ABSTRACT

Dry-cured meat products from Iberian pigs are highly appreciated by consumers due to its high sensory and nutritional quality. The increasing demand of these products leads to the industry to a continuous improvement, not only seeking for new formulations or formats but also by process optimization and through the implementation of new preservation and distribution techniques. The final quality of the dry-cured meat products depends, among other factors, on both the raw matter used and the processing conditions. In this regard, the **fat** is one of the main components affecting quality attributes, fat properties being linked to **the animal breed and rearing system** used. Thereby, the fat content, as well as its physical state (liquid and/or solid), will influence the sensory properties and in particular the texture of the dry-cured meat products. Therefore, it results matter of relevant research to evaluate the **fat content, state and source** in order to evaluate the quality of the dry-cured meat products.

Nowadays, the market of whole pieces of Iberian ham for consumer hand slicing is being replaced by mechanically sliced products which are vacuum packaged before their sale. This type of packaging of dry-cured ham needs the additional use of conventional preservation technologies such as **cold storage** or the use of new technologies, such as **high hydrostatic pressure treatments** (HPT). Thereby, the product self-life is extended, but the product can undergo some changes affecting relevant quality attributes, such as textural properties. As a consequence, the characterization of the changes underwent by dry-cured meat products subjected to preservation technologies, like HPT or cold storage, is also outstanding.

Up to now, several instrumental and sensorial techniques have been used to characterize the fat quality parameters of dry-cured meat products, such as texture, source, content and state at a specific temperature. Notwithstanding, these conventional analytical techniques not only require the sample destruction but also are complex and high time consuming. Thereby, the search for **non-destructive**, easily implemented, on-line and low-cost techniques is interesting from both scientific and technological point of view.

Low-intensity **ultrasound** is considered a non-destructive, fast, easy and non-expensive analytical technique, which can be used as an accurate tool for food characterization and be implemented in processing systems aiming to its optimization. The measurement of the ultrasonic velocity has been reported as an accurate technique to estimate in a non-destructive way the composition and changes underwent of a wide range of foodstuffs, as well as the raw materials used.

Considering the aforementioned, the main aim of this Thesis was to evaluate the reliability of ultrasound for non-destructive characterization of dry-cured meat products from Iberian pigs according to the fat source, content and textural properties. Likewise, its ability to identify the changes underwent by these products after cold storage and high pressure treatment has been also addressed.

In order to reach the overall aim, five different studies have been performed. In the first one, the objective was to test the reliability of ultrasound to characterize the crystallization process of Iberian lard during storage at low temperature and develop a mathematical model to predict the solid fat content. For that purpose, the ultrasonic velocity in Iberian lard was measured during the isothermal storage (11 days) at different temperatures (0, 3, 5, 7, 10 y 20 ºC). In addition, the lard's thermal behavior was studied by Differential Scanning Calorimetry (DSC) and fat hardness was measured by puncture tests. Experimental results showed two steep increases of the ultrasonic velocity during the isothermal storage. This behavior was linked to the crystallization of triacylglycerols with different level of unsaturation, which was also confirmed by the DSC analysis. Textural measurements followed the same pattern, being observed two increases of the hardness. On the other hand, the two-step crystallization pattern was well described by a mathematical model based on the Avrami's equation, being established a good correlation between the ultrasonic velocity and the isothermal storage time (% var>99.9). In addition, a mathematical model was developed to estimate the evolution of the percentage of solid fat content during the isothermal storage, considering the two steep increases observed in the crystallization. Therefore, the ultrasonic measurements allowed adequately characterizing the crystallization pattern of Iberian lard

during the isothermal storage at low temperature, which gave way to the following work.

The second study was carried out with backfat (adipose tissue) obtained from Iberian pigs reared in two different systems (montanera and cebo). The objective was to evaluate the use of ultrasound to assess the textural changes of the adipose tissue during cold storage, as well as to discriminate between both fat sources. For that purpose, the ultrasonic velocity measurement was carried out during 20 days at different temperatures (0, 2, 5, 7 and 10 ºC). During this isothermal storage, the fat hardness was measured by puncture tests. The fatty acid profile and the thermal behavior of both fats were analyzed by gas chromatography and DSC, respectively. Thus in both fats, two steep increases of the hardness were found during the storage at low temperature, following the same trend already observed in the previous study with lard. The textural changes were attributed to the crystallization of triacylglycerols with different level of unsaturation, which was also observed in the DSC curves where two crystallization peaks were found. On the other hand, cebo fat was significantly harder ($p<0.05$) than montanera during the cold storage, which additionally contributed to discriminate between both fat sources. The hardness difference was linked to a higher content of saturated fatty acids in cebo than montanera. Likewise, ultrasonic velocity also showed two steep increases during the crystallization at low temperature, and allowed discriminating between both fats. Thereby, once crystallization of the most saturated triacylglycerols was finalized, the ultrasonic velocity was 2.8 % higher in montanera than cebo, increasing this value to 5.2 % when the triacylglycerols were fully crystallized. Finally, the evolution of hardness and ultrasonic velocity during storage was well described from the Avrami's mathematical equation. Therefore, the experimental results highlighted that ultrasound can be considered a reliable technique in order to non-destructively characterize and discriminate the crystallization of different Iberian backfats, as well as to identify the textural changes underwent by the fatty tissue during cold storage.

From the two previous activities, the reliability of the use of ultrasound to monitor the crystallization process of Iberian fats, which involves textural changes, during cold storage was confirmed, as well as its ability to discriminate

between different fat sources. These previous knowledge allowed addressing the use of ultrasound for estimating the fat content and state in dry-cured meat products, as well as for discriminating according to the fat source used in the formulation. Dry-cured meat products contain lean and fatty tissues and are subjected to a dry-curing process, what makes a difference with the two previous works where fat (lard and montanera and cebo backfats) was almost the unique component. In a first approach, experiments were conducted with model systems formulated with lean and fat (ground and stuffed), this procedure provides a better compositional homogeneity than those found in other dry-cured meat products, such as Iberian ham. Thereby, the third work was carried out with dry-cured sausages formulated with different fat sources and contents. In this case, the objective pursued was to evaluate the usefulness of the use of ultrasound to determine the fat content and state (liquid/solid), as well as to discriminate the sausages according to the fat source used in the formulation. Different dry-cured sausage batches were elaborated with different fat sources (montanera and cebo backfats, lard and sunflower oil) and contents (from 3 to 17 %). The ultrasonic velocity was measured at different temperatures (2, 6, 10, 15, 20, 25 and 30 ºC), the fatty acid profile was determined by gas chromatography, the melting behavior by DSC and finally, the chemical composition was also analyzed. Experimental results showed that the fatty acid profile, and namely the saturation level, affected the melting behavior (temperature and enthalpy) from which the sausage batches could be distinguished. On the other hand, a decrease of the ultrasonic velocity was observed for samples with high fat content when temperature increased from 2 to 30 ºC. The velocity drop was linked to the fat melting observed at this temperature range in the DSC curves. Therefore, the higher the temperature, the greater the melted fat and the lower the ultrasonic velocity. A significant ($p<0.05$) linear relationships were found between the melted fat content and the ultrasonic velocity, which were statistically ($p<0.05$) different according the fat source used in the formulation. Thus, ultrasound measurements involved an accurate estimation of the melted fat content in sausages elaborated with different fat sources and allowed the correct discrimination of the batches.

Finally, the temperature dependence on the ultrasonic velocity made possible an accurate estimation of the composition, and namely of the fat content (% var 96.1), by using the measurement of the ultrasonic velocity at 2 and 25 ºC and applying a semi-empirical model. These results highlight the feasibility of using ultrasound as a non-destructive tool for distinguishing the fat source used in the formulation of dry-cured meat products formulated with lean and fatty tissues, as well as their fat content and state. These aspects largely contribute to a better classification of the dry-cured meat products.

Once it was confirmed that ultrasound could be considered an adequate technique to non-destructively characterize not only the Iberian fat but also the resulting dry-cured meat products formulated by mixing it with lean tissue, the following work addressed the evaluation of this technique on the dry-cured Iberian ham where a greater compositional heterogeneity exists. Thus, the fourth section of this Thesis was focused on testing the feasibility of using ultrasound not only to characterize Iberian ham composition and texture, but also to identify how this product is altered when it is subjected to preservation technologies. In this regard, the feasibility of using ultrasound to estimate the effect of both high pressure treatment and cold storage on the textural properties of Iberian ham was addressed. For that purpose, ultrasonic measurements were carried out in sliced, stacked and vacuum packaged Iberian ham. In a first batch, the effect of high pressure treatment (600 MPa/6 min) was studied from ultrasonic measurements carried out in the range from 2 to 25 ºC (2, 6, 10, 15, 20 and 25 ºC) in two different zones of the ham (punta and babilla). While in a second batch, the effect of the cold storage (6 ºC) was addressed by measuring the ultrasonic velocity at this temperature at the beginning and end of the storage (120 days). In both cases, the ultrasonic measurements were carried on the package surface (direct contact with the plastic film) and were completed with textural analysis. Experimental results showed that the fat content influenced significantly ($R>0.80$; $p<0.05$) on the ham hardness. On the other hand, a significant ($p<0.05$; $R>0.96$) linear relationship was found between the ultrasound velocity and temperature. Thereby, the higher the temperature, the lower the ultrasonic velocity, being greater the velocity drop in those zones with high fat content (-2.5 m/s $ºC^{-1}$ for samples with fat content >36. 34 % w.b.). This fact was linked to the

decrease in the samples of the fat solid/liquid ratio, which increases the liquid fat content and reduces the hardness and as consequence, the ultrasonic velocity. Thereby, a significant ($p<0.05$; $R>0.84$) linear relationship was established between the sample hardness and the ultrasonic velocity, from which it was observed that the higher the hardness, the faster the ultrasound propagation.

Regarding the influence of the preservation technologies, the high pressure treatment involved structural changes increasing the ham hardness from 12 % in babilla (fat content < 21.07 % w.b.) to 18 % in punta (fat content >36.34 %), the difference observed in both ham zones could be linked to the higher fat content in punta than babilla samples. This textural change involved a change of the ultrasonic velocity, which increased 13 and 9 m/s in punta and babilla samples, respectively. Therefore, ultrasound contributed to adequately characterize the textural changes underwent by sliced vacuum packaged Iberian ham after high pressure treatment, as well as to discriminate the packages of two different zones of the Iberian ham. Finally, the cold storage brought about a significant ($p<0.05$) increase of the sample hardness (average 1.10 N) and the ultrasonic velocity (average 70 m/s), which were linked to the fat crystallization and therefore, to the increase of the fat solid/liquid ratio. The aforementioned results highlight that the non-destructive ultrasound measurements allowed evaluating the textural changes occurred during the storage at low temperatures of sliced vacuum packaged Iberian ham. Therefore, ultrasound can be considered a useful tool for non-destructive control and evaluation of the fat content and texture of vacuum packaged Iberian ham, as well as to characterize the modifications occurred in this product as a consequence of high pressure treatments or cold storage.

In this Thesis, it has been stated that the direct contact ultrasonic technology carried out allows for the non-destructive evaluation of the sliced vacuum packaged dry-cured meat products. However, these measurements require the use of a coupling material to assure a good contact between the transducer and plastic film, and so, the coupling material has to be first placed and afterwards removed. In this regard, the use of air-coupled ultrasound techniques may provide a time and resources saving in the ultrasonic analysis of these meat products. On the other hand, in this Thesis, the ultrasonic measurements have been carried out with a 1 MHz transducer aiming to measure

macroscopic properties, such as composition or texture, in a relatively wide area. The use of higher frequencies would provide a better focusing of the measurements, which would contribute to analyze some interesting aspects, like the internal structure of the meat tissues. Thus, a fifth work was carried out aiming to implement other kind of air-coupled ultrasound techniques and evaluate its application in the characterization of dry-cured meat products. By one hand, air-coupled and contact ultrasound measurements were carried out on sliced vacuum packaged Iberian ham, being used different package thicknesses. The air-coupled measurements allowed the simultaneous measurement of the ultrasonic velocity and sample thickness, without an additional instrument to measure the sample height. The average ultrasonic velocity for air-coupled measurements was 1846 m/s, while this figure was of 1842 m/s in direct contact ultrasound measurements. The deviation between both techniques was mostly linked to the structural and compositional heterogeneity of ham samples. Notwithstanding, an adequate correlation was found between both measurements in both the package thickness and ultrasound velocity. Therefore, the reliability of the air-coupled technique was assessed for ham characterization, which makes easier and faster the measurements. By the other hand, scanning acoustic microscopy analysis was carried out in dry-cured ham (thickness 1 mm) and chorizo sausage (thickness 5 mm) at 6 ºC. The obtained images showed the existence of intensity differences in the signal reflection on the sample, being found two different regions. The region with greater reflection intensity corresponded with the lean tissue, while the low intensity was found on the fatty tissue. The measurement presented a spatial resolution of 1 mm on the sample surface (x and y axis) and 300 μm on the depth (z axis). These measurements allow the analysis of samples opaque to the light, like the dry-cured meat products, but where ultrasound can pass through it. Thereby, the acoustic microscopy allowed the characterization of the different tissues existing in the dry-cured meat products, which can facilitate its classification according to its fat content and microscopic distribution. The implementation of both acoustic techniques allowed the non-destructive characterization of dry-cured meat products, improving and optimizing the processing and quality assessment.

Finally, it can be concluded in this Thesis that the ultrasound measurements allowed characterizing the composition and texture of dry-cured meat products, as well as discriminating between different batches according to the fat content and source. Moreover, ultrasound emerges as a reliable, fast and low-cost tool to monitor the textural changes brought about by high pressure treatments and cold storage, which can contribute to the improvement and optimization of the dry-cured meat products elaboration and distribution. The use of air-coupled ultrasound techniques would contribute to shorten the measurement time, removing the need of coupling material and providing an additional measurement of sample thickness.