Ultrasonic assessment of the melting behaviour in fat from Iberian dry-cured hams

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The feasibility of using ultrasounds to characterize the melting properties of fat from Iberian dry-cured hams was evaluated. Differential Scanning Calorimetry (DSC) and ultrasonic measurements were used to characterize the fat melting. The ultrasonic velocity in fat decreased with the increase in temperature, showing four different sections (0–4 °C, 4–10 °C, 10–20 °C and 20–24 °C). Ultrasonic velocity was related (R2 = 0.99) to the percentage of melted fat (%MEF) showing an increase of 5.4 ms-1 for 1% increase of melted fat (%MEF above 60%). The thermal history did not affect the ultrasonic measurements from 10 to 25 °C and, consequently, this range was the most suitable for classifying Iberian dry-cured products with different genetics and feeding backgrounds. Ultrasonic measurements could be a reliable technique to estimate the %MEF and subsequently the related sensory attributes in Iberian dry-cured ham at 10–25 °C, which is the common temperature range for the consumption of Iberian dry-cured products.

1. Introduction

The quality of dry-cured ham from Iberian pigs depends on the fat content and the fatty acid composition of the fatty tissue, which is greatly affected by both the feeding regime of the pigs in the fattening stage (Carrapiso et al., 2003; Cava, Ventanas, Ruiz, Andrés, & Antequera, 2000; Petrón, Muriel, Timón, Martín, Córdoba, Ventanas, & Antequera, 1999). Moreover, the fat content and the fat solid/liquid ratios also affect the fatty acid composition also affects the oiliness, brightness, marbling and consumer acceptance (Carrapiso et al., 2003; Cava et al., 2000; Ruiz, García, Muriel, Andrés, & Ventanas, 2002), due to its influence on the melting point of the fat (Davenel, Riaublanc, Marchal, & Gandemer, 1999). In fact, oiliness and brightness increase as the fat solid/liquid ratio decreases at a given temperature (Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000), with the highest values obtained by dry-cured products with lower fat solid/liquid ratios. Moreover, Ventanas, Ventanas, and Ruiz (2006) suggested that the configuration of the fatty tissue influences the brightness; the less structured and weaker the fatty tissue (adipocyte size and number and connective tissue), the brighter it is.

Carrapiso et al. (2003) reported a close relationship between the fatty acid composition of the subcutaneous fat and the sensory traits (oiliness, brightness and marbling) of dry-cured hams. Moreover, the fat content and the fat solid/liquid ratio also affect the flavor release in meat products (Estévez, Ventanas, & Cava, 2005; Relkin, Marjorie, & Guichard, 2004). Therefore, the analysis of the subcutaneous fat, performed without affecting the integrity of the dry-cured ham, would provide information on the sensory traits of the dry-cured Iberian hams.

Low-intensity ultrasound has been used to assess the physicochemical properties of many foods (Coupland, 2004). Velocity measurements, which depend on temperature, have been used to...
determine the solid fat content (SFC) in solid fats and liquid oils (Martini, Bertoli, Herrera, Neeson, & Marangoni, 2005a; Martini, Herrera, & Marangoni, 2005b; McClements & Povey, 1988b; Singh, McClements, & Marangoni, 2004) and in bulk fats, such as pig’s adipose tissue (Miles, Fursey, & Jones, 1985).

However, the use of ultrasonic velocity measurements in the determination of the SFC in high solid fat content samples involves significant difficulties due to the high attenuation of the signal when travelling across the crystallized fat (McClements & Povey, 1988a; Singh et al., 2004). Moreover, ultrasonic velocities have been found to be dependent not only on the solid content of the sample, but also on the microstructure and polymorphism of the fat crystals. In this regard, studies performed on blends of cocoa butter in canola oil 70% (w/w) (Singh et al., 2004) and in emulsified mixtures of hydrogenated palm oil in sunflower oil (Kloek, Walstra, & VanVliet, 2000) suggested that fats in a more stable polymorphic form exhibit higher velocities than the same fats in a less stable polymorphic form. The fact that the former kind of fat has a higher bulk modulus would explain this phenomenon (Singh et al., 2004). Therefore, the effect of both factors (attenuation and polymorphism) on the velocity measurements should be considered in order to improve this technique’s assessment of the SFC in fats and oils. The advantage of using ultrasonics for SFC assessment, instead of other classical techniques like DSC, is that this technique is non-destructive, fast and inexpensive.

The objective of this study was to evaluate the feasibility of using ultrasonics to characterize the melting properties of subcutaneous fat of Iberian dry-cured hams from different breeds and with differing feeding regimes. The study of the influence of temperature on the ultrasonic measurements was useful to find the optimum conditions for using this technique to classify Iberian dry-cured hams from pigs of different breeds and feeding regimes.

2. Materials and methods

2.1. Animals and diets

Samples of subcutaneous fat of dry-cured hams from three different batches, which differed both as to the feeding regime during the fattening phase and the breed of the animals, were analysed. Ten animals per batch were considered in the study.

The feeding regime of the animals differed as to the fattening phase (from 80 kg until slaughter, at 165 kg which is the usual slaughter weight for Iberian pigs). One batch of pure Iberian pigs was fed intensively (IB IN) on a concentrate feed with low oleic acid content (67% of the total fatty acid content); another batch of pure Iberian pigs were fed on an extensive (IB EX) feeding regime (“montanera”), mainly based on acorns (in which 66% of the total fatty acid content was oleic acid) and grass. Another batch of crossbreed Iberian x Duroc pigs (IBxD IN) was fed intensively on a concentrate feed with lower oleic acid content (31% of the total fatty acid content).

2.2. Sampling procedure

The samples of subcutaneous fat from the dry-cured hams were taken in the curing ham chamber. The skin and the remaining muscle tissue were removed and the samples were kept at –18 °C until they were analysed for their thermal and ultrasonic characteristics. Then, the biceps femoris was also removed, sliced and analysed for the sensory traits related to the characteristics of its fat, since they are commonly used to determine the sensory quality of the dry-cured hams.

2.3. Differential Scanning Calorimetry (DSC)

The thermal behaviour of the samples was analysed by DSC. The instrument for the analysis (DSC5020CU, Seiko Instruments, Torrance, CA, USA) was calibrated with indium at 5 °C/min from 25 to 250 °C. An empty aluminium crucible was used as a reference and liquid nitrogen as the cooling fluid.

The backfat samples from three randomly selected animals per batch were analysed in triplicate (nine measurements per batch). Backfat samples were ground and a 15 mg aliquot was introduced into an aluminium crucible. The temperature was raised from 25 to 40 °C at a heating rate of 5 °C/min. The sample was then tempered at 40 °C for 5 min and, afterwards, it was cooled to −50 °C at a cooling rate of 5 °C/min and held at −50 °C for 5 min. A DSC curve was obtained by heating the sample from −50 to 40 °C at 5 °C/min. Since the samples analysed using ultrasound had been stored for 2 months at 0 °C, and therefore a complete fat crystallization of the triglycerides that crystallize at that temperature was expected, the DSC samples were cooled to −50 °C and held for 5 min to attain a complete crystallization before the start of the heating cycle. The peak maximum temperature (Peak T, °C), onset temperature (Onset T, °C) and latent heat (ΔH, mJ/mg) were computed from the thermograms (Cebula & Smith, 1992). The percentage of melted fat (%MEF) at every temperature was also obtained. This percentage was computed at temperature intervals of 1 °C as the ratio between the accumulated latent heat for each temperature, and the total latent heat for the complete heating cycle was considered.

2.4. Ultrasonic measurements

The experimental set-up consisted of a pair of narrow-band ultrasonic transducers (1 MHz, 0.75” crystal diameter, A314S-U model, Panametrics, Waltham, MA, USA), a pulser-receiver (Panametrics, Model 5058PR, Walthom, USA) and a digital storage oscilloscope (Tektronix, TDS5034, Digital phosphor oscilloscope, Tektronix Inc. Beaverton, Oregon, USA). A digital height gage was designed and built by the research group, and linked to the computer by a RS232 interface to measure the sample thickness, with a precision of ±0.01 mm.

The ultrasonic velocity in the sample was computed from the time of flight obtained from the signal by averaging five signal acquisitions, and the thickness read from the height gage. To determine the system delay, which was considered in the computation of the time of flight, the pulse transit time was measured across a set of calibration cylinders of different thicknesses. The delay time was then obtained from the intercept on the y-axis of the time versus thickness graph (Niñoles et al., 2007). The overall accuracy of the ultrasonic measurements was 0.89 m/s.

The measurements were taken in triplicate from samples of all the animals (10) per batch. The samples were cut into slices (20–30 mm thickness), vacuum packed and placed into a temperature-controlled chamber at the desired temperature until they attained uniform temperature (24 h). Then, the experiments were performed in a temperature-controlled chamber to keep the temperature in the sample constant during the measurements. The same procedure was repeated every 24 h, progressively increasing the temperature in the samples up to 24 °C. The experiments were performed on samples at 0, 2, 4, 6, 8, 10, 12, 14, 20, 22 and 24 °C. The cooling experiments were performed in an analogous way, by progressively cooling the samples from 24 °C back down to 0 °C, changing the temperature every 24 h. The experimental temperatures for this cooling cycle were the same as in the heating one.

A second set of experiments was carried out on another group of samples from the IB EX batch. These experiments were performed following the same above-mentioned procedure. The
temperature of samples stored for 2 months at 0 °C was first raised from 0 to 24 °C then lowered to 0 °C, kept at this temperature for 4 days, and finally the heating cycle was repeated up to 24 °C. The three cycles involved the taking of measurements at the same temperatures used in the previous ultrasonic experiments. Olive oil was used as a couplant in all the experiments. The second heating was performed in order to check if differences for the ultrasonic velocity were found for the two heating cycles with different thermal history, since the holding period at 0 °C was 2 months for the first cycle and 4 days for the second one.

2.5. Sensory analysis

The sensory analyses were performed by 12 members of a trained panel, using a quantitative-descriptive analysis method (Ruiz-Carrascal et al., 2000). A 10 cm unstructured scale was used, the extremes being "very low" and "very high". Three randomly selected hams per batch were analysed in each session until all the hams were analysed (10 hams per batch). All the panelists participated in the sessions, and their order was also randomised. Panelists had a wide experience on sensory evaluation of dry-cured products. Members of the panel had more than 120 h of training in preparation for descriptive analysis. Consistency of panellist's least significant difference (LSD) intervals, at the 95% confidence level, were computed for each parameter. The Statgraphics Plus 5.1 statistics software was used for this purpose.

2.6. Statistical data analysis

A one-way ANOVA was performed on the results from the thermal and sensory analysis in order to study the differences between batches. A two-way ANOVA was performed on the ultrasonic parameters to study the existence of significant differences between batches and thermal history (cooling/ heating cycles). Fisher’s least significant difference (LSD) intervals, at the 95% confidence level, were computed for each parameter. The Statgraphics Plus 5.1 statistics software was used for this purpose.

3. Results and discussion

3.1. Differential Scanning Calorimetry

It is of great interest to determine the thermal behaviour of the dry-cured fat from the Iberian pigs, since the melting behaviour of this product markedly affects some of the sensory properties of the dry-cured ham (Ruiz-Carrascal et al., 2000). Moreover, the melting behaviour of the fat also affects the accuracy of the ultrasonic velocity measurements when used for classification purposes, as the effect of temperature on the ultrasonic measurements is greater for temperature ranges exhibiting higher melting rates (Benedito, Carcel, Rosselló, & Mullet, 2001).

The DSC melting curves performed on the dry-cured fat samples were similar for the three batches analysed (Fig. 1 and Table 1), showing the existence of a melting phenomenon of the fat, with two endothermic peaks, in all the samples analysed.

Table 1 shows the average and the standard deviations for the thermal parameters obtained from the thermograms for the dry-cured fat. No significant differences (p > 0.05) were found between batches for any of the parameters obtained from the curves for the dry-cured samples, and all the thermal parameters were highly variable. These results show that the DSC measurements performed were not useful to sort out dry-cured fat samples from animals differing as to the breed or the feeding regime. This could be due to the fact that only three samples per batch were analysed, and also because the fatty acid composition of these samples was expected to show a high variability, since the composition of samples of fresh backfat from animals of the same breed and feeding regime were also highly variable (Niñoles et al., 2007).

However, the information on the melting behaviour of these samples provided by this technique is of great interest in the assessment of the accuracy of the ultrasonic velocity measurements. The effect of temperature on the ultrasonic velocity measurements would be higher for temperature ranges exhibiting a higher melting rate (−15 to 10 °C and 20–32 °C) due to the higher velocity of ultrasound in solid fat than in liquid one, while temperatures ranging between 10 and 20 °C would have less effect.

The first peak in the DSC analysis (I, Fig. 1) showed an average latent heat for all the batches of 30.9 ± 5.9 mJ/mg and an average maximum peak temperature for all the batches of −3.3 ± 2.8 °C. This first peak has not only been related to the melting of diunsaturated triglycerides (Le Mestre, Cornily, & Simatos, 1984), but also to the melting of the water in the sample. DSC analysis performed on fresh backfat from Iberian pigs of the same breed and following the same feeding regime (Niñoles et al., 2007) also showed the existence of two endothermic peaks. The first peak for this fresh backfat attained a significantly (p < 0.05) higher (64.3 ± 3.2 °C/mg) average latent heat and a significantly (p < 0.05) higher average peak temperature (2.3 ± 0.2 °C) than the ones obtained for the cured fat in the present work. The differences found in this section between the thermograms of fresh and dry-cured fat would mainly be due to the significantly (p < 0.05) higher water content found in the fresh samples (7 ± 0.3%, Niñoles et al., 2007) as compared with the dry-cured fat samples (1 ± 0.2%).

The second peak (II, Fig. 1) in the dry-cured subcutaneous fat melting curve exhibited an average latent heat of 13.9 ± 1.7 mJ/mg, for all the batches and a maximum peak temperature of 27.9 ± 0.9 °C, and it has been related to the melting of monounsatu-
urated triglycerides (Le Mestre et al., 1984). The second peak found in the Iberian pigs’ fresh fat DSC curves from the same animals showed a significantly \((p < 0.05)\) lower average latent heat (11.5 ± 2.0\,\text{mJ/mg}) (Niñoles et al., 2007) and significantly \((p < 0.05)\) higher average peak temperatures (29.3 ± 0.9°C) than those obtained in the DSC of dry-cured fat. The differences found between the thermal parameters of the dry-cured subcutaneous fat from the ham and the fresh backfat could arise from the slight differences in the composition of the fat due to its different location in the animal (Ruiz et al., 1998; Yılmaz & Karakaya, 2009), and to the effect of the lypolysis and the oxidation of the fats that take place during the curing process (Martín et al., 1999).

### 3.2. Ultrasonic measurements

#### 3.2.1. Ultrasonic velocity temperature dependence

The ultrasonic velocity in adipose tissues is highly dependent on the solid/liquid fat ratio (McClements & Povey, 1988b), and this ratio depends on the fatty acid composition of the fat and therefore on its quality and also on the sample temperature. The ultrasonic velocity and the influence of temperature on velocity could be useful to characterize samples of pig’s adipose tissue (Niñoles et al., 2007). Fig. 2 shows the average velocity versus temperature curves obtained in both the heating and the cooling cycles for all the batches analyzed (10 samples per batch). Since the ultrasonic velocity has been previously used to characterize adipose tissue for pigs of different breed and feeding regimes (Niñoles et al., 2007), it is interesting to know if the thermal history of the samples affects the ultrasonic measurements. Four different sections with different behaviours (sections A, B, C and D, for temperature ranges from 0 to 4°C, from 4 to 10°C, from 10 to 20°C and from 20 to 24°C, respectively) were found in the velocity versus temperature curves obtained, in both the heating and the cooling cycles.

During the heating cycle, in the first section of the curve (section A) the curves remained practically constant for all the batches. In this section, the velocity did not behave as expected, since it should decrease with the increase in temperature and, therefore, the increase of melted fat (Benedito et al., 2001; Miles et al., 1985). In fact, the DSC curves obtained in the present study (Fig. 1) showed a continuous and important melting phenomenon in the temperature range from 0 to 4°C.

The unexpected behaviour of the curves in section A could be due to the existence of changes in the polymorphic form of the fat in the sample, which would be masking the effect of the melting phenomena on velocity. These changes would result in more stable polymorphic forms for the fatty acids in the batch and therefore higher velocity values than expected. In line with this, Povey (1997) described a sharp increase in velocity in a 10% hardened palm oil-in-water emulsion, which took place as the temperature in the sample was increased from 47 to 53°C, just prior to melting. This change in velocity was related to a phase transition in the palm oil. Along the same lines, previous studies into blends of cocoa butter in canola oil 70% (w/w) (Singh et al., 2004) and into emulsified mixtures of hydrogenated palm oil in sunflower oil (Kloek et al., 2000) found that the fats which had more stable polymorphic forms exhibited higher velocity values.

A linear regression was performed on the velocity versus temperature data of the different batches in sections B, C and D of the curve considered separately, both in the heating and in the cooling cycles.

A steep decrease in velocity was found in the heating cycle in section B (temperature ranges from 4 to 10°C), with an average velocity temperature coefficient of \(-19.6 ± 2.3\,\text{ms}^{-1}\,\text{°C}^{-1}\). As velocity values are highly dependent on the solid/liquid ratio of the fat (McClements & Povey, 1988b), this steep velocity decrease was probably due to the high melting rate observed in this section in the DSC curves (Fig. 1), which would have resulted in a steep decrease in the solid/liquid fat ratio.

A gentler velocity decrease in line with the temperature increase was found for sections C and D of the heating curve, with average velocity temperature coefficients of \(-7.0 ± 1.2\) and \(-8.3 ± 2.8\,\text{ms}^{-1}\,\text{°C}^{-1}\). This gentler decrease in velocity was in line with the lower melting rate found in these sections in the DSC curves (Fig. 1). In a study performed on fresh backfat from Iberian pigs, Niñoles et al. (2007) also found a change in the slope of the velocity versus temperature curve, with a steeper velocity decrease \((-22.1 \pm 1.2\,\text{ms}^{-1}\,\text{°C}^{-1})\) for temperature ranges (from 0 to 6°C) exhibiting a high melting rate in the DSC curves and a gentler decrease from 6 to 22°C \((-6.8 \pm 1.3\,\text{ms}^{-1}\,\text{°C}^{-1})\). Differences in the slope of the curves between the fresh and the dry-cured fat would be due to differences in the composition of the products (water content and fatty acid composition), which would have resulted in different melting behaviour and, consequently, differences in the velocity versus temperature curves.

As regards the cooling cycle (Fig. 2), sections C and D showed a smooth velocity increase with the decrease in the temperature, with average velocity temperature coefficients of \(-5.9 ± 0.7\) and \(-8.0 ± 1.7\,\text{ms}^{-1}\,\text{°C}^{-1}\), respectively. In section D, no significant \((p < 0.05)\) differences were found between the heating and the cooling cycle, either for the average velocities \((1531.2 ± 24.4\,\text{ms}^{-1}\) in the heating cycle and 1528.6 ± 20.6\,\text{ms}^{-1}\ in the cooling one) or for the velocity temperature coefficients. In section C, no significant \((p < 0.05)\) differences were found between the heating and the cooling cycles for the average velocities \((1587.6 ± 28.9\,\text{ms}^{-1}\) in the heating cycle and 1579.4 ± 21.7\,\text{ms}^{-1}\ in the cooling one). Therefore, this result indicates that the solid/liquid fat ratio for each analysed temperature should have been similar for the cooling and the heating cycles in both sections of the curve (C and D).

As the temperature of the sample decreased (avg. \(-5.4 ± 1.1\,\text{ms}^{-1}\,\text{°C}^{-1}\), the increase in velocity found in the cooling cycle in section B (Fig. 2) was significantly \((p < 0.05)\) lower than the one found in the same section for the heating cycle (avg. \(-19.6 ± 5.1\,\text{ms}^{-1}\,\text{°C}^{-1}\)). This effect is also reflected in the average velocities for this section, with significantly higher \((p < 0.05)\) velocities in the heating cycle \((1673.4 ± 35.0\,\text{m/s})\) than in the cooling one \((1620.1 ± 24.1\,\text{m/s})\). It is well known that the crystallization
and melting of fats are accompanied by changes in their internal structure, morphological properties and molecular packaging, which affect ultrasonic velocities (Awad, 2004). Thus, this phenomenon of hysteresis could be due to the combined effect of the change in the structure of the samples, which would be mainly due to the probable existence of a migration phenomenon of fat from the intracellular to the extracellular media in the heating cycle, and to the supercooling phenomenon taking place in the sample. This supercooling effect would mean that the fat crystals that had been formed by a slow kinetics after long storage periods (2 months) at low temperatures, are not allowed to form during the cooling cycle at the cooling rates used in this study, resulting in lower solid/liquid ratios, a different fat polymorphism (Campos, Sarne, & Marangoni, 2002; Kalnin, Lesieur, Artzner, Keller, & Ollivon, 2005) and, therefore, lower velocities. Singh et al. (2004) also detected this phenomenon of hysteresis when comparing the velocity curves obtained for the cooling and the heating cycles of 70% blends of anhydrous milk fat and cocoa butter in canola oil, which was related to the supercooling effect.

In line with the temperature decrease, a steeper velocity increase was found in the cooling cycle (Fig. 2) in section A ($−9.5 ± 5.4$ m s$^{-1}$ C$^{-1}$) than in section B, probably due to the higher melting rate found in the DSC curve in section A compared to section B (Fig. 1). Section A exhibited the same phenomenon of hysteresis as section B, with significantly higher ($p<0.05$) velocities in the heating cycle ($1732.6 ± 37.1$ m/s) than in the cooling one ($1652.4 ± 25.7$ m/s), which could be due to the previously cited effects of the thermal history of the sample on velocity and to the effect of the polymorphic forms of the fat on the velocity values at low temperatures (0–4°C).

These results showed that temperature has a marked influence on the ultrasonic velocity and, therefore, greatly affects the characterization using ultrasonic techniques. The higher the ultrasonic temperature coefficients, the greater the need for a better temperature control to obtain accurate measurements. Also, the thermal history could affect the structure and the solid/liquid ratio of the fat and, therefore, the ultrasonic measurements.

To complete the study of the influence of the thermal history on the use of the ultrasonic measurements to characterize dry-cured fat from different batches, a second set of experiments was carried out on another group of samples from the IB EX batch (Fig. 3). In this second type of experiment the samples were heated, cooled, rested for four days and subsequently heated again. Fig. 3 shows that the second heating cycle exhibited statistically significant ($p<0.05$) higher velocities than the cooling one at 2°C and at 4°C, probably due to the changes in the polymorphic form of fat at those temperatures, also found in Fig. 2. The second heating cycle exhibited significant ($p<0.05$) lower velocities ($1624.5 ± 17.0$ m/s) than the first heating one ($1675.7 ± 20.7$ m/s), probably because the resting period was not long enough to allow for all the fat in the samples to crystalize and to recover the polymorphic form of the fat existing in the first cycle (2 months’ resting period). In addition, the structure of the fat could be permanently affected by the fat melting in the previous heating cycle. Therefore, the effect of the thermal history on velocities in this section (A–B) should be taken into account for the characterization and classification purposes using ultrasonic techniques. If the samples have been heated and cooled following different patterns, it would be difficult to use ultrasonic velocity measurements in the range 0–10°C as a tool for the classification of fat samples in terms of breed and feeding regime as Niñoles et al. (2008) reported.

However, as shown in Fig. 2, from 10 to 24°C, the thermal history of the samples has no effect on the ultrasonic velocity measurements since no significant differences ($p<0.05$) were found between the velocities obtained in any of the heating and cooling cycles. Therefore, velocity measurements performed at this temperature range would be the most suitable for the characterization of dry-cured fat samples. In fact, significant ($p<0.05$) differences were found between the ultrasonic velocities of the three examined batches (IB IN, IBxD IN and IB EX), for any analysed temperature in the range from 10 to 24°C.

### 3.2.2. Ultrasonic assessment of the melted fat content

Certain sensory traits of Iberian dry-cured ham, particularly oiliness and brightness, are highly dependent on the fat solid/liquid ratio at a given temperature (Ruiz-Carrascal et al., 2000). Therefore, estimating the fat solid/liquid ratio at the different temperatures of analysis using ultrasonics could be of interest to estimate the quality of the hams.

Fig. 4 shows both the effect of temperature on velocity (heating cycle) and the percentage of melted fat (%MEF) in a fat sample at a concentrate feed with high oleic acid content batch. %MEF (percentage of melted fat) versus temperature curves showed four sections with significantly different ($p<0.05$) slopes (Fig. 4). These sections agreed with those obtained in the velocity versus temperature curves for the heating cycle (Figs. 2 and 4). Section A (from 0 to 4°C) showed a steep increase in the %MEF (avg.

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**Fig. 3.** Influence of the thermal history on the ultrasonic velocity measurements of dry-cured subcutaneous fat from the Iberian pigs fed on “montanera” (IB EX).

**Fig. 4.** Temperature dependence of the ultrasonic velocity and percentage of melted fat (%MEF) for a fat sample of the IB IN (pure Iberian pigs fed on a concentrate feed with high oleic acid content) batch. ■ Ultrasonic velocity, △ Percentage of melted fat.
slop 2.5 ± 0.3 % °C⁻¹ for all the batches) as the temperature in the sample increased. This would involve a steep decrease in the solid/liquid ratio and, therefore, a steep decrease in the velocity was expected. On the contrary, as also shown in Fig. 2, in section A, the velocity remained practically constant with the increase in the temperature, probably due to the previously mentioned effect of the polymorphic forms in the fat on velocity.

The increase in the %MEF (avg. slope 1.9 ± 0.2 % °C⁻¹ for all the batches) found in section B resulted in a steep decrease in velocity (avg. value of –21.1 ± 2.0 ms⁻¹°C⁻¹ for all the batches) due to the decrease in the solid/liquid ratio and the changes in the structure of the samples arising from the melting phenomenon.

Section C showed a slightly gentler increase in the percentage of melted fat (avg. slope 1.2 ± 0.1 % °C⁻¹) related to the increase in temperature than sections A and B, which resulted in a gentler decrease in velocity (avg. slope of –6.2 ± 1.1 ms⁻¹°C⁻¹ for all the batches) in line with the temperature increase.

Finally, in section D a new increase in the %MEF (avg. slope 1.7 ± 0.1 % °C⁻¹ for all the batches) was found which resulted in a steeper decrease in velocity (avg. slope of –9.0 ± 1.9 ms⁻¹°C⁻¹ for all the batches).

Two sections were found where the relationship between %MEF and velocity was examined (Fig. 5). Section I in the curve (velocities below 1620 m/s and %MEF above 60%) showed a linear relationship between the %MEF and the ultrasonic velocity. This section in the curve corresponded to temperatures in the sample which were found to be useful when characterizing the fat samples (10–24 °C), as velocity measurements in this temperature range were not influenced by the thermal history of the samples. The linear equations fitted to the experimental data in section I for the different batches showed R² higher than 0.99 in all the batches analysed. Moreover, no statistically significant differences (p > 0.05) were found between batches in the coefficients of these linear equations. Therefore, an equation was obtained considering all the batches analysed in this study (equation 1, R² = 0.99). This equation showed that an increase of 1% of melted fat corresponded to a decrease in velocity (v) of 5.4 m/s.

\[
\%\text{MEF} = -0.18 \times v + 359.0
\]  \hspace{1cm} (1)

The same linear tendency was found in fresh backfat samples from Iberian pigs for the same ranges of velocity and %MEF (Niñoles, 2007). In this fresh product, an increase of 1% of the melted fat corresponded to a velocity decrease of 5.6 m/s.

A higher dispersion in the data was found in section II (for velocities above 1620 m/s and %MEF below 60%) of the curves obtained for the dry-cured fat samples (Fig. 5). The changes in the polymorphic form in the fat and the change in the structure of the fat detected through the hysteresis found in the velocity measurements could be the reason for the scarce relationship found between the velocities and the %MEF. The same dispersion in the data was found in samples of fresh Iberian pig backfat (Niñoles, 2007), for the same velocity ranges.

Along the same lines, Singh et al. (2004) found a linear correlation between the solid fat content and the ultrasonic velocities in anhydrous milk fat, for a solid content below 20%. Martini et al. (2005a) also found a linear relationship between the solid fat content and the ultrasonic velocity in edible fats crystallized at constant temperatures for low solid contents in the sample. In this study, an increase of 1% of the solid fat content resulted in a velocity increase of 2.6 m/s. In both studies, high signal attenuation was found for the higher solid contents in the sample.

Therefore, the ultrasonic measurements could be a reliable technique for estimating the percentage of melted fat in dry-cured fat samples. In the range of 10–25 °C, the thermal history does not affect the ultrasonic measurements and, therefore, these measurements can be used for classification purposes (Niñoles et al., 2008). Furthermore, in this range of temperatures the percentage of melted fat can be assessed, with 10–25 °C being the usual temperatures for the consumption of dry-cured ham and, therefore, the range of interest for the evaluation of the sensory characteristics of the product.

### 3.2.3. Ultrasonic assessment of the sensory traits

The oiliness and the brightness of the Iberian hams are highly dependent on the solid/liquid content in the fat, which could be estimated by the ultrasonic velocity measurements (Eq. (1)). The relationship between the ultrasonic velocities in the dry-cured subcutaneous fat and the sensory traits of the biceps femoris muscles from the dry-cured hams was examined. For this purpose, the average velocity at 22 °C (common temperature for sensory evaluation), the average oiliness and the average brightness values for all the samples in a batch were computed (Table 2).

The IB EX batch exhibited both the lowest average velocity values (1525.2 ms⁻¹) and the highest brightness (6.8) and oiliness (6.1) scores, probably due to the higher percentage of melted fat (Table 1). Whereas the IBxD IN batch exhibited the highest average velocity values (1561.9 ms⁻¹) and the lowest average brightness (4.2), oiliness (4.7) and %MEF.

When considering all the samples in the three analysed batches separately, a significant (p < 0.05) linear relationship (R² = 0.75 and 0.73 for the brightness and oiliness, respectively) was found between these sensory traits and the ultrasonic velocities obtained at the temperature of the sensory analysis (22 °C). The explained variance was not as high as expected, probably because factors other than the solid/liquid ratio of the fat would be affecting the sensory and ultrasonic properties. In line with this, the structure

#### Table 2

<table>
<thead>
<tr>
<th>IB</th>
<th>IBxD IN</th>
<th>IB EX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity (m/s)</td>
<td>1539.4 ± 9.6ab</td>
<td>1554.7 ± 10.2ab</td>
</tr>
<tr>
<td>Oiliness</td>
<td>5.6 ± 0.4ab</td>
<td>4.7 ± 0.5a</td>
</tr>
<tr>
<td>Brightness</td>
<td>5.5 ± 0.7a</td>
<td>4.2 ± 0.7a</td>
</tr>
<tr>
<td>%MEF (22 °C)</td>
<td>77.0 ± 3.0a</td>
<td>75.0 ± 5.2a</td>
</tr>
</tbody>
</table>

a, b and c. Batches with the same letter denote no statistically significant differences between them (p < 0.05). Average ± Standard deviation. IB IN: pure Iberian pigs fed intensively; IBxD IN: Iberian x Duroc pigs fed intensively; IB EX: pure Iberian pigs fed on an extensive feeding regime (“montanera”).
of the adipose tissue could have been affecting both the ultrasonic velocities and the sensory traits. Other authors (Niñoles et al., 2007) have found significant differences between the velocity values of fresh backfat batches of Iberian pigs which were attributed to differences in the composition and the structure of the samples. On the other hand, Ventanas et al. (2006) related the differences found in the brightness scores of dry-cured loin samples to differences in the structure of the adipose tissue. Animals with a less structured adipose tissue would have exhibited a higher migration phenomenon of the fat in the adipocytes and, therefore, higher brightness scores.

4. Conclusions

The DSC measurements showed two temperature ranges with a high melting rate that could affect the accuracy of the ultrasonic velocity measurements when used for classification purposes. Ultrasonic velocity decreased with the temperature increase due to the fat melting which could be used to assess the %MEF from ultrasonic measurements. The %MEF is directly linked to the quality attributes and, consequently, non-destructive ultrasonic measurements could be used to assess the quality of Iberian hams. The thermal history can affect the structure and solid/liquid ratio of the fat and, consequently, the ultrasonic velocity. Using ultrasonics for classification and quality assessment means that the temperature ranges where the thermal history affects velocity must be avoided.

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