Monitoring Physicochemical Modifications In Beef Steaks During Dry Salting Using Contact And Non-Contact Ultrasonic Techniques

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Abstract

In conventional ultrasonic techniques, the necessary contact between the sensor and the product has constrained the implementation of ultrasound for quality control purposes in the meat industry. The use of novel air-coupled ultrasonic technologies provides multiple advantages linked to contactless inspection. Therefore, this study aims to compare the feasibility of contact (C; 1 MHz) and non-contact (NC; 0.3 MHz) ultrasonic techniques for monitoring the physicochemical modifications undergone by beef steaks during dry salting after different times (0, 1, 4, 8 and 24 hours).

Experimental results showed that the ultrasonic velocity increased during salting, which was linked to the reduction in Time-of-Flight ratio (R_{TOF}) and sample shrinkage (velocity C: R^2=0.99; velocity NC: R^2=0.93 and R_{TOF} C: R^2=0.98; R_{TOF} NC: R^2=0.95). In terms of the compositional changes provoked by salting, the velocity variation ($\triangle V$) increased linearly (C: R^2=0.97; NC: R^2=0.95) with the salt content. As for textural parameters, hardness (C: R^2=0.99; NC: R^2=0.97) and relaxation capacity (C: R^2=0.96; NC: R^2=0.94) were well correlated with the $\triangle V$ through power equations. Experimental results reflected that the performance of the non-contact ultrasonic technique was similar to that of the contact technique as regards the monitoring of the physicochemical changes undergone by beef steaks during dry salting.

Keywords: *beef; salting; monitoring; ultrasound; air-coupled; non-destructive*

1. Introduction

Despite the development of alternative preservation techniques, salting is widely used in the meat and fish industry. Beyond its inherent preservative effect, salt also plays a relevant role in meat products, one that is intimately related to consumer perception linked to the development of a characteristic texture, taste and flavor (Matthews & Strong, 2005; Radovanovic et al., 2008). This is why it is no trivial matter for the meat industry to implement efficient salt reduction strategies that regulatory food agencies have been calling for, without compromising either the safety or the quality of cured meat products (Albarracín et al., 2011; Desmond, 2006; European Commission, 2012; Ojha et al., 2016).

Its simplicity makes dry salting one of the most widely used techniques on meat products. It consists of covering the whole piece, placed in mono or multilayer, with coarse salt at a controlled temperature and relative humidity for a period of time dependent on the nature of the product and its thickness (Barat et al., 2004; de Prados et al., 2016). Dry-salting method itself implies variability that stems from the experimental procedure: temperature, humidity, sample location in the multilayer configuration or the size of the salt crystals, among other things (Albarracín et al., 2011; Van Nguyen et al., 2010). In addition, the heterogeneity in the meat samples (weight, shape, size, composition, etc.) strongly impacts the distribution of salt through the piece (de Prados et al., 2016; Matthews & Strong, 2005). Despite its widespread use, a fixed salting time/product mass ratio is not optimal and often results in over and undersalted products. The excess of salt could lead to numerous defects in the final product, such as a salty flavor, meat toughness or even an increase in rancidity due to lipid oxidation (Garcia-Gil et al., 2014). However, undersalted products are also related to product defects, such as microbiological risk or textural defects due to excessive proteolysis (Contreras et al., 2020).

On an industrial scale, the salt content is a daily quality control parameter in dry-cured products, and the conventional analytical techniques of salt content measurement are slow, time consuming and require sample destruction (de Prados et al., 2015; Ruiz-Ramírez et al., 2005; Serra et al., 2005). Some examples of non-destructive techniques applied in the meat industry are near infrared spectroscopy (Collell et al., 2011; García-Rey et al., 2005), X-Ray (Fulladosa et al., 2015), hyperspectral imaging (Liu et al., 2013), computed tomography (Vestergaard et al., 2004) or microwave dielectric spectroscopy (Castro-Giráldez et al., 2010) among others. Some limitations of the aforementioned techniques are the high cost and maintenance of the associated equipment, the difficulty/impossibility of being used on large pieces or implemented in the process line or, in some cases, the low penetration capacity (Feng et al., 2014; Pérez-Santaescolástica et al., 2019).

Low-intensity ultrasound techniques have proven to be particularly useful as a tool for the quality control of industrial processes due to their low-cost, easy maintenance, portability and high degree of adaptability to process lines (Kerhervé et al., 2019; Scanlon, 2004). In the case of the meat industry, previous literature has evidenced the potential of ultrasound techniques for predicting the intramuscular fat in beef longissimus muscle (Park et al., 1994) or carcass composition (Ayuso et al., 2013), as well as for the purposes of assessing the fat content in raw pork hams (Fulladosa et al., 2015) and the salt content in different pork pieces (de Prados et al., 2015, 2016, 2017). In particular, ultrasound velocity has proven to be a useful indicator of the mechanical and/or textural properties of meat (Corona et al., 2013; Llull et al., 2002; Nowak et al., 2015). However, unlike other sectors such as aeronautics or energy, ultrasonic techniques have not been widely deployed since traditional ultrasonic techniques (contact or immersion) do not meet the particular requirements of the food industry in terms of safety or line speed.

More recently, non-contact ultrasound techniques have been successfully applied for the characterization of vacuum-packaged dry-cured ham (Álvarez-Arenas et al., 2009) and burger patties (Fariñas et al., 2021). The industrial application of these techniques based on air-coupled ultrasound sensors is a perfect fit for the aims of Industry 4.0.: it can be easily implemented in production lines, and is completely non-destructive and non-invasive so it dramatically reduces the risk of cross-contamination while enabling data acquisition scanning of the samples in real time (Charoux et al., 2017; Fortin et al., 2003). There are two main drawbacks: the considerable energy loss due to the great mismatch between the acoustic impedances of air and meat and the complex assessment of the actual propagation path of the ultrasonic wave due to the uneven sample surface which can affect the estimation of parameters related to sample thickness, such as the ultrasonic velocity.

Therefore, the objective of this study is to test the feasibility of the non-contact ultrasound technique in the monitoring of physicochemical modifications in beef steaks during dry salting, then comparing these results with those obtained by means of contact ultrasound and textural techniques.

2. Materials and methods

2.1. Raw material and salting

Fifteen deep frozen yearling tenderloin beef steaks were purchased in a local market (Valencia, Spain). Each steak was thawed at 4 ºC for 24 hours. The samples were selected to obtain a steak as a model with which to monitor the salting. Thus, the samples selected were homogeneous in cut and thickness throughout.

Prior to dry salting, the thawed samples were weighed (± 0.01 g; PM4000, Mettler Toledo, Ohio, USA) and their thickness was measured at six different points using a micrometer (±0.01 mm; Mitutoyo, Kawasaki, Japan). The average weight of the beef steaks was 210.73 ± 6.28 g and the average thickness was 14.15 ± 2.02 mm. Additionally, the sample thickness was also measured using the height gauge used for the contact ultrasonic measurements (Table 1).

Dry salting experiments were carried out by covering each beef steak with 5 kg of coarse salt (NaCl moisturized at 10 % w/w) per kg of sample, following the procedure detailed in Garcia-Perez et al. (2019). The beef steaks were salted for different times (1, 4, 8 and 24 h) at 4 ºC to obtain different salt concentrations, according to the optimal salting time followed by Lorenzo et al. (2022): 0.6 day/kg product. Thus, the salting time was chosen to obtain low (LS, 1 h), medium (MS, 4 and 8 h) and over-salted (OS, 24 h) samples. At every salting time, dry-salting was performed in triplicate using one steak per test. Once the salting time finished, the surface salt was carefully removed from the steaks using compressed air.

2.2.Ultrasound measurements

2.2.1. Non-contact

A non-contact ultrasonic (NC) set-up (Figure 1a) comprises a pair of unfocused piezoelectric transducers with 0.3 MHz central frequency (Alvarez-Arenas, 2004), -25 dB peak sensitivity and 20 mm diameter (ITEFI-CSIC, Madrid, Spain). A 400 V square wave semi cycle tuned at 0.3 MHz was driven to the transmitter using a pulser/receiver (5077 PR, Olympus, Houston, TX, USA). The built-in low pass filter (cutoff frequency 10 MHz) was applied to the received signal together with 59 dB amplification. The resulting signal was digitized with an oscilloscope (MDO3024, Tektronix, WA, USA) controlled through pyVISA (Python) and averaged 128 samples at 10 MS/s.

The NC measurements were taken in through-transmission configuration at 4 ºC. Firstly, a blank measurement corresponding to the signal propagated through the air between the aligned pair of transducers was taken as calibration (Figure 2a, ref). For sample measurement, the steak was located on a rigid frame tied with a fishing line network in order to minimize the manipulation of the sample and ensure normal incidence (Figure 1a). Up to 6 ultrasonic measurements were acquired at different points along each steak by smoothly sliding the frame over the platform (Figure 1c), avoiding the edges and areas with fatty and connective tissues.

The time-of-flight method (TOF) was applied to monitor the time taken for the ultrasonic waves to pass through the steaks at different salting times. Prior to this, a Hanning window of 6 cycles in length was applied, centered at the main working frequency of the transducers (0.3 MHz) ensuring that the propagation was taking place along the direct path; this is no trivial matter due to the characteristic heterogeneity of the beef samples. Figure 2a shows normalized measurements taken at different salting times: signal distortion appears due to intrinsic inhomogeneities in the sample and a lack of a plane incident surface. All of these alterations may cause misleading results for TOF calculation purposes. For this reason, three signal analysis methods were applied to compute the TOF: cross-correlation, phase spectrum and edge detection with double threshold (Hull et al., 1984; Papadakis, 1976; Truell et al., 1969). Therefore, the TOF considered in this analysis was the average of those obtained by the three methods.

For the propagation velocity calculation, Eq. 1 was followed (Schindel & Hutchins, 1995):

$$v = \frac{L}{\left(L/v_0\right) + \Delta TOF} \qquad \text{Eq. 1}$$

where v is the ultrasonic velocity through the sample (m/s), L is the sample thickness (m), v_0 is the ultrasonic velocity in air (m/s) and ΔTOF (s) the time-of-flight difference obtained between a steak measurement and the corresponding reference. Due to the great difference between acoustic impedances of beef and air (~1.6 MRayl and ~442 Rayl, respectively), velocity measurements using this NC technique are highly sensitive to small changes in the sample thickness. Therefore, for uneven surfaces, its measurement is complicated and the assessment of the ultrasonic velocity can be misleading (Schindel & Hutchins, 1995).

2.2.2. Contact

The experimental set-up (Figure 1b) consisted of a pair of commercial narrowband transducers centered at 1 MHz (A314S-SU model, Panametrics, Waltham, MA, USA). The receiver transducer was embedded in the sample holder table and the transmitter one attached to a digital height gage, linked to a computer by an RS232 interface, as described by Contreras et al. (2021). The purpose of this device was twofold: it allowed the correct positioning of the sample in-between the aligned transducers for a through transmission measurement ensuring good coupling without permanent deformation; and it was possible to estimate accurately (± 0.01 mm) the thickness of the sample in the measurement location.

Prior to each set of measurements, a blank was taken by placing the transmitter and receiver in close contact using the automatic positioning of the digital height gage, after slightly moistening their radiating surface with water (Figure 2b, ref). Afterwards, the sample was located in-between the transducers, ensuring a perfect coupling using the automatic positioning system and the measurement was repeated at the 6 points along the steak as NC (Figure 1c). A 200 V square wave semi cycle tuned at 1 MHz was driven to the transmitter while attenuating 30 dB at the reception stage to avoid signal saturation. The resulting signal was digitized and averaged 128 samples at 2.5GS/s. The ultrasonic equipment used is the same as that described for NC (section 2.2.1).

Figure 2b shows normalized measurements taken at different salting times using the contact methodology. Unlike NC measurements, no noticeable distortion was observed in the signals. In consequence, TOF was obtained by firstly calculating the cross-correlation between each reference and the signal measured, since this method was previously proven to be accurate enough in these cases (Álvarez-Arenas et al., 2009; Garcia-Perez et al., 2019). Lastly, the ultrasonic velocity was obtained by following Eq. 2 (Truell et al., 1969):

$$v = \frac{L}{\Delta TOF} \qquad \text{Eq. 2}$$

where v is the ultrasonic velocity through the sample (m/s), L is the sample height measured with the digital gage (m) (see Table 1) and ΔTOF is the time-of-flight obtained from the cross-correlation (s) method.

In order to avoid the experimental variability related to temperature fluctuations, the steaks were stored at 4 ºC until the ultrasonic measurements were taken. Altogether, C and NC ultrasonic measurements took less than 5 minutes per sample. It should be noted that the NC measurements were always taken prior to C to avoid any thickness deformation caused by the pressure of the transducers.

2.3.Instrumental texture analysis

The textural properties of the beef steaks were determined experimentally using a texture analyzer (TA. XT2i, Stable Micro Systems, Surrey, UK) equipped with a 10 mm diameter cylindrical probe (SMS P/1K, ANAME, Madrid, Spain) by means of the stress-relaxation method (Bourne, 2002; Landahl et al., 2009). The measurements were taken in the previously tempered steak (4 ºC), avoiding the edges and fatty and connective tissue. The samples were compressed 20 % of their original height parallel to the fiber bundle direction and the further relaxation behavior was monitored for 15 s (Bourne, 2002). This test was selected to describe the viscoelastic properties of dry-cured meat products (Gou et al., 2008; Morales et al., 2007). Between 7 and 10 measurements were taken at different points in the steak at a compression rate of 1 mm/s. The textural parameters obtained were: hardness, computed as the maximum compression force reached at 20 % of strain (F_{max}, N) and the Total Relaxation Capacity (TRC, dimensionless), calculated according to Eq. 3 as the difference between maximum force (F_{max}) and the residual force at 15s of compression (F_{15s}).

$$TRC = \frac{F_{max} - F_{15s}}{F_{max}} \qquad \text{Eq. 3}$$

2.4. Compositional analysis

2.4.1. Moisture content and water activity

The moisture content (X_w) and water activity (a_w) were analysed in fresh and salted samples. Once the ultrasonic and textural measurements were taken, the samples were cut discarding fatty parts. The moisture content (X_w) was determined by a gravimetric method according to AOAC 950.46 (AOAC, 1997). Mainly, it consisted of drying the sample in a convection oven at 105 ºC until constant weight. The X_w was represented as wet basis (%, w.b.). Water activity (a_w) analysis was performed by a dew-point hygrometer at 25 ºC (Sprint th 500, NOVASINA, Switzerland). Both analyses were determined in triplicate at each salting time.

2.4.2. Salt and fat content

The salt content (X_s, % w.b.) was determined following the method described by de Prados et al., (2016). Firstly, a mince sample (0.5 g) was homogenized by ULTRA-TURRAX (T25, IKA Labortechnik, Germany) with 50 ml of distilled water at 8000 rpm for 5 minutes. Then, the homogenized sample was filtered through membrane filters (45 µm) and titrated in Chloride Analyzer equipment (Chloride Meter 926L, Ciba Corning, U.K.) (Cárcel et al., 2007). The X_s was represented as the salt (NaCl) content in wet basis of the sample (%, w.b.). The measurements were taken in triplicate for each salting time.

The fat content of fresh thawed samples was determined according to the AOAC 991.36 method (AOAC, 1997) by using the Soxhlet extraction equipment. This procedure was carried out in triplicate.

2.5.Effective moisture diffusivity

Mass transport modeling was analyzed in order to describe and better understand the rate of water loss (ΔX_w) and salt content gain (ΔX_s) during beef salting. The dry-salting process was considered as only being controlled by diffusion when considering coupled salt and moisture transport. The analytical solution of Fick's second law of salt transport in an infinite slab (Eq. 4) was used to determine the diffusion rate of salt, assuming negligible shrinkage, uniform initial salt distribution and unidimensional diffusion (Crank, 1975). An analogous equation was used for describing moisture transport.

$$X_s = X_{se} + (X_{so} - X_{se}) \sum_{n=o}^{\infty} \frac{8}{\pi^2 (2n+1)^2} \exp\left(\frac{-D_{eff}(2n+1)^2 \pi^2 t}{4L^2}\right) \qquad \text{Eq. 4}$$

Where X_s is the average salt content at a time t (% w.b.), X_{se} is the salt content in the equilibrium, X_{so} is the salt content at initial time, D_{eff} is the effective diffusivity of salt (m^2/s), t is the time (s) and L is the thickness of the steaks (m).

Eq. 4 and the analogous equation for moisture transport were fitted to the experimental salt (X_s) and moisture contents (X_w), respectively, in order to identify the effective diffusivity (D_{eff}) of both components during beef salting. D_{eff} was identified using the generalized reduced gradient (GRG) method from the optimization tool SOLVER available in Excel™ (Microsoft, WA, USA). The estimation of those variables was carried out by minimizing the sum of the squared differences between the experimental and calculated moisture and salt contents (de Prados et al., 2015).

2.6. Statistical analysis

The normality of the textural (maximum force and total relaxation capacity) and chemical parameters (water activity, moisture, salt and fat content) were evaluated by means of the Shapiro-Wilk test. Then, the mean, standard deviation and standard errors were calculated for each parameter considered. The analysis of variance (ANOVA) was applied in order to determine if the textural parameters were significantly affected by the moisture and salt contents of the beef steaks. The comparison of the aforementioned means were performed using Fisher's Least Significant Differences (LSD) test with a 95% confidence interval. Regression models have been applied using linear, exponential and power functions in order to correlate ultrasonic parameters with salting time, salt content and textural parameters. Modelling has been separately addressed for C and NC techniques since the analysis of the individual ability of both techniques was pursued. The statistical analysis was carried out using Statgraphics Centurion XVII (Statgraphics Technologies Inc., VA, USA).

3. Results and discussion

3.1.Physicochemical modifications in beef steaks during salting

3.1.1. Salt gain and moisture loss kinetics

The raw beef steaks presented an average moisture of 73.54 % (w.b.) and a fat content of 1.80 %. The salt content was almost negligible (0.15 % w.b.) and the a_w value was 0.972 (Table 2). Figure 3 shows the evolution of the moisture and salt contents during salting. A progressive dehydration was observed; thus, at 24 h, the samples presented a moisture content of 56.06 % (w.b.), which corresponds to a water loss (ΔX_w) of 10.91 % (w.b.) and a_w of 0.815 (Table 2). Inversely, the salt content increased progressively during salting, and it is possible to differentiate the three groups of samples according to the salt content (LS, MS and OS). Thus, a remarkably high salt content of 14.56 % (w.b.) was reached at 24h in OS samples. At 1h (LS), meanwhile, 4.81 % (w.b.) of salt content was obtained, which is similar to some typical dry-cured meat products, such as beef "cecina" (5-8 %, w.b.) (Lorenzo et al., 2022) and dry-cured ham (3.5-6 % of salt, w.b.) (de Prados et al., 2016). A similar salt content (NaCl) uptake was reported by Żochowska-Kujawska (2016) after salting deer meat (4-8 % NaCl) for 2 days, reaching values from 4.71 % (d.m.) (4 % NaCl) to 11.96 % (d.m.) (8 % NaCl).

Experimental salt gain and moisture loss kinetics were modelled using the diffusion theory (Eq. 4). Figure 3 shows how the evolution of both moisture (X_w, R^2=0.99) and salt (X_s, R^2=0.97) followed a clear diffusion pattern. The effective diffusivities (D_{eff}) computed were 3.69×10^{-10} m^2/s and 7.61×10^{-10} m^2/s for the water and salt diffusion, respectively. Zhao et al. (2020) reported a very similar salt D_{eff} (8.51×10^{-10} m^2/s) during the wet salting (6 % NaCl, 20ºC) of beef samples. Similarly, Aykın-Dinçer & Erbaş (2018) reported a water D_{eff} value of 2.40×10^{-10} m^2/s in the dry-salting (20 g NaCl/100 g d.b.) of beef slices (3.5 mm). Meanwhile, Dimakopoulou-Papazoglou & Katsanidis (2016) reported a higher D_{eff} for water (1.31×10^{-9} m^2/s) than for salt (2.02×10^{-9} m^2/s) in the dry salting of beef parallelepiped samples (28 g).

3.1.2. Changes in textural properties

Figure 4 displays the modifications in the textural properties of the beef steaks during dry salting. As previously reported (Contreras et al., 2021), hardness (computed as F_{max}) increased progressively during salting from 1.07 N (raw sample) to 14.95 N (24 h salting), following a linear pattern (R^2 = 0.99) (Figure 4a). Moreover, both the hardness and the salt content were also satisfactory correlated from a power function (R^2 = 0.99) (Figure 4b). This effect was observed in different cured meat products, such as dry-cured ham (15 mm thick) with a hardness increase from 9.49 N (1.2 % w.b. of X_s) to 12.06 N (4 % w.b. of X_s) (Morales et al., 2007) or in foal "cecina" (10 mm thick), in which case thehardness changed from 27 N (6.5 % d.m. of X_s) to 48 N (10 % d.m. of X_s) (Lorenzo & Carballo, 2015). However, the modification in hardness observed in these studies were not only due to the salting effect, since the moisture loss from the drying and aging steps also contribute greatly to the increase in hardness. During salting, two coupled phenomena are expected: that moisture loss will cause meat tissue hardening, and the salt will bring about protein denaturation (Morales et al., 2007). However, the structural modification linked to the salting was not homogeneous through the sample thickness, making it possible to distinguish between a softer inner part and a harder outer part due to the salt content profile in the steak (Ruiz-Ramírez et al., 2005).

The Total Relaxation Capacity (TRC), which grants insight into the plastic-elastic behavior of the samples, decreased from 0.618 (raw samples) to 0.529 (24 h salting) (Figure 4c), following a linear pattern (R^2=0.94). Thus, the higher the X_s, the more limited the relaxation capacity of the beef steak samples, which become more elastic as a result of the salt uptake (Figure 4d). A linear inverse relationship between TRC and X_s (R^2=0.83) was observed (Figure 4d). The modification in the viscoelastic properties of meat products during salting has also been previously observed. Thus, Gou et al. (2008) reported a change in the TRC (90s of relaxation) from 0.769 in the case of 6 days salting (X_s ,8 % d.m.) to 0.725 after 14 days (X_s ,12 % d.m.). Coll-Brasas et al. (2021), meanwhile, observed a reduction in the TRC (90s) from 0.671 to 0.634 in dry-cured hams salted for 7 (X_s ,14 % d.m.) and 12 days (X_s, 17 % d.m.), respectively.

3.2.Modification of ultrasonic parameters during salting

The evolution of the ultrasonic parameters in beef steaks in line with salting time was assessed for two parameters: the time-of-flight ratio (R_{TOF}, %) and the ultrasonic velocity (v, m/s). R_{TOF} computes the ratio between the TOF in the salted sample and that measured in the raw steak. The reason for the choice of R_{TOF} as parameter is twofold: i) the relative change permits the minimization of the impact of the high degree of heterogeneity in the initial steak and ii) the measurement of the thickness is not necessary, which is particularly intricate for the NC ultrasonic method.

Figure 5a shows the evolution of the average R_{TOF} measured in different points along the samples under study (Figure 1c) for the raw sample (0 h) and for those salted for 1, 4, 8 and 24 h using the two ultrasonic techniques: C and NC. In both cases, a decrease in R_{TOF} during salting was observed, which was well described by hyperbolic functions (C: R^2=0.98, NC: R^2=0.95). A sharp reduction in R_{TOF} is manifested at the beginning of salting, subsequently dropping to -16.5 % for NC and -24.8 % for C. This behavior was reported by de Prados et al. (2017) using the pulse-echo contact technique for monitoring dry salting in whole pieces of pork loins and hams. After that, as illustrated in Figure 5a, the decrease rate slows down. Finally, after 24h of salting, R_{TOF} was -26.5 % for the NC and -31.8 % for the C. The behavior of R_{TOF} during salting corresponded to what was expected: the time taken for the ultrasonic signal to pass through the beef sample shortens as the salting progresses (de Prados et al., 2017). On the one hand, the ultrasonic waves are greatly affected by the mechanical properties of the propagation medium; thus, the increase in the solid content and stiffness of its tissues during salting quickens its propagation (de Prados et al., 2016; Miles & Fursey, 1977). On the other, this observed decrease in TOF includes the effect of the shrinkage that takes place as a result of dehydration (Table 1) (Garcia-Gil et al., 2014). The comparison of predicted R_{TOF} for C and NC techniques is shown in Figure 8a, in which a satisfactory correlation between both approaches was found.

Figure 5b shows the evolution of the ultrasonic velocity during salting. In raw beef steaks, the average calculated velocity was 1622 m/s for the NC and 1584 m/s for the C techniques, which coincides with the figures previously reported for raw beef (Hara et al., 1979; Ludwig, 1950) and beef steaks (Diaz-Almanza et al., 2021; Marcus & Carstensen, 1975). Once the salting began, there was a steep increase in the velocity registered at 1h, with values of 1650 m/s (NC) and 1717 m/s (C), rising to 1732 m/s (NC) and 1754 m/s (C) at 4h. Afterwards, the increasing rate of the velocity diminished, reaching the maximum value at 24h of salting: 1800 m/s and 1875 m/s for NC- and C techniques, respectively. Álvarez-Arenas et al. (2009) observed velocity differences between the C and NC techniques that were in a similar range to those obtained in the present study when characterizing vacuum-packaged dry-cured ham (NC: 1754 ± 28 m/s; C: 1725 ± 29 m/s). It must be remarked that the measurement of the ultrasonic velocity for the NC technique presented a greater variability than for the C (Figure 5), which is associated with the estimation of the actual sample thickness. For both ultrasonic techniques, the evolution of the ultrasonic velocity during salting was satisfactorily described using power functions (Figure 5, NC: R^2=0.93; C: R^2=0.99). Figure 8b illustrates the scores of the predicted ultrasonic velocity using the power functions for C and NC techniques. The pattern found in the ultrasonic velocity coincides with what was previously reported by de Prados et al. (2016), who monitored the salting process in whole pieces of *Longissimus dorsi* pork muscle during dry salting (12 and 24 h) at 2 °C and also showed the increase in the ultrasonic velocity with a sharp change at the beginning and a moderate increase after 2 h of salting.

3.3.Relationships between ultrasonic parameters and physicochemical properties

3.3.1. Salt gain

Figure 6 represents the variation in the ultrasonic parameters, R_{TOF} and velocity increase (△V), with the salt content (X_s) in beef steaks. Figure 6a shows a falling-rate behavior for the R_{TOF}, which was modeled using an exponential equation. Thus, the largest modification in the R_{TOF} takes place below a salt content of 4.81 w.b., which corresponds to the first hour of salting. The final R_{TOF} reached was for NC: -26.5 % and C: -31.8 % for a salt content of 14.56 % w.b.

Figure 6b shows how the ultrasonic velocity increase followed a linear pattern with the salt gain. Thus, △v linearly increased from the first salting hour (X_s=4.81 %, w.b.) to 24 hours (X_s=14.56 %, w.b.) from 27 m/s to 178 m/s for the NC, and from 132 m/s to 291 m/s for the C technique. Thus, the change in △v was slightly greater for C (159 m/s) than for NC (151 m/s), but both techniques presented a similar trend (Álvarez-Arenas et al., 2009; Fariñas et al., 2021). When analysing pork muscle salted at 2 ºC, de Prados et al. (2015) reported velocity increases of 87 m/s for 5 % w.b. of salt gain and 159 m/s for 10 % w.b of salt, which are in the range of the figures found in the present study. Additionally, Kinsler et al. (1999) described an equation for computing the change of the ultrasonic velocity at 22 ºC with the salt content in an aqueous solution, which, as can be seen in Figure 6b, followed a very similar pattern to the linear form of the NC technique (R^2=0.95). However, the velocity increase for the C technique registered a slightly higher slope than the Kinsler and NC relationships. The deviation for predicted △v figures for C and NC ultrasonic techniques is illustrated in Figure 8c. A feasible explanation of this result mismatch for the C technique could be linked to the stress caused by the contact pressure in the meat tissue, which may slightly increase the ultrasonic velocity.

3.3.2. Texture

Figure 7 shows the correlation between the ultrasonic and textural parameters for samples salted for different times. In Figure 7a, the F_{max} evolution was properly described using power functions (NC: R^2=0.97; C: R^2=0.99), which illustrates how, the growth of $\triangle V$ is remarkable at the beginning of salting (from 0 m/s to 109.5 m/s for the NC and from 0 to 170.4 m/s for the C) while F_{max} barely increased (from 1.07 N to 4.12 N). However, at the end of salting, the trend changes and F_{max} grows sharply (from 5.22 N to 14.95 N) while the velocity increase decelerates (from 145.0 m/s to 177.6 m/s for the NC technique and from 211.1 m/s to 291.2 m/s for the C technique). It is worth highlighting that the increase in the growth rate after the aforementioned inflection point is steeper in the case of NC than for the C technique. The non-linear correlation between F_{max} and $\triangle V$ could be explained by the diffusion mechanism that controls the meat salting and causes changes in the structure and composition of the different tissues (de Prados et al., 2016). In the early stages of dry salting, the salt gain and water loss take place mostly in the outer layers of the steak. Thus, the resultant meat hardening linked to salting starts in these external layers. Ultrasonic waves detect the changes in the acoustic impedance linked to the described process; consequently, its propagation velocity will be higher in the outer layers than in the inner, which will result in a shorter TOF compared to that of the raw meat. However, the measurement of F_{max} is not so sensitive during compression due to the fact that the steaks behave as a multilayer material, in which the contribution of the softer inner layers can dramatically reduce its maximum compression force (Ashby & Jones, 2012). Finally, in Figure 7b, it may be observed how the correlation between TRC and $\triangle V$ in beef steaks as a result of the salt uptake was also well described using power functions (NC: R^2=0.94; C: R^2=0.96). Thus, the more elastic the behavior of the samples (TRC reduction) during salting, the greater the $\triangle V$ in both ultrasonic techniques.

4. Conclusions

The performance of contact (C) and non-contact (NC) ultrasonic techniques in the monitoring of dry salting in beef steaks was similar. Thus, ultrasonic techniques permitted an adequate estimation of not only the changes of salt and moisture during salting but also of the structural changes undergone by the samples and manifested in the modification of the textural properties.

As the main novelty of this study, what has to be highlighted is the fact that the non-contact ultrasound technique avoids sample handling, which is highly advantageous for industrial applications, both when considering safety issues and its easy implementation in the process lines. Thereby, future efforts have to made to push the development of the non-contact ultrasonic technique for industrial monitoring, which would allow a real time, non-invasive measurement.

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Figure 1. Ultrasonic set-up for: a) Non-contact (NC) and b) Contact (C) techniques. The experimental set-up comprises: an oscilloscope (1), a pulser-receiver (2), transducers: air-coupled centered at 0.3 MHz (3A); and contact transducers centered at 1 MHz (3B), and the sample holder: frame with fishing line network (4A); and iron-made base with a hole (4B). c) Detail of the distribution of thickness and ultrasound measurement points on beef steaks.

Figure 2. Normalized ultrasound signals measured in beef steaks at different salting times: reference – blank measurement -, 1-, 4-, 8- and 24-hours using non-contact (a) and contact techniques (b). Grey solid and dashed lines show the maximum amplitude and arrival time of the reference, respectively. Black lines show the same parameters for salted beef at different times.

Figure 3. Compositional changes in pork steaks during dry salting: black dots: salt content (X_s; %, w.b.); and grey triangles: moisture content (X_w; %, w.b.). Each marker represents the mean and standard deviations of the experimental measurements. Solid lines correspond to the diffusion models.

Figure 4. Changes in hardness computed as the maximum compression force (F_{max}) versus: a) salting time; b) salt content X_s. Changes in the Total Relaxation Capacity (TRC) versus: c) salting time; d) with salt content X_s. Each marker represents the mean and standard deviations of the experimental measurements.

Figure 5. Evolution of the ultrasonic parameters during salting: a) R_{TOF}: TOF ratio; b) propagation velocity (v). Each marker represents mean and standard errors of the experimental measurements; those in black represent the contact (C) and those in grey the non-contact (NC) ultrasonic techniques.

Figure 6. Relationship between ultrasonic parameters and salt content (X_s): a) R_{TOF}: TOF ratio; b) $\triangle V$: velocity increase during salting. Each marker represents the mean and standard errors of the experimental measurements; those in black represent the contact (C) and those in grey the non-contact (NC) ultrasonic techniques. In b) the dashed black line shows the estimated $\triangle V$ values using the Kinsley equation at 22 ºC.

Figure 7. Relationship between ultrasonic velocity increase ($\triangle V$) and textural parameters during salting: a) hardness computed as the maximum compression force (F_{max}); b) Total Relaxation Capacity (TRC); b) $\triangle V$: velocity increase ($\triangle V$). Each marker represents the mean and standard errors of the experimental measurement; those in black represent the contact (C) and those in grey the non-contact (NC) ultrasonic techniques.

Figure 8. Comparison of average scores of predicted ultrasonic parameters: Time of flight ratio, (RTOF); velocity and variation of velocity during salting, (ΔV) for contact (C) and non-contact (NC) ultrasonic techniques using mathematical models shown in Figures 5, 6 and 7.

Table 1. Mean and standard deviations of the thickness measured in thawed steak samples (N = 15) at different salting times used for velocity calculations for the contact and non-contact ultrasound techniques.

Salting time (h)	**Contact Ultrasound**	**Non-Contact Ultrasound**
	Thickness (mm)	Thickness (mm)
Raw	*13.49 ± 0.99*	14.15 ± 2.02
1	*10.89 ± 0.94*	11.38 ± 0.91
4	*11.01 ± 0.59*	11.69 ± 0.96
8	*10.48 ± 1.20*	10.42 ± 0.85
24	*10.96 ± 0.76*	10.48 ± 0.72

Table 2. Mean and standard error of the compositional parameters measured during beef steak salting.

Parameters	***Salting time***				
	Raw	***1 h***	***4 h***	***8 h***	***24 h***
X_w ***(%, w.b)***	73.54 ± 0.00^{a}	69.94 ± 0.31^{b}	65.26 ± 0.72^{c}	62.63 ± 0.43^{d}	56.06 ± 0.16^{e}
ΔX_w ***(%, w.b)***	-	-3.59 ± 0.31^{d}	-6.19 ± 0.72^{c}	-8.28 ± 0.43^{b}	-10.91 ± 0.16^{a}
X_s ***(%, w.b)***	0.15 ± 0.00^{a}	4.81 ± 0.11^{b}	8.92 ± 0.07^{c}	10.08 ± 0.07^{d}	14.56 ± 0.04^{e}
ΔX_s ***(%, w.b)***	-	4.66 ± 0.46^{a}	5.46 ± 0.08^{b}	8.72 ± 0.08^{c}	9.92 ± 0.05^{d}
a_w	0.972 ± 0.000^{a}	0.939 ± 0.002^{b}	0.901 ± 0.002^{c}	0.873 ± 0.003^{d}	0.815 ± 0.002^{e}

Mean ± standard error (SE)

Subscripts (a,b,c, d, e) denote homogeneous groups for each parameter ($P < 0.05$)

X_w: moisture content (%, w.b), ΔX_w: moisture loss (%), Xs: salt content (%, w.b), ΔXs: salt gain (%, w.b), a_w: water activity (dimensionless).