's thickness. Next, three temperature sensors were introduced; one was placed in the sample, one in the salt and the third

one close to the transducer and the rest of the salt was added until the sample was covered. In this case, the transducers used had a small contact surface (A303S model, 1.77cm^2) so as to maximize the contact area between the meat sample and the salt. The ultrasonic velocity (V) was measured by the through-transmission mode at time intervals of 5min. Due to the fact that the meat sample shrinks during salting, the position of the upper transducer was manually adjusted both initially and during the process with a force of 1N to maintain the contact between the sample and the transducers.

As a consequence of the difficulty of implementing the ultrasonic online measurements in whole hams salted in piles, the V was measured by the through-transmission mode at 2ºC in a temperature-controlled chamber before and after salting in 3 sections of the hams. Thus, 20 measurements were carried out in the cushion (C) and 5 in the fore cushion (FC) and the butt end (BE) sections (Fig. 1). The hams were kept at 2±2°C for 24h before the ultrasonic velocity was measured. The ultrasonic velocity in each ham was calculated as the average of the 30 ultrasonic velocities measured in every ham zone.

The V was computed from the time of flight (TOF) (averaged for 5 signals) and the sample's thickness (T) by using specific software programmed in Visual Basic (VB 6.0 Microsoft™). The variation of ultrasonic velocity (ΔV) was calculated as the difference between the initial V in the samples and the V for a particular time ($\Delta V = V_t - V_{0h}$). The time of flight variation (ΔTOF) was also considered to be related with compositional changes during salting.

2.4. DETERMINATION OF FAT, WATER AND SALT CONTENTS

After dry salting, the excess salt was removed from the surface of the LD and BF samples and a cross slice (SL) of the samples (153.7±44.0g), including the ultrasonic measurement zone, was taken (Fig. 1). Each SL was split into 5 sections for the analytical determinations: sections 1S and 5S (29.9±12.3g) made reference to the end zones, sections 2S and 4S (30.8±9.3g) were the intermediate zones and section 3S (34.4±6.0g) was the central zone where the ultrasonic velocity was measured (Fig. 1). Each section was individually ground and homogenized before the analytical determinations. In the case of the hams, they were washed with water at 15±1ºC and then vacuum-packaged. After 40 d of storage at 3±2ºC, all the hams were dissected into the major parts: bones, skin, lean tissue and fatty tissue. The lean and fatty tissues

were then minced together and homogenized in a bowl chopper for the analytical determinations.

The fat, salt and water contents were determined in the fresh muscles and hams. To this end, a representative piece of each muscle was taken after obtaining the fresh muscle samples. In the case of the hams, 5 additional hams from the same batch were used for the purposes of measuring the initial average fat, salt and water contents of the fresh samples. In addition, the salt and water contents were also analyzed in each section of the salted LD and BF samples and in the mixture of lean and fatty tissues of salted hams. Thus, the fat content was determined by using the Shoxlet extraction method following AOAC 991.36 (AOAC, 1997). The water content was determined by oven drying to constant weight at 102ºC following the standard AOAC method 950.46 (AOAC, 1997). The salt content was analyzed after sample homogenization (1g for fresh samples and 0.5g for salted samples) in 100mL of distilled water at 9500rpm in an ULTRATURRAX (T25, IKA Labortechnik, Germany) for 5min. Supernatant was filtered through membrane filters (45µm) and a 500µl aliquot sample was taken and titrated in a Chloride Analyzer equipment (Chloride Meter 926L, Ciba Corning, U.K.) (Cárcel, Benedito, Bon, & Mulet, 2007). All the analyses were performed in triplicate.

The salt (X_S), water (X_W) and fat (X_F) contents of the fresh samples, the salted LD and BF sections (1S, 2S, 3S, 4S and 5S), the cross slice (SL average of the 5 sections) and the hams, were expressed as percentages (%) in wet basis (w.b.). The salt gain (ΔX_S) and the water loss (ΔX_W) were also calculated for each salting time (6, 12, 24, 36 and 48h in LD-BF muscles and 2, 4, 7, 11 and 16 d in hams).

2.5. STATISTICAL ANALYSIS AND REGRESSION MODELS

The influence that the fresh muscles and hams, X_S, X_W, X_F, Z and T, the salting time and the type of muscle had on the ΔX_S, ΔX_W and ΔV was evaluated by means of an analysis of variance using the Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA). Linear relationships between Z-ΔX_S, Z-ΔX_W, T-ΔX_S and T-ΔX_W were established to determine the effect of the dimensions of the muscles and hams on the compositional changes during salting.

Simple regression models were developed between the dependent variable (ΔV) and the salt gain both for the muscles (considering the LD and BF samples jointly) and the hams. In order to evaluate the capacity of the models for salt content determination, both the BF and LD samples and the hams were split into two sets. The first set (model

calibration, MC), comprising 20 samples (n_{MC}), was used to develop the models. The rest of the samples (n_{MV} = 10) were used for the model validation (MV). The regression analysis was performed by using Statgraphics® Centurion XV (Statpoint Technologies Inc., Warrenton, VA, USA).

The accuracy of each model was estimated by computing the square of the linear regression coefficient (R^2) and the Root Mean Square Error of Prediction (RMSE) value (Eq (1)).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_p - y_i)^2}{n}} \quad (1)$$

where n is the number of samples, y_p is the predicted value and y_i is the experimental value.

3. RESULTS AND DISCUSSION

3.1. FRESH MEAT CHARACTERIZATION

As can be observed in Table 1, non-significant differences ($p>0.05$) were found between the salt, water and fat contents of fresh LD and BF muscles. However, there were significant differences ($p<0.05$) found between all the analyzed parameters of fresh hams and both muscles. Ham and muscle parameters are in the range commonly reported for *Large White* pigs (Schivazappa et al., 2002; Barbin, ElMasry, Sun, & Allen, 2013; Fulladosa, Muñoz, Serra, Arnau, & Gou, 2015b). Table 1 shows that X_S was less variable than the water and fat contents. Similarly, Barbin, ElMasry, Sun, and Allen (2013) found a great variability in the water and fat contents in fresh LD (X_W = 69.1-75.1 and X_F = 0.3-6.3% w.b.) and BF (X_W = 73.6-75.7 and X_F = 1.1-3.5% w.b.) for pork meat. Taking into account that the composition of both fresh muscles and hams varied greatly, it was considered convenient to compute the salt gain (ΔX_S) and the water loss (ΔX_W) in order both to describe the salting kinetics and to relate them with the ultrasonic parameters.

As previously mentioned, the salt absorption in the samples depends on their shape and dimensions, among other things. These factors are characteristic for each fresh ham, BF and LD sample. In the case of hams, the thickness and width of the pieces varied greatly (T = 10.7-12.8cm and Z = 28.7-34.3cm). In that of muscles, the BF samples were not only more irregular than the LD ones (Fig. 1) but also thicker and

wider (T = 4.4-6.4cm and Z = 14.9-18.7cm) than the LD samples (T = 3.6-5.3cm and Z = 10.7-12.0cm) (Table 1).

3.2. SALTING KINETICS IN DRY-SALTED MUSCLES AND HAMS

As can be observed in Table 2, the salt gain (ΔX_S) and water loss (ΔX_W) in the SL slice of LD were significantly ($p<0.05$) greater than in that of BF after 48h of dry salting. Thus, the X_S in the SL slice was 6.9±0.5% w.b. in LD and 4.2±0.1% w.b. in BF and the X_W was 65.0±0.3% w.b. in LD and 69.6±1.2% w.b. in BF after 48h of dry salting. In addition, the ΔX_S and ΔX_W in hams was slower than in muscles. As an example, the salt gain in hams salted for 11 d (2.7±0.3% w.b.) was similar to that found in LD-BF muscles salted for 12h (3.0±0.3% w.b. for LD and 2.2±0.2% w.b for BF). Due to the fact that meat salting is mainly controlled by diffusion, the different compositional changes in both muscles and hams were linked to the different structure/composition, width and thickness of BF, LD and hams (Table 1). In fact, significant ($p<0.05$) relationships were found between the muscle dimensions (T and Z) and the compositional changes (ΔX_S and ΔX_W). Thus, the greater thickness and width of BF could explain its smaller ΔX_S and ΔX_W compared to LD (Table 2).

On the other hand, X_F was not found to be a factor that significantly ($p<0.05$) affected the compositional changes in muscles despite it being well known that fat hinders mass transport in food materials (Røra, Furuhaug, Fjæra, & Skjervold, 2004; Grau, Albarracín, Toldrá, Antequera, & Barat, 2008). That could be due to the narrow experimental range of the fat content covered by the muscles and hams used in the present study (Table 1).

It should also be remarked that a wide experimental dispersion was found, which was more evident for ΔX_W. As an example, the ΔX_W was -6.7±1.6% w.b. and the ΔX_S was 4.5±0.4% w.b. in LD salted for 36h and the ΔX_W was -7.8±2.7% w.b. and the ΔX_S was 2.7±0.3% w.b. in hams salted for 11 d. This experimental dispersion might be mainly ascribed to the heterogeneous dimensions, composition and structure of the fresh meat.

In Fig. 3, the profile of the salt gain and water loss in the sections (from 1S to 5S) of the slice (SL) of the LD and BF samples is plotted at different salting times (6, 12, 24, 36 and 48h). In general terms, the ΔX_S and ΔX_W profiles exhibited a reasonably good symmetry in both muscles (Fig. 3). As expected, the most marked compositional changes (water and salt) took place in the end sections (1S and 5S), the ΔX_S and ΔX_W

in these sections being significantly ($p<0.05$) higher than those in the central (3S) and intermediate (2S and 4S) ones (Fig. 3). As an example, the ΔX_S was 5.8±1.1% w.b. and the ΔX_W was -9.4±1.7% w.b. in the end sections (avg. 1S and 5S) of LD after 36h of dry salting, while the ΔX_S was 4.3±0.3 and 3.1±0.1% w.b. and the ΔX_W was -6.2±1.1 and -4.7±1.1% w.b. in the intermediate (avg. 2S and 4S) and central (3S) sections, respectively, for the same muscle and salting time. The differences between the sections grew as salting progressed, bending the initially flat profiles (Fig. 3). The salt and water profiles also illustrate the fact that the compositional changes in LD were bigger than in BF. Fig. 4 shows the relationship between the composition of SL and that of the zone of ultrasonic measurement (3S) for the salt gain (A) and the water loss (B). As can be observed, the ΔX_S and ΔX_W in 3S were lower than those found in the whole SL slice. Those differences could be explained by considering that, although the thickness of 3S was similar to that of the intermediate sections (2S and 4S), it was thicker than the end sections (1S and 5S) (Fig. 1). Moreover, the contact area between the sample and the salt was larger in the external sections (1S and 5S), which also helps to increase the differences between the SL and 3S salt contents. Additionally, the transducers' surface (A303S model, 1.77cm^2) was in contact with the 3S section, which may hinder the mass transfer (salt and water) due to the fact that it reduces the contact area between the sample and the salt (Fig. 1). Despite the experimental variability, significant ($p<0.05$) relationships were observed between the composition of 3S and SL in both muscles studied, showing high correlation coefficients (avg. R^2 = 0.898) (Fig. 4). These results demonstrate that employing ultrasound to assess the compositional changes occurring in a particular zone could be used to evaluate the changes in the whole meat piece, which would be of great interest for industrial purposes. Relationships like those shown in Fig. 4 are dependent on the shape and dimensions of the meat piece; accordingly, if these factors are sufficiently different from those considered in the present study, new relationships should be developed. Alternatively, a larger number of transducers could be used to assess an average composition of the whole meat piece, as was done in the present study with an irregularly-shaped product, such as ham.

3.3. ULTRASONIC MONITORING OF THE DRY SALTING OF MUSCLES

Fig. 5 shows the change in the ultrasonic velocity (V) of the LD samples during dry salting at 2ºC for 12 and 24h. The same behavior (data not shown) was observed after the LD samples were submitted to the dry salting process for 6, 36 and 48h; in the case of the BF samples, this behavior was observed after every salting time (6, 12, 24, 36

and 48h). The initial V in the fresh muscle (V_0) varied markedly (1558.3±22.7m/s for the LD and 1525.8±10.5m/s for the BF). In previous studies, V has been linked to the meat composition and in particular to its water and fat content (Simal, Benedito, Clemente, Femenia, & Rosselló, 2003; Corona, García-Pérez, Ventanas, & Benedito, 2014). However, in the present study, the V_0 was not significantly ($p>0.05$) related either to the water or the fat contents, probably because the analytical determinations in the fresh sample were not carried out where the V_0 was measured. In addition to composition, the great variability found for V_0 could be explained by the differences in the amount of connective tissue and its distribution among the muscles of different animals, which could lead to differences in their textural properties.

Fig. 5 illustrates how the V increased gradually during dry salting. This behavior could be explained by the fact that ultrasound travels faster in solids, with a high elastic modulus (Benedito, Cárcel, Clemente, & Mulet, 2000), than in liquids (water). Thus, the rise in V is caused by the increase in the solid content, due to the salt gain and water loss that takes place during salting. The same behavior was observed by De Prados, García-Pérez, and Benedito (2015b), who reported an increase in the V in brine-salted cylindrical LD and BF samples. Similarly, Kinsler, Frey, Coppens, and Sanders (1982) observed that the greater the salt concentration in an aqueous solution, the higher the V. De Prados, García-Pérez, and Benedito (2015b) measured the V in small cylindrical samples before and after salting; however, in the present study, the V was measured during dry salting in LD and BF muscle pieces (≈1kg) at intervals of 5min, which proves the feasibility of the online ultrasonic monitoring.

On the other hand, as can be observed in LD salted for 24h (Fig. 5), the V evolved differently in samples under equal salting conditions (temperature, salting time and salt moisture). This could be ascribed to the fact that the salting behavior was different due to the variable dimensions and shape of each fresh muscle (Table 1 and Fig. 1), as well as to the heterogeneity of the fresh meat composition and structure, among other factors.

Despite the progressive increase in ultrasonic velocity during dry salting, some unexpected behavior was observed in the first hours of the process. As an example, Fig. 5 illustrates this behavior for a 24h dry salting trial of LD. A sharp increase in V was found in the first 5h of dry salting (Fig. 5), which was observed in almost all the trials. This behavior is considered to be unexpected because it does not match the normal kinetics for salt and water diffusion in meat. Due to the marked temperature

342 effect on the V (Povey & Scanlon, 1983; Mulet, Benedito, Bon, & Sanjuan, 1999), it is
343 thought that this steep temperature increase could be associated with a possible
344 temperature rise caused by the sample positioning in the ultrasonic experimental set-
345 up. However, a non-significant ($p>0.05$) relationship was found between the measured
346 temperature and the rise in V. Thus, two tests were conducted in order to explain this
347 steep initial increase in V. First, a methacrylate cylinder (6cm in length and 4cm in
348 diameter) was covered with coarse salt (NaCl moisturized at 10% w/w) for 24h and V
349 was measured every 5min. The results showed a constant V (Fig. 6A), which was
350 expected due to fact that the methacrylate cylinder is not affected by salt absorption. In
351 the second test, the V was measured every 5min in the same methacrylate cylinder,
352 but without salt for the first 6h. Afterwards, 1mL of water was added on both flat
353 surfaces of the cylinder, between the sample and the transducers, and subsequently
354 covered with salt. During the first 6h the V was almost constant, followed by an
355 increase for approximately 2h when water and salt were added (Fig. 6B). This behavior
356 could be explained by considering the formation of a salt solution between the
357 transducers and the samples' surfaces. Therefore, the sharp increase in V during the
358 first 5h of dry salting (Fig. 5) could be linked to the formation of a salt solution between
359 the transducer and the meat due to the initial extraction of water from the most external
360 meat layers.

361 **3.4. RELATIONSHIP BETWEEN ULTRASONIC VELOCITY AND COMPOSITIONAL**
362 **CHANGES IN MUSCLES AND HAMS**

363 In order to obtain a good estimator of the dry salting progress based on the ultrasonic
364 parameters and bearing in mind the great variability of V_0, the total velocity variation
365 (ΔV) during salting after 6, 12, 24, 36 and 48h in LD-BF muscles and 2, 4, 7, 11 and 16
366 d in hams was considered (Table 2). The increase in ΔV during dry salting was related
367 to the increase in the solid content of the sample (muscles and hams), as previously
368 mentioned. In muscles, the ΔV value of the LD samples was higher than that of BF, the
369 average ΔV of LD being 59.5m/s and that of BF 30.6m/s after 48h of dry salting. These
370 results are consistent with the greater compositional changes (salt and water) which
371 occur in LD (ΔX_S = 6.7% w.b. and ΔX_W = -9.2% w.b., Table 2) compared to BF. In
372 hams, the ΔV was 46.8m/s after 16 d of dry salting. Different values of ΔV were
373 obtained in hams and LD-BF muscles for a similar salt gain and water loss (Table 2).
374 As an example, the ΔV was 21.9m/s in hams for a ΔX_S of 1.1% w.b. and a ΔX_W = -
375 5.1% w.b. after 2 d of salting. In contrast, the ΔV was 4.5m/s in BF for a ΔX_S of 1.6%
376 w.b. and a ΔX_W = -3.2% w.b. after 6h of salting. De Prados, García-Pérez, and

377 Benedito (2015b) also found higher values of ΔV for a similar salt gain and water loss
378 in LD and BF samples that had been brine-salted for different times (24, 48, 96 and
379 168h). These authors found that cylindrical LD and BF samples reached a ΔV of
380 32.6m/s for ΔX_S = 1.9% w.b. and ΔX_W = -2.3% w.b., after 24h of brine salting. This
381 difference could be linked to the type of ultrasonic measurement. In the present study,
382 the ultrasonic velocity was measured online in LD and BF muscles at time intervals of
383 5min; consequently, transducers and muscles were in continuous contact during
384 salting. This fact prevented the salt from being in direct contact with the sample surface
385 where the ultrasonic velocity was being measured. On the contrary, in the hams used
386 in the present study and in De Prados, García-Pérez, and Benedito (2015b), the V was
387 measured before and after salting; therefore, the meat surface where transducers were
388 located for velocity measurements had been in contact with salt, which can lead to
389 protein denaturation, salt intake and water loss, giving rise to a fast surface textural
390 increase, and, therefore, to a higher initial ΔV.

391 Despite the fact that ΔV reduced the wide dispersion of the V, there was still a great
392 variability for each specific time (Table 2). As previously mentioned, this could be linked
393 to the experimental variability provoked by the heterogeneity of the fresh meat samples
394 and the salting process itself, which resulted in salted samples with different water and
395 salt contents after the same salting time. The relationship between the salt gain (ΔX_S)
396 and the ultrasonic velocity variation (ΔV) in both muscles (LD-BF) is shown in Fig. 7.
397 Since both muscles (LD and BF) exhibited the same behavior in the ΔV *vs* ΔX_S plot, a
398 single relationship was considered for both muscles. Fig. 7 also shows the ΔV *vs* ΔX_S
399 relationship for whole hams, whose slope is quite similar to the one found for muscles.
400 A significant correlation ($p<0.05$) was found between ΔV and ΔX_S in LD-BF and in
401 hams as well as between ΔV and ΔX_W in LD-BF. An increase in ΔX_S produced a rise in
402 ΔV of hams and both muscles (LD-BF) (Fig. 7), whilst the opposite trend was found in
403 the case of an increase in ΔX_W ($\Delta V = -6.4\Delta X_W+2.9$ for LD-BF and $\Delta V = -1.9\Delta X_W+22.6$
404 for hams). However, the goodness of the fit was much more satisfactory for ΔX_S (R^2 =
405 0.903 in both muscles and R^2 = 0.758 in hams) than for ΔX_W (R^2 = 0.611 in both
406 muscles and R^2 = 0.200 in hams). The poorer fit for ΔX_S and ΔX_W in hams could be
407 attributed to the higher degree of variability linked to a greater structural (connective
408 tissue, fat profile, bones and different types of muscles) complexity. Moreover, in hams,
409 the salt and water content determinations were carried out considering the whole
410 muscular tissue of each piece, while the V was measured at 30 points and the initial
411 salt content and moisture are average values for 5 hams from the same batch. In

412 contrast, the ΔV in muscles was related to the compositional changes in 3S (ultrasonic
413 measurement zone).

414 The slope of the linear relationships shown in Fig. 7 indicates that the ΔV increased by
415 13.9±0.9 and 12.7±1.4m/s per 1% increase in ΔX_S for LD and BF muscles and hams,
416 respectively. Moreover, the ΔV decreased by 6.4 and 1.9m/s per 1% increase in ΔX_W
417 for LD and BF muscles and hams, respectively.

418 Although the slopes of the relationships shown in Fig. 7 are similar, a different value for
419 the intercept is observed in muscles (LD-BF) and hams. In hams, the ΔV is much
420 greater at ΔX_S = 1 than in muscles. This difference could be linked to the type of
421 ultrasonic measurement carried out in hams and in muscles. As previously mentioned,
422 the V was measured before and after salting in hams; therefore, the meat surface
423 where the transducers are located has been in contact with salt, which can result in
424 protein denaturation, salt intake and water loss, giving rise to a fast surface textural
425 increase, and, therefore, to a higher initial ΔV. However, for ΔX_S > 1% w.b, the effect of
426 salt on the ham surface in contact with the transducers is negligible, and therefore, the
427 relationship between ΔV and ΔX_S is similar for both samples (muscles and hams).

428 The slope of the ΔV vs ΔX_S relationship was similar to that reported by Kinsler, Frey,
429 Coppens, and Sanders (1982), who found that the ΔV increased by 13.7m/s per 1%
430 increase in ΔX_S in a saline solution at 2ºC. De Prados, García-Pérez, and Benedito
431 (2015b) also found slopes of 12.5m $s^{-1}\%^{-1}$ for BF and 13.7m $s^{-1}\%^{-1}$ for LD for the ΔV *vs*
432 ΔX_S relationship and of -9.8m $s^{-1}\%^{-1}$ (LD) and -8.2m $s^{-1}\%^{-1}$ (BF) for the ΔV *vs* ΔX_W
433 relationship, in brine-salted cylindrical samples at 2ºC. The fact that the slopes of the
434 ΔV *vs* ΔX_S relationship found in this study (for whole hams and two different muscles)
435 and those of Kinsler, Frey, Coppens, and Sanders (1982) and De Prados, García-
436 Pérez, and Benedito (2015b) were similar, indicates that the same increase in the ΔX_S
437 (1%) implies a similar change in the ΔV (≈13-14m/s), regardless of the type of salting
438 process and the structure of the product. Therefore, according to these results, the
439 ultrasonic parameter (ΔV) could be used to monitor the salt gain during dry salting in
440 meat products of great structural complexity, such as hams, or even in products which
441 are different in nature, such as cheese or fish.

442 **3.5. SALT CONTENT PREDICTION IN MUSCLES AND HAMS**

443 Linear regression models for salt prediction in muscles (LD and BF) and in hams are
444 shown in Table 3. Overall, the salt gain during the salting process can be predicted by

simply using the ΔV. In the case of muscles, R^2_{MC} and R^2_{MV} figures were close to 0.9. In contrast, R^2 reached 0.7 in the hams. As previously mentioned, this could be due to the hams being more variable as a result of their more complex nature: the connective tissue, the fat profile, the bones and the different types of muscles, among other factors. However, the Root Mean Square Error of Prediction was almost identical for both samples ($RMSE_{MV}$ 0.43% w.b. for muscles (LD and BF) and 0.44% w.b. for hams). In Fig. 8, the salt gain calculated ($\Delta X_{S\ CAL}$) by using predictive models (Table 3) is plotted against the experimental one ($\Delta X_{S\ EXP}$) for the muscles (BF and LD) and hams of the validation set. Close correlations (R^2 = 0.874 for muscles and R^2 = 0.722 for hams) were found and a random distribution between experimental and calculated values appeared (Fig. 8). Therefore, and considering the estimation errors (Table 3), ultrasound inspection should not be presented as an analytical tool for assessing the salt content in pork meat, but it could be used as an online inspection method for quality control purposes.

3.6. RELATIONSHIP BETWEEN TIME OF FLIGHT AND COMPOSITIONAL CHANGES IN MUSCLES AND HAMS

In addition to V, the time of flight (TOF) is an ultrasonic parameter that can be obtained from the ultrasonic signals, which presents the advantage of not requiring the measurement of the sample thickness. Therefore, the relative increase in the TOF was calculated ($\Delta TOF/TOF_0$) and related to the compositional changes (ΔX_S and ΔX_W) in muscles (LD and BF) (Fig. 9). ΔTOF was divided by the initial time of flight (TOF_0) to account for the initial sample thickness. In hams, this approach was not considered since the points where the ultrasonic measurements were carried out before and after salting were not exactly the same, and therefore, it was not possible to calculate the ΔTOF. Salt and water had an opposite effect on the $\Delta TOF/TOF_0$. Thus, a negative linear relationship was found between $\Delta TOF/TOF_0$ and ΔX_S (Fig. 9), while a positive one was found for $\Delta TOF/TOF_0$-ΔX_W ($\Delta TOF/TOF_0 = 0.0036\Delta X_W - 0.0029$).

Despite the correlation coefficients between the compositional changes and $\Delta TOF/TOF_0$ (R^2 0.859 for ΔX_S and 0.526 for ΔX_W; $RMSE_{\Delta XS}$ 0.46%) being slightly poorer than in the case of the ΔV (R^2 0.903 for ΔX_S and 0.611 for ΔX_W; $RMSE_{\Delta XS}$ 0.36%), the TOF could be considered a good ultrasonic parameter with which to characterize the salting process for online quality control purposes. The main advantage of considering the time of flight is that it is not necessary to measure the sample thickness by means of some electronic gage, something quite difficult to implement in an industrial environment where the pile dry salting is conducted.

Moreover, in this study, the time of flight parameter was calculated by means of the through-transmission mode; however, by changing the ultrasonic arrangement, it could be obtained by means of the pulse-echo mode, which uses a single transducer that acts as emitter and receiver. This would reduce the cost of the industrial devices and would also minimize the impact of the measurements on the salt and water transfer. However, further work is required to test the feasibility of the pulse-echo mode on the continuous monitoring of the salting process in meat products, such as muscles, but mostly in hams, which have a greater anatomical complexity.

4. CONCLUSIONS

Ultrasonic velocity increased progressively during the dry salting of meat due to the salt gain and water loss. As a result of the high degree of variability of the ultrasonic velocity in the fresh samples, the ultrasonic velocity variation is the most appropriate parameter with which to monitor the meat dry-salting process. Ultrasonic velocity variation showed a satisfactory correlation with the salt gain in muscles and hams. Moreover, models used to predict the salt gain in muscles and hams during salting were proven to be accurate enough for industrial online quality control purposes. Thereby, ultrasound may be considered as a fast and reliable technique for non-destructive, non-invasive salt content characterization and for the online monitoring of the dry salting of meat. Velocity variation can be measured online in meat muscles, such as *Longissimus dorsi* and *Biceps femoris*, but further work is required to test the feasibility of employing ultrasonic online monitoring for more complex whole pieces, such as ham. Moreover, future research should address the measurement of the time of flight by means of the pulse-echo mode in order to facilitate the implementation of an industrial online ultrasonic device.

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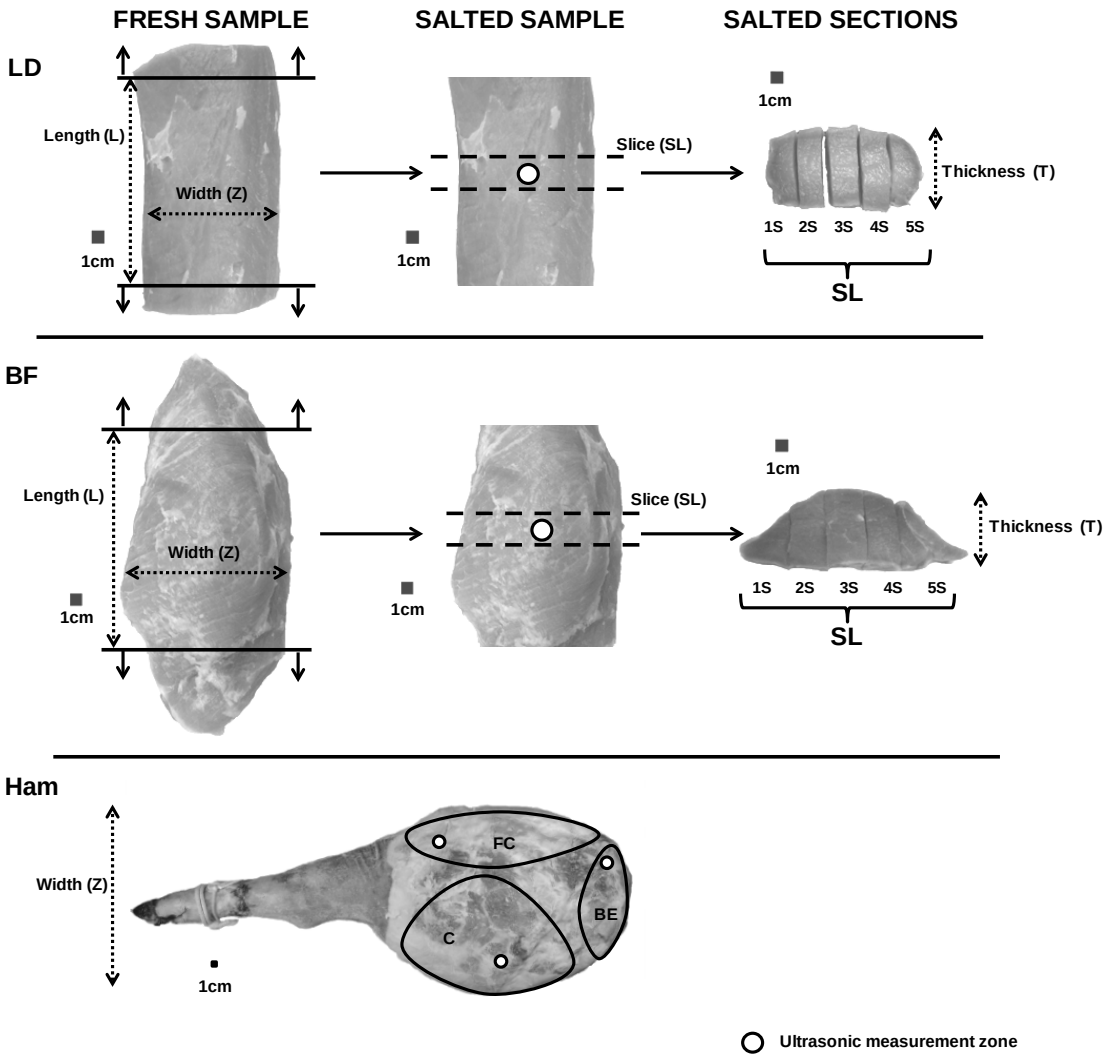
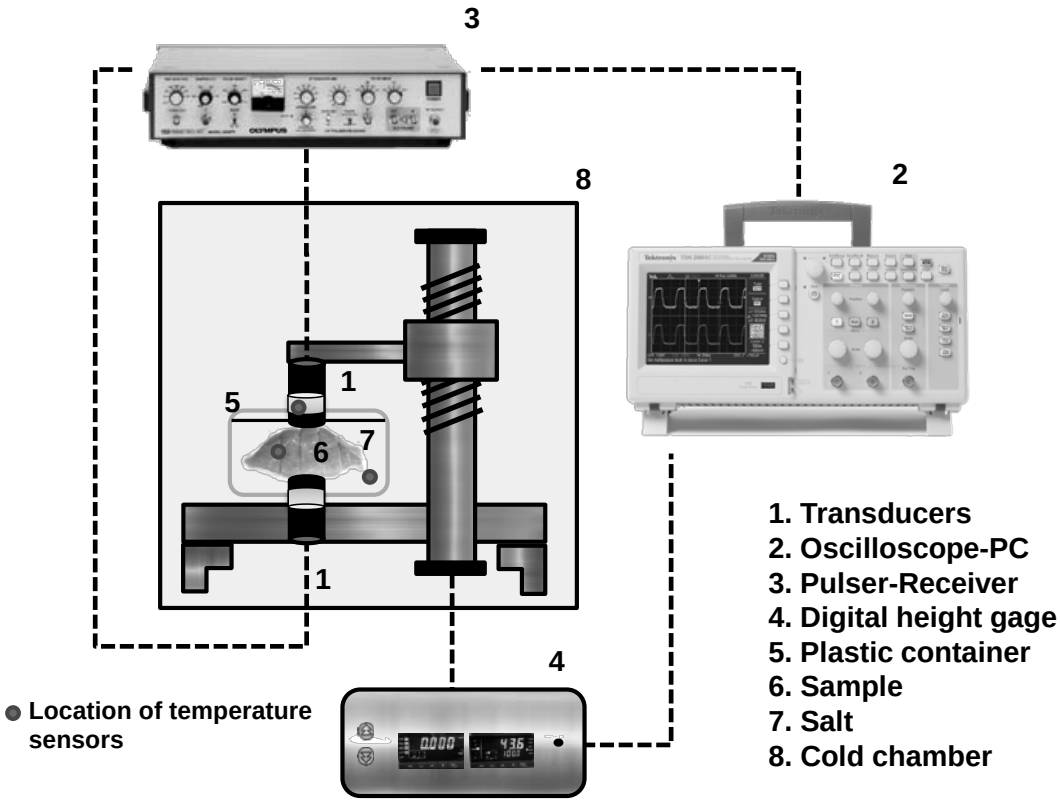
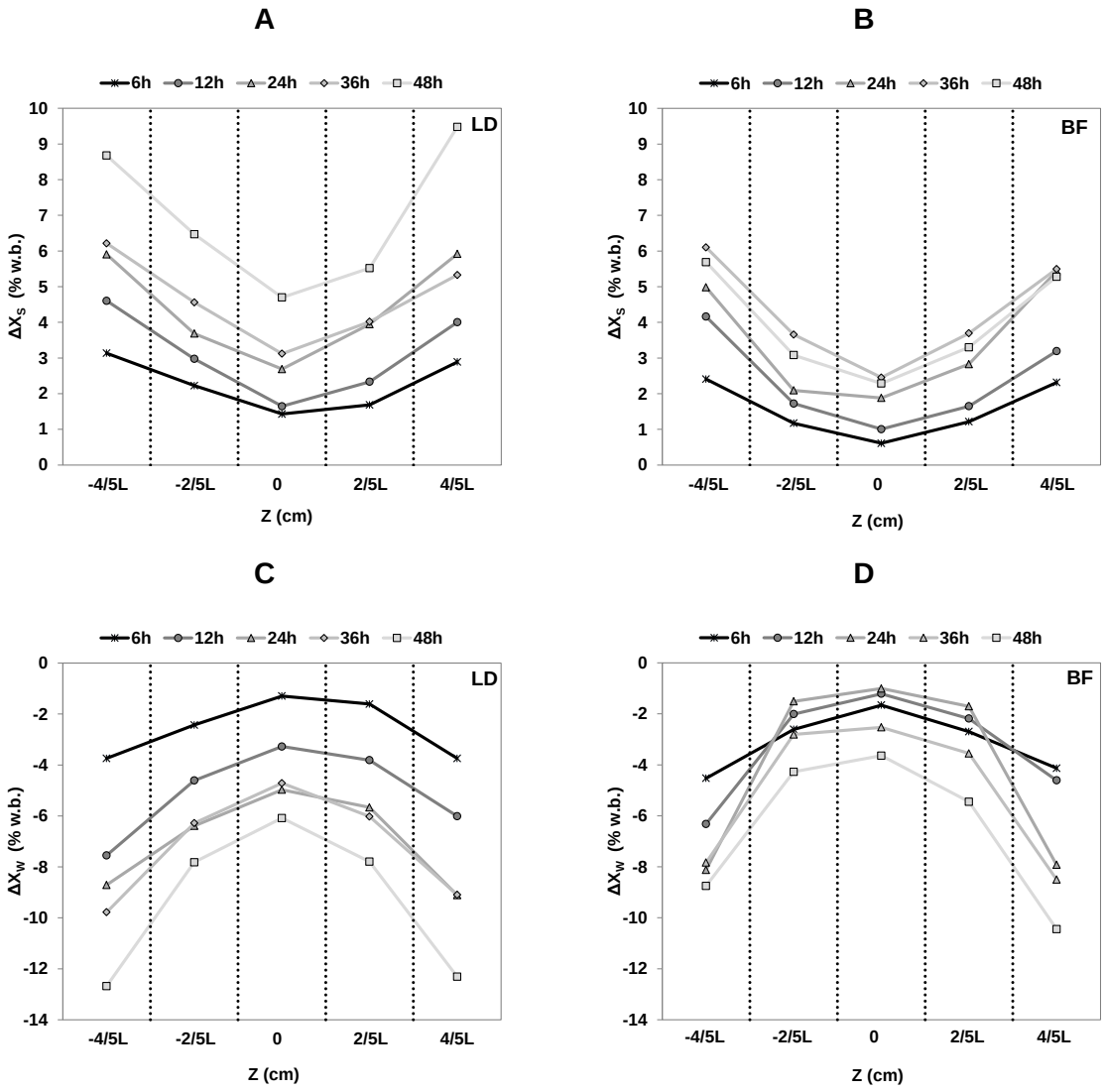
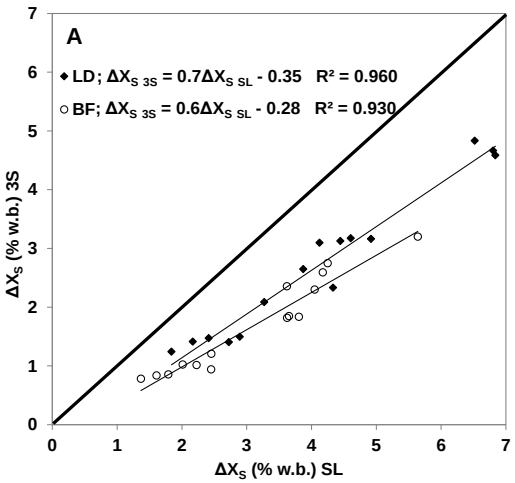
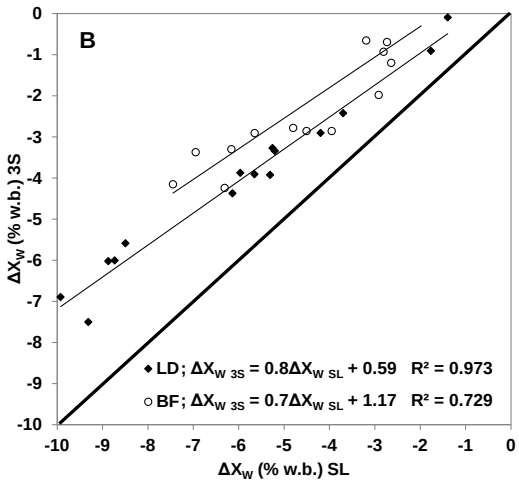
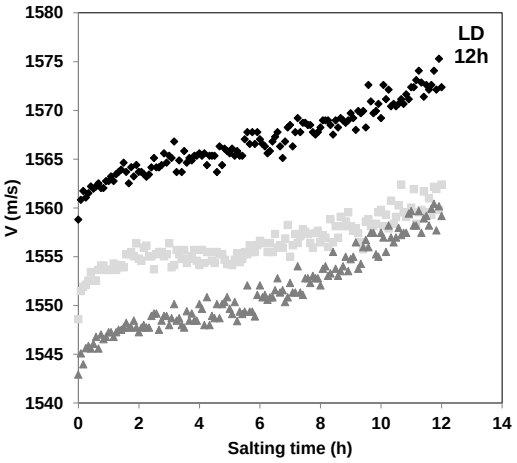

FRESH SAMPLE
SALTED SAMPLE
SALTED SECTIONS
LD
Length (L)
Width (Z)
1cm
1cm
Slice (SL)
1cm
Thickness (T)
1S 2S 3S 4S 5S
SL
BF
Length (L)
Width (Z)
1cm
1cm
Slice (SL)
1cm
Thickness (T)
1S 2S 3S 4S 5S
SL
Ham
Width (Z)
1cm
FC
BE
C
Ultrasonic measurement zone

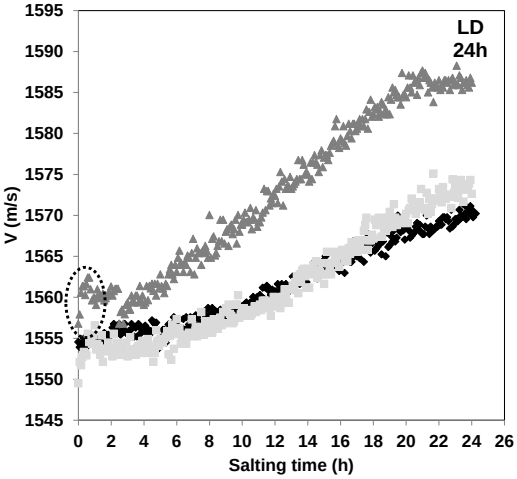

3
8
2
1
5
6
7
1
4
Location of temperature sensors
1. Transducers
2. Oscilloscope-PC
3. Pulser-Receiver
4. Digital height gage
5. Plastic container
6. Sample
7. Salt
8. Cold chamber

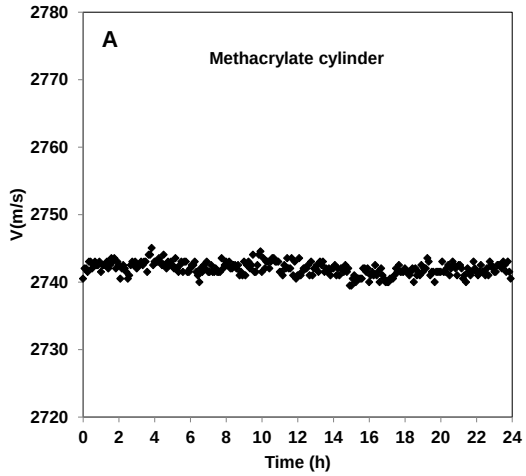

A
B
C
D
6h
12h
24h
36h
48h
LD
BF
ΔXS (% w.b.)
ΔXW (% w.b.)
Z (cm)
-4/5L
-2/5L
0
2/5L
4/5L

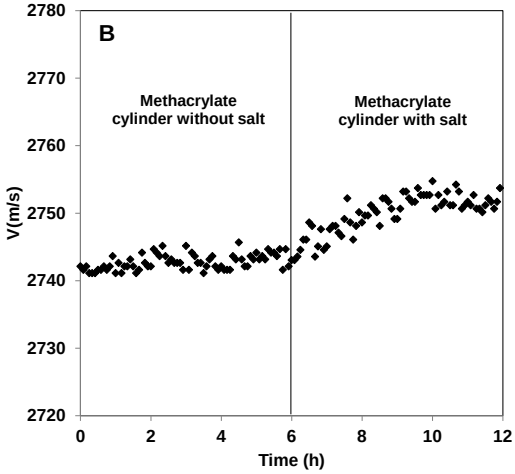

A
LD; ΔX_S 3S = 0.7ΔX_S SL - 0.35 R² = 0.960
BF; ΔX_S 3S = 0.6ΔX_S SL - 0.28 R² = 0.930
ΔX_S (% w.b.) 3S
ΔX_S (% w.b.) SL
0 1 2 3 4 5 6 7

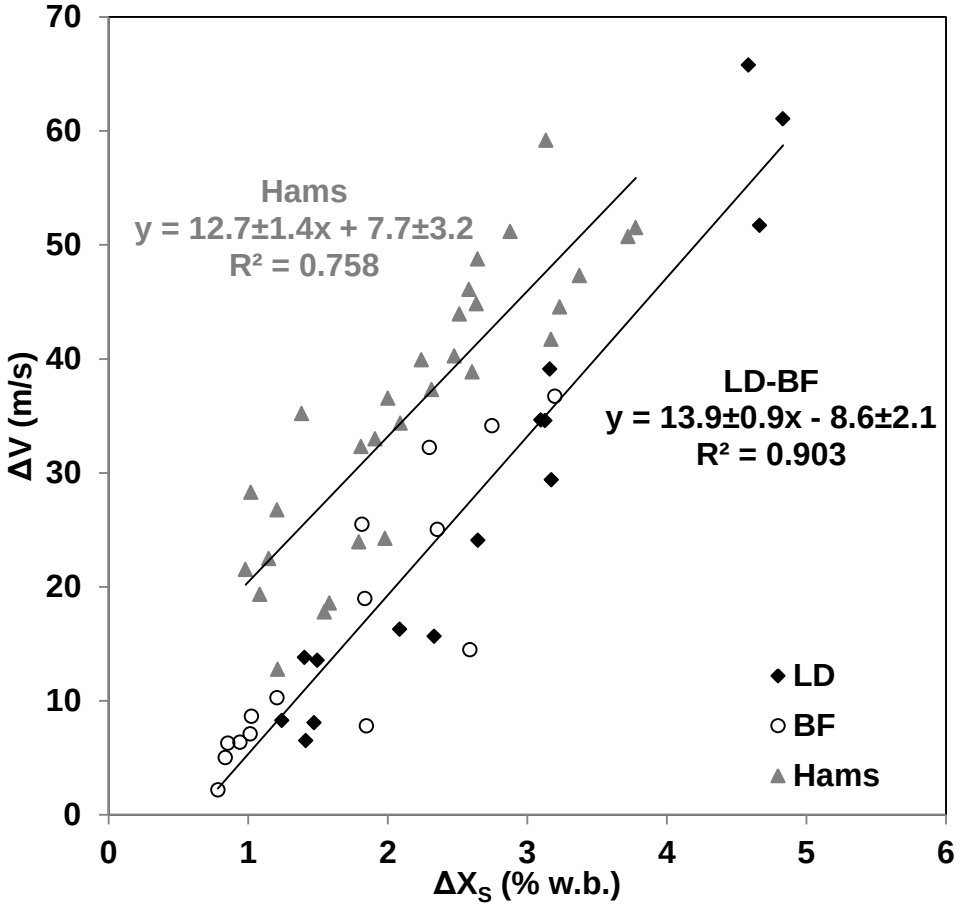

B
LD; ΔX_W 3S = 0.8ΔX_W SL + 0.59 R² = 0.973
BF; ΔX_W 3S = 0.7ΔX_W SL + 1.17 R² = 0.729
ΔX_W (% w.b.) 3S
ΔX_W (% w.b.) SL
0 -1 -2 -3 -4 -5 -6 -7 -8 -9 -10

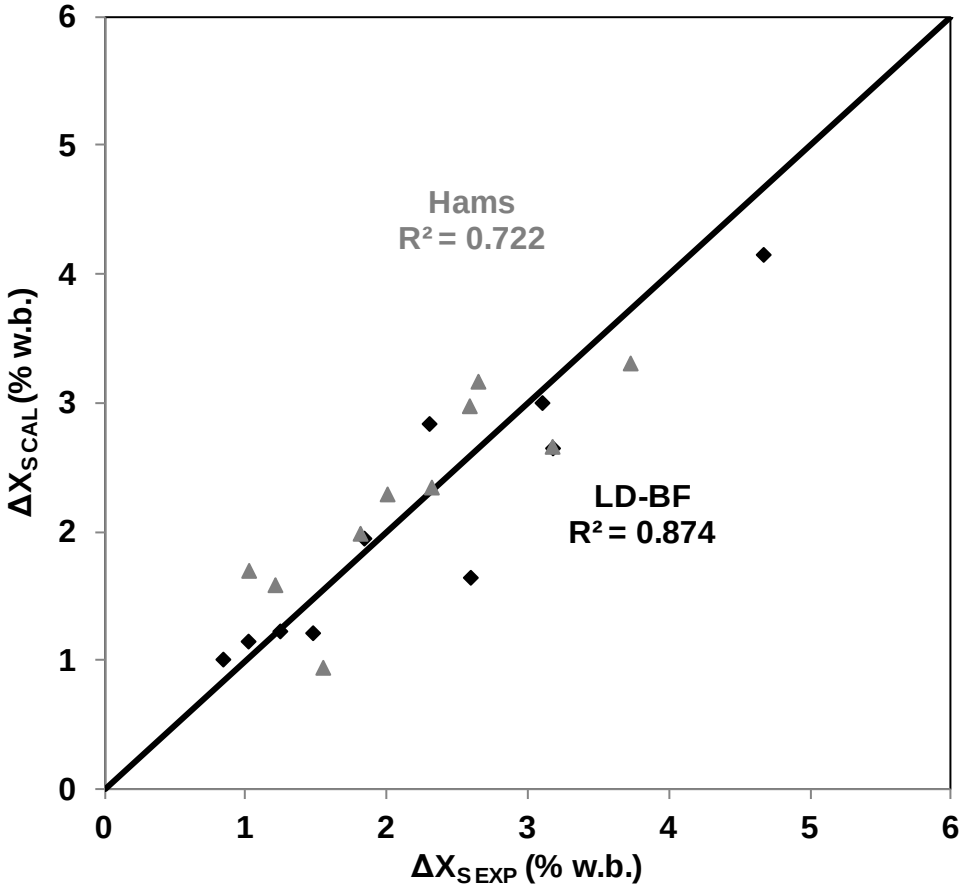

LD
12h
V (m/s)
Salting time (h)
1580
1575
1570
1565
1560
1555
1550
1545
1540
0
2
4
6
8
10
12
14


LD
24h
V (m/s)
Salting time (h)
1595
1590
1585
1580
1575
1570
1565
1560
1555
1550
1545
0
2
4
6
8
10
12
14
16
18
20
22
24
26


A
Methacrylate cylinder
V(m/s)
2780
2770
2760
2750
2740
2730
2720
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (h)


B
Methacrylate cylinder without salt
Methacrylate cylinder with salt
V(m/s)
2780
2770
2760
2750
2740
2730
2720
0 2 4 6 8 10 12
Time (h)


Hams
y = 12.7±1.4x + 7.7±3.2
R² = 0.758
LD-BF
y = 13.9±0.9x - 8.6±2.1
R² = 0.903
LD
BF
Hams
ΔV (m/s)
ΔXS (% w.b.)
0
10
20
30
40
50
60
70
0
1
2
3
4
5
6


Hams
R² = 0.722
LD-BF
R² = 0.874
ΔXS CAL (% w.b.)
ΔXS EXP (% w.b.)
0
1
2
3
4
5
6

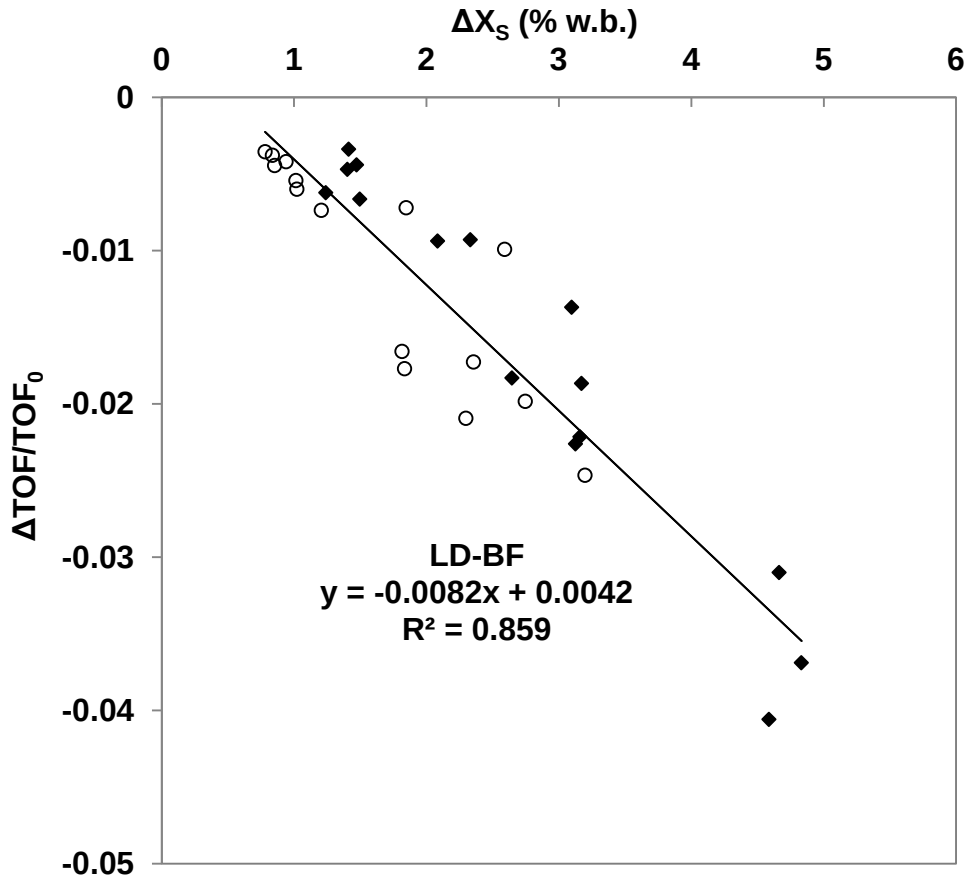

ΔXS (% w.b.)
0
1
2
3
4
5
6
0
-0.01
-0.02
-0.03
-0.04
-0.05
ΔTOF/TOF0
LD-BF
y = -0.0082x + 0.0042
R² = 0.859

Fig. 1. Sample preparation. Fresh sample, salted sample and sections (1S, 2S, 3S, 4S and 5S) of *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles. Ultrasonic measurement zones in muscles and hams. C. Cushion, FC. Fore Cushion and BE. Butt End.

Fig. 2. Experimental set-up for online ultrasonic measurements in *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles during dry salting.

Fig. 3. Profiles of salt gain (ΔX_S) and water loss (ΔX_W) in the slice (SL) of *Longissimus dorsi* (LD) (**A** and **C**) and *Biceps femoris* (BF) (**B** and **D**) muscles during dry salting (6, 12, 24, 36 and 48h) at 2ºC.

Fig. 4. Relationship between the salt gain (ΔX_S) (**A**) and the water loss (ΔX_W) (**B**) in the slice (SL) and ultrasonic measurement section (3S) of *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles during dry salting at 2ºC.

Fig. 5. Ultrasonic velocity (V) evolution in the ultrasonic measurement zone (3S) of the *Longissimus dorsi* (LD) muscle during dry salting (12 and 24h) at 2ºC. Dotted circle indicates an initial sharp increase in the ultrasonic velocity (V) during ultrasonic monitoring. Each series correspond to a different replicate.

Fig. 6. Ultrasonic velocity (V) evolution: (**A**) in a methacrylate cylinder (6cm in length and 4cm in diameter) covered with salt for 24h at 2ºC. (**B**) in a methacrylate cylinder; 6h without salt and 6h with salt and 1mL of water on the transducer's surfaces at 2ºC.

Fig. 7. Relationship between the salt gain (ΔX_S) and the ultrasonic velocity variation (ΔV) in the ultrasonic measurement zone (3S) of the *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles and hams.

Fig. 8. Calculated ($\Delta X_{S\ CAL}$) and experimental ($\Delta X_{S\ EXP}$) salt gain in *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles and hams for the validation set.

Fig. 9. Relationship between the salt gain (ΔX_S) and the relative increase in the time of flight ($\Delta TOF/TOF_0$) in the ultrasonic measurement zone (3S) of the *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles.

Highlights:

-Ultrasound velocity (V) allowed the online monitoring of meat dry salting.

-US could be considered a feasible means of predicting salt content in pork meat.

-Ultrasonic salt content assessment was successful for both muscles and hams.

-Time of flight measurement by pulse-echo could improve industrial applicability.

Table 1 Fat (X_F), water (X_W) and salt content (X_S), thickness (T) and width (Z) of the fresh *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles and hams.

	LD	BF	Hams
X_F (% w.b.)	1.5±1.0[a]	2.6±1.8[a]	12.4±3.2[b]
X_W (% w.b.)	73.9±1.4[b]	74.9±1.7[b]	69.8±2.5[c]
X_S (% w.b.)	0.19±0.06[c]	0.20±0.03[c]	0.26±0.04[d]
T (cm)	4.5±0.5[d]	5.4±0.7[e]	11.5*±0.5[f]
Z (cm)	11.3±0.4[f]	16.7±1.2[g]	31.6±1.3[h]

Mean values and standard deviation.
Different letters in the same row indicate significant differences between LD, BF and hams ($p < 0.05$).
*Mean value between three zones of ham (cushion, fore cushion and butt end)

Table 2 Salt gain (ΔX_S), water loss (ΔX_W) and ultrasonic velocity variation (ΔV) in the slice (SL) of *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles and in hams, during dry salting at 2ºC.

Salting time	ΔX_S (% w.b.)		ΔX_W (% w.b.)		ΔV (m/s)	
	LD	BF	LD	BF	LD	BF
6h	2.1±0.3^{a1}	1.6±0.2^{a1}	-2.4±1.5^{a1}	-3.2±0.7^{a1}	7.6±1.0^{a1}	4.5±2.1^{a1}
12h	3.0±0.3^{b2}	2.2±0.2^{ab3}	-4.7±0.9^{ab2}	-3.1±1.3^{a2}	14.6±1.5^{a2}	7.4±1.2^{a3}
24h	4.3±0.4^{c4}	3.4±0.9^{bc4}	-6.9±2.1^{bc3}	-4.0±1.5^{a3}	23.1±6.9^{b4}	10.9±3.4^{a4}
36h	4.5±0.4^{c5}	4.4±1.1^{c5}	-6.7±1.6^{bc4}	-5.1±2.1^{ab4}	36.1±2.6^{c5}	26.9±9.0^{b5}
48h	6.7±0.2^{d6}	4.0±0.3^{c7}	-9.2±0.7^{c5}	-6.5±0.4^{b6}	59.5±7.2^{d6}	30.6±4.5^{b7}
	Hams		Hams		Hams	
2 d	1.1±0.1^{a}		-5.1±2.3^{a}		21.9±5.6^{a}	
4 d	1.7±0.2^{b}		-3.8±1.7^{a}		25.3±7.1^{a}	
7 d	2.2±0.3^{c}		-6.0±1.4^{ab}		37.6±4.0^{b}	
11 d	2.7±0.3^{d}		-7.8±2.7^{bc}		47.3±7.6^{c}	
16 d	3.3±0.4^{e}		-8.8±2.9^{c}		46.8±3.8^{c}	

Average values and standard deviation. $^{a, b, c, d}$ Values in a column with different letters indicate significant differences between salting times ($p < 0.05$).^{1,2,3,4,5,6,7} Values in a row with different numbers indicate significant differences between muscles ($p < 0.05$).

Table 3 Linear regression models for salt gain (ΔX_S) prediction using ultrasonic velocity variation (ΔV) for *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles and hams.

SAMPLES	MODEL	n_{MC}[a]	$RMSE_{MC}$(%)[b]	R^2_{MC}[c]	n_{MV}[a]	$RMSE_{MV}$(%)[b]	R^2_{MV}[c]
LD-BF	ΔX_S=+0.78+0.062ΔV	20	0.32	0.928	10	0.43	0.874
Hams	ΔX_S=+0.20+0.057ΔV	20	0.41	0.789	10	0.44	0.722

[a] n=number of samples
[b] RMSE=Root Mean Square Error
[c] R^2=coefficient of determination and MC and MV subscripts refer to the calibration and validation set, respectively.